COMPARISON OF GLUTEN VISCOELASTICITY COMPONENTS WITH TRADITIONAL DOUGH AND BAKING TESTS

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DOUGH AND BAKING TESTS

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Abstract:

Quantitate analysis of viscoelastic properties of gluten were done by using mechanical analogs, i.e., spring, spring and dashpot arranged in parallel, and dashpot, to describe the elasticity, delayed elasticity and viscous response. The regressed parameters from nine sets of samples were correlated with dough and bread quality. A surfactant (DATEM) decreased elastic deformation (J0) and increase resistance to flow (η_o) of gluten. While, more deformable gluten (increase in instantaneous elastic compliance J0 and delayed elastic compliance J1) was obtained by treatments of oxidation, reduction of disulfide bonds, and disruption of hydrogen bonds with treatments of ascorbic acid, dithiothreitol, and urea, respectively. The results proved that the contributions of non-covalent bonds which are hydrogen bonds and hydrophobic interactions are as important as disulfide bonds to gluten structure. It also suggested the importance of stable protein aggregation and interactions via a surfactant involving hydrophilic and hydrophobic domains. Deformation (J0 and Jr0) of gluten started to decrease after heating at 45°C, suggesting that non-covalent bonds were affected. After heating up to 65°C, the resistance to flow and recoverability of gluten increased, suggesting that gluten agglomeration and formation of covalent bonds was induced by heating at 65°C. Commercial gluten showed different effects when used in flour substitutions. Gluten B with more acidity (pH=4.2 vs 5.2 or 5.5) deformed gluten structure more than gliadin (a plasticizer). After substituting gluten GB, GC, and gliadin, the resistance to flow of gluten decreased and J0 and J1 increased indicating an increase in gluten deformation. This suggests that no new disulfide bonds were formed. We speculate that native disulfide bonds were diluted by increasing the concentrations of gluten and only hydrophobic interactions and hydrogen bonds were formed by GB, GC, and gliadin. Gluten strength and deformability were the main contributors of the variance in breeder line samples of crop years 2008 to 2011. Gluten recoverability and flour protein also contributed to the variance as second and distant third contributors and were independent of strength and deformability. The viscous coefficients were positively correlated with dough mixing properties.

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CHAPTER I

INTRODUCTION

Macropolymeric gluten protein plays a major role in bread quality due to its unique viscoelastic properties. During the bread making process, the gluten structure is altered by many factors such as additives and temperature. The study of wheat gluten proteins is challenging due to the diversity of their compositions which results in a variation in their structures and properties. This dissertation is a study of viscoelastic properties of gluten by using mechanical modeling to quantify the effect of temperature and additives on the structure of gluten and gluten bonds. The results will reveal relationships between gluten structure at the molecular level and its viscoelastic properties. Gluten is considered as a viscoelastic food material and is formed by glutenin and gliadin. Polymeric glutenins contribute to the elastic properties of dough, while monomeric gliadins give dough viscosity. Consequently, the ratios and the structure of glutenin and gliadin in a gluten system are very critical in dough. Factors such as temperature, mechanical stress, and the presence of additives can directly affect the end product characteristics (e.g., loaf volume, crumb structure, and crust color, etc.) as well as dough properties during processing (e.g., machinability, stickiness, handling stability, and

rate of proof). Currently, the understanding on how these factors affect gluten at the molecular level is still incomplete. Understanding the molecular basis for gluten viscoelasticity could help breeders and manufacturers predict the end product of bread quality and understand processing problems that may arise.

The novelty of this work was the focus on modeling the effect of additives, temperature, and gluten substitution on rheological properties of gluten to improve the analysis of the structures formed. Furthermore, the regressed data were used to investigate an alteration of gluten structure quantitatively in order to enhance the interpretation of the experimental results. The effect of additives and temperature on rheological properties had been studied previously (Ambardekar 2009; Chompoorat 2011), thus, we attempted to further elucidate the results by explaining the possible modifications of gluten structure. We also included a study of breeder samples (hard red winter wheat breeder lines and cultivars) viscoelastic properties; their regressed parameters (from Burgers model) were compared in order to quantitate the contributions from different protein components and the structures attributed to them as suggested by the mechanical model units of springs and dashpots. Moreover, the secondary structure of gluten was studied using Fourier transform infrared spectroscopy (FTIR) with an attempt to provide information regarding changes the structures. The overall results from these studies can help to explain the variations of gluten rheological behavior due to changes at the molecular level.

Objectives

The overall objective is to improve the understanding of the viscoelastic properties of gluten by obtaining regressed parameters from the application of modeling and incorporating these parameters in correlation tests that can improve the interpretation of the experimental results. On each specific test below the experimental results were modeled to obtain more information via the regressed parameters with an attempt to link molecular changes to each mechanical analog used in the model.

The specific objectives for which modeling analysis was applied were:

1) To investigate the effect of diacetyl tartaric acid esters of monoglycerides (DATEM), ascorbic acid (AA), urea, and dithiothrietol (DTT) on viscoelastic properties of gluten, dough, and bread by using creep-recovery, dough mixing, and breadmaking tests. The experimental data of this study was conducted by Amogh Ambardekar and results were modeled by Pavalee Chompoorat to synthesize a direct comparison among the four compounds (DATEM, AA, urea and DTT). Each compound affects specific bonds in the gluten molecules and a comprehensive comparison of the changes measured allowed a direct comparison of the magnitude of change that they produce within the limits of the concentrations used.
2) To investigate the effect of temperature on viscoelastic properties of gluten by using creep-recovery test. The temperature range was increased to include 65°C.
3) To study the effect of commercial gluten and gliadin products substitution in a hard red winter wheat flour on gluten by testing their rheological properties using creep-recovery and compression-recovery.

4) To investigate the effect of commercial gluten and gliadin products substitution in six hard red winter wheat flours on gluten by testing their rheological properties using creep-recovery and compression-recovery.
5) To correlate coefficients of instantaneous elastic deformation, retarded viscoelastic deformation, and pure viscosity with dough extensibility, dough mixing, and breadmaking properties from five breeder sample sets of hard red winter wheat flours by using creep-recovery test, Mixograph test, and breadmaking test.

Hypotheses and Assumptions

Diacetyl tartaric acid esters of monoglycerides (DATEM) act as a surfactant in gluten and dough systems. Previous studies had shown that DATEM decreased gluten compliance at 40 Pa shear stress, air bubble areas of dough, while batter agglomeration and maximum dough development height during fermentation was increased (Ambardekar 2009, Hughes 2011, Lim 2011, Visireddy 2011). Thus, it can be hypothesized that DATEM increased gluten strength and rigidity. We assumed that DATEM interacted with gluten by increasing gluten molecular size polymer with hydrophobic and hydrophilic crosslinks. Ascorbic acid (AA) is an oxidizer which can indirectly promote disulfide bonds in glutenin subunits and gliadins. It had been observed previously that AA did not reveal a trend in viscoelastic properties of gluten but it improved loaf volume of bread (Ambardekar 2009). We hypothesized that AA enhances gluten strength by an increase of disulfide linkages which results in higher elasticity and viscoelasticity. We assumed that AA would enhance elasticity and reduce in viscosity of gluten by increasing the long polymeric glutenin fibrils. Denaturant such as urea has negative effect during mixing by competing with water. Because

water plays an important role on hydrogen bonding with gluten, the stability of gluten via hydrogen bonds will be disrupted by urea addition. It had been reported that urea decreased recovery compliance up to 40% at 0.5 M (Ambardekar 2009). We hypothesized that urea decreases the viscous flow behavior of gluten. We assumed that urea increased the slippage of gluten biopolymer by disrupting the original weak hydrogen and strong hydrophobic bonds of gluten. Lastly, dithiotheritol (DTT) is a reducing agent that disrupts the disulphide bonds converting them to their reduced sulfhydryl form. DTT affected viscosity of gluten by decreasing up to 52.8% at 0.5 mM (Ambardekar 2009). We hypothesized that DTT affected gluten viscoelastic properties by decreasing mainly its elasticity. We assumed that DTT would disrupt disulfide bonds in glutenin subunits which results in a decrease in gluten elasticity. Moreover, molecular weight of gluten would be decreased leading to a lower in gluten elasticity.

Heat treatment will increase kinetic energy in the gluten system and cause a change in conformation by reforming crosslinks of gluten. Previous study showed that heating gluten decreased elastic recoverability starting at temperatures of 45^{o}C to 55^{o}C (Chompoorat 2011). We hypothesized that heating can affect the viscoelastic behavior of gluten by reducing non-covalent bonds by increasing molecular mobility and increasing random covalent bonds during aggregation of gluten. We assumed that gluten conformation will change due to decreasing hydrogen bonds and hydrophobic interactions between gluten components which are high molecular weight glutenin subunits (HMW-GS), low molecular weight glutenin subunits (LMW-GS), and gliadins when exposed to temperature at 45^{o}C to 55^{o}C leading to a decrease in elasticity. After heating gluten at 65^{o}C, the large gluten molecules will have high

energy and increase mobility to reform new cross-links and entanglement; thus, the elasticity of gluten will increase.

The substitution of gluten products and gliadin will alter gluten conformation and affect the viscoelastic of gluten differently. Gluten with all components (HMW-GS, LMW-GS, and glaidin) had shown to increase the elasticity properties of gluten. We hypothesized that gluten products purchased in the market would induce elasticity of gluten, while gliadin would increase viscous flow of a gluten system. We assumed that gluten products and gliadin will interact covalently and non-covalently with the native gluten from a flour creating a larger biopolymer. Therefore, the higher levels of gliadin substitution will significantly increase the viscosity of gluten system, while other gluten products at higher levels of substitution will significantly increase elasticity of a gluten system. If an increase in viscoelasticity is observed, it means that gluten products increase the deformation of gluten by diluting a formation of disulfide bonds. If a decrease in pure viscosity is observed, it can be interpreted that the substitution of gliadin caused weaker molecular interactions and structures resulting in a higher molecular mobility of the polymeric gluten.

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CHAPTER II

LITERATURE REVIEW

1) Background

Gluten is a three dimensional network protein in wheat flour and plays a major role in breadmaking products. Wheat proteins can be classified into four types which are albumin (water soluble), globulin (water insoluble but soluble in salt), gliadin (soluble in 70-90% alcohol), and glutenin (insoluble in water, salt, or alcohol) based on their solubility (Osborne, 1924). Moreover, protein factions can also be categorized as albumins, globulins, prolamins, and glutelins. Gluten protein consists of polymeric glutenin and monomeric gliadin. Both glutenin and gliadin can be further categorized into smaller group in terms of amino acid composition such as high molecular weight (HMW) prolamins, S-rich prolamins, and S-poor prolamins (Shewry et al., 2002) as shown in Table 1. Each group also contains unique repetitive sequences as shown in Figure 1 that contribute to a distinctive gluten structure (Shewry and Halford, 2001) HMW prolamins contain three domains which are short non-repetitive N-terminal domain, short non-repetitive C-terminal domain, and long repetitive central domain

(Shewry and Halford, 2001; Shewry et al., 2002; Shewry et al., 2000;Tatham and Shewry, 2000; Veraverbeke and Delcour, 2002).

Table 1. Partial amino acid composition in prolamins group (Adapted from Shewry and Halford 2001).

Prolamin groups	Gluten compositions	Partial amino acid composition (mol%)					
		Gln	Pro	Gly	Cys	Lys	Phe
HMW prolamins	1) HMW-GS	30-35	10-16	15-20	0.5-1.5	0.7-1.4	0
S-rich prolamins	1) γ-Gliadins 2) β-Gliadins 3) B- and C- type LMW-GS	30-40	15-20	0	2-3	<1.0	0
S-poor prolamins	1) ω-Gliadins 2) D- type LMW-GS	40-50	20-30	0	0-<0.5	0-0.5	8-9

Short non-repetitive N- and C-terminal domains in HMW prolamins have cysteine residues which are responsible for intermolecular covalent bonds (Wieser, 2007). The disulfide linkages between cysteine residues help increase the elasticity of gluten. During hydration, the long repetitive sequences of HMW prolamins with three motifs are contributors to the rod-like β-spiral structure (Belton et al., 1995; Popineau et al., 1994; Wellner et al., 1996). The map of S-rich and S-poor prolamins domains are also depicted in Figure 1 (Shewry et al., 2002). Gliadins are grouped in both S-rich and S-poor prolamins. Gliadins also contain three separate N-terminal, repetitive, and C-terminal domains (Wieser, 2007). The repetitive sequences of S-rich and S-poor prolamins are approximately 40% and 90% of overall wheat prolamins domain, respectively (Fig. 1). Amino acids in these domains also contribute to the gluten conformations where β-turn mostly concentrates in N-terminal domain and α-helix/β-sheet structures predominate at C-terminal domain.

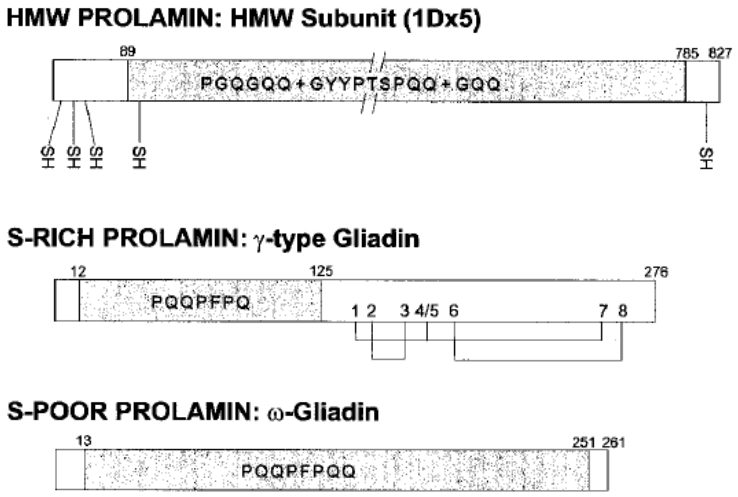

Figure 1. Repetitive amino acid sequences of prolamins group (Adapted from Shewry and Halford (2001).

2) Effect of gluten compositions

The two most important types of gluten are gliadin and glutenin because they are vital to breadmaking performance of wheat flour. Gliadin and glutenin are not soluble in either water or salt solution. They are storage proteins in wheat which contribute to viscoelasticity of dough. Gliadin mainly shows heterogeneous mixture of monomeric polymer and structurally divided into 3 groups which are α-, γ- and ω- types. Glutenin is divided into two groups which are high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). Gliadin is a plasticizer in gluten, while glutenin contributes to elasticity of gluten. Breadmaking quality is mainly depended on both gluten protein quality and quantity as described in Figure 1 (Goesaert et al., 2005). One possible factor that determined gluten protein quantity is the ratio of gliadin to glutenin which results in various glutenin size distribution, structure, and composition.

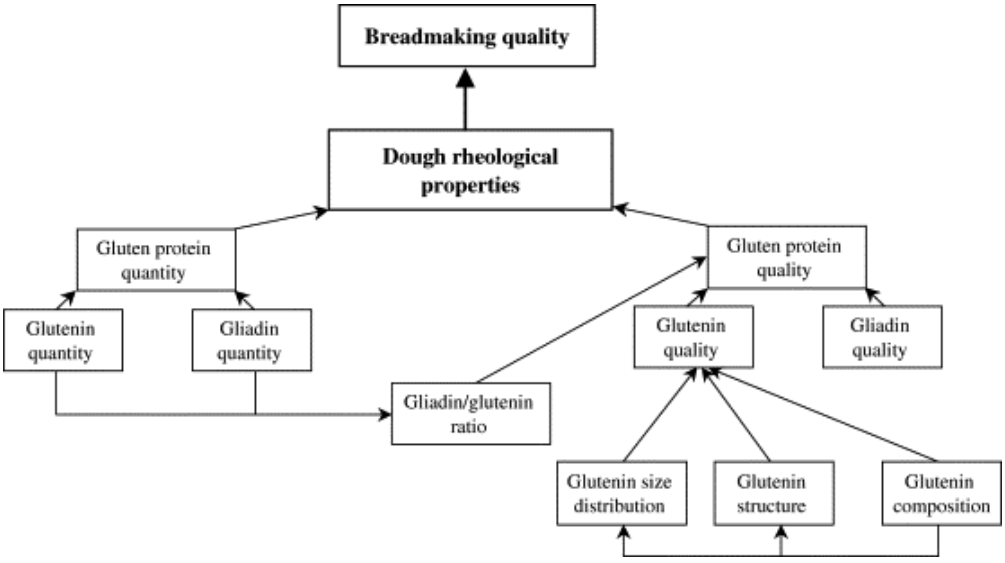

Figure 2. Diagram of howcompositions of gluten affect to breadmaking quality. (Adapted from Goesaert et al., 2005)

Dough strength is positively correlated with loaf volume. Authors support that the ratio of gliadin and glutenin must be balanced in order to have a desirable viscosity and elasticity. However, this ratio has been elusive due to the large variation of quality and cultivars. For glutenin quality, an alteration of glutenin composition can cause the change of non-covalent interactions which is mainly correlated to elasticity of glutenin (Goesaert et al., 2005). As shown by a study of transgene coding for HMW-GS, 1Dx5 transgene subunits increased rheological modulus and cross-linking of glutenin polymers more than 1Ax1 transgene (Popineau et al., 2001).

2.1 High molecular weight glutenin subunits (HMW-GS)

Wheat varieties show more than 20 different HMW-GS. Loci Glu-A1, Glu-B1 and Glu-D1 are genes in which HMW-GS are coded. The molecular weight of HMW-GS is in range of 80,000 to 120,000 Da. HMW-GS is divided into two types which are x-type and y-type according to their molecular weight (x-type has a higher molecular weight

than y-type). Many researchers have attempted to explain how HMW-GS is associated with elasticity of gluten and found that HMW-GS forms the backbone of gluten which has individual subunits cross-linked with disulfide bonds (Eriko et al., 2006; Ng and Bushuk, 1989; Xu et al., 2007). The relationship among size distribution of gluten proteins, surface properties of gluten, dough mixing properties, and baking properties of wheat flours has been intensively studied (Tronsmo et al., 2003). They found that the loaf volume containing HMW-GS 5+10, was positively correlated with oil absorption capacity and hydration capacity of gluten (Tronsmo et al., 2003).

2.2 Low molecular weight glutenin subunits (LMW-GS)

LMW-GS is high in sulfur-containing amino acids and ranges from 30,000-40,000Da. It is approximately 80% of overall glutenin composition. LMW-GS has been divided into three groups which are B- (Mr 42,000-51,000), C- (Mr 30,000-40,000) and D-type (Mr 55,000-70,000) according to their mobilities on SDS-PAGE. To describe quality and quantity of LMW-GS, many researchers have proposed various techniques and hypothesized to explain a different functionality of LMW-GS. Study of viscoelastic properties of durum wheat cultivars with different compositions showed that gluten with both HMW-GS and LMW-GS had positive correlation with overall dough strength (Edwards et al., 2003). When gluten was tested by creep, only gluten with high in LMW-GS showed a change in compliance (Edwards et al., 2003). Maucher et al. (2009) studied the viscoelastic properties of intact wheat kernels of 36 wheat cultivars differing in LMW-GS using load-compression tests. They found that the highest values for gluten strength obtained from SDS-sedimentation and dough mixing time tests corresponded to allelic groups *Glu-A3* d; *Glu-B3* d and g; and *Glu-D3* d, while the lowest strength

corresponded to *Glu-A3* e and *Glu-B3* j (Maucher et al., 2009). Thus there is a large variation of LMW-GS proteins in wheat that contributes to a range in physical and rheological properties.

2.3 Gliadin

Gliadin is divided into four categories which are α-, β-, γ- and ω-gliadins according to their mobility in electrophoresis gel in acidic conditions. The γ-gliadins reveal similar size of 30-40 kDa and similar structure to the LMW-GS. The α-gliadins have amino acid compositions and molecular size similar to γ-gliadins. ω-gliadins are rich in glutamine, proline, and phenylalanine, but contain few or no methionine or cysteine residues (sulphur containing amino acids). However, the α-type and γ-gliadins are relatively rich in sulphur-containing amino acids, and have relatively few proline, glutamine, and phenylalanine residues. Variation in many of gluten functionality can also result from monomeric gliadins. Viscoelastic properties of gliadin was highly depended on their concentration (Xu et al., 2007). Gliadin was suggested an important factor to adjust and control viscoelastic properties of gluten (Xu et al., 2007). The study of gliadin addition showed that total and subgroups of gliadin affected gluten rheological properties. Total gliadin and ω1-gliadin soften gluten, while α-,β-, γ-, and ω2- gliadin stiffen gluten tested by frequency sweep test (Khatkar et al., 2002).

Gluten composition of wheat grain is important in determining the quality and end-use properties of dough. As discussed, various studies revealed that effects of protein quantity, quality, and protein composition are important to distinguish and understand their influence on baking properties.

3) Effect of temperature

Heat treatment increases kinetic energy of the gluten molecules and causes molecular vibration. Many studies reported that elasticity of gluten decreased after heated at 40-45^{o}C, and then increased after 65^{o}C. Glutenin was more heat labile than gliadin because glutenin structure started to alter at 55^{o}C showed by chromatographic examination, while glutenin changed at 75^{o}C (Schofield et al., 1983). Heat increases molecular weight of gluten by polymerization of SH-SS interchange reactions (Schofield et al., 1983). It also increases hydrophobic reaction, chain mobility, and reduces hydrogen bonding. These changes cause a reduction in extractability, deformability (Hayta and Alpaslan, 2001). The secondary structure of gluten at different temperatures was studied and found that moisture content played an important role in the alteration of gluten during heating. Secondary structure of gluten with 0% moisture content did not change after treated with heat, while irreversible changes of gluten secondary structure was observed at 45^{o}C for 47% hydration of gluten (Georget and Belton, 2006). Secondary structure of gliadin was altered by decreasing in α-helical content during heating. α-, β-, γ-, gliadin were stabilized by covalent disulfide bonds and non-covalent hydrogen bonds; however, ω-gliadin was stabilized by strong hydrophobic interaction (Tatham and Shewry, 1985). Other observed gluten behavior with gliadin addition (5% and 10%) during heating and found that thermal stability (200^{o}C) of gluten decreased with an increase of gliadin addition (Khatkar et al., 2013). The rheological properties of gluten during heating (90^{o}C for 0.5 to 6 h) had a higher elastic and viscous modulus when compared with gluten without heating (Apichartsrangkoon, 2002). In addition, gluten was tested with small angle oscillatory deformation at different temperature (25-100^{o}C) and

the results indicated that G' decreased when heated at 60^{o}C and increased at 90^{o}C (Attenburrow et al., 1990).

4) Effect of additives

Various food additives are used to improve bread quality. Diacetyl tartaric acid ester of monoglycerides (DATEM) is a surfactant which can help decreasing surface tension of gas bubble in dough, making it a smaller size. It has been shown that the addition of DATEM, high ester pectin, and transglutanimase helped dough attained a high bread quality by showing suitable dough rheological properties (e.g. high extensibility, optimal resistance to extension, good strain hardening, and longer time of semi-relaxation) (Bollaín and Collar, 2004). Moreover, DATEM can increase resistance to deformation by promoting the interactions among protein, starch, and lipid (Stampfli et al., 1996). DATEM was also shown to affect the glass transition temperature of gluten mainly in rubbery state by softening gluten network (decreasing G' and G") (Toufeili and Kokini, 2004). The secondary structure of gluten was also changed by DATEM as indicated by increased α-helix conformation and decreased in decrease in β-turn and α-helix conformation (Gómez et al., 2013). Ascorbic acid (AA) is used as an ingredient for promoting disulfide linkage via oxidation which improves gas retention ability in dough during fermentation and baking (Wieser, 2007). A denaturant such as urea will disrupt hydrogen bonding by water displacement and increase surface repulsion which destabilizes the overall system (Khatkar, 2005). Therefore, the study that involves urea as additive can be used to find relationships between the degree of hydrogen bonding and the viscoelastic properties. To study the effect of disulfide linkages and the viscoelastic properties, a reducing agent such as DTT can be employed. DTT disrupts disulfide

linkage which will directly affect the molecular weight of gluten due to a reduction in intermolecular and intramolecular bonds and thus reduction of molecular weight (Khatkar, 2005)

5) Burgers model in food systems

Burgers model is commonly applied to study viscoelastic behavior of biopolymers. This model is comprised of Maxwell and Kelvin-Voigt models. Both Maxwell and Kelvin-Voigt models differ in terms of the arrangement of spring and dashpot. Spring represents a Hookean solid which is the elastic component of the material (Steffe, 1996). Dashpot represents Newtonian liquid which is the flow of the material. The combination of Maxwell and Kelvin-Voigt model (i.e., Burgers model) has the ability to describe biopolymers such as gluten during stress and relaxation. Burgers model is also a good tool for investigating molecular response of biopolymers materials. Many researchers have studied the rheological behavior of various food types by modeling the creep-recovery data using Burgers model. For example, studies include the effect of DATEM, ascorbic acid, urea and DTT on gluten (Chompoorat et al., 2013); the relationship among baking quality, glutenin subunit and modulus from modeling (Figueroa et al., 2013); the effect of high and low molecular weight glutenin subunit in wheat kernel (Hernández-Estrada et al., 2012); the effect of water soluble pentosan and ionic strength in gluten (Ma et al., 2012); the effect of creep time, recovery time and shear stress in dough (Van Bockstaele et al., 2011) the effect of high pressure homogenization on tomato juice (Augusto et al., 2013); the effect of gel, emulsions, and hydrocolloid contents on mayonnaise (Dolz et al., 2008); and the effect of resistant starch on biscuit (Laguna et al., 2013).

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CHAPTER III

RHEOLOGICAL CHARACTERISTICS OF GLUTEN AFTER MODIFICATION OF DIFFERENT BONDS BY DATEM, ASCORBIC ACID, UREA AND DTT USING CREEP-RECOVERY TEST

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Abstract

The effects of diacetyl tartaric acid ester of monoglycerides (DATEM), ascorbic acid (AA), urea, and dithiothreitol (DTT) on viscoelastic properties of commercial hard red winter wheat gluten were investigated. A constant shear stress of 40 Pa was applied to gluten during a creep-recovery test. Experimental creep-recovery compliance responses were fitted into a Burgers model with four elements accounting for characteristics of pure elastic (spring), viscoelastic (spring-dashpots elements), and viscous flow (dashpot). DATEM decreased the elasticity and viscoelasticity deformability, and increased pure viscosity (resistance to flow) of gluten. The addition of AA, urea, and DTT, resulted in opposite rheological effects when compared with DATEM. Relationship among physical properties was also studied with principal component analysis (PCA) including gluten viscoelasticity, dough mixing and baking properties. Regressed coefficients from Burgers model accounted for higher percent of explained variance and were independent from flour content, baking and dough mixing properties.

Keywords: Burgers model, creep-recovery test, gluten, gluten and dough rheology, principal component analysis (PCA).

1. Introduction

Gluten is a protein macropolymer in wheat flour that formed in hydrated flour during dough mixing. Gluten plays a major role in viscoelastic properties of breadmaking which is highly correlated to the quality of end product. Glutenins and gliadins are the polymeric and monomeric protein components of gluten, respectively. The high molecular weight glutenin subunits (HMW-GS) are responsible for elasticity of gluten, while low molecular weight glutenin subunits (LMW-GS) for gluten viscoelasticity (Wieser, 2007). Gliadins act as plasticizers by increasing viscous flow to the embedded glutenin polymers. It has been shown that these gluten fractions help holding carbon dioxide and ethanol gases from yeast during fermentation and also provide limited surface activity in dough during proving (Joye et al., 2009). The food industry uses surface active agents and oxidizers in bread formulation to improve interaction between gluten polymers and end product quality.

Diacetyl tartaric acid ester of monoglycerides (DATEM) is one of the most effective surfactants in breadmaking. It is assumed to reduce surface tension resulting in enhancing kinetic stability in gluten and dough system (Gómez et al., 2004). DATEM was also attributed to decrease the surface tension of gas bubbles by interacting with lipids in dough and lead to the formation of smaller bubbles (Hughes, 2011). Presumably, DATEM promotes interactions of protein-starch-lipid, thereby increasing resistance to deformation (Stampfli et al., 1996) and breadmaking functionality such as, dough stability of during proving and volume of bread (Ribotta et al., 2004). While the

effect of DATEM on rheological properties of dough and bread have been reported (Aamodt et al., 2004; Aamodt et al., 2005; Bollaín and Collar, 2004; Jacobsberg et al., 1976; Ponzio et al., 2011; Ying et al., 2009), the underlying understanding of specific changes in the structure of the gluten macropolymers is far from complete. Viscoelastic properties of food and non-food materials are measured by creep-recovery test. It is a rheological test performed by applying an instantaneous constant shear stress to the material and the resulting strain is recorded over time during creep. The shear stress is removed and the residual strain recorded over time during recovery. This test can reveal the alteration of structure at a molecular level. While some reports on the effect of DATEM on viscoelastic properties using creep-recovery test in cereal based foods can be found (Aamodt et al., 2004; Aamodt et al., 2005), no reports have covered the effect of DATEM on viscoelastic properties of gluten using modeling creep-recovery compliance. Ascorbic acid (AA), urea, and dithiothreitol (DTT) are also interesting compounds due to their ability to change protein conformations. The specific interactions of these compounds could yield insights to the relationship between molecular bondings and viscoelastic properties. AA has been widely used as dough improver because of its ability to promote disulfide linkages via oxidation (Wieser, 2007), thus increase dough's ability to retain gas during fermentation and baking. Urea is a denaturant and has the ability to displace water and forms hydrogen bond with amino acids (Khatkar, 2005). It was suggested that urea denatures protein by increasing the surface repulsion which results in structural destabilization. Therefore, by using urea in this study, we could quantify the contribution of hydrogen bonding toward viscoelastic properties. Lastly, DTT disrupts disulfide bond in gluten which will directly

affect both intermolecular and intramolecular bonding of low molecular weight and high molecular weight glutenin subunits (Khatkar, 2005).

In this study we report the structural changes of gluten polymers in the presence of DATEM, AA, urea and DTT, and their relationships to indicators of quality widely used in the baking industry and research laboratories. The gluten was isolated and the protein-protein structures formed were analyzed by modeling their behavior interrogated by creep-recovery compliance.

The objectives of this study were (1) to investigate the effect of DATEM, AA, urea and DTT on viscoelasticity of gluten and apply rheological models to assess structural changes and (2) to determine the relationship between the coefficients obtained from modeling creep-recovery compliance and quality indicators of dough mixing and breadmaking tests. The laboratory experiments in this study were conducted by Amogh Ambardekar.

2. Materials and Methods

2.1 Wheat flours and preparation of DATEM, ascorbic acid (AA), urea, and dithiothreitol (DTT)

Twenty-two commercial hard red winter wheat flour samples (4 controls and 18 treatments of each flour with all levels of all compounds) were analyzed. Flours were obtained from wheat grown in the Southern Great Plains region of United States. We identified the flours as C1, C2, C3, C4, C5, and C6. Flour samples were stored at 0^{o}C and brought to room temperature for 24 h before analysis. Protein, moisture and ash content of flour were determined by near infrared reflectance using a FOSS system model 6500 (FOSS NIR System Inc, Laurel, MD).

Concentrations of each compound were used differently; four levels (0, 0.3, 0.6 and 1.0%, w/w flour basis) of DATEM (AIC DATEM 100, Caravan Ingredients, Lenexa, KS); five levels (0, 50, 100, 150, 200 ppm) of AA (Malinckrodt Baker Inc., Phillipsburg, NJ 08865); four levels (0, 0.5, 1 and 1.5 M) of urea (VWR International Inc., West Chester, PA 19380); and four levels (0, 0.1, 0.25 and 0.5 mM) of DTT (VWR International, West Chester PA, 19380). For a preparation of DATEM, a sonicator was used to heat a 5 mL DATEM solution (0.6, 1.2 and 2 g DATEM in 100 ml of 2% NaCl solution) to 65^{o}C in order to dissolve DATEM. The rest of compounds were directly added to flour as a solution.

2.2 Physicochemical analysis of dough with DATEM, AA, urea, and DTT

Flour with each level of DATEM, AA, urea, and DTT were assessed for 1) dough mixing properties with parameters of development time (DT), stability time (ST), breakdown time (BT) and water absorption (WA) according to Approved Method 54-21.02 (AACC 2000), and 2) baking properties with the optimized straight-dough procedure of Approved Method 10-10.03 (AACC 2000). Parameters of bread quality are dough proof height (PH) and loaf height (LH) measured by a digital proof height gauge (National Mfg. Co. TMCO Inc, Lincoln, NE), loaf volume (LV) from rapeseed displacement, oven spring (OSP) calculated by subtracting loaf height from proof heights, and specific volume (SV) as the ratio of loaf volume to loaf weight. These analyses were performed in duplicates.

2.3 Creep and recovery test of gluten

Five (5) mL DATEM, AA, urea, and DTT solution was added directly to 10 g flour and mixed for 20 sec. Deionized water (5 mL) was used instead of all compounds solution in control samples. Glutomatic system model 2202 (Perten Instruments, Huddinge, Sweden) was used for extracting gluten from wheat flour samples. 10 g of flour sample was added with 0.5 ml of 2% NaCl solution (w/v) in the glutomatic chamber before washing soluble particles with excess 2% NaCl solution through a polyester screen (88 μm) for 6 min. The remaining residue in the chamber was wet gluten which was analyzed with the creep-recovery test.

A creep-recovery method based on Zhao et al. (2010) was used in this study. Mineral oil was applied to the gluten edge in order to prevent moisture loss. Briefly, the gluten was relaxed under a plate of 2.5 kg fitted with 2.5 mm spacers for 60 min at room temperature. A round cutter of 25 mm diameter was used to obtain a gluten disc which was loaded to the rheometer. The test was performed by applying a constant shear stress of 40 Pa for 100 s followed by 1000 s of recovery with parallel plate. The analysis was performed in duplicates. The creep-recovery data was interpolated into 10,000 points before fitting into Burgers model.

3. Burgers model

3.1 Calculation of creep test

During creep test, instantaneous creep (shear) compliance was given to gluten with a constant shear rate and provided changing magnitude of strain as a function of time. Spring and dashpot are two mechanical analogues of rheological behavior. These two elements represent elastic solid (spring) and viscous flow (dashpot) of viscoelastic

materials. Burgers model has been commonly applied to study viscoelastic behavior of soft matter. It is a combination of Maxwell and Kelvin-Voigt models. Maxwell is represented by a spring and a dashpot, while a parallel arrangement between spring and dashpot is used in Kelvin-Voigt model. Equation 1 shows the model during creep:

$$J_c(t) = J_0 + J_1(1 - \exp(-t/t_1)) + t/\eta_0 \quad (1)$$

Our experimental data was fitted into a four-element model of Burgers model. Gluten shows time-dependent behavior during deformation. Therefore, we can study its properties by applying creep (shear) compliance as a function of time ($J_c(t)$). The first element of Burgers model is instantaneous shear compliance (J_0) corresponding to a spring. This element is deflected at the beginning of deformation test and showed gluten pure elasticity with no time delay. The second element is delayed or retarded viscoelasticity (J_1). Retardation time (t_1) is a time of delayed elastic deformation to reach equilibrium at 63.2% of the maximum value of the curve. The last element is pure viscosity of gluten (η_0). This element corresponds to an increase in deformation of dashpot.

Creep-recovery test was applied to investigate the effect of DATEM, AA, urea, and DTT on viscoelastic properties of gluten. Each element of Burger models helped to explain properties of gluten by the coefficients of each curve section. This model is a good tool for investigating molecular response of biological materials. Coefficient values from Burgers model can assist explaining the internal structure of gluten after exposed to DATEM, AA, urea, and DTT in different concentrations.

3.2 Calculation of recovery test

Gluten structure shows non-linear viscoelastic properties due to its ability to recover some structure by storing energy after the applied stress. Shear stress was completely removed during recovery phase. We were able to obtain a reformation value from Burgers model. Equation 2 shows the Burgers model during recovery:

$$J_r(t) = Jr_0 + Jr_1(1 - \exp(-t/tr_1)) \quad (2)$$

Each element in recovery equation corresponds to the described parameters in creep phase (Eq. 1). Eq. 2 contained only 3 elements because there is no dashpot (pure viscous) during recovery phase. In terms of physical changes, tr_1 represents the time it takes the gluten recovery step response to reach 1-1/exp(1) ≈ 63.2% of its final (asymptotic) value. Thus, it is the time required for the elastic recovery of gluten to rise from zero (deformed) to 63.2% of its final value when it varies with time t as 1 – exp(-kt). The time required for elastic recovery to fall to 1/exp(1) (that is 36.8%) of its initial value when it varies with time t as exp(-kt).

4. Statistical analysis

ANOVA was used for testing comparison of means significant differences using Tukey's multiple comparisons test (α=0.05) in SAS program (Version 9.1 SAS Institute Inc., Cary, NC). Principal component analysis (PCA) was performed using Canoco for Windows 4.5 software (Centre for Biometry, Wageningen, The Netherlands) (Braak and Šmilauer, 2002; Legendre and Legendre, 1998).

5. Results and Discussion

5.1 Effect of DATEM, AA, urea, and DTT on viscoelastic properties of gluten

Protein, moisture, and ash content of flour samples are shown in Table 1. Sample C5 was chosen for further study because its protein content closely matched the average of protein content. The creep-recovery curves of selected gluten (C5) with all compounds (DATEM, AA, urea, and DTT) showed typical viscoelastic properties similar to gluten alone (Fig 1). The creep compliance curves of gluten exhibited the same pattern for different levels of all compounds. Compliance was used to describe deformation behavior of viscoelastic material, i.e., the higher the compliance value, the greater deformation and lower rigidity of the material. The result showed that an increase in DATEM concentration significantly reduced the magnitude of maximum compliance, while higher gluten maximum compliance was observed when AA, urea, and DTT were incorporated into gluten system.

Maximum strain (Max strain, γ) at steady-state creep with constant shear rate corresponded to deformation of gluten. In Table 2, max strain (γ) values were obtained directly from the maximum compliance from Fig. 2 in which compliance was converted into strain. After treated gluten with DATEM, gluten showed higher resistance to deformation (γ) compared to control. An increased in rigidity of gluten after treated with DATEM suggested the presence of an end-linked network of high molecular weight and low molecular weight glutenin subunits (HMW-GS and LMW-GS, respectively) infiltrated by gliadin polymers in the form of a resin-like state in which an increase viscosity will account for the increase aggregation of polymers concomitant to a decrease of repulsion forces between polymers caused by DATEM. In comparison, the addition of

AA, urea, and DTT induced significant increase in max strain (γ). An increase in deformation of gluten by urea and DTT can be explained by the disruption of hydrogen bonds and disulfide bonds in gluten system, respectively. It is interesting to note that AA seemed to reduce the deformation of sample C5 which contradicted previous findings because AA was expected to promote disulfide linkages in gluten (Wieser, 2007). Max strain (γ), however, is a function of both elastic deformation (J_0) and viscoelastic deformation (J_1) , and therefore gluten deformation for C5 was significantly affected by a decrease in viscoelastic deformation.

5.1.1 Effect of DATEM, AA, urea, and DTT on gluten coefficients from Burgers model during creep phase

To investigate gluten behavior at the molecular level, the Burgers model was fitted with creep data to obtain rheological parameters. The description of each regressed parameter from Burgers model was described earlier in the methods section. In Table 2, for instantaneous shear compliance (J_0), gluten treated with DATEM resulted in significant decrease in J_0 compared to control, which translated to decrease in elastic deformation and increase in rigidity. When 1% DATEM was added, the parameter J_0 decreased by 18-50% depending on the type of sample (data not shown). The addition of AA, urea and DTT resulted in an opposite behavior when compared to DATEM and they significantly increased elastic deformation (J_0) of gluten. Previously, it has been shown that the elasticity of gluten is mostly attributed to HMW-GS forming the backbone of the polymeric structure via interchain disulfide bonds (Wieser, 2007). Thus, we proposed that a possible explanation for a reduced gluten elastic deformability and the increment of gluten rigidity is the interaction of DATEM with the hydrophobic gluten

domains made largely of HMW-GS and to a less extent by LMW-GS hydrophobic domains. If such interactions lower gluten's original hydrophobicity, the conformation most likely has changed to a lower coil-back potential of the polymer.

The retarded viscoelastic parameter, J_1, obtained from a delayed viscoelastic region of gluten showed that J_1 decreased with an addition of DATEM in gluten, but increased when AA, urea, and DTT were added which is similar to J_0 trend. Viscoelastic properties of gluten is mainly contributed by LMW-GS, therefore, it is possible that all compounds interact with gluten including LMW-GS. Interestingly, increasing the concentration of the compounds did not significantly change retardation time, t_1, even though t_1 is directly related to viscoelastic properties which is similar to J_1. This observation could be due to the insensitivity of exponential term in Burgers model when t_1 was calculated and further suggested that t_1 may not be a suitable parameter for this gluten system of this sample set. Zero shear viscosity (η_0) significantly raised after adding DATEM which indicated the formation of entanglements that resemble increased gluten average molar mass of unlinked polymer (Mezger, 2006). However, the presence of AA, urea, and DTT in gluten decreased η_0. Gliadins have been attributed with the viscous properties of gluten; they do not form interchain disulfide bonds and thus represent the unlinked polymer of gluten. Therefore, physical proximity of gliadin chains have aggregated and arrived to a critical molecular weight for the onset of entanglement. Gliadins are now behaving as larger molecular weight polymer with higher frictional factor and this could have happened by the sum of new hydrophobic and hydrophilic interactions of gliadins-DATEM-gliadins.

5.1.2 Effect of DATEM, AA, urea, and DTT on gluten coefficients from Burgers model during recovery phase

In recovery phase, there was zero shear stress from the rheometer on gluten and gluten molecules naturally regained its relaxed position. In Fig. 1, the result showed that as the higher concentration of DATEM was added, the lower gluten recovery compliance was obtained. In Table 2, Jr_0 (elastic deformation) and Jr_1 (viscoelastic deformation) of gluten during recovery showed significant reduction after treated with DATEM which is similar to creep parameters. For AA, urea, and DTT, the recovery curves were shifted to higher range of compliance (Fig. 1), which indicated that gluten had loss more energy during recovery to its original position compared to control. For Burgers coefficients, the parameters from recovery phase exhibited the same trend in which the values were significantly increased after treated with AA, urea, and DTT. Delayed viscoelastic time of gluten (tr_1) was not significantly different after treating with every compound.

The percent change of gluten rheological properties at lowest concentration and highest concentration of each additive was shown in Table 3. DATEM decreased the parameters that represented the deformation of gluten up to 45% at 1.0% addition. Pure viscosity or resistance to flow of gluten was increased up to 89%. Increasing interactions (hydrophilic/hydrophobic) via surfactant crosslinking made more cohesive gluten which structure resembled larger polymers less compliant during creep and recovery**.** A surprising result is a high increase in pure viscosity meaning the gliadins interacted in a much higher degree. This result suggested that gluten was more viscous. AA decreased viscosity by 56% at 200 ppm. This was a marked difference in the gliadins suggesting that AA at this high dose reduced the intra –S-S– bonds in gliadins and changed their

conformation to be a more fluid with reduced viscous state. AA at higher concentrations (200 ppm) made the gluten more compliant this is due to the non-linear response to the dose of AA in dough. Adding AA beyond the optimum dose, results in a reducing agent. In order to be more compliant the –S-S– bonds have to be reduced and not oxidized as expected (Fig. 1). The urea had similar effect to AA except that the change in the magnitude of viscoelastic properties was lower with the addition of urea. Urea increased max strain, elastic and viscoelastic deformation, meaning the gluten was more compliant (Fig. 1). This suggested that the contributions of hydrogen bonds account for at least 25% of the stability of the gluten structure. Breaking hydrogen bonds decreased pure viscosity of gluten by 22%, this suggested that the contribution of these bonds in gliadins was at least 22% of their stability. DTT had similar effect to AA and urea in which the gluten become more compliant (Fig. 1). A reduction of disulfide bonds makes polypeptides more open and less crosslinked thus increased max strain, elastic and viscoelastic deformation. It also decreased pure viscosity of gluten by 33%. DTT reduced the disulfide bonds to those polymers that can reach. It DTT they did not reduce all the disulfide bonds at the concentrations used suggests that the portion of the disulfide bonds remaining are still contributing to the crosslinked chains. The LMW-GS forming the branches with more mobility would be more susceptible to be reduced but because of their mobility they may reform disulfide bonds again and probably at a higher rate.

5.2 Discrimination of flour samples and relationship of parameters

The mixing and baking properties of treated flour samples were analyzed in order to demonstrate relationship with regressed parameters obtained from Burgers model. The correlation was depicted in a bi-plot graph of principal component analysis (PCA) based

on variation. For each PCA graph, variables with higher contributors were used and variables with low variance contribution (less than 50%) were discarded. For PCA of DATEM, the remaining contributors explained 85.1% of the variance (Fig. 2). The result showed that DATEM affected the viscoelastic parameters (Jr_1 and J_1) during creep and recovery and were the main contributors (PC 1), while, loaf volume (LV) was the secondary contributor (PC 2) to this set of sample variance. Thus, the effect of DATEM was greater on the viscoelastic properties (the gradient on the first component had higher variance) than in loaf volume (LV) (gradient in the second component had lower variance). For PCA of AA, the selected contributors increased total explained variance to 85.2% (Fig. 3). Viscoelastic deformation (J_1) was the main contributor (PC 1) to the variance, while flour protein (FP) was the secondary contributor (PC 2) to this set of sample variance. For PCA of urea, the selected contributors increased total explained variance to 85.7% (Fig. 4). Elastic deformation (J_0) was the main contributor (PC 1), while loaf volume (LV) was the secondary contributor (PC 2) to this set of sample variance. For PCA of DTT, the selected contributors increased total explained variance to 81.5% (Fig. 5). Dough development time (DT) during mixing was the main contributors (PC 1), while viscoelastic deformation (J_1) was the secondary contributor (PC 2) to this set of sample variance. From all of the PCA results, it was demonstrated that coefficients from Burgers model are helpful in discriminating these samples properties because most parameters from Burgers model are the main contributors except loaf volume and dough development time. Overall, maximum strain, J_0, J_1, Jr_0 and Jr_1 were highly correlated to each other and negatively correlated to zero shear viscosity (η_0). These observations are in agreement with previous finding (Van Bockstaele et al., 2011). The regressed

coefficients from Burgers model demonstrated that they were independent of baking and mixing variables and their variances were smaller compared to those of the viscoelastic properties.

6. Conclusions

DATEM affected the viscoelastic properties of gluten differently compared to AA, urea, and DTT. DATEM decreased elastic and viscoelastic deformation, while increased viscosity of gluten. AA, urea, and DTT had opposite effects with increased elastic and viscoelastic deformation and decreased viscosity of gluten. This study confirmed that the Burgers model clearly distinguished elasticity, viscoelasticity, and viscosity portions of gluten in terms of regressed parameters. The model allowed us to directly compare individual portions from creep recovery tests. Furthermore, it was confirmed that parameters from the Burgers model could assist in discriminating gluten samples based on their specific rheological properties and serve as a tool to explain changes in their structures.

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Table 1. Partial proximate analysis of commercial hard red winter flours (means ± SD, n=2)

Flour	Protein (%)	Moisture (%)	Ash (%)
C1	7.9 ± 0.05	11.7 ± 0.02	0.3 ± 0.01
C2	11.2 ± 0.07	10.5 ± 0.03	0.4 ± 0.01
C3	13.7 ± 0.02	10.1 ± 0.02	0.4 ± 0.00
C4	10.4 ± 0.10	12.5 ± 0.02	0.5 ± 0.00
C5	10.6 ± 0.07	12.6 ± 0.00	0.5 ± 0.01
C6	11.4 ± 0.01	12.9 ± 0.04	0.6 ± 0.01

Means ± standard error (n= 2).
Protein and ash values are expressed on 14% moisture basis.

Table 2. Effect of DATEM, Ascorbic Acid (AA), urea and DTT on gluten regressed parameters from Burgers model of creep and recovery phases of a selected flour C5.

			Creep phase				***Recovery phase***		
	Levels	Max Strain, γ (%)	J_0 (10^{-4} Pa^{-1})	J_1 (10^{-4} Pa^{-1})	t_1 (s)	η_0 (10^5 Pa^{-1})	Jr_0 (10^{-4} Pa^{-1})	Jr_1 (10^{-4} Pa^{-1})	tr_1 (s)
DATEM (%)	0	15.0 a	13.5 a	13.4 a	7.4 a	0.9 b	20.1 b	11.6 a	46.2 a
	0.3	15.3 a	13.7 a	13.9 a	7.6 a	0.9 b	23.0 a	11.7 a	76.0 a
	0.6	9.7 b	9.4 b	8.6 b	7.5 a	1.5 a	15.0 c	7.7 b	56.8 a
	1	8.2 b	7.5 c	7.4 b	8.4 a	1.7 a	12.7 c	6.4 c	74.8 a
AA (ppm)	0	14.4 d	12.6 c	13.1 d	7.7 a	0.9 a	20.2 c	11.3 d	59.7 a
	50	22.7 b	18.4 b	21.3 bc	7.9 a	0.6 c	30.8 b	17.9 bc	64.1 a
	100	18.0 c	15.2 bc	17 dc	7.7 a	0.7 b	26.0 bc	14.2 dc	71.0 a
	150	22.8 b	17.1 b	22.2 b	8.2 a	0.5 c	30.6 b	19.2 b	68.8 a
	200	34.0 a	25.7 a	32.9 a	8.1 a	0.4 d	44.5 a	27.3 a	64.8 a
Urea (M)	0	14.4 b	12.6 b	13.1 b	7.7 a	0.9 a	20.2 b	11.3 b	59.7 a
	0.5	19.4 a	16.1 a	18.4 a	8.0 a	0.7 b	25.8 ab	16.1 a	50.9 a
	1	19.5 a	16.1 a	18.4 a	7.9 a	0.7 b	24.9 ab	16.6 a	42.3 a
	1.5	18.9 a	15.8 a	17.7 a	7.8 a	0.7 b	26.9 a	15.2 a	65.7 a
DTT (mM)	0	14.4 d	12.6 c	13.1 d	7.7 a	0.9 a	20.2 b	11.3 d	59.7 a
	0.1	17.6 c	13.5 cb	17.3 c	8.4 a	0.7 b	22.8 ab	15.7 c	57.3 a
	0.25	21.7 a	16.4 a	21.6 a	8.3 a	0.6 b	26.9 a	19.1 a	49.2 a
	0.5	20.3 b	14.8 ab	20.0 b	8.8 a	0.6 b	26.4 a	17.3 b	69.7 a

MaxS = maximum strain during creep, J_0 = instantaneous compliance during creep, J_1= retardation compliance during creep, t_1= retardation time during creep, η_0 = pure viscosity Jr0 = instantaneous compliance during creep, Jr1= retardation compliance during recovery, tr1 = retardation time during recovery. Means with same superscripts in a column are not significantly different ($P \leq 0.05$, n=3).

Table 3. Percent change of gluten rheological properties from control and highest concentration of each additive

Parameters	**DATEM**	**AA**	**Urea**	**DTT**
Max Strain, γ	-45	136	31	41
J0	-44	104	25	18
J1	-45	151	35	53
t1	ns	ns	ns	ns
$\eta 0$	89	-56	-22	-33
Jr0	-45	120	33	31
Jr1	-36	142	35	53
tr1	ns	ns	ns	ns

Positive and negative values indicate percent increase and decrease, respectively. Descriptions are defined in Table 2. ns = non-significant difference.

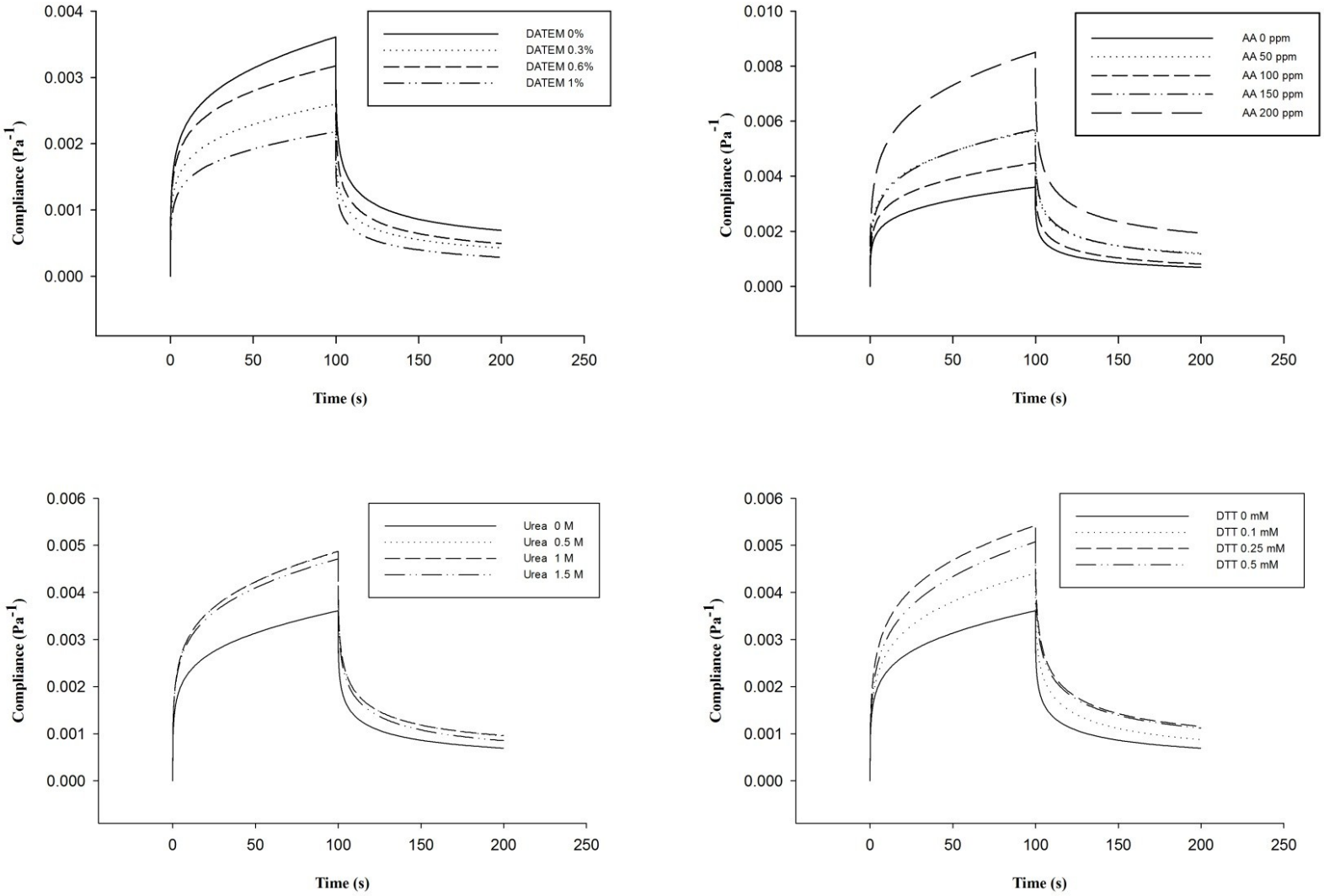

Figure 1. Representative curves of the effect of diacetyl tartaric acid ester of monoglycerides (DATEM), ascorbic acid (AA), urea and dithiothrietol (DTT) on viscoelastic behavior of gluten from a selected flour C5.

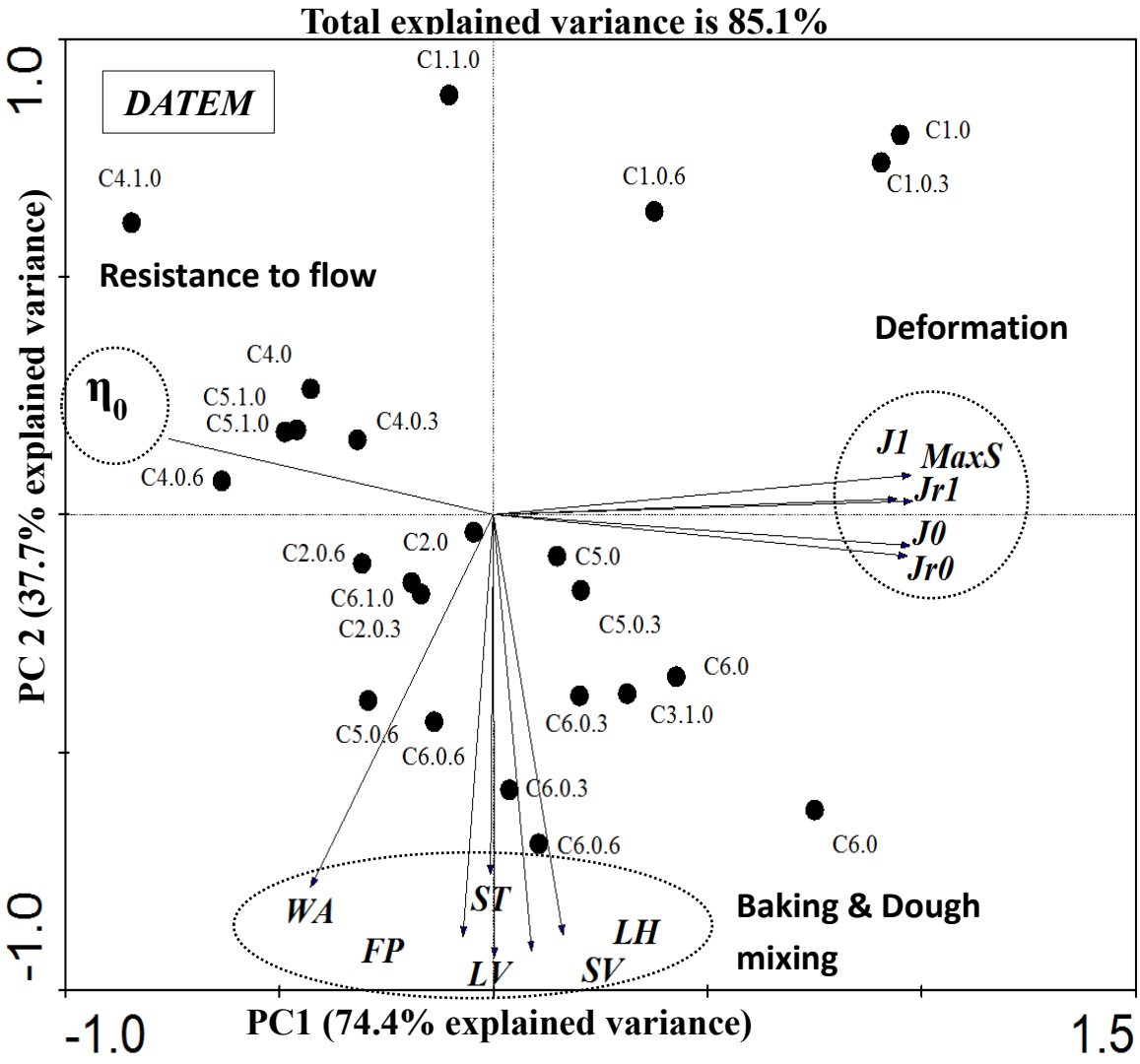

Figure 2. Principal component analysis of gluten from a set of 22 hard red winter wheat samples with DATEM treatment involving 12 indicators of dough and gluten quality (dough mixing and viscoelasticity of gluten) and one indicator of gluten quantity (flour protein) from total of 29 variables. MaxS = maximum strain during creep, J_0 = instantaneous compliance during creep, J_1= retardation compliance during creep, η_0 = pure viscosity Jr0 = instantaneous compliance during creep, Jr1= retardation compliance during recovery, WA = water absorption, ST = stability time, LH = loaf height, LV = loaf volume, SV = specific volume, and FP = flour protein.

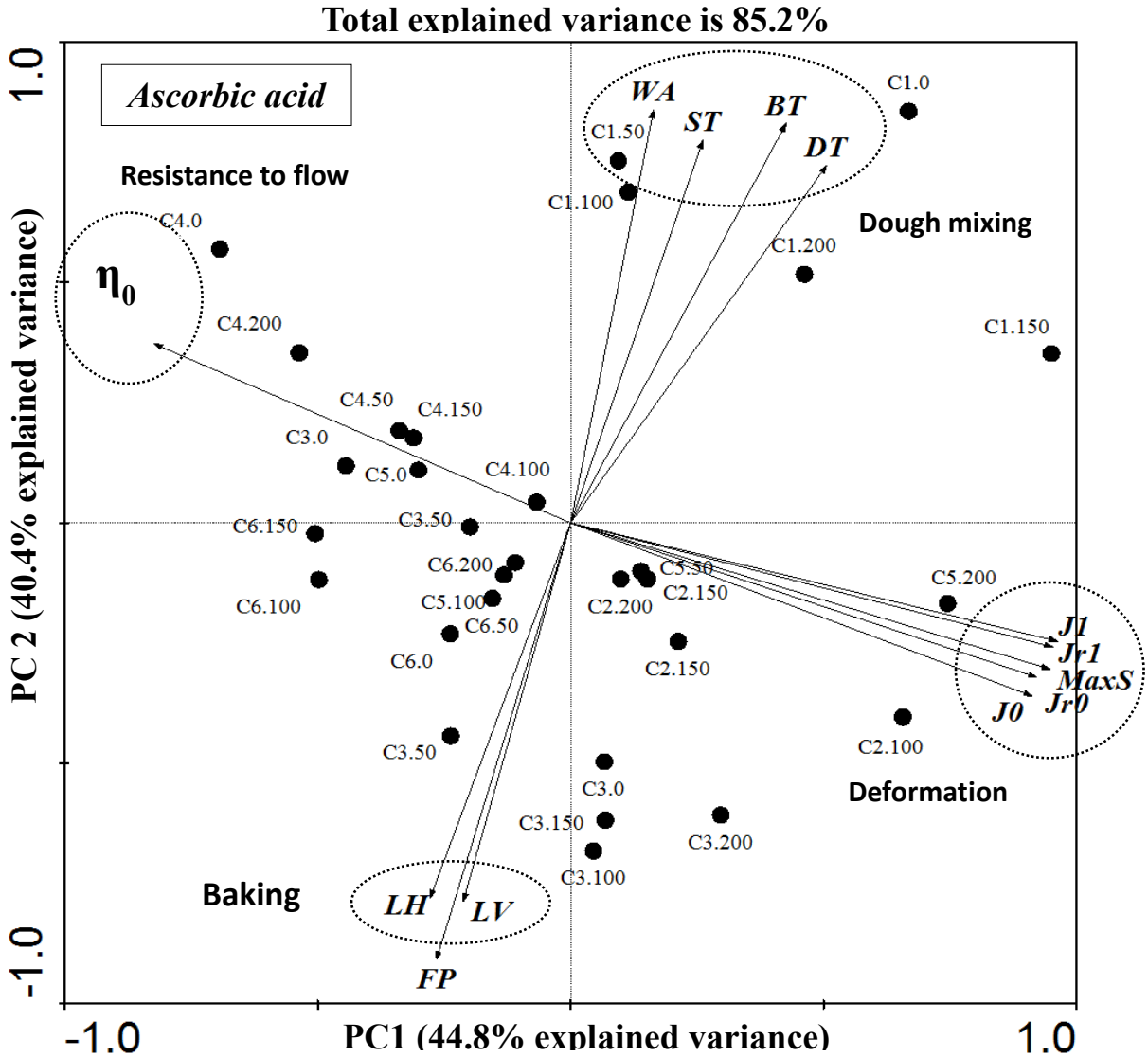

Figure 3. Principal component analysis of gluten from a set of 22 hard red winter wheat samples with ascorbic acid treatment involving 13 indicators of dough and gluten quality (dough mixing and viscoelasticity of gluten) and one indicator of gluten quantity (flour protein) from total of 29 variables. MaxS = maximum strain during creep, J_0 = instantaneous compliance during creep, J_1= retardation compliance during creep, η_0 = pure viscosity Jr0 = instantaneous compliance during creep, Jr1= retardation compliance during recovery, WA = water absorption, ST = stability time, BT = breakdown time, DT = development time, LH = loaf height, LV = loaf volume, and FP = flour protein.

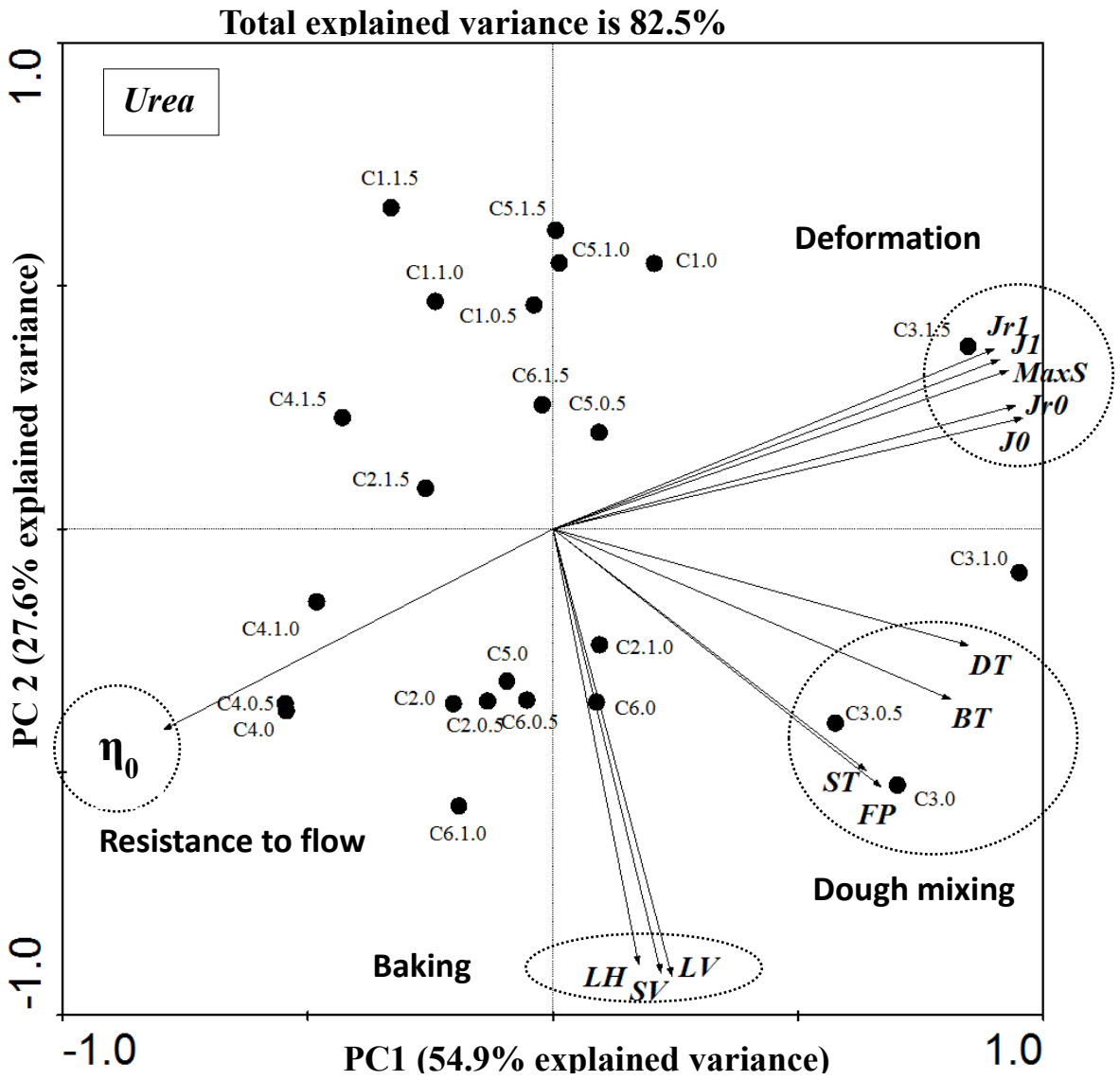

Figure 4. Principal component analysis of gluten from a set of 22 hard red winter wheat samples with urea treatment involving 13 indicators of dough and gluten quality (dough mixing and viscoelasticity of gluten) and one indicator of gluten quantity (flour protein) from total of 29 variables. MaxS = maximum strain during creep, J_0 = instantaneous compliance during creep, J_1= retardation compliance during creep, η_0 = pure viscosity Jr0 = instantaneous compliance during creep, Jr1= retardation compliance during recovery, WA = water absorption, ST = stability time, BT = breakdown time, DT = development time, LH = loaf height, LV = loaf volume, and FP = flour protein.

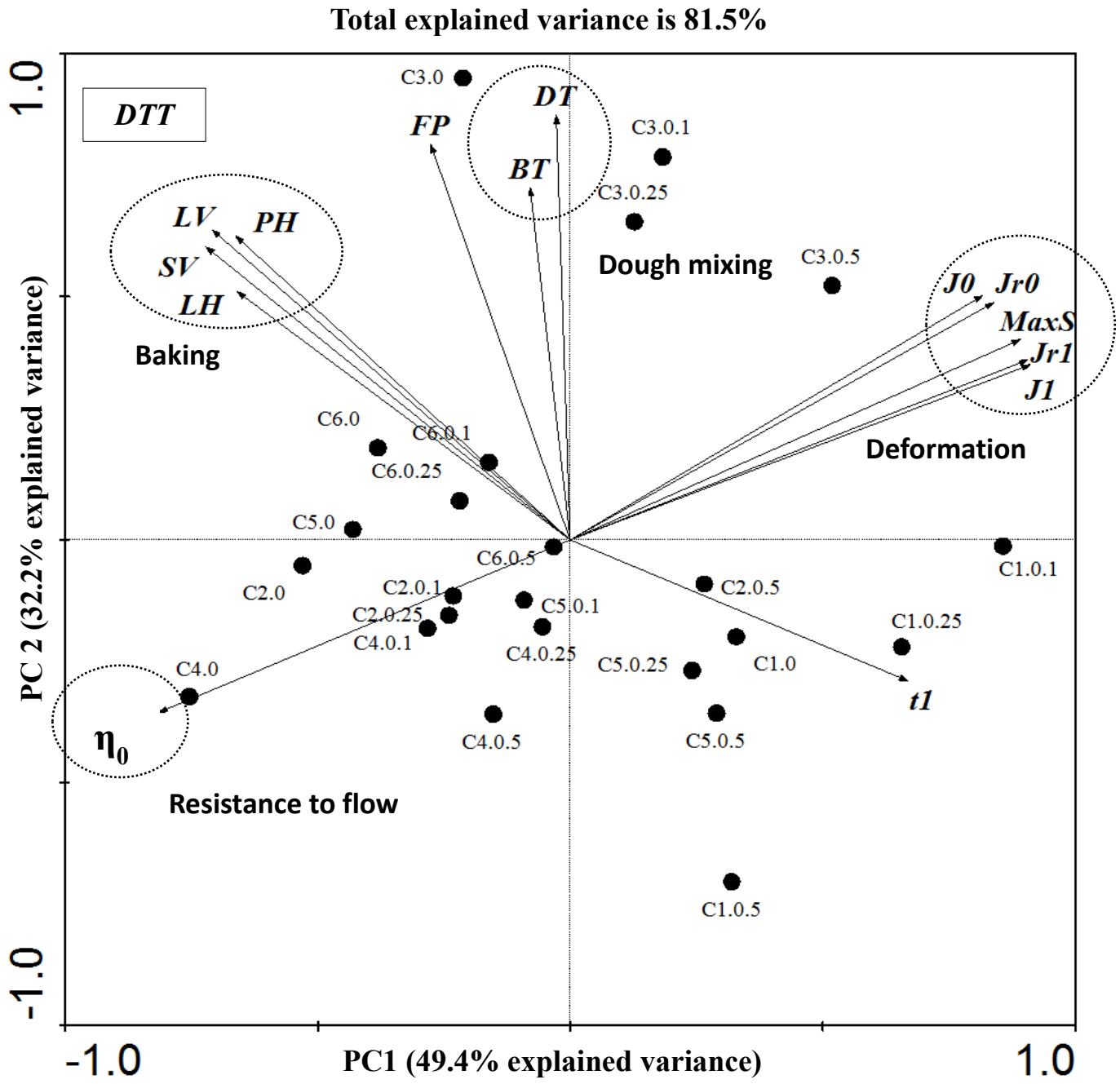

Figure 5. Principal component analysis of gluten from a set of 22 hard red winter wheat samples with DTT treatment involving 14 indicators of dough and gluten quality (dough mixing and viscoelasticity of gluten) and one indicator of gluten quantity (flour protein) from total of 29 variables. MaxS = maximum strain during creep, J_0 = instantaneous compliance during creep, J_1= retardation compliance during creep, η_0 = pure viscosity Jr0 = instantaneous compliance during creep, Jr1= retardation compliance during recovery, BT = breakdown time, DT = development time, LH = loaf height, LV = loaf volume, SV = specific volume, PH = proof height, and FP = flour protein.

CHAPTER IV

MODELING OF GLUTEN VISCOELASTIC PROPERTIES BASED ON CREEP-RECOVERY TEST AT DIFFERENT TEMPERATURES

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Abstract

Creep and recovery test with mathematical modeling of wheat gluten revealed the basic parameters that governed rheological behavior. Commercial flour samples from four U.S. classes (hard red winter, soft red winter, hard red spring, and durum wheat) varying in protein content were studied. Viscoelastic properties of the isolated gluten were measured at 25, 35, 45, 55, and 65°C using a creep and recovery test with a constant shear stress of 100 Pa. To separate the viscous flow and elastic components of the gluten, creep and recovery experimental data was fitted into a Burgers model. Overall, two major transitions of viscoelastic behavior were noticeable at 45 and 65^{o}C. At 45^{o}C an increase in creep compliance (flowability) and a decrease in recovery compliance (elasticity) were observed suggesting that gluten started to denature and became more deformable. At 65^{o}C, however, the trend of flowability and elasticity reversed when compared to the behavior at 45^{o}C which suggested that aggregation of gluten predominated at 65^{o}C. The relationships between samples and parameters were also tested using Principal Component Analysis (PCA) and partial Redundancy Analysis (pRDA).

Keywords: Temperature, creep and recovery compliance, rheological properties, wheat gluten, viscoelasticity

1. Introduction

Gluten protein is an important component of dough because it often associated with bread quality (Attenburrow et al., 1990). Although more difficult to quantitate, gluten protein interactions with other major components such as other proteins, lipids and starch are deemed to have an important impact on the performance of dough and have shown correlation with wheat quality attributes (Kim et al., 2004). Protein interactions are also highly dependent upon temperature during breadmaking which have a wide range 30 to 260°C (Cuq et al., 2000), and alters physicochemical properties of gluten (Madeka and Kokini, 1994). Heat provides energy to gluten system and leads to increased vibrational motion and destabilization of protein by disrupting hydrogen, disulfide bonds and hydrophobic interactions (Tatham and Shewry, 1985). As a result, the disruption of these bonds and interactions dynamically changes the viscoelastic properties of dough and gluten (Hayta and Alpaslan, 2001).

Gliadins and glutenins, the two main components of gluten, are responsible for its viscoelastic properties (Apichartsrangkoon, 2002). It is widely accepted that the elastic properties of gluten are mainly provided by glutenins, while the viscous flow properties of gluten are primarily contributed by gliadins (Xu et al., 2007). When gluten is exposed to temperatures above 45°C, the interaction between glutenins and gliadins are weakened due to decreases in β-sheet, α-helix and hydrogen bonds (Yada, 2004). A number of irreversible crosslinks mainly in the glutenin structure are formed when gluten is exposed to temperature around 50°C (Schofield et al., 1983). Thermal stability of gluten decreased with an increase of 5% and 10% gliadin addition leading to a weak gluten structure

(Khatkar et al., 2013). Several methods such as creep recovery test, protein extractability measurement, and dynamic oscillatory measurements have been used to investigate the effect of heat on gluten and dough structure (Hayta and Schofield, 2005; Mirsaeedghazi et al., 2008; Schofield et al., 1983).

Viscoelastic properties of gluten have been investigated by a creep and recovery test by applying a constant shear stress and measuring creep-recovery compliance as a function of time (Abang Zaidel et al., 2010; Chapman et al., 2012; Chompoorat et al., 2013; Hernández-Estrada et al., 2012). Creep measurement using cone and plate geometry with applying stress of 50 Pa has shown that the elastic component (G') of gluten was lowered during heat treatment at 30-50°C when compared to 70-90°C (Hayta and Schofield, 2005). They found that the compliance in creep test increased at the higher temperature range for both Hereward (good quality wheat gluten) and Riband (poor quality wheat gluten) (Hayta and Schofield, 2005). Heating gluten beyond 40°C caused an increase in solid-like behavior (G') of gluten (Attenburrow et al., 1990; Hayta and Alpaslan, 2001; Hayta and Schofield, 2004). The possible explanation was that the formation of a highly cross-linked gluten structure and induction of the molecule mobility at temperature around 40-50°C had resulted in an increase in rigidity (Attenburrow et al., 1990).

The comparison between Hereward and Riband also showed that the former one had less SDS protein extractability and more SH-SS content than Riband cultivar after heated to 70°C for 15 min (Hayta and Schofield, 2005). This observation was confirmed by another study in which heating gluten from 25 to 90°C for 20 min produced a decrease in free SH-groups, surface hydrophobicity and protein extractability of gluten

(Stathopoulos et al., 2008). Schofield et al. (1983) also reported that exposing winter wheat gluten to temperatures between 55 and 75°C resulted in denaturation and a decrease in gluten extractability yielding poor baking performance. Dynamic oscillatory test at 0.01 to 10 Hz revealed that heating gluten at 90°C for 6 hours caused higher increase in G' and G" compared to the unheated gluten (Apichartsrangkoon, 2002). A decrease in tan δ (ratio of G'/G") of gluten at 60°C by using a temperature sweep test has also been reported (Attenburrow et al., 1990). In the report of Hayta and Schofield (2005), frequency sweep test with gluten heated between 30 to 50°C also revealed a decrease of elastic modulus G'.

A number of techniques have been attempted to study the effect of temperature on viscoelasticity of gluten as discussed above. However, there is limited data about quantitate alteration of gluten structure during heat treatment. Therefore, the objective of this study was to investigate the viscoelastic properties of flour of different U.S. wheat classes (hard red winter, soft red winter, hard red spring, and durum) at temperatures ranging from 25 to 65°C using a creep-recovery test. In this study, creep-recovery test was applied to examine the elastic and viscous elements obtained from Burgers model. The relationships of parameters and samples with heat treatments were also tested by using Principal Component Analysis (PCA) and partial Redundancy Analysis (pRDA)

2. Materials and Methods

A total of nine commercial wheat flour samples were used; six hard red winter wheat named C1, C2, C3, C4, C5, and C6, and three reference samples representing soft red winter wheat flours named SRW, hard red spring wheat flours named HRS, and durum wheat flours named DUR were purchased from a local supplier. Protein, moisture, and ash content of wheat flours were analyzed by using near infrared reflectance in a FOSS NIR System Inc., model 6500-M (Laurel, MD) using manufacturer's procedure. The values were reported as a 14% moisture basis.

2.1 Gluten preparation

Wet gluten was isolated by washing 10 g of flour with 2% (w/v) NaCl solution in a Glutomatic 2200 (Perten Instruments AB, Huddinge, Sweden) according to approved method 38-12.02 (AACC International 2010). Briefly, the flour was mixed for 20 sec and washed for 5 min through 88 μm polyester screen. The wet gluten is the insoluble water protein on the screen.

2.2 Creep and recovery test of gluten

Creep and recovery tests were conducted by following the method described in Chompoorat (2013). In brief, the gluten obtained from the Glutomatic was immediately rolled into a ball-shape and placed under a 2.5 kg plate with 2.5 mm spacing for an hour at room temperature to allow gluten structure to relax. Then, the gluten sample was cut by using a 25 mm diameter round cutter. Gluten was transferred to the lower plate of a rheometer (AR1000, TA Instruments, New Castle, DE), compressed back to 2.5 mm zero gap, and re-trimmed to 25 mm diameter if necessary. To prevent moisture loss during the test, mineral oil was applied to the edge of the gluten. Before the test, the gluten sample

was covered with a metal cover. For conditioning in this creep-recovery test, a constant shear stress of 100 Pa was used for 100 sec to deform the gluten during the creep phase. In the recovery phase, the data was recorded for 100 s without shear stress to allow gluten to recover. The tests were carried out at 25, 35, 45, 55, and 65°C using a peltier plate. The creep-recovery test was able to reveal the viscoelastic behavior of gluten directly based on an empirical observation. Recoverability (RCY) was calculated according to equation (1) and represented elasticity of gluten. J_{max} was the last value of compliance during creep (maximum creep compliance) and Jr_{final} was the last value of compliance during recovery phase.

$$RCY = (Jr_{final} * 100)/J_{max} \quad (1)$$

Delta compliance (J-Jr) reflects the viscous flow of gluten. Maximum strain (MaxS, ε) was the last value of strain during creep, while final strain (FinalS) was the last value of strain during recovery. The MaxS and FinalS were used to measure the deformation of gluten.

2.3 Modeling of rheological properties of gluten

The rheological behavior can be represented by mechanical analogues of spring and dashpot elements. When stress is applied to gluten, the spring represents the elastic behavior in which the sample readily returns to its original form similar to the spring behavior. The dashpot represents the viscosity of the gluten and it does not restore to its original shape. Two basic models commonly used in describing viscoelastic behavior are Maxwell and Kelvin-Voigt models in which the former represents ideal viscoelastic response (spring and dashpot in series) and the latter primary creep (spring and dashpot in parallel). Both of these models, when used separately, are insufficient in describing

biological polymeric materials such as gluten because of its complex behavior. However, when Maxwell and Kelvin-Voigt models are superimposed, they have an exceptional ability to represent gluten behavior during creep and recovery phases. The combination of these two models is developed into the Burgers model that is composed of three elements: spring, spring-dashpot in parallel, and dashpot. Burgers model has been used to investigate the effect of DATEM (Diacetyl tartaric acid ester of mono- and diglycerides), ascorbic acid, dithiothreitol (DTT), and pentosans on gluten rheological behavior (Chompoorat et al., 2013; Ma et al., 2012). The equations for Burgers model are shown below.

For creep phase:

$$Jc(t) = J_0 + J_1(1 - \exp(-t/t_1)) + t/\eta_0$$

For recovery phase:

$$Jr(t) = Jr_0 + Jr_1(1 - \exp(-t/tr_1))$$

Each coefficient quantitates the rheological behavior of gluten. During creep, J_0, J_1, t, and η_0 represent instantaneous elastic deformation, retarded elastic deformation, retardation time, and pure viscous deformation, respectively. During recovery (shear stress = 0), Jr_0, Jr_1 and t represent instantaneous elastic recovery, retarded elastic recovery, retardation time recovery, respectively.

2.4 Statistical analysis

The mean significant difference was performed by using ANOVA (Analysis of variance) and Tukey's multiple comparisons (Statistical Analysis System, SAS Institute Inc., Cary, NC). Principal Component Analysis (PCA) and partial Redundancy Analysis (pRDA) using Canoco for Windows 4.5 (Biometris, Plant Research International,

Wageningen, the Netherlands) were used to show correlations among parameters and treatments.

3. Results and Discussion

3.1 Chemical properties of wheat flour samples from different classes

Hard Red Winter wheat flours ranged in protein content from 8.0 to 13.7%; the reference samples had 11.4, 13.4 and 12% protein for the SRW, HRS, and DUR, respectively (Table 1). The moisture content of Hard Red Winter wheat was between 10.1% and 13.0%; while SRW had moisture content of 11.8%. HRS and DUR had moisture content relatively high which were 14% and 15%, respectively. The ash content of Hard Red Winter wheat was between 0.29% and 0.58%. For the reference samples, SRW, HRS, and DUR had ash content of 0.65, 0.53, and 0.76%, respectively.

3.2 Rheological properties of gluten during heating

The gluten viscoelastic behavior at 25, 35, 45, 55, and 65°C were measured by using creep and recovery test as shown in Figure 1. For representation purposes, C5 was selected to represent the hard red winter wheat as shown in Figure 1 because it has protein content that is close to the average value of this set of samples. Overall, a sample C5 of hard red winter wheat (HRW) gluten had the lowest compliance which indicated creep resistance with lower deformation meaning the material is stiffer or more elastic than other wheat classes. Hard red winter wheat was found to have low value of L (the extensibility of the dough before the bubble breaks) from alveograph which means that it had higher strength compared to other wheat classes (Popper et al., 2006). In contrast, soft red spring wheat gluten (SRW) had the highest compliance which indicated that they had the highest deformation and flowability. Other researchers also observed a similar

trend that SRW gluten had the lowest resistance to stretching and the highest recoverable shear strain compared to HRW gluten (Chapman et al., 2012; Zhao et al., 2010). Every gluten wheat class had the highest maximum compliance at 55°C and followed by a decrease in maximum compliance at 65°C. This suggested that 65°C was a critical temperature for secondary structural changes in gluten. Our data was supported by previous findings that G' (elastic modulus) of gluten tended to increase after 50°C (Hayta and Schofield, 2005). The decrease in maximum compliance after 55°C was likely due to the denaturation followed by aggregation of gluten protein. For HRW gluten, it was interesting to note that at 65°C there was no sign of an attenuation of maximum compliance. However, it was not surprising that rheological properties of HRW gluten samples were different from others. Normally when gluten was exposed to heat, several changes occurred such as 1) unfolding of gluten structure, 2) decrease of hydrophobicity as shown by low gluten extractability and 3) an increase in gluten aggregation. These changes could be depended on gliadin and glutenin fraction, formation of intramolecular covalent bond, intermolecular covalent bond, and also heat levels. Therefore, the notable difference between HRW and other samples could be due to the variation in these factors. Creep-recovery data was used to determine the recoverability (RCY), flowability (J-Jr), and deformability during creep (MaxS) and recovery (FinalS) phase and were shown in Table 2. The recoverability (RCY) of most gluten samples started to decrease after 45°C, with exception to C4 and C6 samples which decreased after 55°C. Although the observed transition temperatures of this set of samples were different, it was not an unusual behavior and other reports have stated that gluten glass transition temperature can range from 45 to 55 °C (Georget and Belton, 2006). The viscous flow (J-Jr) of most gluten

samples started to increase after 45°C, however, DUR wheat gluten increased after 35°C. Heat energy impacted wheat cultivar differently due to variation in vibrational motion and molecular interactions. The hydrogen bonds of DUR wheat gluten weakened at 35°C suggesting that the structure of this sample was different with perhaps lower number of hydrogen bonds than the other samples.

Maximun strain (MaxS) value represents highest deformability of gluten during creep and the maximum strain of most gluten increased from 25°C to 55°C and decreased at 65°C (Table 2). We postulate that the decrease in deformation might be due to an increase in aggregation and protein-protein crosslinks favored by kinetic molecular mobility between 55 and 65°C (Angioloni and Dalla Rosa, 2005; Attenburrow et al., 1990). The deformability of all gluten samples during recovery had an increasing trend as a function of temperature. Overall, gluten of soft red winter wheat (SRW), hard red spring wheat (HRS), and durum wheat (DUR) had a higher deformability (FinalS) compared to hard red winter wheat (HRW) gluten. Although normally HRS and DUR flour samples had a lower deformability compared to HRW, it could depend on a specific cultivars. For example, HRW such as Jagger and Jagalene had a higher degree of recovery (less deformability) than HRS which was consistent with our result (Chapman et al., 2012). Moreover, it had been reported that DUR could have a lower strength compared to HRW as shown by the lower value of W parameter (energy required to disrupt dough bubble) from alveograph (Popper et al., 2006).Burgers model was fitted with creep-recovery experimental data to obtain model parameters that can be used to represent rheological behavior as shown in Table 3. During creep phase, the parameters obtained from Burger model are instantaneous shear compliance (J_0), delayed or retarded

viscoelasticity (J_1), retardation time (t_1), and pure viscosity of gluten (η_0). During recovery phase, the parameters obtained from Burger model are instantaneous shear compliance (Jr_0), delayed or retarded viscoelasticity (Jr_1), and retardation time (tr_1). The J_0 and J_1 of most gluten samples had an increasing trend after 25°C, with exception to C2, C3 and, C6 which decreased after 55°C. Overall, the continuous increase in J_0 and J_1 as a function of temperature indicated that gluten viscoelasticity had a similar trend as recoverability (RCY) which was obtained from experimental data. The t_1 continuously decreased as the temperature increase for five out nine samples, while the rest of the samples showed constant value. The pure viscous component of gluten samples continuously decreased as a function of temperature as indicated by the value of η_0. Furthermore, the result of η_0 even suggested that using our modeling tool was more powerful in describing gluten behavior because a change in flowability (J-Jr) as determined by experiments was not detectable at lower temperatures. The low value of pure viscous deformation (η_0) might be attributed to an increase in the flow of gluten molecule during creep.

Burger model parameters during recovery also yielded new insights to rheological behavior of gluten. The Jr_0 values were higher compared to J_0 and could be due to a partial loss of structure after deformation. This result suggested that elastic region of mechanical model was affected less than viscoelastic region when heated gluten at 25°C to 65°C. It is well known that high molecular weight of glutenin subunits (HMW-GS) and low molecular weight of glutenin subunits (LMW-GS) are responsible to elastic and viscoelastic properties of gluten, respectively via interchain disulphide bonds. Thus, our result suggested that heating gluten at 25°C to 65°C affected mostly in LMW-GS

sturcutre. Most of Jr_0 of gluten increased at 45°C and decreased at 65°C. At this onset temperature of 45°C, it could be an indication of protein rearrangement in gluten specifically in the breakage of non-covalent bond of LMW-GS. However, Jr_1 continued to decrease either at 35 or 45°C depending on gluten samples. The t_1 of HRW wheat gluten were almost constant for every sample. Overall, thermal stress was enough for changes in viscoelastic properties to be detected. It was postulated that heating gluten up to 75^{o}C could induce disulfide bond rearrangement and unfold structure which (Schofield et al., 1983). Our results suggested that an increase in number of cross-link rheologically could be at 65°C indicated by the reduction of Jr0.

3.3 Correlation from principal component analysis (PCA) and partial redundancy analysis (pRDA)

Principal component analysis (PCA) was used to show the relationship of rheological parameters and gluten samples at different temperature. The parameters that were in vicinity to each other were positively correlated; whereas, the parameters that were opposite to each other were negatively associated. Moreover, the parameters that are perpendicular to each other indicated that they were independent. The most important contributors for explaining the variation were the parameters with the highest magnitude and closest to PC1.

The total explained variance of all parameters in this sample set was 81.1% (Figure 2). The maximum strain (MaxS) and instantaneous shear compliance (J_0) were the two parameters with the highest explained variance in the first principal component (PC1), while retardation time parameters during both creep and recovery phases (t_1 and tr_1) were the highest contributors in the second principal component (PC2). The PCA

analysis showed that SRW gluten had rheological behavior that was drastically different from HRW gluten at the temperatures tested. The magnitude of the viscoelastic properties of HRS and DUR gluten tended to be in between the viscoelastic properties of SRW and HRW. DUR gluten at 45 and 55^{o}C had viscoelastic properties close to SRW gluten at 55 and 65^{o}C. The fact that DUR moved into flowability (J-Jr) quadrant (Quadrant 4) at 45 and 55^{o}C suggested that the gluten macromolecules shared similarity with SRW after heat treatment. The SRW and HRS at 25^{o}C and 35^{o}C had a high retardation time in both creep and recovery phases which means that these samples deformed more slowly. When SRW gluten was subjected to 45, 55, and 65^{o}C heat treatment, its rheological properties showed high deformability and elasticity (close to J-Jr, FinalS, J_0, and Jr_0). HRW gluten at 25 ^{o}C and 35^{o}C had a higher recoverability (RCY) and viscosity (η_0) when compared to HRW gluten at 45, 55, and 65^{o}C. Gliadins provide viscous flow or extensibility to gluten system. Thus, we speculated that non-covalent bond between gliadin-gliadin and gliadin-glutenin was broken and rearranged. This cause an increase of aggregation in gluten conformation specific in gliadin structure.The structural changes due to heat around 40-55 ^{0}C was also previously reported (Hayta and Alpaslan, 2001). This alteration was consistent with our data that showed a decrease in elasticity and pure viscosity.

To differentiate the viscoelastic properties of HRW wheat gluten (C1-C6) at different temperatures, we excluded the other wheat class glutens from the PCA analysis (Figure 3). PCA results indicated a trend to a slightly lower total explanation of variance (80.6%) when compared to PCA results from all samples (81.1%). The explained variances of PC1 and PC2 were 60.5% and 20.1%, respectively. The main contributors to the variance (highly correlated with PC1) were MaxS, Jr_0, and J_0 which are variables

associated with deformability and elasticity of gluten. HRW gluten samples were separated into two major groups according to their viscoelastic properties at different temperature as indicated by circles. Gluten samples analyzed at 25, 35, and 45°C were associated mainly with recoverability (RCY) and pure viscosity (η_0). In contrast, the samples analyzed at 55 and 65°C were negatively associated with RCY and η_0. However, C3 sample was separated from these two groups which mean its viscoelastic properties appeared to be independent from the rest of gluten samples.

Partial Redundancy Analysis (pRDA) was used to reveal the intercorrelation between temperature and viscoelastic parameters regardless of gluten samples (Fig. 4). This analysis was performed only on hard red winter wheat gluten ~~depicted in Figure 4~~. pRDA is a multivariate direct gradient analysis which can test the statistical hypothesis and correlation at the same time. Result showed that the axes of pRDA corresponded to recoverability (RCY) (axis 1) and retardation time during recovery (tr_1) (axis 2). The correlation between temperature and viscoelastic parameters was 0.95 in the first axis and 0.82 in the second axis. Monte-Carlo permutation test showed that all canonical axes had significant relations ($P<0.001$), which indicated that temperature significantly affected viscoelastic properties of gluten. The viscoelastic properties of gluten samples at different temperature were significantly different from each other. Gluten samples at 25 and 35°C were mainly located in quadrant 2 indicating that they are positively correlated to retardation time (t1) and pure viscosity (h0). In contrast, gluten samples at 45 and 55°C were negatively correlated to t1 and h0. Only the sample at 65°C was negatively correlated with recoverability (RCY), indicating that the gluten at this temperature had low elasticity.

In order to compare the changes in rheological properties at native state (25°C) and denatured state (65 ^{0}C), the percent changes of experimental parameters and modeling parameters from Burgers model were shown in Table 4. HRW had the lowest change in RCY but highest change in J1 and Jr1. SRW was the only gluten sample that had a decrease in MaxS and also the sample with the lowest change in J1 and η0. Moreover, SRW had the highest increase in J0 and highest decrease in t1 and tr1. For HRS gluten, it had the highest increase in J-Jr and FinalS. Moreover, it had the highest decrease in η0. DUR gluten had the highest decrease in RCY; while other parameters were between the values of extreme samples. Overall, the experimental and modeling parameters could help us compared differences of gluten samples after heat treatment. By quantitatively compared these viscoelastic parameters, the changes in gluten structure could be inferred. J0 (spring element of Burgers model, instantaneous elastic deformation) could represent high molecular weight-glutenin subunit (HMW-GS) backbone which forms a large polymeric structure. For example, the large change in J0 after heating at 65^{o}C in weak sample such as SRW gluten could be due to a large deformation in HMW-GS of SRW. J1 (parallel spring-dashpot element, delayed viscoelastic deformation) could represent changes in low molecular weight-glutenin subunit (LMW-GS) as shown in HRW. LMW-GS most likely form the branches attached to the main backbone of the gluten polymer. Lastly, η_0 (dashpot element, pure viscosity) could represent changes in gliadin structure which were prominent in HRS sample. Monomeric gliadin acts as plasticizer in gluten system. These results can be speculated that HRS had a large amount of monomeric gliadin and thus, showed to have a high deformation in gliadin structure.

4. Conclusions

The viscoelastic properties of gluten were significantly affected by heat based on experimental and modeling parameters. At temperatures above 45°C, most gluten samples were more deformable as clearly shown by an increase in instantaneous elastic deformation and maximum strain. Upon further heating to about 65°C, aggregation became more prominent as shown by a significant decrease in maximum strain (deformation during creep) Thus, a reduction in deformability (MaxS) between 55°C and 65°C can be attributed to an increase in rigidity and aggregation of gluten. According to PCA results, two groups of HRW gluten samples were easily distinguished according to their association with viscoelastic properties when samples were heated from 55 to 65°C. In summary, results showed that mathematical modeling was a powerful tool and could be used to confirm viscoelastic behavior of gluten obtained from experimental parameters.

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Table 1. Partial proximate analysis of commercial wheat flour samples.

Wheat classe	Abbreviation	Protein (%)	Moisture (%)	Ash (%)
Hard Red Winter	**C1**	8.0 ± 0.05	11.7 ± 0.02	0.29 ± 0.01
	C2	11.3 ± 0.07	10.5 ± 0.03	0.38 ± 0.01
	C3	13.7 ± 0.02	10.1 ± 0.02	0.41 ± 0.00
	C4	10.4 ± 0.10	12.5 ± 0.02	0.47 ± 0.00
	C5	10.6 ± 0.07	12.6 ± 0.00	0.48 ± 0.01
	C6	11.4 ± 0.01	13.0 ± 0.04	0.58 ± 0.01
References				
Soft Red Winter	**SRW**	11.4 ± 0.00	11.8 ± 0.00	0.65 ± 0.00
Hard Red Spring	**HRS**	13.4 ± 0.20	14.0 ± 0.30	0.53 ± 0.00
Durum	**DUR**	12.0 ± 0.00	15.0 ± 0.00	0.76 ± 0.02

Means ± standard error (n= 2).
Protein and ash values are expressed on 14% moisture basis.

Table 2. Experimental parameters from creep-recovery test of gluten at different temperature levels of each flour sample.

Wheat type	Flour ID	Temperature levels	RCY (%)	J-Jr (Pa^{-1})	MaxS, γ (%)	FinalS (%)
Hard Red Winter	C1	25	82.7 a	0.6 c	32.1 b	5.5 c
		35	78.5 ab	0.7 bc	34 ab	7.3 bc
		45	80.0 ab	0.8 bc	37.7 a	7.6 bc
		55	73.2 b	1.0 b	36.1 a	9.7 b
		65	59.7 c	1.4 a	35.2 ab	14.3 a
	C2	25	83.2 a	0.4 d	25.7 c	4.4 d
		35	81.5 ab	0.5 d	25.0 c	4.7 d
		45	78.8 b	0.7 c	35.1 a	7.5 c
		55	70.1 c	1.1 a	37.0 a	11.1 a
		65	68.2 c	0.9 b	29.0 b	9.3 b
	C3	25	81.7 a	1.1 c	58.7 b	8.2 c
		35	80.6 a	0.9 c	48.7 c	9.5 c
		45	72.9 b	1.8 b	68.2 a	18.6 b
		55	66.6 c	2.3 a	69.5 a	23.4 a
		65	59.7 d	2.1 ab	53 bc	21.5 ab
	C4	25	85.3 a	0.3 c	21.8 b	3.2 c
		35	86.0 a	0.3 c	20.7 bc	2.9 c
		45	83.1 a	0.4 bc	23.3 a	4.0 bc
		55	73.1 b	0.7 a	24.3 a	6.6 a
		65	74.2 b	0.5 ab	20.0 c	5.2 ab
	C5	25	82.7 a	0.3 c	16.4 b	2.9 c
		35	81.5 ab	0.4 c	20.1 a	6.5 b
		45	75.0 bc	0.6 b	22.4 a	5.6 b
		55	71.1 c	0.6 b	22.4 a	6.5 b
		65	59.4 d	0.9 a	22.6 a	9.2 a
	C6	25	83.9 a	0.3 c	20.8 b	3.4 c
		35	81.5 a	0.4 c	20.6 b	3.8 c
		45	80.4 a	0.5 bc	25.8 a	5.1 bc
		55	71.5 b	0.8 a	28.0 a	8.1 a
		65	69.2 b	0.6 ab	20.3 b	6.3 ab

RCY= elastic recoverability, J-Jr = delta compliance, Max Strain = maximum strain during recovery, and Final Strain = final strain during recovery. Means with different letters are significantly different in each column, $p \leq 0.05$ (n=3).

Table 2. (Continued) Experimental parameters from creep-recovery test of gluten at different temperature levels of each flour sample.

Wheat type	Flour ID	Levels	RCY (%)	J-Jr (Pa^{-1})	MaxS, γ (%)	FinalS (%)
Soft Red Winter	SRW	25	72.6 a	2.6 c	94.7 c	26.1 c
		35	71.0 ab	3.1 c	106.7 c	31.1 c
		45	64.8 b	4.5 b	127.4 b	45.2 b
		55	58.0 c	6.0 a	142.2 a	60.1 a
		65	49.1 d	6.4 a	125.2 b	64.0 a
Hard Red Spring	HRS	25	76.7 a	1.0 c	44.6 c	10.5 c
		35	74.2 a	1.4 bc	55.3 cb	14.5 bc
		45	69.8 b	1.8 b	59.5 cab	18.1 b
		55	61.1 c	3.2 a	83.0 a	32.5 a
		65	51.6 d	3.6 a	73.7 ab	35.9 a
Durum	DUR	25	69.6 a	1.5 d	49.8 d	15.2 d
		35	64.9 ab	2.4 c	67.9 cb	24.1 c
		45	60.7 b	3.0 bc	75.7 ab	30.0 bc
		55	52.7 c	3.8 a	81.2 a	38.6 a
		65	45.0 d	3.5 ab	63.0 c	35.0 ab

RCY= elastic recoverability, J-Jr = delta compliance, Max Strain = maximum strain during recovery, and Final Strain = final strain during recovery Means with different letters are significantly different in each column, $p \leq 0.05$ (n=3).

Table 3. Coefficients and parameters from Burgers model of gluten at different temperatures of each flour sample.

Wheat type	Flour name	Levels	*Creep phase*				*Recovery phase*		
			J_0 (10^{-4} Pa^{-1})	J_1 (10^{-4} Pa^{-1})	t_1 (s)	η_0 (10^5 Pa^{-1})	Jr_0 (10^{-4} Pa^{-1})	Jr_1 (10^{-4} Pa^{-1})	tr_1 (s)
Hard red winter wheat	C1	25	11.4 b	12.3 a	7.5 a	1.1 a	13.8 c	12.2 a	15.8 a
		35	12.0 ab	12.0 a	7.3 ab	1.0 b	14.8 cb	11.4 a	14.2 a
		45	14.1 a	11.7 a	6.1 b	0.8 c	18.1 a	11.4 a	14.1 a
		55	13.6 a	9.6 b	5.8 b	0.8 cd	17.2 ab	8.7 b	13.8 a
		65	12.5 ab	8.3 c	7.1 ab	0.7 d	14.6 cb	6.1 c	15.6 a
	C2	25	9.8 c	9.4 b	7.2 a	1.5 a	11.6 c	9.3 ab	14.4 a
		35	9.1 c	9.0 b	6.5 a	1.4 a	11.2 c	8.8 b	15.3 a
		45	13.4 a	10.5 a	5.7 a	0.9 b	16.9 a	10.2 a	13.0 a
		55	13.0 ab	9.7 ab	5.4 a	0.7 b	16.7 a	8.8 b	14.3 a
		65	11.8 b	6.4 c	5.3 a	0.9 b	14.4 b	5.1 c	14.2 a
	C3	25	20.7 a	22.3 a	7.0 a	0.6 a	24.6 b	22.4 a	14.5 bc
		35	17.6 b	17.3 b	6.8 a	0.7 a	21.4 c	17.1 c	14.2 bc
		45	22.3 a	20.7 a	5.9 b	0.4 b	28.4 a	20.1 b	13.6 c
		55	22.3 a	17.6 b	5.2 b	0.3 b	29.0 a	16.3 c	14.9 b
		65	18.3 b	10.5 c	5.7 b	0.4 b	22.6 c	8.5 d	18.5 a
	C4	25	9.3 a	7.5 a	7.1 a	1.9 ab	10.85 a	7.4 a	14.8 a
		35	8.8 a	7.3 a	6.8 a	2.1 a	10.4 a	7.0 a	15.2 a
		45	10.2 a	6.9 a	6.1 a	1.5 c	12.5 a	6.5 a	14.5 a
		55	9.6 a	6.1 b	4.7 a	1.2 d	12.2 a	5.3 b	15.0 a
		65	9.8 a	4.2 c	5.6 a	1.6 cb	11.3 a	3.4 c	17.9 a

Table 3. (Continued) Coefficients and parameters from Burgers model of gluten at different temperatures of each flour sample.

Wheat type	Flour name	Levels	*Creep phase*				*Recovery phase*		
			J_0 (10^{-4} Pa^{-1})	J_1 (10^{-4} Pa^{-1})	t_1 (s)	η_0 (10^5 Pa^{-1})	Jr_0 (10^{-4} Pa^{-1})	Jr_1 (10^{-4} Pa^{-1})	tr_1 (s)
	C5	25	6.4 b	5.9 a	7.5 a	2.3 a	7.6 b	5.6 a	16.1 a
		35	8.1 a	6.5 a	6.6 a	1.7 b	9.8 a	6.2 a	14.2 a
		45	8.4 a	6.4 a	6.5 a	1.3 c	10.5 a	6.0 a	14.1 a
		55	8.5 a	5.4 a	5.8 a	1.2 c	10.8 a	4.9 b	13.8 a
		65	8.3 a	5.1 a	7.9 a	1.1 c	9.62 ab	3.6 c	14.8 a
	C6	25	8.3 c	7.4 a	7.3 a	1.9 a	9.8 b	7.3 a	15.2 a
		35	8.2 c	6.9 a	6.6 a	1.8 ab	9.8 b	6.6 a	15.0 a
		45	10.6 a	7.4 a	5.9 ab	1.2 c	13.3 a	7.1 a	14.8 a
		55	10.6 a	6.9 a	4.8 b	0.9 c	13.4 a	6.3 a	16.9 a
		65	8.9 b	4.2 b	6.0 ab	1.3 cb	10.4 b	3.5 b	18.7 a
Soft red winter wheat	SRW	25	22.7 c	40.3 b	9.6 a	0.3 a	26.8 c	40.3 a	18.3 a
		35	27.0 cb	43.6 ab	8.8 ab	0.3 ab	31.2 cb	42.7 a	17.0 ab
		45	31.5 ab	48.0 a	8.0 ab	0.2 cb	38.8 ab	41.7 a	14.1 cb
		55	36.4 a	46.7 a	7.5 b	0.2 c	42.5 a	38.0 a	13.2 c
		65	37.7 a	39.4 b	7.5 b	0.2 c	38.2 ab	22.0 b	13.8 cb
Hard red spring wheat	HRS	25	14.5 b	16.2 a	8.4 a	0.7 a	17.2 b	16.2 ab	16.4 ab
		35	17.3 ab	18.2 a	7.5 ab	0.5 b	21.6 b	18.4 a	15.0 b
		45	17.5 ab	15.3 a	5.8 cb	0.4 c	24.2 ab	16.4 ab	13.2 b
		55	23.6 a	19.8 a	5.6 c	0.3 d	30.9 a	18.8 a	14.2 b
		65	22.8 a	14.5 a	7.1 abc	0.3 cd	25.5 ab	11.9 b	19.2 a

Table 3. (Continued) Coefficients and parameters from Burgers model of gluten at different temperatures of each flour sample.

Wheat type	Flour name	Levels	*Creep phase*				*Recovery phase*		
			J_0 (10^{-4} Pa^{-1})	J_1 (10^{-4} Pa^{-1})	t_1 (s)	η_0 (10^5 Pa^{-1})	Jr_0 (10^{-4} Pa^{-1})	Jr_1 (10^{-4} Pa^{-1})	tr_1 (s)
Durum wheat	DUR	25	12.6 c	18.7 b	10.4 a	0.5 a	15.0 c	19.0 c	19.7 a
		35	15.2 b	22.8 a	10.6 a	0.3 b	19.4 cab	23.6 a	18.2 a
		45	16.2 b	22.8 a	10.0 a	0.3 b	21.5 ab	23.3 ab	16.0 b
		55	18.9 a	23.0 a	9.4 a	0.2 b	22.7 a	19.1 cb	13.9 c
		65	17.0 ab	16.4 b	9.4 a	0.3 b	17.1 cb	10.7 d	18.2 a

J_0 = instantaneous compliance during creep, J_1= retardation compliance during creep, t_1= retardation time during creep, η_0 = pure viscosity Jr0 = instantaneous compliance during creep, Jr1= retardation compliance during recovery, and tr1 = retardation time during recovery. Means with same superscripts in a column are not significantly different ($P \leq 0.05$, n=3).

Table 4. Percent change of gluten rheological properties at 25 and 65°C.

Parameters	Hard Red Winter wheat[1] (%)	Soft Red Winter wheat (%)	Hard Red Spring wheat (%)	Durum wheat (%)
Experimental parameters				
RCY	-21.9	-32.4	-32.7	-35.4
J-Jr	123.6	145.6	243.0	129.1
MaxS (γ)	8.2	-87.2	65.9	28.6
FinalS	133.8	145.1	243.0	130.2
Modeling parameters				
J0	7.9	39.8	36.4	26.0
J1	-63.0	-2.3	-11.7	-14.0
t1	1.7	-28.0	-18.3	-10.6
η0	-58.0	-50.0	-133.3	-66.7
Jr0	7.4	29.8	32.7	12.4
Jr1	-103.5	-83.0	-37.3	-76.6
tr1	7.7	-32.6	14.6	-8.2

[1] Average value of six Hard Red Winter (HRW) wheat gluten samples.

Positive and negative values indicate percent increase and decrease, respectively. Descriptions are defined in Table 3.

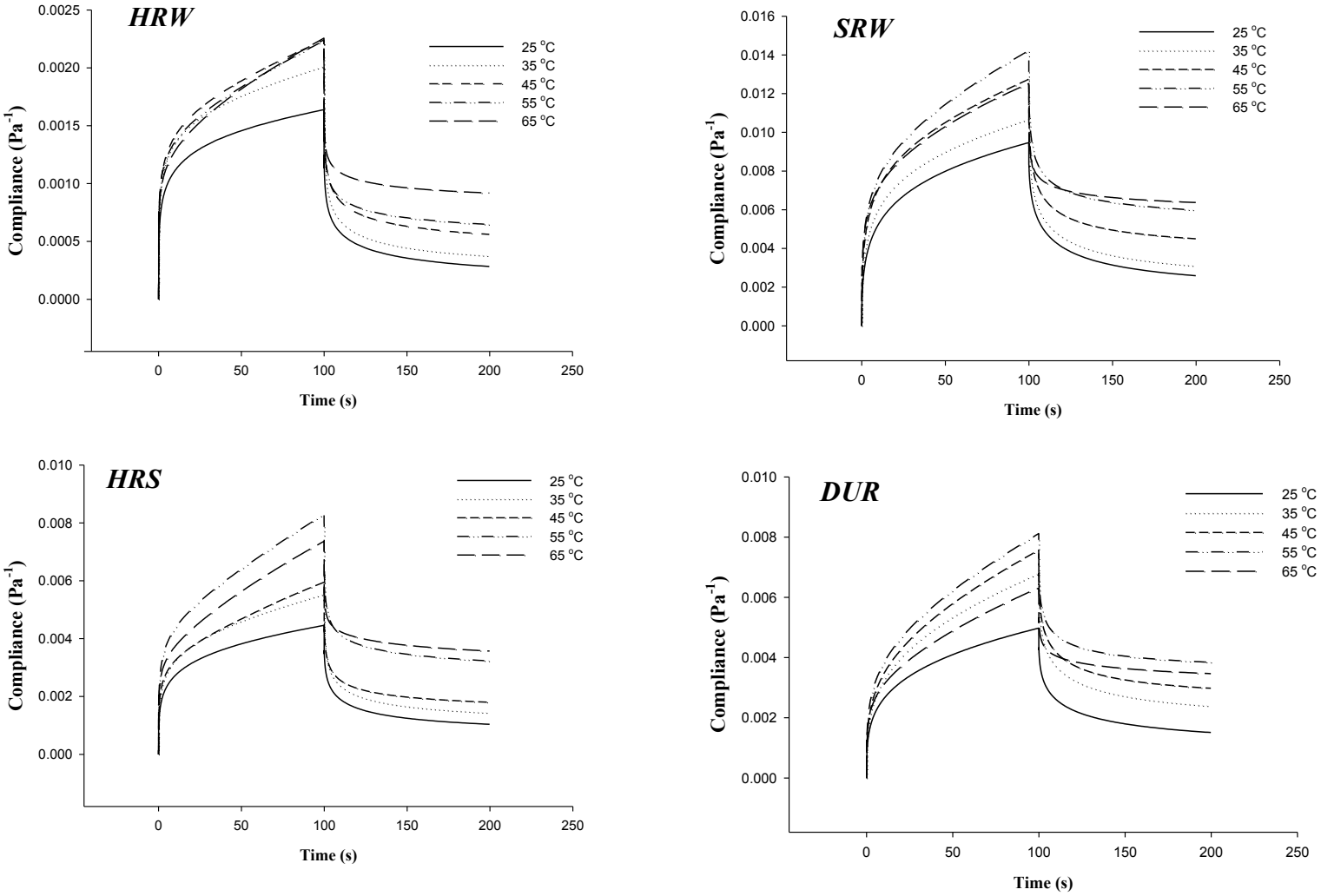

Figure 1. Gluten Creep-recovery curves of tests conducted at 25, 35, 45, 55, and 65°C. Gluten was extracted from four US wheat classes. HRW = Hard red winter wheat flour, SRW = Soft red winter wheat flour, HRS = Hard red spring wheat, DUR = Durum wheat

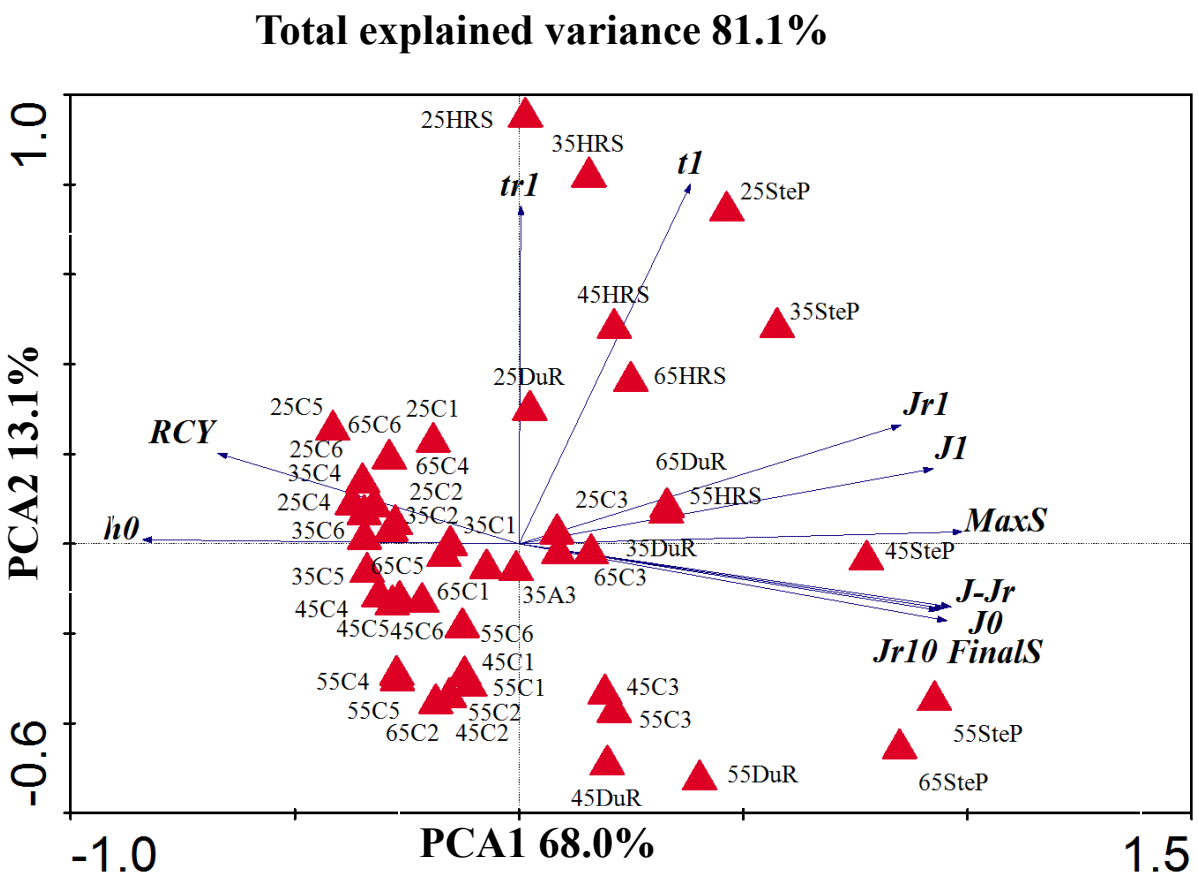

Figure 2. Biplot graph of Principal Component Analysis (PCA) of parameters from creep-recovery test performed at different temperatures of gluten from commercial flour samples from four U.S. wheat classes. Descriptions are defined in Table 3 and Figure 1.

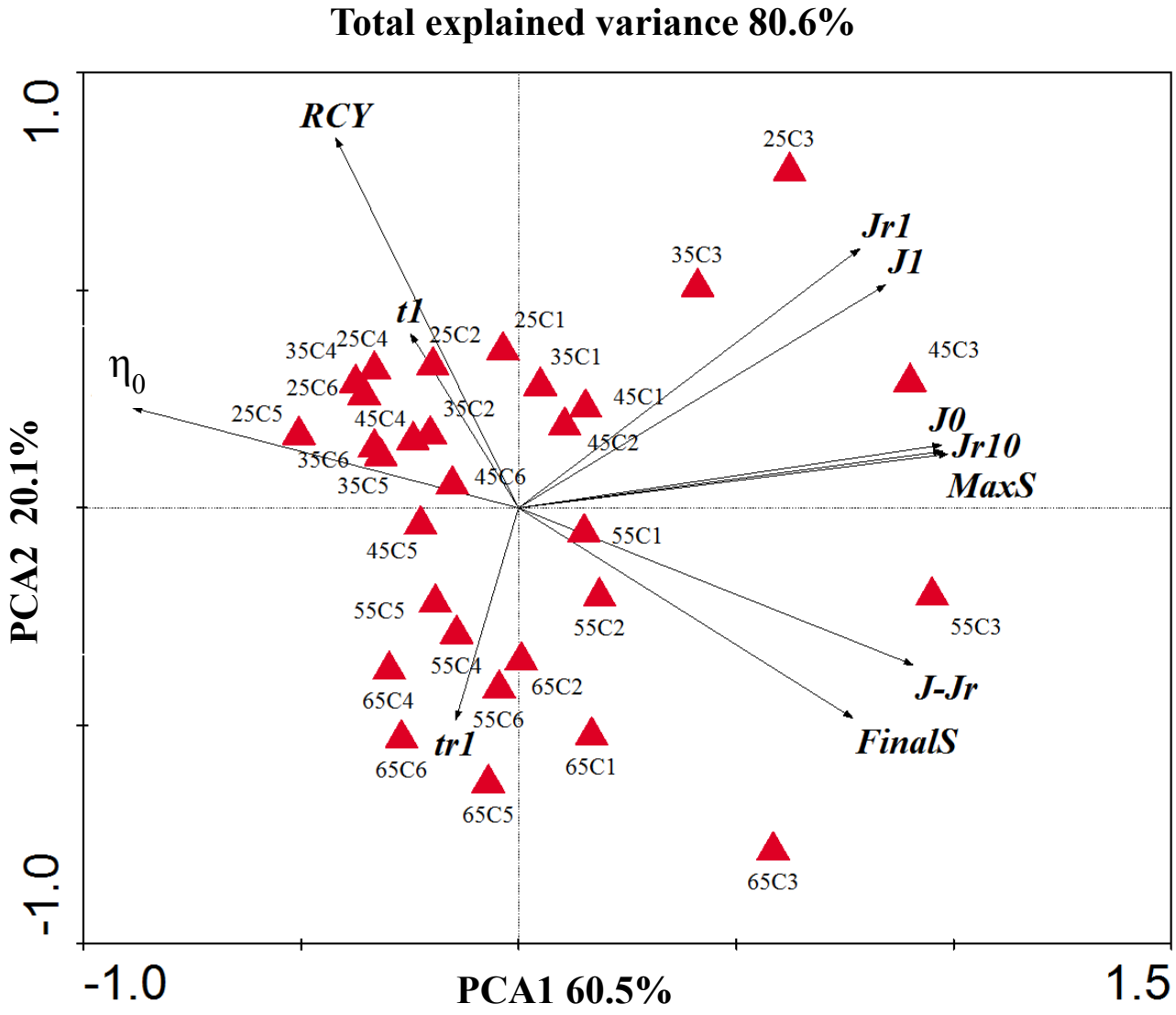

Figure 3. Biplot graph of Principal Component Analysis (PCA) of parameters from creep-recovery test performed at 25, 35, 45, 55, and 65°C for gluten from commercial hard red winter wheat flour samples. Descriptions are defined in Table 3 and Figure 1.

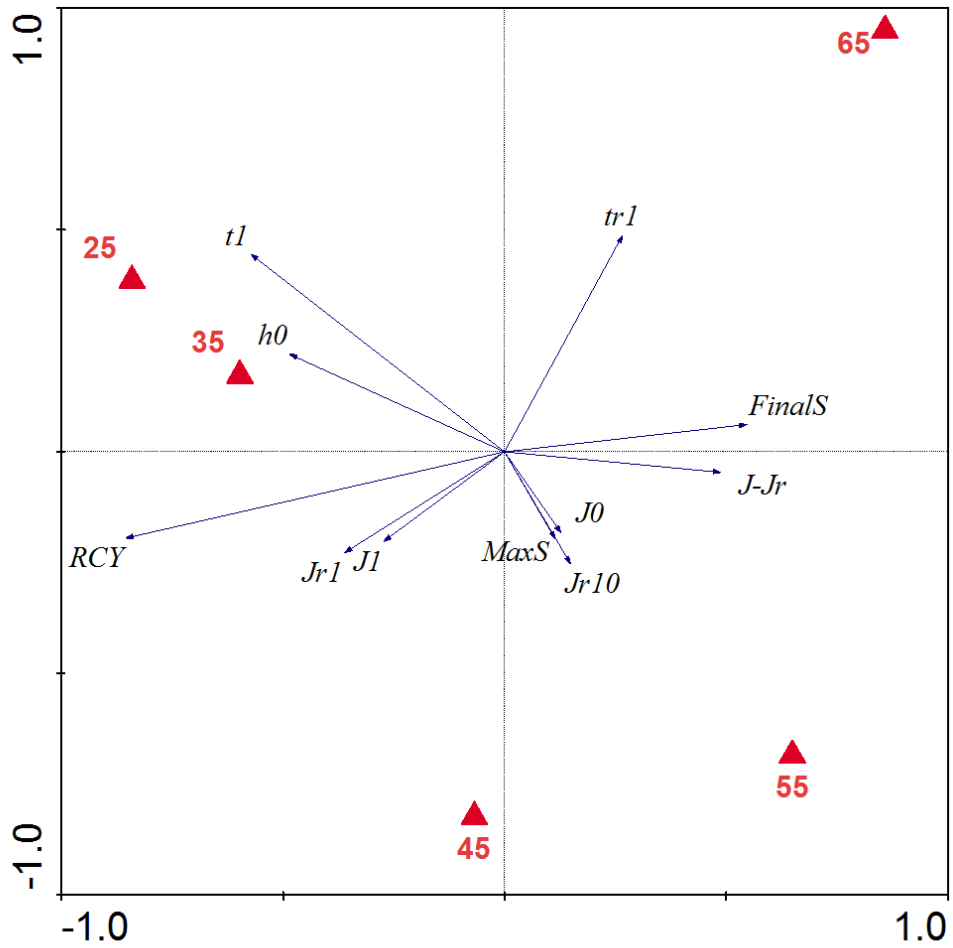

Figure 4. Biplot graph of partial Redundancy Analysis (pRDA) of parameters from creep-recovery test performed at 25, 35, 45, 55 and 65°C of hard red winter wheat gluten. Flour samples were factored out. Descriptions are defined in Table 3 and Figure 1.

CHAPTER V

EFFECT OF GLUTEN SUBSTITUTION ON THE RHEOLOGY OF BASE FLOUR

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Abstract

The effect of gluten substitution on the rheological properties of a gluten system was investigated. One commercial wheat flour, gluten products (A, B, C, and gliadin) and five treatments levels (0, 1, 2, 4, and 8% substitutions) were used to prepare homogenized flour blends. Wet gluten was extracted from the blends and its viscoelastic properties analyzed by creep-recovery (shear stress 100 Pa) and compression-recovery (compression force 10 N) tests. Gluten substitution altered gluten structure by increasing gluten deformation. The experimental data were modeled in order to obtain viscoelastic parameters that can be used to quantitatively compare treatments. Gluten B, C, and gliadin significantly reduced resistance to flow of gluten system by decreasing at 8% level by 75.9%, 13.6%, and 65.0%, respectively. Elastic and viscous character of gluten had similar trends after substituting gluten and gliadin products at all levels of substitution indicated by G0 (elastic modulus), G1 (retarded elastic modulus), η_0 (viscous modulus in elastic region), and, η_1 (retarded elastic modulus in viscoelastic region). Modeling recovery phase of compression-recovery test showed that substituting gluten B and gliadin products at 8% reduced up to 85.7% the instantaneous strain (Ɛ0) and 41.7% retarded strain (Ɛ1). On the other hand, at 8% substitution of gluten C increased Ɛ0 up to 92.8% indicating the gained instantaneous elastic character.

Keywords: Rheological properties, gluten, creep-recovery, compression-recovery, Burgers model

1. Introduction

Gluten is a one of the most important components in breadmaking products because it provides desirable viscoelastic properties. Gluten protein, a combination of glutenin and gliadin, is categorized into one of the larger groups called prolamins. Prolamins are plant storage proteins in wheat and are rich in proline and glutamine (Shewry and Halford, 2001). Prolamins can be further divided into three groups which are sulphur-rich, sulphur-poor, and high molecular weight prolamins. This classification is mainly applied to wheat, barley, and rye. Within these three groups, proteins can either be polymeric or monomeric such as glutenin and gliadin, respectively. Glutenin polymer can be divided into high molecular weight glutenin subunits (MW 60k-90k) and low molecular weight glutenin subunits. High molecular weight glutenin subunits are an elastomeric polymer and are assumed to be a backbone of gluten (Shewry et al., 2000). Low molecular weight glutenin subunits are divided to B-low molecular weight (30k-45k), C- low molecular weight (30k-45k), and D-low molecular weight (30k-75k) glutenin subunits (Shewry and Halford, 2001). Gliadin is considered as a plasticizer in gluten system because it provides viscosity and extensibility characteristics. Both glutenin and gliadin also form into three dimensional networks in dough system during hydration and contribute viscoelastic properties to bread products. However, the three dimensional structure of gluten is still unknown.

Several researchers studied the effect of gluten on viscoelastic properties of bread products. The baking performance of dough has been shown to have a correlation with bubble cell strain hardening properties which indicated an entanglement and long chain branching of high molecular weight glutenin subunits (HMW-GS) (Dobraszczyk, 2004). The low molecular weight glutenin subunit (LMW-GS) fractions, which contributed to elasticity with less extent compared to HMW-GS, have a contribution in breadmaking characteristics as well as HMW-GS, which contributed mainly to elasticity of gluten (Jood et al., 2000b). However, a gluten addition study showed that an increased amount (1.0% flour basis) of low molecular weight glutenin subunits fraction did not improve the quality of bread (Jood et al., 2001). In addition, they also found that the extra-strong wheat with high molecular weight glutenin subunits fraction had a high elastic modulus and a low ratio of viscous to elastic modulus (Jood et al., 2001). The quantity and ratio of glutenin and gliadin is also important for breadmaking. A previous study also showed that the ratio of gliadin and glutenin can impact cookie spread ratio and hardness (Barak et al., 2013a). Gliadin and glutenin ratio also was used to study noodle quality and showed that the ratio had negative correlation to hardness, springiness, cohesiveness, gumminess, and chewiness (Barak et al., 2013b). The addition of wet gluten can also improve the crumb firmness of hamburger buns during storage (Esteller et al., 2005).

Although the effect of gluten on breadmaking has been an area of active research, the study on the alteration of gluten structure at the molecular level in quantitative terms has not been widely reported. The objective of this study was to investigate the effect of substitution of based flour with commercial gluten products on the rheological properties of flour by creep-recovery and compression-recovery tests. The experimental rheological

data were fitted into Burgers model in an attempt to quantitatively explain the time-dependent viscoelastic response of gluten and the structures that may be formed.

2. Materials and Methods

2.1 Flour blends and gluten samples

The study selected one commercial wheat flour (Shawnee Milling, Shawnee, OK) with protein content of 10.8 % as base flour, four gluten products (G1, G2, G3, G4, and gliadin) and five substitution levels (0,1, 2, 4 and 8%) to obtain 11.8, 12.8, 14.8, and 18.8% protein content flour blends. The calculation for the amount of gluten products and flour used in each treatment was explained in Appendix I. Each flour blend treatment was mixed manually with a total of 30 g flour blend in a closed container for 1 min in order to obtain a homogenous blend. The control sample was flour without any substitution of gluten or gliadin.

2.2 Viscoelastic properties of gluten

The viscoelastic properties were evaluated on wet gluten extracted from the flour blends. Wet gluten was extracted by using a Glutomatic 2200 (Perten Instruments AB, Huddinge, Sweden) according to method 38-12.02 (AACC International 2010). Briefly, the flour (10 g) was mixed for 20 sec and washed with 2% NaCl solution for 5 min through 88 μm polyester screen.

Creep and recovery test was used to study the viscoelastic properties after exposed to shear stress. Prior to the test, wet gluten was allowed to relax at room temperature (25°C) for one hour under a 2.5 kg metal plate with 2.5 mm spacing. The creep-recovery test was performed using an AR1000 rheometer (TA instruments, New Castle, DE) equipped with a 25 mm cross hatched round probe and base. The test

consisted in applying a 100 Pa constant shear stress and recording gluten viscoelastic responses for 100 sec in both creep and recovery phases.

Compression and recovery test was performed to study the elastic recovery of wet gluten using a new instrument named Gluten CORE and a Glutomatic 2200 System (Perten Instruments AB, Huddinge, Sweden). Wet gluten was gently recovered and placed in an acrylic cylinder with a special design closed bottom sieve in the centrifuge cassette of the Glutomatic Centrifuge 2015 at 6000 rpm (Perten Instruments AB, Huddinge, Sweden). The gluten compression test consisted in a vertically applied force of 10 N for 30 s (compression phase) and a release of the force for 30 s (recovery phase) while measuring the height of the gluten specimen throughout the test. The data was reported in terms of strain which was derived from the height as a function of time. The recovery strain was calculated according to equation 1.

Recovery strain (t)

= (Height at time t – Height at recovery phase at time zero)/initial height before compression (Eq. 1)

2.3 Modeling of creep-recovery and compression-recovery tests

The experimental values obtained from creep-recovery and compression-recovery tests were fitted using a mechanical analog based model (spring and dashpot). Burgers model was chosen to represent the creep-recovery and compression-recovery behaviors of gluten due to its ability to explain viscoelastic properties. Burgers model contained three important elements that described the viscoelastic behavior including instantaneous elastic deformation (spring), delayed elastic deformation (spring and dashpot), and pure

viscosity behavior (dashpot). The Burgers model equation used for creep-recovery experiments was the following:

$$J(t) = J0 + J1(1 - \exp(-t/t1)) + t/\eta_0$$

Where

J = compliance at time t (Pa^{-1})

J0 = instantaneous elastic deformation (Pa^{-1})

J1 = delayed elastic deformation (Pa^{-1})

t = time (s)

t1 = retardation time (s) and

η_o = pure viscosity (Pa • s).

During the recovery after creep, however, the term t/η_0 was set as zero because the pure viscous component was non-recoverable. The experimental data from creep-recovery test was not fitted very well with Burgers model using 421 original data points in the upper part of the curve compared to the lower part of the curve (Fig. 1a, coefficient of regression $R^2 = 0.96$); therefore, the experimental results were interpolated into 10,000 points and shown to have a better fit than experimental data (Fig. 1b, $R^2 = 0.99$).

The compression-recovery test experimental data were modeled as well, specifically the recovery phase of the test. Similar to the modeling of creep-recovery test, compression-recovery modeling employed the Burgers model, and it was expressed in terms of strain, ε. The following equation was used for modeling gluten viscoelastic behavior from compression-recovery test:

$$\varepsilon(t) = \varepsilon0 + \varepsilon1(1 - \exp(-t/tr1)),$$

where

$\varepsilon(t)$ = strain as a function of time t,

$\varepsilon 0$ = instantaneous strain,

$\varepsilon 1$ = delayed strain,

t = time (s), and

t1 = retardation time (s).

2.4 Statistical analysis

The experimental design for this study was a completely randomized design (CRD). There were four treatments in this study. A flour sample was substituted with three commercial gluten samples (Gluten A, B, and C) and gliadin with five substitution levels (0, 1, 2, 4, 8%) and four replicates per treatment. The experimental data from both creep-recovery and compression-recovery tests were fitted into Burgers model by nonlinear regression analysis using PROC NLIN in SAS program (Version 9.1 SAS Institute Inc., Cary, NC). The mean significant difference was tested by using Tukey's multiple comparison test (α =0.05) in SAS programs (Version 9.1 SAS Institute Inc., Cary, NC).

3. Results and Discussion

3.1 Viscoelastic behavior of commercial gluten products from creep-recovery test

The viscoelastic properties of the four gluten products and gluten from the test flour were analyzed using a creep-recovery test. The samples appeared to separate in three groups (Fig. 2). The interval of creep phase was between 0 and 100 s. Following the creep phase, the strain in the recovery phase was immediately measured from 100 to 200

s. Glutens A, C, and gluten of flour control can be grouped as samples with high rigidity due to low %strain curve (max strain between 15-20%, lower curves). In contrast gluten B was more deformable as shown by a higher %strain curve with max strain of 142%. The third type of curve corr

esponded to gliadin, increasing the %strain up to 78%.

3.2 Effect of gluten substitution on coefficients from creep-recovery test

The effect of gluten substitutions on rheological behavior was investigated by creep-recovery test; examples of the curves obtained are shown in Figure 3 and experimental and modeled parameters are reported in Table 1 and 2.

The substitution of gluten A and C seemed to have similar viscoelastic curves, while the curves of the substitution of gluten B and gliadin seemed to be more separated in each level of substitution (Fig. 3). The max strain was obtained from the end of creep curve, while the final strain was obtained from the end of recovery curve. The change in max strain and final strain were similar in all types of samples (Table 1). After 8% substitution of gluten, gluten A, B, C, and gliadin had percent increase of 21.4, 297.3, not significant, and 185.0% of max strain, respectively. The final strain after adding 8% gluten A, B, C, and gliadin had percent increase of not significant, 302.6, not significant, and 189.7%, respectively. These results indicated that gluten and gliadin substitution affected viscoelastic properties of gluten differently depended on gluten types. The results were in agreement with other authors (Jood et al., 2000a) who found that gluten extracted from various flour types impacted rheological properties of gluten in a different direction. The creep and recovery compliance curves in Fig. 3 were fitted into Burgers model (discussed in Materials and Methods section). The Burgers model creep variables

(J0, J1, t1, and η_0) and recovery variables (Jr0, Jr1, tr1) were reported in Table 1 and 2, respectively. Other creep variables such as G0 (G0= 1/J0), G1 (G1= 1/J1), and η_1 (η_1 = t1*G0) and recovery variables such as Gr0 (Gr0= 1/Jr0), G1 (Gr1= 1/Jr1), and ηr_1 (ηr_1 = t1*G1) were calculated. Overall, instantaneous (J0 and Jr0) and delayed (J1 and Jr1) compliance was increased in both creep and recovery phase after gluten substitution which indicated an increase in deformation of gluten. These results suggested that the substitution of all gluten products formed interactions and perhaps crosslinks with the native protein in the base flour, specifically with high glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). Both HMW-GS and LMW-GS contributed mainly to elasticity of gluten via interchain disulfide bonds. However, there was no significant difference in retardation time (t1) during creep and recovery phase with gluten substitution levels. The substitution of gluten B, C, and gliadin decreased the pure viscosity (η_o) of the sample (at 8% by 75.9%, 13.6%, and 65.0%, respectively) which suggested that the resistance to flow of gluten was reduced. The viscous flow process in gluten structure involved a slippage of non-covalent crosslinks between glutenin molecules. Thus, gluten B, C, and gliadin formed gluten conformation and interactions mainly via non-covalent crosslinks and not disulfide crosslinks. Elastic (G0 and G1) and viscous (η_0 and η_1) modulus decreased in about the same percentage indicating that the viscous and elastic effects from gluten substitution had similar trends during applying and releasing shear stress (Fig. 4). These coefficients, namely, G0, G1, η_1, and, η_2, reflect elastic strength of interfacial network molecule. Thus, the reduction of G0, G1, η_1, and, η_2 after gluten substitutions reflected the formation of a weaker gluten structure. It is accepted that the strength of gluten is obtained from the backbone polymer

formed by high molecular weight glutenin subunit (HMW-GS) and low molecular weight glutenin subunit (LMW-GS) via interchain disulfide bonds. We proposed that after gluten substitutions into based flour, the molecular mobility of the resulting gluten was increased reflecting a more concentrated polymer with a different gluten structural conformation, with more entanglements due to increase in concentration but not increased disulfide bonds. The net effect is a dilution of the original interchain disulfide bonds in the base flour. The increase in concentration most likely forms increased hydrogen bonds and hydrophobic interactions to stabilize the gluten structure. These results are unexpected since the commercial glutens contain in theory HMW-GS and LMW-GS that can potentially form interchain disulfide bonds. The results from gliadin substitution were expected since they are known to act as plasticizers or fillers, filling the spaces of the polymer branches and thus contributing to the viscous flow character of gluten. The behavior of elastic and viscous modulus of gliadin substitution observed in our study was in agreement with a previous study by Khatkar et al. (2002) which showed that G' and G" were reduced after the addition of gliadin fraction (Khatkar et al., 2002).

3.3 Viscoelastic behavior of pure gluten from compression-recovery test

Examples of graphs from compression-recovery test are shown in Figure 5. The test records the thickness of gluten as a function of time. The compression force (10 N) is applied for 30 s (creep phase) followed by a recovery phase for 60 s with zero force applied. Overall, three groups of patterns could be distinguished in the recovery phase. The ranking of recovery is gluten B < gliadin < gluten C and A. The patterns agree with the observations of creep recovery described earlier in which three groups were also distinguished. These observations suggested that different gluten samples can be

differentiated based in the response of their structure to the compression-recovery test. The results are consistent with a gluten compression recovery study that showed discrimination power to distinguish gluten characteristics from hard red winter, hard red spring, soft red winter, hard white, and soft white cultivars (Chapman et al., 2012).

3.4 Compression and recovery behavior of gluten after substitution treatments

The substitution of gluten products and gliadin were also tested by using a large deformation measurement namely compression-recovery test. The thickness was also converted to strain in order to assess the effect of gluten substitution on the compression-recovery behavior. The trend of max and final strain as a function of concentration were reported in Fig. 6 and 7, respectively. The substitution of gluten B and gliadin resulted in the highest max strain value as a function of concentration. The substitution of gluten A did not affect max strain, while the substitution of gluten C resulted in a decrease of max strain as a function of concentration. This trend indicated that gluten B and gliadin substitution increased the deformability of gluten, while gluten C had the opposite effect. The final strain was shown to have similar trends when compared with the max strain results. Max strain, final strain, and recovery index were also summarized in Table 3. The data from the recovery phase of compression-recovery test were fitted using equation discussed in Materials and Methods section and the model parameters (Ɛ0, Ɛ1, t1, and Ɛ0/Ɛ1) were reported in Table 3. Instantaneous strain (Ɛ0) was affected by the substitution of different gluten types and levels except for gluten A. The substitution of gluten B resulted in a decrease in Ɛ0 for 85.7% at 8% substitution. These results of decreasing in elastic character (strength) of gluten after gluten substitution appear to be conflicting with

the literature in which gluten addition should increase gluten strength. A possible explanation for the observations is the different pH of the gluten products. Acidity affected electrostatic behavior of protein by increasing electrostatic repulsion forces. Thus, the gluten with increased acidity exhibited a decrease in resistance to deformation (Galal et al., 1978). Gluten B with a relatively lower pH compared to the other products, can weakened the gluten structure and thus decreasing gluten strength. Gliadin showed a reduction by 28.6% in instantaneous strain (S0) at 8% substitution. The delayed strain (Ɛ1) was lowered after the substitution of gluten B (decreased at 8% substitution by 41.7%) and gliadin (decreased at 8% substitution by 22.2%) which confirmed an increase in deformation from creep-recovery test. The compression-recovery test was unable to detect any changes in the retardation time after gluten substitution. This observation was consistent with the retardation time of gluten samples treated with additives (DATEM, ascorbic acid, DTT, and urea) which were used specifically to affect different bonds in gluten (Chompoorat et al., 2013).

4. Conclusions

Gluten substitution affected viscoelastic properties of gluten system based on viscoelastic behavior and pH level of gluten and gliadin substitutions. Viscoelastic properties of gluten and gliadin products were ranked based on %strain and recoverability from creep-recovery and compression-recovery tests, respectively which were gluten B < gliadin < gluten A and C. The acidity of gluten B can partially explain the highest percent increase at 8% substitution of max strain (up to 297.3%) and final strain (up to 302.2%) from creep-recovery test, which indicated a more deformable of gluten system. The results were in agreement with coefficient parameters from both creep-recovery and

compression-recovery test. The substitutions of the four gluten products of this study increased instantaneous compliance and delayed compliance indicating that the gluten became more deformable. We postulated that the substituted gluten did not form new disulfide bonds and most likely diluted these types of bonds via increased protein concentration and increase of hydrogen bonds and hydrophobic interactions. Almost all of gluten substitution resulted in a decrease in pure viscosity except for gluten A. Moreover, across all samples, the elastic and viscous moduli (G0, G1, η_1, and, η_2) were altered in a similar manner after substituting gluten and gliadin products. The modeling recovery phase of compression-recovery curve allowed us to quantitate contributions to the strength of gluten. It revealed that substituting gluten B and gliadin products reduced the gluten strength up to 85.7% of Ɛ0 and 41.7% of Ɛ1, while gluten C increased gluten strength up to 92.8% of Ɛ0 at 8% substitution.

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Table 1. Experimental and modeling of the creep phase parameters of viscoelastic properties from a creep recovery test of gluten. Gluten extracted from flour blends with treatments of four commercial gluten products and five substitution levels (0, 1, 2, 4, and 8%).

Gluten	Level (%)	Experimental parameters		Modeling creep phase parameters						
		Max strain (%)	Final strain (%)	J0 (1/Pa) x 10^{-4}	J1 (1/Pa) x 10^{-4}	t1 (s)	G0 (Pa) x 10^{3}	G1 (Pa) x 10^{3}	η0 (Pa·s) x 10^{5}	η1 (Pa·s) x 10^{4}
A	0	18.7 bc	3.9 a	6.60 c	6.93 ab	8.56 a	1.52 a	1.45 ab	1.83 ab	1.24 a
	1	18.1 c	3.2 a	6.82 c	6.33 b	7.91 a	1.47 a	1.58 a	1.93 a	1.25 a
	2	20.7ab	4.3 a	7.63 b	7.24 ab	8.40 a	1.31 b	1.38 b	1.64abc	1.16 ab
	4	22.5 a	4.6 a	8.30 a	7.86 a	8.39 a	1.21 c	1.28 b	1.52 bc	1.07 b
	8	22.7 a	4.9 a	8.57 a	7.69 a	8.83 a	1.17 c	1.30 b	1.50 bc	1.15 ab
B	0	18.7 e	3.9 d	6.60 e	6.93 e	8.56 ab	1.52 a	1.45 a	1.83 a	1.24 a
	1	26.7 d	6.0 cd	9.08 d	9.63 d	8.83 a	1.10 b	1.04 b	1.21 b	0.92 b
	2	32.4 c	6.5 c	11.15 c	11.88 c	8.23 ab	0.90 c	0.84 c	1.01 c	0.69 c
	4	55.6 b	13.1 b	17.15 b	21.25 b	8.60 ab	0.58 d	0.47 d	0.56 d	0.40 d
	8	74.3 a	15.7 a	22.55 a	30.38 a	7.89 b	0.44 e	0.33 e	0.44 d	0.26 e
C	0	18.7 bc	3.9 b	6.60 c	6.93 b	8.56 a	1.52 a	1.45 ab	1.83 a	1.24 ab
	1	23.9 a	5.7 a	7.99 a	8.67 a	9.16 a	1.25 c	1.16 c	1.33 c	1.06 b
	2	21.2 b	4.8 ab	7.54 ab	7.46 ab	8.84 a	1.33 bc	1.36 bc	1.55 b	1.19 ab
	4	18.5 c	4.2 b	6.90 bc	6.26 b	8.66 a	1.46 ab	1.61 a	1.80 a	1.39 a
	8	21.2 b	4.5 ab	7.93 a	7.19 b	8.73 a	1.26 c	1.39 ab	1.58 b	1.22 ab
GD	0	18.7 d	3.9 c	6.60 d	6.93 d	8.56 a	1.52 a	1.45 a	1.83 a	1.24 a
	1	21.5 d	4.8 cd	7.51 d	7.70 d	9.06 a	1.33 b	1.30 b	1.51 b	1.18 a
	2	26.3 c	5.9 bc	8.92 c	9.52 c	9.20 a	1.22 c	1.05 c	1.22 c	0.97 b
	4	32.5 b	7.5 b	11.30 b	11.60 b	9.00 a	0.89 d	0.87 d	1.01 c	0.78 c
	8	53.3 a	11.3 a	18.20 a	20.10 a	8.53 a	0.55 e	0.50 e	0.64 d	0.42 d

Commercial gluten product A, B, C, and gliadin (GD). MaxS = maximum strain during creep, FinalS = final strain during recovery, J0 = instantaneous compliance during creep, J1= retardation compliance during creep, t1= retardation time during creep, G0 = instantaneous elastic modulus during creep, G1 = retarded elastic modulus during creep, η_0 = pure viscosity, η_1 = coefficient of viscosity. Means (n=4) with different letters are significantly different within each treatment, $p \leq 0.05$ (n=4).

Table 2. Modeling recovery phase parameters of viscoelastic properties from a creep recovery test of gluten. Gluten extracted from flour blends with treatments of four commercial gluten products and five substitution levels (0, 1, 2, 4, and 8%).

		Modeling recovery phase parameters					
Gluten	**Level (%)**	Jr0 (1/Pa) x 10^{-4}	Jr1 (1/Pa) x 10^{-4}	tr1 (s)	Gr0 (Pa) x 10^{3}	Gr1 (Pa) x 10^{3}	ηr1 (Pa·s) x 10^{4}
A	0	7.71 d	6.78 ab	17.5 a	1.30 a	1.48 ab	2.58 a
	1	8.06 cd	6.52 a	16.9 a	1.24 a	1.54 a	2.59 a
	2	8.86 bc	7.19 ab	16.5 a	1.13 b	1.40 ab	2.30 b
	4	9.66 ab	7.91 a	17.3 a	1.04 bc	1.28 b	2.19 b
	8	9.84 a	7.61 ab	18.0 a	1.02 c	1.32 b	2.36 ab
B	0	7.71 e	6.78 e	17.5 a	1.30 a	1.48 a	2.58 a
	1	10.65 d	9.60 d	17.4 a	0.94 b	1.05 b	1.81 b
	2	13.28 c	12.08 c	16.7 a	0.75 c	0.83 c	1.39 c
	4	20.28 b	21.18 b	16.5 a	0.49 d	0.47 d	0.78 d
	8	26.90 a	30.23 a	15.6 a	0.37 e	0.33 e	0.52 e
C	0	7.71 b	6.78 b	17.5 a	1.30 a	1.48 ab	2.58ab
	1	9.24 a	8.52 a	17.2 a	1.09 b	1.18 c	2.03 c
	2	8.69 ab	7.39 ab	17.2 a	1.15 ab	1.37 bc	2.35 b
	4	7.88 b	6.07 b	16.2 a	1.28 a	1.66 a	2.68 a
	8	9.15 a	7.18 ab	18.0 a	1.09 b	1.40 abc	2.50ab
GD	0	7.71 d	6.78 d	17.5 a	1.30 a	1.48 a	2.58 a
	1	8.73 d	7.63 d	17.9 a	1.15 b	1.31 b	2.34 b
	2	10.43 c	9.53 c	18.9 a	0.96 c	1.05 c	1.98 c
	4	13.10 b	11.40 b	18.0 a	0.76 d	0.88 d	1.59 d
	8	21.18 a	19.75 a	17.3 a	0.47 e	0.51 e	0.88 e

Commercial gluten product A, B, C, and gliadin (GD). Jr0 = instantaneous compliance during creep, Jr1= retardation compliance during creep, tr1= retardation time during creep, Gr0 = instantaneous elastic modulus during creep, Gr1 = retarded elastic modulus during creep, ηr_1 = coefficient of viscosity. Means (n=4) with different letters are significantly different within each treatment, $p \leq 0.05$ (n=4).

Table 3. . Experimental and modeling parameters of viscoelastic properties of gluten from compression-recovery test. Gluten extracted from wheat flour with treatments of four commercial gluten products and five substitution levels (0, 1, 2, 4, and 8%).

		Experimental parameters			Modeling parameters		
Gluten	Level (%)	Max Strain	Final Strain	Recovery (%)	S0	S1	t1 (s)
A	0	0.79 a	0.35 a	55.7 a	0.07 a	0.36 a	6.55 a
	1	0.81 a	0.37 a	54.6 a	0.08 a	0.34 a	5.58 a
	2	0.82 a	0.40 a	51.0 a	0.10 a	0.31 a	6.54 a
	4	0.80 a	0.35 a	56.4 a	0.11 a	0.33 a	5.91 a
	8	0.80 a	0.40 a	49.9 a	0.10 a	0.29 a	6.14 a
B	0	0.79 d	0.35 c	55.7 a	0.07 a	0.36 a	6.55 a
	1	0.74 c	0.35 c	52.6 ab	0.09 a	0.29 ab	6.81 a
	2	0.83 b	0.41 bc	51.0 ab	0.10 a	0.32 b	6.74 a
	4	0.84 b	0.47 b	44.7 b	0.08 a	0.28 b	6.44 a
	8	0.88 a	0.66 a	25.3 c	0.01 b	0.21 c	7.23 a
C	0	0.79 c	0.35 a	55.7 b	0.069 b	0.361 a	6.55 a
	1	0.80 bc	0.29 ab	63.8 ab	0.123 a	0.370 a	5.08 ab
	2	0.78 ab	0.35 a	55.0 b	0.107 a	0.313 a	6.23 a
	4	0.77 a	0.29 ab	62.5 ab	0.119 a	0.346 a	4.46 b
	8	0.73 a	0.22 b	70.2 a	0.133 a	0.363 a	4.31 b
GD	0	0.79 b	0.35 c	55.7 a	0.07 b	0.36 a	6.55 a
	1	0.79 b	0.34 c	57.7 a	0.11 a	0.34 a	6.15 a
	2	0.82 b	0.40 bc	51.2 ab	0.09 a	0.32 ab	7.30 a
	4	0.82 b	0.43 b	47.3 bc	0.09 a	0.29 b	6.83 a
	8	0.85 a	0.52 a	39.1 c	0.05 b	0.28 b	6.70 a

Commercial gluten product A, B, C, and gliadin (GD). Max Strain = maximum strain during recovery, Final Strain = final strain during recovery, Ɛ0 = instantaneous strain, Ɛ 1 = retardation strain, t1 = retardation time, Means (n=4) with different letters are significantly different within each treatment, $p \leq 0.05$ (n=4).

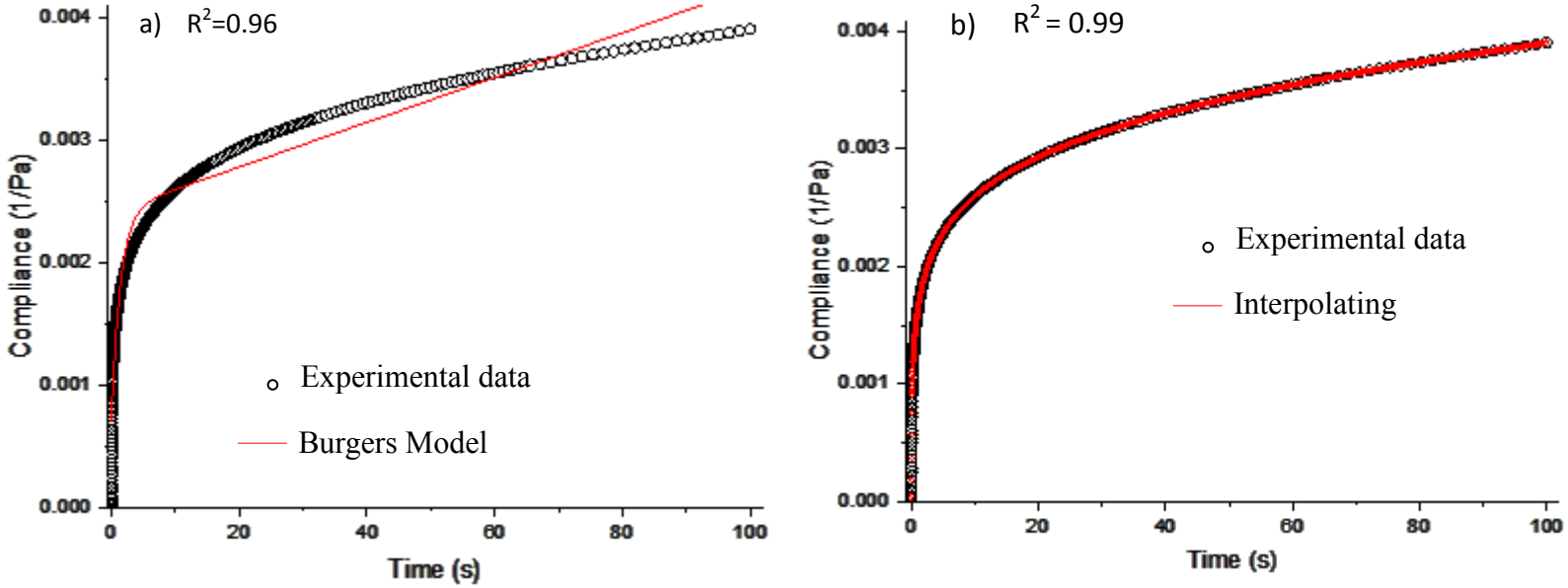

Figure 1. Example of fitting Burgers model with compliance from creep-recovery test (a) and Interpolating compliance from 421 points of original data to 10,000 points and fitting with Burgers model (b).

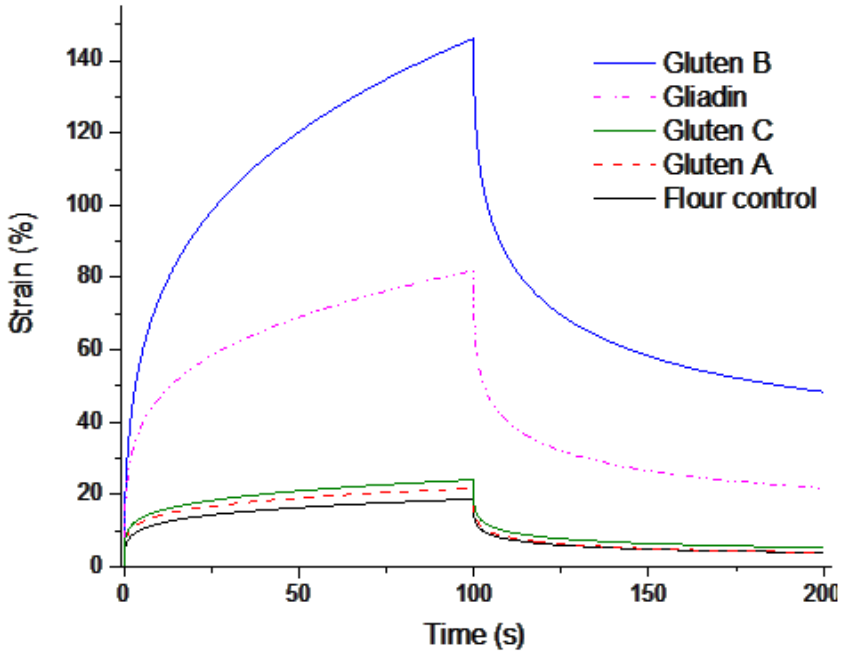

Figure 2. Comparison strain as a function of time of four commercial gluten products and one wheat flour from creep-recovery test.

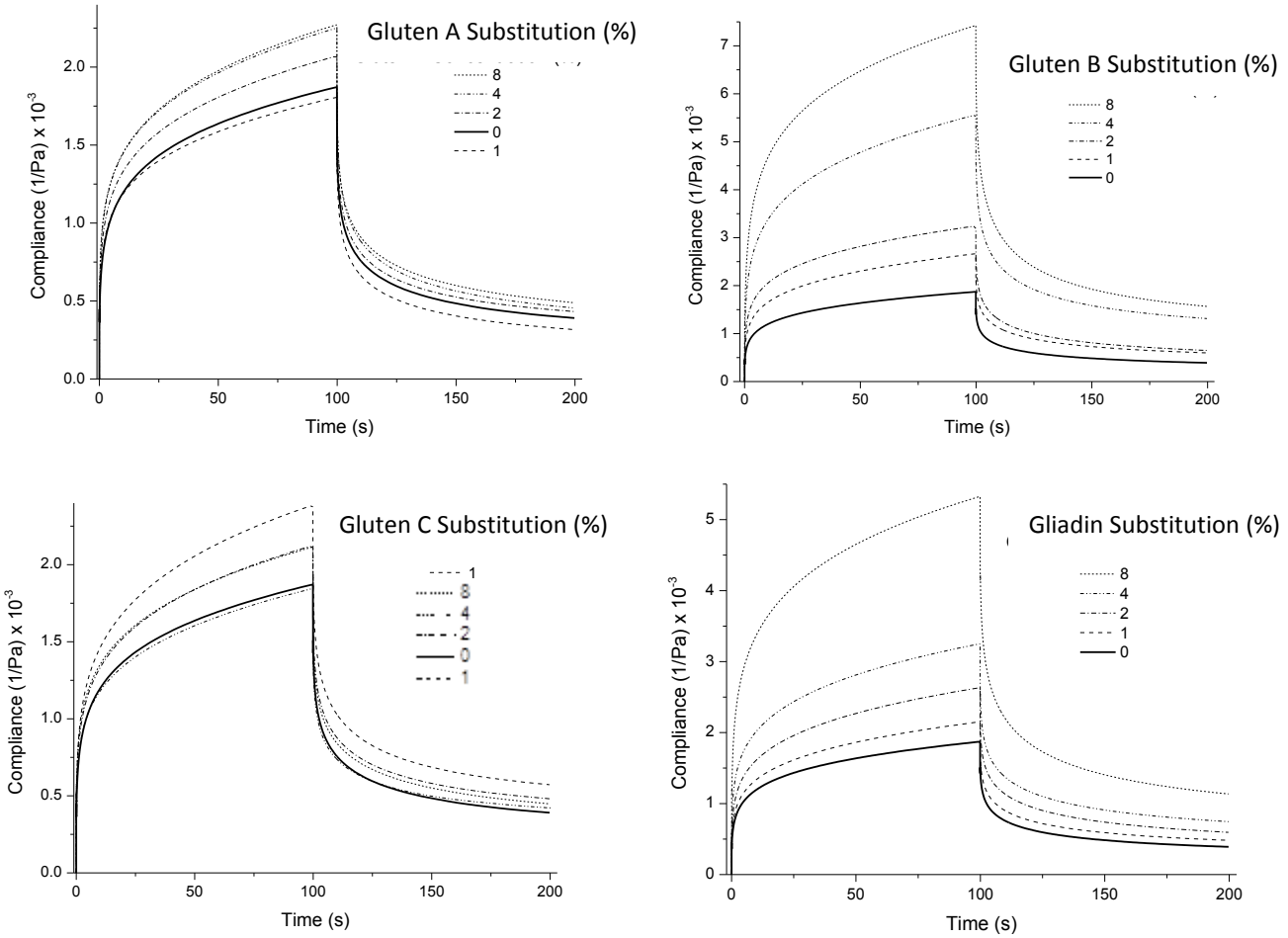

Figure 3. Comparison of gluten compliance from creep-recovery test as a function of time of gluten extracted form a wheat flour blends containing four commercial gluten products and five substitution levels (0, 1, 2, 4, and 8%). Note the different magnitude of the compliance of graphs for Gluten B and gliadin.

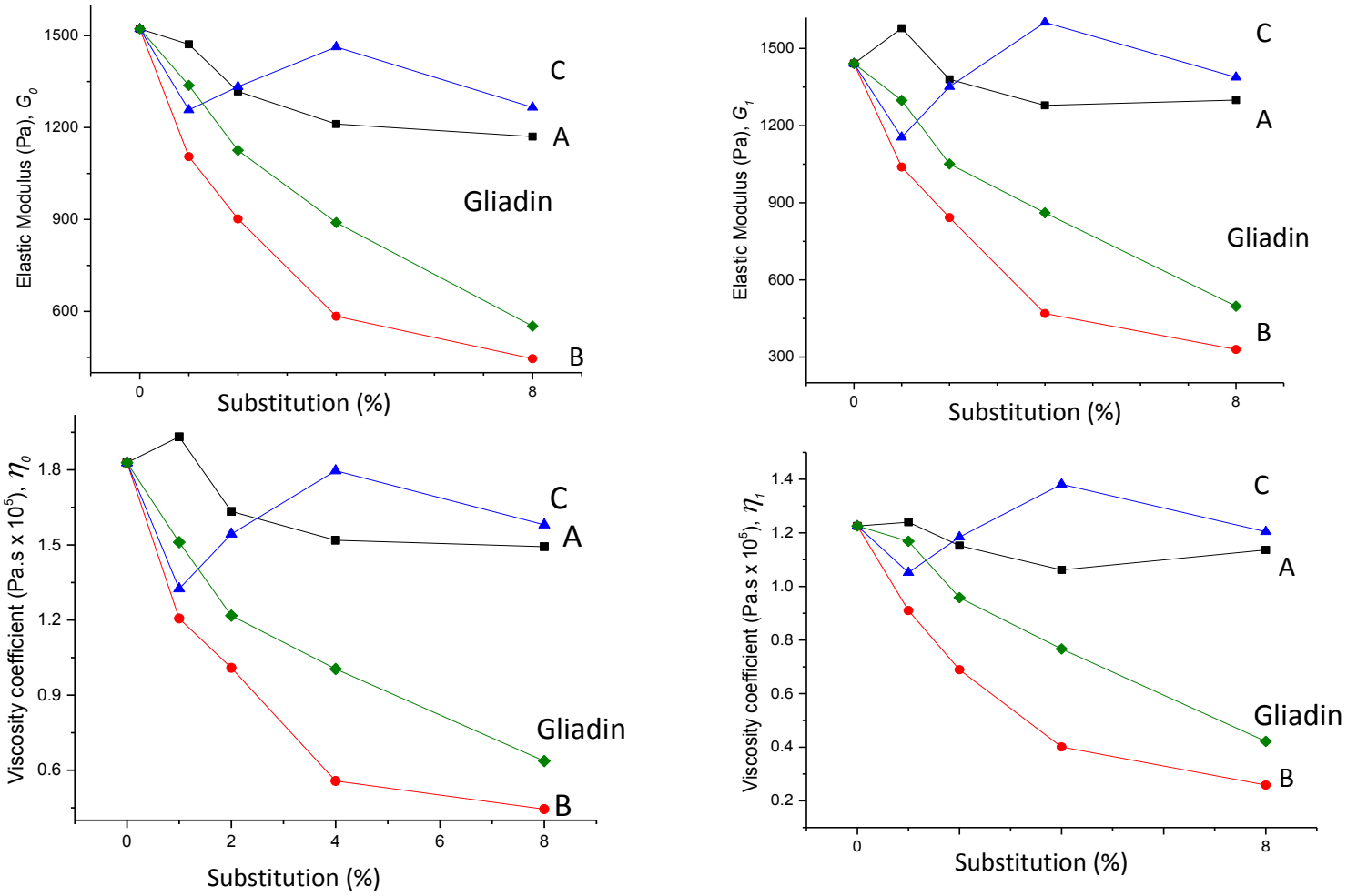

Figure 4. Comparison of elastic and viscous properties from creep-recovery test as a function of substitution levels from flour blends containing four commercial gluten products and five substitution levels (0, 1, 2, 4, and 8%).

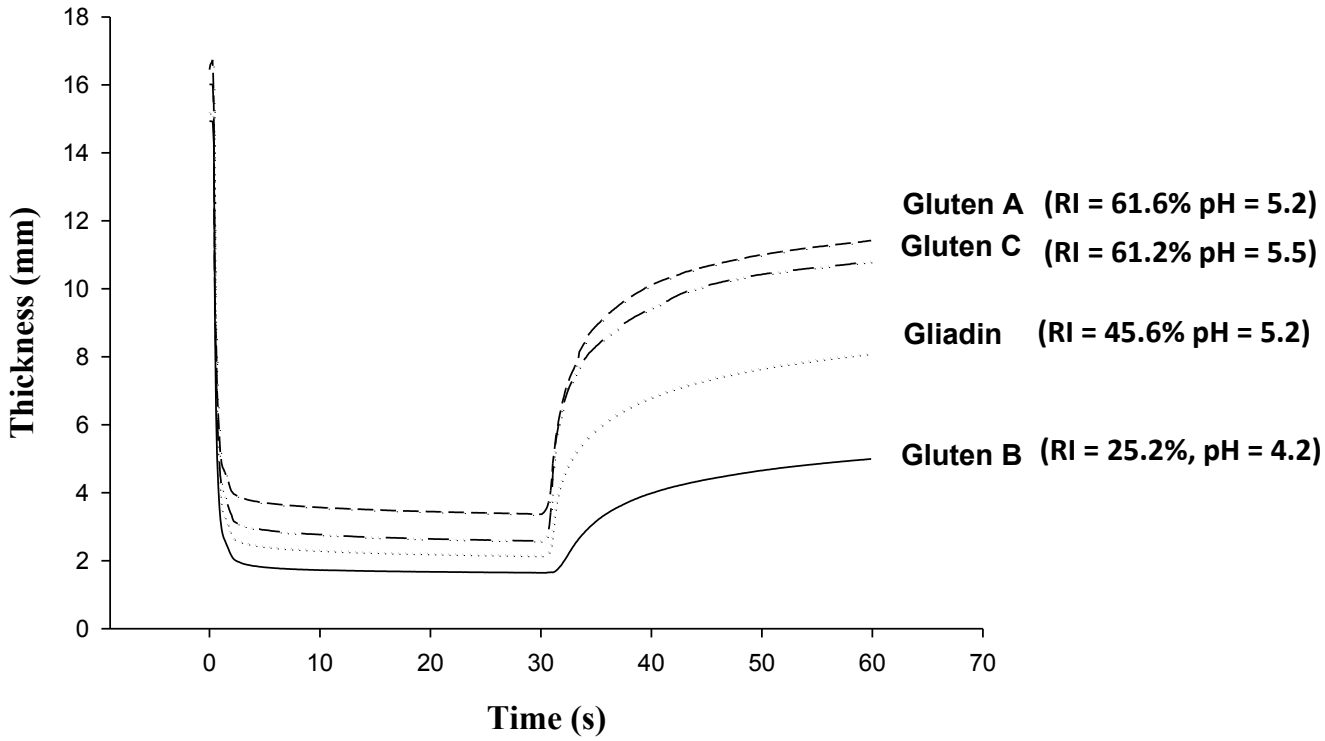

Figure 5. Examples of graphs of gluten thickness as a function of time of four commercial gluten products from compression-recovery test.

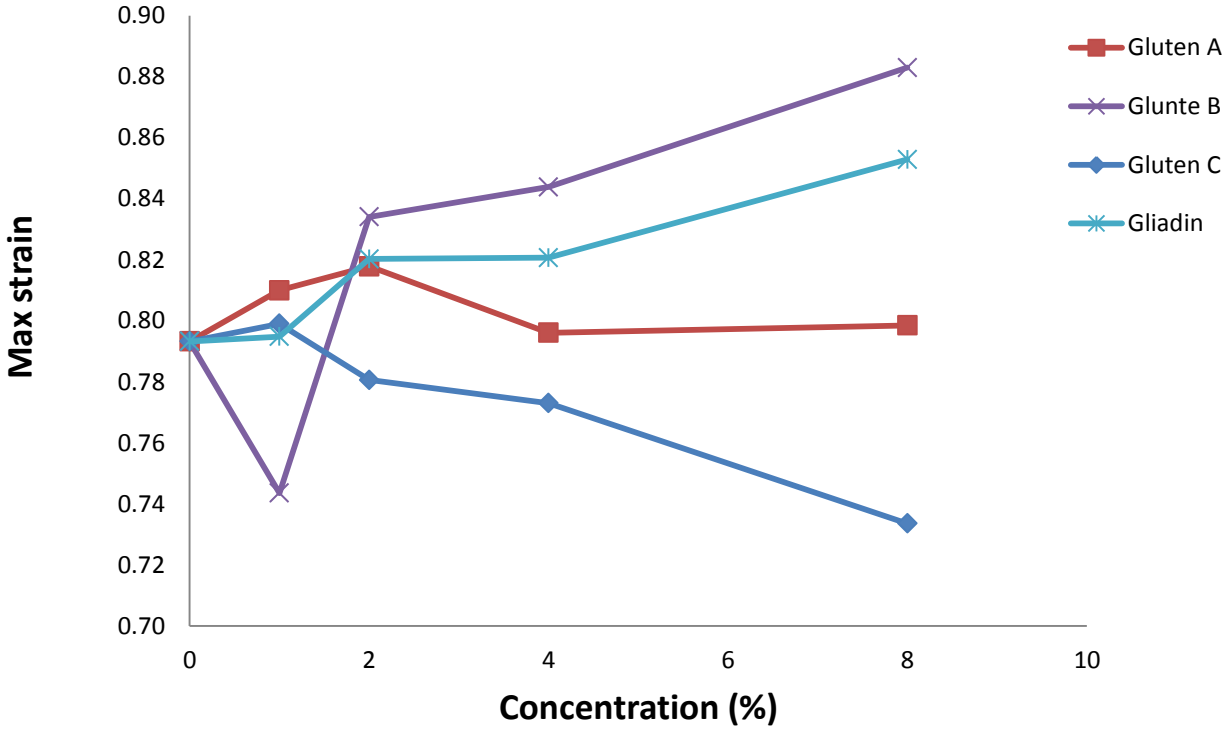

Figure 6. Comparison maximum strain from compression-recovery test as a function of substitution from flour blends containing four commercial gluten products and five substitution levels.

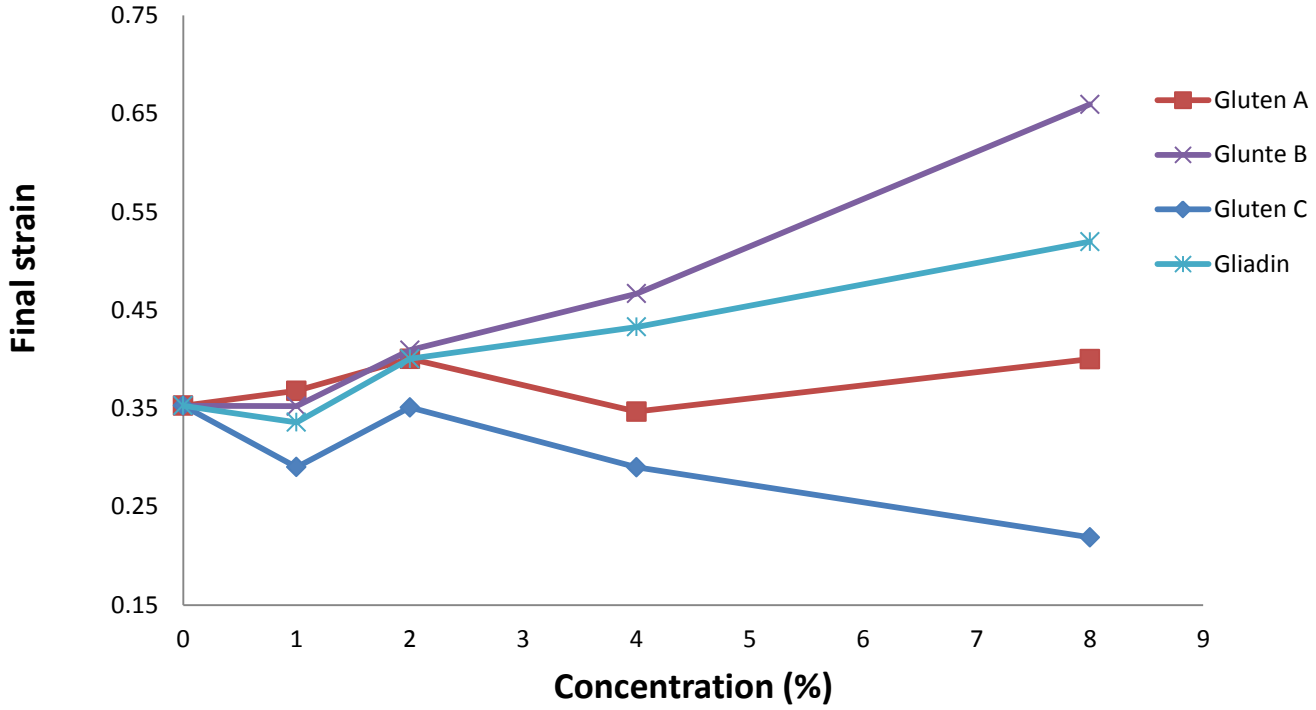

Figure 7. Comparison of final strain from compression-recovery test as a function of substitution from flour blends containing four commercial gluten products and five substitution levels.

CHAPTER VI

MODELING THE EFFECT OF COMMERCIAL GLUTEN PRODUCT SUBSTITUTION ON VISCOELASTIC PROPERTIES OF GLUTEN BY USING CREEP-RECOVERY AND GLUTEN COMPRESSION-RECOVERY TEST

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Abstract

The rheological properties of gluten play a major role in breadmaking process. The quality of the bread is highly correlated with the viscoelastic behavior of gluten. In this work, we investigated the effect of gluten substitution at different levels on viscoelastic properties using creep-recovery and compression-recovery tests. Experimental values were fitted into Burgers model to obtain regressed parameters in order to compare viscoelastic properties of gluten samples. The data were also investigated by principal component analysis (PCA) to reduce dimensions and recognize relationship patterns through their explained variance. Rheological properties of gluten was affected by commercial gluten substitutions depending on their acidity, prolamin-gliadins profile, gluten secondary structure, gluten strength, gluten deformability, and percent level substitution of gluten. The 6% substitution of gluten GB, increased gluten deformation specifically in viscoelastic region indicating by coefficients from creep-recovery test (Jc1, instantaneous elastic deformation, at 6% substitution increased by 64%; and Jr1, retarded elastic deformation, at 6% substitution increased by 53.5%). We speculated that gluten products with more acidity increased gluten deformation by increasing repulsive force in the gluten structure. On the other hand, the 6% substitution of gluten GC increased gluten resistance to flow (η_0) up to 25%. The modeling recovery phase of compression-recovery test (large deformation, 8N) allowed us to detect the changes in gluten viscoelastic properties better than compression-recovery test (small deformation, 100Pa). The compression-recovery test revealed that the substitution of GB and gliadin decreased Ɛ0 (gluten strength) up to 300% and 200%, respectively. The 6% substitution of GB also changed Ɛ1 (retarded strain) by decreasing the value up to 50%,

and Ct1 (retardation time) by increasing the value up to 63.5%. The principal component analysis showed that we can differentiate gluten samples in terms of their strength and deformability after treatments. Regarding percent of gluten substitution, the deformability of gluten was higher after substituting commercial gluten GB and gliadin at higher level (6%).

Keywords: Creep-recovery test, compression-recovery test, gluten rheology, principal component analysis (PCA).

1. Introduction

Wheat flour is the basic ingredient in bakery products and has a unique attributes among other cereal types due to the viscoelastic properties of gluten. When flour is hydrated during mixing, gluten network is formed and provide viscoelastic properties to dough characteristics. Therefore, gluten quality plays a major role in breadmaking process. Gluten structure is comprised of polymeric glutenin and monomeric gliadin (Wieser, 2007) in a still elusive specific three dimensional arrangement. Glutenin mainly contributes to elasticity due to intermolecular disulfide bonds in its structure. High molecular weight-glutenin subunits (HMW-GS) are known to postulate be the backbone of gluten structure, while low molecular weight glutenin subunits (LMW-GS) are branches of HMW-GS. Gliadin acts as a plasticizer and provides viscosity to the gluten system. Protein quantity was positively correlated to bread volume as reported by many researchers (Marchetti et al., 2012). Normally, wheat flour comprises 10-13% gluten. However, protein quantity alone is not sufficient to provide an understanding in bread properties (Barak et al., 2013). Therefore, both quality and quantity of gluten are the important criteria in controlling the quality of breadmaking product.

Many studies have investigated the rheological properties of gluten by using a creep-recovery test as described in Abang Zaidel et al. (2008). Creep-recovery test measures viscoelasticity of material by applying a constant shear stress over time (Abang Zaidel et al., 2008). The deformation of gluten due to shear stress is measured in term of compliance and vary based on its rheological properties (Dobraszczyk and Morgenstern, 2003). Many researchers have attempted to understand the rheological behavior of various food types by modeling experimental data from creep-recovery test using Burgers

model. The moduli from Burgers model which are the combination of Maxwell and Kelvin-Voigt models have been used in different food systems. Examples include studies on the effect of high and low molecular weight-glutenin subunit in wheat kernel viscoelasticity (Hernández-Estrada et al., 2012); relationship among baking quality, glutenin subunits and modeled moduli (Figueroa et al., 2013); DATEM, ascorbic acid, urea and DTT on gluten (Chompoorat et al., 2013); water soluble pentosan and ionic strength in gluten (Ma et al., 2012); creep time, recovery time and shear stress in dough (Van Bockstaele et al., 2011); high pressure homogenization on tomato juice (Augusto et al., 2013); gel, emulsions, and hydrocolloid contents on mayonnaise (Dolz et al., 2008); and resistant starch on biscuit (Laguna et al., 2013).

Another highly effective rheological test was a compression-recovery test which was first described by Chapman et al. (2012). It is a rapid bi-axial compression test that can measure elastic behavior of gluten in terms of recovery degree (Chapman et al., 2012). This test was used to distinguish gluten quality from different cultivars and wheat classes from the U.S. The authors reported that gluten strength from large deformation tensile test had a correlation with degree of recovery from compression-recovery test (Chapman et al., 2012) and offered an improved alternative to the study of gluten and handling the sample

In this work, we discussed the effect of the substitution of commercial gluten products in flour on rheological properties of gluten. We modeled experimental data in order to show the effect on gluten behavior from the molecular contributions of its constituents. The objective of this study was to investigate an effect of gluten products substitution on rheological properties of gluten.

2. Materials and Methods

2.1 Flour preparation

Four base commercial flours named as W, X, Y, and Z with various protein contents were used in this study. These flour samples were stored at -20°C overnight upon arrival followed by 4°C storage. Base flour W, X, Y, and Z consisted of 9.1, 10.8, 11.8, and 13.1% protein content (14% moisture basis), respectively. Combinations of the base flours were made at a fix protein content of approximately 11.0% to obtain blend flour (referred as flours from here on) as follows: flour 1 (F1) (3.04 kg X+ 6.96 kg Z), flour 2 (F2) (3.17 kg X+ 6.83 kg Y), flour 3 (F3) (1.16 kg W+ 8.84 kg Y), flour 4 (F4) (5.20 kg W+ 1.80 kg X+ 3.00 kg Y), flour 5 (F5) (3.00 kg W+ 3.90 kg Z+ 3.10 kg Y), and flour 6 (F6) (10 kg X). Flours were homogenized using an Olsa V-20 mixer (Olsa S.p.A., Milano Italy) for 20 min and stored at -20°C overnight followed by 4°C storage. Flours were brought up to room temperature for 24 h prior to each experiment.

2.2 Gluten preparation

Four different commercial dried glutens (GA, GB, GC, and GD or gliadin) were purchased in the market and substituted flour to make 14 and 17% protein content (14% moisture basis) flour samples. Control flours did not contain commercial gluten substitution and are identified as 11% protein content. In summary, the four commercial gluten products represented 3 and 6% substitution into each flour and for simplicity were labeled as 3.GA, 6.GA, 3.GB, 6.GB, 3.GC, 6.GC, 3.GD, 6.GD. Substitution were prepared in 30 g batches and thoroughly mixed to ensured homogeneity prior to testing.

Wet gluten was extracted based on the method 38-12.02 (AACC 2000). In brief, a Glutomatic system (Perten Instrument, Sweden) was used for dough mixing (20 sec)

and wash(5 min) 10 g flour samples with 2% NaCl solution through an 88 μm polyester screen. The remaining residue within the glutomatic chamber was the wet gluten that was used throughout the study.

2.3 Evaluating pH of gluten

Hydrogen-Ion Activity (pH) of extracted wet gluten was measured by using the electrometric method based on Approved Method 02-52.01 (AACC 2000) . In brief, the dry gluten was suspended in distilled water using magnetic stirrer for 15 min. The solution was allowed to settle for 30 min and the supernatant used for measurement using an Accumet Basic pH meter (Fisher Scientific., Waltham, MA). pH of gluten product A, C, and gliadin was also adjusted to 4 in order to show characteristics of gluten. pH of gluten B was adjusted to 6. 1 N NaOH was used to increase the pH and 6 N HCl was used to decrease the pH. This experiment was conducted to confirm that pH have a profound effect on in gluten characteristic.

2.4 Soluble prolamin protein profile by reverse phase-high performance liquid chromatography (RP-HPLC)

RP-HPLC was used to compare the profile of soluble prolamin proteins extracted from flour sample and gluten products based on surface hydrophobicity. A detailed description of the procedure is reported in the literature (Lookhart et al., 1987). In brief, prolamin gliadins were extracted from the samples using 50% ethanol and loaded into a RP-HPLC. A Waters HPLC system was equipped with a 5060 microprocessor-controlled pump (Varian Associates, CA), a 710A autosampler (Waters Associates, MA), a 970 variable wavelength detector (Tracor Instrument, TX), and a SynChropak RP-P 6.5 μm

particle column (SynChrom, IN). Gliadins were eluted at 1 ml/min, 45°C using a linear gradient program with acetonitrile/0.1% TFA and water/0.1% TFA solvents.

2.5 Fourier transform infrared (FTIR) spectroscopy

FTIR was used to study changes in gluten secondary structures. Wet gluten was extracted as described earlier and freeze-dried (VirTis GPFD 24DX48, The Virtis Company, Gardiner, NY) in order to maintain its structure. Freeze dried samples were stored at -4°C in microfuge tubes tightly closed and inside a polyethylene bag to prevent r moisture changes. Samples of 47% hydrated gluten were prepared prior to analysis by adding water into 100 mg freeze-dried gluten and mixed with a spatula. Fourier self-deconvoluted spectra were obtained using iS50 FTIR spectrophotometer (LabX, ON, Canada). The instrument was set to acquire data between 1725 cm^{-1} to 1580 cm^{-1} region using 1.3 EF and 30 bandwidths.

2.6 Viscoelastic properties of gluten

2.6.1 Creep and recovery test

Creep and recovery test was used to investigate the rheological properties of gluten after shear stress. Prior to measurement, freshly extracted gluten was rolled into a spherical shape and relaxed by a dead load of a 2.5 kg plate with 2.5 mm spacing and at room temperature for one hour. An AR1000 rheometer (TA Instrument, DE) was used to measure the deformation response of gluten samples. The rheometer applied a constant shear stress of 100 Pa for 100s in the creep phase and zero shear stress during recovery phase for 100s. The response of gluten was recorded in terms of compliance which represented deformation. Compliance was shown by J with unit Pa^{-1} and mathematically represented strain over initial stress.

2.6.2 Compression and recovery test

Compression and recovery test was performed to study the elastic recovery of gluten after compression. Sample was prepared by forming a gluten cylinder of 4.5 inches round dimension by placing a specially designed acrylic cylinder on top of the gluten mass in the centrifuge cassette of the Glutomatic Centrifuge 2015 set up which was modified to close the bottom sieve (Perten Instruments AB, Huddinge, Sweden). Gluten sample was centrifuged at 6000±5 rpm for 1 min. The obtained sample was placed at the center of the loading plate of the Gluten CORE Analyzer (Perten Instruments AB, Huddinge, Sweden) and analyzed by applying a 5 s compression phase (8 N compression) and a 55 s recovery phase. The elastic recoverability (reported as recovery index RI) of gluten was recorded throughout the experiment by measuring gluten height during both phases at 1 min interval. The recovery strain was calculated according to equation 1.

Gluten elastic recovery reported ad recovery index (RI):

RI = ((height at final recovery – height after compression)

/(initial height before compression - height after compression))*100 (Eq. 1)

2.6.3 Modeling of viscoelastic properties of gluten

The modeling of gluten rheological properties was based on Burgers model (Steffe, 1996). Burgers model comprised by Maxwell and Kelvin-Voigt models in which both models utilized springs and dashpots as a representation of material behavior. Spring represented a Hookean Solid which is the elastic component of the material (Steffe, 1996). Dashpot represented Newtonian Liquid which is the plastic flow of the material. The difference between Maxwell and Kelvin-Voigt model was the orientation of these mechanical analogs in which the former is series and the latter is in parallel orientation. It

has been reported that the combination of Maxwell and Kelvin-Voigt (i.e., Burgers models) had a much rigorous ability to describe biological systems such as gluten behavior during stress and relaxation (Steffe, 1996). Equation 1 and Equation 2 were based on Burgers model and used to describe creep-recovery behaviors where Jc and Jr = compliance during creep and recovery, respectively (Pa^{-1}), Jo = instantaneous elastic deformation (Pa^{-1}), J1 = retarded elastic deformation (Pa^{-1}), t = time (s), t1 = retarded time (s), and η_0 = pure viscosity (Pa·s). The subscripted c and r stand for creep and recovery, respectively. Equation 3 was also based on Burgers model and used to describe the recovery of gluten after the compression test. Unlike creep-recovery equations, the modeling of compression-recovery was based on the recovery part of the curve with ε = strain, εo = instantaneous strain indicating gluten strength, $\varepsilon 1$ = retarded strain, and Ct1 = retardation time (s) for the recovery phase.

The recovery strain was calculated from height measurements of gluten throughout the test. The initial gluten height before compression and height after recovery were recorded. The gluten height during compression was discarded. The time in recovery phase was reset to begin at zero. The gluten height was converted into strain as shown in Equation 4.

Equation 1 - For creep phase from shear stress:

$$Jc(t) = Jco + Jc1(1 - \exp(-t/t1)) + t/\eta_0$$

Equation 2 - For recovery phase from shear stress:

$$Jr(t) = Jro + Jr1(1 - \exp(-t/tr1))$$

Equation 3 - For recovery phase from compression:

$$\varepsilon(t) = \varepsilon o + \varepsilon 1(1 - \exp(-t/Ct1))$$

Equation 4 – For conversion of recovery strain:

Recovery strain (t):

= (Height at time (t) – Height at recovery phase at time zero)/initial height before compression (Eq. 4)

2.7 Statistical analysis

The experimental design in this study was a split plot design. Flour was the whole unit factor. Three substitution levels (0, 3, 6%), commercial gluten products (Gluten A, B, and C), and gliadin were the split unit factors. Four replicates were performed in this study. Analysis of variance (ANOVA) procedures were used assuming a model in a completely randomized design using SAS program (Version 9.1 SAS Institute Inc., Cary, NC). For principal component analysis (PCA), Canoco for Windows 4.5 software (Centre for Biometry, Wageningen, The Netherlands) will be used to show correlation of each parameter (Braak and Šmilauer, 2002; Legendre and Legendre, 1998).

3. Results and Discussion

3.1 Characteristics of gluten from base flour

RP-HPLC profiles of prolamin fraction soluble in 70% ethanol from the flour F1 and gluten product GA was shown in Figure 1. The prolamin profile was separated based on surface hydrophobicity. The peaks eluting earlier in the chromatogram indicated prolamin gliadins with higher hydrophilicity compared to slower peaks, eluting later in the chromatogram, representing prolamin gliadins with higher hydrophobicity. It should be noted that some low molecular weight glutenin subunits (LMW-GS) could have co-extracted along with gliadins due their similar molecular weight range (28-55kDa). Dough system contains both soluble (monomeric) and insoluble (polymeric) prolamins

and they account for 40-50% and 35 to 45% of the total flour protein, respectively (Cauvain, 2003). The 50% alcohol soluble prolamins are present in higher percentage and contribute to the gluten viscoelasticity by acting as plasticizers since they cannot form a disulfide bonds and crosslink to form the large backbone polymer. Figure 2 showed the prolamin gliadins pattern of gluten product A and flour F1. The patterns for other gluten products were reported in Appendix I. RP-HPLC profile of prolamin gliadins of flour F1 had more hydrophilicity with higher absorbance than gluten product GA. The hydrophobic gliadin of flour F1 also appeared to be higher than gluten product GA. The hydophilicity had a negative charge end, while the hydrophobicity had a positive charge end. Thus, the results indicated that prolamin gliadin of flour F1 had a higher in a negative and positive charge end than prolamin gliadin of gluten product GA.

3.2 Characteristics of commercial gluten products

The pH of commercial gluten products and flour sample are shown in Table 1. Gluten products had a pH range from 4.2 to 5.5. Gluten C (GC) had the highest pH of 5.5 and gluten B (GB) the lowest 4.2 compared to the rest of gluten products. The pH in gluten product GA and GD (Gliadin) was 5.2. The pH of the flour was pH 5.9. After gluten products A (pH 5.2), C (pH 5.5) and gliadin (pH 5.2) were adjusted to pH 4, gluten A and C had the same characteristic (Fig. 7). Both of gluten A and C were not able to form into a gluten ball. Gliadin was separated into two layers after its pH was adjusted from 5.2 to 4. However, after the pH of gluten B was altered from 4.2 to 6, gluten was able to be formed into a cohesive mass. Thus, it was confirmed that pH of gluten affected gluten characteristics. The effect of pH on the electrostatic behavior of protein had shown to impact gluten behavior. During fermentation of sourdough (high acidity condition),

several properties of dough changed, for example, the viscosity, resistance to deformation, dough stability, and mixing time decreased, while extensibility and degree of softening increased (Clarke et al., 2004; Gao et al., 1992; Tsen, 1965).

FTIR was used to evaluate possible changes in the protein secondary structures of gluten samples based on the second derivative spectra (Fig. 3). The peak assignments were based on previous work of Georget and Belton (2006) reported in arbitrary unit (Arb). Major protein secondary structures were clearly identifiable using FTIR such as turns or β-hairpins (1699 cm^{-1}) and β-sheets (1684 cm^{-1}) (Georget and Belton, 2006). Additionally, the spectra also contained weaker peaks such as β-turns (1665 cm^{-1}), random coils and helices (1650 cm^{-1}), intramolecular β-sheets (1630 cm^{-1}), intermolecular β sheets and extended chains (1613 cm^{-1}), and glutamine side chain (1598 cm^{-1}). The second derivative spectra obtained from FTIR showed that all gluten products had similar protein secondary structures (Fig. 2). Secondary structure differences of Gliadin (GD) sample compared to GA, GB, and GC samples were in the β-hairpins and β-sheets; GD had -0.01 Arb and -0.014 Arb, while other samples had -0.008 Arb and -0.012 Arb at 1699 cm^{-1} and 1684 cm^{-1}, respectively. Thus, both β-hairpins and β-sheets might be different in gliadin when compared with other gluten samples. For quantitative comparison, the shift of absorbance peak in β-sheet region of each gluten product and gliadin were shown in Figure 3. Gliadin (GD) and GB had similar frequency in both strongly and weakly hydrogen bonded β-sheets. The GC had the highest frequency of weakly hydrogen bonded β-sheets. Thus, these secondary structure data provided evidence that various types of gluten products and gliadin had differences in secondary structure especially in β-sheets region. These results indicated that a high in viscous flow

properties of gliadin should be due to strongly and weakly hydrogen bonded β-sheets in the structure.

The viscoelastic properties of commercial gluten products by itself (before mixing with flour) were evaluated. This preliminary test was performed to show the differences in rheological properties of commercial gluten products. The data from creep-recovery test was shown in Table 2. In both experimental and modeling parameters, gluten product GA and GC had similar rheological properties ($p<0.05$) except for pure viscosity (η_0) (Table 2). Overall, gluten product GB exhibited highest values in most parameters (MaxS, FinalS, J-Jr, Jc0, Jc1, t1, Jr1, and tr1) indicating that it had the highest deformability, flowability, instantaneous elastic compliance, retarded elastic compliance, retardation time. However, pure viscosity (η_0) and retarded compliance during recovery (Jr0) of GB were not significantly difference from gluten product GD (Gliadin). Although gluten should have lower deformability than gliadin, pH of gluten should be also taken into consideration. The fact that GB had high in acidity (pH = 4.2, Table 1) among the other gluten products, thus, the high in net positive charge of gluten protein affected gluten deformability. At the molecular level, an increase in acidity could enhance electrostatic repulsion forces within gluten (Galal et al., 1978; Tsen, 1965). As amino acids are protonated at carboxyl and amine groups, the overall increase in positively charged sites becomes repulsive near their neighbor molecules. The electrostatic repulsion forces results in a decrease in viscosity and resistance to deformation (Clarke et al., 2004). Furthermore, gluten was reported to decrease β-sheet and increase α-helixes, β-turn, and extended structures in mildly acidic solution (Pézolet et al., 1992). Bonds in this GB gluten structure were broken by shearing showing a high

value in deformation, retardation time, and low in resistance to deformation. We speculate that polymeric gluten GB structure was altered by pH affecting the H-bonding that holds the structures resulting in a high in α-helixes, β-turn, and extended structure.

3.3 Viscoelastic behavior of gluten

Creep-recovery test was used to study the effect of gluten substitution at 3 and 6% levels on the viscoelastic properties of gluten. Typical creep-recovery curves of gluten from flour substituted with commercial gluten products (GA, GB, GC, and GD) are shown in Figure 4. For simplicity graph of F1 is presented here and the rest of the graphs for other samples are reported in Appendix I. During a constant shear stress, gluten flowed (molecules aligned to the stress as they were displaced) and partially recovered in a non-linear deformation behavior as a function of time. Some parts of gluten structure stored energy which resulted in permanent deformation less than the total deformation due to recoil recovery (Steffe, 1996). Substitution of gluten GA and GC increased the elastic component of gluten system by increasing the rigidity as shown by a reduction in maximum creep compliance (i.e., the compliance value at the end of creep phase) (Fig. 4). In contrast, the substitution of commercial gluten GB and GD (Fig. 4) significantly increased maximum creep compliance which indicated that the gluten system responded with increased viscous flow which for breadmaking purposes it could be described as becoming more extensible or weaker. The levels of gluten substitution also affected the compliance curves of the gluten system, except for gluten GC which showed a similar effect from 3% and 6% (Fig. 5). The quantitative data from Burgers model was discussed in this chapter elsewhere.

Gluten elastic recovery was studied with a compression and recovery test and typical curves are shown in Figure 5. The strain was measured at the beginning of recovery phase after the compression force was released. During the test the initial gluten height was taken at the lowest point during compression and the height as a function of time was measured during the recovery phase, therefore, a high value of corresponded to gluten with high recoverability. A high value of the calculated strain in this test means the gluten height was high. The substitution of commercial gluten GA and GC at 3% and 6% showed that the strain increased during recovery phase (Fig. 5). Therefore, gluten GA and GC helped increase the elasticity of gluten system. In contrast, the opposite trend was observed for the addition of gluten GB and GD which made gluten more deformable. The result confirmed the effect of commercial gluten addition which was similar to that obtained with creep-recovery experiments from Figure 4. Creep-recovery test applies small deformation to gluten (100Pa), while compression-recovery test is performed by applying a large deformation (8 N). The consistency of the results from both tests suggested that the substitutions of gluten altered gluten structure which can be detected by its viscoelastic properties applying small or large deformations.

The experimental and modeled parameters from creep-recovery and compression-recovery test were reported in Table 3 and 4. From experimental data of creep-recovery test, maximum strain (MaxS), final strain (FinalS), and delta compliance (J-Jr) of gluten GB substitution at 6% were statistically higher than the control. An increased in both MaxS and FinalS indicated that the gluten system became more deformable and an increase in J-Jr indicated that flowability of gluten was higher with 6% substitution with GB. Concentration of gluten had shown to be an important factor to quality of

breadmaking (Khatkar et al., 2002; Marchetti et al., 2012). It was observed that an increasing concentration of gliadin (5% and 10% addition) significantly reduced the strength of dough indicated by the peak dough height tested by using 4g micro doughlab resulted in a 42.0% and 56.0% decreasing in dough development time and dough stability, respectively (Khatkar et al., 2013). However, the change in recoverability (RCY) was not significant in this study. From the regressed coefficients (Burgers model), GB at 6% Jc1 and Jr1 were significantly higher than the control which means that adding 6% of gluten GB increased the delayed compliance in both creep (64.0%) and recovery (53.5%) phases (Table 3). An increase in Jc1 and Jr1 of 6% GB was consistent with the inherent nature of commercial gluten product GB which was more deformable than other commercial gluten products (Table 2). This observation indicates that the type of structures and the bonds formed with the substitution of GC resulted in an increase pure viscosity response. At the molecular level of gluten, viscosity flow or extensibility is mainly contributed by gliadins which form intrachain disulfide bonds but do not form crosslinks with other gluten polymers (interchain links) that form the backbone thus remaining a monomeric unlinked gluten polymer (Marchetti et al., 2012; Wieser, 2007). The role of gliadins is referred as a plasticizer implying that they are not part of the large backbone gluten structures but fill the area in between branches and backbone. GC may form hydrophobic and hydrophilic interactions of gliadins forming a loosely tied gluten network, which might have caused the increase only in pure viscosity or resistance to flow. However, it seemed that addition commercial gluten products did not affect compliance values of instantaneous elastic and retardation time (Jc0, t1, Jr0, and tr1) during both creep and recovery phases. The lack of effect of substitution of gluten in

flour on the coefficients of Burger model is surprising; however, the creep-recovery test was performed by applying a small deformation (100Pa, for 100s during creep) to gluten. This could partially explain the lack of effect on viscoelastic properties on the flour sample in this study.

The parameters obtained from the compression-recovery experiments which apply higher deformation (8N, for 5s during compression) showed that elastic recoverability (RI) changed with the substitution of gluten GB and GD at 3 and 6% levels (Table 4). A decreased in elastic recoverability (RI) indicated that both gluten GB and GD reduced elasticity of the gluten system. However, this change in elasticity was not detectable in creep-recovery test (100Pa, for 100s during creep) in terms of calculated RCY. When we modeled the recovery phase of the compression-recovery experimental data, gluten GB affected instantaneous elastic strain (Ɛ0) (at 6% decreased by 300%), retarded elastic strain (Ɛ1) (at 6% decreased by 50%), and retardation time (Ct1) (at 6% increased by 63.5%) in both 3 and 6% levels. Moreover, 3% GD and 6% GD (Gliadin) significantly decreased the elasticity of gluten (Ɛ0) (at 6% decreased by 200%). S0 and S1 are associated with elastic and viscoelastic region, respectively, during recovery. It is well known that high molecular weight-glutenin subunits (HMW-GS) polymeric structure mainly contributed to gluten elasticity via interchain disulfide bonds (Shewry et al., 2002; Wieser, 2007). Thus, this result indicated that GB with pH 4.2 and gliadin mainly affected HMW-GS. We proposed that after introducing monomeric gliadin, which acts a plasticizer, it interacted largely of HMW-GS (decreased in Ɛ0) and less extent to LMW-GS by filling itself in the HMW-GS structure exhibiting in a high deformation in elastic portion of mechanical model. In addition, GB with pH 4.2 was higher acidity than

normal flour (pH 5.8-5.9). Gluten is an insoluble protein with a high in hydrophobicity, indicating a high in net positive charge of protein structure. Upon the addition of gluten GB with more positively charge, we speculated that the repulsive force was increased, resulting in a more open gluten polymer structure and less aggregation. Thus, the deformability of gluten increased after we introduced GB to the system. Furthermore, the result of secondary structure of GB and gliadin from FTIR showed that both of them had similar frequency in β-sheet region. This secondary structure of gluten GB and gliadin should have caused in the decreasing of Ɛ0. An increase in retardation time (Ct1) of 3% GB and 6% GB substitution suggested a longer time in delayed deformation in the gluten samples with GB after compression force was released (Steffe, 1996). Thus, the introducing gluten structure with gluten high in positive charge are not only increased the gluten deformation, but also making the gluten take a longer time to reach the equilibrium. In comparison between results from creep-recovery test and compression-recovery test of gluten, our results suggested that RI, Ɛ0, Ɛ1, and Ct1 obtained from compression-recovery test can be useful parameters when analyzing elasticity of gluten because creep-recovery test did not allow us to differentiate these properties (i.e., no change in RCY, Jc0, t1, Jr0, and tr1). In summary, the results from creep-recovery and compression-recovery tests showed the same trends in gluten substitution. At the molecular level of gluten, the results suggested that applying a large deformation is slightly more useful in detecting an alteration of gluten structure with the treatments in this study. This result suggested that gluten structure, which is known for its high complexity, required high stresses to deform structure in order to differentiate its

molecular structure. More studies are needed using higher stresses in the non-linear region of gluten and apply non-linear equations.

3.4 Relationship of viscoelastic properties of gluten

The relationship of viscoelastic properties of commercial gluten (GA, GB, GC, and GD) substitutions into flour F1 was shown in bi-plot graph of principal component analysis (PCA) (Fig. 6). The total explained variance of the first and second principal component was 81.7.6% (71.2% for PCA1 and 10.5% for PCA2). MaxS, FinalS, J-Jr, Jr10, and Jc1 were the major contributors to the variance of the first dimension or PC1 (96.8, 94.3, 94.3, 93.8 %, respectively), while RCY and t1 explained the highest variance in the second dimension or PC2 (60.0 and 59.6%, respectively). The biplot also allowed us to differentiate this sample set in terms of their resistance to flow (ηo, RI, Ɛ0, Ɛ1) versus deformability (FinalS, JJr, MaxS, Jc1, Jr10, Jc0, Jr0, Ct1) from the first dimension or PCA1 and retardation time (t1) during creep versus elasticity (RCY) from the second dimension or PCA2. The negative correlation between pure viscosity (η_0) and deformability had been observed in other study that focused on modeling of dough creep-recovery from 17 cultivars (Van Bockstaele et al., 2011). It is noteworthy that retardation time during creep (t1) and recoverability (RCY) was independent of resistance to flow and flowability. Because these parameters were either from the experimental data or the modeling of creep-recovery and compression-recovery test, it can be concluded that both tests could help differentiate this sample set in terms of their viscoelastic properties. Our result showed that the addition of commercial gluten GB and GD (Gliadin) made gluten more deformable especially with increasing percent levels (6%) (Quadrant 1 and 2). On

the other hand, the addition of commercial gluten GA and GC was closely related to the strength properties and was independent of addition levels (Quadrant 3 and 4).

4. Conclusions

The substitution of commercial gluten and gliadin significantly affected viscoelastic properties of gluten depending on their quality and quantity of gluten such as gluten acidity, prolamin gliadins profile, gluten secondary structure, gluten strength, gluten deformability, and percent level substitution of gluten. The coefficients from Burger model allowed us to study and quantitate the alteration structure of gluten at molecular level. Modeling of creep and recovery phase of gluten substitution showed that the substitution of GB induced deformation in viscoelastic region (Jc1; at 6% increased by 64% and Jr1; at 6% increased by 53.5%). In addition, the substitution of 6% GC also increased pure viscosity up to 25%. The modeling recovery phase of compression-recovery test helped us detecting the changes in all three elements of model which were Ɛ0, Ɛ1, and Ct1. Only the substitution of GB and gliadin decreased Ɛ0 (gluten strength) up to 300% and 200%, respectively. Moreover, only the substitution of GB altered coefficients from compression-recovery test which were Ɛ1 (at 6% decreased by 50%), and Ct1 (at 6% increased by 63.5%). The principal component analysis revealed that resistance to flow and deformability of gluten were the main contributors. In addition, the deformability of gluten was higher after substituting commercial gluten GB and gliadin at higher level (6%). In conclusion, our modeling of rheological behavior allowed us to differentiate the quality between gluten samples.

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Table 1. pH value of gluten product and flour samples

	Sample ID	pH
Gluten products	GA	5.2d
	GB	4.2e
	GC	5.5c
	GD[1]	5.2d
Flour samples	F1	5.9a
	F2	5.8b
	F3	5.8b
	F4	5.8b
	F5	5.8b
	F6	5.8b

[1]GD is enriched gliadin. Gluten product A, B, C, and gliadin flour sample with 11%

Table 2. Experimental and modeling parameters creep-recovery test of commercial gluten products

Gluten product	Experimental parameters				Modeling parameters						
	MaxS (%)	FinalS (%)	RCY (%)	Jc-Jr (Pa^{-1})	Jc0 (10^{-4} Pa^{-1})	Jc1 (10^{-4} Pa^{-1})	t1 (s)	η_0 (10^{5} Pa^{-1})	Jr0 (10^{-4} Pa^{-1})	Jr1 (10^{-4} Pa^{-1})	tr1 (s)
GA	21.7c	3.9c	82a	0.4c	10.0c	10.0c	8.3b	1.6a	10.0b	10.0c	18.0b
GB	146.2a	48.1a	67.2c	4.8a	30.0a	63.3a	10.4a	0.2c	33.3a	60.0a	21.1a
GC	24.1c	5.3c	78.1ab	0.5c	10.0c	10.0c	8.9b	1.4b	10.0b	10.0c	17.7b
GD[1]	81.9b	21.7b	73.6b	2.2b	20b	30b	9.2b	0.3c	30.0a	30.0b	18.7b

[1]GD is enriched gliadin. MaxS = maximum strain during creep, FinalS = final strain during recovery, RCY = elastic recoverability, Jc-Jr = delta compliance Jc0 = instantaneous compliance during creep, Jc1= retardation compliance during creep, t1= retardation time during creep, η_0 = pure viscosity, Jr0 = instantaneous compliance during creep, Jr1= retardation compliance during creep, tr1= retardation time during creep. Means with different letters are significantly different in each column, $p \leq 0.05$ (n=4).

Table 3. Experimental and modeling parameters of gluten system (Flour F1) after adding commercial gluten products at different levels from creep-recovery test

Treatments ID	Levels	Experimental parameters				Modeling parameters						
		MaxS (%)	FinalS (%)	RCY (%)	Jc-Jr (Pa^{-1})	Jc0 (10^{-4} Pa^{-1})	Jc1 (10^{-4} Pa^{-1})	t1 (s)	η0 (10^{5} Pa^{-1})	Jr0 (10^{-4} Pa^{-1})	Jr1 (10^{-4} Pa^{-1})	tr1 (s)
Control	0	45.9bcd	9.9bcd	78.4a	1bcd	17.5ab	20.0bc	8.4a	0.7bcd	20.0ab	20.0bc	16.5a
3GA	3	34.3d	7.1cd	79.6a	0.7cd	12.5b	12.5c	8.3a	1.0ab	12.5b	12.5c	16.8a
6GA	6	38.1cd	8.2bcd	78.6a	0.8bcd	12.5b	12.5c	8.5a	0.9abc	12.5b	12.5c	16.9a
3GB	3	69.1abc	17.1abc	76.1a	1.7abc	17.5ab	25abc	8.7a	0.5d	27.5a	25.0abc	17.2a
6GB	6	91.9a	23.6a	75.3a	2.4a	25.0a	37.5a	9.0a	0.5d	27.5a	35.0a	17.5a
3GC	3	27.6d	5.5d	80.1a	0.6d	10.0b	10.0c	8.3a	1.2a	10.0b	10.0c	17.3a
6GC	6	28.9d	6.7cd	76.8a	0.7cd	10.0b	10.0c	8.7a	1.1a	10.0b	10.0c	16.3a
3GD[1]	3	56.1bcd	13.3bcd	76.8a	1.3bcd	17.5ab	22.5abc	8.8a	0.6cd	20.0ab	22.5abc	17.2a
6GD	6	78.2ab	18.1ab	77.9a	1.8ab	22.5a	32.5ab	8.4a	0.5d	30.0a	32.5ab	16.8a

[1]GD is enriched gliadin. MaxS = maximum strain during creep, FinalS = final strain during recovery, RCY = elastic recoverability, Jc-Jr = delta compliance Jc0 = instantaneous compliance during creep, Jc1= retardation compliance during creep, t1= retardation time during creep, η_0 = pure viscosity, Jr0 = instantaneous compliance during creep, Jr1= retardation compliance during creep, tr1= retardation time during creep. Means with different letters are significantly different in each column, $p \leq 0.05$ (n=4).

Table 4. Experimental and modeling parameters from compression-recovery test of gluten system (Flour F1) from flour blends substituted with commercial gluten products at different levels

Treatments	Levels	Experimental parameter	Modeling parameters		
		RI	Ɛ0	Ɛ1	Ct1 (s)
Control	0	0.70a	0.09a	0.36ab	3.1bc
3GA	3	0.77a	0.11a	0.40a	2.2c
6GA	6	0.76a	0.10a	0.36ab	1.8c
3GB	3	0.49b	0.04b	0.29c	5.2b
6GB	6	0.31c	0.02b	0.20d	8.5a
3GC	3	0.77a	0.11a	0.38a	2.1c
6GC	6	0.80a	0.11a	0.35ab	2.0c
3GD[1]	3	0.56b	0.05b	0.35abc	4.0bc
6GD	6	0.48b	0.03b	0.32bc	4.2bc

[1]GD is enriched gliadin. RI=recovery index, Ɛ0= elastic strain, Ɛ1= viscoelastic strain, Ct1=retardation time. Means with different letters are significantly different in each column, $p \leq 0.05$ (n=4).

Table 5. Percent change of gluten (Flour F1) rheological properties at control (0% gluten substitution) and 6% gluten substitution.

Modeling parameters	GA (%)	GB (%)	GC (%)	GD[1] (%)
Creep-recovery test				
Jc0	-4.5	52.3	-17.3	39.5
Jc1	-17.3	64.0	-34.4	46.3
t1	-8.4	-4.7	-3.4	2.2
η_0	18.2	-125.0	25.0	-80.0
Jr0	1.5	53.5	-17.5	41.5
Jr1	-9.6	65.0	-31.3	46.2
tr1	-5.6	-0.6	4.5	4.5
Compression-recovery test				
S0	10.0	-350.0	10.0	-200.0
S1	0.0	-50.0	0.0	6.3
Ct1	-72.2	63.5	-55.0	26.2

Positive and negative values indicate percent increase and decrease, respectively.
[1]GD is enriched gliadin. Descriptions defined in Table 1-4.

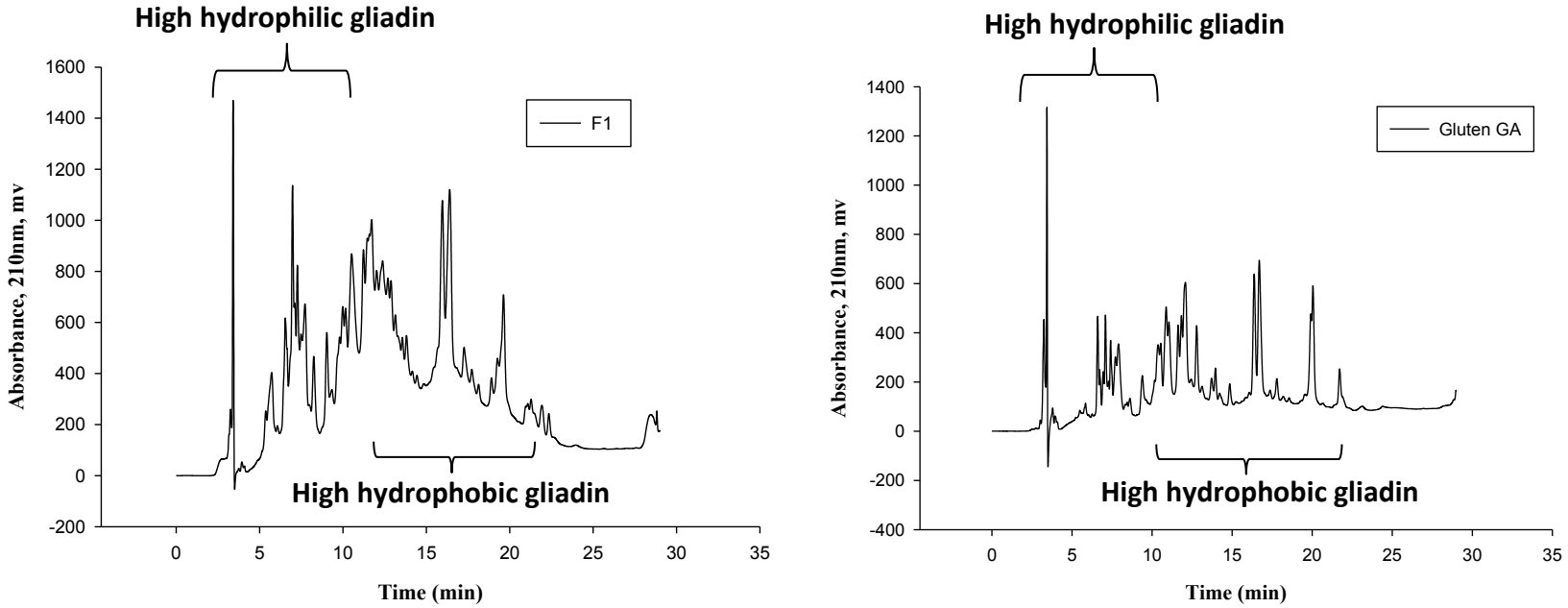


Figure 1. Reverse phase high-performance liquid chromatography profile of the prolamin fraction soluble in 50% ethanol of flour sample F1 (Left) and gluten product GA (Right).

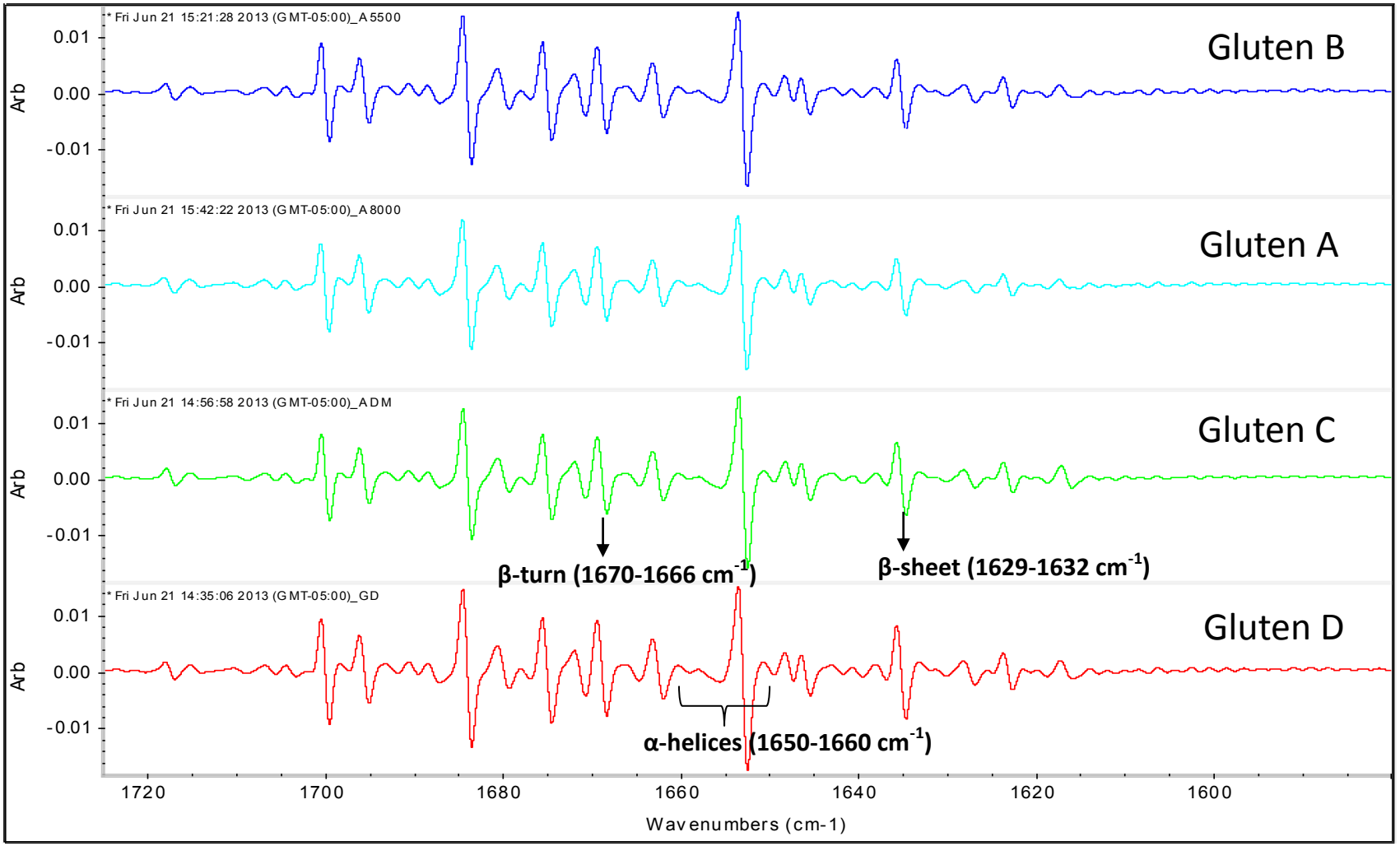


Figure 2. FTIR second-derivative spectra of commercial gluten products.

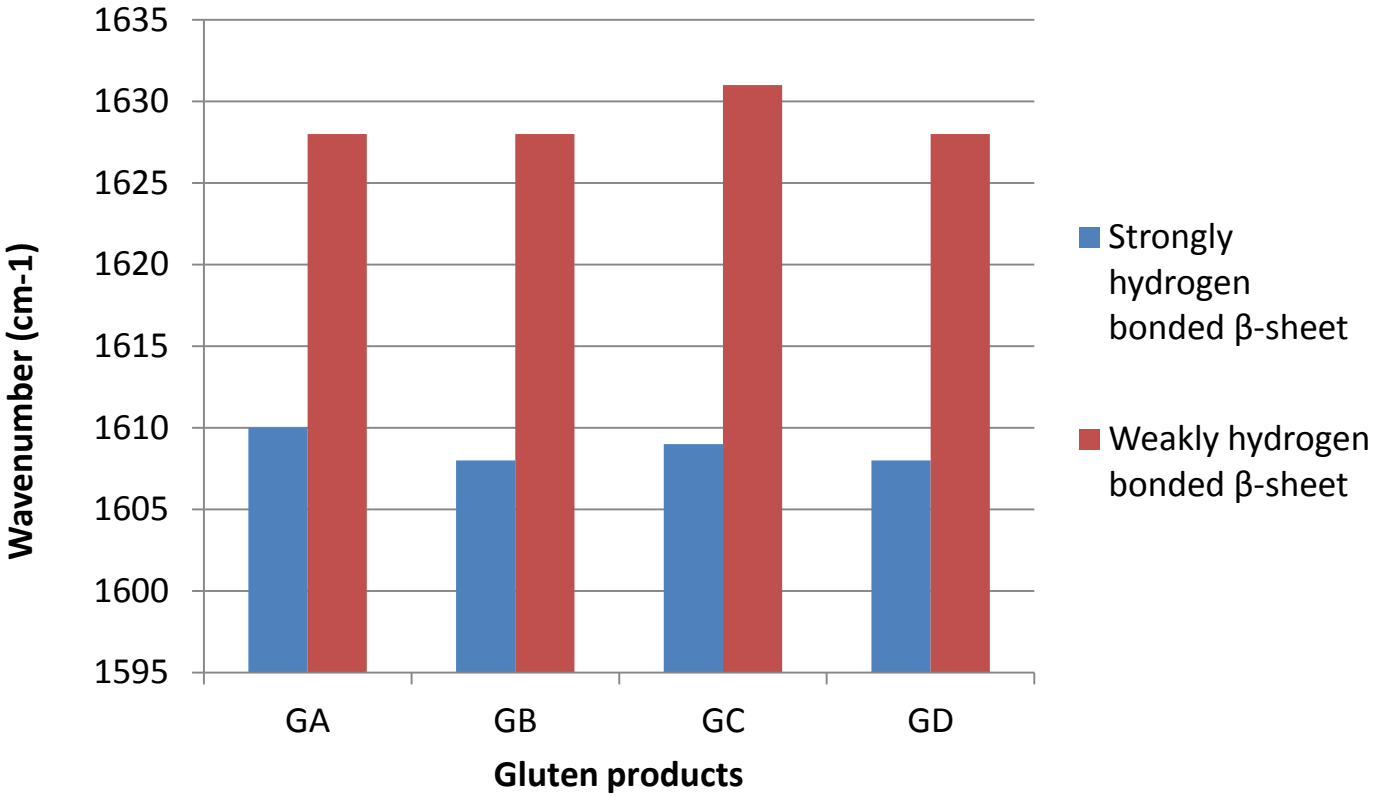


Figure 3. Band shifts in β-sheet region of gluten products.

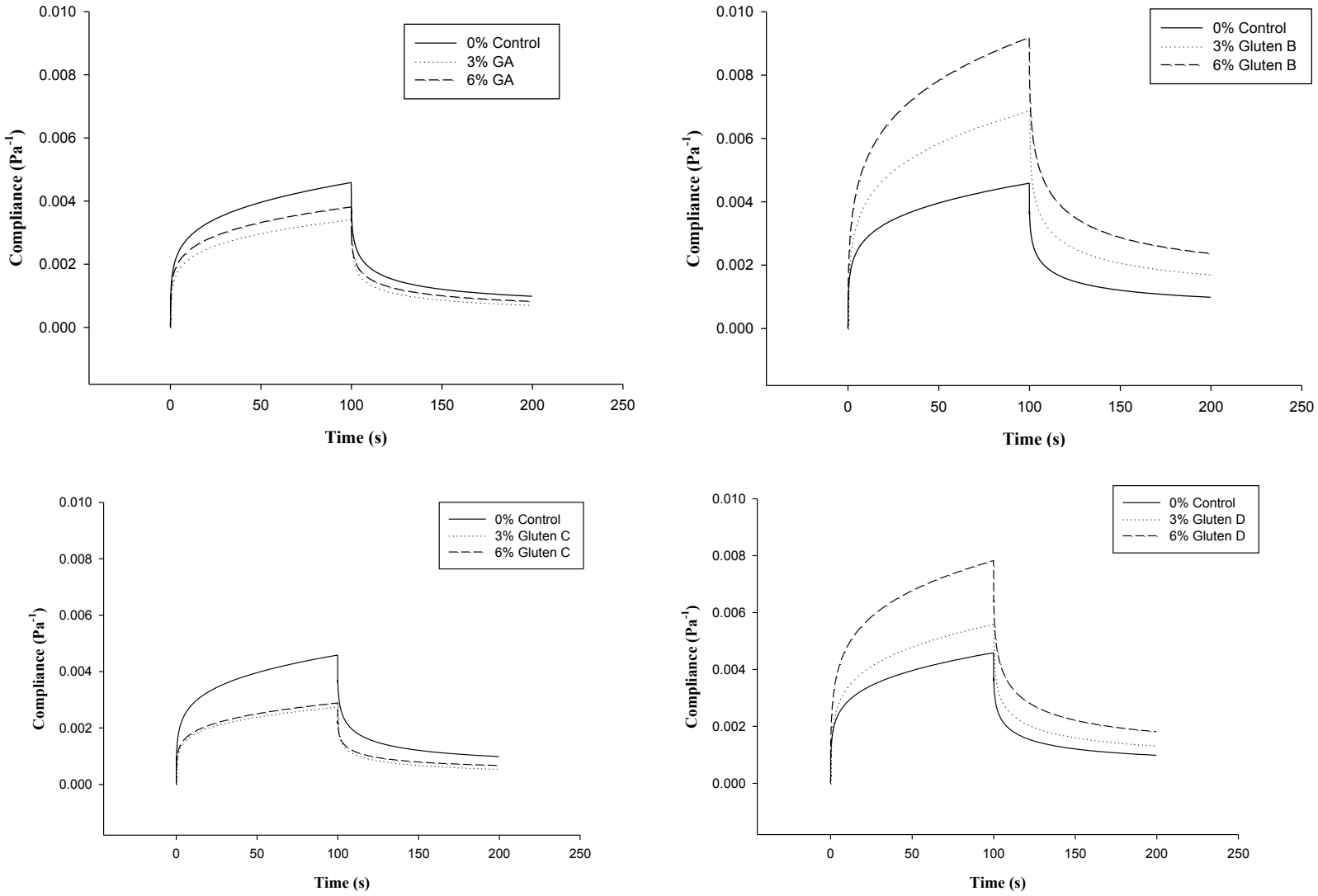


Figure 4. Typical creep-recovery compliance curves of gluten system (Flour F1) with treatments from creep-recovery test.

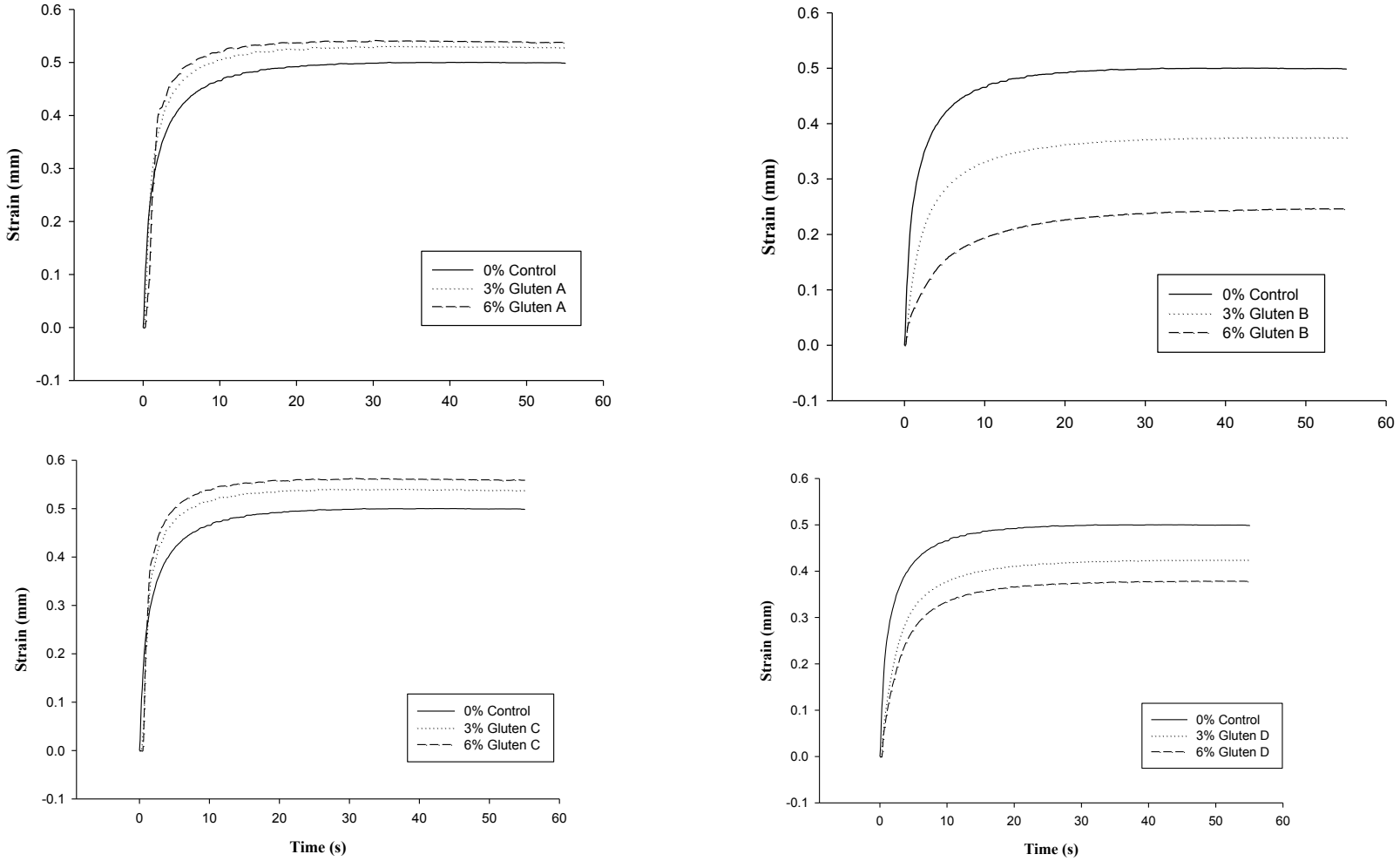


Figure 5. Typical strain curves of gluten system (Flour F1) and two substitution treatments during the recovery of compression-recovery test.

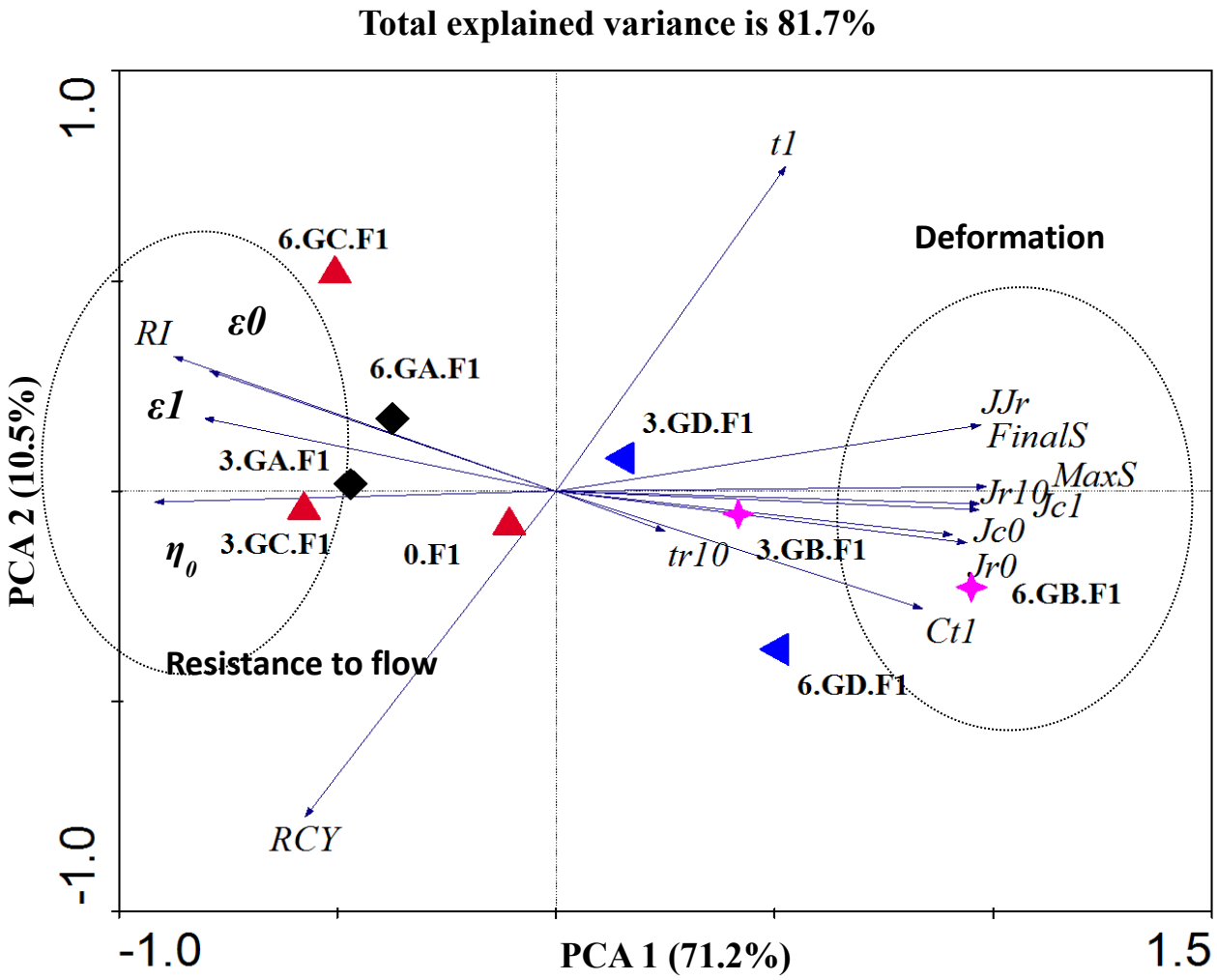
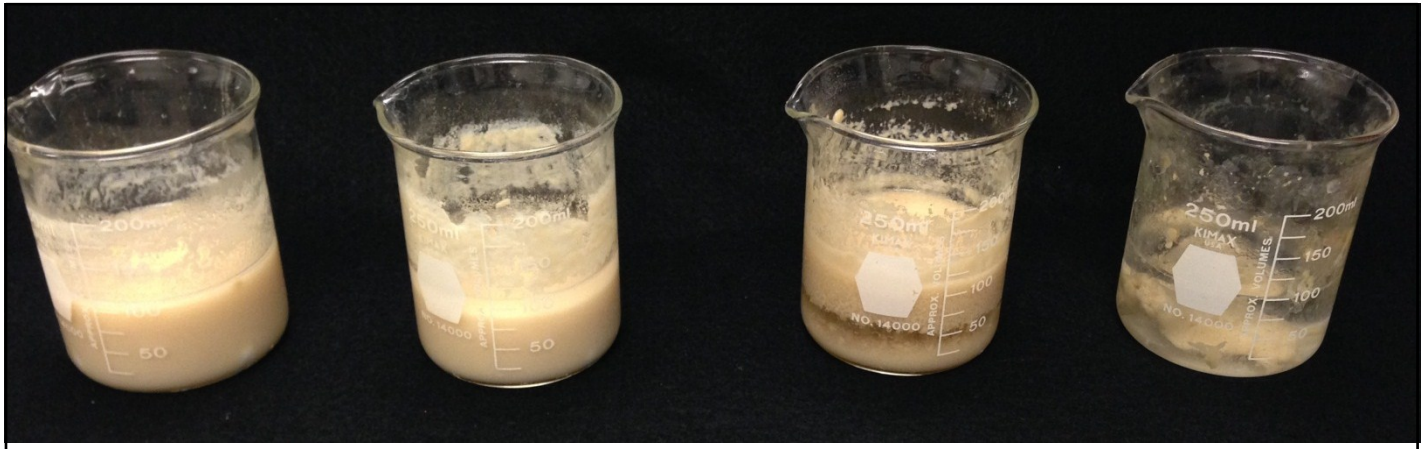


Figure 6. Principal component analysis of gluten containing different protein contents with 15 indicators of gluten quality (viscoelastic properties of gluten). Descriptions are defined in Table 1-4.



(Gluten A) pH 4	(Gluten C) pH 4	(Gliadin) pH 4	(Gluten B) pH 6

Figure 7. Physical state of each gluten product after adjusting the pH. Gluten A pH 5.2, adjusted to pH 4; Gluten B pH 4.2, adjusted to pH 6; Gluten C pH 5.5, adjusted to pH 4; and Gliadin pH 5.2, adjusted to pH 4.

CHAPTER VII

RELATIONSHIP OF GLUTEN AND DOUGH RHEOLOGICAL PROPERTIES AND BREAD CHARACTERISTICS

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Abstract

The correlations of viscoelastic properties of gluten and dough, flour protein, and loaf volume from five breeder sample sets of hard red winter wheat flour (201 samples) grown during 2008 and 2011 were evaluated in this study. The quality of gluten and dough were investigated by using creep-recovery (gluten viscoelasticity), mixing (Mixograph), and breadmaking properties plus flour protein content. The experimental data from creep-recovery test of gluten were fitted into Burgers model to improve the quantitative comparison of its viscoelastic properties. Principal component analysis (PCA) was used to describe the patterns such as distribution and relationship of samples and variables as well as explained variance. The parameters from creep-recovery test and Burgers model (strength and deformability variables) were the main contributors to the variance in the first dimension or principal component 1. Gluten elastic recoverability is an important portion of the variance in the second dimension with a very distant third flour protein. Overall, these two parameters were independent from strength and deformability and the specific relationship among recoverability and flour protein content changed every crop year. Throughout the crop years tested, dough mixing time was positively correlated with viscous parameters calculated from the Burgers model. PCA also helped differentiate samples as either associated to groups or individually. Pearson correlation confirmed that loaf volume, protein content, and dough water absorption were positively correlated. This study showed that the variation in gluten viscoelastic properties adds value to the breeding programs by revealing patterns of samples based on elastic and viscous variability as well as their relation to more traditional tests of mixing properties.

Keywords: Viscoelastic properties, gluten, dough, Burgers model, wheat cultivars

1. Introduction

Wheat is one of the most common crops used as a staple food around the world. Flour from wheat has a unique ability to produce bakery products because of gluten. Three dimensional gluten networks in hydrated dough are formed during dough mixing process. During fermentation, gluten networks expand and retain gas produced by yeast. Gluten networks provide elasticity and viscosity to dough and bakery products. Gluten comprises polymeric glutenin (elasticity) and monomeric gliadin (viscosity) and their balance is important in the quality of all flour based products. The variation of both polymeric glutenin and monomeric gliadin is influenced by environmental factors (Blumenthal et al., 1993) and is attributed in turn with the largest variation of the quality of breadmaking products. Therefore, there is a need to develop an efficient method that will help differentiate wheat quality before use as well as an improvement in the extraction of information from present day analytical equipment. Two studies of viscoelasticity of polymeric gluten protein (i.e., gluten quality) in this dissertation (see the study of additives and the study of correlations between gluten, dough, and bread) had described that regressed parameters from modeling viscoelastic properties of gluten can explain the largest variation of dough and bread properties from traditional testing when principal component analysis (PCA) is applied. Thus, this study was designed to use mathematical modeling of gluten viscoelastic properties using a relatively large number of breeder lines and cultivars from different crop years to analyze trends and relationship to observed values from different quality indicators in dough and bread.

Breadmaking quality parameters are related with several viscoelastic properties of gluten and dough. It has been shown that the quality (elasticity and tenacity) and quantity

of gluten protein tested by large and small deformation tests are important because they can help to understand the gluten network (Tronsmo et al., 2003b). Other important factors such as total glutenins, total ω-gliadin, and ratio of dough resistance to extensibility are potential candidates that differentiate wheat cultivars (Kurtanjek et al., 2008). Loaf volume had been shown to correlate with gluten and dough quality (viscoelastic properties) such as elastic modulus, viscous modulus, and tan δ (ratio of viscous to elastic modulus) (Tronsmo et al., 2003a), dough maximum recovery strain with 54% water absorption (Wang and Sun, 2002), and quantity of gluten such as protein content (Wieser and Kieffer, 2001). Quality of wheat cultivars were also affected by environmental conditions. Soft and hard wheat cultivars grown in a non-optimal climate had a reduced baking quality and also affected flour composition and rheological properties (Mikhaylenko et al., 2000). Seeding at moderately warm temperature could result in a variation of the protein synthesis rate for glutenin and gliadin (Blumenthal et al., 1993). As mention earlier, this variation can significantly affect the quality of bread products. High molecular weight-glutenin subunits (HMW-GS) had been shown to influence dough rheological properties in term of strength (W) and tenacity/extensibility (P/L) obtained with an Alveograph (Peña et al., 2005). Mechanical modeling of gluten viscoelastic properties using Burgers model could allow us to speculate on the type of polymeric gluten structure formed based on regressed parameters.

The objective of this study was to analyze the relationship among gluten and dough rheological properties and loaf volume of flour from breeder lines and check samples of hard red winter wheat. Our work covered a relatively extensive number of samples (five sets, 201 samples) from four crop years (2008-2011). Additionally,

mathematical modeling of creep-recovery experimental data using a viscoelastic based model (Burgers model) was performed and the parameters used for correlation analysis. The results will help differentiate a wide range of wheat quality from breeder samples based on viscoelastic properties of gluten and understand the structure of polymeric gluten protein at molecular level.

2. Materials and Methods

2.1 Flour samples

A total of 201 flours from breeder lines and check samples of hard red winter wheat were obtained from the Oklahoma State University breeding program during the crop years 2008 to 2011 and from breeding programs of the hard red winter region from 2010. The protein content for the OSU breeding program samples were determined by using near infrared reflectance (FOSS NIR System Inc., Laurel, MD).

2.2 Creep-recovery test of gluten

The viscoelastic behavior of gluten was studied by a creep-recovery test. Glutomatic (Perten Instruments AB, Huddinge, Sweden) was used to extract wet gluten from flours. In brief, breeder samples were washed with 2% NaCl solution through an 88 μm polyester screen supported by a metal sieve based on AACC method 38-12.02 (AACC International 2010). After washing for 5 min, wet gluten was obtained as the remaining residue. Prior to the creep-recovery experiment, wet gluten was relaxed under a 2.5 kg load plate with 2.5 mm spacing for 1 h. After the relaxation period, wet gluten was cut using a circular shape cutter into at 25 mm diameter and carefully loaded - onto the lower plate of AR1000 rheometer (TA Instrument, DE). The rheometer was equipped with crossed hatched 25 mm diameter probe and base plate. The test applied 100 Pa of shear

stress for 100 s (creep phase) and released for another 100 s (recovery phase). The viscoelastic response from gluten was recorded and reported in terms of compliance.

2.2.1 Modeling of gluten creep-recovery behavior

Burgers model was used to illustrate the viscoelastic behavior of gluten from the creep and recovery data (Steffe, 1996). Burgers model had been successfully used to represent biological polymer such as gluten because it accounted for spontaneous (spring from Maxwell model), delayed (spring and dashpot in parallel from Kelvin-Voigt model), and non-reversible flow (dashpot from Maxwell model) viscoelastic responses (Mezger, 2006). In this study, the experimental data from creep-recovery test were interpolated to 10,000 points before fitting the model to improve the coefficient of regression. The general form of Burgers model is shown in Equation 1 where J is compliance in Pa^{-1}, J0 was instantaneous compliance in Pa^{-1}, J1 was delayed compliance in Pa^{-1}, t1 was delayed time in s, and η_o was pure viscosity in Pa·s. The experimental data from recovery phase were fitted into equation 2. The pure viscous element was non-recoverable after creep, therefore $t/\eta_0 = 0$ in recovery phase.

Equation 1: $J(t) = J0 + J1(1 - \exp(-t/t1)) + t/\eta_0$

Equation 2: $Jr(t) = Jr_0 + Jr_1(1 - \exp(-t/tr_1))$

2.3 Mixing properties

The mixing properties of dough were determined following Method 54-40.02 (AACC International 2000). A Mixograph (National Manufacturing Co., Lincoln, NE) was used to analyze dough mixing properties in terms of water absorption (Mab) representing optimum dough water absorption corrected to 14% flour moisture basis, mixing time

(Mtime) the time required for optimum dough development, and mixing tolerance (Mtol) the break down behavior of dough.

2.4 Baking properties

An optimized straight-dough method was used to evaluate baking properties using Method 10-10.03 (AACC International 2000). A Swanson-type pin mixer with 100 g capacity (National Manufacturing, Lincoln, NE) was used to determine optimal mixing time. Baking test experiment aimed to measure an important breadmaking parameter which was loaf volume (LV) measured by rapeseed displacement method.

2.5 Statistical analysis

The relationships of gluten and dough rheological properties, protein content, and breadmaking properties were tested by using principal component analysis (PCA) and Pearson correlation. The PCA was performed by using Canoco for Windows 4.5 software (Centre for Biometry, Wageningen, The Netherlands) (Braak and Šmilauer, 2002; Legendre and Legendre, 1998). The Pearson correlation was tested by using CORR procedure in SAS program (Version 9.1 SAS Institute Inc., Cary, NC).

3. Results and Discussion

3.1 Principal component analysis of hard red winter wheat samples

A description of the number of samples per year set and protein content in each set were shown in Table 1. The flour samples with the lowest range of protein content (8.7-11.6%) were in year 2008, while samples in year 2010 and 2011 had a similar range of protein content. Regional flour samples (2010R) were also from the crop year 2010 but grown in different states comprising the hard red winter wheat producing areas. The

2010R flour samples contained the sample with the highest protein content (13.9%). Several environmental factors could affect the protein content of these sample sets. In 2008, the three heavy rainfall events, high humidity, and cold temperature could result in a low protein content of this set (Mikhaylenko et al., 2000).

The average sample set values of parameters from creep-recovery, protein content, bread quality, and Mixograph were shown in Table 2. 2010R sample set had the highest average loaf volume (LV), while sample set from year 2010 had the lowest average loaf volume. Principal component analysis (PCA) revealed relationships among parameters and flour samples based on their variations. PCA allowed a simultaneous overview representation of correlation among parameters and samples thus, finding patterns in our data with high dimensions. It also helped differentiate samples based on high explained variance parameters. The explained variance of parameters from each sample set was reported in Table 3. The results are reported in two dimensions with the highest explained variance. The total explained variance showed a trend to increase from 2008 to 2011 (69.4, 71.7, 73.2, and 78.8%, respectively). 2010R samples had the highest total explained variance of 79.7%. Overall, the highest variance which is represented by the main contributors in the first principal component, (PC1 in the biplot) of the samples was from parameters of creep-recovery test and Burgers model (MaxS, J0, J1, Jr10, G0, G1, and Gr11). All of these contributors were related to the resistance to deformation (elastic component) and deformability (viscous component) of gluten. For the second principal component (PC2), gluten elastic recovery RCY, was the main contributor followed by t1, tr10, FP, and Mab.

Bi-plot graphs of PCA for each year crop containing variables from creep-recovery test of gluten, dough Mixograph, protein content and loaf volume were shown in Figures 1-5. The parameters were shown as vectors, while the samples were depicted using symbols. The highest explained variance showed the longest vector, while the least explained variance showed the shorter length of vectors. When parameters were clustered in the same area or closely related, it indicated that they were positively correlated. In contrast, negatively correlated parameters would be located on opposite side of each other (Dobraszczyk and Salmanowicz, 2008). In every sample set, all biplot graphs indicated a similar trend for PC1 which showed that deformation parameters (MaxS, FinalS, J-Jr, J0, J1, Jr10, and Jr11) were negatively correlated (opposite) to viscosity of gluten (η0, η1, ηr11, G0, G1, Gr10, and Gr11). The result also showed the samples that were separated from other samples and parameters indicating they had higher variation. The viscoelastic parameters from Burgers model were variables that can help discriminate samples efficiently. Burgers model was able to reflect the changes in internal structure of gluten by modeling viscoelastic properties of gluten (Steffe, 1996). Burgers model was also applied to study viscoelastic properties of wheat kernel from different the wheat genotypes (Hernández-Estrada et al., 2012). We speculated that the retardation time in the viscoelastic region reflected the behavior of low molecular weight of glutenin subunits (LMW-GS) which were branches of the high molecular weight of glutenin subunits (HMW-GS) backbone in gluten structure. Gliadin acts as a plasticizer in gluten system via the hydrogen bonding and hydrophobic interactions. Thus, the PCA results indicated that the variation of hard red winter wheat cultivar samples from 2008 to 2011could be explained by variation in gluten compositions (HMW-GS, LMW-GS, and

gliadin). We proposed that regressed parameters from spring from Maxwell model can reflect HMW-GS behavior. LMW-GS behavior can be related to regressed parameters spring and dashpot in parallel from Kelvin-Voigt model. Lastly, regressed parameters from dashpot from Maxwell model can reflect gliadin behavior.

Recoverability of gluten (RCY) had a negative relationship with retardation time (t1) (Fig. 1-5). The retardation time was the time delayed viscoelastic deformation to reach equilibrium at 63.2% of the maximum value of the curves. The results indicated that gluten with high elastic recoverability would show a short retardation time in order to reach the equilibrium. The LMW-GS is a branch of HMW-GS backbone, via disulfide bonds. Thus, the possible explanation was that the LMW-GS in the sample with high elastic recoverability moved faster than the sample with elastic recoverability.

This result was consistent with the study of gluten viscoelastic properties of Norwegian, Portal, and Bastian cultivars. It was found that the cultivar with low breadmaking quality had a long retardation time (Tronsmo et al., 2003a). Dough mixing time from Mixograph had a positive correlation with resistance to deformation parameters in every year crop. In addition, the dough mixing tolerance also showed a positive correlation with resistance to deformation parameters in crop year 2011 (Fig. 4).

The samples from 2008 showed clusters related to the nursery where the samples were grown and parameters related to their variation. Most of the samples from nursery 92 (shown as cross) were distributed in quadrant 1 and 2, while samples of nursery 91 (shown as triangle) and nursery 93 (shown as star) were mostly in quadrant 3 and 4. This result indicated that samples from nursery 92 were correlated to dough water absorption

(45.4% PC2). On the other hand, samples from nursery 91 and 93 were related to gluten elastic recoverability (RCY) (51.4% PC2). In other year crops, samples could be discriminated individually rather than as group-based. This suggests that environmental effects might have been related to specific nurseries. However, this study was not designed to study such differences.

3.2 Correlation between gluten and dough rheological properties, flour protein, and breadmaking

Pearson correlation was used to show the relationship among pairs of parameters across crop years. Table 4 showed the correlation coefficient between parameters from creep-recovery test of gluten, flour protein, loaf volume, and mixing properties. Flour protein (FP) had a positive correlation with deformation parameters MaxS (r=0.42), FinalS (r=0.36), J-Jr (r=0.19), J0 (r=0.23), J1 (r=0.21), and Jr11 (r=0.21) and retardation time (t1, r=0.17), while it had weak negative correlation with resistance to flow parameters η0, η1 (r=-0.18), ηr11 (r=-0.18), G0 (r=-0.21), G1 (r=-0.19), Gr10 (r=-0.19), and Gr11 (r=-0.19). Thus, the results indicated that some samples from each year crop with high protein quantity (FP) tended to have a high deformability of gluten. This result supported PCA that FP had a negative correlation with recoverability of gluten (Fig. 1-5). MaxS and FinalS were the only two parameters from creep-recovery test that had a positive correlation with loaf volume (LV, (r=0.39 and 0.32, respectively)) and water absorption (Mab (r=0.63 and 0.50, respectively)). Dough mixing time (Mtime) and dough mixing tolerance (Mtol) had similar relationship with parameters from creep-recovery test. Both Mtime and Mtol were positively correlated with viscous parameters and negatively correlated with deformation parameters. Table 5 showed the correlation

between the flour protein, dough mixing, and loaf volume. Our result indicated that loaf volume (LV) had a significant positive correlation with flour protein (r=0.57) and Mab (r=0.40).

4. Conclusions

From sample sets of 2008-2011 crop years, regressed coefficients from creep-recovery coefficients explained the highest variance in the first principal component. The strength and deformability were the main contributors. The gluten elastic recoverability and flour protein were the second and third contributors. In addition, the elastic recoverability of gluten and flour protein were independent from strength and deformability. Principal component analysis showed that dough mixing time and viscous parameters were positively correlated across all five year crops which was also supported from Pearson correlation (r=0.50). In 2008, different groups of wheat cultivars could be clearly differentiated based on viscoelastic properties of gluten. In other year crops, samples were more dispersed and must be individually discriminated. In conclusion, a combination of experimental data, Burgers model, and principal component analysis were a useful tool for screening the quality of wheat cultivar based on viscoelastic properties of gluten.

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Table 1. Range of protein content of hard red winter wheat flour representing Oklahoma breeder sample groups of crop year 2008, 2009, 2010, 2011, and regional hard red winter wheat breeders 2010.

Sample sets	Number of samples	Protein content (%)
2008	51	8.7-11.6
2009	51	9.4-12.6
2010	35	10.3-12.4
2011	42	10.4-12.4
WQC	22	10.5-13.9

Protein content expressed on 14% moisture basis.

Table 2. Means of (n=3) breadmaking and rheological properties of gluten and dough from breeder sample groups year 2008, 2009, 2010, 2011, and regional hard red winter wheat breeders 2010

	Variables	**2008**	**2009**	**2010**	**2011**	**WQC**
Creep-recovery test	MaxS (%)	13.4	14.0	17.8	23.0	40.6
	FinalS (%)	2.8	2.8	4.1	5.8	8.9
	RCY (%)	79.4	80.5	77.4	75.5	78.8
	J-Jr (Pa^{-1})	0.7	0.7	1.0	1.5	0.9
	J0 (Pa^{-1})	11.3	11.9	13.9	16.2	13.0
	J1 (Pa^{-1})	12.4	12.8	16.5	21.9	15.1
	t1 (s)	7.8	7.7	8.6	8.9	8.1
	η_0 (Pa.s)	1.2	1.1	0.7	0.6	1.0
	G0 (Pa)	975.3	905.4	753.1	666.2	857.0
	G1 (Pa)	970.3	890.4	661.3	538.1	811.4
	η_1 (Pa.s)	0.7	0.7	0.6	0.5	0.6
	Jr10 (Pa^{-1})	13.8	14.4	16.6	19.4	15.5
	Jr11 (Pa^{-1})	12.5	12.9	17.0	22.6	15.5
	tr10 (s)	15.6	15.7	16.9	17.2	16.0
	Gr10 (Pa)	806.6	749.0	633.0	557.9	722.2
	Gr11 (Pa)	975.4	891.8	647.4	524.3	801.3
	ηr11 (Pa.s)	1.5	1.4	1.1	0.9	1.3

Table 2. (Continue) Means (n=3) of breadmaking and rheological properties of gluten and dough from breeder sample groups year 2008, 2009, 2010, 2011, and WQC of hard red winter wheat flour samples

	Variables	2008	2009	2010	2011	WQC
Protein content	FP (%)	10.4	10.9	11.3	11.3	11.9
Bread quality	LV (cm^3)	818.5	832.4	798.3	885.8	1005
Mixograph	Mab (%)	6.3	6.4	6.4	6.5	6.4
	Mtime (min)	4.2	4.2	4.7	4.3	4.8
	Mtol	2.7	3.7	3.6	3.3	3.5

MaxS = maximum strain during creep, FinalS = final strain during recovery, RCY = recoverability, J-Jr = difference in compliance during creep and recovery, J0 = instantaneous compliance during creep, J1= retardation compliance during creep, t1=retarded compliance during creep, η_0 =pure viscosity, G0 = instantaneous elastic modulus during creep, G1 = retarded elastic modulus during creep, η1 = coefficient of viscosity, Jr10 = instantaneous compliance during creep, Jr11= retardation compliance during creep, tr10= retarded compliance during creep, retardation time during creep, Gr10 = instantaneous elastic modulus during creep, Gr11 = retarded elastic modulus during creep, ηr11 = coefficient of viscosity, FP = flour protein, LV = loaf volume, Mab = Mixograph water absorption, Mtime = Mixograph mixing time, and Mtol = Mixograph tolerance.

Table 3. Principal component analysis (PCA) of flour properties from breeder sample sets year 2008, 2009, 2010, 2011, and regional hard red winter wheat breeders 2010

		2008			2009			2010			2011			WQC		
		PC1	**PC2**	**PC1+2**	**PC1**	**PC2**	**PC1+2**	**PC1**	**PC2**	**PC1+2**	**PC1**	**PC2**	**PC1+2**	**PC1**	**PC2**	**PC1+2**
	Variables	56.2	13.2	69.4	61.4	10.2	71.7	60.8	12.4	73.2	68.6	10.2	78.8	68.1	11.7	79.7
Creep-recovery test	MaxS (%)	90.2	0.6	90.8	**93.4**	1.8	95.2	**97.1**	0.0	97.1	**95.7**	0.2	95.9	90.0	1.9	91.8
	FinalS (%)	42.8	25.4	68.2	80.1	11.0	91.0	86.2	8.4	94.6	89.9	5.3	95.2	85.3	0.8	86.1
	RCY (%)	3.9	**51.4**	55.3	13.7	28.9	42.6	9.0	**63.2**	72.2	43.2	34.9	78.0	27.3	0.8	28.1
	JJr (Pa^{-1})	42.8	25.4	68.2	80.1	11.0	91.0	86.2	8.4	94.6	89.9	5.3	95.2	85.3	0.8	86.1
	J0 (Pa^{-1})	**90.4**	0.5	91.0	**94.0**	0.7	94.7	94.4	2.9	97.2	91.7	3.7	95.4	87.7	3.0	90.7
	J1 (Pa^{-1})	88.4	0.7	89.1	91.6	3.1	94.6	**96.3**	0.0	96.3	**94.9**	0.2	95.1	89.0	1.7	90.7
	t1 (s)	12.7	0.3	13.0	18.5	**65.3**	83.8	9.2	**56.6**	65.8	32.8	**62.0**	94.9	48.9	3.9	52.8
	η_0 (Pa.s)	88.8	0.0	88.8	89.2	0.7	89.9	93.8	1.6	95.4	93.3	0.1	93.4	90.0	0.3	90.4
	G0 (Pa)	**91.4**	0.3	91.8	88.6	8.3	97.0	89.4	4.9	94.3	90.3	7.5	97.8	**90.1**	0.2	90.2
	G1 (Pa)	**91.0**	0.2	91.2	92.4	1.3	93.7	**96.2**	0.2	96.4	**94.3**	1.1	95.4	**91.7**	0.3	92.0
	η_1 (Pa.s)	86.5	0.2	86.7	88.7	7.0	95.7	90.8	5.3	96.2	89.8	6.7	96.5	89.8	0.0	89.8
	Jr10 (Pa^{-1})	89.3	1.2	90.6	**93.4**	1.1	94.5	93.6	3.5	97.2	92.0	3.8	95.7	87.1	3.3	90.4
	Jr11 (Pa^{-1})	87.2	0.7	87.8	89.9	2.9	92.8	94.5	0.0	94.5	93.9	0.2	94.1	88.6	1.7	90.4
	tr10 (s)	0.1	42.1	42.2	0.4	**38.1**	38.5	4.2	7.6	11.9	13.8	**66.5**	80.4	37.8	5.1	42.8
	Gr10 (Pa)	89.7	0.9	90.6	87.0	10.2	97.2	89.2	6.2	95.4	89.9	7.9	97.7	88.8	0.2	88.9
	Gr11 (Pa)	88.8	0.2	89.0	89.3	2.0	91.3	95.3	0.6	95.9	93.6	1.3	94.8	**90.4**	0.4	90.8
	ηr11 (Pa.s)	88.6	0.3	88.9	88.9	5.1	94.0	93.2	1.7	94.9	90.9	5.0	95.9	89.9	0.0	89.9

* The descriptions of each variable were explained in Table 2.

Table 3. (Continue) Principal component analysis (PCA) of breeder sample sets year 2008, 2009, 2010, 2011, and WQC of hard red winter wheat flour

		2008			2009			2010			2011			WQC		
	Variables	**PC1**	**PC2**	**PC1+2**	**PC1**	**PC2**	**PC1+2**	**PC1**	**PC2**	**PC1+2**	**PC1**	**PC2**	**PC1+2**	**PC1**	**PC2**	**PC1+2**
		56.2	13.2	69.4	61.4	10.2	71.7	60.8	12.4	73.2	68.6	10.2	78.8	68.1	11.7	79.7
Protein content	FP (%)	0.1	44.3	44.4	0.1	2	2.1	0.7	41.4	42.1	5.1	8.7	13.8	4.3	**83.1**	87.4
Bread quality	LV (cm^3)	0.3	30.8	31.1	3.7	3.3	7	0	6.2	6.2	6.8	0.7	7.4	14.9	26.9	41.8
Mixograph	Mab	0.9	**45.4**	46.3	0.3	14.3	14.5	0.1	41.4	41.4	2	2.7	4.7	2.4	**87.5**	89.8
	Mtime	45.3	5.8	51.1	53.7	0.5	54.1	17.9	0.4	18.4	76.7	0	76.7	77.1	7.1	84.2
	Mtol	16.8	12.8	29.6	14.4	6.8	21.1	0.3	11.6	11.9	39.2	0.5	39.7	41.5	27.1	68.7

* The descriptions of each variable was explained in Table 2.

Table 4. Relationship among parameters from creep-recovery test, flour protein, breadmaking, and Mixograph of breeder sample sets year 2008, 2009, 2010, 2011, and regional breeders of hard red winter wheat 2010.

	FP	LV	Mab	Mtime	Mtol
MaxS	0.42***	0.39***	0.63***	-0.37***	-0.20***
FinalS	0.36***	0.32***	0.50***	-0.38***	-0.24***
RCY	-0.07	-0.05	0.02	0.14*	0.16*
JJr	0.19*	0.05	-0.02	-0.47***	-0.31***
J0	0.23***	0.03	-0.01	-0.55***	-0.33***
J1	0.21***	0.04	-0.01	-0.55***	-0.32***
t1	0.17*	0.10	-0.05	-0.27***	-0.05
η0	-0.20***	-0.05	0.02	0.50***	0.20***
G0	-0.21***	-0.03	0.02	0.52***	0.27***
G1	-0.19*	-0.04	0.02	0.52***	0.24***
η1	-0.18*	-0.02	0.01	0.52***	0.26***
Jr10	0.22	0.02	-0.02	-0.57***	-0.34***
Jr11	0.21***	0.04	-0.01	-0.55***	-0.32***
tr10	0.13	0.05	-0.05	-0.18	0.06
Gr10	-0.19***	-0.02	0.03	0.54***	0.27***
Gr11	-0.19*	-0.04	0.01	0.51***	0.23***
ηr11	-0.18*	-0.04	0.004	0.51***	0.26***

The descriptions of each variable were explained in Table 2.

*** Correlation is significant at $\alpha < 0.001$, * Correlation is significant at $\alpha < 0.05$.

Table 5. Relationship among parameters from flour protein, breadmaking, and Mixograph of breeder sample sets year 2008, 2009, 2010, 2011, and regional breeders of hard red winter wheat 2010.

	FP	LV	Mab	Mtime	Mtol
FP	1.00				
LV	0.57***	1			
Mab	0.40***	0.64***	1		
Mtime	0.16*	0.24***	0.15*	1	
Mtol				0.46***	1

The descriptions of each variable were explained in Table 2.

*** Correlation is significant at $\alpha < 0.001$, * Correlation is significant at $\alpha < 0.05$.

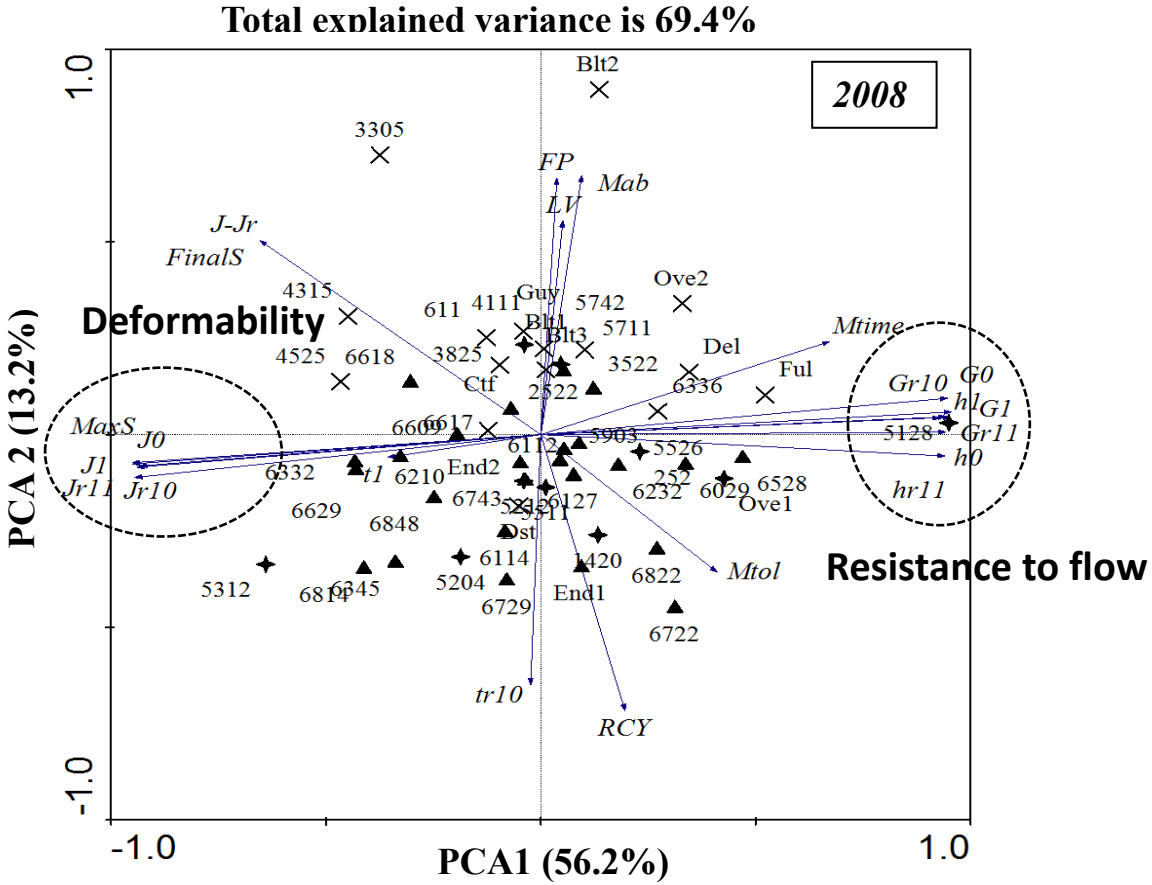

Figure 1. Principal component analysis (PCA) of gluten, dough, and bread from three nurseries of hard red winter wheat breeder samples year 2008 involving 21 indicators of gluten, dough, and bread quality (viscoelasticity of gluten, dough mixing, and loaf volume), one indicator of gluten quantity (flour protein). Triangle symbol indicated samples from nursery 91. Cross symbol indicated samples from nursery 92. Star symbol indicated samples from nursery 93. The descriptions of each variable were explained in Table 2.

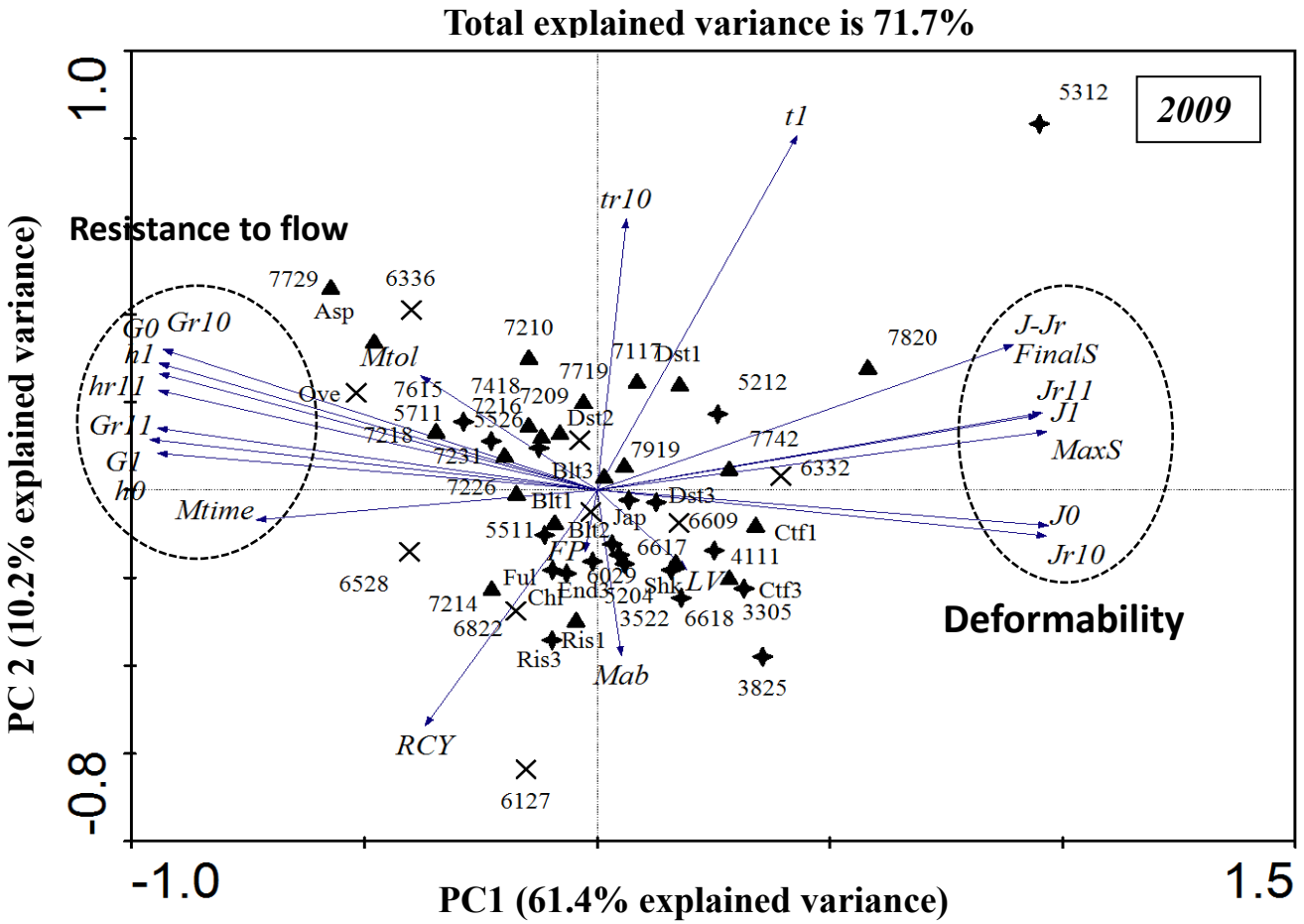

Figure 2. Principal component analysis (PCA) of gluten, dough, and bread from three nurseries of hard red winter wheat breeder samples year 2009 involving 21 indicators of gluten, dough, and bread quality (viscoelasticity of gluten, dough mixing, and loaf volume), one indicator of gluten quantity (flour protein). Triangle symbol indicated samples from nursery 91. Cross symbol indicated samples from nursery 92. Star symbol indicated samples from nursery 93. The descriptions of each variable were explained in Table 2.

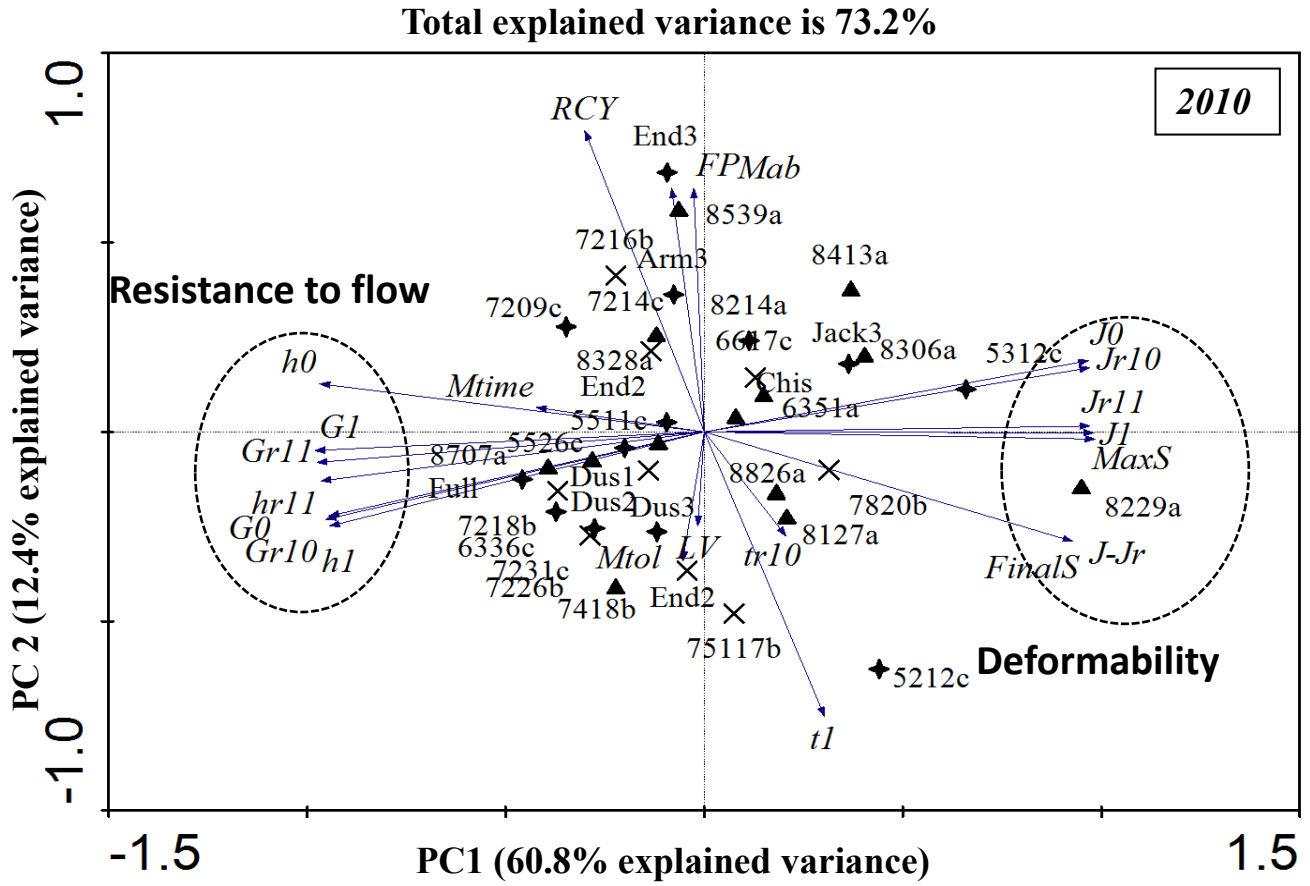

Figure 3. Principal component analysis (PCA) of gluten, dough, and bread from three nurseries of hard red winter wheat breeder samples year 2010 involving 21 indicators of gluten, dough, and bread quality (viscoelasticity of gluten, dough mixing, and loaf volume), one indicator of gluten quantity (flour protein). Triangle symbol indicated samples from nursery 91. Cross symbol indicated samples from nursery 92. Star symbol indicated samples from nursery 93. The descriptions of each variable were explained in Table 2.

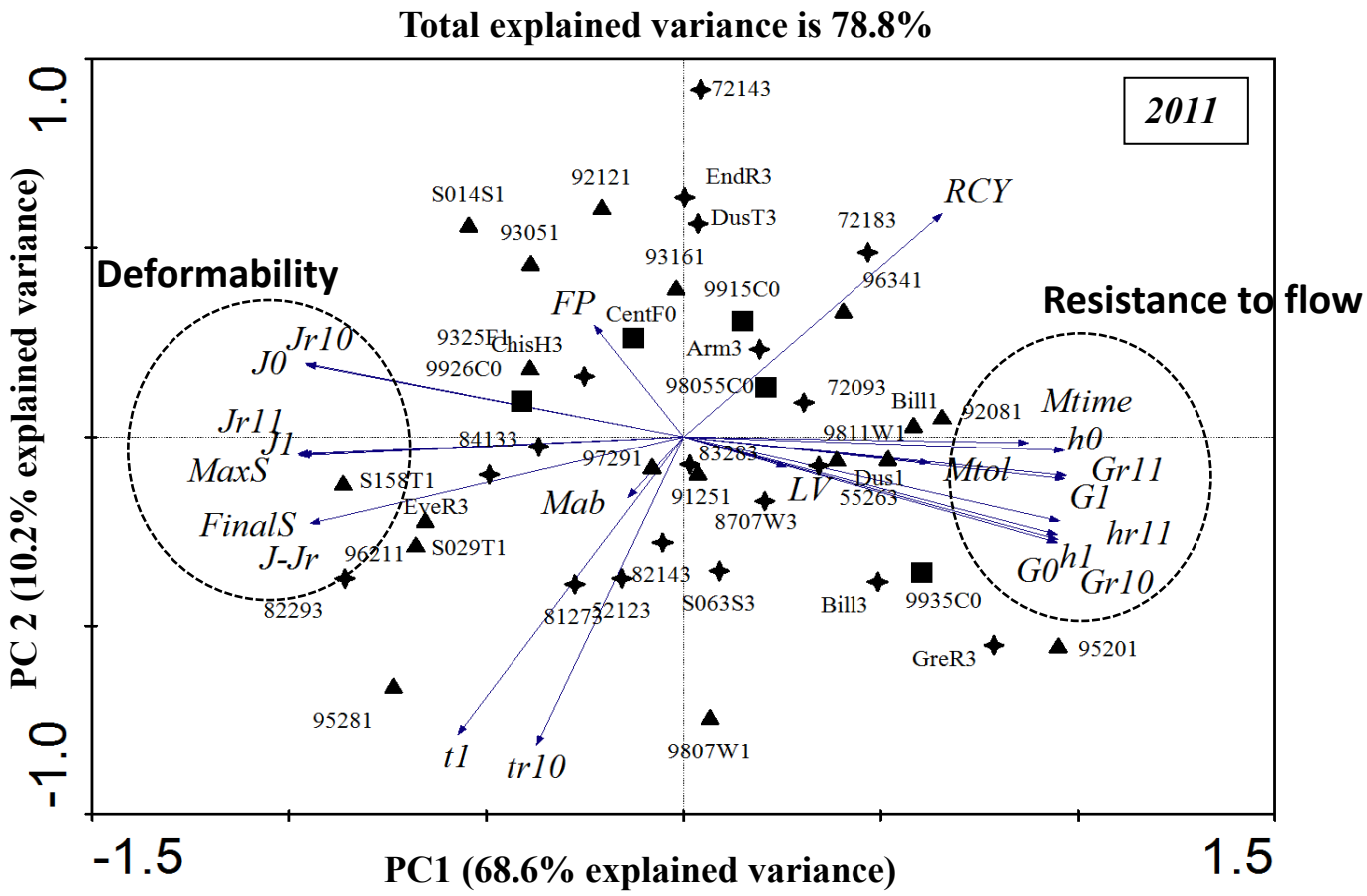

Figure 4. Principal component analysis (PCA) of gluten, dough, and bread from three nurseries of hard red winter wheat breeder samples year 2011 involving 21 indicators of gluten, dough, and bread quality (viscoelasticity of gluten, dough mixing, and loaf volume), one indicator of gluten quantity (flour protein). Square symbol indicated samples from nursery 90. Triangle symbol indicated samples from nursery 91. Star symbol indicated samples from nursery 93. The descriptions of each variable were explained in Table 2.

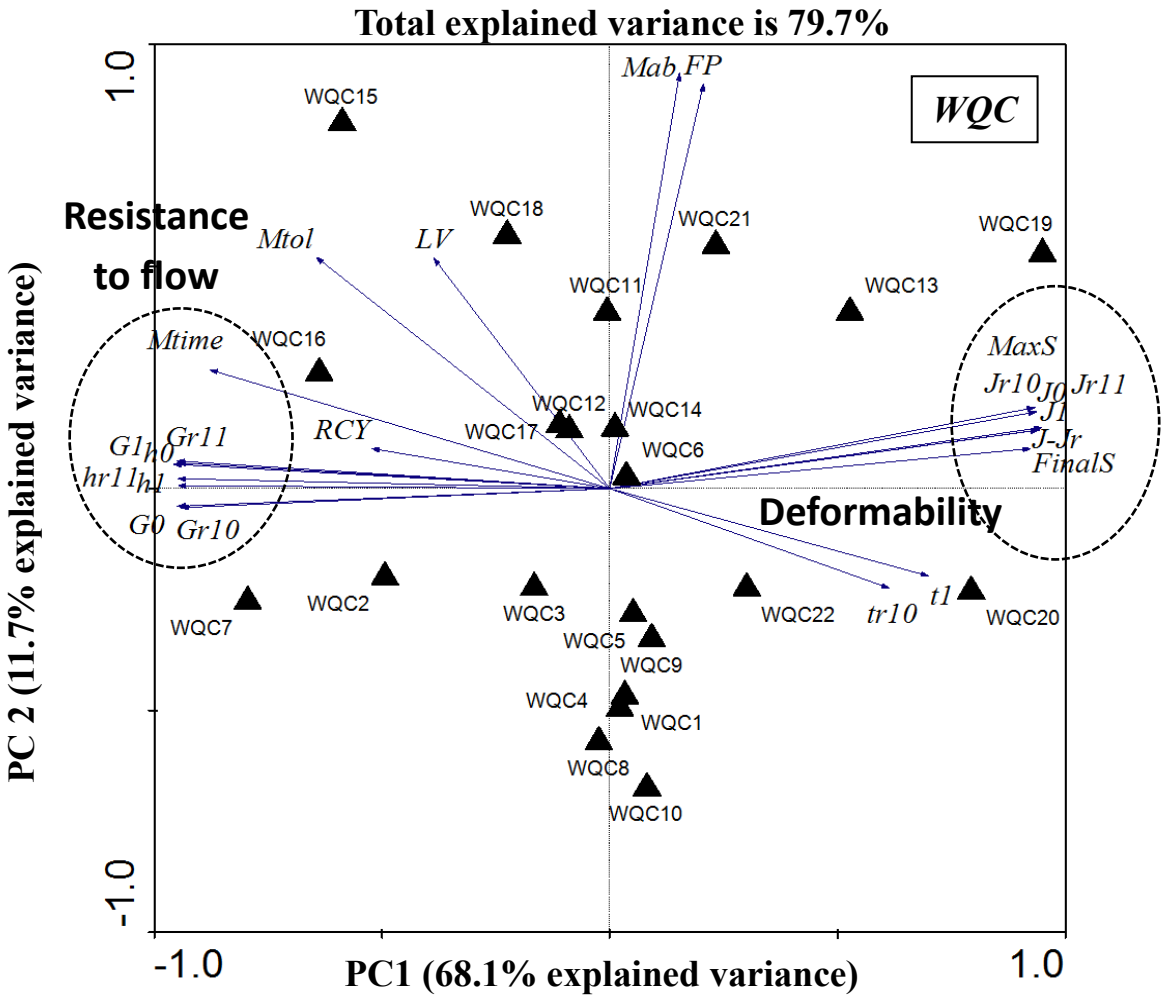

Figure 5. Principal component analysis (PCA) of gluten, dough, and bread from three nurseries of hard red winter wheat breeder lines from regional breeders (2010) involving 21 indicators of gluten, dough, and bread quality (viscoelasticity of gluten, dough mixing, and loaf volume), one indicator of gluten quantity (flour protein). Triangle symbol indicated samples from regional breeders. The descriptions of each variable were explained in Table 2.

CHAPTER VIII

CONCLUSIONS

The polymeric gluten structure was altered by using diacetyl tartaric acid ester of monoglycerides DATEM, ascorbic acid (AA), urea, DTT, heat, and gluten substitution. The modeling viscoelastic properties of gluten were applied to reflect gluten structure. In this study, we attempted to improve the understanding of viscoelastic properties of gluten by interrelating molecular changes to each mechanical analog used in the model. This study also aimed to correlate the regressed coefficients from modeling with dough and bread quality.

DATEM affected viscoelastic properties of gluten differently compared to AA, urea, and DTT. DATEM decreased elastic deformation (J0) (using at 1% DATEM; decreased up to 50%), while increased resistance to flow of gluten (88.8%). We speculated that the hydrophobic gluten domains of DATEM interacted mainly with high molecular weight glutenin subunits (HMW-GS) hydrophobic domains. In contrast, the addition of AA, urea, and DTT at the highest levels (0.5mM) increased elastic deformation (J0) up to 108, 23, and 42%, respectively. A similar trend of increased elastic deformation (J0) was observed with an increased retarded viscoelastic deformation (J1) after adding DATEM, AA, urea, and DTT. DATEM increased resistance to flow (η_0) of gluten, while AA, urea, and DTT decrease it.

In the recovery phase, the elastic and viscoelastic deformation of gluten was decreased after adding DATEM; while the addition of AA, urea, and DTT increased the deformation of gluten during recovery. At the molecular level DATEM decreases surface tension of protein domains and thus effectively favors more molecular interactions. This was reflected by a decreased elastic deformation (J0) and increase resistance to flow of gluten. Comparing to increasing the oxidation level in gluten via formation of disulfide bonds with AA, DATEM is more effective in favoring elastic structures via forming larger molecular weight agglomerates. The disruption of hydrogen bonds and hydrophobic interactions with urea and the reduction of disulfide bonds with DTT yielded gluten structures more compliant during creep (increased deformation) accounted by increased J0 and J1. The interesting finding is that these two different changes in the types of bonds affected produced similar effects. This suggests that non-covalent bonds (hydrogen bonds and hydrophobic interactions) are as important as disulfide bonds in their contributions to the gluten structure.

Heating altered viscoelastic properties of gluten by increasing gluten deformation (J0 and Jr0) starting at 45°C to 55°C. In addition, the Jr0 value of gluten was higher than J0 when it was heated from 45°C to 55°C reflecting that heating at these temperatures affected mostly the LMW-GS structure. We speculated that non-covalent bonds (i.e, hydrogen bonds and hydrophobic interactions) are also as important as disulfide bonds to gluten structure. After heating gluten up to 65°C, deformation (η_0 and recoverability) of gluten during creep was decreased indicating aggregation of gluten Thus, the covalent

bonds of gluten was induced after heating up to 65°C resulting in gluten agglomeration and formation .

Moreover, the effect of heat on viscoelastic properties of gluten depended on wheat class. J0 of a soft red winter wheat used as reference gluten showed a high percent change (39.8%) at 25°C and 65°C indicating a large deformation in HMW-GS of SRW. Gluten substitution affected viscoelastic properties of gluten based on viscoelastic behavior and pH level of gluten and gliadin substitution. The substitution of gluten B (gluten with more acidity, pH=4.2) at 8% increased the deformation up to 302.2% (final strain) of gluten which mainly affected both HMW-GS and low molecular weight glutenin subunits (LMW-GS). The substitution of gluten (GB, GC, and gliadin) at 8% decreased resistance to flow of gluten. Also, the J0 and J1 of gluten after 8% substituting with all gluten products decreased, thus, we speculate that gluten substitution diluted native disulfide bonds and increased hydrophobic interactions and hydrogen bonds.

The quality and quantity of gluten such as gluten pH and percent level substitution of gluten affected gluten viscoelastic properties. Creep and recovery phase of gluten substitution showed that the substitution of GB increased gluten deformation (Jc1; at 6% increased by 64% and Jr1; at 6% increased by 53.5%) partially explained by a more acidic gluten B (pH =4.2). However, pure viscosity of gluten increased up to 25% after substituting gluten with 6% gluten GC. From the recovery phase of compression-recovery test, GB and gliadin decreased gluten strength, Ɛ0 up to 300% and 200%, respectively. The substitution of GB at 6% also decreased Ɛ1 by 50%, and increased retardation time Ct1 by 63.5%.

Overall, coefficients strength and deformability were the main contributors of variability across every crop year from 2008 to 2011. Gluten recoverability and flour protein were the second and distant third contributors to the explained variance and were independent of strength and deformability. The viscous parameters showed a positive correlation with the dough mixing properties. Therefore, including the elastic and viscous variability could add value to breeding program. Although improvements were made in the basic understanding of gluten viscoelastic behavior, the regressed coefficients were independent from loaf volume. Bread loaf volume have low variability compared to the variability of viscoelastic behavior suggesting that during the optimization of bread baking there are factors that are taking into account (such as water absorption and mixing time) to have a more standardized dough consistency (not too swet or dry and well developed). In summary, the bread loaf volume still is the golden standard in bread baking evaluation and has to always be included in comparison made but there are larger variations in gluten viscoelasticity in breeder lines that can be brought to the selection process of new wheat cultivars and enrich it.

CHAPTER IX

FUTURE STUDIES

The study of this dissertation mainly focused on applying mechanical model into the viscoelastic behavior of gluten biopolymers with various treatments in order to quantitate the rheological changes in gluten system. Thus, we can relate the quantitative data to gluten structures at the molecular level. However, a more complete understanding of the molecular basis of gluten and dough rheology still needs to be elucidated.

In the study on the effect of gluten substitution on gluten rheological properties, the work revealed many interesting facts pointing to the lower pH of gluten B (pH=4.2) and the trend of this gluten to decrease elasticity and viscosity. It is therefore essential to study in particular to the effect of pH on the viscoelastic properties of gluten. Regarding the elasticity and viscosity trend, other quality indicators must be measured such as the baking study and other empirical rheological properties in order to consolidate and enhance the understanding. Furthermore, gluten system is a very concentrated protein biopolymer system, thus, the changes in a diluted protein system such as batter, dough, or bread might be also interesting to study in depth. Moreover, the diluted protein system (i.e. dough and batter) will also allow us to study the interactions of protein with other components such as lipid, carbohydrate, and water as well.

It will also be of value to continue this study by measuring the alteration of gluten secondary structure by using Fourier Transform Infrared Spectroscopy (FTIR), which based on molecular vibrational motions. With FTIR information, one can relate back the changes in quantitative information that were obtained from the modeling of viscoelastic behavior of gluten.

Heat and additive treatments are also the two main variables in breadmaking process. The study of secondary structure of gluten by using FTIR combined with the heat and additive treatments will significantly clarify the alteration in secondary conformation. Because gluten was exposed to heat for 200 s (3 min 30 sec) in this dissertation, variation in heating time should be investigated before testing the gluten in order to cover a wider range of physical and secondary structures changes.

Regarding the correlation study between empirical and fundamental rheological properties of gluten, it revealed that deformability along with the resistance to flow of gluten explained the variation in all sets of breeder samples. Because this work contained the results from hard red winter wheat breeder lines and cultivars, it will be a great value to include other cultivars in order to include a wider range of variability for future studies as well.

APPENDIX I

Calculations of the amount of flour and gluten product

$$x + y = 10$$

$$0.108x + wy = 10z$$

$$y = 10 - x$$

$$0.108x + w(10 - x) = 10z$$

$$0.108x + 10w - wx = 10z$$

$$10(w - z) = (w - 0.108)x$$

$$x = 10(w - z)/(w - 0.108)$$

Where;

x = Amount of flour x (Unknown)

y = Amount of gluten product (Unknown)

w = Protein (%) of gluten (Known)

z = Protein (%) of flour (Known)

Note: Assumed the total blend is 10 g. and protein content of flour = 10.8%

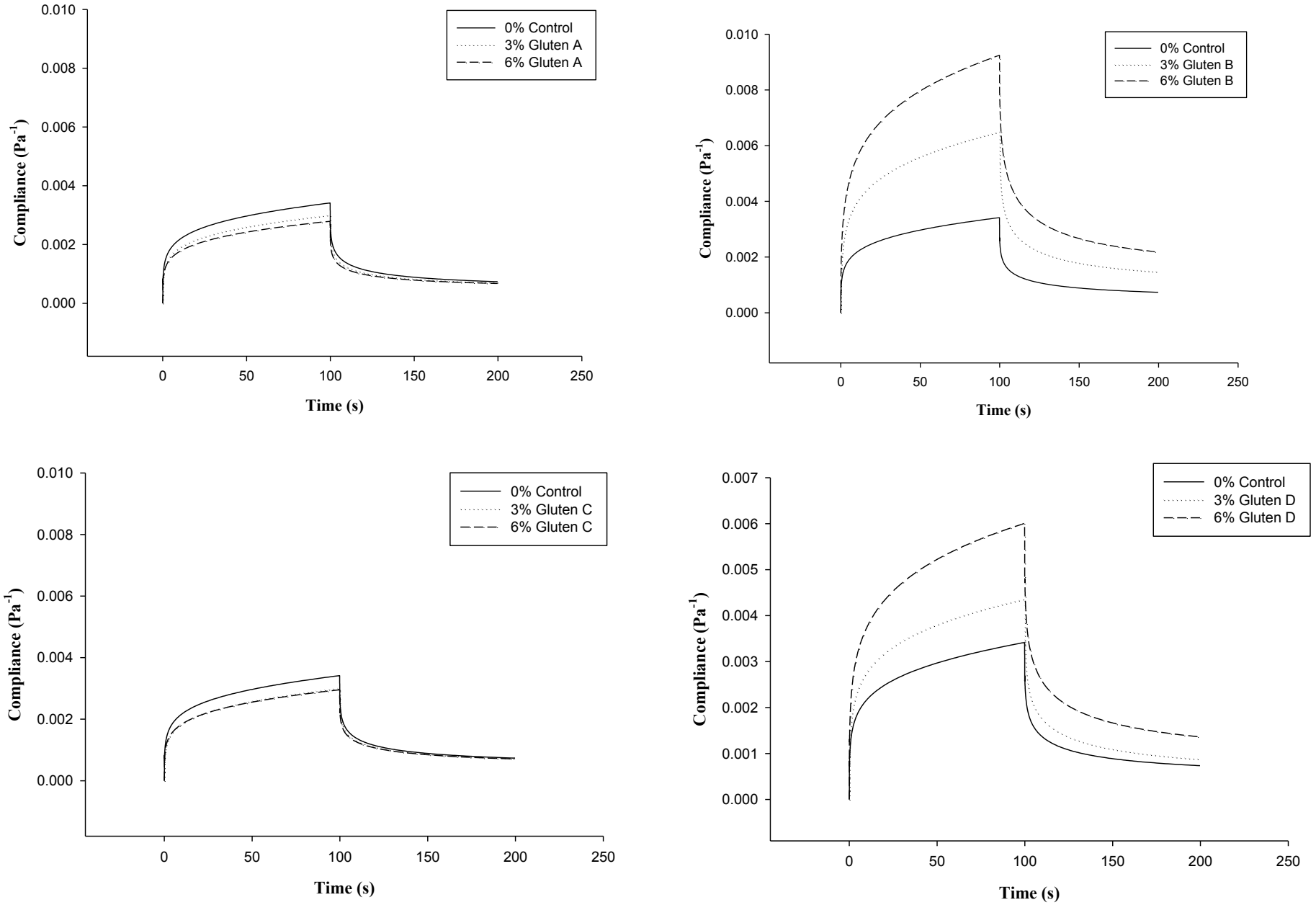

Figure 1. Typical strain curves of gluten system (Flour F2) and two substitution treatments during the recovery of compression-recovery test

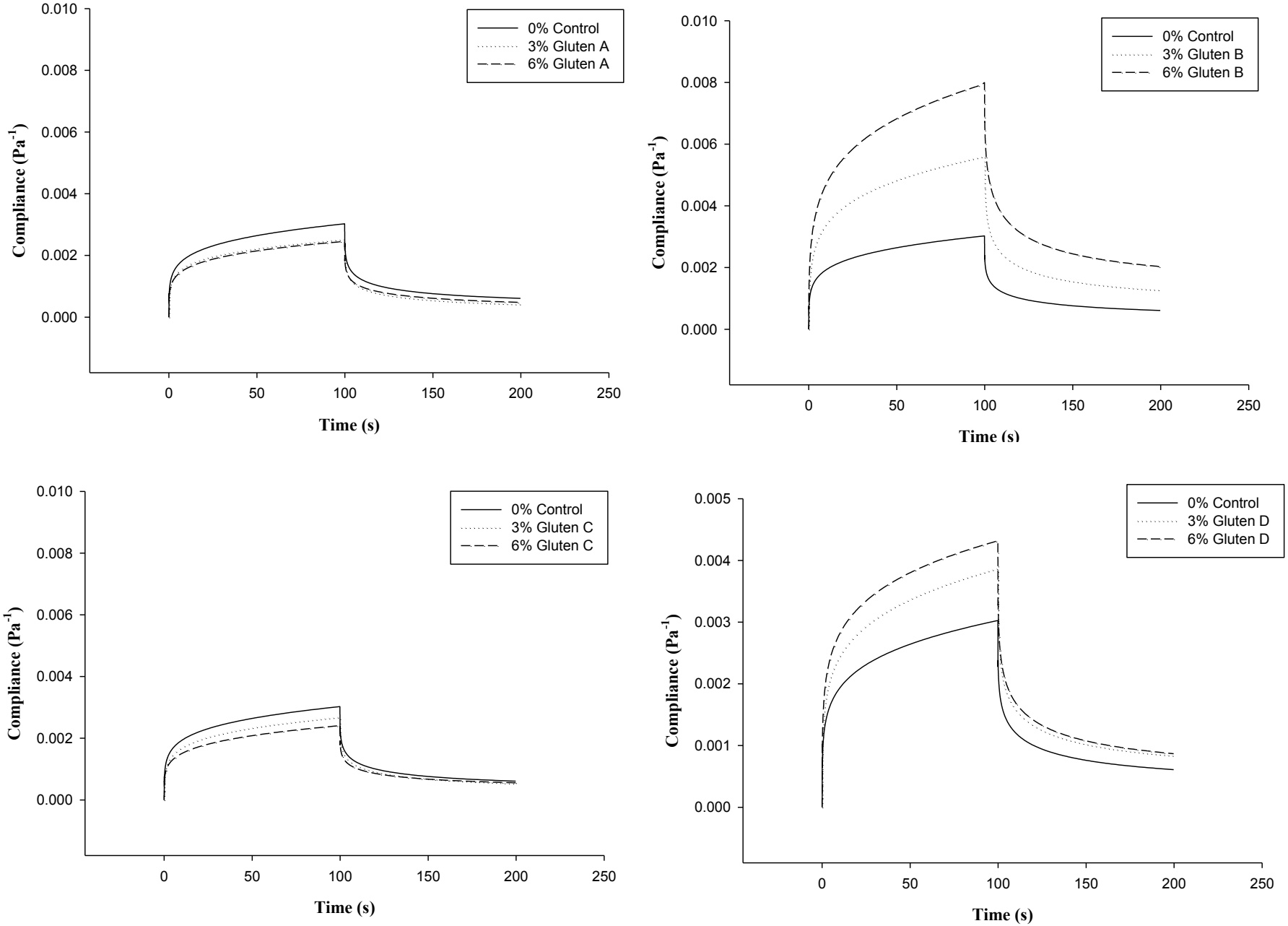

Figure 2. Typical strain curves of gluten system (Flour F3) and two substitution treatments during the recovery of compression-recovery test

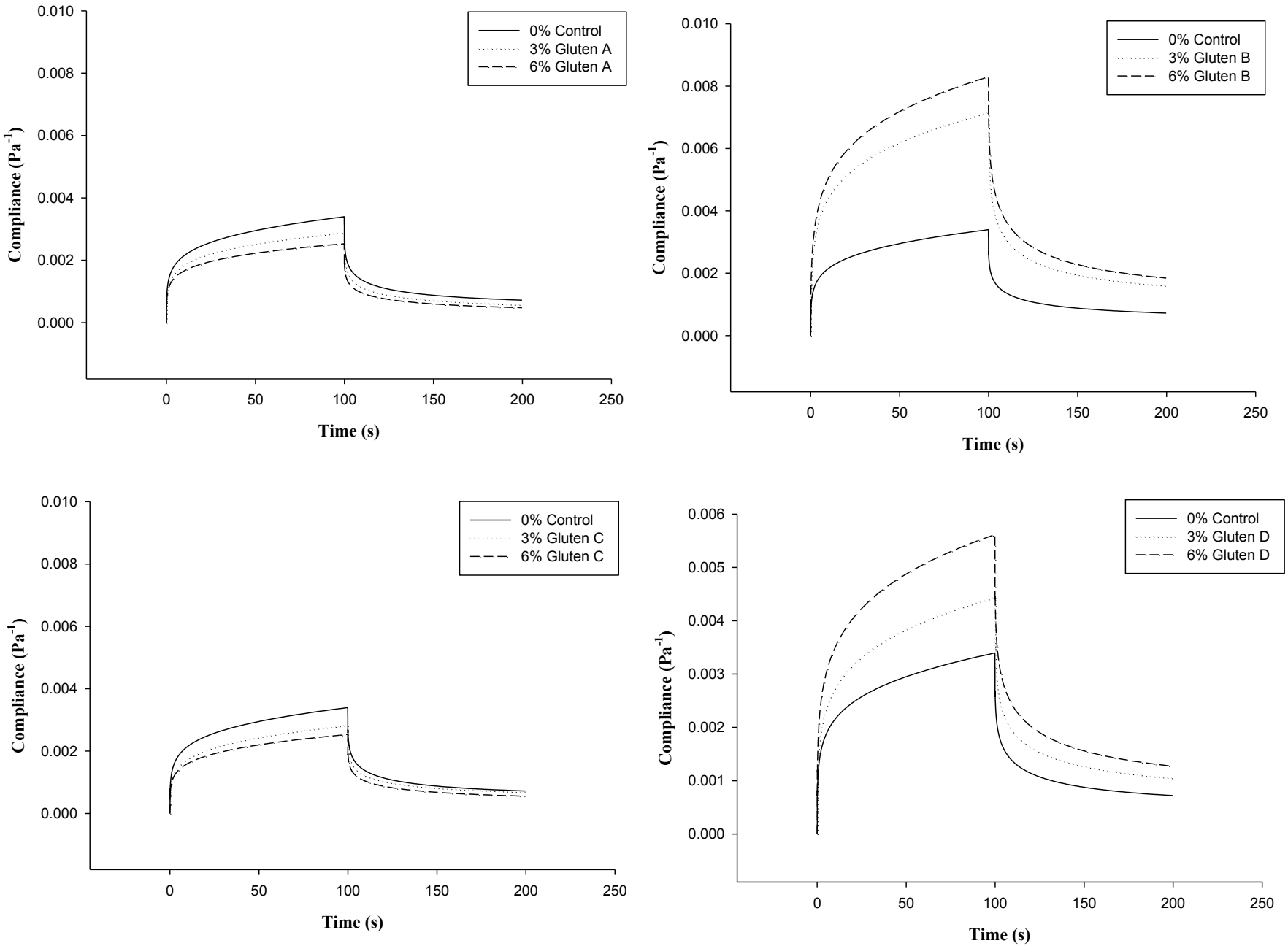

Figure 3. Typical strain curves of gluten system (Flour F4) and two substitution treatments during the recovery of compression-recovery test

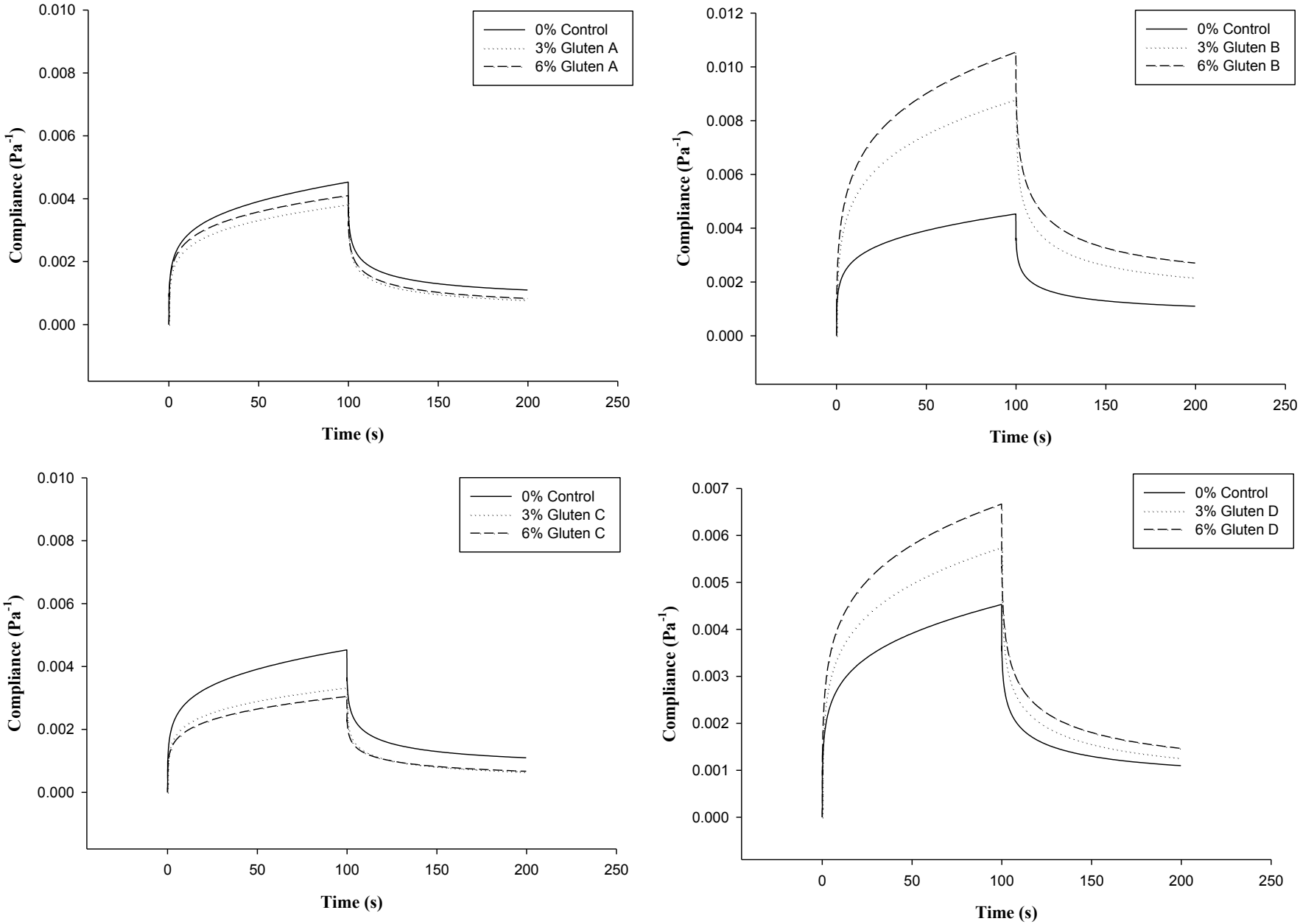

Figure 4. Typical strain curves of gluten system (Flour F5) and two substitution treatments during the recovery of compression-recovery test

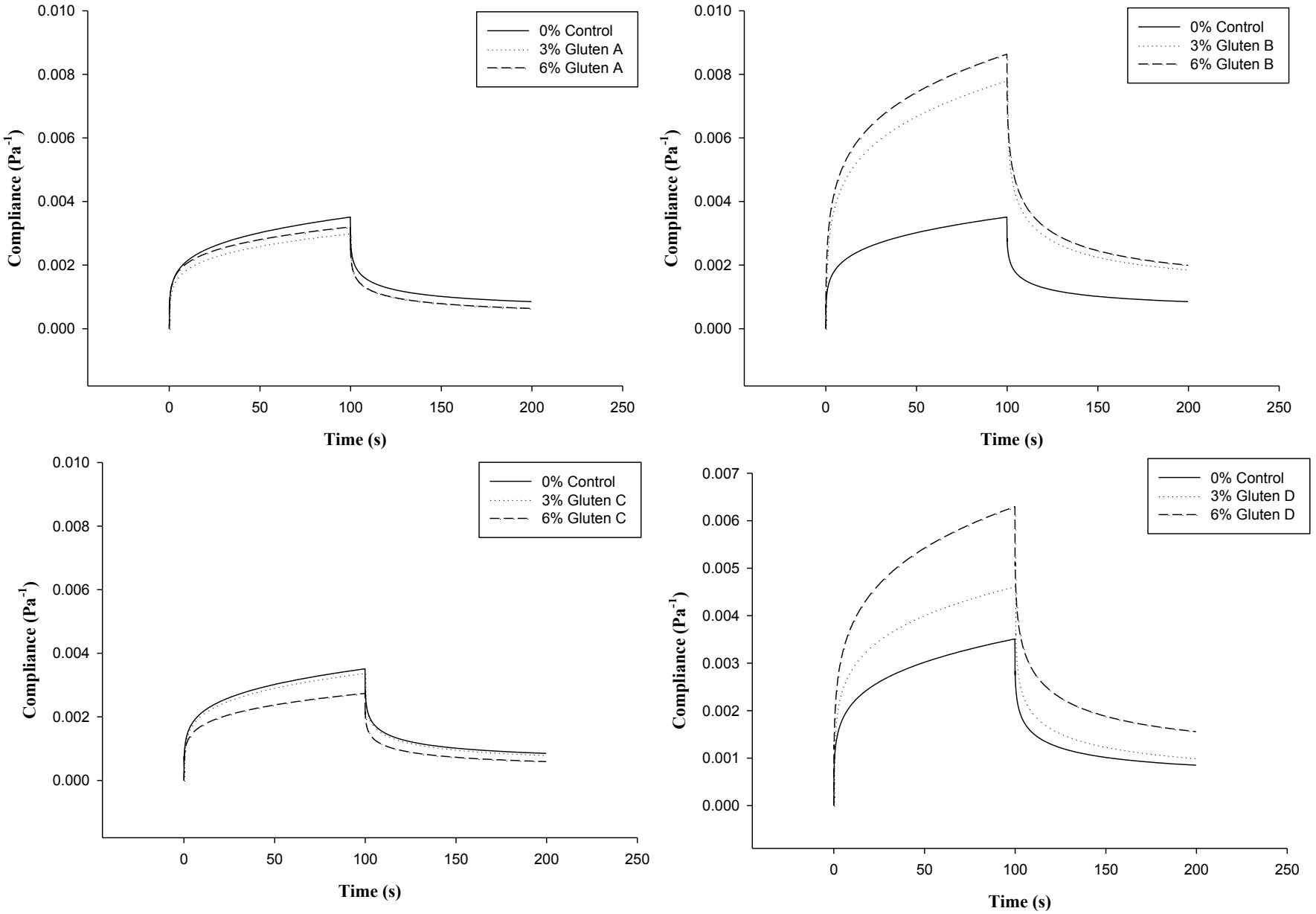

Figure 5. Typical strain curves of gluten system (Flour F6) and two substitution treatments during the recovery of compression-recovery test

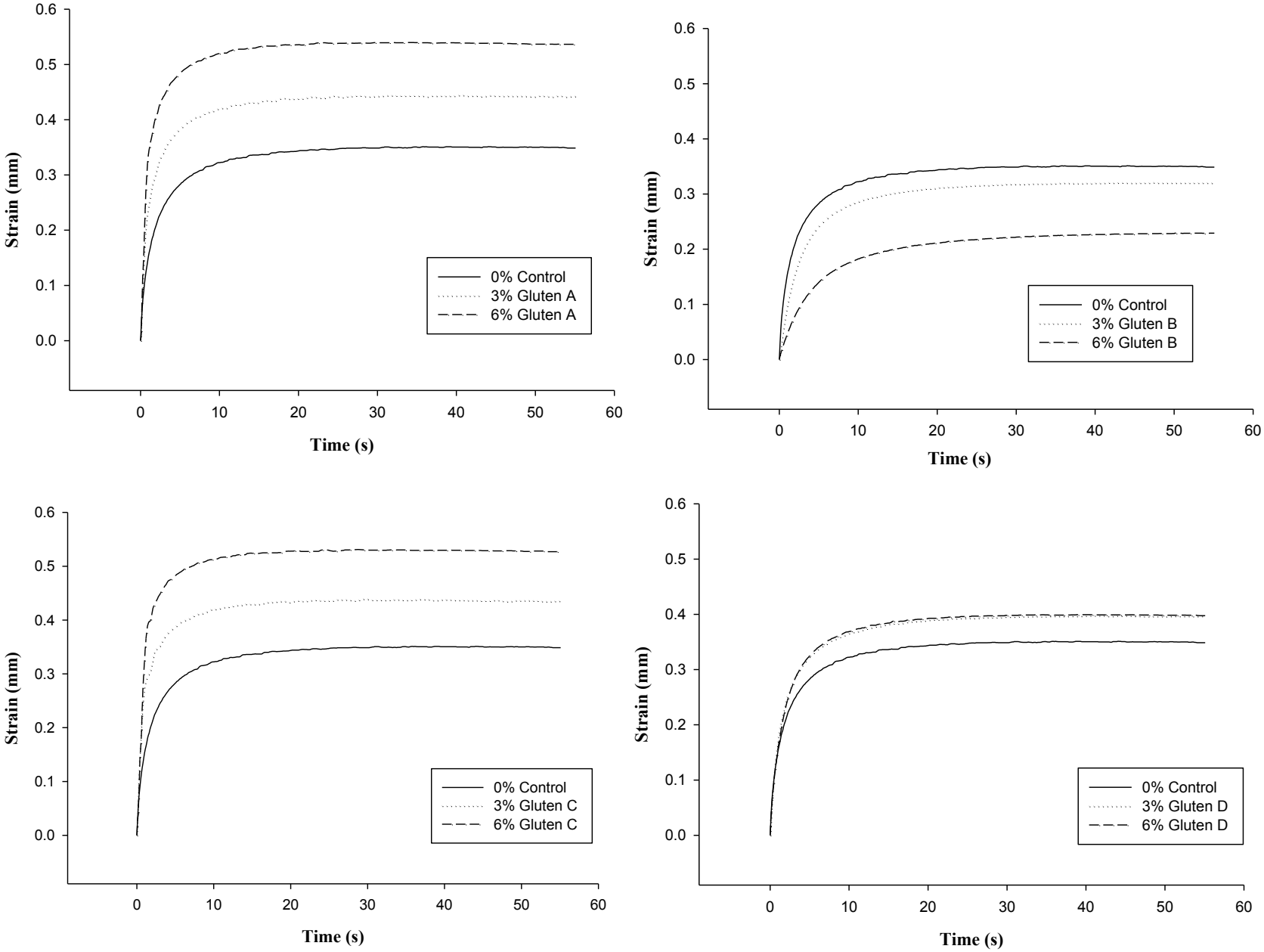

Figure 6. Typical strain curves of gluten system (Flour F2) and two substitution treatments during the recovery of compression-recovery test

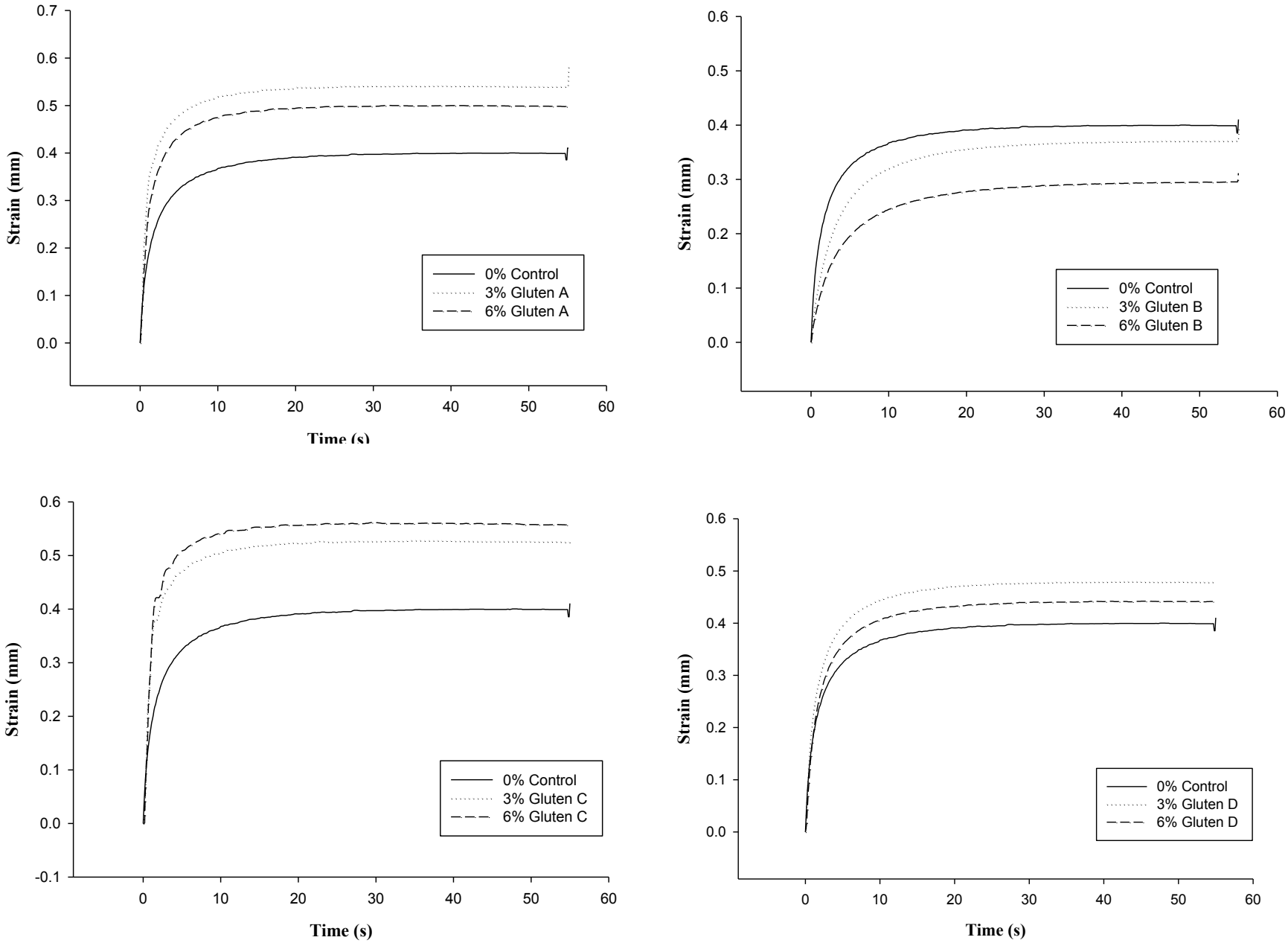

Figure 7. Typical strain curves of gluten system (Flour F3) and two substitution treatments during the recovery of compression-recovery test

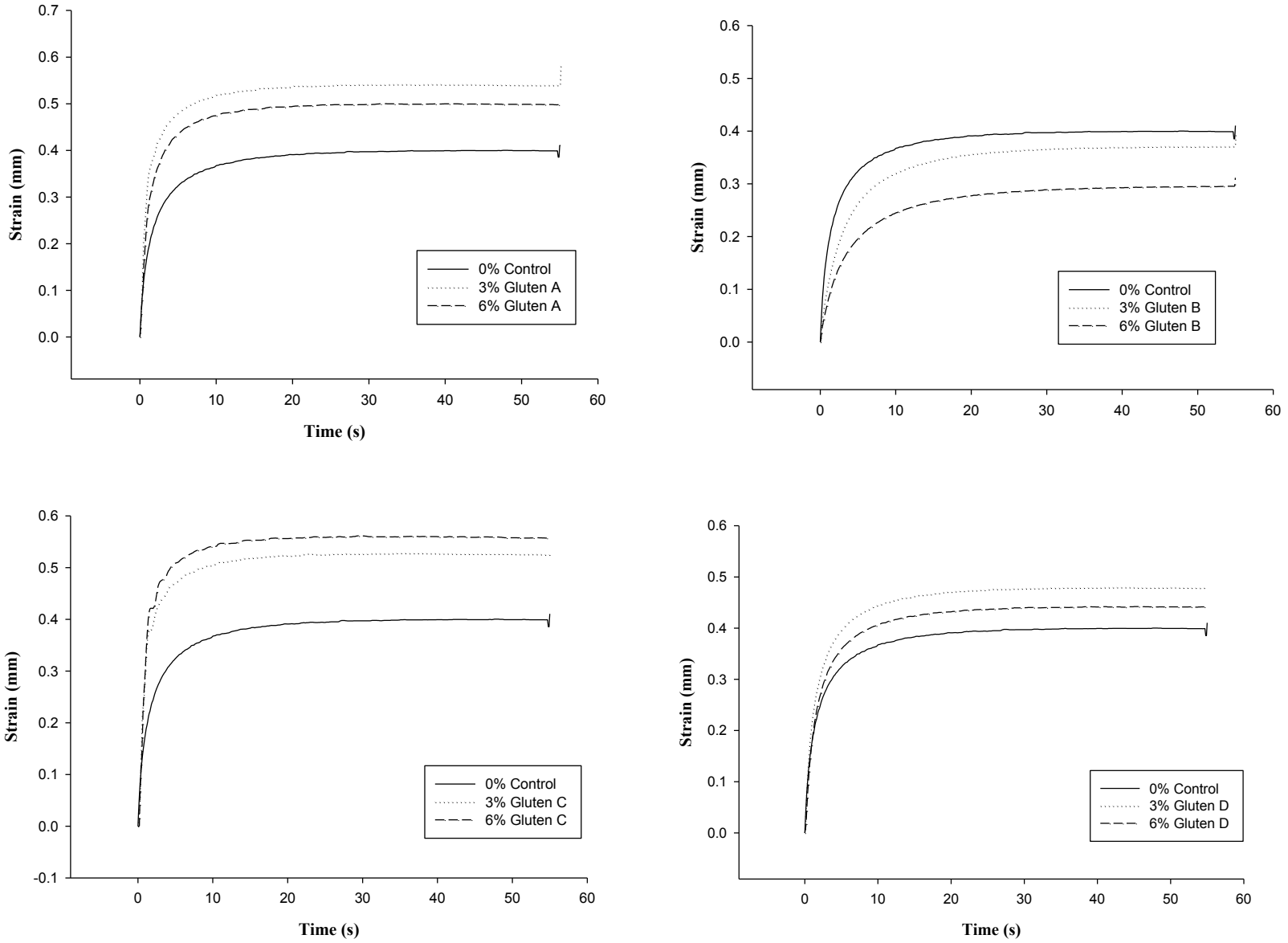

Figure 8. Typical strain curves of gluten system (Flour F4) and two substitution treatments during the recovery of compression-recovery test

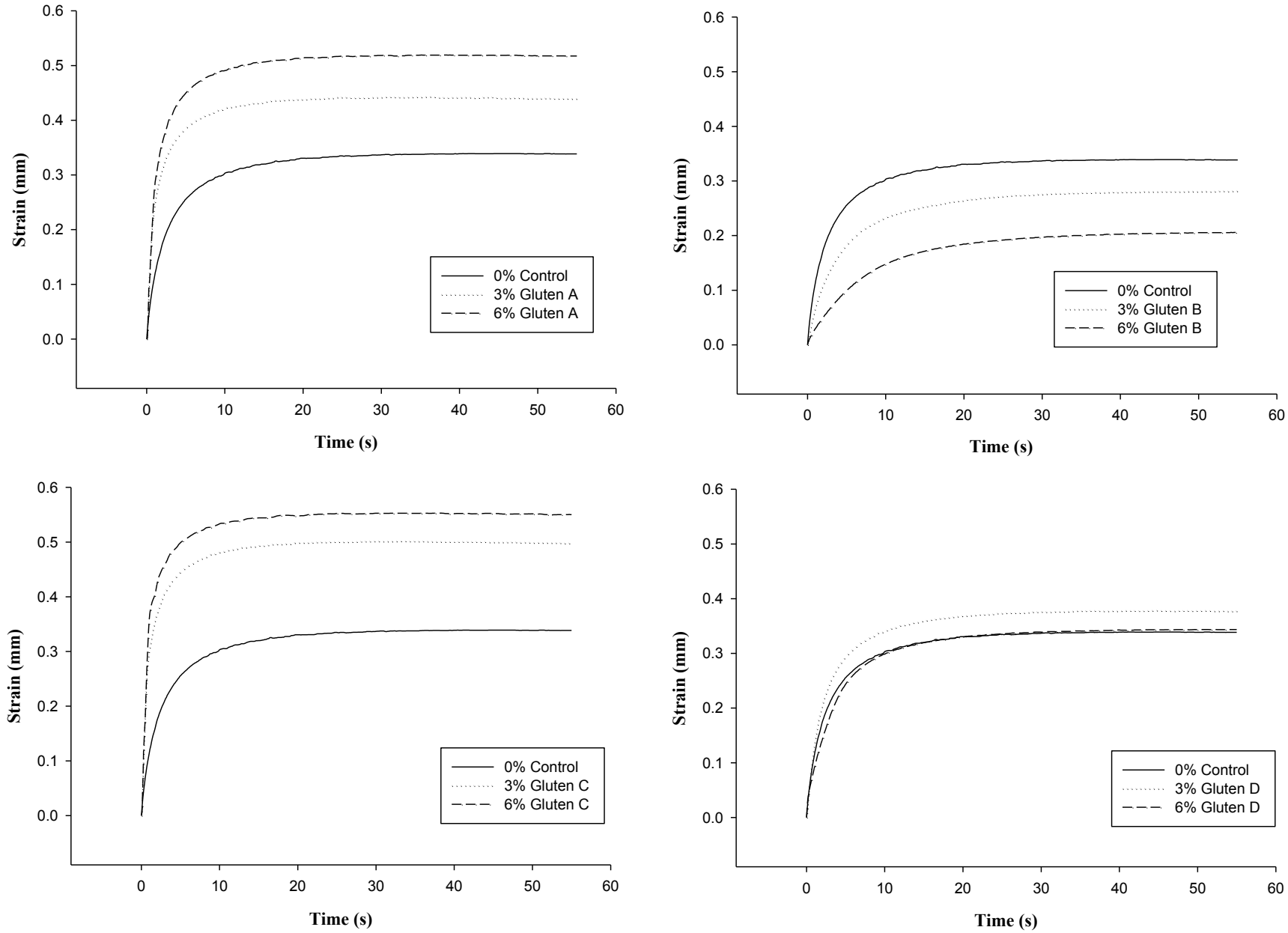

Figure 9. Typical strain curves of gluten system (Flour F5) and two substitution treatments during the recovery of compression-recovery test

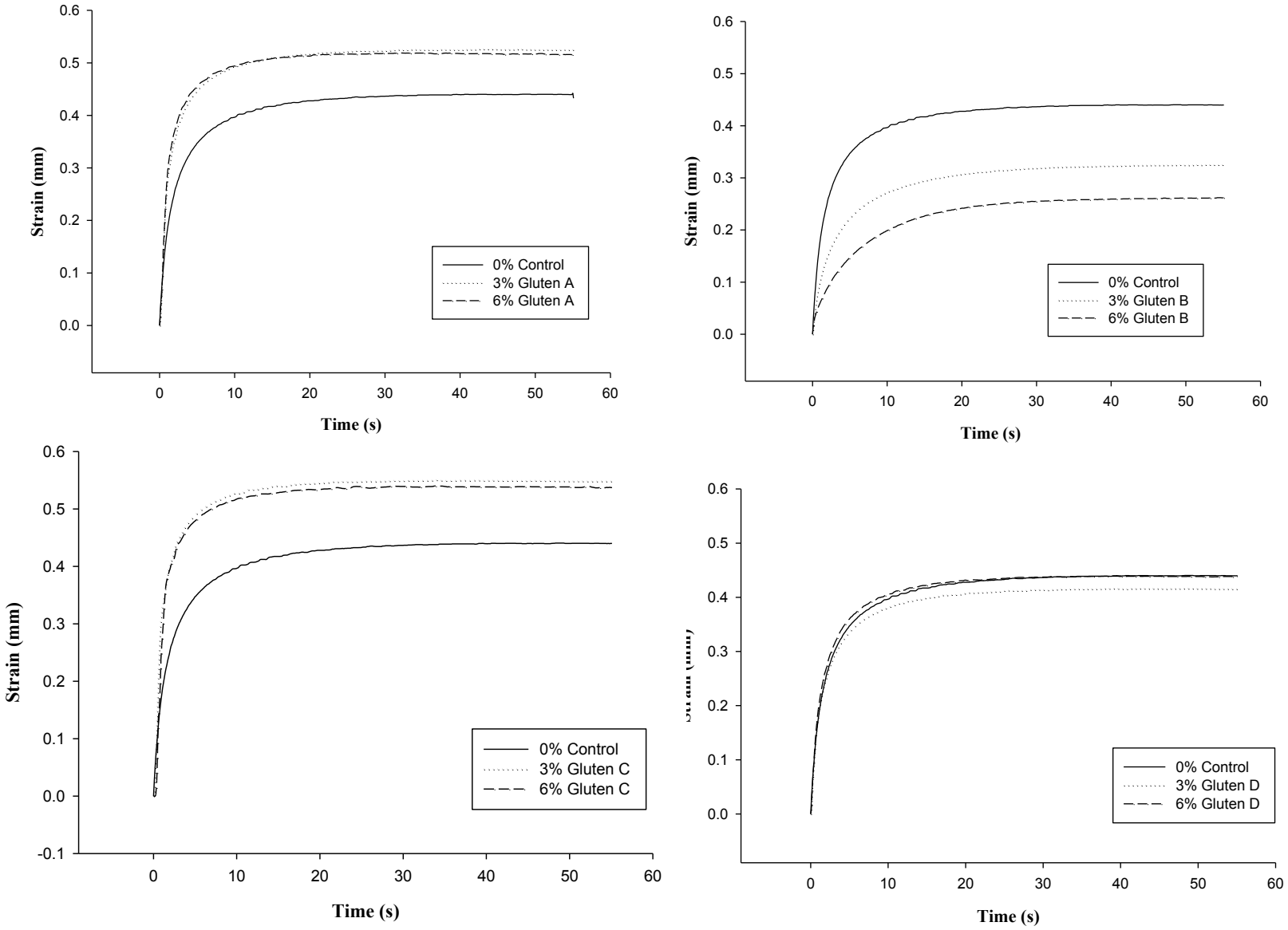

Figure 10. Typical strain curves of gluten system (Flour F6) and two substitution treatments during the recovery of compression-recovery test

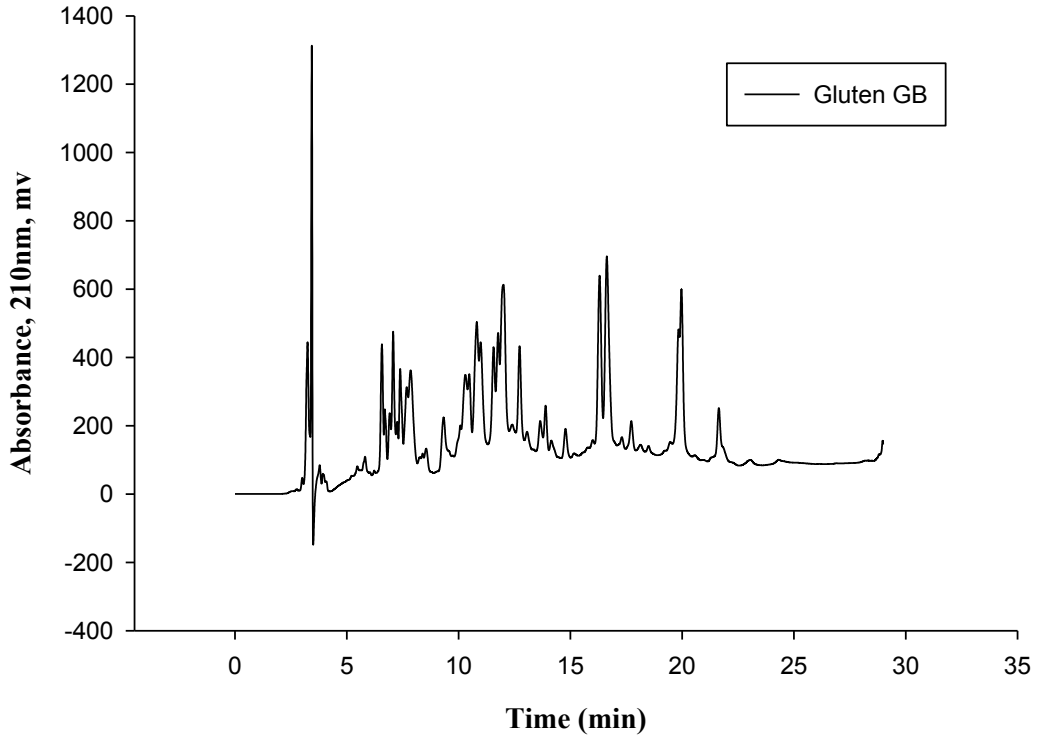

Figure 11. Reverse phase high-performance liquid chromatography profile of the prolamin fraction soluble in 70% ethanol of gluten product GB

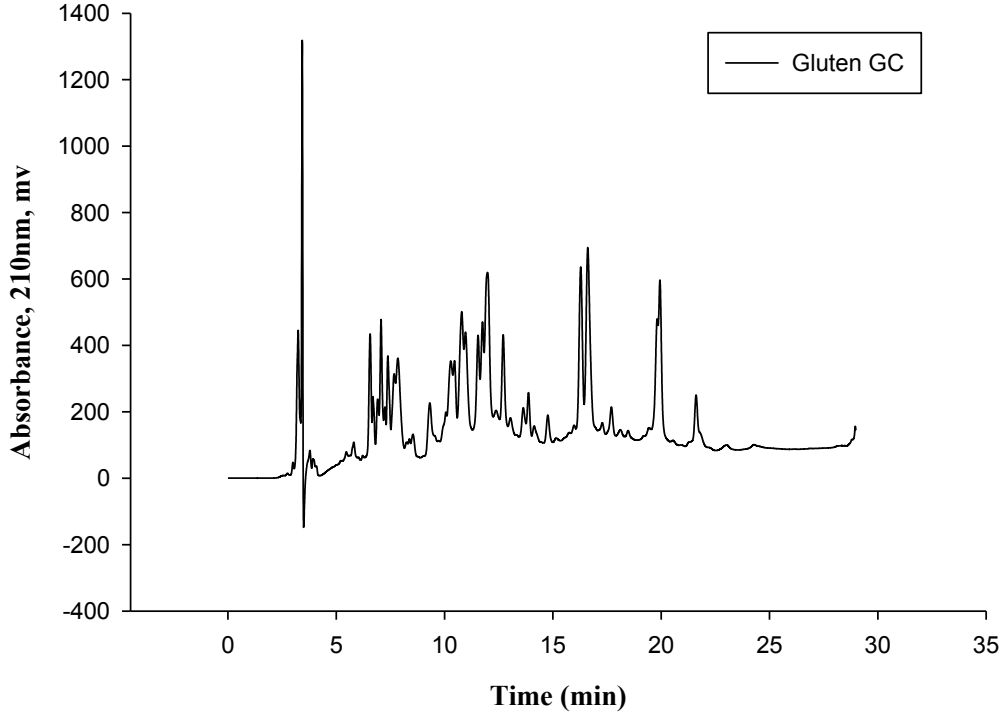

Figure 12. Reverse phase high-performance liquid chromatography profile of the prolamin fraction soluble in 70% ethanol of gluten product GC

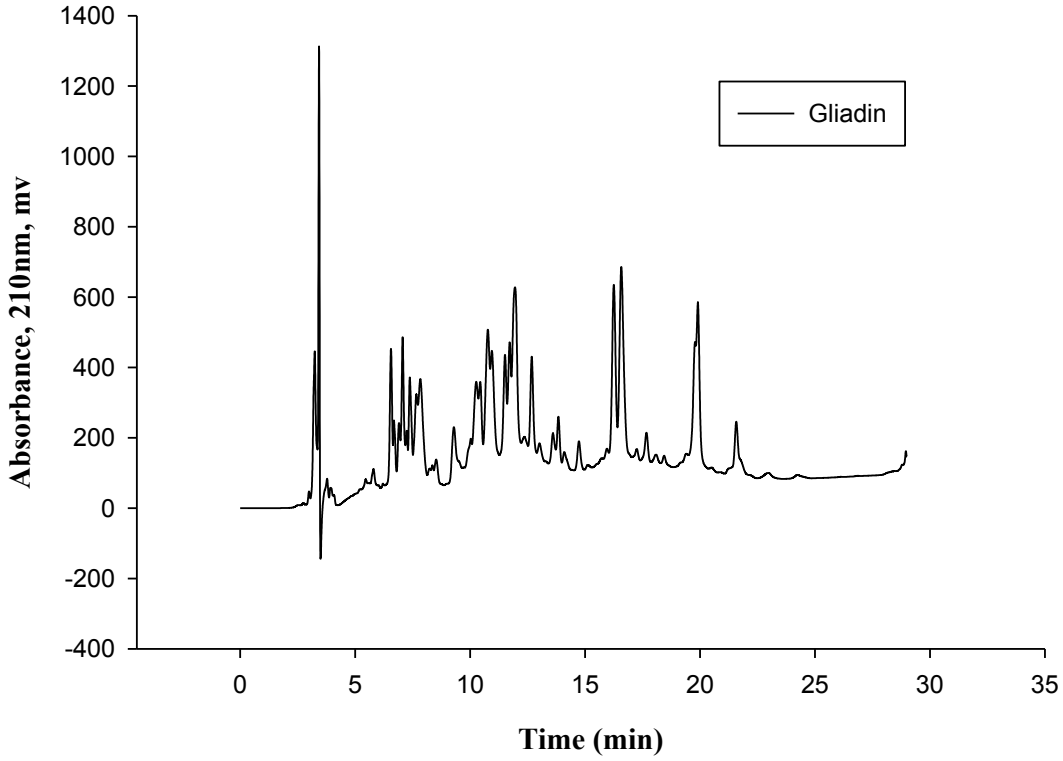

Figure 13. Reverse phase high-performance liquid chromatography profile of the prolamin fraction soluble in 70% ethanol of gluten product gliadin

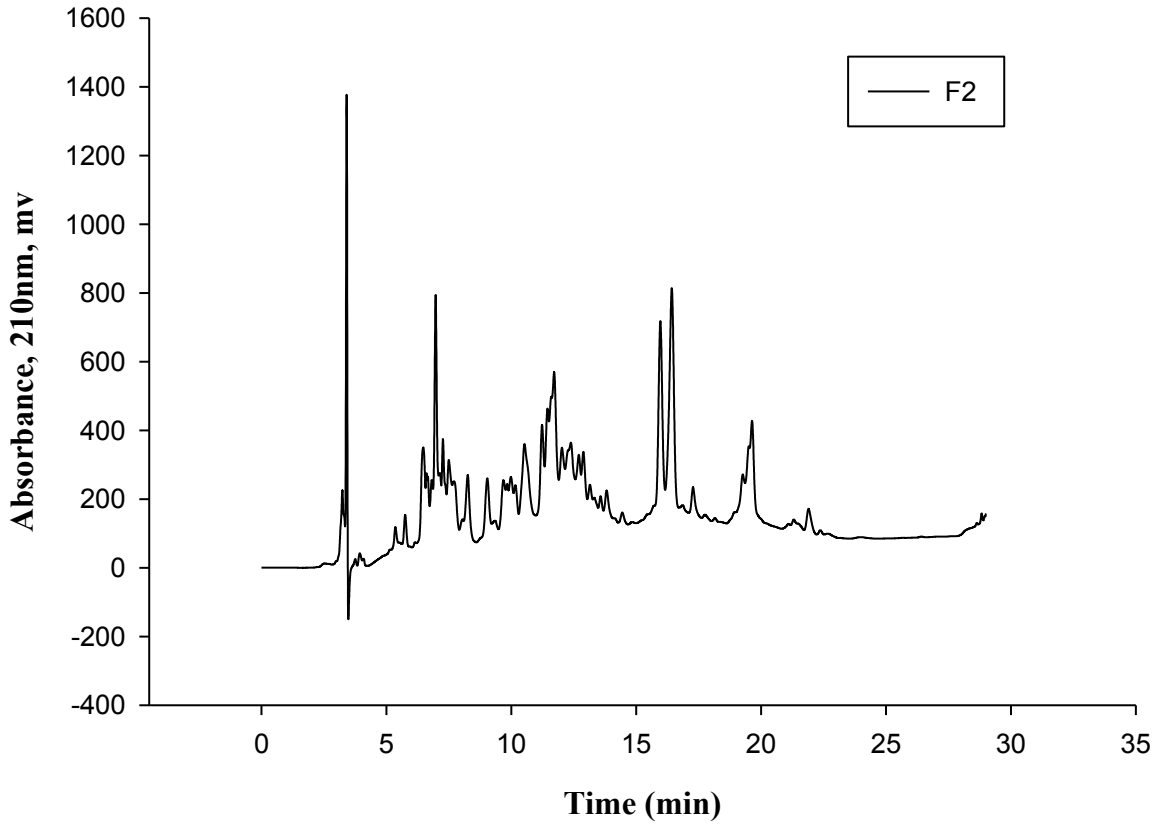

Figure 14. Reverse phase high-performance liquid chromatography profile of the prolamin fraction soluble in 70% ethanol of flour sample F2

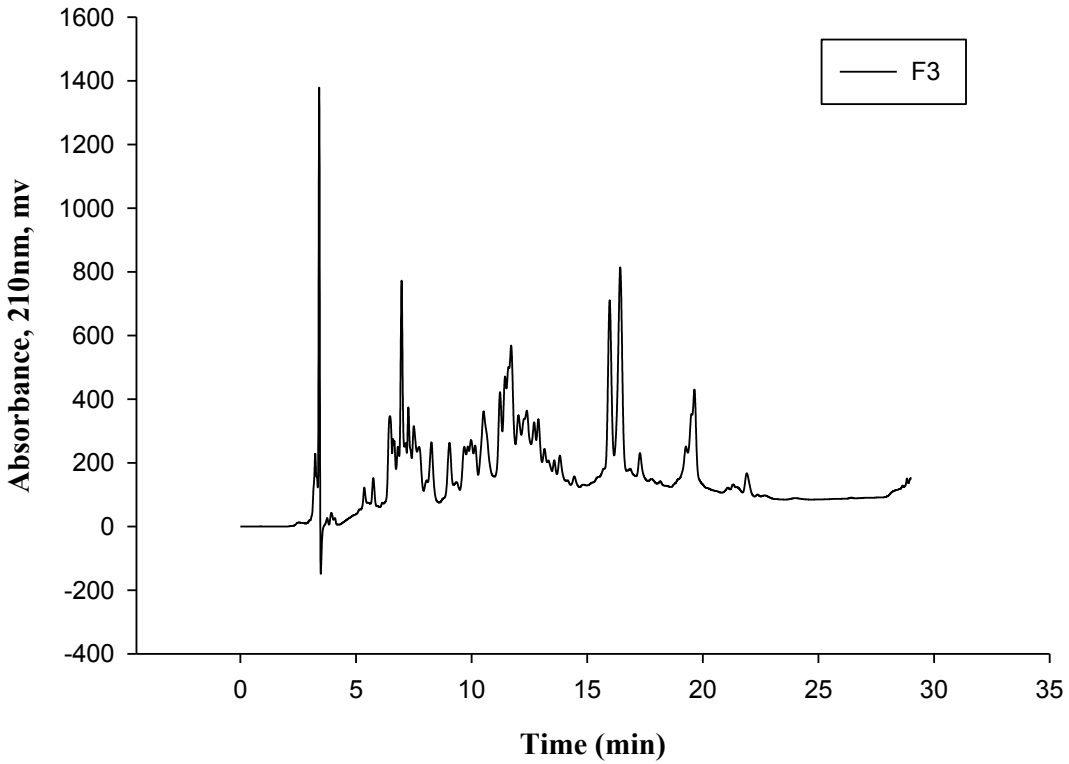

Figure 15. Reverse phase high-performance liquid chromatography profile of the prolamin fraction soluble in 70% ethanol of flour sample F3

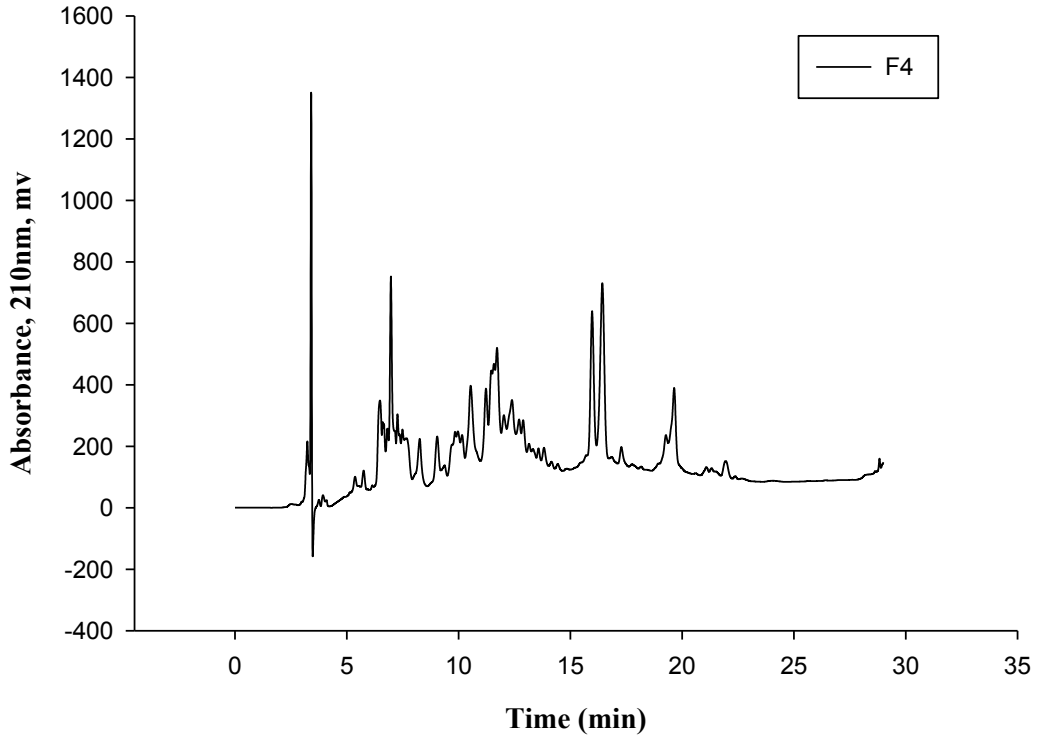

Figure 16. Reverse phase high-performance liquid chromatography profile of the prolamin fraction soluble in 70% ethanol of flour sample F4

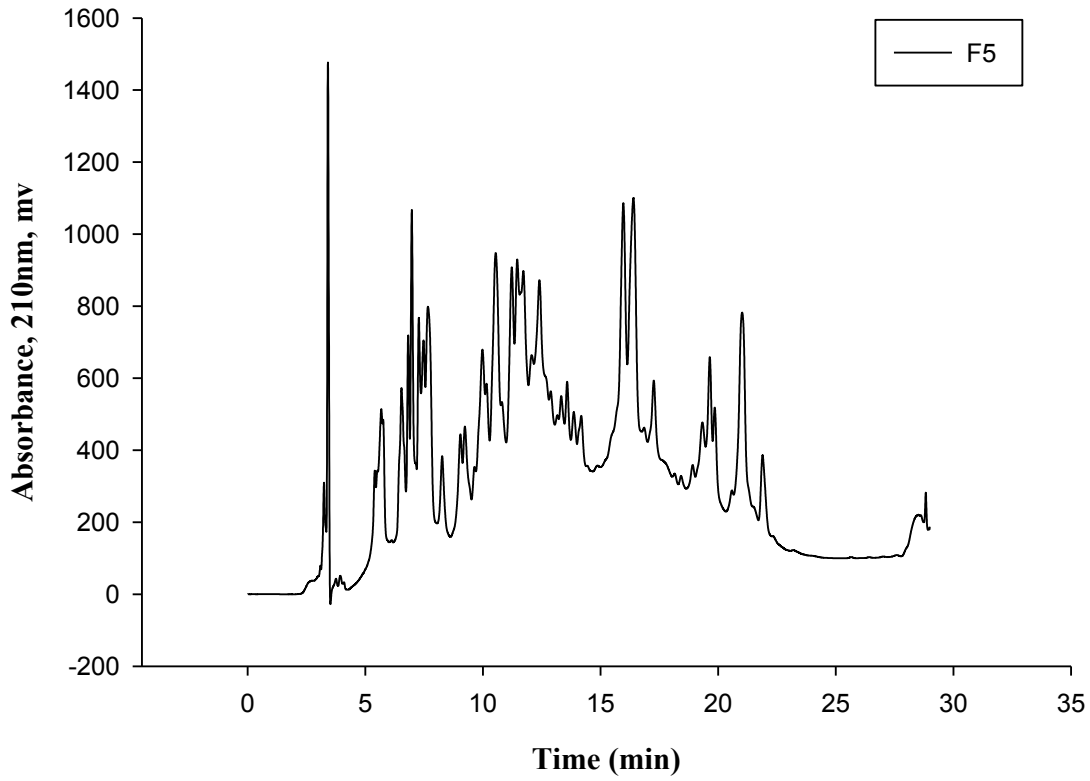

Figure 17. Reverse phase high-performance liquid chromatography profile of the prolamin fraction soluble in 70% ethanol of flour sample F5

APPENDIX II

LITURATURE REVIEW

Gluten

	Citations	Results
1)	(Barak et al., 2013c)	- They studied the relationship between compositions of gluten (i.e., glutenin and gliadin) and properties of gluten, dough and bread. - Gli/Glu ratio had a negatively correlation with dough development time, dough stability, gluten index, and protein content. - They also found that gliadin had a positive relationship with loaf volume.
2)	(Wang and Sun, 2002)	- They showed the relationship between creep recovery test of dough, farinograph, Mixograph, TA-XT2 extension and baking properties. - They found that maximum recovery strain of dough with 54% water absorption had a positive correlation with loaf volume.
3)	(Khatkar et al., 1995)	- Two wheat cultivars (Poor and good bread quality) had different rheological properties of gluten. - Gluten from cultivar with good bread quality had more elastic properties (high G' and low in tan δ) than gluten from poor bread quality. - Gli/Glu ratio had a negative correlation with the elastic of dough.
4)	(Jood et al., 2000)	- Various gluten properties (extra-strong, strong, and weak) were separated into five fractions to study their rheological properties in relation to baking performance. - Both HMW- glutenin and LMW- glutenin subunits were important for bread quality in term of viscosity and elasticity.
5)	(Hovart, 2009)	- Wheat quality was positively affected by HMW-GS 1 and 2* at Glu-A2 and the subunits 5+10 at Glu-D1 loci, and higher proportion of HMW-GS.
6)	(Khatkar et al., 2002)	- Gliadin addition (total and subgroups gliadin) affected gluten rheological properties. - Total gliadin and ω1- gliadin soften gluten, while α-,β-, γ-, and ω2-gliadin stiffen gluten tested by frequency sweep test.
7)	(Marchetti et al., 2012)	- Different qualities of gluten were extracted from flours in order to test the dough properties after adding gluten at different quality. - Low quality flour lacked 8 and 64.5 kDa of glutenin subunits and had low amount of gliadin bands. - After adding gluten from strong flour to medium and inferior flour, dough elasticity was increased.
8)	(Tronsmo et al., 2003b)	- 20 wheat cultivars grown in two different level of nitrogen were studied. - Gluten (good breadmaking quality) had a high in elastic recovery. - Gluten with high in elasticity, viscous modulus (G" and G'), and lower in tan δ had a correlation with loaf volume. - Nitrogen fertilizer level had positive correlation with gliadin (monomeric protein).

9)	(Tronsmo et al., 2003a)	- Relationship between large (SMS/Kieffer dough and gluten extensibility rig), small (stress sweep and creep-recovery test) deformation of gluten and dough and mixing properties was studied. - Elasticity and tenacity from Kieffer dough and gluten were the main contributors in PC1 which showed the quality of protein, while PC2 represented by the variability of protein content.
10)	(Wieser and Kieffer, 2001)	- Fourteen wheat cultivars were measured for their rheological properties and relationship with baking test. - Glutenin subunits and ratio of gliadin to glutenin subunits affected dough maximum resistance and gluten index. - Bread volume had a positive correlation with protein content more than types of gluten.
11)	(Barak et al., 2013b)	- Glutenin and gliadin had an effect on noodle quality. - Glutenin affected on chewiness of noodle, while Hardness, springiness, cohesiveness, gumminess, and chewiness of the noodles were negatively affected by gliadin to glutenin ratio.
12)	(Dobraszczyk, 2004)	- Entanglement and long-chain branching in HMW-GS can be indicated by strain hardening. - Strain hardening also had a positive correlation with breadmaking quality.
13)	(Esteller et al., 2005)	- Wet gluten addition helped improving hamburger buns texture, - Freeze-dried gluten improved shelf life and functional properties of hamburger buns.
14)	(Jood et al., 2001)	- HMW fraction was added into weak wheat cultivars and it improved bread quality of weak wheat cultivars. - However, the addition of LMW fraction did not improve the quality of bread from weak wheat cultivars.
15)	(Barak et al., 2013a)	- The effect of gliadin and ratio of Gli/Glu on cookie was studied. - Spread ratio of cookie had a positive correlation with ratio of Gli/Glu. - Hardness of cookie (breaking force) had a negative correlation with ratio of Gli/Glu
18)	(Sissons et al., 2005)	- Adding gluten in semolina wheat improved pasta quality. - Firmness of cooking pasta increased but the stickiness of cooking pasta decreased when adding gluten protein. - Adding glu/gli ratio to semolina increased Mixograph development time but there was no effect on Mixograph peak resistance.

Effect of temperature on gluten and dough

	Citations	Results
1)	(Tatham and Shewry, 1985)	- Heating dereased α-helical content in gliadin. - α-, β-, γ-, gliadin were stabilized by covalent disulfide bonds and non-covalent hydrogen bonds. - ω-gliadin were stabilized by strong hydrophobic interaction.
2)	(Jansens et al., 2011)	- Glutenin had very low extractability after thermomolding (130-170 ^{o}C). - Cross-linking of gluten mainly based on disulfide bonds during thermomolding but at higher temperature, non-disulfide bonds also provided force to gluten network.
3)	(Angioloni and Dalla Rosa, 2005)	- Starch gelatinization and protein coagulation process in dough were slower at high-speed mixing with salt addition. - G' increased rapidly between 55 and 70 ^{o}C because of gelatinization of starch.
4)	(Apichartsrangkoon, 2002)	- When gluten was heated at 90 ^{o}C for 0.5 to 6 h, G' and G" increased compared with unheated gluten. - They found the formation of disulfide bonds after heating at longer time.
5)	(Attenburrow et al., 1990)	- Gluten was tested with small angle oscillatory deformation at different temperature (25-100 ^{o}C). - G' decreased until 60 ^{o}C and increased after that because of gelatinization of starch. At 90 ^{o}C, G' increased dramatically postulated about an increasing of gluten cross-linking
6)	(Cuq et al., 2000)	- Gluten film was tested their mechanical properties (tensile strength and %elongation) and solubilities in 2% SDS. - Heating gluten film from 80 to 135 ^{o}C, the tensile strength increased, while % elongation and protein solubility decreased.
7)	(Gélinas et al., 2001)	- Heating commercial cookie flour at 80 ^{o}C for 15 min increased bread specific volume and crumb springiness of bread.
8)	(Gélinas and McKinnon, 2004)	- Heating soft wheat flour at 80 ^{o}C for 15 min improved extraction of gluten and dough mixing stability and development time.
9)	(Georget and Belton, 2006)	- Gluten was heated at 25-85 ^{o}C and studied by FTIR. - There was no change in gluten secondary structure at different temperature and at 0% moisture content of gluten. - They suggested that glass transition temperature of gluten was at 45-55 ^{o}C because ratio of β-sheet band intensities altered after exposed to 45 ^{o}C. They also observed irreversible changes at this condition (45 ^{o}C, 47% hydration).
10)	(Lavelli et al., 1996)	- There was no change in gluten after heating at 45 ^{o}C with DTT up to 0.02 mM. - At 65 ^{o}C, disulfide bond of HMW albumin was affected in their linkage to glutenin.

	Citations	Results
11)	(Schofield et al., 1983)	- Heat changed gluten structure at temperature above 55 ^{o}C by unfolding. - Sulphydryl group of glutenin was altered at 55-75 ^{o}C and facilitated a sulphydryl/disulfide interchange between exposed groups. - This phenomena happened with gliadin at temperature above 75 ^{o}C.
12)	(Dreese et al., 1988)	- G' of dough increased at 55 ^{o}C and decreased at 75 ^{o}C.
13)	(Khatkar et al., 2013)	- They observed gluten behavior with gliadin addition (5% and 10%) during heating. - Thermal stability of gluten decreased with an increase of gliadin addition.
14)	(Kim and Cornillon, 2001)	- They studied an effect of temperature and mixing time on molecular mobility in wheat dough. - Gelatinization of starch in dough occurred at 55 and 85 ^{o}C indicated by an increasing in G'.
15)	(Kim et al., 2004)	- Soft and hard wheat flours were suspended in water (30-80 ^{o}C for 20-60 min). - There was positive effect on particle size and temperature level because of starch-protein interactions.
16)	(Stathopoulos et al., 2008)	- Extracability of gluten was decreased after it was heated from 25 – 90 ^{o}C. - Tan delta, free SH groups, and surface hydrophobicity was also decreased begin at 40 ^{o}C.
18)	(Noel et al., 1995)	- The glass transition (Tg) of gluten was tested by using differential scanning calorimetry (DSC). - The Tg of dry gluten was within the range 137 - 144 ^{o}C except for γ-gliadin (123 ^{o}C). - Disulfide crosslink of HMW-GS was more sensitive to plasticization than the gliadin.
19)	(Kieffer et al., 2007)	- Effect of hydrostatic pressure (0.1-800 MPa) and temperature (30-80 ^{o}C) influenced differently in each gluten composition. - Low pressure and temperature increased strength of gluten. - Cohesivity of gluten was lost in 800 MPa with 60 ^{o}C. - Glutenin was strongly affected by hydrostatic pressure and temperature, while gliadin (low thio content) was affected only conformational changes.

Effect of additives on gluten properties

	Citations	Results
1)	(Nagao et al., 1981)	- This study showed the effect of ascorbate and bromate (oxidant agents) at 1200 ppm at different heat levels on gluten properties. - Bromate decreased sulphydryl (SH) content of dough more than ascorbate. - Bromate also helped stabilizing glutenin from deformation. - Glutenin was more unstable in heat than residue protein.
2)	(Hayta and Schofield, 2005)	- Oxidants altered gluten structure to be more deformable (less stiff) at high temperature. - Glutenin was affected by temperature than gliadin. - An increase in elastic modulus of gluten was slower when gluten was treated temperature and bromate.
3)	(Eckert et al., 1993)	- Oxidizing agent altered conformational rearrangements by increasing extended structure and extractability.
4)	(Bollaín and Collar, 2004)	- The addition of DATEM, high ester pectin, and transglutanimase helped dough to perform a high bread quality by showing suitable dough rheological properties (high extensibility, optimal resistance to extension, good strain hardening, and longer time of semirelaxation).
5)	(Toufeili and Kokini, 2004)	- This study showed the effect of surfactant (DATEM, SSL, and monoglyceride (MG) on glass transition behavior and gluten viscoelastic properties. - The surfactants affected gluten mainly in rubbery state indicated by in gluten rheology. DATEM and SSL softened gluten network (low in G' and G"), slowed down the beginning of cross-linking reactions on heating.
6)	(Khatkar, 2005)	- Urea (0.5 M) and urea with DTT (100 ppm) affected on gluten rheological properties. Gluten treated with urea plus DTT had lower in G' than gluten with urea. - Elastic and viscous modulus (G' and G") had a positive relationship with loaf volume. G' and G" explained 73 and 69% of variation in loaf volume, respectively.
7)	(Gao et al., 1992)	- Canadian hard red spring wheat was used in order to study an effect of DTT (20-3,000 μmol) on gluten molecular structure. - After adding DTT at 80-3,000 μmol/50 g of flour, glutenin subunits (2*, 5, 7, 9, and 10) began to reduce gradually.
8)	(Gómez et al., 2013)	- Secondary structure of gluten was induced by DATEM and SSL. - SSL at 1.0% had a greater effect in disorientation and opening gluten than DATEM. - DATEM increased α -helix conformation and decreased in β-turn and α-helix conformation.

Secondary structure of gluten measured by using FTIR

	Citations	Results
1)	(Georget and Belton, 2006)	Protein conformation was shown to be a function of flour type and degree of hydration, and temperature.
2)	(Pézolet et al., 1992)	In mildly acidic solution (0.1 M acetic acid), gluten was reported to decrease β-sheet and increase α-helixes, β-turn, and extended structures
3)	(Georget et al., 2008)	FTIR with combination of creep test using a texture analyzer were shown to be highly sensitive in distinguishing even with wheat gluten grown from different conditions such as dry/hot and wet/cold environment
4)	(Ewoud et al., 2003)	FTIR can also account for conformational changes from dough processing such as kneading and stretching which showed to increase β-sheet structure and decrease α-helixes and β-turn
5)	(Belton et al., 1995)	In dry state, gluten exhibited no secondary structure, after hydration, gluten showed an increase in mobility of protein and β-sheet structure
6)	(Feeney et al., 2003)	In a hydrated state at higher than 76% water content, FTIR showed a reduction in β-sheet and increase of β-turn
7)	(Mejri et al., 2005)	FTIR was also used to study enzymatic hydrolysis of gluten and concluded that there was a decrease in α-helices, and increase in β-turn amount
8)	(Wellner et al., 2005)	- Extension process altered the ratio of β-sheet to random and β-turn structures. - In creep-recovery test, gluten became stiffer during recovery. - The alteration in protein conformation during extension were agree with loop and train model by showing a conversion of β-turn to β-sheet. - The noncovalent intermolecular interactions played a major role in mechanical properties of gluten.
9)	(Li et al., 2006)	- β-sheet of gluten increased continuously from flour to hydrated flour and to hydrated gluten. - β-sheet of gluten increased continuously from soluble gliadin and glutenin to gluten and gel protein. - β-sheet of gluten was higher in gel protein from breadmaking flour than biscuit flour Riband.
10)	(Seabourn et al., 2008)	- Secondary structure of gluten during dough mixing was tested using Fourier transform horizontal attenuated total reflectance (FT-HATR). - β-sheet, α-helices, and β-turn increased during mixing. This result suggested that gluten had more ordered conformation.
11)	(Lambourne et al., 2010)	- The repetitive domain of low molecular weight of gluten had an extended conformation, while the non-repetitive domain had compact globular structure majority in alpha-helix. - Both of repetitive and non-repetitive domains may interact with each

		other based on a more compact conformation.
12)	(Meziani et al., 2011)	- Freezing at -40 ^{o}C reduced elasticity of frozen sweet dough to 12%. - Protein in frozen sweet dough aggregated by decreasing α-helix and increasing β-sheet extended.
13)	(Wellner et al., 1996)	- Secondary structure of ω-gliadin had β-sheet in dry state more than native state. - ω-Gliadin at moisture content higher than 35%, β-sheet content decreased and replaced by extended structure and intermolecular β-sheet structure resulted in more ordered structure.

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