EFFECT OF PHYSICAL CHANGES BY RED-OX STATE AND SURFACE TENSION ON GLUTEN AGGREGATION, VISCOELASTIC, MIXING AND BAKING PROPERTIES OF WHEAT BATTER SYSTEMS

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LIST OF ABBREVIATIONS

Additives

DATEM	Diacetyl tartaric acid ester of monoglycerides
AA	Ascorbic acid
DTT	Dithiothreitol

Instruments

NIR system	Near infrared system
GPT	Gluten peak tester

Agglomeration properties

PMT	Peak maximum time
MT	Maximum torque (strength)
T1	Torque before aggregation
T2	Torque after aggregation
Area	Area under peak (work)

Gluten viscoelastic properties

SeP	Gluten separation time
J-Jr	Delta compliance
RCY	Gluten percent recovery
λ_C	Time constant of creep
λ_R	Time constant of recovery

Dough mixing properties

DT	Dough development time
ST	Dough stability time
BT	Dough breaking time
WA	Dough water absorption

Baking properties

PH	Proof height
LH	Loaf height
LV	Loaf volume
OSP	Oven spring
SV	Specific volume

Flour protein

FP	Flour protein

Statistical analysis

PCA	Principal Component Analysis
PC1	Principal Component 1
PC2	Principal Component 2

CHAPTER I

INTRODUCTION

Statement of problem

Bread is a common food which plays an important role in maintaining good diet for humans. The technology of breadmaking has a long history and undergone a lot of changes in order to fulfill the demand of consumers in the sense of improvementation quality, cost, and convenience and life span products. The important aspects that determine the quality of white pan bread are a high volume and fine crumb structure. These important aspects are influenced by the functional properties of wheat flour since it is the major constituent of bread making. Food additives or dough improvers are used to improve the functional characteristic of wheat flour and also the baking quality. Among the common dough improvers used today are oxidizing reagents such as ascorbic acid and surfactants such as diacetyl tartaric acid ester of monoglycerides (DATEM). The effect of these improvers on dough systems have been evaluated in dough and breads. The effect of DATEM on batter systems has not been examined.

Therefore, the use of a high shear rate mixer was used to measure batter mixing properties when aggregation occurs. Overall, there is limited information regarding the influence of gluten protein on wheat flour batter, especially in the presence of various dough improvers. The study of different dough improvers on wheat flour batter will allow a better understanding of the influence of disulfide bonding wheat flour batter when aggregation is achieved. For this purpose dithiothreitol (DTT) was used. The dithiothreitol (DTT) will reduce disulfide bonds and prevent the aggregation of wheat flour batter. The aggregation properties were studied at high shear rate (3,333 rpm), 35°C and tested systematically varying the percentage of flour used. The aggregation properties of the batter system were compared with baking, mixing and gluten viscoelastic properties of flour samples to investigate possible correlations with the rheological properties of the samples.

Purpose of the study

The objectives of this study are:

1. To study the influence of diacetyl tartaric acid ester of monoglycerides (DATEM), ascorbic acid, and DTT on the aggregation properties of batters and
2. To investigate possible correlation of batter aggregation properties with viscoelastic properties of gluten, and mixing and baking properties of wheat flours.

Hypotheses

1. DATEM improves the viscoelastic properties of the wheat flour batter by favoring the formation of complexes between starch and gluten protein and thus promoting aggregation and improving the mixing and baking properties of wheat flour.
2. Ascorbic acid promotes the formation of disulfide bonds in gluten and improves its viscoelastic properties resulting into improved aggregation and baking performance.
3. DTT acts as a reductant for disulfide bonds in wheat flour batter and prevents aggregation to occur which reduce the mixing and baking properties of wheat flour.

Assumptions

Addition of oxidizing and reducing reagents changes the molecular interactions occurring in the batter and dough systems, affect the aggregation of large polymers and thus the molecular weight species that can be formed. These changes in molecular interactions affect the functionality of gluten in wheat flour. The formation of covalent and non-covalent bonds between glutenin will affect the molecular weight of gluten polymeric proteins as well as the interaction between glutenin and other flour constituents. The reagents used in this study will allow us to evaluate specific interactions and bond types by measuring quantitative and qualitative effects on aggregation characteristics of protein in wheat flour in a model batter system. By comparing the effects on batter versus dough properties, this study will also illustrate any possible correlations with other properties such as baking, mixing and viscoelasticity.

DATEM is an emulsifier with an amphiphilic molecule structure that allows the stabilization of dough due to the reduction of surface tension of different phases such as oil, water and liquid/air phases. DATEM interacts with the gluten protein and native lipid in flour. DATEM will decrease the surface tension of gluten which allows interaction between protein, starch and gas cells that preserve the interphase of dough and gluten structure. It is also suggested that emulsifiers like DATEM could be found in the layer of liquid lamella film between gluten and starch which will improve the film forming properties in gluten. The formation of film by gluten promotes the stability of gas bubbles in bread dough. There are also studies that suggest that DATEM will improve the quality of weak protein flour by increasing the quality of gluten in the system.

Ascorbic acid is an oxidizing agent used in the baking industry to enhance the quality of the dough, i.e., enhances its strength. The presence of covalent and non-covalent bonds between glutenin molecules and interactions between glutenin and other flour constituent play an important role in gluten functionality. Inter and intra disulfide bonds of glutenin subunits are considered very important contributors to the formation of gluten. Oxidizing agents will enhance the thiol-disulfide system that will influence the rheological properties of dough and improve the loaf volume.

DTT is a reducing agent that will weaken the dough structure by reducing the disulfide bonds that binds the high molecular weight glutenin subunits. The addition of DTT in the batters will impact the thiol-sulfide system that influences the viscoelastic properties of gluten. This also allows us to study the effect this type of bonds in batters and breads

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CHAPTER II

LITERATURE REVIEW

Wheat Quality

Wheat is one of the major grains with a wide variety of usage and adaptation to different climates around the world. Quality properties are affected by genotype and environmental conditions, i.e., different wheat cultivars and different environmental conditions produce different quality properties (Kettlewell et al. 2003). The composition of flour protein relies on the genotype but significant interactions with production environment are observed (Graybosch et al. 1996). The interaction between the genotype and environment, have an effect on the connection of flour protein composition to loaf volume (Huebner et al. 1997). Protein content is an important parameter in different wheat cultivars with similar protein quality in determining the end product functionality (Bushuk 1998). For instance, wheat varieties with hardest kernel texture and highest gluten protein are used for pan bread (Bushuk 1998). In comparison, wheat with low gluten protein and weakest kernel texture are used for cakes and cookies (Bushuk 1998). The mixing properties are also important in governing the wheat quality baking performance.

Commercial bakers desire strong flour dough that could handle the harshness of mechanical mixing (Call et al. 1925). A study regarding the comparison of the responses of lower protein modern wheat cultivars and higher protein older cultivar shows that adequate amount of protein could increase the mixing time and mixing tolerance (Fufa et al. 2005). Therefore, adequate protein content (10-13%) is desired for mixing characteristic (Call et al. 1925).

Gluten protein composition and properties

Gluten and starch are important functional ingredients in wheat flour and are related to the widespread application of this ingredient in food and non-food products (Frederix et al. 2004). Gluten is a complex quarternary protein structure created during dough mixing and breadmaking process. However, the quaternary structure of gluten is still not fully understood (Tilley et al. 2001). 80-85% of gluten proteins in wheat are insoluble in water. (Van Der Borght et al. 2005). It is generally accepted that hydration and mixing of gluten will form a strong, cohesive, viscoelastic network (Van Der Borght et al. 2005). Gluten is commonly used in bakery products in order to improve the flour quality (Peighambardoust et al. 2008). The gluten proteins consist of hundreds of protein components, and these proteins have unique amino acid composition (Wieser 2007). There are different groups of gluten protein known and differentiate based on the solubility in alcohol-water solution of gluten (e.g., 60% ethanol) such as the soluble gliadin and insoluble glutenin (Wieser 2007). Both gliadins and glutenins fraction played a major role to the rheological properties of dough (Joye et al. 2009; Van Der Borght et al. 2005; Wieser 2007). Gliadin will contribute to the viscosity and extensibility of the dough and acts as “plasticizer” or “solvent” for glutenins (Wieser 2007). In contrast,

glutenin is contributing mostly to the viscosity and extensibility of the dough (Wieser 2007). Therefore gluten protein will influence the viscoelastic properties of dough and the final products made. The existence of hydrogen bonds, ionic bonds, hydrophobic interactions and disulfide crosslink are crucial components of wheat dough characteristics (Wieser 2007). The SS bonds are the crucial aspect in developing the structure and properties of the three dimensional network of gluten (Joye et al. 2009; Wieser 2007). Additional covalent network bonds formed in breadmaking are tyrosine –tyrosine crosslink between gluten proteins (Tilley et al. 2001). The covalent structure and tyrosine crosslink can be strengthen by non-covalent (hydrogen, hydrophobic and ionic) interactions between wheat proteins (Joye et al. 2009; Wieser 2007). Although, these chemical bonds are not as energetic as covalent bond but they are involved in the formation of gluten aggregation and dough structure (Wieser et al. 2006). The presence of hydrogen bonds in gluten protein can be proved by the dough weakening effect of hydrogen bond breaking agents (e.g., urea) and dough strengthening effect of heavy water compared to ordinary water (Wieser 2007). The influence of ionic bonds in gluten protein can be seen by the strengthening effect of sodium chloride (NaCl) or bipolar ions such as amino acids or dicarboxylic acids (Wieser 2007). Hydrophobic bonds show importance of stabilizing the gluten structure (Wieser 2007). Flour lipid becomes attach or combined with gluten protein through hydrophobic and polar forces when water is present during mixing (MacRitchie 1987).

Gliadins

Gliadins are monomeric low molecular single chain polypeptides that aggregate by interchain disulfide bonds (Sapirstein and Fu 1998). They are also associated by

hydrogen bond and hydrophobic interactions (Shewry et al. 1986). The molecular weight of gliadin is between 30,000 to 80,000 Da (Veraverbeke and Delcour 2002). The alcohol-soluble gliadins have been divided into four subgroups known as α, β, γ, and ω-gliadins (Shimoni and Galili 1996). ω-gliadin contains no cysteine or methionine and have limited amount of essential amino acids (Shimoni and Galili 1996). Meanwhile, α-, β-, and γ -gliadin are rich in glutamine and proline (Shimoni and Galili 1996). The molecular weight of ω-gliadin ranges from 46,000 to 74,000 Da and α-, β-, and ω -gliadin ranges from 30,000 to 45,000 Da (Kasarda et al. 1983). Moreover, gliadin acts as plasticizer that contributes to the viscosity (promoting viscous flow) and extensibility of dough system (Joye et al. 2009; Wieser 2007). The gliadin molecules will bind with one another or glutenins through hydrophobic interactions and hydrogen bonding (Van Der Borght et al. 2005). Therefore, gliadin will also form aggregation in hydrophilic environment with the assistance of hydrophobic interactions and hydrogen bonding (Shewry et al. 1986; Van Der Borght et al. 2005).

Glutenins

Glutenins form multi-chained polymers and differ in MW range from 80,000 to several million Da (Hoseney 1994; Veraverbeke and Delcour 2002). The glutenin fractions are consist of aggregated protein linked by interchain disulfide bond which vary in size ranging from ~500,000 to more than 10 million DA (Wieser et al. 2006). They have been also defined as polydisperse polymers of disulfide bonded polypeptides (Sapirstein and Fu 1998). Glutenins are made up of a mixture of high molecular weight and low molecular weight glutenin subunits (HMW-GS and LMW-GS, respectively). Glutenins are made up of almost 10% HMW-GS and play an important role in baking

good quality breads (Dupont and Altenbach 2003). D'Ovidio and Masni (2004) reported that LMW-GS and HMW-GS are linked together through intermolecular disulfide bonding and are important in the formation of glutenin polymer for pasta making characteristics. The loaf volume of bread depends on the protein content as well as quality and composition of the glutenin (Bushuk 1998). The presence of glutenin provides the resistance to deformation in dough (Van Der Borght et al. 2005).

Effect of alcohol on solubility of gluten

Aggregation properties and low solubility in water are important properties of gluten. These properties occur due to the presence of distribution of amino acid residues with nonpolar and neutral chains and low amount of ionize amino acids (MarRitchie 1996). The ability of gluten proteins (gliadins and glutenins) to separate into fractions are based on their ability to solubilize in alcohol-water solution (e.g. 60% ethanol) (Wieser 2007). The different solubility of gliadin and glutenin in alcohol of difference alkyl-chain shows the difference in hydrophobicity of these proteins (Bean et al. 1998). Aggregation properties in gluten protein are made up of hydrophobic interaction, hydrogen bond and covalent disulfide bonds. These aggregation properties are important for the rheological properties of gluten and dough. It is known that hydrophobic or solvent induced interaction between two or more apolar solute molecules are responsible for stabilizing the conformation of protein in aqueous solution, the stability of micelles and membranes, and the ability of many compounds to form equilibrium in aqueous environment (Tanford 1973). These interactions originate from the three dimensional structure of water and change significantly as the structure of the solvent change (Usha et

al. 2006). The structure of the solvent may change because of the temperature or addition of cosolvents like alcohols (Usha et al. 2006).

Diacetyl Tartaric (Acid) Ester of Monoglyceride (DATEM)

Emulsifiers are active-surface compounds that have a chemical structure with both hydrophobic and hydrophilic moieties (Gómez et al. 2004). The presence of both lipophilic and hydrophilic properties allows the emulsifier to reduce the surface tension between two immiscible phases which facilitates emulsion to occur (Gómez et al. 2004; Stampfli and Nersten 1995). The addition of emulsifier reduces the surface tension in gas bubbles by interacting with the lipids added and creating a large amount of smaller bubbles (Selomulyo and Zhou 2007). The ability of ionization of emulsifiers are based on their electromagnetic charge in aqueous environment, therefore emulsifiers can be classified as ionic or nonionic types depending on the hydrophilic group (Stampfli and Nersten 1995). Nonionic emulsifier such as sucrose esters of fatty acids and ethoxylated mono-diglyceride do not ionize in water and they show good dough strengthening properties (Stampfli and Nersten 1995). Ionic emulsifier can be in the form of anionic (organic acids and their salts) or cationic (base-amines of different degree of replacement) emulsifier; anionic emulsifiers such as DATEM (Xiujin et al. 2007) are used in different baking purposes and cationic emulsifiers are not use in foods (Stampfli and Nersten 1995). Amphoteric emulsifiers have both anionic and cationic groups and the surface-active properties are based on pH (Stampfli and Nersten 1995). The amphoteric characteristic allows the formation of complexes between starch and protein (Gómez et al. 2004). The presence of different types emulsifying agents will influence the mechanism the formation of the dough system occurs.

Diacetyl tartaric acid ester of monoglycerides (DATEM) is an anionic oil-in-water emulsifier (Aamodt et al. 2003). DATEM is a made up of the reaction of mono- and diacetyltartaric acid anhydride with monoacylglycerols or the combination of mono- and diacylglycerols (Kohler and Grosch 1999). It is considered the most common and best dough stabilizer (Stampfli and Nersten 1995). This class of emulsifiers is believed to be able to increase the resistance and decrease the extensibility of dough (Ravi et al. 1999; Stampfli and Nersten 1995). DATEM is suggested to promote aggregation of gluten protein in dough by binding to the protein hydrophobic surface (Selomulyo and Zhou 2007). The formation of strong protein network will result in better texture and increased volume of produced bread (Selomulyo and Zhou 2007).

Ascorbic Acid and Dithiothreitol (DTT)

The quality of the gluten is very important in breadmaking and it is usually determined by the molecular weight of the glutenin subunits present, the presence of the covalent and non-covalent bonds between glutenin molecules and interaction between glutenin with other flour constituents (Goesaert et al. 2005). The presence of disulfide bond to hold glutenin subunits together and other flour constituents that makes disulfide bonds important in the gluten protein functionality in breadmaking (Goesaert et al. 2005). Therefore, the presences of ascorbic acid have strong influence on the thiol-disulfide system that affect the polymerization of glutenin subunit which improves the mechanical and rheological properties of dough. Oxidizing agents are usually added by milling and baking companies to modify the functional properties of the flour by promoting the occurrence of disulfide bond in gluten proteins (Fitchett and Frazier 1986). During mixing, oxidizing agent converts SH groups of the gluten protein to SS linkages between

the closest molecules resulting in a stronger gluten matrix, and thus a stronger dough (Demiralp et al. 2000).

Ascorbic acid is usually added in commercial flour as aging agent and additive blend in baking industry (Aamodt et al. 2003). L-threo-ascorbic acid (L-AA) is the strongest ascorbic acid stereoisomer that improves the strength, handling and baking properties of dough (Goesaert et al. 2005). During mixing, L-ascorbic acid is readily oxidized in the presence of air, copper or iron or enzymatic reactions by oxidases and changes into the form of L-dehydroascorbic acid (L-DHAA) (Carter and Page 1965). L-DHAA is the actual oxidizing improver (Every et al. 1999). L-DHAA is further oxidized into diketogulonic acid and other compounds (Carter and Page 1965). The effect of improving bread quality by L-AA is due to the removal of sulfhydryl (SH) groups in dough (Carter and Page 1965).The formation of disulfide bond that improves the loaf volume are believed to be produced from catalytic oxidation of sulphydryl group in dough by dehydroascorbate reductase (Tsen 1965). Another suggestion regarding the mechanism of L-AA is that the enzyme glutathione reductase (GSH-DH) is oxidized by L-DHAA and forms glutathione (GSSE) in the presence of protein thiols during mixing (Grosch and Wieser 1999). In another study on the effect of L-AA on wheat flour, it was proposed that the oxidation effect on DHA and O_2^- formation during AA oxidation promotes the SH-SS interchange reaction which promotes inter protein disulfide bonds through disulfide-thiols interchange reaction and improves the rheological properties of flour-water dough (Nakamura et al. 1997). The level of L-DHAA of wheat flour samples were measured using enzymatic assay and concluded that the amount of L-DHAA increased rapidly as L-AA was oxidized while mixing (Every et al. 1999). The addition

of ascorbic acid during gluten washing process also increased the stiffness of the gluten obtained (Kieffer et al. 1990). From previous study regarding the effect of ascorbic acid in gluten and baking by Ambardekar (2009), the addition of ascorbic acid at 100 to 150 ppm improved the baking performance in wheat flours and while reduction in baking performance was seen at 200 ppm ascorbic acid added. The mixing properties of wheat flours were negatively correlated to the baking properties and flour protein content with addition of ascorbic acid flours (Ambardekar 2009). As for gluten, addition of ascorbic acid increased the viscous component of gluten and it was closely associated with properties of rise in oven spring (Ambardekar 2009). Oven spring is rapid increase in volume during the first minute of baking, heat from the oven will cause the gas pockets to expand and cause a rapid stretch of the dough. In summary, addition of ascorbic acid during mixing causes oxidation of glutathione (GSH) which will promote the formation of disulfide bonds which improve the mixing properties, maximum resistance to extension and loaf height (Ambardekar 2009).

The treatment with DTT at 500 ppm to strong and weak gluten shows 60% decreased of elasticity on strong gluten and 42% decreased on weak gluten (Khatkar 2005). The effect of DTT addition to gluten and baking, results show that DTT decreased gluten elasticity which in turn affected the performance of baking (Ambardekar 2009). The addition of DTT lead to the reduction of gluten which resulted in decreased of the strength of gluten and affected the loaf properties in all flour tested (Ambardekar 2009).

Aggregation Measurements in Batters - Gluten Peak Tester

The gluten peak tester (GPT) (Brabender GmBh, Duisburg, Germany) is a prototype gluten quality testing instrument that works with liquid batters. This instrument

was developed for the purpose of analyzing the viscosity of flour batters used for wafer and pancake production. There is not an official method to determine the quality of batters. The GPT uses shear force to mix the ingredients (water, sugar and flour) uniformly and measures its consistency and the torque used during the mixing. The sodium chloride and sucrose used in the solution assist the formation of aggregation by forming ionic interactions to solubilize the protein. As the aggregation develops, the shear force will increase as well as the energy required for the mixer (Popper et al. 2006). The measurements are based on the torque required to form aggregation in the batter. In order of study the gluten network formed, five parameters are recorded; lift off time (LOT), peak maximum time (PMT), and maximum torque (MT), before maximum torque (T1) and after maximum torque (T2).

- PMT measures the overall time needed to form the aggregation between the flour protein and solution.
- MT measures the overall maximum torque needed in order to form aggregation.
- T1 measures the torque applied as the aggregation starts to occur between flour and solution, and
- T2 measures the torque applied after the optimum aggregation occurs.

The formation of aggregation between wheat flour protein and other flour constituents shows the quality of the gluten network formed (Popper et al. 2006). The time needed to form aggregation also indicates the water absorption capability of the flour. When the time needed to form aggregation is short (≥ 80s), the flour will have high water absorption capability and better gluten quality (Popper et al. 2006). In contrast, if a

sample required longer aggregation time (> 300s), the flour will have lower water absorption (Popper et al. 2006). Samples that do not have aggregation or late aggregation (> 400s with whole meal flour or 700s with extracted flour) are associated with very low water absorption, low protein and wet gluten content and very poor quality gluten (firm, short or crumbly) (Popper et al. 2006). In addition, the greater the aggregation area the stronger the gluten network formed.

Principal Component Analysis (PCA)

PCA is an indirect ordination method which multivariate data can be evaluated visually in a two dimensional PCA correlation biplot (Everitt 1978). In the correlation biplot, PCA shows direction of the maximum variables which are called principal components (Jackson 1991; Krazanowski 1988). PCA provides the separation of the variance input in order to achieve interesting information from the noise so that hidden information can be obtained from the data (Cocch et al. 2005). In addition, PCA could analyze a variety of samples at the same time (Cocch et al. 2005). The variables with high correlation will have sharp angle between the vectors (Valiranta and Weckstrom 2007). Variables with the greatest variance will project on principle component 1 (PC1) and set of uncorrelated variables will project on principle component 2 (PC2) (Ambardekar 2009). Data input is untransformed, centered and standardized in order to minimize the mean squared (Ambardekar 2009).

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CHAPTER III

EFFECT OF CHANGING OF SURFACE TENSION BY DIACETYL TARTARIC ACID ESTER OF MONOGLYCERIDE (DATEM) ON GLUTEN AGGREGATION, VISCOELASTIC, MIXING AND BAKING PROPERTIES IN BATTERS

Abstract

Aggregation of protein is an important phenomenon in many wheat products. The control of protein aggregation may lead to significant savings and improvement of wheat production of batter systems. The systematic study of key additives can reveal the mechanism of wheat protein aggregation. The objective of this study was to investigate the effect of changes in surface tension by the addition of diacetyl tartaric acid ester of monoglycerides (DATEM) on the aggregation in a batter system. Six commercial flours differing in protein quantity and quality with four DATEM levels (0, 0.3, 0.6 and 1% flour basis) were studied. Aggregation properties were studied with a high sheer apparatus (Gluten Peak Tester (GPT)) at 3,333 rpm, at 35^{o}C and the mixing time was 10 minutes. Aggregation was compared to the amount of protein in the flour, rheological and baking properties describing the comparison of quality parameters. Biplots of principal component analysis (PCA) of agglomeration and flour protein explained 72.2% total variance. In comparison agglomeration, viscoelasticity, mixing and baking properties as well as flour protein explained 56.3% total variance. The first component axis explained

41.6% of variance and dominated by recoverability of gluten and specific volume which explained 97.7 and 93.2% of the variance, respectively. The second component axis explained 14.8% and was dominated by the area under the peak by contributing with 73.8% of the variance. The decrease in surface tension in flour at 43% flour caused an increase in agglomeration work or peak area. Work also was the highest contributor explaining 90.8% of the variance to PC1. Agglomeration strength within all the control samples were negatively associated with 3A (13.7% flour protein) with high agglomeration peak work.

Keywords: DATEM, surface tension, gluten agglomeration test, emulsifier and principal component analysis (PCA).

1. Introduction

Emulsifiers are active-surface compounds that have a chemical structure with both hydrophobic and hydrophilic moieties (Gómez et al. 2004). The presence of both lipophilic and hydrophilic properties allows the emulsifier to reduce the surface tension between two immiscible phases which facilitates emulsion to occur (Gómez et al. 2004; Stampfli and Nersten 1995). The addition of emulsifier reduces the surface tension in gas bubbles by interacting with lipids and creating a large amount of small bubbles (Selomulyo and Zhou 2007). The ability of ionization of emulsifiers is based on their charge characteristics in aqueous environment; therefore emulsifiers can be classified as ionic or nonionic types depending on the hydrophilic groups (Stampfli and Nersten 1995). Nonionic emulsifier such as sucrose esters of fatty acids and ethoxylated mono-diglyceride do not ionize in water and they show good dough strengthening properties (Stampfli and Nersten 1995). Ionic emulsifier can be in the form of anionic (organic acids and their salts) or cationic (base-amines of different degree of replacement) emulsifier. Anionic emulsifiers such as DATEM (Xiujin et al. 2007) are used for different baking purposes and cationic emulsifiers are not used in foods (Stampfli and Nersten 1995). Amphoteric emulsifiers have both anionic and cationic groups and the surface-active properties are based on their pH levels (Stampfli and Nersten 1995). The amphoteric characteristic allows the formation of complexes between starch and protein (Gómez et al. 2004). The presence of different types of emulsifying agents will influence the mechanism by which the formation of the dough system differently.

Surfactants are used as dough strengtheners to improve dough properties and quality of bread as well as dough strength, rate of hydration, tolerance to mixing, crumb

strength, slicing characteristic, shortening reduction, loaf volume and shelf life (Stampfli and Nersten 1995). However, the specific mechanism of surfactant in dough is not fully understood but it is believed that good dough strengtheners are capable to create liquid films and lamella structures between the interface of gluten strand and starch (Stampfli and Nersten 1995). Selomulyo and Zhou (2007) and Gómez and others (2004) explained that the presence of emulsifiers will form complexes with gluten proteins and protein-protein aggregates that improve the strength of gluten matrix resulting in increased dough height during proofing.

Diacetyl tartaric acid ester of monoglycerides (DATEM) is an anionic oil-in-water emulsifier (Aamodt et al. 2003). This emulsifieris made up from the reaction of mono- and diacetyltartaric acid anhydride with monoacylglycerols or the combination of mono- and diacylglycerols (Kohler and Grosch 1999). It is considered the most common and best dough stabilizer (Stampfli and Nersten 1995). This class of emulsifiers is believed to increase the resistance and decrease the extensibility of dough (Ravi et al. 1999; Stampfli and Nersten 1995). DATEM is suggested to promote aggregation of gluten protein in dough by binding to the protein hydrophobic surface (Selomulyo and Zhou 2007). The improved aggregation of protein network will result in better texture and increased volume of produced bread (Selomulyo and Zhou 2007).

Based on a previous study on the effect of DATEM on gluten and baking, DATEM improved the baking properties of high protein content flour and viscoelastic properties of gluten of low protein content flour (Aamodt et al. 2003). Other studies suggested that the presence of DATEM allowed baked pan bread to increase in volume and improve the crumb structure (Ravi et al. 1999). The quality of the pan bread

improved when 0.6% DATEM was treated to the sample but as the level increased to 1.0% DATEM, the quality of the bread reduced (Ambardekar 2009). The addition of DATEM is believed to assist the interaction of lipid with protein and starch, which will increase the loaf volume by improving the gas retention capability in dough system (Jacobsberg et al. 1976). Addition of DATEM also improved the mixing tolerance of dough, gas retention and resistance of the dough collapse (Selomulyo and Zhou 2007; Stampfli et al. 1996). In addition, Köhler (2001) also reported that optimum effect of DATEM in baking test was achieved at concentration of 2 g additives/kg, improvement was seen in rheological properties as the concentration of DATEM increased. However, the optimum concentration failed to detect from the range of concentration that was applied (1-5 g additives/kg) and may suggest that a higher DATEM concentration range is required in order to see the optimum effect. Ambardekar (2009) reported that flour protein content was highly correlated with mixing and baking properties. This was seen when the strength of weak gluten improved due to DATEM addition and the viscoelastic properties of flour with different protein content also improved as higher concentration of this surfactant were present (Ambardekar 2009). Ambardekar (2009) also described that the loaf volume of bread increased with 0.6% DATEM concentration. It was suggested that the addition of DATEM could modify the structure of gluten by improving the gluten quality and the overall baking properties in wheat flours (Ambardekar 2009).

The objective of this study was 1) to analyze the effect of reducing surface tension with the addition of DATEM on the aggregation of batters and 2) to determine a possible correlation between gluten aggregation and flour protein, viscoelasticity of gluten, mixing and baking properties.

2. Materials and Methods

Six commercial hard red winter wheat flours were analyzed in this study. The samples were obtained from two different regional suppliers. The wheat flours contained different cultivars in order to obtain the different ranges of protein content and quality. The samples were labeled as A and B and numbered 1 through 3, in order of increasing protein content, with 1 being the lowest. Flour protein, moisture and ash were obtained by infrared analysis using an NIR system model 6500-M (FOSS NIR Systems Inc, Laurel, MD).

Four concentrations of DATEM (Caravan Ingredients, Lenexa, KS) were used. DATEM was added to the flour at 0, 0.3, 0.6 and 1.0% w/w flour basis as solution. Five mL of DATEM solution (0.6, 1.2 and 2 g DATEM in 100 ml of 2% sodium chloride and 5% sucrose solution) was prepared. The solution with DATEM was heated to 65^{o}C for proper dispersion. Samples with no DATEM, containing 2% sodium chloride and 5% sucrose solution were used as controls. Batters with percent flour ranging from 41 to 51% were systematically analyzed for aggregation patterns with each of the four DATEM concentrations.

2.1. Gluten Agglomeration Test (Gluten Peak Tester)

Hard wheat flour samples with different levels of DATEM (0, 0.3, 0.6 and 1.0% (w/w flour basis)) were analyzed with a high shear mixing apparatus called Gluten Peak Tester (GPT, C.W. Brabender Instruments, Hackensack, NJ). A total weight of 18.5g of wheat flour sample and solution was used for each replicate and the amount of flour in the solution used was reported as percentage (as is basis). The samples were mixed in a

stainless steel cup at 3,333 rpm at 35^{o}C for 10 minutes. The GPT parameters recorded were peak maximum time (PMT), maximum torque (MT), torque before maximum time (T1), and torque after maximum time (T2), and area under peak (AREA, equivalent to work). The peak maximum time is recorded in seconds (s) and torque parameters are recorded in Brabender Equivalent (BE) which is an arbitrary unit used by C.W. Brabender Instruments Inc. All analyses were completed in duplicates.

2.2.Gluten Creep Recovery Analysis

Creep recovery tests were performed by Ambardekar (2009) based on the protocols of Zhao and others (2007) and Liang and others (2007). The gluten extraction was prepared in an automated gluten washer, Glutomatic 2200 (Perten Instruments AB, Segeltorp, Sweden) using approved method 38-12.02 (AACCI 2000). Ten grams of flour was wetted with 5mL of DATEM solution (0.6, 1.2 and 2 g DATEM in 100 ml of 2% sodium chloride) before mixing and washing in Glutomatic for 10 minutes. The gluten extracted from the Glutomatic was gently shaped into gluten ball and relaxed under metal plates (2500 g) for 60 minutes at room temperature (25^{o}C) before the creep recovery measurement.

The gluten prepared was measured by a Rheometer AR1000N (TA Instruments, New castle, DE), and the gap was set at 25 mm at room temperature (25^{o}C). 40 Pa of constant stress was applied in order to shear the gluten and it is maintained at constant stress for 100 s creep test and released to recover for 1000 s. The deformation and recovery of the gluten was measured as compliance. Creep-recovery parameters obtained were: separation time (SeP), delta compliance (J-Jr), % recoverability (RCY), time

constant of creep (λ_C), and time constant of recovery (λ_R). J-Jr and λ_C represents the viscous properties of gluten and SeP, RCY, and λ_R represents the elastic behavior of gluten. All analyses were completed in triplicates.

2.3. Dough Mixing

The dough mixing properties were performed by Ambardekar (2009) according to approved method 54-21.01 (AACCI 2000). Wheat flour samples were analyzed using a 10 g bowl Farinograph-E (C.W. Brabender Instruments, Hackensack, NJ) at 63 rpm at 30^{o}C. Dough development time (DT), stability time (ST), breaking time (BT), and water absorption (WA) adjusted to 14% moisture basis were recorded. DATEM solution (3, 6, 10 g per 100 ml deionized water) is heated at 65^{o}C for proper dispersion. One milliliter of DATEM solution is added to 10 g of wheat flour samples before additional water is added for mixing and hydration. The control samples were prepared with only deionized water. All analyses were completed in duplicates.

2.4.Baking Test

Baking tests were performed by Ambardekar (2009) based on approved method 10-10.03 (AACCI 2000) which is a straight-dough bread making method. Wheat flour samples (100 g) were added with 0.3, 0.6, and 1 g DATEM prepared with 3 g of melted shortening. Samples were mixed to dough with a 100-g mixer Swanson-Working pin type (National Mfg. Co. TMCO Inc, Lincoln, NE). Several mixing and baking tests were performed in order to obtain the optimum mixing time and bake water absorption. Bread quality parameters like dough proof height (PH), loaf height (LH), loaf volume (LV), oven spring (OSP) and specific volume (SV) were measured. The proof height (PH) and

loaf height (LH) of bread were measured with a digital proof height gauge (National Mfg. Co. TMCO Inc, Lincoln, NE). The LV was obtained by rapeseed displacement after the bread was cooled for 10 minutes after taking out from the oven and OSP was calculated by subtracting PH from LH. Specific volume SV was defined as the ratio of loaf volume to loaf weight. All analyses were completed in duplicates.

2.5.Statistical Analysis

The relationship of DATEM, flour types (protein content), the aggregation properties (PMT, MT, T1, T2, Peak Area), viscoelastic variables (J-Jr, SeP, RCY, λ_C, and λ_R), mixing properties (WA, DT, ST and BT), and baking characteristics (LV, PH, LH, OSP, SV) were evaluated with Principal Component Analysis (PCA) using Canoco for Windows version 4.5 (Biometris, Plant Research International, Wageningen, The Netherlands). Data input is untransformed, centered and standardized in order to minimize the mean squared error.

3. Results and Discussion

General composition analysis (protein, moisture and ash content) of the six commercial hard red winter wheat flours previously reported by Ambardekar (2009) are shown on Table 1. The range of protein is representative of hard red winter wheat flour in the Southern Plains region. An example of the recorded curves and representation of the parameters obtained from the gluten peak tester (GPT) is shown on Figure 1. Also, an example of gluten aggregation curves from a sample differing in protein content is shown on Figure 2. An expanded definition of the parameters and their units are presented in Table 2.

3.1.Gluten Agglomeration Test (GPT)

Samples were systematically analyzed from 41 to 51% flour in order to find the concentration of flour at which all or most flours will form agglomeration. Tables 3, 4, 5, and 6 have a summary of the agglomeration results. Agglomeration obtained with different flour percentages cannot be compared directly but the trends of the aggregation describes the effects of protein quality and quantity in the presence and absence of DATEM. For example, 1A sample with 8.0% protein does not aggregate until the flour reaches 49% (Table 3). In comparison 3A flour with 13.7% protein aggregated at 41% flour, the lowest percentage of the control groups. The concentrations of DATEM used in this study and water slurry were based on Ambardekar (2009) who studied the viscoelasticity of gluten and breadmaking properties. The concentration is within the range reported by Kohler and Grosch (1999) who used 0-0.5% DATEM in studying the gluten rheology and baking and Stampfli et al (1996) who reported 1-2% DATEM concentration in gluten using extensograph. Xiujin et al (2007) proposed that at 0.1% DATEM will give the optimal effect on the Chinese steam bun by significantly improving the skin color, skin structure, inner structure and total score of Chinese steam bun. Samples with 43% flour were chosen because they had the highest total explained variance in principal component analysis (PCA) and were the percentage that allowed more samples to obtain aggregation.

The presence of DATEM in the batter system improved the ranges that allow the samples to aggregate. For example, control 1A only aggregated at 49 to 51% flour. However, addition of DATEM allowed the sample to aggregate even at 46% flour. A similar trend can be seen in other samples with and without DATEM (Table 3, 4, 5, and

6). DATEM promotes aggregation of flour protein by forming hydrogen bridges with amidic groups from the gluten proteins (Gaupp and Adams 2007). Anionic emulsifiers connect the hydrophobic emulsifier moieties with non-polar side chains of the proteins (i.e. ethylene chains) by forming intermolecular matrix through hydrogen bridges (Greene 1976).

The gluten aggregation test (GPT) apparatus can measure the gluten strength (MT) and gluten aggregation time (PMT). In principle, PMT is similar to the dough development time with the Farinograph (Huschka et al. 2011) but the systems are totally different, i.e., batter and dough. PMT of sample 3A was the lowest (2.1 times lower compared to the average PMT) among samples (Fig. 3). DATEM levels appear to decrease PMT drastically for the majority samples (4 times lower compared to the controls) except sample 3A (Fig. 3). Peak maximum time differences may be due to protein quality and possible differences in surface tension regions of the proteins. Such differences may affect the interaction of DATEM with the hydrophobic and hydrophilic sites. The delay of gluten aggregation may be influenced by the compounds that tie the water in the system. If the water is strongly bounded for example to arabinoxyloses and damaged starch, it will require more time and energy to free the water for gluten aggregation (Huschka et al. 2011). This shows that DATEM reduces the surface tension of different phases therefore reduces the time.

MT is a measure of the gluten strength in the system. Sample 2A showed a trend to decrease at 1% DATEM concentration (Fig, 4). The gluten strength of 3B also showed a trend to decrease at 0.3% DATEM but increased as the level of DATEM increased (Fig, 4). As for samples 1B and 2B, these samples do not show any trend of changes (Fig. 4).

Sample 3A showed a drastic decrease in strength when DATEM is present in the system (Fig.4). This clearly shows the reducing of surface tension between the starch, fat and protein phases of flour by DATEM and therefore reduces the strength of the gluten network. This also suggests that the effect of reducing surface tension is dependent on the protein quality of the samples. Salehifar et al (2010) reported that higher quality and quantity of protein wheat flour will increase the dough resistance to mixing which is also a measure of dough strength. In a batter system with more water than in a dough system, differences of strength may not be clearly observed compared to mixing dough with water content at about 50%.

The area under the peak (AREA) is an indicator of the amount of work required for the batter to mix to its optimum. The contribution of explained variance for this parameter is the highest in PC1 and PC2 compared to the other gluten agglomeration parameters (Table 7 and 8) (90.5% and 73.5%, respectively). As DATEM is added to the batter system, the area under the peak increased as much as 7.2 times for 2B (Fig. 5). Lang et al. (1992) reported that increased of area under the curve can be due to the longer mixing times. However, there were no significant changes for peak maximum time as a function of DATEM addition. For example the area under the aggregation peak is illustrated in Figure 5. A reduction of surface tension as a result of the increasing levels of DATEM showed an increase in peak area compared to the control (Fig. 5). This means that the addition of DATEM in the system also promoted stability of the interaction of lipid with protein and starch. The addition of DATEM is believed to assist the interaction of lipid with protein and starch, which will increase the loaf volume of bread by improving the gas retention capability in dough system (Jacobsberg et al. 1976). In

addition, Zhou (2007) and Gómez and others (2004) also reported that the presence of emulsifiers will form complexes with gluten proteins and protein-protein aggregates that will improve the strength of gluten matrix resulting in increased of dough height during proofing. The binding of surfactant to gluten may also change the arrangement of polymer chain and affects the crosslinking in the batter system through hydrophobic interactions and electrostatic bonds (Toufeili and Kokini 2004). This means that when the batter system is organized, there will be more physical entanglements of the polymers in the system. Thus, more work is required for gluten aggregation.

3.2.Principal Component Analysis (PCA)

Principal component analysis (PCA) is a mathematical multivariate method used to find a linear combination of the variables which account the total variance by reducing the dimensionality of the data and still containing most of the variation in the data set (Jolliffe 2002; Mukhopadhyay 2009). The reduction is done by showing the direction (principal component) where the highest variation of data is achieved (Ringner 2008). The variables are further represented by the number of principal components instead of the actual value from the original number of variables (Ringner 2008). Samples are plotted to tell the relationships if there are similarities or differences among the samples and also to determine whether they can be grouped (Ringner 2008).

PCA of gluten aggregation and flour protein resulted in an ordination plot which captured 72% explained variance with PC1 and PC2 explaining 48 and 24%, respectively (Table 7, Fig. 6). PC1 was highly dominated by area under the peak contributing with 91% of the variance (Table 7, Fig. 6). PC2 was dominated by MT, and torque before

aggregation contributing with 45 and 44% of the variance, respectively (Table 7, Fig. 6). The control samples were well separated from the rest of the samples and were related to peak maximum time (PMT). The rest of the samples were related mainly to area under peak (area) or work, torque before aggregation (T1) and torque after aggregation (T2) (Fig. 6).

In order to further understand the relationship of GPT parameters with baking, viscoelastic and mixing properties plus protein content, PCA ordination was used to analyze the samples containing DATEM (0, 0.3, 0.6 and 1.0%). Overall, higher total explained variance was observed from samples with 43% flour compared to samples with 41, 46, 49 and 51% flour (data not shown). The two dimensional correlation biplots of 43% flour is presented in Figure 7. The total explained variance achieved for PC1 and PC2 was 56% according to the contribution of the explained variance by each variable (Table 8). The PC1 explained 42% of the total variance and principal component axis 2 (PC2) explained 15% of total variance. The third and forth principal components were not further discussed because of their low percent explained variance. The RCY had a slightly longer vector compared to other variables (Fig. 7) and contributed to the highest explained variance (98%) on the PC1 (Table 8). Other variables that show high contribution to the variance in PC1 were SV, WA, λ_C and J-Jr (93, 86, and 80%, respectively). PC1 showed high correlation to all the variables (especially RCY, WA and LH) except for gluten aggregation test variables which highly correlated to PC2 (Fig. 7). J-Jr, and λ_C (viscoelastic properties), BT and DT (mixing properties), SV (baking properties) and flour protein are related among themselves (right quadrant) and are negatively associated to RCY, WA, and LH. They are in opposite sides (180^o) on PC1.

LV and PH were also related to RCY, WA and LH (Fig. 7, Table 8). T1, T2 and Area (GPT) were positively correlated to PC2 and negatively correlated to PMT and strength. GPT properties are independent to viscoelastic, mixing and baking properties are highly associated with PC2. PC2 is highly influenced by area under peak which explained 74% of the variance. In contrast, LH showed the lowest explained variance on PC2. Control samples were highly associated among each other and were highly related to PMT and strength variables and were negatively correlated to the majority of the samples with reduced surface tension. Sample 2A with 0.3% concentration of DATEM was separated from other samples with the same treatment. The sample was separated to the bottom right of the quadrant and was related to the rate of recovery of gluten (TCR). In dough, DATEM will bind with gluten protein by hydrophobic interactions. The alkyl residues with unpolar side-chains of DATEM will bind to the unpolar side chain amino acid of the gluten and promote aggregation of protein (Greene 1976; Ribotta et al. 2004). Also, high molecular weight (HMW) glutenin is associated with dough strength. LMW-glutenin may also contribute to the pattern of aggregation achieved in this study because the LMW-glutenin has been suggested to also be related to strength and viscoelasticity of the gluten in the flour sample (Edwards et al. 1999).

4. Conclusion

Changing of the surface tension on batter systems significantly increased agglomeration peak area (work) and reduced PMT while the effect on strength varied. . The decrease in the surface tension of batter increased the work needed for protein aggregations and suggests that the core regions of the protein were exposed and actively participate in gluten aggregation. PCA of agglomeration properties and protein content

revealed that control samples were negatively associated with the samples with reduced surface tension and they were associated with peak maximum time (PMT) and strength. Peak area of agglomeration had the highest contribution of the separation of samples (90.8% explained variance). The control and treated samples were clearly separated by the reduction of surface tension. When agglomeration, rheological, mixing and baking properties were analyzed by PCA, RCY contributed the most to PC1 but negatively correlated with SV. Agglomeration peak area is the highest contributor on PC2 and all the control samples were negatively correlated to agglomeration peak area. A decrease in surface tension causes an increase in agglomeration area peak. This suggests that a decrease in surface tension by DATEM influenced the aggregation properties and modify the stability of the interactions between gluten and starch in a batter system.

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

Flours	Protein (%)[a]	Moisture (%)	Ash (%)[a]
1A	8.0 ± 0.05	11.7 ± 0.02	0.29 ± 0.01
2A	11.2 ± 0.07	10.5 ± 0.03	0.38 ± 0.01
3A	13.7 ± 0.02	10.1 ± 0.02	0.41 ± 0.00
1B	10.4 ± 0.10	12.5 ± 0.02	0.47 ± 0.00
2B	10.6 ± 0.07	12.6 ± 0.00	0.48 ± 0.01
3B	11.4 ± 0.01	13.0 ± 0.04	0.58 ± 0.01

SD = Standard deviation.

[a] Protein and ash as is basis.

Ambardekar (2009).

Table 2. Definition of gluten agglomeration test, viscoelastic, mixing and baking properties terms

Variables	Abbreviations	Definitions	Units
Gluten Agglomeration Test	PMT	Peak maximum time defined as the time needed for aggregation to occur.	s
	MT	Maximum torque defined as the torque required when the peak of aggregation occurs. MT is also the measure of gluten strength.	BE
	T1	Torque before aggregation defined as the torque required before peak of aggregation occurs.	BE
	T2	Torque after aggregation defined as the torque required after peak of aggregation occurs.	BE
	AREA	Area under the curve is the total work of agglomeration.	BE.s
Mixing	WA	Water absorption	%
	DT	Time required for the flour to develop into dough	min
	ST	Time for developed dough to remain stable during mixing	min
	BT	Time the dough start to breakdown after mixing	min
Viscoelastic	SeP	Separation time defined as the time at which the creep and recovery split and no longer stay superimposed	s
	J-Jr	Delta compliance defined as the difference in compliance of creep and recovery at 100s.	Pa^{-1}
	RCY	Percent recoverability defined as the elastic ability of gluten recover to original state as stress is removed	%
	λ_R	Time constant of creep defined as the rate of elastic recovery of gluten to reach equilibrium	s
	λ_C	Time constant of recovery defined as the rate of deformation of gluten	s

Table 2. Continued

Variables	Abbreviations	Definitions	Units
Baking	LV	Loaf volume	cm^3
	LH	Loaf height	mm
	PH	Proof height	mm
	OSP	Oven spring	mm
	SV	Specific volume of baked loaves	cm^3/g
Flour protein	FP	Flour protein	%

Note: BE, Brabender Equivalent, arbitrary unit (not SI).

Table 3. Protein aggregation for flour batter systems with different percentages without DATEM (0%, control). Flour protein (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4.

0 % DTM	**Type of flour**					
% Flour	1A	2A	3A	1B	2B	3B
41	-	-	+	-	-	-
43	-	+	+	+	+	+
46	-	+	-	+	+	+
49	+	+	-	+	+	+
51	+	-	-	-	-	-

Table 4. Protein aggregation for flour batter systems with different flour percentages with 0.3% DATEM. Flour protein (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4.

0.3 % DTM	**Type of flour**					
% Flour	1A	2A	3A	1B	2B	3B
41	-	-	+	+	-	+
43	-	+	+	+	+	+
46	+	+	-	+	+	+
49	+	-	-	+	+	+
51	+	-	-	-	-	-

+ and – means aggregation and no aggregation, respectively.

Table 5. Protein aggregation for flour batter systems with different flour percentages treated with 0.6% DATEM. Flour protein (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4.

0.6 % DTM	**Type of flour**					
% Flour	1A	2A	3A	1B	2B	3B
41	-	-	+	-	-	-
43	-	+	+	+	+	+
46	+	+	-	+	+	+
49	+	-	-	+	+	+
51	+	-	-	-	-	-

Table 6. Protein aggregation for flour batter systems with different flour percentages with 1.0% DATEM. Flour protein (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4.

1.0 % DTM	**Type of flour**					
% Flour	1A	2A	3A	1B	2B	3B
41	-	-	+	-	-	-
43	-	+	+	+	+	+
46	+	+	+	+	+	+
49	+	-	-	+	+	+
51	+	-	-	-	-	-

+ and – means aggregation and no aggregation, respectively.

Table 7. Explained variance (%) in PCA of agglomeration and flour protein from commercial flour treated with DATEM.

	PC1	**PC2**	**PC1 + 2**
	48	24	72
PMT	64	15	79
MT	46	45	91
T1	40	44	83
T2	45	32	77
Area	91	5	96
FP	1	7	8

Note: Abbreviations are listed on Table 2.

Table 8. Explained variance (%) in PCA of agglomeration, viscoelastic, mixing, and baking properties as well as flour protein from commercial flour treated with DATEM.

Variables		**PC1**	**PC2**	**PC1+2**
	PC	42	15	56
Agglomeration	PMT	3	54	57
	MT	1	53	54
	T1	1	39	40
	T2	0	30	30
	Area	1	74	75
Viscoelastic	SeP	0	0	0
	J-Jr	80	1	81
	RCY	98	0	98
	λ_R	56	5	62
	λ_C	83	1	84
Mixing	WA	86	1	86
	DT	63	0	63
	ST	16	1	16
	BT	56	7	63
Baking	PH	41	11	53
	LH	82	0	82
	SV	93	1	94
	OSP	3	5	9
	LV	38	3	41
	FP	30	8	38

Note: Abbreviations are listed on Table 2.

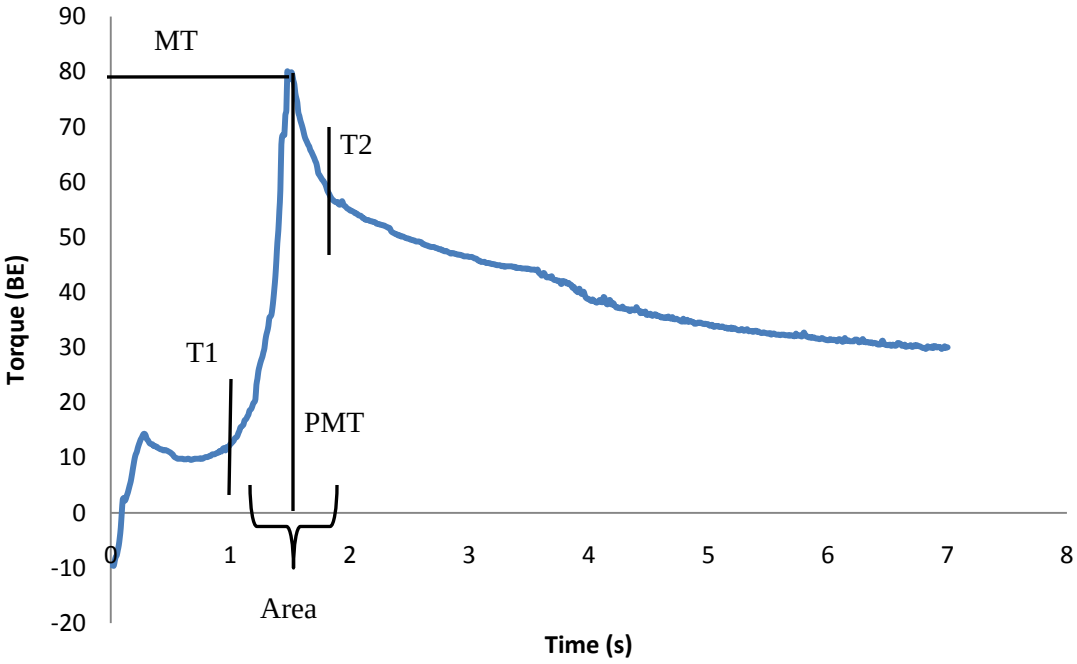

Figure 1. Example of a gluten aggregation test curve obtained with a high speed (3,333 rpm) mixer (Gluten Peak Tester) at 35^{o}C. Parameters recorded were maximum torque (MT), torque before and after peak (T1 and T2, respectively), peak maximum time (PMT) and peak area.

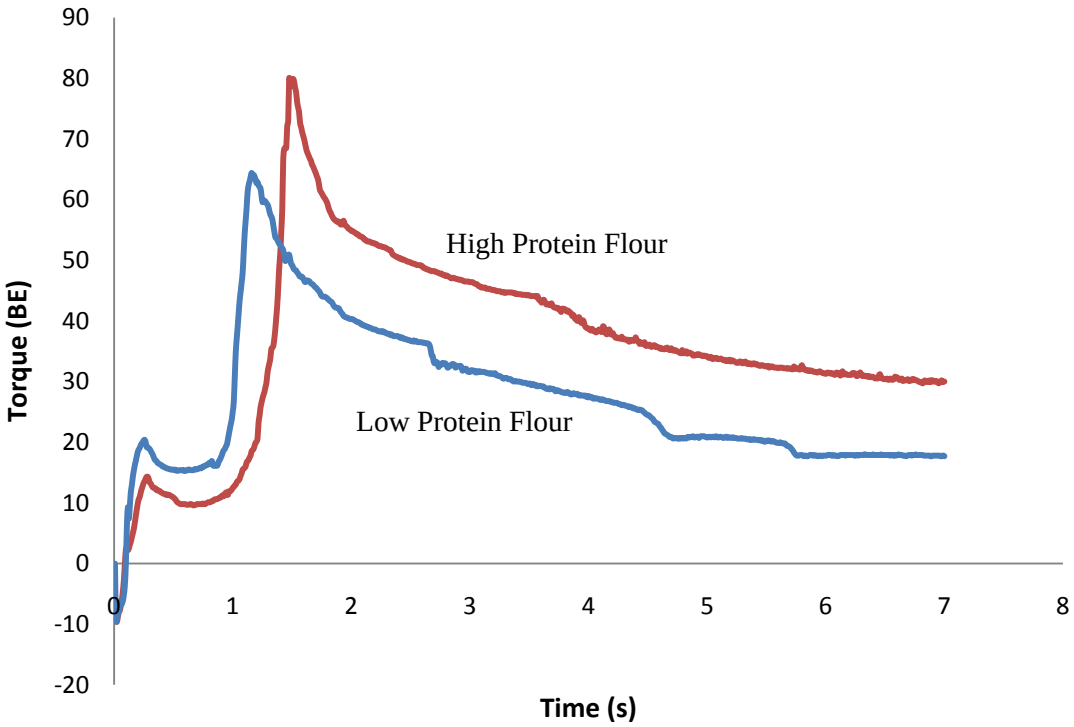

Figure 2. Example of gluten aggregation curves of commercial flours obtained with a high speed (3,333 rpm) mixer (Gluten Peak Tester) at 35^{o}C. High protein flour (A3 = 13.7%) and low protein flour (A1 = 8.0%).

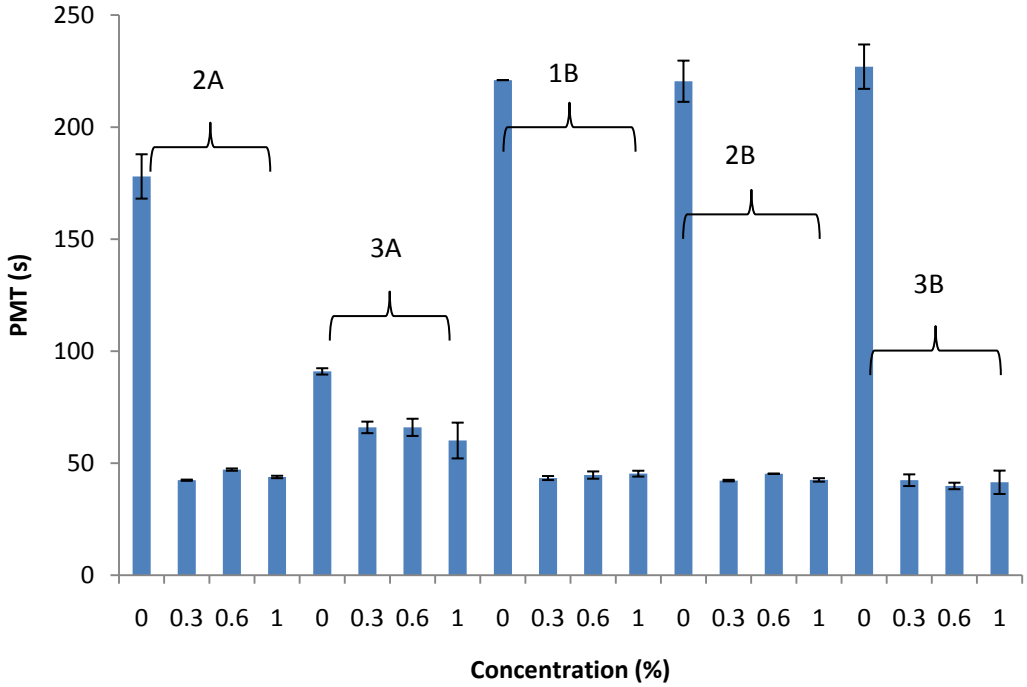

Figure 3. Peak maximum time (PMT) of gluten aggregation test (43% flour) for commercial wheat flours containing four DATEM levels (0, 0.3, 0.6, and 1.0%). Overall A1 did not aggregate. Means (n=2) and standard deviation bar. Flour protein content (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4%.

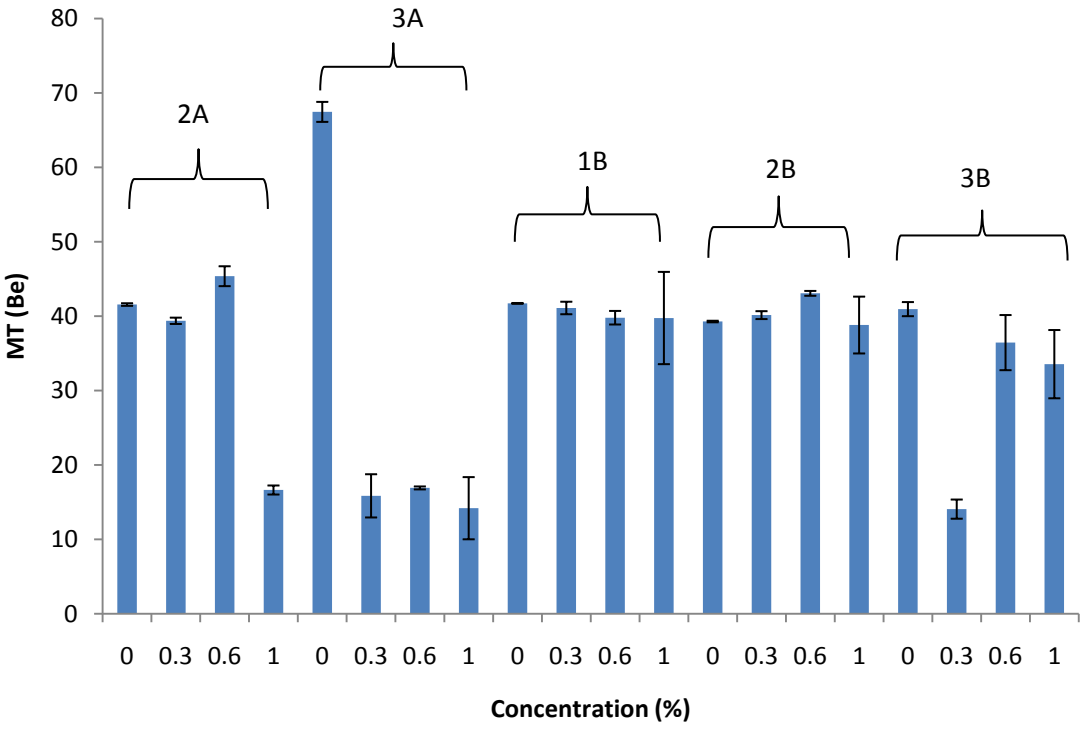

Figure 4. Maximum torque of gluten aggregation test (43% flour) for commercial wheat flours containing four DATEM levels (0, 0.3, 0.6, and 1.0%). Overall A1 did not aggregate. Means (n=2) and standard deviation bar. Flour protein content (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4%.

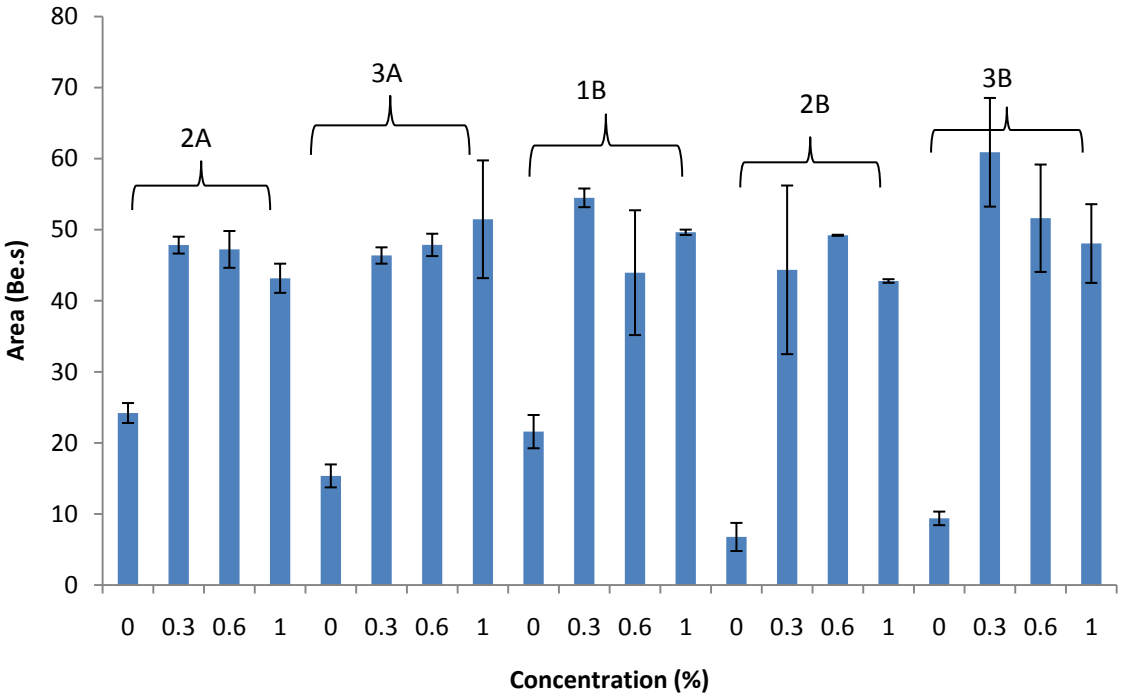

Figure 5. Area under the peak of gluten aggregation test (43% flour) for commercial wheat flours containing four DATEM levels (0, 0.3, 0.6, and 1.0%). Overall A1 did not aggregate. Means (n=2) and standard deviation bar. Flour protein content (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4%.

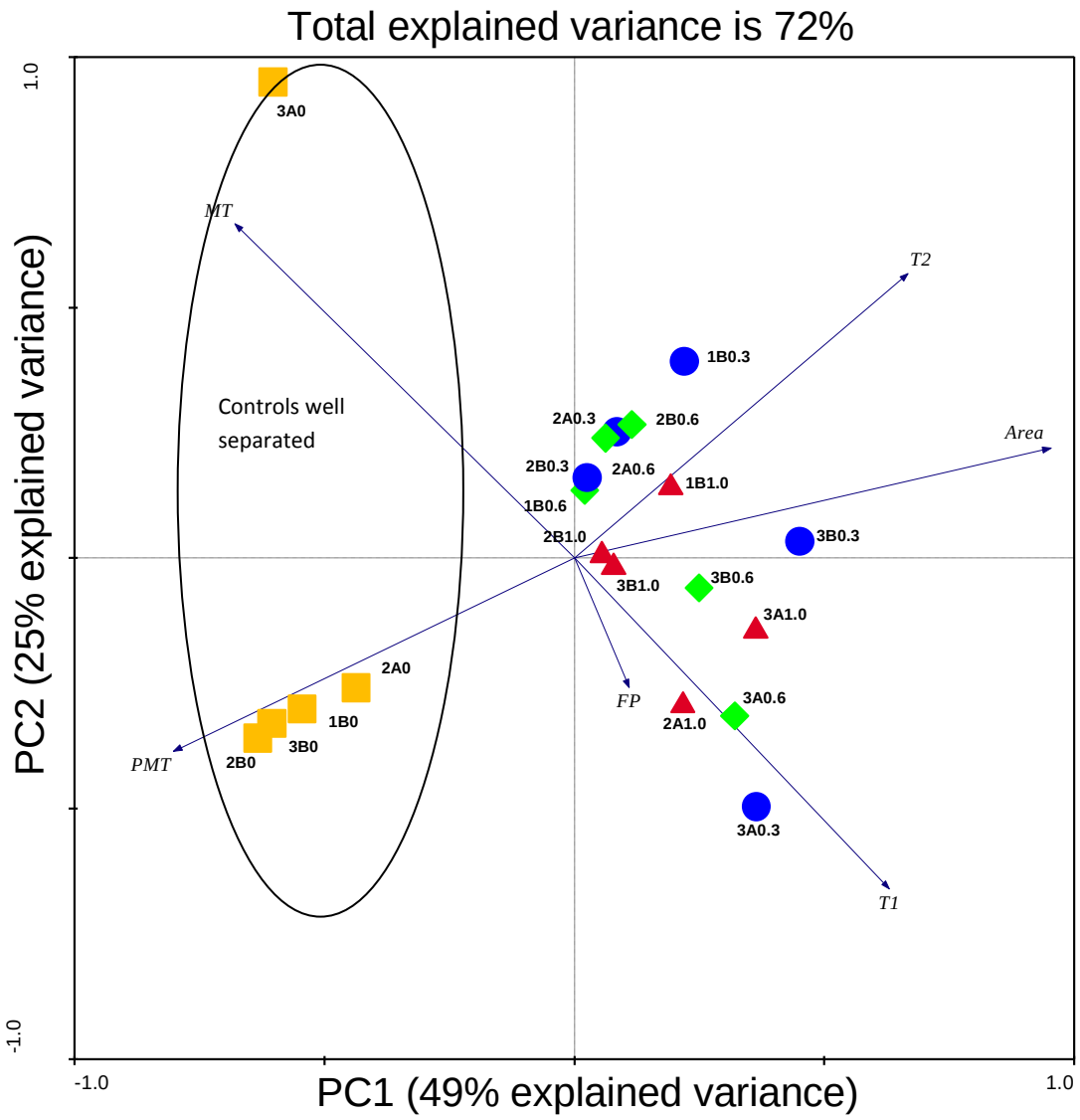

Figure 6. Loading plot of the first two principal components based on gluten agglomeration test properties (GPT) and protein content of six commercial wheat flours containing DATEM (0, 0.3, 0.6, 1.0%). Flour protein content (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4%. ■, ●, ♦, and ▲ represent 0, 0.3, 0.6, 1.0% DATEM, respectively. Abbreviations are listed on Table 2

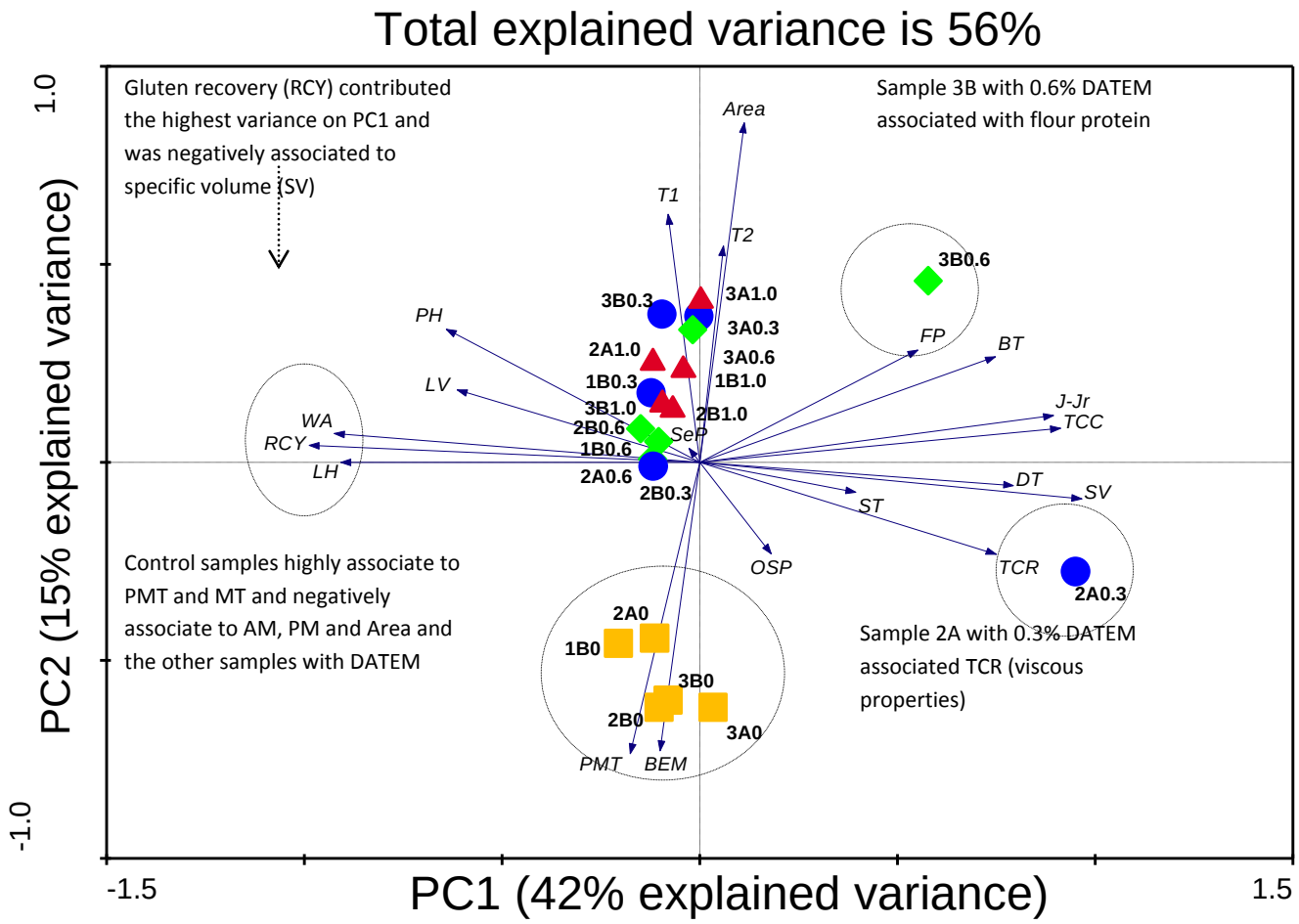

Figure 7. Loading plot of the first two principal components based on gluten agglomeration test, mixing, viscoelastic and baking properties as well as protein content of six commercial wheat flours, containing (0, 0.3, 0.6 and 1.0%) DATEM. ■, ●, ♦, and ▲ represent 0, 0.3, 0.6, 1.0% DATEM, respectively. Flour protein content (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4%. TCC and TCR are listed as λ_R and λ_C.. Abbreviations are listed on Table 2.

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CHAPTER IV

OXIDATION EFFECT OF ASCORBIC ACID ON GLUTEN AGGREGATION, VISCOELASTIC, MIXING AND BAKING PROPERTIES OF BATTER SYSTEM

Abstract

Oxidation of flour in the baking industry is achieved by the addition of ascorbic acid. In this study the effect of oxidation was evaluated on the aggregation properties of dough batters with the addition of ascorbic acid. Six commercial flours with differing protein quantity and quality were treated with five levels of ascorbic acid (0, 50, 100, 150 and 200 ppm) in solution containing 2% sodium chloride and 5% sucrose. Aggregation properties were studied with a high sheer apparatus (Gluten Peak Tester (GPT)) at 3,333 rpm, 35^{o}C and mixing time 10 minutes. The properties measured were strength, time and work of aggregation produced in batters with 43% flour. Agglomeration peak area is increased with 50 ppm of ascorbic acid compared to the control and then decreased steadily as the oxidation level increased. The aggregation parameters were correlated with flour protein, viscoelastic (creep recovery), mixing, and baking properties using principal component analysis (PCA). Agglomeration properties and flour protein explained 89.4% of the total variance. The total explained variance of agglomeration and flour protein was higher compared to the total explained variance of overall variables (flour protein, agglomeration, viscoelastic, mixing and baking properties) (62.5%). The PC1 of overall variables explained 44.3% of variance and showed high contribution by mixing properties such as water absorption (WA), development time (DT) and breaking time (BT). Maximum torque (MT) and torque after aggregation (T2) (gluten

agglomeration test) also highly contribute to PC1. Oven spring (OSP) and separation time (SeP) reflecting the polymer entanglements appeared to be the main contributors in PC2.

Keywords: Ascorbic acid, oxidized batters, oxidation, gluten agglomeration test, principal component analysis (PCA)

1. Introduction

Protein composition played an important role in dough mixing and rheological properties and bread quality (Schofield and Chen 1995; Southan and MacRitchie 1999). The interactions between the number of polymeric glutenin proteins, molecular weights (Every et al. 2008) and the distributions of disulfide bonds are important in the rheological properties of dough (Grosch 1986). Disulfide bonds hold glutenin subunits and other flour constituents together and thus making important contributions in gluten protein functionality in breadmaking (Goesaert et al. 2005).

Ascorbic acid is usually added in commercial flour as additive blend and as an aging agent in baking industry (Aamodt et al. 2003). L-threo-ascorbic acid (L-AA) is the strongest ascorbic acid stereoisomer that improves the strength, handling and baking properties of dough (Goesaert et al. 2005). During mixing, L-ascorbic acid is readily oxidized in the presence of air (oxygen), copper or iron or enzymatic reactions by oxidases and changes into the form of L-dehydroascorbic acid (L-DHAA) (Carter and Page 1965). The L-DHAA is the actual oxidizing improver (Every et al. 1999) but it will be oxidized into diketogulonic acid and other compounds (Carter and Page 1965). The effect of improving bread quality by L-AA is due to the oxidation of sulfhydryl (SH) groups in dough (Carter and Page 1965). The formation of disulfide bonds that improve the loaf volume is believed to be produced by the catalytic oxidation of sulphydryl groups in dough by dehydroascorbate reductase (Tsen 1965). Grosch and Wieser (1999) suggested a mechanism of L-AA with enzyme glutathione reductase (GSH-DH) in which the former will be oxidized by L-DHAA and form glutathione (GSSH) with the presence of protein thiols during mixing. In another study on the effect of L-AA on wheat flour,

the oxidation effect on DHA and O_2 formation during AA oxidation promotes the SH-SS interchange reaction which promotes inter protein disulfide bonds through disulfide-thiols interchange reaction and improves the rheological properties of flour-water dough (Nakamura et al. 1997). The level of L-DHAA of wheat flour samples were measured using enzymatic assay and the amount of L-DHAA increased rapidly as L-AA was oxidized while mixing (Every et al. 1999). The addition of ascorbic acid during gluten washing process also increased the stiffness of the gluten obtained (Kieffer et al. 1990). According to the study on the effect of ascorbic acid in gluten and baking by Ambardekar (2009), addition of ascorbic acid at 100 to 150 ppm improved the baking performance in wheat flours and reduced with 200 ppm of ascorbic acid added to the samples. Based on that study, the mixing properties of wheat flours were negatively associated to baking properties and flour protein content with addition of ascorbic acid to flours (Ambardekar 2009). The addition of ascorbic acid increases the viscous component of gluten which is also closely associated with properties of rise in oven spring (Ambardekar 2009). Oven spring occurs during the first minutes of the dough in the oven when the high temperature causes the gas pocket to expand. The addition of ascorbic acid will oxidize the GSH forming disulfide bonds which strengthen the gluten and stabilize the network (Ambardekar 2009). The formation of disulfide bonds will improve the mixing properties, maximum resistance to extension and loaf height (Ambardekar 2009).

The objectives of this study are to investigate 1) the effect of oxidation by the addition of ascorbic acid on the aggregation properties of slurry flour samples, and 2) to determine a possible correlation between gluten aggregation properties, dough mixing, gluten viscoelastic and baking properties.

2. Materials and Methods

Six commercial hard red winter wheat flours were analyzed in this study. The samples were obtained from two different regional suppliers. The wheat flours contained different cultivars in order to obtain the different ranges of protein content and quality. The samples were labeled as A and B and numbered 1 through 3, in order of increasing protein content, with 1 being the lowest. Flour protein, moisture and ash were obtained by infrared analysis using an NIR system model 6500-M (FOSS NIR Systems Inc, Laurel, MD).

Five levels of oxidation were achieved by the addition of ascorbic acid (Malinckrodt Baker inc., Phillipsburg, NJ) using 0, 50, 100, 150 and 200 ppm. The ascorbic acid was added in solutions made from stock solutions containing 0.05, 0.1, 0.15, and 0.2 g ascorbic acid in 500 mL. The working solutions also contained 2% sodium chloride and 5% sucrose which improved the agglomeration process compared to using water alone (data not shown). Samples with no ascorbic acid but with the solution containing 2% sodium chloride and 5% sucrose were used as controls.

2.1. Gluten Agglomeration Test (Gluten Peak Tester)

The commercial wheat flour samples with different oxidation levels obtained by the addition ascorbic acid (0, 50, 100, 150, 200 ppm) were analyzed with a high shear mixing apparatus, called Gluten Peak Tester (GPT, C.W. Brabender Instruments, Hackensack, NJ). A total weight of 18.5 g of wheat flour sample and solution was used for each replicate and the amount of flour used was reported as percentage (as is basis). Samples were mixed in a stainless steel cup at 3,333 rpm and 35^{o}C for 10 minutes. The

GPT parameters recorded were peak maximum time (PMT), maximum torque (MT), torque before maximum time (T1), torque after maximum time (T2) and area under peak (work). The peak maximum time is recorded in seconds (s) and torque parameters are recorded in Brabender Equivalent (BE) which is an arbitrary unit used by C.W. Brabender Instruments Inc. All analyses were completed in duplicates.

Gluten Creep Recovery Analysis

Creep recovery tests were performed by Ambardekar (2009) based on the protocols of Zhao and others (2007) and Liang and others (2007). The gluten extraction was prepared in an automated gluten washer, Glutomatic 2200 (Perten Instruments AB, Segeltorp, Sweden) using approved method 38-12.02 (AACCI 2000). Ten grams of flour was wetted with 5 mL of ascorbic acid solution (0.05, 0.1. 0.15 and 0.2 g ascorbic acid in 500 ml of 2% sodium chloride solution) before mixing and washing in Glutomatic for 10 minutes. The gluten extracted from the Glutomatic was gently shaped into gluten ball and relaxed under metal plates (2,500 g) for 60 minutes at room temperature (25^{o}C) before the creep recovery measurement.

The gluten prepared was measured on a Rheometer AR1000N (TA Instruments, New castle, DE), with the gap set at 25 mm and at room temperature (25^{o}C). The instrument was set to produce 40 Pa constant stress in order to shear the gluten. The gluten was maintained at constant stress for 100 s creep test and released to recover for 1000 s. The deformation and recovery of the gluten was measured as compliance. Creep-recovery parameters obtained were: separation time (SeP), delta compliance (J-Jr), % recoverability (RCY), time constant of creep (λ_C), and time constant of recovery (λ_R). J-Jr

and λ_C represents the viscous properties of gluten, and SeP, RCY, and λ_R represents the elastic behavior of gluten. All analyses were completed in triplicates.

2.2. Dough Mixing

The dough mixing properties were performed by Ambardekar (2009) and according to approved method 54-21.01 (AACCI 2000). Wheat flour samples were analyzed using a 10 g bowl Farinograph-E (C.W. Brabender Instruments, Hackensack, NJ) at 63 rpm at 30^{o}C. Dough development time (DT), stability time (ST), breaking time (BT), and water absorption (WA) adjusted to 14% moisture basis were recorded. One milliliter of ascorbic acid solution (0.05, 0.1, 0.15 and 0.2 g in 100 mL deionized water delivered the 50, 100, 150, 200 ppm ascorbic acid) was added before additional water was added for mixing and hydration. The control samples were prepared with only deionized water. All analyses were completed in duplicates.

2.3.Baking Test

Baking tests were performed by Ambardekar (2009) based on approved method 10-10.03 (AACCI 2000) which is a straight-dough bread making method. Wheat flour samples (100 g) were added with 1 mL of the previously described working solution to deliver 0.05, 0.1, 0.15 and 0.2 g ascorbic acid and 3 g of shortening. Samples were mixed to dough with a 100-g mixer Swanson-Working pin type (National Mfg. Co. TMCO Inc, Lincoln, NE). Several mixing and baking tests were performed in order to obtain the optimum mixing time and bake water absorption. Bread quality parameters like dough proof height (PH), loaf height (LH), loaf volume (LV), oven spring (OSP) and specific volume (SV) were measured. The proof height (PH) and loaf height (LH) of bread were

measured with a digital proof height gauge (National Mfg. Co. TMCO Inc, Lincoln, NE). The LV was obtained by rapeseed displacement after the bread was cooled for 10 minutes after taking out from the oven and OSP was calculated by subtracting PH from LH. Specific volume SV was defined as the ratio of loaf volume to loaf weight. All analyses were completed in duplicates.

2.4.Statistical Analysis

The relationship of ascorbic acid and flour types (protein content ranging from 8% to 13.7%) with the properties of GPT (PMT, MT, T1, T2, Peak Area), viscoelastic variables (J-Jr, SeP, RCY, λ_C, and λ_R), mixing properties (WA, DT, ST and BT), and baking characteristics (LV, PH, LH, OSP, SV) were evaluated with Principal Component Analysis (PCA) using Canoco for Windows version 4.5 (Biometris, Plant Research International, Wageningen, The Netherlands). Data input is untransformed, centered and standardized in order to minimize the mean square error.

3. Results and Discussion

General composition analysis (protein, moisture and ash content) of the six commercial hard red winter wheat flours previously reported by Ambardekar (2009) were shown on Table 1. The range of protein is a representative of the hard red winter wheat flour in the Southern Plains region. Also, an example of the recorded curves and representation of the parameters obtained from the gluten peak tester (GPT) is shown in Figure 1. Examples of gluten aggregation curves from sample with different protein content and quality is shown on Figure 2. An expanded definition of the parameters and their units are presented in Table 2.

3.1.Gluten Agglomeration Test (GPT)

In order to select a common flour percentage out of the overall samples, five experiments were conducted using 41 to 51% flour slurries. The common flour percentages were selected based on the highest explained variance obtained from PCA because the gluten aggregation occurrence depends on the flour quality and specific range of flour percentages. The aggregation of each percent flours were summarized in Tables 3, 4, 5, and 6. The treatments of different flour percentages treatments cannot be compared directly but the trends of the aggregation describe the effects of protein quality and quantity in the presence and absence of AA. For example, 1A sample with 8% protein does not form agglomeration until the flour reaches 49% in the slurry (Table 3). In comparison, 3A flour with 13.7% protein forms aggregation at 41% flour, the lowest percentage of the control groups. The percent flour in the slurries forming aggregation for 3A sample with ascorbic acid was extended (41 to 46% flour) compared to 3A without ascorbic acid (41 and 43% flour). The oxidation by ascorbic acid of free SH groups promote polymerization and SH/SS reactions between glutenins and thiol compounds. The latter compounds will promote depolymerization of gluten if they are not oxidized (Wieser 2007). There is another possibility that ascorbic acid may promote gluten aggregation with the formation of tyrosin links between the subunits during mixing and baking (Tilley et al. 2001). The result is similar to the SH/SS reaction in covalent bonds form to support the agglomeration of protein. The oxidation effect by the addition of ascorbic acid to support the gluten aggregation on 3A can be seen clearly in Table 3, 4, 5, and 6 by formation of agglomeration in slurries of 41% flour.

A set of 50 samples with 43% flour slurries containing five levels of ascorbic acid (AA) concentration (0, 50, 100, 150 and 200 ppm) was selected. The concentration of ascorbic acid used in this study of flour batters is based on the concentrations used by Ambardekar (2009) who analyzed the mixing, viscoelastic of gluten and breadmaking of the same samples.

The effect of oxidation by ascorbic acid on PMT appears to be complex. The trends suggest that the effect was sample dependent and does not always depend on the level of the dose (Fig. 3). Some samples displayed a trend of no changes in peak maximum time with oxidation/ascorbic acid (3A), increase (1B and 2B), and increase followed by a decrease (2A). The range of PMT recorded was between 91 to 218 seconds. This wide range was useful in the separation of samples as it will be discussed in the Principal Component Analysis section.

Overall, the slurry system behavior of sample 3A agrees with the baking performance of this samples reported by Ambardekar (2009). The loaf volume of 3A with 50 to 150 ppm ascorbic acid was similar to the control and it reduced as much as 8.8% with 200 ppm ascorbic acid. In other words, the loaf volume of this sample does not increase when oxidation agent are added to the samples. In summary, aggregation peak maximum time appears to have the potential in selecting variable which described the differences in flours with different oxidation levels. The usefulness of this variable in samples with other additives or with a wider or narrower flour quality needs to be further investigated.

Oxidation reaction will increase the dough resistance and extensibility (Popper et al. 2006). A balance of these two properties is needed for generating bread volume and overall machine-ability in breadmaking. There is a lack of information on the properties of batter systems and the overall usefulness of the agglomeration properties of flours. This study shows that the effect of oxidation on batter protein aggregation does not have a generalized effect. In contrast, effect of oxidation can be seen when 100 ppm AA is added to dough with only about 50% water and it showed an improvement in baking performance (Ambardekar 2009). The quality of the protein may affect the pattern of aggregation. For example, 3A flour with faster aggregation (shorter PMT) is also the sample with high strength as it will be discussed in the following section (Fig. 4). In a study with batter systems using Canadian flours and the same equipment (GPT), a soft wheat flour revealed faster aggregation (shorter peak max time) compared to hard wheat flour (Huschka et al. 2011). The same study reported that when oil was added to both soft and hard flours, the peak maximum times decreased (aggregated faster) with a higher effect (larger decreased) on the hard flour compared to the soft flour (Huschka et al. 2011). The hard flour had more strength compared to the soft flour. The lower protein flour 1A did not aggregate at 43% flour but it did aggregate at 46% flour when ascorbic acid was added to it (Table 3, 4, 5, and 6). This means that protein quantity and quality of the flour samples influence the pattern and ability of the gluten to aggregate.

Oxidation of the SH side chain in the gluten proteins (Faisy and Neyreneuf 1996) in theory should promote protein aggregation but there are also factors of density of the SH groups. This density cannot be easily quantified and will be part of overall protein quality. There were a number of reports in which the quality of the proteins was

mentioned as explanation of specific parameters, for example Salehifar et al (2010) reported that dough resistance improved with higher quality and quantity of protein wheat flour. Good and poor baking quality flour also affect the aggregation behavior and extractability of gluten protein. Gluten protein from good quality baking flour had higher aggregation and was less extractable compared to poor baking quality flour (Butaki and Dronzek 1979; He and Hoseney 1991). Therefore, the quality of the flour sample will reflect the aggregation behavior of the gluten protein. Thus the aggregation peak recorded by the GPT is due to gluten aggregation. Mixing describes the hydration of flour protein and viscoelastic properties of gluten when gluten network were created and this also showed that the dough is developed (Rouillé et al. 2000). During mixing, the flour protein will interact with other flour constituents (e.g., starch, lipid, non-starch polysaccharides) and also salt that is added to form the gluten matrix (Kulp 1995) by creating SS links. The addition of L-AA decreases the extensibility but improved the elasticity of the dough because L-AA oxidized the SH side chains in the protein to SS and thus a stronger dough network (Rouillé et al. 2000).

The effect of oxidation on agglomeration maximum torque and peak area are presented in Figure 4 and 5. Maximum torque is related to agglomeration strength. The effect of oxidation on strength is similar in most samples except in sample 3A with higher strength. The expected effect of increasing strength with increasing oxidation is not revealed in the batter system. This may be due to the excess water compared to dough sytems with about 50% water. While the effect of oxidation on peak area shows an increase in area with the first oxidation level (50 ppm AA) compared to the control but followed by a decrease as the oxidation level increases (Fig. 5). The increase of area

under the peak also means that more work (compared to the control) was perfomed to develop the gluten and to disintegrate it. This means that instead of the strength reflected by the maximum torque, strength can be related to peak area. The higher the peak, the more strength is associated to the development of the gluten agglomeration in a batter.

3.2.Principal Component Analysis

Principal component analysis (PCA) is a mathematical multivariate method used to find a linear combination of the variables which account the total variance by reducing the dimensionality of the data and still containing most of the variation in the data set (Jolliffe 2002; Mukhopadhyay 2009). The reduction is done by showing the direction (principal component) where the highest variation of data is achieved (Ringner 2008). The samples are further represent by the number of principal components instead of the actual value from the thousands of variables (Ringner 2008). Samples are plotted to tell the relationships if there were similarities or differences among the samples and also to determine whether they can be grouped (Ringner 2008).

The PCA of gluten aggregation and flour protein explained 89% of the total variance where PC1 and PC2 explained 72 and 17% of the variance, respectively (Table 7, Fig 6). These values are high and are very promising in separating the properties of flour samples. PC1 is highly dominated by torque after aggregation (T1), maximum torque (MT), and flour protein by contributing 94, 94 and 90% of the variance, respectively (Table 7). PC2 is dominated by the area under peak by contributing with 97% of the variance (Table 7). The samples were well separated into three different groups (Fig. 6). The control group is negatively correlated to agglomeration peak area;

this means that the areas were small compared to the oxidized samples. As the samples were oxidized by the increase level of AA, they move upward in the biplot and show positive association with maximum torque, torque after the peak (T2), flour protein on the right quadrant and peak maximum time (PMT) on the left quadrant. In summary, oxidation moves the flour towards an association to gluten strength properties and flour protein. The PCA of gluten agglomeration and flour protein also indicated the separation of control 3A and 3A with increasing oxidation. The latter ones were highly correlated to maximum torque or strength (Table 6).

The parameters of gluten agglomeration were further analyzed with PCA ordination to illustrate possible correlations with viscoelastic of gluten, dough mixing and breadmaking. The results are shown as a two dimensional correlation biplot in Figure 6. The total explained variance was 63% with PCA 1 and 2 explaining 44 and 18% of the variance, respectively. PC 3 (10%) and PC 4 (7%) showed low explained variance therefore were not discussed further. According to the contribution of the variance by each variable (Table 8), PC1 was highly influenced by protein content of flour (90%) and mixing properties (WA, 81%). In contrast, loaf height (2%) had the least influence on PC1 axis. PC2 was dominated by SeP (46%) which reflects the entanglement of the polymers and thus its elasticity and OSP (44%) which is related to the elasticity of the dough in the oven. SeP and OSP were negatively correlated meaning that the entangled the polymers will slow down OSP. Protein content of flour was positively correlated with baking properties and T2 and MT or strength of the aggregation; they were negatively correlated with mixing properties. PMT and T1 were highly associated to mixing properties and negatively associated to flour protein. J-Jr, λ_C (both representing the

viscous component of gluten) and λ_R (elastic component of gluten) were positively correlated with agglomeration peak area (GPT) and OSP (baking) but negatively correlated to percent recovery and separation time of the gluten (elastic properties). The gluten separation time represents the physical entanglements of the gluten polymers. Synthetic polymer with more entanglement and more elastic character will have a longer separation time and they are stronger than a polymer with a shorter separation time (Heddleson et al. 1994). In Figure 7, the samples were well separated but there were some groups that can be identified based on protein content, for example 1B and 3A samples. Samples with protein content < 11% were distributed on the left quadrants while samples with protein >11% were plotted on the right quadrants. Sample B with protein content <11% was highly correlated to RCY and SeP of gluten but as the concentration of ascorbic acid increased, the samples moved from top left quadrant to lower left quadrant and showed correlation with mixing properties. On the other hand, sample A with protein content >11% showed correlation with protein content but as the concentration of ascorbic acid increased the sample moved from the top right quadrant to the lower right quadrant and showed correlation with the loaf volume and loaf height. These separations suggest that the agglomeration test can differentiate the oxidized samples due to increasing levels of AA towards the gluten network. The AA in the system will create more SS links and changing the viscoelastic properties of the gluten protein. This also suggests that these chemical crosslink produced in the systems were highly entangled in the system. This chain entanglement is important for the physical properties of batter. The increased of chain entanglement will allow more interaction between protein and water; this means that the structure of the batter is organized therefore increased the

stiffness of the sample. In another word, the more difficult to untangle the chains that interact to the constituent means the stronger and more resilient the properties of the batter, also the higher the molecular weight. The ability of gluten to aggregate can be affected by the oxygen incorporated during the high speed mixing used in the GPT. The presence of AA during mixing will quickly consume the free oxygen radicals within the system in order to oxidize into DHAA and later oxidize to sulfhydryl (-SH). At high concentration of AA, the amount of free oxygen radical might be limited for AA to be oxidized. When the amount of oxygen is limited during mixing, AA could act as reducing reagent and will weaken the gluten network (Li et al. 2000). Therefore, there are many aspects that will influence the effect of AA towards gluten aggregation. In the GPT, we assume an excess of oxygen is introduced in the batter due to the high speed of the test.

The total explained variance of the combined variables in this study (with agglomeration) is lower compared to the report by Ambardekar (2009) (63% vs 66%, respectively). However, the report by Ambardekar did not include the agglomeration test. Therefore, the difference can be explained by the lack of aggregation of the lowest protein content flour (1A) in this study and thus, one sample group is missing from the comparison. The overall trend from Ambardekar (2009) report is similar to this study. An interesting difference is that flour protein contributed slightly more to the explained variance in the present study compared to Ambardekar (2009) (90% vs 87% respectively). In spite of the exclusion of the lowest protein sample (1A) which did not show aggregation. We speculate that flour samples with protein content and similar protein quality will produce similar separation based on their properties when they are oxidized. Ambardekar (2009) reported that there was a clear separation of three groups of

samples in the PCA graph. The 1A group clearly separated from the other two, with the sample with protein > 11% separated further away on the right quadrant and the rest of the samples in the middle of the graph. An improved separation of the properties of the samples was obtained when gluten agglomeration properties were included. Thus, we can conclude that; a) gluten peak aggregation can be used to segregate groups of samples with similar properties and b) the aggregation test can reveal oxidation properties of flours. Figure 6 also demonstrates that as the level of AA increased, the samples are moving upwards toward the direction of flour protein content and this may suggest that the effect of oxidation is highly influenced by the protein content especially when flour protein is higher than 11%. For example, when levels of oxidation increased in sample A3, it moved from the direction of gluten agglomeration properties (PM and MT) to baking properties (LV and LH). Although in Figure 7 the agglomeration properties of sample 1A (8% protein) are missing, low protein samples are represented by samples 1B and 2B (10.5% protein). The trends of these samples showed an improvement by a movement to be closely associated to mixing properties and agglomeration peak maximum time. These observations agree with previous reports of oxidation improving the mixing properties (Ambardekar 2009).

4. Conclusions

The oxidation of batters by the addition of AA influenced the total work required for aggregation. The effect of oxidation on peak area showed a maximum at 50 ppm and a steady decreased with increased of oxidation reaction. While the effect of oxidation on peak maximum time (PMT) appears to be more complex, i.e., sample dependent and not always dose dependent. Agglomeration properties and flour protein separated the samples

with different oxidation states into three groups. When compared to viscoelastic, mixing and baking properties, agglomeration properties, oxidized samples were highly correlated to loaf volume and specific volume of baked products. Thus, agglomeration properties complement the traditional mixing and baking properties plus they have the advantage of being a quick test with a high potential to be further explored in other applications.

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

Flours	Protein (%)	Moisture (%)	Ash (%)
1A	8.0 ± 0.05	11.7 ± 0.02	0.29 ± 0.01
2A	11.2 ± 0.07	10.5 ± 0.03	0.38 ± 0.01
3A	13.7 ± 0.02	10.1 ± 0.02	0.41 ± 0.00
1B	10.4 ± 0.10	12.5 ± 0.02	0.47 ± 0.00
2B	10.6 ± 0.07	12.6 ± 0.00	0.48 ± 0.01
3B	11.4 ± 0.01	13.0 ± 0.04	0.58 ± 0.01

SD = Standard deviation.

Adapted from Ambardekar (2009).

Table 2. Definition of gluten agglomeration test, viscoelastic, mixing and baking properties terms

Variables	Abbreviations	Definitions	Units
Gluten Agglomeration Test	PMT	Peak maximum time defined as the time needed for aggregation to occurs.	S
	MT	Maximum torque defined as the torque required when the peak of aggregation occurs. MT is a measure of gluten strength.	BE
	T1	Torque before aggregation defined as the torque required before peak of aggregation occurs.	BE
	T2	Torque after aggregation defined as the torque required after peak of aggregation occurs.	BE
	AREA	Area under the curved is the total work of agglomeration.	BE.s
Mixing	WA	Water absorption	%
	DT	Time required for the flour to develop into dough	min
	ST	Time for develop dough to remain stable during mixing	min
	BT	Time required for the dough start to breakdown after mixing	min
Viscoelastic	SeP	Separation time defined as the time at which the creep and recovery split and no longer stay superimposed	S
	J-Jr	Delta compliance defined as the difference in compliance of creep and recovery at 100s.	Pa^{-1}
	RCY	Percent recoverability defined as the elastic ability of gluten recover to original state as stress is removed	%
	λ_R	Time constant of creep defined as the rate of elastic recovery of gluten to reach equilibrium	S
	λ_C	Time constant of recovery defined as the rate of deformation of gluten	s

Baking	LV	Loaf volume	cm^3
	LH	Loaf height	mm
	PH	Proof height	mm
	OSP	Oven spring	mm
	SV	Specific volume of baked loaves	cm^3/g
Flour protein	FP	Flour protein	%

BE, Brabender Equivalent, arbitrary unit (not SI).

Table 3. Protein aggregation for flour batter systems with different flour percentages without ascorbic acid (0 M, control). Flour protein (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4.

0 ppm AA	**Type of flour**					
% Flour	1A	2A	3A	1B	2B	3B
41	-	-	+	-	-	-
43	-	+	+	+	+	+
46	-	+	-	+	+	+
49	+	+	-	+	+	+
51	+	-	-	-	-	-

Table 4. Protein aggregation for flour batter systems with different flour percentages with 50 ppm ascorbic acid. Flour protein (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4.

50 ppm AA	**Type of flour**					
% Flour	1A	2A	3A	1B	2B	3B
41	-	-	+	-	-	-
43	-	+	+	+	+	+
46	+	+	+	+	+	+
49	+	+	-	+	+	+
51	+	-	-	-	-	-

Table 5. Protein aggregation for flour batter systems with different flour percentages with 100 ppm ascorbic acid. Flour protein (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4.

100 ppm AA	**Type of flour**					
% Flour	1A	2A	3A	1B	2B	3B
41	-	-	+	-	-	-
43	-	+	+	+	+	+
46	+	+	+	+	+	+
49	+	+	-	+	+	+
51	+	-	-	-	-	-

Table 6. Protein aggregation for flour batter systems with different flour percentages with 150 ppm ascorbic acid. Flour protein (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4.

150 ppm AA	**Type of flour**					
% Flour	1A	2A	3A	1B	2B	3B
41	-	-	+	-	-	-
43	-	+	+	+	+	+
46	+	+	+	+	+	+
49	+	+	-	+	+	+
51	+	-	-	-	-	-

Table 7. Explained variance (%) in PCA of agglomeration and flour protein from commercial flour treated with AA.

	PC1	**PC2**	**PC1 + 2**
	72	17	89
PMT	88	1	89
MT	94	1	95
T1	67	2	69
T2	94	1	95
Area	2	97	99
FP	89	0	90

Table 8. Explained variance (%) in PCA of agglomeration, viscoelastic, mixing, and baking properties from commercial flour treated with ascorbic acid.

Variables		PC1	PC2	PC1+2
	PC	44	18	63
Agglomeration	PMT	69	10	79
	MT	73	19	92
	T1	57	1	58
	T2	73	20	93
	Peak Area	5	15	21
Mixing	BT	71	6	77
	WA	81	2	84
	DT	77	3	80
	ST	40	0	40
Baking	LV	34	1	35
	PH	2	36	38
	LH	30	2	32
	OSP	26	44	70
	SV	15	27	41
Viscoelastic	λ_R	11	27	38
	λ_C	14	32	46
	J-Jr	36	37	73
	SeP	36	46	83
	RCY	45	31	76
Flour Protein	FP	90	5	95

NOTE: Abbreviations are listed on Table 2

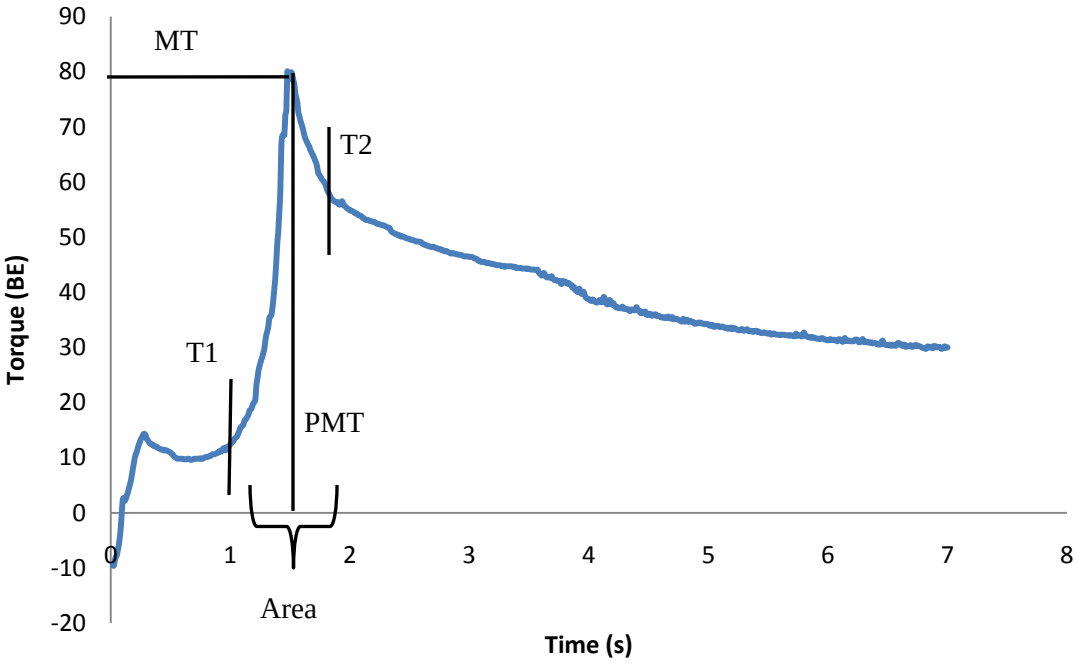

Figure 1. Example of a gluten aggregation test curve obtained with a high speed (3333 rpm) mixer (Gluten Peak Tester) at 35^{o}C. Parameters recorded were maximum torque (MT), torque before and after peak (T1 and T2, respectively), peak maximum time (PMT) and peak area.

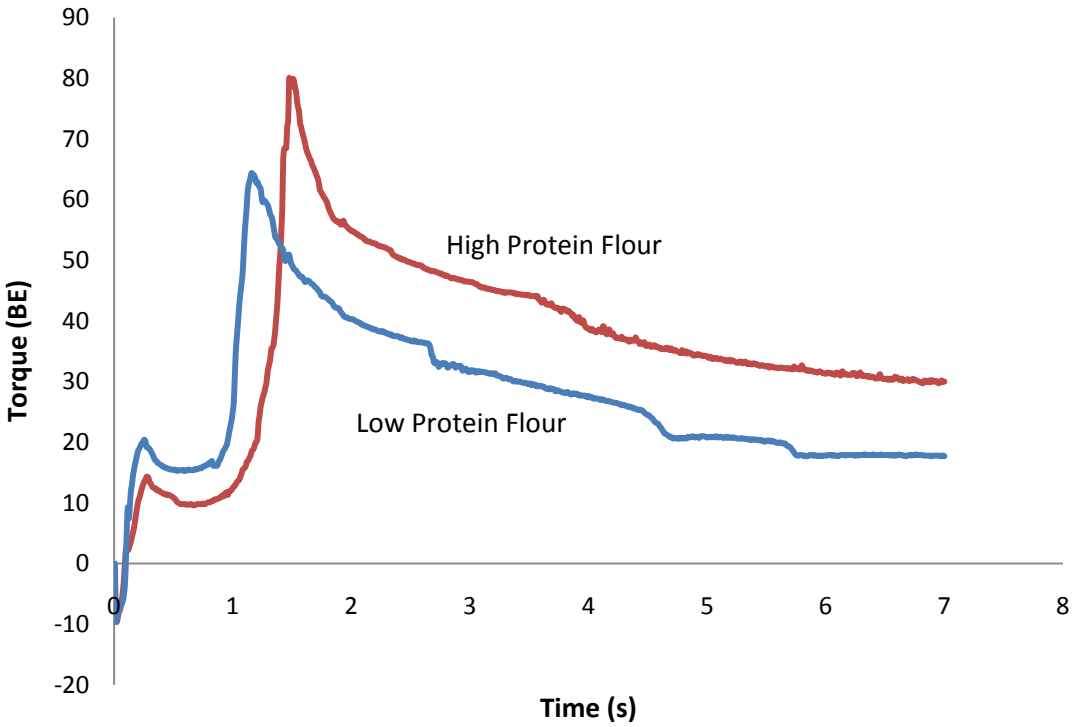

Figure. 2. Example of gluten aggregation curves of commercial flours obtained with a high speed (3333 rpm) mixer (Gluten Peak Tester) at 35°C. High protein flour (A3 = 13.7%) and low protein flour (A1 = 8.0%).

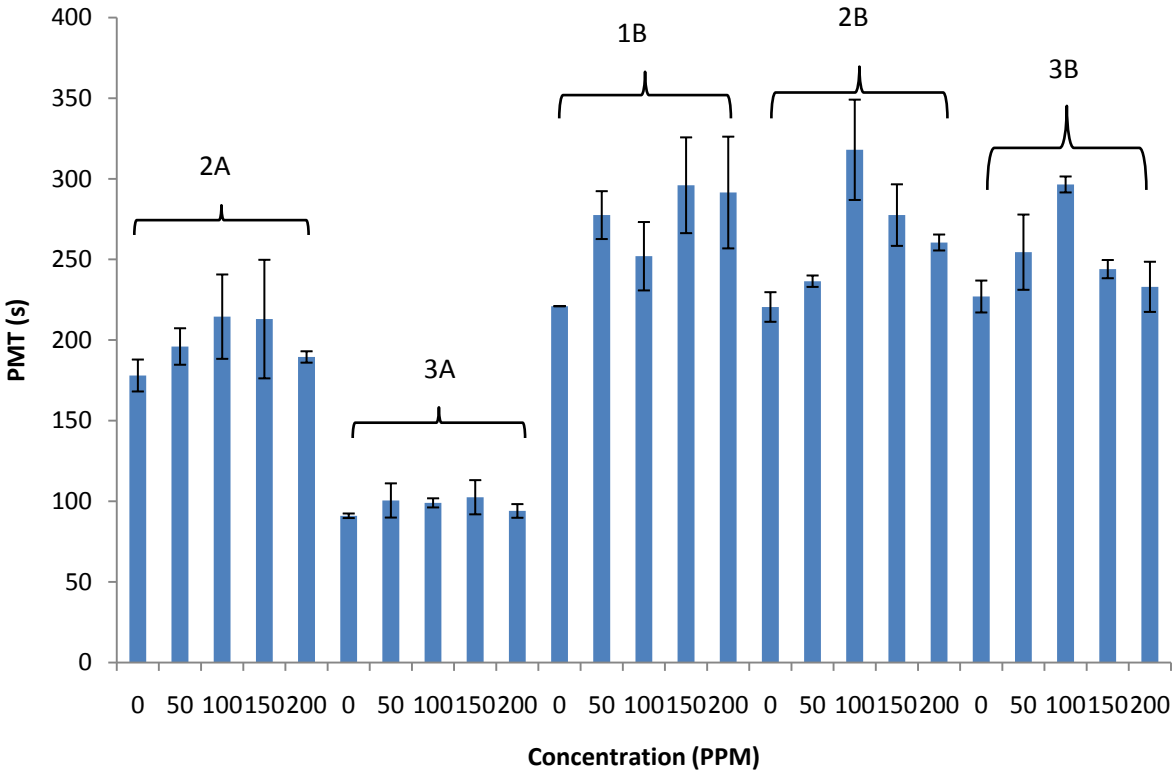

Figure 3. Peak maximum time of gluten aggregation test (43% flour) for commercial wheat flours containing five ascorbic acids levels (0, 50, 100, 150, 200 ppm). Overall samples A1did not aggregate. Means (n=2) and standard deviation bar. Flour protein content (%), A1 = 7.95, A2 = 11.19, A3 = 13.68, B1 = 10.40, B2 = 10.59 and B3 = 11.38.

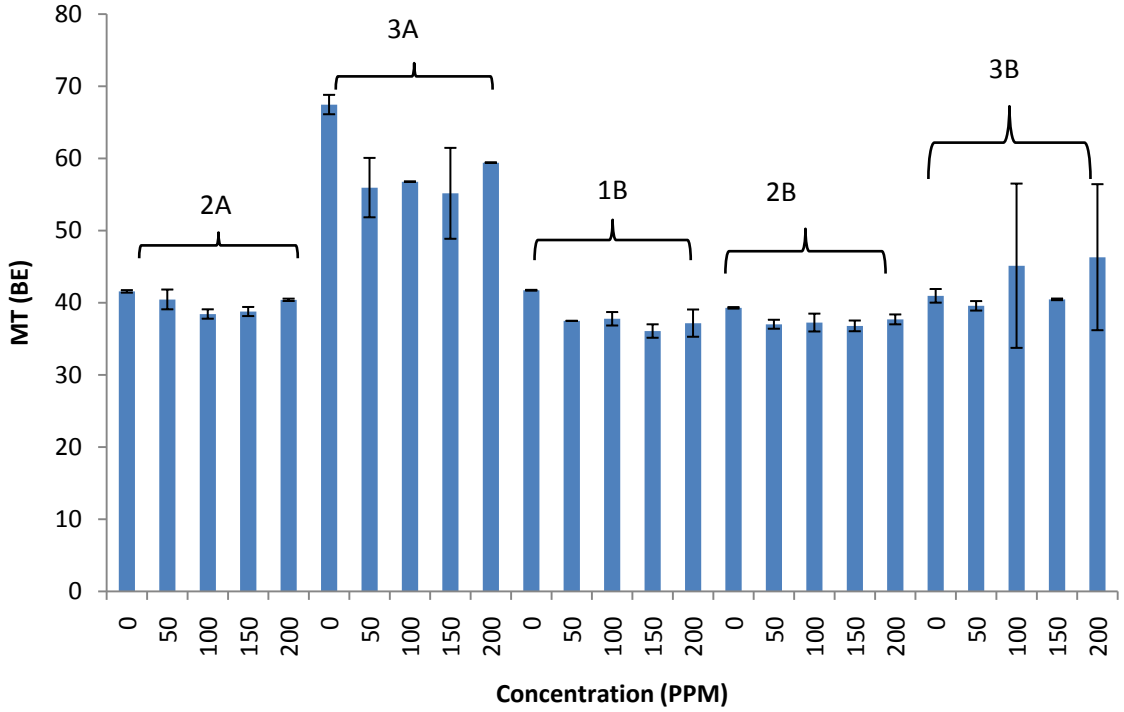

Figure 4. Maximum torque of gluten aggregation test (43% flour) for commercial wheat flours containing five ascorbic acids levels (0, 50, 100, 150, 200ppm). Overall samples 1A did not aggregate. Means (n=2) and standard deviation bar. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.40, 2B = 10.59 and 3B = 11.38.

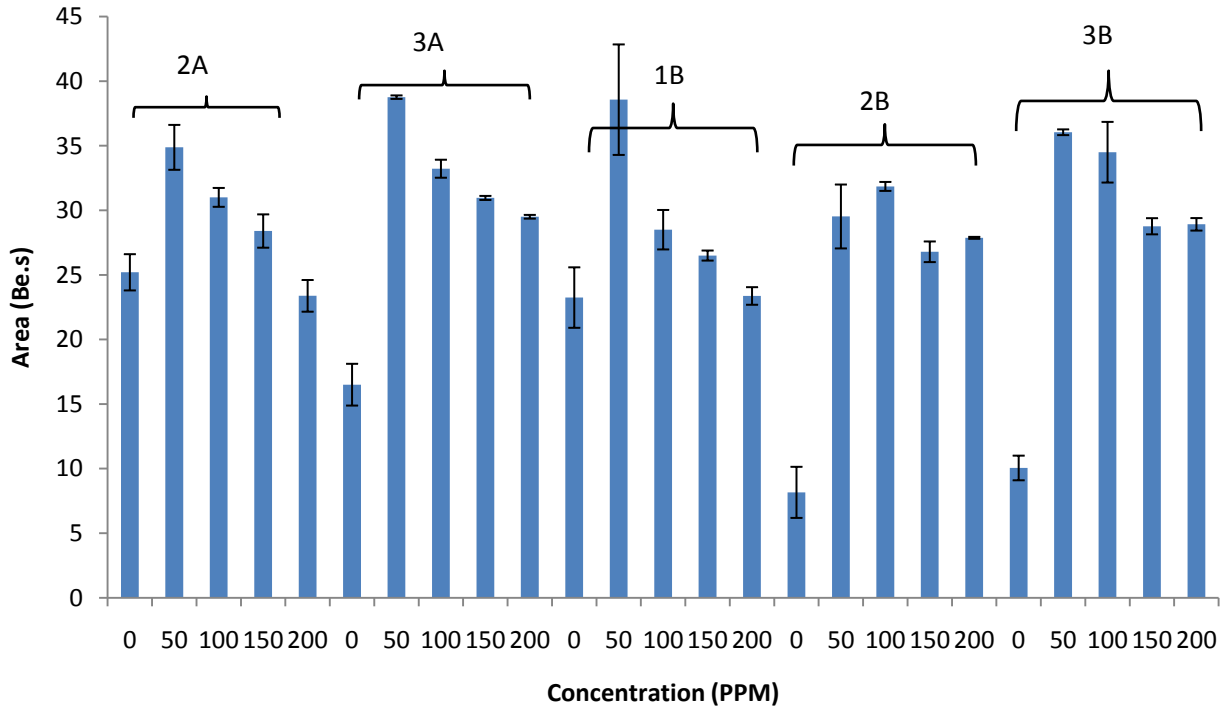

Figure 5. Peak area of gluten aggregation test (43% flour) for commercial wheat flours containing five ascorbic acids levels (0, 50, 100, 150, 200ppm). Overall samples A1did not aggregate. Means (n=2) and standard deviation bar. Flour protein content (%), A1 = 7.95, A2 = 11.19, A3 = 13.68, B1 = 10.40, B2 = 10.59 and B3 = 11.38.

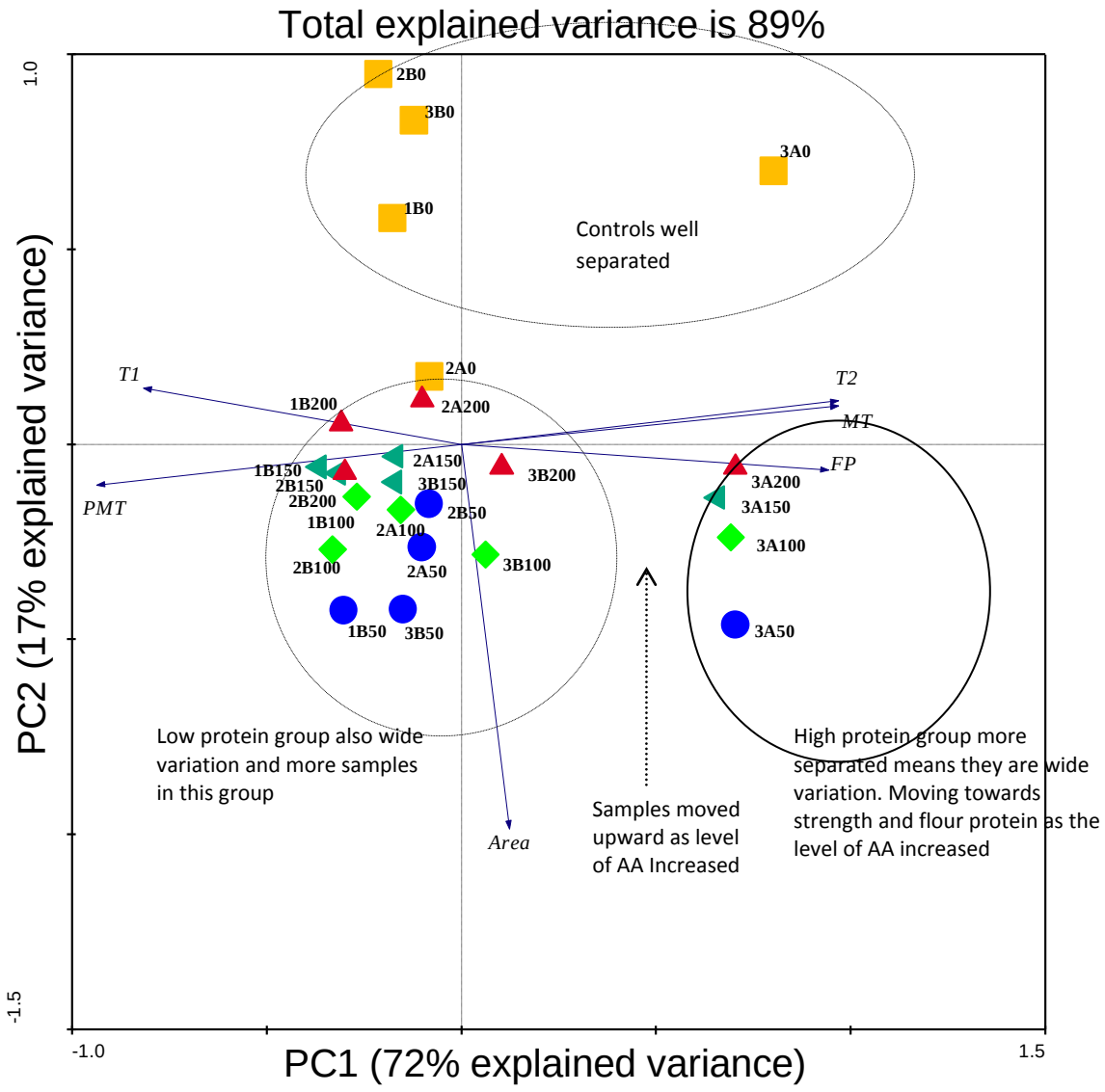

Figure 6. Loading plot of the first two principal components based on gluten agglomeration test properties (GPT) and protein content of six commercial wheat flours, containing (0, 50, 100, 150 and 200 ppm) ascorbic acid. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.40, 2B = 10.59 and 3B = 11.38. ■, ●, ♦, ◀, and ▲ represent 0, 50, 100, 150 and 200 ppm, respectively. Abbreviations are listed on Table 2.

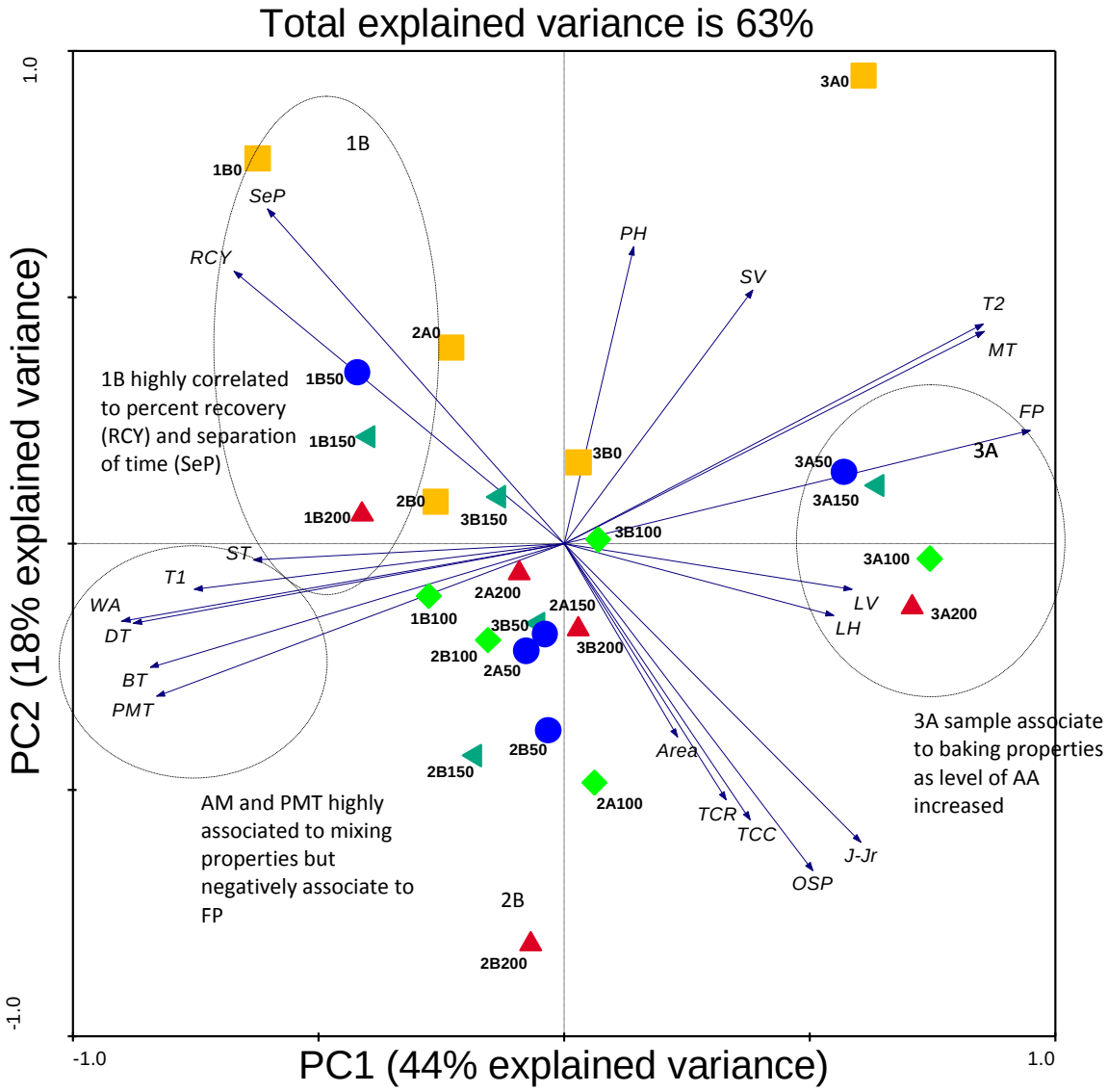

Figure 7. Loading plot of the first two principal components based on gluten agglomeration test, mixing, viscoelastic and baking properties as well as protein content of six commercial wheat flours, containing (0, 50, 100, 150 and 200 ppm) ascorbic acid. ■, ●, ♦, ◄, and ▲ represent 0, 50, 100, 150 and 200 ppm. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.40, 2B = 10.59 and 3B = 11.38. TCC and TCR are listed as λ_R and λ_C. Abbreviations are listed on Table 2.

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CHAPTER V

EFFECT OF DISRUPTING DISULFIDE BONDS BY DITHIOTHREITOL (DTT) ON GLUTEN AGGREGATION, VISCOELASTIC, MIXING AND BAKING PROPERTIES OF WHEAT FLOUR BATTERS

Abstract

Gluten agglomeration is an important step in the development of dough. Aggregation causes the formation of polymeric gluten network which influences the performance aspect of gluten i.e., rheological properties and quality of final product. Gluten proteins consist of monomeric gliadins and polymeric glutenins and are responsible for the gluten viscoelastic properties. These sub-fractions are bound together by intermolecular and intramolecular disulfide bonds. Therefore, Dithiothreitol (DTT), a reducing reagent was used to alter the polymerization of these monomeric and polymeric proteins in order to further study the aggregation of gluten protein in batter systems. The objective of this study is to access the effect of the disruption of disulfide bonds on protein aggregation of six commercial hard red winter wheat flours. Four levels of DTT (0, 0.1, 0.25, and 0.5 mM) were used in this study. A high sheer apparatus (Gluten Aggregation Test, GPT) was used to determine the aggregation properties of batter at 3333 rpm and 35^{o}C for 10 min. The protein quality parameter of aggregation was measured in a batter system with 43% flour. The principal component analysis (PCA) of

agglomeration and flour protein explained 89.7% of total variance and PCA of overall properties (agglomeration, viscoelastic, mixing and baking properties as well as flour protein) explained 69.6% of total variance. The first component axis of the overall properties explained 40.6% of the variance and showed the highest contribution by aggregation properties such as maximum torque (MT) and torque after aggregation (T2). Baking properties such as loaf height (LH), specific volume (SV) and oven spring (OSP) appeared to be the main contributors for the second principal component which explained 29.0% of the variance. Overall, PMT of batters were reduced with a disruption of disulfide bonds, i.e., within necessary levels of DTT. As level of DTT increased to 0.25 mM, samples were correlated to viscoelastic properties suggesting that disulfide bonds disruptions occur at this point, causing them to disaggregate and flow easily.

Keywords: Dithiothreitol (DTT), gluten agglomeration test, viscoelastic properties, disulfide bonds, principal component analysis.

1. Introduction

It is widely acknowledged that the uniqueness of wheat flour is the viscoelastic properties of gluten protein (Veraverbeke and Delcour 2002). These storage proteins consist of monomeric gliadin and polymeric glutenin which are very important in breadmaking (Lagrain et al. 2006). The low molecular weight (M.W. < 90,000, LMW-GS) and the high molecular weight (M.W. > 90,000, HMW-GS) glutenin subunits are two major groups of subunits which form large complexes and created by subunits linked together by disulfide bonds (Wieser 2007). These LMW-GS will interact among themselves and with HMW-GS through interchains and intrachains disulfide bonds, which lead to formation of glutenin polymers (Lagrain et al. 2006). Gupta et al (1995) proposed that the structure of dough and loaf quality is associated to the unextractable high molecular weight subfraction of glutenin. Gliadin and glutenin are responsible for the functionality of gluten network during mixing, dough gas retention during proofing and final bread structure (Lagrain et al. 2006).

Dough mixing increases the extractability of protein in different solvents which may be due to gluten depolymerization (Weegels et al. 1997), conformation rearrangement (Eckert et al. 1993) change of effective surface area and better dissolution (Belton 2005). However all these subjects are not confirmed and are still in debate. The addition of reducing agents affects the SS bonds in dough and reduces the molecular

weight of glutenin protein aggregates (Wieser 2007). The dough mixing characteristic changes with treatment of reducing agents (Gao et al. 1992). Lagrain et al. (2006) also suggested that during hydrothermal treatment, redox reagents such as reduction and oxidation agents will affect the capacity of gluten protein to associate by SH-SS exchange reactions and change in level of free SH groups. These free SH groups can influence the flexibility of glutenin chain and activate the polymerization reaction (Lagrain et al. 2006).

In order to further understand the effect of reducing agents on the viscoelastic properties of gluten and breadmaking, dithiotheritol (DTT) was used to reduce the disulfide bonds in gluten network. Dithiotheritol is commonly used to break the disulfide bonds that bind various glutenin subunits together (Woerdeman et al. 2004). These disulfides bond are located in the hydrophobic region of polymeric glutenin and unreachable to reduction by water soluble reducing agent (Kim and Busuk 1995). Kim and Bushuk (1995) also proposed that one difference in glutenin extracted using DTT may be due to the changes of hydrophobic in the system which promotes the interaction in aqueous environment. Ambardekar (2009) studied the effect of DTT on viscoelasticity of gluten, dough mixing and baking properties of commercial flours. He reported that the gluten treated with DTT treatment have slow recovery and low percent recoverability compared to untreated samples. This caused yeasted product to have poor loaf properties and overall baking performance. Ambardekar (2009) also showed that baking properties of the flour samples were independent of the viscoelastic properties of gluten.

The objectives of this study were 1) to analyze the effect of disrupting disulfide bonds on the aggregation of flour batters by the addition of dithiothreitol (DTT)

treatments and 2) to determine a possible correlation between gluten aggregation properties and protein content, viscoelasticity of gluten, baking and mixing properties.

2. Materials and Methods

Six commercial hard red winter wheat flours were analyzed in this study. The samples were obtained from two different regional suppliers. The wheat flours contained different cultivars in order to obtain the different ranges of protein content and quality. The samples were labeled as A and B and numbered 1 through 3, in order of increasing protein content, with 1 being the lowest. Flour protein, moisture and ash were obtained by infrared analysis using an NIR system model 6500-M (FOSS NIR Systems Inc, Laurel, MD).

Four level concentrations of dithiothreitol (DTT) (VWR International Inc, West Chester, PA) were used at 0, 0.1, 0.25, and 0.5mM and added in solution. Each level of DTT were added using a 100 mM stock solution containing 1.54 g of DTT in 100mL deionized water. Working solutions were prepared for each level of DTT (0.1, 0.25, and 0.5mL of stock solution in 100 mL). All aqueous base solutions contained 2% sodium chloride and 5% sucrose instead of water alone. The addition of 2% sodium chloride and 5% sucrose to the aqueous base solution improved the agglomeration process compared to using water alone (data not shown). Samples with no DTT containing 2% sodium chloride and 5% sucrose solution were used as controls.

2.1.Gluten Aggregation Test (Gluten Peak Tester)

Hard wheat flour samples with different levels of DTT (0, 0.1, 0.25, and 0.5 mM) were analyzed with a high shear mixing apparatus, labeled Gluten Peak Tester (GPT,

C.W. Brabender Instruments, Hackensack, NJ). A total weight of 18.5 g of wheat flour sample and solution were used for each replicate and the amount of flour in the solution used was reported as a percentage (as is basis). The samples were mixed in a stainless steel cup at 3,333 rpm and 35^{o}C for 10 minutes. The gluten peak tester parameters consists of peak maximum time (PMT), maximum torque (MT), torque before maximum time (T1) and torque after maximum time (T2) and area under the peak (work) were recorded. The peak maximum time is recorded in seconds (s) and torque parameters are recorded in Brabender Equivalent (BE), an arbitrary unit used by C.W. Brabender Instruments Inc. All analyses were completed in duplicates.

2.2.Gluten Creep Recovery Analysis

Creep recovery tests were performed by Ambardekar (2009) based on the protocols of Zhao and others (2007) and Liang and others (2007). The gluten extraction was prepared in an automated gluten washer, Glutomatic 2200 (Perten Instruments AB, Segeltorp, Sweden) using approved method 38-12.02 (AACCI 2000). Ten grams of flour was wetted with 5 mL of DTT working solution (0.1, 0.25, and 0.5 mL of 100 mM stock solution added to solution containing 2% sodium chloride) before mixing and washing in Glutomatic for 10 minutes. The gluten extracted from the Glutomatic was gently shaped into a gluten ball and relaxed under metal plate (2500 g) for 60 minutes at room temperature (25^{o}C) before the creep recovery measurement.

The gluten prepared was measured on a Rheometer AR1000N (TA Instruments, New castle, DE), and the gap was set at 25 mm at room temperature (25^{o}C). The instrument was set to produce 40 Pa constant stress in order to shear the gluten. The

gluten was maintained at constant stress for 100 s creep test and released to recover for 1000 s. The deformation and recovery of the gluten was measured as compliance. Creep-recovery parameters obtained were: separation time (SeP), delta compliance (J-Jr), % recoverability (RCY), time constant of creep (λ_C), and time constant of recovery (λ_R). J-Jr and λ_C represents the viscous properties of gluten and SeP, RCY, and λ_R represents the elastic behavior of gluten. All analyses were completed in triplicate.

2.3.Dough Mixing

The dough mixing properties were performed by Ambardekar (2009) and according to approved method 54-21.01 (AACCI 2000). Wheat flour samples were analyzed using a 10 g bowl Farinograph-E (C.W. Brabender Instruments, Hackensack, NJ) at 63 rpm at 30^{o}C. Dough development time (DT), stability time (ST), breaking time (BT), and water absorption (WA) adjusted to 14% moisture basis were recorded. One milliliter of DTT working solutions (containing 0.1, 0.25, and 0.5 mL of 100 mM stock solution in 100 mL deionized water) was added to the flour right before mixing. The rest of the water was added following the normal protocol. The control samples were prepared with only deionized water. All analyses were completed in duplicates.

2.4.**Baking Test**

Baking tests were performed by Ambardekar (2009) based on approved method 10-10.03 (AACCI 2000) which is a straight-dough bread making method. Wheat flour samples (100 g) were added with 0.1, 0.25, and 0.5 mM DTT (0.1, 0.25, and 0.5 mL of 100 mM of stock solution in 100 mL deionized water) at the beginning of mixing. Samples were mixed to dough with a 100-g mixer Swanson-Working pin type (National

Mfg. Co. TMCO Inc, Lincoln, NE). Several mixing and baking tests were performed in order to obtain the optimum mixing time and bake water absorption. Bread quality parameters such as dough proof height (PH), loaf height (LH), loaf volume (LV), oven spring (OSP) and specific volume (SV) were measured. The proof height (PH) and loaf height (LH) of bread were measured with a digital proof height gauge (National Mfg. Co. TMCO Inc, Lincoln, NE). The loaf volume (LV) was obtained by rapeseed displacement after the bread was cooled for 10 minutes after taking out from the oven. Oven spring (OSP) was calculated by subtracting of dough proof heights from bread loaf height. Specific volume (SV) was defined as the ratio of loaf volume to loaf weight of bread. All analyses were completed in duplicate.

2.5.Statistical Analysis

The relationship of Dithiothreitol (DTT), flour types (protein content), the aggregation properties (PMT, MT, T1, T2, Peak Area), viscoelastic variables (J-Jr, SeP, RCY, λ_C, and λ_R), mixing properties (WA, DT, ST and BT) and baking characteristics (LV, PH, LH, OSP, SV) were evaluated with Principal Component Analysis (PCA) using Canoco for Windows version 4.5 (Biometris, Plant Research International, Wageningen, The Netherlands). Data input is untransformed, centered and standardized in order to minimize the mean squared error.

3. Results and Discussion

Dithiothreitol is one of the most commonly used reducing agents. It is used to disrupt the disulfide bonds, causing the dough strength to be weakened which in turn affect the mixing behavior (Zhang et al. 2010). It is expected that DTT will produce

disaggregation of protein by scission of disulfide bonds between protein aggregates through reduction. The presence of DTT will prevent the formation of intermolecular and intramolecular disulfide bonds.

General composition analysis (protein, moisture, and ash content) of the six commercial hard red winter wheat flours is shown in Table 1, as reported earlier by Ambardekar (2009). The range of protein is representative of hard red winter wheat flour in the region. An example of the recorded curves and representation of the parameter obtained from gluten aggregation test is shown on Figure 1. Also, an example of gluten aggregation curve from a sample differing in protein content is shown in Figure 2. An expanded definition of the parameters, flour protein and their units are presented in Table 2.

Protein aggregation properties were systematically analyzed using five flour percentages 41, 43, 46, 49, and 51%. The protein aggregation depends on flour quality and occurs in a specific range of flour protein percentage as can be seen in Table 3, 4, 5, and 6. Treatments of different flour percentages cannot be compared directly because not all samples aggregate at the same percent of flour, for example A1 (8.0% protein) did not aggregate when 41% flour of the sample was analyzed by GPT but A3 (13.7% protein) showed aggregation when 41% flour of the sample was analyzed. This showed that the changes from the treatments were informative although they can't be compared directly. The results were analyzed by PCA for each flour percentage (results not shown) and the explained variance for each percentage was used as criteria for selecting single percent flour. The 43% flour was selected out of the five percentage flours that were analyzed because it had the highest explained variance. A total of 40 samples with 43% flour were

analyzed with four different concentration of dithiothreitol (DTT) (0, 0.1, 0.25, and 0.5 mM) with Gluten Peak Tester (GPT) to disrupt the disulfide bonds of gluten.

3.1.Aggregation Peak Time

The effect of the disruption of disulfide bonds on PMT is reported in Figure 3. Peak maximum time (PMT) is the time required for the protein to hydrate and aggregate. The overall trend was a reduction of PMT, as the protein groups were prevented from forming disulfide bonds. Sample A2 with 0.1 mM DTT treatment required more time to aggregate compared to the control sample. As the concentration of DTT increased the time required for the sample to aggregate reduced (Fig. 3). As for sample 1B and 3B, the time needed for these samples to aggregate decreased as DTT concentrations increased. PMT for sample 3B with 0.5 mM DTT decreased drastically as much as 110 s compared to the control sample.

3.2.Aggregation Torque and Aggregation Peak Area

The maximum torque (MT) is an estimate of gluten strength and it is measured in Brabender Equivalent (BE) units. This unit is not an International System of Unit (SI unit) for torque but can be used to compare relative differences required for each sample to aggregate. Overall maximum torque (MT) for samples with different concentrations of DTT appeared to be similar, suggesting no significant effect of DTT (Fig. 4). However, the aggregation peak area increased as DTT was applied to the batter system (Fig. 5). Aggregation peak area is an estimate of work required for aggregation. Reducing effect of DTT can be seen on the first level test (0.1 mM) compared to the control. The increased in work required for aggregation suggests the disruption of disulfide bonds in

the batter system by DTT, which leads to the opening of polymers hence allowing more interactions to occur. Besides that, disruption of disulfide bonds also helps to prevent chemical crosslink thereby reducing the entanglement in the system. This suggests that the physical properties of batter will be weakened in the presence of DTT. Therefore, more work was required in order to form and maintain the gluten aggregation with presence of DTT.

3.3.Principal Component Analysis

Principal component analysis (PCA) is a mathematical multivariate method used to find a linear combination of the variables which account for the total variance by reducing the dimensionality of the data and still containing most of the variation in the data set (Jolliffe 2002; Mukhopadhyay 2009). The reduction is done by showing the direction (principal component) where the highest variation of data is achieved (Ringner 2008). The samples are further represented by the number of principal components instead of the actual value from the thousands of variables (Ringner 2008). Samples are plotted to tell see if there are similarities or differences among the samples and also to determine whether they can be grouped (Ringner 2008).

PCA of gluten aggregation and flour protein resulted in an ordination plot which captured 90% of the explained variance with both PC 1 and PC 2, explaining 73.2 and 16%, respectively (Fig. 6, Table 7). MT, T2 and flour protein were highly dominated on PC1 by contributing 96, 95 and 85%, respectively (Table 7). PC2 is dominated by area under peak (total work) by contributing 83% of variance.

The samples are clearly well distributed in 3 different groups. High protein sample 3A is separated on the right quadrant and highly correlated to flour protein and lower protein samples (< 12% protein content) were distributed on the left quadrant (Fig. 6). Control samples were distributed on the top corner of the plot with majority samples on the left quadrant and 3A on the right quadrant (Fig. 6).

The results obtained from gluten aggregation test were further analyzed with PCA ordination in order to assess the relationship between aggregation, gluten viscoelastic, mixing and breadmaking properties as well as flour protein (Table 8). This analysis was shown in a two dimensional correlation biplot (Fig. 7) and capturing 70% of total variance. Both PC1 and PC 2 explained 41 and 29% of the total variance, respectively (Table 8). PC 3 (9%) and PC 4 (5%) shows low explained variance therefore were not discussed further. When examining the contribution of explained variance by each variable (Table 8), PC1 was highly influenced by aggregation parameters. Aggregation parameters were dominated by maximum torque with 89% of the variance followed by torque after aggregation and PMT with 89 and 75% of the variance, respectively (Fig. 7, Table 8). Flour protein, MT and T2 were highly associated with protein content of flour had the highest contribution (95%) (Table 8). This suggests that the protein content of flour is important in protein aggregation in batter. It is understandable because MT gives an estimate of the gluten network strength. In contrast, PMT and T1 are negatively correlated to protein content.

Baking properties were highly associated among each other (redundant) and to PC2 with LV (83%) showing the highest contribution of variance followed by SV (79%), LV (73%), OSP (68%) and PH (64%) (Fig.7, Table 8). The rate of deformation and delta

compliance (viscous properties) are highly associated to area under the peak and negatively correlated with percent recovery and separation of time (elastic properties) (Fig. 7). Area under the peak explained the work required for gluten aggregation. This means that the batter become more viscous when DTT was added to the batter system thereby preventing gluten aggregation by disrupting the disulfide bonds that binds the gluten network.

Based on the loading plot, the samples were distributed into three groups, the high protein samples 3A (13.7%) were located on the right quadrant of the plot (Fig. 7). When the level of DTT increased, 3A moved from mixing properties to being positively associated to viscous properties. This group was negatively correlated to elastic properties. This suggests that the presence of DTT increased the viscosity of the sample. Sample 3B located slightly on the middle of the plot also showed negative correlation to baking properties as the level of DTT increased (Fig. 7). Plus, sample 3B with the highest level of DTT (0.5mM DTT) was highly associated to TCR (viscoelastic properties) and area under peak (total work). This suggested that at this level DTT is breaking S-S bonds and hence preventing agglomeration in the batter, this behavior is also seen in sample A3. With the disruption of SS bonds, they are now more related to the viscous parameters, suggesting that they had disaggregated and flowed easily. The low protein samples group is located on the left quadrant of the plot and they were highly associated among themselves (Fig. 7). The proximity of 1B and 2A with 0.1 mM DTT suggested that these two samples were similar. Both the samples were positively correlated to T2 and T1 (gluten aggregation tester) which were related to torque and gluten strength. This supports the high contribution of the explained variance by flour

protein. When agglomeration properties are not taken into account (not included in PCA), these two samples appeared to be more distant as reported by Ambardekar (2009). These suggested that agglomeration properties are useful to understand the role of the disulfide bonds, suggesting that the disruption of disulfide bonds will show how samples can give similar behavior to other samples after the partial disruption of disulfide bonds. The third group was the control samples and these samples located on the lower left quadrant of the plot and were highly associated to the elastic properties (Fig. 7).

It is well reported that DTT treatments disrupt the disulfide linkages and modify the nature and density of crosslink in gluten viscoelasticity and prevent aggregation between glutenin and gliadin (Bean and Lookhart 1998). The disruption of disulfide bonds also unfold and expose the gluten macropolymers' hydrophobic domains. Other factors that can explain the effect of DTT on viscoelastic, mixing, baking and gluten aggregation properties on different group of gluten are the differences in density of disulfide crosslink (Khatkar 2005), type of glutenin viscoelasticity (Southan and MacRitchie 1999), ratio between glutenin and gliadin (Uthayakumaran et al. 1999) and molecular size range and molecular size distribution of gluten properties (Gupta et al. 1995).

4. Conclusions

Total work (area under peak) was highly influenced by the reducing properties exhibited due to the addition of DTT. Disulfide bonds are reduced with the lowest level (0.1 mM) of DTT addition causing an increase in peak area. However, the reducing effect of DTT on PMT appears to be more complex, i.e., sample dependent and not

always dose dependent. Agglomeration properties and flour protein principal component analysis separates the samples into 3 groups. Principal component analysis of viscoelastic, mixing, baking and agglomeration properties indicated that the gluten strength (maximum torque (MT) and aggregation after torque (T2)) were highly associated to flour protein. Flour protein is the variable that explains the highest variance and followed by maximum torque and torque after aggregation. This suggests that protein flour and aggregation properties should be sufficient to explain the variance on the effect of reducing disulfide bonds on wheat batter systems of commercial flours. The reduction of disulfide bonds increases the viscous behavior of all batter systems.

Table 1. Partial analysis (means ± SD, n=2) of six commercial flours.

Flours	Protein (%)	Moisture (%)	Ash (%)
1A	8.0 ± 0.05	11.7 ± 0.02	0.29 ± 0.01
2A	11.2 ± 0.07	10.5 ± 0.03	0.38 ± 0.01
3A	13.7 ± 0.02	10.1 ± 0.02	0.41 ± 0.00
1B	10.4 ± 0.10	12.4 ± 0.02	0.47 ± 0.00
2B	10.6 ± 0.07	12.6 ± 0.00	0.48 ± 0.01
3B	11.4 ± 0.01	13.0 ± 0.04	0.58 ± 0.01

SD = Standard deviation.

Adapted from Ambardekar (2009).

Table 2. Definition of gluten agglomeration test, viscoelastic, mixing and baking parameter terms.

Properties	Abbreviations	Definitions	Units
Agglomeration	PMT	Peak maximum time defined as the time needed for aggregation to occur.	s
	MT	Maximum torque defined as the torque required when the peak of aggregation occurs. MT is a measure of gluten strength.	BE
	T1	Torque before aggregation defined as the torque required before peak of aggregation occurs.	BE
	T2	Torque after aggregation defined as the torque required after peak of aggregation occurs.	BE
	AREA	Area under maximum peak is the total work of aggregation.	BE.s
Mixing	WA	Water absorption	%
	DT	Development time for the flour to develop to dough of 500 BU consistency	min
	ST	Stability time that a dough stayed at a consistency of 500 BU	min
	BT	Breaking time when dough start to breakdown during mixing	min
Viscoelastic	SeP	Separation time defined as the time at which the creep and recovery split and no longer stay superimposed	s
	J-Jr	Delta compliance defined as the difference in compliance of creep and recovery at 100s.	Pa^{-1}
	RCY	Percent recoverability defined as the elastic ability of gluten to recover to its original state as stress is removed	%
	λ_R	Time constant of creep defined as the rate of elastic recovery of gluten to reach equilibrium	s
	λ_C	Time constant of recovery defined as the rate of deformation of gluten	s

Baking	LV	Loaf volume	cm^3
	LH	Loaf height	mm
	PH	Proof height	mm
	OSP	Oven spring	mm
	SV	Specific volume of baked loaves	cm^3/g
Flour protein	FP	Flour protein	%

Note: BE, Brabender Equivalent, arbitrary unit (not SI).

Table 3. Protein aggregation for flour batter systems with different flour percentages without DTT (0 mM, control). Flour protein (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4.

	Type of flour					
% Flour	A1	A2	A3	B1	B2	B3
41	-	-	+	-	-	-
43	-	+	+	+	+	+
46	-	+	-	+	+	+
49	+	+	-	+	+	+
51	+	-	-	-	-	-

Table 4. Protein aggregation for flour batter systems with different flour percentages with 0.1 mM DTT. Flour protein (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4.

	Type of flour					
% Flour	A1	A2	A3	B1	B2	B3
41	-	-	+	-	-	-
43	-	+	+	+	+	+
46	+	+	-	+	+	+
49	+	+	-	+	+	+
51	+	-	-	-	-	-

Table 5. Protein aggregation for flour batter systems with different flour percentages with 0.25 mM DTT. Flour protein (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4.

	Type of flour					
% Flour	A1	A2	A3	B1	B2	B3
38	-	-	+	-	-	-
41	-	-	+	-	-	-
43	-	+	+	+	+	+
46	+	+	-	+	+	+
49	+	-	-	+	+	-
51	+	-	-	-	-	-

Table 6. Protein aggregation for flour batter systems with different flour percentages with 0.5 mM DTT. Flour protein (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4.

	Type of flour					
% Flour	A1	A2	A3	B1	B2	B3
38	-	-	+	-	-	-
41	-	-	+	-	-	-
43	-	+	+	+	+	+
46	+	+	-	+	+	+
49	+	-	-	+	+	-
51	+	-	-	-	-	-

Table 7. Explained variance (%) in PCA of agglomeration and flour protein from commercial flour treated with DTT.

	PC1	**PC2**	**PC1+2**
	73	16	90
PMT	85	3	88
MT	96	1	97
T1	65	10	76
T2	95	1	96
Area	14	83	96
FP	85	0	85

Table 8. Explained variance (%) in PCA of agglomeration, viscoelastic, mixing, and baking properties from commercial flour treated with DTT.

Variables		PC1	PC2	PC1+2
	PC	41	29	70
Agglomeration	PMT	75	3	78
	MT	89	0	89
	T1	53	1	54
	T2	88	0	89
	Area	8	35	43
Viscoelastic	SeP	13	45	59
	J-Jr	50	19	69
	RCY	24	30	54
	λ_R	6	24	31
	λ_C	21	38	59
Mixing	WA	73	2	76
	DT	77	5	82
	ST	30	11	40
	BT	64	0	64
Baking	PH	12	64	76
	LH	7	83	89
	SV	13	79	92
	OSP	2	68	70
	LV	15	73	89
Flour Protein	FP	91	0	91

Noted: Abbreviations are listed on Table 2.

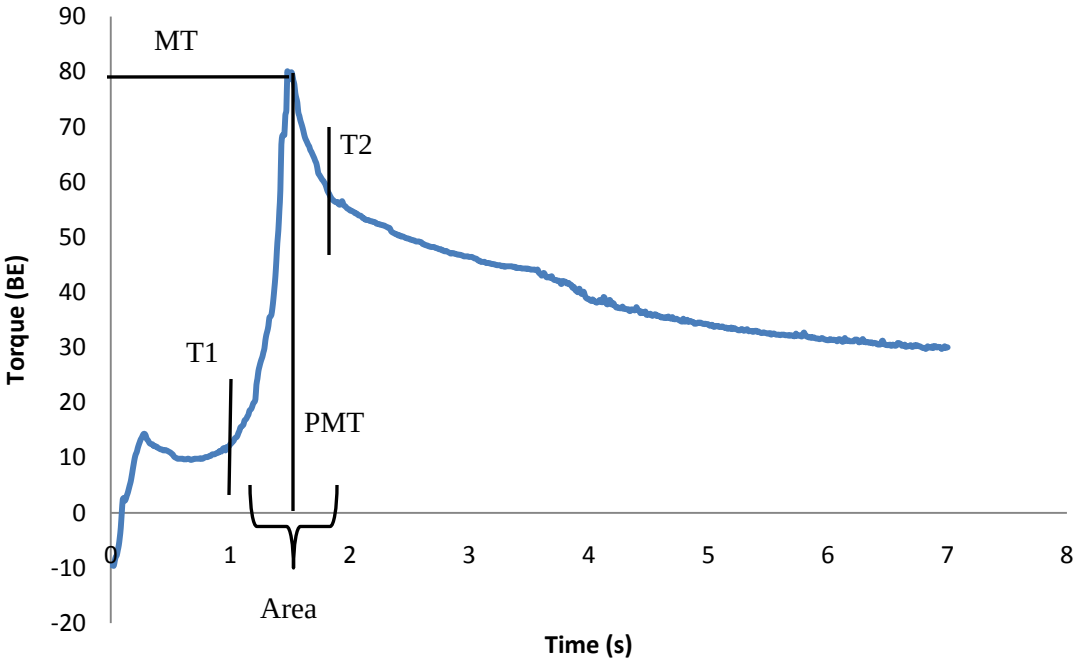

Figure 1. Example of a gluten aggregation test curve obtained with a high speed (3,333 rpm) mixer (Gluten Peak Tester) at 35°C. Parameters recorded were maximum torque (MT), torque before and after peak (T1 and T2, respectively), peak maximum time (PMT) and peak area.

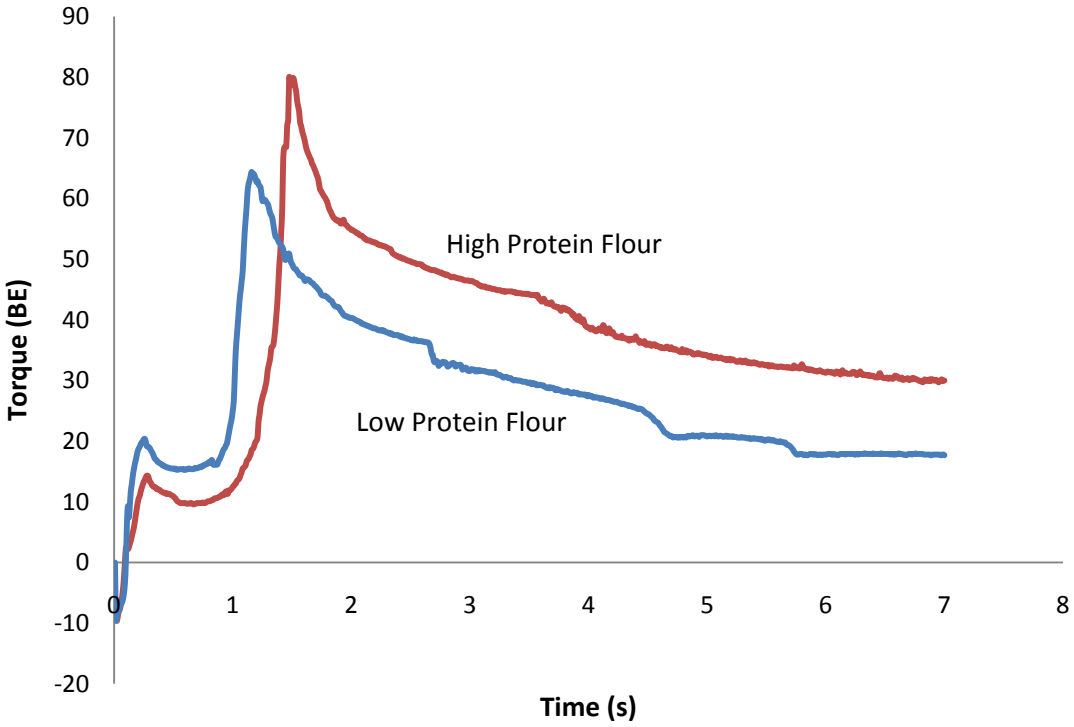

Figure 2. Example of gluten aggregation curves of commercial flours obtained with a high speed (3,333 rpm) mixer (Gluten Peak Tester) at 35^{o}C. High protein flour (3A = 13.7%) and low protein flour (1A = 8.0%).

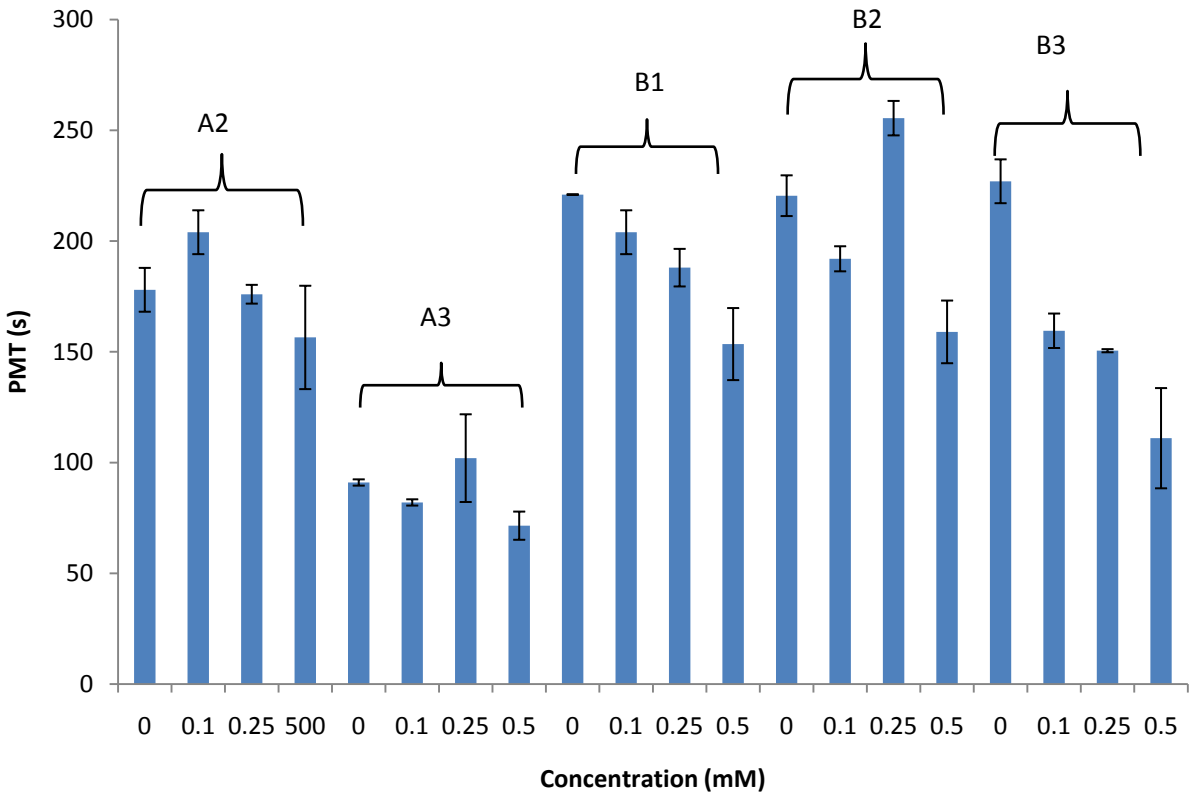

Figure 3. Peak maximum time of gluten aggregation test (43% flour) for commercial wheat flours containing four Dithiothreitol (DTT) levels (0, 0.1, 0.25, and 0.5 mM). Overall samples A1 did not aggregate. Means (n=2) and standard deviation bar. Flour protein content (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4.

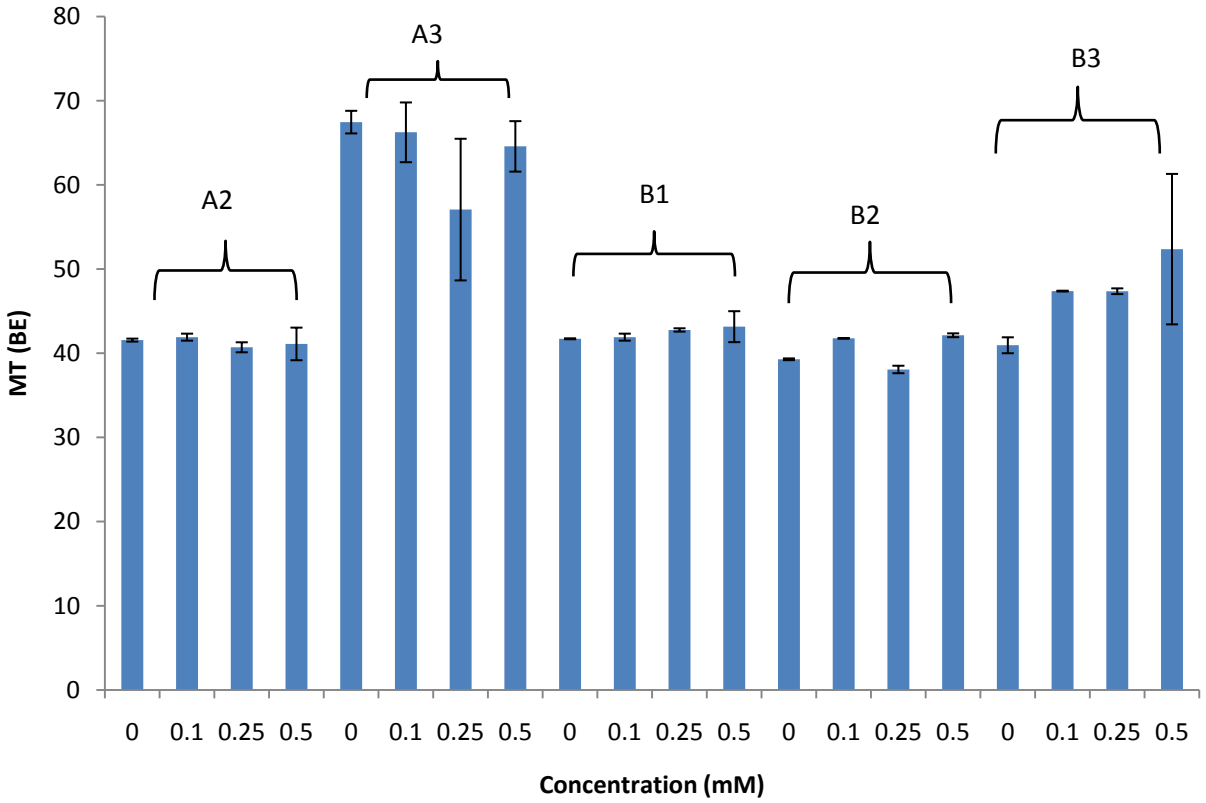

Figure 4. Maximum torque of gluten aggregation test (43% flour) for commercial wheat flours containing four Dithiothreitol (DTT) levels (0, 0.1, 0.25, and 0.5 mM). Samples A1 did not aggregate. Means (n=2) and standard deviation bar. Flour protein content (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4.

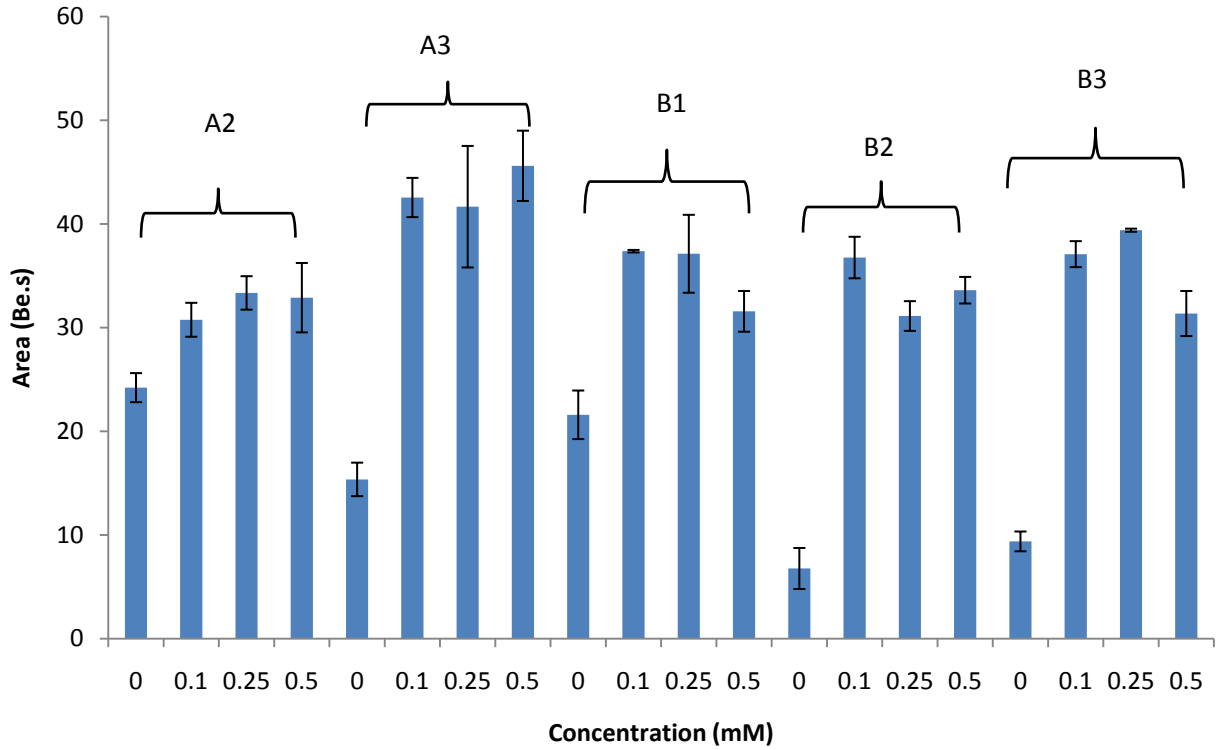

Figure 5. Peak area of gluten aggregation test (43% flour) for commercial wheat flours containing four Dithiothreitol (DTT) levels (0, 0.1, 0.25, and 0.5 mM). Overall samples A1 did not aggregate. Means (n=2) and standard deviation bar. Flour protein content (%), 1A = 8.0, 2A = 11.2, 3A = 13.7, 1B = 10.4, 2B = 10.6 and 3B = 11.4.

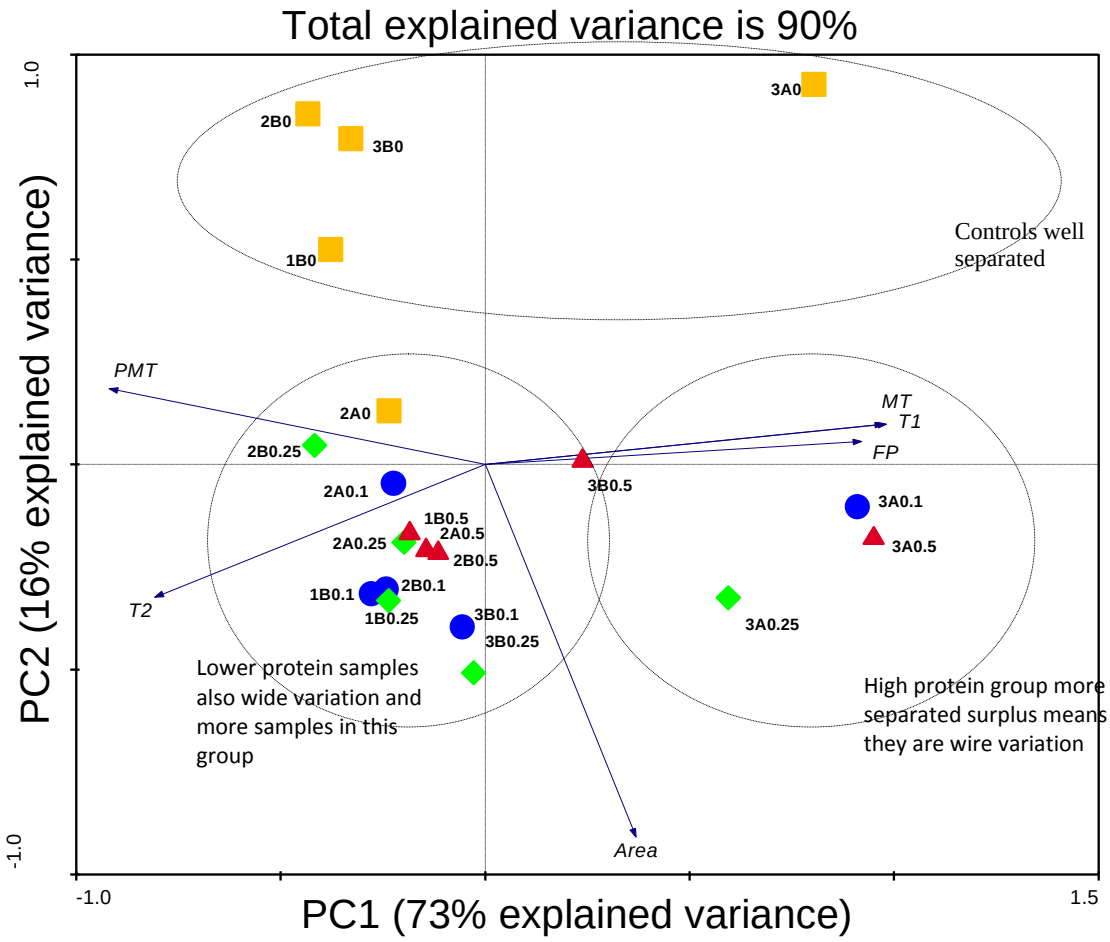

Figure 6. Loading plot of the first two principal components based on gluten agglomeration test properties (GPT) and protein content of six commercial wheat flours, containing (0, 0.1, 0.25, and 0.5 mM) DTT. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.40, 2B = 10.59 and 3B = 11.38. ■, ●, ♦, and ▲ represent 0, 0.1, 0.25, and 0.5 mM DTT, respectively. Abbreviations are listed on Table 2.

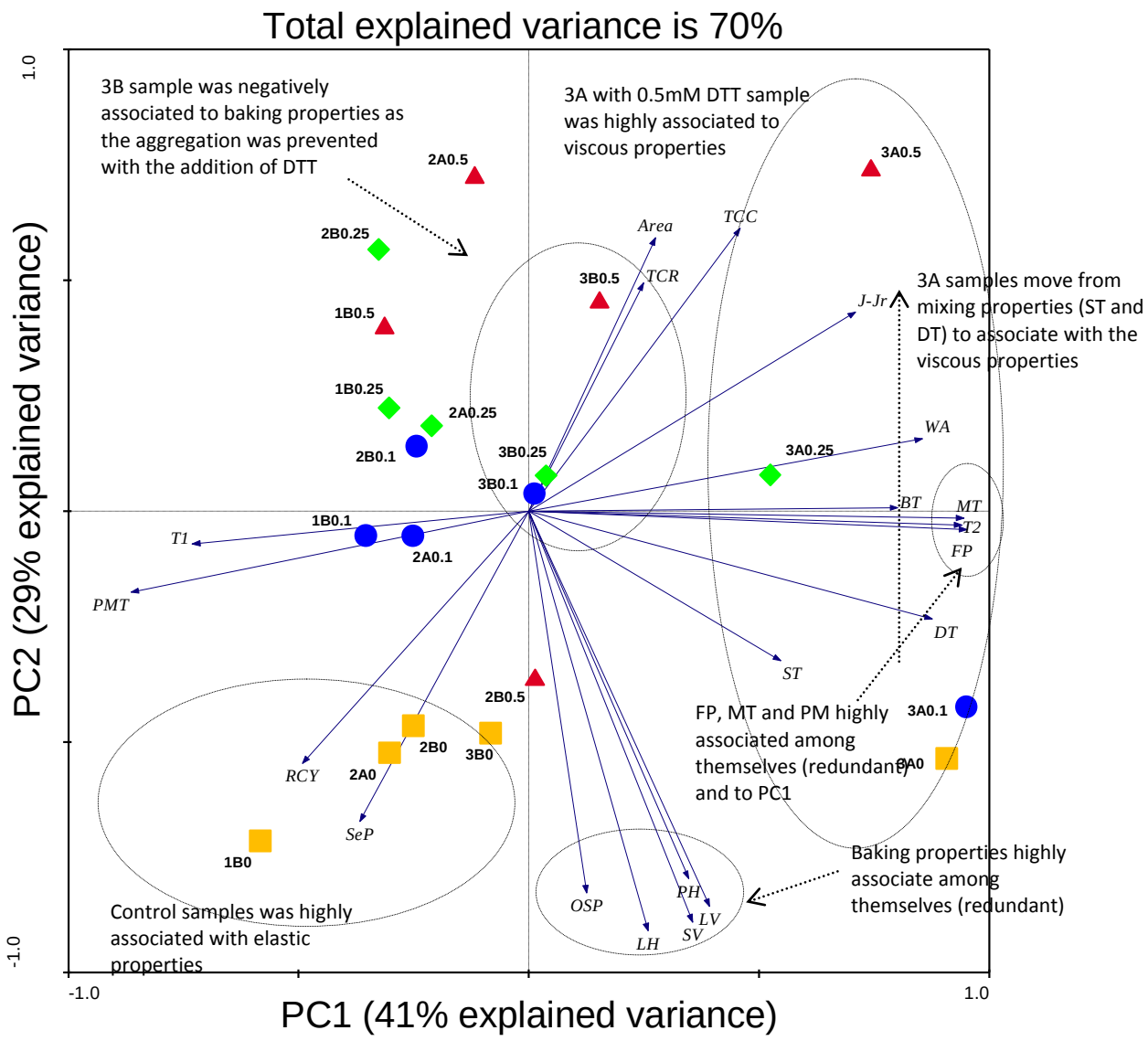

Figure 7. Loading plot of the first two principal components based on gluten agglomeration test, Farinograph, viscoelastic and baking properties as well as protein content of six commercial wheat flours, containing (0, 0.1, 0.25, 0.5 mM) DTT. ■, ●, ♦, and ▲ represent 0, 0.1, 0.25, 0.5 mM DTT respectively. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.40, 2B = 10.59 and 3B = 11.38. TCC and TCR are listed as λ_R and λ_C. Abbreviations are listed on Table 2.

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CHAPTER VI

CONCLUSIONS

The aggregation properties of six commercial flours were studied by modifying the surface tension and disulfide bonds of flour batters by the addition of DATEM, ascorbic acid, and dithiothreitol (DTT). The aggregation properties of batter with 43% flour were analyzed using a gluten agglomeration test.

DATEM reduces the surface tension between different phases (starch, protein and lipids). DATEM greatly increased the work of agglomeration (peak area); while the effect on PMT and strength (MT) appeared to be reduced as the surface tension reduced. The work of agglomeration (peak area) was the main factor that separated the properties of the flour batters. It had the highest contribution (91% explained variance) to the separation of samples on PC1(correlation of agglomeration properties and protein content). Work of agglomeration also had the highest contribution on PC2 of the analysis comparing to several properties (agglomeration, rheological, mixing and baking). Control samples (no DATEM treatments) were negatively correlated to agglomeration peak area, suggesting that they required less work when the gluten aggregates. Different responses to the decrease of surface tension will assist in making inferences on the nature of the different structures and interactions of strength that gluten form in a batter system.

The oxidation of gluten proteins by the addition of ascorbic acid (AA) influenced the work of agglomeration (peak area) the most. Effect of oxidation of gluten proteins on area under the peak (total work) showed a maximum value at 50 ppm AA and a steady decrease with the increase of oxidation level. The effect of oxidation on PMT appears to

be complex and does not depend on the AA dose. More studies are needed with different flour quality to confirm these observations. Samples with different oxidation levels (0, 50, 100, 150, 200 ppm AA) can be separated by agglomeration properties and flour protein through PCA. Overall, PCA suggested that agglomeration properties of flour batters were highly associated to loaf volume and specific volume of baked products.

The reduction of disulfide bonds on gluten protein by the addition of DTT increased the work of agglomeration (peak area) of flour batters. An increase in work of agglomeration was observed with the lowest reduction levels in this study (0.1 mM DTT). The effect of reducing disulfide bonds on PMT and strength (MT) was more complex and cannot be generalized. More studies are needed to confirm these findings. When agglomeration was combined with flour protein, viscoelastic, mixing, and baking in principal component analysis, it revealed that flour protein explained the highest variance followed by batter agglomeration strength (maximum torque) and torque after aggregation. These three variables were highly associated among themselves. Reduction of disulfide bonds on gluten increased the viscous behavior of all batter systems.

Gluten agglomeration test appears to be a promising tool to separate flour based on their quality. Agglomeration is highly dependent on the quantity and quality of protein in flour. The test is relatively fast and uses small sample size, therefore it has some advantages over more time consuming tests used at the present time in flour quality evaluation settings.

CHAPTER VII

FUTURE STUDIES

- Changing the hydrophobicity of the gluten agglomeration in batters appeared to be very useful since it revealed wide differences in the flours. With the highest percent of IPA (30%) only the highest protein sample agglomerated in such increased hydrophobic environment. I recommend adjusting the percentages of IPA to accommodate the lower protein quantity and quality samples. May be a screening using less than 10% IPA could be useful.
- Microscopic visualization of the effect on gluten protein strands from different bond type disturbances or changes (by treatments with IPA, DATEM, AA, urea, and DTT) in batter using confocal laser scanning microscopy (or other less expensive microscopy type) will help our understanding of the localization and interactions of different treatments within the complex batter system.
- Six commercial flour samples were analyzed in this study. I suggest increasing the number of samples with wider range of protein quantity and quality to expand our scope to other behaviors. It will also enhance the present understanding of gluten aggregation with different modifications.

- The temperature (35^{o}C) and speed (3,333 rpm) of gluten agglomeration test were constant in this study. A study with different temperature and speed values can be performed and compared with the present report.

APPENDIX

VITA

Sengwooi Lim

Candidate for the Degree of

Master of Science

Thesis: EFFECT OF PHYSICAL CHANGES ON REDOX STATE AND SURFACE TENSION ON GLUTEN AGGREGATION, VISCOELASTIC, MIXING AND BAKING PROPERTIES OF WHEAT BATTER SYSTEMS.

Major Field: Food Science

Biographical:

Education:

Completed the requirements for the Master of Science in Food Science at Oklahoma State University, Stillwater, Oklahoma in July, 2011.

Completed the requirements for the Bachelor of Science in your major at Oklahoma State University, Stillwater, OK in 2007.

Experience: Graduate Research Assistant, Robert M. Kerr Food and Agricultural Products Center, Oklahoma State University

Professional Memberships: AACC International (formerlyAmerican Association of Cereal Chemists)

Name: Sengwooi Lim Date of Degree: July, 2011

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: EFFECT OF PHYSICAL CHANGES ON RED-OX STATE AND SURFACE TENSION ON GLUTEN AGGREGATION, VISCOELASTIC, MIXING AND BAKING PROPERTIES OF WHEAT BATTER SYSTEMS.

Pages in Study: 124 Candidate for the Degree of Master of Science

Major Field: Food Science

Scope and Method of Study:

The aggregation properties gluten proteins in batters were studied by modifying the surface tension and disulfide bonds of flour batter by the addition of DATEM, ascorbic acid, and dithiothreitol. The properties of aggregation were analyzed with a gluten agglomeration test. Associations of flour protein, agglomeration, viscoelastic, mixing and baking properties were also analyzed by principal component analysis.

Findings and Conclusions:

Decrease in surface tension by the addition of DATEM greatly increased the total work of agglomeration (area under the peak). The different responses due to decrease of surface tension will assist in making inferences on the nature of the different structures and interactions strength that the gluten forms in a batter system. The oxidation of gluten proteins by the addition of ascorbic acid also highly influenced the total work of agglomeration (area under the peak). The effect of oxidation of gluten proteins on area under the peak showed a maximum value at 50 ppm and a steady decrease with increased of oxidation level. Overall, PCA suggested that agglomeration properties of flour batters were highly associated to loaf volume and specific volume of baked pup loaves. The reduction of disulfide bonds on gluten protein by the addition of DTT increased the work of agglomeration (area under the peak) of flour batters with the lowest reduction levels with 0.1 mM DTT. Gluten agglomeration test appears to be a promising tool to separate flour properties. The combined PCA also showed that flour protein explained the highest variance followed by batter agglomeration strength (maximum torque) and torque after aggregation. Reduction of disulfide bonds on gluten also increased the viscous behavior of all batter systems. Overall, the gluten agglomeration test is relatively fast and uses small sample size; therefore it has some advantages over more time consuming tests (mixing and baking tests) used at the present time in flour quality evaluation settings.

ADVISER'S APPROVAL Dr. Patricia Rayas-Duarte