MODIFICATIONS IN VISCO-ELASTICITY OF GLUTEN BY DIACTEYL TARTARIC ACID ESTER OF MONOGLYCERIDE (DATEM), ASCORBIC ACID, UREA AND DITHIOTHREITOL AND ITS EFFECTS ON MIXING AND BAKING PROPERTIES IN COMMERCIAL WHEAT FLOURS

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CHAPTER I

INTRODUCTION

Statement of problem

Variations in annual wheat crops due to genetic and climatic factors result into differences in end use quality in wheat flours. End use quality affects the quality of yeast leavened bread products. In order to maintain the quality of bread, the baking industry uses dough improvers such as surfactants and oxidizing agents. Although the improver effect of these additives is widely studied in dough system, its effect on gluten visco-elasticity has not been examined. Gluten is an important functional ingredient of wheat flours that comprises about 80% of its total protein content. Very little evidence is available on the quantification of fundamental visco-elastic properties of gluten and its correlation to the mixing and baking properties. Gluten is made up of gliadins and glutenins. Gliadins impart viscosity while glutenins are responsible for elastic strength of gluten. Gliadins are low molecular weight monomeric protein molecules while glutenins are made up of low molecular weight as well as high molecular weight polymeric subunits. Surfactants have been reported to improve loaf characteristics and crumb texture but their exact molecular mechanism is not known. Effect of surfactants on visco-elasticity of gluten is poorly studied.

Furthermore, these monomeric and polymeric proteins are entangled and crosslinked together with disulfide linkages as well as secondary noncovalent hydrophilic and hydrophobic bonds. Formation, breakdown and reformation of these disulfide and hydrogen bonds is brought about by surfactants, oxidizing and reducing agents. An effect of oxidizing agents such as ascorbic acid in promoting the disulfide linkages and its correlation to gluten visco-elasticity and baking and mixing properties of flours is not very well understood. Effect of disruption of hydrophilic and hydrophobic bonds with displacement of water molecules with agents like urea on visco-elasticity of gluten has not been studied to understand the importance of noncovalent hydrogen bonds in dough systems. The mechanisms by which reducing agents such as dithiothreitol dislocate disulfide linkages changing the structure and distribution of gluten and its effects on mixing, baking and visco-elasticity of dough and gluten are not understood fully.

Purpose of the study

The objectives of this study are 1) to quantify the visco-elastic properties of gluten extracted from commercial hard red winter wheat flours with different protein content and 2) to measure and correlate the effect of diacetyl tartaric acid ester of monoglyceride (DATEM), ascorbic acid, urea and DTT on the visco-elastic properties of gluten and mixing and baking properties of wheat flours.

Hypotheses

1) DATEM strengthens the gluten by improving its visco-elastic properties and as a result improve the mixing and baking quality of wheat flours.
2) Oxidizing effect of ascorbic acid promotes disulfide bonds in gluten and improves its visco-elastic potential resulting into improved baking performance.

3) Urea disrupts noncovalent hydrophilic and hydrophobic bonds in gluten decreasing the visco-elastic characteristics of gluten and baking and mixing performance of wheat flours.
4) DTT reduces gluten by severing the disulfide linkages within gluten proteins reducing the visco-elastic ability of gluten and mixing and baking properties of wheat flours.

Assumptions

Addition of surfactants and oxidizing agents, the distribution of low and high molecular weight subunits of gluten could be modified which could affect the dough properties of baking, mixing and visco-elasticity.

DATEM is an amphiphilic molecule with hydrophobic and hydrophilic domains. DATEM will orient itself in the gluten and dough with its appropriate moieties. When subjected to practical stress during baking processes, due to the breakdown and formation of different crosslinks present in the gluten and shifting and mobility of polymeric and monomeric subunits of gluten, visco-elastic properties are affected. Structure of gluten is changed with folding and unfolding of gluten which in turn affects dough strength. We assume that DATEM will decrease the surface tension in gluten and dough, align itself in the interface of protein, starch and bubbles in dough or interface of protein and air in gluten and maintain the integrity of the dough and gluten structure. We also assume that DATEM will increase the quality of weak flours with low protein content by strengthening the gluten quality and dough structure.

Ascorbic acid reacts with oxygen during mixing and is oxidized to dehydroascorbic acid (DHA). Dehydroascorbic acid reacts with endogenous glutathione

(GSH) and converts it to its oxidized form (GSSG). The interchange of sulfhydryl (-SH) to disulfide linkages (-SS) in high and low molecular weight glutenin subunits and gliadins in gluten results in improvement of dough strength and enhanced loaf properties. We assume ascorbic acid addition to gluten will promote the disulfide linkages and strengthen it improving the quality.

Urea competes with water to form hydrogen bonds. We assume that addition of urea in gluten and flours, result in displacement of bulk water from the system and disruption of secondary non covalent hydrophilic and hydrophobic crosslinks in gluten proteins. This disruption of non covalent bonds in protein can affect the visco-elastic properties of gluten and the integrity of dough resulting in poor performance in baking and mixing properties.

DTT is a reducing agent that will promote the conversion of disulfide linkages to sulfhydryl (-SH) in gluten. Since disulfide bonds in high molecular weight glutenin subfractions are known to form a backbone of gluten proteins, structure of gluten will change due to formation of gluten proteins into smaller size polymers. This will have a negative effect on the quality of gluten and its visco-elastic properties resulting into reduction of baking and mixing ability of dough.

CHAPTER II

REVIEW OF LITERATURE

1. Wheat Quality

Wheat is one of the primary grains consumed by humans and is grown around the world in diverse environments from cool rain-fed to hot dry-land areas. It has long been recognized that productivity and quality vary considerably as a result of environmental conditions. Among hard-endosperm wheat, protein amount and composition are primary determinants of flour functionality. At the biochemical level, composition of flour protein depends primarily on genotype but significant interactions with production environment are common (Graybosch, Peterson, Shelton & Baenziger, 1996). Both genotype and environment, and their interaction, affect the relationship of flour protein composition to loaf volume (Huebner, Nelsen, Chung & Bietz, 1997). Yield is a major concern for wheat growers, while millers and bakers cite variability in the functional properties of flour as one of their biggest problems. Despite years of research, critical gaps in our understanding of factors controlling yield and quality remains. Protein content is used as important quality parameter in end use of wheat if only similar protein quality cultivars are selected (Bushuk, 1998). The term quality is used to indicate the performance of a cultivar, at a specific protein level, in a test that reflects a specific end product, e.g., bread from hard common wheat, pasta from durum wheat, or cookies from soft common wheat (Peterson, Graybosch, Baenziger & Grombacher, 1992). A study comparing the

responses of higher protein content older cultivars to low protein content modern cultivars in Nebraska reported high tolerance over mixing and average mixing times of the latter with genetic improvements (Fufa, Baenziger, Beecher, Graybosch, Eskridge & Nelson, 2005).

2. Gluten composition and properties

Gluten is one of the important functional ingredients of wheat flours that impart a structural back bone to the bread. Gluten is a composite of two protein groups; gliadins and glutennins. Gliadins are monomeric low molecular weight (28,000 to 55,000 Da) proteins linked by interchain disulfide bonds. Non reduced glutenins on the other hand consists of a mixture of low and high molecular weight proteins (ranging from 500,000 to 10 million Da). Presence of hydrogen bonds, ionic bonds, hydrophobic interactions and disulfide crosslinks are decisive in expression of wheat dough characteristics (Wieser, 2007). Mature wheat contains about 8 to 17 % of protein and gluten constitutes about 80% of the total protein that confer properties of elasticity and extensibility that are essential for the functionality of wheat flours (Shewry, Tatham, Barro, Barcelo & Lazzeri, 1995). The gluten proteins consist of monomeric gliadin components and polymeric glutenin units. High molecular weight subunits (HMW-GS) in glutenins that comprise only about 10 % of total flour protein and act as important determinant of bread making quality (Dupont & Altenbach, 2003). The ability of low molecular glutenin sub units to form intermolecular disulfide bonds with each other as well as with the HMW-GS is also important for formation of glutenin polymers and pasta making characteristics (D'Ovidio & Masci, 2004). A study by Bushuk (1998) established that loaf volumes were not only dependent on protein content but also the quality and composition of the

glutenins. The same study concluded that loaf volumes were inversely related to the proportion of acid soluble glutenin fractions and directly related to acid insoluble glutenin fractions. Significantly high correlations were obtained between relative quantity of unextractable polymeric protein in total protein and dough resistance (r = 0.88) and with loaf volumes (r = 0.74) (Gupta, Khan & Macritchie, 1993). In another approach, Khatkar, Bell & Schofield (1995) reported that elasticity and gliadin to glutenin ratios are inversely related, thus suggesting the importance of the glutenin sub fractions in the visco-elastic of gluten. Low gliadin to glutenin ratios has higher amounts of glutenins and amount of high molecular sub fractions could be higher that could contribute to strength of gluten by offering resistance to deformation.

3. Visco-elasticity of gluten

Since the conventional molecular size distribution techniques such as the size exclusion HPLC are limited in the efficiency to fractionate the insoluble HMW-GS components, other techniques like visco-elastic have been used as a sensitive indicator of changes in the structure of HMW-GS fractions (Dobraszczyk & Morgenstern, 2003). HMW-GS polymers of gluten have shown to have long chain branching structure every 40 to 50 nm. These structures gives rise to the strain hardening (non linear rapid increase in viscosity with increased strain) that is highly sensitive to the degree of entanglement and presence of long chain branching (LCB) in the HMW polymer (Humphris, McMaster, Miles, Gilbert, Shewry & Tatham, 2000). In order to quantify the measurements of viscosity, elasticity and gluten strength, more fundamental rheological methods and instruments are used. Dynamic oscillation testing measures the elastic and viscous moduli of a sample by applying oscillating stress or strain with time. But a major

disadvantage of this test is that it cannot replicate the stress conditions that are actually applied during the process of baking (Bloksma, 1990). Many rheological tests are carried out by small deformations that give information about structure of gluten dough but in order to simulate conditions during actual fermenting process, large deformations tests are performed to obtain information on the mechanical properties of dough (Kokelaar, van Vliet & Prins, 1996). Extensional testing has been performed in two different modes, uniaxial that involves stretching a sample in a single direction and biaxial where a sample is stretched in two opposing direction. This is one of the large deformation tests that apply a large load of stress comparable with forces applied during actual baking.

Creep recovery tests were first used in the 1930s wherein the stress applied is constant and deformation (creep) in the sample is measured along with its recovery when the stress is removed. This method has been found reliable with high protein content and better quality wheat flours in which elasticity was increased with a greater recovery (Wang & Sun, 2002) and maximum creep strain served as a estimate of wheat dough strength in durum wheat flours (Edwards, Dexter, Scanlon & Cenkowski, 1999).

The strain rates used during the actual baking and proofing are much higher ranging in several hundred percent (during gas formation in proofing) in comparison to 1% in dynamic oscillatory tests (Amemiya & Menjivar, 1992). It has been known that the changes in viscosity by shear and small deformations have been similar at lower strain rates but these viscosities drastically change in large deformations. Creep recovery experiments have been performed on bread dough with higher strain rates than the dynamic oscillatory tests and has yielded significant correlations among maximum recovery strain and bread volumes (Wang et al., 2002). Studies using high stresses (250

Pa) on dough had correlation of r = 0.79 among the maximum recovery strains and bread volumes (Van Bockstaele, De Leyn, Eeckhout & Dewettinck, 2008). When creep recovery experiments were conducted at 250 and 50 Pa stresses, the former showed significant correlation ($r = 0.68$, $P < 0.01$) found between maximum recovery and bread volume (Tronsmo, Magnus, Faergestad & Schofield, 2003). No significant differences were found at 50 Pa stress level.

4. Bread quality

Baking technology that consists in producing bread from industrial refrigerated or frozen or non-frozen bakery goods and retailing them to the bakery shops and supermarkets for the final baking, has many advantages and among them the standardization of product quality is very important. Analysis of bread quality includes loaf weight, loaf volume (determined by rapeseed displacement in a loaf volume meter), proof heights that measures the height of leavened dough due to expansion of bubbles during proofing, loaf heights after removing from oven and oven spring which is difference in loaf and proof heights (Rosell, Rojas & Benedito de Barber, 2001). Furthermore quality of bread is also assessed by different grading methods. Evaluation of crust color, crumb color, crumb cell structure similarly, loaf structure, color, shape, texture are attributed to the bread quality determination (Basman, Köksel & Ng, 2002).

5. Surfactants in breadmaking

Emulsifiers are surface-active agents with hydrophilic and lipophilic properties. Surfactants reduce the surface tension between two immiscible phases and forms emulsions. The ratio of hydrophilic domain to lipophilic domain mainly determines the emulsifying potential of the surfactant. This ratio is called hydrophilic lipophilic balance

(HLB) and is scaled from 0 to 20. The surfactant with higher HLB increased the dough extensibility and resistance evaluated using Alveograph measurements (Addo, Slepak & Akoh, 1995). The surfactants are further classified according to their ionization potential; ionic and nonionic. The ionic emulsifiers, namely cationic (not used in foods) and anionic emulsifiers, are used for different purposes during baking. Nonionic surfactants such as sucrose esters of fatty acids and ethoxylated mono-diglycerides do not dissociate in water and exhibit excellent dough strengthening properties (Stampfli & Nersten, 1995). Commonly used surfactants in bakery industry are diacetyl tartaric acid esters of monodiglycerides (DATEM), sodium stearoyl-2-lactylate (SSL) and calcium stearoyl-2-lactylate (CSL). These surfactants are excellent dough strengtheners and anionic in nature (Stampfli et al., 1995).

Although the mechanisms of surfactants in dough strengthening are not fully understood, theories suggests that effective surfactants form a thin interfacial layer in between the gluten and starch granules that improved the integrity of the dough during baking (Stampfli et al., 1995). Bread staling is another undesirable phenomenon that can be ameliorated using surfactants. Mono-diglycerides at 0.3% and SSL at 0.5% w/w flour basis have been shown to be very good crumb softeners as they showed 42% softening of crumb over the controls (Armero & Collar, 1998).

Numerous studies have been carried out to determine the role of surfactants in bread making. Emulsifiers have been suggested to form complexes with gluten proteins and protein-protein aggregates that increase the strength of gluten matrix resulting in increased dough height during proofing (Gómez, del Real, Rosell, Ronda, Blanco & Caballero, 2004). Keller, Orsel and Hamer (1997) reported the ability of gliadin to form

complexes with not only albumin monolayer but also SSL monolayer by inserting itself into lipid monolayer due to competing surface activities. Dough-strengthening effects like enhanced tenacity, visco-elasticity, improved loaf volumes, crumb softness and anti-staling effects were not observed at 0.7% (w/w flour basis) concentrations of emulsifiers (Gómez et al., 2004).

DATEM is produced synthetically by reaction of diacetyl tartaric anhydride with monoacylglycerol with stearic acid as the main hydrophobic component. DATEM components on isolation yield three different components, a monocylglyceride group as major component, two carboxyl groups and a third group of esterified tartaric acid residues, all three play different role in baking activity (Koehler, 2001b). Different mechanisms of action of DATEM could be due to positive role of carboxyl group in visco-elastic of dough and gluten but did not improve the loaf volumes at 0.1% (w/w flour basis) (Koehler, 2001a). Optimum concentration of DATEM in wheat flours suggests that concentrations above 0.5% w/w flour basis produced no significant change in the visco-elasticity, dough properties and baking (Koehler & Grosch, 1999).

The growth and control of gas phase in baking is important determinant of final bread quality and textural attributes. During the proofing stage the bubbles slowly expand, producing increase in volume. As the volume continue to increase, coalescence or rupture of adjacent bubble walls leads to the cessation of bubbles and typical open sponge-like structure we know as bread (Dobraszczyk, 2004). Thus, the integrity of the cell wall structure surrounding the bubbles is extremely important in relation to gas cell stabilization and gas retention during proving and baking, and to the final structure and volume of the baked product. Small air bubbles infused in the dough during mixing give

better crumb texture than large ones. Large bubbles are removed during the punching process of dough. Surfactants reduce the surface tension at the interface of bubbles aiding infusion of small bubbles during mixing and reducing the coalescence (rupture) during proofing thus contributing fine crumb structure (Campbell & Mougeot, 1999). DATEM levels of 0.4 to 0.7% were effective in enhancing bubble breakup during mixing, increasing surface areas for mass transfer and reducing the partial pressure of CO_2 resulting into improved baked volumes (Campbell, Herrero-Sanchez, Payo-Rodriguez & Merchan, 2001).

6. Oxidizing agents and breadmaking

In commercial flours, ascorbic acid is added as an oxidizing agent to the wheat flours to promote disulfide cross-linkages in gluten proteins. Ascorbic acid interacts with oxygen during mixing and is oxidized to dehydroascorbic acid that is mainly responsible for oxidizing the sulphydryl groups in gluten proteins. Improver action of L-ascorbic acid (L-AA) and corresponding oxidized product L-dehydroascorbic acid (L-DHAA) has been studied by various research groups. During mixing L-AA interacts with atmospheric oxygen and is oxidized to L-DHAA (Gerhard Mair, 1979). It is generally accepted that L-DHAA is the actual oxidizing agent. However, there are many postulates as to the ways in which L-DHAA exerts its improver effect. The disulfide bond formation that improves the loaf volumes are believed to be produced by the catalytic oxidation of sulphydryl groups in dough by dehydroascorbate reductase (Tsen, 1965). A more popularly accepted theory was proposed by Grosch and Wieser (1999) suggesting that the enzyme glutathione reductase (GSH-DH) was readily oxidized by L-DHAA to form oxidized glutathione (GSSG) during mixing which reacts with protein thiols. Another assumption

by Kuninori and Nishiyama (1993) states that GSSG promotes inter protein disulfide bonds through disulfide-thiols interchange reactions. A spectroscopic method of measuring the levels of L-DHAA in perchloric acid extract of wheat flour samples at 265 nm was studied concluded (Every, 1996) rapid increase of L-DHAA occurred during mixing when L-AA was oxidized. High molecular weight water soluble flour fractions with reduced glutathione influenced the baked volumes and affected the crumb structure negatively (Every, Simmons, Sutton & Ross, 1999). This could suggest that effect of oxidizing agents is primarily specific to dough mixing properties. Ascorbic acid increased mixing properties, maximum resistance to extension and loaf heights. L-AA had greater dough strengthening effect in form of mixograph peak time and resistance to extension in low quality wheat flours than the high quality ones (Aamodt, Magnus & Faergestad, 2003). A recent study predicted that the improver action of ascorbic acid (100 ppm) on dough rheology, mixing and baking is pronounced on strong wheat containing high percentage of unextractable polymeric protein (%UPP) in both flour and in total polymeric protein (Every, Motoi, Rao, Shorter & Simmons, 2008). Significantly high correlations were obtained between baking score, dough development time and maximum resistance to extension ($r = 0.75$ and $r - 0.57$, respectively) at $P < 0.05$.

Gluten proteins form different bonds types that directly affect the performance of wheat flours. They form cross-links and entanglements with hydrogen bonds and disulfide bonds which play a major role in folding and unfolding of protein matrix (Edwards, Peressini, Dexter & Mulvaney, 2001). Hydrophilic and hydrophobic interaction within gluten moieties during baking, stabilize the bubble formation and influence the quality of baked bread. Although it is widely accepted that disulfide

bonding provides a strong elastic backbone to the dough, interactions between non covalent hydrogen bonds and glutamine residues in protein are also very important in baking quality (Shewry, Halford, Belton & Tatham, 2002). The ability of gluten to form non covalent hydrogen bonds and disulfide bonds during baking in response to DATEM or ascorbic acid may be dependent on the protein content of the flours for the particular year and the quality of gluten and protein (Aamodt et al., 2003).

7. Urea and DTT

Recent studies indicate that hydrogen bonding between adjacent high molecular weight glutenin subunits may play an important role in stabilizing the structure of gluten (Belton et al., 1995). The role of hydrogen bonds explained by Belton (1999) suggests presence of large amounts of glutamine residues in high molecular weight glutenin subunits. These glutamine residues are repeatedly form sequences with amino acids with inter-molecular and intra-molecular hydrogen bonds. On hydration of gluten, hydrogen bonding with water increases. When the gluten is deformed on small extension, the hydrogen bonds break. When the stress is released, the structure relaxes returning to equilibrium compensated by increased entrophy with release of hydrogen bonded water and reformation of hydrogen bonds.

The ability of reduced and disulfide linkage free high molecular weight glutenin fractions to form branched hydrogen bonding structures was estimated with atomic force microscopy (Humphris et al., 2000). Branching arose from intermolecular hydrogen bonding between glutamine side chains and amide groups of polypeptide chains. Thus, the presence of specific amino acid residues in the gluten matrix could facilitate the hydrogen bonding and structural integrity of gluten depending on its composition. Gluten

treated with urea (1 to 5 M) showed increased elasticity when analyzed in the linear visco-elastic region due to disruption of hydrogen bonding (Inda, 1991). A similar study suggested elasticity decreased at urea concentrations of 0 to 3 M and increased at concentration of urea above 3 M. Strong and weak gluten treated with DTT at 500 ppm showed 60% decrease in elasticity in strong gluten compared to 42% decrease in weak gluten (Khatkar, 2005).

The objectives of this study are, 1) to understand the effect of reducing the surface tension using different levels of DATEM on visco-elastic properties of gluten and baking performance of flours of different protein content and quality; 2) to quantify the visco-elastic properties of gluten modified by DATEM, ascorbic acid, urea and DTT and correlate it with the baking performance and dough characteristics of the flours with different protein quantity and quality.

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CHAPTER III

EFFECT OF DIACETYL TARTARIC ACID ESTER OF MONOGLYCERIDE (DATEM) ON VISCO-ELASTIC PROPERTIES OF GLUTEN AND BREAD MAKING PROPERTIES IN COMMERCIAL WHEAT FLOURS.

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Abstract

A study involving incorporation of surfactants in gluten and wheat flours was performed. The objective of the study was to assess the effect of adding diacetyl tartaric acid ester of monoglycerides (DATEM) on the visco-elastic and baking potential of hard red winter wheat flours. DATEM was added to flours and gluten extracted from the flours at 0.3, 0.6 and 1.0% w/w, flour basis. Six commercial hard red winter wheat flours obtained from two different milling sites with different protein content (8 to 13.7%). Flours and gluten with no treatment were used as controls. Visco-elastic properties of gluten were analyzed with a creep-recovery method. Dough mixing properties of flour were measured using a Farinograph and baking properties were evaluated using a straight dough method on pup loaves. DATEM increased (36 to 62% range) the separation time of gluten from all flours significantly ($P < 0.05$). DATEM levels of 1% decreased delta compliance (42 to 66% range) of gluten from most flours significantly ($P < 0.05$). This increase of separation time and decrease in delta compliance of gluten indicated the strengthening of gluten due to DATEM. The levels of 1% DATEM decreased creep-recovery compliance in gluten extracted from most flours by 31 to 50%. DATEM levels of 0.6% in wheat dough showed significant ($P < 0.05$) increase in loaf volumes in all wheat flours regardless of protein content. All loaf volumes dramatically decreased with 1% DATEM. Mixing characteristics showed high correlation with flour protein content as well as baking properties. Increase in dough heights during proofing showed significant negative correlation ($r = -0.57$, $P < 0.01$) with delta compliance (viscosity). Oven spring rise was negatively correlated ($r = -0.69$, $P < 0.01$) to separation time (elasticity) and positively correlated ($r = 0.50$, $P < 0.01$) to delta compliance (viscosity).

Biplots of principal component analysis explain 65.4% of total variance. First principal component axis explained 40.1% variance and was dominated by flour protein content while second component axis explained 25.3% variance and was influenced by delta compliance (viscosity). Visco-elastic characteristics were mostly independent of flour baking properties. DATEM improved the baking potential of high protein flours from both sites and improved the visco-elastic properties of gluten in low protein flours.

1. Introduction

Wheat quality, like any other agricultural products, is subject annual variations due to environmental and genetic factors. Wheat flour quality variations are important factors in quality of baked products produced from flour. The milling and baking industry depend in the production of products made from a high level of uniformity and consistency in flour performance to meet the demands of automated, high-speed, processing facilities (Peterson, Graybosch, Shelton & Baenziger, 1998). In order to overcome the problems faced by inconsistent and non-uniform quality of wheat flours, a variety of dough improving additives are used (Azizi & Rao, 2004). To achieve consistency and uniform quality, blending of commercial gluten with wheat flours to improve dough characteristics and quality of bread is a common practice (Borla, Motta, Saiz & Fritz, 2004). Among functional food additives, surfactants have been used to improve dough properties and the quality of bread including dough strength, rate of hydration, tolerance to mixing, crumb strength, slicing characteristics, reduction of shortening in the formula, loaf volume and shelf life (Stampfli & Nersten, 1995). Diacetyl tartaric acid ester of monoacylglyceride (DATEM) is a surfactant widely used by the bread-making industry. DATEM increases the loaf volumes and improves the handling of wheat dough. Three active fractions of DATEM have been found to improve baking properties in wheat dough (Koehler, 2001a). The major active component was the glycerol molecule with a stearic acid component attached; the second fraction was the diacetyltartaric acid and hydroxyl group on the secondary carbon and the third fraction was the acetylated hydroxyl group on the primary carbon (Koehler, 2001b). Emulsifiers form complexes with gluten proteins and starch (Krog, 1981), form inter-lamellar films in

between starch and gluten and improve the retention of the gas (Stampfli et al., 1995). The polar and non polar lipids were observed in similar areas when gliadins, starch and protein lipid matrix were located when visualized by confocal microscopy (Li, Dobraszczyk & Wilde, 2004). The exact effect of low surface tension brought about by surfactants on protein-starch matrix and bubble interface and its influence on gluten visco-elasticity is not understood very well.

Different rheological methods have been used to study the visco-elastic nature of dough including extensional techniques (BollaÌn & Collar, 2004), shear oscillation (Baltsavias, Jurgens & Vliet, 1997), stress relaxation and creep-recovery (Campos, Steffe & Ng, 1997) over the past few years. Many test methods attempt to measure large deformations using the uniaxial extensional properties of doughs, such as the Simon Research Extensometer, Brabender Extensigraph, Stable Micro Systems Kieffer dough and gluten extensibility rig, but none of these gives rheological data in fundamental units of stress and strain (Dobraszczyk & Morgenstern, 2003). Large deformations are very common in processing of foods. Use of creep recovery visco-elastic testing was introduced by Bloksma (1962) and involves measurement of deformation and recovery of a sample under constant stress. Recent studies by Edwards et al. (1999), Wang et al. (2002) and Van Bockstaele et al. (2008) suggests the use of creep-recovery as a simplified approach for interpretation of visco-elasticity of gluten and its quality compared to other studies.

The objectives of this study are 1) to quantify the effect of increasing DATEM concentrations on the visco-elastic properties of gluten using commercial wheat flours

using creep-recovery technique and 2) to evaluate the effect of DATEM on the bread-making quality using commercial wheat flours.

2. Materials and Methods

Six commercial wheat flours were used in this study. They were obtained from two different sites A and B (locations kept confidential at the supplier's request) in Oklahoma. Blends of wheat were used including different cultivars and types of wheat to obtain the ranges in protein content and different physical dough and bread making potential. The flours were enriched and malted.

Wheat flours were obtained from two sites in Oklahoma (A & B), and represented three levels of flour protein (FP) content (L = low, M = medium and H = high) from each source, and three levels of DATEM (Caravan Ingredients, Lenexa, KS 66515) were used. DATEM was added to the flours at 0.3, 0.6 and 1.0%, w/w flour basis. Flours with no DATEM were used as controls. Thus, site A flours were denoted as 1A0, 1A0.3, 1A0.6 and 1A1; 2A0, 2A0.3, 2A0.6 2A1; 3A0, 3A0.3, 3A0.6, 3A1, respectively. Similarly site B flours were named, 1B0, 1B0.3, 1B0.6 and 1B1; 2B0, 2B0.3, 2B0.6 and 2B1; 3B0, 3B0.3, 3B0.6 and 3B1, respectively. The protein, moisture and ash contents were determined using the NIR system (FOSS NIR Systems Inc, Laurel, MD 20723) and results are shown in Table 1. This design was implemented in gluten visco-elastic, mixing properties in Farinograph and baking tests.

2.1. Gluten extraction

Glutens were prepared in triplicates in an automated gluten washer, Glutomatic 2200 (Perten Instruments, Sweden) from 10 g of flour. Five mL of DATEM solution (0.6, 1.2 and 2 g DATEM in 100 ml of 2% NaCl solution) was heated to 65°C for proper

dispersion. Flour was wetted using DATEM solution and mixed for 20 sec and washed for 10 min with 2% NaCl solution (w/v). Control samples were mixed with 5.0 ml of pure deionized water.

2.2. Creep recovery tests

The creep recovery of gluten were performed using the protocols of Zhao et al. (2007) and Liang et al. (2007). Creep-recovery measurements of flour-water dough were made on a Rheometer AR1000 N (TA Instruments, New castle, DE), using a 25 mm parallel-plate. The test was performed in a controlled temperature environment (25°C). The gluten samples after removing from the Glutomatic 2200 automatic washing system were rounded gently into a ball shape. The sample was relaxed under metallic plates with top plate weighing 2500 g for 60 min at room temperature (25°C) before the creep-recovery measurement.

A 0.5-g gluten sample (30 mm diameter, cut from the relaxed gluten) was loaded between the parallel plate and the gap was set to 2.5 mm. To reduce moisture loss, a plastic cap covered the sample-plate interface and the whole geometry was covered in the holding chamber. In order to maintain the humidity in the chamber, a concentric plastic container with water was placed around the sample. The peltier base attachment and parallel plate geometry were custom made with cross hatch surface to prevent slippage. TA Software for Windows (Rheology Advantage Instrument Control V.5.4) was used to program the creep-recovery experiment. A constant stress of 40 Pa was applied to shear the gluten and maintained for 100 s creep test. The stress was released after 100 s and gluten recovery was measured for 1000 s. The deformation of gluten under the stress and its recovery after the stress removed was measured as compliance by the Rheology

Advantage Data Analysis (software version 5.4.8). Time constants of logarithmic values for creep (TCC) and recovery (TCR) at 63.2% were calculated using a exponential decaying function. The calculations were modified from the method of Chaung & Yeh (2006) to describe the rate at which the creep and recovery reached equilibrium. This study used time constants for both creep and recovery instead of only for recovery that was used by Chaung and Yeh (2006) study. Equilibrium reached at a faster rate was indicated by small time constant values. The creep-recovery measurements from the software were plotted against time (logarithmic scale) for each treatment and controls. The measurements of creep (J) and recovery (J_r) compliance were superimposed on each other to depict the visco-elasticity properties such as the delta compliance at 100s ($J-J_r$) and the separation time (SeP). Rubbery plateau separation time is the time to which J and J_r are no longer superimposed and split (Fig. 2). The higher the value of $J-J_r$, less elasticity and more viscosity behavior is observed. The higher the value of SeP, the more elasticity and less viscous behavior are observed. Recoverability (RCY) in gluten is calculated by following formula: RCY = (compliance of recovery J_r at 100 s/compliance of creep J at 100 s) * 100. A graphical representation of visco-elastic parameters is depicted in Fig. 2.

2.3. Dough mixing properties

Flours were analyzed for optimal dough development time (DT), stability time (ST), breaking time (BT) and water absorption (adjusted to 14% protein content; WA) at 63 rpm and 30°C in a 10-g bowl Farinograph-E (C.W. Brabender Instruments, Hackensack, NJ) according to approved method 54-21 (AACC 2000). DATEM solution (3, 6, 10 g per 100 ml deionized water) was heated at 65° C for proper dispersion and 1

mL was added to 10 g of wheat flour sample prior to addition of pre-calculated amount of water for mixing and hydration.

2.4. Baking tests

Approved method 10-10B (AACC, 2000) consisting of 100-g flour optimized straight-dough bread making procedure was used for baking experiments. DATEM was added to the flour by dissolving 0.3, 0.6 and 1 g in 3 g of melted fat for proper dispersion. A 100-g mixer Swanson-Working pin-type, (National Mfg. Co. TMCO Inc, Lincoln, NE) was used to mix the dough. Optimum mixing times were obtained by using several baking trials. All loaves were weighed and measured for dough proof heights (PH) and loaf heights (LH) using a digital proof height gauge (National Mfg. Co. TMCO Inc, Lincoln NE) and loaf volume (LV) by rapeseed displacement 10 min after they were removed from the oven. Difference between loaf height and proof heights referred as the oven spring (OSP) was calculated (Fan, Mitchell & Blanshard, 1999). Specific volume (SV) was calculated as ratio of loaf volumes to the loaf weights.

3. Statistics

A factorial design within a randomized block design was implemented, with sites as a blocking factor. Within each site, 4 levels of DATEM and 3 levels of flour protein were compared in a 4 X 3 factorial. The significant differences in means were compared using ANOVA with Tukey's comparisons (α =0.05) in SAS programs (Version 9.1 SAS Institute Inc., Cary, NC). Variables of baking, visco-elastic and farinograph were correlated using Pearson's correlation coefficients without blocking the sites at $\alpha = 0.01$ and $\alpha = 0.05$.

The variables from visco-elastic experiments (J-J_r, SeP, RCY, TCC and TCR), dough characteristics (WA, DT, ST and BT) and baking characteristics (LV, PH, LH, OSP and SV) that are possibly correlated, were transformed into principal components. Principal component analysis (PCA) is a mathematical algorithm that reduces the dimensionality of the data (Ringner, 2008). In order to do so, PCA identifies directions of maximum variation in data called principal components. Principal components are linear combination of original variables. Variables that project greatest variance lie on the first co-ordinate called principal component 1 (PC1) and set of uncorrelated variables that project that project second greatest variance lie on PC2. Data is centered and standardized to minimize mean squared error. For each variable, a line that passes in a certain direction through its mean and minimizing sum of squared error is determined and is called as eigenvector. Eigenvector has a scalar value to indicate its magnitude called eigenvalues. An eigenvalue indicates the portion of the variance that is correlated with each eigenvector. The length of eigenvector and its proximity to the component axis is proportional to the amount of variation explained by that variable and its correlation to principal component, respectively (Ringner, 2008). Canoco for windows version 4.5 (Biometris, Plant Research International, Wageningen, the Netherlands) was used to perform PCA.

4. Results

4.1. Visco-elastic properties

Creep recovery experiments performed on the controls indicate significantly low recovery compliance values for gluten from 2A, 2B and 1B flours (Appendix 1, Fig. 1). DATEM treatments significantly reduced the recovery compliance for creep and recovery

in gluten extracted from commercial wheat flour samples from both sites in comparison to controls (Appendix 1, Fig. 2 & 3). Overall, addition of 1 % DATEM reduced the recovery compliance of gluten the most in all flours (Appendix 1, Fig. 2 & 3) except 3A, 3B and 1B which showed no significant differences in reduction at 0.6% and 1%. The ability of DATEM to reduce recovery compliance in gluten from site A was highest in low protein flours (62%) compared to medium (44%) and high (42%) protein flours (Appendix 1, Fig. 2). Recovery compliance reduction by 1% DATEM in site B gluten was 55% for high protein flour, 45% with medium protein flour and 36 % with low protein flour (Appendix 1, Fig. 3).

Significant protein and DATEM treatment interactions were observed in visco-elastic variables within both the sites except for recoverability (RCY) in site A (Appendix 2, Table 1). This means no simple statements can be made that one level of DATEM always produces a predictive effect, regardless of protein content of flour. DATEM levels increased the SeP time (elasticity) in 1A gluten by 36.4% with 0.6% DATEM and 2A gluten by 61.9% at 1% concentration (Table 3). DATEM levels of 0.6% and 1% increased SeP time in 2B and 3B by 59% and 35.8%, respectively (Table 3). Reduction in $J\text{-}J_r$ (elasticity) was brought out by DATEM levels of 1% in gluten from 1A, 2A and 3A by 56.6, 42.1 and 45%, respectively. A similar trend in reduction of $J\text{-}J_r$ with 1% DATEM was observed in 1B, 2B and 3B by 43.9, 47.8 and 66.2%, respectively. No significant differences and interactions in recoverability of gluten were noticed in sites A. (Appendix 2, Table 1). The protein and treatment effects were significant ($P < 0.05$) where the recoverability was high (84.2%) in medium protein gluten and high recoverability (83.2%) for 0.6% treatment effect (Appendix 3, Table 1). In site B, the

recoverability was not affected by treatment, only protein effect of controls was observed. Significantly higher recoverability ($P < 0.05$) of 1B0 (86.5%) was observed compared to 2B0 (80.8%) and 3B0 at 81.2% (Table 4). Time constants of creep were lowered significantly by 1 % DATEM in 1A gluten by 40% and 36% by 0.3% DATEM but no significant differences were observed in other gluten samples from both sites. Time constants for recovery were significantly lowered in 1A by 50% at 1% DATEM (Table 3). Time constants for recovery reduced by 55% and 41% in 2B and 3B treated with 1% DATEM (Table 4). In contrast, time constants increased in 1B by 42% with DATEM levels.

4.2. Mixing properties

Dough characteristics were evaluated by Farinograph measurements as shown in Tables 2 and 3 and statistical analyses in Appendix 2, Table 1. Flour protein content and addition of DATEM significantly affected dough water absorption (WA) and there was a significant interaction of protein and DATEM addition for samples from both sites (Appendix 2, Table 1). No interaction was observed in dough breakdown time (BT) in site A flours (Appendix 2, Table 1). Dough breakdown time for high protein in site B (14.4 min) was significantly high compared to other protein levels (Appendix 3, Table 1). WA was 63.6% in high protein content flours and decreased to 51.2% as protein content reduced as observed in site A (Table 3). In site B, 0.6% and 1% DATEM increased water absorption in dough with all protein contents by 4.8% in 1B and 3B flours and 6% in 2B flours (Table 4). Stability time decreased with 1% DATEM level in flours with all protein contents in site A by 55, 71 and 21% in 1A, 2A and 3A, respectively (Table 3). On the contrary, 1% DATEM increased stability time in low and medium protein flours by 83%

and 32%, respectively in site B (Table 4). No significant interactions of protein and DATEM levels were observed in DT of flours from site B (Appendix 2, Table 1). Significantly lower DT ($P < 0.05$) was observed in 1% DATEM level (treatment effect) with 1.6 min compared to other treatments levels (Appendix 3, Table 1) in site B. Dough development time for high protein in site B was significantly high with 1.96 min compared to other protein levels (Appendix 3, Table 1).

4.3. Baking characteristics

Baking characteristics such as the loaf volumes (LV), loaf height (LH), proof height (PH), oven spring (OSP) and specific volume (SV) and are shown in Table 2 and 3. Significant interactions among flour protein content and DATEM addition were observed in baking properties except for LH in site A and OSP in both sites (Appendix 2, Table 1). Significantly low LH ($P < 0.05$) was observed in 0.6% DATEM level (treatment effect) with 88.5 mm of all treatments levels (Appendix 3, Table 1) in site A. Loaf heights for high protein in site A was significantly high with 96.85 mm of all protein levels (Appendix 3, Table 1). Significantly low OSP ($P < 0.05$) was observed in 1% DATEM (treatment effect) with 13.0 and 9.56 mm of all treatments levels (Appendix 3, Table 1) in sites A and B, respectively. Oven springs for low protein in sites A and B were significantly low with 18.2 and 13.2 mm of all protein levels, respectively. (Appendix 3, Table 1). Increment in DATEM levels up to 0.6 % in baking increased LV but drastically dropped at 1% irrespective of protein content in both sites. In site B flours, 0.6% DATEM increased loaf volumes by 4% in 1B, 7.3% in 2B and 5.7% in 3B. Increase in loaf volumes in 3A (FP = 13.7%) and 3B (FP = 11.4%) were similar (table 4). Proof heights increased significantly only at 1 % DATEM level in all 1A flour by 17%, while

no significant effects on proof heights were observed in other flours (Table 3). Overall no significant changes in loaf heights were observed with addition of DATEM except for 1B which decreased with 1% DATEM (Tables 3 and 4). Increasing DATEM levels decreased OSP in all bread loaves from site A but no significant changes were observed in breads from site B. For the most part, SV increased when DATEM was added at 0.3 and 0.6% and decreased with 1% (Tables 3 and 4), except for 1A flour in which SV increased linearly with addition of 0.3 and 0.6% DATEM (Table 3).

4.4. Correlations and PCA

Pearson's correlation coefficients for variables of visco-elastic, farinograph and baking properties are shown in Table 5. Flour protein was significantly correlated ($P < 0.01$) with baking and dough characteristics (Table 5). A highly significant negative correlation between PH and $J\text{-}J_r$ ($r = -0.57$) suggest the role of increased elasticity in flours and gluten due to surface tension changes. A significant positive correlation of oven spring with $J\text{-}J_r$ ($r = 0.50$, $P < 0.01$) suggests that an increase in viscosity is associated with oven spring. Positive correlation of oven spring with recovery time constants ($r = 0.36$, $P < 0.05$) suggests that faster the rate of recovery induced by DATEM had a positive effect on oven spring. Correlations of proof height with recoverability ($r = 0.46$, $P < 0.05$), rate of deformation or TCC ($r = -0.44$, $P < 0.05$) and $J\text{-}J_r$ ($r = -0.57$, $P < 0.01$) as shown in Table 5 suggested not only the role of both viscosity and elasticity of gluten are important but also the faster rate at which the gluten deformed in the baking process (Table 5).

Principal component analyses were performed on the data sets obtained from visco-elastic, baking and farinograph parameters to get the overview of variability (Fig.

2). The two principal component axes 1 and 2 explained 66.8% variability (Table 6). Principal component axis 1 (PC1) and principal component axis 2 (PC2) explained 39.9% and 26.9% of total variance, respectively. Flour protein content (FP) has a slightly longer vector (Fig. 1) and highest explained variance (87.1%) in the first axis or PC1 (Table 5). Variables related to baking properties, loaf volume (LV), loaf heights (LH) and specific volume (SV) and mixing properties, water absorption (WA) in the order of their variation were projected on PC1. Visco-elastic variables are independent and uncorrelated to the first component axis. These variables were associated with the second component axis PC2. The highest variance (87.1%) on PC2 is explained by J-J_r with the longest eigenvector. The biplot of PC1 to PC2 shows two closely related groups of variables. The lower left quadrant grouping, LV, SV, LH and DT shows baking performance parameters are closely related to dough development time. The second grouping is observed in PC2 lower right quadrant, consists of J-J_r, TCC and TCR. This grouping is related to the visco-elastic properties of gluten. In the first quadrant, medium protein flours from site B were brought closer to PC2 axis which is dominated by visco-elastic properties. Increased DATEM levels up to 0.6% (Fig. 2) clustered the site A high protein flours close to the PC1 axis that is dominated by protein content, baking and dough characteristics as observed in lower left quadrant. DATEM increments increased the proximity of site A low protein flours towards PC1. Site A low protein flour (8% FP) and site B low protein flour with 1% DATEM showed weakest correlation to the variables (component axis) this can be explained in part by their low protein content and inferior quality.

5. Discussions

DATEM altered the visco-elastic properties of gluten by decreasing the creep and recovery compliance with increasing concentrations irrespective of protein content. In this set of samples, gluten strength was independent of the protein content (Appendix 1, Fig. 1 and 2). Low protein flour from site B exhibited stronger gluten in controls while medium protein flour exhibited higher gluten strength from site A. This study observed gluten strengthening from concentrations of 0-1% DATEM using creep-recovery in contrast to the studies of Koehler and Grosch (1999) that reported 0-0.5% and Stampfli, Nersten and Molteberg (1996) reported 1-2% DATEM concentrations in gluten using extensigraph. Changes in creep-recovery compliance of gluten were not specific to the protein content and gluten strength of 1B in site B and 2A in site A were higher than its counterparts. However, this was not found true in case of baking where increased loaf volumes were observed with 0.6% DATEM. Increased protein contents also increased loaf volumes as reported by Farvilli et al. (1997). High DATEM concentrations decreased loaf volumes in this study which agrees with reports by Campbell et al.(2001). Flours obtained from site A, showed improved elasticity (increased SeP and decreased $J\text{-}J_r$, decreased creep-recovery compliance), loaf volumes in low protein content flours with addition of DATEM. But its ability to improve the elasticity of the flour with high protein diminished. It is possible that flours with different protein content may vary with presence of low molecular weight glutenin markers that could act as predictors of dough strength and visco-elasticity of gluten (Edwards, Mulvaney, Scanlon & Dexter, 2003). Taking one specific site of flours at a time, gluten quality of site B flours had improvement directly proportional to their protein content. Similar protein contents

among the site B flours e.g. only a 0.2 % difference in between 1B and 2B showed a great amount of differences in its visco-elastic, dough and baking characteristics evidently suggesting differences in their protein quality over quantity. The stress levels applied to the gluten under creep recovery experiments were 40 Pa that seemed less than practical stress levels that the dough undergoes during actual baking process, but since gluten is only one of the components of the complex dough structure, stress levels in present study seemed appropriate. Liang et al. (2007) tested stress levels from 10 to 300 Pa and reported that stress level of 40 Pa was optimized to test creep recovery of gluten within linear visco-elastic region. Although present study used the measurements from gluten visco-elastic in linear visco-elastic region, it will be interesting to find out if a better correlation is observed at larger deformations and non linear visco-elastic region.

The flours obtained in this study were blend of different varieties with majority of hard red winter wheat (90 to 95%) optimized to certain protein content for better output. With no significant difference in protein contents of low protein content flour in site B (10.4%) and medium protein flours from same site was (10.5 %), loaf volumes of 2B flours were significantly higher than that of 1B flours (Table 4). This clearly demonstrated that along with protein content, quality of protein also influences the loaf volumes. Decrease in stability time in flours of site B with DATEM addition and contrasting effect with increased stability time in flours from site A also showed protein quality affects dough mixing properties in wheat flours.

In agreement with a similar study by Tronsmo et al. (2003) who observed less correlations between visco-elastic and baking properties, few highly significant and strong correlations were observed between visco-elastic properties and baking and dough

characteristics. Our study observed similar trends in which viscosity and elasticity of gluten were independent of most baking properties (Fig. 2). The only visco-elastic parameters correlated with baking parameters were time constants for creep recovery, delta compliance with oven spring and recoverability with proof heights (Tables 5 and Fig. 2). This suggests that added DATEM concentrations could be interacting with specific gliadin sites as well as modifying the low molecular weight glutenins (Edwards, Peressini, Dexter & Mulvaney, 2001) in some flours modifying the viscosity that in turn may increase the baking potential. Previous studies with creep recovery and baking showed very low correlations among the visco-elastic and baking variables (Wang et al., 2002).

Similarly a weak negative correlation of decreased time constants of creep with proof height suggested DATEM increased the rate at which gluten deformed could lead to increased proof height (Table 5). Weaker correlations also suggest that DATEM alone may not be the only factor that could correlate the gluten visco-elasticity to baking and dough characteristics. It is also worth noting that high DATEM concentrations (1%) that were optimum in increasing visco-elastic strength of gluten did not improve baking performance. This could mean that an interaction of high DATEM concentrations with a sole gluten component of flour at molecular level is different than its interaction in a complex colloidal mixture of dough. It is quite possible that efficacy of DATEM level to increase the loaf volumes at levels of 1% may not be stronger as it did with gluten as a single flour component due to the complex composition of dough that has starch, gluten, air , water and other minor components. Although protein content showed the highest significant positive correlation ($P < 0.01$) with dough mixing characteristics, water

absorption in site A was not affected by DATEM. On the contrary, 0.6% and 1 % DATEM increased water absorption in flours from site B. This may be due to the protein quality affecting the ability of DATEM to interact with number of hydrophilic and hydrophobic sites in the flour proteins. The rise in loaf in oven (OSP) was significantly affected by DATEM concentrations up to 0.6 % (Tables 3 and 4) and rather dropped significantly ($P < 0.05$) at 1% DATEM levels. This suggests that there is maximum effectiveness of DATEM during the thermal stages of baking and beyond that level the effect is detrimental. This could be due to the starch gelatinization and protein denaturation that affected DATEM interactions adversely during that stage. Similar adverse effect of high surfactant (DATEM + MGL) levels on mixing and baking properties was reported that weakened the structure of dough by additional adsorption at protein binding sites (Armero & Collar, 1996). Elasticity and viscosity of gluten could be playing a prominent role during different stages of baking as reported by Koehler (2001b). Ability of surfactants to improve the baking potential in flours could be function of not only the flour protein content but also the quality of gluten. High protein flours with protein ranges above 11.5% from both sites improved their baking quality with addition of 0.6% DATEM levels (Fig. 2). DATEM improved the gluten elasticity and loaf volumes of very low content protein flours (site A FP = 8%) as observed in Fig. 2 and Table 3.

6. Conclusions

Gluten strength measured by creep recovery experiments was not related to the protein content of the flours. Ability of DATEM to improve weak gluten was observed to be higher than its ability to improve the strength of stronger gluten. Visco-elastic

properties of gluten showed a strengthening effect with high DATEM concentrations in flours with different protein contents, however, the increase in loaf volumes increased with 0.6% levels. This suggests DATEM interactions in a single functional ingredient as gluten is less complicated than that in the dough. Improvement of gluten visco-elastic properties differed in flours by their location and their protein quality. Although, loaf volumes were a function of protein content with increased DATEM concentrations in each site, protein quality also influences the baking output. Viscosity and elasticity of gluten influenced different processes of baking due to surface tension modifications by DATEM. Viscosity and elasticity of gluten showed correlation to processes of baking such as oven spring and proof heights.

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Table 1. Proximate analysis of flours (means ± SD, n=2) obtained from sites A and B.

Flours	Protein (%)	Moisture (%)	Ash (%)
1A	7.95 ± 0.05	11.69 ± 0.02	0.29 ± 0.01
2A	11.19 ± 0.07	10.51 ± 0.03	0.38 ± 0.01
3A	13.68 ± 0.02	10.14 ± 0.02	0.41 ± 0.00
1B	10.40 ± 0.10	12.54 ± 0.02	0.47 ± 0.00
2B	10.59 ± 0.07	12.57 ± 0.00	0.48 ± 0.01
3B	11.38 ± 0.01	12.98 ± 0.04	0.58 ± 0.01

Table 2. Definitions of visco-elastic, mixing and baking terms.

Abbreviations	Definitions	Units
Visco-elastic		
$J\text{-}J_r$	Delta compliance defined as the difference in compliance of creep and recovery at 100 s. An increase in delta compliance suggests that the viscous component is higher than elastic component by either an increase in viscosity or decrease in elasticity of the gluten structure at 100s.	(J)
SeP	Separation time is time at which the creep and recovery split and no longer stay superimposed (Fig. 1). An increase in separation time suggests that the elastic component is higher than viscous component by either an increase in elasticity or decrease in viscosity of the gluten structure.	(s)
RCY	Percent recoverability is the elastic ability of gluten to recover to its original state after the stress is removed.	(%)
TCC	Rate at which the deformation of gluten reaches its equilibrium. Higher the value of TCC slower the rate of deformation of gluten	(s)
TCR	Rate at which the elastic recovery of gluten reaches its equilibrium. Higher the value if TCR, slower the rate of recovery of gluten	(s)
Mixing		
WA	Ability of flour to absorb water in order to form a convened dough consistency at 500 FU.	(%)
DT	Time required for the flour to develop into dough of convened consistency during mixing.	(min)
ST	Time for which the developed dough remains stable during mixing.	(min)
BT	Time at which the dough starts breaking down after mixing.	(min)
Baking		
LV	Volumes of baked loaf measured at 10 min.	(cm^3)
LH	Heights of baked loaves.	(mm)
PH	Heights of loaves after proofing.	(mm)
OSP	Increase in height of loaves in the oven during baking.	(mm)
SV	Specific volume of baked loaves.	(cm^3/g)
FP	Flour protein	(%)

Table 3. Visco-elastic, farinograph and baking characteristics in commercial wheat flours from site A, treated with DATEM levels. Means with same superscripts in a column are not significantly different (P > 0.05). The standard deviations of means are shown in parentheses. Definitions of visco-elastic, mixing and baking variables described in Table 2. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively.

TRT	Visco-elastic					Farinograph				Baking				
	SeP (s)	J-J_r (J)	% RC (%)	TCR (s)	TCC (s)	WA (%)	DT (min)	ST (min)	BT (min)	LV (cc)	PH (mm)	LH (mm)	OSP (mm)	SV (cc/g)
1A0	4.8^{e}	1.27^{a}	79.3^{c}	8.0^{abc}	9.5^{a}	53.6^{c}	1.0^{c}	2.0^{e}	1.4^{b}	550.0^{i}	59.2^{e}	84.4^{de}	25.2^{a}	4.0^{h}
	(0.3)	(0.1)	(1.0)	(0.9)	(0.7)	(0.2)	(0.1)	(0.0)	(0.1)	(4.1)	(1.5)	(1.3)	(0.2)	(0.02)
1A0.3	4.7^{e}	1.11^{a}	80.8^{bc}	8.3^{ab}	9.6^{a}	51.2^{d}	1.0^{c}	2.0^{e}	1.6^{b}	582.5^{h}	66.8^{d}	86.7^{cde}	19.9^{abcd}	4.4^{g}
	(0.7)	(0.1)	(1.1)	(0.7)	(0.9)	(0.0)	(0.1)	(0.4)	(0.1)	(2.9)	(1.9)	(0.0)	(2.0)	(0.0)
1A0.6	8.2^{d}	0.75^{bc}	82.3^{abc}	6.4^{cde}	6.8^{bc}	52.1^{cd}	0.8^{c}	1.4^{e}	1.5^{b}	648.8^{g}	68.3^{d}	85.7^{de}	17.4^{bcd}	4.8^{f}
	(0.3)	(0.1)	(1.8)	(0.8)	(0.4)	(0.1)	(0.1)	(0.3)	(0.1)	(6.3)	(0.5)	(1.6)	(1.0)	(0.02)
1A1	12.6^{c}	0.55^{cdef}	80.8^{bc}	4.0^{f}	6.8^{bc}	52.7^{cd}	1.1^{c}	0.9^{e}	1.4^{b}	553.8^{i}	71.2^{bcd}	81.4^{e}	10.2^{e}	4.1^{h}
	(1.3)	(0.1)	(1.9)	(0.2)	(0.5)	(0.8)	(0.1)	(0.6)	(0.2)	(4.8)	(0.2)	(1.3)	(1.1)	(0.06)
2A0	16.4^{b}	0.57^{bcde}	82.7^{abc}	4.0^{f}	6.2^{bc}	58.6^{b}	1.8^{c}	8.3^{d}	3.7^{b}	745.0^{e}	71.0^{bcd}	94.5^{ab}	23.5^{ab}	5.4^{d}
	(0.8)	(0.1)	(1.8)	(0.6)	(0.1)	(0.3)	(0.0)	(0.7)	(1.1)	(4.1)	(1.7)	(0.6)	(2.3)	(0.0)
2A0.3	26.5^{a}	0.39^{def}	85.4^{a}	9.7^{a}	5.0^{c}	58.9^{b}	1.6^{c}	13.6^{c}	3.3^{b}	778.8^{d}	70.8^{bcd}	94.3^{ab}	23.5^{ab}	5.7^{c}
	(1.1)	(0.0)	(1.2)	(0.9)	(0.3)	(0.4)	(0.1)	(2.1)	(0.3)	(4.8)	(0.6)	(4.1)	(3.5)	(0.06)
2A0.6	25.8^{a}	0.34^{ef}	85.1^{ab}	4.7^{ef}	6.6^{bc}	59.0^{b}	1.5^{c}	2.4^{e}	2.7^{b}	826.3^{c}	69.7^{cd}	94.3^{ab}	24.6^{ab}	5.9^{bc}
	(0.4)	(0.1)	(2.3)	(0.6)	(0.4)	(0.4)	(0.0)	(0.2)	(0.1)	(4.8)	(1.6)	(2.3)	(0.6)	(0.08)
2A1	25.2^{a}	0.33^{f}	83.6^{abc}	4.9^{ef}	5.5^{bc}	59.9^{b}	2.1^{c}	2.9^{e}	3.0^{b}	695.0^{f}	75.9^{ab}	90.2^{bcd}	14.3^{de}	5.1^{e}
	(2.0)	(0.0)	(1.5)	(0.6)	(0.5)	(0.1)	(0.1)	(0.6)	(0.1)	(12.2)	(1.8)	(2.9)	(1.1)	(0.01)
3A0	10.7^{cd}	1.11^{a}	80.2^{c}	5.8^{def}	7.2^{b}	63.6^{a}	10.8^{a}	21.5^{a}	16.0^{a}	821.3^{c}	74.2^{abc}	97.3^{ab}	23.1^{ab}	5.9^{c}
	(1.0)	(0.1)	(0.5)	(0.7)	(0.5)	(0.9)	(0.1)	(0.9)	(0.2)	(4.8)	(2.6)	(0.3)	(2.8)	(0.03)
3A0.3	9.6^{cd}	0.80^{b}	82.5^{abc}	6.6^{bcd}	7.3^{b}	62.5^{a}	9.3^{b}	15.6^{c}	12.2^{a}	847.5^{b}	75.8^{ab}	97.3^{ab}	21.5^{abc}	6.2^{a}
	(1.3)	(0.1)	(2.0)	(0.6)	(0.1)	(0.4)	(0.4)	(1.6)	(0.1)	(2.9)	(0.9)	(0.7)	(1.6)	(0.04)
3A0.6	10.5^{cd}	0.70^{bc}	82.4^{abc}	4.8^{ef}	6.9^{b}	64.1^{a}	8.0^{b}	19.7^{ab}	15.4^{a}	872.5^{a}	75.0^{abc}	98.8^{a}	23.7^{ab}	6.0^{ab}
	(1.5)	(0.0)	(1.5)	(0.2)	(0.3)	(0.0)	(0.1)	(1.3)	(5.5)	(6.5)	(0.8)	(0.2)	(0.6)	(0.0)
3A1	11.4^{cd}	0.61^{bcd}	82.8^{abc}	5.9^{de}	7.4^{b}	63.2^{a}	8.7^{b}	16.9^{bc}	14.2^{a}	738.8^{e}	79.5^{a}	94.1^{abc}	14.6^{cde}	5.5^{d}
	(1.1)	(0.1)	(0.6)	(0.2)	(0.6)	(0.3)	(1.2)	(0.1)	(2.0)	(4.8)	(0.6)	(2.3)	(1.7)	(0.05)

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Table 4. Visco-elastic, farinograph and baking characteristics in commercial wheat flours from site B, treated with DATEM levels. Means with same superscripts in a column are not significantly different (P > 0.05). The standard deviations of means are shown in parentheses. Definitions of visco-elastic, mixing and baking variables described in Table 2. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively.

TRT	Visco-elastic					Farinograph				Baking				
	SeP (s)	J-J_r (J)	% RC (%)	TCR (s)	TCC (s)	WA (%)	DT (min)	ST (min)	BT (min)	LV (cc)	PH (mm)	LH (mm)	OP (mm)	SV (cc/g)
1B0	29.0^{ab}	0.28^{ef}	86.5^{a}	3.2^{ef}	5.0^{abcd}	59.3^{ef}	1.5^{ab}	1.8^{e}	2.1^{c}	696.3^{d}	76.9^{a}	91.1^{abc}	14.2^{abc}	4.9^{e}
	(2.7)	(0.0)	(1.2)	(0.3)	(0.3)	(0.0)	(0.0)	(0.4)	(0.1)	(4.8)	(1.9)	(1.7)	(0.1)	(0.0)
1B0.3	29.6^{ab}	0.41^{de}	83.6^{ab}	3.2^{ef}	3.2^{d}	59.1^{f}	1.5^{b}	8.3^{d}	2.5^{c}	715.0^{bc}	72.1^{a}	90.0^{bc}	17.9^{abc}	5.0^{de}
	(1.5)	(0.1)	(1.8)	(0.4)	(0.1)	(0.0)	(0.1)	(0.0)	(0.0)	(4.1)	(0.7)	(0.5)	(0.2)	(0.06)
1B0.6	26.7^{b}	0.26^{ef}	83.3^{ab}	5.2^{cd}	7.0^{a}	62.3^{c}	1.8^{ab}	10.5^{d}	3.7^{c}	726.3^{a}	78.7^{a}	90.4^{abc}	11.6^{bcd}	5.1^{de}
	(2.7)	(0.0)	(0.8)	(0.1)	(1.6)	(0.1)	(0.1)	(0.2)	(0.5)	(2.5)	(1.4)	(2.7)	(4.0)	(0.09)
1B1	32.3^{a}	0.23^{f}	83.8^{ab}	5.5^{cd}	4.1^{cd}	62.3^{c}	1.7^{ab}	11.0^{cd}	9.9^{b}	547.5^{gh}	73.0^{a}	77.0^{d}	4.0^{d}	3.9^{g}
	(3.8)	(0.0)	(2.0)	(0.3)	(0.2)	(0.3)	(0.0)	(0.5)	(1.4)	(6.5)	(1.5)	(4.1)	(2.5)	(0.07)
2B0	10.2^{cd}	0.71^{ab}	80.8^{b}	6.2^{bc}	6.2^{ab}	59.6^{ef}	1.9^{ab}	10.4^{d}	4.5^{c}	772.5^{e}	73.3^{a}	94.3^{abc}	21.0^{abc}	5.5^{c}
	(1.4)	(0.1)	(1.7)	(1.1)	(0.5)	(0.1)	(0.2)	(0.7)	(0.1)	(2.9)	(0.1)	(1.0)	(1.0)	(0.02)
2B0.3	13.5^{cd}	0.59^{bc}	82.9^{ab}	6.0^{bc}	6.5^{a}	59.9^{e}	1.5^{ab}	12.8^{e}	2.8^{c}	821.3^{c}	75.9^{a}	98.5^{ab}	22.5^{ab}	5.9^{b}
	(1.2)	(0.0)	(0.4)	(0.9)	(0.4)	(0.2)	(0.0)	(0.1)	(0.1)	(2.5)	(1.9)	(1.9)	(0.1)	(0.07)
2B0.6	24.9^{b}	0.41^{de}	83.6^{ab}	5.5^{cd}	4.4^{bcd}	63.7^{a}	2.0^{ab}	14.1^{bc}	4.0^{c}	833.8^{b}	76.7^{a}	99.4^{a}	22.7^{ab}	6.0^{ab}
	(1.1)	(0.1)	(1.8)	(0.7)	(0.3)	(0.0)	(0.1)	(0.2)	(0.1)	(4.8)	(1.2)	(1.0)	(0.2)	(0.07)
2B1	13.6^{cd}	0.37^{def}	82.7^{ab}	2.8^{f}	6.4^{ab}	63.4^{ab}	2.1^{ab}	15.4^{ab}	12.5^{ab}	627.5^{i}	76.7^{a}	86.8^{c}	10.1^{cd}	4.6^{f}
	(1.0)	(0.1)	(2.8)	(0.8)	(1.1)	(0.0)	(0.1)	(2.5)	(1.4)	(6.5)	(0.9)	(2.7)	(1.8)	(0.07)
3B0	9.3^{d}	0.83^{a}	81.2^{b}	7.7^{ab}	6.5^{a}	60.6^{d}	1.9^{ab}	18.6^{a}	4.2^{c}	798.8^{h}	75.0^{a}	95.5^{abc}	20.5^{abc}	5.9^{b}
	(1.0)	(0.1)	(2.0)	(0.1)	(0.8)	(0.1)	(0.1)	(0.8)	(0.2)	(2.5)	(0.2)	(2.0)	(1.8)	(0.05)
3B0.3	14.3^{c}	0.64^{bc}	83.4^{ab}	6.4^{abc}	5.9^{abc}	61.2^{d}	1.9^{ab}	10.2^{d}	2.9^{c}	831.3^{fg}	73.7^{a}	99.2^{a}	25.6^{a}	6.2^{a}
	(1.4)	(0.0)	(0.5)	(0.5)	(0.2)	(0.0)	(0.5)	(0.4)	(0.6)	(2.5)	(0.3)	(2.8)	(3.1)	(0.0)
3B0.6	15.4^{c}	0.49^{cd}	82.6^{ab}	4.8^{cde}	5.9^{abc}	62.8^{bc}	1.9^{ab}	15.8^{ab}	14.9^{a}	847.5^{f}	73.4^{a}	96.8^{ab}	23.4^{a}	6.1^{a}
	(2.0)	(0.1)	(2.7)	(0.1)	(0.5)	(0.4)	(0.0)	(0.6)	(2.5)	(2.9)	(0.7)	(3.2)	(2.6)	(0.02)
3B1	14.5^{c}	0.28^{def}	85.3^{ab}	4.5^{de}	5.2^{abc}	63.7^{a}	2.2^{a}	15.9^{ab}	13.6^{ab}	707.5^{j}	78.8^{a}	93.4^{abc}	14.5^{abcd}	5.2^{d}
	(0.5)	(0.1)	(1.3)	(0.4)	(0.6)	(0.1)	(0.3)	(0.8)	(1.1)	(9.6)	(0.8)	(1.4)	(2.3)	(0.1)

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Table 5. Pearson's correlation coefficients of the visco-elastic properties of gluten, dough and baking characteristics. Definitions of visco-elastic, mixing and baking variables explained in Table 2.

	SeP	$J\text{-}J_r$	RCY	TCR	TCC	WA	DT	ST	BT	LV	PH	LH	SV	OSP	FP
SeP	1														
$J\text{-}J_r$	-0.81**	1													
RCY		-0.82**	1												
TCR		0.46*		1											
TCC		0.76**	-0.66**	0.44*	1										
WA		-0.40*	0.35*		-0.43*	1									
DT						0.50**	1								
ST						0.78**	0.64**	1							
BT						0.71**	0.73**	0.80**	1						
LV						0.60**	0.44*	0.59**		1					
PH		-0.57**	0.46*		-0.44*	0.77**	0.35*	0.56**	0.46*	0.43*	1				
LH						0.53**	0.41*	0.57**		0.95**	0.41*	1			
SV						-0.54**	0.42**	0.52**		0.99**	0.39*	0.95**	1		
OSP		0.50**		0.36*						0.64*	0.35*	0.71**	0.68**	1	
FP						0.82**	0.80**	0.77**	0.72**	0.73**	0.61**	0.67**			1

*Correlation is significant at $\alpha = 0.05$ level

**Correlation is significant at $\alpha = 0.01$ level

Table 6. Explained variance (%) in PCA of visco-elastic, mixing and baking variables in gluten and flours treated with DATEM. Definitions of visco-elastic, mixing and baking variables explained in Table 2.

DATEM	Axes	PC1	PC2	1+2
	PC (%)	39.9	26.9	66.8
Visco-elastic	SeP	0.31	69.79	70.1
	$J\text{-}J_r$	2.78	87.11	89.89
	RCY	5.14	48.09	53.23
	TCR	1.34	28.22	29.56
	TCC	6.97	60.8	67.77
Farinograph	WA	76.68	9.1	85.78
	DT	46.07	11.8	57.87
	ST	64.66	1.03	65.69
	BT	46.66	0.28	46.94
Baking	PH	46.89	22.9	69.79
	LH	63.62	8.73	72.35
	SV	69.03	6.74	75.77
	OSP	8.68	44.93	53.61
	LV	72.93	3.42	76.35
Protein Content	FP	87.07	0.43	87.5

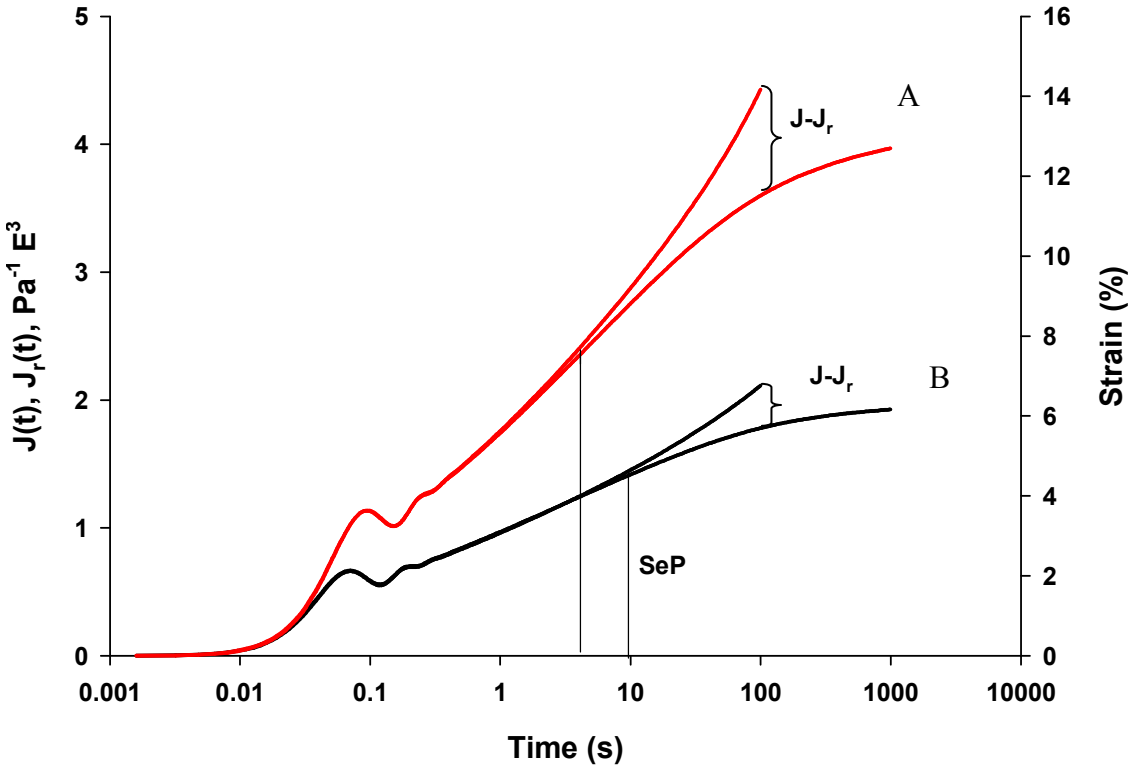

Fig. 1. A graphical representation of creep recovery behavior of gluten showing weak gluten strength in curve (A) and strong in curve (B) is shown. The gluten strength is expressed as compliance for creep measured for 100 s. and recovery for 1000 s. Δ compliance ($J-J_r$) is the difference between compliance of creep and recovery at 100 s. The time at which the creep and recovery components split is called as separation time (SeP).

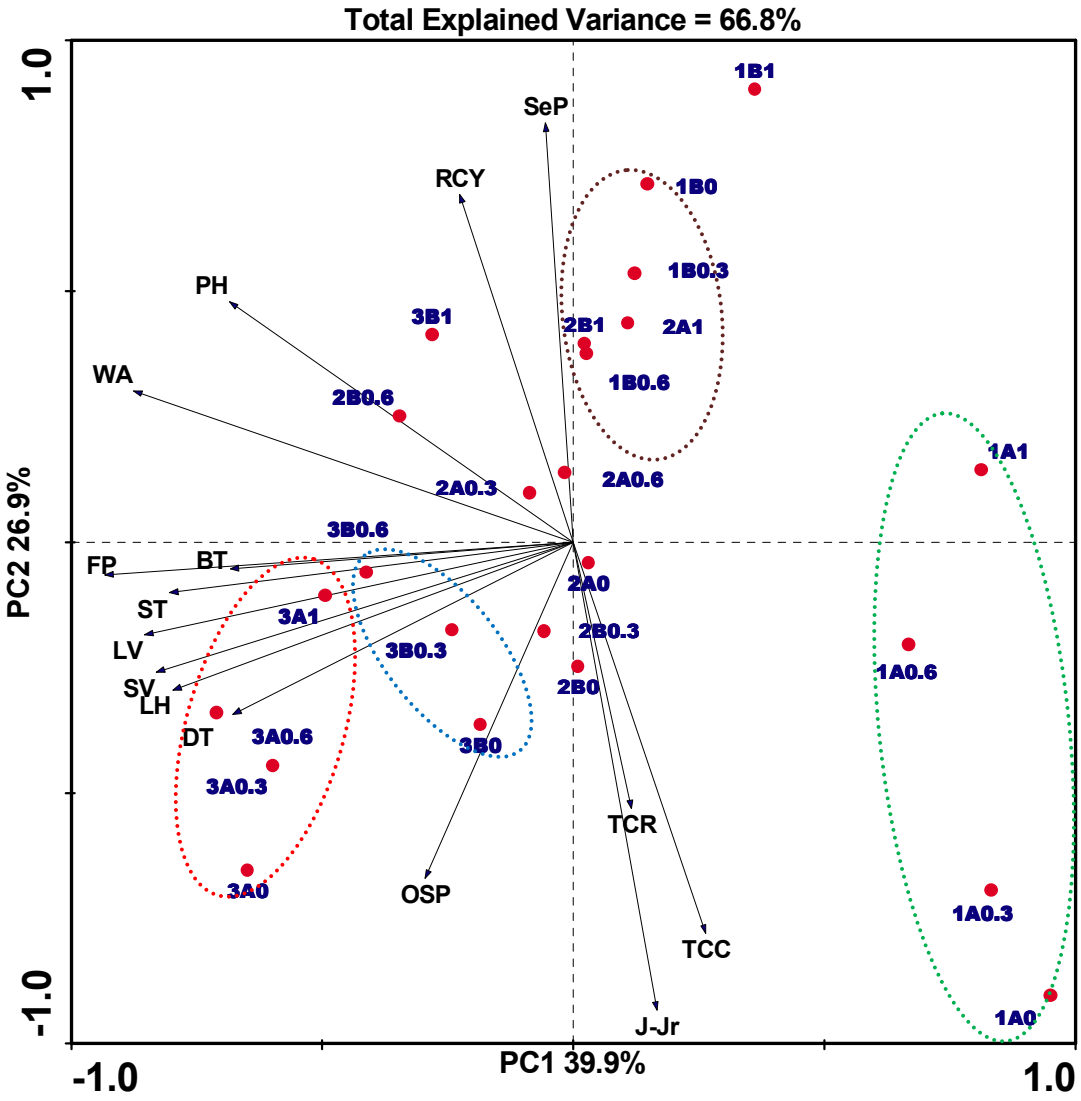

Fig. 2. Loading plot of first two principal components based on baking, visco-elastic and dough properties of six commercial wheat flours obtained from sites A and B, added with three levels of DATEM. Definitions of visco-elastic, mixing and baking variables explained in Table 2. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively.

CHAPTER IV

OXIDIZING EFFECT OF ASCORBIC ACID IN MIXING AND BAKING PROPERTIES OF WHEAT FLOURS AND ITS CORRELATION TO GLUTEN VISCO-ELASTICITY.

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Abstract

Effect of oxidizing agent, ascorbic acid was evaluated on the visco-elastic properties of gluten and mixing and baking properties of dough in hard red winter wheat flours obtained from two different sources. Ascorbic acid was added to gluten and wheat flours at the levels of 0, 50, 100, 150 and 200 ppm. Creep-recovery measurements were performed to investigate the effect of ascorbic acid on visco-elastic properties of gluten. Mixing properties of wheat flours were evaluated using the Farinograph measurements. Baking characteristics were measured after the wheat flours were baked using an optimized straight dough bread making method. No significance changes in creep and recovery compliance were observed except ascorbic acid decreased compliance (increased elasticity) in site A flour with 8% protein content by 35% with 50 ppm and site B flour with 11.5% protein content by 32% with 150 ppm, respectively. No specific trends were observed in separation time and delta compliance of gluten with ascorbic acid addition. An overall significant reduction of recoverability of gluten with ascorbic acid was observed. Rate of creep and recovery indicated by changes in time constants for creep and recovery showed no specific trend with ascorbic acid addition. No clear trend was observed in the mixing properties of flour with addition of ascorbic acid. Loaf volumes, loaf heights, oven springs, specific volumes and proof heights in all flours showed significant ($P < 0.05$) increase at 100 to 150 ppm ascorbic acid levels. Loaf volumes increased in all flours (3 to 13% range) with 100 to 150 ppm ascorbic acid levels. All flours showed a sharp decrease in loaf properties like volume, height, proof height, oven spring and specific volume with 200 ppm. Pearson correlation coefficients and principal component analysis indicated that increase in oven springs were associated

with increase in viscosity of dough and weakening of gluten. Oven spring was significantly ($P < 0.01$) negatively correlated to elasticity properties of gluten, percent recoverability ($r = -0.57$). Separation time showed weak but significant correlation with proof heights ($r = 0.38$, $P < 0.05$) and highly significant correlation with specific volume ($r = -0.61$, $P < 0.01$). All other visco-elastic properties were independent of baking and mixing properties. Mixing properties were found to be negatively correlated to baking properties and flour protein content with addition of ascorbic acid flours. Oxidizing effect of ascorbic acid at the levels of 50 to 100 ppm improved the quality baking performance in wheat flours. Rise in oven spring and specific volumes were closely associated with increase in viscous component of gluten with ascorbic acid addition.

1. Introduction

Flour quality and dough strength are important characteristics of baked bread. Ascorbic acid is used as one of the dough improvement additive by the baking industry. Many studies have been performed to understand the mechanism of ascorbic acid in enhancing the quality of dough. Reduction-oxidation reactions takes place during mixing and water addition involving the sulphydryl (SH) residues and disulfide (SS) linkages in gluten. These reactions modify the polymeric fraction of gluten leading to the changes in gluten visco-elasticity (Chen & Schofield, 1996). The reaction sequence that follows the addition of ascorbic acid to dough was reviewed by Grosch and Wieser (1999). L-Ascorbic acid (L-AA) interacts with oxygen during mixing and is oxidized to L-dehydroascorbic acid (L-DHA). The endogenous glutathione (GSH) in flour is converted to its oxidized disulfide derivative GSSG catalyzed by glutathione reductase GSH-DH and L-DHA as its co-substrate. This causes SH/SS exchange by the reaction of GSSG with SH groups of proteins. The dough improver effect of L-AA is due to the oxidation of GSH to GSSG and rapid blocking of SH groups in gluten (Grosch et al., 1999). Free GSH weakens the dough by cleaving the intermolecular SS bonds in glutenin causing depolymerization (Chen et al., 1996). Another theory proposed by Tilley et al. (2001) suggested that tyrosine cross-linkage along with disulfide linkage could be equally contributing towards the strengthening effect of dough. Dityrosine an isomer of tyrosine was found in sections of glutenin is a source of tyrosine crosslinking among gluten proteins. The microbial enzyme transglutaminase improves the dough properties during mixing via a non oxidative cross linking (Gerrard, Fayle, Wilson, Newberry, Ross & Kavale, 1998). Transglutaminase catalyses the acyl-transfer reaction between the γ-carboxyamide group of peptide bound glutamine residues and various primary amines.

The ε-amino groups of lysine residues in proteins can act as the primary amine, yielding inter- and intramolecular ε -N-glutamyl lysine crosslinks.

There is very little evidence available on the action of ascorbic acid (AA) in visco-elasticity of gluten and its correlation to breadmaking process. The objectives of this study are 1) to investigate the effect of ascorbic acid on visco-elastic properties of gluten extracted from flours with different protein content from different locations; 2) to quantify the effect of ascorbic acid on visco-elastic properties of gluten using creep recovery and 3) to correlate the visco-elastic changes in gluten induced by ascorbic acid with baking performance of the flours.

2. Materials and Methods

The procurement of wheat flour samples are explained in methods and materials section of chapter 3.

Four levels (50, 100, 150 and 200 ppm) of ascorbic acid (Malinckrodt Baker Inc., Phillipsburg, NJ 08865), were added to flours from each source. Thus, site A flours were denoted as 1A0, 1A50, 1A100, 1A150 and 1A200; 2A0, 2A50, 2A100, 2A150 and 2A200; 3A0, 3A50, 3A100, 3A150 and 3A200, respectively. Similarly site B flours were named, 1B0, 1B50, 1B100, 1B150 and 1B200; 2B0, 2B50, 2B100, 2B150 and 2B200; 3B0, 3B50, 3B100, 3B150 and 3B200, respectively. Flours and gluten isolated from flours with no AA were used as controls. The protein, moisture and ash contents were determined using the NIR system (FOSS NIR Systems Inc, Laurel, MD 20723) as shown in Table 1 (Chapter III).

2.1. Gluten extraction

Glutens were prepared in triplicates in an automated gluten washer, Glutomatic 2200 (Perten Instruments, Sweden) from 10 g of flour and 5.0 mL of AA solution (0.05, 0.1. 0.15 and 0.2 g ascorbic acid in 500 ml 2% salt solution) using a mixing time of 60 sec and washing for 10 min with 2% NaCl solution (w/v). Control samples were mixed with 5.0 ml of pure deionized water.

2.2. Creep recovery tests

The creep recovery experiments were carried out as described in Chapter III. The definitions of visco-elastic parameters are explained in Table 2, Chapter III.

2.3. Dough mixing properties

One ml of AA (0.05, 0.1, 0.15 and 0.2 g per 100 ml deionized water) was added to 10 g of wheat flour. Dough mixing properties were evaluated as described in Chapter three. Definitions of the terms used to describe dough mixing properties as explained in Table 2, Chapter III.

2.4. Baking tests

Baking tests were performed as explained in chapter 2. The definitions of baking, dough mixing and visco-elastic are explained in Table 2 (Chapter III).

3. Statistics

Statistical analysis is performed using same methods explained in chapter III.

4. Results

4.1. Visco-elastic properties

Effects of ascorbic acid on the visco-elastic properties of gluten varied and were dependent of protein content, protein quality and source of the flours. Gluten strength

increased in form of recovery compliance reduction by 35% in 1A flours with addition of 50 ppm AA and by 32% in 3B flours with 150 ppm AA (Fig. 4 and 5. Appendix1). No significant gluten strengthening reduction, i.e. decrease in recovery compliance was observed in other flours. AA at 200 ppm weakened the gluten in all flours.

Significant interactions were observed in the visco-elastic properties of gluten extracted from flours of different protein content and ascorbic acid addition (Appendix 2, Table 2) except for time constants for creep in site B. Elasticity decrease estimated as SEP was observed in 1A, 2A, 3A and 1B gluten by 43.1, 83.5, 64.4 and 70% at 100 ppm AA levels, respectively (Tables 1 and 2). Gluten from 2B showed increased viscosity with reduction in SEP at 200 AA levels by 74.5%. Similarly, reduction in $J-J_r$ as a function of elasticity was observed in 1A and 3B at 50 ppm and 150 ppm AA levels by 30 and 40%, respectively (Tables 1 and 2). AA levels of 200 ppm decreased the recoverability in gluten in all flours significantly. Ascorbic acid reduced the time constant for creep by 43% in 1A gluten enhancing its ability to respond to stress at a faster rate compared to creep rates of gluten from other flours (Table 1). A significant protein and treatment effects were observed in TCC (Appendix 2, Table 3). Low protein from site B had significantly low time constant (TCC) of 5.4 min compared to medium protein level while AA level of 200 ppm increased significantly the deformation rate to 11.85 s compared to controls (Appendix 3, Table 2). Time constants for recovery reduced in 1A and 3B flours significantly at 50 and 100 ppm AA levels by 48 and 69%, respectively (Tables 1 and 2). Gluten from other flours showed slow recovery rates at increased AA levels.

4.2. Dough mixing properties

Significant interactions were observed in the mixing properties of flours of different protein content and ascorbic acid addition (Appendix 2, Table 2) except dough development time (DT) in site B. Water absorption in 1A and 3B flours decreased in 6 and 3%, respectively where AA was added at levels of 200 ppm (Tables 1 and 2). In contrast, 3A flour had 2.3% higher water absorption at 200 ppm AA concentrations (Table 1). Dough stability increased in 1A flour by 52.3% at 50 ppm ascorbic acid addition (Table 1). In site B flours, dough stability decreased in high protein flour with the addition of AA, with 80% decrease at 200 ppm. Similarly, dough breakdown time showed no major differences in flours from sites A and B with ascorbic acid treatment. The development time of high protein in site A decreased by 56% with 50 ppm AA (Table1). No significant differences were observed in the flour protein and ascorbic acid treatment interaction in dough development time as well as the protein and ascorbic acid treatment effects in site B flours (Appendix 2, Table 2).

4.3. Baking characteristics

Significant interactions of flour protein content and ascorbic acid addition in baking properties were observed except proof heights in both sites and specific volumes in site A (Appendix 1, Table 2). The addition of AA to flours from both sites A and B produced an increase in bread loaf volume up to 150 ppm (Table 1 and 2.). With the addition of 200 ppm, the bread volume decreased. The increased loaf volumes were obtained with 100 ppm and 150 ppm of AA, except for one sample, 3A flour, in which loaf volume was obtained with 50 ppm AA (Table 1.). Breads from 1A, 2A and 3A flours showed loaf volume increase up to 13, 9 and 7% respectively with addition of AA. Loaf

volumes increased in 1B, 2B and 3B with 100 ppm AA by 6.5, 8.7% and 2.9%, respectively. Significantly high PH ($P < 0.05$) was observed in controls (treatment effect) with77.01 and 77.77 mm of all treatments levels (Appendix 3, Table 2) in sites A and B, respectively. High protein (protein effect) had significantly high proof height (76.1 mm, $P < 0.05$) among all protein contents (Appendix 3, Table 2). Loaf heights (LH) increased up to the addition of 100 ppm AA and decreased with higher concentrations in all the flours in sites A and B (Tables 1 and 2). Loaf height increased with AA additions by 7%, 4% and 5% in 1A, 2A and 3A, respectively at 100 ppm. In site B flours, increase in loaf heights with 100 ppm AA was observed in 1B, 2B and 3B was 9%, 3.6% and 2%, respectively. Oven springs also known as oven spring, increased with 50 ppm AA levels in all flours from site A (56, 40 and 34% in 1A, 2A and 3A, respectively) and 100 ppm levels in flours from site B (53, 12 and 19% in 1B, 2B and 3B, respectively). No significant differences were observed in the flour protein and ascorbic acid treatment interaction in specific volumes as well as the protein and ascorbic acid treatment effects (Appendix 2, Table 2). Specific volumes significantly increased in all site B flours at 100 ppm AA levels (Table 2).

4.4. Correlations and PCA

Significant negative correlations were observed among baking properties and dough mixing characteristics (Table 3). The most dough improvement and gluten strengthening were observed at AA levels of 50 ppm. Effects of levels of AA higher than 100 ppm were negatively influencing the baking properties and weakened gluten by increasing its compliance. Dough mixing properties were highly negatively correlated to flour protein content. Weak negative correlation between $J\text{-}J_r$ and PH ($r = -0.33$ at $P <$

0.05) indicated the response of proof height during fermentation was inversely related to increased elasticity. Elasticity of gluten in form of separation time (SeP) had weak positive correlation with proof heights ($r = 0.38$, $P < 0.05$) and strong negative correlation to specific volume ($r = -0.61$, $P < 0.01$) as shown in Table 3. Increase in oven spring (OSP) could be contributing to increase the viscosity in gluten at AA levels above 100 ppm as significant correlations were observed with J-J_r ($r = 0.36$ at $P < 0.05$) and RCY ($r = -0.57$ $P < 0.01$), respectively (Table 3).

Principal component analysis (PCA) was performed in order to classify the samples on basis of visco-elastic performance of gluten, mixing characteristics of the flours and baking properties of the flours with addition of AA. PCA grouped the linear combinations visco-elastic, farinograph and baking variables into principal components captured maximum variance. Principal components 1 and 2 (Fig.1) accounted for 66.2% of the total variance with 39.9% variance explained by PC1 and 26.3 % variance explained by PC2. The majority of PC1 was influenced by linear combinations of flour protein content (87.3% explained variance), followed by dough breakdown time, dough development time, loaf heights and loaf volume (Table 4). PC2 was clearly related to the visco-elastic properties dominated by SeP at 80.7% explained variance followed by delta compliance and recoverability (RCY) at 78.4 and 73.1% explained variance, respectively (Table 4). Confirming the results from Pearson's correlation coefficient (Table 3), flour protein was highly positively correlated to baking properties and negatively correlated to the dough mixing properties. Visco-elastic properties were independent of baking and mixing properties with one exception. The oven springs were found to positively correlate with Δ compliance (viscosity) and negatively correlated to recoverability.

Low protein site A flours showed higher positive correlation to dough mixing properties and negative correlation to baking properties at 0 and 50 ppm. Similarly, high protein flours from site A showed very high positive correlations with baking properties and negative correlations at 50 ppm. As AA levels increased 1A and 3A samples became independent of mixing and baking properties. Low protein site B flours added with AA were independent of baking and mixing properties but were highly correlated to the elasticity of gluten.

5. Discussion

The oxidizing effect of ascorbic acid had a variable effect in the visco-elastic, mixing and baking properties on the set of samples analyzed. The ability of AA to improve the loaf volumes differed with protein content and the sites of procurement of flours. Conforming to our study, the ability of ascorbic acid to strengthen the dough from lower protein flours more than higher protein flours was also observed in hearth loaves bread (Aamodt, Magnus & Faergestad, 2003). Overall, the flour protein quality as measured by mixing baking and visco-elastic properties, improved by the oxidation of sulphydryl to disulfide bonds due to blending of local cultivars. The concentration of AA in our study in different processes of baking was found to be optimum at 50 -100 ppm that could be the highest levels of AA to convert the GSH to GSSG and bring a positive effect on dough strength. The results agreed with Koehler (2003b) who reported that AA levels of 125 ppm were optimum to improve on dough structure and visco-elastic. Oxidizing agents promote SS bond formation and, hence, minimize SH/SS interchange with positive impacts on oven spring and oven spring time (Joye, Lagrain & Delcour, 2009). Our study agreed that ascorbic acid treatments affected the oven springs positively

which could be due to promotion of SS bond formation. Rise in oven spring was one of the prominent baking properties that were highly correlated to decreased elasticity in our study. Increased oven spring along with loaf volumes was extensively observed with AA additions of 15 ppm (Yamada & Preston, 1994). High concentrations of AA increased gluten viscosity may be responsible for the increasing oven spring as observed in the baking performance of flours. The baking process in this study was carried out at room temperature. However, a study by Li et al. (2004) compared the formation of oxidized glutathione GSSG at 25 C and 40 C, reported 70% higher GSSG formation at 40° C. Gluten strength of low protein flour from site A (FP = 8%) improved with 50 ppm AA. Similar trend was reflected in baking properties where increase in loaf volumes and loaf heights was more pronounced in low protein content flours. Oxidized AA (DHAA) reacts rapidly with GSH to form GSSG and inhibits depolymerization of glutenin intermolecular disulfide linkages that makes the dough weak (Joye et al., 2009). Koehler (2003a) reported that AA levels of 100 and 125 ppm decrease the GSH and increased cysteine residues that bind to protein SH groups and found a strong correlation between flour protein quality and sulfahydril (SH) concentration. Koehler (2003a) reported similar ability of AA at optimized concentrations to improve the quality of lower protein content flours with increased concentration of SH groups of glutenins than high protein content. During mixing ascorbic acid rapidly consumes free oxygen radicals and gets oxidized to DHAA which in turn oxidize reform sulfyhydril (-SH) bonds to disulfide linkages. But at high concentration of AA and limited mixing time can yield limited oxygen for reactions thus allowing only partial oxidation of AA to DHAA. The non oxidized AA could act as reducing agent and reverse the reaction of disulfide interchange back to glutathione. The

bread qualities in form of loaf volumes and heights as well as visco-elastic properties of gluten were negatively affected at 150 to 200 ppm of ascorbic acid levels in this study. Concentration of oxygen available after mixing can be limited due to competition between ascorbic acid and yeast (Lu & Seib, 1998). Lu and Seib (1998) reported that only 33% of DHAA was formed from 200 ppm ascorbic acid level due to limiting oxygen availability during mixing. The study of Lu and Seib (1998) further reported that addition of 25 ppm of DHAA produced loaf volumes of 930 cc while 25 ppm of non oxidized AA produced loaf volumes of 730 cc. Thus the improver action of ascorbic acid depended mostly on the availability of oxygen during mixing. It is possible that gluten being a single isolated functional ingredient of flour may require low amounts of ascorbic acid concentrations to bring about strengthening effect. The ability of ascorbic acid to bring to improve visco-elasticity in gluten depends on the quality of gluten of which available SH residues in the glutenin sub fractions is an important factor. Significant negative correlations of baking parameters with dough mixing properties (Table 3 and Fig. 4) could suggest that the ability of AA at higher levels diminishes due to increased competition to react with oxygen. When oxygen is limiting, AA has a reducing effect during mixing, and weakening of the gluten network occurs (Li, Li, Tsiami & Schofield, 2000). Decreased proof heights explained minimal role of AA in gas stabilization. Wikstrom and Eliasson (1998) reported higher increase in loaf volumes in low protein (FP = 8%) and medium protein (FP = 11.2%) winter wheat flours similar to the protein contents of 1A and 3B by 19% and 51%, respectively. Wikstrom and Elliasson (1998) also reported that stress relaxation measurements after a large strain decreased relaxation rate in dough from high, medium and low protein flours with the effect less pronounced

in low protein flour. Our study in contrast with Wikstorm and Eliasson's (1998) observation that rate at which gluten deformed and recovered accelerated to reach equilibrium at AA levels of 150 ppm and above in low protein flours and showed no differences in higher protein flours.

6. Conclusions

Effect of ascorbic acid as a dough improver is effective at concentrations less than 100 ppm. Ascorbic acid improved baking performance of low protein flours effectively. Response of the flours to ascorbic acid treatment was a function of quality of the protein as well as the content. Addition of ascorbic acid modified gluten viscosity which in turn increased the rise of loafs in the oven. Gluten strengthening by ascorbic acid was effective at low concentrations in low protein content flours and higher concentrations as protein level increased. Relationship of gluten strengthening was observed with baking performance in low protein flours to be more efficient than higher protein. Although, visco-elastic and baking variables had very few individual correlations, flours from both sites with range of 10.5 to 11% protein content and ascorbic acid concentrations of 50 to 150 ppm were found to be correlated to visco-elastic changes in gluten.

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Table 1. Visco-elastic, farinograph and baking characteristics in commercial wheat flours from site A, treated with ascorbic acid levels. Means with same superscripts in a column are not significantly different (P > 0.05). The standard deviations of means are shown in parentheses. Definitions of visco-elastic, mixing and baking variables described in Table 2, Chapter III. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively.

TRT	Visco-elastic					Farinograph				Baking				
	SeP (s)	J-Jr (J)	RC (%)	TCR (s)	TCC (s)	WA (%)	DT (min)	ST (min)	BT (min)	LV (cc)	PH (mm)	LH (mm)	OSP (mm)	SV (ccg^{-1})
1A0	4.8^{ef}	1.27^{b}	79.3^{abc}	8.0^{abc}	9.5^{bcd}	53.6^{d}	1.0^{d}	$2.0f^{g}$	1.4^{d}	620.0^{j}	70.5^{de}	81.5^{i}	11.0^{e}	5.9^{a}
	(0.3)	(0.1)	(1.0)	(0.4)	(0.7)	(0.2)	(0.1)	(0.0)	(0.1)	(9.1)	(1.1)	(0.3)	(1.0)	(0.1)
1A50	6.7^{de}	0.86^{ef}	80.1^{ab}	4.2^{e}	5.4^{g}	52.7^{d}	1.0^{d}	4.2^{f}	1.5^{d}	637.5^{i}	59.2^{g}	84.4^{hi}	25.2^{a}	5.9^{a}
	(1.2)	(0.0)	(0.8)	(0.8)	(0.8)	(0.1)	(0.1)	(0.6)	(0.3)	(9.6)	(1.5)	(1.3)	(0.1)	(0.03)
1A100	2.7^{f}	1.26^{bcd}	77.7^{bcd}	5.7^{de}	9.0^{cd}	51.0^{e}	0.9^{d}	1.0^{g}	1.3^{d}	707.5^{fg}	65.0^{ef}	86.3^{gh}	21.3^{abc}	6.3^{a}
	(0.3)	(0.2)	(3.8)	(0.4)	(0.5)	(0.1)	(0.1)	(0.4)	(0.2)	(5.0)	(1.0)	(1.0)	(1.0)	(0.03)
1A150	3.5^{f}	2.13^{a}	75.5^{cd}	9.1^{a}	10.3^{bc}	50.5^{e}	1.0^{d}	1.7^{fg}	1.6^{d}	712.5^{f}	63.8^{fg}	82.9^{i}	19.1^{bcd}	6.2^{a}
	(0.3)	(0.1)	(0.6)	(0.6)	(0.2)	(0.1)	(0.2)	(0.8)	(0.4)	(2.9)	(0.1)	(1.4)	(2.4)	(0.00)
1A200	5.3^{def}	1.22^{bcd}	79.6^{abc}	8.9^{ab}	12.7^{a}	50.2^{e}	0.8^{d}	2.2^{fg}	1.5^{d}	655.0^{h}	63.6^{fg}	87.9^{fg}	21.1^{ab}	5.3^{a}
	(0.9)	(0.1)	(0.7)	(1.0)	(1.0)	(0.3)	(0.1)	(0.1)	(0.0)	(4.4)	(0.7)	(1.0)	(4.6)	(0.06)
2A0	16.4^{a}	0.57^{f}	80.8^{ab}	4.0^{e}	6.2^{fg}	58.6^{c}	1.8^{d}	8.3^{e}	3.7^{d}	728.8^{e}	79.8^{ab}	94.0^{de}	14.2^{de}	5.5^{a}
	(0.8)	(0.1)	(1.7)	(0.6)	(0.1)	(0.3)	(0.0)	(0.7)	(1.0)	(10.3)	(2.3)	(1.5)	(0.8)	(0.1)
2A50	11.7^{b}	0.92^{de}	78.0^{abcd}	5.2^{de}	7.9^{def}	57.9^{c}	1.7^{d}	10.6^{de}	3.1^{d}	745.0^{d}	71.0^{d}	94.5^{cd}	23.5^{abc}	5.4^{a}
	(1.0)	(0.1)	(0.2)	(0.4)	(0.6)	(0.1)	(0.1)	(0.3)	(0.7)	(4.0)	(1.7)	(0.6)	(1.0)	(0.00)
2A100	2.7^{f}	1.92^{a}	77.0^{bcd}	6.9^{bcd}	7.7^{def}	58.2^{c}	1.5^{d}	10.4^{de}	4.0^{d}	800.0^{b}	75.1^{bcd}	97.9^{ab}	22.8^{abc}	6.0^{a}
	(0.1)	(0.1)	(0.2)	(0.2)	(0.2)	(0.0)	(0.1)	(0.5)	(0.4)	(4.0)	(1.2)	(0.3)	(0.1)	(0.02)
2A150	7.6^{d}	1.30^{bc}	79.0^{abcd}	5.8^{de}	7.0^{fg}	58.3^{c}	1.6^{d}	3.5^{fg}	3.0^{d}	801.3^{b}	72.0^{cd}	89.9^{f}	17.8^{cd}	5.9^{a}
	(1.5)	(0.0)	(1.4)	(0.4)	(0.8)	(0.4)	(0.1)	(0.3)	(0.1)	(2.5)	(0.4)	(0.5)	(0.2)	(0.01)
2A200	4.7^{def}	0.95^{cde}	82.4^{a}	5.8^{de}	6.9^{fg}	58.3^{c}	1.6^{d}	3.0^{fg}	2.7^{d}	696.3^{g}	71.5^{cd}	90.8^{ef}	18.4^{abcd}	5.1^{a}
	(0.7)	(0.1)	(1.6)	(0.5)	(0.2)	(0.0)	(0.1)	(0.7)	(0.1)	(4.8)	(0.1)	(0.4)	(0.2)	(0.2)
3A0	10.7^{bc}	1.12^{bcde}	80.2^{ab}	5.8^{de}	7.2^{ef}	63.6^{b}	10.8^{a}	21.5^{a}	16.0^{a}	773.8^{c}	80.8^{a}	95.9^{bcd}	15.1^{de}	5.9^{a}
	(1.0)	(0.1)	(0.5)	(0.7)	(0.5)	(0.9)	(0.1)	(0.9)	(0.2)	(7.5)	(1.0)	(0.7)	(0.0)	(0.1)
3A50	7.8^{cd}	0.90^{e}	79.7^{abc}	6.3^{cd}	8.9^{cde}	63.4^{b}	4.7^{c}	16.9^{b}	15.2^{ab}	821.3^{a}	74.2^{bcd}	97.3^{bc}	23.1^{abc}	5.9^{a}
	(0.7)	(0.0)	(0.5)	(0.6)	(0.6)	(0.2)	(0.2)	(1.4)	(1.9)	(4.8)	(2.6)	(0.2)	(1.8)	(0.03)
3A100	3.8^{ef}	1.37^{b}	77.3^{bcd}	6.3^{cd}	11.4^{ab}	64.9^{ab}	7.5^{b}	14.8^{bc}	12.6^{bc}	833.8^{a}	76.0^{abcd}	101.1^{a}	25.2^{a}	6.3^{a}
	(0.4)	(0.0)	(0.1)	(0.3)	(0.0)	(0.5)	(0.8)	(0.0)	(0.7)	(2.5)	(2.6)	(0.2)	(0.6)	(0.03)
3A150	4.1^{ef}	1.40^{b}	76.8^{bcd}	6.7^{cd}	9.0^{cde}	65.1^{a}	7.8^{b}	11.9^{cd}	10.4^{c}	833.8^{a}	76.9^{abc}	95.9^{bcd}	19.0^{bcd}	6.2^{a}
	(0.8)	(0.0)	(0.7)	(0.1)	(0.0)	(0.4)	(0.4)	(0.5)	(0.6)	(2.5)	(0.4)	(0.4)	(1.7)	(0.00)
3A200	4.7^{def}	1.82^{a}	74.8^{d}	6.5^{cd}	13.2^{a}	65.1^{a}	7.9^{b}	11.0^{de}	10.3^{c}	748.8^{d}	72.7^{cd}	94.9^{bcd}	20.9^{abc}	5.3^{a}
	(0.4)	(0.0)	(0.1)	(0.4)	(0.3)	(0.6)	(0.2)	(1.8)	(0.9)	(2.5)	(0.1)	(0.3)	(1.3)	(0.06)

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Table 2. Visco-elastic, farinograph and baking characteristics in commercial wheat flours from site B, treated with ascorbic acid levels. Means with same superscripts in a column are not significantly different (P > 0.05). The standard deviations of means are shown in parentheses. Definitions of visco-elastic, mixing and baking variables described in Table 2, Chapter III. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively.

TRT	Visco-elastic					Farinograph				Baking				
	SeP (s)	J-J_r (J)	RC (%)	TCR (s)	TCC (s)	WA (%)	DT (min)	ST (min)	BT (min)	LV (cc)	PH (mm)	LH (mm)	OSP (mm)	SV (ccg^{-1})
1B0	29.0^{a}	0.28^{h}	86.5^{a}	3.2^{h}	5.0^{b}	59.3^{b}	1.5^{a}	1.8^{e}	2.1^{c}	680.0^{g}	77.0^{abc}	87.2f^{g}	10.2^{f}	5.0^{e}
	(2.7)	(0.0)	(1.2)	(0.3)	(0.3)	(0.3)	(0.0)	(0.4)	(0.1)	(4.1)	(0.7)	(0.3)	(0.8)	(0.07)
1B50	16.7^{b}	0.49^{gh}	86.1^{ab}	2.3^{h}	5.6^{b}	59.9^{ab}	1.9^{a}	3.2^{ef}	2.8^{bc}	696.3^{f}	76.9^{abc}	91.1^{defg}	14.2^{def}	4.9^{e}
	(1.7)	(0.0)	(0.1)	(0.1)	(0.3)	(0.3)	(0.1)	(1.3)	(0.5)	(4.8)	(1.8)	(1.7)	(0.2)	(0.00)
1B100	8.7^{def}	0.77^{def}	81.6^{bc}	4.6^{fg}	5.3^{b}	59.8^{ab}	1.8^{a}	3.5^{def}	3.2^{abc}	727.5^{de}	74.0^{abcde}	96.0^{abcd}	21.9^{ab}	5.4^{cd}
	(1.8)	(0.0)	(1.0)	(0.4)	(0.3)	(0.1)	(0.4)	(0.1)	(0.2)	(6.5)	(1.0)	0.1)	(2.0)	(0.01)
1B150	16.6^{b}	0.54^{fg}	84.7^{ab}	6.1^{de}	6.3^{b}	59.2^{b}	1.8^{a}	4.7^{cdef}	3.1^{abc}	717.5^{e}	74.5^{abcd}	87.5^{efg}	13.0^{ef}	5.2^{d}
	(2.3)	(0.0)	(1.3)	(0.6)	(0.3)	(0.2)	(0.3)	(1.8)	(0.1)	(6.5)	(3.4)	(1.0)	(1.6)	(0.06)
1B200	14.7^{bc}	0.54^{g}	80.1^{bc}	3.5^{fgh}	5.0^{b}	59.7^{ab}	1.9^{a}	3.1^{ef}	3.0^{abc}	643.8^{h}	68.3^{e}	84.6^{g}	14.6^{cde}	4.5^{f}
	(0.6)	(0.1)	(1.6)	(1.0)	(0.3)	(0.1)	(0.1)	(1.8)	(0.3)	(8.5)	(0.5)	(2.7)	(4.3)	(0.07)
2B0	10.2^{cde}	0.71^{defg}	80.8^{bc}	6.2^{cde}	6.2^{b}	59.6^{ab}	1.9^{a}	10.4^{b}	4.5^{ab}	728.8^{de}	76.9^{abc}	96.7^{abcd}	19.8^{abc}	5.4^{cd}
	(1.4)	(0.1)	(1.7)	(1.1)	(0.5)	(0.1)	(0.2)	(0.7)	(0.1)	(4.8)	(3.7)	(4.5)	(0.8)	(0.1)
2B50	6.2^{ef}	1.20^{b}	78.1^{c}	9.6^{a}	7.8^{ab}	59.7^{ab}	1.6^{a}	8.9^{bc}	4.0^{abc}	772.5^{c}	73.3^{bcde}	94.3^{bcd}	21.0^{abc}	5.5^{c}
	(0.1)	(0.1)	(1.1)	(0.4)	(0.2)	(0.1)	(0.1)	(0.5)	(1.3)	(2.9)	(0.1)	(1.0)	(2.3)	(0.02)
2B100	7.6^{def}	0.81^{cde}	82.0^{abc}	7.2^{bcd}	7.2^{b}	59.7^{ab}	1.9^{a}	8.8^{bc}	3.2^{abc}	798.8^{b}	77.8^{ab}	100.4^{ab}	22.6^{ab}	6.0^{b}
	(0.3)	(0.1)	(1.2)	(0.2)	(0.4)	(0.4)	(0.5)	(1.6)	(0.4)	(2.5)	(0.2)	(0.3)	(1.5)	(0.06)
2B150	6.6^{ef}	1.17^{b}	79.4^{c}	8.4^{ab}	10.4^{ab}	59.2^{b}	1.9^{a}	8.2^{bcd}	4.9^{a}	793.8^{b}	74.1^{abcde}	94.8^{abcd}	20.7^{abc}	5.9^{b}
	(0.2)	(0.0)	(0.6)	(0.4)	(0.3)	(0.0)	(0.0)	(0.6)	(0.6)	(4.8)	(0.1)	(0.0)	(0.1)	(0.01)
2B200	2.6^{f}	1.94^{a}	77.2^{c}	8.1^{ab}	10.0^{ab}	59.5^{b}	1.6^{a}	10.3^{b}	3.7^{abc}	673.8^{g}	70.4^{e}	91.1^{defg}	20.7^{abc}	4.9^{e}
	(0.3)	(0.1)	(0.3)	(0.5)	(1.0)	(0.2)	(0.1)	(0.2)	(0.3)	(2.5)	(0.5)	(0.7)	(0.7)	(0.1)
3B0	9.3^{de}	0.83^{cd}	81.2^{bc}	7.7^{bc}	6.5^{b}	60.6^{a}	1.9^{a}	18.6^{a}	4.2^{ab}	807.5^{b}	79.5^{a}	98.9^{abc}	19.4^{abc}	6.1^{b}
	(1.0)	(0.1)	(2.0)	(0.1)	(0.7)	(0.1)	(0.0)	(0.9)	(0.2)	(6.5)	(0.7)	(0.7)	(1.7)	(0.07)
3B50	8.1^{def}	1.03^{bc}	78.0^{c}	4.8^{ef}	8.0^{b}	59.5^{b}	1.6^{a}	7.6^{bcde}	4.2^{ab}	798.8^{b}	75.0^{abcd}	95.5^{abcd}	20.5^{abc}	5.9^{b}
	(1.1)	(0.0)	(0.2)	(0.6)	(0.5)	(0.5)	(0.1)	(0.3)	(0.7)	(2.5)	(0.2)	(2.0)	(2.9)	(0.04)
3B100	12.6^{bcd}	0.73^{defg}	77.3^{c}	2.5^{h}	8.0^{ab}	59.6^{ab}	1.8^{a}	10.7^{b}	4.4^{ab}	831.3^{a}	77.3^{abc}	101.3^{a}	24.0^{a}	6.3^{a}
	(2.0)	(0.1)	(2.7)	(0.3)	(1.0)	(0.1)	(0.4)	(1.8)	(0.1)	(4.8)	(0.2)	(0.4)	(2.4)	(0.06)
3B150	11.4^{bcde}	0.57^{efg}	80.6^{bc}	3.0^{gh}	9.0^{ab}	59.6^{ab}	1.7^{a}	6.0^{bcdef}	3.3^{abc}	830.0^{a}	75.8^{abcd}	94.2^{bcde}	18.4^{bcd}	6.3^{a}
	(0.3)	(0.1)	(1.4)	(0.1)	(0.8)	(0.1)	(0.3)	(1.1)	(0.5)	(4.1)	(0.8)	(0.8)	(0.8)	(0.05)
3B200	8.2^{def}	1.04^{bc}	77.6^{c}	6.2^{cde}	8.2^{b}	59.8^{ab}	1.7^{a}	3.6^{def}	4.0^{abc}	738.8^{d}	71.3^{cde}	92.3^{cdef}	20.5^{abc}	5.4^{cd}
	(1.0)	(0.0)	(0.4)	(0.4)	(0.2)	(0.4)	(0.1)	(0.1)	(0.5)	(13.1)	(1.0)	(1.8)	(1.9)	(0.00)

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Table 3. Pearson's correlation coefficients of the visco-elastic properties of gluten, dough and baking characteristics with ascorbic acid. Definitions of visco-elastic, mixing and baking variables explained in Table 2, Chapter III.

	SeP	$J\text{-}J_r$	RCY	TCR	TCC	WA	DT	ST	BT	LV	PH	LH	SV	OSP	FP
SeP	1														
$J\text{-}J_r$	-0.77**	1													
RCY	0.73**	-0.79**	1												
TCR	-0.64**	0.66**	-0.40*	1											
TCC	-0.50**	0.68**	-0.55**	0.49**	1										
WA						1									
DT				0.33*		0.70**	1								
ST						0.66**	0.68**	1							
BT						-0.73**	0.91**	0.79**	1						
LV						-0.58**	-0.57*	-0.65**	-0.66**	1					
PH	0.38*	-0.33*				-0.55**	-0.64**	-0.45**	-0.73**	0.60**	1				
LH						-0.61**	-0.68**	-0.58**	-0.72**	0.95**	0.41*	1			
SV	-0.61**												1		
OSP		0.36*	-0.57**							0.35*	-0.31*	0.45*		1	
FP						-0.96**	-0.78**	-0.77**	-0.85**	0.70**	0.66**	0.71**	0.32*		1

*Correlation is significant at $\alpha = 0.05$ level

**Correlation is significant at $\alpha = 0.01$ level

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Table 4. Explained variance (%) in PCA of visco-elastic, mixing and baking variables in gluten and flours treated with ascorbic acid. Definitions of visco-elastic, mixing and baking variables explained in Table 2, Chapter III.

Ascorbic acid	Axes	PC1	PC2	1+2
	PC (%)	39.89	26.30	66.19
Visco-elastic	SeP	1.52	80.74	82.26
	$J\text{-}J_r$	1.76	78.45	80.21
	RCY	0.11	73.13	73.24
	TCR	4.63	44.27	48.90
	TCC	0.49	41.93	42.42
Farinograph	WA	70.38	3.35	73.73
	DT	77.81	2.88	80.69
	ST	65.17	2.96	68.13
	BT	87.04	0.57	87.61
Baking	PH	63.43	6.58	70.01
	LH	60.97	10.89	71.86
	SV	70.88	3.34	74.22
	OSP	2.11	43.67	45.78
	LV	4.76	0.01	4.77
Protein content	FP	87.30	1.69	88.99

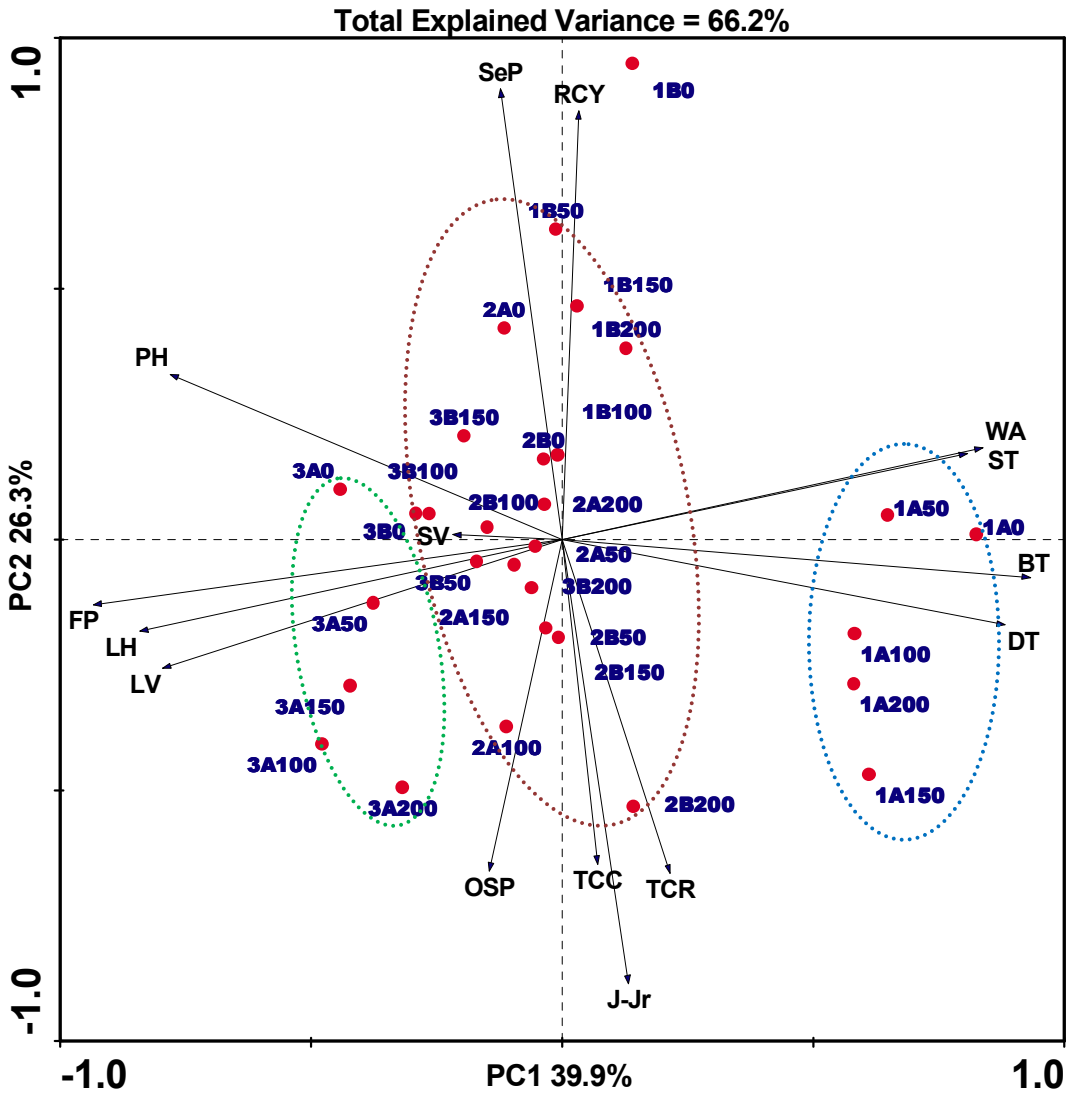

Fig. 1. Loading plot of first two principal components based on baking, visco-elastic and dough properties of six commercial wheat flours obtained from sites A and B, added with four levels of ascorbic acid. Definitions of visco-elastic, mixing and baking variables explained in Table 2, Chapter III. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively.

CHAPTER V

EFFECT OF UREA ON MIXING AND BAKING PROPERTIES IN WHEAT FLOURS AND ITS CORRELATION TO GLUTEN VISCO-ELASTICITY.

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Abstract

Effect of urea was evalulated on the visco-elastic properties of gluten and mixing and baking properties of dough in hard red winter wheat flours obtained from two different sources. Urea was added to gluten and wheat flours at the levels of 0, 0.5, 1 and 1.5 M concentrations. Creep-recovery measurements were performed to investigate the effect of urea on visco-elastic properties of gluten. Mixing properties of wheat flours were evaluated using the Farinograph measurements. Baking characteristics were measured after the wheat flours were baked using an optimized straight dough bread making method. An overall increase (25 to 27% range) in recovery compliance of gluten with urea in most flours with few exceptions indicated the weakening of gluten. Overall significant decrease (33 to 65%) in separation time of gluten in all flours with few exceptions was observed ($P < 0.05$). Changes in delta compliance due to urea addition to gluten did not indicate a specific change. Time constants for creep and recovery increased (25 to 50% range) indicating a slowdown in rates at which gluten deformed and recovered in most cases except a few. Urea addition resulted in a general decrease in dough stability in most flours except in site A flour with 11.5% protein content which showed increase in stability by 53% at 1 M urea. Water absorption ability of all flours decreased (1.5% to 4% range) with urea addition. Dough development time was found to be significantly correlated to delta compliance ($r = -0.57$, $P <0.01$) indicating decrease in development time could be attributed to increase in viscous component of gluten induced by urea addition. Loaf characteristics such as loaf heights, loaf volumes, specific volumes, oven springs and proof heights decreased significantly ($P <0.05$) with urea addition. A sharp significant decrease in loaf volumes (18 to 38% range) and oven

springs (79 to 87% range) was observed with urea addition. Weak but significant correlations ($P < 0.05$) of visco-elastic properties such as creep and recovery time constants and percent recoverability of gluten with proof heights and loaf heights were observed. Correlation of recoverability of gluten with proof heights ($r = 0.38$) and loaf heights ($r = 0.39$) indicated decrease in elastic recovery in gluten could be associated with decreased proof and loaf heights of dough due to urea addition. A negative correlation of recovery time constants of gluten with proof heights ($r = -0.40$) and loaf heights ($r = -0.39$) suggested that baking properties were affected due to the rate at which the gluten recovered was slowed down by urea addition. Urea addition in gluten extracted from all flours decreased its overall strength and rate at which the gluten deformed and recovered which could be attributed to the poor baking performance and loaf qualities of the wheat flours.

1. Introduction

Urea is widely used as denaturant agents, but it is still not clear by which molecular mechanism they denature proteins. It is well known that the solubility of most protein side chains and backbone increases with denaturant concentration It has been shown that these urea concentrations are high enough (i) to destabilize many proteins and enzymes (Finer, Franks & Tait, 1972), (ii) to interfere with protein–ligand interactions (Yancey & Somero, 1978), (iii) to perturb conformation and assembly state of urea-sensitive proteins (Yancey & Somero, 1980), and (iv) to offer competitive inhibition of enzymes (Yancey et al., 1978). Urea is understood to disturb the ability of water to maintain the tetrahederal hydrogen bonding (Caballero-Herrera, Nordstrand, Berndt & Nilsson, 2005). Urea competes with water molecule and has a tendency to form hydrogen bonds with peptide units faster than water (Tobi, Elber & Thirumalai, 2003). It has also been suggested that urea induces a denaturation process of electrostatic character by adhering on the surface of charged residues, leading to repulsion between residues. The result of the repulsion is an opening to water into the protein interior that will provoke the unfolding (Tobi et al., 2003).It has been generally accepted that gluten play a key role in determining the unique baking quality of wheat by conferring water absorption capacity, cohesivity, viscosity and elasticity on dough. The visco-elastic properties of gluten enable the wheat dough to be processed into a range of food products including bread, pasta, and noodles. Gluten is made up of monomeric gliadins and polymeric glutenin fractions. The gliadins are considered to impart viscous properties to dough and the polymeric glutenins elastic properties. One group of glutenin proteins, the HMW subunits, has been shown to play a major role in determining dough elasticity (Shewry, Halford, Belton & Tatham,

2002b). A large number of gene sequences are now available for HMW subunits, showing that they typically comprise between 630 and 820 amino acids, with M_r ranging from 67,500 to 88,000 (Shewry, Halford, Tatham, Popineau, Lafiandra & Belton, 2003). Their sequences can be divided into three domains; an extensive central domain consists of repeated sequences based on two or three peptide motifs, hexapeptides, nonapeptides and tripeptides which vary in length from 420 to 700 residues. These repetitive domains are flanked by shorter non-repetitive domains which vary in length between 81 to 104 residues at the *N*-terminus and 42 residues at the *C*-terminus. The non-repetitive *N*- and *C*-terminal domains contain most or all of the cysteine residues available for inter-chain covalent bonding. The repetitive domains of the molecules contain many hydrophilic glutamine residues that can interact with the solvent (water) or form intermolecular hydrogen bonds, leading to nonentropic interactions (Feeney et al., 2003). High-MW glutenin subunits join end-to end through disulfide bonds to provide a sort of backbone to the gluten complex. Low-MW glutenin subunits are also crosslinked through disulfide bonds into the protein network. The smaller spherical gliadin molecules are incorporated into gluten primarily through noncovalent (hydrogen and hydrophobic) bonds (Bietz & Lookhart, 1996). The ultimate structure and properties of gluten may depend on the amounts and types of specific proteins that are present. Thus, even slight changes in type or amount of key subunits can markedly change gluten's quality or functionality. Belton et al. (1995b) proposed a model for structure and function of glutenin. This model postulated that repetitive domains of glutenin that do not interact with other chains are called as loops and are mobile sections of polymer. During extension (or dough mixing) the subunits become aligned, resulting in the formation of more rigid intermolecular β-

sheet also termed as train structures stabilized by interchain hydrogen bonds and corresponding decreases in β-turn (loop) structures (Belton, 1999). The glutenin structure as a whole is made up of entanglements of long peptide chains end linked by disulfide bonds to N and C terminals and crosslinked by hydrogen bonding among glutamine residues of repetitive portions of chains. Working or stretching of the dough extends the loops, and the trains are pulled apart, allowing the proteins to slide along one another (Edwards et al., 2003). Reestablishment of the train-loop equilibrium, driven by conformational entropy of the loops and the enthalpy of the inter-chain hydrogen bonds, provides elastic recovery. However, if the secondary cross-links are completely disrupted during processes of baking by temperatures and pressures, the quality of the loaf will be affected. There are very few studies that investigated the effect of disrupted non covalent bonds on the visco-elastic properties of gluten and performance of wheat flours.

The objective of this study was assess the effect of urea on visco-elastic properties of gluten using creep-recovery, mixing and baking properties of commercial hard red winter wheat flours by breaking the non-covalent hydrogen bonding in dough.

2. Materials and Methods

The procurement of wheat flour samples are explained in materials and methods section of chapter III.

Three levels (0.5, 1 and 1.5 M) of urea (VWR International Inc., West Chester, PA 19380), were added to flours from each source. Thus, site A flours were denoted as 1A0, 1A0.5, 1A1 and 1A1.5; 2A0, 2A0.5, 2A1 2A1.5; 3A0, 3A0.5, 3A1, 3A1.5, respectively. Similarly site B flours were named, 1B0, 1B0.5, 1B16 and 1B1.5; 2B0, 2B0.5, 2B1 and 2B1.5; 3B0, 3B0.5, 3B1and 3B1.5, respectively. Flours and gluten

isolated from flours with no AA were used as controls. The protein, moisture and ash contents were determined using the NIR system (FOSS NIR Systems Inc, Laurel, MD 20723) as shown in Table 1 (Chapter III). This design was implemented in gluten visco-elastic, dough farinograph tests and baking tests.

2.1. Gluten extraction

Glutens were prepared in triplicates in an automated gluten washer, Glutomatic 2200 (Perten Instruments, Sweden) from 10 g of flour and 5.0 mL of urea solution (3, 6, and 9 g urea in 100 ml 2% salt solution) using a mixing time of 60 sec and washing for 10 min with 2% NaCl solution (w/v). Control samples were mixed with 5.0 ml of pure deionized water.

2.2. Creep recovery tests

The creep recovery experiments were carried out as described in Chapter III.

2.3. Dough mixing properties

One ml of urea (3, 6 and 9 g per 100 ml deionized water) was added to 10 g of wheat flour. Dough mixing properties were evaluated as described in Chapter III.

2.4. Baking tests

Five ml of urea solution (3, 6 and 9 g urea in 100 ml deionized water) was added to the flour. Baking tests were performed as explained in chapter 2. The definitions of baking, dough mixing and visco-elastic are explained in Table 2 (Chapter III).

3. Statistics

Statistical analysis is performed using same methods explained in chapter III.

4. Results

4.1. Visco-elastic properties

Urea levels affected the recovery compliance of gluten extracted from site A flours very differently with different protein levels. A significant decrease in recovery compliance ($P < 0.05$) by 39% with 1.5 M urea was observed in 1A gluten (Appendix 1, Fig. 6). In 2A and 3A gluten, 0.5 M urea significantly increased the recovery compliance by 27% (Appendix 1, Fig. 6). No significant differences in recovery compliance of gluten extracted from 1B were seen with urea addition. Urea level of 1.5 M increased the recovery compliance of gluten from 2B flours significantly by 25% (Appendix 1, Fig 7). Gluten extracted from high protein flour from site B strengthened with decrease in recovery compliance by 40% with 0.5 M urea and 20% with 1 M urea, respectively (Appendix 1, Fig 7).

Significant interactions among flour protein contents and urea levels in the visco-elastic properties of gluten were observed except for recovery time constants in site A (Appendix 2, Table 3). Delayed elasticity of gluten from 2A, 3A and 1B decreased in form of separation time (SeP) by 32.3, 65.4, and 33.1% with urea addition of 1, 0.5 and 1.5 M, respectively (Tables 1 and 2). On the contrary, SeP increased in 3B with 0.5 M urea by 46.8% (Table 2). Delta compliance ($J-J_r$) decreased in gluten from 1A suggesting increased elasticity by 42% with 1.5 M urea concentration (Table 1). No significant change was observed in $J-J_r$ of gluten from 2A with urea addition. Gluten elasticity of 3A decreased with 1.5 M urea levels as delta compliance increased by 23% (Table 1). No significant changes were observed in delta compliance of gluten from 1B. Elasticity increased in gluten from 3A as SeP increased by 79.4% and $J-J_r$ decreased by 44% with 0.5 M urea concentrations (Table 2).

An increase of 5% in percent recoverability was observed in gluten from 3A flour at 1 M urea level (Table 1). No significant differences were seen in percent recoverability of gluten extracted from other flours from both sites with urea. There was no significant interaction in recovery time constants (TCR) in gluten from site A flours (Appendix 2, Table 3). A significant protein effect was observed in which mean recovery rates (TCR) of gluten from 1A flours were 50 and 34% longer than those of gluten from 1A and 3A, respectively (Table 1). The rate at which the gluten recovered in 2B flours was 28.7% slower than the controls at 1.5 M urea (Table 2). Faster recovery rates of gluten by 41.5% were observed with 0.5 M urea over controls in 3B flours (Table 2). Significant protein effect ($P < 0.05$) indicated that rate of recovery of gluten with urea was 7.93 s in low protein, 5.62 s in high protein and 4.4 s in medium protein (Appendix 3, Table 3) The rate at which the gluten deformed (TCC) showed no significant differences with urea addition except for gluten from 2B flour. Deformation rate of gluten slowed as time constants for creep increased in comparison controls by 22.5% with 1.5 M urea level in 2B (Table 2).

4.2. Dough mixing properties

Significant interactions of flour protein content and urea levels in mixing properties of flours were observed with the exception of dough development time (DT) in site B 9Appendix 2, Table 3). Water absorption (WA) of 3A flour increased by 4.6% at 1.5 M urea levels (Table 1). Urea concentration of 1 M increased WA in 1B, 2B and 3B flours by 2.5, 1.5 and 2%, respectively (Table 2). Dough development time (DT) increased in 1A flours by 28.5% and decreased in 3A flours by 16.6% with 0.5 M urea (Table 1). Significant protein and treatment effect were observed in DT of flours in site

B. Dough development time was significantly high in flours in site B with 1.5 M urea levels (2.1 min) compared to controls and 1 M urea (Appendix 3, Table 3) while high protein flour gad significantly high DT (2.06 min) compared to other protein levels (Appendix 3, Table 3). Dough stability time (ST) showed increase of 53% in 2A flour at 1 M urea level (Table 1). Dough stability of 3A and 2B decreased by 28 and 35%, respectively with 1.5 M urea and 35.5% in 3B with 0.5% urea levels (Tables 1 and 2). Time required to breakdown the dough after mixing (BT) decreased by 26% with 0.5 M urea in 3A flour and further increased with increasing concentration of urea (Table 1). High protein flour from site B showed increase in BT by 53.3% at 1 M urea level (Table 2).

4.3. Baking properties

Significant interactions of flour protein content and urea treatment in baking properties were observed (Appendix 2, Table 3). Addition of urea decreased the baking quality of bread. All baking parameters such as loaf volumes (LV), loaf heights (LH), proof heights (PH), oven springs (OSP) and specific volumes (SV) showed a sharp decrease with 1.5 M urea level in all the breads from sites A and B. Loaf volumes in 1A, 2A, 3A, 1B, 2B and 3B decreased by 26.1, 31.3, 34.3, 34.2, 46 and 40%, respectively with 1.5 M urea (Tables 1 and 2). Decrease in LH in 1A, 2A, 3A, 1B, 2B and 3B breads with 1.5 M urea was observed to be 38.5, 31.1, 31.8, 24.3, 45.7 and 28.5%, respectively (Tables 1 and 2). Urea addition of 1.5 M retarded the proof heights in 1A, 2A, 3A, 1B, 2B and 3B by 23.6, 32.6, 31.2, 32.7, 31.9 and 20%, respectively (Tables 1 and 2). Oven springs decreased in 1A by 78% with 0.5 M urea (Table 1). Urea addition of 1.5 M decreased OSP in 2A and 3A by 26.3 and 33.7%, respectively (Table 1). OSP decreased

in 1B bread by 79.5% with 1 M urea level but no difference in OSP was observed at 1.5 M urea from the control (Table 2). No oven spring rise during baking was observed at 1 and 1.5 M urea levels, i.e. the loaves collapsed in the oven such that their heights were lower than proof heights which gave negative values for OSP (Table 2). Negative drop in 2B dough OSP was 63.1% higher at 1M urea than 1.5 M urea. No significant change in OSP of 3B bread was observed with urea addition. Specific volumes of 1A, 2A, 3A, 1B, 2B and 3B breads dropped by 30, 35.1, 40.6, 34.6, 47.2 and 44%, respectively at 1.5 M urea concentrations. Drop in LV, LH and SV was higher in 2B flour in comparison to bread baked from other flours. Only 2B gluten had lower rates of deformation and recovery (TCC and TCR, respectively) compared to the gluten from other flours with urea addition. Gluten strength decreased with increase in compliance with addition of urea in gluten from 2B flour.

4.4. Correlations and PCA

The relationship of visco-elastic, baking and mixing parameters affected by urea addition is shown in Table 3. Although separation time (SeP), a function of elasticity and delta compliance ($J-J_r$), function of viscosity and elastic behavior, showed no correlations with baking properties, its correlations with mixing properties were observed. Delta compliance ($J-J_r$) was significantly negatively correlated ($P < 0.01$) to DT and positively correlated to BT ($r = -0.59$ and 0.54, respectively). Urea levels decreased the dough development time and increased viscosity of gluten as observed in $J-J_r$ and DT measurements of 3A flours (Table 1). Visco-elastic parameters like creep and recovery time constants (TCC and TCR, respectively) and percent recoverability (RCY) showed weak correlations ($P < 0.05$) with baking parameters (Table 3). Elasticity in the form of

percent recoverability of gluten was positive correlated to proof heights (r = 0.37) and loaf heights (r = 0.38). This could be due to increased recoverability (RCY) in case of 2A gluten which showed a decrease in loaf heights (LH) and proof heights (PH) of 2A dough at 1.5 M urea (Table 1). Decrease in proof heights, loaf heights and loaf volumes were associated to longer recovery rates (TCR) with urea addition (Table 3). Significantly negative correlations ($P < 0.05$) of TCR with LV (r = -0.34), LH (r = -0.39) and PH (r = -0.40) were observed. Significant increase in 2B and 1B gluten TCRs showed drastic drop in LV, LH and PH values in their breads (Table1). Similarly, rate of deformation (TCC) had weak negative correlations ($P < 0.05$) with LV, LH, PH and SV (Table 3). Urea addition increased the rate at which the gluten deformed (TCC) which decreased PH (r = -0.43), LH (r = -0.44), LV (r = -0.42) and SV (r = -0.40), respectively ($P < 0.05$).

Fig. 1. depicts principal component analysis of visco-elastic, mixing and baking characteristics in hard red winter wheat flours from sites A and B. Principal component axis 1 (PC1) and 2 (PC2) cumulatively explained 69.5% of total variance with 41.4% and 28.1% individually. Principal component axis 1 was dominated by loaf volumes (79.4%) followed by specific volume (73.4%) and loaf heights (73.6%) as reported in Table 4. Delta compliance ($J-J_r$) explained the highest variance (86.2%) on PC2. Upper left quadrant showed a group of closely related variables, PH, LH, SV, OSP and LV. These are all baking variables and are very closely related to PC1. Lower left quadrant showed that all mixing parameters along with flour protein content (FP) are very closely correlated. This was also confirmed by Pearson's correlations of FP with WA, DT, ST and BT respectively in Table 3. Percent recoverability of gluten had a positive correlation with baking characteristics such as proof heights and loaf heights as confirmed by

Pearson's correlations (Table 3). Creep and recovery rates (TCC and TCR) placed in the lower right quadrant had negative correlations (also refer Table 3) with baking characteristics.

Addition of urea did not bring any improvement in 1A flour visco-elastic, mixing or baking performance. Urea addition to 3A flour showed improved water absorption and dough development time at 0.5 and 1 M concentration while 1.5 M concentration linked 3A flour to viscosity ($J\text{-}J_r$) (Fig. 1). Urea addition decreased the elastic (SeP) performance of 1B flour at 1.5 M urea concentration (Fig. 1). Increased urea levels in 2B oriented it away from upper left quadrant to lower right quadrant which explains the longer recovery rates (TCR) and poor baking performance.

5. Discussion

Effect of urea addition on the baking characteristics of flours from both sites had a negative effect on the loaf quality. Visco-elastic properties of gluten measured using creep recovery tests showed varied results on addition of urea. Increase in recovery compliance in gluten from site A low protein flour and site B high protein flour with urea addition suggested that non-covalent crosslinks in between glutamine residues of repeat sections of high molecular weight glutenin fractions may not have been completely broken. With release of stress, hydrogen bonding could have formed back imparting the elastic strength. The concentrations of urea in this study may have brought partial disruptions in hydrogen bonding in gluten of some wheat flours. Inda and Rha (1982) noticed similar partial disruptions at the urea concentrations up to 3 M and complete disruptions of hydrogen bonds were obtained at 8 to 10 M concentrations when gluten was subjected to tensile testing at constant strain. The increase in elasticity as observed in

dynamic visco-elasticity measurements of gluten added with 1 to 5 M urea levels could be due to unfolding of protein polypeptides that allows freedom to the β-sheets to extend (Inda & Rha, 1991). Increment in the length in β-sheets provides mobility to the entanglements or loops and under these circumstances an increased elasticity is observed as longer times are required for the entanglements to move under external stress (Inda et al., 1991). Khatkar (2005) observed that disruption of hydrogen bonds by concentrations of urea less that 3 M decreased the elasticity and increased viscosity in a controlled stress rheometry on gluten using dynamic oscillatory measurements. However, gluten exhibited increased resistance to the deformation at the levels higher than 3 M up to 9 M concentrations. This increased strength of gluten was attributed to exposure of sulfahydryl (SH) group due to urea that reacted with disulfide linkage. Khatkar (2005) reported that shear storage modulus (G') and shear loss modulus (G'') of gluten treated with urea and measured showed significant positive relationships with bread-making performance, explaining 73 and 69% of the variation in loaf volume, respectively. Our study agreed with the observations where elastic recovery of gluten was found to be positively correlated to the baking parameters (Fig. 1). The secondary structure change during gluten deformation were studied on a highly developed gluten network isolated protein bodies from developing wheat endosperm subjected to biaxial tensile tests (Wellner et al., 2004). The tests revealed the deformation of isolated protein bodies slowed down by 20% in comparison to gluten that slowed by 10% after five test cycles (Wellner et al., 2004). Secondary crosslinks in gluten were well developed during mixing of dough and isolation of wet gluten. However, isolated protein bodies from developing wheat endosperm aggregated to give a cohesive mass upon membrane removal were

believed to have the least amount of hydrogen bonding. In this study gluten took longer time to deform and recover (TCC and TCR, Table 3) in comparison to control which could be due to a decreased ability of gluten to recover faster due to disruption of secondary bonding in glutenin subunits. During baking the starch gelatinizes and protein gets denatured and that affects the interactions of hydrogen bonding in protein and starch. Hydrogen bonds are continuously broken and reformed during heating due to the interaction of water and amylose from starch that forms crosslinks with amylopectin (Kuo & Wang, 2006). McGrane et al. (2004) used various hydrogen bond-forming and breaking agents to study the visco-elastic properties of amylose gels. They reported that the use of intermolecular hydrogen bond breaking agents such as urea reduced gel strength significantly, presumably by decreasing the intermolecular network formation between water and amylose. Steep hindrance in the ability of 2A flour dough to rise during oven spring that lead to negative values of oven springs at urea concentrations of 1 and 1.5 M could be attributed to loss in viscosity due to loss of gel strength in starch.

6. Conclusions

Urea caused noncovalent hydrophilic bond disruption in gluten and lowered the baking performance of all the flours used in this study. Visco-elastic changes in gluten containing urea appeared to vary with the quality of protein, but overall, urea slowed the rate at which gluten deformed or recovered. Although urea addition affected the baking performance negatively, elasticity of gluten was not always reduced by hydrophilic bond disruption.

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Table 1. Visco-elastic, farinograph and baking characteristics in commercial wheat flours from site A, treated with urea levels. Means with same superscripts in a column are not significantly different (P > 0.05). The standard deviations of means are shown in parentheses. Definitions of visco-elastic, mixing and baking variables described in Table 2, Chapter III. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively.

TRT	Visco-elastic					Farinograph				Baking				
	SeP (s)	J-J_r (J)	RCY (%)	TCR (s)	TCC (s)	WA (%)	DT (min)	ST (min)	BT (min)	LV (cc)	PH (mm)	LH (mm)	OSP (mm)	SV (cc/g)
1A0	4.8^{d}	1.27^{ab}	79.3^{d}	8.0^{a}	9.5^{a}	53.6^{f}	1.0^{e}	2.0^{d}	1.4^{cd}	550.0	59.2^{d}	84.4^{c}	25.2^{a}	4.0^{c}
	(0.3)	(0.1)	(1.0)	(0.9)	(0.7)	(0.2)	(0.1)	(0.0)	(0.1)	(4.1)	(1.5)	(1.3)	(0.2)	(0.02
1A0.5	8.6^{cd}	0.77^{def}	79.4^{cd}	8.2^{a}	7.6^{abc}	52.3^{fg}	1.4^{cd}	1.0^{d}	1.9^{cd}	566.3	66.4^{c}	71.8^{d}	5.4^{e}	4.0^{c}
	(0.1)	(0.0)	(0.4)	(0.9)	(0.9)	(0.4)	(0.5)	(0.4)	(0.5)	(4.8)	(0.4)	(0.4)	(0.1)	(0.04
1A1	4.7^{d}	0.95^{cd}	80.7^{bcd}	8.4^{a}	8.9^{ab}	50.8^{g}	1.3^{cd}	1.4^{d}	1.8^{cd}	488.8	59.5^{d}	66.2^{e}	6.8^{e}	3.5^{d}
	(0.6)	(0.1)	(1.1)	(0.8)	(1.4)	(0.4)	(0.2)	(0.1)	(0.4)	(2.5)	(0.8)	(1.7)	(2.5)	(0.00
1A1.5	8.7^{cd}	0.74^{def}	79.7^{cd}	7.2^{ab}	7.6^{abc}	51.1^{g}	1.1^{de}	1.2^{d}	1.3^{d}	406.3^{i}	44.0^{f}	51.9^{f}	8.0^{e}	2.8^{e}
	(0.6)	(0.0)	(0.8)	(0.0)	(0.5)	(0.1)	(0.2)	(0.3)	(0.5)	(2.5)	(0.0)	(0.4)	(0.5)	(0.05
2A0	16.4^{a}	0.57^{f}	82.7^{abc}	4.0^{cd}	6.2^{cd}	58.6^{de}	1.8^{cd}	8.3^{c}	3.7^{cd}	745.0	71.0^{bc}	94.5^{a}	23.5^{ab}	5.4^{b}
	(0.8)	(0.1)	(1.8)	(0.6)	(0.1)	(0.3)	(0.0)	(0.7)	(1.1)	(4.1)	(1.7)	(0.6)	(2.3)	(0.0)
2A0.5	17.1^{a}	0.65^{def}	84.8^{a}	4.9^{bc}	5.6^{d}	58.7^{de}	1.9^{cd}	15.9^{b}	3.3^{cd}	735.0	72.7^{ab}	92.2^{b}	19.5^{abc}	5.4^{b}
	(4.6)	(0.1)	(0.7)	(0.2)	(0.4)	(0.4)	(0.2)	(2.2)	(1.0)	(12.9)	(2.7)	(3.0)	(0.7)	(0.04
2A1	11.1^{bc}	0.66^{ef}	80.8^{bcd}	3.7^{d}	6.1^{cd}	58.8^{de}	2.2^{cd}	17.8^{ab}	3.7^{cd}	737.5	72.6^{ab}	90.9^{b}	18.4^{bcd}	5.3^{b}
	(1.0)	(0.0)	(1.5)	(0.5)	(0.4)	(0.3)	(0.2)	(1.2)	(0.4)	(8.7)	(1.3)	(0.2)	(1.5)	(0.14
2A1.5	14.8^{ab}	0.60^{ef}	79.4^{cd}	5.0^{bc}	6.6^{cd}	59.9^{d}	2.3^{c}	15.3^{b}	4.2^{c}	511.3	47.8^{ef}	65.1^{e}	17.3^{cd}	3.5^{d}
	(1.7)	(0.0)	(1.4)	(0.3)	(0.0)	(0.1)	(0.1)	(2.1)	(0.2)	(2.5)	(0.6)	(0.9)	(0.3)	(0.02
3A0	10.7^{bc}	1.12^{bc}	80.2^{cd}	5.8^{bc}	7.2^{bcd}	63.6^{b}	10.8^{a}	21.5^{a}	16.0^{a}	821.3	74.2^{ab}	97.3^{a}	23.1^{abc}	5.9^{a}
	(1.0)	(0.1)	(0.5)	(0.7)	(0.5)	(0.9)	(0.1)	(0.9)	(0.2)	(4.8)	(2.6)	(0.3)	(2.8)	(0.03
3A0.5	3.7^{d}	1.43^{a}	81.3^{abc}	5.8^{bc}	7.4^{bcd}	63.1^{bc}	9.0^{b}	18.4^{ab}	11.8^{b}	772.5	79.0^{a}	98.1^{a}	19.1^{abc}	5.5^{a}
	(0.2)	(0.0)	(0.6)	(0.3)	(0.3)	(0.4)	(0.4)	(0.1)	(0.2)	(2.9)	(1.4)	(0.6)	(2.0)	(0.14
3A1	4.3^{d}	0.87^{cd}	84.2^{ab}	5.3^{bc}	6.9^{bc}	64.1^{b}	9.5^{b}	18.3^{ab}	12.5^{b}	746.3	73.9^{ab}	91.8^{b}	17.8^{bcd}	5.3^{b}
	(0.7)	(0.1)	(0.6)	(0.0)	(0.5)	(1.0)	(0.4)	(1.6)	(0.6)	(4.8)	(1.8)	(0.4)	(1.5)	(0.06
3A1.5	6.3^{cd}	1.45^{a}	80.6^{bcd}	5.6^{bc}	8.1^{ab}	66.7^{a}	9.6^{b}	15.5^{b}	14.1^{a}	531.3^{f}	51.0^{e}	66.3^{e}	15.3^{d}	3.5^{d}
	(0.6)	(0.1)	(0.1)	(0.3)	(0.1)	(0.4)	(0.5)	(1.9)	(1.6)	(4.8)	(0.5)	(0.5)	(0.0)	(0.01

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Table 2. Visco-elastic, farinograph and baking characteristics in commercial wheat flours from site B, treated with urea levels. Means with same superscripts in a column are not significantly different (P > 0.05). The standard deviations of means are shown in parentheses. Definitions of visco-elastic, mixing and baking variables described in Table 2, Chapter III. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively.

TRT	Visco-elastic					Farinograph				Baking				
	SeP (s)	J-J_r (J)	% RC (%)	TCR (s)	TCC (s)	WA (%)	DT (min)	ST (min)	BT (min)	LV (cc)	PH (mm)	LH (mm)	OSP (mm)	SV (cc/g)
1B0	29.0^{a}	0.28^{e}	86.5^{a}	3.2^{fg}	5.0^{cde}	59.3^{fg}	1.5^{bc}	1.8^{f}	2.1^{c}	696.3^{d}	76.9^{ab}	91.1^{bc}	14.2^{bcd}	4.9^{c}
	(2.7)	(0.0)	(1.2)	(0.3)	(0.3)	(0.0)	(0.0)	(0.4)	(0.1)	(4.8)	(1.9)	(1.7)	(0.1)	(0.0)
1B0.5	23.0^{ab}	0.33^{e}	85.6^{ab}	4.6^{ef}	3.8^{e}	59.4^{efg}	1.9^{abc}	1.4^{f}	2.2^{bc}	706.3^{d}	75.8^{ab}	88.3^{c}	12.5^{cd}	5.1^{c}
	(1.4)	(0.0)	(1.5)	(0.3)	(0.2)	(0.4)	(0.3)	(0.1)	(0.1)	(4.8)	(0.7)	(2.0)	(2.7)	(0.05)
1B1	21.7^{b}	0.37^{de}	81.3^{bc}	4.1^{ef}	5.1^{cde}	60.8^{bc}	1.7^{abc}	2.5^{f}	2.5^{bc}	625.0^{e}	79.3^{a}	82.1^{d}	2.9^{e}	4.4^{d}
	(1.0)	(0.0)	(0.5)	(0.1)	(1.1)	(0.3)	(0.3)	(0.5)	(0.4)	(4.1)	(0.3)	(1.1)	(0.9)	(0.02)
1B1.5	19.4^{bc}	0.41^{de}	84.3^{abc}	4.2^{ef}	4.4^{de}	60.4^{cde}	1.9^{abc}	1.9^{f}	2.8^{bc}	457.5^{h}	51.7^{e}	68.9^{f}	17.1^{abc}	3.2^{f}
	(2.6)	(0.1)	(1.9)	(0.2)	(0.8)	(0.0)	(0.1)	(0.4)	(0.3)	(6.5)	(2.1)	(0.3)	(1.8)	(0.01)
2B0	10.2^{de}	0.71^{bc}	80.8^{c}	6.2^{bcd}	6.2^{bcd}	59.6^{defg}	1.9^{abc}	10.4^{cd}	4.5^{b}	772.5^{b}	73.3^{bc}	94.3^{ab}	21.0^{a}	5.5^{ab}
	(1.4)	(0.1)	(1.7)	(1.1)	(0.5)	(0.1)	(0.2)	(0.7)	(0.1)	(2.9)	(0.1)	(1.0)	(1.0)	(0.02)
2B0.5	8.3^{e}	0.95^{a}	80.4^{c}	7.1^{abc}	4.7^{cde}	59.1^{g}	1.9^{abc}	12.5^{bc}	3.6^{bc}	587.5^{f}	66.3^{d}	76.7^{e}	10.4^{d}	4.2^{e}
	(0.6)	(0.1)	(0.9)	(0.1)	(0.4)	(0.4)	(0.1)	(1.0)	(0.4)	(8.7)	(0.7)	(0.6)	(1.3)	(0.06)
2B1	8.6^{e}	0.96^{a}	80.3^{c}	5.7^{cde}	7.8^{ab}	60.5^{cde}	1.5^{c}	8.2^{de}	2.2^{c}	455.0^{h}	67.8^{cd}	58.3^{g}	-9.5^{f}	3.2^{f}
	(0.7)	(0.0)	(0.2)	(0.3)	(0.1)	(0.4)	(0.1)	(0.6)	(0.9)	(9.1)	(2.7)	(2.0)	(0.7)	(0.07)
2B1.5	10.3^{e}	0.87^{ab}	81.7^{bc}	8.7^{a}	8.7^{a}	62.2^{a}	2.1^{ab}	6.7^{e}	3.9^{bc}	417.5^{i}	49.9^{e}	51.2^{h}	-3.7^{e}	2.9^{g}
	(2.0)	(0.1)	(1.1)	(0.6)	(0.6)	(0.0)	(0.2)	(0.1)	(0.4)	(6.5)	(0.3)	(0.4)	(0.2)	(0.04)
3B0	9.3^{e}	0.83^{ab}	81.2^{bc}	7.7^{ab}	6.5^{bc}	60.6^{cd}	1.9^{abc}	18.6^{a}	4.2^{bc}	798.8^{a}	75.0^{ab}	95.5^{ab}	20.5^{a}	5.9^{a}
	(1.0)	(0.1)	(2.0)	(0.1)	(0.8)	(0.1)	(0.1)	(0.8)	(0.2)	(2.5)	(0.2)	(2.0)	(1.8)	(0.05)
3B0.5	17.5^{bcd}	0.47^{de}	82.8^{abc}	4.5^{ef}	4.9^{cde}	60.2^{cdef}	2.0^{abc}	12.0^{c}	4.0^{bc}	760.0^{bc}	80.3^{a}	95.2^{a}	16.4^{abcd}	5.4^{ab}
	(1.2)	(0.0)	(1.4)	(0.7)	(0.9)	(0.2)	(0.0)	(0.2)	(0.1)	(8.2)	(0.4)	(0.5)	(0.4)	(0.12)
3B1	10.7^{e}	0.57^{cd}	84.4^{abc}	4.6^{ef}	5.5^{cd}	61.8^{a}	2.1^{abc}	18.4^{a}	9.0^{a}	755.0^{c}	75.4^{ab}	96.6^{ab}	19.8^{ab}	5.4^{b}
	(3.2)	(0.0)	(0.3)	(0.1)	(0.4)	(0.1)	(0.1)	(1.4)	(1.4)	(4.1)	(2.4)	(0.8)	(2.9)	(0.00)
3B1.5	12.7^{cde}	0.70^{bc}	83.0^{abc}	4.7^{def}	6.5^{bc}	61.7^{ab}	2.3^{a}	15.2^{b}	4.0^{bc}	478.8^{g}	60.0^{ae}	68.3^{f}	16.3^{abcd}	3.3^{f}
	(1.0)	(0.0)	(0.9)	(0.1)	(0.5)	(0.4)	(0.1)	(1.3)	(0.8)	(6.3)	(0.1)	(0.7)	(0.8)	(0.02)

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Table 3. Pearson's correlation coefficients of the visco-elastic properties of gluten, dough and baking characteristics with urea addition. Definitions of visco-elastic, mixing and baking variables explained in Table 2, Chapter III.

	SeP	$J-J_r$	RCY	TCR	TCC	WA	DT	ST	BT	PH	LH	SV	OSP	LV	PC
SeP	1														
$J-J_r$	-0.83**	1													
RCY	-0.64**	-0.58**	1												
TCR	-0.69**	0.57**	-0.55**	1											
TCC	-0.66**	0.70**	-0.69**	0.65**	1										
WA				-0.42*		1									
DT		-0.59**				0.62**	1								
ST						0.63**	0.59**	1							
BT		0.54**				0.73**	0.95**	0.71**	1						
PH			0.37*	-0.40*	-0.43*					1					
LH			0.38*	-0.39*	-0.44*			0.43*		0.85**	1				
SV					-0.40*			0.50**	0.39*	0.87**	0.97**	1			
OSP								0.46*	0.37*		0.68**	0.61**	1		
LV				-0.34*	-0.42*			0.53**	0.41*	0.86**	0.97**	0.99**	0.61**	1	
FP			-0.37*			0.88**	0.82**	0.82**	0.85**		0.43*	0.48**	0.37*	0.51**	1

*Correlation is significant at $\alpha = 0.05$ level

**Correlation is significant at $\alpha = 0.01$ level

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Table 4. Explained variance (%) in PCA of visco-elastic, mixing and baking variables in gluten and flours treated with urea. Definitions of visco-elastic, mixing and baking variables explained in Table 2, Chapter III.

Urea	PC (%)	PC1	PC2	1+2
	Axes	41.39	28.11	69.50
Visco-elastic	SeP	2.34	73.60	75.94
	$J\text{-}J_r$	0.04	86.20	86.24
	RCY	17.45	36.85	54.30
	TCR	25.62	29.27	54.89
	TCC	22.35	46.67	69.02
Farinograph	WA	43.49	5.77	49.26
	DT	34.00	44.13	78.13
	ST	49.28	22.42	71.70
	BT	43.76	42.07	85.83
Baking	PH	50.27	10.23	60.50
	LH	73.59	4.35	77.94
	SV	76.45	2.38	78.83
	OSP	37.15	0.31	37.46
	LV	79.39	1.82	81.21
Protein content	FP	65.65	15.59	81.24

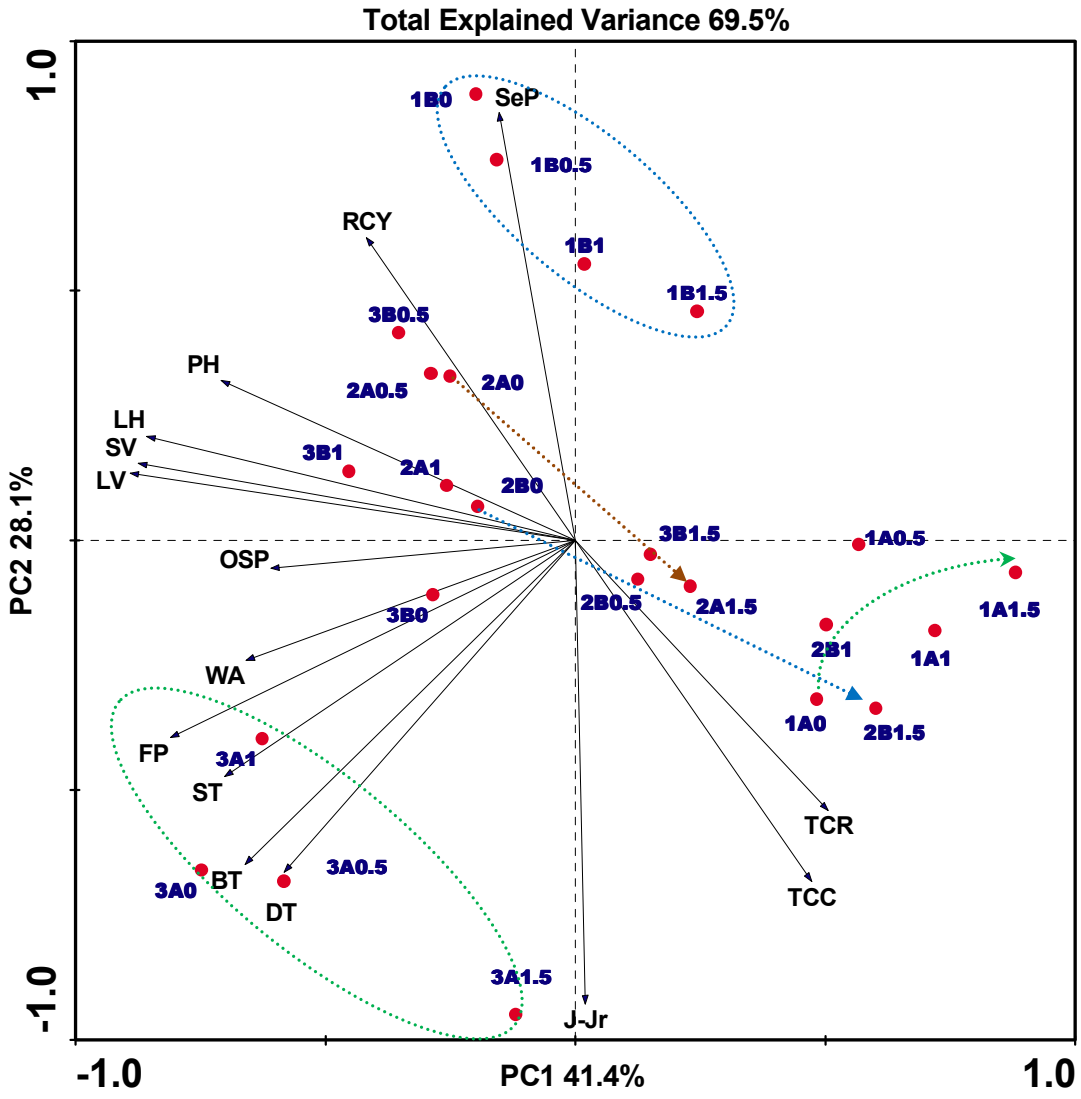

Fig. 1. Loading plot of first two principal components based on baking, visco-elastic and dough properties of six commercial wheat flours obtained from sites A and B, added with three levels of urea. Definitions of visco-elastic, mixing and baking variables explained in Table 2, Chapter III. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively.

CHAPTER VI

EFFECT OF DITHIOTHREITOL (DTT) ON MIXING AND BAKING PROPERTIES IN WHEAT FLOURS AND ITS CORRELATION TO GLUTEN VISCO-ELASTICITY.

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Abstract

The redox state of dough systems are key determinants of their functionality during mixing and baking. Naturally occurring and added reducing agents ultimately affect the polymerization of monomeric and polymeric proteins that form gluten. The objective of the study was to determine visco-elastic properties of gluten, mixing and baking properties of flours containing different levels of reducing agent. Six commercial flours were obtained from two sites (A and B) in Oklahoma; three samples from each site. Dithiotheritol (DTT) was added at the levels of 0, 0.1, 0.25 and 0.5 mM to the flour during isolation of gluten. Creep-recovery experiments were performed to evaluate the visco-elastic properties of gluten. Mixing and baking were evaluated using a Farinograph and a 100 g flour bake test. DTT increased (12 to 54% range) the recovery compliance of gluten from most of the flours, indicating the weakening of gluten structure. Visco-elastic properties of gluten such as separation time decreased (60 to 865 range) andd delta compliance increased (40 to 67% range) indicating weakening of gluten structure due to DTT. The rate at which gluten deformed and recovered indicated by time constants for creep and recovery significantly slowed by 20 to 55% and 15 to 53%, respectively with DTT. Mixing properties of wheat flours were evaluated using the Farinograph measurements. Overall dough stability and water absorption decreased with addition of DTT with a few exceptions. Loaf volumes in all flours decreased (18 to 39% range) with 0.5 mM DTT addition. Significantly positive correlations of separation time of gluten an estimate of delayed elasticity with proof heights ($r = 0.56$, $P < 0.01$) and loaf heights ($r = 0.53$, $P < 0.01$) suggested that DTT decreased gluten elasticity affecting the performance of baking. A principal component analysis (PCA) performed on viscoelastic properties

of gluten and mixing and baking properties of flours showed that addition of DTT resulted in loss of elastic properties like separation time and percent recoverability of gluten and oriented the flours towards viscous attributes such as increased delta compliance and time constants for creep and recovery of gluten in all the flours. Reduction of gluten by DTT resulted in decreased strength and affected the loaf properties in all flours.

1. Introduction

The variation in dough visco-elastic and bread making performance between wheat cultivars is largely determined by differences in protein quantity and composition. Gliadins and glutenins make up the storage or gluten proteins. Glutenins are present as large complexes formed by subunits linked together by disulphide bonds. The two major groups of subunits are the low molecular weight glutenin subunits (LMW-GS) and the high molecular weight glutenin subunits (HMW-GS) (Wieser, 2007). These subunits are associated through disulfide bonding, forming the glutenin macropolymer which is responsible for the viscoelastic properties characteristic of dough. The separation of high molecular weight subunit and low molecular weight subunit is brought about by the reduction of inter-chain disulfide bonds within the glutenin sub-fraction (Shewry & Tatham, 1997). It is well understood that the dough structure and loaf quality have been correlated to the presence of unextractable high molecular weight subfraction of glutenin (Gupta, Popineau, Lefebvre, Cornec, Lawrence & MacRitchie, 1995). Chemical depolymerization of the gluten macropolymer has been studied (Kawamura, Matsumura, Matoba, Yenozawa & Kito, 1985; Matsumura, Kawamura, Matoba & Yonezawa, 1984), but little evidence is available on the effect of reducing agents on the visco-elasticity of gluten and mixing and baking properties of dough. Gao et al. (1992) used 0.02 to 3 mM concentrations of DTT during dough mixing and concluded that disruption of disulfide bonds begin at 0.08 mM and an increased dough stickiness started at 3 mM DTT level. Visco-elastic studies on sodium dodecyl sulfate (SDS) insoluble protein gel extracted from two Canadian hard red winter wheat flours with protein contents of 6.8 and 9.6%

showed decrease in storage modulus (elastic component) by 79 and 97%, respectively, with 0.1 mM DTT concentration (Kim & Bushuk, 1995).

The objective of this study was to assess the effect of DTT on the visco-elastic properties of gluten using creep-recovery, as well as mixing and baking properties of commercial hard red winter wheat flours

2. Materials and Methods

The procurement of wheat flour samples are explained in Materials and methods section of Chapter III.

Three levels (0.1, 0.25 and 0.5 mM) of DTT (VWR International, West Chester PA, 19380), were added to flours from each source. Thus, site A flours were denoted as 1A0, 1A0.1, 1A0.25 and 1A0.5; 2A0, 2A0.1, 2A0.25 2A0.5; 3A0, 3A0.1, 3A0.25, 3A0.5, respectively. Similarly site B flours were named, 1B0, 1B0.1, 1B0.25 and 1B0.5; 2B0, 2B0.1, 2B0.25 and 2B0.5; 3B0, 3B0.1, 3B0.25and 3B0.5, respectively. Flours and gluten isolated from flours with no DTT were used as controls. The protein, moisture and ash contents were determined using the NIR system (FOSS NIR Systems Inc, Laurel, MD 20723) as shown in Table 1 (Chapter III).

2.1. Gluten extraction

A stock solution of 100 mM of DTT was prepared containing 1.54 g of DTT in 100 mL deionized water. Working solution of DTT was prepared by containing 0.1, 0.25 and 0.5 mL of stock solution in 100 mL of 2% NaCl solution. Glutens were prepared in triplicates in an automated gluten washer, Glutomatic 2200 (Perten Instruments, Sweden) from 10 g of flour and 5.0 mL of DTT solution using a mixing time of 20 sec and

washing for 10 min with 2% NaCl solution (w/v). Control samples were mixed with 5.0 ml of pure deionized water.

2.2. Creep recovery tests

The creep recovery experiments were carried out as described in Chapter III.

2.3. Dough mixing properties

Working solutions of DTT were prepared containing 0.1, 0.25 and 0.5 mL of stock solution in a total of 100 mL of 2% deionized water. One ml of DTT working solution was added to 10 g of wheat flour at the beginning of mixing. Dough mixing properties were evaluated as described in Chapter III.

2.4. Baking tests

Working solutions of DTT were prepared containing 0.1, 0.25 and 0.5 mL of stock solution in 100 mL of 2% deionized water. Five ml of the same DTT working solution as described earlier was added to the flour at the beginning of mixing. Baking tests were performed as explained in chapter III. The definitions of baking, dough mixing and visco-elastic parameters are explained in Table 2 (Chapter III).

3. Statistics

Statistical analysis is performed using same methods elaborated in Chapter III.

4. Results

4.1. Visco-elastic properties

DTT addition significantly ($P < 0.05$) weakened the gluten in all flours by increasing its compliance except for one instance, where 0.5 mM of DTT decreased the recovery compliance of 1A flour by 21.5% (Appendix 1, Fig. 8). Increase in recovery compliance of 1A gluten by 0.1 and 0.25 mM of DTT was 31.1 and 12%, respectively

(Appendix 1, Fig. 8). DTT level of 0.5 mM increased the recovery compliance in 2A flours by 43.5%. Recovery compliance of gluten in 3A flours increased by 20 and 32.1% with DTT addition of 0.1 and 0.5 mM levels (Appendix 1, Fig. 8). No significant increase in the recovery compliance of gluten of 3B flours was observed with DTT addition. Recovery compliance of gluten from 1B flour increased by 41, 54 and 43% with 0.1, 0.25 and 0.5 mM of DTT, respectively (Appendix 1, Fig. 9). Recovery compliance of 2B flours increased at 0.1 and 0.25 mM DTT levels by 16.5 and 30.5%, respectively (Appendix 1, Fig. 9).

Viscoelastic properties of gluten had significant interaction among flour protein content and DTT levels in all the flours except percent recoverability (RCY) in site B (Appendix 1, Table 4). Significant ($P < 0.05$) reduction in separation time (SeP) was observed in gluten of 1A, 2A and 3A flours (Table 1) at 0.5 mM DTT level by 77.1, 59.1 and 85.9%, respectively. Reduction in SeP in 1B and 2B gluten by 75.1 and 71.4% with 0.25 mM DTT levels respectively was observed (Table 2). Significant increase in delta compliance ($J\text{-}J_r$) was observed in gluten from all flours with the addition of DTT (Tables 1 and 2). Viscosity in form of $J\text{-}J_r$ increased at 0.5 mM DTT concentrations in 2A and 3A gluten by 52.8 and 40.1%, respectively. An increase in delta compliance suggests that the viscous component is higher than elastic component by either an increase in viscosity or decrease in elasticity of the gluten structure at 100s. Increase in viscosity of gluten from 1A flour with 0.1 and 0.25 mM DTT by 45.4 and 33.1%, respectively. No significant differences in delta compliance of gluten in 3B gluten were observed. Viscosity ($J\text{-}J_r$) of gluten in 1B flour increased by 53 and 65.8% with 0.1 and 0.25 mM DTT concentrations

(Table 2). Increase of $J-J_r$ in gluten of 2B flour with 0.25 and 0.5 mM addition was 39.1 and 36.9%, respectively (Table 2).

No significant differences were observed in percent recoverability of gluten extracted from flours from sites A and B with DTT addition. Although no significant interaction in recoverability of gluten from site B flours was observed (Appendix 2, Table 4), significant treatment effects were observed ($P < 0.05$). The estimated percent recovery of gluten with was significantly high (82.8%) in controls ($P < 0.05$) (Appendix 3, Table 3). The rate at which the gluten from all flours deformed and recovered (TCC and TCR, respectively) was reduced significantly by DTT addition ($P < 0.05$). Time constants for recovery of gluten from 1A, 2A, 1B and 2B flours increased with 0.5 mM DTT by 14.8, 52.9, 56.1 and 35.4%, respectively, thus slowing down the rate at which the recovery reached equilibrium (Tables 1 and 2). DTT at of 0.1 mM increased TCR of gluten from 3A flour by 26.6% (Table 1). No significant differences in recovery rates were observed in gluten from 3B flour. The rate at which the gluten of 2A flour deformed (TCC) was reduced by 25.3 and 29.7% compared to the control at 0.25 and 0.5 mM DTT levels, respectively (Table 1). No significant differences were observed in rates of gluten deformation of 1A flours. An increase of 28% in time constants of creep of gluten from 3A flour was observed at 0.5 mM DTT (Table 1). Time constants for creep in gluten from 1B flour increased with 0.5 mM concentration by 24.2% (Table 2). Rate of deformation (TCC) slowed in gluten from 2B flour with 0.25 and 0.5 mM DTT levels by 26.2 and 54.7%, respectively (Table 2). Rate at which the gluten from 3B flour deformed slowed down by 30.8 and 20.7% with 0.1 and 0.5 mM DTT, respectively.

4.2. Dough mixing properties

Significant interactions among flour protein contentas and DTT levels in mixing properties of gluten were observed except water absorption in site B (Appendix 2, Table 4). Water absorption (WA) of 1A flour decreased with 0.1 mM DTT level by 4.3% (Table 1). No significant changes were observed in water absorption of 2A and 3A flours. Significant treatment and protein effects ($P < 0.05$) were observed in water absorption of flours in site B where 0.5 mM treatment level had 61.6% water absorption while high protein had 61.1% water absorption (Appendix 3, Table 3). Dough development time (DT) decreased in 3A flour by 17.6, 37 and 55.5% with addition of 0.1, 0.25 and 0.5 mM DTT, respectively (Table 1). DTT concentration of 0.25 mM increased DT in 2A flour by 35.7% (Table 1). No significant differences in DT were observed in site B flours. Stability of 2A and 1B flours significantly ($P < 0.05$) increased with addition of DTT while 3A, 2B and 3B flours showed decrease in stability with DTT addition (Tables 1 and 2). DTT levels of 0.1 and 0.5 mM (Table 1) increased the stability of 2A and 1B dough by 54.8 and 52.2% and 81.8 and 66%, respectively (Tables 1 and 2). Stability time decreased by 30.1 and 66% in 3A dough and by 37.5 and 49% in 2B dough with 0.25 and 0.5 mM DTT respectively (Tables 1 and 2). Dough stability of 3B flour decreased with 0.1 and 0.25 mM DTT concentration by 36.5 and 54.8%, respectively (Table 2). Dough breakdown time (BT) of all flours increased significantly ($P < 0.05$) with addition of DATEM except the dough from 3A flour (Tables 1 and 2). Time required for the dough to breakdown after mixing decreased in 3A dough with 0.5 mM of DTT by 56.8% (Table 1). Breakdown time increased by 64 and 60.6% in 2A dough and by 53.3 and 51.1% in

3B dough with 0.25 and 0.5 mM DTT respectively (Tables 1 and 2). Dough breakdown time increased in 2B flour with 0.5 mM DTT level by 37.5% (Table 2).

4.3. Baking characteristics

DTT treatment and protein content interactions were significant for all baking properties of flours from sites A and B (Appendix 2, Table 4). Addition of DTT at each concentration to the dough decreased the baking quality of bread. All baking parameters such as loaf volumes (LV), loaf heights (LH), proof heights (PH), oven springs (OSP) and specific volumes (SV) showed a sharp decrease with 0.5 mM DTT level in all the breads from sites A and B. Loaf volumes in 1A, 2A, 3A, 1B, 2B and 3B decreased by 22.9, 29.3, 26.1, 18.5, 38.5 and 21.6%, respectively with 0.5 mM DTT (Tables 1 and 2). Decrease in LH in 1A, 2A, 3A, 1B, 2B and 3B breads with 0.5 mM DTT was observed to be 22.4, 20.3, 8.4, 23.6, 25.1 and 12.6%, respectively (Tables 1 and 2). DTT addition of 0.5 mM retarded the proof heights in 1A, 2A, 3A, 1B, 2B and 3B by 40.7, 35.4, 27, 32.1, 38.8 and 27.7%, respectively (Tables 1 and 2). Oven springs decreased in 1A, 2A, 3A, 1B, 2B and 3B breads by 82.9, 81.3, 87.4, 78.9, 86.6 and 82.9% with 0.5 mM DTT (Table 1 and 2). Specific volumes of 1A, 2A, 3A, 1B, 2B and 3B breads dropped by 26.8, 34, 34.4, 21.5, 40 and 24.5%, respectively at 1.5 mM DTT concentration.

4.4. Correlations and PCA

The relationship of visco-elastic, baking and mixing parameters affected by DTT addition is shown in Table 3. Separation time (SeP), a function of elasticity showed highly significant correlation ($P < 0.01$) with proof heights ($r = 0.56$) and loaf heights ($r = 0.53$) and less significant correlations ($P < 0.05$) with specific volume ($r = 0.51$) and loaf volume ($r = 0.48$). Delta compliance ($J\text{-}J_r$) showed weak but significant negative

correlations with specific volume (r = -0.37). Decrease in elasticity in form of percent recoverability of gluten (RCY) was significantly ($P < 0.05$) correlated to decreased loaf heights (LH), proof heights (PH) and loaf volumes (LV). Positive correlations of RCY were observed with PH (r = 0.48), LH (r = 0.44) and LV (r = 0.46), respectively. Recoverability had strong significant correlation ($P < 0.01$) with specific volume (SV) with r = 46. Visco-elastic parameters like creep and recovery time constants (TCC and TCR, respectively), showed weak correlations ($P < 0.05$) with loaf height, loaf volume and specific volume (Table 3). Decrease in loaf heights and loaf volumes can be attributed to longer recovery rates (TCR) with DTT addition (Table 3). Significant negative correlations ($P < 0.05$) of TCR with LV (r = -0.41), LH (r = -0.37) and SV (r = -0.44) were observed. Significant increase in 1A, 2A, 1B and 2B gluten TCRs showed decrease in LV, LH and SV values (Table1) while the rate of deformation (TCC) had weak negative correlations ($P < 0.05$) with LV, LH, and SV (Table 3). DTT addition increased the rate at which the gluten deformed (TCC) and this rate was related to LH (r = -0.37), LV (r = -0.42) and SV (r = -0.44), respectively. This suggests that the rate of recovery appears to be more important than the rate of viscous deformation in gluten with reference to bread quality.

Principal component analysis on visco-elastic, mixing and baking characteristics in hard red winter wheat flours from sites A and B, added with DTT is shown in Fig. 1. Principal component axis 1 (PC1) and 2 (PC2) explained 64.36% of total variance, with each explaining 44.58 and 19.78%, of the total variance respectively. Principal component axis 1 was dominated by loaf volumes (90.52%) followed by specific volume (88.29%) (Table 4). Time required for the dough to breakdown after mixing (BT)

explained for the highest variance (53.32%) on PC2. Upper right quadrant showed all mixing parameters along with flour protein content (FP) to be very closely correlated. Lower right quadrant showed a group of closely related variables, PH, LH, SV, OSP and LV. The closely grouped variables in upper and lower right quadrants confirmed significantly high correlations in Table 3. Loaf volumes, proof heights and specific volumes were closely placed to PC1. Percent recoverability of gluten was found to have positive correlation with baking characteristics such as oven springs and loaf heights (Fig. 1). Creep and recovery rates (TCC and TCR) placed in the upper left quadrant had negative correlations (also refer Table 3) with baking characteristics.

DTT disrupted the disulfide linkages in dough from 2A, 3A, 1B, 2B and 3B samples and oriented them from the lower right quadrant dominated by elasticity parameters such as SeP and RCY to the upper left quadrant dominated by creep and recovery rates and viscosity parameters such as delta compliance. DTT levels in 1A flours appear to group the samples closer to the PC1 axis suggests that this group was completely independent of their visco-elastic properties and negatively correlated to their baking characteristics. 1A is the flour from site A with lowest protein content (8%).

5. Discussion

Disruption of disulfide bonds in gluten by use of DTT showed increased recovery compliance that was attributed to the weakening of gluten in wheat flours. Khatkar (2005) studied the effect of 100 ppm DTT added to gluten extracted from British winter wheat flours. He observed loss of elasticity in stronger gluten by 37% versus 71% decrease in weak gluten in form of elasticity modulus using dynamic oscillatory measurements at 25 Pa stress level. Our study showed consistent decrease in elasticity in

form of increased compliance of gluten, increased delta compliance, decreased separation time and increase in rate at which the structure of gluten from all flours deformed and recovered. Baking performance deteriorated in form of loaf volumes, heights, proof heights, specific volumes and oven springs with increasing levels of DTT. Elasticity of gluten in form of recoverability and separation times was related to the decreased in baking characteristics of the flours. Similarly viscosity in form of delta compliance and rate at which gluten deformed and recovered were inversely related to baking parameters.

Lu & Seib (1998) reported that addition of DTT at 1.3 mM in flours with 11.5 and 13 % protein levels did not have significant effect on water absorption but increased the mixing times by 9 and 19% respectively. Our study revealed that water absorption cannot be explained by protein content and DTT addition. While the protein contents of 2A (11.5%) and 3A (13.5%) flours were similar to that of the protein contents of flours described by Lu & Seib (1998), dough development time increased in Am and decreased in 3A with DTT. Disruption of disulfide bond with DTT could increase the hydrophobicity in the dough and affect water absorption. Similar effects were observed when 1% DTT and 5% β-mercaptoethanol salt in propanol were used to extract the glutenin subfractions in a stepwise reduction of gluten (Bean & Lookhart, 1998). The study reported that reducing agents disrupted the disulfide crosslinks and solubilized the high molecular weight subunits thus preventing the aggregation of gliadins and glutenins. Disruption of disulfide bonds cause unfolding of gluten macropolymer and expose the hydrophobic domains that will repel water causing decrease in water absorption. Quantitative differences in the magnitudes of visco-elastic, mixing and baking characteristics among different groups of glutens may be attributable to differences in the

density of disulfide cross-links (Khatkar, 2005), in addition to variation in the glutenin visco-elasticity (Southan & MacRitchie, 1999) gliadin/glutenin ratio (Uthayakumaran, Gras, Stoddard & Bekes, 1999), and the molecular size range and molecular size distribution of the glutenin polymers (Gupta et al., 1993). Reduction of gluten with DTT could be complete or partial depending on the quality of the flour protein. DTT concentrations upto 100 mM used to reduce the gluten from Chinese spring wheat released the glutenin subunits in form of dimers and oligomers as well as small glutenin aggregates in a stepwise reduction (Lindsay & Skerritt, 1998). Size exclusion HPLC and SDS-PAGE analyses indicated that release of low and high molecular weight subunit dimers were released at low DTT concentrations of 0.3 to 0.7 mM while complete depolymerization took place at 20 mM DTT levels releasing larger oligomers. The order of depolymerization of glutenin subunits was consistent with all flours used and rheologically effective bonds were broken down at concentrations of DTT less than 1 mM (Lindsay et al., 1998). The decrease in visco-elastic performance of gluten added with DTT was independent of their protein content. This could be due to the rate at which the glutenin depolymerized with addition of DTT that affected the magnitude in changes of visco-elastic, baking and mixing properties in the flours in this study.

6. Conclusions

DTT levels increased the recovery compliance in the gluten extracted from all flours making it weaker. Reduction of gluten strength, slow recovery rates of gluten, decreased percent recoverability could be associated with the decrease in loaf properties and poor baking performance of wheat flours. An increase in delta compliance and its close negative correlations with baking properties suggested DTT increased the viscosity

in dough and decreased the quality of loaves. Mixing properties of the flours were independent of visco-elastic properties of gluten.

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Table 1. Visco-elastic, farinograph and baking characteristics in commercial wheat flours from site A, treated with DTT levels. Means with same superscripts in a column are not significantly different (P > 0.05). The standard deviations of means are shown in parentheses. Definitions of visco-elastic, mixing and baking variables described in Table 2, Chapter 2. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively.

TRT	Visco-elastic					Farinograph				Baking				
	SeP (s)	J-J_r (J)	RCY (%)	TCR (s)	TCC (s)	WA (%)	DT (min)	ST (min)	BT (min)	LV (cc)	PH (mm)	LH (mm)	OSP (mm)	SV (ccg^{-1})
1A0	4.8^{d}	1.27^{c}	79.3^{abc}	8.0^{abc}	9.5^{a}	53.6^{c}	1.0^{h}	2.0^{e}	1.4^{g}	550.0^{h}	59.2^{d}	84.4^{b}	25.2^{a}	4.1^{f}
	(0.3)	(0.1)	(1.0)	(0.9)	(0.7)	(0.2)	(0.1)	(0.0)	(0.1)	(4.1)	(1.5)	(1.3)	(0.2)	(0.02)
1A0.1	2.2^{e}	2.33^{a}	75.1^{c}	7.8^{abcd}	9.8^{a}	51.3^{d}	1.1^{gh}	1.7^{e}	1.5^{g}	527.5^{i}	56.1^{de}	63.1^{f}	7.0^{bcd}	3.8^{g}
	(0.0)	(0.3)	(3.4)	(0.4)	(0.9)	(0.0)	(0.1)	(0.4)	(0.4)	(6.5)	(0.2)	(1.3)	(1.5)	(0.06)
1A0.25	0.9^{e}	1.90^{ab}	75.4^{bc}	6.4^{cdef}	8.4^{ab}	51.0^{d}	1.2^{fgh}	1.4^{e}	1.7^{g}	494.8^{j}	52.4^{e}	59.3^{g}	6.9^{bcd}	3.6^{g}
	(0.1)	(0.0)	(0.9)	(0.2)	(0.6)	(0.1)	(0.3)	(0.4)	(0.1)	(4.1)	(0.3)	(0.0)	(0.3)	(0.03)
1A0.5	1.1^{e}	0.98^{cde}	79.3^{abc}	9.4^{a}	8.9^{ab}	51.4^{d}	1.0^{h}	1.7^{e}	1.6^{g}	423.8^{k}	45.9^{f}	50.2^{h}	4.3^{d}	3.0^{h}
	(0.0)	(0.1)	(0.7)	(0.5)	(0.1)	(0.4)	(0.1)	(0.4)	(0.1)	(4.8)	(0.3)	(0.3)	(0.0)	(0.03)
2A0	16.4^{a}	0.57^{e}	82.7^{a}	4.0^{g}	6.2^{d}	58.6^{b}	1.8^{fg}	8.3^{d}	3.7^{fg}	745.0^{b}	71.0^{ab}	94.5^{a}	23.5^{a}	5.4^{b}
	(0.8)	(0.1)	(1.8)	(0.6)	(0.1)	(0.3)	(0.0)	(0.7)	(1.1)	(4.1)	(1.7)	(0.6)	(2.3)	(0.01)
2A0.1	10.5^{b}	0.72^{de}	82.0^{a}	4.9^{fg}	6.6^{cd}	58.7^{b}	1.9^{f}	18.8^{b}	4.7^{ef}	648.8^{e}	65.4^{c}	77.1^{c}	11.7^{b}	4.7^{d}
	(1.0)	(0.0)	(1.1)	(0.3)	(0.3)	(0.3)	(0.1)	(0.4)	(1.4)	(4.8)	(1.3)	(1.4)	(0.1)	(0.01)
2A0.25	11.0^{b}	0.66^{de}	81.6^{abc}	4.0^{g}	8.3^{abc}	58.3^{b}	2.8^{e}	17.4^{b}	10.3^{bc}	623.8^{f}	60.1^{d}	67.1^{e}	7.0^{bcd}	4.5^{e}
	(0.3)	(0.1)	(2.9)	(0.5)	(0.0)	(0.6)	(0.4)	(0.5)	(0.7)	(4.8)	(0.4)	(1.4)	(0.9)	(0.03)
2A0.5	6.7^{c}	1.21^{c}	79.1^{abc}	8.5^{ab}	8.8^{ab}	59.5^{b}	1.9^{f}	10.9^{c}	9.4^{cd}	526.3^{i}	56.6^{de}	61.0^{fg}	4.4^{d}	3.7^{g}
	(0.2)	(0.1)	(1.9)	(0.2)	(0.5)	(0.3)	(0.1)	(0.4)	(0.6)	(7.5)	(1.7)	(0.2)	(0.0)	(0.05)
3A0	10.7^{b}	1.12^{cd}	80.2^{abc}	5.8^{ef}	7.2^{bcd}	63.6^{a}	10.8^{a}	21.5^{a}	16.0^{a}	821.3^{a}	74.2^{a}	97.3^{a}	23.1^{a}	6.1^{a}
	(1.0)	(0.1)	(0.5)	(0.7)	(0.5)	(0.9)	(0.1)	(0.9)	(0.2)	(4.8)	(2.6)	(0.3)	(2.8)	(0.06)
3A0.1	4.7^{cd}	1.51^{bc}	79.1^{abc}	7.9^{abc}	8.1^{ab}	62.3^{a}	8.9^{b}	18.5^{b}	13.2^{ab}	707.5^{c}	70.6^{ab}	81.5^{b}	10.9^{bc}	5.1^{c}
	(0.1)	(0.1)	(1.8)	(0.1)	(0.4)	(0.8)	(0.1)	(0.1)	(1.1)	(5.0)	(0.7)	(0.4)	(1.1)	(0.01)
3A0.25	6.5^{cd}	1.18^{cd}	82.1^{ab}	5.9^{def}	7.1^{bcd}	63.2^{a}	6.8^{c}	16.8^{b}	13.8^{a}	693.8^{d}	70.2^{ab}	76.7^{c}	6.5^{cd}	4.9^{d}
	(0.3)	(0.1)	(1.3)	(0.1)	(0.1)	(0.1)	(0.3)	(1.6)	(1.1)	(4.8)	(0.3)	(1.1)	(0.8)	(0.02)
3A0.5	1.5^{e}	1.88^{ab}	77.9^{abc}	7.5^{bcde}	10.0^{a}	64.0^{a}	4.8^{d}	7.3^{d}	6.9^{de}	606.3^{g}	68.0^{bc}	71.0^{d}	2.9^{d}	4.0^{f}
	(0.0)	(0.4)	(3.2)	(0.5)	(0.9)	(0.2)	(0.1)	(0.3)	(0.6)	(4.8)	(0.4)	(1.0)	(0.6)	(0.02)

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Table 2. Visco-elastic, farinograph and baking characteristics in commercial wheat flours from site B, treated with different levels of DTT. Means with same superscripts in a column are not significantly different (P > 0.05). The standard deviations of means are shown in parentheses. Definitions of visco-elastic, mixing and baking variables described in Table 2, Chapter 2. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively.

TRT	Visco-elastic					Farinograph				Baking				
	SeP (s)	J-J_r (J)	RCY (%)	TCR (s)	TCC (s)	WA (%)	DT (min)	ST (min)	BT (min)	LV (cc)	PH (mm)	LH (mm)	OSP (mm)	SV (ccg^{-1})
1B0	29.0^{a}	0.28^{e}	86.5^{a}	3.2^{d}	5.0f^{g}	59.3^{e}	1.5^{c}	1.8^{f}	2.1^{e}	696.3^{d}	76.9^{a}	91.1^{b}	14.2^{bc}	5.1^{bc}
	(2.7)	(0.0)	(1.2)	(0.3)	(0.3)	(0.0)	(0.0)	(0.4)	(0.1)	(4.8)	(1.9)	(1.7)	(0.1)	(0.04)
1B0.1	7.9^{bcde}	0.61^{d}	83.2^{ab}	4.1^{cd}	4.4^{g}	60.0^{bcde}	2.0^{bc}	9.9^{bc}	4.2^{d}	646.3^{f}	62.2^{f}	75.0^{de}	12.8^{bc}	4.8^{e}
	(0.3)	(0.0)	(0.5)	(0.5)	(0.3)	(0.5)	(0.3)	(1.3)	(0.1)	(6.3)	(0.4)	(0.5)	(0.2)	(0.07)
1B0.25	7.2^{bcde}	0.83^{bcd}	81.9^{ab}	6.5^{bc}	5.4^{efg}	61.0^{abc}	1.9^{bc}	7.6^{cde}	3.1^{de}	610.0^{h}	59.7^{fg}	71.0^{ef}	11.3^{c}	4.4^{f}
	(0.7)	(0.0)	(0.9)	(0.6)	(0.7)	(0.3)	(0.2)	(0.2)	(0.0)	(4.1)	(0.6)	(0.2)	(0.8)	(0.02)
1B0.5	12.0^{b}	0.60^{d}	83.2^{ab}	7.3^{b}	6.6^{de}	61.3^{ab}	2.0^{bc}	5.3^{e}	6.3^{c}	567.5^{i}	58.7^{g}	61.8^{g}	3.0^{e}	4.0^{g}
	(0.3)	(0.1)	(1.6)	(0.6)	(0.3)	(0.1)	(0.1)	(0.1)	(0.4)	(6.5)	(1.1)	(0.9)	(0.2)	(0.05)
2B0	11.2^{bc}	0.71^{cd}	80.8^{b}	6.2^{bc}	6.2^{ef}	59.6^{cde}	1.9^{bc}	10.4^{bc}	4.5^{d}	772.5^{b}	73.3^{bc}	94.3^{ab}	21.0^{a}	5.5^{a}
	(1.4)	(0.1)	(1.7)	(1.1)	(0.5)	(0.1)	(0.2)	(0.7)	(0.1)	(2.9)	(0.1)	(1.0)	(1.0)	(0.02)
2B0.1	8.0^{bcde}	0.87^{abcd}	80.2^{b}	6.5^{bc}	6.5^{def}	59.4^{de}	1.9^{bc}	9.7^{bcd}	3.7^{de}	665.0^{e}	68.8^{d}	74.2^{de}	5.3^{de}	4.8^{de}
	(0.6)	(0.1)	(2.1)	(0.5)	(0.0)	(0.6)	(0.1)	(1.6)	(0.3)	(4.1)	(0.2)	(0.3)	(0.5)	(0.04)
2B0.25	3.7^{e}	1.15^{a}	78.8^{b}	6.6^{b}	8.4^{bc}	60.7^{bcde}	1.7^{c}	6.5^{de}	3.2^{de}	526.3^{j}	55.3^{h}	68.9^{f}	13.6^{bc}	3.8^{h}
	(0.7)	(0.0)	(0.5)	(0.7)	(0.4)	(0.7)	(0.2)	(0.1)	(0.1)	(2.5)	(0.2)	(1.2)	(0.9)	(0.01)
2B0.5	6.0^{cde}	1.11^{ab}	79.7^{b}	9.6^{a}	13.7^{a}	61.4^{ab}	2.0^{bc}	5.3^{e}	7.2^{bc}	475.0^{k}	54.9^{h}	57.7^{g}	2.8^{e}	3.3^{i}
	(0.4)	(0.2)	(2.9)	(0.5)	(0.9)	(0.4)	(0.1)	(0.5)	(0.1)	(7.1)	(0.8)	(0.3)	(0.5)	(0.05)
3B0	9.3^{bcd}	0.83^{bcd}	81.2^{b}	7.7^{ab}	6.5^{ef}	60.6^{bcde}	1.9^{bc}	18.6^{a}	4.2^{d}	798.8^{a}	75.0^{ab}	95.5^{a}	20.5^{a}	5.7^{a}
	(1.0)	(0.1)	(2.0)	(0.1)	(0.8)	(0.1)	(0.1)	(0.8)	(0.2)	(2.5)	(0.2)	(2.0)	(1.8)	(0.05)
3B0.1	4.9^{de}	0.94^{abc}	80.4^{ab}	7.2^{ab}	9.4^{b}	60.8^{bcd}	2.1^{bc}	11.8^{bc}	3.1^{de}	727.5^{c}	70.2^{cd}	84.6^{c}	14.4^{b}	5.3^{b}
	(0.1)	(0.2)	(2.4)	(0.2)	(0.2)	(0.1)	(0.2)	(0.4)	(0.4)	(6.5)	(0.1)	(0.6)	(0.4)	(0.06)
3B0.25	8.5^{bcde}	0.84^{abcd}	80.5^{b}	7.2^{ab}	7.1^{cde}	60.7^{bcde}	2.4^{ab}	8.4^{cde}	9.0^{a}	702.5^{d}	69.2^{d}	76.7^{d}	7.5^{d}	5.0^{cd}
	(0.9)	(0.1)	(0.3)	(1.0)	(0.7)	(0.1)	(0.3)	(1.4)	(1.4)	(2.9)	(0.3)	(0.8)	(0.5)	(0.03)
3B0.5	4.9^{de}	0.99^{abc}	79.3^{b}	7.2^{ab}	8.2^{bcd}	62.4^{a}	2.0^{bc}	8.4^{cde}	8.6^{ab}	626.3^{g}	65.5^{e}	69.0^{f}	3.5^{e}	4.3^{f}
	(0.3)	(0.0)	(0.3)	(0.6)	(0.2)	(0.4)	(0.1)	(0.6)	(0.1)	(13.8)	(1.4)	(0.9)	(0.5)	(0.1)

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Table 3. Pearson's correlation coefficients of the visco-elastic properties of gluten, dough and baking characteristics with DTT addition. Definitions of visco-elastic, mixing and baking variables explained in Table 2, Chapter III.

	SeP	J-J_r	RCY	TCR	TCC	WA	DT	ST	BT	PH	LH	SV	OSP	LV	FP
SeP	1														
J-J_r	-0.74**	1													
RCY	0.83**	-0.86**	1												
TCR				1											
TCC				0.98**	1										
WA			0.42*			1									
DT						0.54**	1								
ST						0.57**	0.62**	1							
BT						0.63**	0.84**	0.72**	1						
PH	0.56**		0.48*			0.60**	0.43*	0.56**		1					
LH	0.53**		0.44*	-0.37*	-0.37*	0.36*		0.45*		0.88**	1				
SV	0.51*	-0.37*	0.47**	-0.43*	-0.44*	0.48**	0.47*	0.66**		0.91**	0.92**	1			
OSP										0.47**	0.84**	0.65**	1		
LV	0.48*		0.46*	-0.41*	-0.42*	0.55**	0.47**	0.67**	0.37*	0.94**	0.90**	0.99**	0.59**	1	
FP						0.87**	0.78**	0.75**	0.81**	0.64**	0.41*	0.55**		0.61**	1

*Correlation is significant at $\alpha = 0.05$ level

**Correlation is significant at $\alpha = 0.01$ level

Table 4. Explained variance (%) in PCA of visco-elastic, mixing and baking variables in gluten and flours treated with DTT. Definitions of visco-elastic, mixing and baking variables explained in Table 2, Chapter III.

DTT	PC (%)	PC1	PC2	1+2
	Axes	44.58	19.78	64.36
Visco-elastic	SeP	28.25	29.5	57.75
	$J\text{-}J_r$	8.61	21.81	30.42
	RCY	25.71	15.16	40.87
	TCR	8.15	14.43	22.58
	TCC	8.87	15.6	24.47
Farinograph	WA	47.51	18.47	65.98
	DT	37.5	37.8	75.3
	ST	56.07	15.06	71.13
	BT	29.45	53.32	82.77
Baking	PH	84.01	1.42	85.43
	LH	72.05	11.65	83.7
	SV	88.29	3.49	91.78
	OSP	26.38	24.13	50.51
	LV	90.52	1.61	92.13
Protein Content (%)	FP	57.39	33.27	90.66

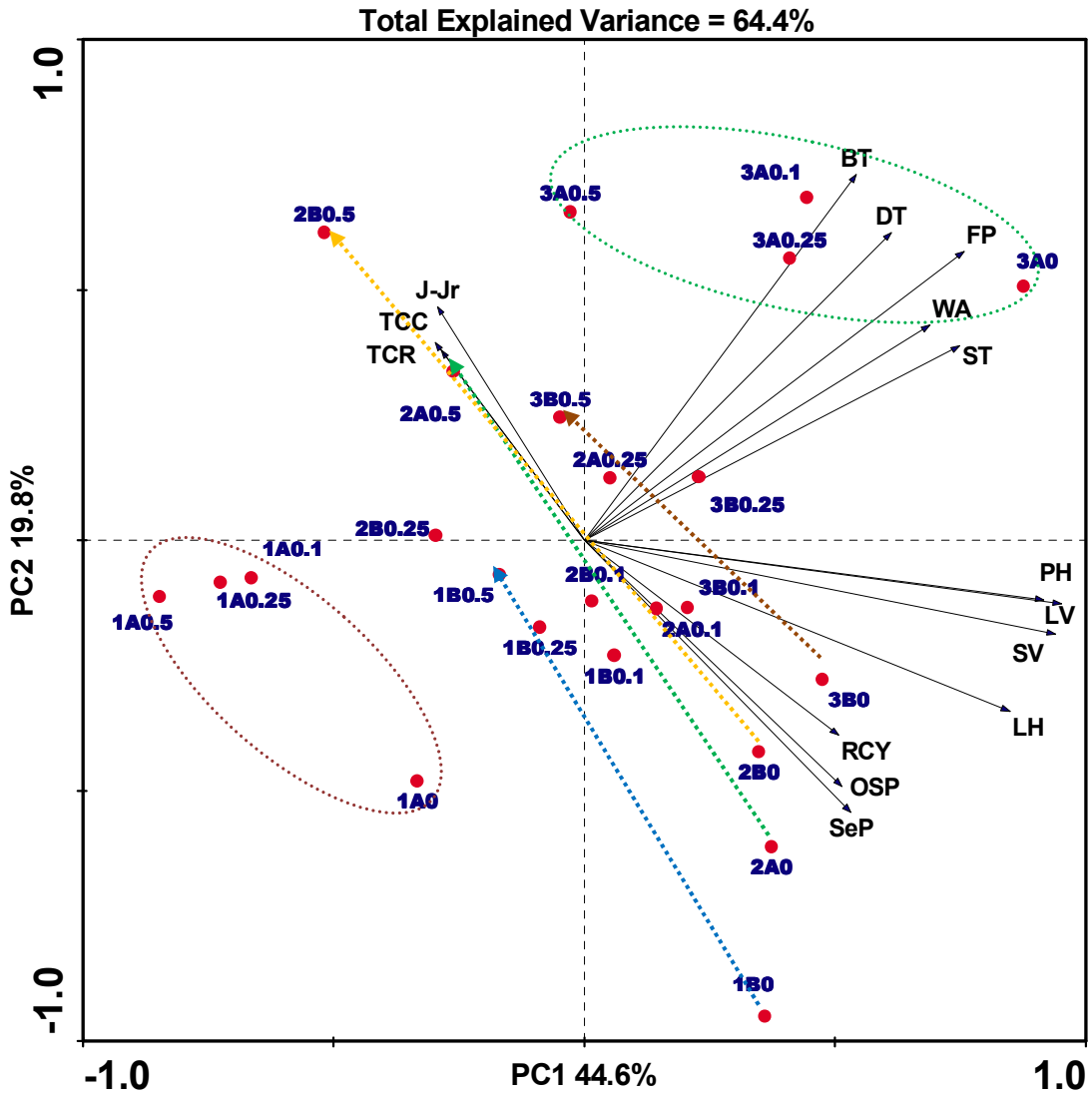

Fig. 1. Loading plot of first two principal components based on baking, visco-elastic and dough properties of six commercial wheat flours obtained from sites A and B, added with three levels of DTT. Definitions of visco-elastic, mixing and baking variables explained in Table 2, Chapter III. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively.

CHAPTER VII

CONCLUSIONS

The structure of gluten was modified by adding diacetyl tartaric acid ester of monoglycerides (DATEM), ascorbic acid, urea and DTT and the changes in it visco-elasticity were quantified along with the changes in bread quality in this study. The study attempted to correlate the changes in gluten visco-elasticity due to structural modifications with mixing and baking properties of wheat flours.

Addition of DATEM improved the strength of gluten extracted from all the flours used in this study. The DATEM level of 1% (w/w flour basis) showed highest decrease in creep and recovery compliance of gluten in all wheat flours. The ability of DATEM to improve gluten strength was observed to be specific to the quality of gluten. Bread quality was improved in all flours at 0.6% levels. No specific trends were observed in mixing characteristics of wheat flours added with DATEM. Bread quality decreased with 1% DATEM addition. Highly significant correlations were observed among flour protein content and mixing and baking properties of wheat flours. For the most part visco-elastic properties of gluten were independent of bread quality with the exception of few. Thus DATEM could have modified the structure of gluten that improved its quality as well as the overall quality of baking in all wheat flours.

Gluten with ascorbic acid addition did not show any specific trend in changes in its visco-elasticity in all wheat flours. Similarly no specific tendency was observed in mixing characteristics of ascorbic acid. Baking characteristics showed improvement with

ascorbic levels up to 100 ppm in most wheat flours and 150 ppm in the rest. Reduction in the quality of bread was observed at 200 ppm. Overall rheological properties of gluten were independent of baking and mixing characteristics. Availability of oxygen could be a limiting factor in oxidizing high levels of ascorbic acid to dehydroascorbic acid which is solely responsible for promoting disulfide linkages and bread quality.

Structure of gluten modified by urea decreased overall gluten strength by increasing its plasticity and decreasing its rate of deformation and recovery. A general decrease in dough stability was observed with urea addition. Baking performance decreased with urea. Decrease in bread quality was positively correlated to decrease in gluten elasticity in form of recoverability and separation time while negatively correlation to delta compliance and time constants. Visco-elasticity of gluten affected by disruption of hydrophobic and hydrophilic bonds in glutenin subfractions by urea contributed to the decreased baking performance. Thus the presence of secondary non covalent cross links in gluten is important for bread quality.

Reducing effect of dithiothreitol (DTT) reduced gluten strength by increasing creep and recovery compliance, slowed the rate of creep and recovery and decreased separation times of gluten extracted from all flours. The baking performance was reduced dramatically with DTT addition. Significant correlations were observed among baking properties and visco-elasticity of gluten that suggested reduced gluten strength due to depolymerization of glutenin sub units as a result of cleavage of disulfide bonds affected the baking negatively.

Overall structural modifications in gluten by DATEM, ascorbic acid, urea and DTT changed the quality of gluten and its functionality in bread making process.

CHAPTER VIII

FUTURE STUDIES

This study was a preliminary step in understanding the ability different additives in improving or deteriorating the physical structure of dough as well as gluten. The study focused on modification in visco-elastic properties of gluten and baking and mixing properties of flours with DATEM (surfactant), ascorbic acid (oxidizing agent), urea (non covalent hydrogen bond disruption in glutenin) and DTT (disulfide linkage disruption in glutenin). Major focus of this study was to measure the changes in visco-elastic properties of gluten, mixing properties of dough and baking performance of dough from hard red winter wheat flours with variable protein content. Correlations were identified among visco-elastic, baking, mixing properties in gluten and dough to establish the relationship between visco-elastic properties of gluten and baking and mixing performance with modifications by application of various treatments. The visco-elastic analysis was performed in the linear visco-elastic region with small deformations using creep-recovery techniques.

Although different levels of DATEM, ascorbic acid, urea and DTT were used in this study, optimized concentrations were not identified in this study. This can be achieved in a separate study with appropriate experimental design and statistical modeling using response surface methodology. Ratios of gliadin to glutenins, composition of low molecular weight and high molecular weight subunits of glutenin sub-fractions and changes in its distribution with optimized treatments of DATEM,

ascorbic acid, urea and DTT is required to be studied. Such study will increase our understanding of response of gluten proteins to the treatments on a molecular level. Microscopic visualization of DATEM in proofed dough and baked bread under confocal microscopy will help understand the localization and interaction of surfactant within the complex dough system. It will be interesting to correlate the visco-elastic measurements performed on gluten in non linear visco-elastic region under large deformations using unaxial extension or rupture tests with baking and mixing properties of the flours.

APPENDIX 1

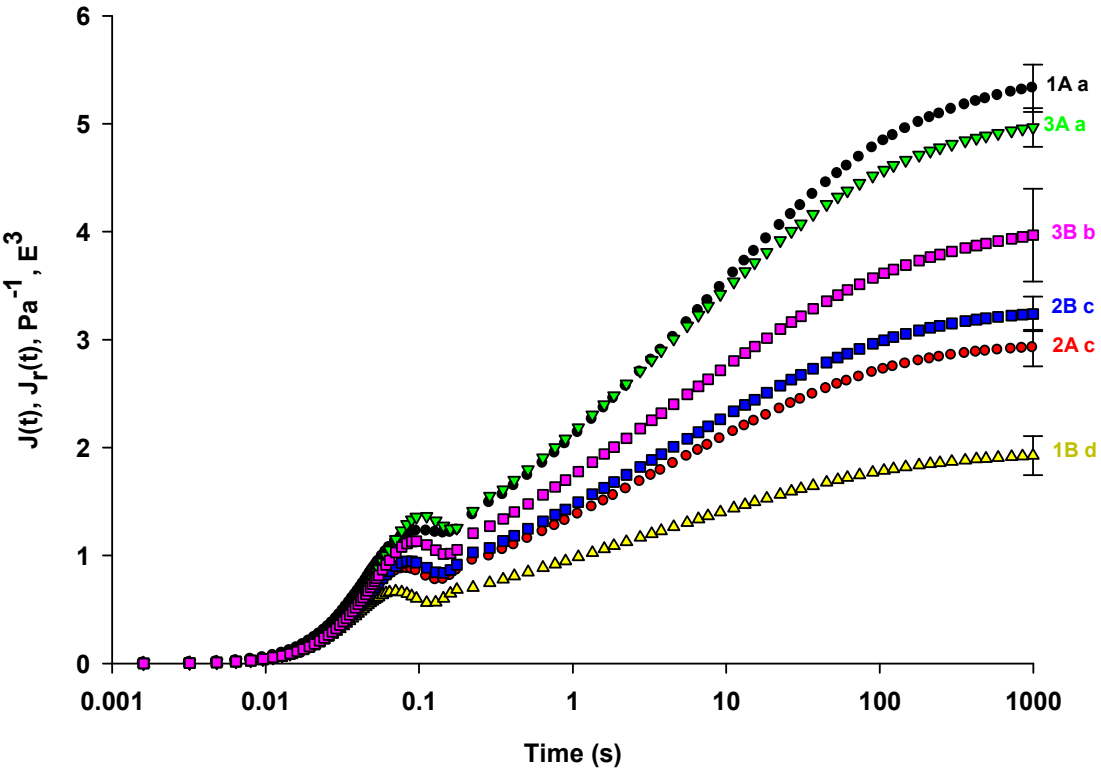

Fig. 1. Recovery compliance of gluten with no treatments applied in all wheat flours. Recoveries (means ± SD, n= 4) with similar letters are not significantly different ($P \leq 0.05$).

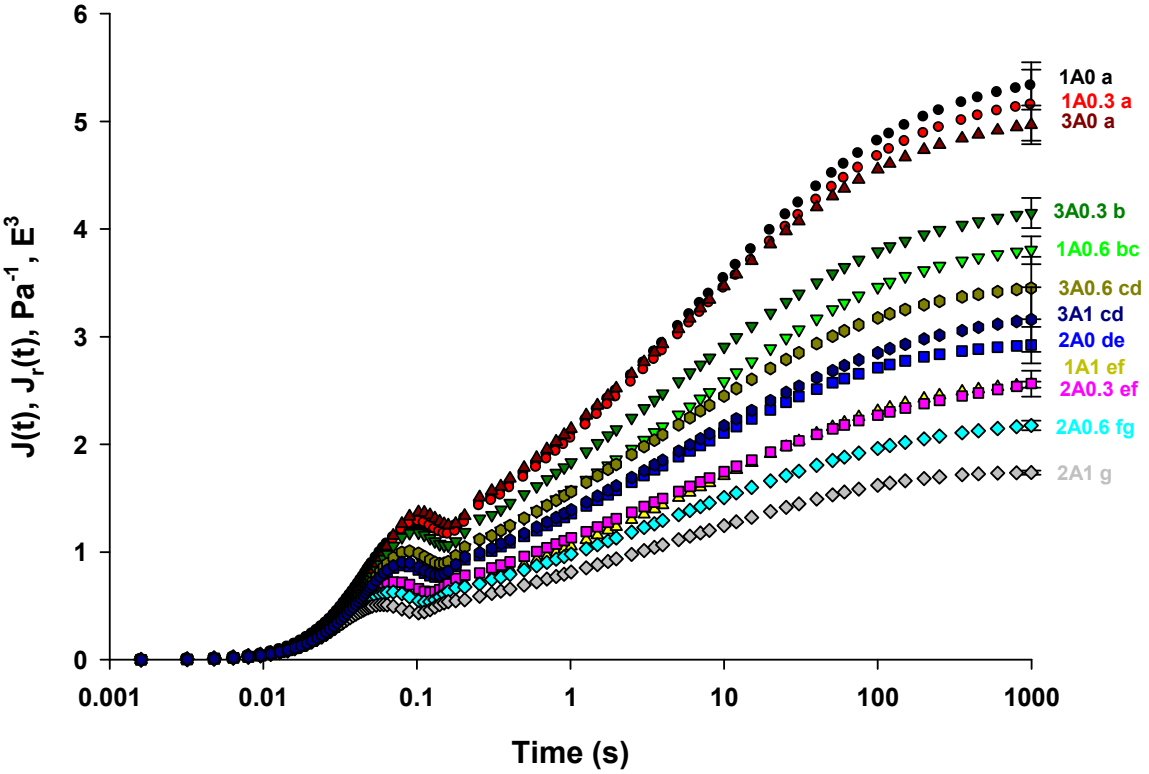

Fig. 2. Recovery compliance of gluten extracted from wheat flours mixed with DATEM and obtained from site A. Recoveries (means ± SD, n= 4) with similar letters are not significantly different ($P \leq 0.05$).

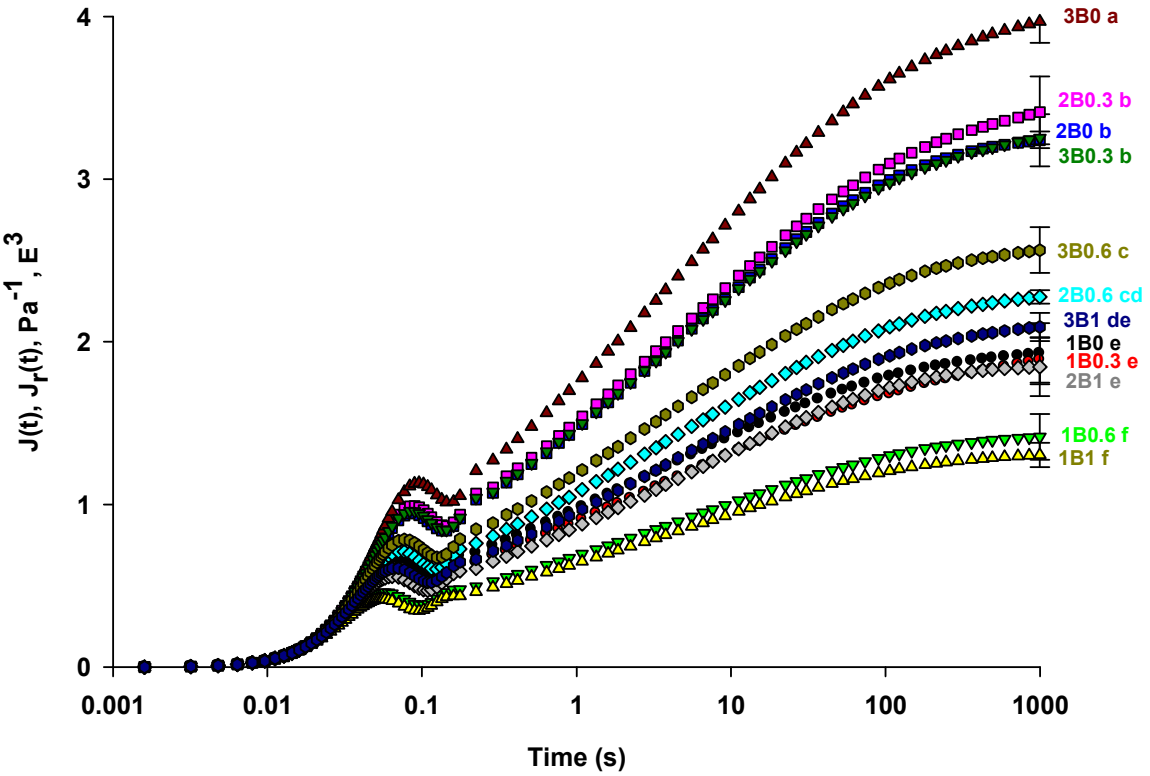

Fig. 3. Recovery compliance of gluten extracted from wheat flours mixed with DATEM and obtained from site B. Recoveries (means ± SD, n= 4) with similar letters are not significantly different ($P \leq 0.05$).

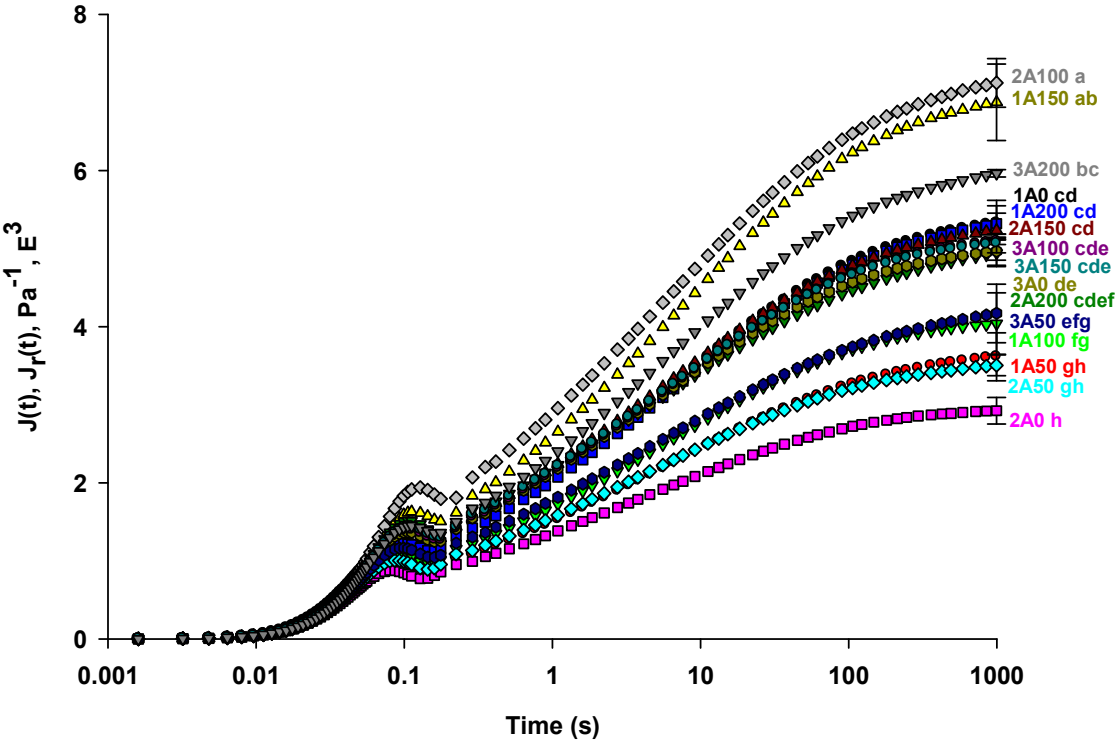

Fig. 4. Recovery compliance of gluten extracted from wheat flours mixed with ascorbic acid and obtained from site A. Recoveries (means ± SD, n= 4) with similar letters are not significantly different ($P \leq 0.05$).

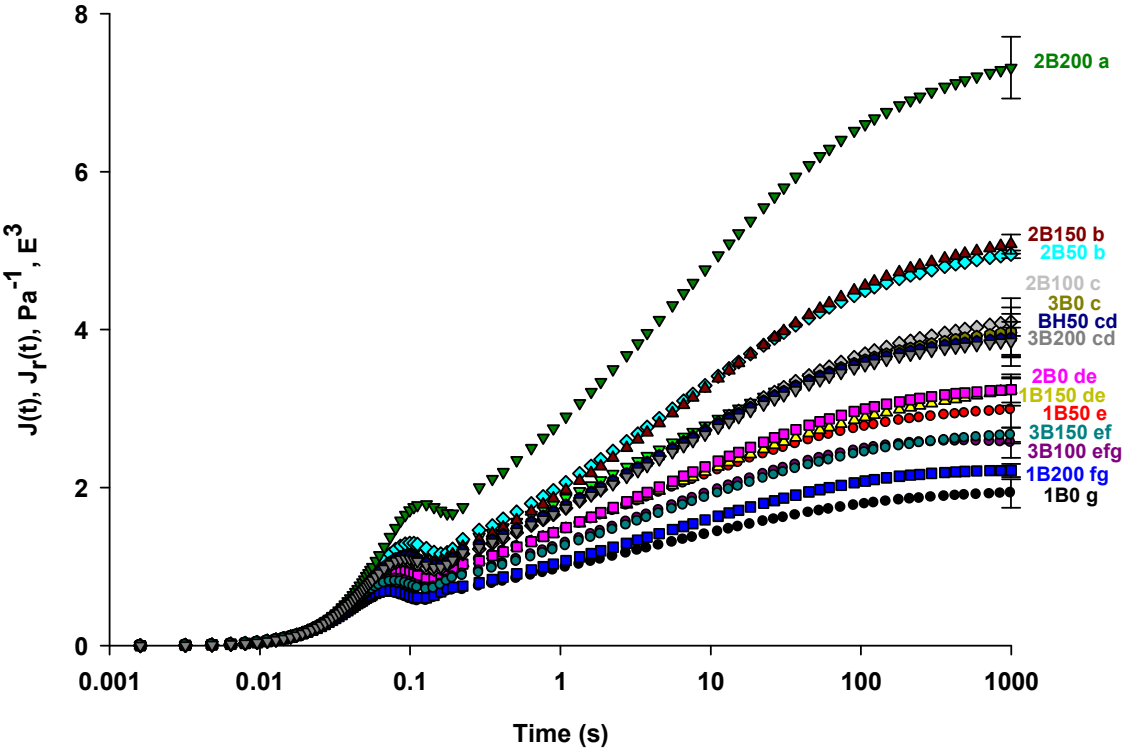

Fig. 5. Recovery compliance of gluten extracted from wheat flours mixed with ascorbic acid and obtained from site B. Recoveries (means ± SD, n= 4) with similar letters are not significantly different ($P \leq 0.05$).

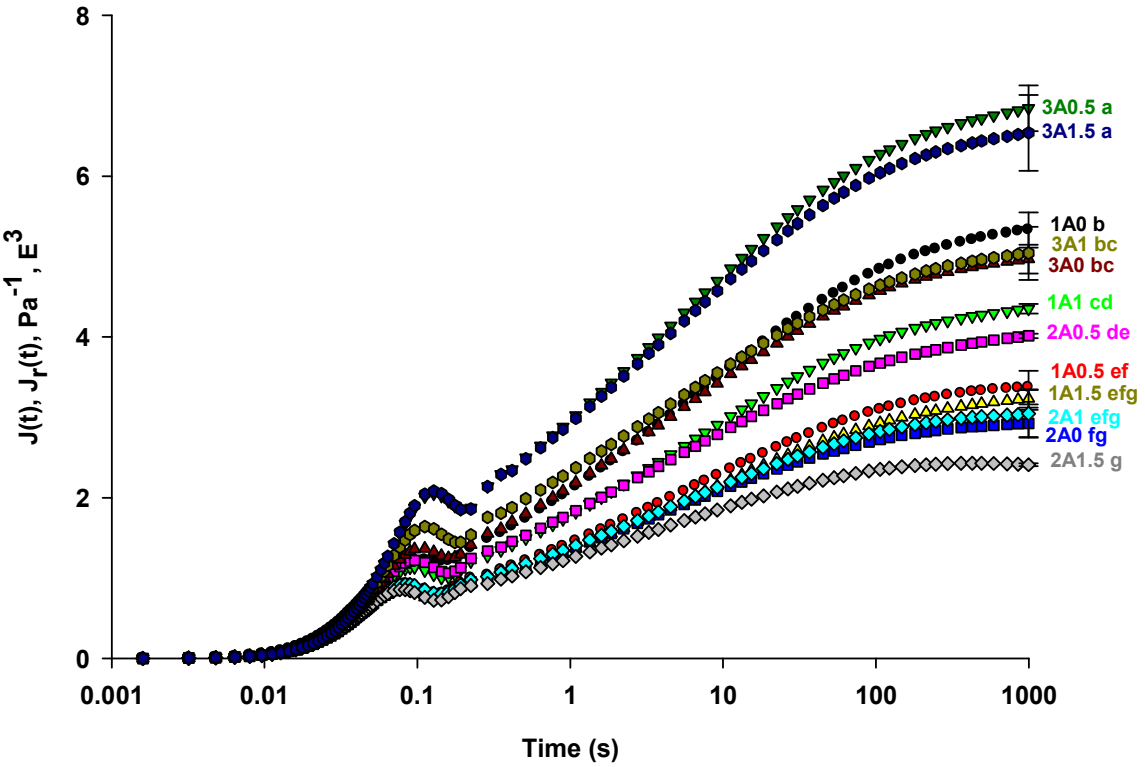

Fig. 6. Recovery compliance of gluten extracted from wheat flours mixed with urea and obtained from site A. Recoveries (means ± SD, n= 4) with similar letters are not significantly different ($P \leq 0.05$).

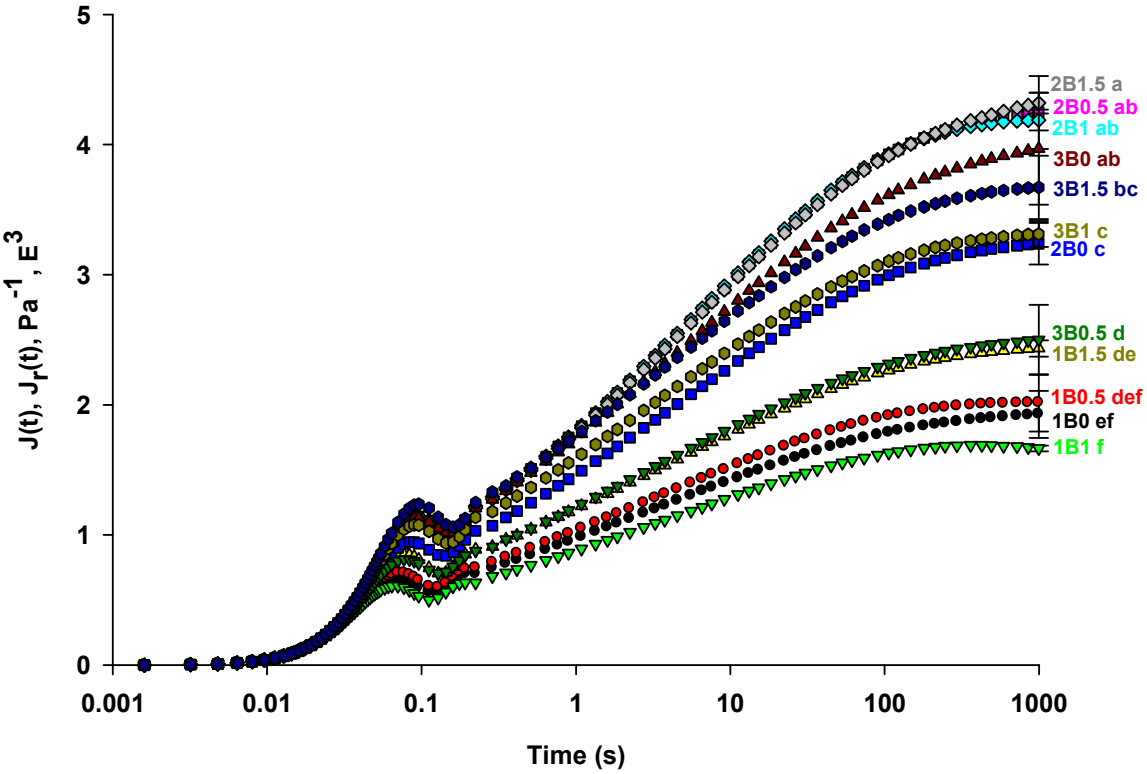

Fig. 7. Recovery compliance of gluten extracted from wheat flours mixed with urea and obtained from site B. Recoveries (means ± SD, n= 4) with similar letters are not significantly different ($P \leq 0.05$).

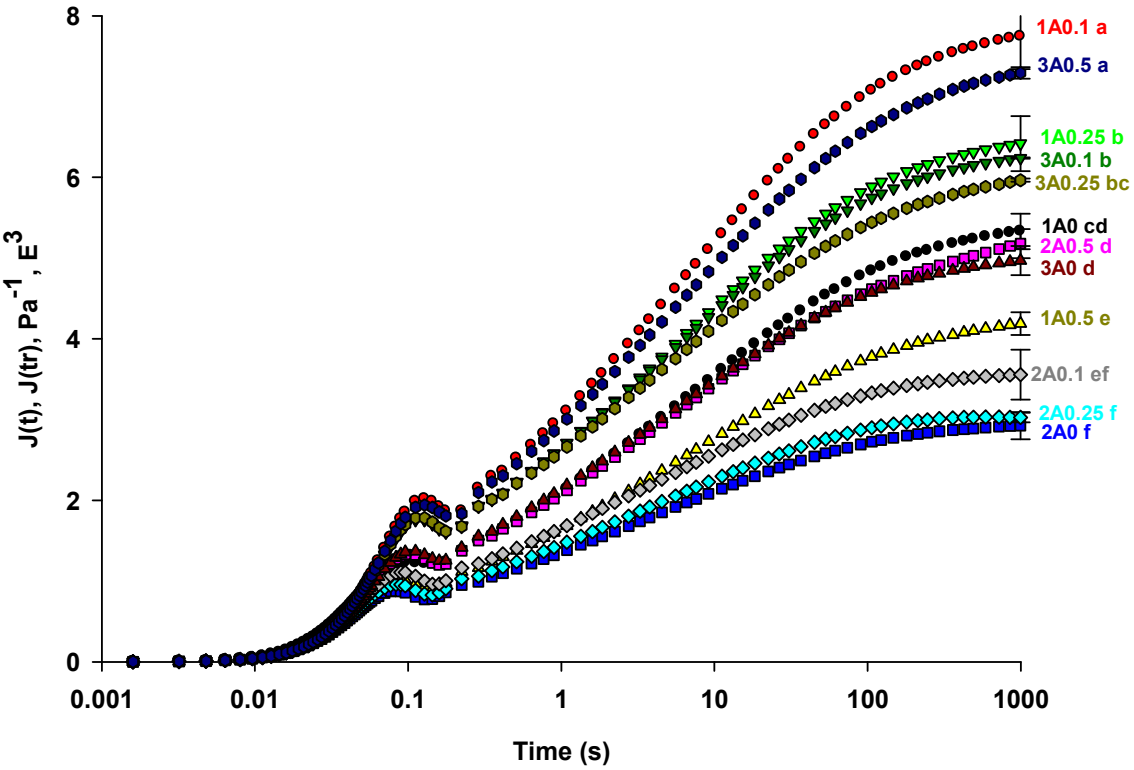

Fig. 8. Recovery compliance of gluten extracted from wheat flours mixed with DTT and obtained from site A. Recoveries (means ± SD, n= 4) with similar letters are not significantly different ($P \leq 0.05$).

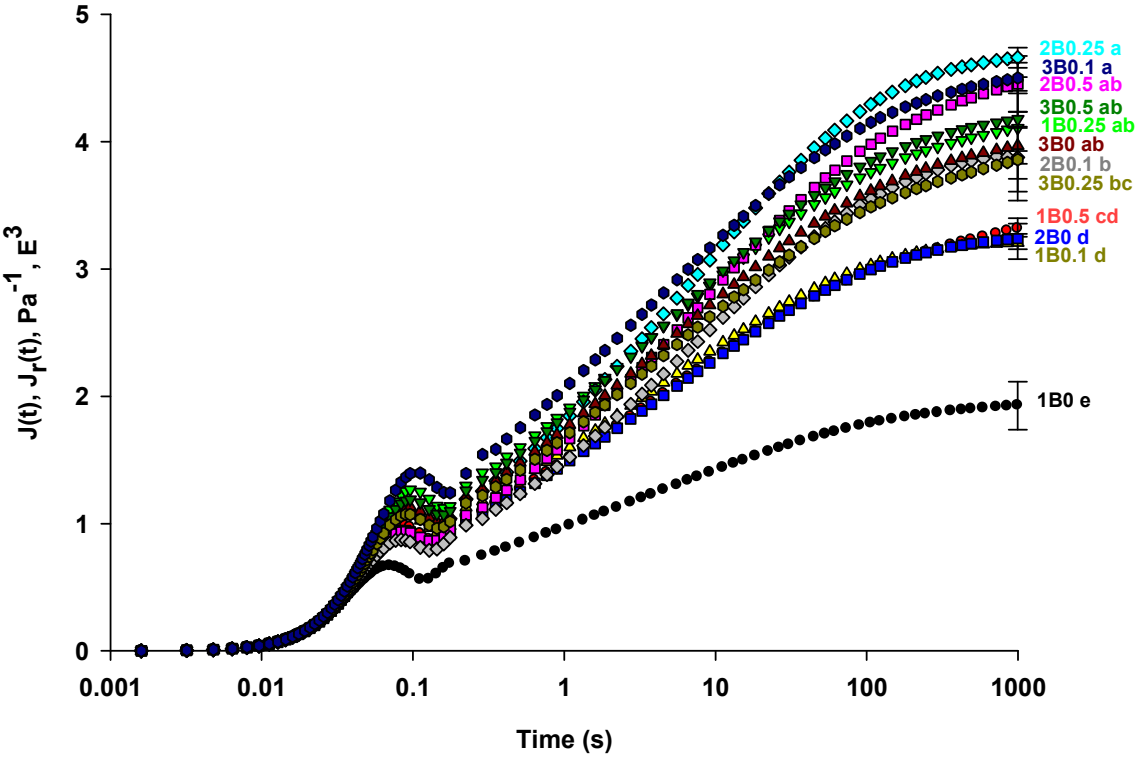

Fig. 9. Recovery compliance of gluten extracted from wheat flours mixed with DTT and obtained from site B. Recoveries (means ± SD, n= 4) with similar letters are not significantly different ($P \leq 0.05$).

APPENDIX 2

Table 1. Analysis of variance for visco-elastic, mixing and baking properties of wheat flours from sites A and B, treated with DATEM. TRT=DATEM treatment, Prot=flour protein content, TRT*Prot = interaction

Variable	Effects	Site A				Site B			
		Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
SeP	TRT	3	24	311.33	<0.0001	3	24	1286.15	<0.0001
	Prot	2	24	452.52	<0.0001	2	24	3665.47	<0.0001
	TRT*Prot	6	24	202.22	<0.0001	6	24	684.72	<0.0001
$J\text{-}J_r$	TRT	3	24	67.18	<0.0001	3	24	50.10	<0.0001
	Prot	2	24	138.77	<0.0001	2	24	75.12	<0.0001
	TRT*Prot	6	24	7.64	0.0001	6	24	9.09	<0.0001
RCY	TRT	3	24	4.82	0.0091	3	24	0.56	0.6470
	Prot	2	24	15.46	<0.0001	2	24	3.20	0.0584
	TRT*Prot	6	24	0.51	0.7979	6	24	2.81	0.0324
TCC	TRT	3	24.3	4.31	0.0142	3	24.8	2.30	0.1021
	Prot	2	24.3	37.73	<0.0001	2	24.8	9.53	0.0008
	TRT*Prot	6	24.3	9.62	<0.0001	6	24.8	11.23	<0.0001
TCR	TRT	3	24.3	54.20	<0.0001	3	24.8	18.04	<0.0001
	Prot	2	24.3	8.57	0.0015	2	24.8	11.63	0.0003
	TRT*Prot	6	24.3	19.33	<0.0001	6	24.8	40.88	<0.0001
WA	TRT	3	11.9	8.44	0.0028	3	11.9	625.07	<0.0001
	Prot	2	11.9	1366.90	<0.0001	2	11.9	116.60	<0.0001
	TRT*Prot	6	11.9	5.40	0.0066	6	11.9	18.48	<0.0001
DT	TRT	3	11.9	8.44	0.0028	3	11.9	4.69	0.0220
	Prot	2	11.9	1174.99	<0.0001	2	11.9	7.11	0.0093
	TRT*Prot	6	11.9	6.59	0.0029	6	11.9	0.77	0.6089
ST	TRT	3	11.9	22.27	<0.0001	3	11.9	81.57	<0.0001
	Prot	2	11.9	638.21	<0.0001	2	11.9	140.93	<0.0001
	TRT*Prot	6	11.9	27.00	<0.0001	6	11.9	41.38	<0.0001
BT	TRT	3	11.9	0.59	0.6349	3	11.9	108.70	<0.0001
	Prot	2	11.9	133.10	<0.0001	2	11.9	39.45	<0.0001
	TRT*Prot	6	11.9	0.69	0.6631	6	11.9	17.64	<0.0001
LV	TRT	3	35.9	931.07	<0.0001	3	35.9	3296.84	<0.0001
	Prot	2	35.9	7338.19	<0.0001	2	35.9	2892.90	<0.0001
	TRT*Prot	6	35.9	51.16	<0.0001	6	35.9	55.49	<0.0001
LH	TRT	3	11.9	6.98	0.0058	3	11.9	25.45	<0.0001
	Prot	2	11.9	89.17	<0.0001	2	11.9	35.74	<0.0001
	TRT*Prot	6	11.9	0.28	0.9372	6	11.9	3.16	0.0432
PH	TRT	3	11.9	28.57	<0.0001	3	11.9	5.80	0.0111
	Prot	2	11.9	96.16	<0.0001	2	11.9	0.42	0.6669
	TRT*Prot	6	11.9	5.62	0.0056	6	11.9	11.77	0.0002
OSP	TRT	3	11.9	42.81	<0.0001	3	11.9	22.44	<0.0001
	Prot	2	11.9	7.25	0.0087	2	11.9	15.86	0.0004
	TRT*Prot	6	11.9	2.77	0.0632	6	11.9	1.68	0.2098
SV	TRT	3	11.9	350.97	<0.0001	3	11.9	472.76	<0.0001
	Prot	2	11.9	2801.65	<0.0001	2	11.9	660.29	<0.0001
	TRT*Prot	6	11.9	28.09	<0.0001	6	11.9	5.30	0.0071

Table 4. Analysis of variance for visco-elastic, mixing and baking properties of wheat flours from sites A and B, treated with Ascorbic acid. TRT= Ascorbic acid treatment, Prot=flour protein content, TRT*Prot = interaction.

Variable	Effects	Site A				Site B			
		Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
SeP	TRT	4	22.8	869.80	<0.0001	4	23	87.14	<0.0001
	Prot	2	22.8	698.70	<0.0001	2	23	204.59	<0.0001
	TRT*Prot	8	22.8	733.77	<0.0001	8	23	99.12	<0.0001
$J\text{-}J_r$	TRT	4	18.8	83.44	<0.0001	4	18	79.59	<0.0001
	Prot	2	18.8	18.98	<0.0001	2	18	293.40	<0.0001
	TRT*Prot	8	18.8	41.17	0.0001	8	18	48.25	<0.0001
RCY	TRT	4	21	8.18	0.0004	4	18.7	10.13	0.0002
	Prot	2	21	5.64	0.0109	2	18.7	37.80	<0.0001
	TRT*Prot	8	21	5.92	0.0005	8	18.7	2.88	0.0285
TCC	TRT	4	24.8	49.80	<0.0001	4	23	2.62	0.0612
	Prot	2	24.8	88.15	<0.0001	2	23	5.81	0.0090
	TRT*Prot	8	24.8	28.97	<0.0001	8	23	2.13	<0.0752
TCR	TRT	4	24.8	14.62	<0.0001	4	23	6.42	0.0013
	Prot	2	24.8	24.97	<0.0001	2	23	222.43	<0.0001
	TRT*Prot	8	24.8	14.72	<0.0001	8	23	45.11	<0.0001
WA	TRT	4	15	3.17	0.0449	4	15	2.99	0.0530
	Prot	2	15	2879.29	<0.0001	2	15	3.14	0.0727
	TRT*Prot	8	15	18.49	<0.0001	8	15	3.81	0.0124
DT	TRT	4	15	45.56	<0.0001	4	15	0.35	0.8400
	Prot	2	15	2081.89	<0.0001	2	15	0.04	0.9573
	TRT*Prot	8	15	44.96	<0.0001	8	15	1.02	0.4620
ST	TRT	4	15	64.33	<0.0001	4	15	13.07	<0.0001
	Prot	2	15	714.33	<0.0001	2	15	81.42	<0.0001
	TRT*Prot	8	15	22.37	<0.0001	8	15	17.31	<0.0001
BT	TRT	4	15	10.99	0.0002	4	15	0.17	0.9527
	Prot	2	15	744.55	<0.0001	2	15	19.49	<0.0001
	TRT*Prot	8	15	9.01	0.0002	8	15	3.15	0.0266
LV	TRT	4	45	560.17	<0.0001	4	45	562.63	<0.0001
	Prot	2	45	2891.40	<0.0001	2	45	1688.45	<0.0001
	TRT*Prot	8	45	45.66	<0.0001	8	45	20.96	<0.0001
LH	TRT	4	15	41.16	<0.0001	4	15	27.74	<0.0001
	Prot	2	15	621.09	<0.0001	2	15	53.55	<0.0001
	TRT*Prot	8	15	14.02	<0.0001	8	15	1.80	0.1552
PH	TRT	4	15	35.98	<0.0001	4	15	22.91	<0.0001
	Prot	2	15	194.29	<0.0001	2	15	3.36	0.0625
	TRT*Prot	8	15	1.86	0.1431	8	15	1.98	0.1208
OSP	TRT	4	15	49.09	<0.0001	4	15	24.90	<0.0001
	Prot	2	15	2.20	0.1452	2	15	74.23	<0.0001
	TRT*Prot	8	15	3.14	0.0268	8	15	4.68	0.0004
SV	TRT	4	15	1.00	0.4374	4	15	327.20	<0.0001
	Prot	2	15	1.08	0.3631	2	15	885.02	<0.0001
	TRT*Prot	8	15	1.01	0.4684	8	15	6.95	0.0007

Table 3. Analysis of variance for visco-elastic, mixing and baking properties of wheat flours from sites A and B, treated with Urea. TRT=Urea treatment, Prot=flour protein content, TRT*Prot = interaction.

Variable	Effects	Site A				Site B			
		Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
SeP	TRT	3	13.5	240.78	<0.0001	3	16.5	75.33	<0.0001
	Prot	2	13.5	1121.32	<0.0001	2	16.5	168.46	<0.0001
	TRT*Prot	6	13.5	347.07	<0.0001	6	16.5	49.81	<0.0001
$J\text{-}J_r$	TRT	3	15.3	5.88	0.0071	3	15.6	2.17	0.1330
	Prot	2	15.3	137.11	<0.0001	2	15.6	183.72	<0.0001
	TRT*Prot	6	15.3	24.11	0.0001	6	15.6	14.26	<0.0001
RCY	TRT	3	15.3	5.40	0.0099	3	15.6	0.77	0.5270
	Prot	2	15.3	11.20	0.0010	2	15.6	16.99	0.0001
	TRT*Prot	6	15.3	6.58	0.0014	6	15.6	4.18	0.0107
TCC	TRT	3	16.1	2.77	0.0754	3	19	23.11	<0.0001
	Prot	2	16.1	35.25	<0.0001	2	19	32.38	<0.0001
	TRT*Prot	6	16.1	4.50	0.0073	6	19	8.32	0.0002
TCR	TRT	3	16.1	0.76	0.5300	3	19	16.53	<0.0001
	Prot	2	16.1	83.23	<0.0001	2	19	109.78	<0.0001
	TRT*Prot	6	16.1	1.84	0.1537	6	19	19.80	<0.0001
WA	TRT	3	11.9	16.19	0.0002	3	11.9	74.61	<0.0001
	Prot	2	11.9	1261.30	<0.0001	2	11.9	35.63	<0.0001
	TRT*Prot	6	11.9	17.27	<0.0001	6	11.9	9.32	0.0006
DT	TRT	3	11.9	2.25	0.1358	3	11.9	6.00	0.0099
	Prot	2	11.9	2107.36	<0.0001	2	11.9	7.34	0.0084
	TRT*Prot	6	11.9	6.64	0.0029	6	11.9	2.04	0.1382
ST	TRT	3	11.9	3.16	0.0647	3	11.9	11.14	0.0009
	Prot	2	11.9	405.30	<0.0001	2	11.9	713.44	<0.0001
	TRT*Prot	6	11.9	13.44	0.0001	6	11.9	23.22	<0.0001
BT	TRT	3	11.9	4.42	0.0262	3	11.9	5.73	0.0116
	Prot	2	11.9	668.95	<0.0001	2	11.9	51.97	<0.0001
	TRT*Prot	6	11.9	5.35	0.0068	6	11.9	18.79	<0.0001
LV	TRT	3	35.9	3435.02	<0.0001	3	35.9	5546.08	<0.0001
	Prot	2	35.9	5745.18	<0.0001	2	35.9	2136.96	<0.0001
	TRT*Prot	6	35.9	159.06	<0.0001	6	35.9	527.58	<0.0001
LH	TRT	3	11.9	831.28	<0.0001	3	11.9	659.82	<0.0001
	Prot	2	11.9	683.71	<0.0001	2	11.9	451.88	<0.0001
	TRT*Prot	6	11.9	24.90	<0.0001	6	11.9	78.14	<0.0001
PH	TRT	3	11.9	267.15	<0.0001	3	11.9	432.25	<0.0001
	Prot	2	11.9	111.27	<0.0001	2	11.9	59.21	<0.0001
	TRT*Prot	6	11.9	4.28	0.0157	6	11.9	11.82	0.0002
OSP	TRT	3	11.9	59.69	<0.0001	3	11.9	89.46	<0.0001
	Prot	2	11.9	70.51	<0.0001	2	11.9	135.56	<0.0001
	TRT*Prot	6	11.9	13.37	0.0001	6	11.9	47.80	<0.0001
SV	TRT	3	11.9	942.19	<0.0001	3	11.9	1976.24	<0.0001
	Prot	2	11.9	1100.23	<0.0001	2	11.9	701.30	<0.0001
	TRT*Prot	6	11.9	45.62	<0.0001	6	11.9	182.75	<0.0001

Table 4. Analysis of variance for visco-elastic, mixing and baking properties of wheat flours from sites A and B, treated with DTT. TRT=DTT treatment, Prot=flour protein content, TRT*Prot = interaction.

Variable	Effects	Site A				Site B			
		Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
SeP	TRT	3	15.6	1544.17	<0.0001	3	15.9	91.72	<0.0001
	Prot	2	15.6	3580.99	<0.0001	2	15.9	60.29	<0.0001
	TRT*Prot	6	15.6	1577.37	<0.0001	6	15.9	77.65	<0.0001
$J\text{-}J_r$	TRT	3	15.6	18.07	<0.0001	3	15.9	24.25	<0.0001
	Prot	2	15.6	83.49	<0.0001	2	15.9	53.99	<0.0001
	TRT*Prot	6	15.6	20.66	<0.0001	6	15.9	5.33	0.0034
RCY	TRT	3	15.6	2.26	0.1213	3	15.9	4.49	0.0.181
	Prot	2	15.6	12.44	0.0006	2	15.9	16.92	0.0001
	TRT*Prot	6	15.6	2.87	0.0439	6	15.9	0.86	0.5430
TCC	TRT	3	15.6	13.57	0.0001	3	15.9	70.29	<0.0001
	Prot	2	15.6	25.52	<0.0001	2	15.9	32.38	<0.0001
	TRT*Prot	6	15.6	10.56	<0.0001	6	15.9	8.32	0.0002
TCR	TRT	3	15.6	47.09	<0.0001	3	15.9	19.37	<0.0001
	Prot	2	15.6	62.47	<0.0001	2	15.9	31.21	<0.0001
	TRT*Prot	6	15.6	10.13	0.0001	6	15.9	8.60	0.0003
WA	TRT	3	11.9	10.25	0.0013	3	11.9	30.06	<0.0001
	Prot	2	11.9	1363.06	<0.0001	2	11.9	12.38	0.0012
	TRT*Prot	6	11.9	6.15	0.0039	6	11.9	2.49	0.0853
DT	TRT	3	11.9	102.64	<0.0001	3	11.9	9.01	0.0022
	Prot	2	11.9	2601.04	<0.0001	2	11.9	4.65	0.0323
	TRT*Prot	6	11.9	122.06	<0.0001	6	11.9	9.70	0.0005
ST	TRT	3	11.9	114.90	<0.0001	3	11.9	37.98	<0.0001
	Prot	2	11.9	1184.46	<0.0001	2	11.9	210.72	<0.0001
	TRT*Prot	6	11.9	98.88	<0.0001	6	11.9	43.68	<0.0001
BT	TRT	3	11.9	13.81	0.0004	3	11.9	91.55	<0.0001
	Prot	2	11.9	419.41	<0.0001	2	11.9	53.41	<0.0001
	TRT*Prot	6	11.9	38.97	<0.0001	6	11.9	30.48	<0.0001
LV	TRT	3	35.9	2738.10	<0.0001	3	35.9	2382.75	<0.0001
	Prot	2	35.9	6918.85	<0.0001	2	35.9	1304.22	<0.0001
	TRT*Prot	6	35.9	91.34	<0.0001	6	35.9	216.50	<0.0001
LH	TRT	3	11.9	1248.55	<0.0001	3	11.9	960.00	<0.0001
	Prot	2	11.9	707.33	<0.0001	2	11.9	135.43	<0.0001
	TRT*Prot	6	11.9	11.88	0.0002	6	11.9	11.15	0.0003
PH	TRT	3	11.9	111.18	<0.0001	3	11.9	431.27	<0.0001
	Prot	2	11.9	490.27	<0.0001	2	11.9	162.28	<0.0001
	TRT*Prot	6	11.9	8.36	0.0010	6	11.9	46.98	<0.0001
OSP	TRT	3	11.9	299.64	<0.0001	3	11.9	414.94	<0.0001
	Prot	2	11.9	1.20	0.3358	2	11.9	4.65	0.0322
	TRT*Prot	6	11.9	3.11	0.0452	6	11.9	53.21	<0.0001
SV	TRT	3	11.9	1992.39	<0.0001	3	11.9	931.60	<0.0001
	Prot	2	11.9	3159.37	<0.0001	2	11.9	336.49	<0.0001
	TRT*Prot	6	11.9	71.28	<0.0001	6	11.9	62.53	<0.0001

APPENDIX 3

Table 1. Significant differences (P < 0.05) in main effects in absence of an interaction (Appendix 2, Tables 1 to 4). Main effects: flour protein (H = high, L = low and M = medium) and treatment (DATEM = 0, 0.3, 0.6 and 1%) in flours obtained from sites A and B. Means are separated using Tukey's test.

Chapter	Site	Variable	Effect	Level	Mean	Std Error	Letter Grouping
III	A	RCY (%)	DATEM	0	80.74	0.50	B
				0.3	82.91	0.50	A
				0.6	83.26	0.50	A
				1	82.41	0.50	AB
			Protein	H	82.00	0.44	B
				L	80.79	0.44	B
				M	84.21	0.44	A
III	A	BT (min)	Protein	H	14.43	0.60	A
				L	1.45	0.60	B
				M	3.23	0.60	B
III	A	LH (mm)	DATEM	0	92.04	0.77	A
				0.3	92.76	0.77	A
				0.6	92.94	0.77	A
				1	88.56	0.77	B
			Protein	H	96.85	0.67	A
				L	84.56	0.60	C
				M	93.32	0.60	B
III	A	OSP (mm)	DATEM	0	23.93	0.74	A
				0.3	21.64	0.74	A
				0.6	23.93	0.74	A
				1	13.00	0.74	B
			Protein	H	20.72	0.64	A
				L	18.17	0.64	B
				M	21.48	0.64	A
III	B	DT (min)	DATEM	0	1.75	0.07	AB
				0.3	1.60	0.07	B
				0.6	1.88	0.07	AB
				1	1.98	0.07	A
			Protein	H	1.96	0.06	A
				L	1.61	0.06	B
				M	1.83	0.06	AB
III	B	OSP (mm)	DATEM	0	20.25	1.17	A
				0.3	22.00	1.17	A
				0.6	19.25	1.17	A
				1	9.56	1.17	B
			Protein	H	21.01	1.02	A
				L	13.20	1.02	B
				M	19.09	1.02	A

Table 2. Significant differences ($P < 0.05$) in main effects in absence of an interaction (Appendix 2, Tables 1 to 4). Main effects: flour protein (H = high, L = low and M = medium) and treatment (Ascorbic acid = 0, 50, 100, 150 and 200 ppm) in flours obtained from sites A and B. Means are separated using Tukey's test.

Chapter	Site	Variable	Effect	Level	Mean	Std Error	Letter Grouping
IV	A	PH (mm)	Ascorbic acid	0	77.01	0.57	A
				50	68.11	0.57	D
				100	72.03	0.57	B
				150	70.90	0.57	BC
				200	69.28	0.57	CD
			Protein	H	76.10	0.44	A
				L	64.42	0.44	C
				M	73.87	0.44	B
IV	B	TCC (s)	Ascorbic acid	0	5.95	1.28	B
				50	7.12	1.38	AB
				100	6.84	1.47	AB
				150	8.55	1.47	AB
				200	11.85	1.47	A
			Protein	H	7.96	1.08	AB
				L	5.44	1.04	B
				M	10.98	1.17	A
IV	B	LH (mm)	Ascorbic acid	0	94.23	0.68	B
				50	93.63	0.68	B
				100	99.20	0.68	A
				150	92.17	0.68	BC
				200	89.32	0.68	C
			Protein	H	96.44	0.53	A
				L	89.26	0.53	B
				M	95.43	0.53	A
IV	B	PH (mm)	Ascorbic acid	0	77.77	0.61	A
				50	75.04	0.61	B
				100	76.37	0.61	AB
				150	74.78	0.61	B
				200	70.00	0.61	C

Table 3. Significant differences ($P < 0.05$) in main effects in absence of an interaction (Appendix 2, Tables 1 to 4). Main effects: flour protein (H = high, L = low and M = medium) and treatment (Urea = 0, 0.5, 1 and 1.5 M) in flours obtained from sites A and B. Means are separated using Tukey's test.

Chapter	Site	Variable	Effect	Level	Mean	Std Error	Letter Grouping
V	A	TCR (s)	Protein	H	5.62	0.20	B
				L	7.93	0.19	A
				M	4.40	0.19	C
V	B	DT (min)	Urea	0	1.75	0.06	B
				0.5	1.93	0.06	AB
				1	1.75	0.06	B
				1.5	2.10	0.06	A
			Protein	H	2.06	0.05	A
				L	1.75	0.05	B
				M	1.83	0.05	B

Table 4. Significant differences ($P < 0.05$) in main effects in absence of an interaction (Appendix 2, Tables 1 to 4). Main effects: flour protein (H = high, L = low and M = medium) and treatment (DTT = 0, 0.1, 0.25 and 0.5 mM) in flours obtained from sites A and B. Means are separated using Tukey's test.

Chapter	Site	Variable	Effect	Level	Mean	Std Error	Letter Grouping
VI	B	RCY (%)	DTT	0	82.84	0.54	A
				0.1	81.59	0.66	AB
				0.25	80.37	0.66	B
				0.5	80.17	0.66	B
			Protein	H	80.61	0.54	B
				L	83.69	0.52	A
				M	79.43	0.54	B
VI	B	WA (%)	DTT	0	59.81	0.15	C
				0.1	60.03	0.15	C
				0.25	60.80	0.15	B
				0.5	61.66	0.15	A
			Protein	H	61.11	0.13	A
				L	60.37	0.13	B
				M	60.25	0.13	B

APPENDIX 4

Table 1. Description of important studies cited in this dissertation.

Author	Description	Conclusion
Aamodt et al. 2003	Norwegian wheat cultivars (10 to 13% FP), hearth loaves baked, SE-HPLC Carried out for mono and polymeric fractions and their ratios, extensibility tests using Kieffer and gluten extensibility rig, controls had no additives, DATEM 0.45% (w/w flour), ascorbic acid (AA) (30 ppm)	DATEM and AA improved bread characteristics. Loaf area and form ratios proportional to the amount of largest glutenin polymers. No increase in ratio of monomeric to polymeric protein with increase in protein content. DATEM increased Rmax/ Ext ratio, increased elasticity no change in viscosity. AA increased Rmax and MPT
Armero et al. 1996	Spanish wheat (11.13% FP), 100 g loaves, DATEM (0.3%), MGL (0.3%), SSL (0.5%) and controls. Rheological (extensibility, Rmax), baking, mixing by Farinograph	DATEM and SSL increased loaf volumes (11.6 and 10.8%, respectively) and oven spring heights (21.1 and 12.5%, respectively). SSL improved the mixing properties like stability (25%). DATEM added in combination of MGL reduced the Rmax by 31.2%
Koehler and Grosch 1999	German wheat cultivar (10.5% FP), DATEM 0.1 to 0.5% w/w flour basis, micro-scale baking, rheology by micro-extensograph	Loaf volumes increased 55 to 60% with 0.3 to 0.5% DATEM concentrations, respectively. Resistance increased by 50% with 0.5% DATEM compared to controls.
Campbell et al. 2001	DATEM (0, 0.4 and 0.7% w/w) British wheat cultivar Riband (8.1% FP). Loaf volumes, gas content, air content, dough density measured	DATEM and SSL levels of 0.4% improved loaf volumes (31.8% and 40%, respectively).DATEM (0.4%) and SSL (0.7%) increased air content (11.6 and 13.1%, respectively) and gas-free dough density (1.1 and 0.35%, respectively) than controls.
Stampflii et al 1996	Norwegian wheat (13% FP), DATEM 0 to 2% w/w flour basis, Farinograph and extensograph	Extensibility decreased by 10 and 22% with 1 and 2% DATEM, Water absorption increased 2.3% with 1.5% DATEM. Resistance to constant deformation after 90 min resting time of dough increased by 32% with 2% DATEM.
Koehler 2003	Ascorbic acid levels 0 to 200 ppm added to the dough (French and German	Ascorbic acid levels of 125 ppm effectively reduced 1 to 35% free GSH in the flour.

	wheat cultivars wit 8 to 13% FP), determination of GSH and GSSG	
Yamada and Preston 1994	Canadian western red spring wheat flour (11.7% FP) AA (0 to 150 ppm), loaf volumes oven rise and crumb characteristics measured	Ascorbic acid treatments of 15 ppm increased loaf volumes by 4.7% and oven rise by 14.9%. No significant increase in loaf volumes and oven rise observed with levels higher than 15 ppm.
Li et al 2004	Thirty eight wheat grain samples (9.3 to 11.8% FP), GSSG content in flours determined using HPLC after solubilizing with 0.025 M DTT, pH 9 at 25 °C and 40 °C	The derivatization yield was 70% higher for GSSG and 68% higher for GSH at 40 °C than 25 °C
Wikstrom et al 1998	Ascorbic acid (0 to 500 ppm), Swedish wheat flours (8.2 to 13.8% FP), stress relaxation study on dough, loaf volumes	Loaf volumes increased by 19 to 52% with 75 ppm AA levels. Initial stress levels increased by 44% with 50 ppm AA on flour with 13.8% FP. No significant increase in initial stress observed on other flours with 50 ppm AA. The rate at which the dough relaxed was 14.1% faster on low protein (8.2% FP) dough at 50 ppm AA and 15% slower in high protein flour at same level.
Inda and Rha 1982	Gluten isolated from wheat flours. Gluten subjected to extensibility and rupture tests, 0 to 8 M urea concentrations used	The rate at which the stress relaxed was faster for gluten treated with 5 M urea (29%) compared to 1 M urea (11%). Constant strain modulus decreased by 40.6% at 1 M urea compared to control but increased by 35.7% again at 5 M urea. Similarly derived stress relaxation modulus decreased by 42.7 % with 1 M urea from control and again increased 42.7% at 5 M urea.
Inda and Rha 1991	Dynamic oscillation test with 1 M to 5 M urea on gluten isolated from commercial wheat flour	Rate at which loss modulus increased with increased frequency was 5 % slower for gluten treated with 5 M urea than control. Loss tangent increased by 60% with 5 M urea with increasing frequency compared to 10% increase of control.
Khatkar 2005	Dynamic oscillatory test 1Hz frequency and 25 Pa stress amplitude used, 0 to 10 M urea concentrations	Storage and loss modulus were positively correlated to loaf volumes r = 0.69 and 0.73, respectively). Decrease in elastic moduli with 3 M urea was 25 and 50% with strong and

	used, 0 to 500 ppm DTT used, loaf volumes, gluten from strong and weak varieties isolated.	weak gluten respectively, but 66.6 and 40% increase with 8 M urea. DTT levels of 500 ppm decreased elastic modulus by 66 and 50% in strong and weak gluten
McGrane et al 2004	Urea concentration of 50% (w/w) on amylose gel, dynamic oscillatory testing	Viscous modulus (G'') and elastic modulus (G') increased by 91% and 80% respectively.
Lu and Seib 1998	Hard red winter wheat (11.5% FP) 1.3 mM DTT added to flour to convert DHAA to AA, mixograph and loaf volumes	3.2% decrease in loaf volumes observed. Ascorbic acid efficient at pH above 4, mixing time increased by 19%.

VITA

Amogh Arun Ambardekar

Candidate for the Degree of

Doctor of Philosophy

Dissertation: MODIFICATIONS IN VISCO-ELASTIC OF GLUTEN BY DIACETYL TARTARIC ACID ESTER OF MONOGLYCERIDE (DATEM), ASCORBIC ACID, UREA AND DITHIOTHREITOL AND ITS EFFECTS ON LOAF VOLUMES IN COMMERCIAL WHEAT FLOURS.

Major Field: Food Science

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ADVISER'S APPROVAL: Dr. Patricia Rayas-Duarte

Name: Amogh Ambardekar Date of Degree: December 2009

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: Modifications in visco-elasticity of gluten by diacetyl tartaric acid ester of monoglycerides (DATEM), ascorbic acid, urea, dithiothreitol (DTT) and its effects on baking and mixing properties in commercial wheat flours.

Pages in Study: 146 Candidate for the Degree of Doctor of Philosophy

Major Field: Food Science

Scope and Method of Study: Quality and structure of gluten is one of the determining factors of baking quality in wheat flours. Changes in structure and quality of gluten at molecular level are dependent on different covalent and non covalent crosslinks, redox states and folding and unfolding of gluten during baking processes. Surfactants, oxidizing agents, reducing agents and chemicals like urea modify the structure of gluten at a molecular level changes the visco-elastic as well as reactive properties with other components of the flour resulting into improved or adverse effect on baking. DATEM, ascorbic acid, urea and dithiothreitol (DTT) were added to the gluten and the dough at different levels to measure the changes in visco-elasticity in gluten and mixing and baking properties of wheat flours with different protein content and quality. Six flours of different protein content and quality were procured from two sites in Oklahoma, three from each site. Visco-elastic properties of gluten extracted from flours were measured using creep recovery tests. Mixing properties were evaluated using a Farinograph and baking characteristics were assessed using standard 100 g flour pup loaves baking method.

Findings and Conclusions: DATEM treatments strengthened the structure of gluten as observed with various visco-elastic parameters during creep recovery experiments. While most of the visco-elastic properties were independent of baking and mixing properties of flours, oven spring rise was closely related with rheological parameters that explained increased viscosity like delta compliance ($r = -0.57$, $P < 0.01$) due to DATEM. The addition of ascorbic acid had no specific effect on the visco-elastic properties of gluten and mixing properties of flour. However, loaf quality improved with 100-150 ppm ascorbic acid showing significant correlations between recoverability of gluten and oven spring ($r = -0.57$, $P < 0.01$). An overall weakening of the gluten strength resulted into dramatic decrease in baking and mixing characteristics of wheat flours with urea. Decrease in baking performance was related to slow rates of deformations and recovery of gluten due to urea indicated by significant correlations between loaf volume and creep time constants ($r = -0.42$, $P < 0.05$). Reducing effect of DTT on gluten weakened the gluten structure due to disulfide bond destruction resulting into poor baking supported by strongly significant ($P < 0.01$) correlations of separation time with proof and loaf heights ($r = 0.56$ and 0.53, respectively). The changes in visco-elasticity of gluten, mixing and baking properties of commercial wheat flours due to structural modification of gluten with different compounds were quantified. For the most part visco-elastic properties of gluten were independent of mixing and baking properties of flours with exceptions of few weak but significant correlations. This study concluded that 1 and 0.6% (w/w flour basis) DATEM strengthened gluten structure and improved bread quality, respectively. Ascorbic acid levels up to 100 ppm promoted disulfide linkages in gluten and improved baking. All levels of urea and DTT weakened the gluten strength due to disruption of hydrophilic and hydrophobic non-covalent bond and disulfide linkages resulting into poor baking performance.

ADVISER'S APPROVAL: Patricia Rayas-Duarte