RHEOLOGICAL PROPERTIES OF WHEAT DOUGH USING A NOVEL COMPRESSION RECOVERY TECHNIQUE

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Lena Susan Halabi

January 2012

ABSRACT

Ranking wheat flour quality by class or grade does not reveal functional quality attributes relevant to the end user. This has resulted in a continuous effort to find more effective ways to measure quality across the wheat value chain. In line with these efforts, a novel rheology instrument, the CORE, was introduced as a simple and rapid quality test for gluten. The instrument applies a biaxial compression force followed by a free recovery, to measure the elastic behavior of gluten samples. Although designed for gluten, the instrument exhibits potential to reveal valuable data using dough as a more realistic test material.

The CORE was optimized for dough, resulting in new test parameters where dough is compressed at 1 Newton (N) for 5 seconds, followed by a 55-second free recovery. To gain a deeper understanding of its characterization abilities, this test was applied on three large sample sets of flour. It showed a wide range of degrees of elasticity (DE) across different wheat classes and within two sets of Hard Red Winter (HRW) wheat. In addition, the test revealed a new measureable material property, firmness, represented by a sample's resistance to the applied compression force (RC). This new value was strongly negatively correlated with DE, at r^2=0.89, indicating that samples which are highly elastic are also difficult to compress.

Values for DE and RC showed inconsistent correlations with some physicochemical data, but strong agreement with rheological data of the farinograph and alveograph, where multivariate correlations exceeded 0.80. The CORE was capable of detecting a significant increase in DE and RC upon treatment of flour with dough-enhancing enzyme transglutaminase. However, the enzyme's effect varied among cultivars. Similarly, the CORE was successful in detecting improved elasticity upon blending strong flour with weaker flour. Yet, the extent of elasticity imparted by the donor flour was cultivar-specific, and not mathematically predictable.

BIOGRAPHICAL SKETCH

Lena Susan Halabi was born in Alexandria, Virginia on September 24, 1987. She was the third child among three sisters. She completed her K-12 schooling in many countries, including Canada, Saudi Arabia, and Lebanon.

Lena earned her Bachelor's degree from the American University of Beirut, Lebanon (AUB) in Food Science and Management in 2008. She proceeded to work in a food manufacturing company specializing in processed meats, named Al-Taghziah S.A.L. Her responsibilities were primarily oriented towards Quality Assurance, however also included roles in Research and Development.

Lena returned to the US in 2010 to pursue her Master's degree at Cornell University in Food Science and Technology. Amidst her graduate work, she spent one summer in Minneapolis, MN, working as a Product Development Intern at General Mills Inc. Lena wishes to complete her Master's degree in January 2012, and return to General Mills to work in the position of Scientist I.

Lena's passion-driven food science career is balanced with other cherished activities in her life. Among these are sports that include jogging, spinning, skiing, and swimming, as well as indulgences in the arts, family values, social outings, and cultural artifacts.

To all the Lebanese who have bravely left their homeland in search for more…

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to Dr. Steven Mulvaney, for serving as my thesis committee chair, and granting me the opportunity to pursue my Masters studies under his guidance. I would also like to thank Dr. Andrew Novakovic for his time and support as a member of my committee, as well as his assistance in the field of Applied Economics and Management.

This work would not have been achievable without the faith of my father and mother, who passed on their values of education and hard work. Their support was coupled by that of my two older sisters, Zeina and Dima, whom I continue to learn from every day. I would like to extend my familial gratitude to Rami Raad for his invaluable advice, as well as the encouragement of Mahassen and Kamal Chanouha throughout every stage of my degree.

My final thank you belongs to my dear friends, who span all time zones, and have collectively made every aspect of graduate school manageable. More specifically, I would like to recognize Randa Adra and Delbert Abi-Abdallah, whose irreplaceable friendship lives in this thesis, and in my memory of Cornell, forever.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

CORE	Compression Recovery
DE	Degree of Elasticity
DR	Degree of Recovery
FGIS	Federal Grain Inspection Services
GMP	Glutenin macropolymer
HDWH	Hard White
H_f	Final height of sample
H_i	Initial height of sample
H_m	Minimum height of sample
HRW	Hard Red Winter
HRS	Hard Red Spring
MC_{wb}	Moisture Content on a wet basis
NBS	Nominal Bowl Size
OTA	Office of Technology Assessment
PCA	Principal Component Analysis
RC	Resistance to Compression
REML	Residual Maximum Likelihood
SRW	Soft Red Winter
SWH	Soft White
TG	Transglutaminase

INTRODUCTION

1.1 Wheat Harvest and Flour Production

Wheat is one of the principal grains grown throughout the world, with an annual average of 576.3 metric tons (Mt) in the last ten years (FAO 2004). The grain's ability to form a nutritious viscoelastic mass upon grinding and mixing with water separates it from other common grains that do not exhibit these properties. The wheat crop is part of the 'grass' family *Gramineae*, of genus *Triticum*. Wheat commonly used for baked goods is named *Triticum aestivum*, while wheat used for pasta applications is known as *Triticum durum* (Hui and Corke 2006).

Harvested wheat kernels must go through several steps before becoming a useful baking ingredient. The most notable of these is the milling process. After harvest, wheat is transported and stored in a grain elevator. Prior to milling, it is transferred to large silos, after which the grain is cleaned and separated from foreign materials by sieving, dusting, and chaffing, then exposed to magnetic separators and destoners for any remaining foreign material. After ensuring the removal of these materials, the grains are tempered by means of spraying with water and resting in their wet state in conditioning bins. This contributes to more effective milling by preventing pulverization during milling and facilitating separation of the bran (Wheat Marketing Center 2008).

The milling process itself may take on different forms, however the general scheme involves pre-breaking the grain, running it through adjacent breaking rolls, which rotate in such a way to scrape off the bran and separate it from the endosperm as the grain passes through. Afterwards, this intermediate product is sifted, sending ready flour and coarser particles on two different paths. The coarser particles, consisting mainly of semolina and wheat bran, are re-

processed to further grind the semolina into flour, while separating the germ and bran out of the system. Each of the three components, flour, bran, and germ, enter their designated path on the supply chain, whereby they are kept separate or combined to produce a variety of baked goods or animal feed. Flours of varying source and quality may be blended to enhance properties such as protein content and quality. Similarly, different parts of the wheat grain may be recombined to produce whole wheat flours (Wheat Marketing Center 2008).

The meaning of whole grain embodies the three main components of the wheat kernel: the bran, endosperm, and germ. A diagram representing these three constituents is shown in Figure 1. As shown, the endosperm comprises the largest portion of the kernel, about 83% by weight, and contains most of the nutrients, namely protein, carbohydrates, and iron, as well as some of the major B vitamins. The outer layer bran makes up about 14% of the kernel weight, and is known for its insoluble fiber content and health benefits. The germ is contained inside the kernel, and is usually separated from flour due to its high fat content, which poses a threat to shelf-life (Cauvain, Young et al. 2007).

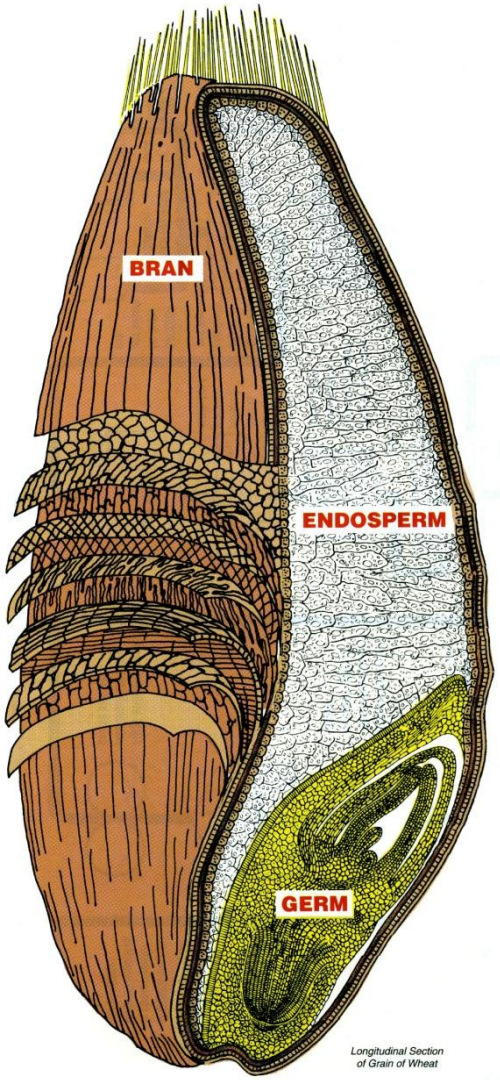


Figure 1 Diagram showing main components of wheat kernel (Wheat Marketing Center, 2004)

1.2 Components of the Wheat Endosperm

1.2.1 Starch

Starch, the storage carbohydrate in plants, is the main component of wheat flour, comprising about 65% of regular flour (14% moisture basis). Starch is a polysaccharide of two types: 23% found in the linear form of α-1,4 linked glucose units, named amylose, and the remaining 73% contained in a highly branched structure known as amylopectin. These two structures exist in the form of starch granules that do not play an active role during dough mixing, however find significant influence on dough elasticity and baking performance. The most common quality parameters attributed to starch for milling and baking purposes is 'damaged starch,' which may represent up to 15% of starch granules that have been cracked or fractured during milling and cleaning processes. These are known for their increased water absorption capabilities and susceptibility to the action of the hydrolytic enzyme α-amylase. While the name indicates 'damage,' the presence of damaged starch is actually a parameter to be optimized and controlled, rather than eliminated (Cauvain, Young et al. 2007).

1.2.2 Protein

Osborne (1907) pioneered the attempt to understand the complex nature of wheat flour proteins. Osborne first characterized proteins by sequential fractionation according to their solubility in various solutions. This resulted in three groups of proteins:

1. Albumins and globulins, soluble in salt solutions
2. Prolamins, soluble in 70% aqueous ethanol
3. Glutelins, insoluble in both salt and 70% ethanol solutions

However, this method did not provide an accurate quantification due to overlapping solubilities of different fractions shown in Size-Exclusion High Performance Liquid

Chromatography (SE-HPLC) assays. Moreover, solubility properties were sensitive to the type of alcohol used, the surrounding temperatures, and other external factors (F and John 1992).

Upon further investigation, studies showed that size-based classification may be a superior method of classification to the solubility approach. The use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) effectively divided proteins into polymeric and monomeric categories, with glutenin, tricitins, and HMW albumins representing the former, and gliadins, albumins, and globulins representing the latter. The principle polymeric protein, glutenin was further separated by its subunits based on their mobility in the SDS-PAGE assay. This generated a structural understanding of low-mobility compounds that became known as high molecular weight glutenin subunits (HMW-GS), and low molecular weight glutenin subunits LMW-GS, which were more volatile, and similar in size to monomeric gliadins. The second portion of gluten proteins consisted of four types of monomeric gliadins that differed in their order of mobility, with α-gliadins being the most mobile, followed by β, γ, and ω. The mobility of these compounds as shown in an SDS-PAGE assay is portrayed in Figure 2. (F and John 1992).

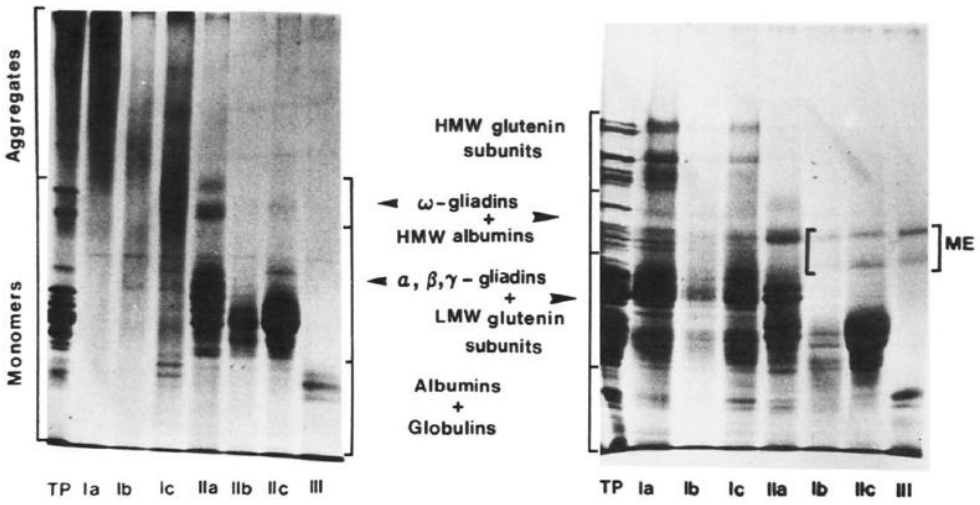


Figure 2 SDS-PAGE assay of unreduced (left) and reduced (right) gluten proteins (F and John 1992)

The third form of classification is the most relevant to wheat functionality, as it divides proteins into three types based on their amino acid sequences, and more specifically, their sulfur groups that are responsible for creating structural crosslinks. Building on previous research, the first group of prolamins is the high molecular weight compounds (HMW), accounting for approximately 10% of gluten proteins. These are found in two forms, X and Y, both of which exhibit a repetitive domain in the middle of the polypeptide chain, which coils into what is known as β-turns, capable of deforming and reforming under stress relaxation parameters. Its cysteine residues lie on both the N and C terminals, allowing for inter and intra-molecular crosslinking(Shewry, Halford et al. 2002). The X types move more slowly up the SDS-PAGE assay, due to their larger MW, usually ranging from 80,000 to 83,000. The Y types exhibit higher mobility up the assay, with MW in the range of 67000 to 74000 (Shewry, Napier et al. 1995). These two HMW structures are illustrated in Figure 3.

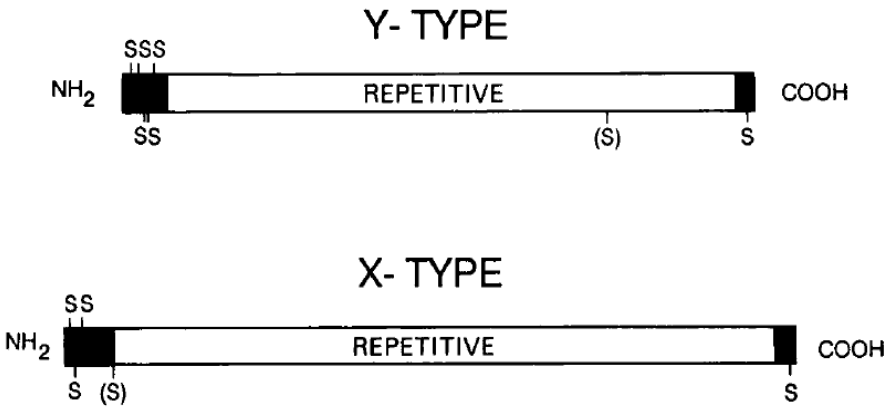


Figure 3 Representations of the X- and Y-type HMW glutenin subunits (F and John 1992)

The second group of prolamins is the sulfur-poor (S-poor), which are the ω-gliadins, whose complete amino acid sequence remains unknown. As for the third group, these are a heterogeneous mix of α, β, and γ-gliadin, along with LMW glutenins, which all have common

elements of structure. These groups all possess two domains: the N terminal, which is rich in glutamine and proline, and responsible for forming β-reverse turn structures – and the α-helical C terminals, which are not repetitive, and contain most of the cysteine residues (Shewry, Halford et al. 2002).

Polymeric glutenins and monomeric gliadins jointly form the complex gluten protein structure that is responsible for dough's viscoelastic properties. HMW-GS have been shown to exhibit elastic behavior, while gliadins and LMW glutenins are known for their contributions to viscosity. Thus, the ratio of the two partly determines the dough's material properties (Wieser 2007). Other issues that factor into gluten quality is the extent of polymerization, and the qualitative genetic makeup of HMW-GS unit, with some alleles contributing a higher 'quality score' than others (Lasztity and Abonyi 2009).

The predominant covalent bonds in dough are cysteine-induced disulfide bridges that take place within a protein or between proteins, as shown in Figure 4. The other covalent bond relies on tyrosine-tyrosine crosslinks between the gluten proteins. Non-covalent bonds of hydrogen, ionic, and hydrophobic nature supplement this system, providing a different type of linkage that contributes to dough stability during mixing, handling, and baking. The prevalence and location of these bonds largely affect the formation and retention of the protein network (Cauvain, Young et al. 2007).

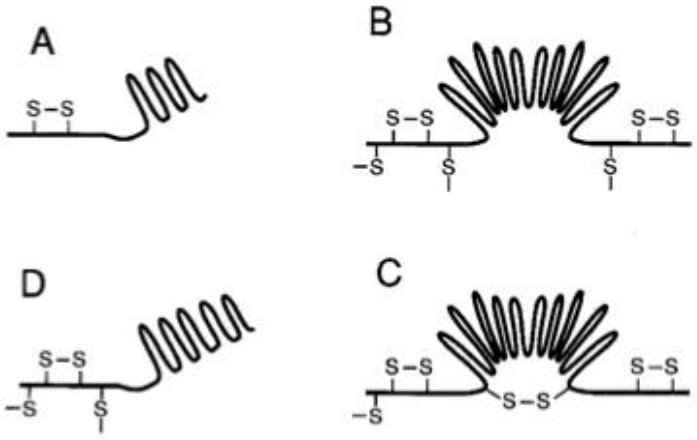


Figure 4 Schematic illustration of gluten proteins (A) Gliadin; (B) HMW glutenin subunits showing β-turns as molecular spring; (C) HMW glutenin subunits showing β-turns linked by disulfide bond preventing spring; (D) LMW glutenin subunit (Cauvain, Young et al. 2007)

1.2.3 Lipids

Despite the slight appearance that lipids make in wheat flour, their presence at 2.5% certainly contributes to flour's mixing, handling, and baking characteristics. Lipids are found in both polar and non-polar forms, with the former consisting of galactosyl glycerides (0.6%) and phospholipids (0.9%), and the latter comprising the remaining 1% of triglycerides, diglycerides, free fatty acids, and sterol esters (Cauvain, Young et al. 2007).

Studies have shown that lipids interact with gluten proteins during mixing, and provide additional support for the newly formed gluten network. This is made evident through the significant decrease in amount of extracted lipids, from 98% in flour to 36% in dough, by means of a petroleum-ether solvent-extraction method (Pomeranz 1991). Although regular and defatted flours showed no difference in their mixogram output, the effects of bound lipids play a role in the later stages of baking. In addition, indigenous lipids may act as substrates for added enzymes such as lipoxygenase, the oxidation of which produces hydroperoxides that oxidize sulfhydryl groups of flour proteins, and hence exhibit changes in rheological properties of dough (O.K. Chung 1978).

1.3 The US Wheat Supply Chain

The US wheat industry is a nation-wide system of interdependent activities that relies on quality control measures to ensure the safety and integrity of the grain and final product. The system involves many levels, including plant breeders, farmers, and milling plants, to transform harvested wheat into a finished product. In a staff paper written for the Upper Great Plains Transportation Institute (UGPTI), Barber and Titus classify the post-harvest supply chain into three sequential steps: elevators, milling, and baking, whereby elevators collect, store, and even mix wheat from varying sources, and transfer them to milling plants. At the plant, wheat is

grinded and sifted into flour for human consumption, or mill feeds for animal feed. These domestic mills supply flour to wholesale baking industries, composed of manufacturers of bread, cake, cookies, crackers, and other wheat-based products (Barber and Titus 1995).

An earlier report for the UGPTI notes that the key to the success of US wheat flow, for both internal and external trade, lies in the intricate transportation network. This consists of over 25,000 miles of waterways that serve major wheat export locations, as well as 191,000 miles of rail and track and 3.9 million miles of roads, which effectively transport product from farms gates to elevators, milling plants, and eventually the consumer (Houghe 1994). The role of quality control testing is interwoven into nearly every step of the supply chain, on both a private and government-regulated scale. Government inspection services are mandatory for all exported grains. These are carried out upon loading of the vessel to ensure quality criteria match the customer's purchase contract (USDA).

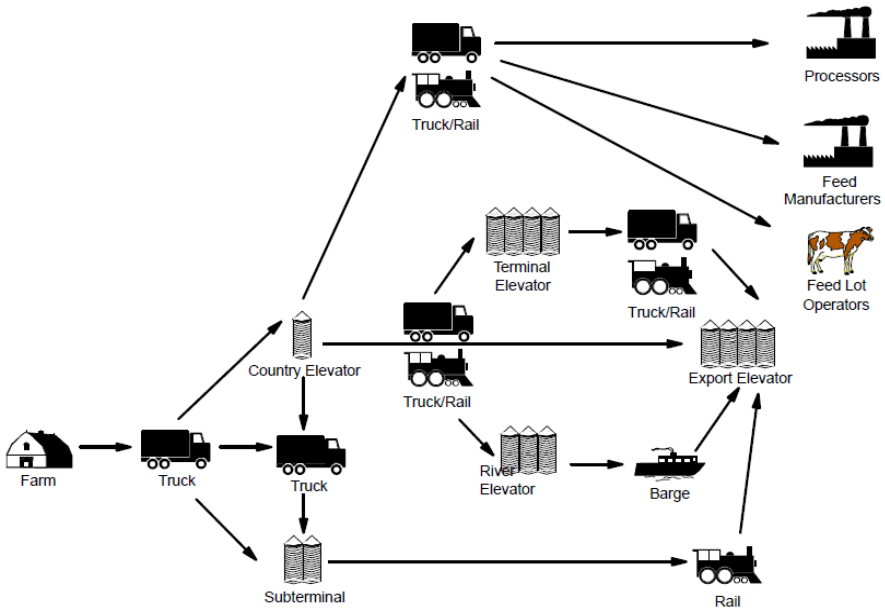


Figure 5 A general representation of the US wheat supply chain (Houghe 1994)

1.4 US Wheat Grades and Requirements

The Official US Standards for Grain represents the legal obligation of the government to define terms and standards necessary for effective domestic and international trade of grains. In 1976, Congress created the Federal Grain Inspection Services (FGIS), under the US Department of Agriculture's Grain Inspection Packers and Stockyards Administration (GIPSA), in order to manage nationwide grain quality. The tests done on wheat center around sanitary and physical attributes, including: damaged kernels, shrunken and broken kernels, foreign material, dockage, and live insects. Minimum and maximum limits for these attributes have been established as the 'US Standards for Wheat,' and are used for determining U.S grades of flour, which span grades 1, 2, 3, 4, and 5 (shown in Table 1). Assigning grades to wheat is mandatory for all exported lots.

Table 1 US wheat grades and grade requirements

	Minimum Limits of -		Maximum						
	Test Weight per bushel		Damaged Kernels					Wheat of other classes **2/**	
Grade	Hard Red Spring Wheat or White Club Wheat (pounds)	All other classes and subclasses (pounds)	Heat damage (part of total) (percent)	Total (percent)	Foreign material (percent)	Shrunken and broken kernels (percent)	Defects **1/** (percent)	Contrasting classes (percent)	Total **3/** (percent)
U.S. No. 1	58.0	60.0	0.2	2.0	0.4	3.0	3.0	1.0	3.0
U.S. No. 2	57.0	58.0	0.2	4.0	0.7	5.0	5.0	2.0	5.0
U.S. No. 3	55.0	56.0	0.5	7.0	1.3	8.0	8.0	3.0	10.0
U.S. No. 4	53.0	54.0	1.0	10.0	3.0	12.0	12.0	10.0	10.0
U.S. No. 5	50.0	51.0	3.0	15.0	5.0	20.0	20.0	10.0	10.0

U.S. Sample Grade:

U.S. Sample Grade is wheat that:

(a) Does not meet the requirements for grades U.S. No.1, 2, 3, 4, or 5; or
(b) Contains 4 or more stones or any number of stones which have an aggregate weight in excess of 0.1 percent of the sample weight, 1 or more pieces of glass, 3 or more crotalaria seeds (Crotalaria spp.), 2 or more castor beans (Ricinus communis L.), 4 or more particles of an unknown foreign substance(s) or a commonly recognized harmful or toxic substance(s), 2 or more rodent pellets, bird droppings, or an equivalent quantity of other animal filth per
1,000 grams of wheat; or
(c) Contains 5 or more animal filth, castor beans, crotalaria seeds, glass, stones, or unknown foreign substance(s) in any combination; or
(d) Has a musty, sour, or commercially objectionable foreign odor (except smut or garlic odor); or
(e) Is heating or otherwise of distinctly low quality.
(f) Contains more than 31 insect-damaged kernels in 100 grams.

1/ Defects include damaged kernels (total), foreign material, and shrunken and broken kernels. The sum of these three factors may not exceed the limit for defects for each numerical grade.
2/ Unclassed wheat of any grade may contain not more than 10.0 percent of wheat of other classes.
3/ Includes contrasting classes.

1.5 Non-Grade Attributes of Wheat Quality

The FGIS also measures several non-grade attributes, including dockage, moisture content, and protein content. Dockage refers to non-wheat material that is large enough to remove through screens, scalping, or aspiration. Moisture is the water content of the grain, and has potential links to shelf-life considerations, given that higher moisture levels promote the growth of spoilage organisms such as mold. Protein is measured using an approved near infrared transmittance (NIRT) instrument calibrated against a Combustion Nitrogen Analyzer (CNA). These three parameters do not affect the numerical grade of the wheat lot, yet are still considered a part of the lot's quality assessment (Wheat Marketing Center 2008).

Unlike other crops, wheat is not a commodity. Regardless of grade, it is a differentiated product, due to its unique ability to exhibit a large variation in its intrinsic properties. This fluctuation directly influences its value and price (Noel and Bengt 1996), thus having economic implications. Traditionally, the variation expressed by wheat cultivars was attributed to the class they belonged to. Wheat classes are a culmination of three qualities: kernel hardness, kernel color, and growing season. The six main US wheat classes are: Hard Red Winter (HRW), Hard Red Spring (HRS), Soft Red Winter (SRW), Soft White (SW), Hard White (HW), and Durum (DU). Each class is known to exhibit certain general characteristics, which represents the first tier of 'differentiation' for the crop. Hard wheat has high protein content, and is generally used for yeast-leavened products that can withstand gas expansion without collapsing, such as breads, bagels, and croissants. Soft wheat exhibits lower protein contents, usually between 8 and 10%, and is used for chemically-leavened or non-leavened goods, creating an inner structure that gives suitable mouthfeel and texture properties in bakery products such as cookies and cakes (Hui and Corke 2006). Each class is native to different regions throughout the US, with approximately

two-thirds of all wheat being grown in the Great Plains, known as the region extending from Texas to Montana (EPA 2009). The map in Figure 6 shows the geographical distribution of wheat classes across the country.

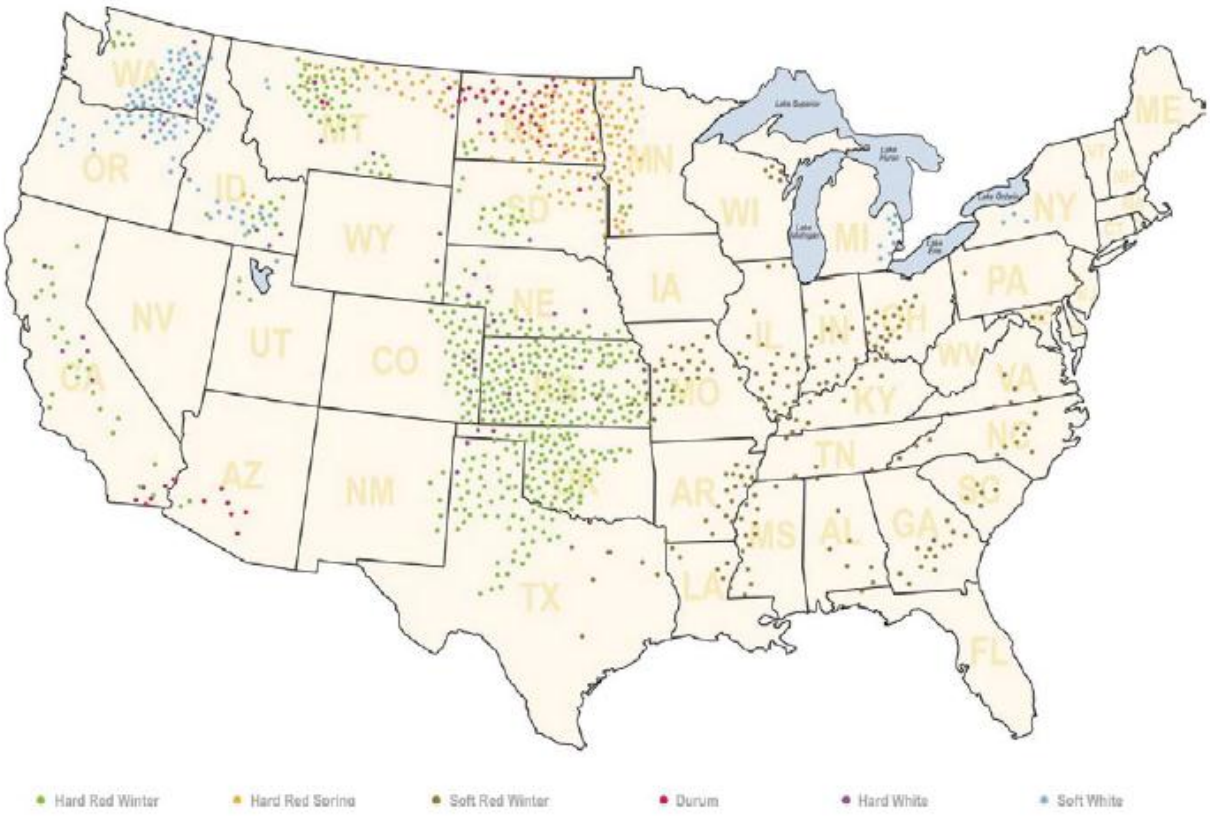


Figure 6 US wheat cultivars, by region (Wheat Marketing Center 2008)

As part of a survey conducted by the Office of Technology Assessment (OTA) in 1988, domestic and overseas millers were asked whether wheat class is a good indicator of wheat quality. Both groups responded negatively, confirming that additional data is required to define wheat quality. In order to identify properties that were most valued by millers, the survey listed 28 attributes for local millers and 22 for international participants, and asked them to rank the importance of each attribute for their purposes. The highest ranking attributes in terms of significance among domestic millers were protein content, pesticide residue, mycotoxins, insects, and baking tests, which all ranked at 6.0 or above on a scale from 1.0 to 7.0. Overseas millers differed greatly by region, however, agreed on the importance of protein content (62% would include it in a contract), falling number (45%), and farinograph results (36%). The overseas market seemed to recognize the significance of rheological testing more than the domestic

market. When asked what additional tests ought to be included in wheat standards, the overseas market mostly recommended rheological measurements, the most popular of which was the amylograph, a measure of starch quality and sprout damage. Both groups equally voiced their desire for the inclusion of falling number and pesticide residue in wheat standards (United States. Congress. Office of Technology 1989).

The market's inclination to value one attribute over another points to the potential of using a hedonic model for understanding wheat price. More specifically, this entails recognizing the value of each wheat component, or measure of quality, and accounting for that value in the final price. In a case study on the hedonic pricing of milk, a multiple-component pricing system that included new and relevant qualities of protein and lactose replaced a traditional approach of pricing, which ineffectively relied primarily on butterfat to define milk's worth (Gillmeister, Yonkers et al. 1996).

Similarly, another economic study attempted to valuate six quality characteristics normally measured for wheat by the FGIS. These characteristics were test weight per bushel, percentage of foreign materials, percentage of shrunken and broken kernels, percentage dockage, moisture content, and protein content. Other relevant quality factors such as milling rate, falling number, and wet gluten were not included in the study due to a lack of supporting data. The study concluded that average price did not equally account for the value that each characteristic represented; instead, the six variables were able to explain 80% of the variation in price for exported wheat. Of these six characteristics, the only two that showed to have a statistically significant effect on price were test weight and protein content. These results were consistent with previous studies, such as Wilson (1989) and Larue (1991), which also demonstrated the positive influence of protein content on wheat price (Noel and Bengt 1996).

1.6 The Evolution of Wheat Quality Testing

In an increasingly competitive and technology-driven industry, producers of baked goods are aware of the properties they require in flour for the success of their end products. Although indicative of certain physical characteristics, wheat grading, wheat class, and protein content, are not sufficient in describing flour quality from an end use standpoint. Due to this, an arms race began for the development of more rapid and comprehensive methods of measuring wheat quality. This began with biochemical tests, and proceeded instrumental capabilities.

1.6.1 Rapid Laboratory Tests

The following is table summarizes some of the common rapid laboratory tests done to measure various aspects of wheat quality. The validity of these tests tends to be verified by the extent to which they correlate with baking quality parameters, most notably that of bread loaf volume (BLV).

Table 2 Summary table of most common wheat quality laboratory tests

Test Name	Method	Output	Significance
Protein Content	A sample of flour is weighed and placed in a Combustion Nitrogen Analysis (CNA) instrument. Flour is burned and the amount of nitrogen gas released is measured.	A formula is used to convert the amount of nitrogen gas into protein content, measured as percentage protein in flour or wheat (%)	Knowing the amount of protein provides a quantitative indication of certain flour quality parameters, including water absorption, dough mixing, and dough strength during baking.
Falling Number	It measures the time required for a stirrer to fall through a heated hydrated flour slurry.	Time (seconds)	The test measures the slurry's viscosity. A small falling number (below 250) indicates a large presence of enzymes, resulting in a lower amount of starch, thereby affecting baking quality. High enzyme activity is an indication of sprout damage.
Sedimentation volume	A small sample of flour is mixed with water and lactic acid, allowing gluten proteins to swell and precipitate as sediment.	Sedimentation volume (ml)	This test provides a good indication of the amount of gluten protein in the flour. The test has been shown to correlate well with dough strength or bread loaf volume.
Wet Gluten	A small sample of flour is mixed with a 2% salt solution to wash away starch and other soluble materials from the hydrated flour	Percentage of gluten on a 14% moisture basis (%)	The test gives a good estimation of the amount of gluten in flour.
Gluten index	A sample of wet gluten mass is centrifuged in a special chamber containing a sieve	Percentage of gluten remaining on the sieve (%)	The test gives a good estimation of the amount of gluten in flour.

1.6.2 Wheat Quality: A Rheological Approach

Rheology is the study of the flow and deformation of a given material. It is usually used to understand the material properties of a subject in an objective, consistent, and mathematically sound manner. Deformations may be small or large, and applied in a manner that is static or dynamic. The type of test done usually mimics a large-scale unit operation in a manufacturing setting, thereby allowing us to predict the behavior of a material.

Rheology found a wide application in the measure of both dough and gluten quality due to their unique viscoelastic nature. Researchers hoped that by applying existing rheological principles, and calculating parameters such as the storage, loss, or bulk modulus from obtained stress and strain values, they may be able to extrapolate results and predict raw material and end product quality. Traditionally, tests done for these purposes have included shear stress, creep recovery, stress-relaxation, and extension (Uthayakumaran, Newberry et al. 2000).

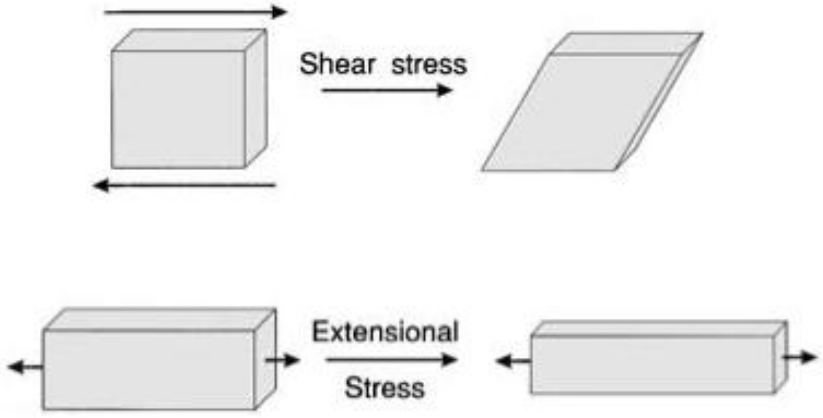


Figure 7 An illustration of two fundamental rheology principles (Cauvain, Young et al. 2007)

However, although "fundamental tests" are able to accurately measure and quantify dough properties, their application in understanding dough remains highly disadvantageous due to their unrealistic test parameters as well as dough's complex composition, that is both inherently variable and susceptible to external factors such as time and temperature

(Dobraszczyk and Morgenstern 2003). This resulted in the shift to "empirical tests" which rely on rheological principles that imitate baking processes, and yield results in arbitrary units that describe and predict dough behavior.

1.6.3 Dough as a Viscoelastic Material

The formation of dough begins with the hydration of flour with a fixed amount of water, and is only accomplished once the two are mixed together for a set period of time. This blending allows for the formation of an integrated network of gluten proteins, surrounded by lipids, and polysaccharides, until the thick liquid-like mixture is transformed into a smooth viscoelastic mass (Cauvain, Young et al. 2007). Gluten proteins hold the greatest responsibility for the formation of this structure. Upon hydration and physical aggregation, the protein structure unfolds, and reacts with its surroundings to form new disulfide and non-covalent bonds. The strength of this network rests in the flour's protein composition, more specifically, in the amino acids and genetic code that determine the amount, extent, and type of bonding available (Skerritt, Hac et al. 1999).

1.6.4 Examples of Dough Testing using Fundamental Rheology

In order to understand how dough strength has been characterized using rheology, one must examine the efforts that have been done towards this. Janssen et al. investigated two fundamental tests on dough and their relation to bread-making performance. The two methods were oscillatory dynamic tests using a rheometer over an angular frequency range, ω, of 0.03 to 3 rad/s, resulting in stress-strain curves and calculation of the storage and loss moduli , G' and G" respectively. The second fundamental test used an Overload Dynamics material testing instrument fitted with a loading cell of mixed force (200 and 2000 N) to biaxially compress dough at three different crosshead speeds. Although the loss modulus tan δ (G"/G') and other

material properties were revealed, results indicated that "differences determined in small deformations in shear did not relate well to differences in loaf volumes." Although fundamental and empirical rheological tests were in agreement with one another, the information they revealed regarding baking performance did not constantly correlate with loaf volume. It was concluded that more than one aspect of rheological performance is required to adequately predict dough's baking performance (Janssen, van et al. 1996)

In another study, rheological characterizations of dough using dynamic tests in an oscillatory rheometer were carried out on the linear viscoelastic region of the dough sample. Rheograms were able to characterize the elastic and viscous components of the dough exposed at different mixing times. Dough with more insoluble protein residue (IPR) showed higher elastic moduli, while dough showing a high content of gliadins consistently exhibited higher viscosity (Puppo, Calvelo et al. 2007). This study, among many others, highlights the potential of using fundamental rheology to understand the interactions taking place on a molecular level, more specifically the contribution of gliadins to viscosity and IPR to elasticity.

Still in the domain of fundamental testing, researchers began to incorporate new and innovative principles to learn dough properties. In a recent study in 2006, an Instron 5567 was used to compress dough samples of a known volume, to a maximum compression stress of 4.97 MPa at a constant speed of 0.05 mm/s. As expected, the extent of strain was not constant, and depended on the applied pressure. The volume strain was calculated by accounting for the diminution of the total volume in respect to the initial volume. Diminution of volume increased rapidly between compression stresses of 0 to 1MPa, after which it reached a plateau, indicating that compressibility of dough is significantly diminished after a stress of 1 MPa. Using values of stress and strain for various levels of stress, the bulk modulus (K) was calculated. Values of K

were consistent with results, with an initial slow increase until about 10 MPa, followed by a sharp increase to 66MPa, representing rapid stiffening of dough. This work attempted to study compressibility of dough on a small scale in order to understand how dough might react to compression during processing. Although measurements were fundamental, this experiment drew a parallel between dough properties and manufacturing parameters (Chunguang Wang 2006).

1.6.5 Empirical Rheology: An Adaptation of Fundamental Rheology

Understanding the behavior of dough in a standard manner is certainly important, however results often seem to answer general questions as opposed to solve practical problems faced in the flour manufacturing and baking settings. As a result, new methods were adapted from fundamental rheology, which used fundamental principles in the dough-specific context of baking. These 'customized' tests were able to mimic baking processes and phenomena, and consequently provide predictive information regarding the quality of a viscoelastic dough in a large scale manufacturing setting.

1.6.5.1 Farinograph

Measurements and Outputs:

This instrument involves the addition of water to a flour sample in a recording mixer, whereby the amount of water required to reach the standard 500 Brabender Unit (BU) line, is deemed the **absorption**, and expressed as a percentage. The amount of time required for the mixing curve to reach this line is known as the **arrival time**, while the time in which the curve leaves this line is known as **departure time**. The **stability time** of the dough is the time it remains at the line, calculated by the difference between departure and arrival. Stability time reflects the consistency of the dough during mixing. Other parameters include **peak time**,

expressed as the time required for the dough to reach its maximum consistency, and **Mixing Tolerance Index (MTI)**, the difference in BU units between the curve peak and 5 minutes later (Wheat Marketing Center 2008). Diagrams illustrating these parameters for a strong and weak dough are shown below.

Significance in Baking

The farinograph allows for the understanding of several quality parameters of the dough, namely processing requirements, effects of additives, and final product texture. It is widely used in the industry.

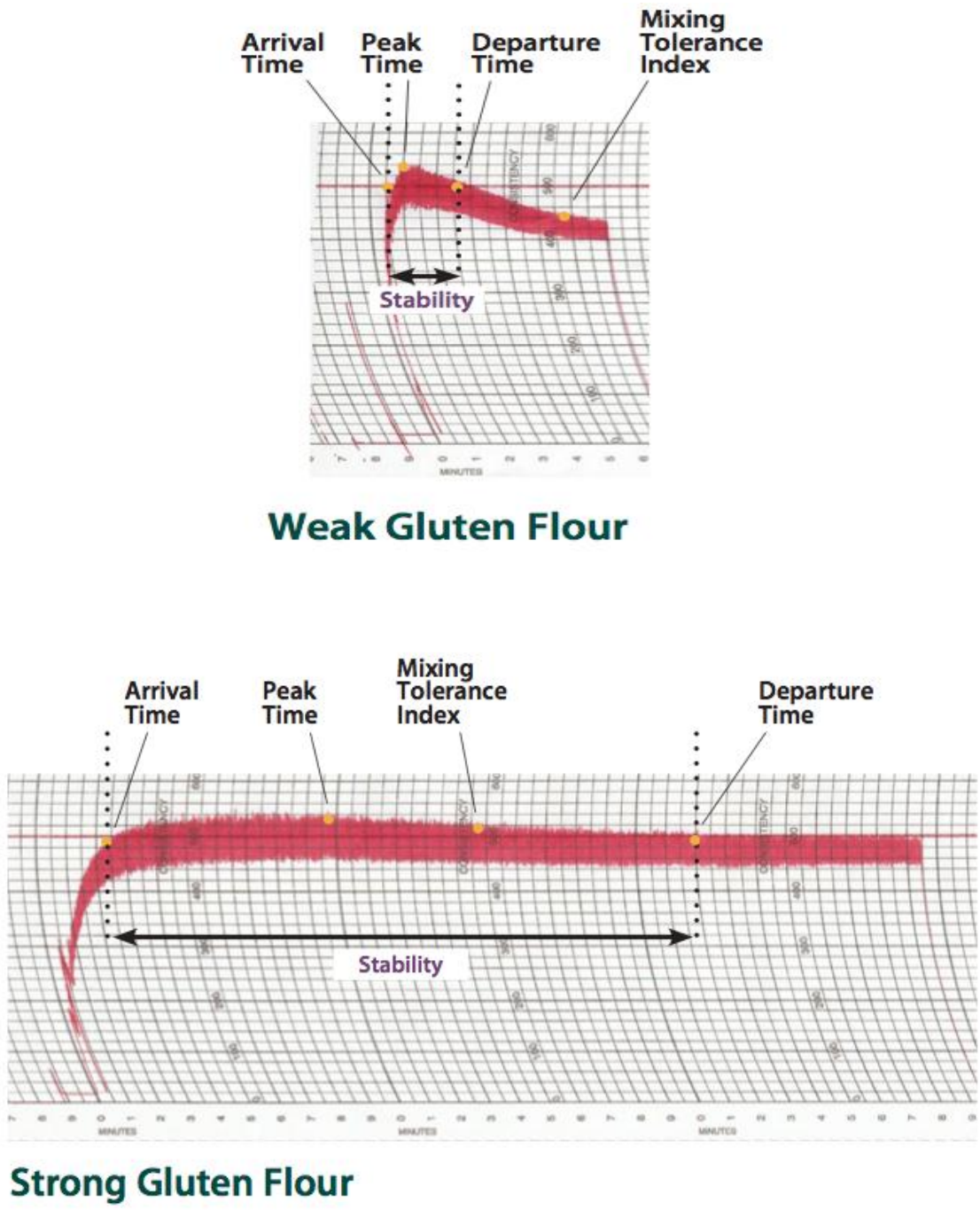


Figure 8: A farinogram for a weak flour (above) and a strong flour (below) (Wheat Marketing Center 2008)

1.6.5.2. Mixograph

Measurements and Outputs:

Similar to the farinograph, this instrument is also a mixing recorder, however, its mode of operation is more open-ended than that of the farinograph. Water is added to a flour sample in a mixing bowl, and the instrument measures the increasing resistance of the hydrated flour to mixing in torques. The time required to reach the peak of the curve is known as the **peak time**, Another output of the instrument is **mixing tolerance**, which measures the resistance to breakdown as the dough continues to be mixed passed its peak (Wheat Marketing Center 2008).

Significance to baking:

Peak time is an indication of the optimal mix time in a baking process for a specific dough type, while mixing tolerance represents the resistance of a dough to breakdown. Both parameters correlate positively with strong doughs, and may describe the strength of the gluten network in the dough, or the effect of dough-enhancing additives and enzymes. The main advantage of using both the mixograph and farinograph is their ability to rapidly test samples while providing meaningful information.

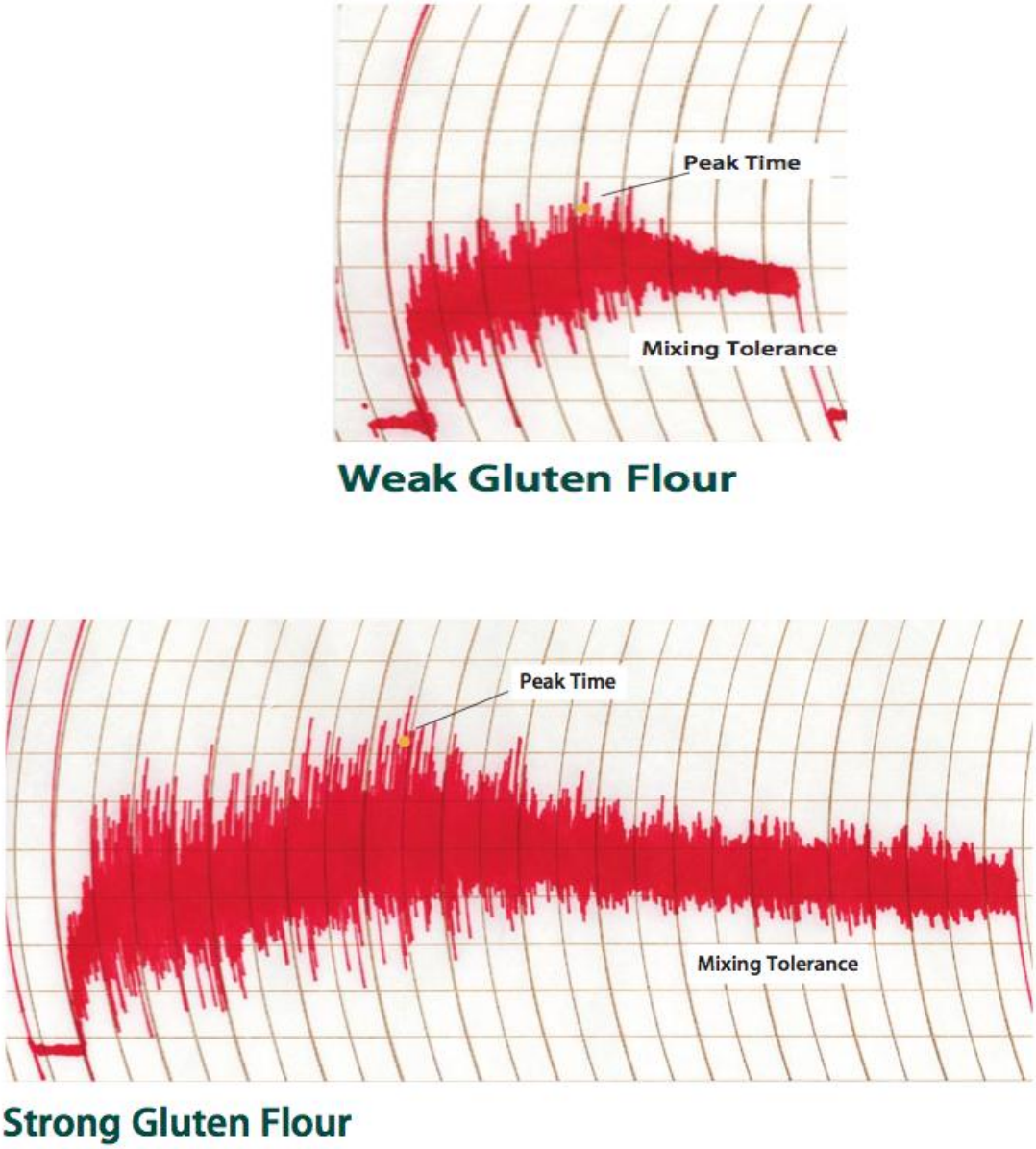


Figure 9 Mixogram for weak flour (above) and strong flour (below) (Wheat Marketing Center 2008)

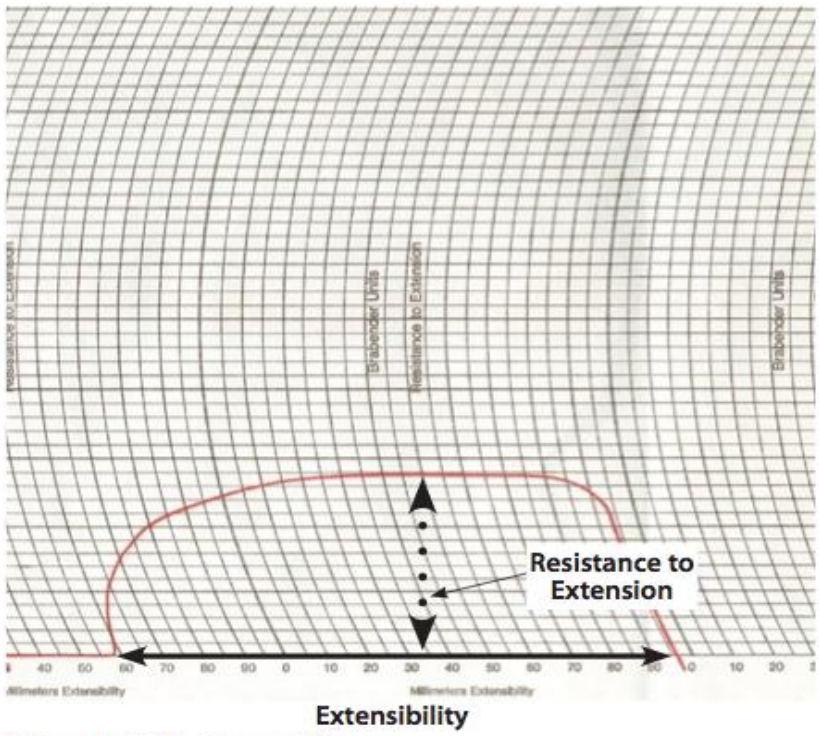
1.6.5.3 Extensigraph

Measurements and Outputs:

This instrument relates to the act of stretching a dough, as is usually done in bakeries. A large flour sample (300 grams) is formed into a dough, rolled into a ball, and eventually placed in a dough "cradle" where a hook runs through its center, and stretches it downwards. The sample's **resistance to extension (R)** is an indication of the dough strength, or more specifically, its firmness. This value may be measured in centimeters (cm), Brabender units (BU), or Extensigraph units (EU). The second parameter is **extensibility (E)**, shown on the graph as the length of the curve, expressed in centimeters (cm) or millimeters (mm). The third parameter extracted from the curve is a combination of R and E, calculated by the **area under the curve**, and expressed in squared centimeters (cm^2). Results for these parameters with strong and weak flours are shown below (Wheat Marketing Center 2008).

Significance to baking:

The actions carried out on dough from this instrument are very similar to those done in real life bakeries or manufacturing settings. This approximate replication of industrial processes allows us to measure and predict a flour's behavior, and is important for blending operations, where properties such as extensibility may be quantified and optimized prior to large scale manufacturing. The main disadvantage of this method lies in its inefficiency, both in amount of sample, and time-consuming nature.



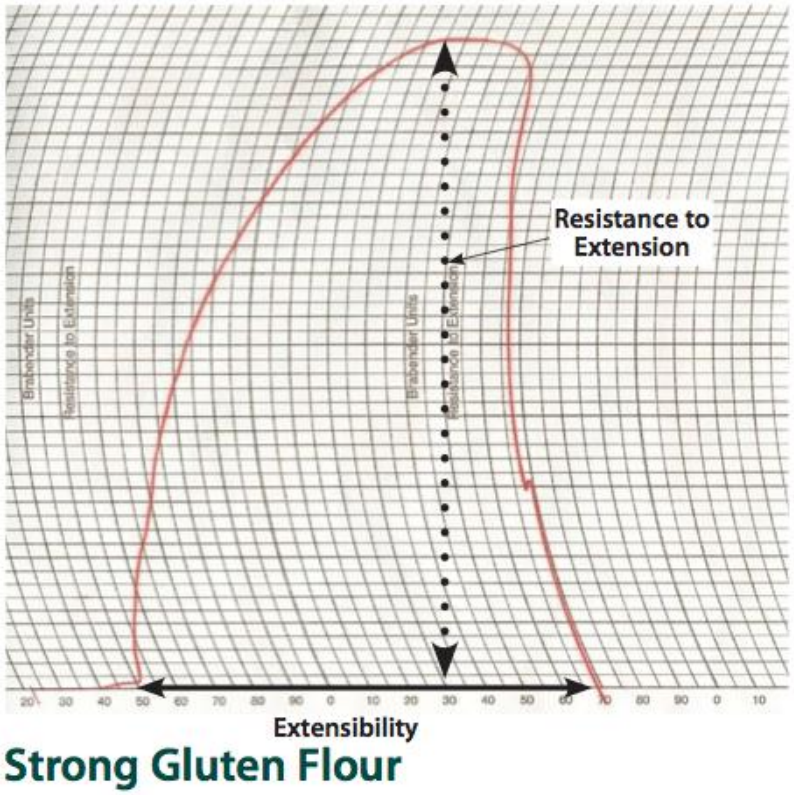


Figure 10 Extensigram for weak flour (above) and strong flour (below) (Wheat Marketing Center 2008)

1.6.5.4 Alveograph

Measurements and Outputs

This instrument measures the force required to blow air into a sheet of dough, forming a bubble that will eventually rupture. The amount of force required to create the bubble is denoted by the **P-value**, while the extensibility of the dough before rupture is the **L-value**. The area under the curve, **W-value**, is a combination of the two, expressed in Joules (Wheat Marketing Center 2008).

Significance to Baking

This instrument is designed to mimic the expansion of carbon dioxide gas caused by yeast or chemical leavening during oven baking. Its data gives a good indication of a flour's ability to withstand expansion, and is once again helpful in blending operations, which match baking performance parameters with end product considerations.

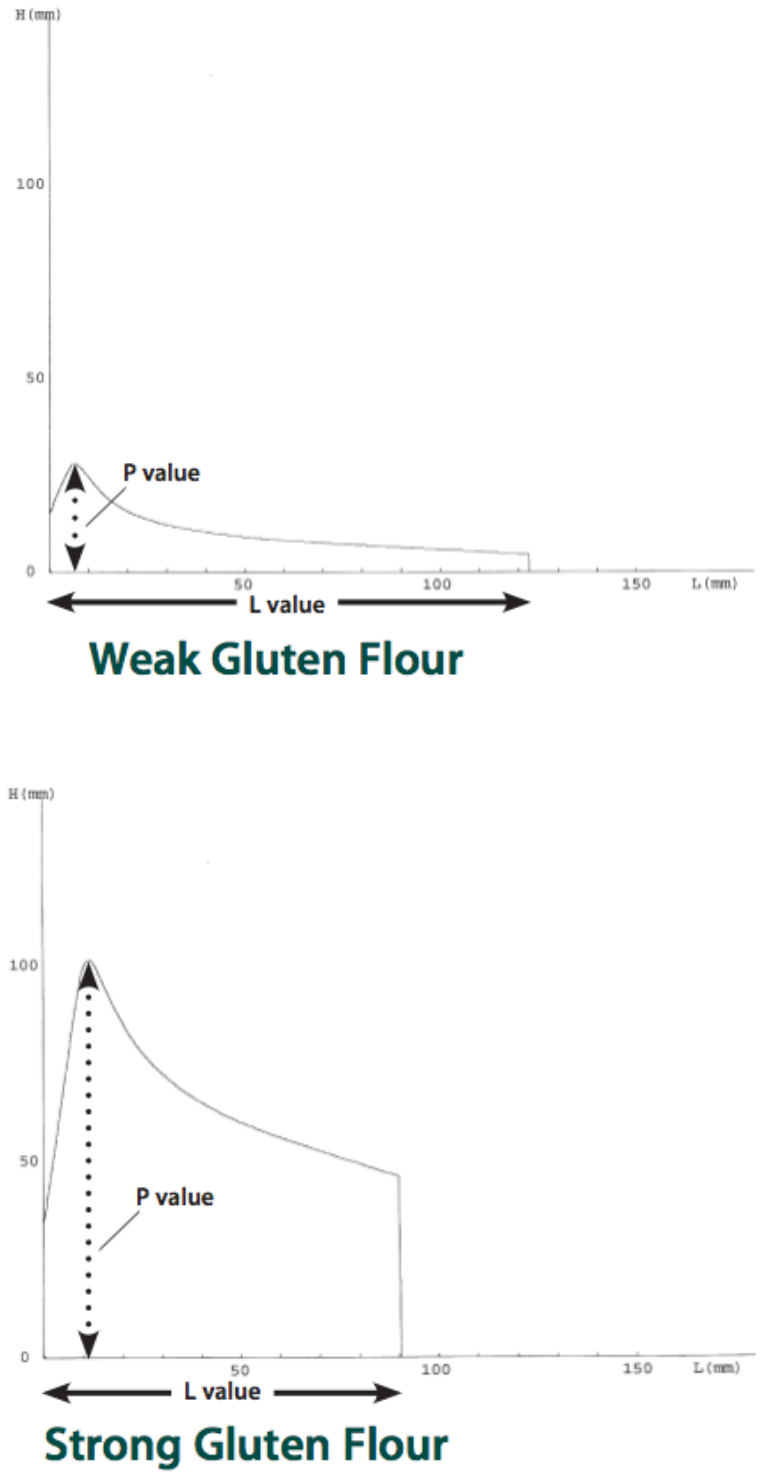


Figure 11 Alveogram for weak flour (above) and strong flour (below) (Wheat Marketing Center 2008)

1.6.6 Rheology and its Relation to Breadmaking Performance

Results from laboratory tests, spanning physical, biochemical, and rheological tests are usually verified against baking tests, to confirm their predictive abilities. Baking tests usually feature BLV, but may include bread shape, crust color, crumb structure, and crumb texture. The BLV of 4340 mL (based on an 800 g loaf) as determined by Janssen represents a common standard for a "good" quality dough. However, BLV may not always be an accurate representation, due to differences in dough formulation, such as the exclusion of common enzymes and additives. Studies that have linked both fundamental and empirical rheology to breadmaking performance recognize the need for multiple tests to holistically represent different baking aspects of a high quality bread (Kokelaar, van Vliet et al. 1996).

Objectives

Over the years, research in wheat quality testing has developed an array of methods that measure one or more aspects of wheat flour quality. However, many of these methods suffer disadvantages, such as being expensive, time-consuming, or incapable of generating meaningful and practical results that are relevant to wheat breeders, producers, millers, and bakers. Therefore, the overall objective of this study is to investigate the use of a novel compression recovery instrument, the CORE (Perten Instruments AB), and its potential to accurately evaluate rheological aspects of wheat dough quality in a rapid, simple and effective manner. The study includes the following four objectives:

1. To optimize the CORE for dough testing, instead of gluten.
2. To evaluate the CORE's ability to characterize flours of varying cultivars, based on measuring functional properties and examining their relation to documented quality tests.
3. To evaluate the instrument's ability to identify effects of adding the dough-enhancing enzyme, transglutaminase.
4. To evaluate the effect of blending strong and weak flours in the CORE.

CHAPTER TWO

OPTIMIZATION OF A COMPRESSION RECOVERY TEST FOR DOUGH

2.1 Part 1: Optimization of Test Parameters

2.1.1 Introduction

In an ongoing effort to identify and understand wheat flour quality parameters relevant to baking, Perten Instruments AB (Huddinge, Sweden) developed the CORE instrument, which evaluates gluten samples for their elastic properties. The instrument measures the ability of a gluten sample to recover freely after being subject to a biaxial compression force of 8 N for 5 seconds. Preliminary tests showed that values for degree of recovery (DR) were highly correlated with results of gluten strength from tensile tests, at a correlation coefficient of 0.855. These tensile tests, in turn, showed some strong positive correlations with mix time and gluten index.

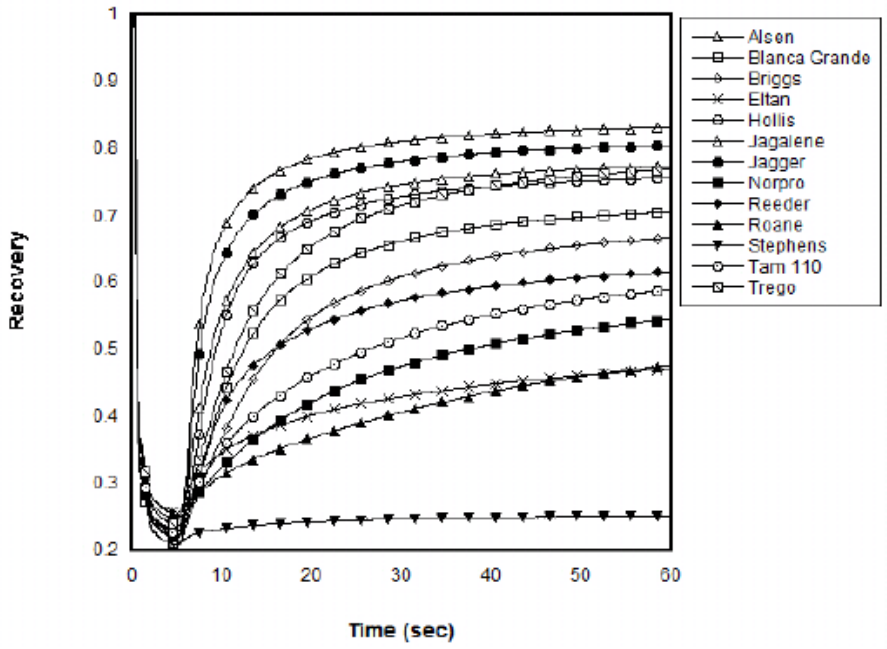


Figure 12 Recovery curves obtained from testing gluten from various wheat in the CORE (Chapman 2011)

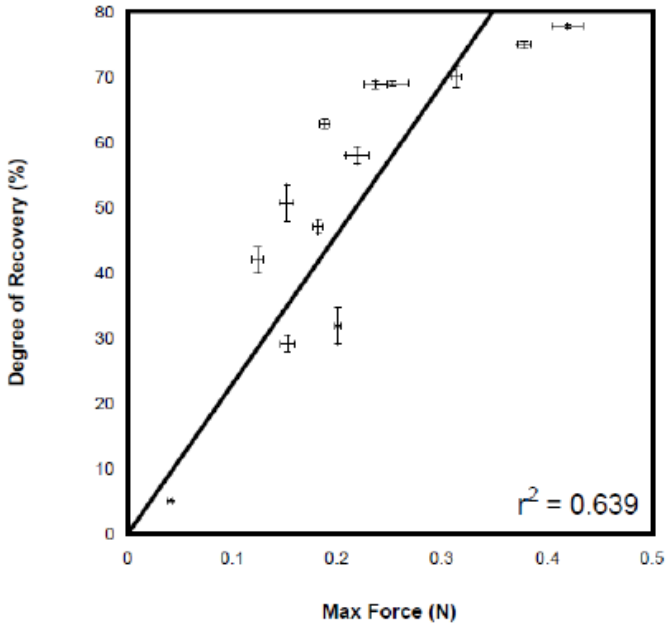


Figure 13 Correlation between CORE Degree of Recovery and F_{max} from a tensile test at 500% extension (Chapman 2011)

The employment of the CORE for the first time was a valuable step in understanding how the instrument may be further used for wheat quality evaluation. However, this first trial had two main limitations. The experiment failed to show a relationship between DR and documented cultivar information, such as physicochemical properties or rheology test results. Moreover, the test was limited to gluten, an indirect material to bakers who deal with dough. The idea of using dough, rather than gluten, as a testing material, was the subject of this study.

In order to accommodate dough testing, the existing method for measuring gluten strength in the CORE required adjustment. Preliminary experiments showed that the intense time and force combination of 8 N for 5 seconds compressed the dough beyond its critical strain. Therefore, the objectives of the following experiments were to carry out a rigorous optimization process for the CORE that would result in a method for obtaining the most meaningful rheological data from dough samples. This was done in two steps:

Part 1: Identifying the best test parameters for the instrument, consisting of a new time and force combination.

Part 2: Revaluating the existing techniques for sample preparation to ensure reliable, reproducible, and accurate measurements from samples.

2.1.2 Materials and Methods

2.1.2.1 Materials

Six wheat cultivars of certified seed were selected from a set of fifteen cultivars harvested in 2005. For identification purposes, this set of fifteen flours was named set A. The six cultivars were chosen to represent at least one of the five US wheat classes, including Hard Red Winter (HRW), Hard Red Spring (HRS), Soft Red Winter (SRW), Hard White (HDWH), and Soft White (SWH), in order to test the scope of the instrument across wheat classes. Fortunately,

cultivars in set A were highly characterized, in terms of protein content, Zeleny Sedimentation volume, pup loaf volume, and other relevant parameters, which would be useful for correlating with the response variable from the CORE. The six flours used for this experiment were Alsen, Tam110, Reeder, Trego, Eltan, and Roane. Some of the documented physicochemical properties of these samples and the remaining set may be founds in Table 3.

Table 3 Physicochemical properties of 15 US wheat cultivars representing the five wheat classes HRW, HRS, HDWH, SRW, SWH (Set A)

	HMW-GS Alleles				Glutenin Macropolymer			
Cultivar	1A	1B	1D	Protein Content (%)*	Yield (%)	Weight (g)	Protein Quantity (mg)	BLV (mL)
HRW								
Tam 110	2*	7+8	2+12	14.04±0.05	9.12	1.44	18.43	918.75 ± 9.38
Jagger	1	17+18	5+10	11.21±0.02	12.22	1.51	20.69	832.25 ± 5.98
Jagalene	1/2*	17+18	5+10	9.98±0.07	9.92	1.18	11.68	768.75 ± 9.38
HRS								
Alsen	2*	7+9	5+10	15.96±0.07	9.71	1.92	29.76	918.75 ± 12.88
Briggs	1/2*	7+9	5+10	13.52±0.06	12.06	1.63	26.57	825.00 ± 5.10
McNeal	1	17+18	5+10	14.28±0.05	11.34	1.93	31.27	956.25 ± 7.86
Reeder	2*	7+9	5+10	13.11±0.04	8.31	1.39	15.15	856.25 ± 5.98
Hollis	2*	17+18	5+10	13.01±0.04	12.22	1.60	25.44	881.25 ± 23.59
Norpro	2*	7+9	5+10	12.04±0.1	8.64	1.36	14.14	787.50 ± 6.25
HDWH								
Blanca Grande	1	17+18	5+10	13.15±0.08	11.63	1.98	30.29	912.50 ± 3.61
Trego	2*	14+15	5+10	10.34±0.04	19.05	1.32	26.00	743.75 ± 20.01
SRW								
Patterson	1	7	5+10	8.49±0.09	20.26	0.94	16.17	737.50 ± 14.88
Roane	null	7+8	2+12	7.69±0.02	16.64	1.14	14.59	687.50 ± 8.07
SWH								
Stephens	null	7+9	2+12	11.62±0.03	8.18	1.01	9.60	675.00 ± 7.22
Eltan	1	7	5+10	10.93±0.04	13.08	1.35	19.31	862.50 ± 11.97

* 14% Moisture basis

2.1.2.2 Sample Preparation

The flour of each cultivar was mixed with distilled water at room temperature in a 35-gram Mixograph (National Manufacturing Div., TMCO, Inc., Lincoln, NE) using approved method 5440A (AACC International., 2009). The flour and water components were weighed in grams, and were each calculated according to the following 'Tenmarq' equation for the Mixograph, assuming a wet basis moisture content of 14%:

$$\text{Flour Wt. (g)} = \text{NBS} * (100\text{-}14)/ (100\text{-Flour MC}_{wb})$$

$$\text{Water Wt. (g)} = \text{NBS} * (A/100) + (\text{NBS-Flour Wt.})$$

where NBS=Nominal Bowl Size, Flour MC_{wb}=documented moisture content, and A= flour absorbance rate. In this case, the absorbance rate was kept constant across cultivars, at 58%. The

nominal bowl size was 35, based on the size of the mixing bowl. The ratios of flour and water are shown in table 4.

Table 4 Mixing parameters for 6 wheat cultivars from Set A

Flour Cultivar	M.C (%)	Bake Absorption	Flour Weight	Water Weight	Midline Peak Time
Alsen	13.30	58.00	34.72	20.58	5.33
Tam110	12.85	58.00	34.54	20.76	2.78
Reeder	11.50	58.00	34.01	21.29	3.07
Trego	13.65	58.00	34.86	20.44	3.52
Eltan	12.70	58.00	34.48	20.82	2.46
Roane	13.10	58.00	34.64	20.66	1.59

The hydrated flour samples were placed in the mixograph and worked to their individual peak development time. Mix times were established experimentally in a Mixograph, whereby flour samples were over-mixed to 10 minutes, and their midline peak time, identified.

After optimal mixing was complete, the dough mass was rolled out, and divided into fifteen small spherically-shaped samples, weighing exactly 3.00 grams each. These newly portioned pieces of dough served as samples for testing in the CORE. Samples were coated with petroleum jelly and covered in a plastic film wrap to prevent loss of moisture and drying out. Samples were allowed to rest for 45 minutes to reach a state of equilibrium by way of complete stress relaxation on a plastic board. A visual scheme of the sample preparation method is shown in Figure 14.

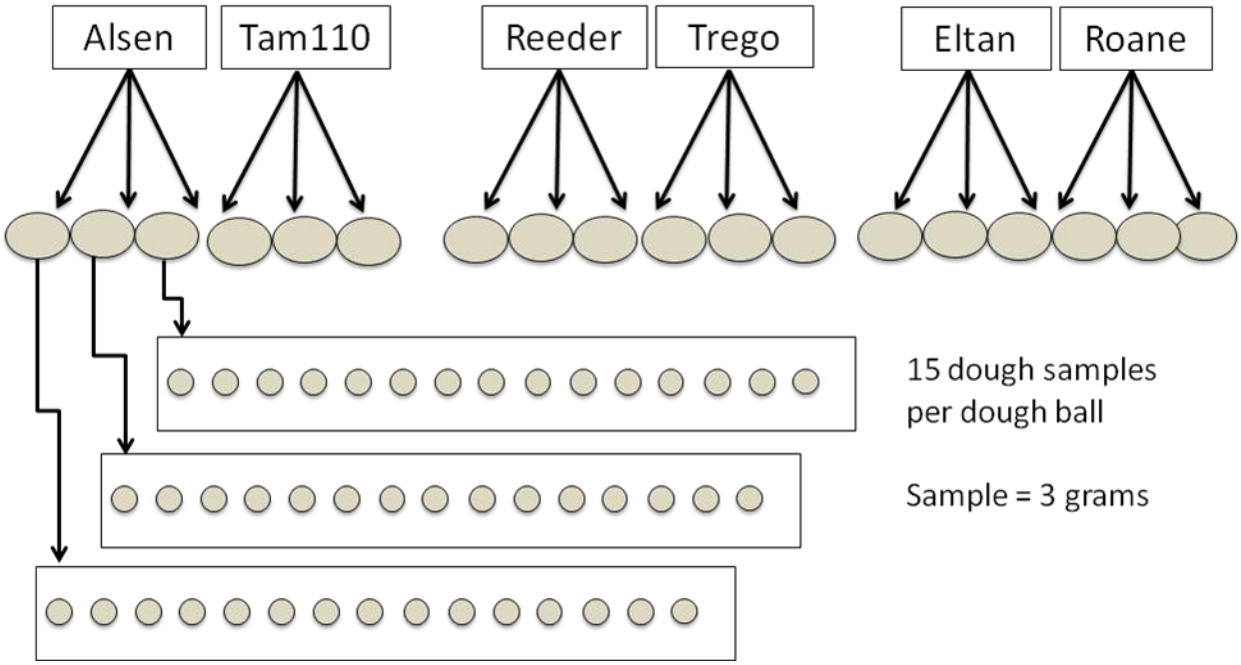


Figure 14 Visual scheme of sample preparation

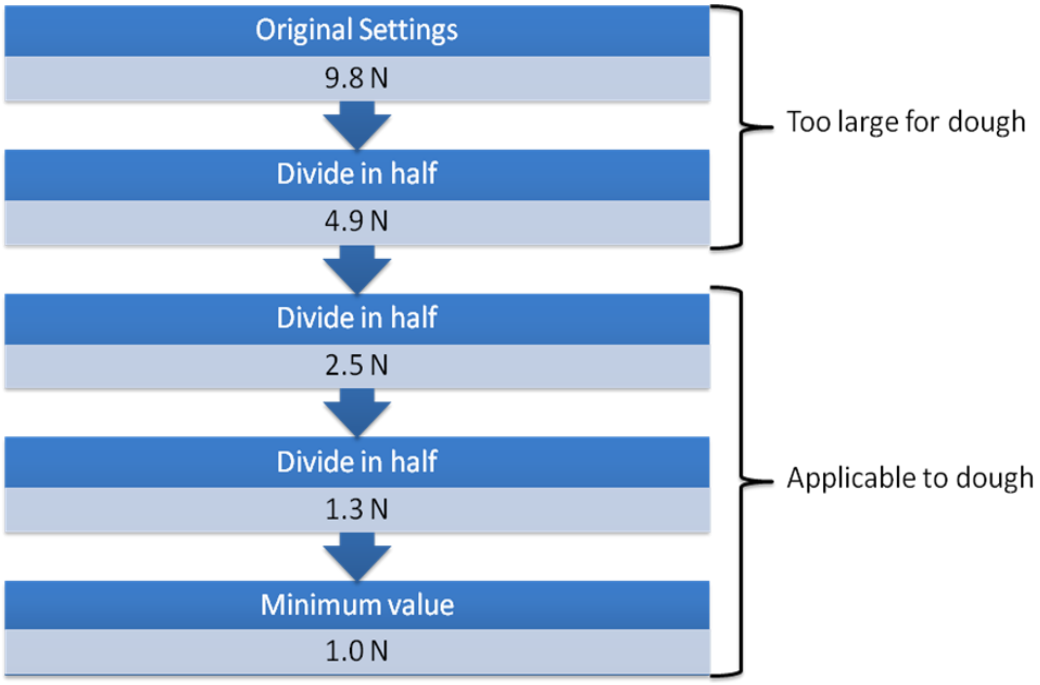
Prior to compression testing, a pair of samples were placed in a Gluten Index Centrifuge 2015 (Perten Instruments AB, Huddinge, Sweden), and centrifuged simultaneously for 5 minutes at 6000 RPM. This created two uniformly-shaped cylindrical samples, which were suitable for the CORE's compression chamber, while contributing to the consistency and reproducibility of data collected. All samples were prepared in a temperature-controlled room at 21° C.

2.1.2.3 Compression Recovery Test

Each dough sample was tested separately in the CORE (Perten Instruments AB, Huddinge, Sweden). The instrument was calibrated once daily, before use in experimentation. Samples were placed in the compression chamber, and tests were carried out at the designated levels, in randomized order. Each level represented a time and force combination, whereby the instrument compressed the dough at a specific force, measured in Newton's (N), and held this force for a specific time, measured in seconds (s). After compression, the force was released, allowing the sample to gradually and freely recover until the end time of 60 seconds.

In order to find the best parameter for dough testing, a wide range of time and force combinations were investigated. These were chosen based on the capability of the instrument to apply a force, as well as the dough's ability to recover from it. After a preliminary screening, a force of 2.5 N was found to be the maximum threshold that average quality dough could still recover from. This force was then approximately halved, at 1.3 N, for the second level. The third level represented the minimum force exerted by the instrument, 1 N.

As for the time, referring to the duration of compression, the highest value was chosen at the standard 30 seconds, based on the previously identified method in Chapman 2011. Proceeding levels were taken at ten-second intervals, resulting in the times of 30, 20, and 10 seconds, as well as two lower levels, 5 and 2 seconds. A 2-second test was the minimum time the instrument required to reach any given level of force. Figure 15 represents the rationale behind the selection of the parameters.



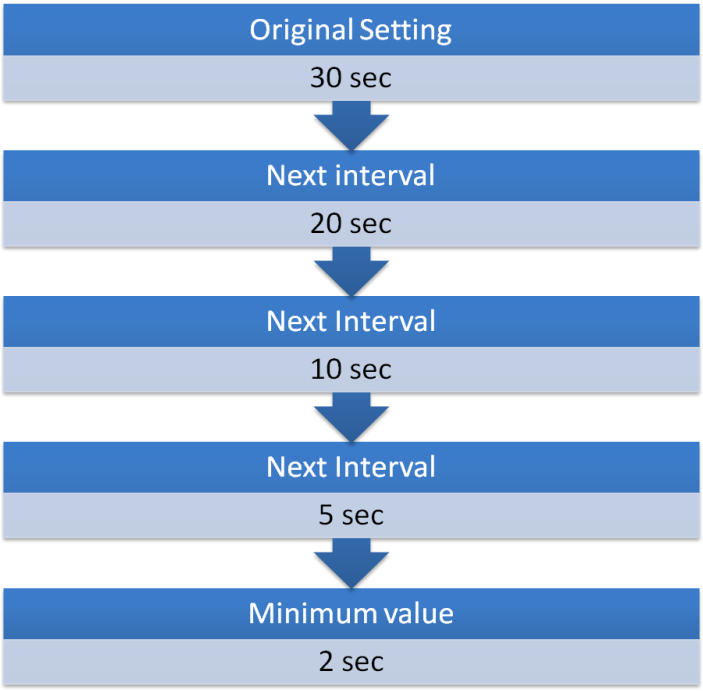


Figure 15 Selection process for force (above) and time (below) parameters for dough testing

Data was collected in an Excel file, whereby the height of the dough sample, measured in millimeters (mm), was tracked from the beginning of the test until the 60 second end time. Results were calculated as Degree of Elasticity (DE), using the following equation:

$$DE = H_f\text{-}H_m/H_i\text{-}H_m$$

where H_f= final height at 60 seconds, H_m= minimum height reached throughout compression test, and H_i= initial height of sample. Subtracting H_m resulted in a normalized value for the degree of elasticity across samples.

2.1.2.4 Statistical Analysis

Each of the six cultivars was tested in triplicate. The statistical software JMP® (SAS Institute Inc., USA) was used to analyze data. Analyses included descriptive statistics, analysis of variance (ANOVA), and simple bivariate Pearson correlations with select parameters.

2.1.3 Results and Discussion

Following rheological testing of samples, four criteria were established to determine which of the fifteen levels would provide the most accurate, reliable, and reproducible data from the CORE. Data from each level was analyzed in the context of how well it met the four conditions, listed below in Table 5. The numbering of each condition does not reflect its order of significance.

Table 5 Selection criteria for choosing an optimum test parameter in the CORE

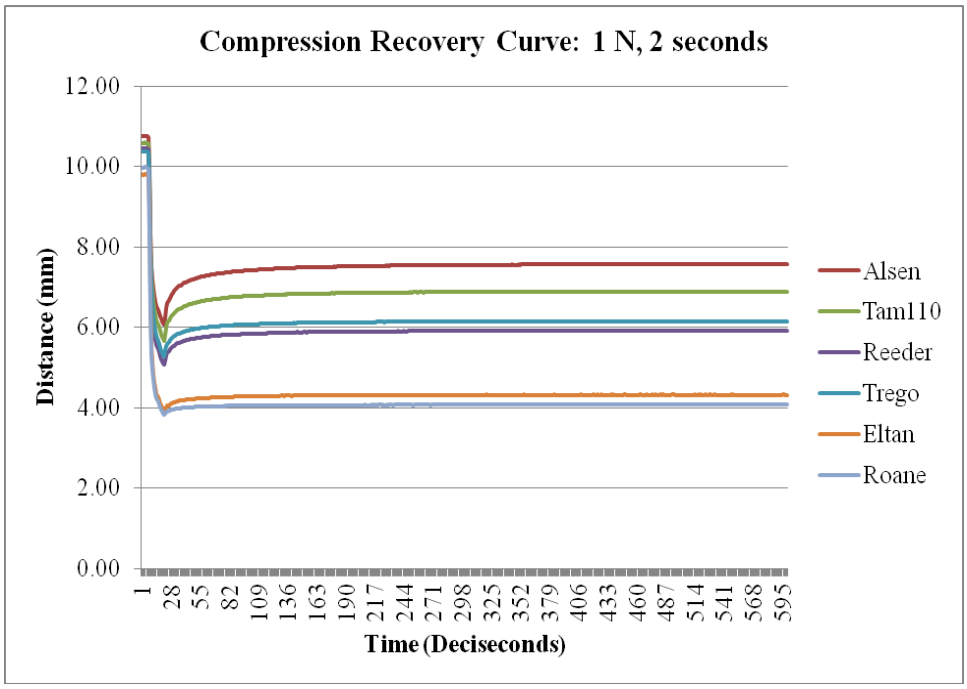
Criteria Number	**Selection Criteria**	**Reasoning behind Selection Criteria**
1	The optimal level must yield empirically evident groupings among cultivars of similar strength	To evaluate ability of instrument to discriminate between cultivars of varying quality
2	The optimal level must yield relatively high values for 'Degree of Elasticity'	To ensure that samples are not damaged, and data sets are analyzable
3	The optimal level must exhibit an intermediate amount of variation	To ensure that samples are exposed to a treatment that is neither harsh nor negligible
4	The optimal level must yield data that is statistically different from neighboring levels	To ensure that the data from one treatment is unique and meaningful.

Criteria Number 1:

Line graphs traced the output of each sample's compression and recovery path from 0 seconds to 60 seconds. Examples of these graphs are shown in Figure 16. Regardless of what is known about each cultivar, a good test will succeed in differentiating cultivars based on similarities in their rheological behavior. Based on all graphs produced from the 15 possible levels, compression at 1 N for 5 seconds was able to best group 'similar' cultivars based on their elastic recovery by displaying three pairs of perfectly-aligned curves for six cultivars. Each pair represented two cultivars that behaved similarly, in terms of their elastic recovery.

Based on the graphs, treatments at 2 seconds did not allow sufficient time to truly characterize the dough samples. This is apparent through the lack of a sharp minimum point, and lack of aligned curves. As for the more intense treatments for time, force, or both, samples were seemingly damaged, and therefore unable to exhibit similar behavior. Intense treatments resulted in the failure of the CORE to characterize similar cultivars. They were detrimental to their molecular structure, resulting in a permanent deformation into their viscous realm, and rendering elastic recoverability impossible.

Figure 16 CORE output showing recovery curves for five different test parameters


Compression Recovery Curve: 1 N, 2 seconds
Distance (mm)
12.00
10.00
8.00
6.00
4.00
2.00
0.00
1
28
55
82
109
136
163
190
217
244
271
298
325
352
379
406
433
460
487
514
541
568
595
Time (Deciseconds)
Alsen
Tam110
Reeder
Trego
Eltan
Roane

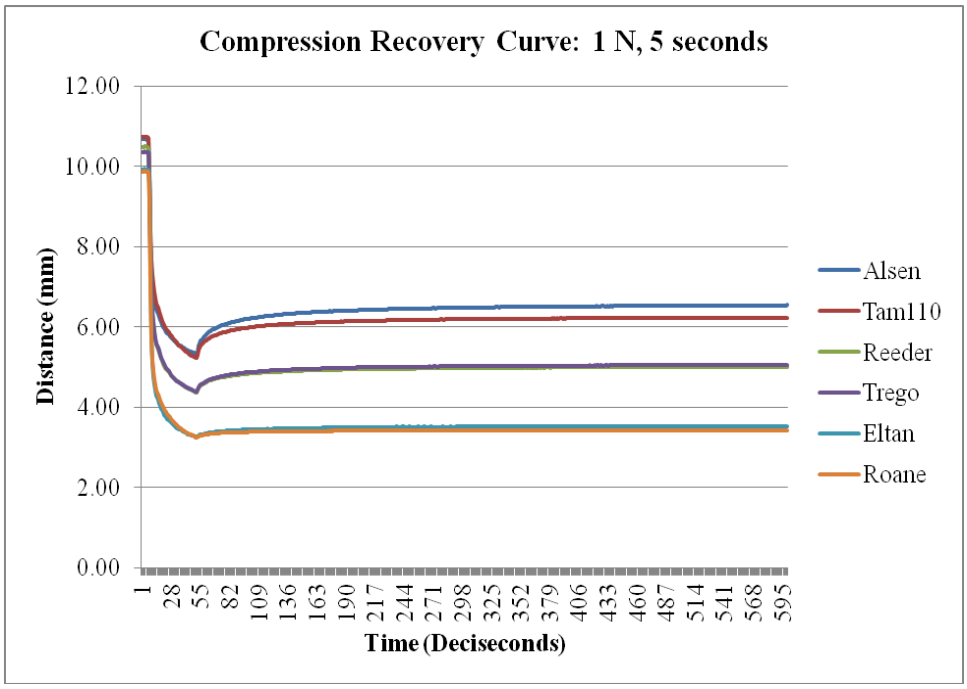
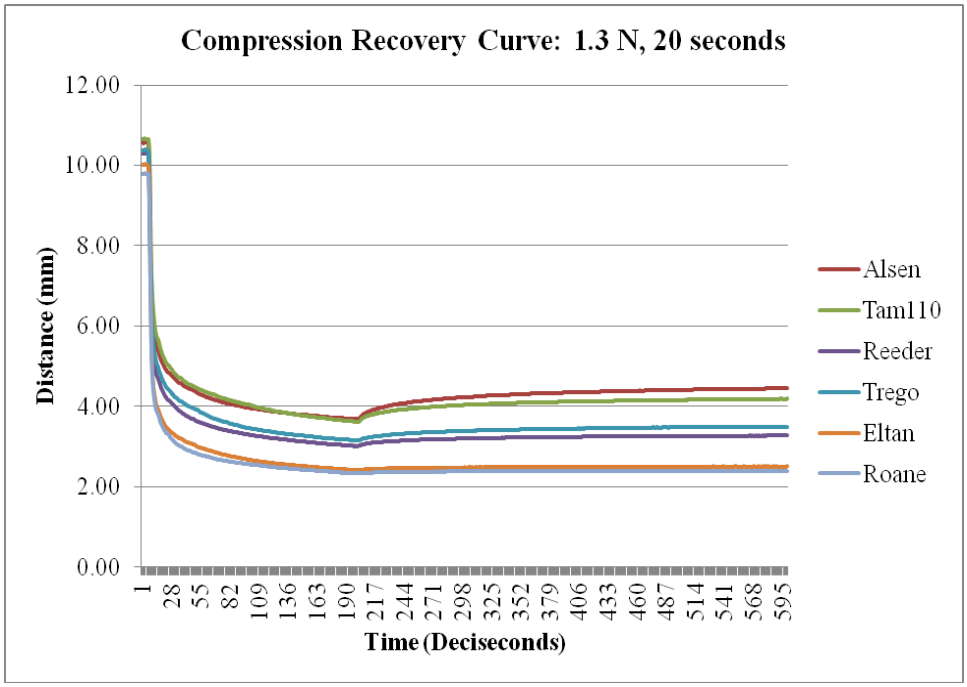

Compression Recovery Curve: 1 N, 5 seconds
Distance (mm)
12.00
10.00
8.00
6.00
4.00
2.00
0.00
1
28
55
82
109
136
163
190
217
244
271
298
325
352
379
406
433
460
487
514
541
568
595
Time (Deciseconds)
Alsen
Tam110
Reeder
Trego
Eltan
Roane

Figure 16 (continued)



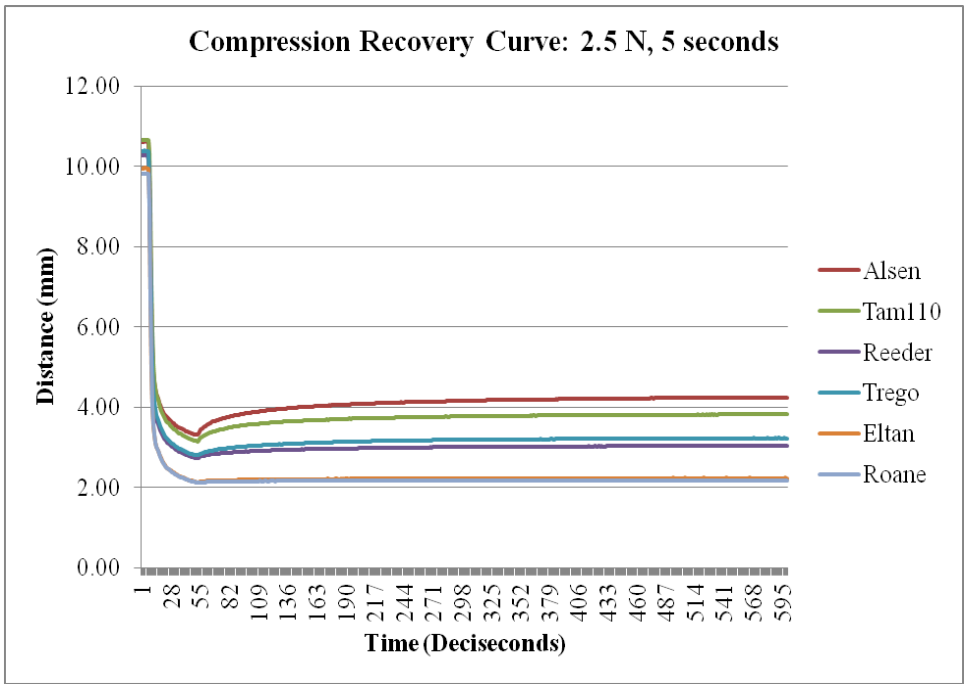
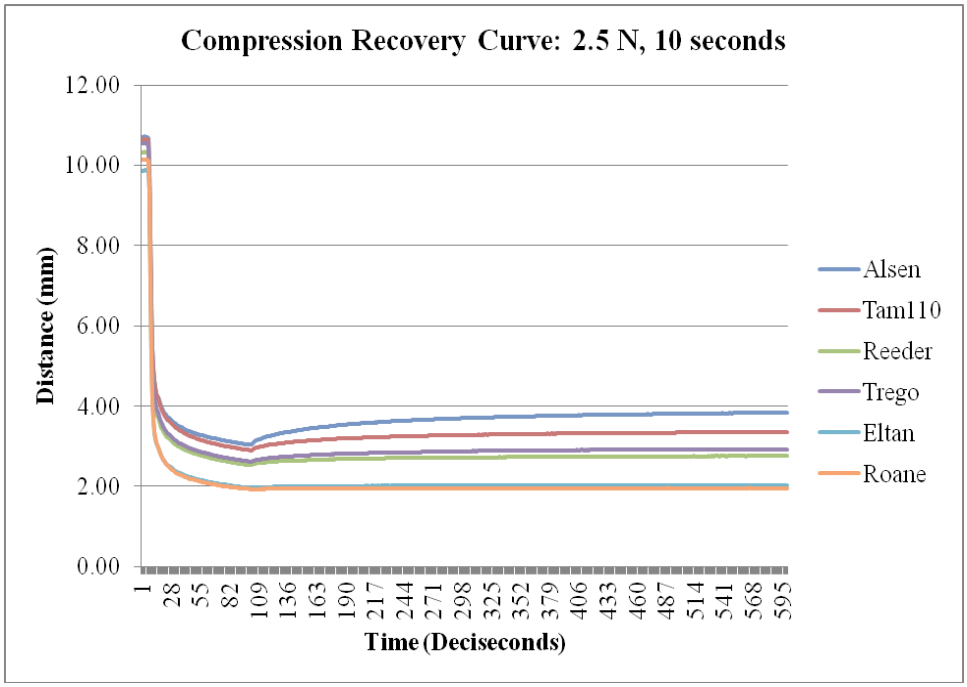


Figure 16 (continued)



Criteria Number 2:

Dough is a fragile material, one that is prone to being damaged at severe conditions. Given that the CORE was designed for gluten applications, its ability to apply a large deformation force may potentially compress dough into an irrecoverable viscous state. This form of damage would prevent samples from elastic recovery, and therefore hinder the instrument's attempt at characterizing cultivars based on their elastic recovery.

In this case, harsh treatments ultimately destroyed samples, resulting in consistently low degrees of elasticity. Cultivars of similar quality could not be spotted, nor could those of different quality, because the extreme treatment exceeded the threshold for recoverable elastic behavior of all samples. Therefore, screening for an optimum level required choosing one that is accommodating to the viscoelastic nature of dough. Consequently, levels that prevented relatively high magnitudes of elastic recovery, namely all 30 second and all 2.5N treatments, were eliminated; and levels that yielded high recoveries were reserved for further consideration.

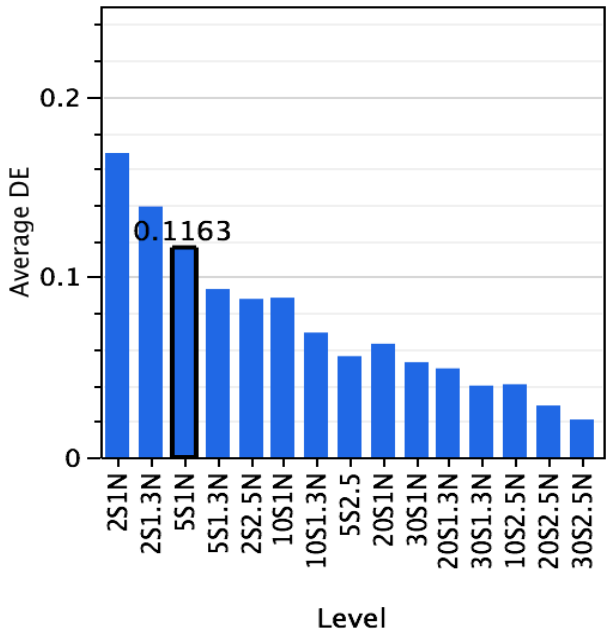


Figure 17 Bar graph showing average DE of six cultivars across all test levels

Criteria Number 3:

The third rule was created in response to the second. Although treatments yielding high values for DE were desirable, they were also prone to being highly variable. Such inconsistency indicated a lack of proper characterization of similar and dissimilar cultivars. For example, treatments of 1 N and 1.3 N at 2 seconds gave replicate DE's that were highly variable for the same cultivar. It became clear that 2 seconds of compression did not provide the dough with enough time to react to the new stress, resulting in overblown recoveries and misrepresented degrees of elasticity for all cultivars. Graphical representations of the variability across treatments is shown in Figure 18 below. The chosen level (5 seconds 1 N), exhibited mediocre variability.

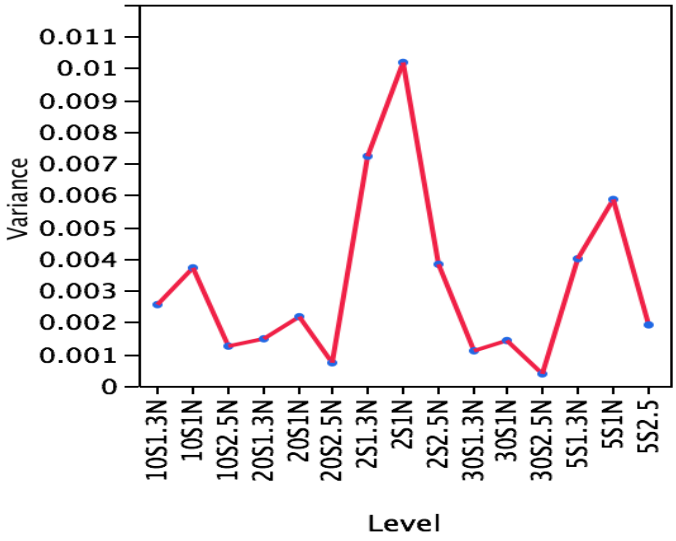


Figure 18 Graphical depiction of variance among replicates of six cultivars across all test levels

Criteria Number 4:

Two ANOVA tests were done to identify statistically significant responses across treatments. The first test was a mixed effects test, which accounted for the random effect of the cultivar, and focused on measuring the fixed effect of time, force, and the possible interaction effect of time with force, on the response, DE. Results showed that both time and force had a significant effect on DE, with time being the more influential of the two, given its larger F-value. The effect of the triplicate samples was also tested. Triplicates were not statistically different from one another. F-ratios for the fixed effects are shown in Table 6.

Table 6 Results of F-test for the significance of time, force, and their interaction effect on DE

Fixed Effect	Degrees of Freedom	F-Ratio	Prob>F		Significant?
Time	4	521.1457	<.0001	*	Yes
Force	2	479.1684	<.0001	*	Yes
Force*Time	8	1.9801	0.0494	*	Yes

The second ANOVA test treated each force-time combination as one level, and analyzed the difference amongst those treatments. The F-ratio of 17.76 showed that the levels had a significant effect on the response (DE). Using the method of Least Square Means, a Tukey HSD test was done to simultaneously compare the means of different levels, and group them into separate categories. The results assigned an alphabetical letter to each significantly different level, shown in Table 7. The 5 second 1 N grouping stood on its own, preceded by the unsuccessful 2-second treatments, and followed by a group of less-discriminatory parameters. Its unique effect on samples, and high LS Mean, made it a strong candidate for testing purposes.

Table 7 Results of Tukey HSD test showing alphabetical grouping of statistically different means

Level	Grouping	Least Squares Mean
2S1N	A	0.3905
2S1.3N	B	0.3537
5S1N	C	0.3208
5S1.3N	D	0.2856
10S1N	D	0.2784
2S2.5N	D	0.2761
10S1.3N	E	0.2431
20S1N	E F	0.2313
5S2.5	F G	0.2160
30S1N	F G	0.2127
20S1.3N	G H	0.2035
30S1.3N	H	0.1813
10S2.5N	H	0.1799
20S2.5N	I	0.1487
30S2.5N	I	0.1282

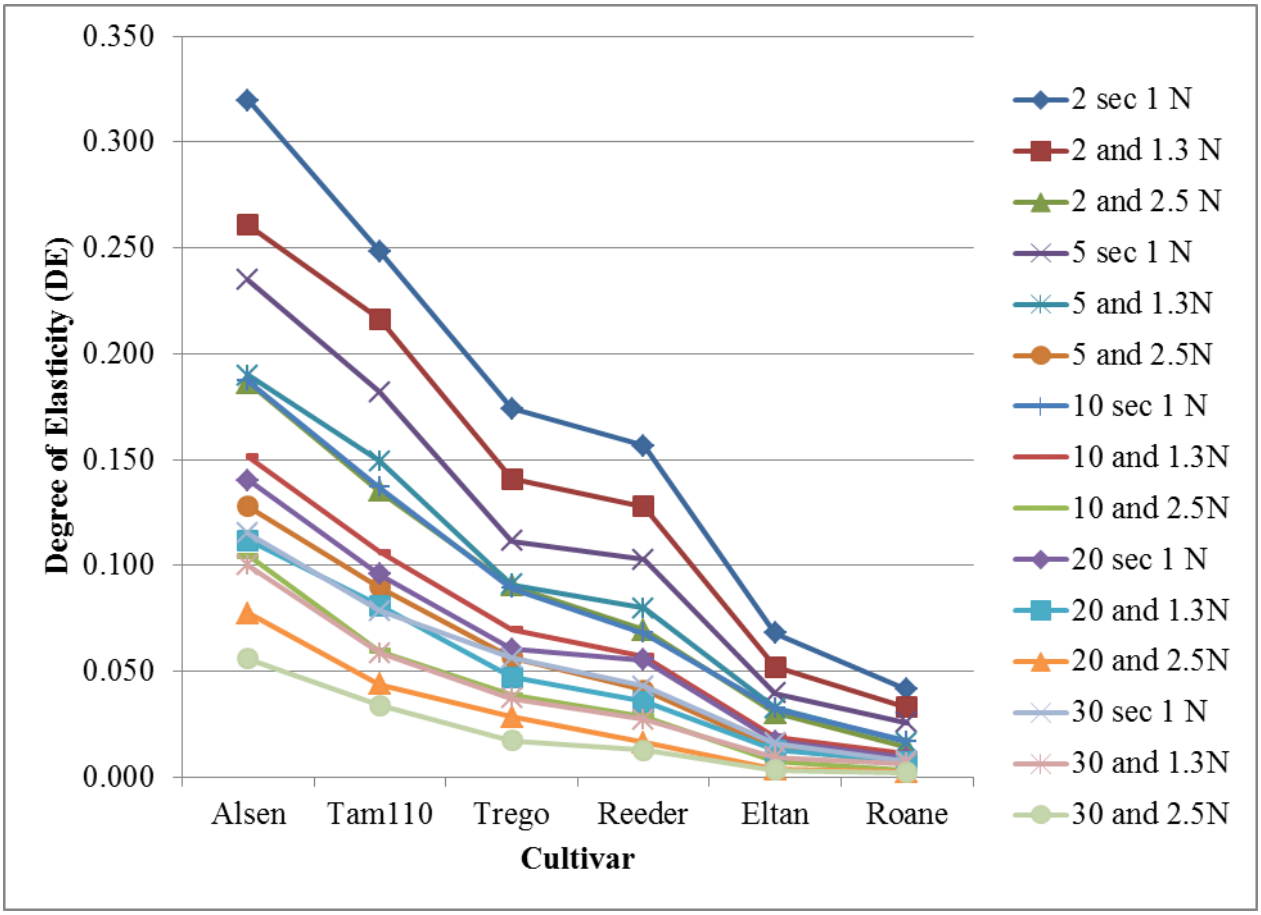


Figure 19 DE values for six cultivars, in order of decreasing flour strength, across all treatments levels

2.1.4 Conclusion

In an attempt to optimize the CORE instrument for use with dough as a test material, four criteria measures were used to separately evaluate fifteen potential treatment levels for their efficacy in characterizing a set of cultivars by their material properties. Hence, the level that was able to create evident groupings among similar flours, while exhibiting acceptable magnitude and variation in DE values, was the medium-intensity level at 1 N for 5 seconds. This level was deemed the ‘optimum’ level for dough application in the CORE.

2.2 Part 2: Revaluation of Existing Sample Preparation Method

2.2.1 Introduction

The first optimization experiment sought to find an optimum level in which to test dough in the CORE. However, it utilized a previously identified method to prepare samples and run the tests. This method was initially designed for gluten, not dough. Therefore, in order to complete the optimization of the CORE, a second series of tests were done to confirm the validity of dough sample preparation.

A set of experiments were done to uncover the effects of three variables on final DE values. These three variables were:

1) The absorbance rate
2) The customary 45-minute dough resting period
3) The reproducibility of centrifuged samples

2.2.2 Materials and Methods

2.2.2.1 Materials

The three experiments utilized two independent sets of flour. The first consisted of the same set of fifteen used in the aforementioned optimization process, i.e. set A. The second consisted of 21 HRW cultivars, including some experimental types. These 21 were milled using a Buhler Mill model MLU-202, as per approved method 26-21A (AACCI 2000), and were chosen as a good representative set for flours grown in the Midwest region of the US. They have also been partially characterized in terms of protein content and other basic physicochemical parameters, as shown in Table 8. For identification purposes, the second set was named Set B.

Table 8 Physicochemical properties for 21 HRW cultivars (Set B)

Cultivar	Protein Content (%)*
1-UT9743-42	11.17
GARLAND	11.81
IDO653	13.12
IDO651	13.01
UI DARWIN	11.95
WA7975	10.85
FINLEY	10.74
IDO621	12.5
BOUNDARY	11.62
UT9325-55	11.84
PROMONTORY	11.69
Millenium	12.34
NH03614	11.355
OK Bullet	12.79
OK00514-05806	11.955
OK03522	11.11
OK02405	12.4
SD01058	12.185
SD0111-9	12.72
SD01273	11.375
MT0495	11.175

* 14% Moisture basis

2.2.2.2 Sample Preparation

Although some factors in the sample preparation method were altered, other aspects remained constant. All flours were mixed as described in the previous section, using distilled water at calculated weights in a 35-gram Mixograph (National Manufacturing Co. Ltd). All samples were removed from the Mixograph, and as previously described, divided into smaller 3.00-gram samples, which were coated with petroleum jelly. Samples were made uniform in shape using the Centrifuge 2015 (Perten Instruments AB, Huddinge, Sweden)

1) Absorbance rate

Six individual cultivars from set A were randomly selected to test for differences in mixing properties. The control samples were mixed at 58% absorbance rate, while the test

samples were mixed at their optimum absorbance rates. The flour and water weights were calculated using the mixograph equation. Proportions for optimally mixed doughs are shown in Table 9.

Table 9 Mixing parameters for remaining six cultivars from Set A

Cultivar Name	M.C (%)	Absorbance (%)	Flour Weight (g)	Water Weight (g)
Blanca Grande	13.7	64	34.88	22.52
Briggs	13.6	64.5	34.84	22.74
Norpro	14.1	65	35.04	22.71
Jagger	12.05	60	34.22	21.78
Jagalene	13.75	63.5	34.90	22.33
Hollis	13.45	63.5	34.78	22.45

2) Dough Resting

Using all 21 flours of set B, control samples followed the established protocol of resting dough for 45 minutes prior to testing. On the other hand, test samples were not rested. Instead, they were immediately centrifuged after being divided into 3-gram samples, and placed into the CORE thereafter.

3) Reproducibility of centrifuged "replicates"

Using both sets A and B, dough samples were prepared in the same way as the first optimization experiment. No variables were altered.

2.2.2.3 Compression Recovery Testing

Samples for all three experiments were compressed in the CORE at the newfound optimal level for dough testing: 1 N and 5 seconds.

2.2.2.4 Statistical Analysis:

Samples of each experiment were tested in replicate. The statistical software JMP® (SAS Institute Inc., USA) was used to analyze differences in elasticity, expressed as DE, between control and test samples for each experiment.

2.2.3 Results and Discussion

1. Absorbance rate

An absorbance rate of 58% was chosen throughout all experimentations, for ease of testing and to gain control over an additional source of variability. It was hypothesized that this absorbance would yield higher recoveries, yet would still represent the functional properties of optimally mixed flours. In order to verify this, six cultivars were randomly selected and tested in the CORE. Their results showed that flours mixed at 58% (control) experienced overall higher degrees of elasticity than the optimum absorbance flours (test). Results of an ANOVA test showed that this difference was significant at an alpha level of 0.05. Likewise, a Student's LS Means t-test placed the two in separate categories, with a least squares mean of 0.157 for control samples, and 0.105 for test samples.

Despite the apparent and statistically significant difference between the absorbencies, DE values of the two levels still correlated strongly with one another, at r^2=0.895. This strong relation indicated that doughs mixed at 58% absorbance were still capable of representing the more realistic scenario of doughs mixed at their optimum. Therefore, the 58% absorbance was accepted as the standard absorbance for future experiments.

2. Resting Time

The second follow up experiment investigated the tradition of allowing a dough sample to rest for 45 minutes prior to rheological testing. Although this rest period is customary, its effect on elastic recovery was nevertheless investigated. An ANOVA was done to test the difference in DE between rested (control) and non-rested (test) dough samples. Although rested samples exhibited a greater overall mean of 0.198, as compared to 0.171 for non-rested samples, the

difference between the two treatments was not significant. Similarly, a student's LS Means t-test placed the two outcomes in the same letter category.

Regardless of different rest periods, the two sets were highly correlated at a Pearson correlation coefficient of r^2=0.885. This indicated a consistency among cultivars, regardless of permissible resting time. The implication of this relationship highlights the potential to carry out immediate and therefore more rapid testing with the CORE. Eliminating the relaxation stage in industry-scale testing can save a substantial amount of time, making the CORE a more appealing approach to wheat quality testing. Although immediate testing was feasible, subsequent experiments adhered to the 45-minute resting time.

Table 10 Least square means and results of F-test for the significance of absorbance rate and rest time

Fixed Effect	Control Mean DE	Test Mean DE	F-Ratio	Prob>F	Significantly Different?	Correlation
Absorbance Rate	0.157	0.105	13.94	0.0135 *	Yes	0.895
Rest Time	0.198	0.171	1.927	0.1691	No	0.885

3. Reproducibility of "Replicate" Samples

The final experiment confirmed the reproducibility of replicate samples. The question of their repeatability was due to the one-minute delay in CORE testing after they have been simultaneously centrifuged as a pair. It was hypothesized that no significant difference exists between replicate samples. An ANOVA test confirmed this, with very low F-ratios indicating no significant difference between control and test samples.

Table 11 Results of F-Test to determine reproducibility of replicates

Sample Set	1st Replicate	2nd Replicate	F-Ratio	Significantly Different
A	0.1345	0.1377	0.0101	No
B	0.1985	0.1985	0.0000	No

2.2.4 Conclusion

In conclusion, the mission to optimize the sample preparation and test methodology for rheological testing of dough in the CORE was successful. Sample replicates gave reproducible results, while a constant absorbance rate of 58% and resting time of 45 minutes yielded consistent data. When screened across an array of fifteen potential force and time combinations for the CORE, the level most capable of distinguishing dough samples of differing quality was the level applying a test force of 1 N for 5 seconds, before allowing the sample to recover. Established as the method for dough testing in the CORE, these parameters for sample preparation and CORE testing were adhered to throughout all succeeding experimentations.

CHAPTER THREE

EVALUATING THE ABILITY OF THE CORE TO CHARACTERIZE DIFFERENT WHEAT CULTIVARS

3.1 Introduction

Wheat nomenclature utilizes three main properties to categorize different cultivars. These are kernel hardness, seed color, and growth season. Cultivars in the same category are known to have varied milling, baking, and other rheological parameters. External factors such as environmental conditions and genetic composition, may affect wheat properties from year to year. In addition to this inevitable variation, process parameters such as sorting, milling, and storage, may further alter the end characteristics of flour, thereby affecting the functional properties that are relevant to bakers and industry members.

In order to test functional properties of wheat flour and obtain true predictive values of its bread-making quality, one must go beyond general categorization systems, and look at reliable, measurable, and reproducible data that has been proven to correlate positively with specified measures of "quality". Although many instruments have been designed for these purposes, the CORE introduces a new form of testing, using biaxial compression, followed by free recovery. It is an empirical method that is advantageous in its simplicity of use and time-effective means of obtaining results. Compression testing is not typical on dough systems, because it does not imitate any single unit operation in baking, as most empirical tests do. However, its promising results with gluten in Chapman 2011, as well as its novelty, reveal its potential to provide meaningful data regarding bread-making quality.

The following experiment investigated the CORE's ability to characterize the behavior of a variety of wheat cultivars from a functional standpoint, using dough as a practical testing

material. The primary functional component was elastic recovery, in which the 'elastic' property of the dough is hypothesized to contribute to the holistic definition of 'dough strength'. A secondary functional component was identified as 'firmness,' which was investigated by observing the sample's resistance to compression (RC), defined as the extent to which dough resists the compression force exerted upon it. The success of the instrument will depend on its ability to distinguish properties of both elasticity and firmness, for cultivars belonging to different classes, as well as those belonging to the same class.

In order to evaluate the CORE's potential to characterize sample strength, output values of both DE and RC, were analyzed in two ways:

1) Graphical representations of data (trends and distributions)

2) Correlations with existing cultivar data spanning:

a. Results from documented laboratory tests (protein content, wet gluten, dry gluten, gluten index, and Zeleny Sedimentation)

b. Results from rheology tests, including Farinograph, Mixograph, Alveograph, and Extensigraph

3.2 Materials and Methods

3.2.1 Materials

Three sets of flour were used in this experiment. Set A consisted of the same well-characterized set used in chapter one, which spanned five classes of flour. Set B included the group of 21 HRW samples, followed by Set C, another group of 22 HRW flours, which were highly characterized in the 61st Report on Wheat Quality (Hard Winter Wheat Technical Board of the Wheat Quality Council, 2010). Set A was chosen to demonstrate differences in strength

across flours of different categories. The second two, on the other hand, were used to highlight any in-class variations existing within the HRW class.

3.2.2 Sample Preparation

The samples were prepared in the same manner as described in chapter one. Flours were mixed to their peak development times in a 35-gram Mixograph (National Manufacturing Div., TMCO, Inc., Lincoln, NE) using approved method 5440A (AACC International., 2009) at a constant absorbance of 58%. Each dough mass was then divided into equal 3.00 gram samples and allowed to rest for 45 minutes before being centrifuged for uniformity of shape.

3.2.3 Compression Recovery Test

After resting, all samples underwent a compression recovery test using the CORE (Perten Instruments AB, Huddinge, Sweden). Samples were compressed at 1 N for 5 seconds, and then allowed to gradually recover for the remaining 55 seconds. Data was tabulated and DE values were calculated for each cultivar using the same formula described in Chapter One. In addition, resistance to compression (RC) values were calculated, using the following formula:

$$RC = H_i\text{-}H_{min}/H_i$$

where H_i = the initial height of the centrifuged sample before compression, and H_{min} = the minimum height reached after compression. The RC value represents a percentage that can be compared across samples, regardless of their initial height.

3.2.4 Statistical Analysis

Samples were tested in duplicate. The statistical software JMP® (SAS Institute Inc., USA) was used to analyze results. Analyses included descriptive statistics for DE and RC values, bivariate Pearson correlations with select parameters, and multivariate correlations across rheological data. Multivariate correlations were estimated using the default Residual Maximum

Likelihood (REML) approach of the software, and to account for the random effects of the cultivar. A principal component analysis (PCA) was carried out on the multivariate correlations to reduce the dimensionality of the data, and find groupings of correlated variables among the various rheological instruments.

3.3 Results and Discussion

3.3.1 Trends and Distribution Analysis

In the first experiment, flour set A was tested in the CORE. The hard flours recovered better than the soft flours, and therefore exhibited overall higher degrees of elasticity. However, considerable differences in elasticity were observed within the broad class of hard wheat, as well as within specific classes, such as Hard White, Hard Red Spring, and Hard Red Winter. For example, the DE of Blanca Grande was more than double that of Trego, despite both of them belonging to the Hard White class of flour. Likewise, Alsen and Reeder, two Hard Red Spring cultivars, also differed greatly in elasticity, with Alsen reaching a DE of 0.24, and Reeder a mere 0.10. These varying responses prove that material testing may provide a more accurate representation of a cultivar's performance than its commonplace classification by kernel strength, color, and season.

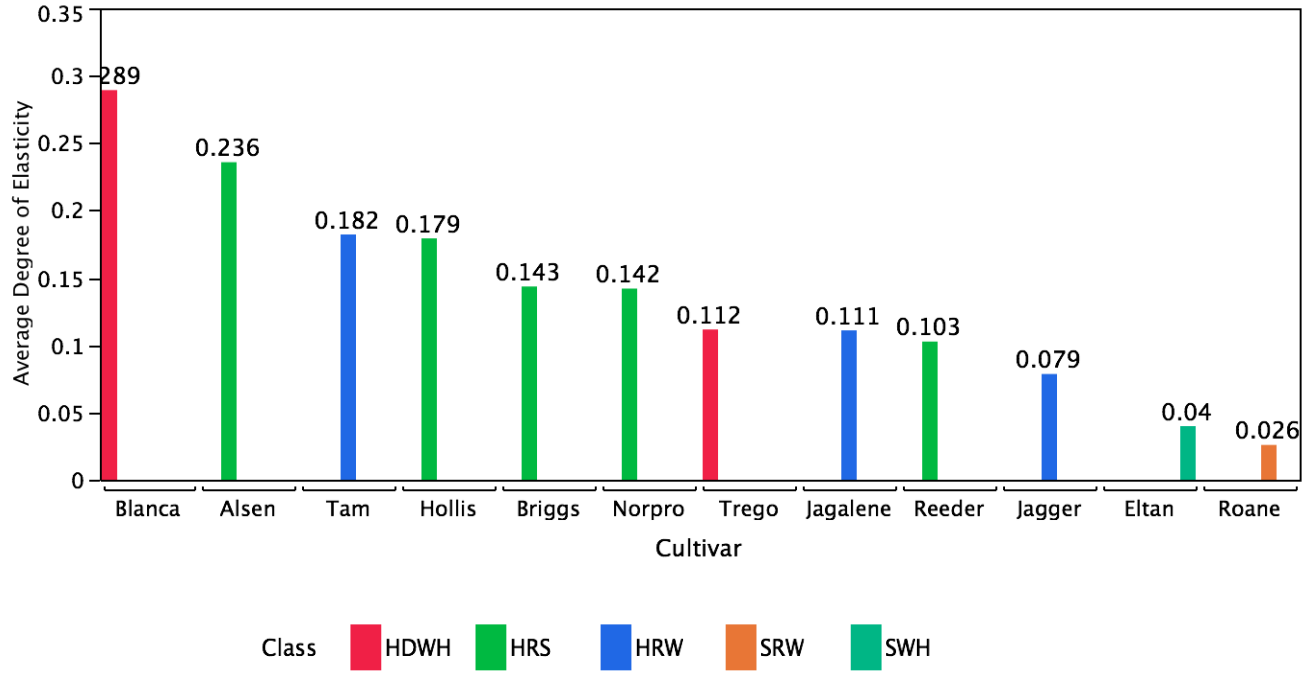


Figure 20 DE values for twelve cultivars across varying wheat classes

In a subsequent experiment, further investigation of elastic properties of flours within the same class was carried out with two sets of HRW flour, Set B and C. These two sets of flour were treated and tested in the CORE under the same conditions. The range of responses can be seen in Figure 21, and confirm a sample's ability to perform differently from its class members. The two sets of HRW samples, B and C, experienced similar range breadth in elastic recovery. Both had a strong outlier with a high DE, followed by a gradual decrease in flour strength, and ending with weaker cultivars. In Set B of 21 HRW cultivars, dough samples experienced generally higher elastic recoveries than the second Set C of 22 HRW cultivars. The strongest flour of B recovered at a DE of 0.45, whereas the strongest flour of C recovered at a mere 0.22.

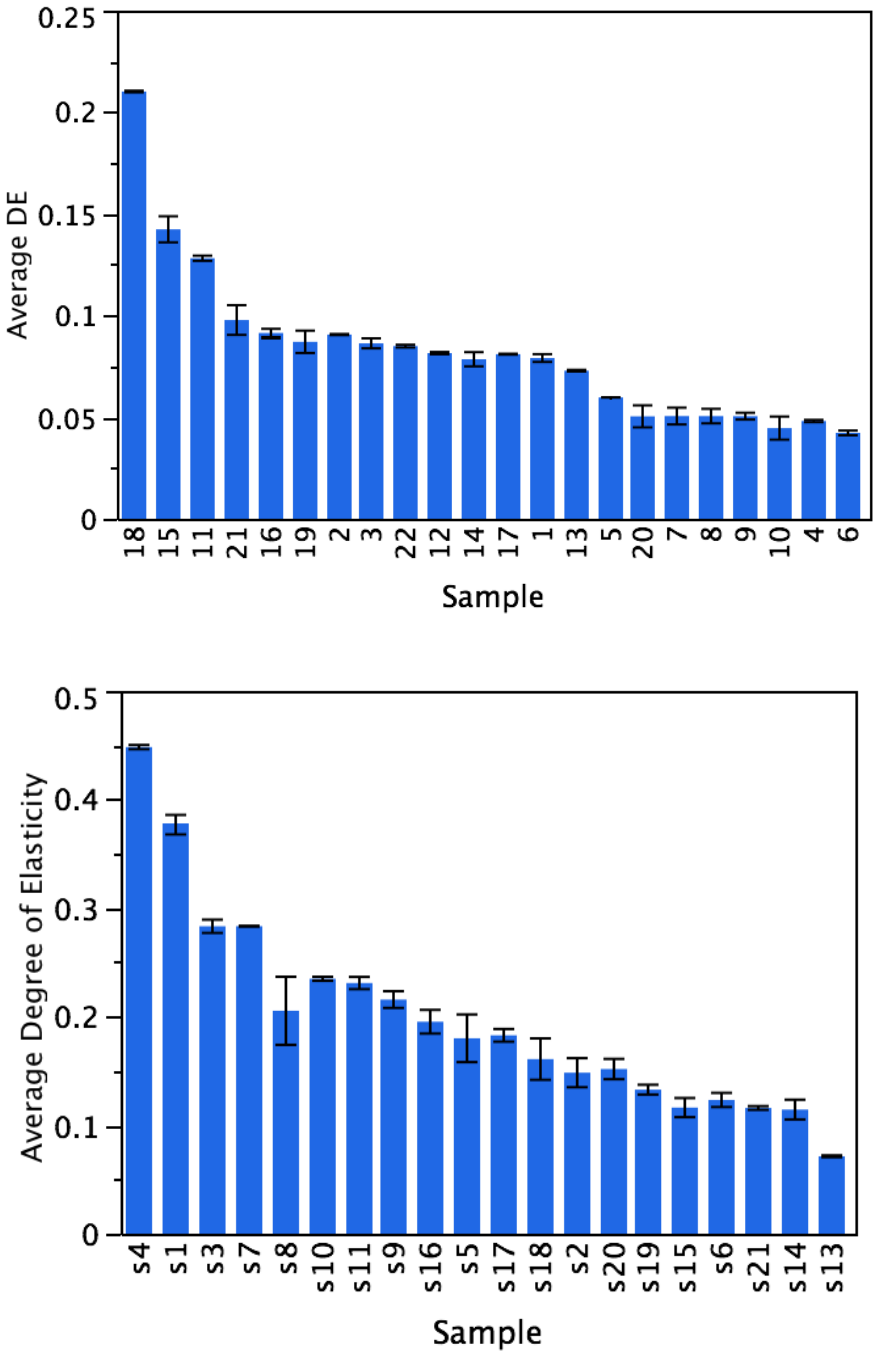


Figure 21 Average DE values across HRW class set B (above) and C (below)

The general relationship between dough and the CORE was examined across all three sets combined (A, B, and C). Values for DE were not normally distributed; rather, they were skewed towards lower values. The minimum value was 0.02, and ranged up to 0.44. The mean of the three sets was 0.14, with a standard deviation of 0.08. The mode of DE responses was 0.08, with the majority of responses between 0.05 and 0.10, as seen in Figure 22. The number of outliers with superior elasticity was limited, implying that the CORE succeeded at distinguishing exceptionally elastic samples, but was less discriminatory with 'mediocre' samples, which were lumped together on the lower end of responses.

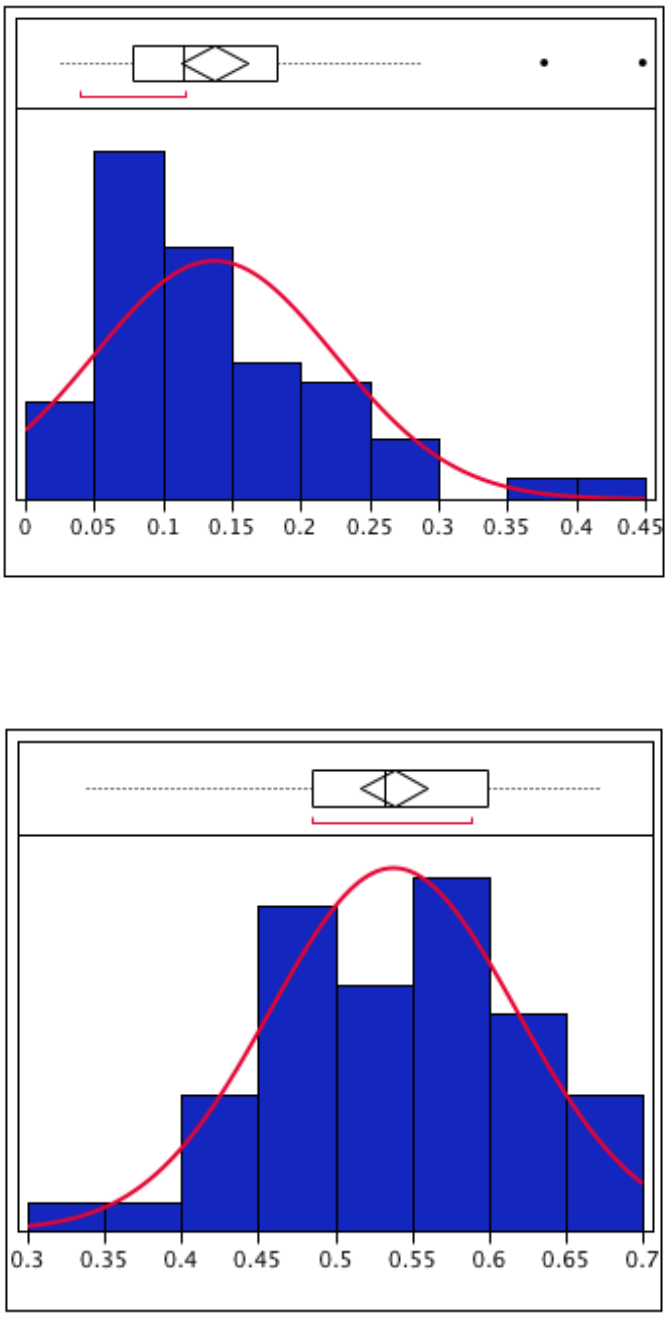


Figure 22 DE values for all sets showing skewed distribution (above) and RC values for all sets showing more normal distribution (below)

Another measurement that may be extracted from the CORE is the resistance to compression (RC). This secondary output seems to represent dough firmness, since it measures the extent to which the dough resists the biaxial force of compression, just like it may resist the biaxial force of tensile extension, commonly referred to as tenacity. For bakers, this is perceived as resistance to extend dough. RC values were more normally distributed than those of DE, indicating that the instrument is more sensitive to this measurement. It had no outliers and was able to capture and express the firmness of all samples. Ranging from 0.33 to 0.67, its mean was 0.54, with a standard deviation of 0.08. As expected, RC and DE were highly negatively correlated with one another, at $r^2 = -0.85$, correctly implying that more elastic doughs are also more difficult to compress. This relationship can also be seen in Figure 23.

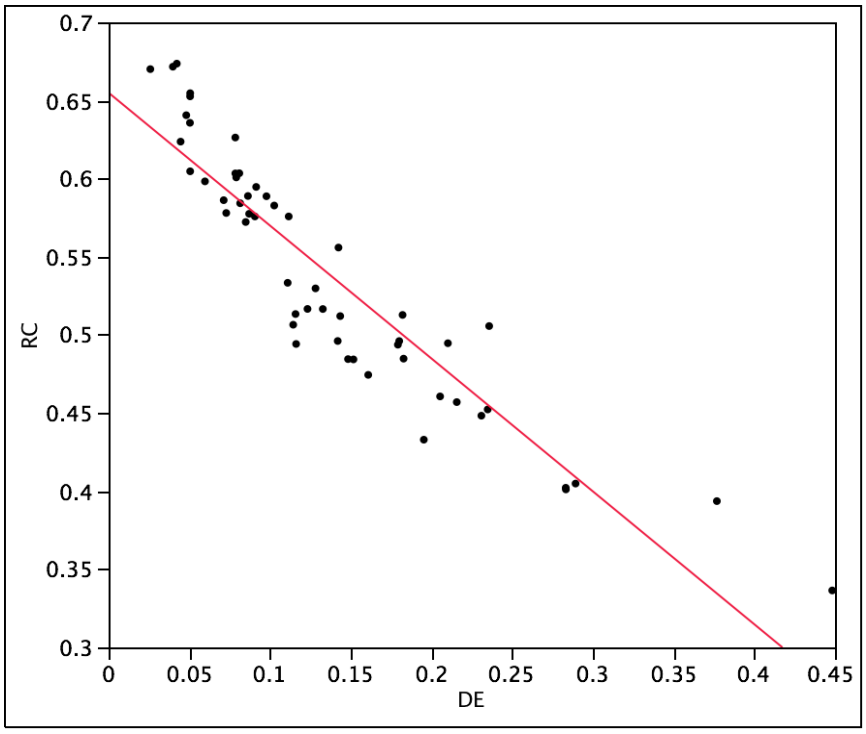


Figure 23 Scatterplot showing strong negative correlation between DE and RC

3.3.2 Relationship to Documented Laboratory Tests

The amount of protein in the homogenous HRW sets of B and C were much narrower in scope of response when compared to the CORE's DE values. The two sets had an identical mean protein content of 11.9%, with the set C showing a maximum protein value of 13.9%, compared to 13.1%, the maximum of set B. This indicates that protein content does not correctly depict rheological properties of dough samples.

To expand on this notion, two comparatively strong HRW flours, Sample 4 (IDO651) of set B, and sample 21 (TX05A001822) of set C, were both composed of 13.0% protein, however their DE values were 0.45 and 0.09, respectively. Likewise, Figure 24 depicts an entire range of elasticity, from 0.02 to 0.4, for cultivars that all have 11% protein.

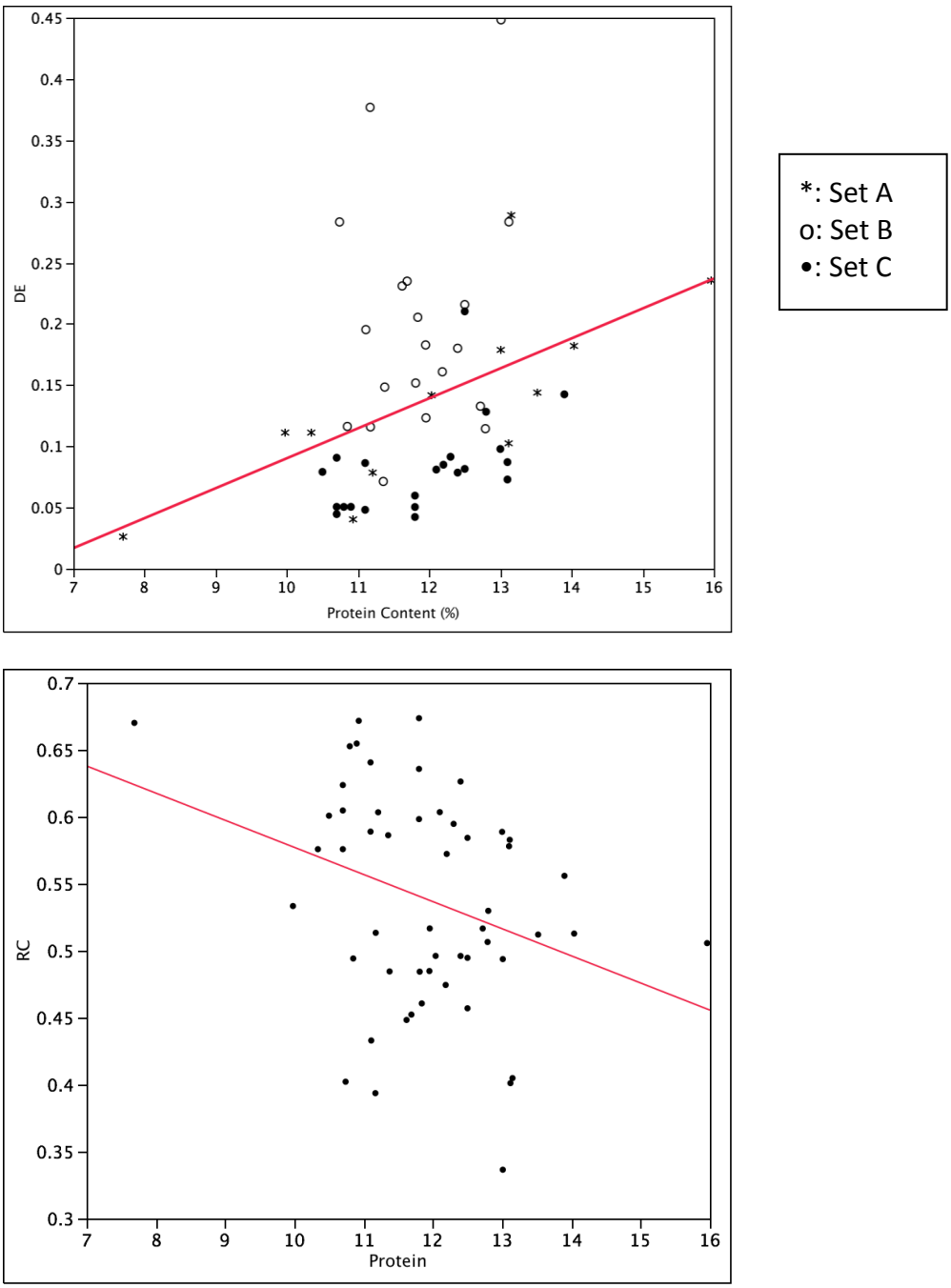


Figure 24Correlation of DE (above) and RC (below) with protein content across all tested cultivars

Therefore, it can be said that two flours may exhibit vastly distinctive elastic strengths, even while having the same protein content. A parallel relation exists between RC and protein content. A scatter plot showed that there was no relation between the two variables, given an r^2 of 0.1.

The correlation between protein content and dough strength has been repeatedly addressed in studies on wheat quality. With strength as an indication of quality, research has shown that protein content alone cannot predict flour quality. Rather, one must look deeper into protein composition and other physical properties to answer questions of a cultivar's performance. Measures such as Zeleny Sedimentation and GMP quantity have shown to more accurately portray dough strength (Chapman 2011).

To investigate the relationship between a cultivar's DE and corresponding chemical analyses (other than protein) independently from one another, bivariate Pearson correlations were carried out on flour sets A and C with a number of select parameters. These two sets were chosen based on availability of their supporting data. Statistical analyses were carried out separately for each set because they represented complex experiments that were done in separate laboratories.

Set A exhibited a promising relationship between DE and documented values of three measurements. These included wet gluten content, dry gluten content, and Zeleny sedimentation values, which expressed significantly positive correlation strengths of 0.56, 0.60, and 0.60 respectively. DE values for Set C showed a much weaker relationship with those same variables, none of which were significantly correlated with wet or dry gluten content, gluten index, and Zeleny sedimentation volume. Data for set B was not available for analysis. A table of correlations may be found in Table 12.

Table 12 Pearson coefficients for DE with select quality tests

Quality Tests	Set A	Set C
Wet Gluten (%)	0.56 *	0.06
Dry Gluten (%)	0.60 *	0.09
Gluten Index	0.01	0.02
Zeleny Sedimentation (ml)	0.60 *	0.24
GMP Quantity	0.43	N/A
Mixograph Mix Time	0.31	N/A
Glu/Gli Ratio	N/A	0.008
%IPP	N/A	0.01

* Correlation value is significant at p=0.05 (two-tailed)
N/A: Data not available

This dissimilarity in correlation strengths between the two sets of flour may have occurred because Set A was composed of five different wheat classes, including both soft and hard, allowing it to exhibit it a wider assortment of behaviors. Soft wheat cultivars such as Roane and Eltan exhibit greater extremities in both physical properties and functionality. Two skewed outcomes, such as low gluten content and low DE, tend to pair together, hence strengthening the correlation. On the other hand, Set C was composed solely of HRW wheat samples, which may vary in functionality but will only deviate slightly from expected physical and chemical properties. Moreover, samples that were tested with the CORE were mixed at a constant absorbance rate of 58%, while all tests of chemical properties were carried out on dough mixed to optimum peak times. Such incongruence in methodologies may disrupt an otherwise positive correlation.

3.3.3 Relationship to Documented Rheological Data

Preliminary correlations with basic properties, in this case protein content, showed no direct link to elastic strength. This was followed by a more extensive look into other tests like Zeleny Sedimentation and GMP quantity, which showed some association to CORE elasticity. However, the most relevant comparison was hypothesized to occur among rheology tests. If the ability of the CORE to test dough quality is to be examined, it must be done in the context of existing rheological test methods.

Much like the CORE, instruments such as Farinographs, Alveographs, Mixographs, and Extensigraphs also show a large scope of responses within one class of wheat flour. For example, Farinograph stability time for the all-HRW Set C yielded values as low as 10 and as high as 32 minutes. Similarly, observed values for "W", the alveograph measure of dough strength, ranged from 145 to 457 with a standard deviation of 71.58 for the same set of HRW flours. Similar breadth in responses would appear with other parameters from farinograph, extensigraph, or mixograph variables. Therefore, the CORE is not unusual in its wide array of responses, from a DE of 0.04 to 0.21, within Set C alone.

In order to investigate the relationship between DE, RC, and existing rheological data, a multivariate Principal Component Analysis (PCA) was carried out for flour sets A and C combined. A loading plot showed that DE associated most strongly with Farinograph WA and Development Time, as well as Alveograph W (strength) and P (firmness) values, as these variables were all within close proximity to one another on the plot. Furthermore, W was located in between P and L (extensibility), which is an accurate depiction, since W is defined by P and L. The two forms of WA, at 500 BU and at 14%, were also closely positioned.

Visual illustration of the data in the form of a loading plot was very helpful in grouping related variables together (Figure 25). Additionally, a multivariate correlation matrix indicated that correlations of DE with W and P were significantly high, at 0.82 and 0.81 respectively. RC resembled a similar strength. These two correlations revealed a consistency among data from the CORE and the Alveograph. As for W, this value provides an overarching idea of where dough stands in terms of both firmness and extensibility, hence representing overall dough strength, as cited in literature. While the alveograph does not provide a direct measure of elasticity, the CORE seems to do so, thereby playing a supplementary role to existing data.

The PCA was successfully able to reduce dimensionality of the data, with two principal components explaining more than 75% of the variance. It was difficult to characterize the key attributes of each principle component for a system as complex as dough. However, points of the loading plot still gave a good indication of the nature of relationships among variables. Moreover, considering samples were tested in different laboratories and using different absorbance rates, positive correlations of DE, Farinograph, and Alveograph variables indicated potential for an even stronger relationship. Extensigraph data did not blend in as expected. When an attempt was made to examine the relationship of 'Extensibility' with L, the two were poorly correlated.

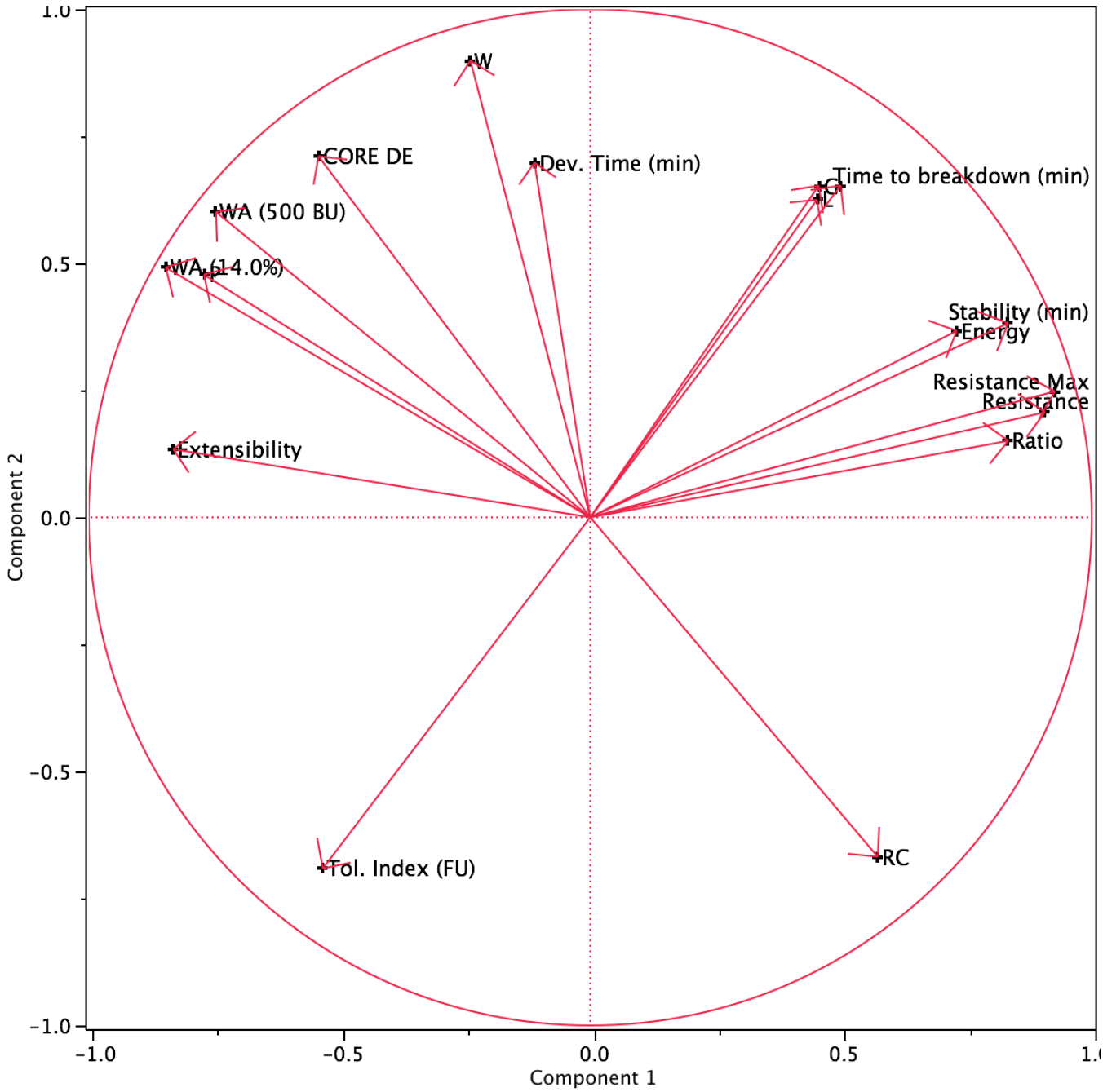


Figure 25 PCA loading plot showing the relationships of various documented rheological testing data across all samples. Variables that are in close proximity to one another on the plot are highly correlated across all samples.

Table 13 Extract from matrix of multivariate correlations of rheological tests

	Correlation with DE		Correlation with RC	
WA (500 BU)	0.80	*	-0.78	*
WA (14.0%)	0.80	*	-0.79	*
Dev. Time (min)	0.41	*	-0.37	*
Stability (min)	-0.15		0.21	
Tol. Index (FU)	-0.16		0.13	
Time to breakdown (min)	0.16		-0.07	
P	0.81	*	-0.82	*
L	0.08		-0.04	
G	0.09		-0.06	
W	0.82	*	-0.77	*
Resistance	-0.24		0.29	
Extensibility	0.39		-0.48	*
Energy	-0.21		0.15	
Resistance Max	-0.29		0.29	
Ratio	-0.19		0.26	

* Correlation value is significant at p=0.05 (two-tailed)

3.4 Conclusion

In rheology, dough ‘strength’ or ‘quality’ is a culmination of three key attributes, whose relative proportions predict a flour’s end use characteristics. Most ‘good quality’ dough will require a balance between extensibility, tenacity (or firmness), and elasticity. With some instruments revealing the former, the CORE does a fine job at providing information regarding both elasticity and firmness, for flours of equivalent or separate wheat classes. This information can be useful for blending purposes, whereby flours that have been characterized for their elasticity and firmness using the CORE, may be added with confidence to a flour mix lacking in these two properties. For practical purposes, critical values for DE or RC must be developed, so that users interested characterizing the elasticity or firmness of a flour sample may distinguish superior values from inferior ones.

CHAPTER FOUR

EVALUATING THE EFFCT OF DOUGH-ENHANCING ENZYME TRANSGLUTAMINASE ON DOUGH STRENGTH

4.1 Introduction

The use of enzymes in food processing has played a significant role in improving the quality of products. Transglutaminase (TG), an example of such additives, has found applications in protein-rich foods, including meat, dairy, and wheat products. Its ability to act on protein substrates, and create new or enhanced structures through agglomeration and polymerization, gives it the ability to improve certain aspects of functionality in a food. Incorporation of TG in wheat dough has shown to impart benefits such as improved elasticity, volume, and texture for a wide scope of wheat-based products (Kuraishi, Yamazaki et al. 2001).

The mechanism for the influence of TG in wheat dough systems has been characterized as a cross-linking reaction between the carboxyamide of a glutamine fraction and a primary amine of a lysine protein. Despite the low lysine content of gluten, these new cross-links are formed, creating a gel-like network that is both heat and acid-resistant, leading to numerous new advantages in the performance of the wheat dough (Kuraishi, Yamazaki et al. 2001). These include an improvement in extensibility, stickiness, and water-holding capacity, which are all highly relevant to dough handling and end product integrity (Tseng and Lai 2002).

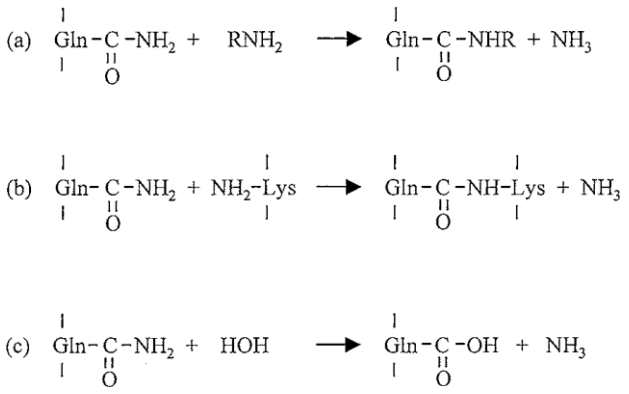


Figure 26 The three main reactions induced by the addition of transglutaminase in foods. (a) acyl-transfer reaction; (b) cross-linking reaction; (c) deamidation (Kuraishi, Yamazaki et al. 2001)

Given the inherent variability in protein composition throughout the wheat endosperm, it is important to understand how different flour varieties will respond to the addition of TG, from a rheological standpoint. The following experiment aims to investigate the sensitivity of the CORE to the addition of TG by looking at differences in elastic recovery between enzyme-treated and untreated samples.

4.2 Materials and Methods

4.2.1 Materials

Two sets composed of solely HRW flours were used for this experiment. These were the same two used in previous experiments: Set B and C. Set C has been extensively tested for milling and baking characteristics. This data was obtained from the 61st Report on Wheat Quality, compiled by the Hard Winter Wheat Technical Board of the Wheat Quality Council. Data spanned results of chemical analyses, such as ash and protein curves, as well as conventional rheological tests, such as farinographs, extensigraphs, mixographs, and alveographs.

Microbial Transglutaminase, commercially known as ACTIVA® TI, was obtained from Ajinomoto Food Ingredients LLC (Chicago, IL, USA). The enzyme contained 100 Units of

enzyme activity per gram of powdered preparation (U/g). The enzyme was in powder form, and was stored in properly sealed bags at room temperature. Open bags were frozen for later use.

4.2.2 Sample Preparation

Transglutaminase was added on a dry basis, to the wheat flour, at a concentration of 2000 ppm. This value was chosen as a reasonable amount that would be realistically added in the baking industry, as well as a common quantity investigated in previous scientific studies. The dry ingredients were adequately mixed in a 35-gram Mixograph mixing bowl (National Manufacturing Div., TMCO, Inc., Lincoln, NE) resulting in a 'pre-blend' prior to hydration. Water was then added, at a constant absorbance rate of 58%. The adjusted weights were calculated using the same equation used in chapter one for flour-water calculations.

The hydrated pre-blends were mixed according to approved method 5440A (AACC International., 2009), to their peak development times. Samples were subsequently divided into the customary 3.00 gram samples, coated with petroleum jelly, and allowed to rest for 45 minutes.

4.2.3 Compression Recovery Test

Samples were tested in the CORE instrument at the optimum 1 Newton 5 second parameter. Results were tabulated, and values for DE and RC for each sample were subsequently calculated.

4.2.4 Statistical Analysis

Samples were tested in replicate. The statistical software JMP® (SAS Institute Inc., USA) was used to analyze differences between responses in the control and test set for each experiment. Data was normalized using the square root method for a more accurate analysis. An ANOVA was done to test for a significant difference of DE and RC values between samples

treated with transglutaminase and untreated control samples. The ANOVA accounted for the fixed effect of the treatment, as well as the random effect of the cultivar, resulting in a mixed REML model of analysis.

4.3 Results and Discussion

As hypothesized, the addition of TG to flour samples had an influence on the elastic recovery of the dough. Treated samples for both sets of flour recovered at higher magnitudes than untreated samples, as shown in Figure 27. Set B, with a control mean of 0.19 and a TG mean of 0.31, was more strongly affected by the enzyme than Set C, whose control mean of 0.08 only increased slightly to 0.12 after treatment.

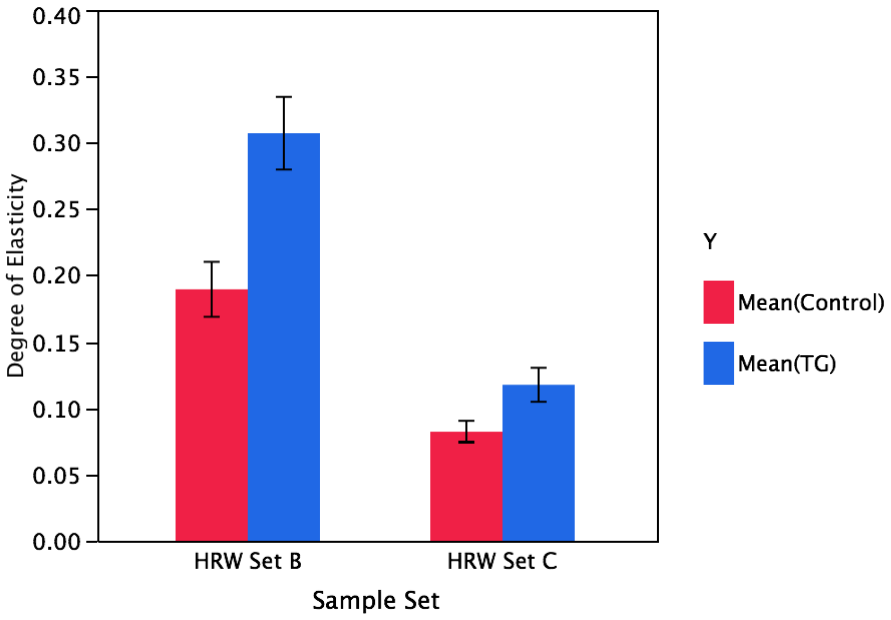


Figure 27 Average DE values of sets B and C, before and after addition of TG

The difference in DE responses between treated and untreated samples were examined as absolute values and percentages for each set. As seen in Figure 28, the percentage of enhanced elasticity was much greater than the actual elasticity, on a scale from 0 to 1. This noticeable relative increase proves that TG may alter the elasticity of dough, however, dough remains limited in its capacity to reach much higher DE values. Hence, elasticity experiences an improvement by means of a notable percent increase, however DE values remain modest since dough cannot extend beyond a certain limit.

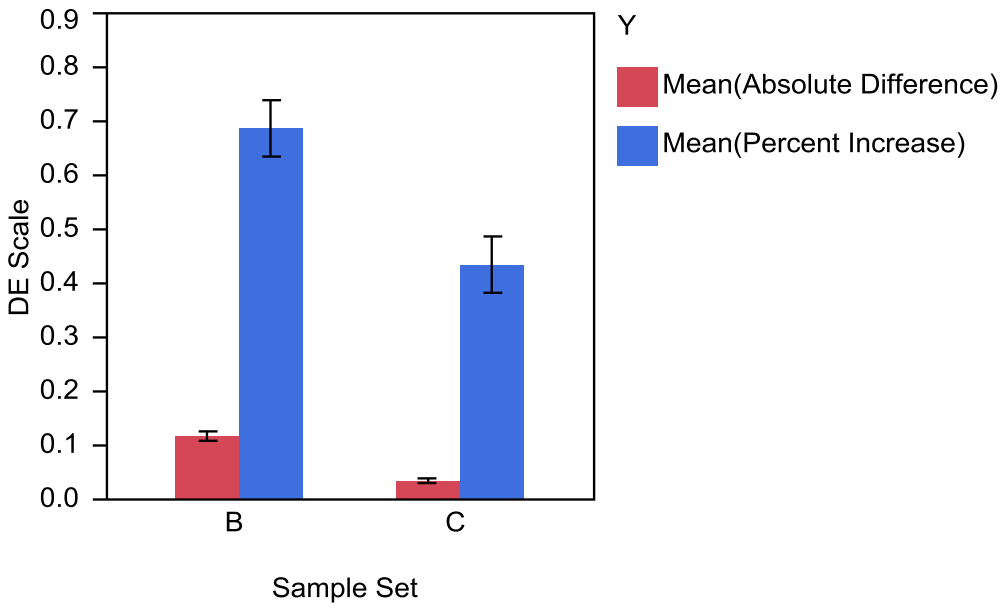


Figure 28 Difference between absolute and percent difference in sample sets B and C

An ANOVA was done on each set to explore whether observed differences between control and treated samples were significant or not. For Set B, the difference between the normalized DE responses for the two treatments was significantly different, at an F-ratio of 310.06 for 53 degrees of freedom. Moreover, a Least Squares Means (LSM) student's t-test also detected a significant difference, at an alpha level of 0.05, with a least squares mean of 0.54 for the TG samples and 0.42 for the control samples.

Using the same approach to ANOVA, set C also portrayed a significant difference between TG-treated and control samples. The fixed effect of the treatment yielded a smaller F-ratio of 87.71 at 65 degrees of freedom, qualifying as a significant effect for this test. Furthermore, the LSM student's t-test grouped treated and untreated responses in two separate groups, indicating a significant difference between them at an alpha level of 0.05. Although Set

C was affected to a lesser extent by the enzyme, it nevertheless experienced a similar noticeable and statistically significant change in response as set B.

Another way of looking at the effects of TG in dough samples is through observing differences in RC values. Upon treatment, RC values decreased slightly for both sets. Unlike elastic recovery, resistance to compression was less sensitive to the effect of the enzyme.

The two sets experienced a similar degree of decrease in RC. In order to find out whether this discrepancy represented a significant difference, a REML ANOVA was done on each set. Results of the ANOVA indicated a significant difference between RC values for control and TG samples, however, the magnitude of significance did not match the larger effect seen in DE results. This indicates that TG affects properties of elasticity more readily than it affects those of firmness.

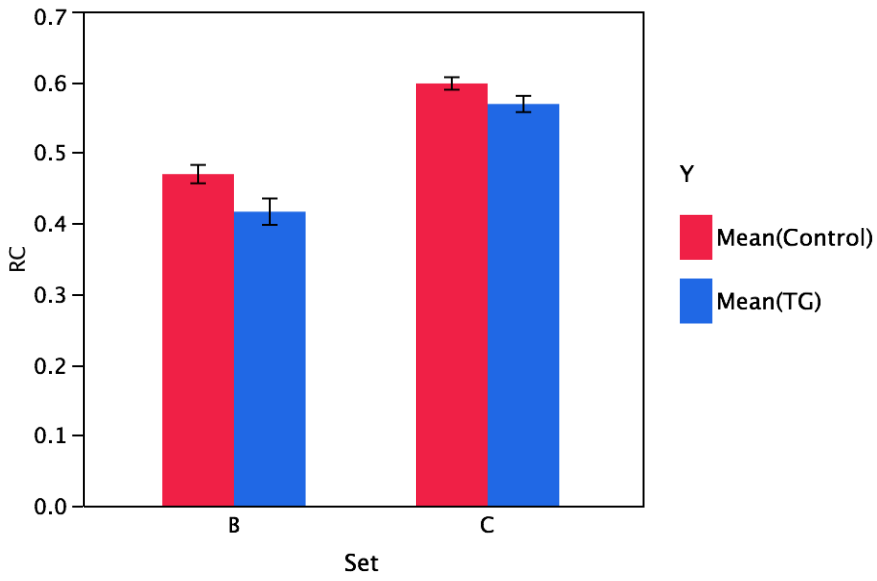


Figure 29 Average RC values of sets B and C, before and after addition of TG

Table 14 Results of F-test to determine significance of exposure to TG on elastic recovery (DE) and firmness (RC) of dough

ANOVA	Set	DF	F Ratio	Prob >F
DE	B	1	310.0586	<.0001*
	C	1	87.7116	<.0001*
RC	B	1	26.7794	<.0001*
	C	1	18.7838	0.0003*

As a first step to understanding the effect of TG on flour samples, a bivariate Pearson correlation was done on DE values of samples that were exposed to TG, and those that were not. Both sets B and C showed significantly strong correlations at respective r-squared values of 0.76 and 0.83. This indicates that samples with a high initial DE are more responsive to the enzyme, due to their corresponding high DE after treatment with the enzyme. This also indicates that the molecular components responsible for dough elasticity, in this case gluten, are the same ones involved in the catalytic polymerization reaction of TG.

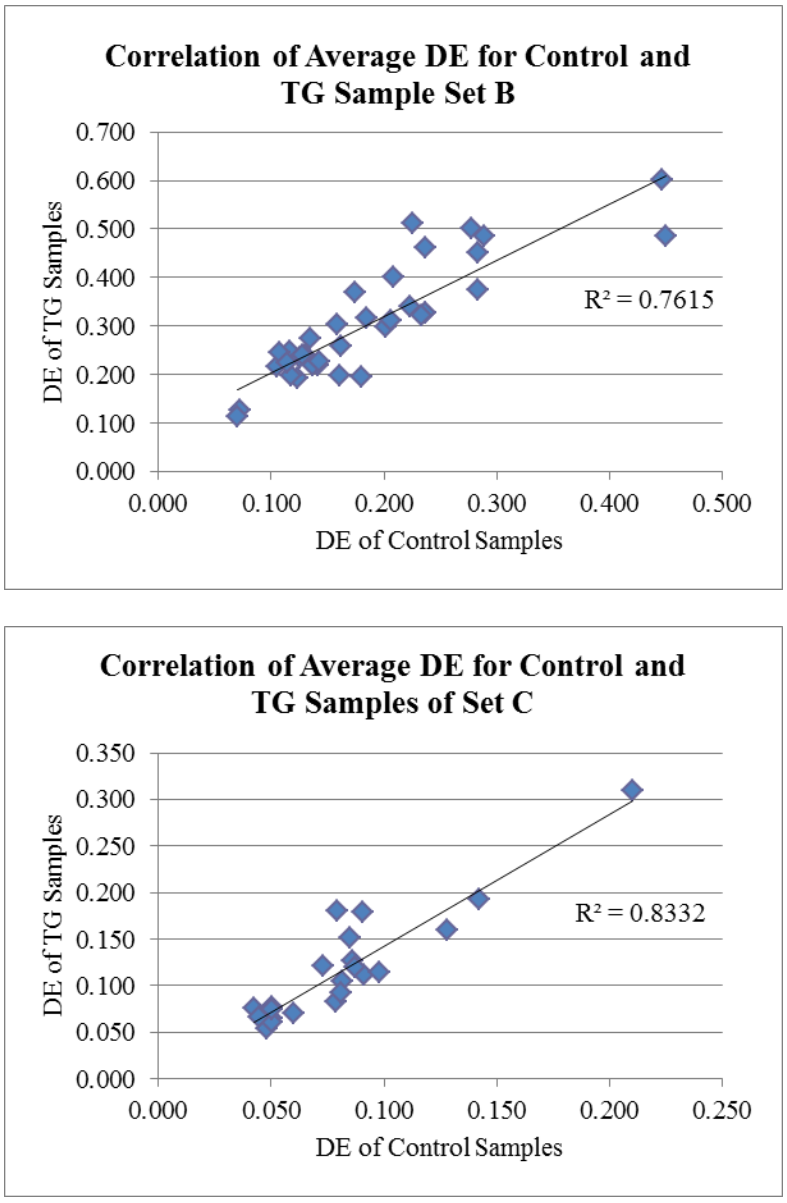


Figure 30 Scatterplots showing correlations of average DE values for Control and TG-treated samples in set B (above) and set C (below)

After observing the change in DE and RC upon treatment with TG enzyme, another bivariate Pearson correlation was done for the two responses. In the non-treated state, these two outputs for both sets B and C combined, had experienced a strong correlation of 0.86, as seen in chapter two. Following the addition of the enzyme, DE and RC continued to act consistently with one another, at an even higher correlation of 0.92. Samples with a higher TG-induced elastic response expressed higher resistance to the applied compression force, again suggesting that factors responsible for DE and RC are interrelated. This stronger correlation in the enzyme-treated state highlighted the overall enhancement in quality that occurred after addition of TG on both fronts of elasticity and firmness.

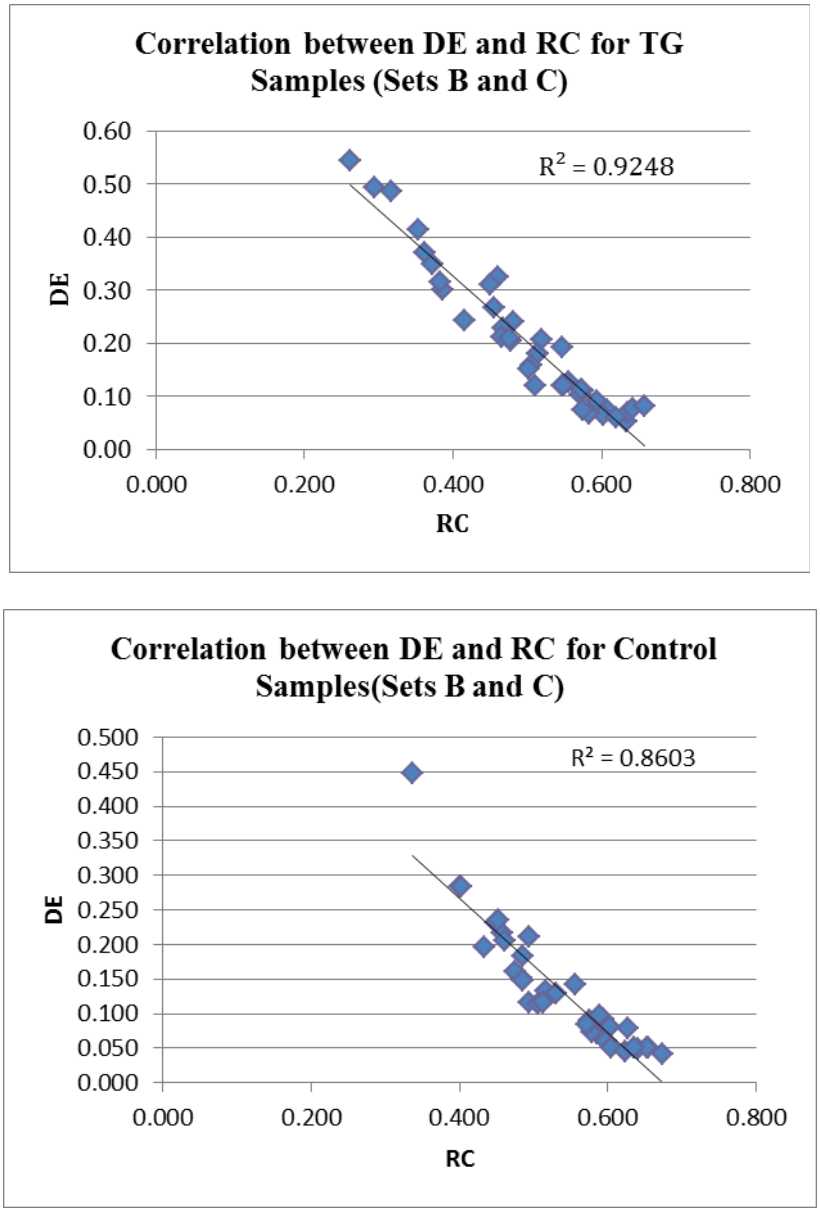


Figure 31 Scatterplot showing correlation between DE and RC for TG-treated samples (above) and control samples (below)

Although consistently imparting increased dough elasticity, results reveal that TG may affect some cultivars to a greater extent than others. This may be useful to bakers who might want to be selective regarding which flours to add a dough-enhancing enzyme to. In Set B, the effect of the enzyme seems to be independent of the initial elastic strength of the dough sample, as no correlation was found between DE and the percent that DE increased, per sample (r^2=0.001). To expand on this, sample 4, previously shown to exhibit superior elastic behavior at a DE of 0.45, was the least affected by TG. Sample 7, another strong flour with an initial DE of 0.30, was more affected. Sample 15 and 11 were both improved to the same extent, although sample 15 had a poorer starting DE than 11. This seemingly random effect of TG was apparent in Set C as well, with a very poor bivariate correlation of 0.17 between the DE of treated cultivars and their corresponding improvement. Cultivars which exhibited similar low initial DE values, such as samples 2, 3, 4, and 5, each reacted differently to the added enzyme.

An attempt was made to relate the percent increase in DE to the available supporting data of the cultivars. For Set B, correlations of enhanced elasticity and protein content were negligible. Given the larger scope of data available for Set C, more comparisons of existing data, such as IPP, wet gluten, and gluten to gliadin ratio were done with the DE percent increase. However, no relationship existed with these given parameters. In fact, all correlations neared an r squared value of zero. Therefore, predicting the extent to which dough may improve with addition of TG based on its original degree of elasticity, or its documented physical or chemical properties, is a difficult task. Besides the poor correlations, outcomes seemed to be largely variable for each cultivar, as shown from the error bars reflecting the wide-ranging scope of average increase per sample.

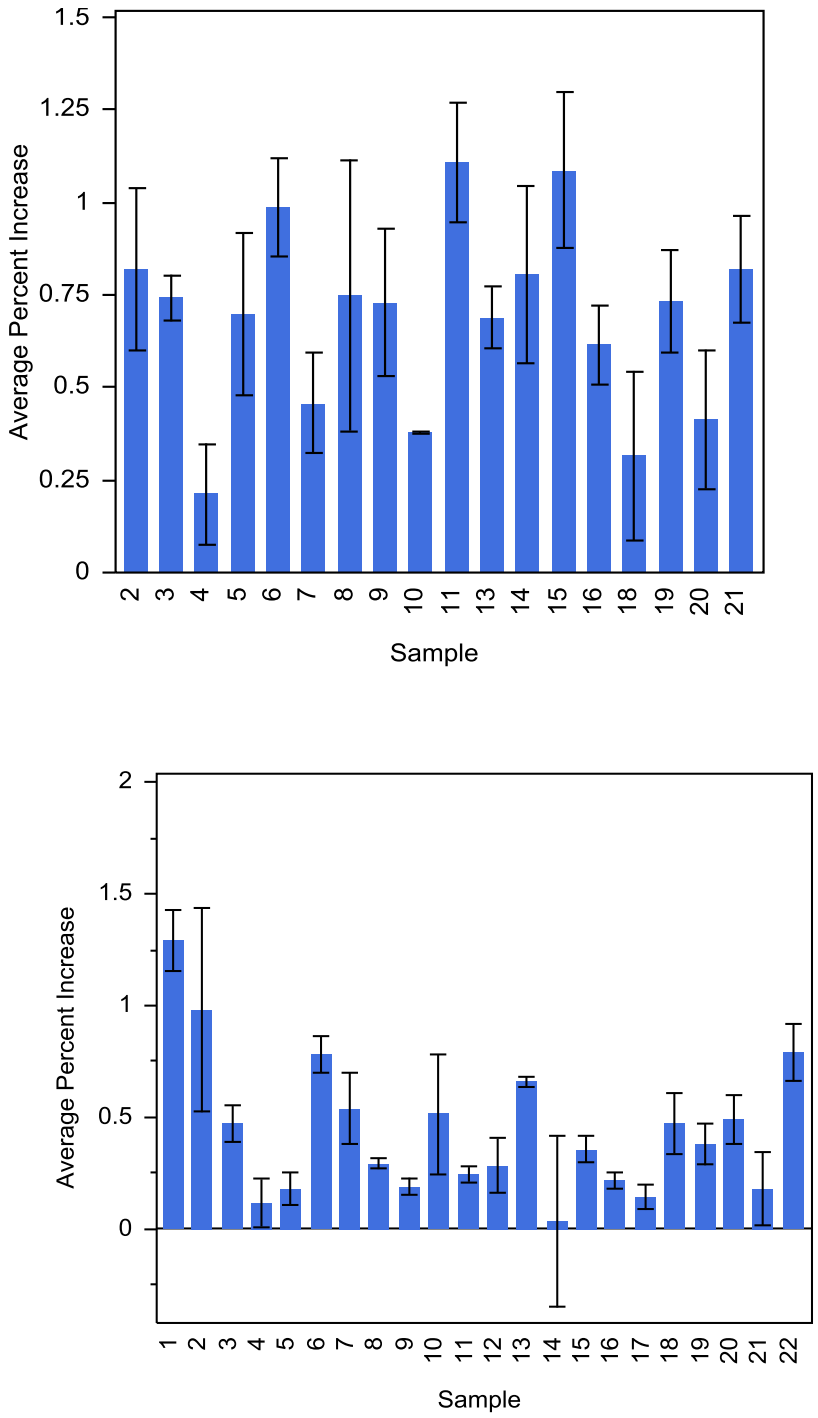


Figure 32 Average percent increase of sample replicates upon treatment with TG for Set B (above) and Set C (below)

Lastly, protein analysis data for Set C was examined in the context of DE for both control and TG-treated samples. The D1 locus is known for its indicative role in wheat quality characterization, whereby the "5+10" gene is attributed to good quality flours, and the "2+12" gene is a sign of poor quality flour. When the DE of these two groups was compared, the cultivars with a "5+10" gene had greater elastic recoveries than the "2+12" cultivars. However, upon treatment with TG, both groups experienced the same extent of increase in elasticity. In other words, the reaction that occurs upon addition of TG does not depend on the D1 locus of wheat protein quality.

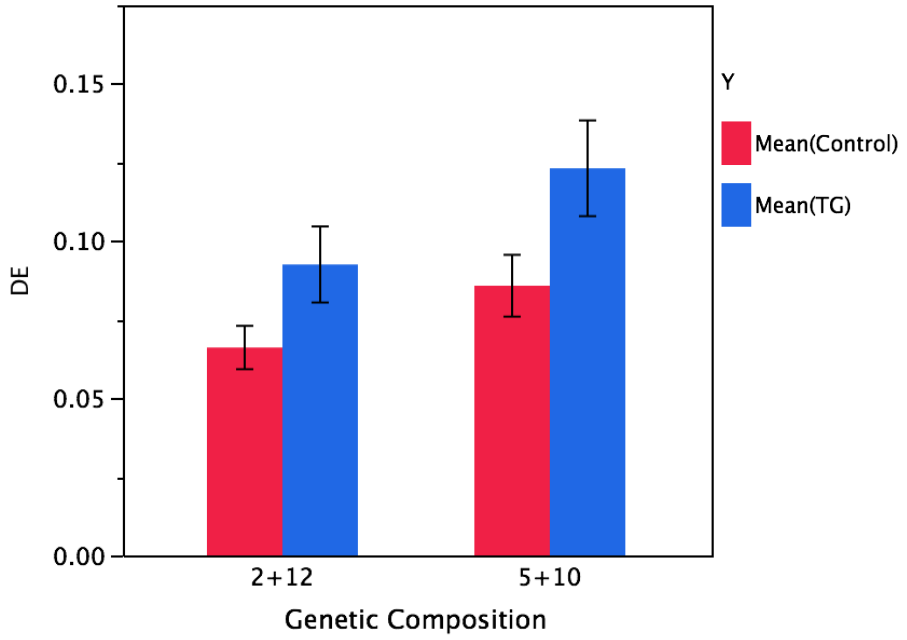


Figure 33 Elastic recoveries of control and TG-treated samples, grouped by the D1 characteristic

4.4 Conclusion

Previous research has shown that added transglutaminase may drastically improve the strength of a protein-rich food structure. When tested with wheat dough, the CORE was able to detect the molecular changes taking place, and translate them into a rheological measurement of increased degree of elasticity. In addition, it was able to show a slight increase in dough firmness through the resistance of the sample to the compression force, expressed through lower RC values. Although there was no direct correlation to what is known about the protein composition and chemical properties of the tested wheat cultivars, it stands true that the instrument can detect increases in material properties among cultivars of the same wheat class. However, in the context of the CORE, a cultivar's relation to TG will most likely remain an empirical one, that must be tested rather than predicted from existing data.

CHAPTER FIVE

THE EFFECT OF FLOUR BLENDING ON DOUGH STRENGTH

5.1 Introduction

In the milling industry, it is customary to combine flours of varying quality in a process known as "blending." Blending allows millers to select for specific characteristics expressed in different cultivars, and obtain a final flour product with targeted end characteristics. It is important for millers to recognize the quality parameters that are relevant to a blending operation, and factor them into their blending procedure. With an increased reliance on chemical, genetic, and rheological data to understand baking characteristics of flour cultivars, millers may now use this flour "characterization" data to further define and optimize their blending operations.

In a study on the optimization of flour blends, a multiple linear regression was carried out to select the smallest number of available factors that were able to best predict bread loaf volume, a common quality indicator of milled flour. As a result, Particle Size Index (PSI), dough volume, and falling number, were chosen as the factors for blending, and were successfully optimized in a computerized model to yield desired end products at a lower cost (Hayta and ÇAkmakli 2001). Other experiments tried to determine whether trusted quality indicators, such as gluten content and Zeleny sedimentation volumes remained accurate representations of a flour's baking quality, after blending non-wheat flours with wheat flours (Dhingra and Jood 2004).

Selecting relevant quality variables for highly variable wheat flours has remained a difficult task for the milling industry. Elasticity and firmness are two quality parameters which have been shown to correlate with some rheological tests, as well as a number of chemical and

physical analyses. Given that the CORE provides information regarding these two attributes, this experiment sought to evaluate the instrument's ability to detect differences in both elasticity (DE) and firmness (RC) upon blending varying proportions of a strong "donor" flour with a set of weaker ones.

5.2 Materials and Methods

5.2.1 Materials

Seven wheat flour varieties from sample Set C, a set of 22 HRW wheat flours, were used to test the effects of flour blending on dough strength. These cultivars were selected based on their elastic performance, measured as DE in the CORE instrument. The strongest flour, Sample 18 (Yellowstone), was the outlier with the greatest DE value among its class members. As for the six weak flours, these were chosen as the flours with DE values in the lowest quartile of the entire set, resulting in a cut off value of DE=0.072. The weak values consisted of samples 2 (SD05118-1), 3 (SD06158), 4 (Hatcher), 5 (CO050303-2), 10 (NE04490), and 13 (OK05212).

5.2.2 Sample Preparation

Dough samples of the above-mentioned flour cultivars were prepared at three separate blend ratios. The blend ratio consisted of blending the strong flour with each of the weak flours on a dry basis. Blends were calculated for an absorbance rate of 58% for all flours, using the Tenmarq equation. Initial calculations were carried out for each cultivar using this equation, after which a combination calculation was done to account for the desired blend proportions. The three ratios of strong to weak flour were 25:75, 50:50, and 75:25.

Table 15 Mixing parameters for three blend ratios: 25:75, 50:50, and 75:25

Test Entry Number	Sample Identification	Blend Ratio (% by weight)	Flour Wt. (g)	Water Wt. (g)
2	SD05118-1	75%	25.252	16.223
3	SD06158	75%	25.308	16.167
4	Hatcher (check)	75%	25.308	16.167
5	CO050303-2	75%	25.308	16.167
10	NE04490	75%	25.280	16.195
13	OK05212	75%	25.280	16.195
18	Yellowstone (check)	25%	8.417	5.408

Test Entry Number	Sample Identification	Blend Ratio (% by weight)	Flour Wt. (g)	Water Wt. (g)
2	SD05118-1	50%	16.834	10.816
3	SD06158	50%	16.872	10.778
4	Hatcher (check)	50%	16.872	10.778
5	CO050303-2	50%	16.872	10.778
10	NE04490	50%	16.853	10.797
13	OK05212	50%	16.853	10.797
18	Yellowstone (check)	50%	16.834	10.816

Test Entry Number	Sample Identification	Blend Ratio (% by weight)	Flour Wt. (g)	Water Wt. (g)
2	SD05118-1	25%	8.417	5.408
3	SD06158	25%	8.436	5.389
4	Hatcher (check)	25%	8.436	5.389
5	CO050303-2	25%	8.436	5.389
10	NE04490	25%	8.427	5.398
13	OK05212	25%	8.427	5.398
18	Yellowstone (check)	75%	25.252	16.223

Once the proportions of flour and water were determined for each blend ratio, dough samples were prepared using a 35-gram Mixograph (National Manufacturing Div., TMCO, Inc., Lincoln, NE) using approved method 5440A (AACC International., 2009), as described in chapter one. Each blend was thoroughly mixed to create a homogenous 'pre-blend' of two different cultivars. After one minute of manually mixing the two flours, water was added at the calculated amount.

The combined flour and water was mixed to the optimal mix time of the strong flour: 6 minutes and 15 seconds, as documented in the 61st Report on Wheat Quality (Hard Winter Wheat Technical Board of the Wheat Quality Council). After mixing was complete, samples were divided into equal 3.00 gram samples, and allowed to rest for 45 minutes prior to centrifuging and compression testing.

5.2.3 Compression Recovery Testing

Dough samples underwent a compression recovery test using the CORE (Perten Instruments AB, Huddinge, Sweden), in the same manner as described in previous chapters. Data was recorded in Excel. Both DE and RC values were calculated as outputs of the instrument.

5.2.4 Statistical Analysis

Blended samples were tested with the CORE in duplicate. The statistical software JMP® (SAS Institute Inc., USA) was used to analyze results. New DE values from blends were compared across treatments using Analysis of Variance (ANOVA). A paired t-test was also carried out to examine the difference between measured DE values and expected values for each sample, to reveal whether the measured DE is a product of the two initial DE's at the corresponding proportions. Other graphical representations were used to visualize and compare the diverse effects of blending among individual cultivars.

5.3.1 Results and Discussion

5.3.1 Evaluation of blending effects across blend ratios

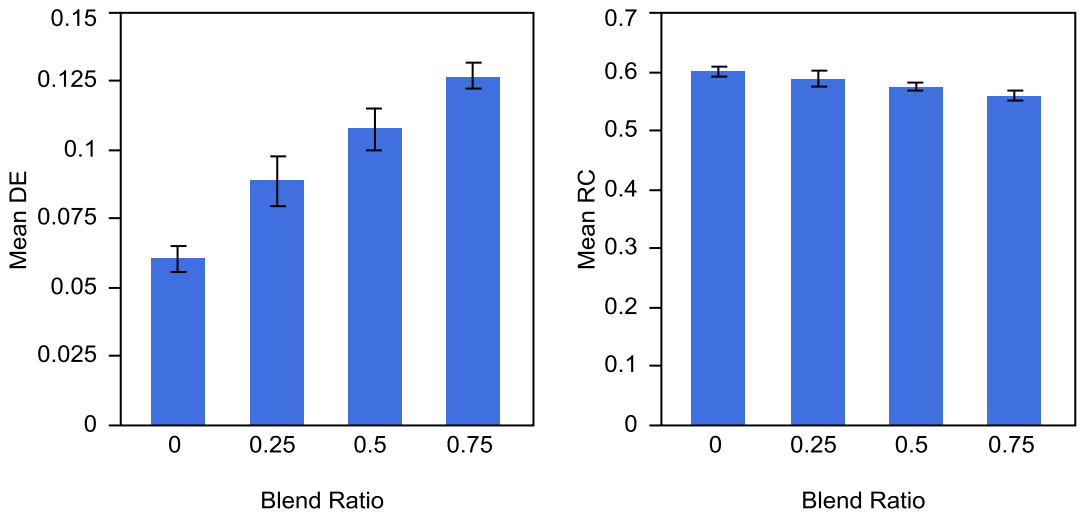


Figure 34 Effects of blending on DE (left) and RC (right)

Addition of the donor flour (Sample 18) to six weak flour cultivars resulted in an apparent increase in dough elasticity. The mean DE for all cultivars exhibited a dose-dependent effect, as a noticeable and nearly constant increase of 0.02 was added with every 25% incremental upgrade of the blend ratio. The same cannot be said for RC, whereby the effect of blending seemed negligible on the sample's firmness. This indicates that the primary attribute of DE, elasticity, and that of RC, firmness, may be separated with the CORE. The instrument is more capable of detecting changes in elastic recovery of a dough sample.

In order to numerically evaluate the significance of the impact of the three blend ratios on the two responses DE and RC, an ANOVA was carried out on the raw data. Unlike previous ANOVA tests which used an REML method, the random effect of the cultivar was not taken into account. Instead, only the fixed effect of the treatment was considered. This approach was

adopted to account for the presence of possible unknown synergistic effects resulting from combining cultivars and mixture effects.

Results of the ANOVA confirmed that differences detected in both DE and RC across varying blend ratios were significantly different from one another. Incremental differences among DE were more visible than those of RC, validated by a higher F-ratio for DE and therefore greater significance.

Response	Sum of Squares	F Ratio	Probability >F		Significant
DE	0.0286	53.5467	<0.0001	*	Yes
RC	0.0118	11.7089	0.0013	*	Yes

Figure 35 Results of F-test for the significance of blending on DE and RC

5.3.2 Evaluation of Blending Effects across Individual Cultivars

To gain a deeper understanding of the implications of blending a strong flour with individual weak varieties in the CORE, it is necessary to observe the outcomes of DE, which represent the new elastic strength of the blended flours. According to DE values shown in Figure 35, the incorporation of a fixed amount of strong "donor" flour has different effects on different weak flour samples. For example, sample 13, previously observed as the most elastic of the weak flours, with a DE of 0.0729, experienced a large boost in DE upon addition of donor flour at 25%. However, this effect was not extrapolated with the second and third blend ratios, which exhibited a much smaller boost in DE. This suggests a threshold circumstance, whereby the recipient flour experienced a synergistic effect up to a certain concentration of donor flour.

On the other hand, samples 3, 4, and 5 exhibited a nearly linear effect, with the dose response being almost constant with each bump in blend ratio. There was no threshold effect, as these weak flours seemed to welcome the donor flour, and increased proportionally with its

integration. Sample 2 exhibited this linear response only after being blended at 50%; a 25% incorporation of flour 18 did not lead to such an increase, however 50% and 75% did.

The most unique response was that of sample 10, whereby the sample first experienced a DE lowering effect at 25%, followed by a linear increase at 50 and 75%. However, sample 10 seemed to be the least cooperative with sample 18; it started off as the second strongest flour from the weak set, and remained the lowest regardless of addition of strong flour. Both sample 10 and 2 required a large amount of donor flour to be affected in terms of elasticity, indicating a weak interaction with the strong flour on a molecular level.

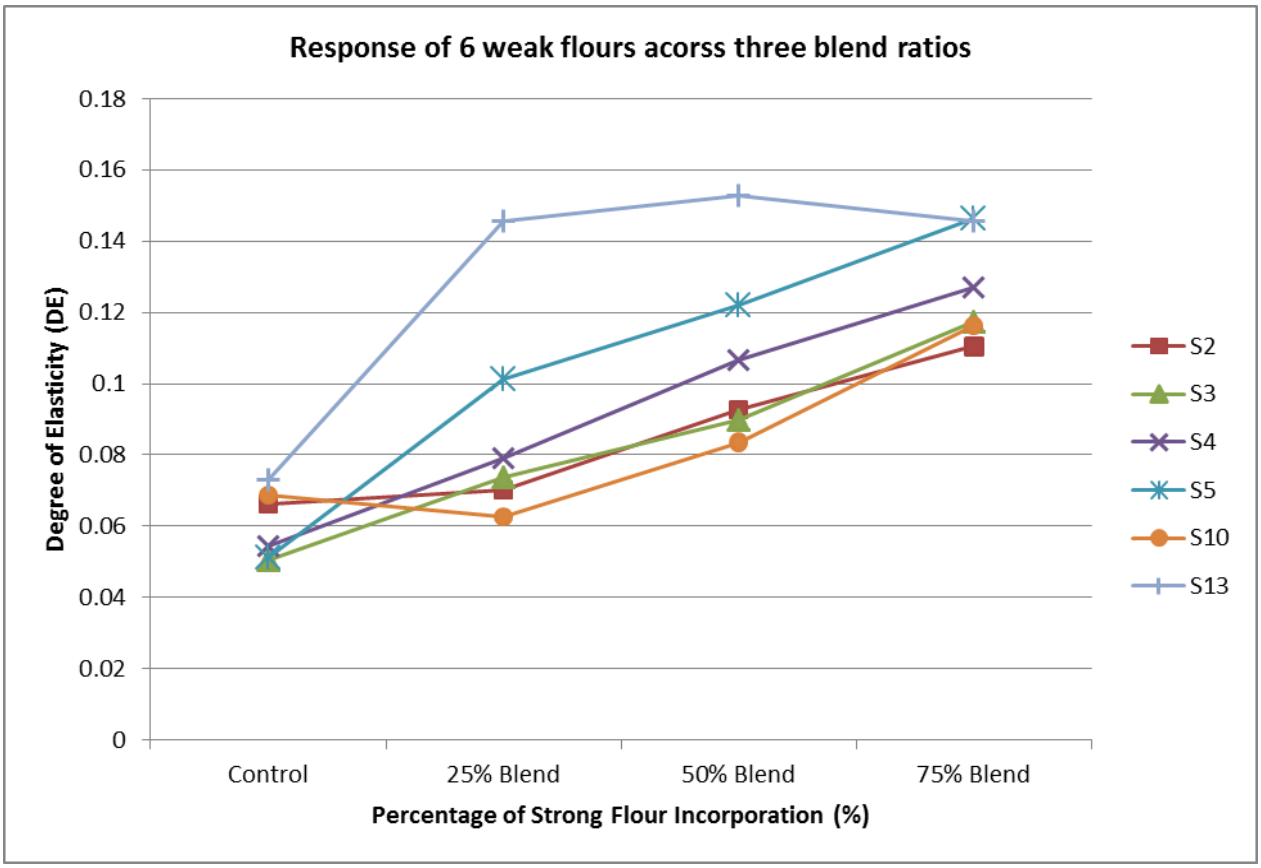


Figure 36 DE values of 6 weak flour cultivars across three blends

In looking at the overall effect of blending on different "weak" or low-elasticity flours, one can get an idea of the complexity involved in the molecular interactions between the two newly-meshed networks. From a strong synergy, to a maximum threshold for interaction, adding strong flour certainly exhibits variable effects that rely heavily on the recipient flour's internal composition.

When referring to effects of blending on dough structure and elasticity, it is customary to attribute change in material properties to the available storage proteins, namely HMW-GS composition. In this case, compositional changes were translated into the rheological measurement DE, resulting in an examination of this new data through the lens of corresponding protein composition (documented in Table 16). Accordingly, both samples 5 and 13, which experienced the highest overall enhancement in elasticity, had the same HMW composition. Their point of differentiation from other samples was their 2+12 trait on the 1D chromosome. This suggests that the 'weak' 2+12 gene was the most reactive with proteins from the donor flour. Additionally, their identical genetic composition did not result in an equal final response, indicating that the HMW proteins are not the sole factor affecting elasticity in blending operations.

As for the flours that did not perform as well, sample 10, which remained the least affected, was unique in its 17+18 gene on its 1B chromosome. A solid explanation cannot be based on one sample; however the relationship between this cultivar and the strong 18 stood out as a poor one. Similarly, sample 2 also experienced a minimal overall effect from blending, increasing only 0.0442 in DE. Sample 2 differed from 10 in its 7+9 1B chromosome. Although this gene was present for better performing flours, such as 5 and 13, its presence with the 5+10 does not seem to help its elastic performance.

The 1A chromosome seems to play a negligible role in the determination of dough elasticity enhancement. Its presence as 1 or 2* seem to be disregarded, as sample 3 and 4 experience similar elastic enhancement but differ in their 1A chromosome.

In conclusion, and based on the available data for HMW-protein composition, it seems that the Glu-1D chromosome is the most significant factor in determining a recipient flour's enhancement in elastic performance after blending with a specific donor flour: sample 18. The second most relevant player for improved elasticity lies in the Glu-1B. The 1A chromosome seems to be impartial in this molecular intervention. However, it must be noted that the role of these HMW proteins in weak flour samples only applies when blending is carried out with donor sample 18. Blending effects will need to be reevaluated for different donor flour samples.

Table 16HMW-GS composition of blended cultivars and their corresponding DE values across blend ratios

Protein Analysis	Glu-1A	Glu-1B	Glu-1D	Control DE	DE 25%	DE 50%	DE 75%	Final Difference
S2	2	7+9	5+10	0.0663	0.0703	0.0926	0.1104	0.0442
S3	2	7+8	5+10	0.0504	0.0739	0.0900	0.1172	0.0668
S4	1	7+8	5+10	0.0544	0.0790	0.1065	0.1271	0.0728
S5	2	7+9	2+12	0.0512	0.1013	0.1221	0.1462	0.0949
S10	2	17+18	5+10	0.0688	0.0625	0.0835	0.1161	0.0474
S13	2	7+9	2+12	0.0729	0.1456	0.1528	0.1455	0.0727
S18	1	7+8	5+10	0.210				

5.3.3 Evaluation of Differences between Observed and Expected Outcomes

Another approach to understanding the effects of blending is to determine whether the final DE of a blended sample is equivalent to the original DE values of its two components at the specified blend ratio. These "expected" DE values are a purely computational approach to blending, however they may help us understand the relationship between blended flours by analyzing where and why discrepancies between measured and expected values occur.

In order to determine whether a significant difference existed between expected and observed values, a two-tailed paired t-test was carried out at alpha level of 0.05. This test revealed a difference between the two values, with the expected DE generally exceeding the observed, across the three treatments, resulting in a negative mean difference (refer to Figure 36). The t-ratio was equal to -4.268 for 17 degrees of freedom, indicating that the probability of achieving a critical t-value less than -4.268 is 0.0003, which represents a significant difference between the two data sets.

Table 17 Actual and expected results of DE based on blend proportions

Sample	Actual DE				Expected DE		
	25%	50%	75%		25%	50%	75%
2	0.070	0.093	0.110		0.102	0.138	0.174
3	0.074	0.090	0.117		0.090	0.130	0.170
4	0.079	0.106	0.127		0.093	0.132	0.171
5	0.101	0.122	0.146		0.091	0.131	0.170
10	0.062	0.084	0.116		0.104	0.139	0.175
13	0.146	0.153	0.146		0.107	0.142	0.176

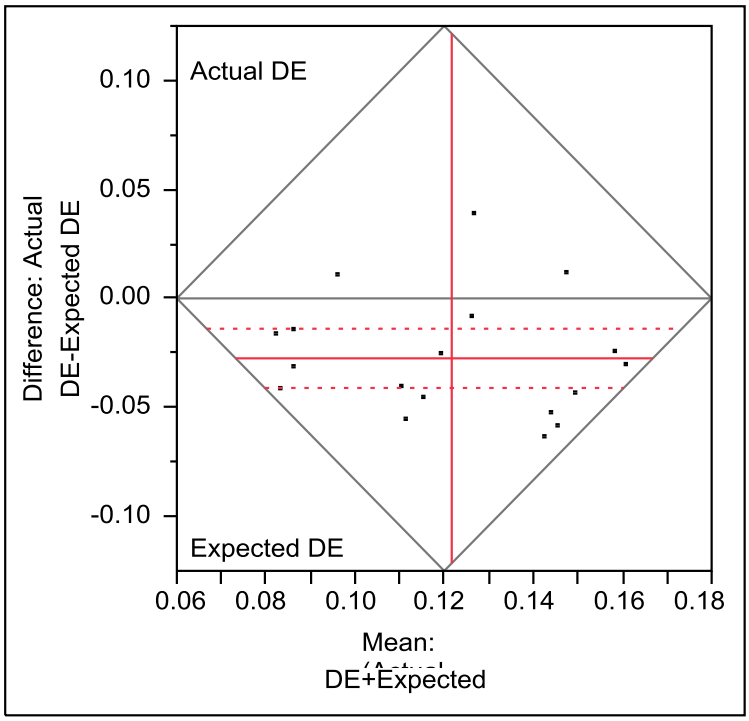


Figure 37 Results of a paired t-test showing a negative mean difference, as expected DE values generally exceeded observed DE values

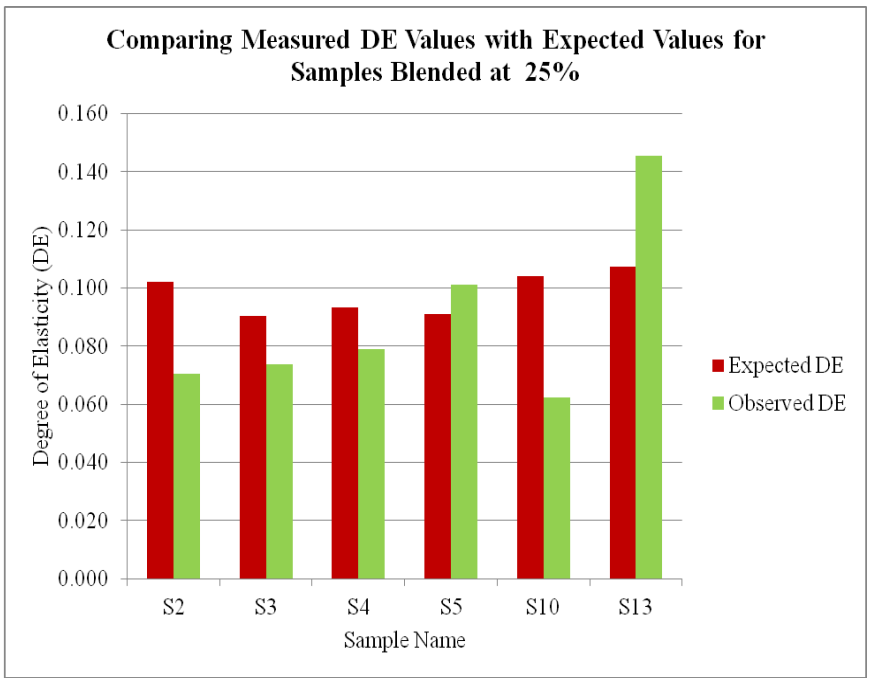
Although an overarching difference exists between predicted and observed values across all treatments, these discrepancies need to be examined in the context of separate treatments and individual cultivars. In the first blend, with 25% integration of donor flour, some samples recovered below expected values, others recovered at higher DE values. Sample 13, which previously showed dramatic improvement in elastic recovery, was the only flour to exceed its expected DE by a large extent. Sample 5 exceeded expectations slightly, with samples 2, 3, and 4 falling behind target. As for sample 10, we continue to see that its relationship with the strong flour was the farthest from what was expected of it.

The second blend ratio, a mix of 50% weak and strong flour, showed different relationships between actual and calculated values from the 25% ratio samples. In the 50% blend, the gap between measured DE's and calculated ones grew larger. No samples matched up to expected values, with the exception of sample 13, which only barely exceeded the target DE. This shows a non-linear relationship, whereby increases in elasticity are not directly proportional to the blend ratio at hand. As the proportion of donor flour goes up, it becomes more difficult for some flours, such as 2 and 3, to match the higher ideal DE value. Sample 5 was almost able to live up to its projected value, followed by sample 4, which indicates a nearly proportional relationship for these two flours with incremental blend ratios. This was consistent with previous data representations for these two cultivars. Sample 10 remained equally uncooperative as before.

The final blend ratio was the most severe treatment, with 75% of the dry four consisting of donor sample 18. In this case, no measured DE for any flour sample was able to exceed or approach the expected DE values. This indicates that dough strength cannot simply be translated into numbers, and that molecular interactions between two flour components in dough in fact

hinder the progression of a continued incremental increase. At 75%, sample 5 remained the most capable of catching up to its expected value, with its measured DE surpassing that of sample 13. However all flours virtually fell behind, increasing only to an extent they were physically able to.

Figure 38 Graphical representations of expected and observed values for each cultivar, at three blend ratios


Comparing Measured DE Values with Expected Values for Samples Blended at 25%
0.160
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
Degree of Elasticity (DE)
S2
S3
S4
S5
S10
S13
Sample Name
Expected DE
Observed DE

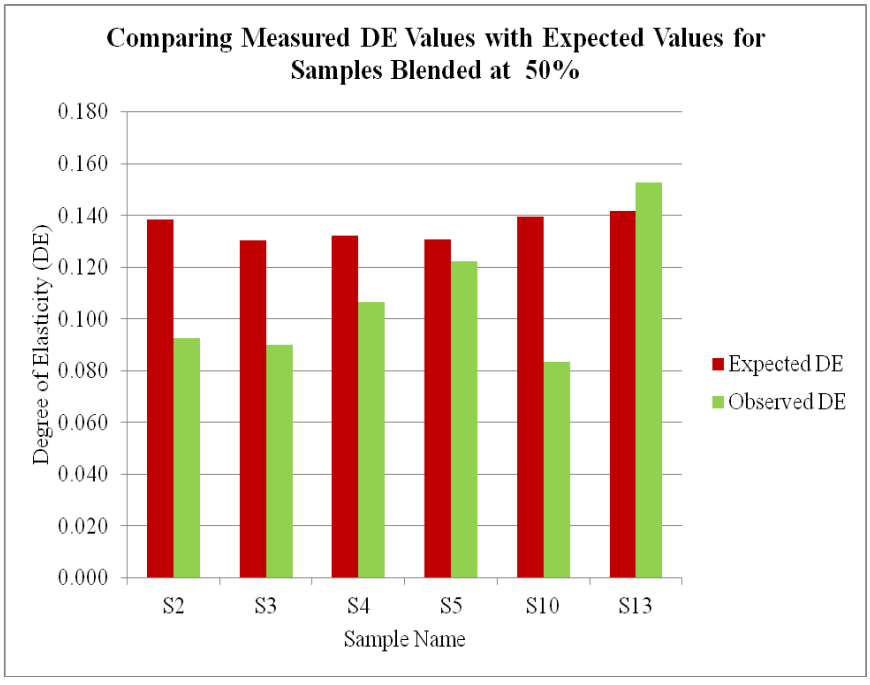
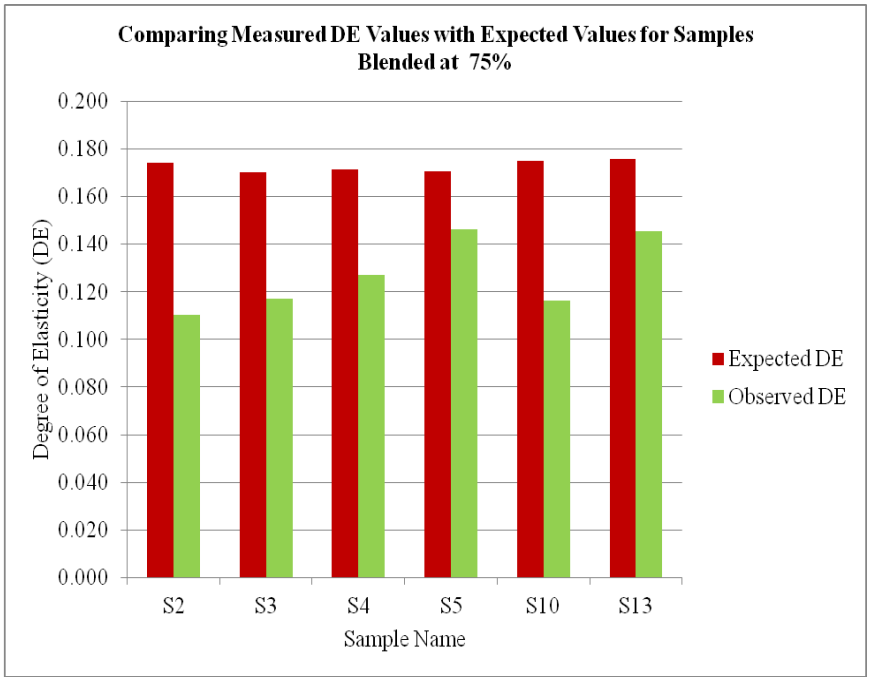

Comparing Measured DE Values with Expected Values for Samples Blended at 50%
0.180
0.160
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
Degree of Elasticity (DE)
S2
S3
S4
S5
S10
S13
Sample Name
Expected DE
Observed DE

Figure 37 (continued)



5.4 Conclusion

The implications of blending two flours of varying strength can be explored in general, and on an individual basis. In a broad sense, it can be said that blending a strong cultivar with a weaker one does effectively improve the elastic strength of the corresponding dough. The firmness of the dough, measured by a sample's resistance to the compression, is affcted to a lesser degree than elasticity, measured by DE. Overall, samples did not recover as much as was expected of them, with the gap between projected and achieved DE widening with increasing blend ratios. Individual cultivars were distinctively affected by blending, shown by differing degrees of enhanced elasticity across samples. This can be explained by the differences in each flour's network-forming composition, which may result in a weak reaction, or a synergistic one. Nevertheless, it is difficult to forecast the improvement in elasticity based solely on a computation of DE and blend proportions.

BIBLIOGRAPHY

Barber, J. and M. Titus (1995). Structure of the U.S. wheat supply chain. Fargo, N.D., Upper Great Plains Transportation Institute, North Dakota State University.

Cauvain, S. P., L. S. Young, et al. (2007). Principles of Dough Formation. Technology of Breadmaking, Springer US**:** 299-332.

Chapman, S. (2011). Large deformation stress relaxation and bi-axial compression recovery of gluten representing different wheat classes. Food Science. Ithaca, Cornell University. **Masters:** 78.

Chunguang Wang, S. D. a. R. I. T. (2006). "On the compressibility of bread dough." Korea-Australia Rheology Journal **18**(3): 5.

Council, W. Q. (2010). 61st Report on Wheat Quality Milling and Baking Test Results for Hard Winter Wheat. B. Handcock. Pierre, South Dakota, The Wheat Quality Council.

Dhingra, S. and S. Jood (2004). "Effect of flour blending on functional, baking and organoleptic characteristics of bread." International Journal of Food Science & Technology **39**(2): 213-222.

Dobraszczyk, B. J. and M. P. Morgenstern (2003). "Rheology and the breadmaking process." JOURNAL OF CEREAL SCIENCE **38**(3): 229-245.

EPA (2009). "Major Crops Grown in the United States." from http://www.epa.gov/agriculture/ag101/cropmajor.html.

F, M. and E. K. John (1992). Physicochemical Properties of Wheat Proteins in Relation to Functionality. Advances in Food and Nutrition Research, Academic Press. **Volume 36:** 1-87.

FAO (2004). "Food Outlook Global Market Analysis ". from http://www.fao.org.

Gillmeister, W. J., R. D. Yonkers, et al. (1996). "Hedonic Pricing of Milk Components at the Farm Level." Review of Agricultural Economics **18**(2): 181-192.

Hayta, M. and U. ÇAkmakli (2001). "OPTIMIZATION OF WHEAT BLENDING TO PRODUCE BREADMAKING FLOUR." J Food Process Engineering Journal of Food Process Engineering **24**(3): 179-192.

Houghe, J. (1994). Logistics of the U.S. Wheat Industry. Fargo, Upper Great Plains Transportation Institute.

Hui, Y. H. and H. Corke (2006). Bakery products : science and technology. Ames, Iowa, Blackwell.

Janssen, V. van, et al. (1996). "Fundamental and Empirical Rheological Behaviour of Wheat Flour Doughs and Comparison with Bread Making Performance." Journal of Cereal Science **23**(1): 43.

Kokelaar, J., T. van Vliet, et al. (1996). "Strain Hardening Properties and Extensibility of Flour and Gluten Doughs in Relation to Breadmaking Performance." JOURNAL OF CEREAL SCIENCE **24**(3): 199.

Kuraishi, C., K. Yamazaki, et al. (2001). "Transglutaminase: Its Utilization in the Food Industry." FOOD REVIEWS INTERNATIONAL **17**: 221-246.

Lasztity, R. and T. Abonyi (2009). "Prediction of Wheat QualityPast, Present, Future. A Review." Food Revs. Int. Food Reviews International **25**(2): 126-141.

Noel, D. U. and H. Bengt (1996). "The market valuation of wheat quality characteristics." Journal of Economic Studies **23**(3): 44.

O.K. Chung, Y. P., and K.F. Finney (1978). "Wheat Flour Lipids in Breadmaking." CEREAL CHEMISTRY **55**(5): 598-618.

Pomeranz, K. A. a. Y. (1991). "Lipid Binding and Fatty Acid Distribution in Flour, Dough, and Baked and Steamed Bread." CEREAL CHEMISTRY **68**(6): 570-572.

Puppo, M. C., A. Calvelo, et al. (2007). "Physicochemical and Rheological Characterization of Wheat Flour Dough." Cereal chemistry. **1**: 173.

Shewry, P., N. Halford, et al. (2002). "The structure and properties of gluten: an elastic protein from wheat grain." Philosophical Transactions: Biological Sciences **357**(1418): 133-142.

Shewry, P. R., J. A. Napier, et al. (1995). "Seed Storage Proteins: Structures and Biosynthesis." The Plant Cell **7**(7): 945-956.

Skerritt, J. H., L. Hac, et al. (1999). "Depolymerization of the Glutenin Macropolymer During Dough Mixing: I. Changes in Levels, Molecular Weight Distribution, and Overall Composition." Cereal chemistry. **76**(3): 395.

Tseng, C. S. and H. M. Lai (2002). "Physicochemical Properties of Wheat Flour Dough Modified by Microbial Transglutaminase." Journal of Food Science **67**(2): 750-755.

United States. Congress. Office of Technology, A. (1989). Enhancing the quality of U.S. grain for international trade. Washington, DC, Congress of the U.S., Office of Technology Assessment : For sale by the Supt. of Docs., U.S. G.P.O.

USDA. "FGIS Inspection and Weighing Services." from http://www.gipsa.usda.gov/fgis/inspweigh.html.

Uthayakumaran, S., M. Newberry, et al. (2000). "Basic Rheology of Bread Dough with Modified Protein Content and Glutenin-to-Gliadin Ratios." Cereal chemistry. **77**(6): 744.

Wheat Marketing Center, K. S. U. (2008). Wheat and Flour Testing Methods: A Guide to Understanding Wheat and Flour Quality: Version 2.

Wieser, H. (2007). "Chemistry of gluten proteins." Food Microbiology Food Microbiology **24**(2): 115-119.