RHEOLOGICAL CHARACTERIZATION OF FOUR KANSAS HARD RED WINTER WHEAT FLOUR-WATER DOUGH SYSTEMS

by

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Abstract

Kansas is the top wheat-producing state, providing about 1/5 of the yearly wheat crop in the U.S. Therefore, the quality of wheat grown in Kansas is a primary concern of the milling and baking industry. Quality of wheat flour is measured through analysis of protein, dough rheology, and baked product characteristics. This study characterized four commonly-grown Kansas hard red winter wheat cultivars chosen to span the largest possible range of protein contents and baking qualities. Flour protein content and moisture was determined by NIR, and composition was assessed using SE-HPLC. Dough empirical rheological and mixing characteristics were determined by farinograph and mixograph recording dough mixers. Rheological measurements of fundamental dough properties were performed through strain sweeps, frequency sweeps, temperature sweeps, creep-relaxation, and stress relaxation on a rheometer. All cultivar flours were baked to assess baking quality through evaluation of loaf volume, texture profile analysis (TPA), C-cell, and x-ray microtomography (XMT).

Overley and Karl 92 have the two highest protein contents, respectively, and are not significantly different in percent of unextractable polymeric protein (UPP). Generally, cultivars with higher protein and percent UPP (Overley and Karl 92) gave larger loaves, much more expanded air cells, thinner cell walls, greater void fractions, and better mixing properties. Lower TPA firmness was found for Overley, corresponding with its larger XMT fragmentation index, existence of large air cells, and high void fraction. In contrast, 2137 gave the lowest XMT fragmentation index, low void fraction, larger cell wall thicknesses, and a significantly firmer ($P < 0.05$) crumb structure. Protein content was found to have an inverse relationship with the elastic nature of dough in fundamental rheological measurements since small amplitude measurements generally do not give good correlations to baking quality. Stress relaxation gave the most useful information about flour quality through its relaxation spectra. Flours with high total polymeric protein percentages could be identified through their higher relaxation spectra. Starch gelatinization properties of the flours were different for RVA and rheometer temperature sweeps. All of these tests have helped characterize the four Kansas wheat cultivars chosen for this study.

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Dedication

For Brad, the best friend I could ever ask for

And to living adventurously

CHAPTER 1 - Introduction

Kansas is the top wheat-producing state in the U.S., providing nearly 1/5 of the yearly U.S. wheat crop, or 400 million bushels, with a value of 1 billion dollars annually (Kansas Department of Agriculture FAQ 2010). With such a large part of the Kansas economy and the U.S. food supply dependent on Kansas wheat, it is important that the wheat being produced is of good quality. Wheat breeders rely on empirical measures of wheat flour-water doughs and analytical bread baking techniques to characterize their cultivars and ultimately decide which varieties should be released for production. The milling and baking industry also rely on empirical measures of dough rheology to determine how a flour will work in their processes. However, empirical measures of rheology only measure bulk rheology, and analytical baking shows the final performance of a flour for breadmaking, not its processability. Fundamental rheological studies can help to show the behavior of dough under small deformations, such as those seen during dough resting and relaxation. Dough rheology is measured in stress and strain in fundamental rheology, and effects of cultivar, dough ingredients, and additives on dough at small deformations scan be studied.

The overall intent of this research is to provide better understanding of the relationship between flour composition, large deformation rheology, small deformation rheology, and end-product characteristics. The experimental plan and procedures (Figure 1.1.) were designed with the following objectives.

- Characterization of four Kansas wheat cultivar flours through proximate analysis, pasting, baking, and end-product analysis.
- Characterization of flour-water doughs for the same four cultivars through empirical rheological measures and fundamental rheological measures.
- Achievement of understanding of the interrelationship between flour composition, dough large and small rheological properties, and baked product quality.

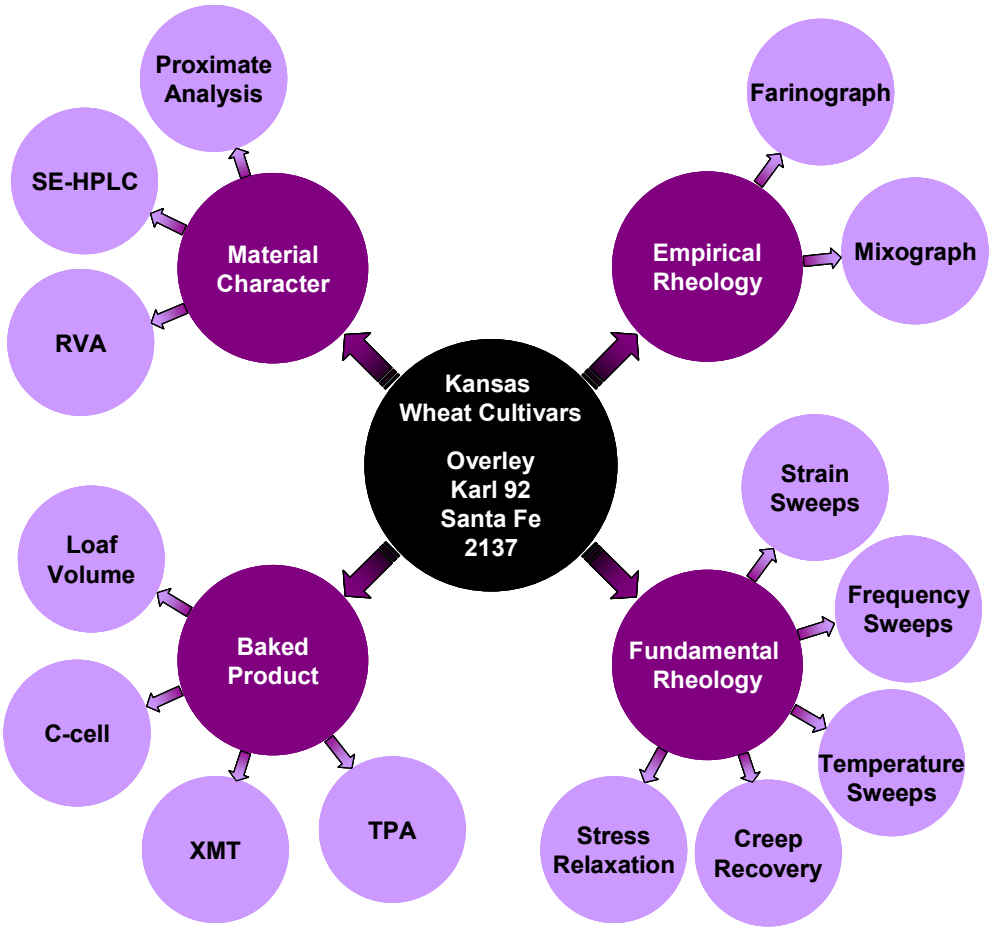


Figure 1.1 Diagram showing all facets of the wheat flours measured in this study.

1.1 References

Anonymous. 2010. Kansas Department of Agriculture Frequently Asked Questions. Available at http://www.ksda.gov/kansas_agriculture/faq/id/56. Kansas Department of Agriculture. Topeka, KS.

CHAPTER 2 - Literature Review

2.1. Rheology and the Breadmaking Process

The complexity of wheat flour dough has always presented a challenge to researchers who aim to better control its processing by characterizing its components and material properties. Originally, dough quality was determined by individual bakers' experience and physical examination. Later, empirical methods of dough characterization were developed, and most recently, fundamental techniques have furthered the understanding of dough quality and processing by studying its rheology. After many decades of studying fundamental rheometry of dough, it is recognized that quantitative description is difficult due to the inhomogeneity and complexity of wheat flour dough. Even understanding dough deformation and flow on a physiochemical basis is a formidable task, but novel products and processes will result from application of this knowledge.

2.1.1. Rheometry

Rheology is the study of the flow of materials, or more precisely, the mode of response by materials to specific deformation strains or stresses (Steffe 1996). When a deformation is applied, individual polymers will produce distinct responses to stress or strain, and measuring the differences can provide valuable information about the makeup and structure of that material. The intent of rheological characterization is to be able to compare different test methods, sample sizes and conformations, and even different samples by reproducibly measuring material properties (Dobraszczyk and Morgenstern 2003). Steffe (1996) outlines several areas in the food industry where rheological characterization is useful. These include calculations in process engineering for pipelines, pumps, extruders, mixer, heat exchangers, and other equipment, ingredient functionality evaluation for product development, quality control, relation of food texture data to sensory data, and study of rheological constitutive equations. Since rheometry can reveal so many integral characteristics of a material, it has great potential for the study of wheat flour dough and regulation of dough processing performance.

Dough consistency and material properties can be assessed both empirically and fundamentally. However, only fundamental characterization can measure dough rheology in

terms of stress and strain, thereby allowing comparison between testing methods and sample sizes. Effects of flour cultivar, ingredients and dough additives can be studied through testing. A significant amount of research has been conducted on cultivar and ingredient effects in wheat flour dough rheology (Faubion and Hoseney 1990).

2.1.2. Breadmaking Process

Wheat flour dough is a multi-component composite material with complex rheology. It is an incompressible, viscoelastic, soft-solid material that can be considered a hydrated elastomeric protein network filled with starch granules that behaves nonlinearly during large deformations (Tanner et al 2008). Primary ingredients in bread include wheat flour and water, but minor ingredients such as sugar, salt, malt, yeast, shortening, and ascorbic acid are commonly added to improve palatability and bread loaf volume and texture. Within wheat flour, there are many components. Gluten protein is made up of glutenin and gliadin proteins of different molecular weights, and the entanglements of these different proteins during mixing and their interaction with starch molecules have the greatest effect on the bread dough and final bread quality (Bloksma 1990a). Starch granules in the protein network of dough absorb water and swell at temperatures below its gelatinization temperature. Above the gelatinization temperature, (i.e. during baking) the starch granule fragments, amylose, and exudate from the disseminated starch granules forms a filamentous network and increases the system viscosity (Olkku and Rha 1978). Lipids native to the wheat flour can affect the starch gelatinization temperature by complexing with the starch amylose to inhibit swelling of the starch granule, thereby increasing starch gelatinization temperature (Morrison 1995). Along with components of flour, processing conditions are important for bread quality. The three main processing steps of breadmaking are mixing, proofing or fermentation, and baking.

2.1.2.1. Mixing

Mixing, at the macro level, is the process of combining all the ingredients, plus air, into a homogeneous dough through sufficient energy input, rates of shear and extension, torque, and mixing time (Bloksma 1990a). Development of dough and air bubble occlusion and subdivision are further processes performed during mixing (Bloksma 1990a). The entanglement of gluten proteins into an organized network and hydration of starch qualifies the development of dough.

Creation and of gas cell nuclei in the dough allows for subsequent expansion of the dough during fermentation and baking.

2.1.2.2. Proofing

Proofing or fermentation of the dough allows air cells to expand and the volume of gas to grow, and use of punching or molding can increase the number of gas cells by further dividing existing bubbles (Bloksma 1990a). This is important, because it is the last step where the number of bubbles can increase. A rise in the number of cells through subdivision often creates a smoother texture and finer crumb grain in the baked bread loaf.

2.1.2.3. Baking

The final baking process applies heat to the dough and further expansion of the dough occurs, called oven rise. Starch gelatinization and protein denaturation coupled with the reduction of the moisture content set the bread loaf structure (Bloksma 1990a). Both fermentation and oven rise are primarily due to biaxial expansion of gas cells. In oven rise, the gas cell growth is due to continued production of carbon dioxide by yeast as well as steam produced from evaporating water (Bloksma 1990a). A significant portion of the loaf volume is gained from oven rise. All of these steps have an impact on the final product quality.

2.1.3. Dough Rheology

The purpose of defining dough rheology is to have better control over each processing stage as well as the final products of the breadmaking process. This can be accomplished by relating rheological performance to product functionality during mixing, sheeting, and baking through rheological tests (Dobraszczyk and Morgenstern 2003). By understanding what the rheology of the dough is doing and how each component of dough contributes to the overall material properties, it becomes easier to manipulate the dough and get consistent, desirable results (van Vliet et al 1992). For the production of good quality bread there are three requisite rheological properties: extensibility and viscosity (which together determine dough's viscoelasticity), and strain hardening. Extensibility refers to the dough's ability to stretch and lengthen without rupture to its structure, and viscosity is the resistance to flow of the dough. Strain hardening is a more complex property; it is the increase of stress at a rate larger than proportional to the strain (van Vliet et al 1992). Extensibility must endure throughout baking to

prevent gas cell membranes from fracturing prematurely, viscosity of the dough must be high enough to arrest gas cell ascension (Bloksma 1990b), and strain hardening must surpass a certain level to ensure proper retention of gas cells that expand during fermentation and baking. Extension of long molecules, such as glutenin, from their native state into elongated conformations is achieved through input of a sufficient amount of mechanical energy into the dough, thereby giving dough its good machinability and gas retention properties (Bloksma 1990a). Each of these rheological properties is described below.

2.1.3.1. Strain Hardening

Biaxial extension occurs during the fermentation and baking phases of breadmaking. During these periods, the dough must remain extensible enough to allow further expansion of gas cells and elastic enough to prevent failure of the loaf structure. The ability of a cell to undergo biaxial extension and not rupture is called strain hardening and has a large influence on the stability of gas cells (Sroan and MacRitchie 2008). It occurs as dough stress increases more than the strain at constant strain rate and increasing strain, and it must be exhibited up to high strain levels to provide adequate protection against fracture for good quality bread (van Vliet et al 1992; Dobraszczyk 1999; Kokelaar et al 1996). This process can be described by the Hencky strain (Eqn. 1). The phenomenon of strain hardening is especially important because it provides the dough a mechanism that allows for expansion during proofing and oven spring without catastrophic fractionation within the dough. Without strain hardening, thin areas of a developing dough would get thinner during bubble growth after reaching a maximum strain and ultimately fatally fracture the dough structure and cause collapse of the loaf.

$$\varepsilon_h = \ln\left(\frac{L_1}{L_0}\right) \qquad \text{[Eqn. 1]}$$

where L_0 is the length of the test piece before testing, and L_1 is the length after testing

2.1.3.2. Viscoelasticity

Viscoelasticity exists when a material has an intermediary rheological consistency between a viscous liquid and an elastic solid. Dough viscoelasticity is especially important because it has a great influence over the dough machinability, texture characteristics, and final product stability (Uthayakumaran et al 2000). Viscoelastic qualities have been attributed mainly to the gluten protein fraction of dough, specifically the glutenin to gliadin ratio. Although

determinations of the viscoelastic properties are performed through fundamental and empirical measurements, only suitable fundamental methods can specifically correlate the rheological viscoelastic behavior during processing with the material makeup (Bloksma 1990b; Walker and Hazelton 1996). While gluten has been credited with creating the viscoelasticity found in dough, it cannot form a viscoelastic dough without significant input of energy from mixing (Kilborn and Tipples 1972).

2.1.4. Mixing Effects on Rheology

Since many gluten network developments that affect final product quality occur during mixing, it is not surprising that a great deal of the research on dough has involved the relationship between mixing, rheology, and baked bread quality (Dobraszczyk and Morgenstern 2003). Mixing combines the bread ingredients and causes a multitude of concurrent processes that "develop" the dough. The gluten matrix is formed by the hydration of proteins and through mixer shear. As the dough develops, its consistency increases and becomes less lumpy, transforming it into a homogeneous mass (Bloksma 1990a). High shear rate dough mixers aid hydration of flour particles through exposure of the particle surfaces to water and create gas cell nuclei that provide the aerated structure of bread. Adequate speed and torque in mixing is required for dough to reach its maximum consistency; at this point, the dough is said to be mixed to its optimum. The optimum mixing parameters vary depending on the type of wheat flour and the mixer and mixing conditions used. Dough strengthens and stiffens until a maximum point, past which the consistency decreases continually. The break down of the dough results from mixing past optimum and produces a dough that is sticky, wet, and cohesive. The development of dough can be considered at the molecular level as well. Molecular bonds form and rearrange during mixing to develop dough. Molecular weight of proteins involved, number and strength of entanglements and crosslinks, and their proximity to one another all affect the viscoelastic behavior of the gluten phase (Cornec et al 1994; Song and Zheng 2007).

2.1.5. Measuring Rheological Effects in Dough

The great number of variables included in the baking process that affect proofing and baking performance of dough (wheat flour composition, water absorption, mixing, and rheological properties) created the need for bakers to quantitatively describe the properties of the dough at each of the processing stages. In-depth studies on wheat flour dough rheology have

been conducted for many decades, and instrumental measures have been used since the 1930s (Tronsmo et al 2003b). Using rheology to study bread dough is relatively new to the centuries-old baking industry, but dough properties have been a subject of investigation in rheological characterization almost since its inception (Newberry et al 2002). Rheological tests were devised for the purpose of simulating processing in order to reveal how flow behavior relates to material composition, as well as describing mechanical properties of the dough for quality control purposes. Data obtained from rheological characterization can be useful in the development of food products through ingredient selection, product improvement and optimization, choosing and optimizing manufacturing techniques, and developing packaging and storage methods.

The use of both small and large deformation measurement of dough is critical for a complete understanding of dough. Large deformation methods simulate stress-strain conditions found in commercial processing and therefore can disclose food textural properties under those conditions as well as indicate final breadmaking quality (Dobraszczyk and Morgenstern 2003 Davidou et al 2008). Small deformation techniques are most useful for exposing viscoelastic properties of dough (Angioloni and Collar 2009). The empirical descriptive techniques are generally better accepted than fundamental methods. Although basic rheometry has provided a massive amount of important knowledge and experience in dough rheology, fundamental techniques have been considered to be time-consuming and labor intensive. Researchers also observed that fundamental techniques often do not provide good correlations with final bread quality (Weipert 1990). Yeasted doughs lengthen and complicate fundamental rheological testing (Newberry et al 2002). To minimize irreproducible results due to material variability, primarily nonyeasted doughs are used in rheological testing.

2.2. Ingredient Effects

Wheat flour dough can be described as a filled elastomeric network composed of a gluten-water phase structure supporting starch granules possessing the ability to interact with both themselves and the elastomer network, all enveloped by a continuous water phase (Amemiya and Menjivar 1992; Edwards et al 2002; Tronsmo et al 2003b). The primary factors affecting the rheological properties and workability of dough are flour components (gluten's glutenin to gliadin ratio and starch) and their arrangements formed during mixing (Bloksma 1972; Uthayakumaran et al 2000; Larsson et al 2000; Collar et al 2007) and other integral dough

components (starch, water, yeast, and air). Some ingredients have large effects on flow properties even though they are present in small amounts. Pentosans, salts, shortening or other fats, dough conditioners, emulsifiers, and enzymes all have the potential to alter dough rheology in different stages of dough processing.

Wheat flour-water dough is often used for research, especially when studying rheology, to avoid complications of yeast and effects of other constituents during testing. While Newberry et al (2002) reported that yeasted and nonyeasted doughs performed similarly in rheological experiments, Connelly and McIntier (2008) found significant differences. Using nonyeasted doughs without salt, sugar, fat, or malt simplifies the rheological characterization. Typical doughs consist of 40-45 g starch, 6-10 g protein, and 45-50 g water per 100 g of dough. Having a simpler dough makeup is critical because dough rheology can be affected by water, starch, and protein content, wheat genetics, mixing procedures, temperature, and resting time (Bagley et al 1998; Tanner et al 2008). When fewer ingredients are involved, effects seen in the dough's rheology can be better interpreted in terms of the flour constituents. Each of the constituents in dough has a unique structure and function. Quantifying the flour constituents can be very useful for dough evaluation. One often-used method of determining a flour's gluten makeup is by size exclusion high performance liquid chromatography (SE-HPLC).

One of the most commonly used way to evaluate starch pasting is through rapid viscoanalysis (RVA), which determines the gelatinization temperature of a flour and its peak viscosity and can be helpful information when explaining flour rheological behavior during temperature ramps at small strains. To adequately understand how the dough structure is formed and interacts, it is helpful to know about the structure of each individual part of the dough and quantify the constituents of the flour.

2.2.1. Gluten

It is widely recognized that the gluten in wheat flour is what gives dough its unique viscoelasticity. Gluten is made up of a mixture of monomeric and polymeric proteins that include gliadin, glutenin, albumins, and globulins. Since gluten protein-protein interactions are considered the most influential force acting upon dough rheological properties in non-linear large deformations (Kasarda 1988; Kokelaar et al 1996; Kieffer et al 1998; Sliwinski et al 2004b), much of the research conducted on wheat flour dough has concentrated on overall gluten and its

fractions of gliadin and glutenin. Gluten proteins in general have been closely linked to loaf volume and bread form ratio (height to width ratio) (Tronsmo et al 2003a). This close relationship between protein, dough rheology, and baking quality concerns protein composition as well as the overall amount of protein (MacRitchie 1992). Therefore, it is critical to understand the various fractions of gluten and their function in dough.

2.2.1.1. Monomeric Proteins

Monomeric gluten proteins that are soluble in alcohol-water solution are called gliadins (Kasarda 1989). Gliadins have a molecular weight range of 30,000 to 80,000 and are known to provide extensibility and contribute to the viscous nature of dough, as well as acting as a plasticizer in gluten (Cornec et al 1994; Khatkar et al 1995; Tronsmo et al 2003a; Tronsmo et al 2003b; Sliwinski et al 2004a). There are four types of gliadins: α-, β-, and γ-gliadins, which all have disulfide bonds that intramolecularly link their polypeptide chains, and ω-gliadins which are missing cysteine in their structure and cannot form disulfide bonds (Kasarda 1989). It has been shown that increases in the total protein content of a flour increases the proportion of monomeric proteins (Gupta et al 1992). The classes of monomeric proteins with lower molecular weights (20,000-30,000) are called albumins and globulins, and together they comprise about 10% of the protein in wheat flour.

2.2.1.2. Polymeric Proteins

The link between the number of disulfide bonds and sulfhydryl groups in protein and the stability of the protein network and resulting rheological properties has been widely accepted (Dong and Hoseney 1995). By creating highly crosslinked and entangled structures, polymeric proteins provide most of the elasticity found in wheat flour dough (Cornec et al 1994; Khatkar et al 1995; Tronsmo et al 2003b). Polymeric proteins consist of mainly glutenins, and they are distinctly different from monomeric proteins because they have intermolecular disulfide bonds between subunits as opposed to only intramolecular disulfide bonds. Glutenins vary greatly in molecular weight because they are made up of linked subunits to create polymers that can differ in length (Sliwinski et al 2004a). Polymeric proteins can be categorized into high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). HMW-GS can have molecular weights in the range of 80,000-120,000, while the two classes of LMW-GS have molecular weight ranges of 40,000-55,000 and 30,000-40,000. It has been

shown that better breadmaking quality wheats have higher amounts of HMW-GS (Huebner and Wall 1976; Gupta et al 1993; Sliwinski et al 2004a), and HMW-GS influences dough rheological properties (Uthayakumaran et al 2002). LMW-GS have also been shown to have a positive effect on dough's mixing stability (Edwards et al 2003). Although wheat flour has an average HMW-GS to LMW-GS ratio of about 3:1, there is variability among cultivars, and so as the ratio increases, the molecular weight distribution shifts toward higher molecular weights (MacRitchie, 1992). Having a higher ratio of HMW-GS to LMW-GS gives flour better mixing and baking properties (Kasarda 1989).

There is one more fraction of polymeric protein that was defined by Gupta et al (1993) called unextractable polymeric protein (UPP). This fraction cannot be solubilized without sonication, and is made up of large molecular weight polymeric proteins. Good correlations were made between percent UPP of total polymeric protein, which is essentially a determination of polymeric protein molecular weight distribution, good dough properties (Gupta et al 1993), and eventually good breadmaking qualities (Tronsmo et al 2003a). Figure 2.1 shows the different fractions of protein in SE-HPLC, and it shows how UPP is determined by subtracting the first peak of extractable protein (orange peak) from the first peak of total polymeric protein (red peak).

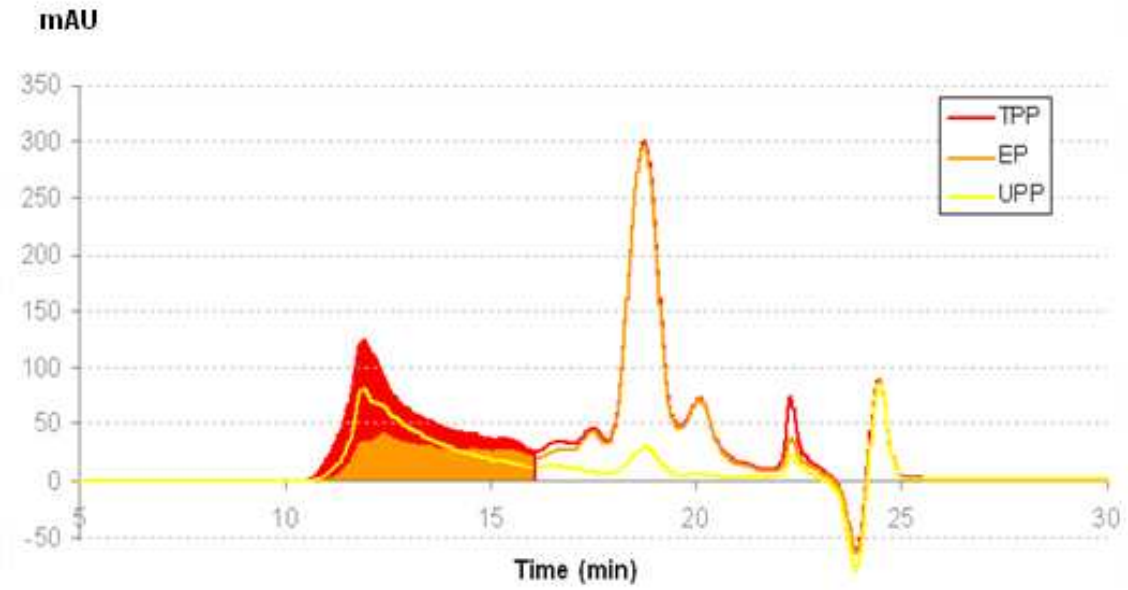


Figure 2.1. Size exclusion high performance liquid chromatography analysis of proteins: Total polymeric protein (TPP), extractable protein (EP), and unextractable polymeric protein (UPP).

2.2.1.3. Glutenin to Gliadin Ratio

Dough relies upon the ratio of glutenin and gliadin to create the correct balance of viscous and elastic properties to make workable dough and good bread. The glutenin to gliadin ratio is the primary factor influencing gluten dough rheological behavior at constant protein content (Jansssen et al 1996c), and several observations have been made about dough properties by altering it. Increases in the glutenin to gliadin ratio have been known to cause increased mixograph mixing time and tolerance to overmixing, increases in resistance to extension in uniaxial extension tests, and decreased extensibility (Uthakumaran et al 1999; Sliwinski et al 2004a). Large deformation measurements provide valuable information about the viscoelasticity of dough, which can be used to determine if the ratio of glutenin to gliadin is appropriate for the application for which the flour is to be used.

2.2.2. Starch

Starch is a significant portion of wheat flour volume (60-80%), so it is important to understand its behavior in dough. Although dough is considered a filled elastomer network, it has long been recognized that starch plays an important role in the viscoelastic and water absorption properties of dough (Hibberd 1970b), especially with changes in temperature as is typically found during baking. While it is more susceptible to amylase activity, damaged starch in flour is desirable to a certain extent because it increases water absorption and aids in proofing by swelling and absorbing more water than intact starch during increases in temperature. Wheat starch gelatinization occurs at about 60 ºC, but it could vary ±10 ºC depending on the sensitivity of starch, amount of available water, and presence of solutes or amylase activity (Dogan 2002; Salvador et al 2006). During gelatinization, the starch granule continues uptake of water until granules that are fully hydrated rupture and increase the dough viscosity by forming a starch network (Olkku and Rha 1978). Starch determines flour pasting characteristics, which can be measured by rapid viscoanalysis (RVA). Parameters measured by RVA are shown in Figure 2.2 and include pasting temperature (PT, starch gelatinization temperature), peak viscosity (PK, hot paste maximum viscosity), trough (TR, minimum viscosity of the flour after heating), breakdown (BD, difference between the PK and TR), and final viscosity (FV, viscosity after cooling).

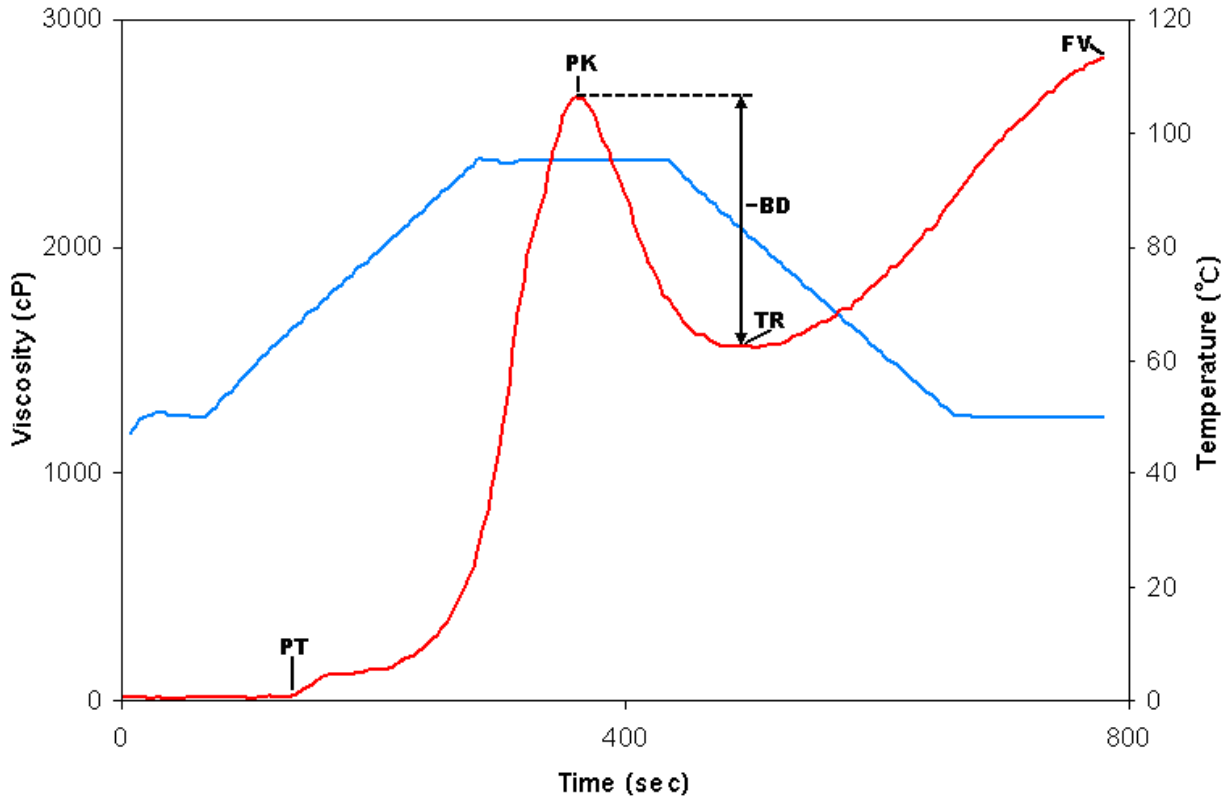


Figure 2.2. Typical Rapid visco-analysis pasting curve (red) with superimposed temperature profile (blue), showing common measurements.

2.2.3. Water

Water plays a very essential role in the rheology of wheat flour dough. By creating a continuous water phase in the dough and hydrating both the protein and starch, it acts as a lubricant and allows protein to entangle and form a strong network and swells starch. Water is essential to this process because it can donate hydrogen ions and form hydrogen bonds to facilitate protein-protein and protein-starch interactions that are not able to occur otherwise. Just like protein and starch, there is an ideal amount of water for dough, but the amount of water depends on the amounts and types of protein and starch contained in the flour. Too little water will leave the gluten under-hydrated and the dough unable to develop properly during mixing, and too much water results in a sticky dough because water acts as a lubricant and allows the viscous component of dough to dominate. Water reduces the dynamic moduli of dough during rheological testing because it has a lubricating effect that increases dough relaxation (Masi et al 1998). Sliwinski et al (2004a) found a positive correlation between flour gluten content and

water addition in the resulting dough which indicated that a larger water addition allowed more extensibility in the dough.

2.2.4. Molecular Interactions

There are three levels of resolution commonly used to depict the interactions and structure of wheat flour dough: Macroscopic (greater than 1 mm), microscopic (greater than 1 μm), and molecular (greater than 0.1 nm). Looking at dough from a macroscopic level, it appears as a homogeneous mixture comprised of a distributed gas phase within the continuous aqueous dough phase (Amemiya and Menjivar 1992). At the microscopic level, the continuous dough phase appears inhomogeneous, and can be seen as a hydrated protein network containing the dispersed solid starch granules. On the molecular level, the dough is revealed to have multiple coexisting phases that consist of a continuous crosslinked gluten phase with adsorbed lipids and a continuous "free" water phase containing starch granules and dissolved soluble proteins, lipids, and carbohydrates (Bloksma 1990b).

In wheat flour, gluten proteins are randomly oriented and have only weak non-covalent bonds between them. When water is added and mechanical mixing action is applied, the gluten protein chains unfold, allowing them to entangle and cause a large amount of their length to overlap and form covalent and noncovalent bonds to create a network (Amemiya and Menjivar 1992). There are three main types of molecular interactions that occur in dough: protein-protein, starch-protein, and starch-starch interactions.

The three-dimensional gluten network is the basis for dough development and gas retention, and it is connected by protein-protein interactions in the form of covalent disulfide bonds that cross-link the network and increase gluten protein size to enable entanglements and noncovalent bonds (hydrogen bonds, van der Waals interactions, hydrophobic interactions) that work at shorter range (Amemiya and Menjivar 1992; MacRitchie 1992). The starch granules are able to form a continuous network through starch-starch interactions when surrounded by the "free" water phase and also interact with the protein network (Amemiya and Menjivar 1992).

Small and large deformation rheological measurements on dough give very different results because the amount of strain applied determines which interactions will be dominant. Rheological measurements at small deformations often do not give good correlations with final bread quality because the interactions occurring in the dough cannot be resolved (Amemiya and

Menjivar 1992; Janssen et al 1996b; Safari-Ardi and Phan-Thien 1998; Khatkar and Schofield 2002b). At low stresses within the linear viscoelastic region, starch-starch and starch-protein interactions dominate rheology, but at large deformation and higher stresses, protein-protein interactions determine dough rheology and explain why breadmaking quality is correlated better with empirical dough quality instruments (Amemiya and Menjivar 1992; Safari-Ardi and Phan-Thien 1998; Khatkar and Schofield 2002a). This was reinforced by Tanner et al (2008) when they observed that doughs containing starch had 20 times the small-strain G′ storage modulus of gluten alone. Also, Khatkar and Schofield (2002b) determined that the starch fraction caused the non-linearity of dough at most strains because gluten-starch blends had much narrower linear viscoelastic ranges than gluten alone. Since starch-starch interactions are primarily hydrogen bonding and van der Waals forces, which are both short-range interactions, they will be more significant when smaller strains and deformations are applied to the dough (Tronsmo et al 2003b).

Protein has a large effect on rheology in heating until the starch gelatinization point is reached, then the gelatinized starch has the dominant role in rheology (Weipert 1990; Chiotelli et al 2004). This is due to the increased starch swelling at its gelatinization temperature and starch granule rupture to form a more viscous gel from starch molecules freed from the granules.

2.3. Empirical Measures of Rheology

The first instruments developed for assessment of dough quality were empirical because they evolved from the traditional methods of dough quality assessment which involved bakers kneading and stretching the dough by hand. Empirical tests such as the farinograph, mixograph, extensograph, and alveograph are used in industrial and laboratory settings to perform quality control analyses. They are simple to use, rugged instruments that provide information about dough performance during processing. Due to the nature of empirical tests, the descriptive test results vary by instrument, size, and geometry of the sample and analysis conditions. Large amounts of data are disregarded because only a single point from a range of data is used to determine a parameter, and then that parameter is correlated to performance (Wikstrom and Bohlin 1996; Dobraszczyk and Schofield 2002), which disallows any other extrapolation of the data to conditions other than those used for testing (Dobraszczyk and Morgenstern 2003; Tronsmo et al 2003a; Tronsmo et al 2003b). Often the testing instruments do not control strain

and strain rate and have undefined or variable sample geometries, and therefore they lack fundamental units of measure, making comparisons with processing performance or between different instruments not possible (Dobraszczyk and Morgenstern 2003).

2.3.1. Recording Mixing Instruments

Recording dough mixers are the most basic type of empirical testing instrument. They measure torque generated on the mixing blades and can monitor gluten network formation during dough development (Collar et al 2007). The farinograph and the mixograph are the two most common types of recording dough mixers.

2.3.1.1. Farinograph

The farinograph was invented in the 1930s—the first of the physical dough testing instruments (Janssen et al 1996c; Walker and Hazelton 1996) - and it is the most popular of the empirical rheological instruments (Mondal and Datta 2008). The farinograph is one of the most widely used recording dough mixers. The two Z-shaped blades of the farinograph mixer rotate at constant speeds and subject the dough to mixing at constant temperature. The farinograms (Figure 2.3) generated from testing a flour are analyzed to obtain quantitative information on arrival time (time required for the top of the curve to cross the 500 Brabender Unit (BU) line), peak time (time required for dough to reach maximum consistency without breakdown), departure time or time to breakdown (time lapsed until the top of the curve permanently drops below the 500 BU line), stability (time difference between arrival time and departure time), mixing tolerance index (MTI, the difference in BU's between the peak time and peak time plus 5 min), and water absorption. Farinographs are commonly used for determining the water absorption of flour, especially in industrial settings.

2.3.1.2. Mixographs

The mixograph was developed to mimic the more vigorous mixer action used in U.S. bakeries to contend with stronger U.S. wheats (Walker and Hazelton 1996). The farinograph was not adequate for the task because it had been developed for weaker European wheats.

The result of running a mixograph is the mixogram as shown in Figure 2.4. From examination of the mixogram, characteristics of the dough are measured by the parameters: peak time (T), peak height (H), developing slope (D), weakening slope (W), and angles of the

developing and weakening slopes. Peak time is when the dough reaches its maximum resistance to extension (peak height), or optimum mixing time. Mixing dough to its peak has been correlated to high loaf volume. Different wheat cultivars can produce widely varying optimum mixing times.

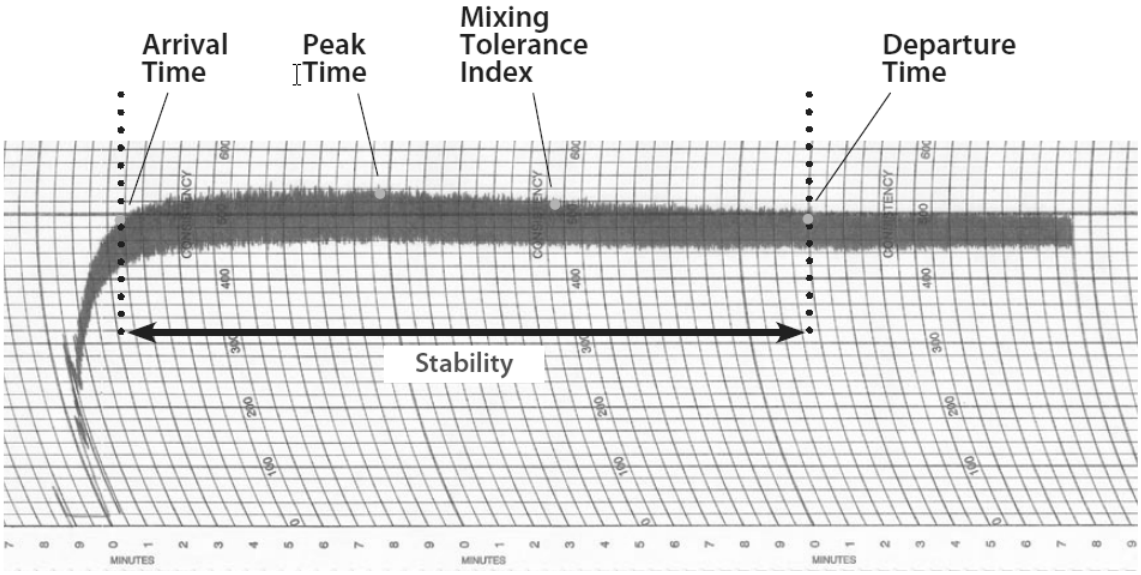


Figure 2.3. Typical farinogram of a strong dough indicating where parameters are measured (adopted from Wheat and Flour Testing Methods 2004).

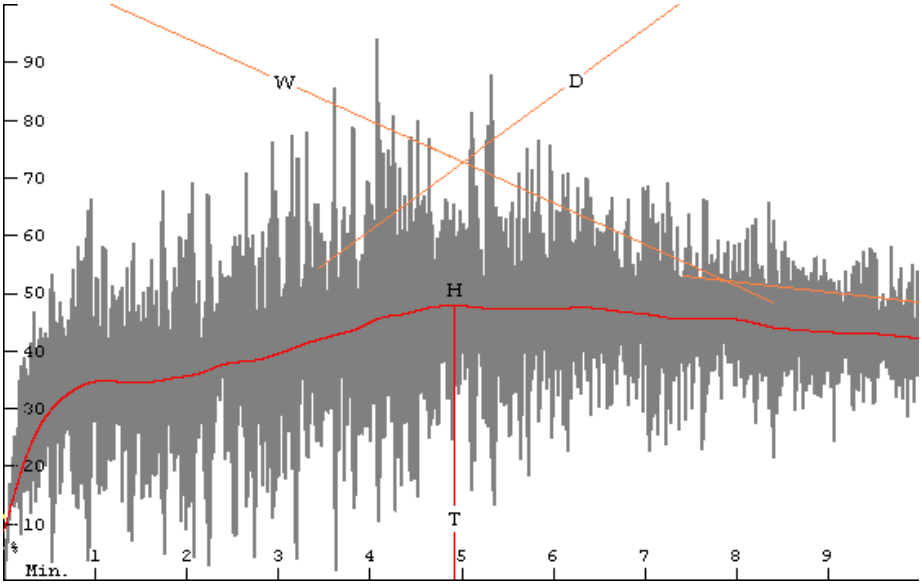


Figure 2.4. Typical mixogram of a strong wheat flour dough showing the peak time (T), peak height (H), developing slope (D), and weakening slope (W).

2.3.2. Biaxial and Uniaxial Deformation

Another category of instruments uses elongational deformations to measure stress-strain relationships in dough. These include extensional instruments like the extensograph and gluten extensibility rig (Gras et al 2000; Newberry et al 2002; Sliwinski et al 2004b and 2004c), bubble inflation techniques such as the alveograph and dough inflation system (Hlynka and Barth 1955; Charalambides et al 2002; Dobraszczyk et al 2003), and lubricated squeezing flow (Huang and Kokini 1993; Janssen et al 1996a and 1996b; Nasseri et al 2004). Extensional flow is important because it occurs in processing activities such as sheeting, bubble growth due to CO_2, die swell expansion in extrusion, and squeezing to spread a product (Padmanabhan 1995; Huang and Kokini 1999), and its direct relation to deformations in mixing and bubble growth during fermentation and oven rise correlate baking performance with rheological properties (Huang and Kokini 1993; Dobraszczyk et al 2003).

The extensograph and gluten rig subject dough to uniaxial extension while the alveograph and dough inflation system use biaxial extension to test the dough. Uniaxial elongation rheology best describes the deformations that dough undergoes during mixing and sheeting. Biaxial extension is useful for predicting final bread quality because biaxial stretching flow is the dominating type of deformation in gas cell expansion occurring during loaf volume development (Bloksma and Nieman 1975; de Bruijne et al 1990; Davidou et al 2008). Since parameters from uni- and biaxial extension give valuable dough behavior data and can be correlated with baked product quality when tests are run at deformations and rates similar to the baking process (Bloksma 1990a; Dobraszczyk and Roberts 1994; Bollaín and Collar 2004; Collar et al 2007), knowledge of both types of flow is essential for predicting behavior in the real processes.

2.3.2.1. Extensograph

Resistance to stretching and dough extensibility in uniaxial extension can be tested using the extensograph. Wheat flour dough with salt and adjusted water content is mixed in the farinograph before being shaped into a cylinder and clamped into the extensograph. The dough is stretched uniaxially by a hook to obtain an extensogram curve as shown in Figure 2.5. It gives data on resistance to extension (R_5) at 5 cm extension, maximum resistance to extension (R_m), extensibility or length of the curve (E), energy required for extension (area, A), the ratio number (extensibility over resistance, E/R_m), and the ratio of extensibility (L) to maximum peak.

2.3.2.2. Alveograph

The alveograph measures the properties of dough by subjecting it to biaxial extension through bubble inflation. This method is popular in the dough industry because it simulates deformations occurring during expansion of cells in the fermentation and early baking stages of dough processing (Bloksma and Nieman 1975). Dough preparation in the alveograph emulates the stages of the baking process; the dough is mixed, sheeted, cut, relaxed, then clamped into the inflation device and inflated using pressurized air. Bubble wall thickness during inflation varies, but the maximum deformation is always near the pole, and the minimum is at the rim (Figure 2.6). Knowledge of the thickness around the bubble allows calculations of strain in the axisymmetric direction. Data obtained is in terms of the parameters maximum pressure (P), curve length (L), and work input or area of the curve (W) as shown in Figure 2.7. More recently, it has been suggested that the rates of biaxial extension in actual dough processing differ by several orders of magnitude from those used in the alveograph (Bloksma 1990a; Launay and Michon 2008). Despite this discrepancy, the alveograph is still a very useful tool for characterizing extensional properties of dough.

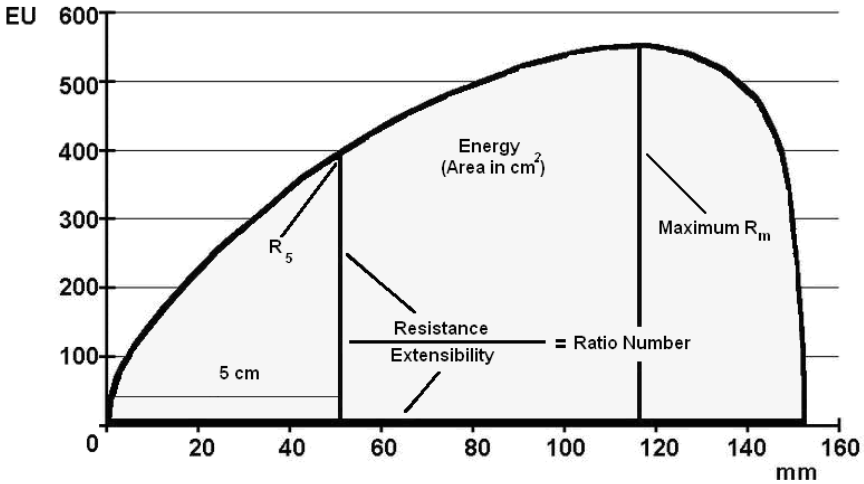
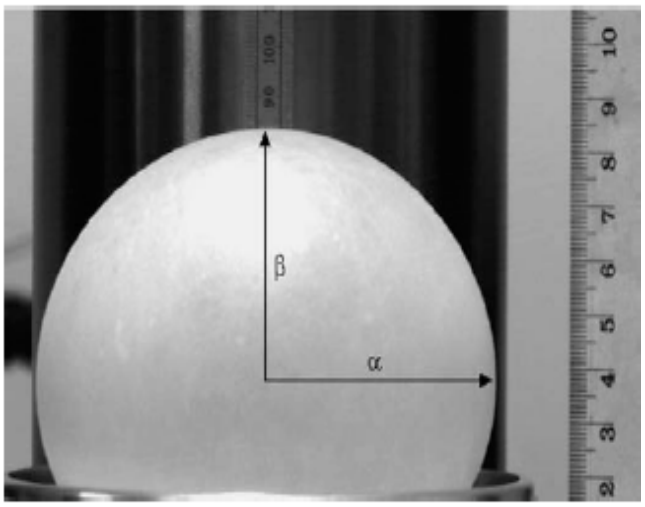


Figure 2.5. Typical extensogram showing the extensibility (E), resistance (R), and maximum resistance (Rm) of dough to extension (adopted from Brabender.com 2010).

(a) 

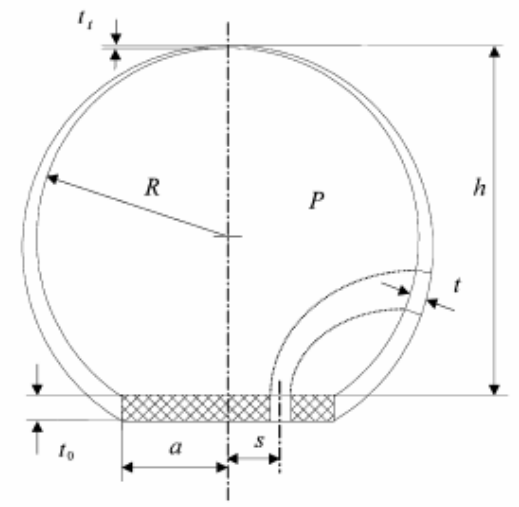
(b) 

Figure 2.6. Dough inflation system (a) inflated dough sample, (b) geometry of bubble inflation (adopted from Charalambides et al 2002).

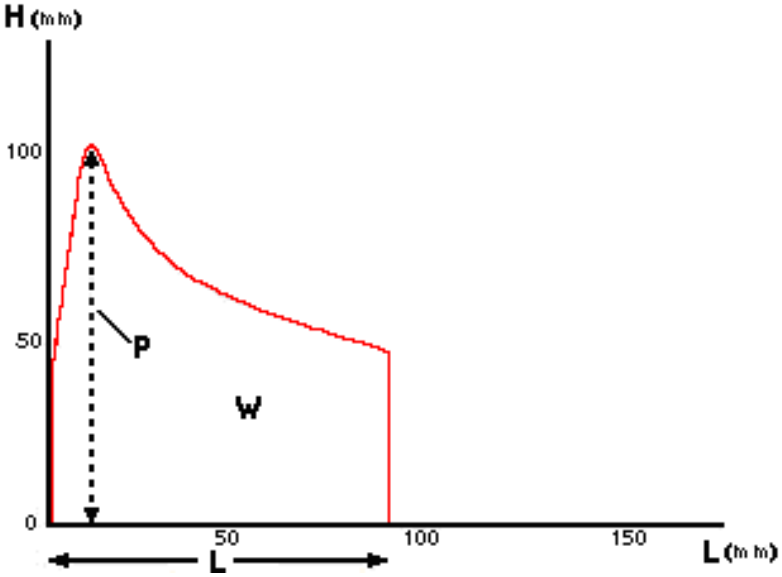


Figure 2.7. Typical alveogram and parameters for maximum length (L), maximum pressure (P), and total work input (W) (Wheat and Flour Testing Methods 2004).

2.3.2.3. Kieffer Gluten Extensibility Rig

The Stable Micro Systems Kieffer dough and gluten extensibility rig is an empirical instrument, but data can be converted into fundamental units. This means that the Kieffer rig is easy to use, but can also give some data in fundamental units; in essence, it is a great compromise between empirical and fundamental measurement. The uniaxial deformation and shear component used is similar to that of the extensograph, so much so that the Kieffer rig can

be called a micro extensograph. The extensograph force and extension are not expressed in terms of fundamental units, and high rate deformation data from the extensograph may not be relevant to baked product quality (Dunnewind et al 2004). The advantages of the Kieffer rig over the extensograph are that the Kieffer rig can be attached to several different material testing machines, uses much smaller sample sizes, allows testing at lower and controllable (more relevant to baking) strain rates, and data obtained can be expressed in terms of strain and stress (Dunnewind et al 2004). A curve of force versus displacement (extension) is obtained by the Kieffer rig (Dobraszczyk and Morgenstern 2003), and parameters collected include resistance to extension (Rmax), maximum extensibility (E), and area under the curve (A) as shown in Figure 2.8. It has been shown that Kieffer rig testing can predict loaf volume from dough and gluten properties (Kieffer et al 1998).

2.3.2.4. Dough Inflation System

The Dobraszczyk-Roberts dough inflation system (D/R DIS) attachment for the texture analyzer is similar to the Kieffer rig in that it is also a further development of an empirical method, the alveograph. The D/R DIS software takes measurements in fundamental units, but it is as easy to use as the empirical method. The DIS can inflate bubbles at a controlled, constant strain rate similar to that occurring in baking expansion.

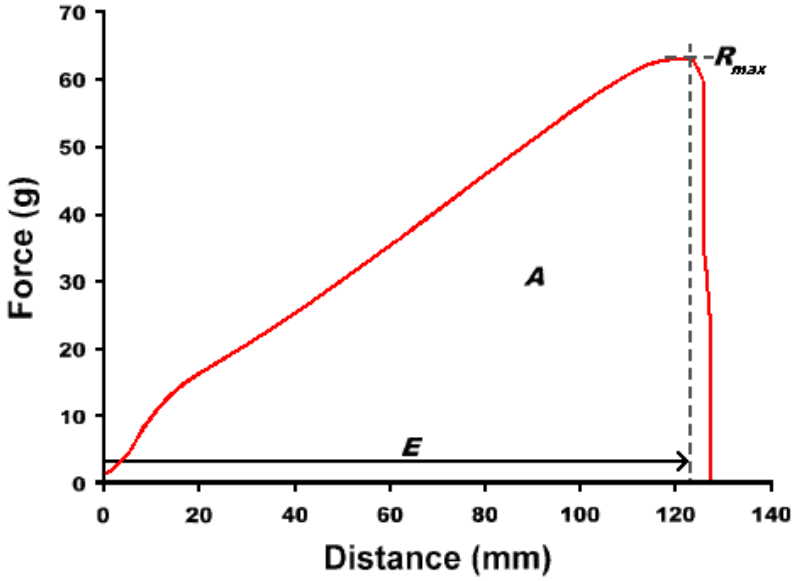


Figure 2.8. Typical force-displacement curve obtained by the Kieffer uniaxial extension test (adopted from Grausgruber and Schöggl 2002).

Biaxial extensional of a sample occurs when a sample is stretched in two directions simultaneously and at the same rate (Dobraszczyk and Morgenstern 2003), and it can be used to measure strain hardening (Kokelaar et al 1996). The texture analyzer drives air into the dough bubble using a piston; pressure is measured by a pressure transducer, and dough bubble volume is calculated from the displacement of the piston. Data in the form of inflation curves are plotted as stress versus Hencky strain, and the strain hardening index is calculated (Figure 2.9).

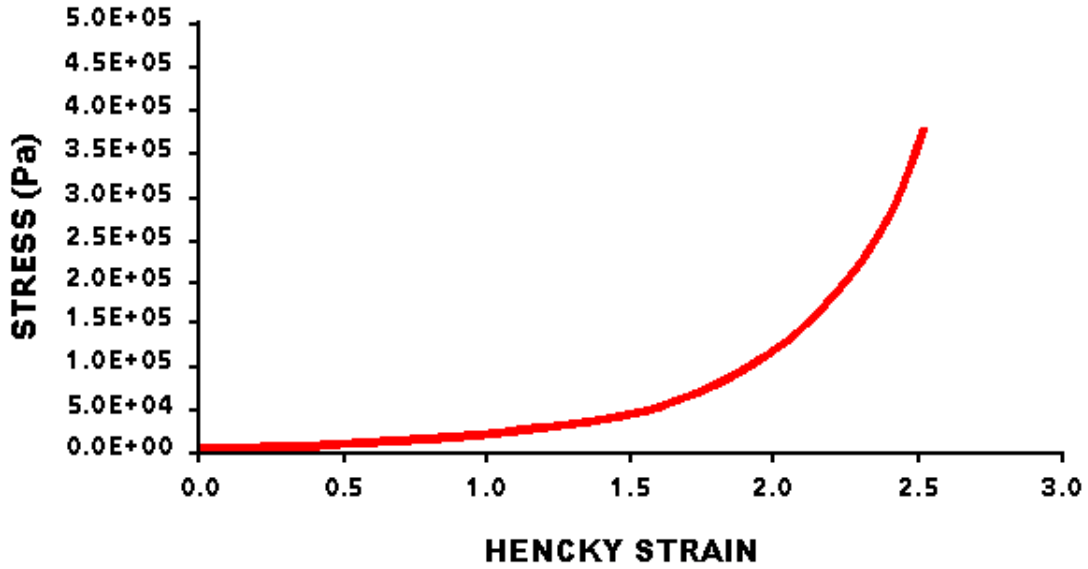


Figure 2.9. Dough inflation system curve showing stress versus constant Hencky strain at 0.1/sec for a strong, good quality wheat flour dough (adopted from Dobraszczyk et al 2003).

2.4. Fundamental Measurements of Rheology

Measurements on dough have evolved from traditional methods to empirical measures and finally into quantitative fundamental rheological testing. As mentioned before, fundamental measurement of rheology defines the sample deformation in terms of stress and strain. Fundamental methods have relatively recently become feasible for the study of dough rheology and yet they are responsible for the bulk of the scientific knowledge and experience in that area (Weipert 1990). Fundamental measurements have been originally used to link rheology of synthetic polymers to their molecular properties such as size distribution, cross-linking, and type of cross-linking (Ferry 1980). More recently, description of dough structure and understanding of dough component interactions has significantly benefited from these fundamental characterizations.

Methods often used in fundamental testing include the large deformation extensional measurements, small and large deformation shear creep/creep recovery/stress relaxation, small amplitude deformation dynamic oscillation, and flow viscometry (Dobraszczyk and Morgenstern 2003). Large deformation extensional instruments include the Kieffer gluten and dough extensibility rig and the Dobraszczyk/Roberts dough inflation system as discussed in the previous section. Creep, creep recovery, and stress relaxation as well as small amplitude oscillatory measurements (strain sweep, frequency sweep, and temperature sweep) are commonly performed on a rheometer. Flow viscometry includes both capillary viscometers and squeezing flow viscometers. Each of these instruments is capable of measuring dough's elastic and viscous properties by examining the relationship between stress and strain and strain rate in time-dependent experiments. Stress relaxation and creep tests instantaneously apply a constant strain (or stress) to the test sample and measure changes in stress (or strain) as a function of time. Dynamic testing consists of applying an oscillatory stress (or strain) to the test sample and determining its strain (or stress) response as a function of frequency. Since they are related, all linear viscoelastic measurements can be calculated from one another (Ferry 1980; Macosko 1994).

One of the drawbacks of basic rheometry is the difficulty of use. Fundamental testing requires a high level of technical skill, is laborious, time-consuming, expensive, and the difficulties are often compounded by the arduousness of result interpretation (Weipert 1990; Dobraszczyk and Morgenstern 2003). These shortcomings are countered by the high quality of information gleaned from fundamental measurements about the stress-strain properties of the material.

2.4.1. Dynamic Oscillatory Measurements

Small amplitude dynamic oscillatory measurements have been used extensively to determine wheat flour dough's fundamental mechanical characteristics (Faubion and Hoseney 1990; Amemiya and Menjivar 1992). During dynamic testing, samples held in various geometries are subjected to oscillatory motion. The cone and plate as well as parallel plate are the most commonly used geometries. During dynamic measurements, a sinusoidal strain (or stress) is applied to the sample and the resulting sinusoidal stress (or strain) is measured (Weipert 1990; Steffe 1996). The magnitude of strain used in the test is very small, usually on the order of

0.1-2%, where the material is in the linear viscoelastic range. There are two types of dynamic oscillatory instruments: controlled stress instruments have fixed stress amplitude and measure deformation, and controlled strain instruments that have a fixed strain rate and measure stress (Steffe 1996). Controlled stress rheometry (CSR) was used successfully to illustrate the fundamental rheological properties of glutens and their sub-fractions (Khatkar et al 1995), but the more common method in oscillatory measurement of rheology is the controlled strain instrument. Each of these methods produces similar results, and often controlled stress instruments can be used as controlled strain instruments through software manipulation.

The oscillatory method is the most useful technique for determining the viscoelastic properties of materials that cannot be investigated by steady-shear instruments due to their shear-sensitivity, such as hydrocolloid solutions, batter, dough, and starch solutions. Oscillatory testing has an advantage over many other types of rheological evaluation. It possesses a well-developed theoretical background, testing equipment is readily available, and measurement of both the elastic (solid-like) and viscous (liquid-like) properties of the dough is separately performed (Dobraszczyk and Morgenstern 2003; Connelly and McIntier 2008). The nondestructive nature of the test allows numerous measurements to be done on the same sample using an extensive range of conditions of temperature, strain, and frequency (Gunasekaran and Ak 2000; Dobraszczyk and Morgenstern 2003).

In spite of their favorable attributes, dynamic oscillatory measurements possess a number of inconvenient deficiencies. Oscillatory tests must be performed in the linear viscoelastic range of frequencies in order to be accurate and reproducible. This plateau range has shown a low sensitivity to polymer molecular weight differences, and since large molecular weight glutenin proteins have the largest bearing on final bread quality, oscillatory methods are not applicable to predictions of breadmaking performance (Dobraszczyk and Morgenstern 2003). While the linear viscoelastic range limits the sensitivity in differentiating between proteins, oscillatory measurements are overly sensitive to polymer concentration changes associated with water and other diluents (Ferry 1980). Also, small amplitude oscillatory measurements are performed at strain rates and frequencies not relevant to practical processing conditions occurring during dough mixing, expansion, and oven rise. Proofing and oven rise extensional rates are on the order of $5x10^{-3}$ and $5x10^{-4}$ sec^{-1}, several orders of magnitude smaller than the rates utilized in oscillatory testing (Bloksma 1990a). To be within the linear viscoelastic range, the magnitude of

strain applied during measurement is in the range of 0.1-2%, but the strains in gas cell expansion during fermentation have been revealed to be several hundred percent (Amemiya and Menjivar 1992). Since most of the common dough processing operations, such as extrusion, sheeting, fermentation, and oven rise, are large-strain and extensional in nature, the oscillatory tests will yield entirely different results than large deformation extensional measurements (Ferry 1980; Padmanabhan 1995; Dobraszczyk and Morgenstern 2003).

Although there seems to be many disadvantages of dynamic oscillatory testing, it can be a very useful technique if applied in the correct situations. The very low strains that are not suitable for predicting breadmaking quality are useful for characterizing time- and temperature-dependent alterations occurring in dough because they do not change or destroy dough structure (Weipert 1990). The linear viscoelastic region is important because it allows for simplified interpretation of resulting data. Assessment of physical properties and dough ingredient function becomes easier and more reliable in the linear region because the mathematical equations describing those properties are less complex, and continuous testing of time-dependent changes is made possible by the non-destructive nature of the testing (Weipert 1990; Song and Zheng 2007). Characterization of dough at the molecular level is possible using dynamic oscillation methods (Tronsmo et al 2003a), which is an integral part of the multi-resolution characterization that is critical when examining a composite with as many components as wheat flour (Connelly and McIntier 2008). This unique and critical use of small amplitude oscillatory testing ensures that this method will continue to be as useful and powerful in the future as it is currently (Weipert 1990; Salvador et al 2006).

2.4.1.1. Equations and Parameters

As mentioned previously, in small amplitude oscillatory flow experiments, a sinusoidal oscillating stress or strain with a frequency (ω) is applied to the material and the oscillating strain or stress response is measured along with the phase difference between the oscillating stress and strain. The input strain (γ) varies with time according to the relationship

$$\gamma = \gamma_0 \sin \omega t \qquad \text{[Eqn. 2]}$$

and the rate of strain is given by

$$\dot{\gamma} = \gamma_0 \omega \cos \omega t \qquad \text{[Eqn. 3]}$$

where γ_0 is the amplitude of strain. The corresponding stress (τ) can be represented as

$$\tau = \tau_0 \sin(\omega t + \delta)$$ [Eqn. 4]

where τ_0 is the amplitude of stress and δ is shift angle (Figure 2.10).

$\delta = 0$ for a Hookean solid

$\delta = 90°$ for a Newtonian fluid

$0 < \delta < 90°$ for a viscoelastic material.

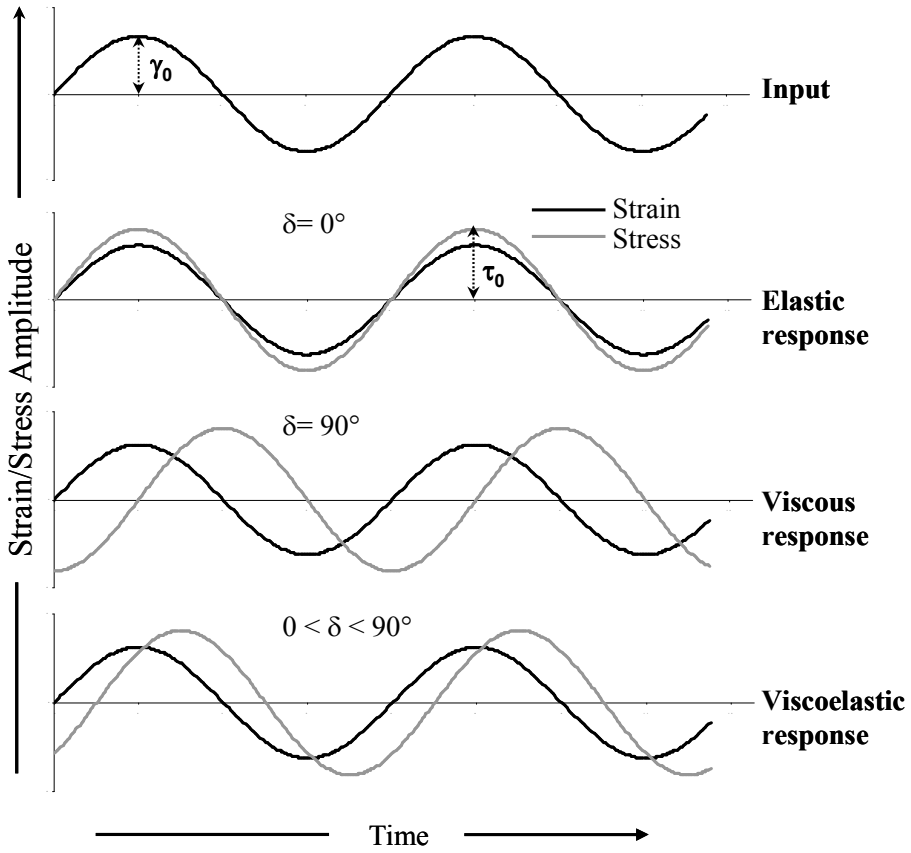


Figure 2.10. Input and response functions differing in phase by the angle δ (Darby 1976).

A perfectly elastic solid produces a shear stress in phase with the strain. For a perfectly viscous liquid, stress is 90° out of phase with the applied strain. Viscoelastic materials, which have both viscous and elastic properties, exhibit an intermediate phase angle, between 0 and 90°. A solid like viscoelastic material exhibits a phase angle smaller than 45° while a liquid like viscoelastic material exhibits a phase angle greater than 45°.

Two rheological properties can be defined as follows:

$$G'(\omega) = \frac{\tau_0}{\gamma_0}\cos\delta \qquad \text{[Eqn. 5]}$$

$$G''(\omega) = \frac{\tau_0}{\gamma_0}\sin\delta \qquad \text{[Eqn. 6]}$$

The storage modulus, G′, is related to the elastic character of the fluid or the storage energy during deformation. The loss modulus, G″, is related to the viscous character of the material or the energy dissipation that occurs during the experiment. Therefore, for a perfectly elastic solid, all the energy is stored, i.e. G″ is zero and the stress and the strain will be in phase. However, for a perfect viscous material all the energy will be dissipated, i.e. G′ is zero and the strain will be out of phase by 90°. The complex modulus, $G^*(\omega)$, is defined as

$$G^*(\omega) = \sqrt{(G'(\omega))^2 + (G''(\omega))^2} \qquad \text{[Eqn. 7]}$$

Another commonly used dynamic viscoelastic property, the loss tangent, $\tan\delta(\omega)$, denotes ratio of viscous and elastic components in a viscoelastic behavior:

$$\tan\delta(\omega) = \frac{G''}{G'} \qquad \text{[Eqn. 8]}$$

For fluid-like systems, appropriate viscosity functions can be defined as follows:

$$\eta' = \frac{G''}{\omega} \quad \text{and} \quad \eta'' = \frac{G'}{\omega} \qquad \text{[Eqn. 9]}$$

where η′ represents the viscous or in-phase component between stress and strain rate, while η″ represents the elastic or out-of-phase component. The complex viscosity η^* is equal to

$$\eta^* = \sqrt{\left(\frac{G'}{\omega}\right)^2 + \left(\frac{G''}{\omega}\right)^2} \qquad \text{[Eqn. 10]}$$

2.4.1.1.1. Relationship between Oscillatory Data and Dough Properties

Dynamic oscillatory data can be interpreted in an uncomplicated manner when testing is performed within the linear viscoelastic region. Although breadmaking performance cannot be determined directly by dough rheology, other observations and interpretations of data are allowable. Many researchers have tried to correlate the rheological results with baking quality, but this has resulted in conflicting reports about whether higher or lower G′ in doughs and glutens indicates better baking quality (Dobraszczyk and Morgenstern 2003). Since protein interactions are not as influential on the results of small amplitude oscillatory testing, it is not

suitable for judging final bread quality, and this could be producing the conflicting correlations. Almost all studies on wheat dough have reported that the G′ storage modulus is always larger than the G″ loss modulus in the linear range, indicating that dough is a viscoelastic soft-solid (Cornec et al 1994; Khatkar et al 1995; Sofou et al 2008; Tanner et al 2008; van Bockstaele et al 2008). Tan delta has been reported to be generally lower in good quality wheat flour dough than in poor quality flour dough (He and Hoseney 1991; Janssen et al 1996c; Kokelaar et al 1996). The focus of previously published research on wheat flour dough rheology centered on how rheological properties were affected by major components in flour (gluten, starch, and water) and flour cultivar (Faubion and Hoseney 1990). Valuable knowledge has been gained on the effects of mixing time and temperature, rest time after mixing, dough moisture content, and some additives through the application of dynamic oscillatory testing.

2.4.1.1.2. Dough Preparation and Resting

Resting dough after mixing and before loading into an oscillatory testing apparatus is crucial for reproducible results. Allowing dough to rest before testing gives it time to undergo stress-releasing rearrangements which may be related to relaxation of mixing-generated stresses, such as water redistribution, enzymatic modification of gluten and starch, and sulfhydryl-disulfide interchange decreasing average gluten molecular weight (Dong and Hoseney 1995). Any of these actions could be occurring directly after mixing, and if dough is not allowed to rest for a period of time, forces created from these processes could affect dough rheology and experiment reproducibility. Doughs allowed to rest before testing have shown lower G′ and larger loss tangent than doughs tested immediately after mixing (Dong and Hoseney 1995). Similarly, other researchers have shown that doughs rested less than a minimum of 15 or 20 min are not sufficient to obtain reproducible data (Dong and Hoseney 1995; Phan-Thien and Safari-Ardi 1998; Newberry et al 2002). This illustrates that a minimum amount of resting time must be allowed before loading the dough into the testing apparatus to avoid any variable effects.

2.4.1.2. Linear Viscoelastic Range

Strain sweeps are performed to determine the linear viscoelastic region for a particular sample. A range of strains are applied keeping a constant frequency (usually 1Hz), and the resulting stresses are measured. The G′ and G″ moduli are constant within the linear viscoelastic region, and at strains above that region, the moduli significantly decrease (Figure 2.11). For

wheat flour dough, strain of 0.1% is typically used as the endpoint of the linear viscoelastic region (Weipert 1990; Tanner et al 2008).

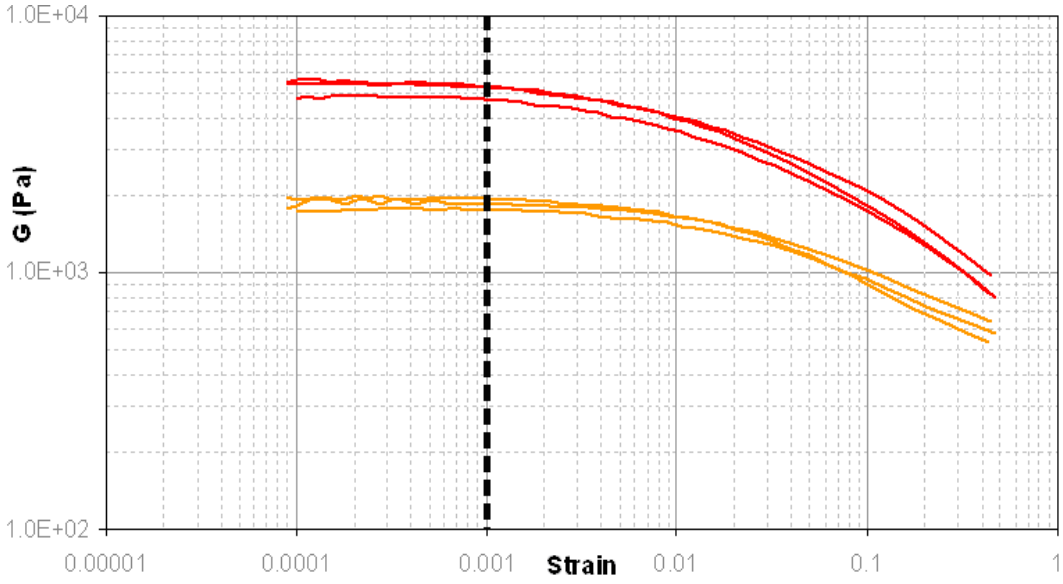


Figure 2.11. Strain sweeps of wheat flour dough with a line marking the end of the linear viscoelastic region at a strain of .001.

2.4.1.3. Frequency Sweeps

There has been a great deal of research done using the frequency sweep method in small amplitude oscillatory testing where the applied strain remains constant while the frequencies increase. Some frequency sweep tests used to study dough rheological changes run from 0.1 to 10 Hz are at a strain of 0.2% (Angioloni and Dalla Rosa 2007; Connelly and McIntier 2008), and others span a larger range of frequencies or use a lower strain rate (usually on the order of 0.1%). Frequency sweeps have demonstrated the greatest aptitude for explaining the importance of component interactions and their role in the processibility of wheat flour dough (Song and Zheng 2007). Through its ability to measure the changes in viscous and elastic behaviors of materials corresponding with increases in applied strain or stress, frequency sweep has become the most popular method used for oscillatory testing (Steffe 1996). Defining the storage and loss moduli of a particular substance is very useful for comparing it to other materials. One detriment to the practicability of frequency sweeps is that they tend to have large variability between replicates. Typical range of the coefficient of variation for frequency sweeps is between 10-15%. Phan-

Thien and Safari-Ardi (1998) reported one of the lowest variations at 10%, and Connelly and McIntier (2008) and Lefebvre et al (2006) found similar variations at 14.6% and 15%, respectively. This variable reproducibility due to the nature of dough hinders the ability of frequency sweeps to give good correlations between rheology and material composition.

2.4.1.4. Temperature Sweeps

Temperatures are very important in dough systems because dough displays different rheological characteristics depending on the ambient or applied temperature. Temperature sweeps are performed in oscillatory testing by keeping the frequency and applied strain constant and running a temperature profile on the rheometer. Temperature profiles are easily programmable on rheometers and can be set to have any number of temperature ramps and cooling periods to best measure the material (Weipert 1990; Salvador et al 2006). This allows for a simulation of rheology changes due to temperature during baking. As would be expected, lower temperatures provide easier testing and fewer difficulties than higher temperatures.

Oscillatory testing from 20-40 ºC is below the gelatinization point of wheat flour dough; therefore, dough shows a high frequency dependence and changes over this temperature range are reversible (Salvador et al 2006; Song and Zheng 2007). Higher temperatures show irreversible changes as the dough approaches its gelatinization temperature, especially when temperatures near 80 ºC. At high temperatures (after dough's gelatinization temperature of about 60 ºC), the dough system becomes stronger and the G′ becomes much larger than the G″ (Weipert 1990; Salvador et al 2006). This transition of the dough into a more solid system could be caused by sulphhydryl/disulfide exchange (Song and Zheng 2007), or starch granule rupture to form a gel amylase matrix coupled with protein denaturation (Salvador et al 2006). The starch gelatinization that occurs at elevated temperature has a great effect on the viscoelastic properties of the dough, and temperature sweeps in oscillatory testing are a useful way to study these changes. The only restriction in using this method is that it cannot be used above 90 ºC. Higher temperatures and cooling from higher temperatures causes extra loss of moisture and shrinkage of the sample, which has the effect of the sample pulling away from the measurement apparatus and inconsistency of measurements (Weipert 1990).

2.4.2. Stress Relaxation

Stress relaxation has been commonly used to study food systems, and extensive work on dough rheology has been performed using it (Bloksma 1990a). In a stress relaxation test, a constant strain (γ_0) is applied to the material at time t_0 and the change in the stress over time, $\tau(t)$, is measured (Darby 1976; Macosko 1994). Ideal viscous, ideal elastic, and viscoelastic materials show different responses to the applied step strain. When a constant stress is applied, viscoelastic materials respond with an initial stress growth which is followed by decay in time (Figure 2.12). Upon removal of strain, viscoelastic fluids equilibrate to zero stress (complete relaxation) while viscoelastic solids store some of the stress and equilibrate to a finite stress value (partial recovery).

The relaxation modulus, *G(t)*, is an important rheological property measured during stress relaxation. It is the ratio of the measured stress (τ) to the applied initial strain at constant deformation (γ_0).

$$G(t) = \frac{\tau}{\gamma_0} \qquad \text{[Eqn. 11]}$$

From the theory of linear viscoelasticity, the linear response to any type of deformation can be predicted using the relaxation modulus, G(t), in the linear viscoelastic region. Constitutive equations can be used to simulate the linear relaxation modulus. If the spectrum of relaxation times is continuous, the relaxation spectrum, $H(\lambda)$, can be obtained from relaxation modulus:

$$G(t) = \int_{-\infty}^{+\infty} H(\lambda)\, e^{-t/\lambda}\, d(\ln \lambda) \qquad \text{[Eqn. 12]}$$

The relaxation time spectrum contains the complete information on the distribution of relaxation times which is very useful in describing a material's response to a given deformation history.

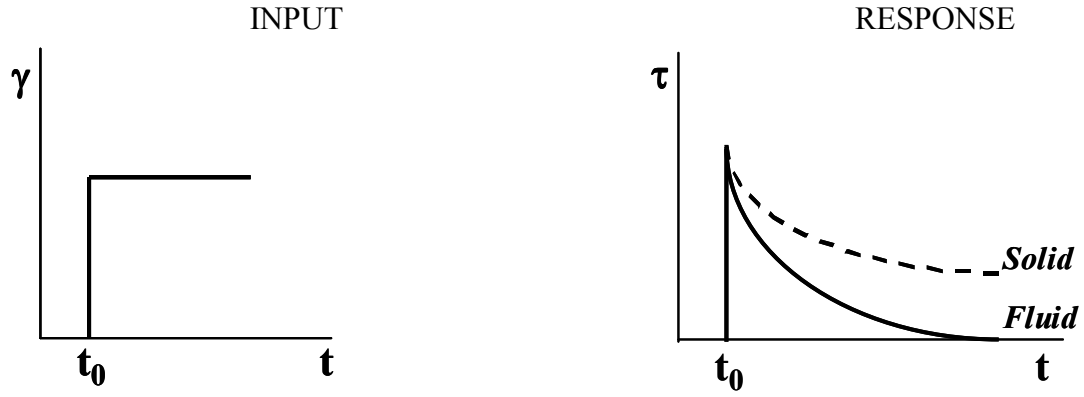


Figure 2.12. Response of viscoelastic materials to imposed step strain (Darby, 1976).

There are a number of causes for stress relaxation: chain cleavage reducing molecular weight due to oxidative degradation or hydrolysis, rearrangement of molecular chains to lowest stress conformation, slippage of chains past each other to cause viscous flow, and glass transition temperature molecular relaxation (Dong and Hoseney 1995). It has been found that different wheat cultivar doughs produce distinctly different relaxation moduli at high strains where small strains (0.1% and less) do not show significant differences (Safari-Ardi and Phan-Thien 1998; Dobraszczyk and Morgenstern 2003). From the large strain data, it was determined that the doughs with stronger dough properties had a higher magnitude of modulus; in fact, the doughs were found to be in the order of extra strong> strong > medium> weak dough in terms of elastic moduli. So for relaxations performed at large strains, molecular weight distributions correlate well with relaxation properties, and this data can be used to differentiate between hard and soft wheat cultivars as well as wheats with different types and amounts of protein that produce varying baked product qualities. Although small-strain stress relaxations have not been linked to bread quality, the relaxation spectrum does give some interesting information about the dough. Relaxation spectrums are bimodal, showing two major relaxation times for the dough. The shorter relaxation is for lower molecular weight proteins, and the longer relaxation curve is related to high molecular weight polymer relaxation (Rao et al 2000; Dobraszczyk and Morgenstern 2003). This information can be linked to the SE-HPLC data to determine protein sizes and relaxation times.

2.4.3. Creep Recovery

In a creep test, a constant stress (τ_0) is applied at time t_0 and removed at time t_1, and the corresponding strain $\gamma(t)$ is measured as a function of time. As in the case with stress relaxation, various materials respond in a different way: A viscoelastic material responds with a non-linear strain (Figure 2.13). Strain level approaches a constant rate for a viscoelastic fluid and a constant magnitude for a viscoelastic solid. When the imposed stress is removed at t_1, the solid recovers completely at a finite rate, but the recovery is incomplete for the fluid (Darby 1976).

The rheological property of interest is the ratio of strain to constant stress as a function of time is the creep compliance, *J(t)*:

$$J(t) = \frac{\gamma(t)}{\tau_0} \qquad \text{[Eqn. 13]}$$

Creep compliance describes how compliant a material is. The greater the compliance, the easier it is to deform the material. A typical creep-recovery curve is shown in Figure 2.14. Maximum creep compliance (J_{max}) and maximum recovery compliance (J_m) are two of the most basic parameters measured during creep recovery. Percent recovery (J_m/J_{max}) is an indicator of damage done to the dough structure with a larger recovery percent showing less damage (van Bockstaele et al 2008).

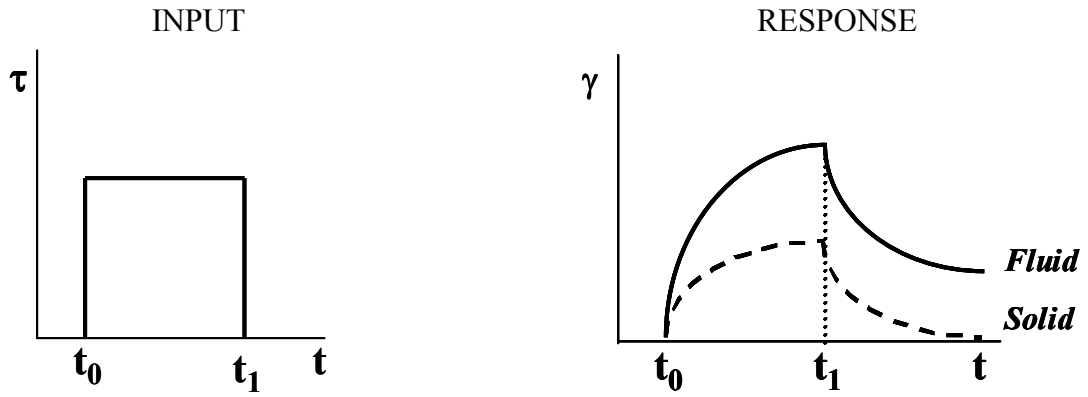


Figure 2.13. Response of viscoelastic materials to imposed instantaneous step stress (Darby 1976).

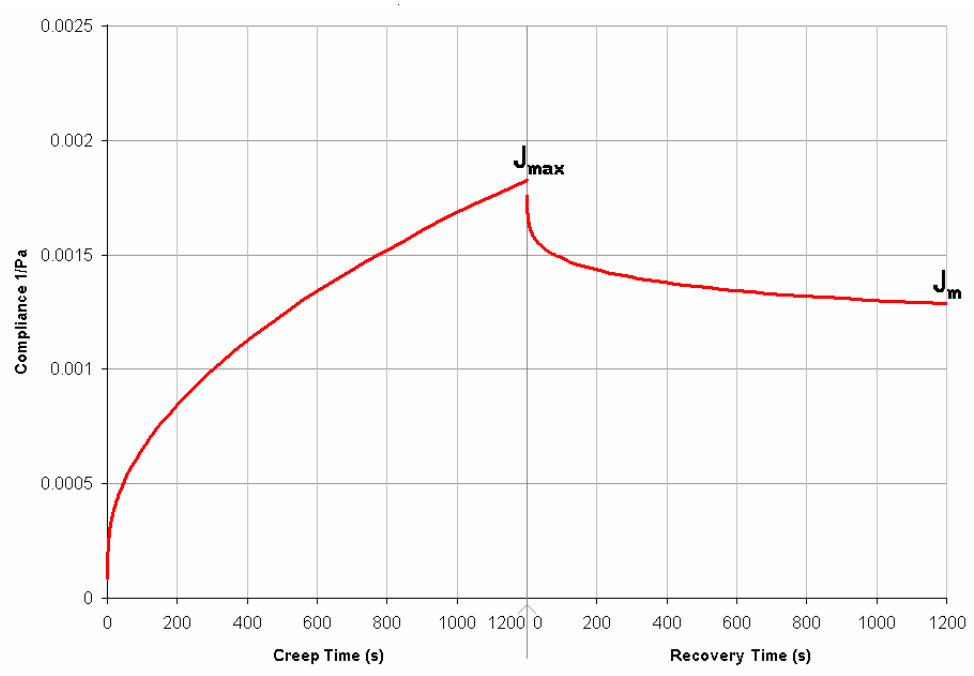


Figure 2.14.Typical creep recovery curve, creep/recovery compliances versus time

The first section of the creep curve generated is correlated to structural bonds in the dough undergoing rapid elastic deformation, the second part is when gluten structural bonds break and reform, and finally the plateau of recoverable compliance occurs after which, any increases in creep compliance are entirely due to flow (Rouillé et al 2005). Dough behaves differently under high and low creep stresses. Creep recovery at large stresses has shown correlations between stronger flours, larger glutenins, and larger amounts of unextractable polymeric protein (UPP), but small deformation creep recovery is not able to differentiate between flours (Tronsmo et al 2003a, b). This is similar to oscillatory data-when the measurement is performed in dough's linear viscoelastic region, starch-starch and starch-protein interactions are dominant and results generally do not correlate well with gluten strength or baking quality.

2.4.4. Lubricated Squeezing Flow

Lubricated squeezing flow is a method for measuring biaxial deformation in response to applied stress. A sample coated with a lubricant is placed between two rheometer plates and squeezed out to measure biaxial deformation and determine extensional flow. This method is one of the most useful for measuring dough rheology because deformations and strain rates used

are similar those that occur in gas cell expansion during fermentation and oven rise (Dobraszczyk and Morgenstern 2003). Because a lubricated system is used, theoretically, all shear is eliminated and the only forces acting on the sample are compression. The biaxial extension viscosity can be described by Eqn. 14 for an incompressible material

$$\eta_b = \frac{\sigma_b}{\varepsilon_b} \qquad \text{[Eqn. 14]}$$

where σ_b is normal stress and ε_b is the biaxial strain rate(Launay and Michon 2008).

2. 5. End-Quality Parameters

As discussed previously, correlations between final bread quality and dough rheological characteristics have been made. Analysis of baked breads made from different wheat cultivars is valuable because it can help to additionally differentiate between cultivars. Even if correlations cannot be directly made between rheology and final baked products, comparisons of wheat flour composition and baking quality can be conducted.

2.5.1. Loaf Volume

Once the structure of the loaf has been set in the oven through baking, the final loaf quality can be observed and measured. Typically, the loaf volume is measured directly after removing the bread from the oven using the rapeseed displacement method. Many analytical bakers then give a score to the crumb grain of 0-6, (0 being unsatisfactory and 6 being outstanding) for its structure depending on its uniformity and how open the crumb structure appears, which is in accordance with the methods employed by the Wheat Quality Council and has proven reproducible (Park et al 2004). Crumb color and loaf shape are examined in a similar manner to give an overall indication of baking quality for each particular flour.

2.5.2. Texture Profile Analysis

The texture of many foods has always been a concern, and the first known instrumental measurement of food texture was performed in 1861 (Bourne 1978). Softness of pan bread has perpetually been of importance to bakers since consumers prefer a softer crumb texture. Texture profile analysis (TPA) was developed by Szczesniak (1963), which provided measurement of a food by a two “bite” instrumental method to yield the parameters of fracturability, hardness, cohesiveness, adhesiveness, and springiness, as well as the derived parameters gumminess and

chewiness. Parameters that have been commonly used for bread and bread dough include: Firmness (the peak force during the first bite in Newtons), cohesiveness (ratio of peak2 area / peak 1 area), springiness (height in mm that the bread recovers during the time lapse between bites), and chewiness (hardness*cohesiveness*springiness in joules) as shown in Figure 2.15 (Carr and Tadini 2003). TPA has the distinct advantage of producing data that is easy to understand and can be utilized fundamentally and practically by researchers and product development people (Szczesniak 1998). Simple instrumental methods for measuring food properties are desirable because they can provide repeatable information without requiring a highly-skilled operator, and texture analysis can provide a link between bread structure and consumer-desired texture.

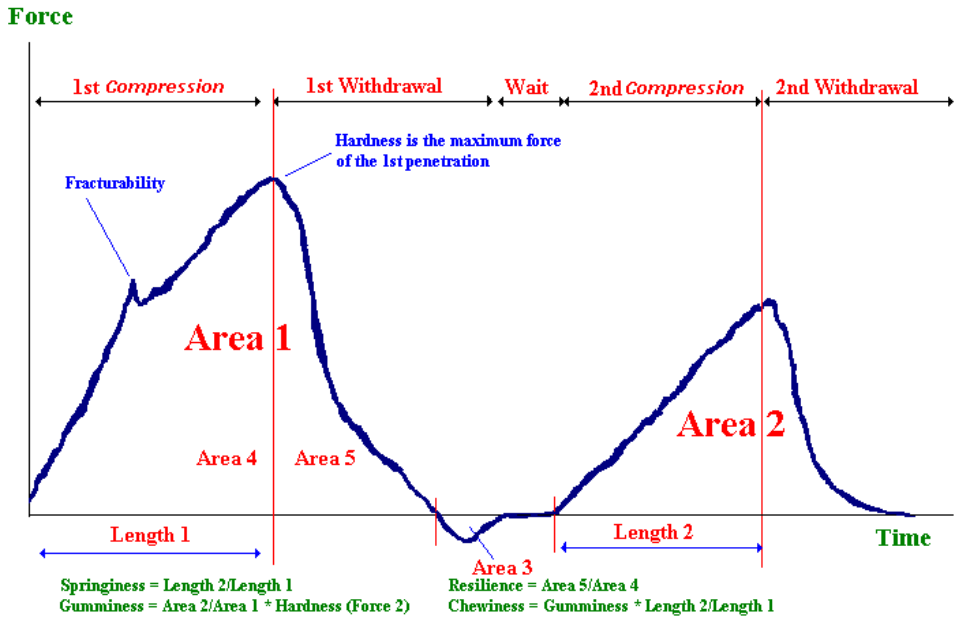


Figure 2.15. Standard "two bite" TPA force-time curve and textural parameters (adopted from Texture Technologies 2010).

2.5.3. Crumb Structure

New methods of crumb analysis are beginning to be implemented that do not rely entirely on human judgment. The C-cell has been utilized to measure the structure on the surface of a loaf's center slice through digital imaging. A range of information can be obtained using C-cell including: slice area, number of cells, cell diameters, sizes of large air occlusions (holes), cell wall thicknesses, crumb color, loaf shape, cell shape, and other derived volume measurements.

Since the 2-D techniques of sample structure analysis such as scanning electron microscopes (SEM), light microscopy, and C-cell damage the sample by cutting the surface to be measured, the data obtained is not as accurate as in 3-D imaging techniques (Babin et al 2006; Pickett 2009). Generally 2-D methods are less accurate because they only measure the surface of a sample; a cut surface does not always intersect the center of the air bubbles, so the data on cell sizes varies depending on what cross-sectional part of the semi-spherical air cell is being measured as well as the actual cell sizes in the sample. 3-D imaging measures the entire cell, giving a more complete view of the actual cellular structure of the food foam measured, as well as their spatial distribution within a particular food matrix.

2.5.4. X-Ray Microtomography

A newer technique called x-ray microtomography (XMT) has been developed to non-destructively measure the 3-D structure of food foams. It is based on the same principles as medical CAT scans and uses an x-ray source and charged-coupled device (CCD)-camera to capture radiographs of the sample through 180-360º of sample rotation with an adjustable rotation step. The radiographs are then digitally combined to create a 3-D image of the sample, which can be sliced noninvasively to create hundreds of 2-D cross-sectional images. Multiple cross sections are then analyzed to determine average air cell size, cell size distribution, cell wall thicknesses, cell wall thickness distribution and other data. The technique of XMT been applied to frozen bread dough by Whitworth and Alava (1999) and Pickett (2009), to dough during baking by Babin et al (2006), and to baked bread by Pickett (2009). XMT imaging has some very great advantages over 2-D imaging techniques. Its non-destructive nature and sensitive imaging that captures the relatively open and fragile structure of baked bread and allows discrimination between solid and air makes it an attractive method for the analysis of air cells and the structure of bread.

2.6. References

AACC International. Approved Methods of Analysis, 11th Ed. AACC International: St. Paul, MN.

Amemiya, J., and Menjivar, J. 1992. Comparison of small and large deformation measurements to characterize the rheology of wheat flour doughs. J. Food Eng. 16: 91-108.

Angioloni, A., and Collar, C. 2009. Small and large deformation viscoelastic behaviour of selected fiber blends with gelling properties. Food Hydrocolloid. 23: 742-748.

Angioloni, A., and Dalla Rosa, M. 2007. Effects of cysteine and mixing conditions on white/whole dough rheological properties. J. Food Eng. 80: 18-23.

Anonymous. 2004. Wheat and Flour Testing Methods: A Guide to Understanding Wheat and Flour Quality: Version 2. Available at www.wheatflourbook.org. Wheat Marketing Center, Inc. Portland, OR.

Babin, P., Della Valle, G., Chiron, H. Cloetens, P., Hoszowska, J., Pernot, P., Reguerre, A.L., Salvo, L. and Dendievel, R. 2006. Fast x-ray tomography analysis of bubble growth and foam setting during breadmaking. J. Cereal Sci. 43: 393-397.

Bagley, E., Dintzis, F., and Chakrabarti, S. 1998. Experimental and conceptual problems in the rheological characterization of wheat flour doughs. Rheol. Acta. 37: 556-565

Bloksma, A.H. 1990a. Rheology of the breadmaking process. Cereal Food World. 35: 228-236.

Bloksma, A.H. 1990b. Dough structure, dough rheology, and baking quality. Cereal Food World. 35: 237-244.

Bloksma, A.H., and Nieman, W. 1975. The effect of temperature on some rheological properties of wheat flour dough. J. Texture Stud. 6: 343-361.

Bollaín, C., and Collar, C. 2004. Dough viscoelastic response of hydrocolloid/enzyme/surfactant blends assessed by uni-and bi-axial extension measurements. Food Hydrocolloid. 18: 499-507.

Bourne, M. 1978. Texture profile analysis. Food Technology. 32(7): 62-66.

Brabender. 2010. Available at . C.W. Brabender Instruments, Inc., South Hackensack, NJ.

Carr, L., and Tadini, C. 2003. Influence of yeast and vegetable shortening on physical and textural parameters of frozen part baked French bread. Lebensm.-Wiss. Technol. 36: 609-614.

Charalambides, M.N., Wanigasooriya, L., Williams, J.G., Chakrabarti, S. 2002. Biaxial deformation of dough using the bubble inflation technique. I. Experimental. Rheol. Acta. 41: 532-540.

Chiotelli, E., Rolée, A., and Meste, M. 2004. Rheological properties of soft wheat flour doughs: Effect of salt and triglycerides. Cereal Chem. 81: 459-468.

Collar, C., BollaínBollain, C., and Rosell, C. 2007. Rheological behavior of formulated bread doughs during mixing and heating. Food Sci. Technol. Int. 13: 99-107.

Connelly, R., and McIntier, R. 2008. Rheological properties of yeasted and nonyeasted wheat doughs developed under different mixing conditions. J. Sci. Food Agric. 88: 2309-2323.

Cornec, M., Popineau, Y., and Lefebvre, J. 1994. Characterization of gluten subfractions by SE-HPLC and dynamic rheological analysis in shear. J. Cereal Sci. 19: 131-139.

Darby, R. 1976. Viscoelastic Fluids. Marcel Dekker Inc., New York.

Davidou, S., Michon, C., Thabet, I., and Launay, B. 2008. Influence of shaping and orientation of structures on rheological properties of wheat flour dough measured in dynamic shear and biaxial extension. Cereal Chem. 85: 403-408.

de Bruijne, D.W., de Loof, J., and van Eulem, A. 1990. The rheological properties of breads dough and their relation to baking. In: Rheology of Food. Pharmaceutical and Biological Materials with General Rheology. R.E. Carter, ed. Elsevier Applied Science: London, UK.

Dobraszczyk, B.J. 1999. Measurement of biaxial extensional rheological properties using bubble inflation and the stability of bubble expansion in bread doughs. In: Bubbles in Food. G. Campbell, C. Webb, S. Pandiella, and K. Niranjan, eds. AACC International: St. Paul, Minnesota.

Dobraszczyk, B.J. and Roberts, C.A. 1994. Strain hardening and dough gas cell-wall failure in biaxial extension. J. Cereal Sci. 20: 265-274.

Dobraszczyk, B.J., and Morgenstern, M. 2003. Rheology and the breadmaking process. J. Cereal Sci. 38: 229-245.

Dobraszczyk, B.J., and Schofield, J. 2002. Rapid assessment and prediction of wheat and gluten baking quality with the 2-g direct drive mixograph using multivariate statistical analysis. Cereal Chem. 79: 607-612.

Dobraszczyk, B.J., Smewing, J., Albertini, M., Maesmans, G., and Schofield, J.D. 2003. Extensional rheology and stability of gas cell walls in bread doughs at elevated temperatures in rations to breadmaking performance. Cereal Chem. 80: 218-224.

Dong, W., and Hoseney, R. 1995. Effects of certain breadmaking oxidants and reducing agents on dough rheological properties. Cereal Chem. 72: 58-64.

Dunnewind, B., Sliwinski, E., and Grolle, K. 2004. The Kieffer dough and gluten extensibility rig- An experimental evaluation. J. Texture Stud. 34: 537-560.

Edwards, N., Mulvaney, S., Scanlon, M., and Dexten, J. 2003. Role of gluten and its components in determining durum semolina dough viscoelastic properties. Cereal Chem. 80: 755-763.

Faubion, J. and Hoseney, R. 1990. The viscoelastic properties of wheat flour doughs. In: Dough Rheology and Baked Product Texture. H. Faridi and J. Faubion, eds. Van Nostrand Reinhold Publishers: New York.

Ferry, J. 1980. Viscoelastic Properties of Polymers. John Wiley: New York.

Gras, P.W., Carpenter, H.C., and Anderssen, R.S. 2000. Modelling the Developmental Rheology of Wheat- Flour Dough using Extension Tests. J. Cereal Sci. 31: 1-13.

Grausgruber, H., and Schöggl, G. 2002. Investigations on the validity of the micro-extensograph method to measure rheological properties of wheat doughs. Eur. Food Res. Technol. 214: 79-82.

Gunasekaran, S., and Ak, M.M. 2000. Dynamic oscillatory shear testing of foods - Selected applications. Trend Food Sci. Technol. 11: 115-127.

Gupta, R.B., Khan, K. and MacRitchie, F. 1993. Biochemical basis of flour properties in bread wheats I. Effects of variation in the quantity and size distribution of polymeric protein. J. Cereal Sci. 18: 23-41.

He, H., and Hoseney, R. 1991. Differences in gas retention, protein solubility, and rheological properties between flours of different baking quality. Cereal Chem. 68: 526-530.

Hibberd, G. 1970. Dynamic viscoelastic behavior of wheat flour doughs. Part III: The influence of the starch granules. Rheol. Acta. 9: 501-505.

Hlynka, I., and Barth, F.W. 1955. Chopin alveograph studies I. Dough resistance at constant sample deformation. Cereal Chem. 32: 463-471.

Huang, H., and Kokini, J.L. 1993. Measurement of biaxial extensional viscosity of wheat flour doughs. J. Rheol. 37(5): 879-891.

Huang, H., and Kokini, J.L. 1999. Prediction of dough volume development which considers the biaxial extensional growth of cells. In: Bubbles in Food. G. Campbell, C. Webb, S. Pandiella, and K. Niranjan, eds. AACC International: St. Paul, MN.

Huebner, F., and Wall, J. 1976. Fractionation and quantitative differences of glutenin from wheat varieties varying in baking quality. Cereal Chem. 53: 258-269.

Janssen, A.M., van Vliet, T., and Vereijken, J.M. 1996a. Rheological behaviour of wheat glutens at small and large deformations. Comparison of two glutens differing in bread making potential. J. Cereal Sci. 23: 19-31.

Janssen, A.M., van Vliet, T., and Vereijken, J.M. 1996b. Rheological behaviour of wheat glutens at small and large deformations. Effect of gluten composition. J. Cereal Sci. 23: 33-42.

Janssen, A.M., van Vliet, T., and Vereijken, J.M. 1996c. Fundamental and empirical rheological behavior of wheat flour doughs and comparison with bread making performance. J. Cereal Sci. 23: 43-54.

Kasarda, D. 1989. Glutenin structure in relation to wheat quality. Pages 277-302 in: Wheat is Unique. Y. Pomeranz, ed. AACC International: St. Paul, MN.

Khatkar, B., and Schofield, J. 2002a. Dynamic rheology of wheat flour dough. I. Nonlinear viscoelastic behaviour. J. Sci. Food Agric. 82: 827-829.

Khatkar, B., and Schofield, J. 2002b. Dynamic rheology of wheat flour dough. II. Assessment of dough strength and bread-making quality. J. Sci. Food Agric. 82: 823-826.

Khatkar, B., Bell, A., and Schofield, J. 1995. The dynamic rheological properties of glutens and gluten sub-fractions from wheats of good and poor bread making quality. J. Cereal Sci. 22: 29-44.

Kieffer, R., Wieser, H., Henderson, M., and Graveland, A. 1998. Correlations of the breadmaking performance of wheat flour with rheological measurements on a micro-scale. J. Cereal Sci. 27: 53-60.

Kilborn, R., and Tipples, K. 1972. Factors affecting mechanical dough development. I. Effect of mixing intensity and work input. Cereal Chem. 49: 34-47.

Kokelaar, J., van Vliet, T., and Prins, A. 1996. Strain hardening properties and extensibility of flour and gluten doughs in relation to breadmaking performance. J. Cereal Sci. 24: 199-214.

Launay, B., and Michon, C. 2008. Biaxial extension of wheat flour doughs: lubricated squeezing flow and stress relaxation properties. J. Texture Stud. 39: 496-529.

Lefebvre, J., and Mahmoodi, N. 2006. The pattern of linear viscoelastic behaviour of wheat flour dough as delineated from the effects of water content and high molecular weight glutenin subunits composition. J. Cereal Sci. 45: 49-58.

Leman, P., Bijttebier, A., Goesaert, H., Vandeputte, G., and Delcour, J. 2006. Influence of amylases on the rheological and molecular properties of partially damaged wheat starch. J. Sci. Food Agric. 86: 1662-1669.

MacRitchie, F. 1992. Physiochemical properties of wheat proteins in relation to functionality. Pages 1-87 in: Advances in Food and Nutrition Research, vol. 36. J. Kinsella, ed. Academic Press, Inc.: San Diego, CA.

Masi, P., Cavella, S., and Sepe, M. 1998. Characterization of dynamic viscoelastic behavior of wheat flour doughs at different moisture contents. Cereal Chem. 75: 428-432.

Mondal, A., and Datta, A., 2008. Bread baking-A review. J. Food Eng. 86: 465-474.

Morrison, W. 1995. Starch lipids and how they relate to starch granule structure and functionality. Cereal Food World. 40: 437-446.

Nasseri, S., Bilston, L., Fasheun, B., and Tanner, R. 2004. Modeling the biaxial elongational deformation of soft solids. Rheol. Acta. 43: 68–79.

Newberry, M.P., Phan-Thien, N., Larroque, O.R., Tanner, R.I., and Larsen, N.G. 2002. Dynamic and elongation rheology of yeasted bread doughs. Cereal Chem. 79: 874-879.

Olkku, J., and Rha, C. 1978. Gelatinisation of starch and wheat flour starch-a review. Food Chemistry. 3: 293-317.

Padmanabhan, M. 1995. Measurement of extensional viscosity of viscoelastic liquid foods. J. Food Eng. 25:311-327.

Park, S., Wilson, J., Chung, O., and Seib, P. 2006. Size distribution and properties of wheat starch granules in relation to crumb grain score of pup-loaf bread. Cereal Chem. 81: 699-704.

Phan-Thien, N., and Safari-Ardi, M. 1998. Linear viscoelastic properties of flour-water doughs at different water concentrations. J. Non-Newton Fluid Mech. 74: 137-150.

Pickett, M. 2009. Study of gas cell stability during breadmaking using x-ray microtomography and dough rheology. MS Thesis, Kansas State University.

Rao, V., Mulvaney, S., and Dexter, J. 2000. Rheological characterization of long- and short-mixing flours based on stress-relaxation. J. Cereal Sci. 31: 159-171.

Rouillé, J., Della Valle, G., Lefebvre, J., Sliwinski, E., vanVliet, T. 2005. Shear and extensional properties of bread doughs affected by their minor compounds. J. Cereal Sci. 42: 45-57.

Safari-Ardi, M., and Phan-Thien, N. 1998. Stress Relaxation and Oscillatory Tests to Distinguish Between Doughs Prepared from Wheat Flours of Different Varietal Origin. Cereal Chem. 75: 80-84.

Sliwinski, E., Kolster, P., Prins, A., and van Vliet, T. 2004a. On the relationship between gluten protein composition of wheat flours and large-deformation properties of their doughs. J. Cereal Sci. 39: 247-264.

Sliwinski, E.L., Kolster, P., and van Vliet, T. 2004b. Large-deformation properties of wheat dough in uni- and biaxial extension. Part I. Flour dough. Rheol. Acta. 43: 306–320.

Sliwinski, E.L., van der Hoef, F., Kolster, P., and van Vliet, T. 2004c. Large-deformation properties of wheat dough in uni- and biaxial extension. Part II. Gluten dough. Rheol. Acta. 43: 321-332.

Sofou, S., Muliawan, E., Hatzikiriakos, S., and Mitsoulis, E. 2008. Rheological characterization and constitutive modeling of bread dough. Rheol. Acta. 47: 369-381.

Song, Y., and Zheng, Q. 2007. Dynamic rheological properties of wheat flour dough and proteins. Trends Food Sci. Technol. 18: 132-138.

Sroan, B. and MacRitchie, F. 2008. Mechanism of gas cell stability in breadmaking. Pages 299-306 in: Bubbles in Food 2: Novelty, Health and Luxury. G. Campbell, M. Scanlon, and D. Pyle, eds. AACC International: St. Paul, MN.

Steffe, J. 1996. Rheological Methods in Food Process Engineering. 2nd ed. Freeman Press: East Lansing, MI.

Szczesniak, A. 1963. Classification of textural characteristics. J. Food Sci. 28: 385-389.

Szczesniak, A. 1998. Sensory texture profiling--Historical and scientific perspectives. Food Technol. 52(8): 54-57.

Tanner, R., Qi, F., and Dai, S.-C. 2008. Bread dough rheology and recoil I. Rheology. J. Non-Newton Fluid Mech. 148: 33-40.

Texture Technologies. 2010. Available at . Texture Technologies. Scarsdale, NY.

Tronsmo, K., Magnus, E., Baardseth, P., Schofield, J., Aamodt, A., and Faergestad, E. 2003b. Comparison of small and large deformation rheological properties of wheat dough and gluten. Cereal Chem. 80: 587-595.

Tronsmo, K., Magnus, E., Faergestad, E., and Schofield, J. 2003a. Relationships between gluten rheological properties and hearth loaf characteristics. Cereal Chem. 80: 575-586.

Uthayakumaran, S., Beasley, H., Stoddard, F., Keentok, N., Phan-Thien, N., Tanner, R., and Bekes, F. 2002. Synergistic and additive effects of three high molecular weight glutenin subunit loci. I. Effects on wheat dough rheology. Cereal Chem. 79: 294-300.

Uthayakumaran, S., Newberry, M., Keentook, M., Stoddard, F., and Bekes, F. 2000. Basic rheology of bread dough with modified protein content and glutenin-to-gliadin ratios. Cereal Chem. 77: 744-749.

van Bockstaele, F., De Leyn, I., Eeckhout, M., and Dewettinck, K. 2008. Rheological properties of wheat flour dough and the relationship with bread volume. II. Dynamic oscillation measurements. Cereal Chem. 85: 753-761.

van Vliet, T., Janssen, A., Bloksma, A., and Walstra, P. 1992. Strain hardening of dough as a requirement for gas retention. J. Texture Stud. 23: 439-460.

Walker, C., and Hazelton, J. 1996. Dough rheological tests. Cereal Food World. 41: 23-28.

Weipert, D. 1990. The benefits of basic rheometry in studying dough rheology. Cereal Chem. 67: 311-317.

Whitworth, M., and Alava, J. 1999. The imaging and measurement of bubbles in bread doughs. Pages 221-231 in: Bubbles in Food. G. Campbell, C. Webb, S. Pandiella, and K. Niranjan, eds. AACC International: St. Paul, MN.

Wikstrom, K., and Bohlin, L. 1996. Multivariate analysis as a tool to predict bread volume from mixogram parameters. Cereal Chem. 73: 686-690.

CHAPTER 3 - Materials and Methods

3.1 Materials

Wheat of three common Kansas varieties (Overly, Karl 92, and 2137) from the 2009 crop year were obtained from KSU Foundation Seed, Kansas State University. One other 2009 crop year certified Kansas wheat variety, Santa Fe, was obtained from Phillips Seed Farms Inc. Karl 92 and Overley are characterized as high protein content flours with exceptionally good baking qualities, and Karl 92 is strong enough that it requires blending with lower quality flours for optimal bread production (Kansas State University 2009). 2137 and Santa Fe are classified as having acceptable baking qualities, but 2137 has a lower protein content and Santa Fe has a medium protein content (Kansas State University 2009). In 2009 Overley had the highest percentage of acres of wheat grown in Kansas (13.7%), Santa Fe had the third highest (9.5%), 2137 had 2.9%, and Karl92 had 0.8% (Kansas State University 2009). These wheat cultivars were chosen because they are of varying protein contents and commonly grown in Kansas.

All wheat samples were milled on a Buhler mill model MLU-202 (Bühler AG, Switzerland) into untreated, unbleached straight-grade flour (AACC method 26-21A) and stored in airtight plastic bags at -20 ºC until used.

3.2. Physico-Chemical Analysis of Wheat Flours

3.2.1. Proximate Analysis

Flour moisture and protein contents were determined by near infrared analysis on a diode array 7200 NIR from Perten Instruments (Springfield, IL, USA). Ash content of the flour was determined by the muffle furnace method (AACC method 08-02).

3.2.2. Protein Quality

Sizes and relative proportions of gluten proteins in each wheat flour were evaluated by size exclusion high performance chromatography (SE-HPLC) (Hewlett-Packard 1100 Agilent HPLC, Agilent Technologies, Santa Clara, CA, USA). A Biosep SEC-4000 column (Phenomenex, Torrance, CA, USA) was used to fractionate gluten proteins. Total, extractable, and unextractable polymeric proteins were determined using separate samples. A mobile phase

of phosphate/SDS buffer at pH 6.9 was injected with a flow rate of 0.5 mL/min and total injection volume of 20 μL/sample. Detection of proteins in SE-HPLC was via absorption at 214 nm. All data was analyzed using ChemStation (Agilent Technonlogies, Santa Clara, CA, USA) software.

3.2.2.1. Total Protein Analysis

To prepare samples for total protein analysis, the method developed by Singh et al (1990) was used. In this method, 1 mL of phosphate/SDS buffer and 10 mg flour was weighed into a 1.7 mL microfuge tube vortexed for 5 min to suspend the flour in solution. Sonication of the mixture was performed at an output of 6 watts for 15 sec at room temperature (60 Sonic Dismembrator, Fisher Scientific, Pittsburgh, PA, USA) to ensure that the largest molecular size fraction was solubilized. The solutions were then centrifuged at 12,000 x *g* for 20 min. The supernatant was filtered (0.45 μm pore) before transferring to an HPLC vial.

3.2.2.2. Extractable and Unextractable Protein Analysis

Sample preparation for extractable protein analysis was completed by weighing 10 mg flour and 1 mL phosphate/SDS buffer into a 1.7 mL microfuge tube, vortexing for 5 min, and centrifuging at 12,000 x *g* for 20 min. Supernatant liquid was passed through a 0.45 μm pore filter and used for SE-HPLC analysis. The pellet remaining from centrifugation was then prepared for unextractable protein analysis. The pellet was added to 1mL of phosphate/SDS buffer in a 1.7 mL microfuge tube and vortexed for 10 min. Sonication was then performed for 25 sec at 6 watts to reduce the size of proteins of high molecular weight. Samples were centrifuged at 12000 x *g* for 20 min, and the supernatant was filtered and used for analysis by SE-HPLC.

3.3. Pasting Characteristics (RVA)

AACC method 76-21 was used to perform rapid visco analysis on an RVA-4 (Perten Instruments AB, Kungens Kurva, Sweden) and provide measurements on flour pasting characteristics for the four cultivars (at a solid content of 10%) in this study. Information on flour pasting temperature, peak viscosity, holding strength, and final viscosity were collected by the software program, ThermoCline for Windows (version 3) that controls the RVA.

3.4. Dough Mixing Properties

The farinograph and mixograph were used to determine dough mixing properties. AACC method 54-21 was used to determine farinograph consistency, water absorption (adjusted to 14% MC), development time, stability, mixing tolerance index (MTI), time to breakdown, and farinograph quality number

A modified version of AACC method 54-40A was used for mixograph testing that used the water absorption determined during the farinograph test instead of the normal mixograph water procedure. The 10 g mixograph (National Manufacturing Co., Lincoln, NE, USA) was used to measure peak time, peak height, peak band width, peak work input, developing slope, weakening slope, and the angle created by the developing and weakening slopes. The peak time was used to determine mixing time for each cultivar dough.

3.5. Rheological Properties

A stress controlled rheometer (Bohlin C-VOR, Malvern Instruments, Malvern, Worcestershire, UK), equipped with a parallel plate measuring system (20 mm diameter, gap 2.0 mm) and plate temperature held constant at 30 ºC, was used to measure the small deformation rheology of dough samples.

3.5.1. Sample Preparation

Dough samples were prepared by mixing 10 g flour (adjusted to 14% moisture) using a mixograph (National Manufacturing Co., Lincoln, NE, USA) at optimum water absorption levels determined previously by the farinograph using AACC method 54-21 and mixing times determined by the previous mixograph testing using a modified version of AACC method 54-40A where the water absorption determined during the farinograph test was used instead of the normal mixograph water procedure. Two small balls of approximately 2 g were made from each mixed dough sample and placed on parchment paper in an uncovered plastic bowl, then rested in a humidity and temperature-controlled cabinet set at 30 ºC and 85 % relative humidity for 10 min. After resting, a lid was placed on the bowl to transport the sample to the rheometer located in the same room. The dough piece was removed from the parchment paper using a sharp plastic knife to avoid excess deformation of the dough and placed on the rheometer's bottom plate.

The rheometer was lowered to a gap of 2.0 mm, and the excess sample was trimmed. Trimming was done with a sharp plastic blade in a downward motion to avoid excess deformation of the dough while cutting it even with the edge of the top plate. Silicone oil was used to keep the edges of the dough from drying. After trimming, samples were allowed to rest for 20 min prior to testing as suggested by Phan-Thien and Safari-Ardi (1998).

3.5.2. Strain Sweep (Linear Viscoelastic Region)

Strain sweeps were performed to determine the linear viscoelastic region of the wheat flour doughs. Samples were prepared, rested, and loaded. Tests were done at 30 ºC at varying strain rate of $5x10^{-5}$ to $5x10^{1}$ and at a constant frequency of 1.0 Hz.

3.5.3. Frequency Sweep

Frequency sweeps were performed at frequencies of 0.1 to 500 Hz with a constant strain rate of 0.001 s^{-1}at 30 ºC. At least three replicates (separate dough batches) were performed for each wheat variety. Data for elastic modulus (G′), viscous modulus (G″), complex modulus (G*), shear stress, phase angle, and complex viscosity were collected and used to compare the wheat varieties.

3.5.4. Temperature Sweep

Temperature sweeps were performed from 30-90 ºC at a heating rate of 1.5 ºC/min with a constant strain rate of 0.001 s^{-1} and a frequency of 1.0 Hz. At least three replicates using separately prepared doughs were performed for each flour type. Cultivars were compared using data for elastic modulus (G′), viscous modulus (G″), complex modulus (G*), shear stress, phase angle (δ), and complex viscosity (η*).

3.5.5. Creep Recovery

Creep recovery measurements were performed on each flour type using an isothermal temperature of 30 ºC and shear stress of 50 Pa over a creep time of 1200 sec and recovery time of 1200 sec. For each cultivar, at least three separately prepared doughs were measured for replication. Data for the maximum creep strain (J_{max}), maximum recovery strain (J_m) and percent recovery (recovery strain expressed as a percent of J_{max}) were collected to compare cultivars.

3.5.6. Stress Relaxation

At least three replicates of each cultivar were analyzed by stress relaxation using separately prepared doughs. Constant temperature of 30 ºC and strain of 0.1% were used for the 250 sec stress relaxation test. Relaxation modulus, G(t), and relaxation spectrum were collected and analyzed to compare wheat varieties.

3.6. Test Baking

The straight-dough procedure (AACC method 10-10B) using 100 g (flour weight) was used to bake each cultivar. Each dough was mixed in a 100 g pin mixer (National Manufacturing Co., Lincoln, NE, USA) using optimized mixogram water absorptions and mix times as described in the method. Proofing was performed at 30 ºC and 95% relative humidity for 90 min. Throughout proofing, doughs were punched twice, then molded on a specialized pup loaf molder. After molding, panning, and final proofing, the doughs were baked for 20 min at 210 ºC in a reel oven (National Manufacturing Co., Lincoln, NE, USA). Directly after being removed from the oven, loaf volume was measured with a calibrated rape seed displacement meter (AACC Method 10-05).

3.7. Bread Morphology

3.7.1. Imaging of Crumb Structure

Baked loaf crumb structure was determined 24 hr after baking using C-Cell image analysis software and equipment (Calibre Control International Ltd., Appleton, Warrington, UK). In preparation for analysis, bread loaves were sliced to a thickness of 15 mm using a rotary disk food slicer (Chef's Choice International), and the middle three slices were retained for C-Cell. Image analysis was performed to provide data including: number of cells, average cell wall thickness, and cell diameter.

3.7.2. Bread Microstructure

Bread samples were scanned using a high resolution desktop x-ray microtomograph (XMT) (Skyscan 1072, Aartselaar, Belgium) consisting of a rotatable sample stage, an x-ray tube, an x-ray detector, and a 12-bit, cooled CCD-camera (1024x1024 pixels).

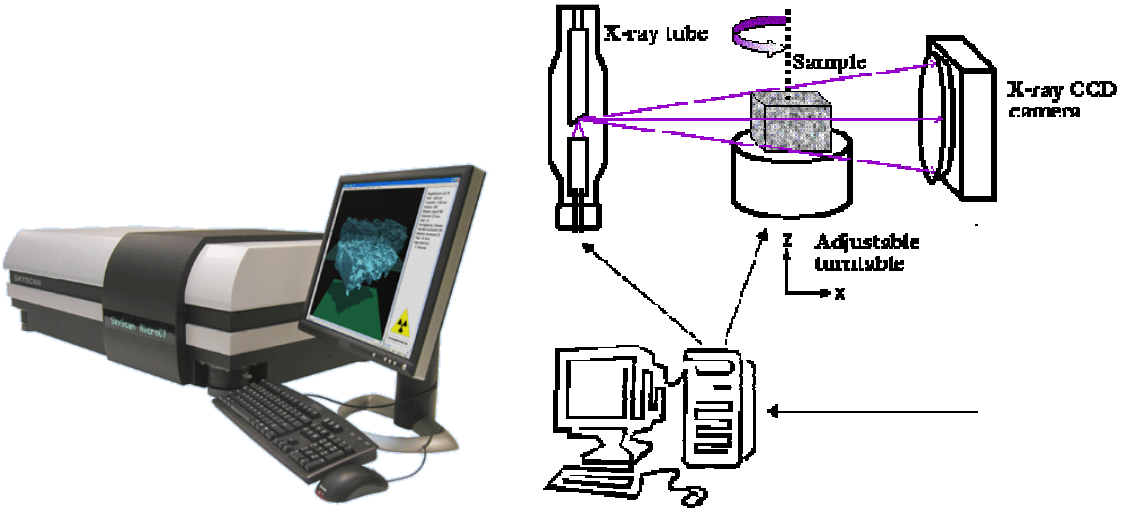


Figure 3.1. Diagram of the x-ray microtomograph's mechanics.

3.7.2.1. Sample Preparation

In preparation for XMT scanning and analysis, a small (8 mm x 8 mm x 15 mm) rectangular section of the central bread loaf slice (used for C-Cell) was cut using a sharp knife while the bread was frozen. The cut specimen was then placed with the bottom part of the sample downward in a clear plastic tube (15 mm diameter) to prevent drying of the bread during scanning. The bread samples were adhered to a double sided self-adhesive disc to stabilize the specimen. Then the plastic tube was secured on the rotatable sample stage using double sided tape.

3.7.2.2. Scanning

Scanning was performed at 40 kV/248 μA at 20x magnification (resulting in 13.7 μm/pixel resolution) at 1.35º scan steps with an exposure of 1.88 sec through 180º of rotation. Total scanning time was around 15 min/sample. The scanning process was controlled by SkyScan 1072-TomoNT control software (version 3N.5).

3.7.2.3. Image Reconstruction

Sets of 138 2-D radiographs (shadow images) per sample were rendered into 3-D objects using a filtered back-projection algorithm by the NRecon reconstruction software (V1.5.1.) which were subsequently digitally sliced to create hundreds of 2-D cross-sectional images that could be used for quantitative analysis. Samples were rotated before reconstruction using the CS

rotation function of the program to orient the 2-D cross-sections parallel to the screen. To minimize erroneous indications of solid particles caused by beam hardening, the beam hardening correction was set to 40%. A dynamic image range of 0.015-0.06 1/mm (attenuated coefficient) was selected in the gray-scale histogram to give an optimized clear reconstruction of the object. These reconstruction parameters yielded 975 cross-sectional images with a thickness of 14 μm.

3.7.2.4. Image Analysis

Image analysis was performed using CT-analysis processing and analysis software (CTAn, v.1.7). A 5.02.mm x 6.02 mm rectangular region of interest (ROI) was defined in the center of the bottom slice. This rectangular section was then interpolated across the selected 600 layers (i.e. reconstructed 2-D cross-sectional images) with a total thickness of 8.21 mm to define the volume of interest (VOI). This created a 3-D volume of interest that avoided misrepresentative data caused by ragged cut edges of the bread sample. By using a VOI smaller than the originally scanned specimen, it was possible to avoid large air inclusions (make-up holes) that would have skewed the data.

Images were then converted from grayscale to pure black and white (binary images) for analysis. Grayscale images have pixels with values that range from 1-255. The range of 0-64 was converted to pure black, representing void areas or gas cells. Pixels in the range of 65-255 were converted to pure white to represent cell wall structures. After conversion to binary images, cross-sections were despeckled to eliminate pixels considered as instrumental noise. Black speckles of 5 or fewer pixels and white speckles of 20 or fewer pixels were removed.

3.7.2.5. Quantitative Analysis

CTAn uses the marching cubes algorithm and marching cubes surface construction algorithm for quantitative analysis of morphometric parameters including void volume, average cell size, cell size distribution, average cell wall thickness and thickness distribution, fragmentation index, and structure model index. Specific information on the algorithm methods used can be found in Feldcamp et al (1984) and Lorensen and Cline (1987). The software features include:

Structure Separation (St. Sep): Average of the thickness of the spaces between structures (cell walls) i.e. measure of air cell size (mm). Analysis also provides air cell size distributions in the form of histograms.

Structure Thickness (St. Th): Average of the thicknesses of solid structure, used as a measure of average cell wall thickness (mm). Analysis also provides cell wall thickness distributions in the form of histograms.

Void Fraction (VF): The ratio of void volume to total volume within the VOI; measure of the percentage of the sample occupied by air.

Fragmentation Index (FI): Measure of the connectivity between air cells; calculated by the relative concavity or convexity of the structure surface with concavity indicating connectivity and convexity indicating isolated, disconnected structures. More connected structures (open cells) cause a lower FI.

3.8. Texture Analysis

Texture profile analysis (TPA) was performed using a TA-XT2 texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) on slices from the baked loaves. Slices were analyzed 24 hr after baking, at the same time that C-cell measurements were taken. At least 6 measurements were performed (2 from each loaf) per wheat variety using a 1 inch diameter probe at a constant speed of 1.0 mm/sec during the pre-test, test, and post-test. A compression of 10 mm was used, which corresponded to 40% of the sample thickness, and a trigger force of 5.0 g was specified. Between the first and second compression, a rest time of 10 sec was allowed. Resulting force-rime curves were used to determine hardness (kg), fracturability (kg), springiness, cohesiveness, chewiness, and resilience values of the bread samples.

3.9. References

Feldkamp, L., Davis, L., and Kress, J. 1984. Practical cone-beam algorithm. J Opt Soc Am A. 1: 612–619.

Kansas Performance Tests with Winter Wheat Varieties Report of Progress 1018, 2009. Kansas State University Agricultural Experiment Station and Cooperative Extension Service. Contribution number 10-008-S. Available at .http://kscroptests.agron.ksu.edu.

Lorensen, W. and Cline, H. 1987. Marching cubes: A high resolution 3D surface construction algorithm. Comput. Graphics. 21: 163-169.

Phan-Thien, N., and Safari-Ardi, M. 1998. Linear viscoelastic properties of flour-water doughs at different water concentrations. J. Non-Newton. Fluid. Mech. 74: 137-150.

Singh, N., Donovan, G., Batey, I., and MacRitchie, F. 1990. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. I. Dissolution of total proteins in the absence of reducing agents. Cereal Chem. 67: 150-161.

CHAPTER 4-Results and Discussion

4.1. Physicochemical Analysis of Wheat Flours

4.1.1. Protein Content/Quality

Tables 4.1 and 4.2 present the findings of the compositional and SE-HPLC analysis of the four flours. Protein contents of the wheat flours were almost in line with the protein valuations given by the Kansas Performance Tests with Winter Wheat Varieties (Kansas State University 2009), the only difference being that the protein content of Santa Fe was lower than 2137 for the flour in this study whereas 2137 was reported as having a lower protein rating in the performance tests. Starch contents were not measured in this method. Overley and Karl 92 have almost 2 percent more protein than Santa Fe, and 2137 has about 1.5 percent more protein than Santa Fe. A wide range of protein contents (9.27-11.55%) is shown for Kansas hard red winter wheat varieties since hard red winter wheats generally have protein contents of 10-13%.

The gluten proteins are quantitatively the most important protein fraction in dough. It has been suggested that gliadins generally contribute to the viscosity of dough and gluten, whereas glutenins contribute to their elasticity. Percent total polymeric proteins (TPP) ranged from 40.16 (Karl 92) to 43.00 (2137), and had groupings of Overley and Karl 92, Overley and Santa Fe, and Santa Fe and 2137 that were not significantly different. Data from analysis of gluten fractions (Table 4.2) showed that 2137 was significantly ($P< 0.008$) the lowest in percent monomeric proteins (MP) with Santa Fe the second lowest, but Santa Fe and Karl 92 were not significantly different. Karl 92 and Overley were not significantly different, but they were lower than the other flours in percent MP.

Monomeric proteins (gliadins) provide the viscous nature of the dough and act as a plasticizer (Cornec et al 1994; Khatkar et al 1995; Tronsmo et al 2003a; Tronsmo et al, 2003b; Sliwinski et al 2004), and high quality dough requires a sufficient amount of extensibility to allow gas bubble expansion. Addition of total gliadins has been reported to decrease mixing time, peak height, extensograph R_{max}, and loaf height, and increase resistance to breakdown and extensibility. The investigations of the effects of glutenin subunits on dough properties have shown that flour with a higher HMW-GS to LMW-GS ratio has better bread-making quality than a lower ratio. Recently, Uthayakumaran et al (1999, 2001) also reported that when the HMW-GS

to LMW-GS ratio increased, mixing time, peak height, R_{max} and loaf height increased; but resistance to breakdown and extensibility decreased.

Table 4.1. Chemical composition of the four Kansas wheat cultivar flours.

	% Moisture	% Total Protein*, **	% Ash*, **
Overley	12.77±0.21	11.55	0.44
Karl 92	12.98±0.27	11.20	0.40
2137	12.95±0.20	10.82	0.49
Santa Fe	12.60±0.09	9.27	0.51

[*] *Protein and ash were determined by NIR, then calculated to 14% flour moisture content, so standard deviations were not obtained for them.*
***14% moisture basis*

Table 4.2. Size exclusion high performance liquid chromatography analysis of flours.

	TPP (%)	MP (%)	SP (%)	UPP (%)	Glutenin to Gliadin ratio
Overley	40.53±0.84 [ab]	49.92±0.77 [a]	9.56±0.07 [a]	54.14±1.82 [a]	0.81 [a]
Karl 92	40.16±0.01 [a]	49.14±0.01 [ab]	10.50±0.31 [b]	56.06±1.61 [a]	0.82 [ab]
2137	43.00±0.14 [c]	45.12±0.12 [c]	11.89±0.09 [c]	48.42±0.22 [b]	0.95 [c]
Santa Fe	42.13±0.16 [bc]	48.08±0.05 [b]	9.71±0.15 [a]	48.97±00.1 [b]	0.88 [b]

TPP *Total polymeric proteins (Glutenins)*
MP *Monomeric proteins (Gliadins)*
SP *Soluble proteins (Albumins+Globulins)*
UPP *Unextractable polymeric proteins*
[a] *Values with the same letter in the same column are not significantly different at (P <0.05).*
[b] *SE-HPLC results are the average of two separate runs.*

The total protein content and glutenin to gliadin ratio of flour are known to affect dough and baking properties independently (Uthayakumaran et al 1999). When glutenin to gliadin ratio is constant, mixing time, peak height, $R_{max,}$ E_{xt} and loaf volume increases as the protein content increases. At constant protein content, increases in glutenin to gliadin ratios have been associated with increases in mixing time, peak height, $R_{max,}$ and loaf volume, and with a decrease in extensibility. In addition, several authors have shown positive correlations between UPP content of flours and extensibility and loaf volume (Newberry et al 2002; Tronsmo et al 2003a).

The higher protein flours, Overley and Karl 92, had significantly ($P<0.008$) higher unextractable polymeric protein (UPP) than the lower protein content flours 2137 and Santa Fe. UPP is the portion containing the highest molecular weight proteins (Gupta et al 2003), and

therefore is positively associated with loaf volume (Newberry et al 2002; Tronsmo et al 2003a). Some small amounts of protein may have been left in the pellet after extractions, so there could be varying amounts of unextractable polymeric proteins that were not accounted for in each of the different wheat flours.

Soluble proteins (SP, albumins and globlulins) were significantly ($P < 0.05$) the highest in 2137, followed by Karl 92, then Overley and Santa Fe. The glutenin to gliadin ratio is important because dough must have a good ratio of elasticity to extensibility, which allows air bubbles to expand while keeping them from coalescing or escaping, and provides a good baked product (Kasarda 1989). 2137 having the highest percentage of SP, highest glutenin to gliadin ratio, and the lowest UPP showed that it had a lot of low molecular weight proteins compared to the other wheats, yet it maintained the highest ratio of high molecular weight to low molecular weight proteins. No specific ratio of gliadin to glutenin is known to be ideal for breadmaking. The dough must simply be extensible enough to allow gas bubbles to expand and elastic enough to keep the gas from escaping the dough.

4.2. Dough Mixing Properties

4.2.1. Farinograph

The Brabender farinograph is one of the most widely used recording dough mixers. The resulting farinogram provides a range of information on mixing behavior of dough samples in relation to their strength. Dough development time and stability time increase with increasing strength of flour. Whereas mixing tolerance index (MTI) and degree of softening decrease with increasing strength of flour. Stronger flours with higher protein content and better gluten quality are characterized by higher water absorption. The 500 Brabender unit (BU) line is considered the optimum consistency for dough, so the middle line of the farinogram curve is desired to be centered over the 500 BU line. The width of the farinogram, as indicated by difference between the top and bottom lines, gives the strength of the dough. Stronger, stiffer doughs will yield wider farinogram curves.

The farinograms in Figure 4.l show the different mixing qualities of the four cultivars used in this study. All farinograms have the same dough strength until the 5 min mark as indicated by their similar width. After 5 min, 2137 and Santa Fe began to show a decrease in the band width as well as a departure of the midline from the 500 Brabender unit (BU) line, showing

their breakdown. Karl 92's midline dropped below 500 BU at about 7 min, and Overley's at about 8 min, demonstrating that Overley had the greatest dough strength and Karl 92 was the second strongest. Even though after 5 min 2137 and Santa Fe showed greater breakdown than the other cultivars, they still showed more strength than would be expected of a weak flour. This is likely because they are still hard red winter wheats, despite their lower protein content.

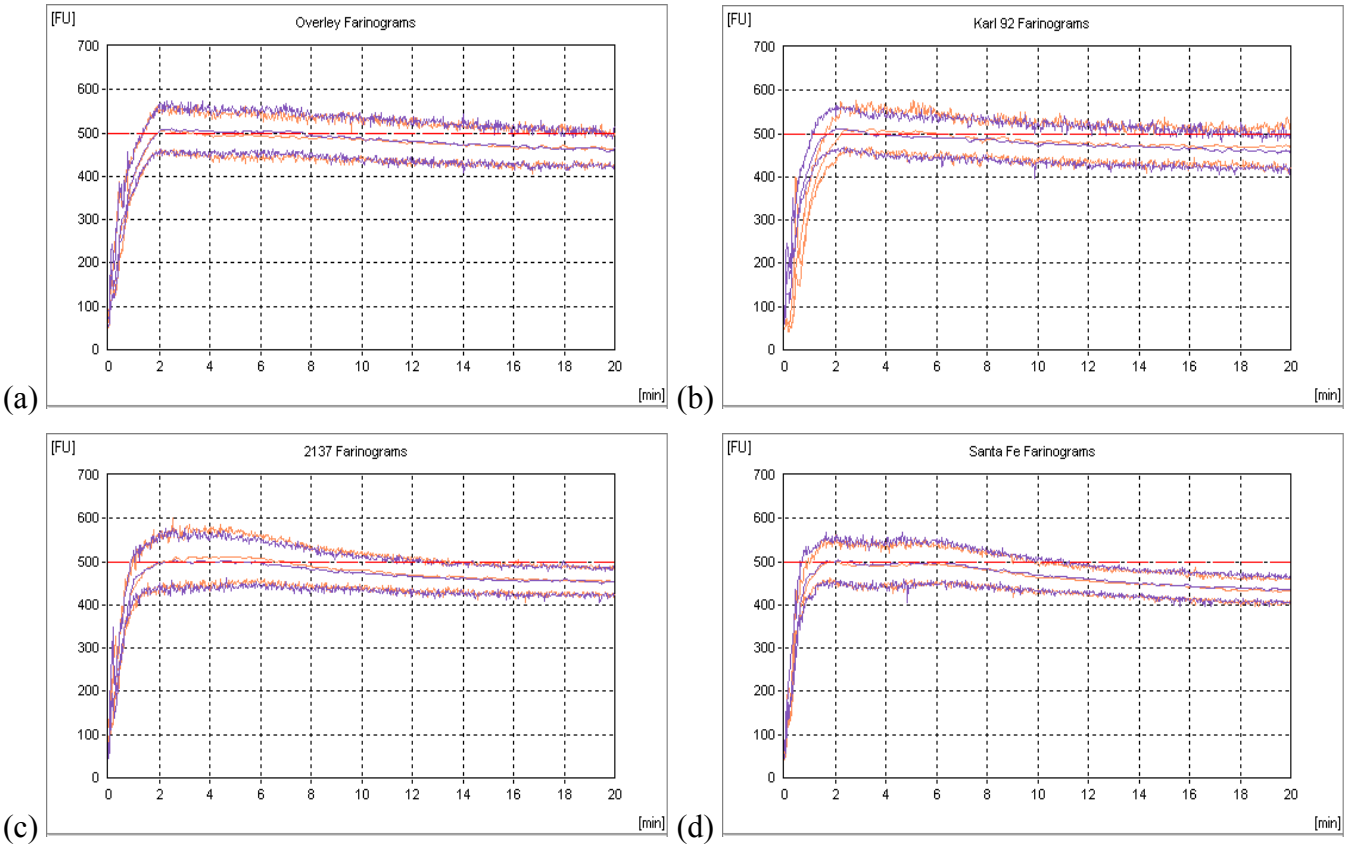


Figure 4.1. Farinograms for wheat flours (a) Overley, (b) Karl 92, (c) 2137, and (d) Santa Fe.

Table 4.3. Farinogram mixing parameters.

	Absorption* (%)	Development time (min)	Stability (min)	Mixing Tolerance Index	Time to breakdown (min)	Farino Quality Number
Overley	60.25±0.07 [a]	2.45±0.07 [a]	16.60±1.41 [a]	13.50±6.36 [a]	12.00±0.71 [a]	120.00±7.07 [a]
Karl 92	61.10±0.00 [b]	3.00±0.71 [a]	16.70±2.12 [a]	22.00±1.41 [ab]	8.35±0.78 [b]	83.50±7.78 [b]
2137	58.40±0.14 [c]	4.20±1.41 [a]	10.15±0.35 [b]	28.50±4.95 [b]	9.25±0.07 [b]	92.50±0.71 [b]
Santa Fe	60.35±0.07 [a]	2.00±0.28 [a]	9.35±0.64 [b]	11.50±2.12 [a]	9.35±0.35 [b]	93.50±3.54 [b]

[a] *Values with the same letter in the same column are not significantly different at (P <0.05).*
[b] *Data are the result of three replicates.*
** 14% moisture basis*

The farinograph quality number of 120±7 and time to breakdown of 12.0±0.7 min shown in Table 4.2 for Overley were significantly larger than the other wheats, illustrating Overley's relative strength. Overley and Karl 92 had very similar farinograms (Figure 4.1a and b) because they had much better stability times (16.6±1.41 and 16.7±2.12 min respectively) than 2137 (10.15±0.35 min) and Santa Fe (9.35±0.64 min). Higher stability values suggest stronger doughs (Autio et al 2001), so farinograms of Overley and Karl indicated that they were good quality flours that created good doughs. Karl 92 had the highest absorption value at 61.1% with values for Overley (60.3%) and SantaFe (60.4%) falling in the middle, and 2137 retaining the lowest absorption of 58.4%. Absorption values are influenced by both protein and starch content, including the amount of damaged starch in flour, which can be a direct result of the milling process. However, all samples were milled using the same process and equipment, so only their inherent differences would cause differing amounts of damaged starch. Typically higher protein and damaged starch contents increase the water absorption capacity. Although Santa Fe had the lowest protein content, its water absorption was found to be as high as that of Overley which may indicate presence of damaged starch in Santa Fe.

4.2.2. Mixograph

The mixograph is another widely used recording mixer. The mixing action is provided by four planetary pins revolving about three stationary pins attached to the bottom of the mixing bowl. The mixing can be described as a pull, fold, and repull action, which is more severe than that produced by the farinograph. The shape of a mixogram can be characterized by indices similar to those defined for the farinograph (AACC Method 54-40). Peak mixing time is similar to dough development time. Peak height (%) provides information about flour strength and absorption. Resistance to breakdown is similar to MTI. A lower resistance to breakdown and smaller slopes of the ascending and descending portions of the curve at the peak indicate a greater tolerance to overmixing. A higher tolerance to overmixing and overall flour strength can also be judged from the area under the curve.

Mixograms presented in Figure 4.2 illustrate the relative strength of Karl 92 (b) and weakness of 2137 (c) optimally mixed doughs. Walker and Hazelton (1996) stated that high protein hard winter and spring wheats take longer and require more work input to reach their mixograph peak and result in larger peak heights than lower protein, soft wheats. The peak

mixing times did not show a direct relation to protein content in this study, as Overley, Karl 92, and Santa Fe had similar mixing times, which were not significantly different. All the wheats in this study were hard red winter wheats, and even though Karl 92 had the largest values for work input, it was not significantly different from the other flours. These results suggested that all four flours required a large amount of mechanical torque to be mixed to their optimal consistency. Peak heights ranged from the highest, Karl 92 at 50.35±0.03 %, to the lowest, Santa Fe at 44.86±0.42 %, and the trend was Karl 92 > Overley > 2137 > Santa Fe.

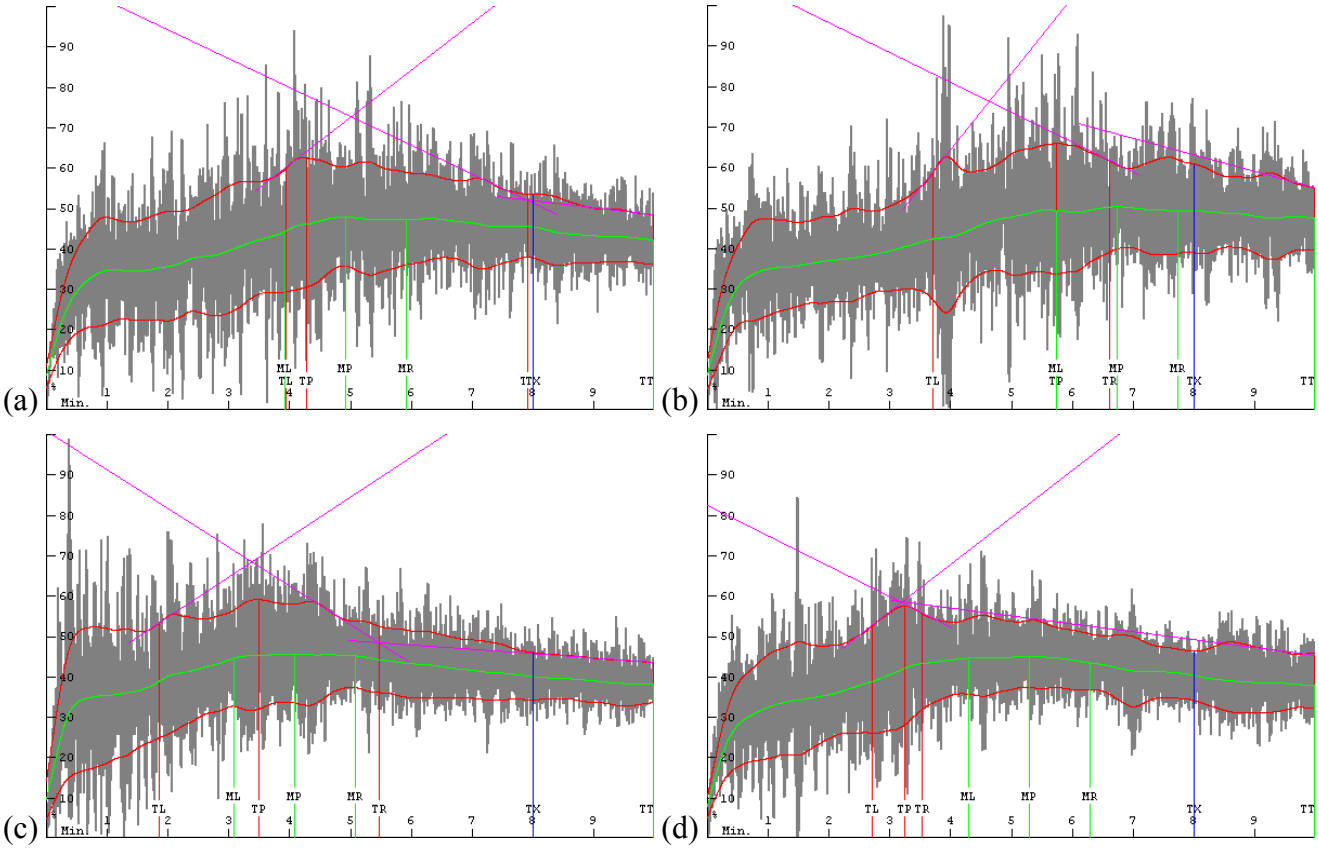


Figure 4.2. Mixograms for wheat flours (a) Overley, (b) Karl 92, (c) 2137, (d) Santa Fe at water absorptions determined by farinograph measurements.

Table 4.4. Mixograph mixing parameters for midline analysis of four Kansas wheat cultivar flours.

	Water Absorption (%)	Peak Mixing Time (min)	Peak Height (%)	Descending Slope (%/min)	Peak Band Width (%)	Peak Work Input (%Tq*min)
Overley	60.25±0.07 [a]	5.03±0.16 [ab]	48.65±1.13 [ab]	-0.27±0.74 [a]	27.46±4.02 [a]	191.6±10.5 [a]
Karl 92	61.10±0.00 [b]	6.15±0.83 [a]	50.35±0.03 [a]	-0.53±0.52 [a]	25.43±6.63 [a]	244.6±41.3 [a]
2137	58.40±0.14 [c]	3.72±0.51 [b]	46.28±0.95 [bc]	-0.52±1.11 [a]	25.39±1.25 [a]	141.0±23.5 [a]
Santa Fe	60.35±0.07 [a]	4.99±0.42 [ab]	44.86±0.42 [c]	-1.78±0.39 [a]	17.40±1.22 [a]	185.7±17.4 [a]

[a] *Values with the same letter in the same column are not significantly different at (P <0.05)*
[b] *Results are the averages of two replicates.*

4.3. Pasting Characteristics

Rapid viscoanalysis (RVA) is a commonly used method to evaluate pasting characteristics of starch and flour solutions to determine their gelatinization temperature and peak viscosity. RVA pasting curves for the four cultivars are typical for wheat flour (Figure 4.3). Initial pasting temperatures (66-67 °C) (Table 4.4) indicated that the beginning of starch gelatinization and was not significantly different ($P < 0.05$) between cultivars.

The maximum viscosity of the mixture during heating was measured by the peak viscosity, and flours studied ranged from 2200 centipoise (cP) for Santa Fe to 2651 cP for Karl 92. Viscosities at 50 °C during cooling were lower for Overley, Santa Fe and 2137 which could indicate relatively higher α-amylase activity in these flours as compared to that of Karl 92. Confirmation of α-amylase activity would be required via inhibitor assay to definitively conclude α-amylase is the cause of the differences in viscosity. However, the starch content of each flour sample was not the same since only whole flour amounts were weighed out, and the amount of damaged starch in these flours was unknown, both of which could have greatly influenced pasting viscosities.

Karl 92 had the highest peak viscosity and the highest trough viscosity and final viscosity ($P<0.05$) signifying that it had better starch-protein interactions than the other cultivars. The lower values for peak, trough, and breakdown viscosities seen for Santa Fe showed that it had more viscous character during heating than the other wheat flours. Leon et al (2006) reported that the flour viscosity during pasting decreases as damaged starch content increases. Lower peak viscosity of Santa Fe could be associated with its starch quality. Low peak viscosities have

been further correlated to lower loaf volume and lower form factor (proper pan bread shape) by Repeckiene et al (2001), which explains the low baking performance of Santa Fe reported in section 4.5.1.

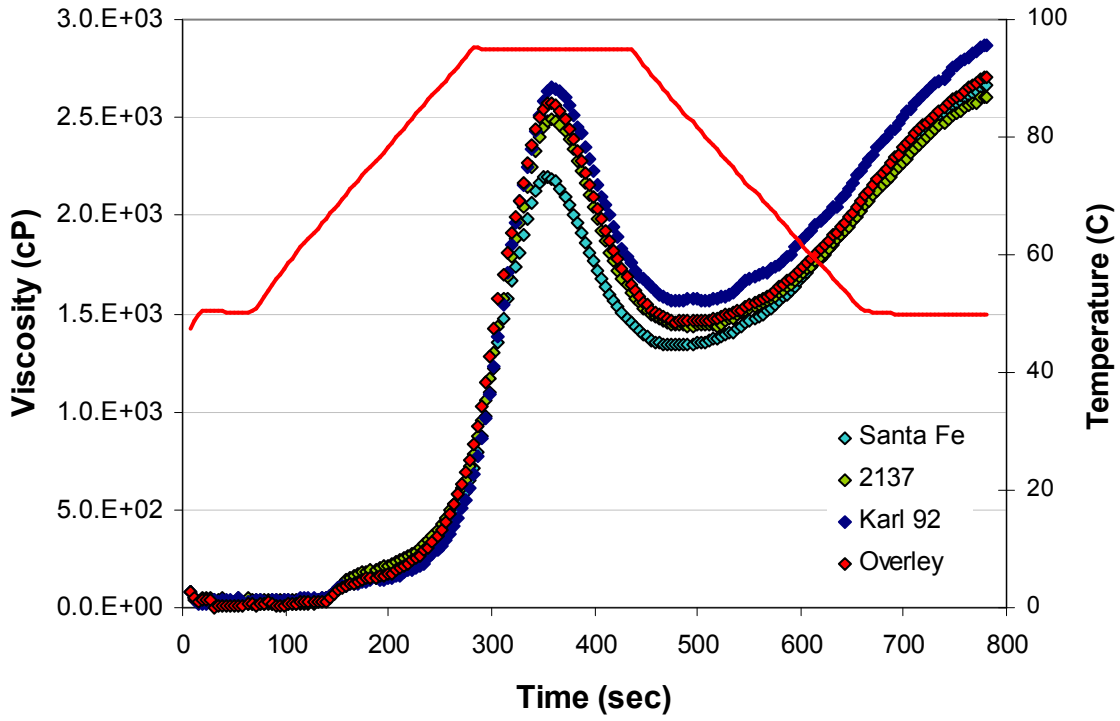


Figure 4.3. Rapid visco analysis pasting profiles of wheat flours at 10% solid content.

Table 4.5. Rapid visco analysis pasting characteristics of the four Kansas wheat cultivars studied.

	Pasting temperature (ºC)	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown viscosity (cP)	Final viscosity (cP)
Overley	66.80±0.43 [a]	2571±59 [a]	1452±53 [a]	1120±8 [a]	2703±55 [a]
Karl 92	65.67±0.46 [a]	2651±33 [a]	1558±31 [a]	1093±7 [a]	2865±29 [b]
2137	66.43±0.46 [a]	2631±153 [a]	1451±13 [a]	1184±161 [a]	2709±103 [a]
Santa Fe	65.93±1.89 [a]	2200±12 [a]	1335±18 [a]	864±25 [b]	2665±3 [a]

[a] *Values with the same letter in the same column are not significantly different at (P <0.05)*
[b] *Results are the averages of three replicates.*

4.4. Rheological properties

4.4.1. Strain sweep (Linear viscoelastic region)

Before performing any dynamic measurements, the limit of linear viscoelastic region (LVR) was first determined by a set of strain sweep tests, performed at constant frequency of 1 Hz. It can be seen in Figure 4.4. the value of the storage and loss moduli were relatively constant for strain values less than 0.1%, whereas moduli started to decrease at higher values, indicating the onset of nonlinear behavior. Drop of elastic modulus, G′, started to occur above 0.1% strain and became large above 1% strain, indicating the breakdown of the dough structure beyond this deformation level. Similarly, it has been previously found that wheat flour-water doughs exhibit linear viscoelasticity at strain levels lower than 0.1-0.25% (Phan-Thien and Safari-Ardi 1998; Safari-Ardi and Phan-Thien 1998; Weipert 1990).

Although some studies suggest that oscillatory measurements in the linear viscoelastic region can segregate wheat doughs differing in strength (Edwards et al 1999; Weipert 1990), the dynamic rheological parameters of dough show little or no relationship with the functionality during processing and end-use performance. The low deformation conditions used for these measurements are often inappropriate to practical processing situations, because they are carried out at rates and conditions very different from those experienced by the dough during processing or baking expansion. However, low strains, which allow measurements but do not disturb or destroy inherent structure, are of great value in studying the influence and action of additives, such as hydrocolloids in dough systems (Weipert 1990), because dynamic mechanical parameters are highly sensitive to changes in polymer type and concentration (Ferry 1980).

The dough is defined as a viscoelastic soft-solid because more elastic than viscous character is exhibited in the strain sweeps by G′ being much higher than G″ in the linear region and up to 0.3% strain. Figure 4.4a shows G′ and G″ crossover in the region between 0.3 and 0.5% strain, indicating a greater viscous character of dough at higher strains. In LVR, G′ and G″ values for Santa Fe and 2137 were higher in comparison with that of Karl 92 and Overley indicating stiffer dough from these flours. These results may look contradictory as higher protein content is expected to cause larger consistency since increasing intermolecular cross-linkage causes higher G′ and lower loss tangent in dough. However, the protein-protein interactions play a more significant role on the rheological properties of doughs. Despite their

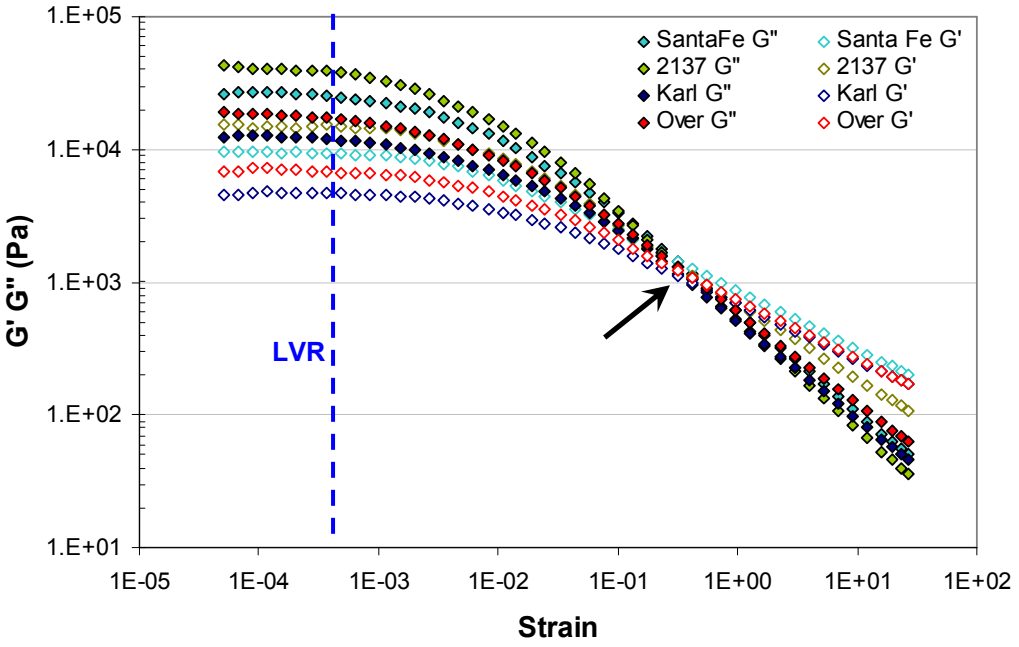
lower protein contents, Santa Fe and 2137 have significantly higher TPP (glutenins). A recent study reported by Kieffer (2007) clearly indicates that G′ and G″ of glutenin are much larger than that of gluten (Table 4.6). Storage modulus of dough samples tested in this study was found to be 2137 > Santa Fe > Overley > Karl 92, which is the same order observed for the TPP of these flours.

Table 4.6. Rheological properties of gliadin, glutenin, and gluten (Kieffer 2007).

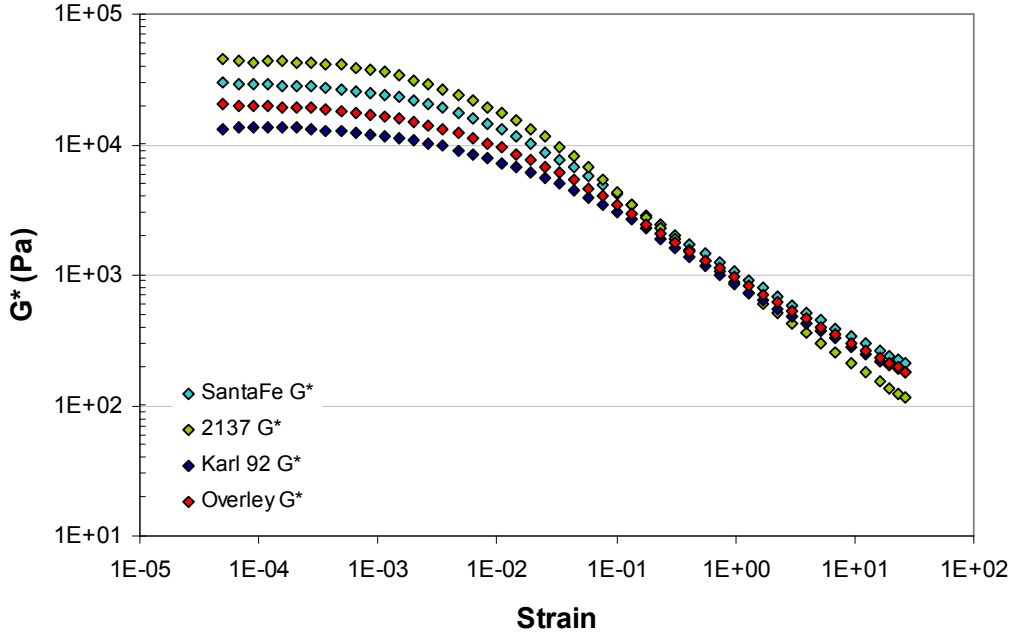
	Storage modulus, G′ (Pa)	Loss modulus, G″ (Pa)	Tan δ
Gliadin	561	657	1.2
Glutenin	38580	8986	0.2
Gluten	2506	1321	0.5

[a] *Results of dynamic testing at 1 Hz and 0.15% deformation.*

G′ and G″ values presented an opposite order after the crossover point, i.e. Overley > Karl 92 ≈ Santa Fe > 2135. Data obtained at large strains show a greater relation to end-product quality since deformations are closer to those used in the breadmaking process. At the largest strains measured in the strain sweeps, G′ values were largest for Overley, and Overley having the largest loaf volume value shows that it has the best baking quality as will be discussed later (section 3.5.1). Moreover, phase angles (Figure 4.4c) started to differ from one another after the crossover point, before which all flour samples displayed similar values. More viscous behavior is observed for Karl 92 and Santa Fe at high deformations as indicated by slightly higher phase angles compared to those of 2137 and Overley. The change in behavior of wheat flour doughs with increasing strain is expected since starch-starch and starch-protein interactions in the linear region give way to protein-protein interactions at larger deformations. The trend seen in the strain sweeps (Figure 4.4a) of the four Kansas wheat cultivars in this study is consistent with the findings of He and Hoseney (1991) and Safari-Ardi and Phan-Thien (1998) in which they found that lower breadmaking quality cultivars gave higher modulus values than good breadmaking quality wheats in the linear region. 2137 and Santa Fe are of relatively lower baking quality than Overley and Karl 92 as reported in the 2009 Kansas Performance Tests with Winter Wheat Varieties Report of Progress 1018 (Kansas State University 2009).


1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
G' G" (Pa)
SantaFe G"
Santa Fe G'
2137 G"
2137 G'
Karl G"
Karl G'
Over G"
Over G'
LVR
1E-05
1E-04
1E-03
1E-02
1E-01
1E+00
1E+01
1E+02
Strain

(a)


1E+05
1E+04
1E+03
1E+02
1E+01
G* (Pa)
SantaFe G*
2137 G*
Karl 92 G*
Overley G*
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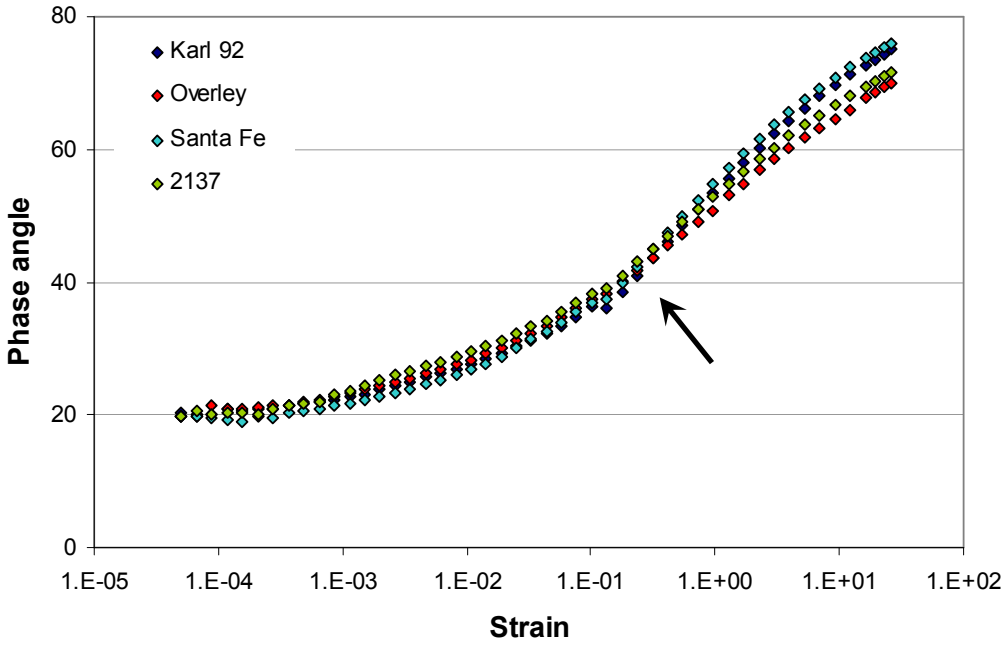
1E-03
1E-02
1E-01
1E+00
1E+01
1E+02
Strain

(b)



(c)

Figure 4.4. Strain sweeps performed to determine the linear viscoelastic region. (a) Storage and loss moduli, G′ and G″, (b) Complex modulus, G*, (c) Phase angle, δ.

4.4.2. Frequency sweep

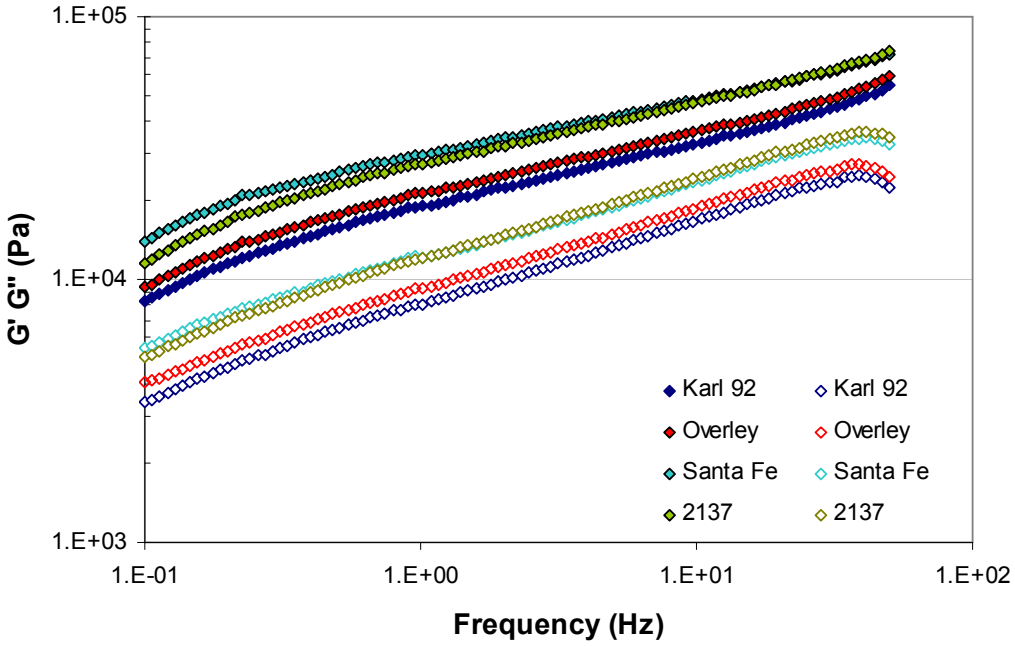
Frequency sweeps involved increasing the frequency of oscillation while keeping strain constant at 0.1 %. Results for the four cultivars (Figure 4.5) reveal that G′ is greater than G″ over all frequencies and therefore the dough has a viscoelastic soft-solid nature, and phase angles below 45º reinforce the slightly more solid-like material property of dough. Higher values of all moduli for Santa Fe and 2137 over the entire frequency range (Figure 4.5a and b) fit with observations by other authors that higher dough strengths correlate with lower moduli values at small deformations (Safari-Ardi and Phan-Thien 1998; Uthayakumaran et al 2002).

The frequency scans showed that all the dough samples displayed higher values of G′ and G″ at higher frequencies compared with low frequencies. These results indicate that the recovery of the stressed dough network was a slow process; that is, the network was not completely elastic. Dreese et al (1988) reported a similar trend that G′, G″, and tan delta of flour dough were frequency dependent and increased with increasing frequency.

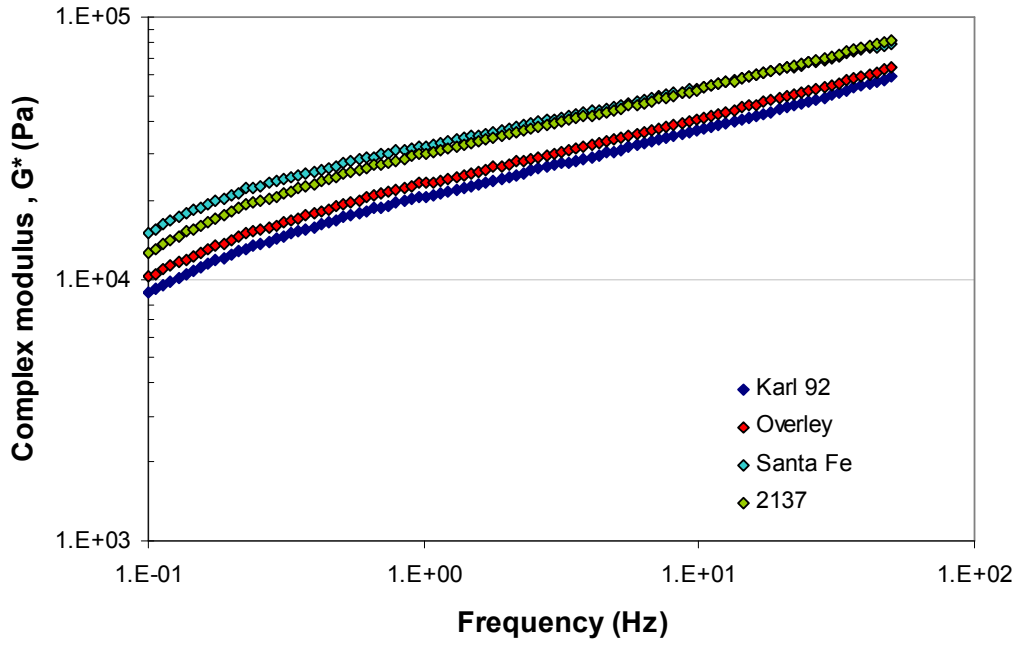
Phase angle in Figure 4.5c is much lower for Karl 92 than the other cultivars. Lower values for phase angles are indicative of a more elastic structure, so Karl 92 has the greatest

elastic nature at small deformations across a range of frequencies. All cultivars showed increases in their phase angles with increasing frequency, and higher phase angle values indicate that larger frequencies cause the viscous nature of the samples to increase (become more fluid-like). Since phase angle is the ratio of G″ to G′, an increase in the phase angle reflects that G″ has a greater slope than G′, and is therefore increasing at a faster rate. This results from the increase in viscous nature of the dough. Complex viscosity η* (Figure 4.5d) also supports the conclusion that the dough is becoming more liquid-like with increasing frequency because η* steadily decreases with increasing frequency, and a lower complex viscosity means a more free-flowing fluid material.

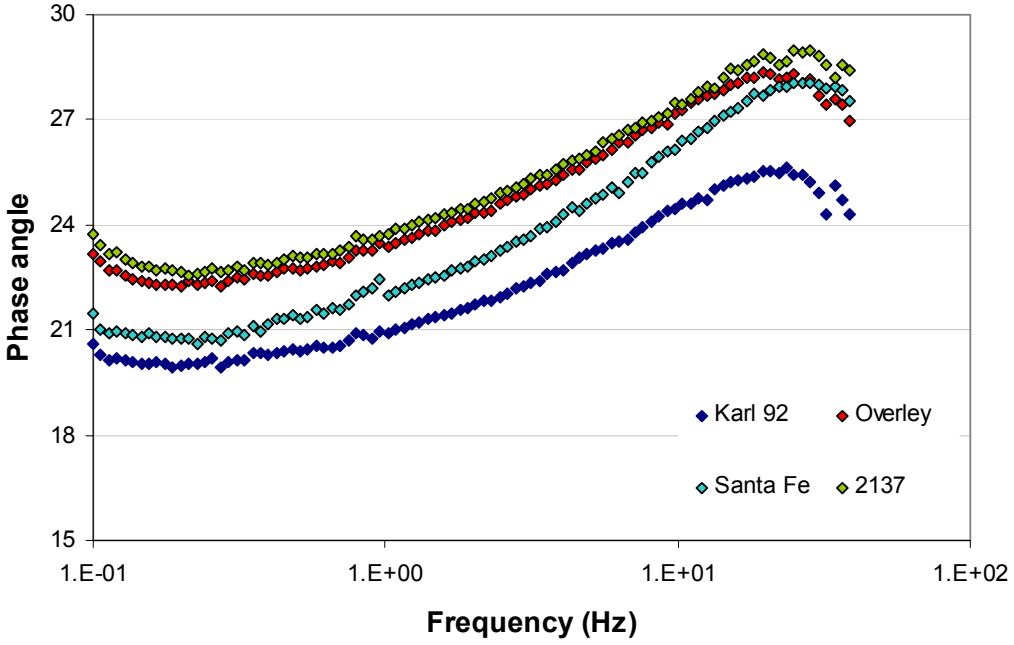
Differences in protein composition can explain the higher moduli of Santa Fe and 2137 versus the moduli of Karl 92 and Overley. The high percent of overall protein, low percentage of TPP, and significantly higher ($P < 0.05$) percent UPP in Overley and Karl 92 give lower moduli at the low strain (0.01%) used for frequency sweeps because starch-starch and starch-protein interactions dominate at small strains. A higher overall protein content means a smaller amount of starch is present in Overley and Karl 92, which provides for fewer starch interactions. Since the differences between cultivars is slightly greater in percent UPP than in TPP, the significantly higher level of UPP in Karl 92 and Overley than in 2137 and Santa Fe overrides the lower values of TPP in Overley and Karl 92 (Gupta et al 1993). This means that they should have better baking qualities due to their high level of high molecular weight proteins (UPP) that are mainly responsible for bread quality and have good correlations with baking qualities when dough is measured under large deformations. High UPP translates to a lower G′ and G″ in small deformation measurements such as oscillatory frequency sweeps.


1.E+05
1.E+04
1.E+03
G' G" (Pa)
1.E-01
1.E+00
1.E+01
1.E+02
Frequency (Hz)
Karl 92
Karl 92
Overley
Overley
Santa Fe
Santa Fe
2137
2137

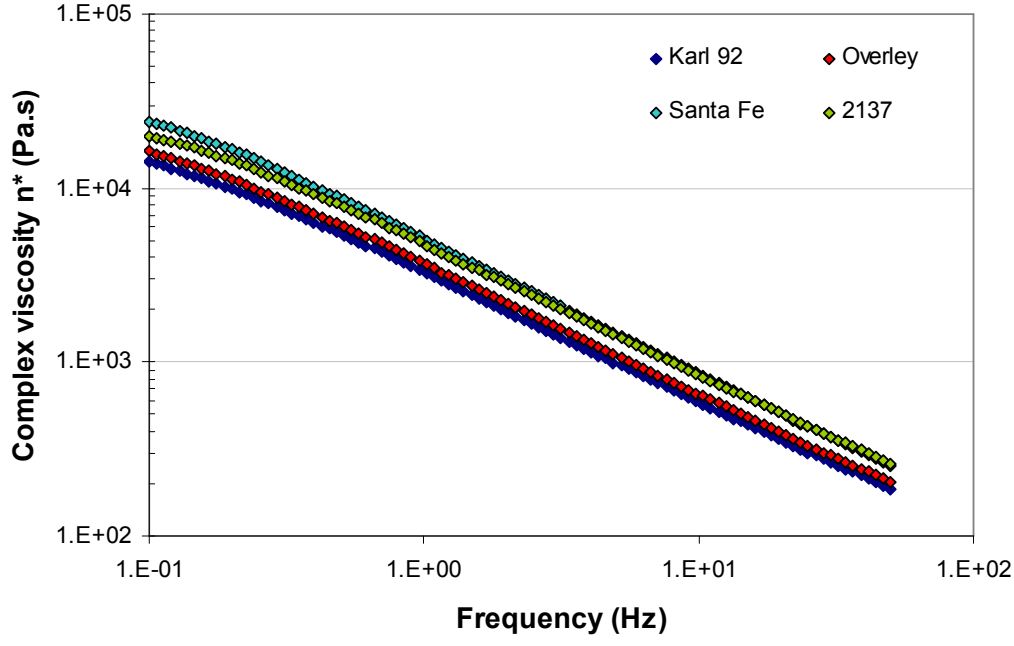
(a)


1.E+05
1.E+04
1.E+03
Complex modulus , G* (Pa)
1.E-01
1.E+00
1.E+01
1.E+02
Frequency (Hz)
Karl 92
Overley
Santa Fe
2137

(b)



(c)



(d)

Figure 4.5. Frequency sweeps of the dough samples. (a) Storage modulus (G′, solid symbols) and loss modulus (G″, open symbols), (b) Complex modulus, G*, (c) Phase angle, δ, (d) Complex viscosity, η*.

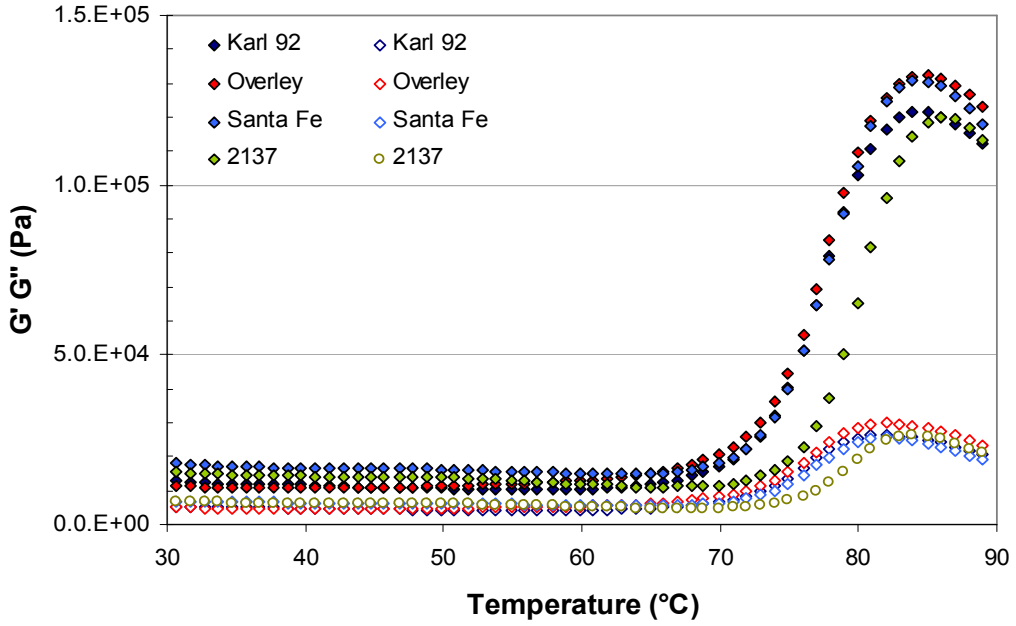
4.4.3. Temperature sweep

Temperature sweep were performed between 30-90 ºC at a heating rate of 1.5 ºC/min with a constant strain rate of 0.1% and a frequency of 1 Hz to mimic the initial part of the baking process. Temperature sweeps have advantage over RVA as the test conditions more similar to baking (i.e. a higher meal concentration and without heavy shearing).

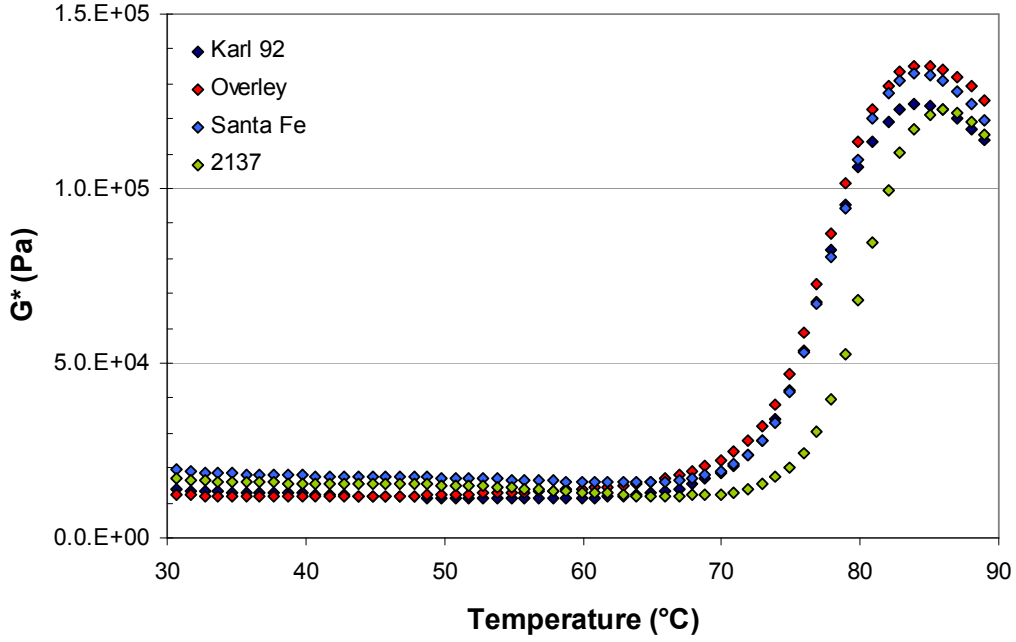
Figure 4.6 shows the temperature-induced changes in moduli, viscosity, and phase angle in four dough samples. At 30°C, the storage modulus G′ was slightly lower for Overley and Karl 92 than Santa Fe and 2137, similar to what was observed in frequency sweeps (Figure 4.6a). There was a slight or no initial decrease in G′ and G″ until around 60°C, as has been generally observed for wheat flour doughs (Bloksma 1980). This might be attributed to the swelling of starch during initial stages of heating. Slight decreases in all moduli of Santa Fe and 2137 between 30 and 60°C indicate softening of the dough until the gelatinization temperature is reached around 70°C. The decreases are likely due to α-amylase acting on starch and releasing some absorbed water thereby reducing dough interactions (Dogan 2002; Salvador et al 2006). The action of cereal amylase is more visible in Santa Fe and 2137 as a result of their higher proportion of starch.

The moduli vs. temperature curves (Figure 4.6a and b) indicated increase in G′, G″, and G*. with increasing temperature. The peak in G″ (81-83°C) occurred at a lower temperature than the peak in G′ (85-86°C). After 85°C, viscosities start to decrease due to starch breakdown. Complex viscosity data indicated that the initial pasting temperature was >68°C. It is evident that at conditions more similar to baking, the development of viscosity starts at slightly higher temperatures than those observed in RVA.

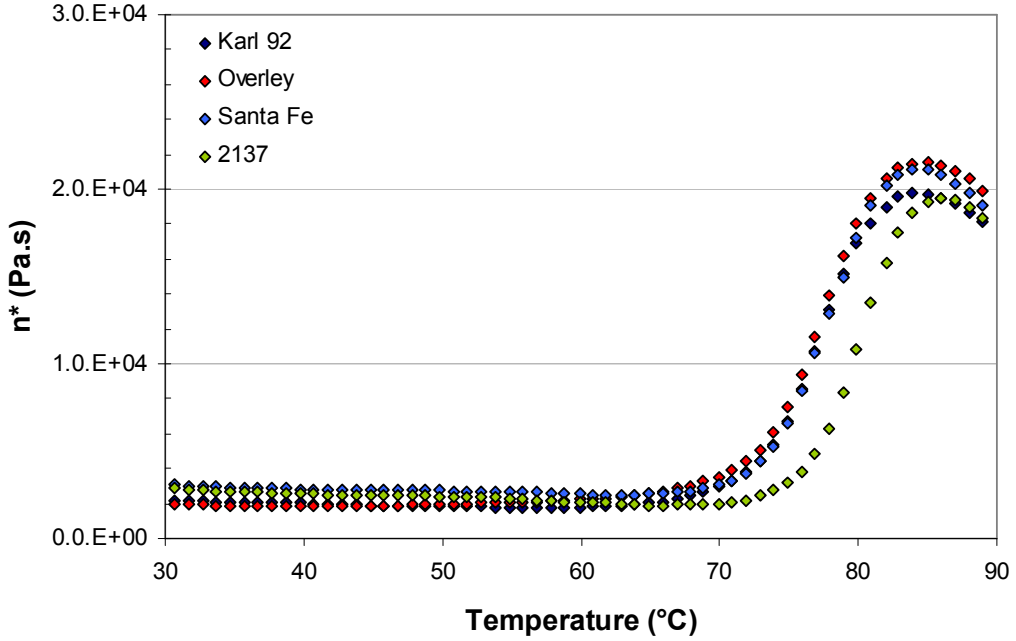
Gelatinization of starch, the most abundant carbohydrate in dough, is an essential step in the transformation of dough into bread. Gelatinization of native starch grains embedded in the glutinous matrix of bread dough leads to formation of a porous, elastic crumb. According to Sandsted (1961), starch in bread dough dilutes the gluten to a desired consistency; furnishes sugar through amylase action; becomes flexible during partial gelatinization, thereby permitting further stretching of the gas-cell film; takes water from the gluten by gelatinization, thus causing the film to set and become rigid. In the gelatinization process, wheat starch can take up several times its weight in water from the gluten and retain the moisture, allowing the surrounding dehydrated gluten matrix to maintain a semi-rigid form.


1.5.E+05
1.0.E+05
5.0.E+04
0.0.E+00
G' G" (Pa)
Karl 92
Overley
Santa Fe
2137
Karl 92
Overley
Santa Fe
2137
30
40
50
60
70
80
90
Temperature (°C)

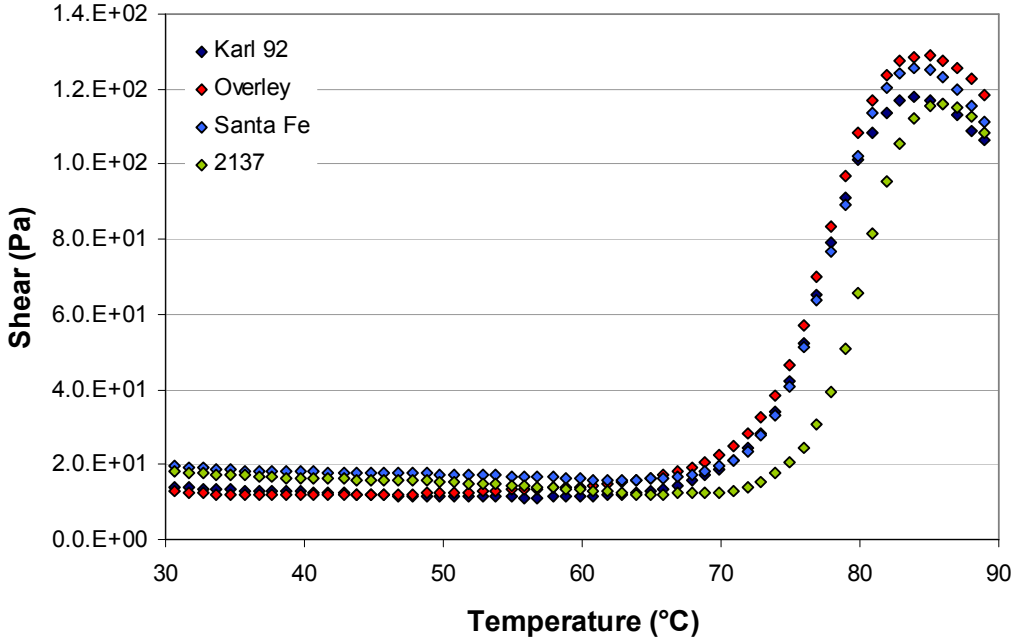
(a)


1.5.E+05
1.0.E+05
5.0.E+04
0.0.E+00
G* (Pa)
Karl 92
Overley
Santa Fe
2137
30
40
50
60
70
80
90
Temperature (°C)

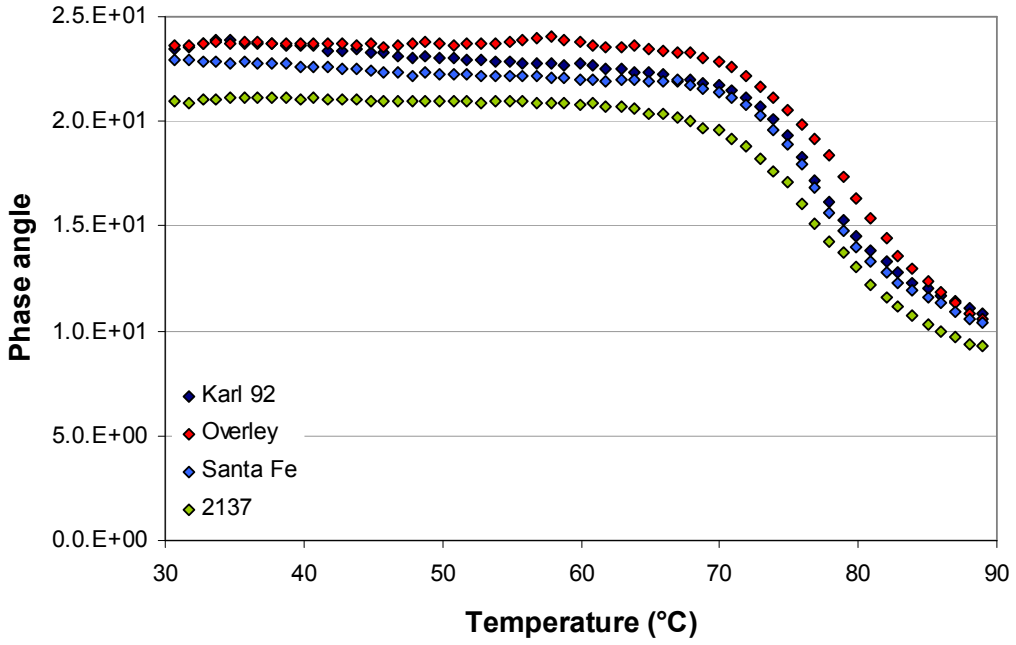
(b)


3.0.E+04
2.0.E+04
1.0.E+04
0.0.E+00
n* (Pa.s)
Karl 92
Overley
Santa Fe
2137
30
40
50
60
70
80
90
Temperature (°C)

(c)


1.4.E+02
1.2.E+02
1.0.E+02
8.0.E+01
6.0.E+01
4.0.E+01
2.0.E+01
0.0.E+00
Shear (Pa)
Karl 92
Overley
Santa Fe
2137
30
40
50
60
70
80
90
Temperature (°C)

(d)



(e)

Figure 4.6. Temperature sweeps of four flour-water dough samples (a) Storage modulus (G′, solid symbols) and loss modulus (G″, open symbols), (b) Complex modulus, G*, (c) Complex viscosity, η*, (d) Shear stress, and (e) Phase angle, δ .

Table 4.7. Temperature sweep parameters determined by analysis of four Kansas wheat cultivar flour-water doughs.

	Peak G′ (Pa.s)	Peak G′ temp (°C)	Peak G″ (Pa.s)	Peak G″ temp (°C)	Peak η* (Pa.s)	Peak η* temp (°C)
Overley	132237±4947 [a]	84.8±0.50 [ab]	29799±1467 [a]	81.75±1.26 [a]	21522±814 [a]	84.75±0.50 [a]
Karl 92	121810±2346 [b]	84.0±0.82 [a]	26604±716 [b]	81.25±0.96 [a]	19813±385 [b]	84.00±0.82 [a]
2137	120202±2658 [b]	86.0±0.82 [b]	26474±612 [b]	84.00±0.82 [b]	19545±434 [b]	86.00±0.82 [b]
Santa Fe	130982±6206 [a]	84.5±0.58 [a]	25647±1401 [b]	82.25±0.50 [a]	21206±1007 [a]	84.50±0.58 [a]

[a] *Values with the same letter in the same column are not significantly different at (P <0.01)*
[b] *Results are the averages of four replicates.*

Increases in G′ can be attributed to cross-linking interactions induced in gluten during the formation of network structure. Protein-protein interactions would begin to provide an increasingly highly cross-linked structure resulting in higher G′ and generally lower G″ values. Starch gelatinization, gluten cross-linking, or both are the most commonly accepted explanations for the thermally induced rheological changes during baking (Dreese et al 1988). Amylose and amylopectin that leach out from the starch granules during gelatinization increase the viscosity

(and therefore the G′ value). One possible effect of gelatinization may be to provide an opportunity for increased hydrogen bonding between gluten polypeptides and starch molecules. As reported by Dreese et al (1988), below 55 °C, the amount of native, unmodified starch present in gluten-starch dough had only a small effect on G′; however, above 55 °C, the magnitude of change in G′ was proportional to the amount of starch present in the dough.

Eliasson (1983) showed protein to increase starch gelatinization temperature; therefore, the delay in pasting of 2137 is congruent with it having the significantly ($P < 0.05$) greatest amount of SP because proteins soluble in water could more easily interact with the starch granule surface to slow starch swelling and increase gelatinization temperature. Since starch gelatinization in limited water systems (like bread dough) can be affected by any change in water availability (Zamponi et al 1990), the presence of a larger amount of proteins that will interact with water would likely shift the gelatinization temperature as seen in Figure 4.6 for 2137. Table 4.7 shows significantly larger ($P < 0.01$) G*, G′, and η* for Overley and Santa Fe, meaning that they have higher pasting viscosities in dough systems than Karl 92 and 2137. 2137 has also the highest TPP and the lowest MP content which may interfere with starch gelatinization. The glutenin fraction of gluten has been found to be more sensitive to heat than the gliadin fraction; on heating up to 75 °C, glutenin protein unfolds and disulphide/sulphhydryl interchange reactions are promoted (Angioloni and Dalla Rosa 2005). In addition to proteins, residual α-amylase activity, amylose to amylopectin ratio, and amount of damaged starch are also known to affect the pasting temperature. For this purpose, further studies need to be carried out, including falling number analysis of flour, a-amylase assay, and amylase/amylopectin analysis..

The loss tangent of all the flour dough treatments experienced little change until about 65-68 °C, beyond which it rose rapidly suggesting a dominating viscous feature of the heated products (Figure 4.6e). The viscous characteristic of the dough structure seemed to be augmented by the presence of higher MP which could be the reason for Overley and Karl 92l having slightly higher phase angle values at all temperature range tested. All of the flour samples exhibited more elastic response above 70 °C. The sharp decline in tan δ indicated predominant elastic properties of a viscoelastic solid ($G' > G''$) because of starch granules swelling, which reinforced the gluten gel network. The compositional difference between the flours reflected the subtle variations in the G″ and G′ of the dough matrix after being heated to

high temperatures. At 90 °C, Karl 92, Overley, and Santa Fe all reached the same phase angle values which were slightly higher than that of 2137.

4.4.4. Creep recovery

Creep recovery measurements were performed on each flour type constant temperature of 30 ºC and shear stress of 50 Pa over a creep time of 1200 sec and recovery time of 1200 sec. The creep-recovery curves of the doughs (Figure 4.7) exhibited a typical viscoelastic behavior combining both viscous fluid and elastic components similar to the corresponding curves obtained previously for wheat doughs (Edwards et al 1999; Rouille et al 2005; Sivaramakrishnan et al 2004; Wang and Sun 2002). During the application of the stress for creep recovery (the creep period), the doughs' creep compliances increase very quickly at first, then they reach a steady state of almost linear creep compliance. When the stress is removed (the recovery period), all cultivars have an immediate drop in their recovery compliance, followed by a rapid recovery period, then an almost linear steady state of recovery compliance. Magnitudes of the creep-recovery curves in Figure 4.7 (Overley > Karl 92 > 2137 > Santa Fe) follow the same order of complex viscosity found in the frequency sweeps.

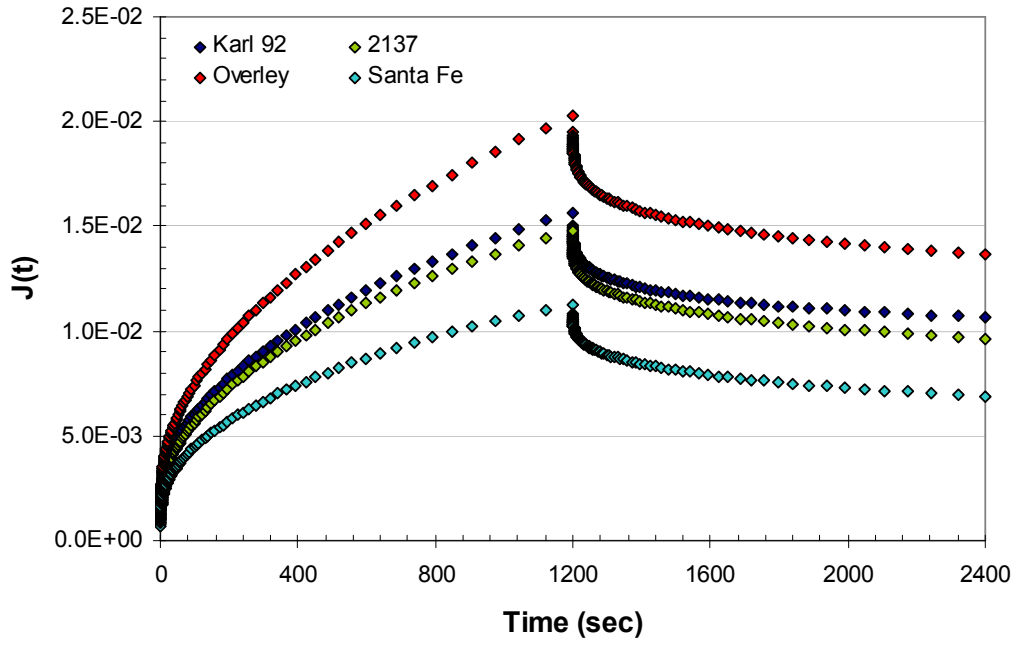


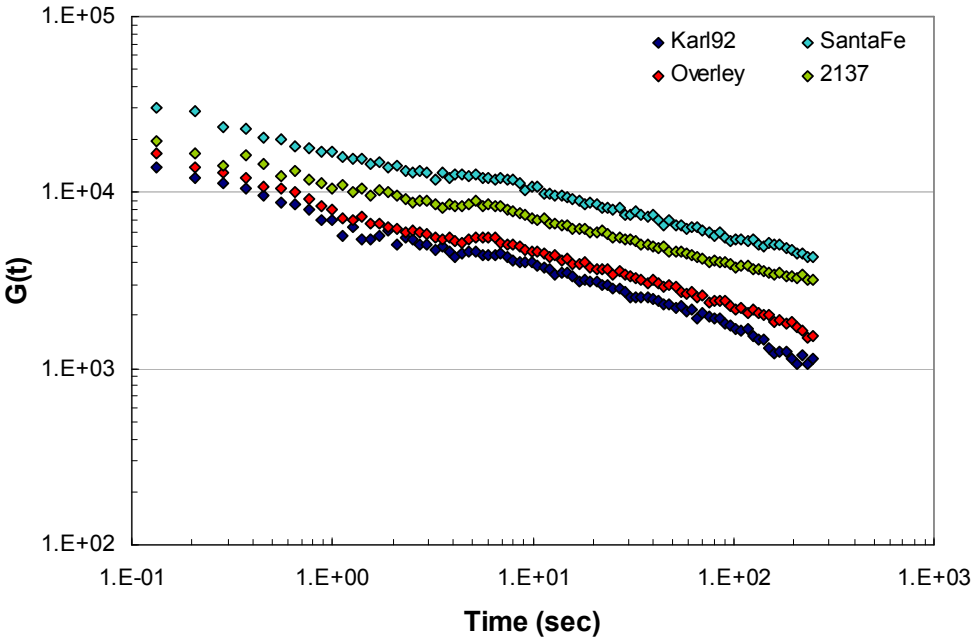
Figure 4.7. Creep and recovery compliance of the dough samples.

Large strain measurements of creep recovery have given correlations with UPP and large molecular weight glutenins (Tronsmo et al 2003a and 2003b). The data shown in Figure 4.7 indicate that small deformation creep recovery measurements are also related to protein composition. The interactions among protein molecules play a significant role on the rheological properties of wheat flour doughs. Despite their lower protein contents, Santa Fe and 2137 have significantly higher TPP (glutenins), as discussed previously. Due to the fact that moduli of glutenin are much larger than that of gluten (Kieffer 2007), Santa Fe exhibited higher resistance to deformation as shown by lower maximum creep percent strain (i.e. strain at the end of creep phase. The ratio of J_m to J_{max} gives the percent recovery of the dough, which was higher for 2137 (34.5%) and Santa Fe (38.9%). This could relate to 2127 and Santa Fe having higher amounts of TPP, giving them a more elastic nature at small deformations. Higher percent recoveries indicate little damage was done to the dough structure (van Bockstaele et al 2008). Lower percent recoveries Karl 92 and Overley (31.7% and 32.4%, respectively) show more viscous flow and breakdown in the dough. These values are similar to those reported by Sivaramakrishnan et al (2004).

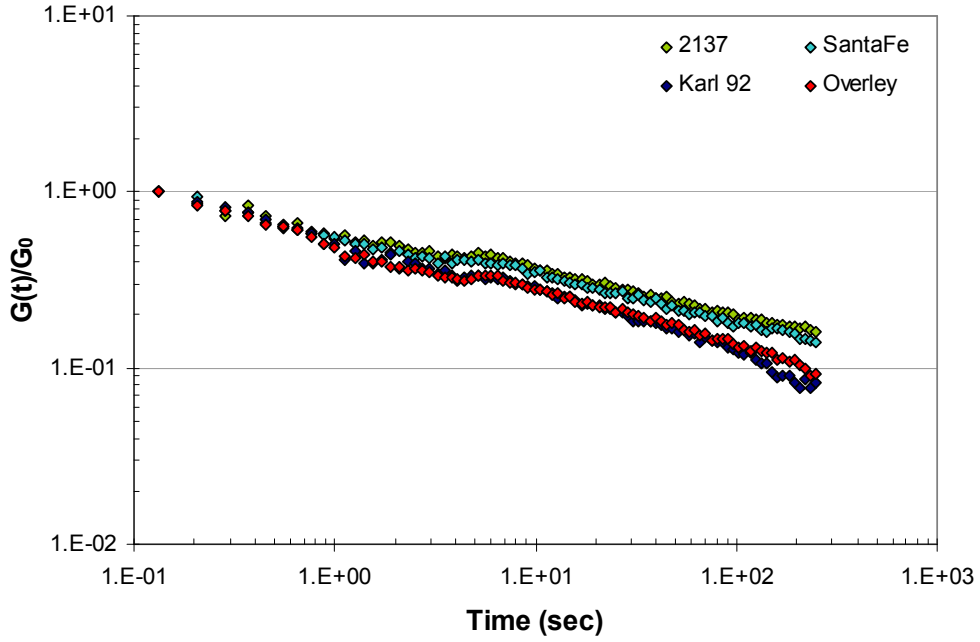
4.4.5. Stress relaxation

Stress relaxation was used to determine the time dependence of the viscoelastic properties of doughs at times up to 250 sec. Figure 4.8b shows the stress relaxation curves obtained at 30 °C, plotted as $G(t)/G_0$ versus time, where G(t) is the relaxation modulus at any time and G_0 is the initial modulus. This normalized stress relaxation moduli indicated that all cultivars had similar behavior until after 10 sec of relaxation. However, the data showed a split in behavior between the higher protein wheats (Karl 92 and Overley, and the lower protein wheats (2137 and Santa Fe) after 10 sec. Time required for modulus to decay one-half of its original values (t_{50}) was found to be 0.9 sec for Overley and Karl 92, while it was 1.4 and 1.9 sec for Santa Fe and 2137, respectively. t_{75} values followed the same order: 13.0, 13.9, 33.7 and 39.6 sec for Karl 92, Overley, Santa Fe and 2137, respectively. Quicker relaxations for Karl 92 and Overley were indicated by the greater descending slope of their moduli and slightly slower relaxation for Santa Fe and 2137 are reflected in their moduli's smaller descending slopes. The faster relaxation of Overley and Karl 92 was due to their slightly lower levels of TPP. Although UPP was higher in Overley and Karl 92, TPP was more influential in this case because flours with a larger quantity

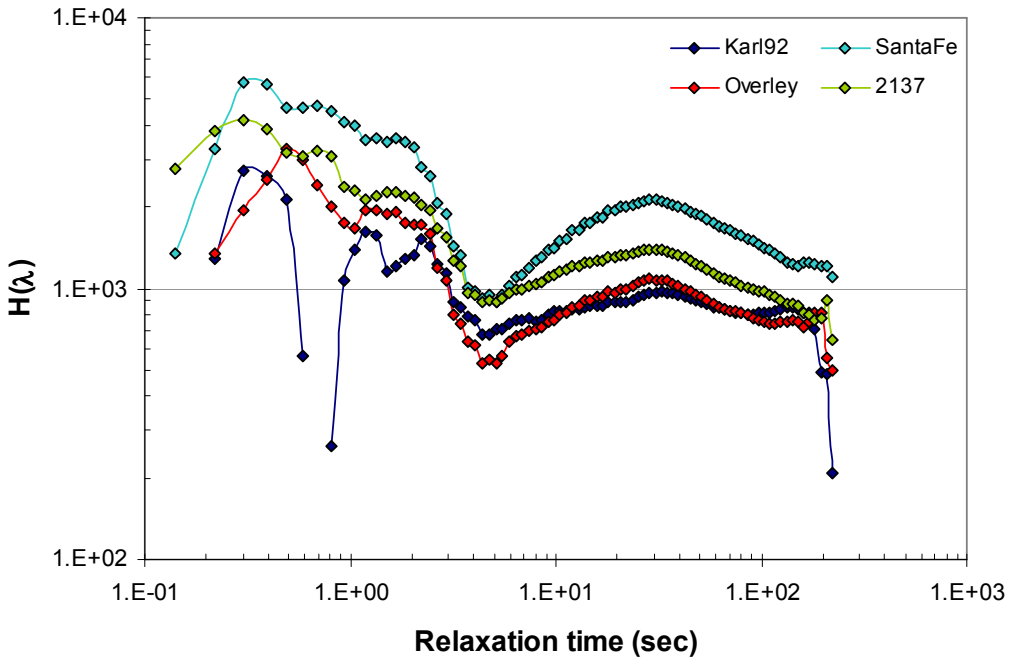
of high molecular weight proteins take longer to relax than flours with fewer high molecular weight proteins. Larger proteins needed more time to rearrange into lower energy conformations than small proteins.



(a)



(b)



(c)

Figure 4.8. Stress relaxation parameters for the four Kansas wheat cultivar flour-water doughs (a) Relaxation modulus, G(t), (b) Normalized relaxation modulus, G(t)/G0, and (c) Relaxation spectra, H(λ).

All cultivars showed bimodal behavior in their relaxation spectra, which indicated two distinct groups of proteins that relaxed at specific time intervals. These bimodal relaxation spectra (Figure 4.8c) for all four cultivars were in accordance with results found by Rao et al (2000). The first peak was centered at approximately 0.7 sec and spanned the time period from 0 to 4 sec, and the second peak spanned the time period of 4 to >100 sec with a peak at approximately 30 sec. Rao et al (2000) attributed the bimodal distribution of relaxations times to a blend of low and high molecular weight polymers and suggested that the first of these two peaks represents entanglements between low molecular weight proteins, possibly with some high molecular weight proteins, while the second peak was due to high molecular weight entanglement relaxation. The second relaxation peak is related to the entanglement properties of high molecular weight insoluble glutenin polymers, and has been shown to be directly related to the insoluble fraction of the high MW glutenins (Lee and Mulvaney 2003). The much larger distribution of relaxation times of the longer process (sec peak) is consistent with the reported broader molecular weight distribution of the glutenins, while the much narrower distribution of

relaxation times of the faster relaxing mode (first peak) is consistent with the description of gliadin. The glutenin fractions itself is also polydisperse**.**

The first peak of the relaxation spectrum is therefore related to the percent MP in the flours. Percent TPP is the primary influence on the second peak, indicated by the lower relaxation spectrums of Overley and Karl 92 in this peak. The end of the Santa Fe and 2137 relaxation spectra show a steady relaxation, but Overley and Karl 92 show a flat section at the end of their spectra which could indicate presence of unrelaxed proteins probably due to their higher levels of UPP.

Although, small strain measurements of relaxation modulus often cannot differentiate wheat cultivars, relaxation properties (determined at optimum deformation levels) of doughs relate well to MWD and particularly to entanglements of HMW glutenin polymers and may be used as a rapid method of discriminating variations in MWD between varieties which vary in baking quality. Several researchers have shown that a slower relaxation time is associated with good baking quality (Bloksma 1990; Launay 1990; Wang and Sun 2002). Measurements of large-deformation creep and shear stress relaxation properties were found to be useful in discriminating between different wheat varieties of varying quality, and were found to be closely associated with baking volume (Safari-Ardi and Phan-Thien 1998; Wikstrom and Eliasson 1998) and strength of durum wheat varieties (Edwards et al 1999, 2001). At small strain amplitudes (0.1%), doughs with different baking quality showed almost no differences in relaxation behavior, but at a range of large strains (up to 29%) their creep and relaxation behavior was closely correlated with the baking behavior of dough (Safari-Ardi and Phan-Thien 1998).

4.5. Baked Product Characteristics

4.5.1. Loaf volume

Loaf volumes for wheat cultivar breads (Table 4.8) decreased with decreasing total protein content. Overley and Karl 92 had the highest loaf volumes, which is congruent with their high protein contents and significantly higher percent UPP ($P < 0.05$). Previous correlations between total protein content, loaf volume, and all protein fractions determined in SE-HPLC were made by Huebner et al (1999). High molecular weight protein entanglements are known for their ability to stretch without rupturing air cell walls during the strains of proofing via strain hardening. Since UPP is the largest molecular weight fraction of protein, it contributes

significantly to the successful expansion of air bubbles, allowing bread dough to form an expanded structure and resulting in larger loaf volumes.

Table 4.8. Loaf volume of bread samples.

Average Loaf Volume (cc)			
Overley	943.3	± 11.6	[a]
Karl 92	911.7	± 20.2	[ab]
2137	888.3	± 5.8	[b]
Santa Fe	830.0	± 13.2	[c]

[a] *Values with the same letter are not significantly different at (P <0.05).*

In order to produce bread with good volume quality, two factors should be considered: Dough should have a high viscosity to prevent gas cells rising and dough should remain extensible at high level to prevent sudden breakage in gas cell membranes (Sliwinski et al 2004). Glutenin-to-gliadin ratio of Overlay and Karl 92 (0.81-82) seemed to be at well balanced levels leading to better end-product characteristics. 2127 and Santa Fe had significantly higher ratios indicating increased elasticity which limits air bubble expansion during proofing and/or baking. The damaged starch was not measured and could have contributed to a lower loaf volume for Santa Fe.

4.5.2. C-cell

C-cell data provided in Table 4.9 shows Santa Fe's slice area was significantly ($P< 0.05$) lower than all the other cultivars and had a smaller height ($P< 0.05$) than Overley and Karl 92. Cell numbers ranged from 4771±248 for Karl 92 to 4388±214 in Santa Fe, and only Karl 92 and Overley were significantly different from Santa Fe at $P< 0.05$. Although Karl 92 had the highest number of cells and the lowest cell diameter of all the cultivars, the differences between different flours was not statistically significant. Loaf slice images in Figure 4.9 showed a more uniform texture and better loaf shape for Karl 92 than the other wheats flours, which was expected since it was described in the Kansas Wheat Report (Kansas State University 2009) as having the best bread baking quality--suitable for mixing with weaker flours.

Table 4.9. Crumb structure of baked loaves measured using C-cell.

	Slice area (mm^2)	Number of cells	Cell density (#cells/mm^2)	Mean cell wall thickness (mm)	Mean cell diameter (mm)	Cell thickness to radius ratio
Overley	5910±139 [a]	4620±248 [a]	0.782±0.005 [a]	0.399±0.005 [a]	1.605±0.069 [a]	0.249±0.005 [a]
Karl 92	5769±127 [a]	4771±248 [a]	0.827±0.025 [a]	0.390±0.005 [b]	1.536±0.070 [a]	0.254±0.008 [a]
2137	5909±76 [a]	4753±152 [a]	0.804±0.019 [a]	0.395±0.003 [ab]	1.565±0.059 [a]	0.253±0.008 [a]
Santa Fe	5392±206 [b]	4388±214 [b]	0.814±0.027 [a]	0.397±0.003 [a]	1.547±0.054 [a]	0.257±0.008 [a]

[a] *Values with the same letter in the same column are not significantly different at ($P < 0.05$).*
[b] *Number, area, and volume of holes was not significant between varieties (data not shown).*

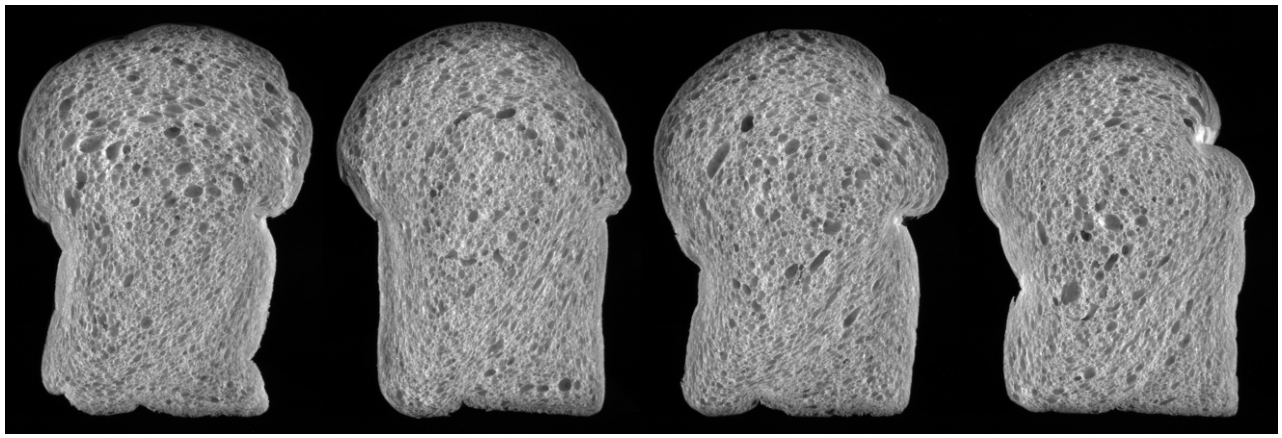


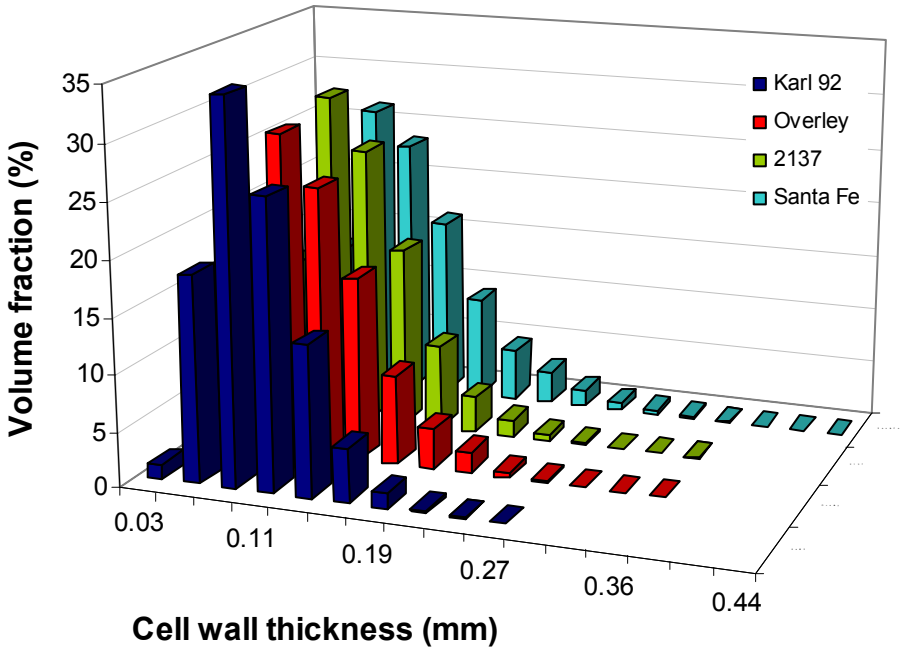
Figure 4.9. C-cell bread scan images from central slices of loaves of (a) Overley, (b) Karl 92, (c) 2137, (d) Santa Fe. All loaves are pictured at the same scale.

4.5.3. X-ray Microtomography (XMT)

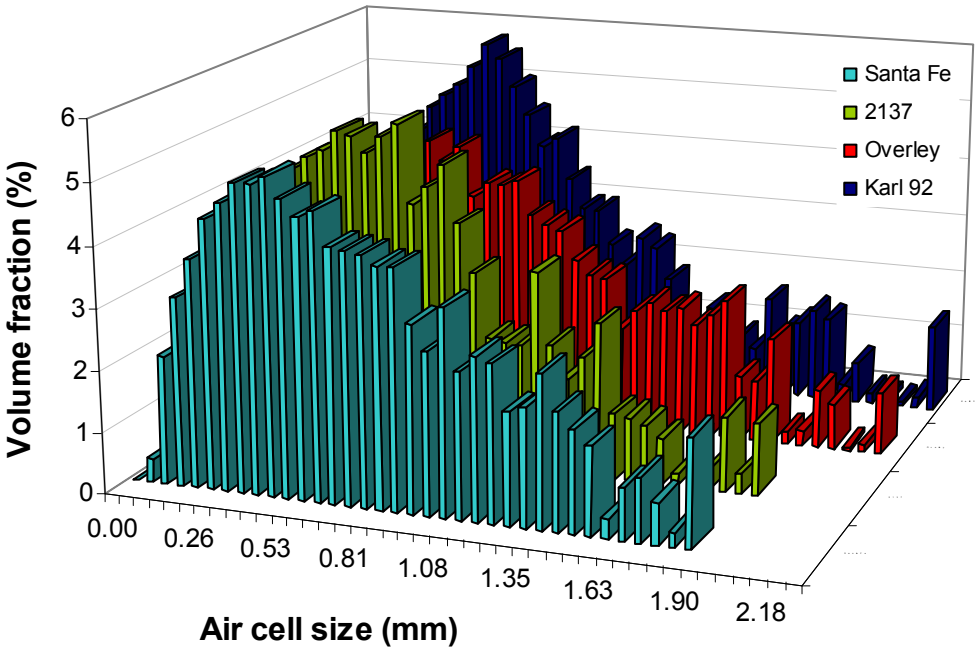
C-cell and XMT methods both measure the structure of baked bread, but they use different resolutions. C-cell gathers data using a 2-D surface image of the bread and takes measurements from the entire slice, including both the center and edges. Any 3-D parameters reported in C-cell are derived from the surface data using algorithms, not measured directly. This 2-D macro approach gives useful data on the overall loaf characteristics and crumb texture, but location of the slice, large inclusions or air holes, and cutting method/position can all significantly effect the data produced. XMT measures the bread crumb on a micro level, allowing for more specific 3-D measurements of cell wall thicknesses and air cell sizes. Sample location can significantly effect data, so samples are chosen from the same general area of the loaf that do not include large air holes or other abnormalities. Data from these two methods can differ greatly. C-cell data only provides an average cell size, but XMT shows the average cell

size as well as the cell size distribution. The same is true for cell wall thicknesses; XMT data can show the relative number of cell walls of each thickness. Distributions are much more useful for analyzing baking performance and differentiating between cultivars because they show the relative number of air cells or cell wall thicknesses of each size to give a more accurate picture of the crumb structure. This is important because simple averages often do not show differences between cultivars' cell sizes and cell wall thicknesses.

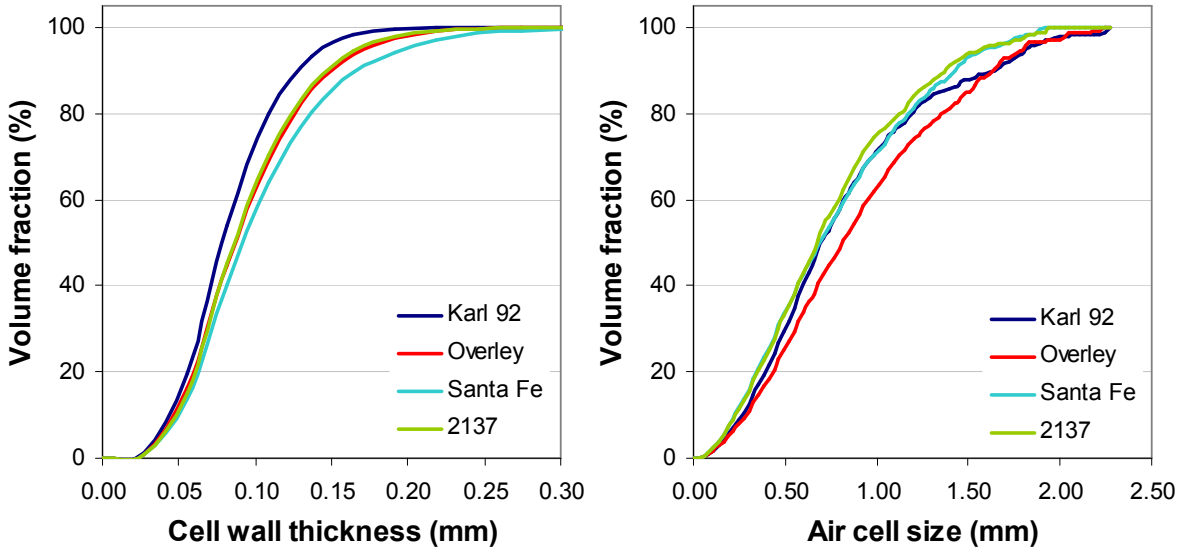
Cell wall thickness distributions determined by XMT (Figure 4.10) indicated Karl 92 had the greatest proportion of thin cell walls and no cell walls thicker than 0.27 mm. The bread cross-sectional images in Figure 4.11b illustrate the thinness of Karl 92's cell walls. Santa Fe had the greatest proportion of thick cell walls, with some ranging up to 0.44 mm. Table 4.10 shows that it had a significantly ($P< 0.05$) larger structure thickness than the other flours. The thickness differences in cell walls could also be seen in Figure 4.11, where Santa Fe and 2137 showed a much thicker structure in places than the other breads. Cell wall distributions of Overley and 2137 were almost identical, which was echoed by Table 4.10 showing their structure thicknesses were not significantly different at $P< 0.05$. Cell size distributions showed that Karl 92 had the greatest portion of small cells. Overley had a more spread out distribution of air cell sizes with fewer small air cells than the other cultivars, and a large proportion of its cells were in the 1.18 to 1.75 mm range. This was probably due to greater expansion of all air cells formed in Overley compared to other cultivars. Wider cell size distrubutions and higher meant strctures separation values observed in Karl 92 and Overley supported the fact that these two had higher loaf volumes than 2137 and Santa Fe.


Volume fraction (%)
35
30
25
20
15
10
5
0
0.03
0.11
0.19
0.27
0.36
0.44
Cell wall thickness (mm)
Karl 92
Overley
2137
Santa Fe

(a)


Volume fraction (%)
6
5
4
3
2
1
0
0.00
0.26
0.53
0.81
1.08
1.35
1.63
1.90
2.18
Air cell size (mm)
Santa Fe
2137
Overley
Karl 92

(b)



(c)

Figure 4.10. X-ray microtomography distribution curves for the four flours showing (a) Cell wall thickness distribution, (b) Cell size distribution, and (c) Cumulative distribution curves.

Table 4.10. Bread microstructure measured by x-ray microtomography for the four wheat cultivars' flours.

	Fragmentation index (1/mm)	Mean structure thickness (mm)	Mean structure separation (mm)	Void fraction (%)
Overley	4.77±1.27 [a]	0.11±0.00 [ab]	0.90±0.00 [a]	88.47±0.00 [a]
Karl 92	6.53±1.54 [a]	0.10±0.00 [a]	0.83±0.00 [a]	89.37±0.01 [a]
2137	3.87±0.22 [a]	0.11±0.00 [a]b	0.76±0.00 [a]	86.01±0.01 [b]
Santa Fe	4.01±0.43 [a]	0.12±0.00 [b]	0.78±0.00 [a]	85.56±0.01 [b]

[a] *Values with the same letter in the same column are not significantly different at (P <0.05).*

Together, the cell wall thicknesses and cell sizes helped to determine the void fraction of the bread samples, and a larger fraction indicated a more airy, expanded structure. Karl 92 had the largest void fraction of 89.37, but Overley's was less than 1% smaller and equivalent at P < 0.05. Fragmentation index is a measure of the interconnectedness of air cells within the bread structure, with a higher number indicating a more open structure. None of the cultivars showed significant differences, but the trend indicated that in openness, Karl 92 > Overley > Santa Fe > 2137. Cumulatively, the XMT data indicated that Karl 92 had the most airy, connected structure with the thinnest cell walls, Overley had the greatest number of its air cells expanded to larger sizes, Santa Fe had the thickest cell walls with the least airy structure while still maintaing the

largest portion of its air cells as small sizes, and 2137 had the most connected structure with mostly small air cell sizes.

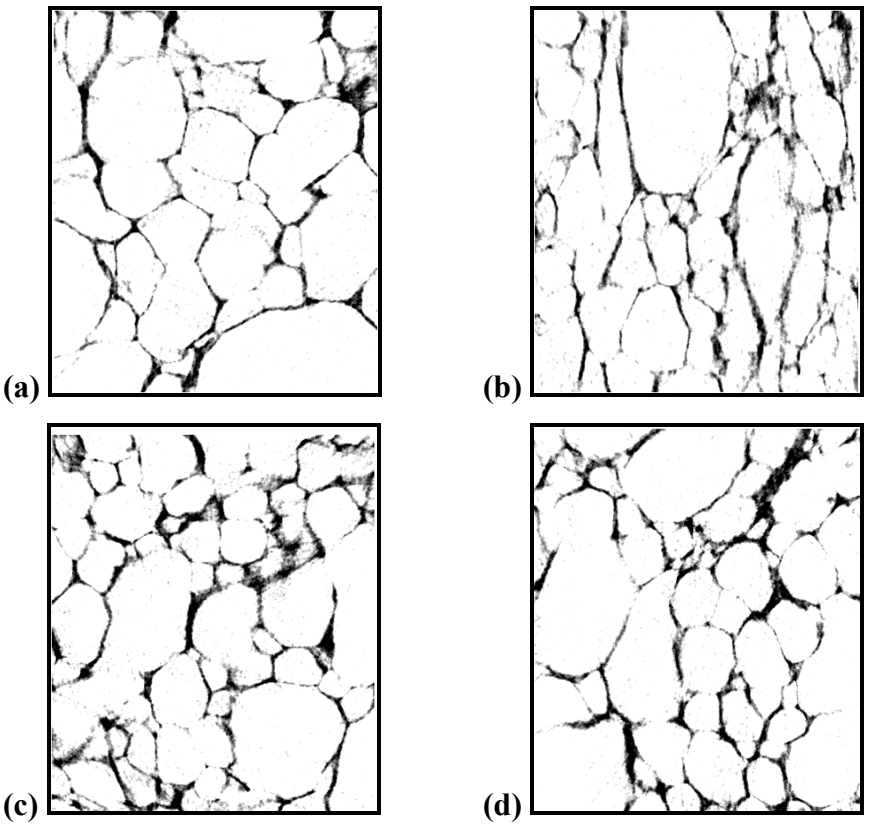


Figure 4.11. X-ray microtomography cross sectional images of the four wheat cultivars' bread samples (a) Overley, (b) Karl 92, (c) 2137, (d) Santa Fe.

4.5.4. Texture Analysis

Texture profile analysis (TPA) force-time curves (Figure 4.12) of the bread samples indicated that 2137 required the most force to compress, followed by Santa Fe and Karl 92 which were very similar, then Overley. Data derived from the TPA curves are shown in Table 4.8. Firmness of the doughs was of the order 2137 > Santa Fe > Karl 92 > Overley. Santa Fe and Karl 92 were very close (555 and 534 g, respectively). Greater firmness means a less compressible crumb, often indicating a less-expanded structure. Firmness generally had an inverse relationship to protein content; Overley had the least amount of firmness and the highest total protein content. TPP, MP, and glutenin to gliadin ratio were all negatively related to firmness. Protein composition had positive relationships with loaf volume, implying that a greater protein

content gave more expanded loaves and led to a more expanded crumb structure. Springiness of all cultivars was not significantly different, indicating that the internal bond strength of all the bread loaves were similar. Overley was significantly lower in cohesiveness than the other wheat varieties. It had a high void fraction and the second-highest fragmentation index indicating an open structure that contributes to its compressibility. Because chewiness was a derivative of all the other parameters, it reflects the other measurements. Chewiness of 2137 was higher because it had the greatest firmness.

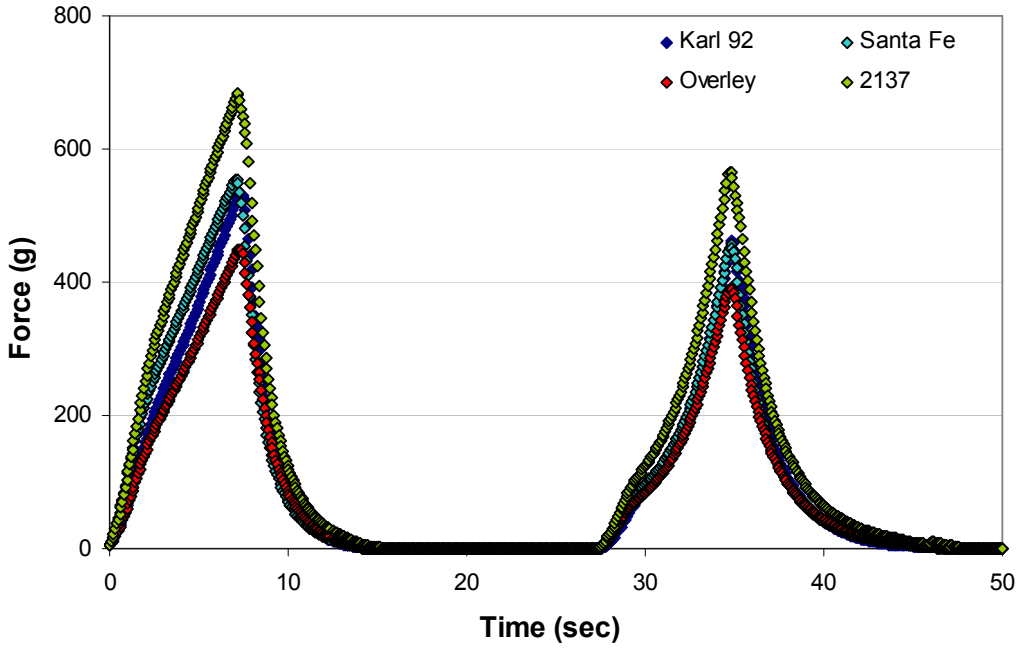


Figure 4.12. Texture analysis curves for all cultivars showing the double compression.

Table 4.11. Texture profile analysis (TPA) data for the four Kansas wheat flour breads.

	Firmness (g)	Cohesiveness (-)	Springiness (-)	Gumminess (g)	Chewiness (g)	Resilience (-)
Overley	449±37 [a]	0.822±0.021 [a]	1.01±0.02 [a]	369±25 [a]	372±24 [a]	0.357±0.009 [a]
Karl 92	534±94 [a]	0.783±0.044 [a]	1.02±0.05 [a]	418±62 [a]	426±60 [a]	0.416±0.023 [b]
2137	684±155 [b]	0.757±0.053 [a]	1.01±0.06 [a]	518±98 [a]	522±94 [a]	0.429±0.030 [b]
Santa Fe	555±68 [a]	0.727±0.028 [a]	1.03±0.04 [a]	403±41 [a]	416±40 [a]	0.421±0.016 [b]

[a] *Values with the same letter in the same column are not significantly different at (P <0.05).*
[b] *Data are the result of at least three replicates.*

4.6. Interrelationships between composition-rheology-microstructure-texture

This study characterized four commonly-grown Kansas hard red winter wheat cultivars chosen to span the largest possible range of protein contents and baking qualities. All of four cultivars were known to have acceptable baking performance and end-product qualities. Although Karl 92 and Overley, and Santa Fe and 2137 were observed to exhibit two slightly distinct compositional, mixing, rheological, and microstructural characteristics, all four flour cultivars performed at more or less the same level.

The high percentage of UPP and overall protein in Overley and Karl 92 can be related to their significantly better Farinograph stabilities, higher loaf volumes, and larger XMT void fractions. Farinograms generally have good correlations with dough processing behavior and baked bread quality (Bloksma 1990; Dobraszczyk and Morgenstern 2003; Tronsmo et al 2003b, Rosell et al 2007). This is confirmed by the volumes of the baked loaves where it was observed that Overley and Karl 92 had the two highest loaf volumes. UPP and total protein content were the only protein parameters directly related to loaf volume since Overley and Karl 92 had the two highest protein contents, respectively, and were not significantly different in percent of UPP or loaf volume. Loaf volume has shown significant positive relationships with protein content in previous studies (Uthayakumaran et al 1999. Mean air cell sizes from XMT had the inverse order of the firmness values for TPA (2137 < Santa Fe < Karl 92 < Overley) which suggested that air cell size is negatively correlated with TPA. Greater loaf volumes and XMT-measured thinner cell walls, air cells of the larger sizes, and greater void fractions were all negatively related with firmness since more expanded structures gave less resistance to compression, resulting in smaller firmness values. 2137 had the lowest XMT fragmentation index (FI) meaning it had the least connected structure with the most closed (less expanded and therefore having unbroken cell walls) air cells. The connected nature of its structure caused it to be more resistant to deformation under compression, leading to higher firmness values. Attenburrow et al (1989) found that hardness of food sponges was highly influenced by crumb density and cell walls, so a more connected cell wall structure would lead to greater crumb firmness.

Fundamental rheological tests are also influenced by flour composition so that strain sweeps and frequency sweeps done at small deformations result in flour with lower protein contents giving higher elastic moduli as reported by Khatkar et al (2005). The order of the moduli, highest to lowest, (2137 > Santa Fe > Overley > Karl 92 in strain sweeps; Santa Fe >

2137 > Karl 92 > Overley in frequency sweeps) showed higher protein content flours having larger viscous character. This could be explained by starch-starch and starch-protein interactions having a greater effect on dough rheology at small strains than protein-protein interactions, resulting in flours with a higher portion of starch producing larger moduli. Greater elasticity in Karl 92, as shown by its lower phase angle, could explain the thinner cell walls and a large air cell sizes found in XMT analysis.

Stress relaxation shows the best link to protein composition of any small deformation rheological measurement performed in this study. Faster relaxation for Karl 92 and Overley moduli was related to lower TPP since smaller quantities of high molecular weight proteins relax quicker. The bimodal relaxation spectrum found for all cultivars shows that wheat flour dough is a mixture of high and low molecular weight protein entanglements (Rao et al 2000), and the lower molecular weight proteins relaxed quickly (0 to 4 sec), while the higher molecular weight proteins relaxed slower (4 to >100 sec). Therefore, MP influenced the first relaxation, and TPP influenced the second relaxation. Although stress relaxation gave information about the proteins in the four wheat flour doughs studied, it did not correlate well with breadmaking quality. This is expected since stress relaxation is a small deformation rheological measurement, and small deformation rheology does not correlate well with final product quality.

The conflicting results from the temperature sweeps and RVA were likely due to having a different degree of hydration and mixing mode in these systems. RVA basically measures the pasting behavior of slurries made of flour and water up to 8% solid content. The doughs used in oscillatory sweeps on the rheometer have an optimal amount of water added to produce the best dough and bread properties possible, while RVA adds the same amount of water to all flour samples, adjusting only for moisture content. Proteins can affect the starch gelatinization temperature of starch in a system (Eliasson 1983), and limited water systems such as bread dough affect starch gelatinization temperature through changes in available water (Zamponi et al 1990).

4.7. References

Angioloni, A., and Dalla Rosa, M. 2005. Dough thermo-mechanical properties: influence of sodium chloride, mixing time and equipment. J. Cereal Sci. 41: 327-331.

Anonymous. 2004. Wheat and Flour Testing Methods: A Guide to Understanding Wheat and Flour Quality: Version 2. Available at . Wheat Marketing Center, Inc. Portland, OR.

Attenburrow, G. E., Goodband, R. M., Taylor, L. J., and Lillford, P. J. 1989. Structure, mechanics and texture of a food sponge. J. Cereal Sci. 9: 61-70.

Autio, K., Flander, L., Kinnunen, A., and Heinonen, R. 2001. Bread quality relationship with rheological measurements of wheat flour dough. Cereal Chem. 78: 654-657.

Bloksma, A.H. 1990. Rheology of the breadmaking process. Cereal Food World. 35: 228-236.

Chiotelli, E., Rolée, A., and Meste, M. 2004. Rheological properties of soft wheat flour doughs: Effect of salt and triglycerides. Cereal Chem. 81: 459-468.

Cornec, M., Popineau, Y., and Lefebvre, J. 1994. Characterization of gluten subfractions by SE-HPLC and dynamic rheological analysis in shear. J Cereal Sci. 19: 131-139.

Dreese PC, Faubion JM, and Hoseney RC. 1988. Dynamic rheological properties of flour, gluten, and gluten-starch doughs. I. Temperature-dependent changes during heating. Cereal Chem. 65: 348-353.

Dobraszczyk, B.J., and Morgenstern, M. 2003. Rheology and the breadmaking process. J. Cereal Sci. 38: 229-245.

Dogan, I. 2002. Dynamic rheological properties of dough as affected by amylases from various sources. Nahrung. 46: 399-403.

Edwards, N., Dexter, J., Scanlon, M., and Cenkowski, S. 1999. Relationship of creep recovery and dynamic oscillatory measurements to durum wheat physical dough properties. Cereal Chem. 76:638-45.

Edwards, N., Peressini, D., Dexter, J., and Mulvaney, S. 2001. Viscoelastic properties of durum wheat and common wheat dough of different strengths. Rheol. Acta. 40:142-53.

Eliasson, A.C. 1983. Differential scanning calorimetry studies on wheat starch-gluten mixtures: I. Effect of gluten on the gelatinization of wheat starch. J. Cereal Sci. 1: 199-205.

Gupta, R.B., Khan, K. and MacRitchie, F. 1993. Biochemical basis of flour properties in bread wheats I. Effects of variation in the quantity and size distribution of polymeric protein. J. Cereal Sci. 18: 23-41.

He, H., and Hoseney, R. 1991. Differences in gas retention, protein solubility, and rheological properties between flours of different baking quality. Cereal Chem. 68: 526-530.

Huebner, F., Nelsen, T., Chung, O., and Bietz, J. 1999. Protein distributions among hard red winter wheat varieties as related to environment and baking quality. Cereal Chem. 74: 123-128.

Kansas State University Agricultural Experiment Station and Cooperative Extension Service. 2009. 2009 Kansas Performance Tests with Winter Wheat Varieties Report of Progress 1018. Contribution number 10-008-S. Available at .

Kasarda, D. 1989. Glutenin structure in relation to wheat quality. Pages 277-302 in: Wheat is Unique. Y. Pomeranz, ed. AACC International: St. Paul, MN.

Khatkar, B., Bell, A., and Schofield, J. 1995. The dynamic rheological properties of glutens and gluten sub-fractions from wheats of good and poor bread making quality. J. Cereal Sci. 22: 29-44.

Kieffer, R. 2007. The role of gluten elasticity in baking quality of Wheat, Chapter 14 in Future of Flour: A Compendium of Flour Improvement, L. Popper, W. Schäfer, W. Freund (Eds.) AGRIMEDIA Publishers, Bergen, Germany, ISBN 978-3-86037-309-5.

Launay, B. 1990. A simplified nonlinear model for describing the viscoelastic properties of the wheat-flour doughs at high shear strain. Cereal Chem. 67: 25-31.

Leon A.E., Barrera G.N., Perez G.T., Ribotta P.D., and Rosell C.M. 2006. Effect of damaged starch levels on flour-thermal behavior and bread staling, Eur. Food Res. Technol. 224(2): 187-192.

Lee, C., and Mulvaney, S. 2003. Dynamic viscoelastic and tensile properties of gluten and glutenin gels of common wheats of different strength. J. Agric. Food Chem. 51: 2317-2327.

Newberry, M., Phan-Thien, N., Larroque, O., Tanner, R., and Larsen, N. 2002. Dynamic and elongation rheology of yeasted bread doughs. Cereal Chem. 79: 874-879.

Repeckiene, A., Eliasson, A.-C., Juodeikiene, G., and Gunnarsson, E. 2001. Predicting baking performance from rheological and adhesive properties of rye meal suspensions during heating. Cereal Chem. 78: 193-199.

Rao, V., Mulvaney, S., and Dexter, J. 2000. Rheological characterization of long- and short-mixing flours based on stress-relaxation. J. Cereal Sci. 31: 159-171.

Rosell, C., Collar, C., and Haros, M., 2007, Assessment of hydrocolloid effects on the thermo-mechanical properties of wheat using the Mixolab, *Food Hydrocolloids*, 21: 452-462.

Safari-Ardi, M., and Phan-Thien, N. 1998. Stress Relaxation and Oscillatory Tests to Distinguish Between Doughs Prepared from Wheat Flours of Different Varietal Origin. Cereal Chem. 75: 80-84.

Salvador, A., Sanz, T., and Fiszman, S. 2006. Dynamic rheological characteristics of wheat flour-water doughs. Effect of adding NaCl, sucrose, and yeast. Food Hydrocolloid. 20: 780-786.

Sandsted, R.M. 1961.The function of starch in the baking of bread. Baker's Digest. 35: 36-44.

Sivaramakrishnan, H., Senge, B., and Chattopadhyay, P. 2004. Rheological properties of rice dough for making rice bread. Journal of Food Engineering, 62: 37-45.

Sliwinski, E., Kolster, P., Prins, A., and van Vliet, T. 2004. On the relationship between gluten protein composition of wheat flours and large-deformation properties of their doughs. J. Cereal Sci. 39: 247-264.

Steffe, J. 1996. Rheological Methods in Food Process Engineering. 2nd ed. Freeman Press: East Lansing, MI.

Tronsmo, K., Magnus, E., Faergestad, E., and Schofield, J. 2003a. Relationships between gluten rheological properties and hearth loaf characteristics. Cereal Chem. 80: 575-586.

Tronsmo, K., Magnus, E., Baardseth, P., Schofield, J., Aamodt, A., and Faergestad, E. 2003b. Comparison of small and large deformation rheological properties of wheat dough and gluten. Cereal Chem. 80: 587-595.

Uthayakumaran, S., Gras, P., Stoddard, F., and Bekes, F. 1999. Effect of varying protein content and glutenin-to-gliadin ratio on the functional properties of wheat dough. Cereal Chem. 76: 389-394.

Uthayakumaran, S., Tomoskozi, S., Tatham, A.S., Savage, A.W.J., Gianibelli, M.C., Stoddard, F.L., and Bekes, F. 2001. Effect of gliadin fractions on functional properties of wheat dough depending on molecular size and hydrophobicity. Cereal Chem. 78: 138-141.

Uthayakumaran, S., Beasley, H., Stoddard, F., Keentok, M., Phan-Thien, N., Tanner, R., and Bekes, F. 2002. Synergistic and additive effects of three high molecular weight glutenin subunit loci. I. Effects on wheat dough rheology. Cereal Chem. 79: 294-300.

Walker, C., and Hazelton, J. 1996. Dough rheological tests. Cereal Food World. 41: 23-28.

Wang, F.C., and Sun, X.S. 2002. Creep-recovery of wheat flour doughs and relationship to other physical dough tests and breadmaking performance. Cereal Chem. 79:567-71.

Weipert, D. 1990. The benefits of basic rheometry in studying dough rheology. Cereal Chem. 67: 311-317.

Wikstrom, K. and Eliasson, A.C. 1998. Effects of enzymes and oxidizing agents on shear stress relaxation of wheat flour dough: additions of protease, glucose oxidase, ascorbic acid and potassium bromate. Cereal Chem. 75:331-337.

Zamponi, R., Giner, S., Lupano, C., and Añon, M. 1990. Effect of heat on thermal and functional properties of wheat. J. Cereal Sci. 12: 279-287.

CHAPTER 5-Conclusions

This study compared four Kansas hard red winter wheat cultivars of different protein contents and baking qualities using protein analysis, recording dough mixers, quantitative baking, textural and structural analysis of baked loaves, and small deformation fundamental rheological measurements. This characterization provided a better understanding of the relationship between flour composition, large deformation rheology, small deformation rheology, and end-product characteristics.

Dough mixing properties and baking quality were heavily dependent on overall protein content and percent UPP. Overley and Karl 92 were good bread quality wheats with high protein contents (11.55% and 11.20%, respectively) and significantly higher percent UPP. Santa Fe and 2137 had lower protein contents (9.27% and 10.82%, respectively) with acceptable baking qualities. RVA pasting properties for all wheats varieties were similar, but Santa Fe showed the significantly ($P< 0.05$) lowest peak viscosity, while Karl 92 gave the significantly highest ($P <0.05$) final viscosity.

Farinograph and mixograph measurements, loaf volumes, and XMT void fractions were generally higher, and firmness was lower for flours with higher protein and UPP contents (Overley and Karl 92). Karl 92 also had the largest portion of thin cell walls in its distribution and the thinnest average cell walls in XMT measurements. Santa Fe had the lowest protein content, smallest loaf volume, and least expanded structure according to XMT data revealing the lowest void fraction and structure thickness, and it was the only cultivar with part of its cell wall thickness distribution in the thickest range of 0.36-0.44 mm. Santa Fe also showed the smallest C-cell slice area of all the cultivars measured. Overley had the most compressible structure (smallest TPA firmness) and it was the most cohesive of these flours. 2137 was the firmest bread with the greatest chewiness, and Karl 92 and Santa Fe were very similar in firmness. Internal bond strength of all cultivars was similar as indicated by their similar TPA springiness. Karl 92 had the lowest resilience, which correlates well with its large number of thin cell walls and high void fraction measured by XMT.

Large deformation measurements of dough had better correlations with baking quality than small deformation measurements. Protein quantity and quality had an impact on all dough and bread measurements even though small deformation oscillatory measurements are highly

dependent on the starch-starch and starch-protein interactions. Protein content inversely affects small deformation measurements because flours with lower protein contents generally have larger percentages of starch.

The linear viscoelastic region of the dough was found to end at .01% strain. Dynamic oscillatory and creep recovery measurements showed that Overley and Karl 92 had higher amounts of viscous character than 2137 and Santa Fe during small deformation measurements, indicative of higher amounts of total polymeric protein in 2137 and Santa Fe. Temperature sweeps revealed similar pasting temperatures for Karl 92, Overley, and Santa Fe, but 2137's gelatinization temperature was several degrees (Celsius) higher than the other wheat flours, likely from interactions between starch and the higher level of soluble protein in 2137 compared to the other flours. Stress relaxation rheological measurements indicated a bimodal relaxation spectrum, characteristic of mixed high and low molecular weight proteins. Stress relaxation moduli give a connection between TPP and relaxation time. Greater percent TPP as in Santa Fe and 2137 gave a slower relaxation, and Overley and Karl 92 relaxed faster since they had a smaller percentage of TPP, even though they had a greater overall protein content and higher percent UPP.

Collectively, the protein characterization and empirical and fundamental measures of rheology gave good characterizations of these four Kansas wheat cultivars. Although some significant differences were found, much of the data indicated the similarity, rather than the difference, of these flours. Since they are all hard red winter wheat cultivars that have been developed for breadmaking purposes, it is not surprising that these flours give similar responses to characterization.

CHAPTER 6 - Future Studies

1. Small strain rheological instruments are useful for determining the small forces in bread dough, but not predicting baked bread quality. Large deformation measurements that give results in terms of fundamental parameters of stress and strain would give better correlations between dough properties and breadmaking quality. In order to better link dough behavior with final bread quality, biaxial and uniaxial dough measurements as well as oscillatory measurements all employing larger strains should be used. The Dough Inflation System or alveograph, and Kieffer Rig or extensograph would be good instruments for this task.

2. Mixolab measurements would provide a better understanding of the relationship between flour protein content, starch pasting, water addition, and mixing parameters. This should help clarify the behavior of different cultivars during rheometer temperature sweeps and RVA.

3. Analysis of starch composition and gelatinization properties as well as α-amylase activity would clarify the reasons for flour pasting behaviors.