Compression-Recovery Analysis of Gluten Extracted from Whole-Meal Wheat Flours and of Wheat Gluten in the presence of Selected Oat Bran Fractions

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

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Abstract

I. Compression Recovery (CORE) Analysis, as a candidate test of the baking quality and elastic strength of wheat protein, was investigated by evaluating gluten samples extracted from whole meal flours from 15 cultivars of bread wheat of varying baking qualities. It was found that whole meal flour was a valid (albeit troublesome) starting material for the isolation of gluten samples for CORE Analysis. Multivariable regression indicated that CORE Recovery Index, a measurement of gluten elasticity, was significant ($p=0.0123$) when considered along with gluten sample moisture content ($p=0.0036$), and flour protein content ($p=0.0001$), in predicting the baking quality of flour from these cultivars. Trends in Moisture Content, Half-Recovery Time, Recovery Index, and Percent Compression results were are considered in terms of wheat gluten chemistry.

II. An interaction between oat bran materials of varying β-glucan content, and wheat gluten, was characterized by constructing dose-response curves of CORE Recovery Index, Half-Recovery Time, Percent Compression, and Moisture Content, of gluten samples treated with variable doses of three β-glucan containing oat bran materials. Significant increases in measured sample elasticity were observed, which mirrored decreases in sample moisture content. Evidence that these effects could be due to an interaction occurring within the protein phase of the gluten samples was provided by comparing the results of both a “washed” and “unwashed” treatment series. Evidence that Oat β-glucan could be the oat bran component involved in this interaction was provided by comparing dose response behavior with the estimated β-glucan content of each treatment material. Applications of these findings for product development purposes are suggested, and a hydrogen-bonding based explanation of these observations is proposed.

Biographical Sketch

Susanna (Susie) Rose Kahn was born on April 18th, 1985, in Portland, OR., USA, to parents Marc and Fay Kahn. She grew up in Vancouver, WA., USA, with one younger sister, Celeste.

In 2004, one year after completing high school with broad interests in the musical, visual, and culinary arts, Susie enrolled in Clark College's Baking and Pastry program. During this program of study, she developed a strong interest in Bakery Technology and Food Chemistry, so decided to continue her community college education by enrolling in relevant fundamental science courses.

In 2009, Susie graduated from Clark College with both an Associate of Applied Science culinary degree, and an Associate of Science transfer degree. She then moved across the country, to Ithaca, NY, to study Food Science at Cornell University. In addition to embracing her Food Science coursework, she became active in Cornell's Food Science Club and IFTSA College Bowl (Food Science Trivia) Team, completed undergraduate research projects in the Regenstein Lab and Mulvaney Lab, and pursued elective coursework in biochemistry and physical chemistry. She received her Bachelor of Science degree in May of 2011, fulfilling requirements for both the Food Science and the Biological Science (Biochemistry) Majors.

Susie began graduate studies in the Fall of 2011 as a member of the Mulvaney Lab, in the Graduate Field of Food Engineering, at Cornell University. Over the next two years, while enrolled as a graduate student, she completed the research described in this thesis, served as a TA in Food Chemistry and Food Packaging courses, traveled to India to study food production there as part of a course in International Agriculture and Development, completed a challenging Chemical Engineering course to gain a fuller understanding of polymer science concepts, and helped Cornell's IFTSA College Bowl Team become 2013's National Champions.

In August of 2013, Susie accepted a full time position as the R&D Manager at New Hope Mills Manufacturing, Inc., in Auburn, NY. Over the 18 months she has now spent in this role, she has contributed to the realization of over 30 new products, and has been involved in implementing many changes and innovations at this small but growing company. During this time, Susie has also spent many evenings and weekends working toward a final draft of this thesis.

Dedicated to my grandmother, Elayne Kahn, whose influence, support, and high expectations will always continue to inspire me in pursuit of my dreams.

Acknowledgements

I thank Dr. Steven Mulvaney, my committee chair, for guiding and advising this research, as well as my interest in cereal and protein chemistry. Congratulations on your retirement, Dr. Mulvaney! I thank Dr. John Brady for his insights into thermodynamics and chemistry, which helped me interpret and understand the results of this research, and also for the academic guidance he provided as my minor committee member. I also thank both of the above, as well as the following exceptional teachers, all of whom have been heroes to me over the course of my Food Science education: Alicia Orta-Ramirez, Joe Regenstein, Carmen Moraru, Dennis Miller, Syed Rizvi, Karl Bailey, April Mixon, Ian Titterton, Jean Williams, and Per Zeeberg.

I thank all of my Mulvaney-Lab labmates: Monthakan (Dutch) Boonpermpol, Agnes Lim, Lena Halabi, Stephen Chapman, and Dongjun Zhao, for all of the help, inspiration, and input they have provided me with during my completion of graduate school, this research, and the writing of this thesis. In addition to members of the Mulvaney Lab, I also thank Joseph McKay, Michael Bono, Diwakar Gupta, Diane Schmitt, Yifan Cheng, Veronica Pillar, Janette Robbins, Douglas Weed, Steven Wellauer, and James Amie for the various support, input, and crisis assistance each provided during the researching and writing of this thesis.

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I thank Perten Instruments, Inc., as well as The Department of Food Science at Cornell University, for providing funding, materials and support for this research. I also thank Scott Dumler of Oat Ingredients, LLC, Darren Schubert and Blossom Van Kinkle of Grain Millers, Inc., Bassam Faress of Garuda International, and Brooke Rodriguez of USDA's GIPSA, for donating materials used in this research. Finally, I thank Dave Barbano and members of his Lab at Cornell University, for carrying out several Kjeldahl analyses on my behalf, which helped greatly in the interpretation of these research results.

Table of Contents

Terms and Abbreviations

Abbreviation	Meaning	See
BLV	*Baked Loaf Volume*	Zhao et al., 2010
CORE	*Compression-Recovery (a rheological testing mode)*	
GIPSA	*Grain Inspection, Packers & Stockyard Administration*	
HDWH	*Hard White (a type of wheat)*	
HMW-GS	*High Molecular Weight Glutenin Subunits*	Page 14
HRS	*Hard Red Spring (a type of wheat)*	
HRT	*Half-Recovery Time (a Gluten CORE variable)*	Page 27
HRW	*Hard Red Winter (a type of wheat)*	
LMW-GS	*Low Molecular Weight Glutenin Subunits*	Page 14
MC	*Moisture Content*	Page 33
PC	*Percent Compression (a Gluten CORE variable)*	Page 27
PM	*Percent Moisture*	Page 33
RF	*Refined Flour*	Page 29
RI	*Recovery Index (a Gluten CORE variable)*	Page 27
SRW	*Soft Red Winter (a type of wheat)*	
SWH	*Soft White (a type of wheat)*	
USDA	*United States Department of Agriculture*	
WMF	*Whole Meal Flour*	Page 29

Term	Meaning	See
Flour Protein	*Percent weight of protein in a flour, via Kjeldahl Nitrogen x 5.7*	Zhao et al., 2010
Gluten CORE	*An apparatus developed by Perten, Inc., for CORE analysis of gluten samples.*	Page 22
Gluten Index	*A measure of the fraction of HMW-GS in gluten*	Zhao, 2008
Glutomatic	*A gluten-washing apparatus, developed by Perten, Inc., for extraction of gluten from flour.*	Page 31
Glutork	*A gluten-drying apparatus, developed by Perten, Inc., that dries gluten by flattening it between two heated Teflon plates.*	Page 33
Sedimentation Volume	*A traditional combined measure of gluten quality and quantity*	Zhao et al., 2010

Chapter 1. Introduction

1.1 Gluten Viscoelasticity and the Cereal Industry

1.1.1 Why does gluten viscoelasticity matter?

Triticum aestivum, known as "bread wheat", is one of the most important crops in the world. Humans have been growing and using wheat as a food source since our hunter gatherer ancestors first developed agriculture. Since this time, the crop has proven adaptable to many continents, climates, and growing seasons, and wheat has gained great historical and cultural importance. It is a staple food for 30% of the world's growing population (Eversole et al., 2014), and is utilized today in many different food traditions. A tour of the world's food cultures and traditions would involve a wide variety of iconic wheat based loaf breads, flat breads, pastries, noodles, and other starchy staples, representing cultures from all over the world. An investigation into the preparation method of all of them would reveal that our culinary traditions have come to depend on the unique properties of gluten, the protein fraction of wheat. Cookie dough and cake batter viscosity depends on the water-binding properties of gluten, and the baked structure of these products depends greatly on its ability to form cross-links when heated (Delcour et al., 2012). Yeast leavened hearth breads, sandwich breads, pizza doughs, raised donuts and other sweet doughs, owe their producibility to gluten's viscoelasticity and excellent gas holding and structure-setting properties.

1.1.2 Microstructures of wheat: from kernels to baked bread.

Gluten proteins are synthesized in the endoplasmic reticulum of cells within a growing wheat grain endosperm, and then transported to protein bodies which form in the cell alongside starch granules. As the grain matures, starch granule growth squeezes the protein bodies such that they coalesce into a continuous phase around them (Shewry et al., 1995).

Different milling protocols can separate the bran, germ, and endosperm. Refined flour is the product of finely milling the isolated wheat endosperm, whereas whole meal flour is the product of milling the entire kernel, including the bran and germ. When flour is hydrated, flour particles absorb water. This allows the glassy dehydrated gluten in the flour to become hydrated and pliable. Starch granules from flour also take up a small amount of water, but it is the gluten proteins which are mainly responsible for the water-binding capacity of a flour (Delcour et al., 2012). Gluten reaches a maximum water-binding capacity at around a 65% moisture content, however, because it is not soluble. In a dough, freshly hydrated gluten is able to interact with neighboring hydrated gluten particles to begin the formation of a gluten network. Network strength and alignment can then be improved via kneading or otherwise imparting mechanical energy to the dough, which is known to bakers as the process of "gluten development". In bread dough, the continuous protein phase of a developed gluten network surrounds and binds together starch granules. This protein phase can be purified by working developed dough in the presence of flowing water, allowing the starch to be washed away while the insoluble gluten network becomes condensed.

1.1.3 Gluten biochemistry

The gluten network in dough is a complex material consisting primarily of hydrated protein. If isolated gluten is washed with 60-70% ethanol (Delcour, 2012), two fractions are obtained. The class of protein soluble in the alcohol is known as gliadin, and the class of proteins insoluble in the alcohol is known as glutenin. Gliadin and glutenin however, are not pure fractions, but are still complex assortments of different gene products. Figure 1-1 illustrates the various types of gliadin and glutenin proteins in terms of their overall structure.

Schematic structures of gliadin proteins are shown in yellow in Figure 1-1. Gliadin proteins do not have free cysteine residues available for participation in intermolecular disulphide bonds, and are therefore soluble in alcohol because the solvent reduces the strength of non-covalent protein-protein interactions between these "monomeric" proteins and the covalently cross-linked gluten network (Shewry et al., 1995).

Individual glutenin proteins, unlike gliadins, have free cysteine residues which are able to form intermolecular covalent disulphide cross-links (Delcour et al., 2012). Glutenin proteins are classified as either High Molecular Weight Glutenin Subunits (HMW-GS) or Low Molecular Weight Glutenin Subunits (LMW-GS). Schematic structures of LMW-GS are shown in blue in Figure 1-1, and HMW-GS are shown in grey. LMW-GS have molecular weights of 30,000 to 45,000, while HMW-GS have molecular weights of 70,000 to 90,000 (Delcour et al., 2012). The HMW-GS have been generally recognized as the gluten component that is most important in wheat's breadmaking quality for some time (Anjum et al., 2007; Shewry et al., 1995). Their importance is illustrated in the model of the gluten network that is shown in Figure 1-2.

Individual HMW-GS structures consist of two globular terminal domains separated by a long, hydrophilic, repetitive central domain. Cysteine residues are concentrated in the terminal domain, allowing these to form cross-linking nodes.

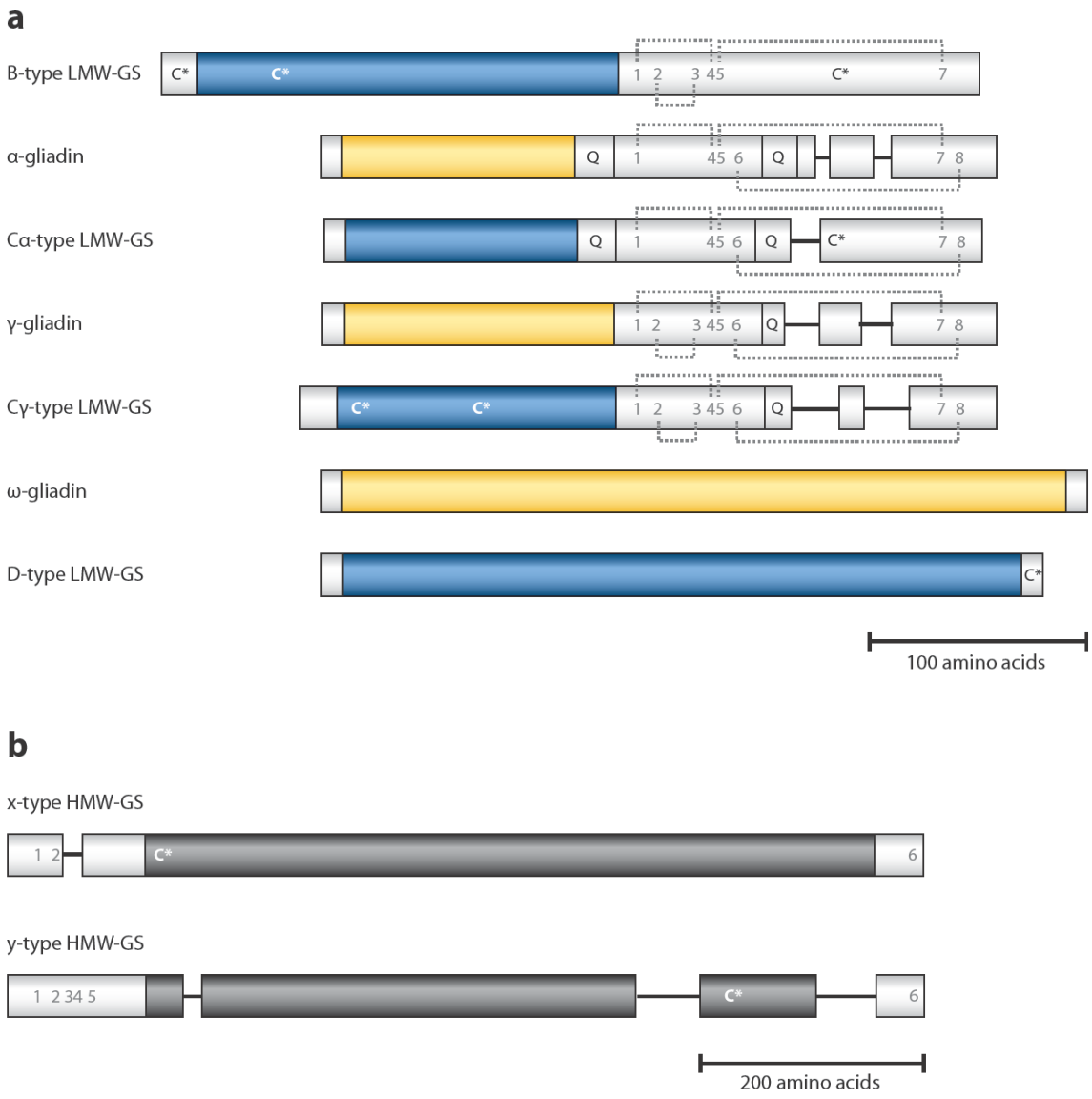


Figure 1

(*a*) Structures of α-, γ-, and ω-gliadin (*yellow*) and B, C, and D low molecular weight–glutenin subunits (LMW-GS; *blue*). (*b*) Structure of x- and y-type high molecular weight (HMW)-GS. Repetitive domains are colored and nonrepetitive domains are white. Positions of the conserved cysteine residues are indicated with Arabic numerals. Additional unconserved cysteine residues are indicated with C*. Intramolecular disulfide bonds are indicated by the gray dotted lines. Q represents a polyglutamine sequence. Similar polypeptide regions are aligned based on their amino acid sequences.

Figure 1-1 The proteins of gluten. (From Delcour et al., 2012)

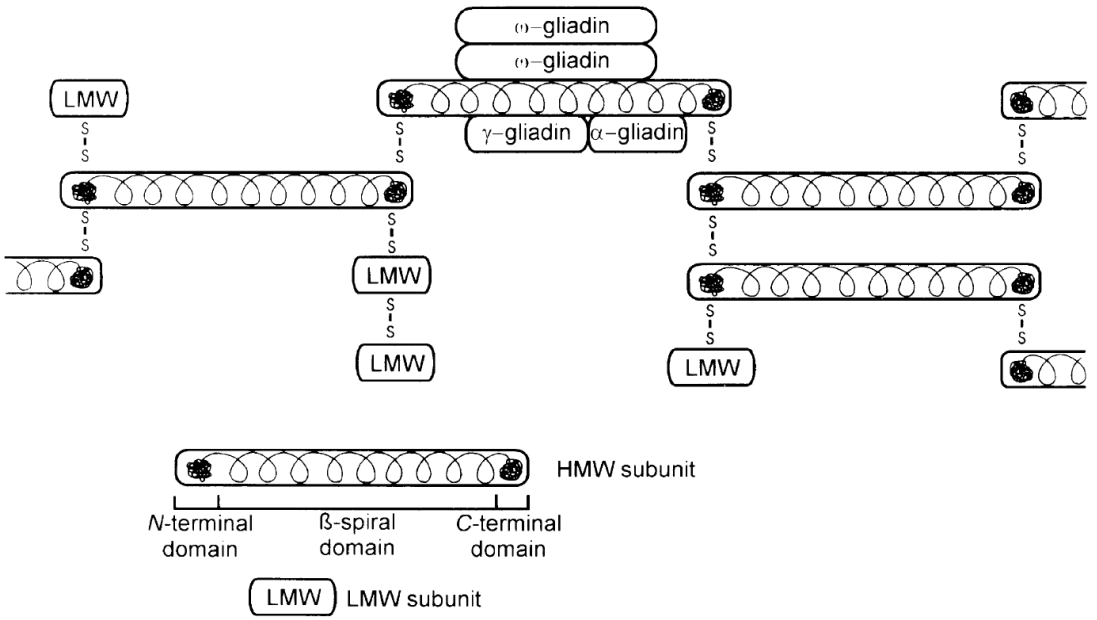


Figure 1-2 A model of protein organization within the gluten network. (From Shewry et al., 2001)

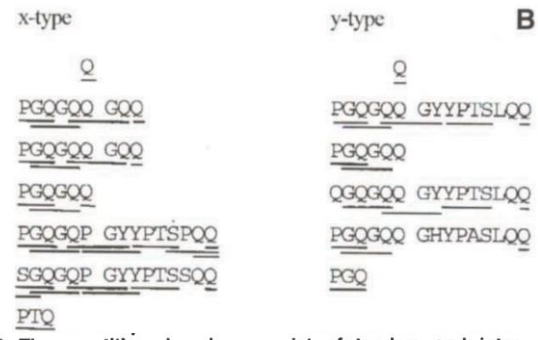
1.1.4 Gluten biodiversity in modern cultivars of wheat.

Wheat domestication by humans was integral to the development of agriculture during the Neolithic period, in the Fertile Crescent of the Levant. Wheat precursor species contain both glutenin and gliadin type proteins, which function in the wild seed as storage proteins for providing nutrients to the plant upon germination. There is no known biological benefit for the wheat plant associated with the viscoelastic properties of these proteins, so it is speculated that it is only a lucky accident that they happen to exhibit viscoelastic gas-holding properties when ground, hydrated and worked. Domestication of wheat would have primarily resulted in increased yield and other agriculturally useful traits, but also appears to have improved food processing traits. Domesticated wheat, for example, expresses an improved ratio of glutenin to gliadin compared to its wild

ancestors, giving it better bread-making properties. Over the past 7,000 to 10,000 years, selective breeding has resulted in the establishment of wheat cultivars adapted to a wide variety of climates and growing seasons (Shewry et al., 2001), as well as varieties that have optimized properties for different culinary applications.

Modern bread wheat, or *Triticum Aestivum*, is a grass. A chromosome-based sequencing of its 17 gigabase genome has only recently been published (Eversole et al., 2014; IWGSC, 2014). The hexaploid genome consists of three diploid sub-genomes, known as A, B, and D, each of these consisting of 7 sets of homologous chromosomes (IWGSC, 2014). The A, B, and D subgenomes were each contributed by a different diploid ancestor grass, via a series of allopolyploidizations (Marcussen et al., 2014).

The genetics of the wheat-quality-associated HMW-GS has been the subject of much investigation. Each of the 1A, 1B, and 1D chromosomes (that is, the "1" chromosome of each of the A, B, and D subgenomes), of any given wheat cultivar has 1 "x-type" and 1 "y-type" HMW-GS allele (Shewry et al., 1995). However, the 1Ay subunits are almost always silenced, while the 1By and the 1Bx subunits are sometimes silenced. As a result, bread wheat cultivars usually have phenotypes with between 3 and 5 HMW-GS subunits expressed (Anjum et al., 2007). Both the x and y type HMW-GS genes are encoded at a single locus, so each set of x and y alleles are always inherited together (Anjum et al., 2007). Allelic variation of HMW-GS that influences quality variation in wheat can involve differences in the number and location of cysteine residues, and differences in the repeating sequences found in the central hydrophillic domain (Shewry et al., 1995). Some examples of these repeat sequences are shown in Figure 1-3.



B. The repetitive domains consist of tandem and interspersed repeats based on two or three short peptide motifs. These are hexapeptides and nonapeptides in the y-type subunits, with additional tripeptides in the x-type subunits. Secondary structure prediction indicates the formation of regular β-reverse turns, both within individual motifs and spanning the junction between adjacent motifs. The figure shows sequences derived from the repetitive domains of typical x-type and y-type HMW subunits, aligned to demonstrate the repeat structure. Predicted β-reverse turns, which involve four amino acid residues, are underlined. Standard single letter codes for amino acid residues are used. The non-repetitive N-terminal and C-terminal domains are predicted to be rich in α-helix (not shown).

Figure 1-3 Examples of repeat sequences found in HMW-GS center domains. (From Shewry et al., 1995)

1.1.5 The Chemistry of Gluten Viscoelasticity

Numerous physical and chemical phenomena are involved in the rheology of gluten. Generally, gluten elasticity is a thermodynamically driven phenomenon that results from the unfavorableness of the increased molecular order of the polymer matrix when it is extended. The unfavorable entropy loss associated with extension of a gluten network can be resolved by elastic recovery of the material to a non-extended conformation. The viscous property of gluten viscoelasticity is due to the ability of cross-links to interchange and entanglements to become resolved (untangled), which with enough time restores the entropy of the network without the need for an elastic recovery (Delcour et al., 2012). Strain hardening is a property exhibited by gluten when the deformation applied stretches segments of the gluten network to the extent that they are pulled taut between network nodes, as illustrated in Figure 1-4. The strain hardening

behavior of gluten is important to the baking quality of wheat because it is thought to help stabilize growing air cells in dough (Dobraszczyk & Morgenstern, 2003).

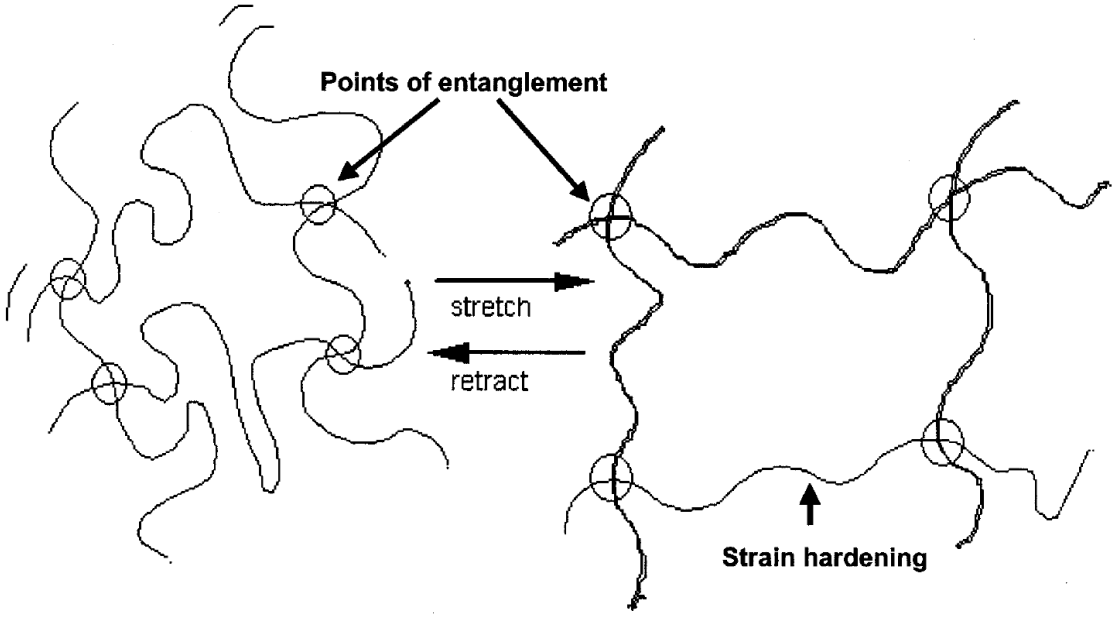


Figure 1-4 Network entanglements and strain hardening. (From Dobraszczyk & Morgenstern, 2003)

Covalent disulphide cross-links between glutenin subunits were the first to be identified as a source of network elasticity and strain hardening (Delcour et al., 2012). The reactions responsible for disulphide cross link formation and interchange are shown in Figure 1-5.

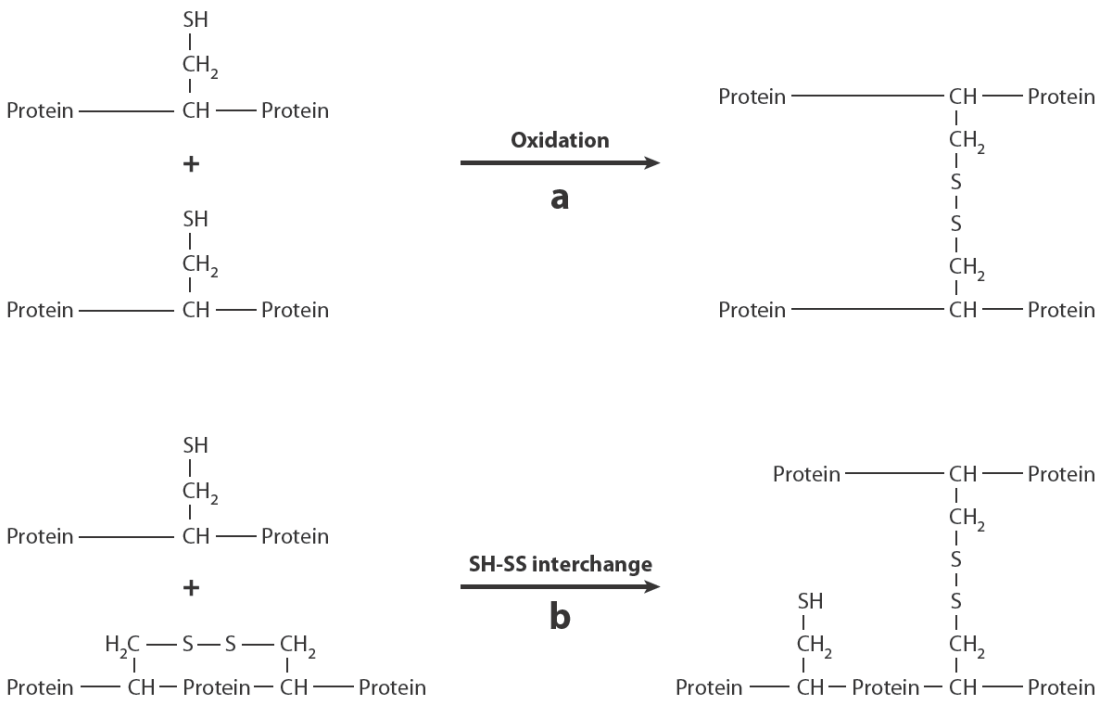


Figure 1-5 The formation and interchange of protein-protein disulphide bonds in gluten. (From Delcour et al., 2012)

In addition to disulphide cross-links, it is also speculated that covalent dityrosine and isodityrosine cross links that can form between gluten tyrosine residues in the central repetitive domains of HMW-GS also contribute to dough strength (Delcour et al., 2012). Non-covalent interactions such as hydrogen bonds and electrostatic interactions are thought to be an additional source of elasticity, but not strain-hardening. Hydrated central domains of HMW-GS can form hydrated loops but also condensed protein-protein trains, which act as transient cross-links and therefore contribute to elasticity (Belton, 1999).

1.2 CORE Analysis

1.2.1 The Gluten CORE

Figure 1-6 The Gluten CORE, Gluten Sample, and Sample Shaping Mold for Gluten Centrifuge.

Figure 1-6 shows a prototype of the "Gluten CORE", a novel apparatus developed by the Perten Company for measuring the elasticity of a gluten sample via a "Compression Recovery" (CORE) testing mode. The objective of the development of the Gluten CORE was to create a method of analysis that would quickly yield information about the quality of an isolated sample of gluten that could be used to make inferences about the protein quality of the wheat the gluten was extracted from, so that the gluten quality could as easily be measured as the gluten quantity. "Quality", for the purposes of this project, was determined to be best defined as "elasticity", and the Gluten CORE procedure protocol constants – time and force – were optimized to give results with the best discrimination in the "recovery index" measurement (Chapman, 2011).

It is hoped that grain millers and flour blenders might use the Gluten CORE Recovery Index as a quality control specification, to help ensure predictable and consistent sheeting, machining, bubble expansion, and baking performance of their various flour products. Another potential application of the Gluten CORE assay is that the Recovery Index measurements it produces could be used by wheat breeders and geneticists to help identify and characterize alleles associated with superior wheat quality.

Table 1-1 summarizes work on which development of the Gluten CORE has been based, as well as preliminary work that has used versions of the instrument. Chapman et al., 2012, characterized large deformation tensile stress relaxation behavior and established a relationship between these fundamental (but time consuming) rheological measurements and the applied (but more rapid) Gluten CORE assay using the constant "8 Newton, 5 seconds" compression cycle, which had been chosen to give optimal

discrimination across the wide range of elasticity represented by different wheat classes (Chapman et al., 2012).

The CORE Recovery Index data reported by Chapman et al., 2012 had good correlations with the results of earlier tensile and creep recovery analyses of Zhao and Liang, but, other established parameters, and flour protein content, remained much more predictive of Baked Loaf Volume (BLV) than Chapman's reported Tensile Strength, or CORE Recovery index, or Zhao's "Degree of Elasticity" after 500% extension.

Halabi (2012) reported the results of a CORE assay that had been modified to measure the elasticity of hydrated, developed dough instead of isolated gluten. An improvement in correlation between this dough elasticity measurement over the elasticity measurements of isolated gluten was observed. However, the hydrated dough was much more fragile and difficult to work with to obtain a standardized CORE elasticity measurement, such that the discrimination between glutens of different quality was impaired.

Despite a lower than hoped for correlation between the CORE Recovery Index and BLV, it was determined that the development of a rapid measurement of isolated gluten's elastic behavior could still be valuable to wheat breeders and the industry, particularly with respect to the machinability of dough, where there is a need for maintaining a constant expected degree of "snapback" that sheeted, extruded, or otherwise shaped dough pieces might undergo in any particular production process.

Table 1-1 Summary of selected previous wheat and gluten rheological studies from the Mulvaney Lab at Cornell University

Citation	*Material Evaluated*	*Testing Apparatus*	*Testing Mode*	*Outcome variable of used for representing "gluten quality"*	*Bivariate Correlation with BLV*
Liang, 2006	Whole gluten from cultivars with different protein	Rheometer	Long-time; Small-deformation; Shear;	N/A	N/A

	chemistry		Creep Recovery		
Zhao et al., 2010	HMW-GS fraction of Gluten from refined flour representing 15 US wheat cultivars.	Rheometer	Short-time; Small-deformation; Shear; Creep Recovery	"Recoverability" = "Recovered strain / total strain"	0.3095
Zhao et al., 2010	HMW-GS fraction of Gluten from refined flour representing 15 US wheat cultivars.	TA-XT+	Large deformation; Tensile; Cyclic extension-recovery	"Degree of Elasticity" = recoverable work / work of extension *100	0.6485
Chapman et al., 2012	Whole gluten from refined flour representing 15 US wheat cultivars.	TA-XT+	Large deformation; Tensile; Stress-relaxation	"Degree of Elasticity" = F_{max} / F_{eq} *100	0.4206
Chapman et al., 2012	Whole gluten from refined flour representing 15 US wheat cultivars. (excluded 2)	Perten Gluten CORE prototype	Standard 5s, 8N compression, followed by 55s recovery period.	"Recovery Index" = recovery distance / compression distance	0.5466
Halabi, 2012	Constant hydration developed dough prepared from refined flours representing 15 US wheat cultivars (excluded 3)	Perten Gluten CORE prototype	Standard 5s, 1N compression, followed by 55s recovery period.	"Recovery Index" = recovery distance / compression distance	0.6870

1.2.2 Gluten CORE Used in This Thesis

Figure 1-7 on page 26 illustrates the basic procedure used in the Gluten CORE assay. A gluten sample that has been cylindrically shape-standardized is placed on the compression platform, and subjected to a 5 second, 8 Newton compression period, followed by a 55 second recovery period. Sample thickness data is taken and used to calculate compression and recovery distances. Measured parameters and calculated outcome variables from the CORE analysis are defined in Table 1-2 on page 27. It should be noted that "Degree of Recovery" is interchangeably used with the term "Percent Recovery" throughout this thesis. The term "Recovery Index" specifically refers to the Degree of Recovery observed at the *end* of the full 55 second recovery period, whereas

Degree of Recovery could apply to the apparent recovery observed at any point in time during the 55 second recovery period, as it does in the construction of Average Recovery Curve graphs.

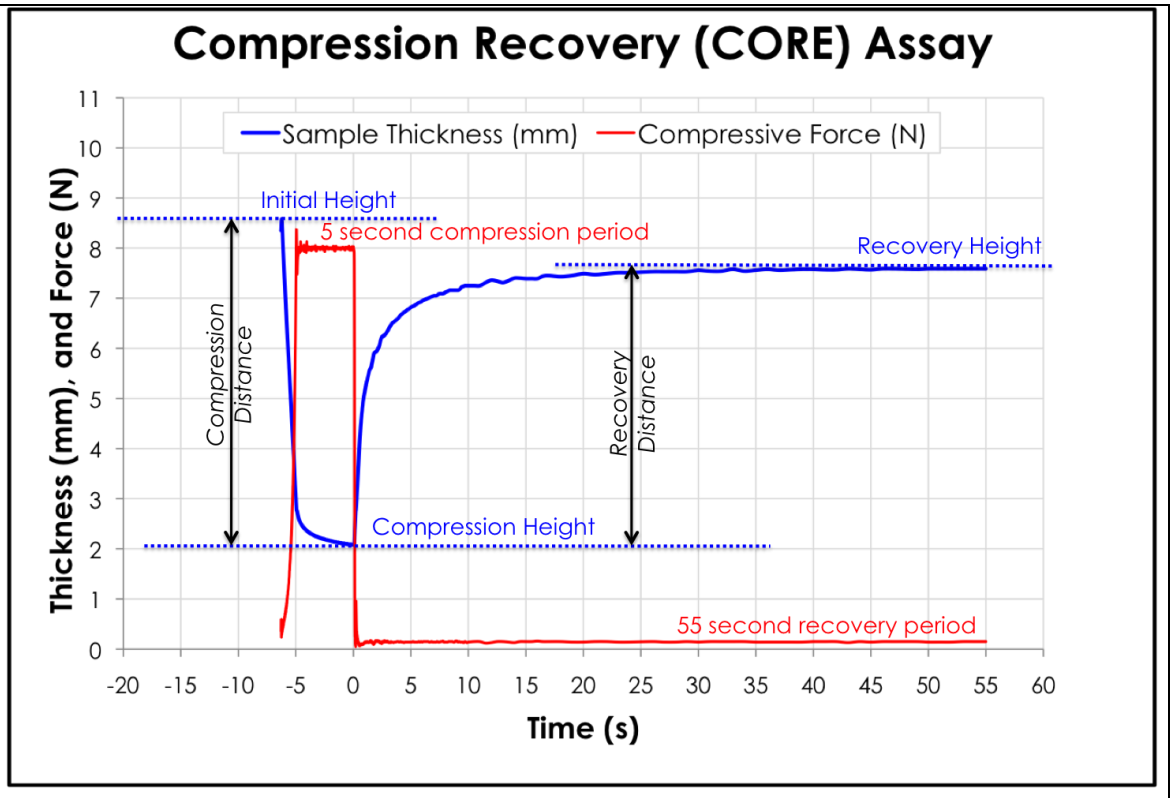


Figure 1-7 Gluten CORE Measurement Plot with Explanation of Calculated Parameters

Table 1-2 Measurements and calculated parameters used in Gluten CORE analysis

Parameter/ Variable	**Unit**	**Definition**
Initial Height	mm	Measured sample height at start of compression cycle
Compression Height	mm	Measured sample height at end of compression cycle
Recovery Height	mm	Sample height attained during recovery period as a function of time (for apparent recovery curves), or, sample height at end of the 55 second recovery period.
Compression Distance	mm	*= Initial Height – Compression Height*
Recovery Distance	mm	*= Recovery Height – Compression Height*
Degree of Recovery	%	*= (Recovery Distance / Compression Distance) x 100*
Recovery Index (RI)	%	Degree of Recovery observed at the end of the 55 second recovery period.
Percent Compression (PC)	%	*= (Compression Distance / Initial Height) x 100*
Half-Recovery Height	mm	*= (Compression Height + Final Recovery Height) / 2*
Half-Recovery Time (HRT)	s	Time, in seconds, taken by sample to reach Half-Recovery Height

Chapter 2. CORE Analysis of Gluten from Whole Meal Flours

2.1 Introduction

2.1.1 Background & Motivation

One of the motivations behind the development of Perten's Gluten CORE assay is its potential usefulness as a quality control test for the wheat production and milling industries. Wheat, as a raw agricultural commodity, is subject to natural variations in composition and quality. To produce flour which performs reliably for their customers, grain millers must evaluate this variable raw material to determine which grinding, separating, and blending processes are necessary to ensure their finished flour product conforms to the required specifications. An important quality test used by most millers is measurement of a grain's protein content, which is usually calculated from a measurement of the nitrogen content using the Kjeldahl factor for wheat. However, simple quantification of the protein does not reflect variation in its functional quality (Dobraszczyk & Salmanowicz, 2008). Therefore, there is a need for a meaningful measurement of wheat protein quality that can be used by millers and their customers.

It was shown by Chapman (2011) that Perten's Gluten CORE assay could be used to measure the elastic recovery of gluten extracted from refined flour, allowing for reproducible differentiation across a variety of wheat cultivars – a promising result for

the possibility of using the Gluten CORE as a test for gluten quality. Grain is typically purchased by millers as intact, whole grains. To carry out the Gluten CORE assay as described by Chapman, et al., however, whole grains must first be refined and milled before the gluten can be extracted with Perten's standard Glutomatic gluten washing system. Whole grains can be converted to simple whole meal flour by direct grinding, however, which makes the choice of whole meal flour as a starting material for Gluten CORE analysis as a quality control protocol attractive.

2.1.2 Hypotheses

This study was primarily designed to test the hypothesis that the extraction of gluten using a Glutomatic extraction procedure with whole meal flour as a starting material will result in the production of a wet gluten that is representative – or at least indicative – of the quality of the gluten that could be extracted from refined flour made from the same wheat.

During the early stages of data collection of this experiment, it was observed that Perten's Glutomatic gluten washing procedure produces gluten samples with non-controlled moisture content, since gluten samples are allowed to retain whatever amount of the washing fluid they are able to bind at their equilibrium hydration level, since the wash fluid is exposed to samples during the washing process in vast excess. It was hypothesized that variations in this effective equilibrium moisture content of a gluten sample could be responsible for variations in sample behavior during CORE analysis. To investigate this hypothesis, it was decided that moisture content measurements should be made of all remaining gluten samples, after CORE analysis.

2.1.3 Objectives

The most basic objective of this study was to determine if gluten extracted from whole meal flour is a valid material for evaluating a wheat harvest in terms of protein quality. This was accomplished by studying the same set of cultivars studied by Chapman (2011), so that results could be directly compared to Chapman's measurements of gluten extracted from refined flour. In addition, the use of this set of 15 highly-characterized cultivars allowed for a direct comparison of results to a wide range of established wheat physicochemical and baking quality parameters. A secondary objective of this study was to test the hypothesis that the moisture content of each individual gluten sample could be important to the interpretation of Gluten CORE results. Finally, this study aimed to further explore the analytical capabilities of the new Gluten CORE prototype by evaluating two additional CORE outcome measurements besides Recovery Index: Percent Compression, and Half-Recovery Time, in order to more fully examine the relationship between wheat gluten physicochemical properties and information provided by Gluten CORE analysis.

2.2 Materials and Methods

2.2.1 Materials

2% Sodium Chloride solution was prepared by dissolving 200.00g of fine crystalline NaCl to a final solution volume of 10 L. ACS Grade Sodium Chloride was obtained from Fisher Chemical (Agawam, MA).

Whole Meal Flours representing a set of 15 wheat cultivars were obtained from the United States Department of Agriculture Grain Inspection, Packers & Stockyard Administration (USDA GIPSA). The 15 cultivars included Hard Red Winter (HRW), Hard Red Spring (HRS), Soft Red Winter (SRW), Soft White (SWH), and Hard White (HDWH) wheats, and are described in Table 2-1. Whole meal flours were stored in zip-sealed polyethylene bags at room temperature for a period of 6-16 months before use.

Table 2-1 Background information about the 15 wheat cultivars studied

Wheat Class	Cultivar	Flour Protien Content (%) [a]	Baked Loaf Volume (mL) [a]	Gluten Index [a]	Sed. Value (mL) [a]	Degree of Elasticity after 500% extension Gluten (%) [a]	CORE Degree of Recovery Gluten (%) [b]	CORE Degree of Recovery Dough (%) [c]
HDWH	Blanca Grande	12.9	913	93.4	49	34.3	62.7	28.9
	Trego	10.4	744	97.6	38	31.5	41.4	11.2
HRS	Alsen	15.7	919	95.6	57	38.9	68.9	23.6
	Briggs	13.4	825	93.1	43	35.5	57.9	14.3
	Hollis	12.9	881	96.3	50	37.8	68.7	17.9
	McNeal	12.9	956	99.4	56	35.8	-	-
	Norpro	11.8	788	88.6	36	37.6	41.8	14.2
	Reeder	12.9	856	88.4	52	40.5	50.5	10.3
HRW	Jagalene	10	769	98.5	36	30.5	77.5	11.1
	Jagger	11	831	99.6	36	35.9	74.8	7.9
	Tam110	13.8	919	81.9	55	34.5	47.0	18.2
SRW	Patterson	8.5	738	65.1	15	33.4	-	-
	Roane	7.7	688	92.8	17	32.9	35.5	2.6
SWH	Eltan	11.1	863	81.5	27	35.4	29.1	4.0
	Stephens	11.4	675	42.7	22	28.2	5.0	-

a. Zhao et al., 2010
b. Chapman et al., 2012
c. Halabi, 2012.

2.2.2 Extraction of Gluten from Whole Meal Flour

Four to six replicate gluten samples were extracted for CORE Analysis from whole meal flour of each of the 15 cultivars studied. Glutomatic extraction was carried out using a modified version of the procedure developed by Chen (2011) for whole meal flours. 10.00g samples of each whole meal flour were hydrated with 6.5g of 2% NaCl, and transferred to a Glutomatic chamber equipped with a fine mesh sieve. The beginning

of the first Glutomatic wash cycle was manually controlled by restricting the flow of the wash solution to keep the wash feed rate of input from outpacing the rate at which the starchy effluent was able to drain. Once enough starch had been rinsed from the Glutomatic chamber that effluent was able to drain at a sufficient rate, the automatic Glutomatic "Wash 1" cycle was allowed to proceed as normal, separating starch from gluten and bran. The Glutomatic was stopped at the end of the "Wash 1" cycle to transfer partially washed material to clean Glutomatic chambers equipped with a large mesh filter of sufficient pore size to allow bran particles through. The Glutomatic was then restarted to complete the "Wash 2" cycle, yielding finished washed gluten samples for CORE analysis

2.2.3 Preparation of Gluten for CORE Analysis

Washed gluten was removed from the Glutomatic, formed into a ball, placed in a small airtight plastic container coated with petroleum jelly on the interior, and allowed to rest for 30 to 45 minutes at room temperature. Prior to CORE analysis, gluten samples were transferred to Perten centrifuge holders lubricated with petroleum jelly and centrifuged for 5 minutes to standardize their dimensions for the CORE analysis.

2.2.4 CORE Analysis

CORE analysis was carried out using the protocol described in Chapman (2011), using the Gluten CORE apparatus and software, calibrated according to Perten's protocol. Samples were expelled from centrifuge holder/shapers directly onto the petroleum jelly lubricated CORE platform. Gluten compression used a force of 8 Newtons, for 5 seconds, followed by a recovery period of 55 seconds. Height measurements of samples were recorded by the Perten software every 0.1 seconds during

the CORE cycle. Calculated outcome variables from the CORE analysis, and the measured parameters used in their calculation, are defined in Table 1-2 on Page 27.

2.2.5 Moisture Content Determination

It was determined mid-way into data collection for this experiment that the moisture contents of individual gluten samples were of interest to this study. Unfortunately, samples tested by CORE analysis before this revelation had already been discarded, and could not be tested for moisture content (MC). However, measurements were still obtained for the moisture content of at least two samples representing each cultivar. When moisture measurements were obtained, they were carried out after CORE analysis by weighing gluten samples before and after sample dehydration. Gluten dehydration was carried out by heating in a Perten "Glutork" gluten drying apparatus for at least the four minute standard heating cycle, or longer if necessary. Completion of drying was determined by stability of the dried sample weight against further heating cycles and by observation that the sample underwent a glassy transition. Moisture Content, MC (%), which is also expressed as Percent Moisture (PM) in this thesis, was determined as the sample's measured moisture loss divided by the initial sample mass.

2.2.6 Statistical Analyses

Averages and Standard Deviations of Recovery Index (RI), Percent Compression (PC), Half-Recovery Time (HRT), and Moisture Content (MC) measurements were calculated for each set of replicates. Student's t-tests were performed to determine whether average measurements for each set of replicates were significantly different from one another, using $\alpha = 0.05$.

CORE Thickness Average Curves were constructed for gluten from each cultivar by averaging thickness data for all CORE test replicates at each time point during the compression and recovery periods.

Apparent Percent Recovery Average Curves were constructed for gluten from each cultivar by averaging the Degree of Recovery (%) for all replicates at each time point during the 55 second recovery period.

Relationships between Measured Outcome Variables were explored by creating a scatter plot matrix with all individual measurements of Recovery Index (RI), Percent Compression (PC), Half-Recovery Time (HRT), and Moisture Content (MC). Correlations between outcome variables across all replicates of all cultivars were calculated by linear regression. Scatter plots were also examined qualitatively for non-linear relationships and trends.

Relationships between CORE Analysis Outcome Variables and other Wheat Quality Measurements were explored by examination of scatter plots and calculation of correlations between Gluten Recovery Index averages (of whole meal flour extracted gluten), and values for Gluten Index, Baked Loaf Volume, Protein Content, and Sedimentation Value (all pertaining to refined flour milled from the same cultivars.)

2.3 Results and Discussion

2.3.1 Summary of Results

Table 2-2 gives a summary of average measurements, replicate counts and standard deviations, and identifies groups of results within each column which do not differ significantly. Information from Table 2-2 is also shown visually in Figure 2-1.

Of the three CORE variables analyzed, Recovery Index demonstrates the widest range of variation as well as the smallest relative standard deviations. This supports the choice of Recovery Index as the CORE parameter of interest, as it is able to discriminate well between gluten samples sourced from different cultivars.

The results of the partial Moisture Content analysis indicate significant differences between the levels of water bound in gluten samples from different cultivars. All moisture contents measured were between 61 and 68%, but the moisture content for each set of replicates appears to fall within a narrower hydration level that can be assumed to be characteristic of the cultivar.

Table 2-2 Summary of RI, PC, HRT, and MC data for gluten extracted from whole meal flour of 15 wheat cultivars.

Class	Cultivar	n	Recovery Index (RI)	Percent Compression (PC)	Half Recovery Time (HRT)	Moisture Content (MC)
HDWH	Blanca Grande	4	38.2 ± 5.3 *f*	73.0 ± 1.5 *c,d*	3.60 ± 0.67 *a,b,c,d*	66.0 ± 1.1 (n=3) *b*
	Trego	4	53.2 ± 8.0 *c,d*	72.3 ± 1.2 *c,d,e*	3.18 ± 0.70 *b,c,d*	64.7 ± 0.3 (n=2) *c,d,e*
HRS	Alsen	4	56.4 ± 1.9 *c*	70.8 ± 1.1 *e*	2.90 ± 0.29 *d,e*	64.0 ± 0.1 (n=2) *e*
	Briggs	4	54.6 ± 2.7 *c*	72.4 ± 0.9 *c,d,e*	3.23 ± 0.43 *b,c,d*	64.7 ± 0.6 (n=2) *c,d,e*
	Hollis	4	47.9 ± 6.4 *d,e*	73.7 ± 2.0 *c,d*	3.88 ± 0.82 *a,b,c*	65.4 ± 0.3 (n=2) *b,c,d*
	McNeal	4	67.9 ± 4.7 *a,b*	71.8 ± 1.8 *d,e*	1.78 ± 0.17 *f,g*	64.5 ± 0.4 (n=2) *d,e*
	Norpro	4	25.9 ± 3.9 *h,i*	77.0 ± 0.9 a	4.18 ± 0.41 *a*	65.8 ± 0.0 (n=2) *b,c*
	Reeder	4	54.8 ± 3.3 *c*	73.5 ± 0.7 *c,d*	3.78 ± 0.75 *a,b,c*	64.7 ± 1.0 (n=3) *c,d,e*
HRW	Jagalene	4	71.2 ± 1.4 *a*	73.1 ± 1.2 *c,d*	1.23 ± 0.17 *g*	62.3 ± 1.0 (n=2) *f*
	Jagger	4	63.4 ± 3.1 *b*	73.8 ± 0.8 *c,d*	2.28 ± 0.43 *e,f*	64.2 ± 0.6 (n=2) *d,e*
	Tam110	4	45.0 ± 1.7 *e*	73.1 ± 1.2 *c,d*	4.10 ± 0.75 *a*	66.6 ± 0.2 (n=2) *a,b*
SRW	Patterson	4	28.9 ± 7.6 *g,h*	76.9 ± 2.6 *a*	3.93 ± 0.65 *a,b*	64.6 ± 0.2 (n=2) *c,d,e*
	Roane	4	34.4 ± 0.8 *f,g*	74.1 ± 1.5 *b,c*	4.10 ± 0.89 *a*	66.4 ± 0.5 (n=3) *b*
SWH	Eltan	6	22.7 ± 5.3 *i*	75.8 ± 1.9 *a,b*	3.17 ± 0.14 *c,d*	67.6 ± 0.3 (n=6) *a*
	Stephens	4	9.0 ± 0.8 *j*	77.3 ± 0.4 *a*	1.15 ± 0.37 *g*	63.5 ± 0.8 (n=4) *f*

Values given as "±" are standard deviations. Within each column, results not connected by the same letter are significantly different at $\alpha = 0.05$.

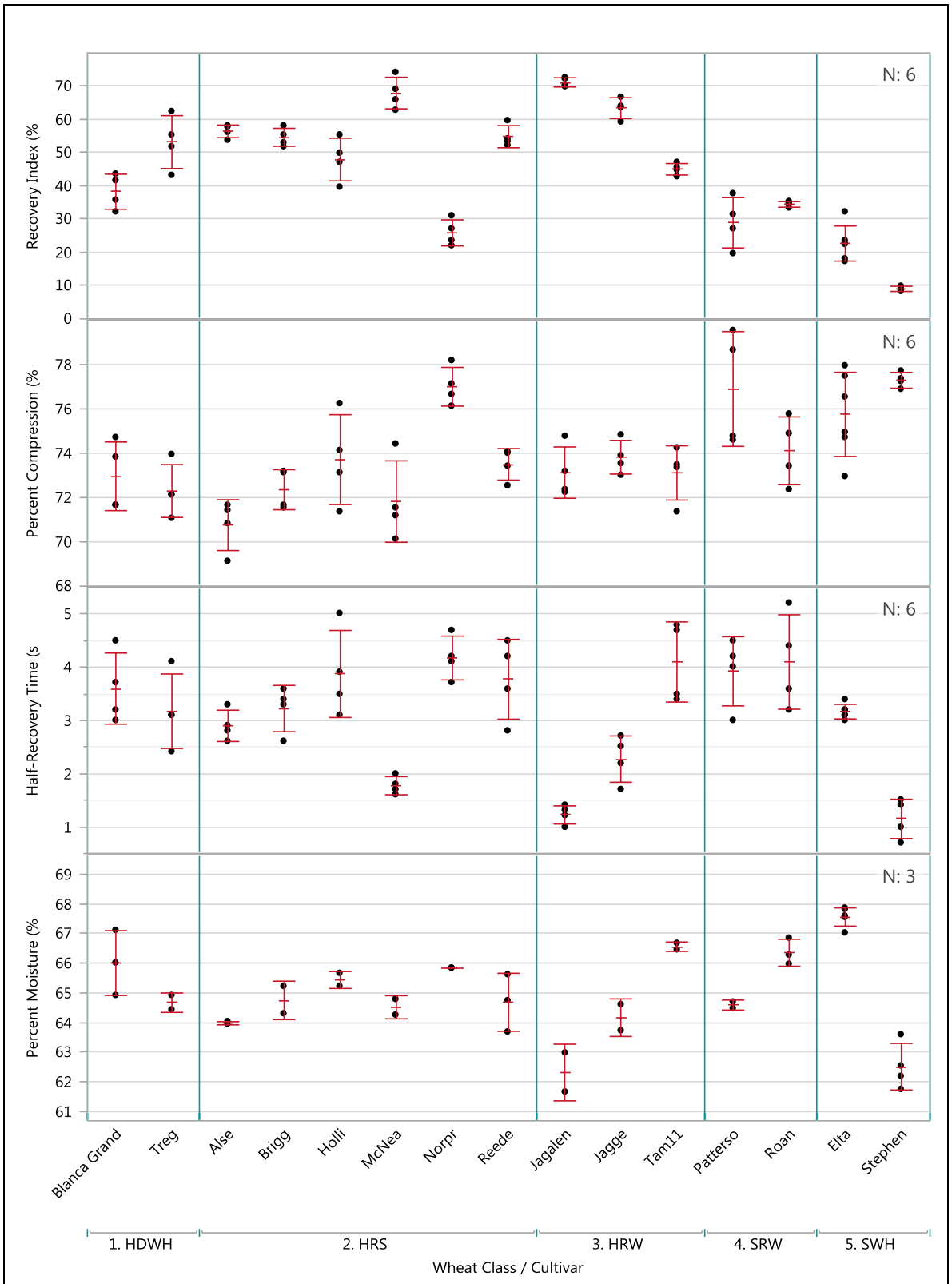


Figure 2-1 Visualization of replicate measurements, averages and standard deviations of RI, PC, HRT and PM for gluten extracted from whole meal flour of 15 wheat cultivars.

2.3.2 Average Curves from CORE Analysis

Figure 2-2 shows Average Thickness Curves from CORE analysis, which were obtained by averaging the Degree of Recovery (%) for all replicates of each cultivar, at each time point during the 5 second compression period and the 55 second recovery period. Each cultivar's average initial sample thickness and final sample thickness are indicated by the shaped markers. The wide variation in each cultivar's initial thickness can be attributed to the wide range in wet gluten yield obtained from the 10g whole meal flour, which likely largely due to variability in cultivar protein content and protein hydration. Variation in the percent of yield loss characteristic of different cultivars' gluten during gluten extraction could also affect these values.

Figure 2-3 shows Average Recovery Curves from CORE analysis, which were constructed by averaging the Degree of Recovery (%) for all replicates at each time point during the 55 second recovery period, and plotting these results with time on a log axis. Shaped markers indicate each cultivar's average final Recovery Index, as well as each cultivar's average "Half-Recovery Time" point.

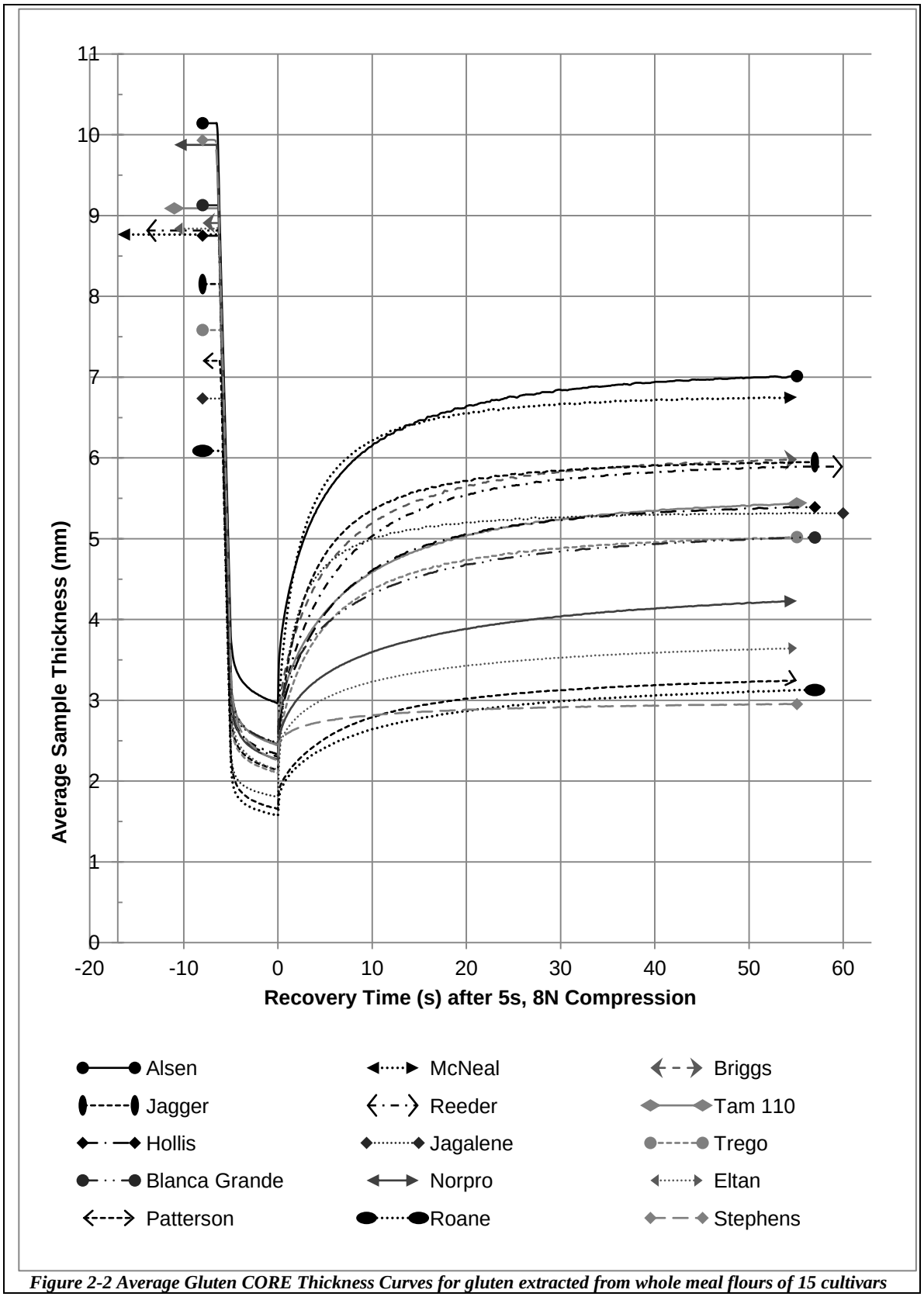


Figure 2-2 Average Gluten CORE Thickness Curves for gluten extracted from whole meal flours of 15 cultivars

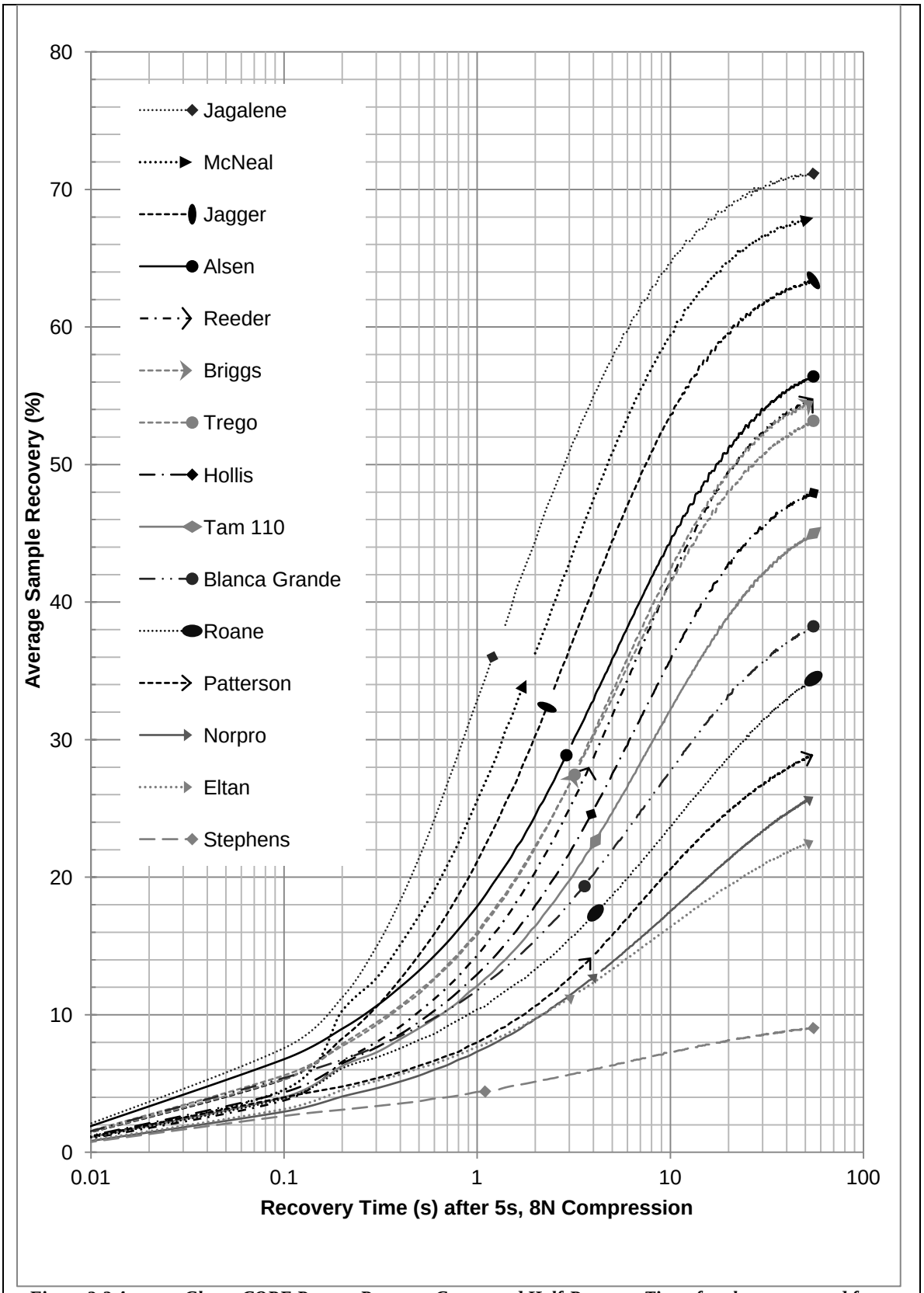


Figure 2-3 Average Gluten CORE Percent Recovery Curves and Half-Recovery Times for gluten extracted from whole meal flours of 15 wheat cultivars

2.3.3 Relationships between Measured Outcome Variables

Figure 2-4 shows a scatterplot matrix with individual sample measurement data points shown by cultivar and overall correlations for RI, HRT, PC, and MC. There appears to be no relationship between Half-Recovery Time and Percent Compression data, indicating that the majority of factors affecting these two parameters are independent. A complicated relationship can be seen between Recovery Index and Half-Recovery Time data. Gluten samples with the highest Recovery Indexes appears to exhibit faster Half-Recovery Time measurements, while gluten of mid-range elasticity appears to have perhaps slower, yet more variable Half-Recovery Time measurements. Finally, evidence for two linear relationships can be seen: Recovery Index with Percent Compression, and Half Recovery Time with Moisture Content.

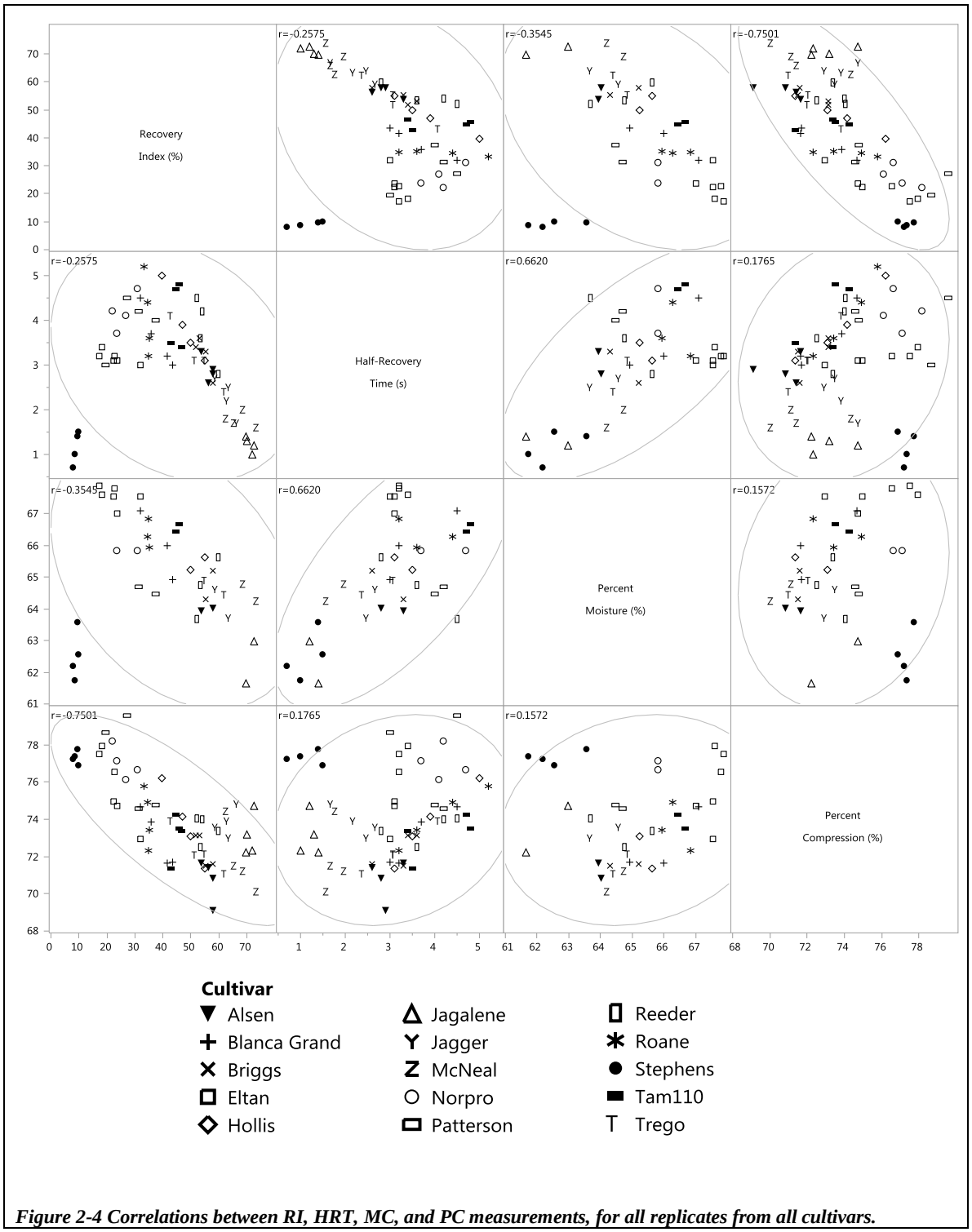


Figure 2-4 Correlations between RI, HRT, MC, and PC measurements, for all replicates from all cultivars.

Gluten Samples with Higher Hydration had Longer Half-Recovery Times. Half-Recovery Time and Percent Moisture correlate better with each other than either does with Recovery Index or Percent Compression. A consideration of the role of hydrogen bonds in the rheology of hydrated gluten may help to explain this result. The water bound in a gluten matrix is associated with glutamine-rich, hydrophillic interior domains in the HMW glutenin subunits, which lie between disulphide cross-linking end domains. Although single hydrogen bonds are much weaker than covalent bonds, the collective cohesive effect of many hydrogen bonds between neighboring glutenin subunits can be significant, and in fact the overall insolubility of gluten can be attributed to this (Belton, 1999). In a low hydration gluten network, the glutamine-rich interior domains of glutenin are forced to hydrogen-bond to each other, forming "trains" rather than "loops". At higher hydrations, free water becomes available to act as an alternative hydrogen-bonding partner, allowing highly hydrated loops to form (Shewry et al., 2001) This is illustrated in Figure 2-5, below. The lower moisture gluten samples observed in this study, then, might be expected to have a lower loop-to-train ratio and therefore more protein-protein hydrogen bonding contributing to the gluten network's cohesive forces.

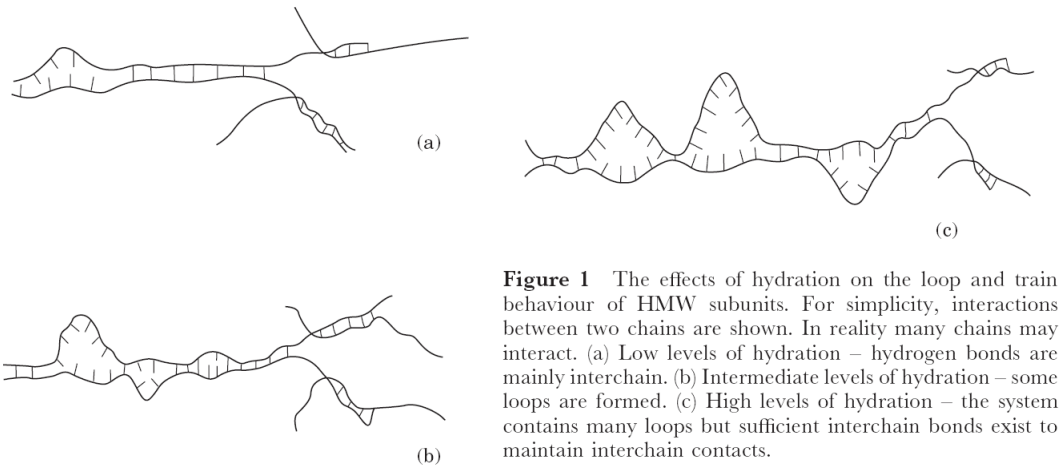


Figure 1 The effects of hydration on the loop and train behaviour of HMW subunits. For simplicity, interactions between two chains are shown. In reality many chains may interact. (a) Low levels of hydration – hydrogen bonds are mainly interchain. (b) Intermediate levels of hydration – some loops are formed. (c) High levels of hydration – the system contains many loops but sufficient interchain bonds exist to maintain interchain contacts.

Figure 2-5 Loop to train ratio increases with gluten hydration. (From Belton, 1999)

Uniaxial compression of the gluten matrix extends the HMW-GS network biaxially. The hydrated loop regions of the HMW-GS domains are likely the most easily deformed. Once the loops have been pulled taut, the sticky protein-protein hydrogen-bonded train regions will begin to slide past one another, eventually restoring the previous loop-train equilibrium and returning the extended protein chains to a favorable, free conformation. This sequence of response to deformation is illustrated in Figure 2-6, below. Similarly, but on a longer time scale, disulphide crosslink interchange will also proceed, and can be expected to eventually restore the covalently bonded protein network to a favorable, non-extended, conformation. If the deforming force is removed from the gluten matrix before the hydrogen-bonded sticky train regions and/or the covalent crosslinks have finished re-associating into the deformed shape, however, a restoring force will result to restore the conformational entropy of the network.

Figure 2-6 Gluten loops and trains undergoing deformation: hydrated protein loops (a) are deformed first (b), followed by the slippage of protein-protein trains (c). (Adapted from Belton, 1999)

The time required for the gluten samples observed in this experiment to recover half their ultimate recovery distance ranged from approximately 1 second to 5 seconds. This time scale is comparable to the 5 second compression period that was used. The 55 second recovery period which samples had to reach their full "recovery", however, is an order of magnitude longer than both the compression period and the half-recovery time measurements. A possible explanation of the observed positive correlation between Half-Recovery-Time and Percent Moisture, then, could be that the greater proportion of trains in the less hydrated gluten samples are effectively giving the gluten network more "short term" crosslinks, which increases the degree with which the recovery force can act during the first few seconds of the recovery period. As the longer time scale of the full 55 second recovery period proceeds, however, the effective crosslink contribution of the hydrogen bonded trains is attenuated, due to re-association of the sticky train regions into the new, partially recovered, gluten sample shape and dimensions. This hypothesis would be an interesting topic for further investigation.

Gluten Samples with higher Recovery Indexes had lower Percent Compressions. Percent Compression and Recovery Index correlate better with each other than either does with Half-Recovery Time or Percent Moisture. This result is not unexpected, since both the "resistance to compression" and the "elasticity" of a gluten sample might be generally interpreted as descriptors of "gluten strength". A heavily cross linked gluten sample that undergoes significant strain hardening to counteract the constant 8N, 5s deforming force would exhibit a lower "percent compression" measurement, and would also be expected to exhibit a stronger elastic recovery.

2.3.4 Understanding Wheat Quality: What does Gluten Extracted from Whole Meal Flour and CORE Analysis have to offer?

Is gluten extracted from whole meal flour a valid experimental material for wheat quality testing?

A potential problem with the prospect of adapting whole meal flour as a material useful in rapid testing of elasticity with the Gluten CORE is the difficulty of the whole meal flour gluten extraction procedure used in this study. The modification to the Glutomatic gluten extraction procedure (which is based on a method designed for refined flour), which was used in this experiment, is not optimized. Manual control of the wash fluid flow rate at the beginning of the first Glutomatic wash cycle was required to prevent the Glutomatic chamber from overflowing; this was not only difficult to carry out and tedious, but also introduced an unwanted source of potential variation to the results of this experiment. Furthermore, manually transferring partially extracted wheat gluten to a wash chamber equipped with a coarser mesh halfway through each gluten extraction cycle was also a source of procedural difficulty and potential yield loss. Even if whole meal flour is determined to be a valid source material for gluten to be used in wheat quality testing, improvements to the method of gluten extraction should be investigated.

It was seen in Section 2.3.1 that whole meal flour derived gluten produced reproducible measurements of Recovery Index and other outcome variables, which supports the hypothesis that whole meal flour derived gluten is a valid material of study. Figure 2-7 compares the CORE Recovery Index measurements obtained in this study for gluten extracted from whole meal flour with CORE Recovery Index measurements from Chapman et al. (2012), for gluten from refined flours of the same set of cultivars (excluding McNeal and Patterson, which were not reported by Chapman). Error bars

show similar standard deviations across the two datasets, suggesting that whole meal flour derived gluten is no more problematic than refined flour derived gluten in terms of CORE Recovery Index reproducibility. A best-fit line equation, shown in the figure, demonstrates that overall, gluten samples derived from whole meal flour have proportionally lower CORE Recovery Indexes than gluten samples derived from refined flour. This result is understandable, because the whole meal flour derived samples were observed to retain a small percentage of insoluble wheat bran flakes, and it is possible that lipids from wheat germ could have also been taken up by the gluten matrix. Both of these impurities would not be expected in gluten derived from refined flour, yet would be expected to reduce the cohesiveness of the gluten network, and therefore also the gluten sample's potential for elastic recovery.

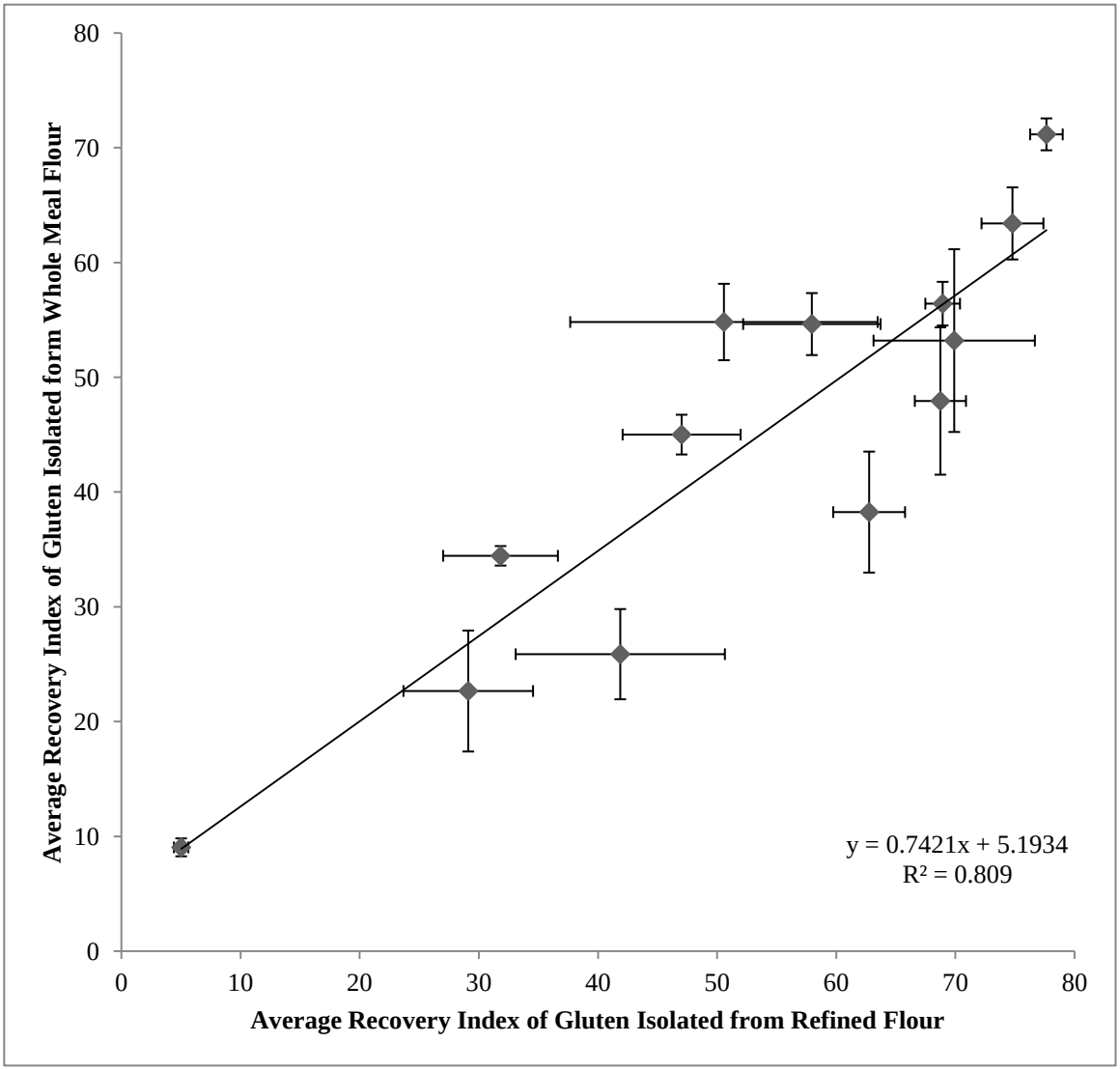


Figure 2-7 Comparison of CORE Recovery Index measurements of gluten extracted from Whole Meal Flour to Gluten extracted from Refined Flour.

Comparison of results to established wheat quality measurements. Figure 2-8 shows outcome variables from this study compared to four common measurements of wheat quality, and Table 2-3 lists the correlations observed between and within these 8 variables.

The Outcome Variable that Gluten Index correlates highest with (r = 0.783) is WMF Recovery index. “Gluten Index” is a measurement of the relative proportion of macropolymeric glutenin present in gluten, and is determined by centrifugally separating

gluten using a sieve though which macropolymeric material will not pass. Since gluten with a higher polymeric component would have a higher crosslink and entanglement density, it would in fact be expected to be more able to store the energy of deformation as recoverable, thus resulting in higher Recovery Index measurements.

The Outcome Variable that Baked Loaf Volume correlates highest with (r = 0.5811) is Percent Compression. Percent Compression describes the degree to which the 8 Newton compression force is able to reduce the thickness of the gluten within the 5 second compression period. The bi-axial compression of the gluten sample in the CORE analysis is comparable to the mode of deformation a layer of dough surrounding a growing air cell undergoes. The percent compression of a gluten sample should indicate the gluten's potential for strain-hardening, and thus the ability of the gluten network surrounding an air cell in dough to provide resistance to the expanding gas to allow the loaf to reach greater volume without collapsing.

Baked Loaf Volume is more highly correlated with Flour Protein Content than any outcome variable from CORE analysis. If BLV is defined as the most relevant wheat quality measurement method, then Flour Protein Content and Flour Sedimentation Volume (which is a method of approximating flour gluten content), with their high correlations with BLV of 0.803 and 0.855, respectively, should be taken to be better indicators of a flour's potential baking quality overall than CORE Recovery Index or Percent Compression, which have correlations with BLV of only 0.454 and -0.581.

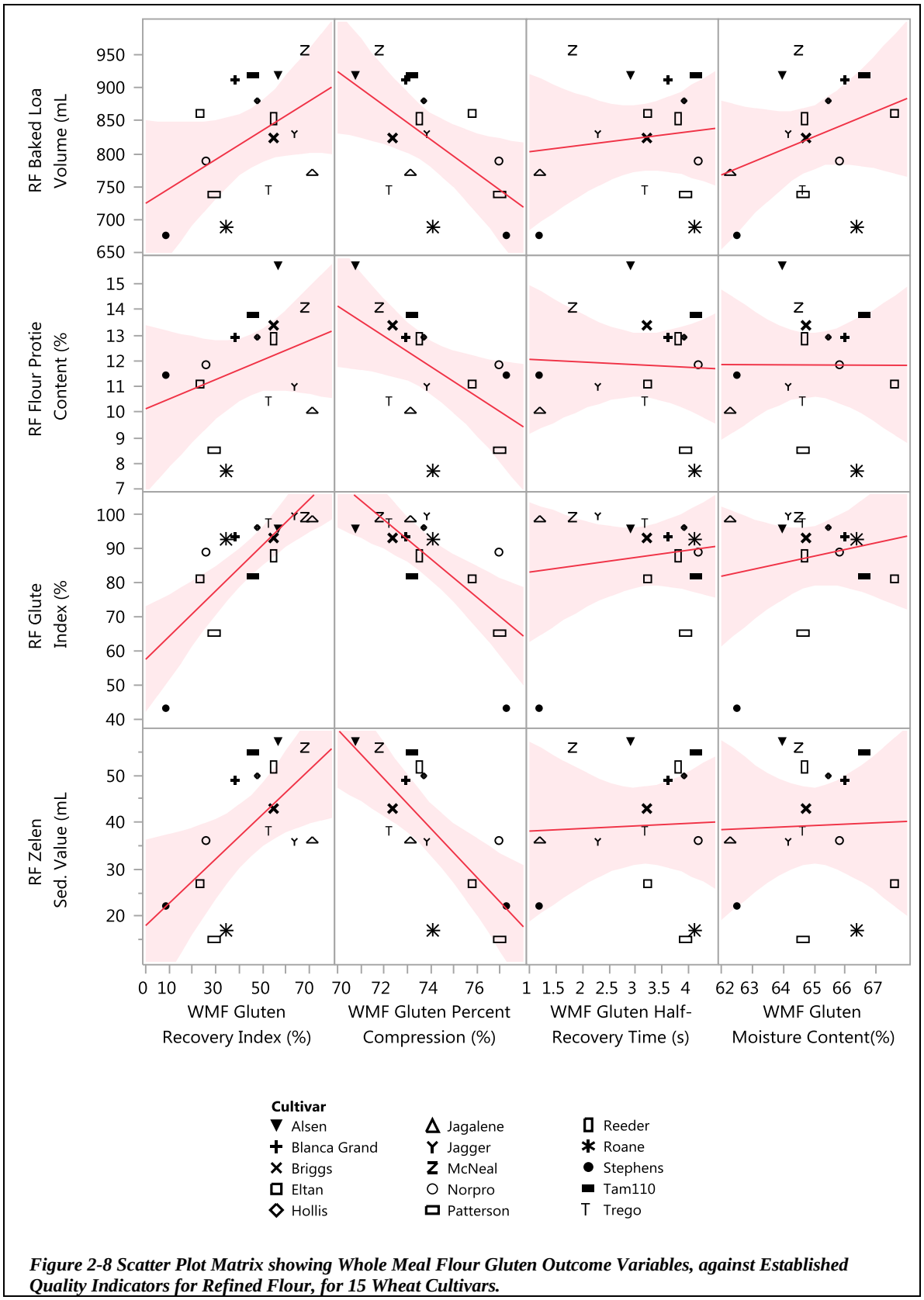


Figure 2-8 Scatter Plot Matrix showing Whole Meal Flour Gluten Outcome Variables, against Established Quality Indicators for Refined Flour, for 15 Wheat Cultivars.

Table 2-3 Correlations between average measured WMF Gluten RI, PC, HRT, and MC and other wheat quality indicators across 15 wheat cultivars.

	Refined Flour Quality Indicators				*WMF Gluten Outcome Variables*			
	BLV	**Flour Protein**	**Gluten Index**	**Sed. Vol.**	**RI**	**PC**	**HRT**	**MC**
BLV		**0.809**	**0.483**	**0.855**	**0.454**	**-0.581**	**0.119**	**0.314**
Flour Protein	**0.809**		**0.225**	**0.890**	**0.318**	**-0.544**	**-0.050**	**-0.003**
Gluten Index	**0.483**	**0.225**		**0.531**	**0.783**	**-0.732**	**0.144**	**0.182**
Sed. Vol.	**0.855**	**0.890**	**0.531**		**0.602**	**-0.737**	**0.040**	**0.030**
RI	**0.454**	**0.318**	**0.783**	**0.602**		**-0.811**	**-0.260**	**-0.317**
PC	**-0.581**	**-0.544**	**-0.732**	**-0.737**	**-0.811**		**0.092**	**0.078**
HRT	**0.119**	**-0.050**	**0.144**	**0.040**	**-0.260**	**0.092**		**0.762**
MC	**0.314**	**-0.003**	**0.182**	**0.030**	**-0.317**	**0.078**	**0.762**	

Could combining measurements of flour protein quantity with quality be an improvement over looking at just one or the other measurement? Figure 2-9 shows the WMF Recovery Index average results obtained in this study plotted against BLV averages and a best fit line calculated by linear regression. The Flour Protein Content of each cultivar is represented in this figure by the darkness of the data point. It is intriguing that in general, the higher protein (darker) cultivars tend to fall above the line of best fit, while the lower protein (lighter) cultivars fall below. This would suggest that perhaps, Baked Loaf Volume could be considered some function of flour protein *quantity* combined with the intrinsic elastic *quality* of a cultivar's gluten.

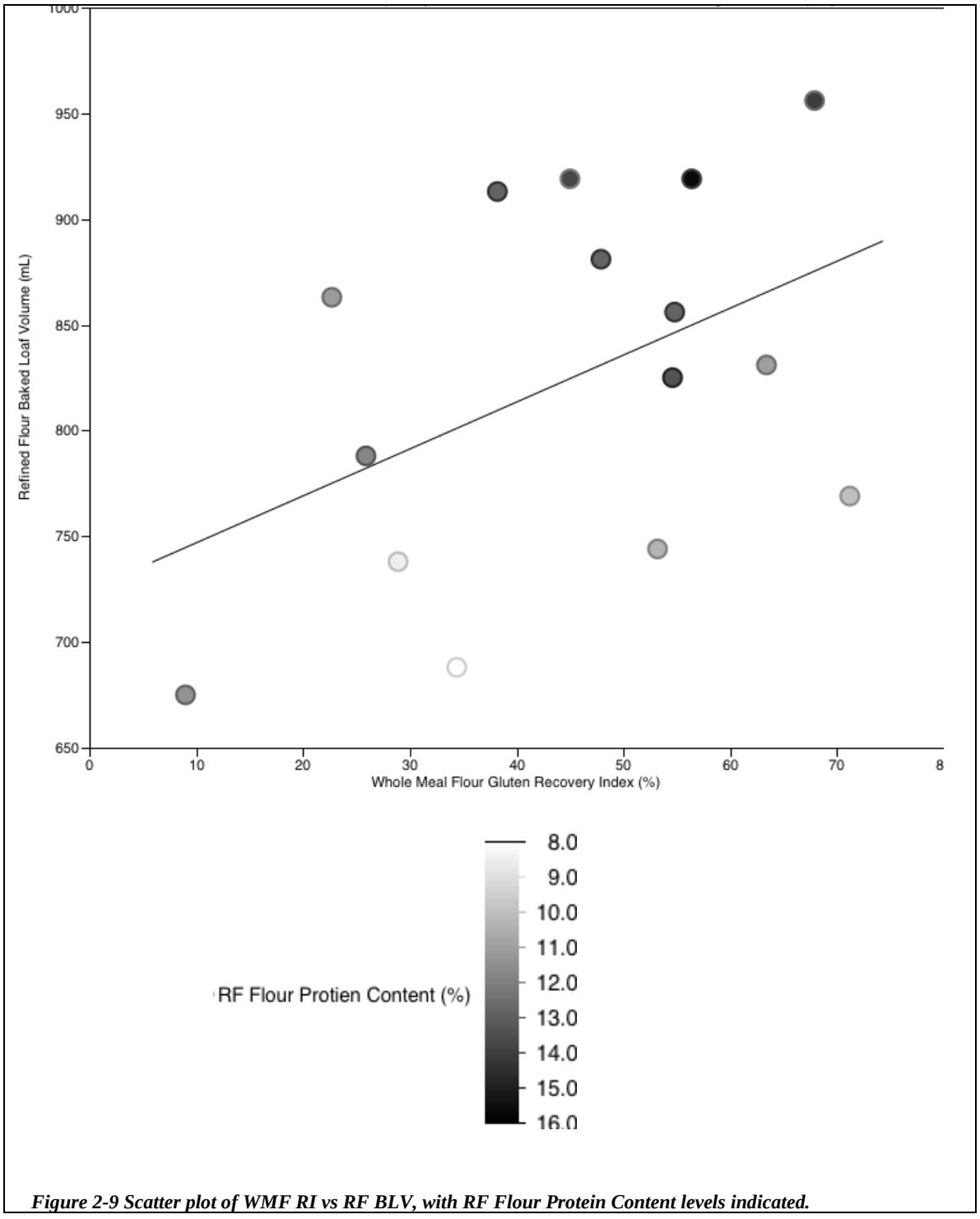


Figure 2-9 Scatter plot of WMF RI vs RF BLV, with RF Flour Protein Content levels indicated.

Considerations for a multivariable model of wheat quality. Figure 2-10 (F) shows the best fit line of BLV vs Refined Flour Protein Content for the 15 cultivars studied. Figure 2-10 (A) shows the same best fit line's BLV residuals. These residuals represent the variation in BLV that is *not* explained by Flour Protein Content. Since Protein Content is easily and routinely measured in flour, and correlates well with BLV, it assumed here to be a good choice for use as a first-order predictor of BLV. To explore the possibility of using the four outcome variables obtained in this study as second-order predictors of BLV, WMF Gluten outcome variables RI, PC, HRT, and MC are shown plotted against the BLV residuals from Protein Content, in Figure 2-10 (B-E).

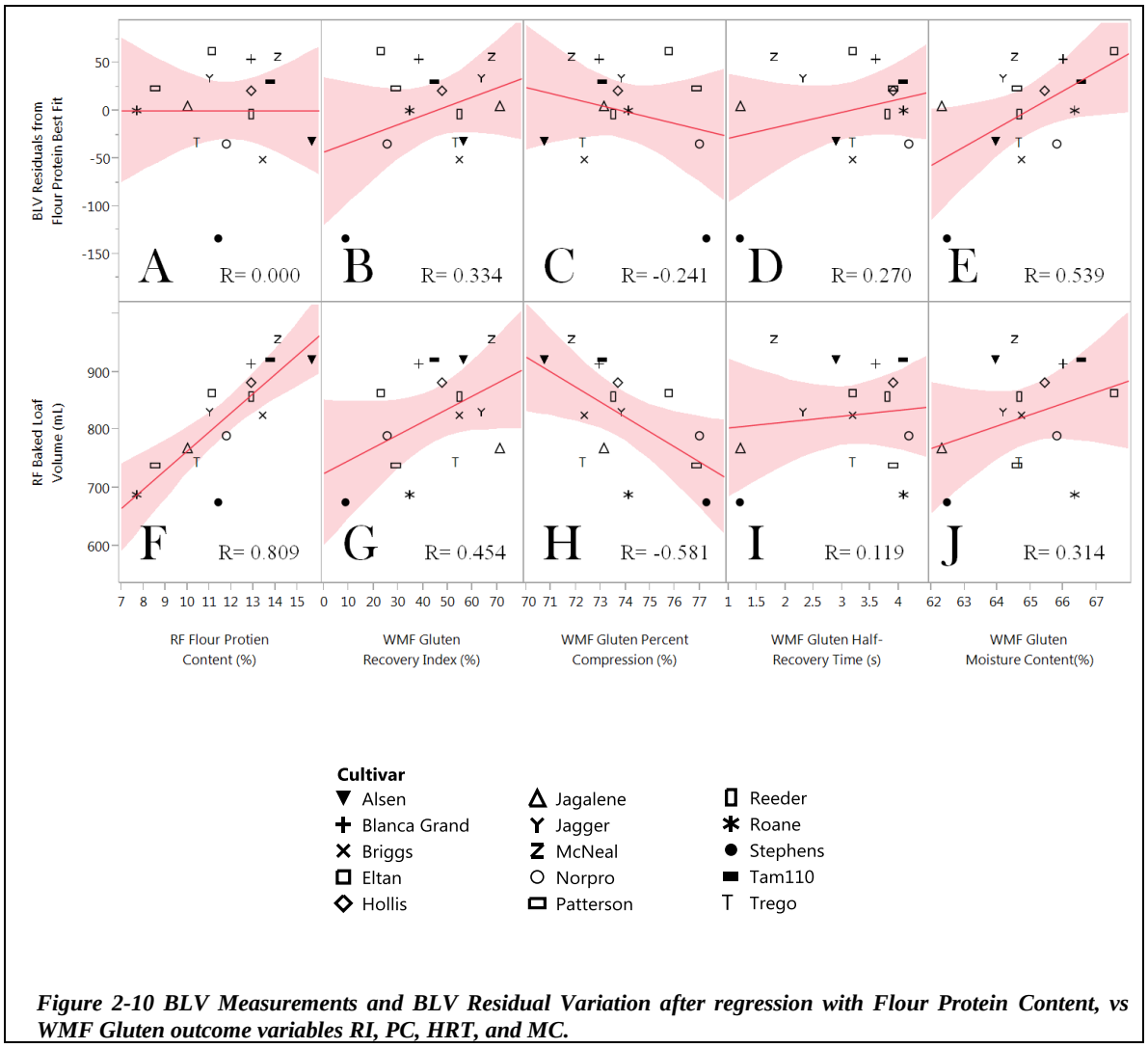


Figure 2-10 BLV Measurements and BLV Residual Variation after regression with Flour Protein Content, vs WMF Gluten outcome variables RI, PC, HRT, and MC.

Figure 2-10 (E) shows highest correlation between BLV Residuals from Protein Content and WMF Gluten outcome variables is Moisture Content (r=0.539). This suggests that, of all the outcome variables measured in this study, the WMF Gluten Moisture Content is the best choice of a second order predictor of BLV after Flour Protein Content. A potential reason WMF Gluten MC correlates with BLV residuals after regression against Flour Protein Content is that flour that has gluten with higher water-binding capacity will require more water to reach optimum dough hydration. Since BLV is measured using standard loaves baked from dough prepared with a constant amount

(100g) of flour, the doughs prepared from flour containing high-hydration gluten, when hydrated to "optimum" levels, will weigh more and have a greater volume. These more voluminous doughs would then be expected to be more able to expand into higher volume bread loaves.

Figure 2-10 (B) shows the second highest correlation between BLV Residuals after Protein Content among WMF Gluten outcome variables is Recovery Index (r=0.334). It is interesting that although the Percent Compression variable correlated better with BLV alone than Recovery Index, Recovery Index had a better correlation with the BLV Residuals than did Percent Compression. This suggests that among CORE calculated outcome variables, Recovery Index could actually be the most meaningful metric, when considered along with flour protein content, in terms of BLV prediction.

Refined Flour BLV can be modeled with a high correlation using three variables: Flour Protein Content, WMF Gluten MC, and WMF Gluten RI. A multivariable model was fitted using a standard least squares method of multiple regression. RF Baked Loaf Volume was used as the outcome role variable, and RF Flour Protein Content, WMF Gluten Moisture Content, and WMF Gluten Recovery Index were used as Model Effects. This regression was carried out using direct measurements of individual WMF replicate gluten RI and MC samples, but average values pertaining to each cultivar's BLV and refined flour protein content. All three model effects were found to be significant at $\alpha = 0.05$.The resulting best fit equation, with a high correlation of r=0.9524 (r^2=0.86) follows:

$$\textbf{\textit{Refined Flour BLV}} = -1328 + (28.24 \times \textbf{\textit{Refined flour protein content}}) + (1.815 \times \textbf{\textit{WMF Gluten Recovery Index}}) + (26.74 \times \textbf{\textit{WMF Gluten Moisture Content}})$$

Figure 2-11 shows the predicted vs actual BLV plot and a residual plot from this best fit regression, as well as the leverage plots for each of the contributing variables: Flour protein content, WMF Gluten RI, and WMF Gluten MC.

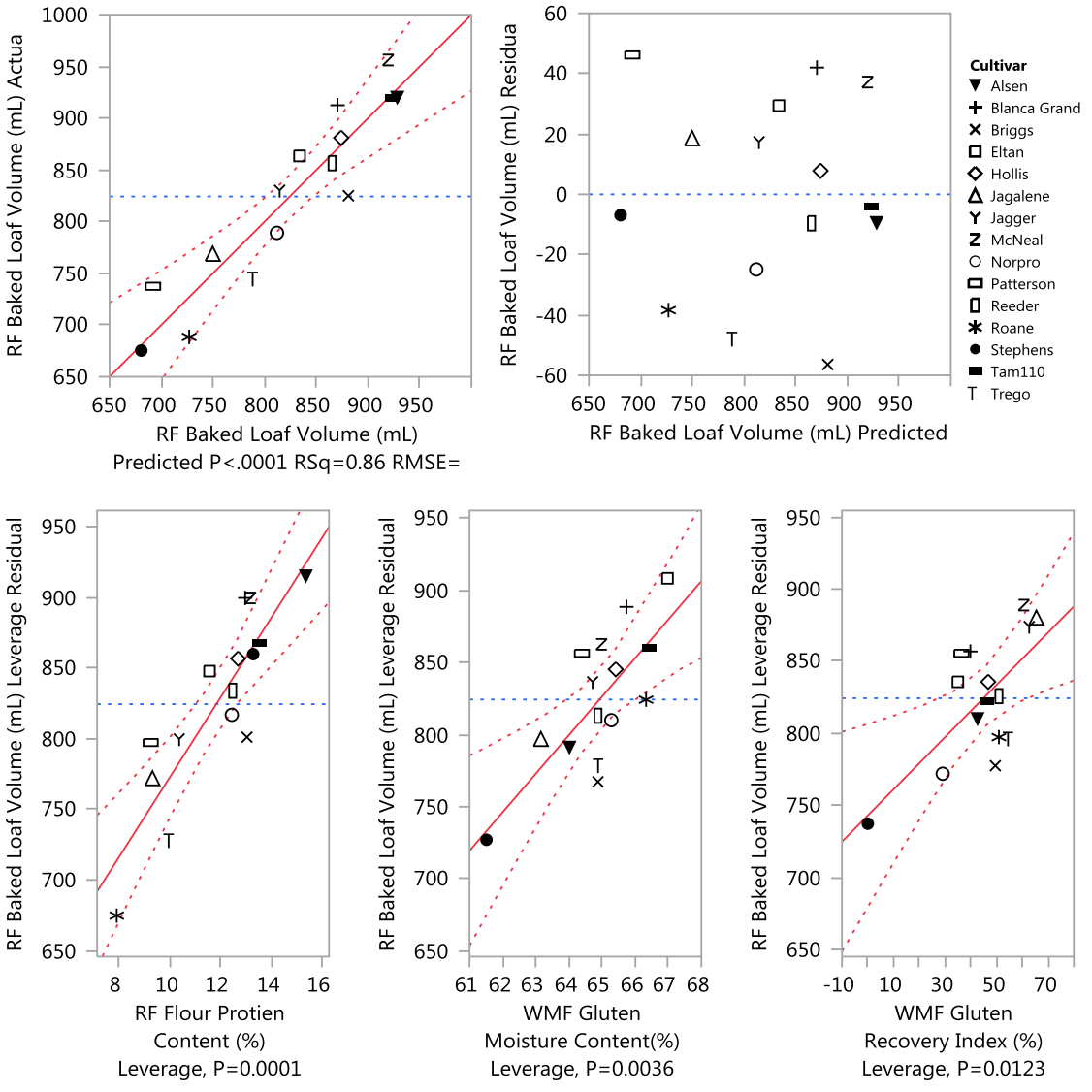


Figure 2-11 Predicted vs Actual BLV Plot, Predicted vs Actual Residuals, and Model Leverage Plots for RF Protein Content, WMF Gluten RI, and WMF Gluten MC.

Although correlation values for multivariable regression models should not be directly compared to correlation values for two-variable regressions, such as those found

in Table 2-3 (on page 50), or Table 1-1 (on page 24), this result is still exciting. The significance assigned to all three variables in this analysis suggests a way in which Gluten CORE analysis could be used to establish a meaningful 3-measurement summary of both the protein quality and quantity that could be useful in defining the bread-making properties of a harvest of wheat.

Considering the results of this analysis, in hindsight, it seems understandable that a hydrated gluten sample's CORE behavior might be only considered applicable to the properties of the wheat the gluten has been extracted from if both: (1) the water content of the hydrated gluten, and (2) the protein content of the flour the gluten has been extracted from, are known. Gluten moisture content can be considered relevant to interpreting CORE behavior because gluten with a strong elastic response while also maintaining a high hydration should be more valuable to bread-making than gluten with a equivalent elastic response that does not hold as much moisture. Similarly, the protein content of the flour from which the gluten is extracted can be considered relevant to interpreting CORE behavior since even if the gluten in a wheat sample has very strong elastic and moisture holding abilities, a low level of protein could still result in an overall weakened and dilute gluten network and therefore low baking quality. In summary, a proposed three-step model for evaluating the likely quality of a wheat flour could consist of three questions, shown in Figure 2-12, allowing the Gluten CORE Recovery Index measurement to be converted, using additional information, to a prediction of Baked Loaf Volume. The predictive ability of the multivariable regression model this conceptual model is based on should be tested in future research, using additional cultivars, and

perhaps using more traditionally-extracted gluten samples, to further explore and verify the results obtained in this study.

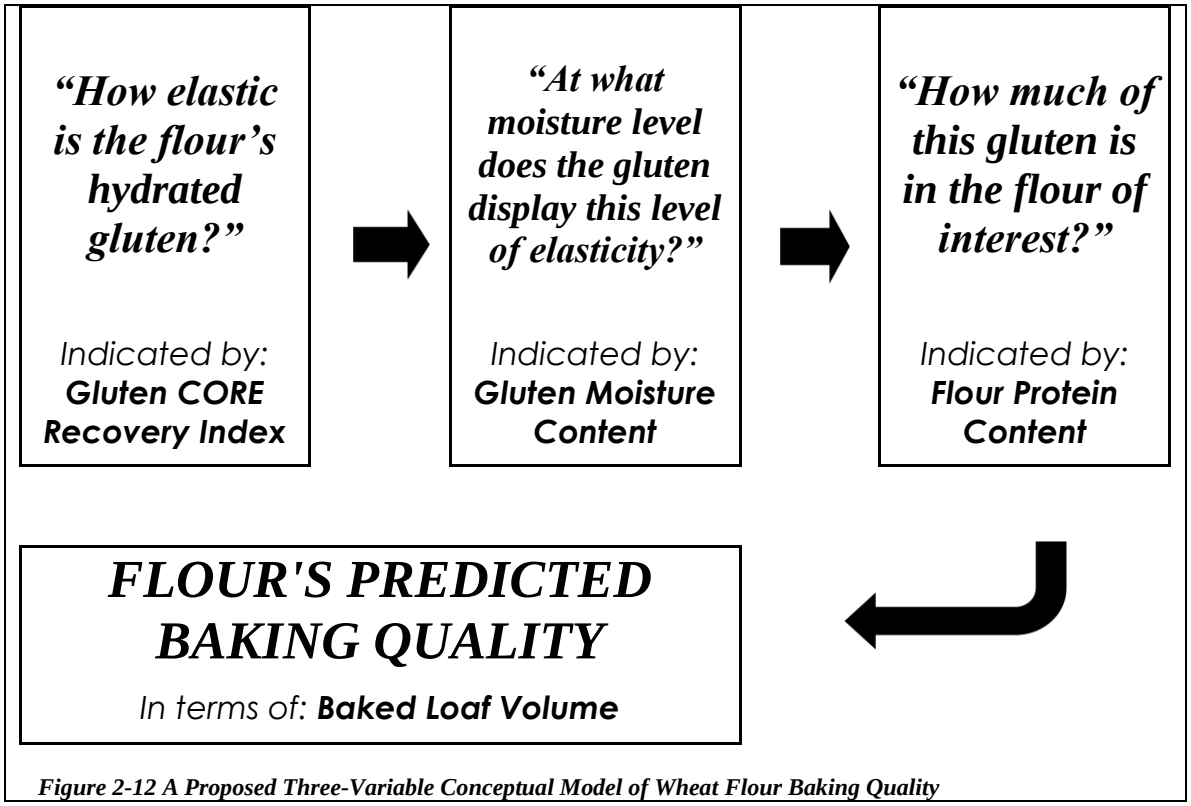


Figure 2-12 A Proposed Three-Variable Conceptual Model of Wheat Flour Baking Quality

2.4 Conclusions

This study has shown that gluten extracted from whole meal flour exhibits CORE behavior comparable to gluten extracted from refined flours from the same wheat cultivars. The practicality of the use of whole meal flour as a source material for Gluten CORE analysis will depend, however, on the development of a more reliable extraction procedure than that used in this study.

Wet Gluten Moisture Content and Flour Protein Content may be useful factors when interpreting the Wet Gluten CORE Recovery Index in terms of flour baking quality.

Multivariable analysis of these three factors could prove to be predictive of a flour's bread making quality, but further study and verification will be required.

Analysis of gluten sample Moisture Content and CORE outcome variables suggests that gluten samples with lower hydration levels undergo elastic recovery more quickly, which is possibly due to a greater contribution of deformed hydrogen-bonded "trains" to the recovering force than is found in gluten samples with higher hydration.

Chapter 3. Characterization of Changes in CORE Behavior of Gluten Treated with Variable Doses of β-Glucan-Containing Oat Bran Materials

3.1 Introduction

3.1.1 Background & Motivation

(1-3,1-4)-β-D-glucans found in the soluble fiber of oats (*Avena sativa*) have been shown to impart numerous health benefits, such as reduction of LDL cholesterol, improved satiety, and the reduction of the glycemic response (Singh et al., 2013; Niba, 2012). Development of cereal products that utilize β-glucan-containing oat ingredients alongside gluten-containing wheat ingredients to allow for the combination of β-glucans' health benefits with wheat gluten's functional benefits has been a research target of many studies, with mixed results. Decreased baked good volume, toughness, and poor texture have been reported as outcomes of fortifying wheat products with oat based ingredients (Aravind et al., 2012; Brennan & Cleary, 2005; Hager et al., 2011; Kim & Yokoyama, 2011; Lee et al., 2005; Mohamed et al., 2008; Moriartey et al., 2011; Peymanpour et al. 2012; Reider et al., 2012; Sivam et al., 2010; Zhou et al., 2011).

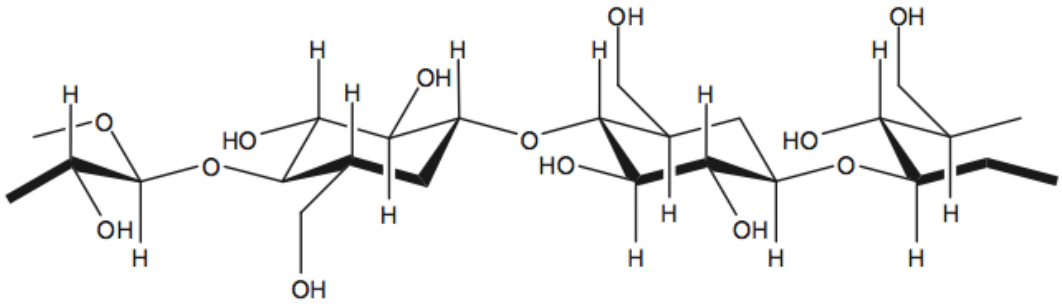


Figure 1 Structure of β-glucan with $\beta 1 \longrightarrow 4$ and $\beta 1 \longrightarrow 3$ linkages.

Figure 3-1 Oat β –glucan structure. (From Singh et al., 2013)

Figure 3-1 shows the basic structure of (1-3,1-4)-β-D-glucan . The 1-3,1-4)-β-D-glucans found in oat are linear polymers of glucose with single 1-3 linkages separating groups of two or three (but sometimes more) 1-4 linkages (Lazaridou & Biliaderis, 2007). As a neutral linear polysaccharide, oat gum is somewhat similar to guar gum in physicochemical properties (Lazaridou & Biliaderis, 2007).

3.1.2 Hypothesis

During the course of preliminary experimentation with oat β-glucan-containing materials and gluten, it was noticed that preparation of gluten in a Glutomatic in the presence of added oat β-glucan-containing ingredients resulted in a qualitatively observed increase in elasticity. Based on this, it was hypothesized that there was an interaction between wheat gluten proteins and one or more component of the oat bran materials (suspected to be the β-glucan) that was occurring within the isolated gluten matrix.

3.1.3 Objectives

The present experiment was designed to both detect and characterize changes in the hydration and CORE behavior of gluten samples treated with variable doses of β-

glucan containing materials. To combat the high variability inherent in Gluten CORE output variables, 5 or 6 gluten sample replicates per each treatment dose were determined to be necessary, so that significant differences between control and treatment gluten could be determined, and so that trends could be visualized by plotting average measurements for each set of replicates. In order to pinpoint the location of the hypothesized interaction to the gluten network itself, complimentary sample preparation procedures were used. A "Washed" sample preparation method was devised with the intention of carrying away solubles not bound to the insoluble gluten matrix itself, so that any dose response observed could be assumed to be intrinsic to the gluten network. A complimentary "Unwashed" sample preparation procedure was developed to balance the unknown degree with which solubles were removed from the washed samples, so full control over the amount of treatment materials present in a sample could be maintained. Funding restrictions limited the choice of oat bran β-glucan materials available for use as treatments to crude, commercially available ingredients. To preserve the potential for chemical interpretation of results, three different materials were chosen as treatments that represented a range of compositions, so that dose response behavior could be compared to each material's concentration of β-glucan and other components. The use of commercially available materials also allowed for the production of results more immediately applicable to the baking industry, which is generally limited to the use of industrially available ingredients in new product formulation.

3.2 Materials and Methods

3.2.1 Materials

Crude Dry Powdered Wheat Gluten (catalog No. G5004-500G, batch SLBD0196V) was obtained from Sigma-Aldrich, Inc (Milwaukee, WI). Protein content was estimated to be 77.4% by triplicate Kjeldahl Analysis using the wheat conversion factor of 5.70. Gluten powder was stored in a sealed jar at room temperature.

Organic Stabilized Oat Bran Powder, a finely powdered oat bran product (Lot 1201120) was obtained from Grain Millers, Inc. (Eugene, OR). According to the manufacturer's estimate, the product contained 5.5% β-glucan by weight, but this value was determined using the USDA nutritional database and not a direct measurement of the actual material. Protein content was estimated to be 14.6% by triplicate Kjeldahl Analysis using the oat conversion factor of 5.83. Oat Bran Powder was stored in a sealed PET jar at room temperature for a period 6-12 months before use.

Oatwell 28, an air-fractionated β-glucan isolate from oat bran was obtained from CreaNutrition via the US distributor Oat Ingredients, LLC (Boulder, CO). According to the manufacturer's certificate of analysis, the material (Batch 1126, Lot 6), contained 27.2% β-glucan by weight. Protein content was estimated to be 20.5% by triplicate Kjeldahl Analysis using the oat conversion factor of 5.83. Oatwell was stored in zip-sealed polyethylene bags at room temperature for a period 6-12 months before use.

O-Glucan 70, a wet-fractionated, spray-dried β-glucan isolate from oat bran was obtained from Garuda International. According to the manufacturer's Certificate of Analysis, the material contained 70% β-glucan by weight. Protein content was estimated to be 3.34% by triplicate Kjeldahl Analysis using the oat conversion factor of 5.83. O-

Glucan 70 was received in airtight manufacturer packaging at room temperature, and used immediately upon receipt, although the material was likely stored by the manufacturer for a period of up to one year before shipment.

The wet-fractionated, spray-dried O-Glucan 70 material, unlike the dry-processed oat bran or Oatwell 28 materials, was not readily dispersible in water because of the size of the dense spray-dried particles, and could therefore not be simply intermingled as a powder with the dry gluten in order to prepare treated samples. However, the O-Glucan 70 could be solubilized by gentle heating, so O-Glucan 70 was prepared for use by pre-solubilization into 2% NaCl solution.

A stock solution of 27.8 g/L O-Glucan 70 in 2% NaCl was prepared by heating an O-Glucan 70 sample, in 2% NaCl solution, in an underfilled volumetric flask on a hot plate at just below the boiling point for one hour, then cooling and filling the flask to volume with additional 2% NaCl solution.

2% Sodium Chloride Solution was prepared by dissolving 200.00g of fine crystalline sodium chloride (NaCl) to a final solution volume of 10 L. ACS Grade Sodium Chloride was obtained from Fisher Chemical (Agawam, MA).

3.2.2 Preparation of gluten samples

"Washed" gluten samples treated with Oatwell 28 or Oat Bran were prepared using a modified version of the extraction procedure developed by Chen (2011) for whole meal flours. 1.5g of Sigma dry vital wheat gluten flour was intermingled with a variable experimental dose of either fine ground oat bran or Oatwell oat bran isolate, hydrated with 3 mL 2% NaCl solution, and transferred to a Glutomatic chamber equipped with a fine mesh sieve. The Glutomatic Wash 1 cycle was allowed to proceed as normal,

developing the gluten while washing away solubles and fine particles. The Glutomatic was stopped at the end of the "Wash 1" cycle to transfer partially washed material to new Glutomatic chambers equipped with a large mesh filter of sufficient pore size to allow coarser material through. The Glutomatic was then restarted to complete the "Wash 2 cycle", yielding finished washed gluten samples ready for CORE analysis

"Unwashed" gluten samples treated with Oatwell 28 or Oat Bran were prepared using a modified version of the standard Glutomatic procedure for extraction of gluten from refined flour. 1.5g of Sigma dry vital wheat gluten flour was intermingled with a variable experimental dose of either fine ground oat bran or Oatwell oat bran isolate, hydrated with approximately 3 mL 2% NaCl solution, and transferred to a Glutomatic chamber equipped with a fine mesh sieve. The Glutomatic "wash 1" and "wash 2" cycles were allowed to proceed without allowing wash solution to flow through the Glutomatic chambers. This was accomplished by removing the feed tubes from the wash fluid reservoir. To ensure gluten was hydrated to its saturation level, small amounts of additional 2% NaCl solution (less than 1 mL) were added to each Glutomatic chamber during the mixing cycle.

"Unwashed" gluten samples treated with O-Glucan 70 were prepared differently than unwashed Oat Bran and Oatwell 28 treated samples, since treatment with the O-Glucan 70 required the use of a pre-dissolved stock solution. 1.5g of Sigma dry vital wheat gluten flour was hydrated with variable doses of the O-Glucan 70 stock solution combined with sufficient additional 2% NaCl solution to provide approximately 3.6 mL total. Hydrated gluten samples were transferred to a Glutomatic chamber equipped with a fine mesh sieve. The Glutomatic "wash 1" and "wash 2" cycles were

allowed to proceed without allowing wash solution to flow through the Glutomatic chambers. This was accomplished by removing the feed tubes from the wash fluid reservoir. To ensure gluten was hydrated to its saturation level, small amounts of additional 2% NaCl (less than 1 mL) were added to each Glutomatic chamber during the mixing cycle.

3.2.3 Preparation of gluten samples for CORE analysis

Developed washed and unwashed gluten samples were removed from the Glutomatic, formed into a ball, placed in a small airtight plastic container coated with petroleum jelly on the interior, and allowed to rest for 10 to 45 minutes at room temperature. Prior to CORE analysis, gluten samples were transferred to Perten centrifuge holders lubricated with petroleum jelly and centrifuged for 5 minutes to standardize their dimensions for the CORE analysis.

3.2.4 CORE Analysis

CORE analysis was carried out using the protocol described in Chapman (2011), using the Gluten CORE apparatus and software, calibrated according to Perten protocol. Samples were expelled from centrifuge holder/shapers directly onto the petroleum jelly lubricated CORE platform. Gluten compression used a force of 8 Newtons, for 5 seconds, followed by a recovery period of 55 seconds. Height measurements of samples were recorded by the Perten software every 0.01 seconds during the CORE cycle.

Measured parameters and calculated outcome variables from the CORE analysis are defined in Table 1-2 on Page 27.

3.2.5 Moisture Content Determination

After CORE analysis, the moisture contents of gluten samples were determined by weighing gluten samples before and after sample dehydration. Gluten dehydration was carried out by heating in a Perten Glutork apparatus for either the default four minute heating cycle or longer if necessary, based on dried sample glassiness and weight stability. Percent Moisture (PM), which is also expressed as Moisture Content (MC) in this thesis, was determined as the sample's measured moisture loss divided by the initial sample mass.

3.2.6 Statistical Analyses

Averages and standard deviations for four outcome variables; Recovery Index (%), Percent Compression (%), Half-Recovery Time (s), and Percent Moisture (%); were calculated for each set of n replicates. Significant ($\alpha = 0.05$) differences between control and treatment results within each treatment series were identified by carrying out a Students' T-Test on each set of replicate treatment and control data.

CORE Thickness Average Curves were constructed for each treatment by averaging thickness data for all CORE test replicates at each time point during the compression and recovery periods.

Apparent Percent Recovery Average Curves were constructed for each treatment by averaging the Degree of Recovery (%) for all replicates at each time point during the 55 second recovery period.

3.3 Results and Discussion

3.3.1 Summary of Results for Washed and Unwashed Dose Response Series

Table 3-1 summarizes replicate counts, average outcome variable measurements, standard deviations, and identifies treatment results significantly different than control results. Figure 3-2 and Figure 3-3 shows dose response curves constructed using average values per group of replicates, plotted separately for each treatment series, as a function of the treatment dose used.

Overall, standard deviations listed in Table 3-1 can be assumed to indicate the degree of variability inherent in the gluten sample preparation and Gluten CORE analysis method used in this study. It should be noted that these standard deviations are uncomfortably similar in magnitude to some of the differences observed between control and treatment average measurements. Because of this, the use of a high number of replicates per sample was necessary to obtain support for the hypothesis that there is an interaction between wheat gluten proteins and the oat bran materials. Using a Student's T-test at the 0.05 alpha significance level, significant differences between control and (at least some) treatment doses, within all 5 treatment series, were identified. Furthermore, the Central Limit Theorem states that many independent replicate measurements will converge on a true average value, which validates the plotting of average measurements as a function of the treatment dose for all dose response series, as is done in Figure 3-2 and Figure 3-3. These plots of average measurements for all three of the unwashed treatment series, and for both of the washed treatment series, show the same patterns of response behavior for three of four outcome variables recorded: Recovery Index, Moisture Content, and Half Recovery Time. That these patterns of dose response

behavior are found across five separate dose response studies is compelling evidence that the effects observed are the result of the hypothesized interaction, and not accidental.

Recovery Index increases with increasing treatment dose. Recovery Index is a measure of gluten elasticity. Generally, RI measurements for all five treatment series increase as a function of treatment dose. This indicates that oat bran materials interacted with the gluten somehow to give it a more elastic character. The "unwashed" dose response series, which uses much smaller doses of oat treatment materials, reaches higher RI levels than does the washed series, but then it shows a leveling off followed by a slight reversal of the RI response, as treatment doses reach their upper limits. This was experimentally observed to be caused by the proliferation of a second phase containing viscous oat soluble fiber, which inhibited sample cohesiveness, causing the apparent RI to decrease.

Moisture Content decreases with increasing treatment dose. Moisture Content (MC) is a measurement of degree with which a gluten sample binds water at it's equilibrium hydration, as each gluten sample was allowed to take on as much 2% NaCl solution as was required for it to reach saturation hydration. Generally, MC measurements for all five treatment series decrease as a function of treatment dose. It is interesting to note that examination of all five sets of graphs in Figure 3-2 and Figure 3-3 shows that the increases in RI with increasing dose appears to mirror the decreases in MC. As was discussed in section 2.3.3 on page 40, gluten that binds less water will be expected to have a greater proportion of condensed protein-protein trains than hydrated loops, so will be expected to have a greater elastic response. Therefore, at least some of

the increases in the RI measurements of treatment samples might be directly attributed to these changes in their equilibrium moisture content.

Half-Recovery Time decreases with increasing treatment dose. Half-Recovery Time (HRT) is a measurement of how fast a gluten sample recovers the first half of its ultimate recovery distance, and this was theorized in the previous chapter to potentially relate to the contribution of short-term forces such as entanglements and protein-protein hydrogen bonded train interactions, as opposed to contributions from long-term forces such as covalent cross links, to the overall recovering force. In all five treatment series, HRT and MC are observed to decrease with increasing dose, supporting the theory that shorter HRT values are related to the greater prevalence of trains rather than loops. However, it should also be noted that the very large decrease in the HRT measurements for even the lowest doses of the unwashed treatment samples contrasts a great deal with the moderate differences in HRT behavior observed across the washed treatment series' dose response curves. This is suspected to represent a lubricating effect from the second slippery phase, described above, observed to form in the unwashed treatment samples. This observation is also discussed more in depth in a discussion on page 100.

Percent Compression (PC) shows no clear dose response directional behavior. Percent compression is a measure of the gluten network's tendency to resist the compression during the 5s 8N compression force applied during CORE analysis. Although there are some replicate sets that have significant differences between each other, overall, there is no pattern of PC changes that occurs across all five treatment series. This indicates that whatever effect or interaction these oat bran materials are

exerting, it does not generally have a significant influence on the gluten network's resistance to compression.

Table 3-1 Summary of Treatment Doses, Replicates, RI, PC, HRT, and PM data for Gluten Samples from Washed and Unwashed Treatment Series

Treatment Series	Treatment Dose (g) per 1.5g dry gluten	n	Recovery Index (%)	Percent Compression (%)	Half Recovery Time (s)	Percent Moisture (%)
Unwashed OGlucan70 (Presolubilized)	**0.000**	**6**	**60.9 ± 2.8**	**76.4 ± 0.8**	**4.71 ± 0.92**	**64.7 ± 0.8**
	0.011	5	64.9 ± 2.8	80.0 ± 1.2 *	2.58 ± 0.73 *	61.3 ± 0.3 *
	0.022	6	64.1 ± 2.4	78.6 ± 0.4 *	3.13 ± 0.53 *	61.9 ± 0.2 *
	0.028	6	71.0 ± 3.8 *	80.0 ± 0.6 *	2.42 ± 0.63 *	60.4 ± 0.9 *
	0.033	6	78.0 ± 3.5 *	76.3 ± 0.8	1.70 ± 0.24 *	60.8 ± 0.7 *
	0.067	6	78.0 ± 4.9 *	75.6 ± 0.8	1.73 ± 0.27 *	61.2 ± 0.3 *
	0.100	6	77.0 ± 4.6 *	75.4 ± 0.9 *	1.88 ± 0.38 *	61.3 ± 0.7 *
Unwashed Oat Bran	**0.00**	**6**	**60.9 ± 2.8**	**76.4 ± 0.8**	**4.71 ± 0.92**	**64.7 ± 0.8**
	0.03	6	63.7 ± 7.6	78.4 ± 0.9 *	3.42 ± 1.13 *	64.4 ± 1.1
	0.06	6	81.5 ± 5.7 *	73.3 ± 0.9 *	1.78 ± 0.89 *	62.8 ± 0.9 *
	0.09	6	79.8 ± 5.3 *	75.0 ± 1.3 *	1.50 ± 0.77 *	63.3 ± 1.1 *
	0.12	6	83.0 ± 3.3 *	74.0 ± 1.1 *	1.36 ± 0.26 *	62.8 ± 0.6 *
	0.18	6	79.6 ± 2.2 *	75.0 ± 0.6 *	1.26 ± 0.15 *	61.4 ± 0.6 *
	0.24	6	77.8 ± 2.3 *	74.6 ± 0.5 *	1.60 ± 0.39 *	61.6 ± 0.9 *
	0.30	6	73.1 ± 3.7 *	75.9 ± 1.1	1.85 ± 0.39 *	61.8 ± 0.8 *
Unwashed Oatwell 28	**0.00**	**6**	**60.9 ± 2.8**	**76.4 ± 0.8**	**4.71 ± 0.92**	**64.7 ± 0.8**
	0.01	6	72.9 ± 8.5 *	77.0 ± 1.1	2.37 ± 0.92 *	64.6 ± 0.8
	0.02	6	76.8 ± 5.8 *	75.4 ± 0.8	1.99 ± 0.7 *	64.6 ± 0.5
	0.03	6	78.8 ± 3.2 *	75.6 ± 1.3	1.44 ± 0.34 *	63.8 ± 1.0 *
	0.04	6	80.9 ± 4.1 *	74.5 ± 1.0 *	1.36 ± 0.36 *	62.4 ± 0.6 *
	0.06	6	79.2 ± 2.5 *	74.3 ± 0.7 *	1.42 ± 0.19 *	62.5 ± 0.7 *
	0.08	6	74.9 ± 2.0 *	76.6 ± 0.6	1.62 ± 0.34 *	62.0 ± 0.6 *
	0.10	6	74.3 ± 4.2 *	76.2 ± 0.9	1.67 ± 0.25 *	62.1 ± 0.3 *
Washed Oat Bran	**0.00**	**6**	**61.1 ± 2.3**	**75.8 ± 0.8**	**2.91 ± 0.28**	**67.4 ± 0.5**
	0.60	6	65.8 ± 1.6 *	74.8 ± 0.5 *	2.77 ± 0.15	66.5 ± 0.3 *
	1.20	6	63.8 ± 4.9	75.2 ± 0.7	2.95 ± 0.45	66.0 ± 0.7 *
	1.80	6	70.3 ± 5.0 *	74.9 ± 1.0 *	2.20 ± 0.62 *	66.2 ± 0.9 *
	2.40	6	74.7 ± 5.8 *	73.0 ± 0.8 *	1.78 ± 0.36 *	65.2 ± 0.6 *
	3.00	6	78.3 ± 2.5 *	74.6 ± 0.8 *	1.56 ± 0.28 *	64.9 ± 0.8 *
Washed Oatwell 28	**0.00**	**6**	**61.1 ± 2.3**	**75.8 ± 0.8**	**2.91 ± 0.28**	**67.4 ± 0.5**
	0.20	6	60.3 ± 3.8	75.6 ± 1.2	3.17 ± 0.61	66.9 ± 0.4
	0.40	6	67.9 ± 5.3*	75.9 ± 1.9	2.60 ± 0.53	66.2 ± 0.7 *
	0.60	6	65.4 ± 5.4 *	75.4 ± 1.1	2.53 ± 0.40	66.2 ± 0.4 *
	0.80	6	67.8 ± 4.9 *	75.6 ± 0.6	2.38 ± 0.51	65.6 ± 0.2 *
	1.00	6	70.2 ± 2.3 *	74.7 ± 0.9	2.13 ± 0.25 *	65.2 ± 0.4 *

** Values are significantly different from the series' control value via Student's T-Test, $\alpha = 0.05$.*

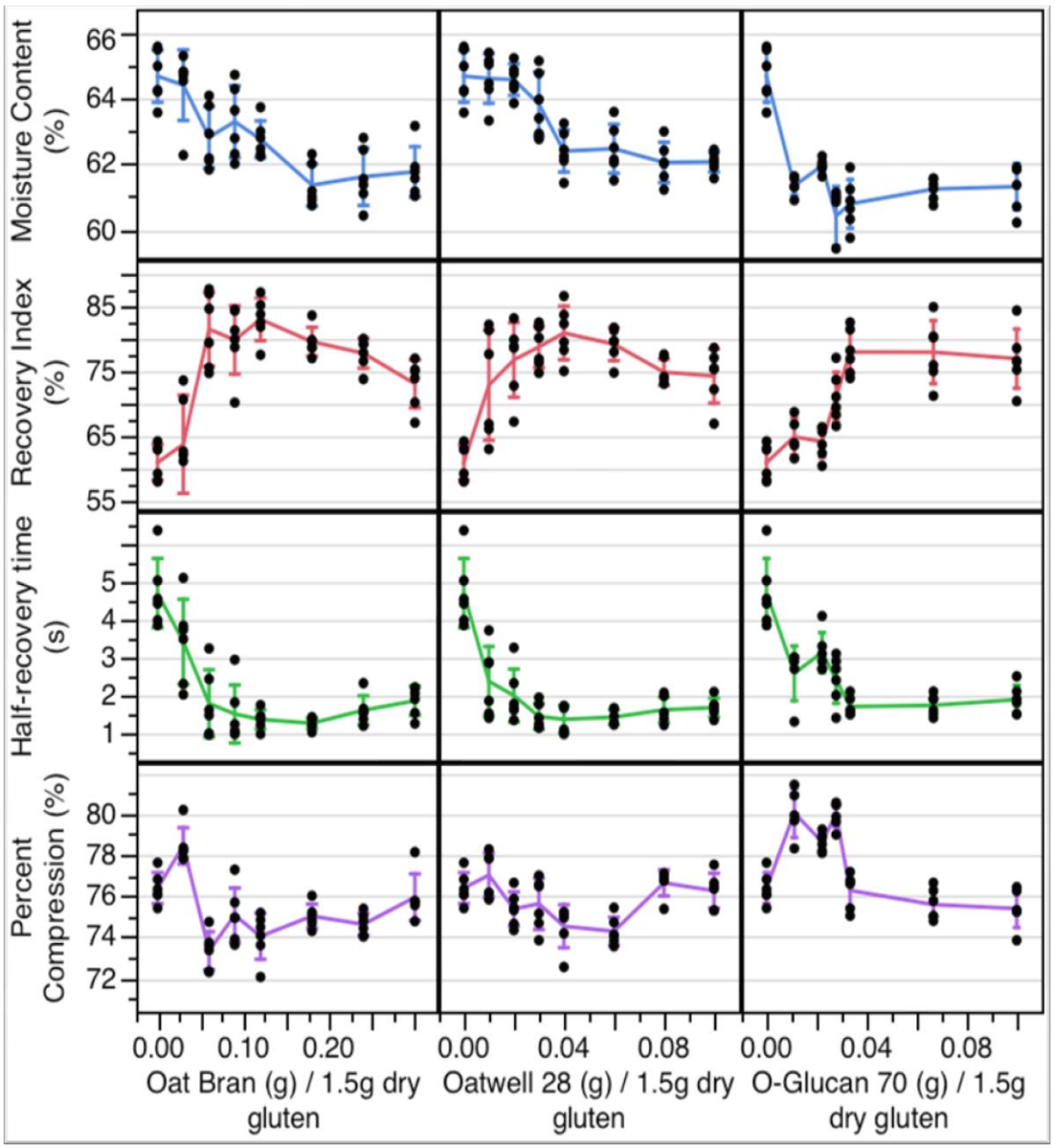


Figure 3-2 Unwashed Treatment Series Dose Response Data, Averages, and Standard Deviations

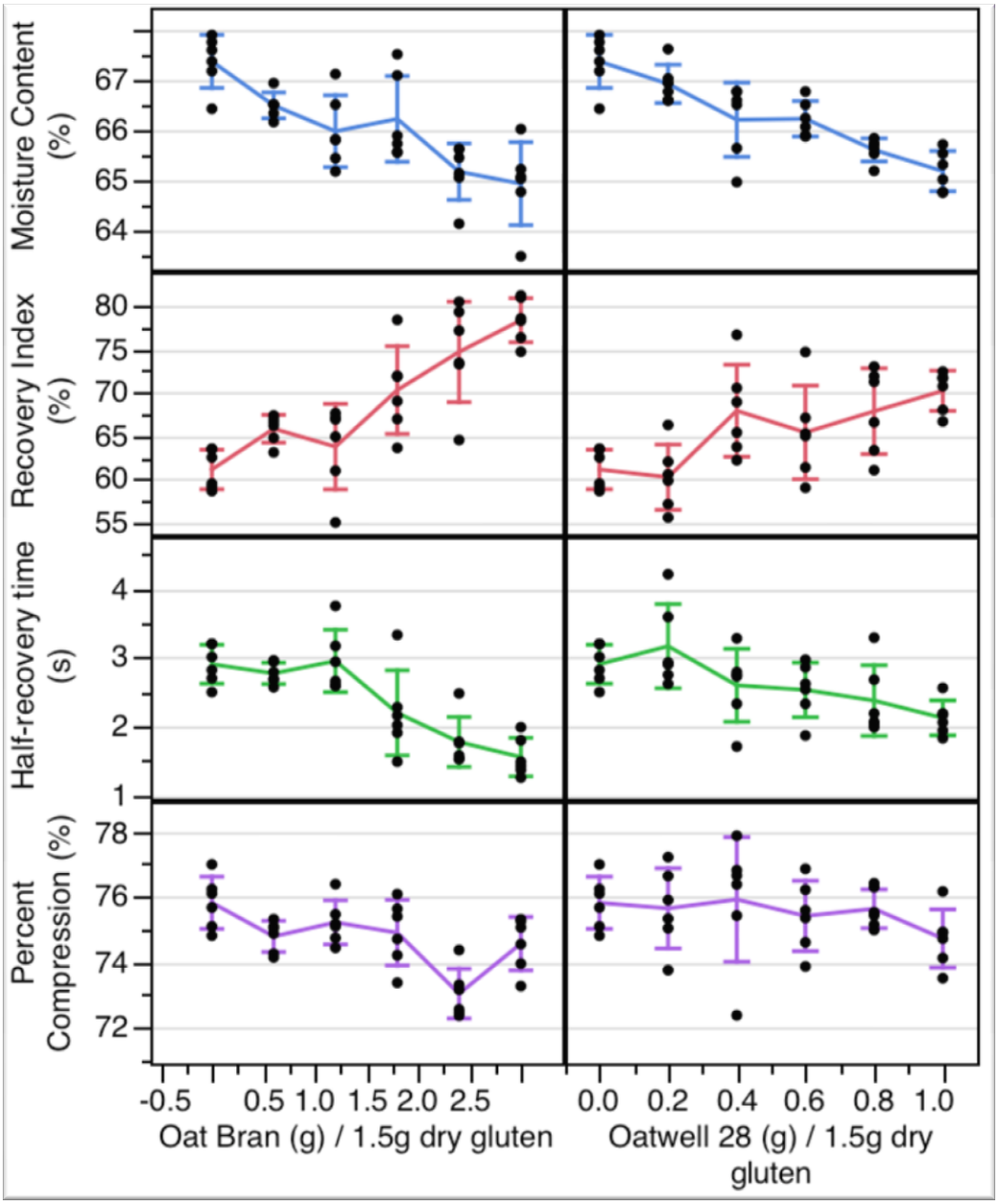
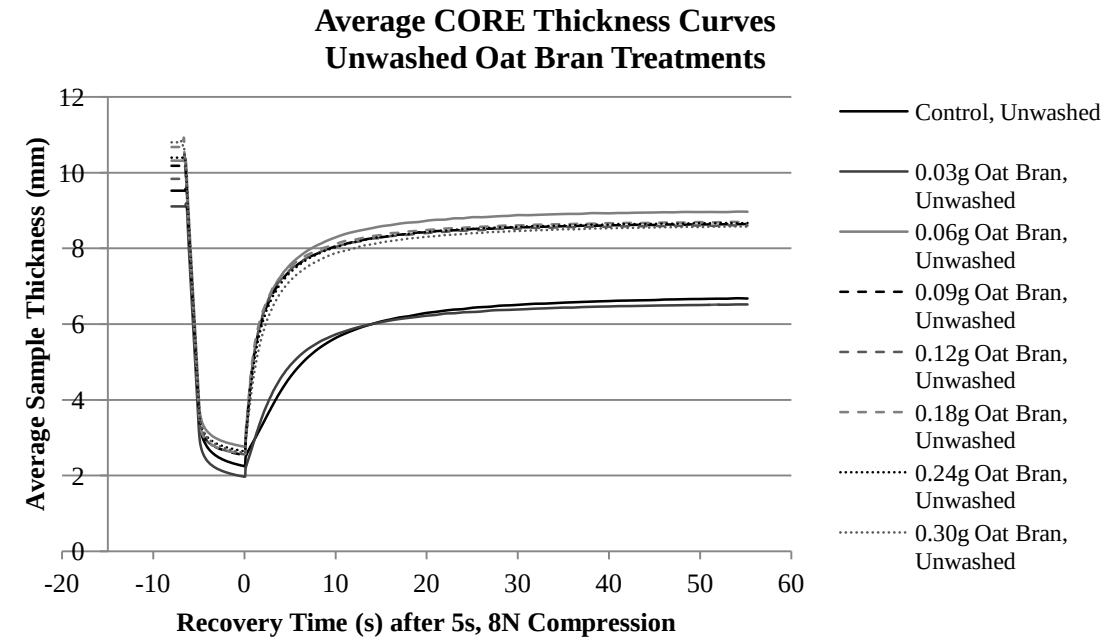


Figure 3-3 Washed Treatment Series Dose Response Data, Averages, and Standard Deviations

3.3.2 Visualization of CORE Behavior, by Treatment Series: Average Sample Thickness and Recovery Curves over time.

Figure 3-4, Figure 3-5, Figure 3-6, Figure 3-7 and Figure 3-8 show Gluten CORE Average Thickness Curves and Average Recovery curves with Half Recovery Times for all five dose response treatment series. In all series, the control is shown as a solid black line and treatments with increasing dose are shown using progressively lighter colors and a greater degree of dashing.



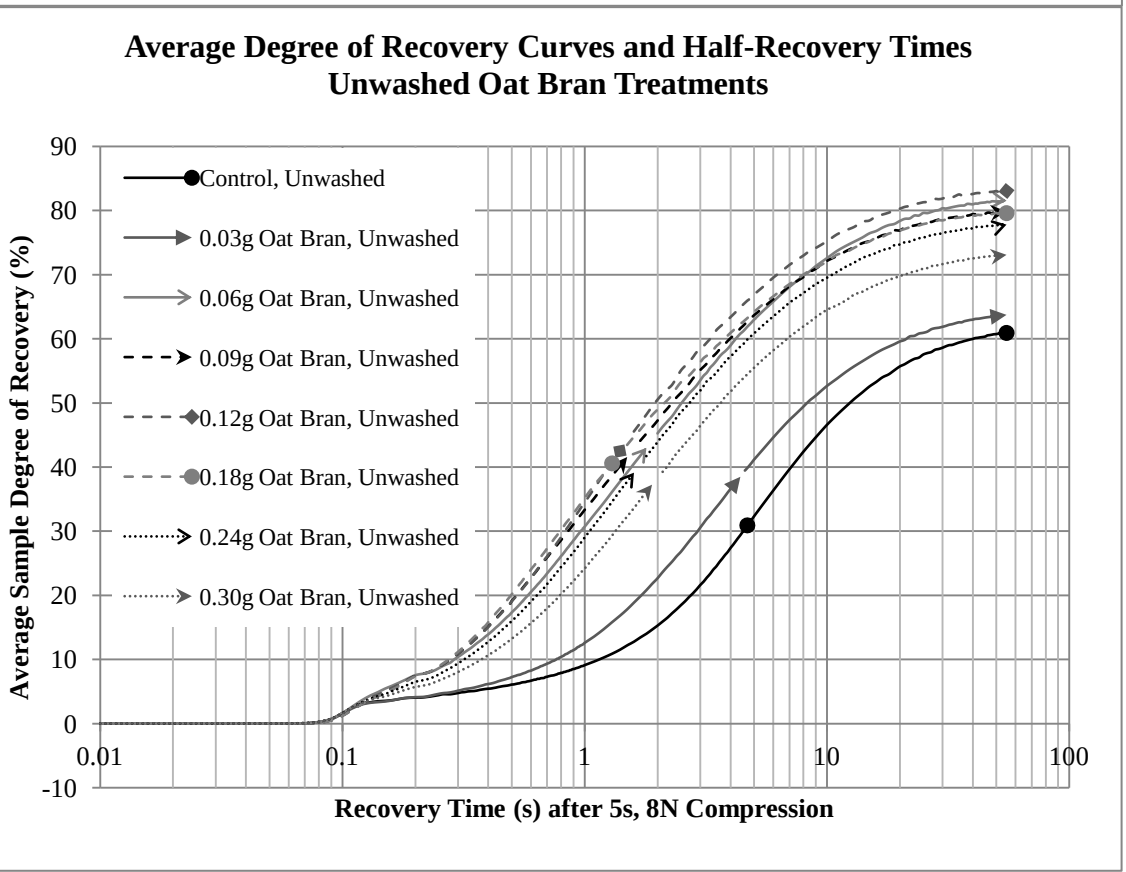
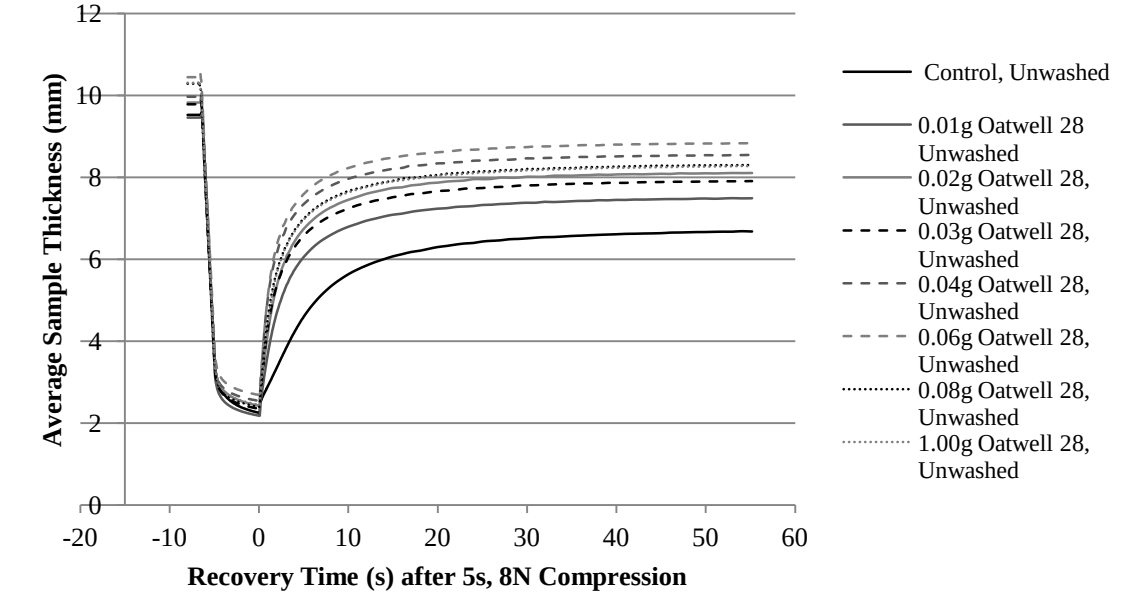


Figure 3-4 Average Thickness and Recovery Curves for Unwashed Oat Bran treatment series



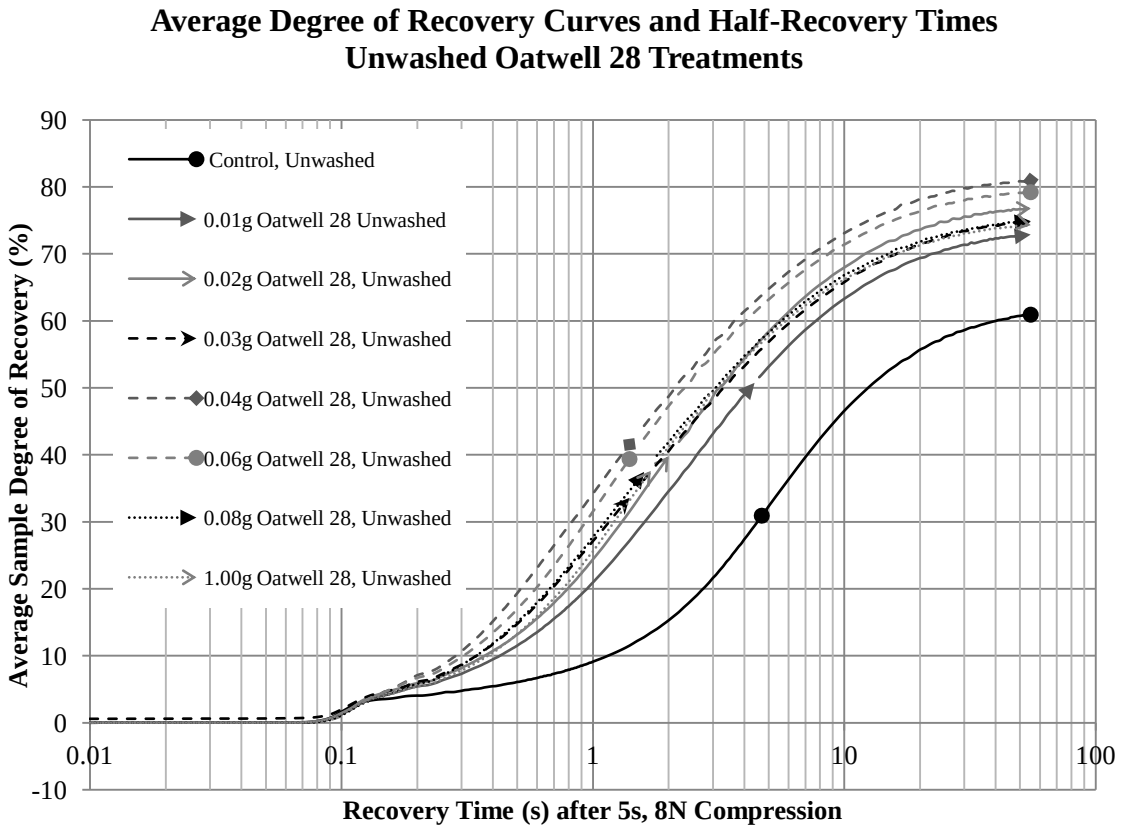
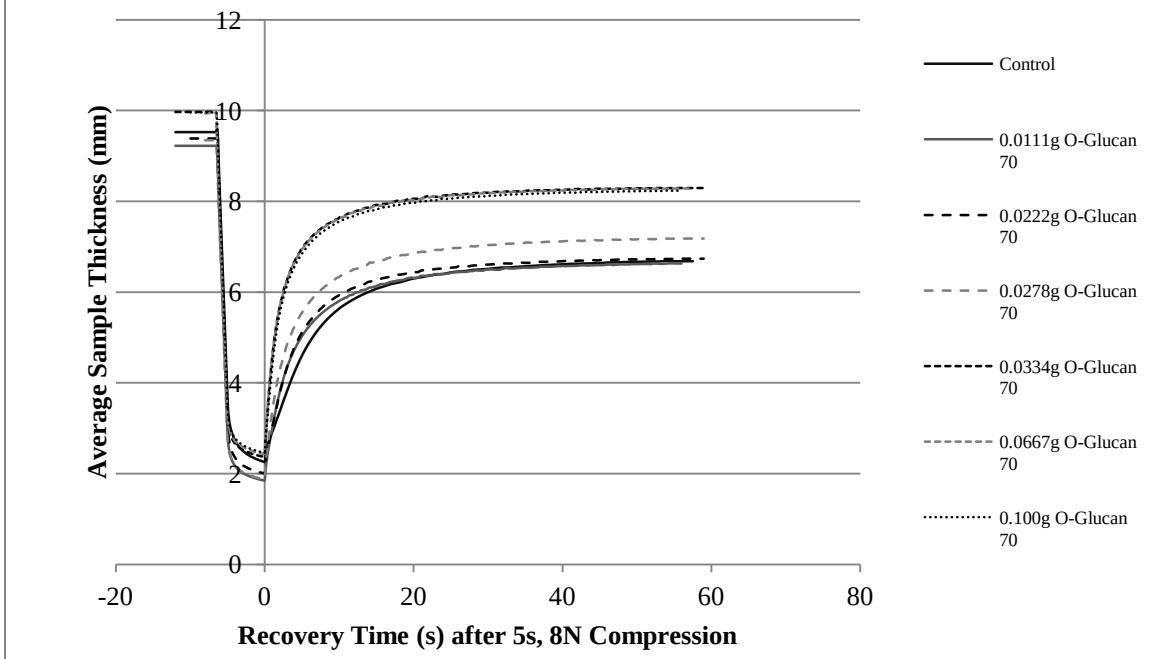


Figure 3-5 Average Thickness and Recovery Curves for Unwashed Oatwell 28 treatment series



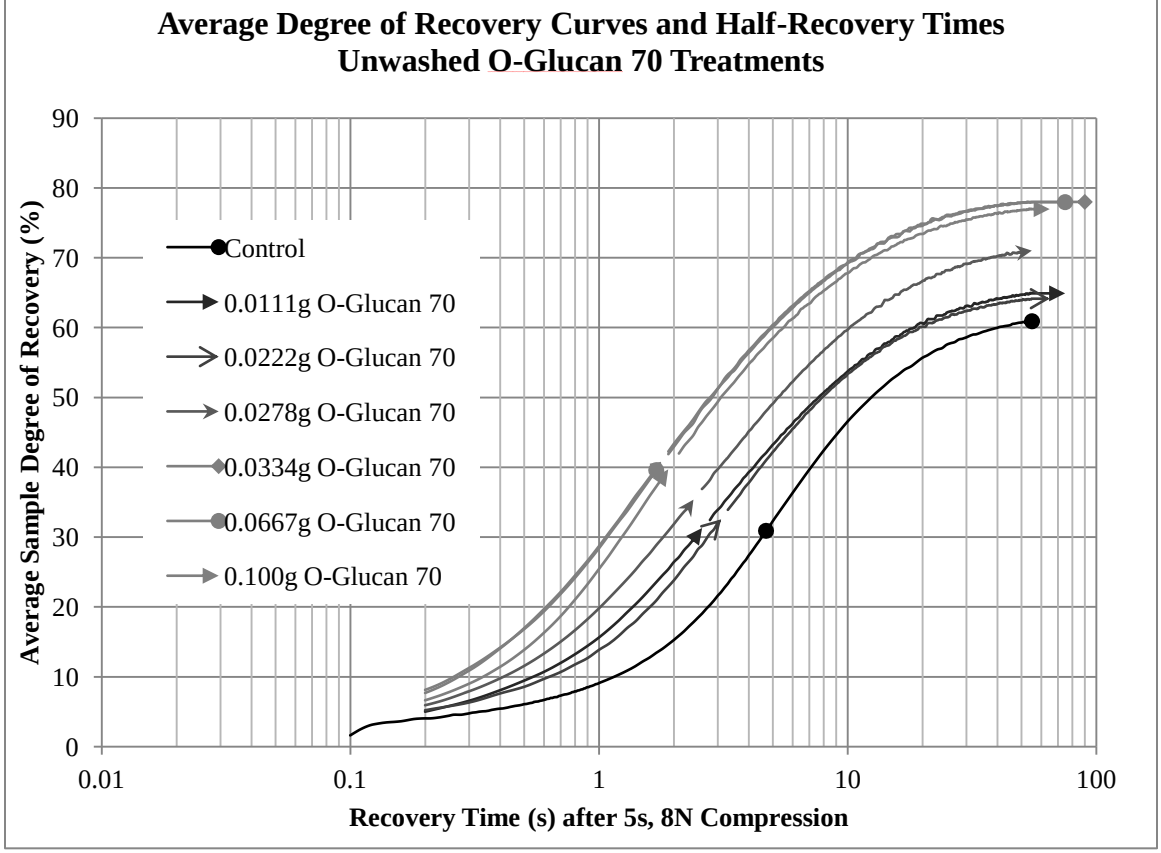


Figure 3-6 Average Thickness and Recovery Curves for Unwashed O-Glucan 70 treatment series

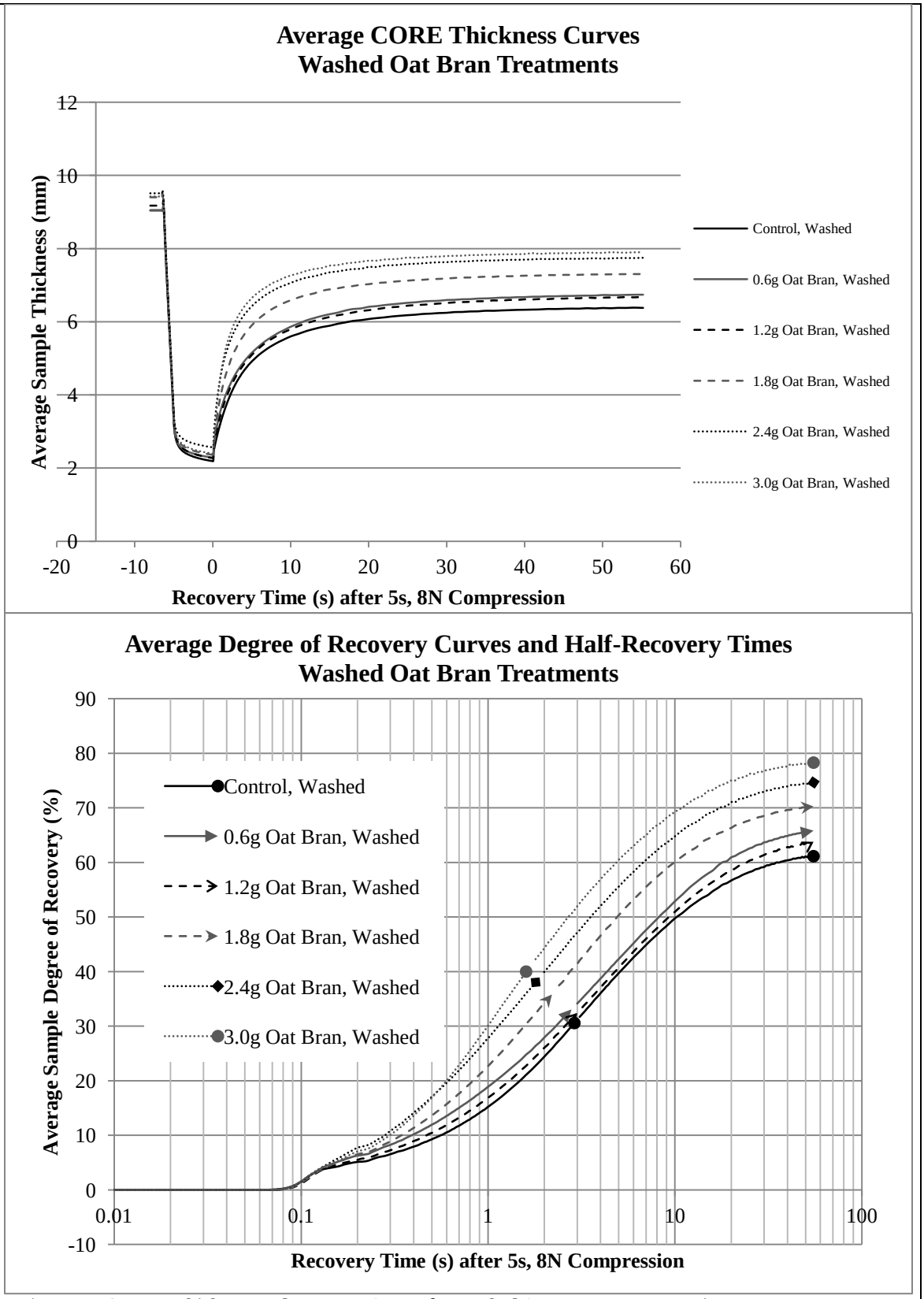
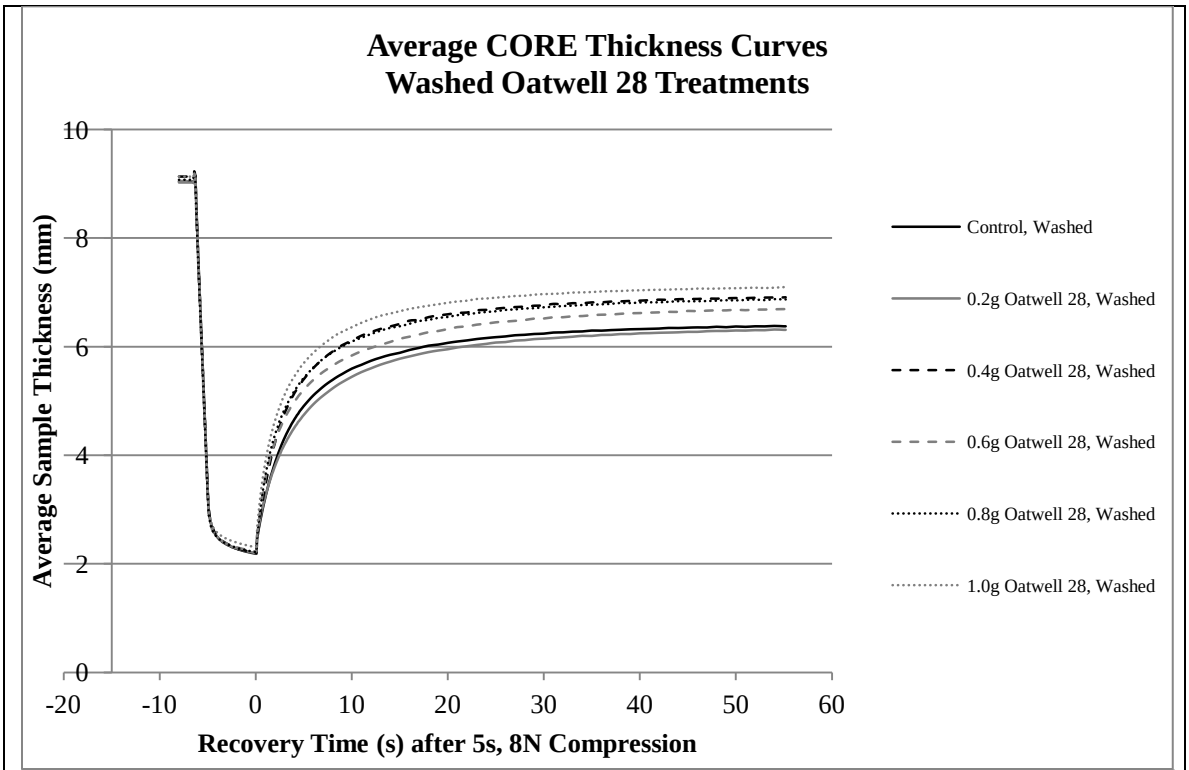


Figure 3-7 Average Thickness and Recovery Curves for Washed Oat Bran treatment series



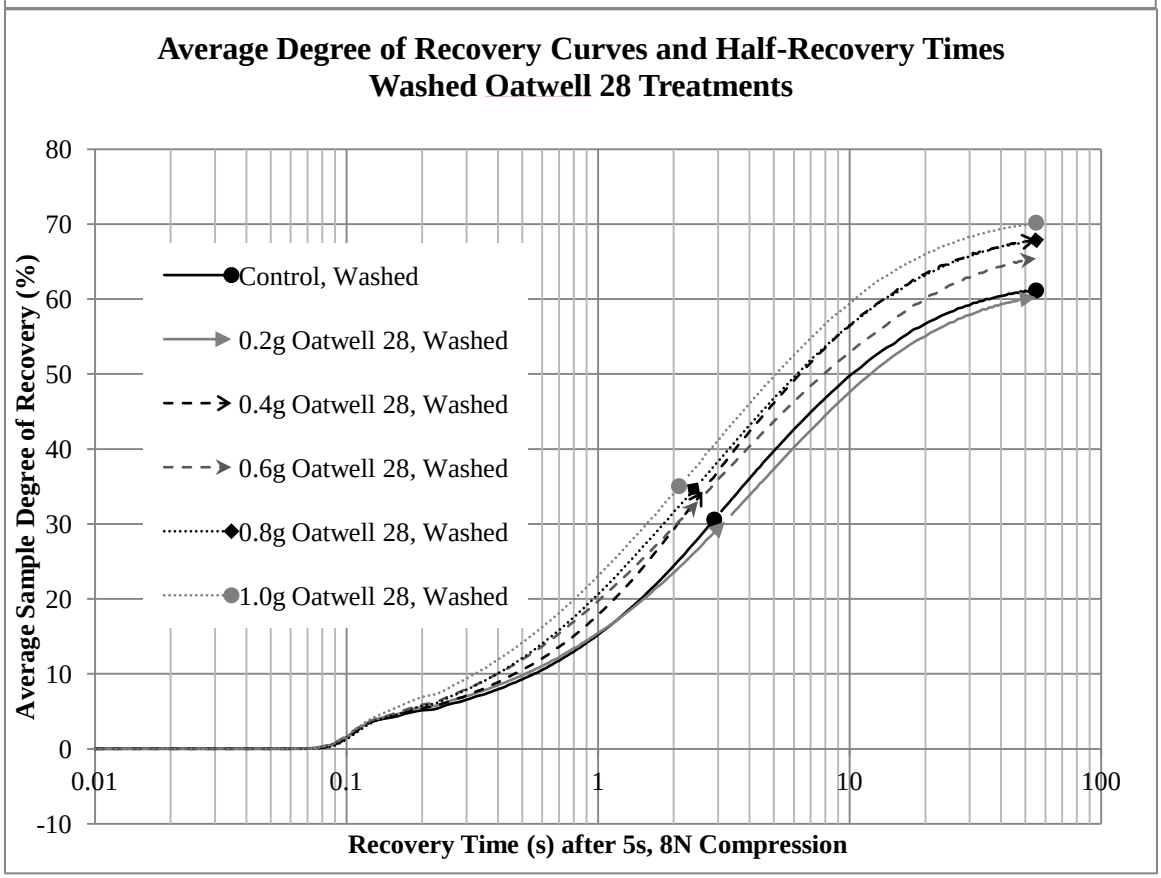


Figure 3-8 Average Thickness and Recovery Curves for Washed Oatwell 28 treatment series

3.3.3 Comparison of Washed and Unwashed Dose Response Behavior

That the "washed" as well as the "unwashed" dose response series both show significant changes in average outcome variables supports the hypothesis that the oat bran/wheat gluten interaction is localized to the gluten network itself, since the washed dose response series was expected to show only effects on the gluten phase, as non-gluten associated materials would have been washed away during sample preparation.

3.3.4 Interpretation of results using compositional information about treatment materials

Figure 3-9 compares the estimated amount of β-glucan delivered by each of the treatments to the response behaviors across the two washed treatment series and the three unwashed treatment series. Average and standard deviations of outcome measurements are shown connected by a smoothed line generated by the JMP statistics software. Estimated β-glucan doses are calculated using compositional information provided by manufacturers of each of the three oat bran materials used. (Compositional information for each treatment material can be found in Section 3.2.1, on page 62.) That these estimated β-glucan doses of each treatment series appears to produce RI and MC responses of similar magnitude provides some support for the hypothesized identity of β-glucan as the active component of interest. However, this is not definitive. The overlay of the three graphs of estimated β-glucan dose is not perfect, and that could be due to some unidentified non-β-glucan component, perhaps which happens to be co-extracted with them, actually being responsible. If β-glucan is in fact responsible, variation could be due to β-glucan concentrations being incorrectly reported by manufacturers, or because co-interactions between different oat bran components are involved. It is also interesting that

in Figure 3-9 the estimated β-glucan doses from the oat bran series exhibited a stronger apparent effect on the RI than does the Oatwell 28, which exhibits a stronger apparent effect on the RI than does the O-Glucan 70. If β-glucan is the responsible component, and if the estimated doses of β-glucan are correct, these differences could be explained by probable differences in the MW of β-glucan found in each material. The finely powder oat bran would be expected to have the highest MW since it is relatively less processed compared to the air fractionated Oatwell 28, which is relatively less processed compared to the O-Glucan 70.

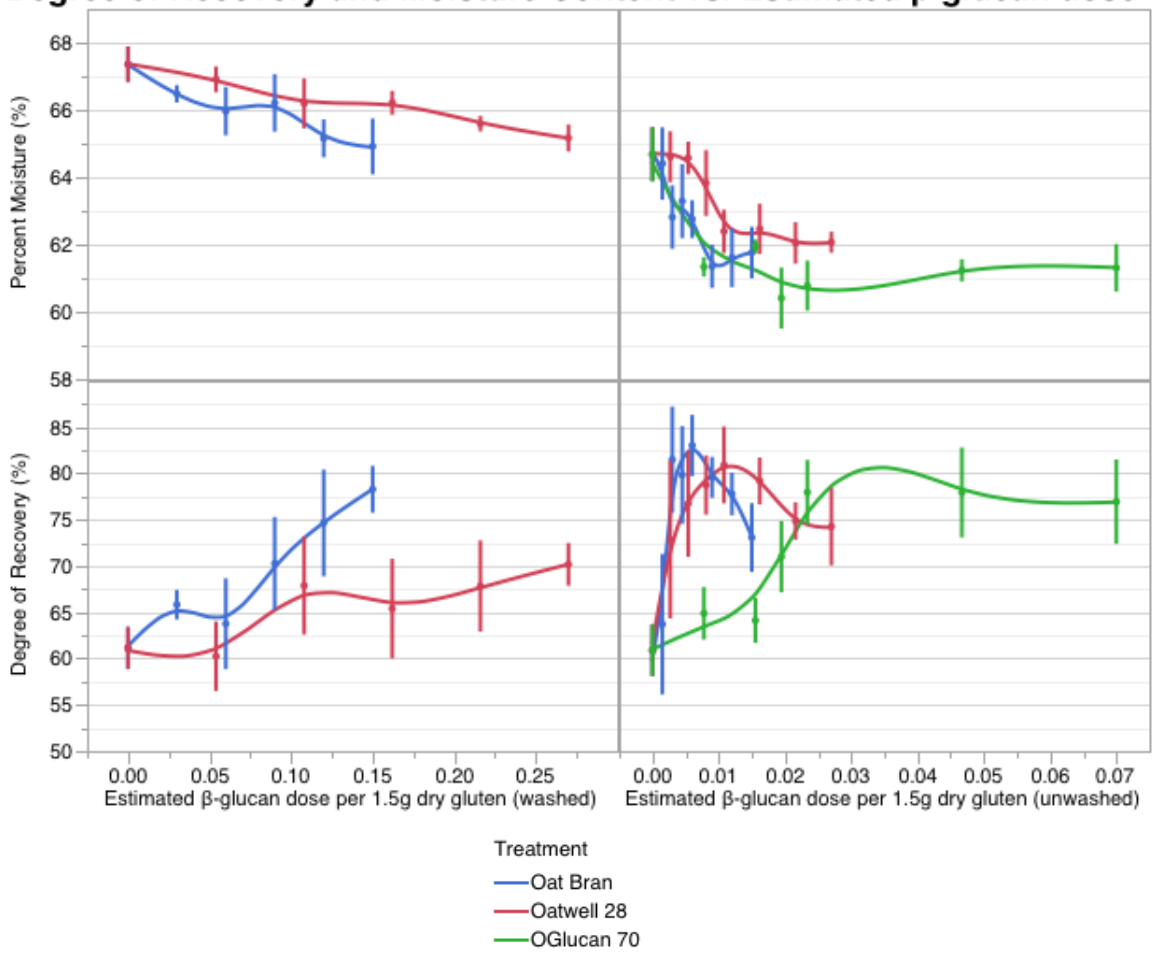


Figure 3-9 Comparison of Estimated β-glucan dose to Degree of Recovery Response for Washed and Unwashed Treatment Series

3.3.5 What is causing the observed effects? A proposed hydrogen bonding explanation.

The results of this study show that oat bran materials increase both washed and unwashed gluten samples' elasticity while decreasing their equilibrium hydration. That the washed as well as the unwashed dose response series exhibit these effects suggests that this could be due to an interaction that occurs within the gluten network itself, between some part of the gluten network and some component (or components) of the oat bran materials. Furthermore, β-glucan is implicated as a candidate for the active oat component based on the partial convergence of Recovery Index and Percent Moisture effects when estimated β-glucan doses for each treatment series are directly compared, as in Figure 3-9 on page 81. A possible explanation of these observations might be found by looking at the chemistries of wheat gluten and of oat β-glucans. As discussed in Section 1.1.3 on page 14, the repetitive central domains of HMW-GS are largely responsible for the water-binding properties of gluten, since they are heavy in hydrogen-bonding residues. β-glucans are soluble linear polymers of glucose, and as such are also capable of participating in hydrogen bonds. A potential explanation of the observed effects is that oat β-glucans may be able to associate with the gluten network via hydrogen bonding, where they could indirectly decrease the hydration by displacing water as hydrogen-bond partners to protein in hydrophilic portions of the gluten network. This dehydration would be expected to result in a less plasticized, more elastic material, as less water available to act as a gluten plasticizer would slow the resolution of entanglements, etc. If the gluten network were only able accommodate up to a certain concentration of β-glucan, however, excess soluble fiber would be forced out to form a second, disruptive phase that inhibits

gluten network cohesiveness, as was observed in higher doses of the unwashed treatment series.

3.4 Conclusions

The results of this study strongly suggest that there is an interaction between wheat gluten proteins and oat bran components, and that this interaction occurs within the gluten network itself. The interaction appears to cause gluten samples to absorb less water, become more elastic, and undergo faster elastic recovery. Oat β-glucan may be a possible candidate for the oat bran component responsible for these effects, as it's concentration in three treatment materials corresponds with their relative effects. A hydrogen-bonding, water displacement theory explaining these results is proposed.

Further study will be required, however, before oat β-glucan can be conclusively identified as the active oat bran component, or a mechanistic explanation of these phenomena can be confirmed. Support for the identification of oat β-glucan as a the active oat bran component responsible for the effects observed in this study could be provided by checking to see if the observed effects can be eliminated by treating samples with a β-glucanase enzyme. Fluorescent labeling of oat β-glucan and gluten HMW-GS central domains would allow the hypothesized hydrogen bonding based explanation of these effects to be tested via fluorescence microscopy. It would also be interesting to investigate the effect of oat β-glucan with variable - or at least known - degrees of polymerization, on the rheology and moisture-binding properties of treated gluten.

Based on the results of this study, it can also be suggested that the fortification of wheat products with oat bran ingredients will be best accomplished if a decreased gluten water holding capacity can be counteracted by dough improvers, or if applications are selected where an increased gluten elasticity coupled with decreased gluten water-binding, is desirable. Another possible solution for product developers wishing to incorporate oat bran ingredients into wheat based items would be to formulate these with a cultivar or blend of wheat containing gluten which naturally binds high levels of moisture, so that upon dehydration by interaction with oat bran materials, it might reach a more optimum level of hydration. An unknown aspect of the potential applications of these results to baked goods is the behavior of excess β-glucan when a starchy phase is present, such as is found in dough, and this could also be an interesting topic of future study.

Chapter 4. Effect of β-Glucan Treatment on Gluten from Selected Wheat Cultivars, Measured by CORE Analysis

4.1 Introduction

4.1.1 Background & Motivation

The results of the experiment described in Chapter 3 suggested there is an interaction between β-glucan containing oat bran materials and wheat gluten that occurs in the gluten phase itself which results in a net dehydration of the gluten network along with an increase in the elastic behavior of the gluten. It is suggested that product developers seeking to introduce the health benefits of oat bran β-glucan into wheat based baked goods might have good results by seeking out ways to counteract the oat-bran-treated gluten network's tendency toward dehydration, specifically. Dough conditioners such as enzymes, sulfites, or simply reducing the level of sodium chloride, may be found useful here. Another strategy would be to find wheat that has gluten which naturally binds high levels of moisture so that upon dehydration by interaction with oat bran materials, it might reach a more optimum level of hydration. It was decided that it would be interesting to look at differences in the response to oat bran materials of gluten of different qualities.

4.1.2 Hypothesis

This study was designed to test the hypothesis that the oat bran/wheat gluten interaction observed in Chapter 3 would persist when the oat bran ingredients were incorporated into dough used as a starting material for gluten extraction, but might vary across gluten samples extracted from wheat of different qualities.

4.1.3 Objectives

This experiment was designed to attempt to replicate the effect of oat bran materials on wheat gluten that was observed in Chapter 3, using gluten extracted from a wide variety of wheat cultivars. Because only flours from different wheat cultivars were available, and not pre-isolated powdered gluten, only a "washed" sample preparation type of method could be used for this study. In order to compare an interaction between gluten protein and oat bran materials across gluten samples extracted from different flours, it was decided that a constant dose of the oat bran materials per expected dry gluten yield of the flour in each sample should be used. In order to maximize the probability that significant differences in outcome variables could be detected, maximum doses of each oat bran material that could be used with all of the selected flours were determined experimentally, since it was found that higher doses of the oat bran materials prevented the Glutomatic gluten washing machine from draining correctly.

4.2 Materials and Methods

4.2.1 Materials

Refined Flours were selected from a set of 15 refined flours corresponding to the same cultivars tested as whole meal flours in Chapter 2. Cultivars were selected to

represent the full range of wheat strength as measured by Recovery Index. Selected cultivars were: Jagalene (HRW), Briggs (HRS), Norpro (HRS), Eltan (SWH), and Stephens (SWH). Table 4-1 gives some background results from previous work conducted by the Mulvaney lab and collaborators for these five selected cultivars. Refined flours produced from these cultivars were obtained from GIPSA and stored in zip-sealed polyethylene bags at room temperature for a period of 3-4 years before use.

Table 4-1 Background information about the 5 selected wheat cultivars

Cultivar	Flour Protien Content (%) [a]	Baked Loaf Volume (mL) [a]	Gluten Index [a]	WMF Gluten RI [d]	WMF Gluten MC [d]	CORE Degree of Recovery: Gluten (%) [b]	CORE Degree of Recovery: Dough (%) [c]
Jagalene	10	769	98.5	71.2	62.3	77.5	11.1
Briggs	13.4	825	93.1	54.6	64.7	57.9	14.3
Norpro	11.8	788	88.6	25.9	65.8	41.8	14.2
Eltan	11.1	863	81.5	22.7	67.6	29.1	4
Stephens	11.4	675	42.7	9	63.5	5	-

a. *Zhao et al., 2010*
b. *Chapman et al., 2012.*
c. *Halabi, 2012.*
d. *Chapter 2 of this thesis.*

Organic Stabilized Oat Bran Powder, a finely powdered oat bran product (Lot 1201120) was obtained from Grain Millers (Eugene, OR). According to the manufacturer's estimate, the product contained 5.5% β-glucan by weight, but this value was determined using the USDA nutritional database and not a direct measurement of the actual material. Protein content was estimated to be 14.6% by triplicate Kjeldahl Analysis using the oat conversion factor of 5.83. Oat Bran Powder was stored in a sealed PET jar at room temperature for a period 6-12 months before use.

Oatwell 28, an air-fractionated β-glucan isolate from oat bran was obtained from CreaNutrition via the US distributor Oat Ingredients, LLC (Boulder, CO). According to the manufacturer's certificate of analysis, the material (Batch 1126, Lot 6), contained 27.2% β-glucan by weight. Protein content was estimated to be 20.5% by triplicate

Kjeldahl Analysis using the oat conversion factor of 5.83. Oatwell was stored in zip-sealed polyethylene bags at room temperature for a period 6-12 months before use.

2% Sodium Chloride Solution was prepared by dissolving 200g crystalline NaCl to a final solution volume of 10.0 L. ACS Grade Sodium Chloride was obtained from Fisher Chemical (Agawam, MA).

4.2.2 Preparation of control gluten samples

Untreated control gluten samples were prepared using a modified version of the extraction procedure described by Chen (2011) for wholemeal flours described in Chapter 2. Ten grams of refined flour corresponding to each of the chosen cultivars were used as a starting material. Even though the control samples consisted only of refined flour, the whole meal extraction procedure was used so that established preparation protocol would be appropriate for use with the oat-bran material treated samples. The average yield of dry gluten obtained upon drying each control sample was then used in the calculation of treatment levels for each flour.

4.2.3 Determination of Oat Bran and Oatwell 28 treatment levels

A maximum treatment of Oatwell 28 and Oat Bran that could be used successfully with 10g samples of each flour was determined by trial and error, and these maximums were compared. Levels of both Oatwell 28 and Oat Bran that could be successfully added to 10g Glutomatic batches of all five selected experimental flours, on a constant “per gram dry gluten” basis, were then determined. Final doses of Oat Bran and Oatwell 28 to be used for each cultivar were then calculated using the average yield of dry gluten obtained during analysis of the control samples.

4.2.4 Preparation of treated gluten samples

Treated gluten samples were prepared using a modified version of the preparation method used for the control samples. 10g of refined flour was intermingled with the appropriate calculated dose of either fine ground oat bran or Oatwell oat bran isolate and hydrated with exactly 5.0mL 2% NaCl solution, and transferred to a Glutomatic chamber equipped with a fine mesh sieve.

4.2.5 Preparation of control and treated gluten samples for CORE analysis

Washed gluten samples were removed from the Glutomatic, formed into a ball, placed in a small airtight plastic container coated with petroleum jelly on the interior, and allowed to rest for 10 to 45 minutes at room temperature. Prior to CORE analysis, gluten samples were transferred to Perten centrifuge holders lubricated with petroleum jelly and centrifuged for 5 minutes to standardize their dimensions for the CORE analysis.

4.2.6 CORE Analysis

CORE analysis was carried out using the protocol described in Chapman (2011), using the Gluten CORE apparatus and software, calibrated according to Perten protocol. Samples were expelled from centrifuge holder/shapers directly onto the petroleum jelly lubricated CORE platform. Gluten compression used a force of 8 Newtons, for 5 seconds, followed by a recovery period of 55 seconds. Height measurements of samples were recorded by the Perten software every 0.01 seconds during the CORE cycle. Measured parameters and calculated outcome variables from the CORE analysis are defined in are defined in Table 1-2 on Page 27.

4.2.7 Moisture Content Determination

After CORE analysis, the moisture content of gluten samples was determined by weighing gluten samples before and after sample dehydration. Gluten dehydration was carried out by heating in a Perten Glutork apparatus for either the default four minute heating cycle, or longer if necessary. Percent Moisture (PM), which is also expressed as Moisture Content (MC) in this thesis, was determined as the sample's measured moisture loss divided by the initial sample mass.

4.2.8 Statistical Analyses

Averages and Standard Deviations of Recovery Index (%), Percent Compression (%), Half-Recovery Time (s), and Fraction Water measurements, were calculated for each set of replicates. Student's t-tests were performed to determine whether treatment average measurements for each cultivar were significantly different from control average measurements of the same cultivar, using $\alpha = 0.05$.

CORE Thickness Average Curves were constructed for each treatment by averaging thickness data for all CORE test replicates at each time point during the compression and recovery periods.

Apparent Percent Recovery Average Curves were constructed for each treatment by averaging the Degree of Recovery (%) for all replicates at each time point during the 55 second recovery period.

4.3 Results and Discussion

4.3.1 Summary of Results for Control and Treated Gluten Samples

Table 4-2 summarizes the control dry gluten yield measurements and dose calculations that were carried out in order to ensure treatment doses for each flour were proportional to the amount of gluten present.

Table 4-3 summarizes replicate counts, average outcome variable measurements, standard deviations, and identifies treatment results that were significantly higher or lower than their corresponding control results. The same data are shown visually in Figure 4-1, with box plots representing quantiles.

Significant increases in Recovery Index were observed for some, but not all, wheat cultivars. Jagalene, which has the highest baseline RI, appears to have the least observable interaction with the oat bran treatments in terms of RI. Mid-elasticity cultivars Briggs, Norpro, and Eltan did show significant increases in RI from at least one of the two oat bran treatments. Stephens, the cultivar with the lowest baseline RI, showed increases in the average RI measured, but these were not significant in this study. The results of Half-Recovery Time followed the same pattern of significant decreases as was seen for the significant increases of Recovery Index.

Moisture Content results were generally observed to mirror the results of Recovery Index trend-wise, but there appears to be a higher degree of noise in the Moisture data here. The high variation in Moisture Content measured in Jagalene gluten replicates, for instance, could be related to this cultivar's low gluten yield.

It is interesting that Percent Compression measurements in this study exhibit significant decreases for at least one of the two treatment samples across all of the

cultivars examined, since a clear decrease in Percent Compression was not one of the effects observed in the dose response study of Chapter 3.

Overall, these data suggest at a conclusion that cultivars of wheat with a low baseline elasticity may be particularly well suited to fortification with oat bran materials, but the lack of significance across all treatments, as well as the poor agreement from gluten MC results, indicate that further study will be necessary to confirm this hypothesis. Since the "washed" sample preparation method used here has shown to be problematic with higher doses of oat bran materials, it is suggested that a new sample preparation method be devised to improve the detectability of the gluten/oat bran material interaction. A dosed dough, rather than gluten extracted from a dosed dough, may be an interesting and highly industrially relevant model to examine, although this would prevent conclusions from being made about the gluten network itself, since the presence of a starchy phase would make the system more complicated. A modification to the "washed" sample preparation method used here that is suggested would be to use a gluten washing solution consisting of both 2% NaCl and the soluble fraction of the oat materials of interest. If the interaction between the oat materials and the gluten is in fact based on hydrogen bonds between gluten and β-glucan, as is proposed in Chapter 3, this sample preparation method would yield much more significant changes in both MC and RI.

Table 4-2 Control Yield Averages and Treatment Dose Determination

Cultivar	Average Dry Yield Gluten Obtained from 10g Flour Control Sample (g)	Oat Bran Treatment Dose per 10g Flour Sample (g) *(= Control Yield x 1.25)*	Oatwell 28 Treatment Dose per 10g Flour Sample (g) *(= Control Yield x 0.75)*
Jagalene	0.81 ± 0.02	1.01	0.60
Briggs	1.22 ± 0.01	1.53	0.92
Norpro	1.10 ± 0.01	1.38	0.83
Eltan	1.05 ± 0.01	1.31	0.79
Stephens	1.14 ± 0.04	1.43	0.86

Table 4-3 Summary of Control and Treated Gluten Sample Measurements

Cultivar	Treatment	n	Recovery Index (%)	Percent Compression (%)	Half Recovery Time (%)	Moisture Content (%)
Jagalene	**Control**	**6**	**87.2 ± 2.9**	**76.3 ± 1.1**	**0.94 ± 0.24**	**62.1 ± 0.3**
	Oat Bran	4	83.9 ± 9.2	73.6 ± 2.5 *↓	1.22 ± 0.51	64.2 ± 4.7
	Oatwell 28	6	88.0 ± 1.5	73.9 ± 0.8 *↓	0.74 ± 0.06	62.9 ± 0.7
Briggs	**Control**	**6**	**71.8 ± 2.5**	**78.0 ± 0.5**	**2.11 ± 0.25**	**66.0 ± 0.7**
	Oat Bran	5	66.9 ± 6.2	76.0 ± 0.7 *↓	2.87 ± 1.02	67.2 ± 1.7
	Oatwell 28	6	86.8 ± 5.5 *↑	73.9 ± 1.2 *↓	1.09 ± 0.37 *↓	65.4 ± 0.9
Norpro	**Control**	**7**	**55.4 ± 4.4**	**78.8 ± 1.7**	**4.74 ± 0.85**	**67.1 ± 0.8**
	Oat Bran	6	71.3 ± 5.7 *↑	75.5 ± 1.0 *↓	2.49 ± 0.62 *↓	66.9 ± 0.8
	Oatwell 28	6	78.6 ± 4.9 *↑	75.8 ± 1.0 *↓	1.69 ± 0.57 *↓	66.2 ± 0.5 *↓
Eltan	**Control**	**6**	**44.4 ± 3.4**	**80.6 ± 0.6**	**5.91 ± 1.05**	**68.6 ± 0.8**
	Oat Bran	6	65.7 ± 9.6 *↑	76.5 ± 2.0 *↓	3.90 ± 1.12 *↓	66.3 ± 0.9 *↓
	Oatwell 28	6	52.2 ± 9.6	78.1 ± 0.8 *↓	5.63 ± 1.99	68.6 ± 1.5
Stephens	**Control**	**6**	**15.3 ± 4.3**	**83.9 ± 1.0**	**8.47 ± 2.94**	**63.5 ± 0.8**
	Oat Bran	6	23.7 ± 7.0	84.3 ± 1.2	10.10 ± 1.38	66.7 ± 0.6
	Oatwell 28	6	21.9 ± 12.0	81.6 ± 1.6 *↓	8.35 ± 3.09	65.4 ± 2.7 *↑

Asterisks followed by arrows indicate whether treatment averages are significantly higher (↑) or significantly lower (↓) than control averages.

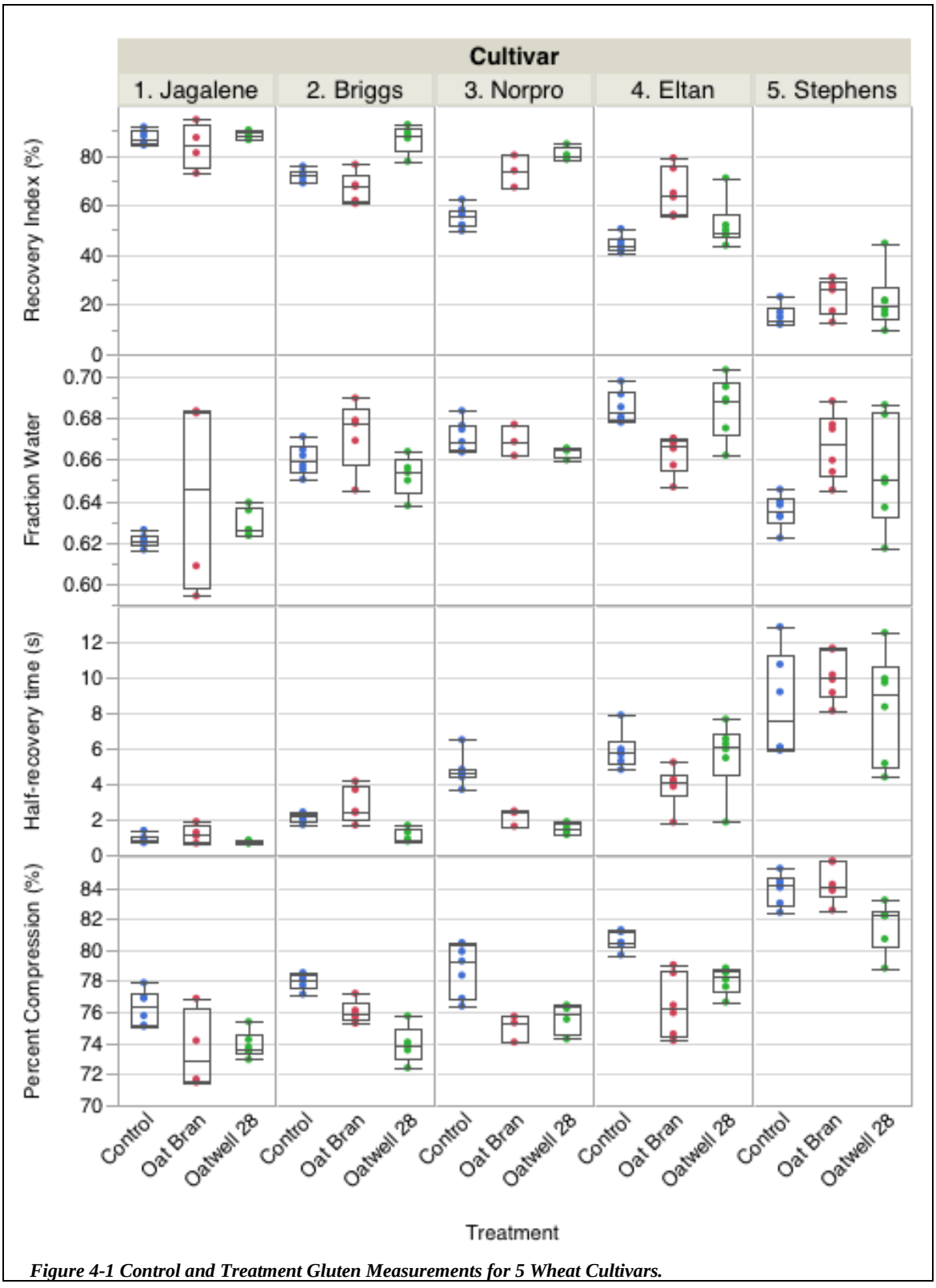


Figure 4-1 Control and Treatment Gluten Measurements for 5 Wheat Cultivars.

4.3.2 Visualization of CORE behavior of Control and Treatment Samples: Average Sample Thickness and Recovery Curves over Time.

Figure 4-2 shows Gluten CORE Average Thickness Curves for all five control and treatment sets. Figure 4-3 shows and Average Recovery curves with Half Recovery Times for the same five control and treatment sets. Control averages are shown with black lines and treatment averages are shown with lighter lines. Control and treatment lines from the same cultivars are shown with the same degree of dashing, and matching arrow heads.

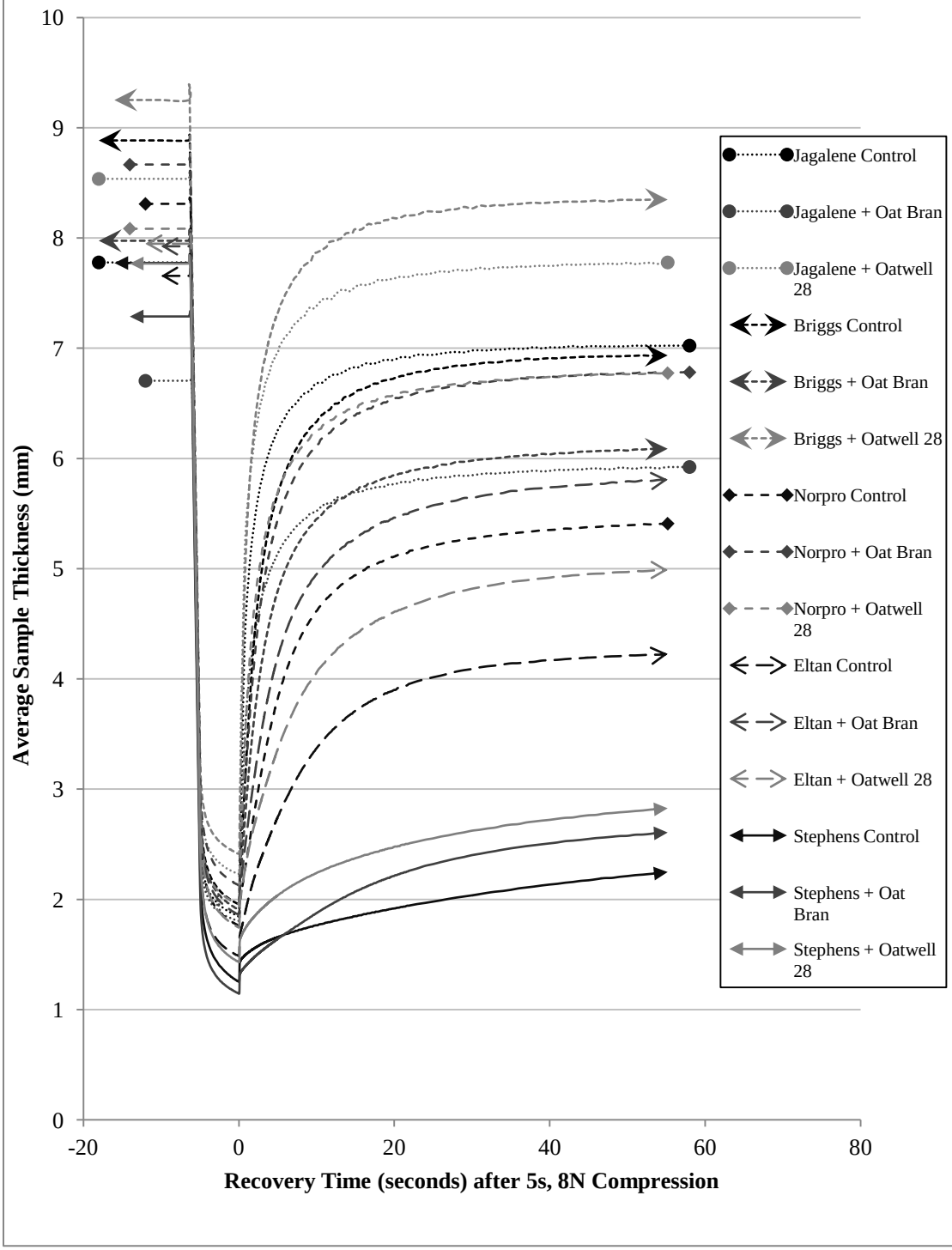


Figure 4-2 Average CORE Thickness Curves for Control and Treated Gluten from Five Wheat Cultivars

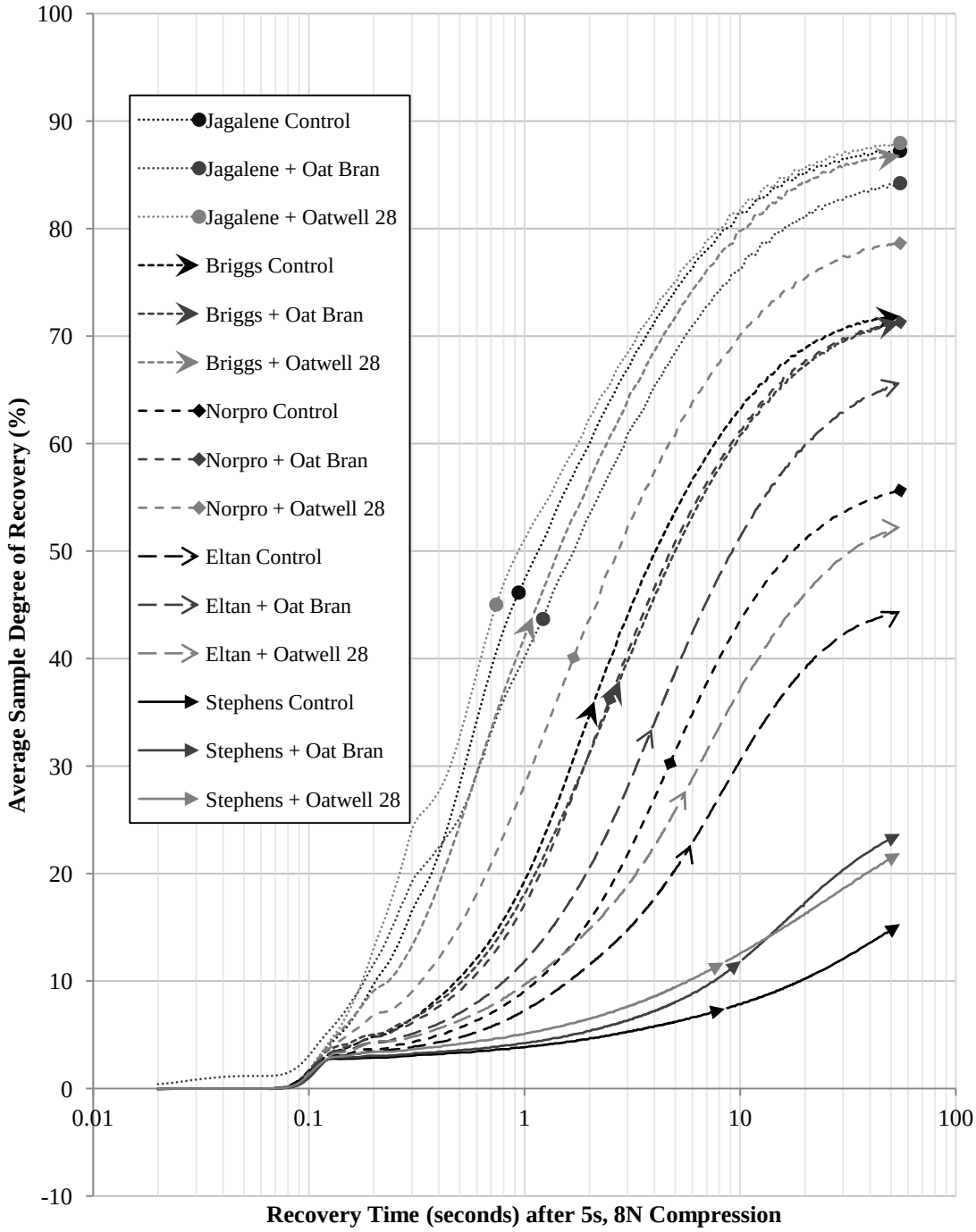


Figure 4-3 Average Degree of Recovery Curves and Half-Recovery Times for Control and Treated Gluten from Five Wheat Cultivars

4.4 Conclusions

It is possible that the oat-bran/wheat gluten interaction observed in Chapter 3 could be taken advantage of to impart simultaneously improved health and baking properties to flour with poor native elasticity, but this is only suggested by the results of this study. A better sample preparation method would allow higher effective doses of oat bran materials to be used so that more significant changes in gluten Moisture Content and Recovery Index could be observed. Also, more applied studies characterizing the rheological effect of oat bran materials on gluten in complex dough systems will be useful.

Appendix. Recommendations for future work with the Gluten CORE

Recommendation #1 - Gluten sample moisture content measurement will allow for a more meaningful interpretation of Gluten CORE measurements.

Chapter 2 of this thesis suggests that the baking quality of a flour should be evaluated using a combination of 1) protein quantity, and 2) protein quality. This study has suggested that hydrated gluten is only a valid model for examination of wheat protein quality *if* the moisture content of that hydrated gluten is recorded on an individual sample basis, which previous work with the Gluten CORE did not do.

It is suggested that the best way to proceed with the Gluten CORE project will be to begin measuring sample moisture content. Another advantage of moisture content measurement is that the Glutork sample drying procedure preserves each sample, making it available for reference in case any later analyses are determined to be useful.

Recommendation #2 – Gluten CORE sample temperature should be controlled.

Liang, 2006, reported significant changes in gluten rheology across a temperature range of 25 degrees to 60 degrees C. Although the "room temperature" that this and previous Gluten CORE research did not vary this widely, it may be prudent for further researchers to either ensure gluten samples are formed and analyzed at a constant specified temperature, or to record measurements of individual sample temperatures during Glutomatic extraction and CORE analysis, so that effects of any temperature variation can be evaluated.

Recommendation #3 – Gluten CORE sample lubrication technique should be improved.

The difference in the calculated "half recovery time" observed for gluten extracted from the Stephens wheat cultivar using the different procedures in Chapter 2 and Chapter 4 highlights the potential of friction effects to obscure CORE analysis results without the implementation of an optimized and standardized sample lubrication procedure. The research conducted for this thesis made use of a petroleum jelly coating on the sample contact surfaces of the Gluten CORE compression plates, but it was observed that there was still sometimes variation in sample tackiness, which likely resulted in differences in friction losses across test samples. The extremely low sample tackiness experimentally observed for treatment samples in Chapter 3's "unwashed" dose response series suggests there may be better lubricant choices available for the Gluten CORE procedure. The Chapter 3 treated unwashed samples' low tackiness was due to the formation of an aqueous phase made viscous enough by excess β-glucan to maintain a thin layer of stickiness-preventing water on the surface of the gluten samples so they did not stick to the petroleum jelly surface of the plates at all. It may be useful to identify an appropriate hydrocolloid solution to use to thinly coat gluten samples immediately prior to Gluten CORE analysis. Perhaps a heavily side-chained or branched neutral polymer such as xanthan gum or gelatinized amylopectin would be an ideal choice, since these may be sterically hindered from infusing into - and potentially interacting with - the sample gluten network.

Recommendation #4 – Gluten CORE output files containing data collected at 0.01s intervals should list every 0.01s interval, to allow averaging of replicate trials.

In the experiments described in Chapters 3 and 4 of this thesis, sample thickness and force data was collected by Perten's Gluten CORE software every 0.01 seconds during compression and recovery, as opposed to the experiment described in Chapter 2, which used a setting to collect data every 0.1 seconds. It was found that the 0.01s data was preferable, because it provided better resolution of sample behavior during periods of rapid change, such as at the start of the compression period and the start of the recovery period. However, using the 0.01s setting proved to be problematic in creating "Average Thickness Curves" and "Average Recovery Curves", since it was found that random time points were occasionally missing from the raw data, making it difficult to manually average data from replicates because the missing data points prevented replicate time points from lining up with each other in a spreadsheet. To resolve this issue, raw data was processed using a Pick BASIC program, shown in Table A-1, which was developed by Marc Kahn. The program identified missing time points in the raw CORE data for each replicate, created "filler" time points using thickness and force data from the previous recorded time point, and marked the new filler data points with an asterisk so they could be reviewed. The output of this program allowed data collected from all replicates in a series to be averaged easily in a spreadsheet. Future work with Perten's Gluten CORE should seek another way to allow for high resolution data collection without requiring further processing to align replicate data in a spreadsheet for averaging.

Table A-1 Pick BASIC program used to align raw CORE data with random missing time points.

```
* NORMALIZE.SUSIES.DATA
*
* Written by Marc Kahn, July 4, 2013
*
* Susie has collected data in a time series from a mechanical analysis machine
*  which generated data in .01 second increments.  However, there are some
*  missing samples at random places within the data series.  These need to be
*  filled in so that the various series which need to be compared can all
*  be synchronized chronologically.
*
*-----------------------------------------------------------------------------
* Mainline Routine
*-----------------------------------------------------------------------------
*
GOSUB A100.INITIALIZE
END.OF.LIST = FALSE
LOOP
  READNEXT INPUT.FILE.NAME FROM INPUT.FILE.NAME.LIST ELSE END.OF.LIST
= TRUE
UNTIL END.OF.LIST DO
  GOSUB B100.PROCESS.A.FILE
REPEAT
CRT "ALL DONE!"
STOP
*
*-----------------------------------------------------------------------------
* Subroutines:
*
* A100.INITIALIZE
*
* B100.PROCESS.A.FILE
*
*-----------------------------------------------------------------------------
A100.INITIALIZE:
*
AM = CHAR(254)
VM = CHAR(253)
SVM = CHAR(252)
TAB = CHAR(09)
CR = CHAR(13)
TRUE = 1
FALSE = 0
OTHERWISE = 1
NONE.OF.THE.ABOVE = 1
*
INPUT.FILE.FOLDER = "D:\CUBS\CCI\MARCDATA\SUSIE\BEFORE\"
OUTPUT.FILE.FOLDER = "D:\CUBS\CCI\MARCDATA\SUSIE\AFTER\"
*
OPENPATH INPUT.FILE.FOLDER TO INPUT.PICK.FILE ELSE
  CRT  "Unable to OPENPATH on ": INPUT.FILE.FOLDER
  STOP
END
OPENPATH OUTPUT.FILE.FOLDER TO OUTPUT.PICK.FILE ELSE
  CRT "Unable to OPENPATH on ": OUTPUT.FILE.FOLDER
  STOP
END
*
SELECT INPUT.PICK.FILE TO INPUT.FILE.NAME.LIST
*
* Data structures for data manipulation
DIM INPUT.DATA (6300,8) ;* Max 6300 spreadsheet rows by 8 data series
DIM OUTPUT.DATA (6300,8)
* Each variable within the above arrays will contain 3 attributes:
TIME.AMC = 1
THICKNESS.AMC = 2
```

```
            FORCE.AMC = 3
            *
            RETURN
            *
            *-----------------------------------------------------------------------------
            B100.PROCESS.A.FILE:
            *
            READ INPUT.FILE FROM INPUT.PICK.FILE, INPUT.FILE.NAME THEN
              CRT "Processing ": INPUT.FILE.NAME : " - ": TIMEDATE()
            END ELSE
              CRT "Unable to read ": INPUT.FILE.NAME
              INPUT JUNK
              RETURN
            END
            *
            * Data comes in with CR row delimiters instead of expected CRLF.  Convert
            *  CRs to AMs.
            CONVERT CR TO AM IN INPUT.FILE
            *
            MAT INPUT.DATA = ""
            MAT OUTPUT.DATA = ""
            *
            * Load input data array from data in rows 13+ from the input file
            DATA.ROW.IDX = 0
            MAX.INPUT.FILE.AMC = DCOUNT(INPUT.FILE, AM)
            FOR INPUT.FILE.AMC = 13 TO MAX.INPUT.FILE.AMC
              DATA.ROW.IDX += 1
              ROW.DATA = INPUT.FILE<INPUT.FILE.AMC>
              CONVERT TAB TO AM IN ROW.DATA
              FOR COLUMN.IDX = 1 TO 8
                BASE.COLUMN.AMC = ((COLUMN.IDX - 1) * 5) + 2
                TIME.VALUE = ROW.DATA<BASE.COLUMN.AMC>
                THICKNESS.VALUE = ROW.DATA<BASE.COLUMN.AMC + 1>
                FORCE.VALUE = ROW.DATA<BASE.COLUMN.AMC + 2>
                BUILD = ""
                BUILD<TIME.AMC> = TIME.VALUE
                BUILD<THICKNESS.AMC> = THICKNESS.VALUE
                BUILD<FORCE.AMC> = FORCE.VALUE
                INPUT.DATA(DATA.ROW.IDX, COLUMN.IDX) = BUILD
              NEXT COLUMN.IDX
            NEXT INPUT.FILE.AMC
            *
            * Navigate input data array, one column at a time, populating the output
            *  data array with raw data, but also filling in for missing time slices
            FOR COLUMN.IDX = 1 TO 8
              BEGINNING.TIME.VALUE = INPUT.DATA(1, COLUMN.IDX)<TIME.AMC>
              * Most files only have 6 data columns.  In order to not populate columns
              *  7 and 8 on those guys, detect an empty input column and escape the loop.
              IF BEGINNING.TIME.VALUE EQ "" THEN
                CONTINUE
              END
              PREVIOUS.TIME.VALUE = BEGINNING.TIME.VALUE - .01 ;* Initialize for first loop
iteration
              PREVIOUS.THICKNESS.VALUE                =                INPUT.DATA(1,
COLUMN.IDX)<THICKNESS.AMC>
              END.OF.THIS.COLUMN = FALSE
              INPUT.ROW.IDX = 0
              OUTPUT.ROW.IDX = 0
              LOOP
                INPUT.ROW.IDX += 1
                SAMPLE.DATA = INPUT.DATA(INPUT.ROW.IDX, COLUMN.IDX)
                TIME.VALUE = SAMPLE.DATA<TIME.AMC>
                IF TIME.VALUE EQ "" THEN
                  * We've gone past the last data point for this column
                  END.OF.THIS.COLUMN = TRUE
                END
              UNTIL END.OF.THIS.COLUMN DO
                EXPECTED.TIME.VALUE = PREVIOUS.TIME.VALUE + .01
                LOOP UNTIL TIME.VALUE EQ EXPECTED.TIME.VALUE
                  * Here's where we fill in missing data
```

```
      OUTPUT.ROW.IDX += 1
      BUILD = ""
      BUILD<TIME.AMC> = EXPECTED.TIME.VALUE
      BUILD<THICKNESS.AMC> = PREVIOUS.THICKNESS.VALUE
      BUILD<FORCE.AMC> = "*" ;* Indication that this is inserted data
      OUTPUT.DATA(OUTPUT.ROW.IDX, COLUMN.IDX) = BUILD
      EXPECTED.TIME.VALUE += .01
    REPEAT
    *
    OUTPUT.ROW.IDX += 1
    OUTPUT.DATA(OUTPUT.ROW.IDX, COLUMN.IDX) = SAMPLE.DATA
    PREVIOUS.THICKNESS.VALUE = SAMPLE.DATA<THICKNESS.AMC>
    PREVIOUS.TIME.VALUE = TIME.VALUE
  REPEAT
NEXT COLUMN.IDX
*
* Now, we build the output file...
*
NAME.LENGTH = LEN(INPUT.FILE.NAME)
OUTPUT.FILE.NAME = INPUT.FILE.NAME[1, (NAME.LENGTH - 4)]: "_Normalized.txt"
OUTPUT.FILE = ""
*
* Replicate first 12 rows of spreadsheet file
FOR AMC = 1 TO 12
  OUTPUT.FILE<AMC> = INPUT.FILE<AMC>
NEXT AMC
*
OUTPUT.ROW.IDX = 0
COLUMN.1.TIME.VALUE = -.01 ;* Initial value, so first line becomes zero
LOOP
  OUTPUT.ROW.IDX += 1
  COLUMN.1.TIME.VALUE += .01
  ROW.DATA = COLUMN.1.TIME.VALUE
  END.OF.DATA = TRUE
  FOR COLUMN.IDX = 1 TO 8
    SAMPLE.DATA = OUTPUT.DATA(OUTPUT.ROW.IDX, COLUMN.IDX)
    TIME.VALUE = SAMPLE.DATA<TIME.AMC>
    THICKNESS.VALUE = SAMPLE.DATA<THICKNESS.AMC>
    FORCE.VALUE = SAMPLE.DATA<FORCE.AMC>
    *
    BASE.COLUMN.AMC = ((COLUMN.IDX - 1) * 5) + 2
    ROW.DATA<BASE.COLUMN.AMC> = TIME.VALUE
    ROW.DATA<BASE.COLUMN.AMC + 1> = THICKNESS.VALUE
    ROW.DATA<BASE.COLUMN.AMC + 2> = FORCE.VALUE
    *
    IF TIME.VALUE NE "" THEN
      END.OF.DATA = FALSE
    END
  NEXT COLUMN.IDX
UNTIL END.OF.DATA DO
  CONVERT AM TO TAB IN ROW.DATA
  OUTPUT.FILE<OUTPUT.ROW.IDX + 12> = ROW.DATA
REPEAT
*
WRITE OUTPUT.FILE ON OUTPUT.PICK.FILE, OUTPUT.FILE.NAME
*
RETURN
*
END
```

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