DETERMINATION OF THE BINDING PROPERTIES AND RHEOLOGICAL BEHAVIOR OF REDUCED GLUTENIN SUBFRACTIONS IN THE PRESENCE OF CARBOHYDRATES

By

MA. CRISTINA SUCGANG ESCOBER

Bachelor of Science

University of the Philippines

Los Baños, Laguna

1998

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2003

DETERMINATION OF THE BINDING PROPERTIES AND RHEOLOGICAL BEHAVIOR OF REDUCED GLUTENIN SUBFRACTIONS IN THE PRESENCE OF CARBOHYDRATES

Thesis Approved:

Patricia Rayas Duarte
Thesis Adviser

Daniel Bellmer

Andrew Mort

Gordon Emslie
Dean of the Graduate College

ACKNOWLEDGEMENTS

I would like to express my gratitude to my adviser, Dr. Patricia Rayas-Duarte for her guidance and generosity. The invaluable knowledge imparted by Dr. Andrew Mort and Dr. Danielle Bellmer is also acknowledged for the completion of this research. My sincere appreciation also extends to Dr. Guadalupe Davila-El Rassi and Dr. Juntanee Uriyapongson for their encouragement, suggestions for my research, and friendship. The professional assistance and moral support provided by Frances Griffin, Wang Zheng Guang and Menchu Wang are also sincerely appreciated.

I have drawn my inspiration to further my education from the love and support of my family and KK. Most importantly, I owe this opportunity of obtaining education at OSU to the kindness and generosity of my Uncle Brendan and Aunt Nanette O'reilly, to whom I am truly indebted.

Finally, I would like to thank the faculty and staff of Oklahoma Food and Agricultural Products Research and Technology Center for their support during these years of study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS USED

AUFS	absorbance units full scale
ACN	acetonitrile
BSA	Bovine Serum Albumin
CE	capillary electrophoresis
ChE	Chain extension/extender/propagators
ChT	Chain termination/terminator
cm	centimeter
CV	Coefficient of Variation
cv.	cultivar
CZE	capillary zone electrophoresis
Da	Daltons
d.b.	dry basis
DTT	dithiothreitol
EOF	electro-osmotic flow
FZCE	free zone capillary electrophoresis
g	grams
G'	storage modulus, Pa
G"	loss modulus, Pa

h	hour
HCl	Hydrochloric acid
HMW-GS	high molecular weight-glutenin subunits
HPLC	high performance liquid chromatography
Hz	Hertz
i.d.	internal diameter
J	Joules
K_b	binding constant
kDa	kilo Daltons
KIO_3	potassium iodate
kV	kilovolts
LMW-GS	low molecular weight-glutenin subunits
LVR	linear viscoelastic region
min	minutes
µl	microliter
mM	millimolar
µM	micromolar
µm	micrometer, microns
MW	molecular weight
NaOH	sodium hydroxide
NIR	near infrared
nm	nanometer

°C	degrees Celsius
Pa	Pascals
R^2	Coefficient of Correlation
RP-HPLC	reverse phase-high performance liquid chromatography
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl-sulfate polyacrylamide gel electrophoresis
sec	seconds
S-S	disulfide
TFA	trifluoroacetic acid
UV	ultraviolet
vs.	versus
w/v	weight per volume
$\geq$	greater than or equal to
$\simeq$	approximately equal to
$>$	greater than
$<$	less than

CHAPTER I

INTRODUCTION

Flours milled from different wheat cultivars can vary widely in work input required for optimum dough development. The fundamental reason for this is not fully understood. It is believed that inter-protein interactions are involved in the observed differences among cultivars (Bushuk, 1998).

Knowledge of basic biochemical properties of cereal proteins is valuable in many ways. The ability to differentiate cultivars is important during breeding, marketing, utilization, and research. Generally, this differentiation is highly relevant for quality prediction and for identification of specific cultivars. It is well established that protein amount alone is not sufficient to provide such information due to its heterogeneity and complexity (Bean *et al.*, 1998). Evidence of wheat protein's complexity is shown in its inability to establish pure subfraction recovery during separation based on the Osborne solubility method.

Physico-chemical characterization is used to predict end-use quality of wheat flour. The focus of such methods is mainly to correlate the amount of proteins with its composition and other functional characteristics. Wheat flour can contain between 6% and 14% protein, most of which is in the form of gluten, a highly extensible polymeric material when hydrated. Both the quantity and quality of gluten protein are key indicators of wheat quality, especially in relation to bread making (Stauffer, 1998).

Gluten proteins are thought to be responsible for the gas retention properties of bread dough during baking. Thus, baking quality is determined largely by quantitative and compositional differences in the proteins that make up the gluten. The unique properties of gluten are the main determinants of quality variation among different wheat cultivars (Dobraszczyk, 2001). However, the gluten matrix itself poses another challenge. The events that occur when gluten proteins are hydrated and mixed are not completely understood. The proteins are continually interacting with other flour components, both soluble and insoluble, as well as additional ingredients in the dough (Stauffer, 1998).

Thus, other methods may still be essential in providing a better understanding of the wheat protein matrix of the dough. As suggested by studies of Weegel, *et. al* (1998), these tests may predict the end-use quality of the flour. The suggested methods include the following: gel electrophoresis, capillary electrophoresis, rheology, immunoassay tests, mass spectroscopy and DNA analysis, to name a few.

SIGNIFICANCE OF THE STUDY

The overall acceptability of a baked product relies equally on its intrinsic and extrinsic qualities. Bread volume, followed by grain characteristics, crust formation, and color are given full consideration in categorizing baking qualities. Prior to baking, fundamental and empirical methods are used to predict the baking performance. Although very useful in predicting handling qualities (e.g. mixing time, water absorption, and dough strength), such tests have disadvantages such as the need for large sample quantities and length of analysis.

Gluten proteins govern most of the baking properties of wheat. Fractionation of gluten produces gliadin and glutenin. Gliadin is believed to contribute primarily to viscosity and extensibility (Gianibelli *et al.*, 2001). Glutenin, on the other hand, has a substantial influence on baking strength. Thus, more emphasis on breadmaking qualities is given to the glutenin fraction. Glutenin is further classified as high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS).

Cereal scientists have proposed means to predict baking qualities by consolidating rapid test methods. Common physico-chemical analyses include the use of reverse phase high performance liquid chromatography (RP-HPLC), sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), capillary zone electrophoresis (CZE) and rheometry. These analyses have been correlated to breadmaking qualities based on comparisons of the hydrophobic nature, molecular weight distribution, net surface charge and viscoelastic properties of wheat glutenins. Presently, these tests are consolidated to derive more meaningful aspects of defining wheat quality.

Separation of glutenin based on hydrophobicity was exemplified by studies of Burnouf and Bietz (1984). The RP-HPLC revealed distinct molecular weight distributions of glutenin fractions in accordance with the level of organic solvent.

CZE, on the other hand, has the potential to characterize the charge to size ratio. Another application of capillary electrophoresis, one which may best benefit the characterization of cereal proteins, is determining the binding constants using affinity capillary electrophoresis. Connors (1987) and Hulme (1992) expressed the importance of the binding interactions of ligand to receptor proteins particularly in chemistry, biochemistry, and molecular medicine. Measurements of the binding stoichiometries and

equilibrium constants are equally valuable. Results obtained from these analyses provide insight into the mechanism and specificity of interactions at the molecular level. The principle is that there is a change in the electrophoretic mobility of a receptor protein upon binding to a charged ligand present in the electrophoresis buffer mainly due to changes in charge to size ratio. The binding constants reflect inherent intermolecular interactions in quantitative terms (Chu *et al.*, 1994).

Davila-El Rassi *et al.* (2003) reported qualitative molecular association of glutenins and maltodextrin using CZE. The hypothesis was that the maltodextrin, a neutral polymer, would form a complex with glutenins. Such a complexation was assumed to reduce the electrophoretic mobility of glutenins due to changes in charge to size ratios. The glutenin fractions were highly heterogeneous and the mobility curves did not exhibit the usual rectangular hyperbolic forms of 1:1 binding isotherms.

Dynamic changes in molecular configuration of subsets of polypeptides in glutenin can result in modification of its physical properties. Hence, understanding a model starch-protein gel system by means of fundamental dynamic rheology can assess the viscoelastic behavior of individual or groups of glutenin subfractions.

OBJECTIVES OF THE STUDY

The objectives of this study were:

1. To characterize two hard red winter wheat cultivars based on hydrophobicity, molecular weight distribution, and net surface charge of the reduced soluble glutenin fractions using RP-HPLC, SDS-PAGE and CZE.

2. To determine the binding constants between maltodextrin with average molecular weight of 2,000 and reduced soluble glutenin fractions from RP-HPLC using affinity capillary electrophoresis (ACE).

3. To investigate the fundamental viscoelastic properties of soluble glutenin fractions in the presence of starch treated with a heat and reduction-oxidation systems.

For the percent peak areas, binding constants and rheological properties (G', G" and tan delta) of the glutenin subfractions, contrast analyses were conducted to detect whether (a) there were significant differences between the enriched HMW-GS and LMW-GS within each cultivar and (b) the differences between the enriched HMW-GS and LMW-GS subfractions were significant upon comparing the two cultivars.

CHAPTER II

REVIEW OF LITERATURE

MOLECULAR INTERACTIONS IN THE DOUGH

Dough is a dynamic system attributed to the covalent and non-covalent bonds resulting primarily from glucosidic bonds of starch and intermolecular disulfide bonds of storage proteins. The α(1-4) bond, which forms the backbone of the starch molecules, is susceptible to hydrolysis by α- and β-amylases. Some of these α(1-4) bonds are continuously being broken down during mixing to produce fermentable sugars until the amylases are inactivated during baking. The disulfide bonds play a key role in forming strong cross-links among polypeptide chains and stabilize the hydrophobic and hydrogen bonds (Bushuk, 1998).

The thiol groups may affect the disulfide bonds in two ways: (a) by oxidation, new disulfide bonds may be formed; and (b) interchange reaction may occur between S-S bonds and thiol groups, causing a dynamic change in the distribution of disulfide linkages (Bloksma, 1980). On the contrary, reduction of disulfide bonds causes an increase of sulfhydryl (SH) groups. Oxidation strongly affects the rheological properties of dough since it removes thiol groups and forms new disulfide bonds. Lásztity (1995) contradicted this and suggested that the quantity of oxidizing agents used for improving the rheological properties of dough is very small and the precondition for the formation of new disulfide bonds is sterically favorable for the positioning of two thiol groups.

Therefore, the quantity of the newly formed S-S bonds may be extraordinarily small. Moreover, certain polymeric storage proteins of wheat responsible for the disulfide interchanges, are the main determinants of the dough functional properties (Bekes et al. 1994).

Bloksma and Bushuk (1988) proposed a hypothetical re-orientation of protein chains in dough under the influence of mixing stress through disulfide interchange interaction. In dough development, sulfhydryl groups, along with rheologically effective disulfide bonds, facilitate the alignment of protein chains by mixing to form a structure optimal for a particular baking process. The authors suggested that this hypothesis should not be overemphasized due to the complexity of the interactions in the dough.

Non-covalent bonds also occur in the dough system. According to studies conducted by Wehrli and Pomeranz (1969), the overall effect of ions is promotion of the association of dough components resulting in increased dough rigidity and reduced dough extensibility. Other bonds among polypeptide chains include hydrogen bonds, van der Waals and hydrophobic bonds. These categories of molecular interactions are present in large numbers, however, such interactions are not given extensive consideration because of their presumed weaker significance to the dough formation compared to disulfide bonds.

GLUTEN PROTEIN

The target for wheat studies or protein-protein interactions is gluten, which is known as the viscoelastic mass that gives wheat dough its unique properties (Wrigley et al., 1998). The fractionation of the cereal proteins based on solubility has retained its

importance to the present time. It gives relatively reproducible results that provide some information about the proteins. However, the fractions obtained are mixtures of different proteins. Each group have subgroups, none of the groups consist of a single protein (Lásztity, 1996).

GLIADINS

Osborne classification (Osborne, 1907) described gliadins as the fraction of gluten soluble in 70% ethanol. Woychik *et al.* (1961) stated that approximately 50% of storage proteins found in wheat are gliadins. Gliadins are further subdivided into ω-, γ-, β-, and α-gliadins based on their mobility detected in acid polyacrylamide gel electrophoresis (Shewry and Tatham, 1990, and Metcham *et al.*, 1978). Based on molecular weight distribution, the ω-gliadins are sulfur poor monomers with molecular weight ranging from 60,000 - 80,000 Da, while γ-, β-, and α-gliadins are apparently the sulfur rich polypeptides with about 40,000 to 80,000 Da.

The ω-gliadins have virtually no sulfur-containing amino acids and are limited to non-covalent interactions in the dough. The γ-, β-, and α-gliadins, which has a capacity intrachain disulfide bonds, are restricted in the involvement of disulfide linkages (Wrigley *et al.*, 1998). The γ- and α-gliadins have eight and six cysteine residues, respectively. There are no free cysteines, and all S-S linkages are intramolecular, preventing gliadins from participating in the polymeric structure of glutenin (Gianibelli *et al.*, 2001). This reduces their capacity to take part in the formation of gluten polymers. However, the S-S bonds are critical in retaining the folding structure that determines the nature of non-covalent bonding (Wrigley et al., 1998).

Concentrated solutions of gliadins are highly viscous with little measurable elasticity (Stauffer, 1998). Hydrophobic gliadins (γ-gliadins) were found to increase the loaf volume, while ω-gliadins decreased this bread property. (Weegels *et al.*, 1990, van Lonkhuijsen *et. al.*, 1992).

Bietz, *et. al.* (1973) showed that gliadin molecules are small, more globular and symmetrical and have reduced surface area. Thus, these proteins have fewer tendencies than glutenins to interact with other proteins. Excessive gliadins in a dough would cause it to be too extensible to yield a good finished product.

GLUTENINS

Previous studies on wheat proteins, particularly the gluten matrix, were generally focused on the giant molecule hypothesis. Later, stronger evidence was shown by Ewart (1977) that the gluten structure is due to the long, linear glutenin polymers. More experimental evidence has indicated that most interchain junctions between glutenin subunits consist of a single disulfide bond (Ewart 1988) leading to a general hypothesis that aggregated glutenin is a series of linear molecules. This linear structure, largely consisted of HMW glutenin subunits with LMW branching from the polymer, was also formed by disulfide bonds (Graveland *et al.*, 1985).

Two major subgroups comprise glutenin fractions and this classification is based on their molecular weight distribution. According to Stauffer (1998), HMW-GS is from 80-120 kDa, while LMW-GS from 40-55 kDa. The approximate molar ratio of HMW and LMW is 2:1 or higher but these are roughly equal on weight basis.

The LMW-GS has sulfhydryl groups that can form both intrachain and with other glutenin subunits in aggregated glutenin (Wrigley *et al.*, 1998). In HMW-GS, the cysteine groups are near each end of the chain and with a long stretch of other amino acids between these two ends. These were postulated to form spirals, which can apparently turn and fold like a coil spring. LMW subunits have similar concentration of cysteine residue as HMW-GS, but only at the C terminal. (Panozzo *et al.*, 1994). Compared to the LMW-GS, HMW-GS have abilities to polymerize on both ends (Wrigley *et al.*, 1998). However, upon application of work, such intramolecular disulfide bonds can also restrain springing during dough development and most likely break down during this process (Stauffer, 1998).

Within the context of improving protein quality by wheat breeding, e.g. appropriate extensibility and dough strength, research has shown strong evidence of the importance of HMW-GS (Gupta and MacRitchie 1991; Popineau *et al.*, 1994). Such studies stressed the importance of glutenin and gave it more weight in terms of its influence to the overall functionality in the bread making process. A strong relationship has been established between dough strength and baking quality and the average molecular size or the distribution of the polymeric glutenin protein contents (Dachkevitch and Autran, 1989). HMW-GS have been found to contribute to extensibility and dough strength (Gupta and MacRitchie 1991; Popineau *et al.* 1994).

Isolated glutenin, when rehydrated, forms an elastic, rubbery mass that has almost no viscous flow characteristics (Stauffer, 1998). Jones *et al.*, (1974) postulated that only 3% of the bonds in flour affect the rate of dough development, and about 12% of these bonds are involved in resistance during mixing. Glutenins are large linear polymers and

interaction between glutenin chains in dough also occurred through non-covalent forces, namely hydrogen and hydrophobic bonds (Ewart, 1977).

Similar disulfide bonding has been demonstrated to accompany heat-induced damage during the commercial drying of wet gluten (Weegels *et al.*, 1994). The exposure of hydrophobic groups of the glutenin fraction caused aggregation and irreversible conformational changes. The weakening of the dough associated with disulfide bonds rupture is accompanied by a decrease in molecular weight distribution (Bekes *et al.*, 1994).

GLUTENIN CHARACTERIZATION

Physico-chemical techniques used in analyzing glutenins practically give more evidence of its complexity. Thus, it would be best to correlate such properties to the varietal differences of cultivars, which would exhibit good baking properties and solve other problems connected with production of wheat (Lásztity, 1995).

Lásztity (1995) has shown that different subunits differ in their tendency to polymerize, and once polymerized, have different capacities for interactions with other constituents. Thus, the mechanism and control of disulfide bond formation makes handling of wheat proteins more challenging.

HYDROPHOBIC CHARACTERISTICS AND MOLECULAR WEIGHT DISTRIBUTION

One of the most powerful techniques in analyzing glutenin is RP-HPLC. The mechanism of separation involves a nonpolar and hydrophobic stationary phase with a

polar initial mobile phase. Analytes, therefore, elute in the order of increasing hydrophobicity or decreasing net charge, degree of ionization and ability to participate in hydrogen bonding (Cunico et al., 1998). Combined with high resolution and technological innovation, RP-HPLC has become one of the most widely utilized method in optimizing separation and characterization of glutenin fractions. Most chromatograms obtained by researchers showed an overlap between the LMW-GS and hydrophobic gliadins (Payne *et al.* 1981 and Moonen *et al.*, 1985). Kruger *et al.*, (1988) found a correlation between the proportion of HMW-GS versus LMW-GS and dough strength using peak area on chromatograms. Certain peaks of interest in RP-HPLC identified by SDS-PAGE were used to determine the HMW-GS to LMW-GS ratio. A higher ratio value was directly related to dough strength. Although a potentially useful technique, the ratio of glutenins based on molecular weight has limitations since relative amounts of HMW polypeptides were not directly related to the breadmaking quality (Marchylo *et al.*, 1989).

Burnouf and Bietz (1984) indicated that reduced and alkylated storage proteins eluted during RP-HPLC in the order of: ω-gliadins, HMW glutenin subunits, LMW (α- and β-) gliadins, LMW ethanol soluble glutenin subunits and γ-gliadins. Reduced but unalkylated proteins behave similarly but generally are more hydrophobic, eluting later in the HPLC chromatograms. It is best to have a reference sample because glutenin chromatograms may contain overlapping residual gliadins (Payne and Lawrence, 1983).

Using different stacking gel concentrations of SDS polyacrylamide gel, Khan and Huckle, (1992), and Wrigley *et al.* (1993) were able to demonstrate improved direct analysis of molecular weight distribution of glutenin. Bushuk *et. al.* (1980) provided a

schematic presentation, postulating that glutenins are composed of two types of molecules: (1) subunits of 68,000 molecular weight and lower named glutenin I and (2) glutenin II composed of subunits with greater than 68,000 molecular weight and that are joined to each other through at least one intersubunit disulfide bond. Presently, there are four classifications as specified in the literature, the A-, B-, C- and D- regions based on SDS-PAGE. A-region (80-120 kDa) is HMW-GS (Payne and Corfield, 1979), B-region (42-51 kDa) and C-region (30-40 kDa) are low molecular weight glutenin subunits supposedly to γ- and α gliadins (Payne *et al.*, 1985; Thompson *et al.* 1994) and D-region contains the highly acidic polypeptides believed to be related to ω-gliadins (Masci *et. al.*, 1993).

Amino Acid Content and Genetic Studies

The amino acid sequence of the glutenins consists of three separate domains: N-terminal non-repetitive, central repetitive and C-terminal non-repetitive domains. Cysteine residues are mainly located in the terminal domains. The central repetitive domain, consisting of tandem or interspersed repeats based on the peptide motifs rich in proline and glutamine residues, can undergo deformation or reformation under stress or relaxation (Tamás *et al.*, 2002).

In a growing polymer system, the glutenins need at least two cysteine residues. The HMW-GS and LMW-GS have even number of cysteine residues and therefore considered as chain propagators or chain extenders (ChE) (Kasadra, 1989; Shewry *et al.*, 1989). However, modified gliadin subunits are incorporated into the glutenin polypeptide which contained an odd number of cysteine residues (e.g. ω-gliadins). These are modified subunits since normal gliadins usually have an even number of cysteine

residues or none at all. These subunits are classified as chain terminators (ChT) which behave as glutenin subunits but shift the molecular weight distribution of the isolated glutenins to lower values (Gianibelli *et al.*, 1996; Masci *et al.*, 1999). A novel polymeric protein identified by Gianibelli *et al.* (2002) was classified as ω-gliadins (71,000 Da) and a ChT. The authors proposed that there may be more than one chain terminator in a certain glutenin sequence and were speculated to be in the LMW-GS.

The HMW-GS are encoded at the *Glu-1* loci on the long arms of group 1 chromosomes (1A, 1B and 1D). These loci are *Glu-A1*, *Glu-B1* and *Glu-D1*(Payne *et al.*, 1984 and 1987). Each locus includes two genes linked together encoding two different types of HMW-GS: x- and y-type subunits (Payne, 1987 and Shewry *et at*, 1992). The HMW-GS have high content of glutamic acid, proline and glycine. However, they have low content of lysine. The amino acid composition of HMW-GS indicates the hydrophilic nature of the central repetitive domain and hydrophobic characteristics of the N- and C- terminals (Shewry *et al.*, 1989).

The LMW-GS are controlled by genes at *Glu-A3*, *Glu-B3* and *Glu-D3* loci on the short arms of the chromosome 1AS, 1BS and 1DS, respectively (Gianibelli *et al.*, 2002). It is assumed that the genes that control the LMW-GS are closely linked and similar to those controlling the gliadin blocks (Gupta and Shepherd 1990a,b; Lagudah *et al.*, 1991). Seven main types of LMW-GS were identified by N-terminal sequences of the proteins according to the first amino acid present in this region. Three of these LMW-GS were identified with N-terminal sequences similar to those of α-, γ- and ω-type gliadins while another three were reported to have an odd number of cysteine residues, allowing intermolecular disulfide bonds in the gluten protein (Kasadra, 1989).

Net Surface Charge

Capillary Electrophoresis (CE) combines the high resolution of electrophoresis with the automation and ease of use of HPLC, thus giving a high resolution and rapid separations of proteins (Bean and Lookhart, 1998). Similar to gel electrophoresis, the separation of species depends upon differential migration in an electric field. Unlike the gel electrophoresis, capillary electrophoresis is performed using a free solution and shorter analysis time. Its resemblance to HPLC is evident in the use of concentration sensitive detectors and the presentation of data is in the form of peaks, which can be readily quantitated (Cunico *et al.*, 1998).

Capillary zone electrophoresis (CZE) is performed under the influence of an electric field. Charged species travel inside the fused silica capillary. In this case, migration time of the species is determined by a combination of its electrophoretic mobility and the velocity of the electro-osmotic flow (EOF) (Baker, 1995). Neutral compounds are not separated, whereas ions are separated on the basis of their charge to size ratios (Baker, 1995). The greater the charge to size ratio, the faster the electrophoretic mobility and for a given applied electric field and buffer, the higher the velocity. Small highly charged molecules move through the capillary the fastest and large molecules with lower charge move slower. Electrophoretic velocity, however, is dependent only on mobility and electric field, while electrophoretic mobility is dependent on solute and buffer properties and is independent of the applied electric field (Baker, 1995).

Several reports of excellent separation involve the use of different buffers on free zone capillary electrophoresis (FZCE). Lookhart and Bean, (1995), among others, have devised a 100mM phosphate buffer containing a polymeric additive, hydroxymethylpropyl cellulose, which can differentiate closely related wheat cultivars.

VISCOELASTIC BEHAVIOR

Viscoelasticity is the ability of materials to exhibit both viscous (flow) and solid like elastic behavior, depending on conditions such as temperature, time and deformation applied. A viscoelastic material can flow if deformed over a long period of time or at elevated temperature but may behave as rigid, brittle material, if deformed very quickly or at low temperature. The gliadins are viscous and extensible molecules. Doughs made from gliadins alone behave like a highly viscous liquid with little resistance to deformation (elasticity). Consequently, during the process of baking a dough, more gas bubbles will form but these will not remain entrapped for a very long time. A dough containing only glutenin lacks extensibility but exhibits substantial strength and elasticity. Gas will be retained longer but the dough will not expand. Glutenins are the wheat proteins which exhibits viscoelasticity, whereas suspensions of gliadins form viscous liquids. Therefore, dough viscoelasticity and development is partially explained by the glutenin network formed (Dobraszczyk et al., 2001).

Physical and chemical properties of the flour or dough primarily affect its rheological behavior. In a flour system, the application of mechanical work causes the formation of crosslinking and disruption of bonds simultaneously to form a dough. As mentioned earlier, the viscoelastic properties are believed to be due to the gluten matrix

and macromolecular interactions of dough components such as starch, lipids, sugars and pentosans. These interactions may change the resistance of the dough to deformation.

In food systems, rheometry is characterized as either descriptive or empirical and fundamental approach. One of the primary considerations in using the empirical approach in rheometry is practicality (Bourne, 1982). However, in some cases, there is a greater need for physical or rheological properties, thus, a more sensitive means of analysis are applicable (Weipert, 1992; Bourne, 1982). The challenge for the present study is to express the viscoelastic properties of glutenin subfractions in the form of a gel.

The rheometer has the advantage of a well developed theoretical background, readily available instrumentation and simultaneous measurement of elastic and viscous moduli. Most importantly, its nondestructive nature of the test enables multiple measurements to be performed while temperature, strain, or frequency is varied (Ferry, 1980). Using a rheometer, it is possible to extrapolate dynamic oscillation measurements over a much wider range of frequencies using the time-temperature superposition principle (Tsiami *et al.* 1997). This means that time of frequency and temperature are interdependent. If the time to deform the polymeric material is short (high frequency), the polymer molecules have little time to adjust to the imposed deformation, and the material is interpreted as stiff and apparently solid-like or has an elastic behavior. If the deformation occurs over long time (low frequency), the polymer molecules have time to flow, and the material is recognized as less stiff and a fluid-like or viscous behavior. Temperature has an equivalent effect to time by changing the speed at which the polymer molecule responds: low temperature decreases the molecular mobility and is equivalent to increasing frequency or decreasing time. Therefore, the elastic zone is extended into

the lower frequency region. By conducting rheological measurements over a fixed range of frequency at a series of temperatures, it is possible to extend the frequency range by several orders of magnitude to much lower frequencies relevant to baking expansion (Dendy and Dobraszczyk, 2001).

Conventional oscillatory shear rheological tests usually function in the linear region at small strains in the order of up to 1%, whereas strain in gas cell expansion during proofing is over 100%. Most rheological tests are carried out in shear, whereas most large-strain deformations in dough (sheeting, proofing, baking) are extensional in nature (Dendy and Dobraszczyk, 2001). Polymer melt fluid dynamics show that very different results are possible in shear than in extension because of the different physical effects these deformations can have on networks of molecules (Cogswell, 1981; Padmanabhan, 1995).

CHAPTER III

DETERMINATION OF THE PHYSICO-CHEMICAL PROPERTIES OF GLUTENIN FROM WHEAT CULTIVARS OF CONTRASTING BAKING QUALITY

ABSTRACT

It is widely accepted that the proteins that form the gluten matrix are responsible for most of the bread making qualities of wheat flour. Among the wheat storage proteins, the glutenins have been correlated to most of the functional breadmaking characteristics.

The objective of this study was to characterize selected physico-chemical properties of the reduced and soluble glutenin subfractions of hard red winter wheat Jagger and Big Dawg *cv*. Wheat samples were analyzed for protein content, extraction rate and empirical rheological properties as measured by an alveograph. Extraction of the glutenin was based on a modified method of Lookhart and Bean (1995) and Bean and Lookhart (1998). The physico-chemical properties of the soluble and reduced glutenin extracts were analyzed with semi-preparative RP-HPLC, SDS-PAGE and FZCE.

Seven subfractions were identified from semi-preparative RP-HPLC to be enriched with glutenin subunits and some residual gliadins. Glutenin subfractions 2, 3 and 4 were enriched with high molecular weight glutenin-subunits, HMW-GS, (92,000 to 146,000 molecular weight) and subfractions 1 and 5 to 7 were enriched with low molecular weight glutenin-subunits, LMW-GS (72,000 to 84,000). LMW-GS enriched subfractions which eluted with 35-45% acetonitrile, were more hydrophobic, except for

subfraction 1 which was hydrophilic and enriched with LMW-GS. Although varying in hydrophobic nature, the glutenin subfractions contain some subunits with similar molecular weight and FCZE maps with peaks of similar charge to size ratio.

Strong aggregative tendencies were observed in more hydrophobic subfractions which were enriched with LMW-GS. Subfraction 1 was assumed to contain ω-gliadin due to the apparent highly negative net surface charge in FZCE and their low molecular weights from 44,000 to 82,000.

The differences found in percent peak areas of semi-preparative HPLC between enriched HMW-GS and LMW-GS, in Jagger and Big Dawg, were attributed to the differences in composition and quantity of extractable glutenin components. These observations were found in Jagger enriched HMW-GS subfractions, wherein these were 1.2x as large as Big Dawg enriched HMW-GS subfractions. In additon, more separated peaks in the more hydrophobic region were evident in Big Dawg crude extract than in Jagger.

Introduction

The prospect of understanding the role of glutenin subunits has grown consistently due to the strong indication of their importance to overall baking quality. A positive correlation between dough strength and the proportion of large-size glutenin has been reported (Weegels, et. al, 1996; Southan and MacRitchie, 1999). Thus, better understanding of bread making qualities can be generated by further characterizing the glutenin. Presently, there is no sufficient evidence to show that differentiation based on the physico-chemical properties of glutenins can differentiate fine differences in the

functionality of flour. In this study, hard red winter wheat Jagger and Big Dawg *cv.* were analyzed.

Jagger was developed cooperatively by the Kansas Agricultural Experiment Station and the Agricultural Research Service, USDA. The cultivar was released by Dr. J.M. Moffatt in 1984 and the foundation seed was distributed in Kansas by 1994 (Sears et al., 1995). Jagger is classified as a strong blending wheat. Jagger flour is generally blended with weak flours to obtain desirable baking quality. It was reported as wheat of exceptional quality, based on the properties like large kernels, high protein content, good milling and bread-making performance. Big Dawg is a cultivar released by AgriPro from Junction City, KS. It is considered as wheat of acceptable quality, with acceptable milling and bread-baking attributes but not outstanding in all properties (Bennett *et al.*, 2002).

Jagger is the most popular wheat cultivar grown in Oklahoma and Kansas. The percent seeded acreage from 1999 to 2003 in Oklahoma ranged from 29.5-46.1% and 0.2-0.6%, for Jagger and Big Dawg respectively (National Agricultural Statistics Service, 2003). In Kansas, for the same number of years as Oklahoma, the percent seeded acres were 29.2-45.2% and 0.2-0.5% for Jagger and Big Dawg, respectively (Anonymous, 2003).

The aim of this study was to characterize the physico-chemical properties of the reduced and soluble glutenin subfractions from wheat flours of different baking qualities. The molecular weight distribution and net surface charge of glutenin subfractions from Jagger and Big Dawg, separated according to surface hydrophobicity, were compared.

MATERIALS AND METHODS

CHEMICALS

Dithiothreitol (DTT), glycine, potassium iodate (KIO_3), and sodium dodecyl sulfate (SDS) were purchased from Fisher Scientific (Fairlawn, NJ). The glacial acetic acid, n-propanol and methanol were from Pharmco Science (Brookfield, CT). Acetonitrile (EM Science, Gibbstown, NJ) and Trifluoroacetic acid (TFA), (Sigma Aldrich, Milwaukee, WI) were both HPLC grade. Sodium hydroxide (NaOH) was purchased from Malinckrodt Chemical Inc. (Paris, Kentucky), while the phosphoric acid came from Spectrum (New Brunswick, NJ). A bicinchoninic acid protein assay kit (Sigma Chemical Co., St. Louis, MO) was intended for total protein determination. Chemicals for the preparation of polyacrylamide gels were obtained from Sigma Chemical Co. (St. Louis, MO). Nanopure water was acquired from a milliQ water purification system (Millipore, Milford, MA).

FLOUR SAMPLES AND ALVEOGRAPH ANALYSIS

Two hard red winter wheat cultivars with differences in baking qualities were used in this study. Jagger *cv.* was obtained from Oklahoma Seed Stock, Inc., Oklahoma State University, Stillwater, OK, and Big Dawg *cv.* was from Phillips Seed Farm, Salina, KS. Both were milled with a Brabender Quadrumat Sr. Mill and the flours obtained were sifted using a nominal sieve opening of 0.149 mm. Moisture and protein content were determined with a NIR FOSS Model 6500 (Silver Spring, MD). The baking strength

(W), tenacity (P) and extensibility (L) were analyzed using three replicates by a constant hydration method from an Alveograph (CHOPIN, Cedex, France).

GLUTENIN EXTRACTION

Glutenin fractionations were performed using a revised method based on Lookhart and Bean (1995) and Bean and Lookhart (1998). A slurry of 50 g flour and 25% (w/v) nanopure water was mixed for 5 min in a Vortex Genie 2, speed #4. The supernatant obtained by centrifugation (Sorvall RC 5C, Newtown, CT) at 385xg, 5 min and 4°C, was discarded since it contained albumins, globulins and soluble carbohydrates. This procedure was repeated three times and the residual pellet was treated with 200 ml 50% n-propanol. The same mixing, centrifugation, and volume ratio parameters were again applied. The supernatant containing gliadin fractions was collected and stored at -20°C. This process was repeated once and the supernatants pooled. The residual pellet containing glutenins was treated with 200 ml 50% n-propanol containing 1% DTT and mixed for 45 min. After centrifugation (385xg, 5 min, 4°C), the supernatant was collected and enrichment of glutenins followed after treatment with 50 ml n-propanol containing 1% DTT. The suspension was mixed for 1 h and stored at 4°C for 72 h. The enriched glutenin fraction was collected after centrifugation (1951xg, 5 min, 4 °C) and freeze dried, pulverized, and stored at -20°C.

REVERSE PHASE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

HPLC analyses were performed using a Waters 2695 Separations Module equipped with Waters 996 Photodiode Array Detector and Gilson FC 203B Fraction Collector. A 10 mg glutenin sample was solubilized using 1 ml 50% n-propanol (v/v)

containing 1% DTT (w/v). Sonication for 45 min was used to solubilize the proteins. After spinning at 3,000 rpm (735xg) for 5 min in an Eppendorf microcentrifuge, the supernatant was filtered with 0.25 µm Titan membrane and injected into a 250 x 4.6 mm Vydac C18 Protein and Peptide column (The Nest Group, MA). The mobile phases (1.0 ml/min flowrate) were water and acetonitrile, with both solvents containing 0.06% TFA (v/v). The gradient was comprised of (a) 30% acetonitrile (ACN), 5 min; (b) 35% ACN, 10 min; (c) 50% ACN, 15 min; and (d) 80% ACN to wash the column.

Semi-preparative fractionation was done in a Waters Delta 600 HPLC system equipped with a Waters 2487 Dual Absorbance Detector and Waters Fraction Collector II. The same sample preparation and gradient employed in analytical HPLC was employed for semi-preparative RP-HPLC separation. A 250 x 10 mm Vydac C18 protein and peptide column was used for the subfractionations. Samples were collected in batches and dried using a Thermosavant speed vacuum with maximum vacuum pressure of 5 torr. Dried samples were then stored at -20°C and used again for another collection cycle. The total protein content was determined using a bicinchoninic acid protein assay kit from Sigma.

Gel Electrophoresis

A 15% polyacrylamide resolving gel was used to estimate the molecular weight distribution of glutenin subfractions. Freeze dried samples were solubilized in a reducing buffer containing 26% 1 M Tris-HCl, 5% SDS, 1% bromophenol blue in 0.1 N NaOH, 1% DTT, and 42% glycerol. The tank buffer consisted of 0.3% Tris, 1.44% glycine, and 0.05% SDS. The gels were stained with Bio-Rad Silver Stain Plus (Catalog Number 161-0449). A 5% acetic acid was used as a stopping solution.

Free Zone Capillary Electrophoresis

The net surface charge of the reduced and soluble glutenin fractions was determined on a Beckman P/ACE 2000 (San Ramon, CA) using a 27 cm fused-silica capillary (20 cm to the detector, 50 μm i.d.) from Polymicro Technologies Inc. (Phoenix, AZ).

A 10 mg dried glutenin subfraction was resuspended in 1 ml 50% n-propanol containing 1% DTT. The column was rinsed for 1 min and at 20 psi pressure with 0.1 N NaOH and 0.5 M acetic acid consecutively. A nanopure water rinse preceded each solvent rinsing. The column was saturated with the running buffer containing 100 mM phosphate (pH 2.4), 20% (v/v) ACN, 0.4% (w/v) glycine and 0.05% (w/v) hydroxymethylpropylcellulose. An electric field of 15 kV was applied after a 5 sec injection of the sample. Three different extractions for each cultivar were used as replicates for the analyses.

Statistical Analysis

Statistical Analysis Software (SAS) version 8.1 was used to determine significant differences in the percent peak area of the glutenin fractionated by semi-preparative RP-HPLC. A one-way treatment structure and an additional nested factor of subfractions of each cultivar were utilized. There were two different cultivars as treatment levels, seven subfractions within a cultivar, and 14 replications per cultivar. Contrast statements were defined, namely: (a) whether there was a significant difference between the mean percent peak areas of the enriched HMW-GS (F2, F3 and F4) and LMW-GS (F1, F5, F6, and F7)

subfractions and (b) whether the difference between Big Dawg and Jagger in percent peak areas of the enriched HMW-GS and LMW-GS was significant.

Results and Discussion

Physical Properties of the Wheat Flour

The average protein contents of Jagger and Big Dawg flours were 15.1% and 13.2% d.b., respectively (Table 1). The flour extraction recovery from Jagger was higher (63.2%) than that from Big Dawg (58.2%). The average alveographic measurements revealed that Jagger has higher values, namely 86 mm extensibility, 311 x 10^{-4} J baking strength, and an elasticity index of 72.9% as compared to Big Dawg cv. with 56 mm, 247 x 10^{-4} J, and 64.7%, respectively. However, the maximum pressure required to deform the sample was higher in Big Dawg; 105 mm H_2O, than in Jagger, 85 mm H_2. For both cultivars, the physical properties obtained could be attributed to the compositional differences in the gluten, in particular, the glutenin.

Hydrophobic Nature and Molecular Weight Distribution

Preliminary studies of RP-HPLC analytical separation (Fig. 1) have revealed that the less hydrophobic region within an elution time of 15 to 18 minutes (33% ACN) was enriched with HMW-GS, while the more hydrophobic region, 21-26 minutes elution time (35-45% ACN), was composed of enriched LMW-GS subfractions. A greater number of peaks was observed in the Jagger crude glutenin extract than in the Big Dawg extract (10 and 8 peaks, respectively). The peak areas were different, particularly in the peaks

eluting at 17-19 min; Jagger contained two distinct peaks within this migration time while Big Dawg had one large peak followed by a small residual. The more hydrophobic peaks of Big Dawg (20-26 min) were more separated than those of Jagger at the same retention time.

Unlike the analytical RP-HPLC separation, which revealed multiple peaks in the chromatogram (Fig. 1), both cultivars revealed seven glutenin subfractions with the same retention time using semi-preparative RP-HPLC (Fig. 2). The chromatogram can be further classified into three regions, namely hydrophilic (A), less hydrophobic (B) and more hydrophobic (C) regions.

Figures 3, 4 and 5 show the molecular weight distribution of glutenin fractions in SDS-PAGE. Region A (Fig. 3) contained subfraction F1 for both cultivars. JF1 contained polypeptides with prominent molecular weights of 72,000, 85,0000 and 92,000 while the major polypeptides of BF1 had 44,000, 72,000 and 85,000 molecular weights. Both cultivars revealed a residual subunit of 18,000 molecular weight. BF1 and JF1 might have albumins coeluting during glutenin extraction. Figure 4 shows the molecular weight of Big Dawg subfractions 2 to 7. The less hydrophobic subfractions (BF2, BF3 and BF4) were enriched with HMW-GS. These subfractions had low quantities of proteins with molecular weights in the range of 104,000 to 146,000 but had a common protein in BF2 and BF3 (146,000 molecular weight). This common subunit was more prominent in BF3. Aside from containing low quantities of Type A glutenin subunits, BF4 was a mixture of glutenin subunits containing prominent molecular weights of 26,000, 64,000 and 84,000. The more hydrophobic Big Dawg subfractions (BF5, BF6 and BF7) were enriched with LMW-GS. BF5 and BF6 subfractions contained proteins

with molecular weights of 94,000, 84,000 and 78,000, with residual proteins of 35,000 and 26,000 for BF5 but only 26,000 for BF6. Subfraction BF7, however, had two bands with 94,000 and 78,000 molecular weights.

Similar to the observed trend in Big Dawg RP-HPLC subfractions, the less hydrophobic Jagger glutenin subfractions JF2 and JF3 contained similar molecular weights of 144,000, 122,000 and 101,000 (Fig. 5), except that JF3 had an additional 82,000 subunit. JF4, however, contained unique proteins with molecular weights of 135,000, 97,000, 92,000, 82,000, 74,000 and 18,000. The more hydrophobic Jagger subfractions (JF5, JF6 and JF7) were enriched with LMW-GS. The common molecular weight subunits among the three subfractions were 82,000 and 28,000. Both JF6 and JF7 contained a common subunit of 92,000. However, JF7 had additional subunits with molecular weights of 72,000 and 15,000.

Similar molecular weight distribution in subfractions belonging to the same region of hydrophobicity was due to the overlapping of the fractions during separation in RP-HPLC (Fig. 2). However, this separation was observed to not be exclusive to the region. Thus, reduced and soluble glutenin subcomponents in each cultivar may have similar molecular weight of subunits that vary in hydrophobic nature.

Both RP-HPLC glutenin subfractions of Jagger and Big Dawg exhibited subunits with molecular weights of 15,000 to 28,000. These low molecular weight proteins cross-contaminated the glutenin extracts. Some studies have reported that RP-HPLC chromatograms of hydrophobic LMW-GS usually contain hydrophobic gliadins (Bietz and Wall, 1973; Bean *et al.*, 1998). These studies suggest that during glutenin extraction, sulfur rich gliadins, which are hydrophobic in nature, were not completely isolated.

However, the albumins and ω-gliadins detected in SDS-PAGE may have interacted with glutenin prior to extraction. During extraction, the glutenins were only partially reduced by 50% n-propanol containing 1% DTT, causing random and some specific cleavage of the disulfide bonds, which allowed non-glutenin components to be recovered together with the glutenin fraction.

Bietz and Wall (1972) reported the apparent molecular weight distribution in the range of 10,000-70,000 Da and ~ 80,000 to 130,000 Da for LMW-GS and HMW-GS, respectively. The molecular weight classification used for the current study is based on the presence of type A-region glutenins (predominantly >100,000 molecular weight) from Payne and Corfield (1979). Therefore, the molecular weight classification of the semi-preparative glutenin subfractions (Fig. 2) used in this study were (a) enriched HMW-GS subfractions (BF2, BF3, BF4, JF2, JF3 and JF4) and (b) enriched LMW-GS (BF1, BF5, BF6, BF7, JF1, JF5, JF6 and JF7).

The mean percent peak areas of the RP-HPLC glutenin subfractions ranged from 2.3 to 33.3% for Big Dawg and 0.7 to 42.3% for Jagger (Table 2). The variation in the percent peak area (Appendix A) was not explained by the cultivars ($F_{1,144} > 0.00$, $P \leq 0.9996$) but by the subfractions of each cultivar ($F_{12,91} = 449.41$, $P \leq 0.0001$). The differences between enriched HMW-GS and LMW-GS subfractions were significant within each cultivar: (a) Jagger: $F_{1,91} = 1144$, $P \leq 0.0001$ and (b) Big Dawg : $F_{1,91} = 1821$, $P \leq 0.0001$ (Table 2). The highest percent peak areas were due to subfractions BF3, BF4, JF3 and JF4. The percent peak areas of subfractions enriched with HMW-GS were approximately 5 and 2.5 times greater than the peak areas of subfractions enriched LMW-GS in Jagger and Big Dawg, respectively (Table 2). The difference in percent peak areas

of enriched HMW-GS and LMW-GS between the two cultivars were significant ($F_{1,144}$ = 71.32, $P \leq 0.0001$), since Jagger enriched HMW-GS subfractions were 1.2x as large as Big Dawg enriched HMW-GS subfractions. These results indicated that although there were similarities in glutenin extraction and apparent surface hydrophobicity based on the elution times in RP-HPLC of the glutenin subfractions, the two cultivars may have amino acid compositional differences and qualitative and quantitative differences in the soluble glutenin prior to HPLC analysis.

Comparison of the Net Surface Charge of the Subfractions

The electropherograms in FZCE showed that the crude extract of Jagger and Big Dawg *cv.* contained several proteins with a range in net surface charges (Fig. 6). The Big Dawg glutenin exhibited thirteen major peaks while Jagger had seven major peaks. Ten of the peaks were unique to Big Dawg while five (indicated by asterisks) were unique to Jagger. Peaks with the same number have relatively the same retention time. These peaks infer similarities in charge to size ratios. Proteins in Big Dawg glutenin have more negative net surface charge compared to proteins in Jagger glutenin. The smaller number of peaks detected in Jagger may be due to the presence of unextractible glutenin subunits in the crude extract not solubilized during sample preparation.

In Jagger, the less hydrophobic and enriched HMW-GS (JF2, JF3 and JF4) subfractions contained five to seven major peaks in FZCE (Fig. 7). Up to five of the peaks in the crude extract were not identified. Among the enriched HMW-GS, JF2 and JF3 both contained peaks which were unique to the subfraction (indicated by arrows), which indicated that concentrating and solubilizing glutenin subfractions caused

modification of the net surface charge of the polypeptides due to reassociation and partial reduction.

The more hydrophobic Jagger subfractions (JF5, JF6 and JF7) among those enriched with LMW-GS presented a challenging separation. They exhibited an area of broad and unevenly shaped peaks with an undefined number of peaks that co-migrated (Fig. 8). JF1, characterized as the more hydrophilic and enriched LMW-GS with a molecular weight range of 72,000 to 92,000, had five major peaks of which three were not identified in the crude extract. The FCZE maps of enriched LMW-GS subfractions (Fig. 8) contained peaks with more negative net surface charge (JF1 and JF6, 16-20 min migration time) than did the maps of enriched HMW-GS (Fig. 7). JF1 contributed to the highest number of prominent peaks with more negative surface charge and a more hydrophilic nature.

Figure 9 shows the Big Dawg FCZE maps of less hydrophobic and enriched HMW-GS subfractions (BF2, BF3 and BF4). These subfractions contained four to eleven major peaks, with five to nine peaks not identified in the crude extract. Among the enriched HMW-GS, BF2 and BF3 contained peaks which are unique to these subfractions (indicated by arrows). The composition of BF3 was the most complex showing more polypeptides with diversity in charge density. However, this diversity was not apparent in their molecular weight distribution, suggesting the presence of polypeptides with similar molecular weights but different charge to size ratios.

In Figure 10, the FCZE maps of the more hydrophobic and enriched LMW-GS subfractions in Big Dawg (BF5, BF6 and BF7) also exhibited the same challenges at separation, showing clusters of poorly separated peaks as in the Jagger subfractions at the

same RP-HPLC retention times (Fig. 8). Not all the major peaks in BF1 were identified in the crude extract. In comparison to Jagger enriched LMW-GS in which JF1 and JF6 had more negatively charged species, Big Dawg was observed to have these negatively charged species mostly in BF1 and BF7. This observation suggests that there are fine differences between the two cultivars, with Big Dawg containing negatively charged species which are also more hydrophobic in nature than Jagger.

Region B (Fig. 2) contained the less hydrophobic subfractions in both Jagger and Big Dawg. BF2 and JF2 FZCE maps revealed that these subfractions contained more positive net surface charged proteins than did the other enriched HMW-GS in Region B for Big Dawg and Jagger. BF3 and JF3 FZCE maps contained peaks with more negative net surface charge than did BF2 and BF4 maps for Big Dawg and JF2 and JF4 for Jagger. Both BF4 and JF4 did not have significant amounts of Type A glutenins (>100,000 molecular weight) but exhibited similar FZCE profiles to the rest of the subfractions in the less hydrophobic and enriched HMW-GS for Big Dawg and Jagger.

Both Jagger and Big Dawg RP-HPLC subfractions exhibit several peaks with similar FZCE migration times within each cultivar. Thus, some of the reduced and soluble glutenin components with differences in hydrophobic nature (retention time in RP-HPLC) have the same charge to size ratio. These findings suggest that these polypeptides could be very similar or the same on both cultivars.

The more hydrophilic subfraction in each cultivar (JF1 and BF1) contained major peaks with more negative net surface charge than the other subfractions. This was an indication that they contained ω-gliadins, since the trend in elution time in RP-HPLC and net surface charge corresponds to the behavior of ω-gliadins in the two-dimensional

contour plots studied by Bean *et al.* (1998). The FZCE maps combined with RP-HPLC migration time revealed that the most hydrophilic fraction contained the ω-gliadins, the most negative net surface charged molecules among the gliadin family. Thus, the removal of gliadin fractions during crude extraction of wheat glutenins, using 50% n-propanol, did not completely solubilize the ω-gliadin component. Such overlap between enriched LMW-GS and ω-gliadins was found in gel filtration studies by Beckwith *et al.* (1966). Nielsen *et al.* (1968) considered these proteins to be glutenins of low molecular weight because they behaved like glutenins in showing a drastic change in viscosity after the reduction of disulfide bonds.

The clustering of enriched LMW-GS is assumed to be due to their tendency to aggregate as observed by the difficulty in completely solubilizing concentrated Region C RP-HPLC glutenin subfractions using 50% n-propanol containing 1% DTT. This difficulty might be due to the tendency of LMW-GS to polymerize upon exposure to molecular oxygen, a slow-oxidizing agent (Verbruggen *et. al,* 2003). More efficient polymerization was observed in LMW-GS than in HMW glutenin subunits, which required higher concentrations of molecular oxygen or other stronger oxidizing agents. Thus, the RP-HPLC subfractions in the more hydrophobic Region C (Fig. 2), have more tendency to polymerize upon concentrating in large amounts and air exposure since these are enriched with LMW-GS.

The subunits found in FZCE maps of the glutenin subfractions were generally greater in number than the subunits in SDS-PAGE. This difference suggests more heterogeneity in net charge than in molecular weight of the subunits forming the glutenin subfractions. There are also differences in the detection method. At 200nm, the

absorbance of peptide bonds is compared to the binding capacity of the proteins detected with silver nitrate.

Effect of Enrichment of the Glutenin RP-HPLC Subfractions

During sample preparation prior to RP-HPLC, the soluble polymeric glutenins were obtained by random cleavage of sulfhydryl bonds of the glutenin polypeptide. The sample preparation gave different proportions of HMW-GS and LMW-GS in RP-HPLC. Subfractions of reduced and soluble glutenin polypeptides enriched by semi-preparative RP-HPLC separation revealed some subunits with similar molecular weights and FZCE maps.

The common molecular weights found in SDS-PAGE were 94,000 and 84,000 in Big Dawg, and 92,000 and 82,000 in Jagger. These glutenin subunits were found in the less hydrophobic region of RP-HPLC (BF2, BF3, and JF4). These two glutenin subunits from the two cultivars might be the same, since their estimated molecular weights are similar. Confirmatory studies are recommended to detect whether these two subunits are indeed the same in the two cultivars and whether they are responsible for the similarities in the FZCE maps of the glutenin subfractions with a different hydrophobic nature.

In theory, wheat contains six different HMW-GS but due to the "silencing" of some genes, common wheat cultivars contain three to five HMW-GS (Giannbelli *et. al*, 2001) Big Dawg contains four distinct molecular weight subunits that can be categorized as HMW-GS (125,000, 117,000, 104,000 and 94,000), while Jagger has five (122,000, 115,000, 101,000, 97,000 and 92,000). The differences in the number of HMW-GS can be attributed to differences in genetic composition and expression,

specifically in effective transcription and probably the more efficient translation of HMW-GS in Jagger.

It is proposed that when LMW-GS's were exposed to an acetonitrile-rich eluent (35-45%) and concentrated during the series of RP-HPLC separations, a change in their conformation took place, rendering them more susceptible to oxidation when exposed to ambient oxygen. This change could account for the aggregation of subunits that were not resolved as revealed in FZCE maps of subfractions 5 to 7 in both cultivars (BF5-7; JF5-7). These subfractions contained stabilized polymers via disulfide bonds, hydrophobic interactions, and H-bonds.

CONCLUSION

Jagger exhibited a higher protein content, extraction rate, baking strength, extensibility, and elasticity index than did Big Dawg. The two hard red winter wheat cultivars showed seven glutenin subfractions based on hydrophobicity with similar migration times but different molecular weight distributions. Subfractions JF1 and BF1, collected at 30 to 33% acetonitrile in RP-HPLC and designated as Region A, contained ω-gliadins, demonstrated by more negative net surface charge than any of the subfractions and low molecular weight distributions. Less hydrophobic subfractions (BF2, BF3, BF4, JF2, JF3 and JF4) eluted at 33% acetonitrile in RP-HPLC (Region B) were enriched with HMW-GS (92,000 to 125,000 molecular weights). The more hydrophobic subfractions (BF5, BF6, BF7, JF5, JF6 and JF7), eluting at 35-45% acetonitrile in RP-HPLC or Region C, were considered as enriched LMW-GS (72,000 to

84,000 molecular weights). Some of the reduced and soluble glutenin subcomponents have some similar molecular weights but differ in their hydrophobic nature.

The enriched LMW-GS subfractions from both cultivars appeared to have considerable aggregative tendencies. This was probably due to their tendency to polymerize more rapidly than did the enriched HMW-GS. The degree of agglomeration was evident in FZCE, where the polypeptides were not separated but co-migrated. The agglomeration caused clustering of an undefined number of peaks that formed broad, unevenly shaped areas. FZCE maps of glutenin subfractions revealed proteins with similar charge to size ratios. Therefore, glutenin subfractions with a different hydrophobic nature also contained subcomponents with similar charge to size ratios.

The percent peak area of the reduced and soluble glutenin subfractions separated by RP-HPLC was different in two cultivars. This difference was mainly attributed to the significant proportion of enriched HMW-GS subfractions. Subfractions of Jagger enriched HMW-GS were 1.2x as large as those of Big Dawg mainly due to compositional differences, i.e., amino acid composition, the amount of extractable glutenin for RP-HPLC analysis, and the differences in the number of HMW-GS's in the two wheat cultivars.

Table 1. Protein, moisture, flour extraction and alveographic properties of two hard red winter wheat cultivars

Parameter	Big Dawg	Jagger
Protein (%), d.b.[1]	13.2	15.1
Moisture (%)[1]	14.4	15.5
Flour Extraction Recovery (%)	58.2	63.2
Alveograph[2]		
P (mm H_2O)	105	85
L (mm)	56	86
G	16.6	20.7
W (10^{-4} J)	247	311
P/L	1.89	.99
Ie (%)	64.7	72.9

[1] Analyzed by near infrared spectroscopy, d.b. = dry basis.

[2] Average of three independent samples, where P = tenacity; L = extensibility; G = index of swelling; W = baking strength, Ie = elasticity index and P/L = configuration ratio of the curve.

Table 2. Mean percent peak area of glutenin subfractions eluted from semi-preparative RP-HPLC

Subraction[1,2]	Main Component	Peak Area (%)	
		Big Dawg	Jagger
1	LMW-GS	14.52	7.36
2	HMW-GS	8.94	8.33
3	HMW-GS	33.32	42.27
4[3]	HMW-GS	29.00	32.09
5	LMW-GS	7.79	5.51
6	LMW-GS	4.10	3.78
7	LMW-GS	2.32	0.66
Sum of HMW-GS		71.26	82.69
Sum of LMW-GS		28.73	17.31

[1] Subfractions indentified in Figures 3-5 as BF and JF, followed by the number.
[2] n = 14 replicates.
[3] Subfraction 4 was a mix of HMW-GS and LMW-GS with more prominent bands in <100,000 and traces of protein bands >100,000.

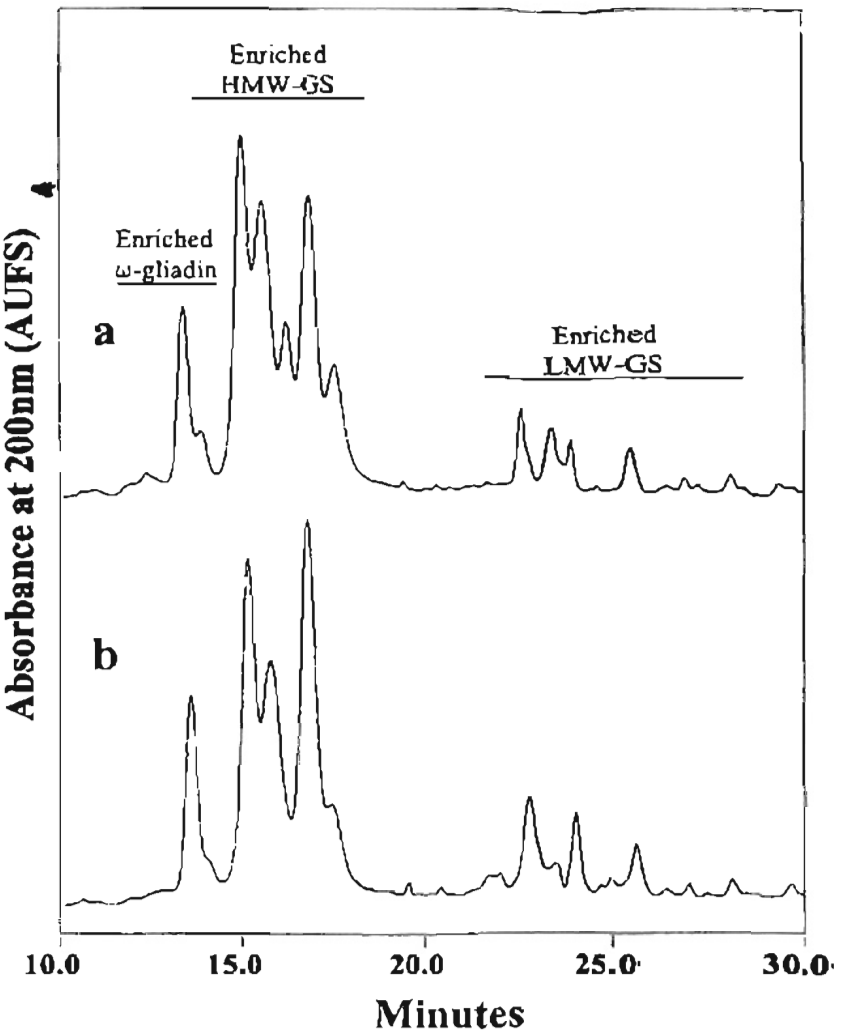

Figure 1. Analytical RP-HPLC separation of glutenin crude extract of two hard red winter wheat (a) Jagger and (b) Big Dawg solubilized in 50% n-propanol containing 1% DTT. Glutenin polypeptides eluted at 33-50% acetonitrile containing 0.06% TFA and detected at 200 nm absorbance.

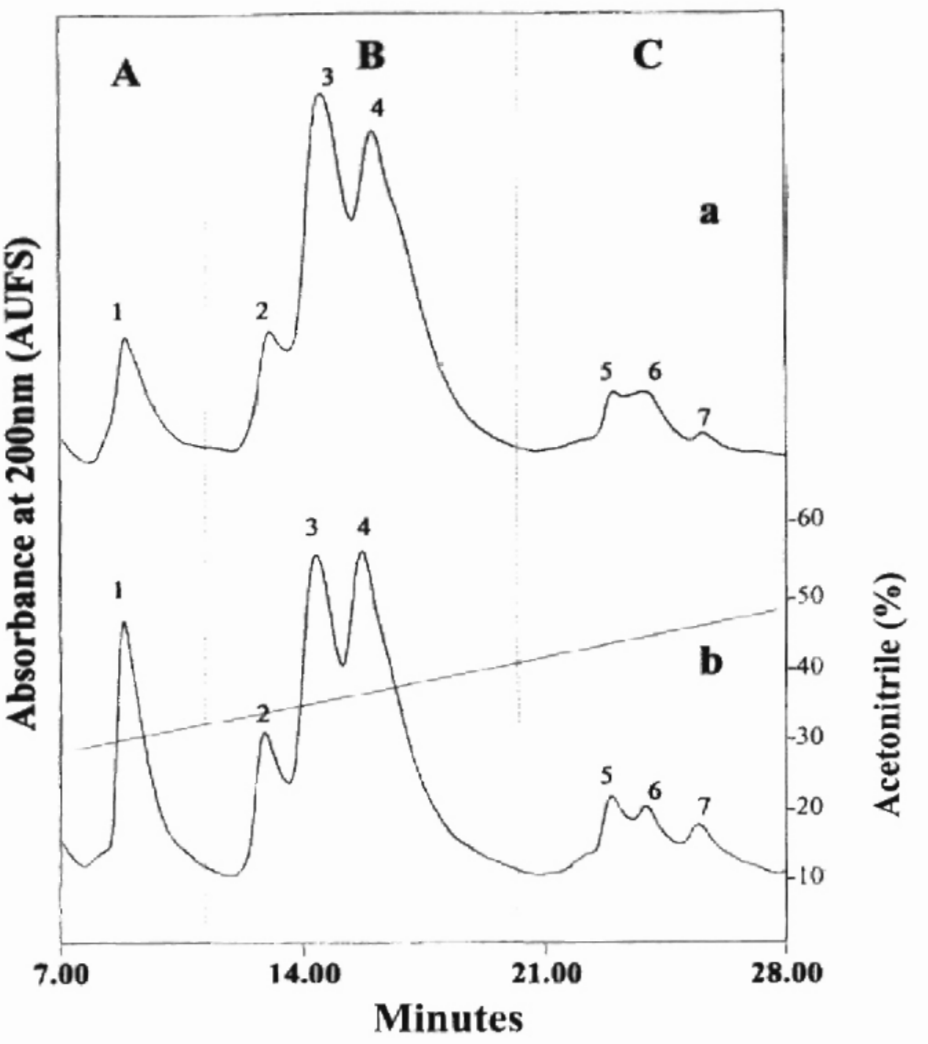

Figure 2. Semi-preparative RP-HPLC separation of glutenin crude extract of (a) Jagger and (b) Big Dawg solubilized in 50% n-propanol containing 1% DTT. Glutenin polypeptides eluted at 33-50% acetonitrile containing 0.06% TFA and detected at 200 nm absorbance. A = hydrophilic, B = less hydrophobic and C = more hydrophobic regions.

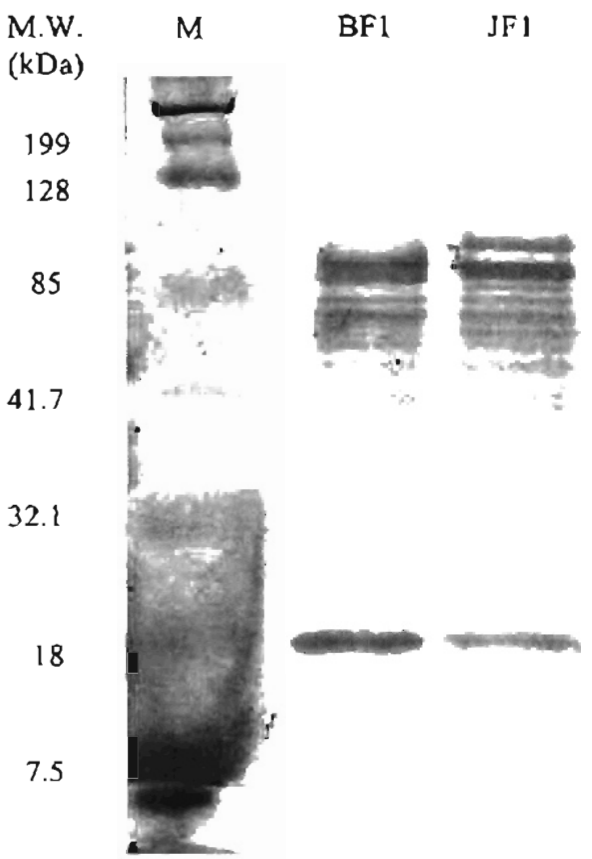

Figure 3. Molecular weight distribution of Big Dawg and Jagger Region A, subfraction 1. Freeze-dried samples were resolubilized in reducing buffer and analyzed using 15% SDS-polyacrylamide gel electrophoresis. M.W. and M are molecular weight markers.

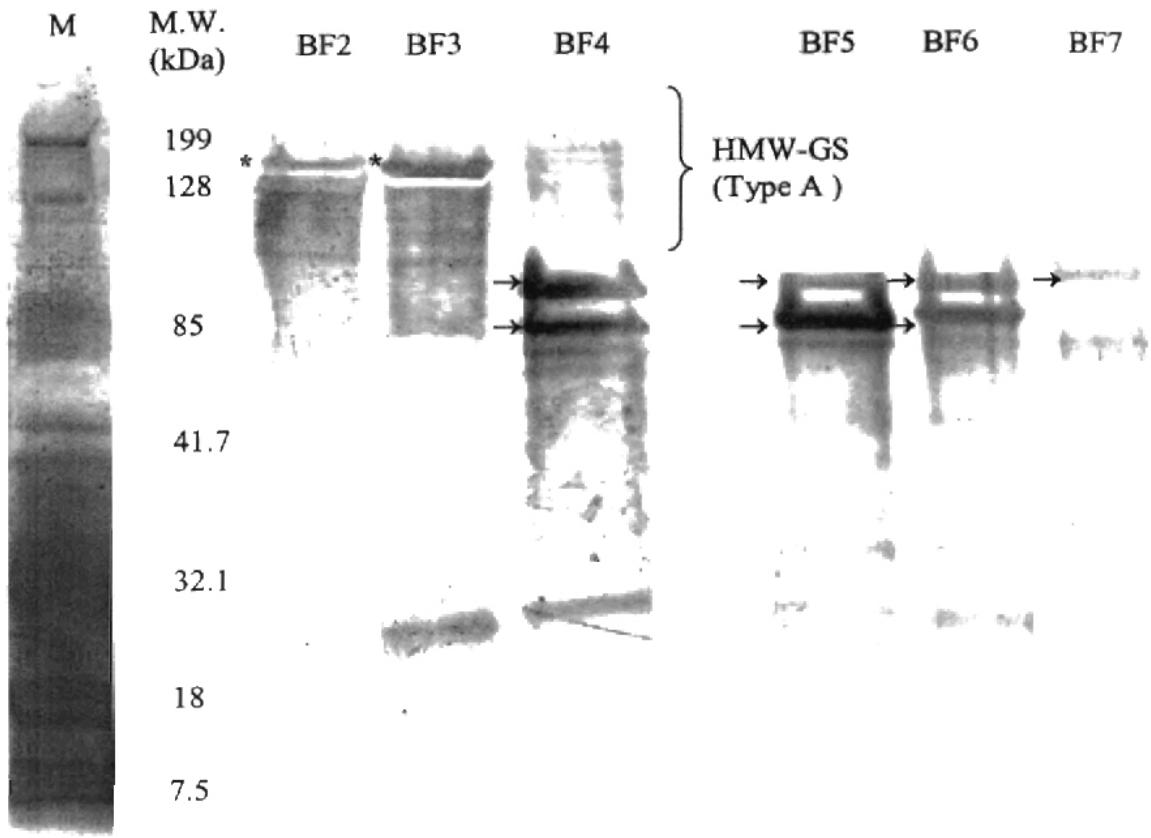

Figure 4. Molecular weight distribution of Big Dawg Regions B and C, subfractions 2 to 7. Subfractions 2 to 4 were categorized as enriched HMW-GS while subfractions 5 to 7 as enriched LMW-GS. Conditions were as stated in Fig. 3. M.W. or M are molecular weight markers. Arrows (→) indicate subunits 94,000 (upper) and 84,000 (lower) molecular weights. Asterisks (*) indicate the common 146,000 molecular weight.

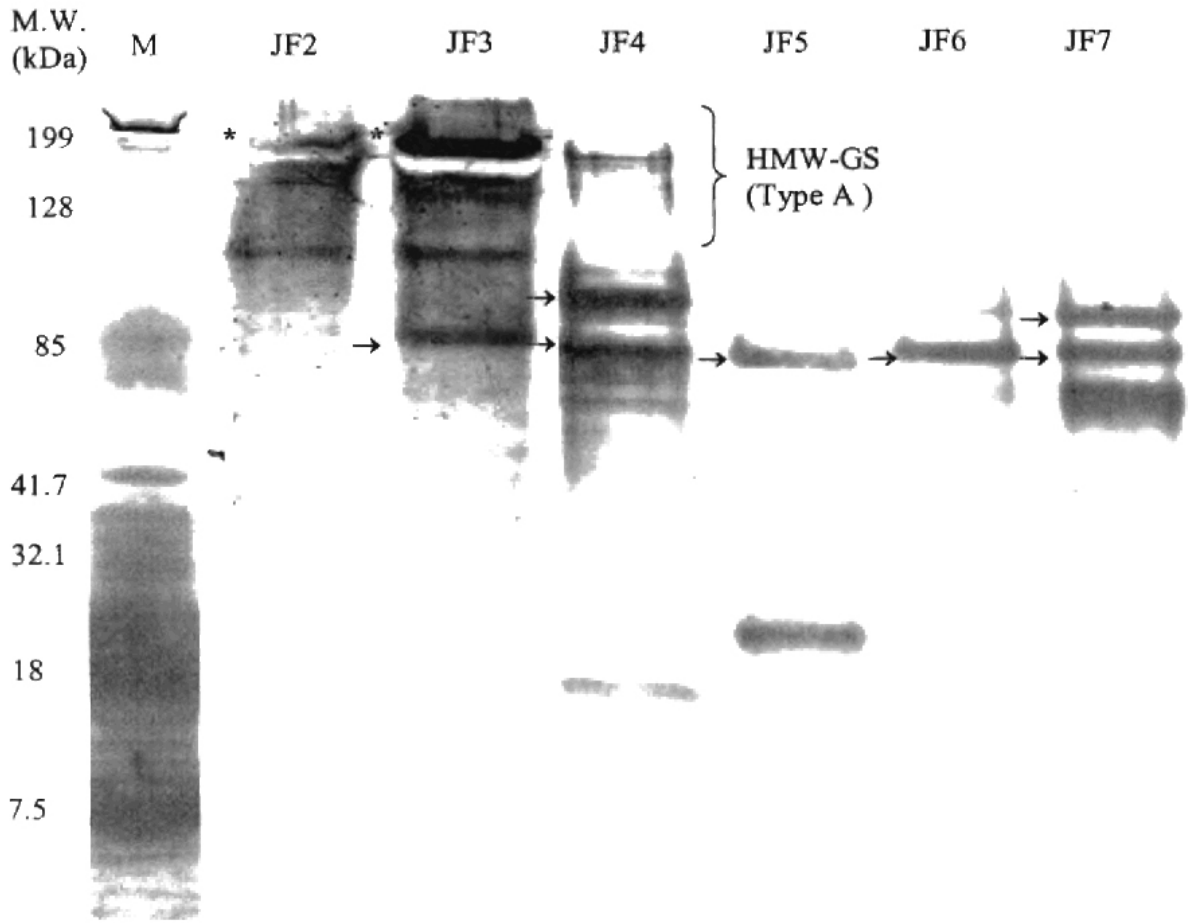

Figure 5. Molecular weight distribution of Jagger Regions B and C, subfractions 2 to 7. Subfractions 2 to 4 were categorized as enriched HMW-GS while subfractions 5 to 7 as enriched LMW-GS. Conditions were as stated in Fig. 3. M.W. or M are molecular weight markers. Arrows (→) indicate subunits 92,000 (upper) and 82,000 (lower) molecular weights. Asterisks (*) indicate the common 144,000 molecular weight.

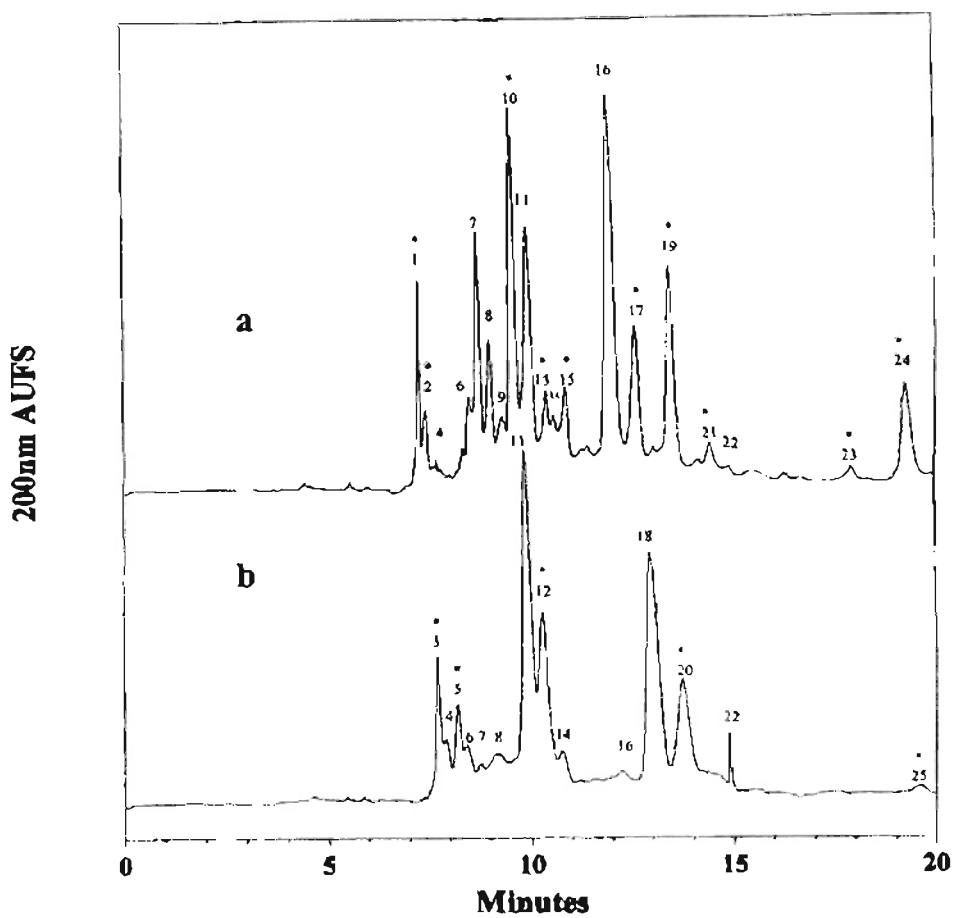

Figure 6. Electropherograms of glutenin subunits from hard red winter wheat (a) Big Dawg and (b) Jagger *cv.* extracted with 50% n-propanol containing 1% DTT, sonicated for 45 min. Capillary, uncoated fused silica 27 x 50 μm i.d.; voltage, 15 kV; temperature, 25 °C; running buffer, 100 mM phosphate buffer (pH 2.5) with 20% acetonitrile, 0.05% HMPC and 0.4% glycine; injection, pressure at 0.5 psi for 5 sec; detection, 200 nm. Asterisks (*) indicate peaks that are unique to the wheat cultivar.

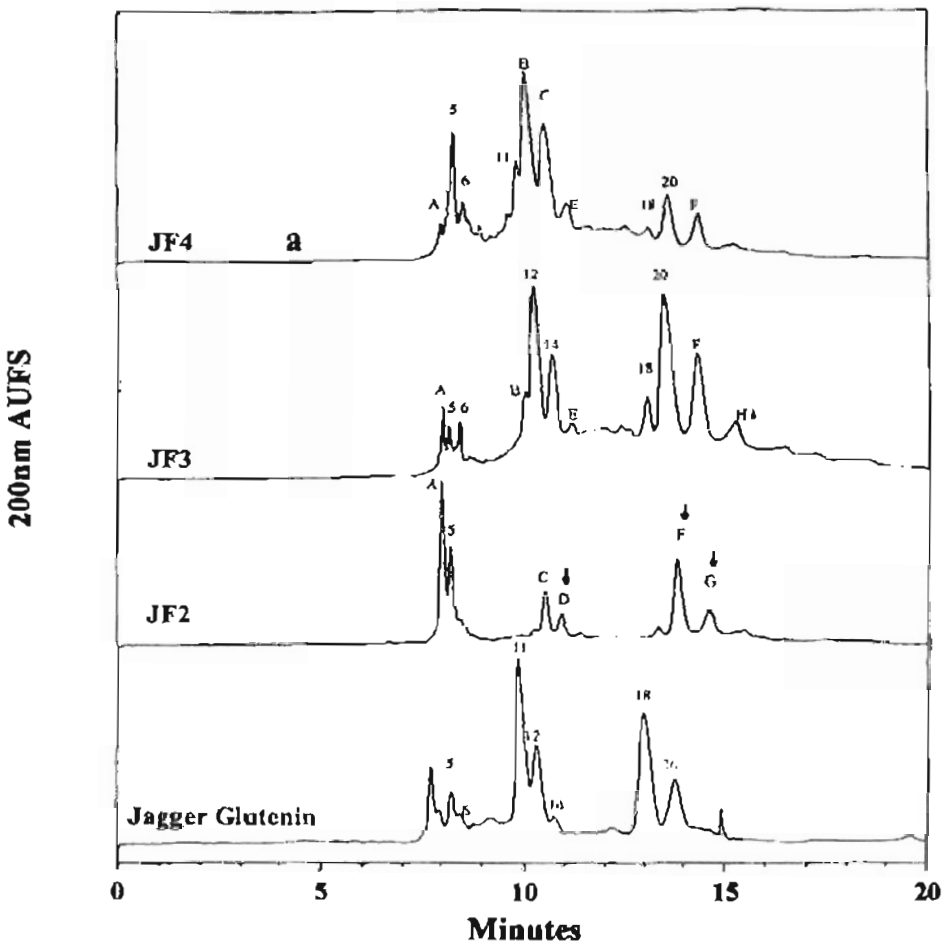

Figure 7. Electropherograms of crude extract and subfractions characterized as enriched HMW-GS from Jagger *cv.*, separated by RP-HPLC as described in Fig. 2. FZCE conditions were as specified in Fig. 6. Arrows (↓) indicate peaks which are unique to the subfractions.

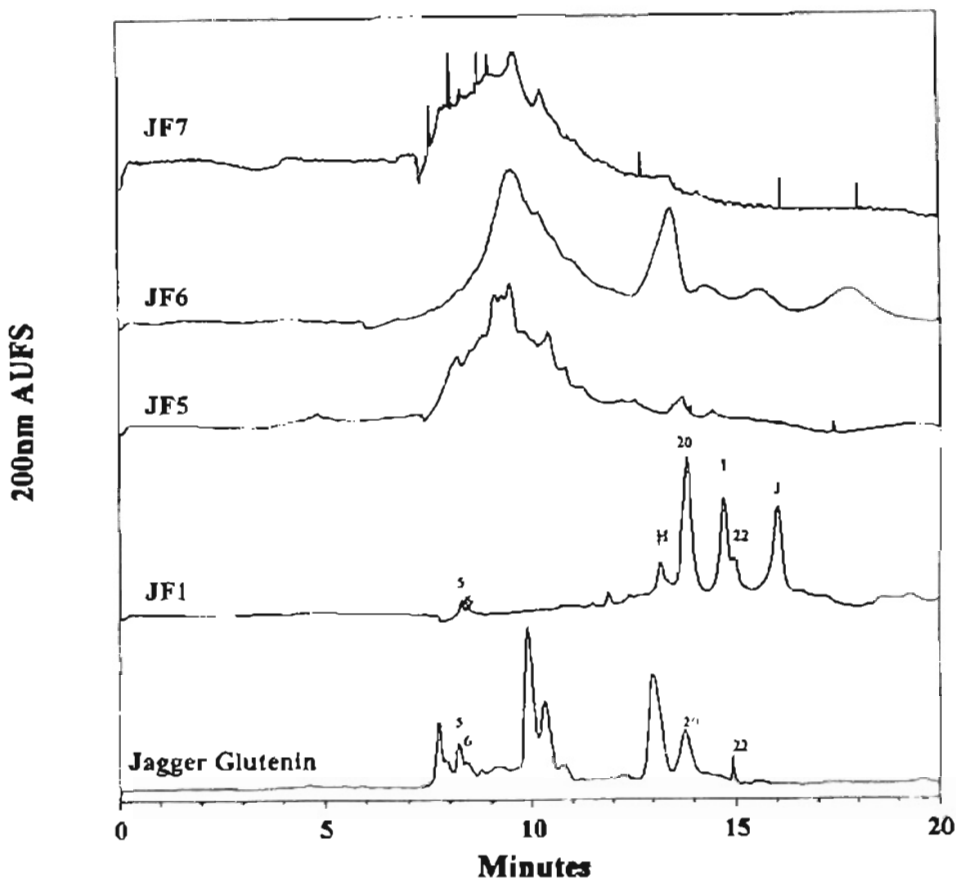

Figure 8. Electropherograms of crude extract and subfractions characterized as enriched LMW-GS from Jagger *cv.*, separated by RP-HPLC as described in Fig. 2. FCZE conditions were as specified in Fig. 6.

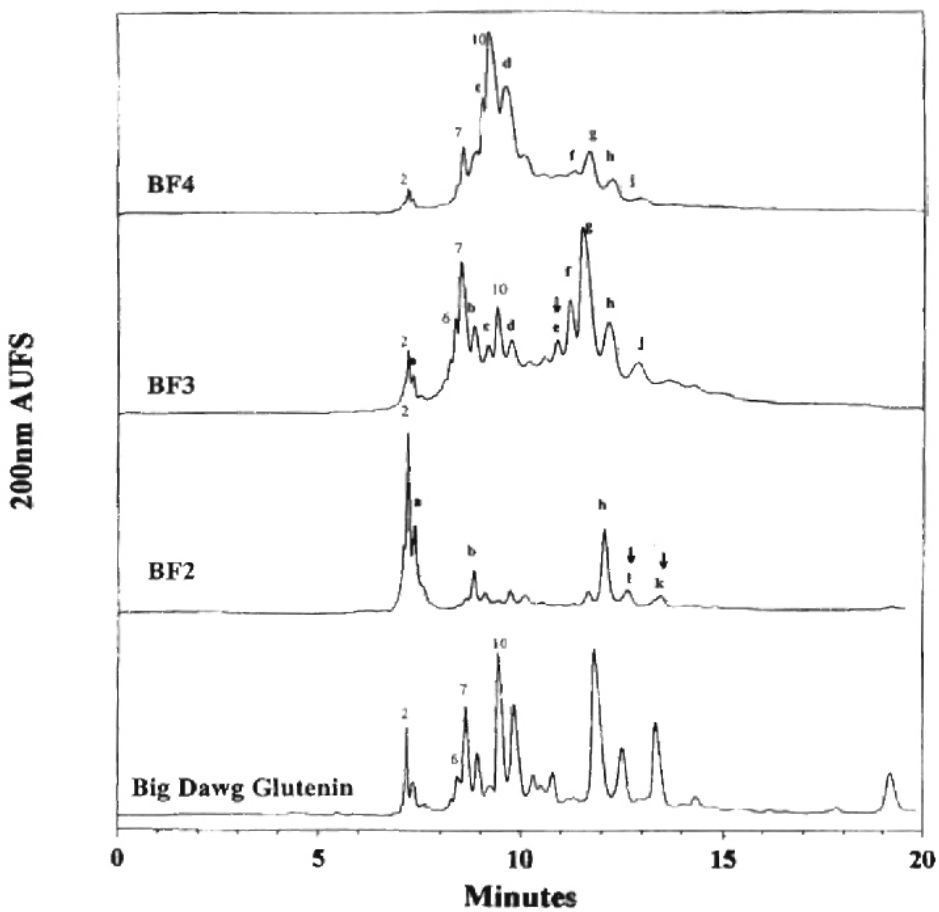

Figure 9. Electropherograms of crude extract and subfractions characterized as enriched HMW-GS from Big Dawg *cv.*, separated by RP-HPLC as described in Fig. 2. FCZE conditions were as specified in Fig. 6. Arrows (↓) indicate peaks which are unique to the subfractions.

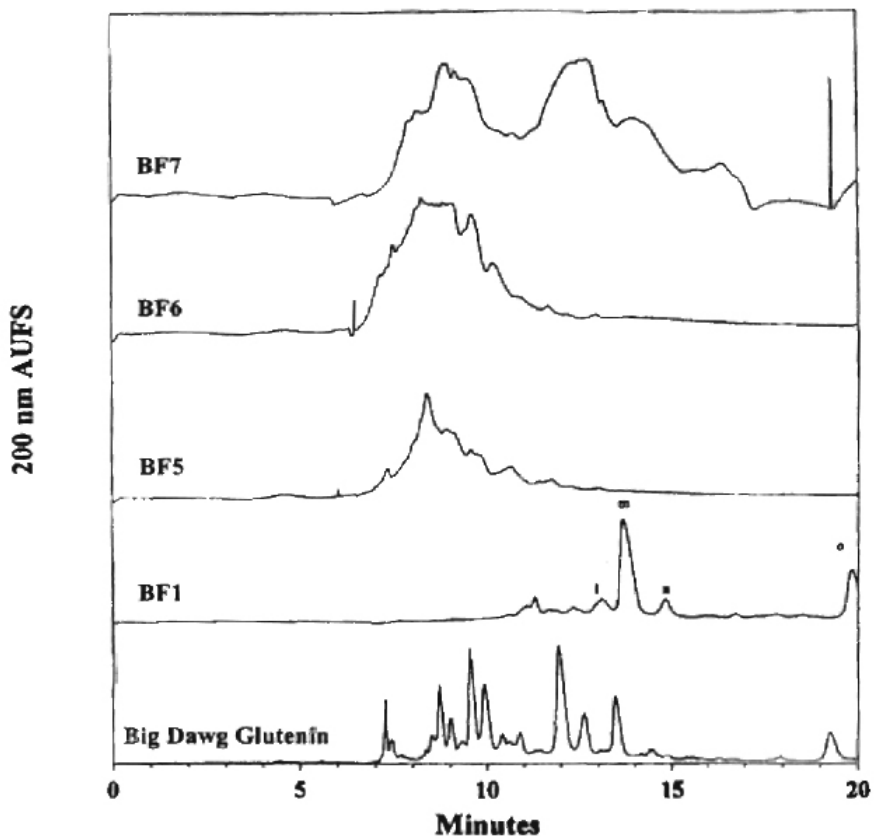

Figure 10. Electropherograms of crude extract and subfractions characterized as enriched LMW-GS from Big Dawg *cv.*, separated by RP-HPLC as described in Fig. 2. FCZE conditions were as specified in Fig. 6.

CHAPTER IV

BINDING ASSOCIATIONS OF REDUCED AND SOLUBLE GLUTENIN SUBFRACTIONS WITH MALTODEXTRIN IN SOLUTION

ABSTRACT

Dough is a complex system due to the synergistic effects of the wheat flour components and added ingredients. Determining the molecular associations of the wheat proteins may define the characteristic affinity of glutenin subfractions such as HMW-GS or LMW-GS to other molecules. Glutenin subfractions from two hard red winter cultivars separated by RP-HPLC as indicated in Chapter III were used in this study. The binding associations of the glutenin subfractions with maltodextrin (2,000 average molecular weight) were analyzed using affinity capillary electrophoresis (ACE).

Analyses were carried out using reduced and soluble subfractions of HMW-GS and LMW-GS with a standard concentration of 1 mg/100 µl. The column was saturated with a buffer containing 100 mM phosphate (pH 2.4), 20% (v/v) acetonitrile, 0.4% (w/v) glycine, and different maltodextrin concentrations (0, 5, 10, 25, 50 and 100 μM). An electric field of 15 kV was applied after a 5 sec injection of the sample.

Due to the heterogeneity of the glutenin subfractions, the binding constants (K_b) were analyzed according to the dominant glutenin polypeptide and the mean or

hypothetical K_b. The analysis of the dominant glutenin polypeptide was based on the largest peak area while hypothetical K_b was derived from the mean K_b of all the peaks in the electropherogram. Analyses of the dominant polypeptide revealed that JF3 and JF4 have strong affinity with maltodextrin ($1.98x10^5$ and $2.41x10^5$ M^{-1}, respectively). However, these K_b's were not significantly different from the K_b of other glutenin subfractions. For the hypothetical K_b, the variation in binding constants was due to the subfractions within each cultivar. Significant differences in the binding constants of enriched HMW-GS vs. LMW-GS subfractions were only observed in Jagger. A strong affinity with maltodextrin was exhibited by soluble glutenin components with less hydrophobic properties containing enriched HMW-GS (JF3 = $1.73x10^5$ M^{-1} and JF4 = $2.30x10^5$ M^{-1}), while the lowest affinity was found in the more hydrophobic region containing enriched LMW-GS subfractions (JF6 = $1.70x10^4$ M^{-1}). JF4 had a wider range of molecular weights, primarily 92,000 and 82,000, than did JF3. This difference suggests the importance of a particular proportion of HMW-GS to LMW-GS, which resulted in strong molecular associations of glutenin subfractions with maltodextrin.

INTRODUCTION

Scanning electron microscopy and magnetic resonance imaging have been utilized for protein-carbohydrate interactions based on microscopic observations of protein fibrils and starch during mixing, fermentation, and baking of the wheat dough (Greenwell and Schofield, 1986; Lindsay and Skirret, 1999; Kokeelar *et al.*, 1996; Ishida *et al.*, 2001). These associations are qualitative in nature while results from capillary electrophoresis provide a quantitative investigation of these associations.

In capillary electrophoresis, charged species travel inside the fused silica capillary under the influence of an electric field. The migration time of the species is determined by a combination of their electrophoretic mobility and the velocity of the electro-osmotic flow (EOF) (Baker, 1995). Molecular associations using capillary zone electrophoresis are more promising than other methods, i.e., gel electrophoresis or liquid chromatography, due to a low surface-to-volume ratio, large separation power, reasonable speed, and good automation prospects (Kraak, *et. al*, 1992).

Binding constants can be determined by different capillary electrophoresis methods, namely vacancy affinity capillary electrophoresis, Hummel-Dryer, frontal analysis, the vacancy peak method, and affinity capillary electrophoresis (ACE). ACE can only determine the binding constant while the rest of the above-mentioned methods determine both binding constants (K_b) and absolute numbers of the different binding sites (Busch *et. al*, 1997a). The advantage of ACE as compared to the other methods is the ease of analysis with more straightforward and less complex means of determining K_b.

For proteins that are capable of multiple or competitive binding sites, the binding constant values contain some discrepancies depending on the apparatus and method of analysis. An example is bovine serum albumin (BSA) which contains competitive binding sites for warfarin and indole. K_1 and K_2 are referred to as the binding constants for warfarin and indole sites (Erim and Kraak, 1998). Kraak *et al.*, (1992), compared Hummel-Dreyer, vacancy and frontal analysis using laboratory made capillary electropresis apparatus. They obtained K_1 and K_2 values of (a) 1.0×10^5 M^{-1} and 6.7×10^3 M^{-1}; (b) 0.7×10^5 M^{-1} and 1.4×10^3 M^{-1} and (c) 1.3×10^5 M^{-1} and 2.1×10^3 M^{-1} (Hummel-Dreyer, vacancy and frontal analysis, respectively). In comparison to using laboratory

made apparatus, using vacancy capillary electrophoresis in a commercial system resulted to K_1 and K_2 values of $1.2x10^5$ M^{-1} and $2.3x10^3$ M^{-1}, respectively (Erim and Kraak, 1998).

Recent studies of glutenin molecular association with maltodextrin have shown that the usual rectangular hyperbolic forms of 1:1 binding isotherms were not observed (Davila-El Rassi *et al.*, 2003). These researchers used a non-linear fitting method and reported limitations due to the limiting value of ligand-protein complex mobility. Linear methods may give a solution to such impediments whereas appropriate linearization may reveal the stability of the chosen model (Bowser *et al.*, 1997).

The objective of this study is to determine the binding constants of reduced and soluble glutenin subfractions with maltodextrin as analyzed by ACE. The binding constants were determined using a Y-reciprocal method.

The K_b of all the peaks found in the electropherograms of the subfractions were calculated. The heterogeneity of the subfractions prompted an investigation to understand the binding association of the analyte with the largest peak area or the dominant glutenin polypeptide and the hypothetical K_b of the glutenin subfraction. To derive the hypothetical K_b, two methods of analyses were compared in the statistical analyses. For method 1, the analyte with the highest K_b among the peaks with multiple analytes per replication, including subfractions with a single peak, was used as an experimental unit. Method 2 used the average K_b of all the analytes found in a replication. The methods for statistical analysis of hypothetical K_b considered that (a) the analyte with the largest peak area may not have the highest K_b (b) the subfractions may have multiple binding sites, and (c) the composition of glutenin subfractions may be heterogeneous.

MATERIALS AND METHODS

SAMPLE SOURCES

Two hard red winter wheat cultivars, Jagger and Big Dawg, were used in the study. Extraction of the soluble glutenins, semi-preparative RP-HPLC separation and capillary electrophoresis instrumentation was as described in Chapter III, Materials and Methods section. The maltodextrin for the starch binding constants in the ACE was a gift from Grain Processing Corporation (Muscatine, IA).

DETERMINATION OF BINDING CONSTANTS

Estimations of the binding constants of glutenin subfractions and maltodextrin were derived using affinity capillary electrophoresis. A 27 cm uncoated fused-silica capillary (20 cm to the detector, 50 μm i.d.) from Polymicro Technologies, Inc. (Phoenix, AZ) was used. The analysis was performed using a Beckman P/ACE 2000 (San Ramon, CA) capillary electrophoresis.

Dried glutenin subfractions were solubilized in 50% n-propanol containing 1% DTT and 4% acetic acid and sonicated for 45 min. The samples injected were adjusted to a protein concentration of 1 mg/100 µl. An aliquot of 120 μl was drawn and used for the actual analysis. The column was rinsed consecutively with 0.1N NaOH and 0.5 M acetic acid for 1 min at 20 psi. A nanopure water rinse preceded each solvent rinsing. The column was saturated with the running buffer containing 100 mM phosphate (pH 2.4), 20% (v/v) acetonitrile, 0.4% (w/v) glycine and different maltodextrin (average M.W. 2,000) concentrations (0, 5, 10, 25, 50 and 100 μM). An electric field of 15 kV was

applied after a 5 sec injection of the sample at 0.5 psi. The migration time was obtained from the electropherogram. Binding constants from peaks of interest were determined using the Y-reciprocal method (Rundlett and Armstrong, 1996).

Assuming that the glutenin and maltodextrin has a 1:1 binding association, the change in solute mobility with changing ligand concentration can be used to calculate the binding constant using the following equation:

$$\mu_i = \frac{\mu_f + \mu_c K[L]}{1 + K[L]} \qquad [1]$$

Where μ_i is the experimentally measured electrophoretic mobility of the solute, μ_f is the mobility of the free (uncomplexed) solute, μ_c is the electrophoretic mobility of the solute-ligand complex, K is the binding constant, and [L] is the equilibrium ligand concentration. Binding constants can be estimated by varying the ligand concentration at constant solute concentrations and fitting the data to equation 1 (Rundlett and Armstrong, 2001). This general formula can be further transformed in equations 2 and 3 and plotted as the Y-reciprocal method (Rundlett and Armstrong, 1997):

$$\frac{[L]}{(\mu_i - \mu_f)} = \frac{1}{(\mu_c - \mu_f)}[L] + \frac{1}{(\mu_c - \mu_f)K} \qquad [2]$$

(y axis) (x axis)

$$\frac{[L]}{(\mu_i - \mu_f)} \text{ vs. } [L] \qquad K_b = \frac{\text{slope}}{\text{intercept}} \qquad [3]$$

Statistical Analyses

To analyze the binding constants based on the dominant glutenin polypeptides (largest peak area) and the over-all hypothetical binding constant in each glutenin subfraction, two separate statistical analyses were made. A complete randomized design, with the cultivar as the main treatment effect and an additional factor of subfraction nested in the cultivar, was used to determine the binding constant of the dominant glutenin polypeptides. Three individual replicates were analyzed for each subfraction.

To determine the hypothetical binding constant of the glutenin subfractions, a complete randomized design, a 2 x 2 factorial treatment structure with two cultivars and two methods of analysis, was used. An additional factor, seven subfractions, was used as nested within the cultivar. Three replications were done in each set of six concentrations of maltodextrin per subfraction to provide three sets of K_b per analysis for each subfraction. Two methods of analysis and two cultivars were used as treatment effects. The use of two methods allowed SAS to correct the error degree of freedom estimation. Statistical Analysis Software (SAS) version 8.1 was used to analyze the binding constants (K_b) of glutenin subfractions.

The data set was transformed to $\log_e K_b$ to control the high values of K_b and unequal variances. Contrast statements were defined for specific research questions, namely (a) whether there was a significant difference between the mean K_b of the enriched HMW-GS (F2, F3 and F4) and LMW-GS (F1, F5, F6, and F7) subfractions; and (b) whether the difference between Big Dawg and Jagger in mean K_b of the HMW-GS and LMW-GS enriched subfractions was significant.

RESULTS AND DISCUSSION

PRELIMINARY STUDIES

Preliminary analyses indicated that the electrophoretic mobility increased when ≥ 500 μM maltodextrin concentration was used (Fig. 11). These analyses contrasted with the theory of molecular association of ACE in which electrophoretic mobility of the protein decreases upon forming a complex with the ligand (Busch, *et. al*, .1997a and 1997b, Chu *et. al*, 1994). Rundlett and Armstrong (1997) reported that increasing the ligand concentration can cause changes in the viscosity, conductivity, and ionic strength of the running buffer solution, and in turn cause changes in the electrophoretic mobilties (Rundlett and Armstrong, 1997). Therefore, concentrations from 5 to 100 μM of maltodextrin (ligand) were used.

To understand the binding properties of the glutenin subfractions, two analyses were utilized. First, the binding constants based on the dominant glutenin polypeptides, particularly for electropherograms with multiple peaks of interest, were determined. These provided information regarding the affinity of the major glutenin components of a particular subfraction to maltodextrin. Second, the hypothetical binding constant values were determined because of the heterogeneous nature of the subfractions.

ANALYSIS OF K_b FROM DOMINANT GLUTENIN POLYPEPTIDES

Table 3 shows the binding constants of the dominant peak in the glutenin subfractions obtained by ACE analysis (Method 1). The lowest mean K_b was obtained from BF1 (1.6×10^4 M^{-1}) and highest mean K_b from JF3 and JF4 (19.8×10^4 and 24.1×10^4

M^{-1}, respectively). The variation in the mean K_b was not due to the cultivar and subfractions used (Appendix B). The mean K_b based on the dominant peak, showed no significant effects due to the cultivar and the subfraction unique to the cultivar ($F_{1,28}$ = 3.25, P=0.0822 and $F_{1,28}$ = 1.68, P=0.1267, respectively). The mean K_b of the enriched HMW-GS was significantly different from that of the LMW-GS ($F_{1,28}$ = 4.61, P=0.0406) in Jagger but was not observed in Big Dawg ($F_{1,28}$ = 0.04, P=0.8522). The difference in K_b's of enriched HMW-GS and LMW-GS between the two wheat cultivars was not significant.

The results suggest that the dominant glutenin polypeptides from JF3 and JF4 have strong affinity with maltodextrin, but statistically the K_b's of these polypeptides were not different from the K_b of the other glutenin subfractions. In addition, the K_b of the enriched HMW-GS were different from the K_b of LMW-GS in Jagger but overall were not significantly different from those of Big dawg subfractions. The dominant peak in the electropherograms of the glutenin subfraction with multiple peaks did not always show the highest K_b.

Average Hypothetical K_b of the Glutenin Subfractions

Table 3 summarizes the properties of the glutenin subfractions analyzed by ACE. The molar concentrations were calculated based on a sample protein concentration of 1 mg/100 µl and an average estimated molecular weight in SDS-PAGE for each subfraction (67,100 to 115,500). The concentrations of the subfractions ranged from 0.087 to 0.182 mM (Table 3). The separation of glutenin subunits was poorer, i.e., lower numbers and clustered peaks, in ACE (Appendix C) compared to FZCE (Figures 7 to 10).

Subfractions BF5, BF6, JF1, JF5 and JF7 revealed only one peak. Two peaks were found in BF4 and JF4. The remainder of the subfractions contained three peaks in ACE analysis (Appendix C). Unlike the sample buffer in FZCE, the ACE sample buffer contained 4% acetic acid which, in addition to the 100 mM phosphate (pH 2.5) in the running buffer, protonates the glutenin subfractions and therefore changes the net surface charge of the proteins. The acetic acid was added to the sample buffer to completely solubilize the glutenin subfractions. Another factor in the number of peaks and net surface charge differences was the absence of a sieving additive (HMPC) in the affinity capillary electrophoresis running buffer. A polymeric additive was not included to avoid competition with the binding sites of the glutenin subunits. However, these additives not only serve as sieving media to improve the resolution, but also reduces the adsorption of proteins in the capillary walls (Baker, 1995). The ACE peaks were clusters of multiple peaks of an undefined number of subcomponents which changed in electrophoretic mobility as the maltodextrin concentration in the buffer increased. As an example, electropherograms of JF1 (Appendix D), eluted with different concentrations of maltodextrin in the running buffer, suggest that the molecular associations of glutenin components with maltodextrin were most likely due to a group of polypeptides and not to a single glutenin subunit of the subfraction.

Figure 12 and Table 3 exhibit the hypothetical mean K_b (Method 2) of glutenin subunits with maltodextrin obtained from ACE. The method and the wheat cultivar did not have a significant effects on the K_b ($F_{1,56}$ 0.03, P =0.871, Appendix E). However, there was a significant main effect of the subfraction within the cultivar ($F_{12,56}$ = 2.58, P 0.0084). These findings suggest that the hypothetical K_b values were not affected by

using two methods and two wheat cultivars in the statistical analysis but the differences were due to the glutenin subfractions. The variation was due to JF3, JF4 and JF6 with mean K_b of $17.2x10^4$, $22.9x10^4$ and $1.7x10^4$ M^{-1} respectively. These values indicate that the reduced and soluble glutenin components of Jagger, particularly the less hydrophobic polypeptides enriched with HMW-GS, form strong molecular associations with maltodextrin.

Figures 13, 14, and 15 show the Y-reciprocal plots of JF3, JF4, and JF6. The highest mean K_b (JF3 and JF4), were enriched with HMW-GS while the lowest mean K_b, JF6, belonged to the group of more hydrophobic and predominantly LMW-GS. The coefficient of correlation revealed a good linear fit using the Y-reciprocal method (R^2 = 0.9648 to 0.9996). Peak A from the JF4 subfractions, with the lowest coefficient of correlation (R^2 = 0.7564), was analyzed with three concentrations of maltodextrin compared to four for the rest of the subfractions.

The seven Big Dawg subfractions appeared to have similar molecular associations with maltodextrin (average K_b $6.3x10^4$ M^{-1}) regardless of hydrophobic differences and the different molecular weights of the subunits in each subfraction. In contrast, Jagger subfractions appeared to have three groups with different molecular associations with maltodextrin. In the group with a strong molecular association with maltodextrin were JF3 and JF4 (K_b $17.2x10^4$ and $22.9x10^4$ M^{-1}, respectively) which were enriched HMW-GS with a less hydrophobic nature. These K_b's were higher than those derived from BSA-warfarin ($1.2x10^5$ M^{-1}) and lectin-polysaccharide ($5.6x10^3$ M^{-1}) using vacancy peak analysis and ACE, respectively (Erim and Kraak, 1998; Winzor, 1995). On average, the hypothetical K_b's of JF3 and JF4 were 3.2 and 4.2x, greater respectively than those of the

other subfractions. In the group with intermediate molecular association were subfractions JF1, JF2, JF5 and JF7 (average K_b $8.0x10^4$ M^{-1}) which contained glutenin subunits enriched with a wide range of surface hydrophobicity and molecular weights. This group had molecular associations with maltodextrin similar to those of Big Dawg subfractions. Only one glutenin subfraction, JF6, with relatively high hydrophobicity, had low molecular association with maltodextrin (K_b $1.7x10^4$ M^{-1}). JF6 was considered enriched LMW-GS.

The molecular interaction between maltodextrin and the reduced, soluble glutenin subunits in solution is believed to be mostly hydrophilic in nature. Maltodextrin and soluble glutenin subunits have a wide range of OH-rich domains that can act as hydrogen bond donors and acceptors. We speculate that the small molecular-weight maltodextrin molecules form a complex with an assumed random coil structure of soluble glutenin as they come in contact in the capillary.

The differences in affinity of the glutenin subunits with maltodextrin were speculated to be caused, in part, by the presence of glutenin subunits known as chain extenders (ChE) and chain terminators (ChT). ChE are glutenin subunits that have at least two cysteine residues available for intermolecular disulfide bonds, which in turn support the growth of the glutenin polymer. A ChT, on the other hand, has only one cysteine residue, which blocks the growth of the glutenin polymers. The availability of these cysteine residues is important in establishing the folded conformations of the glutenin polypeptide (Gianibelli *et al.*, 2001; Gianibelli *et al.*, 2002). The conformation of these glutenin polypeptides, as shown during ACE analyses, was essential in providing the available binding sites for maltodextrin association. This study assumed that the long

glutenin polypeptide chains, in addition to providing a favorable conformation (exposure of more binding sites) might have been the reason for the strong molecular association with maltodextrin. Thus, in JF3 and JF4, there may have been considerable amounts of chain extenders, probably HMW-GS, and the conformation of the polypeptides allowed the exposure of more binding sites which enhanced their interaction with maltodextrin. The seven subfractions of Big Dawg and the Jagger subfractions categorized as having medium molecular association (JF1, JF2, JF5 and JF7) may have considerable amounts of ChE and ChT, but the conformation of the glutenin polypeptides provided fewer binding sites than did the conformation of JF3 and JF4. The subfraction JF6, however, may have smaller amounts of ChE or it may have folded in a manner that exposed fewer binding sites than in the other glutenin subfractions, which reduced its capacity to bind with maltodextrin.

When the molecular associations of glutenin with maltodextrin were analyzed by comparing the differences between subfractions enriched with HMW-GS vs. those enriched with LMW-GS, only Jagger showed significant differences ($F_{1,56}$ = 10.22, P=0.0023, Appendix E). The differences in enriched HMW-GS and LMW-GS between the Jagger and the Big Dawg subfractions were significant ($F_{1,56}$ = 4.33, P=0.0419) due to subfractions JF3, JF4 and JF6. These observations suggest that the surfaces of Jagger glutenins form stronger bonds and a larger number of bonds with maltodextrin than do the surfaces of Big Dawg glutenins. The higher affinity with maltodextrin could be significant if it correlates with similar associations with starch. Glutenin subunits and starch could then form clusters of structures that remain bound during the extension of dough structure occurring during fermentation and baking. Although only physical

evidence of the interactions of gluten proteins with starch is available in the literature (McMaster and Bushuk, 1983; and Zawistowska *et al.*, 1985), it is assumed that those interactions stabilize the delicate foam structure of the dough.

LIMITATIONS IN DETERMINING THE K_b OF GLUTENIN SUBFRACTIONS

It is important to take into consideration that 1:1 binding isotherms are observed in molecular associations using capillary electrophoresis; otherwise, certain corrections of K_b should be utilized based on the number of available binding sites or the use of other methods. Inaccurate K_b calculation is caused by assuming a 1:1 binding isotherm in compounds with multiple binding capacities. Winzor (1995) discussed problems associated with assuming a 1:1 binding stoichiometry for multivalent lectins and charged polysaccharides. Because lectins contain separate, equivalent polysaccharide binding sites, when the ligand (polysaccharide) concentration increases, the difference in the mobilities of lectin-polysaccharide complexes differs by an almost constant value. The K_b can be corrected by determining the intrinsic binding constant based on non-linear regression analysis. These constants were 5.6×10^{3} M^{-1} and 0.094 for the intrinsic binding constant and the incremental change in mobility of free lectin, respectively. In this study, the differences in mobilities between the successive complexes were not constant with glutenin-maltodextrin association. For example, in Peak A of BF2 the differences in electrophoretic mobilities, based on the Y-reciprocal method, were -1.1×10^{-6}, -1.7×10^{-6}, -1.9×10^{-6}, -5.2×10^{-6}, and -9.5×10^{-6} for 5, 10, 25, 50 and 100 mM maltodextrin, respectively (Appendix F). In addition, co-migration of glutenin polypeptides was detected. Therefore, the adjustment for the intrinsic binding constant suggested by Winzor (1995) cannot be estimated. Hence, the binding constants derived from glutenin-maltodextrin

molecular associations were hypothetical values and relied heavily on available surface binding sites of the several polypeptides that comprised the subfraction.

This study acknowledges the restrictions of the method and model used for determining the molecular association of soluble glutenins. Thus, the hypothetical K_b's of the glutenin subfractions reported here represent the first attempt to estimate their affinity with maltodextrin.

To the best of our knowledge, this study is the first report of quantitative evaluation of glutenin subfractions with a carbohydrate. It is speculated that Big Dawg glutenin subfractions differ in charge density from Jagger subfractions. Perhaps the amino acid sequence or conformation of subunits forming subfractions of mainly HMW-GS form domains with favorable molecular associations with carbohydrates. The soluble glutenins from Big Dawg appeared to have lower affinities for carbohydrates than did those from Jagger. It is important to highlight that only the soluble glutenins were studied in this report. Because of this limitation, it is not known if the molecular associations reported here can be extended to the insoluble glutenins.

CONCLUSION

The electropherograms of the glutenin subfractions analyzed in ACE were heterogenous and do not exhibit 1:1 binding isotherms. Analysis of the predominant analyte in the glutenin subfraction revealed that JF3 and JF4 have the highest mean K_b's (1.2×10^5 and 2.4×10^5 M^{-1}, respectively). However, these were not statistically different from the K_b's of predominant polypeptides from other glutenin subfractions.

For the hypothetical K_h, the molecular associations between soluble and reduced glutenin subfractions and maltodextrin were mainly affected by the specific subfractions within a cultivar. Similar to the analysis of the predominant analyte of the glutenin subfraction in ACE, the highest binding constants were found with the enriched HMW-GS subfractions of Jagger (JF3 and JF4) which have K_h's of $1.7x10^5$ and $2.3x10^5$ M^{-1}. The molecular weight distributions of these subfractions ranged from 86,000 to 122,000 for JF3, while JF4 subunits ranged from 74,000 to 122,000 in molecular weights. The lowest molecular association was found in subfraction JF6 (K_h = $1.7x10^4$ M^{-1}), which contained mainly LMW-GS and had a strong tendency to polymerize with itself instead of associating with maltodextrin. HMW-GS enriched subfractions from Jagger contained polypeptides that had a stronger affinity to maltodextrin than did LMW-GS enriched subfractions. This difference in affinity was not observed in Big Dawg. It is speculated that Jagger contains a particular group of soluble glutenin components and enriched with HMW-GS with less hydrophobic properties which are responsible for the strong molecular associations.

It is also speculated that strong molecular associations of soluble glutenin components with carbohydrates are present in specific wheat cultivars with characteristic HMW-GS which are less hydrophobic. More studies are needed to improve the accuracy of the analysis and the appropriate model for determining the K_h

Table 3. Molecular weight, concentration, number of peaks and binding constants (K_b) obtained from dominant glutenin polypeptide and hypothetical K_b based on the mean K_b of the peaks of interest in a glutenin subfraction from two hard red winter wheat cultivars using affinity capillary electrophoresis.

Cultivar/ Subfraction	Estimated Molecular Weight ($x10^4$)[1]	Concentration (mM)	Number of Peaks[2]	ACE Analysis K_b[3] ($x10^4\ M^{-1}$)	
				Method 1[4,6]	Method 2[5]
Big Dawg					
BF1	8.7	0.115	2	3.1[b]	1.6
BF2	11.6	0.087	3	4.3[b]	3.0
BF3	10.5	0.095	3	7.7[b]	8.8
BF4	8.9	0.112	2	7.4[b]	3.4
BF5	6.8	0.147	1	7.9[b]	7.9
BF6	6.8	0.147	1	7.5[b]	7.5
BF7	8.6	0.116	3	6.0[b]	5.7
Jagger					
JF1	9.5	0.105	1	7.1[b]	7.1
JF2	11.3	0.089	3	7.6[b]	6.1
JF3	10.6	0.094	3	17.2[c]	19.8
JF4	8.6	0.116	2	22.9[c]	24.1
JF5	5.5	0.182	1	10.9[b]	10.9
JF6	6.7	0.149	3	1.7[a]	1.8
JF7	6.8	0.146	1	6.5[b]	6.5

[1] Molecular weight obtained from SDS-PAGE
[2] Peaks of interest resolved within a subfractions obtained from RP-HPLC
[3] Average of three independent samples
[4] K_b of the dominant glutenin polypeptides based on peak area
[5] Hypothetical K_b of the glutenin subfractions based on mean K_b of the peaks of interest
[6] Means followed by the same letter within the K_b column are not significantly different at α=0.05

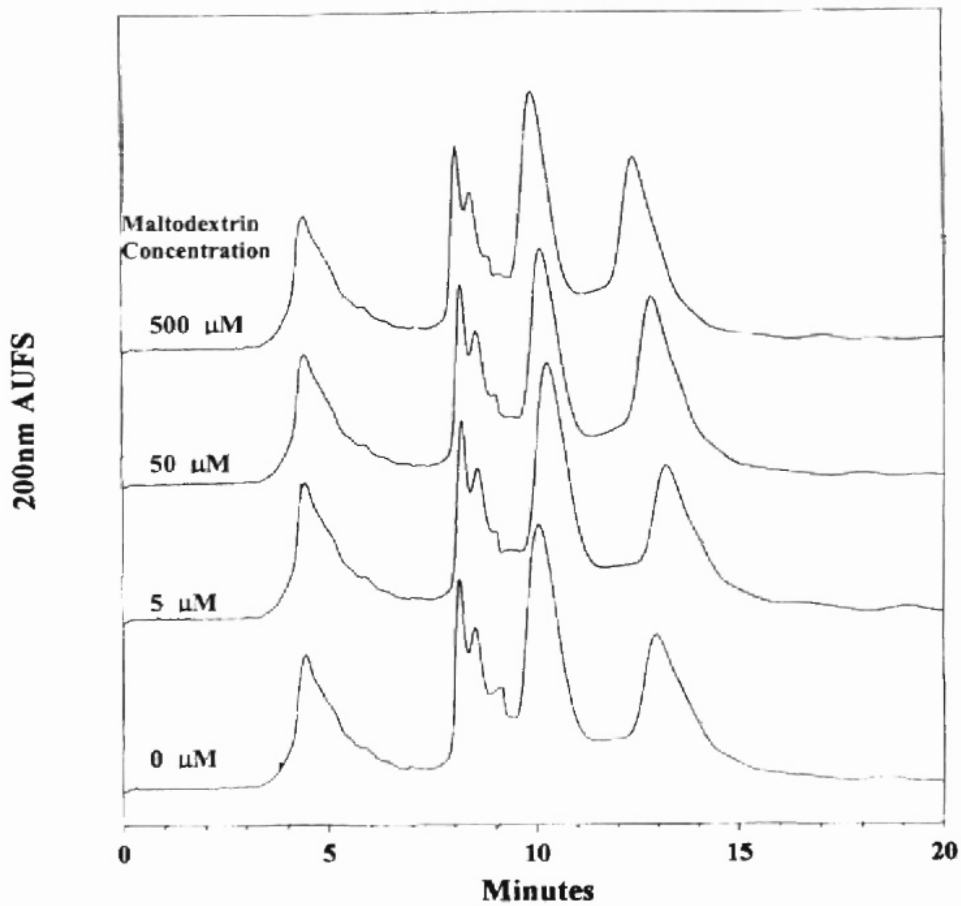

Figure 11. Electropherograms of hard red winter wheat Jagger cv. glutenin (total protein = 10 mg/ml), extracted with 50% n-propanol containing 1% DTT and 4% acetic acid, sonicated for 45 min. Capillary, uncoated fused silica 27 x 50 µm i.d.; voltage, 15 kV; temperature, 25°C; run buffer, 100 mM phosphate buffer (pH 2.5) with 20% acetonitrile, 0.4% glycine and different concentrations of maltodextrin (0, 5, 50 and 500 µM); injection, pressure at 0.5 psi for 5 sec; detection, 200 nm. Changes in electrophoretic mobility were recorded.

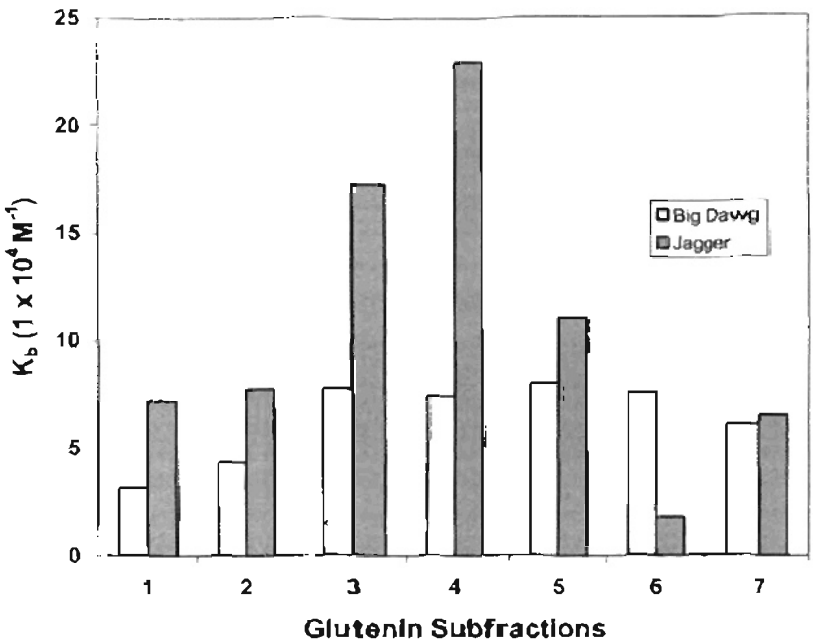

Figure 12. Mean binding constants (K_b) obtained from Y-reciprocal method of glutenin subfractions from hard red winter wheat Big Dawg and Jagger *cv.* Freeze dried samples were solubilized in 50% n-propanol containing 1% DTT and 4% acetic acid (total protein 1 mg/100 μl), injected for 5 sec at 0.5 psi and separated in 100mM phosphate buffer (pH 2.5) containing 20% acetonitrile, 0.4% glycine and maltodextrin (0, 5, 10, 25, 50 and 100 μM), under 15 kV and detected at 200 nm absorbance.

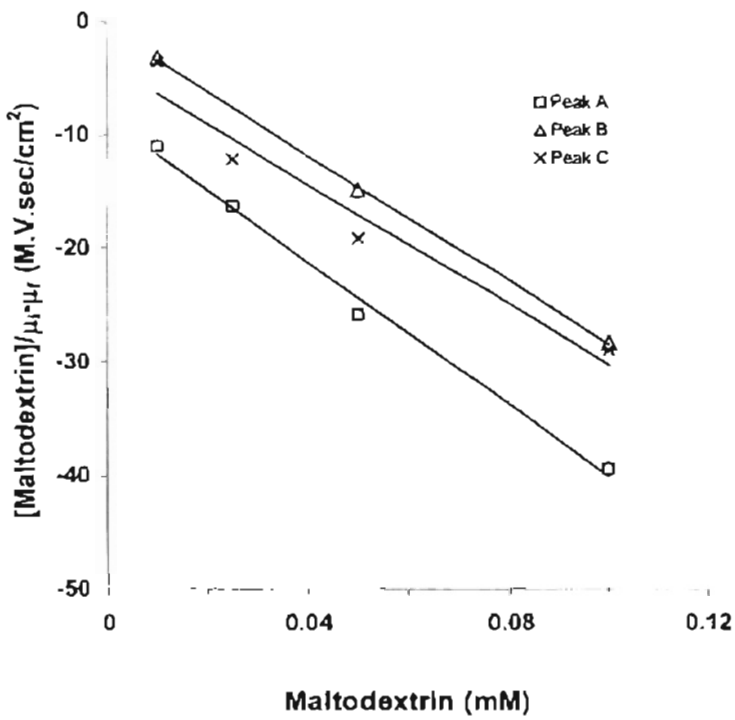

Figure 13. Y-reciprocal plot of Jagger glutenin subfraction JF3. The R^2 and y intercepts are (a) 0.9935, -8.64; (b) 0.9996, -0.6299 and (c) 0.9514, -3.756 for Peaks A, B, and C, respectively. Conditions were as specified in Fig. 12.

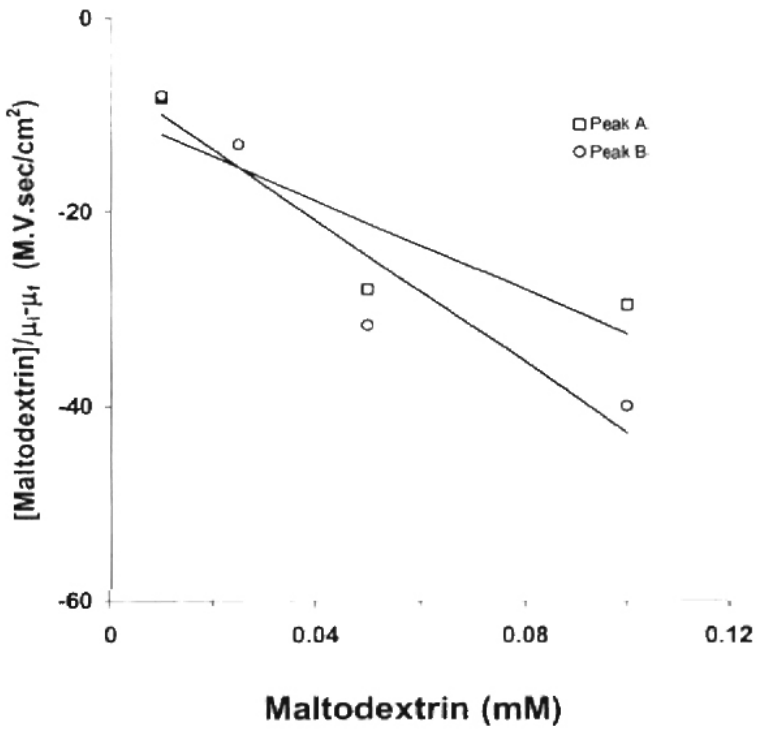

Figure 14. Y-reciprocal plot of Jagger glutenin subfraction JF4. The R^2 and y intercepts are (a) 0.7564, -9.7246 and (b) 0.9827, -4.94, for Peaks A and B, respectively. Conditions were as specified in Fig. 12.

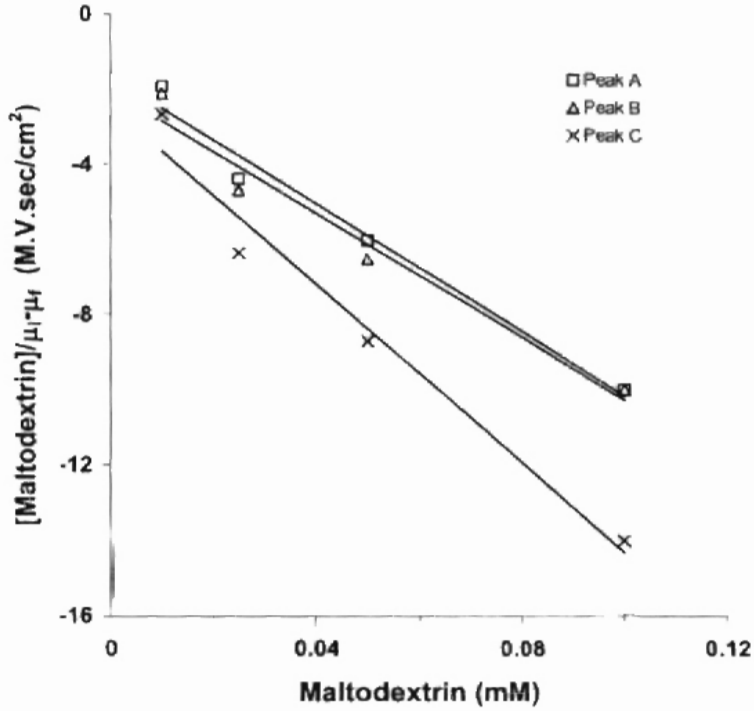

Figure 15. Y-reciprocal plot of Jagger glutenin subfraction JF6. The R^2 and y intercepts are (a) 0.9781, -1.675; (b) 0.9648, -2.04 and (c) 0.9695, -2.48 for Peaks A, B, and C, respectively. Conditions were as specified in Fig. 12.

CHAPTER V

VISCOELASTIC PROPERTIES OF GLUTENIN SUBFRACTIONS MIXED WITH STARCH VIA REDUCTION-OXIDATION SYSTEM

ABSTRACT

Differences in molecular association of glutenin subfractions found in the two hard red winter wheat cultivars (Chapter IV) suggest that specific wheat cultivars contain a group of glutenin polypeptides with strong molecular association with maltodextrin. These polypeptides were found to elute in the less hydrophobic region of RP-HPLC and to be enriched with HMW-GS. The physical interactions of glutenin with other molecules can influence the viscoelastic properties. The objective of this study was to determine the viscoelastic behavior of the starch-glutenin subfraction using a model gel system and compare it to the viscoelastic behavior of the flour-water dough (Chapter I).

Seven subfractions from Jagger and Big Dawg *cv.* with different hydrophobic characteristics were analyzed in the presence of starch. In order to promote interaction and polymerization, a 1.1% total solids starch-glutenin mixture (10:1 w/w ratio) was heated (80 – 90°C) and oxidized with 5 mM KIO_3. Dynamic rheology measurements were performed with frequency sweep tests in one log cycle (1-10 Hz) and a controlled stress of 0.2 Pa.

Throughout the frequency range the trend of the dynamic moduli were that that the storage modulus (G') slightly exceeded the loss modulus (G"). The viscoelastic

behavior of the dilute starch-glutenin gels (1.1% solids) did not increase when enriched HMW-GS and LMW-GS subfractions were added at a ratio of 10:1 (starch/glutenin). The starch-glutenin mixtures formed a weak gel with viscoelastic properties mainly dominated by the starch component. Higher concentrations (greater than 1.1% total solids) and a starch:glutenin 10:1 ratio (w/w) are needed to affect the viscoelastic properties of the diluted starch gels.

INTRODUCTION

Polymer physical theory describes the importance of concentration and the properties of the components forming a network. The network is stabilized by a combination of covalent and non-covalent bonds (Don *et al.*, 2003). In a dilute polymer solution, there are no entanglements of the individual molecules and they can be considered essentially isolated from each other. The appropriate viscoelastic functions for dilute polymer solution are the dynamic rheological properties extrapolated to infinite dilutions (e.g., $<10^{-2}$ g mL^{-1}). These intrinsic dynamic rheological properties are the storage modulus G' and the loss modulus G", and the dynamic viscosities η' and η'' (Ferry, 1980 and Rao, 1999).

Most experiments have investigated the effects of water, temperature, mixing conditions, and added ingredients on the dynamic moduli of the dough. In dynamic rheological studies, a small amplitude oscillatory shear is applied. A sinusoidal stress or strain with a specific frequency is applied to a material. The phase difference between the oscillating stress and strain, including the amplitude ratio, is measured. For deformation within the viscoelastic range, the generated stress can be expressed as elastic

or storage modulus (G') and viscous or loss modulus (G''). G' provides information about the magnitude of stored energy in the material or the recovery per cycle of deformation. G'' is a measure of the energy that is lost as viscous dissipation per cycle of deformation. Tan delta is the loss tangent and is calculated as the ratio of G' to G'', (Rao, 1999). For intrinsic dynamic rheological studies, the dynamic viscosities η' and η'' are derived from the complex viscosity (η^*). The real part of the complex viscosity (η') is equal to the ratio of G' and frequency, while the imaginary part (η'') is the ratio of G'' and frequency (Rao, 1999).

Intrinsic dynamic rheological properties are useful for determining molecular models (i.e., rigidity or flexibility of the molecules) (Rao, 1999). Although such studies are important, this study was intended to determine the dynamic moduli based on the interactions of starch and glutenin without inducing isolation of the molecules by infinite dilutions. Therefore, gels were utilized since these exhibit viscoelastic behavior suitable for dynamic rheological studies. A typical cross-linked gel usually indicates a predominance of elastic properties (G') over viscous modulus (G'') (Ross-Murphy, 1984).

There are two classifications of gels according to the resulting three-dimensional network of biopolymer gels. A system that is free-standing and has a well developed network is known as a true gel. Systems that form a tenuous gel-like network that can be easily broken down when submitted to high enough stress are called weak gels (Doublier *et al.*, 1992). Based on mechanical spectroscopy, Clark and Ross-Murphy (1987) further described strong gels as networks with finite energy and weak gels as transient in time. Their dynamic rheological tests showed that strong gels have networks with fewer molecular rearrangements over time, with G' dominating the entire frequency range and

being almost independent of frequency. In weak gels, there is a higher dependence on frequency and less difference between the moduli values (G' and G"). These findings suggest the existence of relaxation processes occurring at short time scales and a lower percentage of the stored energy being recovered.

In order to establish a gel system with a dilute sample, heating and a reduction-oxidation system were utilized in this analysis. In the actual breadmaking process, reducing and oxidizing agents are usually added to dough to enhance its handling and baking properties. The reducing agents immediately accelerate dough development during mixing (Evans *et al.*, 1981) and decrease development time (Henika and Rodgers, 1965), making the dough more extensible and easier to process. In this study, a reducing agent (DTT) was used to expose the sulfhydryl groups and solubilize the glutenin subunits. With the introduction of oxidizing agents, polymerization would take place due to reformation of the disulfide bonds. Oxidizing agents strengthen the dough during mixing, proofing and initial baking stages. They act by inhibiting disulfide exchange reactions and perhaps by forming intermolecular disulfide bonds which improve baking qualities without long aging periods (Fratner *et al.*, 1960). The use of oxidizing agents was shown in studies by Weegels *et al.* (1996), who reported that after an increase in extractability of glutenin during mixing, a decrease in extractability occurred during resting. This suggested that during resting, polymerisation of the glutenin polymer takes place. Therefore, inducing polymerization of glutenin by application of an oxidizing agent during mild mixing conditions can stabilize the starch-glutenin network, particularly for dilute gel concentrations.

Thermal fluctuations in a gel may cause hydrogen bonds and other non-covalent interactions to break and reform again (Tsiami et al., 1997). As a consequence, the gel is not a rigid structure but may actually flow on long time scales. The time scale at which the gel may flow depends on the number of non-covalent bonds between partially covalent cross-linked structures in the system. Upon heating, the G' and G" moduli increase over the full range of frequencies of glutenin fractions. This suggests association (Tsiami et al., 1997), which is ideal for rheological studies. Nakai and Li-Chan (1988) and Tanford (1961) reported that combination of hydrophobic interactions and hydrogen bonding explain the temperature dependence of the G' and G" moduli during heating: hydrophobic interactions become stronger with increasing temperature.

The objective of this study is to determine the viscoelastic behavior of glutenin subfractions mixed with starch and treated with heat and reducing and oxidizing agents to induce polymerization.

Materials and Methods

Sample Sources

Two hard red winter wheat cultivars, Jagger and Big Dawg, were used in the study. Extraction of the wheat glutenins and semi-preparative RP-HPLC separation were described in Chapter III, Materials and Methods section. The starch was a laboratory isolate from Shawnee commercial flour (Shawnee Milling Inc., Shawnee, OK).

Starch Isolation

A 500 g commercial flour was mixed with 2.5 liters of nanopure water and centrifuged at 500xg for 20 min. The supernatant and the sludge layer were removed. The pellet was recovered and kneaded manually while washing with nanopure water (1:5, starch to water w/v). The mass of gluten was separated and discarded while the starch suspension was centrifuged at 500xg for 15 min. The kneading, washing, and centrifugation were repeated until the recovered pellet was completely white.

Sample Preparation

Starch-glutenin gels were prepared by a reduction-oxidation method using a modified method based on Verbruggen *et al.*, (2001). A 100 µl aliquot of glutenin subfraction (10 mg/100 µl) solubilized in 50% n-propanol containing 1% DTT and 4% acetic acid, was mixed with 9.8 ml of starch stock solution containing 100 mg starch. The mixture was heated to 80-90°C for 1 minute with constant stirring. The samples were oxidized with the addition of 100 µl of 5 mM KIO_3, followed by incubation with constant stirring for 4 minutes (80-90°C). Samples were cooled to 25°C for 5 minutes prior to loading a 6 ml aliquot unto a concentric cylinder cup.

Dynamic Rheological Measurements

Fundamental rheological analyses were done in a controlled stress/strain rheometer, AR-1000N (TA Instruments, Surrey, England). The geometry for the analysis was a stainless steel recessed-end concentric cylinder with dimensions of 15 and 14 mm for inner and outer radii, respectively. The cylinder immersion height was 42

mm. The analyses were done using a 4000 μm gap. Samples were conditioned at 25°C for 3 min and 0.2 Pa pre-shear for 20 sec. The oscillatory stress sweep tests were performed within 0.001 to 100 Pa stress and a constant frequency of 1 Hz to determine the linear viscoelastic region (LVR). The frequency sweep was performed in the linear viscoelastic region with a frequency range of 1 to 10 Hz and 0.2 Pa as a controlled variable for both starch-glutenin gels and 1.1% starch gels. The crude glutenin extracts (1.1% total solids) were also tested at 1 to 10 Hz with 0.25 Pa controlled stress. Overall, the rheological tests resulted to a range in 0.15 to 28% strain.

Statistical Analysis

A complete randomized design, with starch-glutenin subfraction gels as the main sources of treatment, was used to determine the significant differences in the elastic modulus (G'), viscous modulus (G") and tan delta at 9 Hz frequency and the variables were analyzed in Statistical Analysis Software (SAS) version 8.1. Starch and Big Dawg crude glutenin extracts (1.1% total solids) were used as controls.

Contrast statements were defined due to specific research questions, namely (a) whether there were significant differences between the mean G', G" and tan delta of the enriched HMW-GS (F2, F3, and F4) and LMW-GS (F1, F5, F6, and F7) subfractions and (b) whether the differences in G', G" and tan delta of the enriched HMW-GS and LMW-GS subfractions were significant between Big Dawg and Jagger.

RESULTS AND DISCUSSION

The storage and loss moduli (G' and G", respectively) as a function of frequency from starch and glutenin extracts from hard red winter wheat Jagger and Big Dawg are shown in Figure 16. Due to limited amounts of protein in the subfractions obtained from RP-HPLC, all samples were analyzed at 1.1% total solids. Both glutenin and starch G' and G" appeared to be frequency dependent within the ranges tested. The G' and G" profiles seemed similar up to about 7 Hz when starch appears more frequency dependent, showing a sharper slope than the glutenin extracts. These profiles imply that the 1.1% starch formed a stronger gel network than the glutenin extract and could sustain more solid-like properties at higher frequencies than could the 1.1% glutenin gels since the 1.1% starch gel had a higher G' and G" at the higher frequency range (7-10 Hz) than the two glutenin extracts. Starch formed a stronger gel than the glutenin crude extracts primarily due to the better gelling capacities of starch after heating. Thus, samples of starch and glutenin alone were used as controls in this study.

Figures 17 and 18 show the storage (G') and loss (G") moduli of the Jagger glutenin subfractions. At lower frequencies, the G" obtained were not higher than the G', giving tan delta with values less than 1. Differences in the moduli were evident only at higher frequencies (6-10 Hz). The G' of the starch-glutenin, crude extract, and starch gels at 4 Hz (Table 4) ranged from 0.32 to 1.23 Pa while the G" ranged from 0.05 to 0.55 Pa. The tan delta were consistently less than 1 (0.08 to 0.62) except for BF7 with mean tan delta of 5.32 since the G'<G" (0.32 and 0.46, G' and G" respectively). Due to the

high coefficient of variation (up to 146, 150 and 140% for G', G" and tan delta, respectively), the results are discussed as general trends.

Table 5 shows the storage and loss moduli of the Jagger starch-glutenin gels at 9 Hz, with a range of G' and G" of 0.98-2.27 and 0.15-2.56 Pa, respectively. These values also exhibited a high coefficient of variation, 60-140% and 42-159%, for G' and G" respectively. The Jagger starch-glutenin gels suggest a trend of more solid-like characteristics (0.1-0.66 tan delta values), except for the gel containing JF1 (1.53). These tan delta values also have a high coefficient of variation (8-146%).

Storage (G') and loss (G") moduli of Big Dawg glutenin subfractions are illustrated in Figures 19 and 20, respectively. The same observations as for Jagger starch-glutenin subfractions were evident in the viscoelastic properties of Big Dawg glutenin RP-HPLC subfractions at 9 Hz. The G' (0.79-2.63 Pa) and G" (0.54-1.69 Pa), in Table 5, have high coefficients of variation (47-109% and 43-155%, for G' and G" respectively). The tan delta (Table 5) showed that the starch-glutenin gels from Big Dawg generally exhibited solid-like properties (0.34-0.85 tan delta). Tan delta values also have a high coefficient of variation (35-146%).

The mean tan delta values for most of the 1.1% starch-glutenin gels indicate that the gels exhibited trends to relatively less viscous and more elastic characteristics at 9 Hz. The blends formed a weak gel and not a viscoelastic solid because the magnitude of the storage modulus, G' was not significantly higher than that of the loss modulus, G" (Clark and Ross-Murphy, 1987). Other indicators that the starch-glutenin mixtures exhibited a weak gel were the narrow linear viscoelastic region (LVR) obtained from stress sweep tests. True gels usually have broader viscoelastic regions than do weak gels.

According to the flour-water dough studies of Khatkar and Schofield (2002a), starch has an amplitude-dependent behavior while gluten is amplitude-independent. Therefore, the starch component is more responsible for the non-linearity in the cyclic stress sweeps of a flour-water dough, which suggests that in the presence of starch, the dynamic moduli are dependent on the stress applied. For this reason, empirical rheological measurements are useful for flour-water systems, since after application of a significant amount of force (i.e., with an alveograph), the viscoelastic properties due to the gluten are revealed. In this study, the starch-glutenin samples were simplified models of flour-water dough. The starch concentration in the samples was ten times greater than the protein and the total solids were only 1.1%. At very low stress (0.2 Pa), the frequency sweep tests primarily detected the viscoelastic properties of the starch since this component dominated the moduli at low amplitudes and was also present in higher concentrations.

The G' and G" (Appendix G) of the starch-glutenin gels were not significantly different from the 1.1% starch and Big Dawg crude glutenin (controls) primarily due to the high coefficient of variation of the measurements ($F_{15,3,21}$ = 4.83, P=0.0993 and $F_{15,2,13}$ = 2.94, P=0.2701, for G' and G" respectively). The tan delta of the starch-glutenin gels was also similar ($F_{15,3,74}$ = 5.27, P=0.0680). Due to the high variability in the rheological measurements, no differences were observed in the samples.

The sources of error can be systematic errors caused by inaccurate protein content determination of the glutenin subfractions, the temperature sensitivity of certain glutenin polypeptides, and the undefined oxidizing agent requirement of the subfractions. Experimental errors may have been caused by disruption of the cross-linked gel during

pipetting, inefficient temperature control to induce polymerization, and significant wall and end effects in the concentric cylinder during the frequency sweeps.

The over-all trends suggest that dilute concentrations of starch and RP-HPLC glutenin subfractions appeared to have a higher G' than G" for the entire frequency range. The contrast analyses did not reveal significant differences when the enriched HMW-GS and LMW-GS were compared within each cultivar. No differences were observed when enriched HMW-GS and LMW-GS were compared between the two cultivars.

The structure formed by the starch-glutenin gel system at 1.1% total solids contained starch-protein networks that were weak. As seen in Chapter III, the alveograph parameters of the flour-water dough from Jagger and Big Dawg exhibited differences in baking strength, extensibility, and elasticity index. The interaction in a concentrated flour-water dough (≥70% total solids) is mainly due to covalent bonds which are stronger and more stable than the predominantly hydrogen bonds in the starch-glutenin mixtures studied. The viscoelastic behavior of the Jagger and Big Dawg starch-glutenin gels was not significantly different due to high variability in the measurements. The challenge of fundamental rheology to accurately distinguish varietal differences was discussed by Khatkar and Schofield (2002b). At low stress amplitude (<100 Pa), where most LVR are found, the elastic properties of the weak flours were greater than those of the strong flours. These results are inconsistent with those of the frequency sweep tests. Hydrated gluten, in place of the flour-water dough, exhibited the characteristic elastic properties of the strong flours (G'>G") and better correlation to loaf volume. Therefore, inclusion of other components that exhibit viscoelastic properties, like starch, masks the dynamic

rheological properties of the glutenin subfraction. The accuracy of empirical rheological techniques (i.e. alveograph) in distinguishing breadmaking qualities based on baking strength relies on the use of high stress amplitude (>100 Pa), which is not applicable for the rheometer since because it is not in the LVR (Khatkar and Schofield, 2002b).

More studies are needed to reduce the variability of the rheological measurements such as determination of (a) the optimal starch to glutenin proportion to reveal differences in viscoelastic properties within the LVR, (b) the optimal KIO_3 utilized as an oxidizing agent and (c) the optimal temperature required for starch-glutenin mixtures to establish a true gel. Proportions of starch to glutenin could also be studied to discover whether there is a ratio at which dynamic rheology reveals differences with low coefficients of variation (10-20%). Large scale fractionation is also suggested to obtain higher amounts of glutenin subunits in each subfraction.

CONCLUSION

The starch-glutenin, Big Dawg glutenin extract, and starch gels (1.1% total solids) have dynamic moduli properties which were dependent on the frequencies used (1-10 Hz). For the entire frequency range, the general trend was for the storage modulus to be higher than the loss modulus, indicating that the starch-glutenin gels have more solid-like properties. However, at 1.1% total solids, the dynamic moduli of the gels incurred significant variation (>100%) due to several systematic and experimental errors caused by the method, concentration of the samples, and limitations of the rheological test used.

The starch-glutenin mixtures revealed characteristics of a weak gel. These gels have narrow LVR, and deformation was observed in short time-scales and low frequency range.

The trends suggest that the mean values of the starch-glutenin, Big Dawg glutenin extract, and starch gels (1.1% total solids) have mean G' values of 0.79 to 2.63 Pa and mean G'' of 0.15 to 2.56 Pa at 9 Hz. The magnitude of the moduli is due to the limited concentration and low frequency range (1-10 Hz). The viscoelastic properties of the starch-glutenin gels were not differentiated based on the presence of the glutenin subfractions due to the high variability in the measurements under the conditions used in this study.

The data suggest that the viscoelastic properties of the starch-glutenin gel were mainly governed by starch. Excessive dilution dispersed the molecules, forming a weak and unstable network of starch-starch and some starch-protein interactions. Unlike the determination of the flour-water doughs in empirical rheological techniques, fundamental rheological analysis was performed at low stress amplitudes (<100 Pa). Thus, the conditions used in this study were not highly suitable for the analysis of the viscoelastic properties of the protein component in starch-protein mixtures. Therefore, the dynamic fundamental rheological techniques need to be performed at higher solid concentrations than 1.1%.

Table 4. Mean rheological properties of starch-glutenin and starch gels as a control (1.1% total solids) using recessed-end concentric cylinder geometry analyzed at 4 Hz.

Cultivar/ Sample[2]	Mean ± Standard Deviation[1]			Coefficient of Variation (%)[1]		
	G' (Pa)	G" (Pa)	Tan delta	G'	G"	Tan delta
Big Dawg						
BF1	0.71 ± 0.62	0.35 ± 0.44	0.38 ± 0.20	88	128	52
BF2	1.01 ± 1.29	0.12 ± 0.06	0.25 ± 0.15	127	45	61
BF3	0.94 ± 1.00	0.12 ± 0.16	0.09 ± 0.08	107	138	81
BF4	0.72 ± 0.82	0.09 ± 0.03	0.25 ± 0.17	114	27	67
BF5	0.78 ± 0.81	0.15 ± 0.12	0.26 ± 0.13	105	76	51
BF6	0.44 ± 0.31	0.09 ± 0.04	0.25 ± 0.16	70	44	65
BF7	0.32 ± 0.37	0.46 ± 0.69	5.32 ± 7.42	116	149	140
Jagger						
JF1	0.47 ± 0.45	0.14 ± 0.08	0.39 ± 0.17	97	57	43
JF2	0.79 ± 1.11	0.17 ± 0.15	0.62 ± 0.59	141	91	96
JF3	0.55 ± 0.56	0.09 ± 0.09	0.19 ± 0.17	100	93	89
JF4	0.48 ± 0.49	0.12 ± 0.08	0.14 ± 0.04	104	67	35
JF5	0.54 ± 0.57	0.24 ± 0.26	0.48 ± 0.20	105	109	41
JF6	0.76 ± 0.93	0.16 ± 0.18	0.23 ± 0.13	123	117	54
JF7	1.23 ± 1.80	0.55 ± 0.82	0.41 ± 0.04	146	150	10
Starch	0.32 ± 0.20	0.05 ± 0.04	0.10 ± 0.05	62	79	50
Glutenin[3]	0.55 ± 0.02	0.05 ± 0.04	0.08 ± 0.07	3	90	87

[1] Average of three independent samples.
[2] BF1-7 and JF1-7 are subfractions identified in RP-HPLC fractionation of gltuenin extracts of Big Dawg and Jagger, respectively.
[3] Big Dawg Glutenin extract.

Table 5. Mean rheological properties of starch-glutenin and starch gels as a control (1.1% total solids) using recessed-end concentric cylinder geometry analyzed at 9 Hz.

Cultivar/ Sample[2]	Mean ± Standard Deviation[1]			Coefficient of Variation (%)[1]		
	G' (Pa)	G" (Pa)	Tan Delta	G'	G"	Tan delta
Big Dawg						
BF1	1.34 ± 1.10	0.63 ± 0.94	0.41 ± 0.33	82	149	81
BF2	1.09 ± 0.71	0.99 ± 0.85	0.71 ± 0.51	65	86	72
BF3	0.90 ± 0.58	1.13 ± 1.75	0.85 ± 1.17	64	155	138
BF4	1.20 ± 1.00	0.66 ± 0.86	0.44 ± 0.27	83	131	61
BF5	1.26 ± 1.06	0.54 ± 0.23	0.34 ± 0.29	84	43	87
BF6	2.63 ± 2.87	1.69 ± 2.47	0.43 ± 0.30	109	146	70
BF7	0.79 ± 0.37	0.56 ± 0.47	0.64 ± 0.40	47	83	63
Jagger						
JF1	2.10 ± 2.95	2.56 ± 3.42	1.53 ± 1.30	140	133	85
JF2	2.12 ± 1.46	1.89 ± 3.02	0.66 ± 0.96	69	159	146
JF3	0.98 ± 0.59	0.31 ± 0.33	0.28 ± 0.16	60	106	58
JF4	1.25 ± 0.96	0.15 ± 0.08	0.21 ± 0.20	77	57	93
JF5	1.27 ± 1.31	0.77 ± 0.63	0.58 ± 0.04	103	82	8
JF6	1.18 ± 0.91	0.16 ± 0.07	0.16 ± 0.06	77	42	35
JF7	1.52 ± 1.53	0.48 ± 0.41	0.39 ± 0.29	101	85	75
Starch	2.27 ± 0.34	1.10 ± 0.71	0.52 ± 0.35	110	64	68
Glutenin[3]	1.49 ± 0.41	0.57 ± 0.22	0.42 ± 0.23	28	39	54

[1] Average of three independent samples.
[2] BF1-7 and JF1-7 are subfractions identified in RP-HPLC fractionation of gltuenin extracts of Big Dawg and Jagger, respectively.
[3] Big Dawg Glutenin extract.

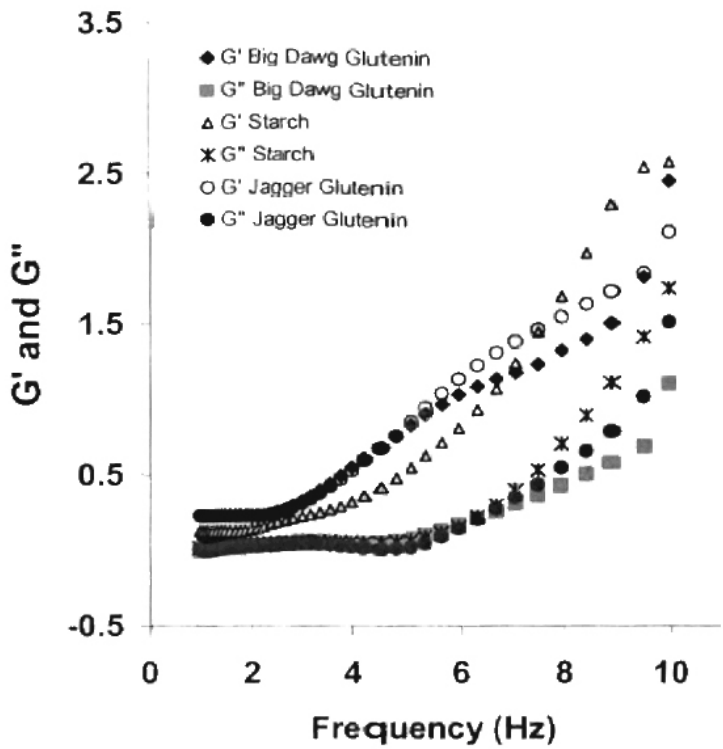

Figure 16. Frequency dependence of the storage (G') and the loss (G") moduli of crude glutenin extracts from hard red winter wheat Jagger and Big Dawg versus starch (1.1% total solids), treated with 100 μl of 50% n-propanol containing 1% DTT and 100 μl of 5mM KIO_3. Frequency sweep tests, with a recessed-end concentric cylinder, were done using 1-10 Hz with 0.25 and 0.2 Pa, for starch and glutenin extracts respectively (n=3).

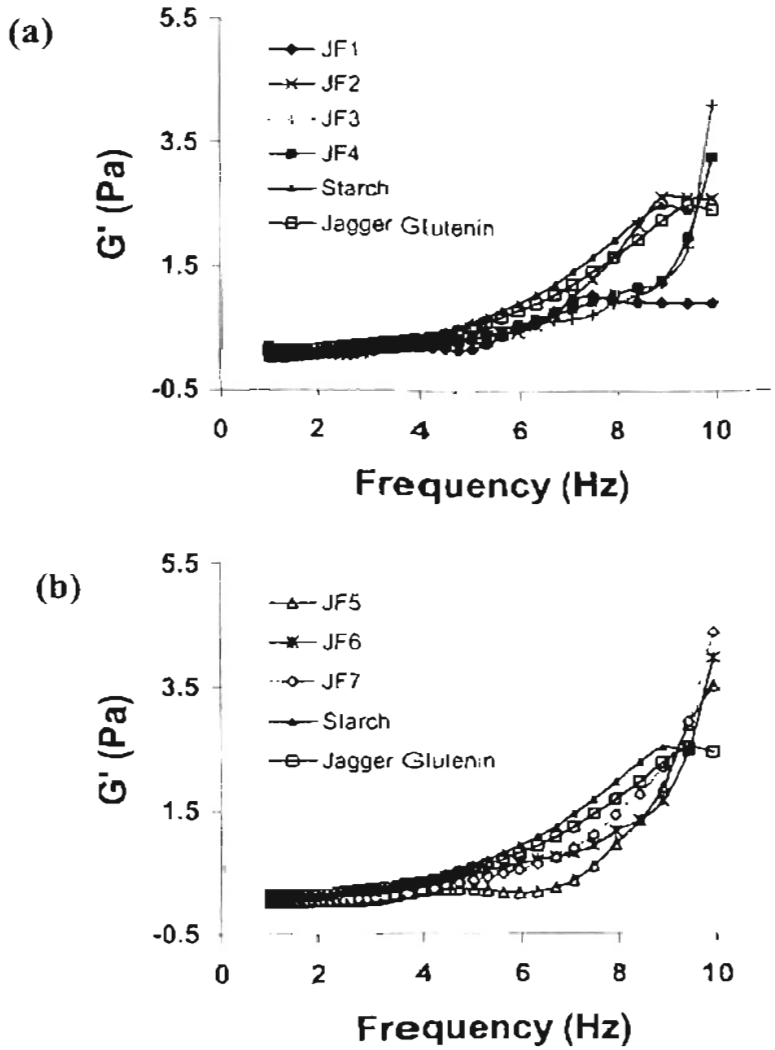

Figure 17. Frequency dependence of the elastic modulus (G') reduced glutenin subfractions (a) JF1-4 and (b) JF5-7, from hard red winter wheat Jagger *cv.* separated by RP-HPLC, Jagger glutenin extract and laboratory isolated starch. Reduced glutenin subfractions (10 mg/100 µl) were added to 1 % starch solutions and treated with 100 µl of 5mM KIO_3. Frequency sweep tests, with recess-end concentric cylinder, were done using 1-10 Hz at 0.2 Pa. Laboratory isolated wheat starch and Big Dawg glutenin extract were used as controls (n=3).

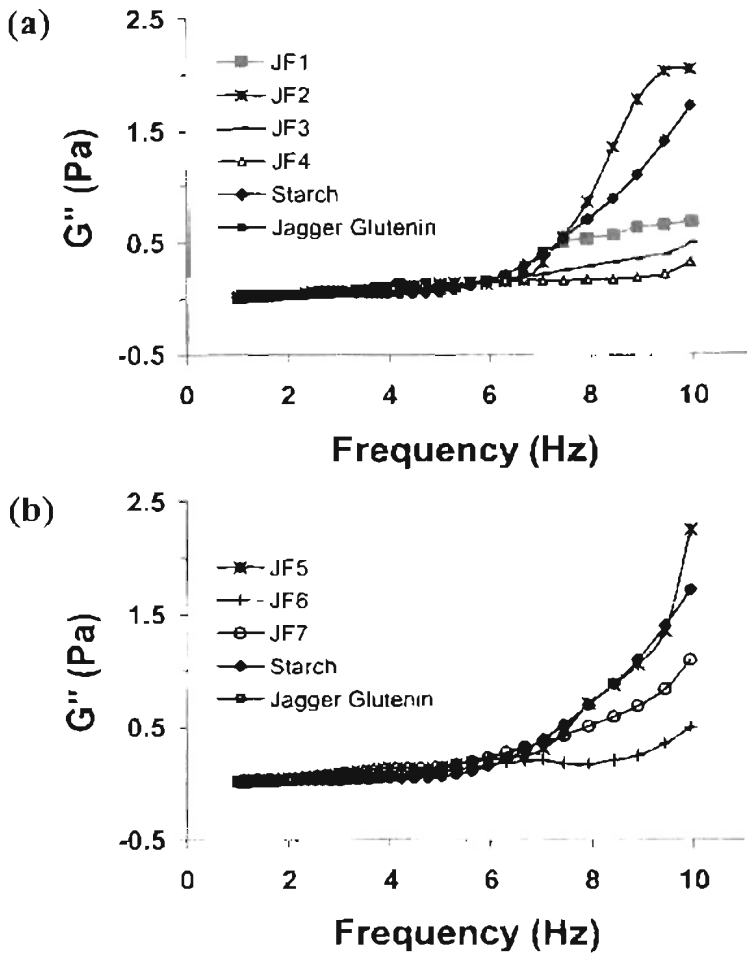

Figure 18. Frequency dependence of the viscous modulus (G") of reduced glutenin subfractions (a) JF1-4 and (b) JF5-7, from hard red winter wheat Jagger *cv.* separated by RP-HPLC, Jagger glutenin extract and laboratory isolated starch. Conditions were as specified in Fig. 17, (n=3).

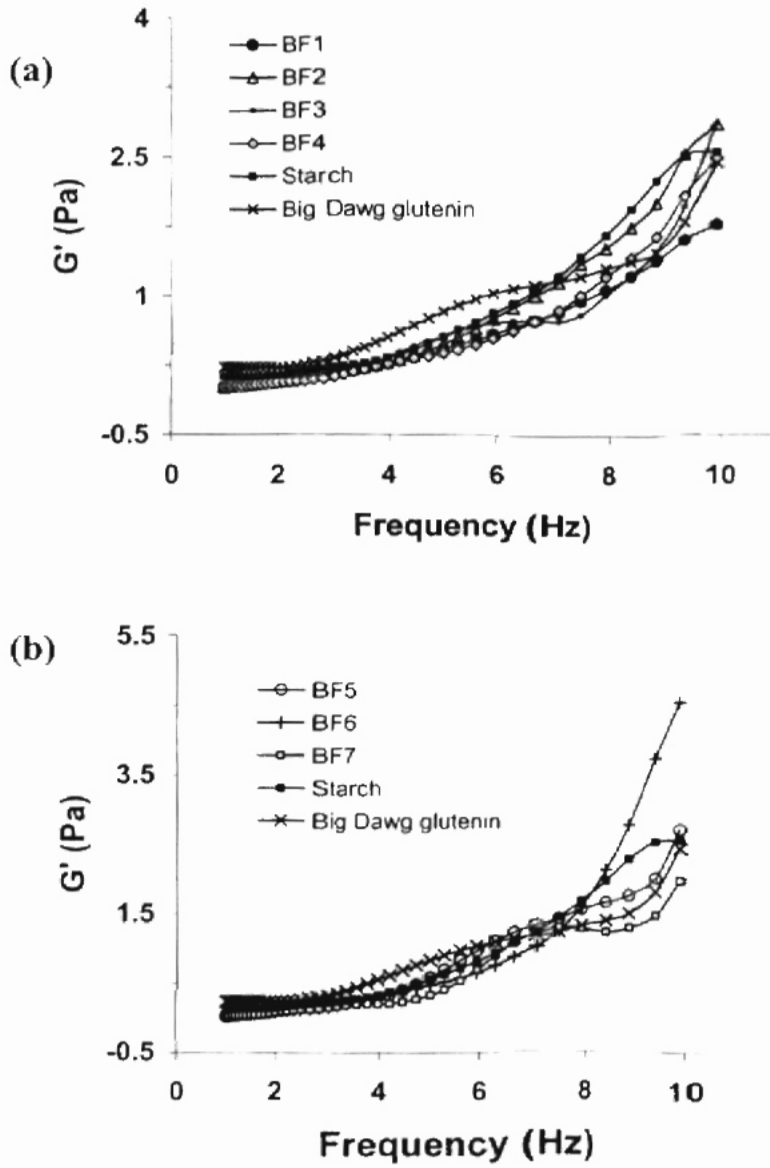

Figure 19. Frequency dependence of the elastic modulus (G') reduced glutenin subfractions (a) BF1-4 and (b) BF5-7 from hard red winter wheat Big Dawg *cv.* separated by RP-HPLC, Big Dawg glutenin extract and laboratory isolated starch. Conditions were as specified in Fig. 17, (n=3).

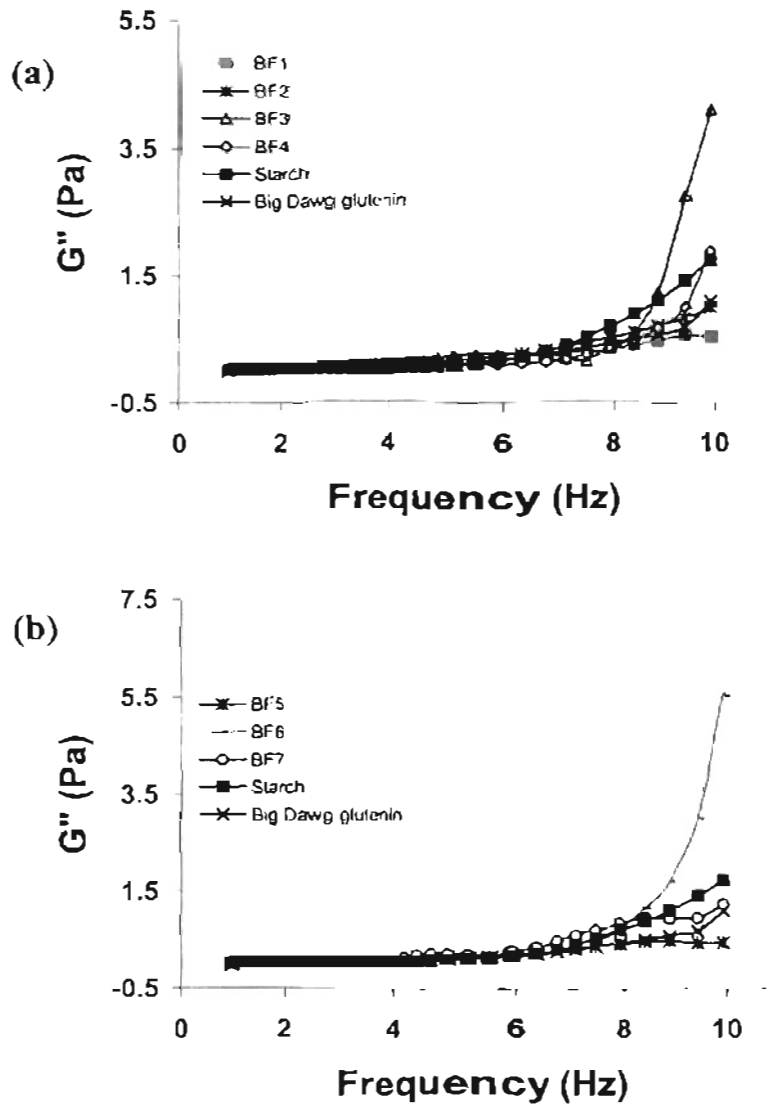

Figure 20. Frequency dependence of the viscous modulus (G") of reduced glutenin subfractions (a) BF1-4 and (b) BF5-7 from hard red winter wheat Big Dawg *cv.* separated by RP-HPLC, Big Dawg glutenin extract and laboratory isolated starch. Conditions were as specified in Fig. 17, (n=3).

CHAPTER VI

RELATIONSHIP OF THE PHYSICO-CHEMICAL PROPERTIES OF THE SOLUBLE GLUTENINS FROM WHEAT CULTIVARS OF CONTRASTING BAKING QUALITIES

The two hard red winter wheat cultivars, Jagger and Big Dawg, exhibited differences in the protein content, percent extraction rate, and empirical rheological properties. Jagger, a better baking quality flour, showed a higher protein content, extraction rate, baking strength, extensibility, and elasticity index, than did Big Dawg. Most of the baking properties of wheat flour are attributed to the gluten matrix, in particular the quality and quantity of glutenin. The soluble glutenin components in the two wheat cultivars were compared in this study.

The semi-preparative scale separation of the reduced and soluble glutenin components based on hydrophobic properties revealed that both Jagger and Big Dawg generated seven glutenin subfractions with relatively similar migration times. Three major categories were defined based on hydrophobicity, namely hydrophilic (subfraction 1), less hydrophobic (subfractions 2, 3 and 4) and more hydrophobic (subfractions 5, 6 and 7).

The SDS-PAGE revealed that the subfractions contained more than one glutenin subunit. Both cultivars exhibited two sub-groups, which were identified based on the presence of glutenin subunits greater than 100,000 molecular weight, namely enriched

HMW-GS (subfractions 2, 3, 4) and enriched LMW-GS (subfractions 1, 5, 6 and 7). Jagger contained one unique HMW-GS not present in Big Dawg. This difference may be due to genetic composition or post-translational modifications during genetic expression of the HMW-GS. Two common glutenin subunits in each cultivar were found with molecular weights (SDS-PAGE) of 94,000 and 84,000 in Big Dawg and 92,000 and 82,000 in Jagger. Since these are estimated molecular weights, these glutenin subunits may actually be the same.

The presence of ω-gliadins in the FCZE maps of subfractions BF1 and JF1 were easily identified since they matched previous reports of more negative net surface charged analytes in FZCE and eluted with the more hydrophilic LMW-GS in RP-HPLC. The net charge of all the glutenin subfractions can be sufficiently described based on their FZCE maps, including the poorly resolved peaks of enriched LMW-GS subfractions. The enriched HMW-GS of Big Dawg contained more peaks than enriched HMW-GS of Jagger, which suggests that there are more extractable glutenin components in Big Dawg than in Jagger. The more hydrophobic region, enriched with LMW-GS, has FCZE maps with poor separation and with co-migrating peaks that formed clusters with similar charge to size ratios. This clustering was assumed to be due to the tendency of LMW-GS to aggregate, making it more difficult to re-solubilize. The FZCE maps of soluble glutenin subfractions with different hydrophobicities revealed peaks with similar migration times. Thus, soluble glutenin components with different hydrophobic characteristics and molecular sizes exhibit similar charge to size ratios.

The physico-chemical properties of the soluble glutenin components from the two wheat cultivars have identified more distinctive characteristics of the less hydrophobic

subfractions enriched with HMW-GS. Jagger contained soluble glutenin components with a stronger affinity to maltodextrin (JF3 and JF4) than Big Dawg. These soluble glutenins with strong affinity are less hydrophobic and are enriched with HMW-GS. These observations may help to explain why specific HMW-GS's found in a given cultivar may contribute to the functionality of the subunits related to different performances in yeast doughs.

The starch-glutenin subfraction gels studied (1.1% total solids) formed weak structures in which no differences were observed among the glutenin subfractions added. Although differences in hydrophobicity and molecular weight were apparent in each subfraction, the weak gel, low solids concentration and the high variability during rheological measurements precluded any possible correlation between the presence of glutenin subfractions and the viscous and elastic behavior of the starch-glutenin gels.

CHAPTER VII

FUTURE RESEARCH

To achieve the objective of characterizing of the physico-chemical properties of glutenin proteins and correlating their performance during baking, the testing of large amounts of enriched glutenin subfractions is needed. Enriched glutenin subfractions in the range of gram quantities are needed to evaluate the rheological analysis and actual baking tests. Upon concentrating the glutenin subfractions, the next challenge is to improve the solubilization of the more hydrophobic and enriched LMW-GS's that strongly aggregate and appeared to have limited interactions with other molecules.

The common molecular weights found in SDS-PAGE were 94,000 and 84,000 in Big Dawg and 92,000 and 82,000 in Jagger. More studies are needed to detect whether these two subunits are indeed the same in the two cultivars and whether they are responsible for the similarities in the FZCE maps of the glutenin subfractions with different hydrophobic natures.

Ideally, in the determination of molecular association using ACE, a single compound is used and it is typically homogeneous. The heterogeneity of the glutenin RP-HPLC subfractions, as reported by Davila-El Rassi *et al.* (2003), was also observed in this study. The K_b's of single or groups of two or three polypeptides per subfraction were determined. But further research is needed to determine the K_b's of single polypeptides.

Six subfractions (BF2-4 and JF2-4) were enriched with HMW-GS while eight (BF1, JF1, BF5-7 and JF5-7) were categorized as enriched with LMW-GS. The two glutenin subunits with strong binding association (JF3 and JF4) contain two and three polypeptides and more studies are needed to find out the binding association of each polypeptide. Particularly for JF4, which has a wider range of molecular weights than JF3 and for which more peaks were detected in FZCE, the molecular association may be due to the synergistic effects of the HMW-GS and LMW-GS, which comprised the peaks found in ACE. Furthermore, the molecular association of soluble glutenins should also be tested using other analytical methods and analyzed with different models to determine the accuracy of the calculated K_b's in the present study.

Since one of the subunits identified in this study has close molecular weight close to that of the ω-gliadin of 71,000 reported by Gianibelli *et al.* (2002), it would be interesting to determine if this polypeptide is the same. The mentioned authors suggest a likely role of chain terminators (ChT) because the 71,000 ω-gliadin is assumed to have one cysteine group. The importance of ChT is shown in the studies of Uthayakumaran *et al.* (2000) in which the presence of substantial amounts of ω-gliadins decreased loaf height and peak dough resistance. It is also suspected that a glutenin sequence contains more than one ChT and these ChT's were assumed to be in the class C and D glutenin subunits. Since more similarities in molecular weight and charge to size ratio have surfaced in the physico-chemical analysis of the RP-HPLC glutenin subfractions, the identification of ChE and ChT subunits could partially explain the conformation of the glutenin polypeptides and the exposure of binding sites as a result of the conformational change.

LITERATURE CITED

Anonymous. 2003. State Statistical Report : Wheat Variety. Kansas Agricultural Statistics Service. pages 1-4.

Antes, S. and Wieser, H. 2001. Effects of high and low molecular weight glutenin subunits on rheological properties and breadmaking quality of wheat. Cereal Chemistry 78:157-159.

Baker, D.R. 1995. Capillary Electrophoresis. John Wiley & Sons, Inc. New York, NY pp. 19-20, 40, 54.

Bean, S. and Lookhart, G. 1998. Faster capillary electrophoresis separation of wheat proteins through modifications to buffer composition and sample handling. Electrophoresis 19:3190-3198.

Bean, S.R., Bietz, J.A. and Lookhart, G.L. 1998. Review: High Performance capillary electrophoresis of cereal proteins. Journal of Chromatography A 814:25-41.

Beckwith, A.C., Nielsen, H.C., Wall, J.S. and Huebner, F.R. 1966. Isolation and characterization of a high-molecular-weight protein from wheat gliadin. Cereal Chemistry 43:14-28.

Bekes, F., Gras, P.W., and Gupta, R.B. 1994. Mixing properties as a measure of reversible reduction and oxidation of doughs. Cereal Chemistry 71: 44-50.

Bennett, R., Chung, O.K., and Herrman, T.J. 2002. Milling and bread-baking qualities of hard winter wheat varieties. 2002 Kansas Update. Kansas State University, Agricultural Experiment Station and Cooperative Extension Service. pp. 1-2.

Bietz, J.A. and Wall, J.S. 1972. Wheat glutenin subunits: Molecular weights determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chemsitry 49:416-430.

Bietz, J.A. and Wall, J.S. 1973. Isolation and characterization of gliadin like subunits of glutenins. Cereal Chemistry 50: 537-540.

Bloksma, A.H. 1980. Rheology and Chemistry of Dough. Page 523 in: Wheat Chemistry and Technology, Vol 1, 2nd ed. Y. Pomeranz, ed. American Association of Cereal Chemists. St. Paul, MN.

Bloksma, A.H. and Bushuk, W. 1988. Rheology and Chemistry of Dough. Page 131-217 in: Wheat Chemistry and Technology, Vol II, Y. Pomeranz, ed. American Association of Cereal Chemists. St. Paul, MN.

Bourne, M.C. 1982. Food Texture and Viscosity: Concept and Measurement. Academic Press, NY. pp. 280-289.

Bowser, M.T., Sternberg, D.E. Chen, D.D.Y. 1997. Quantitative description of migration behavior of porphyrins based on the dynamic complexation model in a non-aqueous capillary electrophoresis system. Electrophoresis 18:82-91.

Burnouf, T. and Bietz, J.A. 1984. Reversed-phase high performance liquid chromatography of reduced glutenin, a disulfide bonded protein of wheat endosperm. Journal of Chromatography 299:185.

Busch, M.H.A, Kraak, J.C. and Poppe, H. 1997a. Principles and limitations of methods available for the determination of binding constants with affinity capillary electrophoresis. Journal of Chromatography A 777:329-353.

Busch, M.H.A., Carels, L.B., Boelens, H.F.M., Kraak, J.C. and Poppe, H. 1997b. Comparison of five methods for the study of drug-protein binding in affinity capillary electrophoresis. Journal of Chromatography A 777:311-328.

Bushuk, W. 1998. Interactions in wheat Doughs. Pages 8-9 in: Interactions : The keys to cereal quality. R. Hamer and R.C. Hoseney, eds. American Association of Cereal Chemists. St. Paul, MN.

Bushuk, W., Khan, K., and McMaster, G.J. 1980. Functional glutenin: a complex of covalently and non-covalently linked components. Annales de Technologie Agricole 29:279.

Chu, Y-H., Lees, W.J., Stassinopoulos, A. and Walsh, C.T. 1994. Using affinity capillary electrophoresis to determine binding stoichometries protein-ligand interactions. Biochemistry 33:10616-10621.

Clark, A.H. and Ross-Murphy. 1987. Structural and mechanical properties of biopolymer gels. Advances in Polymer Science. 83:57-192.

Cogswell, F.N. (1981) Polymer melt rheology. George Godwin Ltd, London. page 82.

Connors, K.A. 1987 Binding constants: The measurement of molecular complex stability. John Wiley & Sons, Inc., New York. pp. 370-371.

Cunico, R., Gooding, K., and Wehr, T. 1998. Basic HPLC and CE of Biomolecules . Bay Bioanalytical Laboratory, Inc., Richmond, CA. Page 145.

Dachkevitch, T. and Autran, J.C. 1989. Prediction of baking quality of bread wheat in breeding programs by size exclusion high performance liquid chromatography. Cereal Chemistry 66:448-456.

Davila-El Rassi, G.M., Mitchum, J.A. Escober, M.C., Rayas-Duarte, R., El Rassi, Z. 2003. Studies of Molecular Association of Glutenins and Maltodextrin in Solution by Capillary Electrophoresis. Journal of Liquid Chromatography & Related Technologies 26:2465-2479.

Dendy, D. 2001. Bread: A unique Food. Page 121 in: Cereal and Cereal Products, Chemistry and Technology. D. Dendy and B.J. Dobraszczyk, eds. Aspen Publishers Inc. Gaithersburg, MD.

Dendy D, Dobraszczyk B. 2001. Wheat and Flour. Page 121 in: Cereals and Cereal Products, Chemistry and Technology. D. Dendy and B.J. Dobraszczyk, eds. Aspen Publishers Inc.

Dobraszczyk, B.J., Campbell, G.M. and Gan, Z. 2001. Bread: A unique Food. page 203 in: Cereal and Cereal Products, Chemistry and Technology. D. Dendy and B.J. Dobraszczyk, eds. Aspen Publishers Inc. Gaithersburg, MD.

Don, C., Lichtendonk, W., Plijter, J.J., Hamer, R.J. 2003. Glutenin Macropolymer: a Gel Formed by Glutenin Particles. Journal of Cereal Science 37:1-7.

Doublier, J.L., Launay, B., and Cuvelier, G. 1992. Viscoelastic properties of food gels. Pages 371-343 in: Viscoelastic properties of Foods. M.A. Rao and J.F. Steffe, eds. Elsevier Science. Barking, England.

Dreese, P.C., Faubion, J.M. and Hoseney, R.C. 1988. Dynamic rheological properties of flour, gluten, and gluten-starch systems. I. Temperature dependent changes during heating. Cereal Chemistry 65:348-353.

Erim, F.B. and Kraak, J.C. 1998. Vacancy affinity capillary electrophoresis to study competitive protein-drug binding. Journal of Chromatography B 710:205-210.

Ewart, J.A.D. 1988. Studies on disulfide bonds in glutenin. Cereal Chemistry 65:95-100

Ewart J. A.D. 1977. Re-examination of the linear gluten hypothesis. Journal of the Science of Food and Agriculture 28:191-9.

Evans, L.G. Pearson, A.M. and Hooper, G.R. 1981. Scanning electron microscopy of flour-water doughs treated with oxidizing and reducing agents. Scanning Electron Microscopy. pages 583-592.

Ferry, J.D. 1980. Viscoelastic properties of polymers. New York: John Wiley and Sons.

Fratner, R., Hird, F.J.R., Moss, H.J. and Yates, J.R. 1960. A role for thiol and disulfide groups in determining the rheological properties of dough made form wheaten flour. Nature. 186:451-454.

Gianibelli, M.C. Larroque, O., and MacRitchie, F. 1996. Purification and characterization of a novel polymeric endosperm protein from wheat (*T. aestivum*). Pages 267-271 in Gluten 96. C.W. Wrigley, ed. RACI, Melbourne.

Gianibelli, M.C., Larroque, O.R., MacRitchie, F. and Wrigley, C.W. 2001. Biochemical, genetic and molecular characterization of wheat endosperm proteins. Cereal Chemistry 78:635-646.

Gianibelli, M.C., Masci, S., Larroque, O.R., Lafiandra, D. and MacRitchie, F. 2002. Biochemical characterization of a novel polymeric protein subunit from bread wheat (*Triticum aestivum L.*). Journal of Cereal Science 35:265-276.

Graveland, A., Bosveld, P., Lichtendonk, W.J., Marseille, J.P., Moonen, J.H.E., and Scheepstra, A. 1985. A model for the molecular structure of the glutenins from wheat flour. Journal of Cereal Science. 3:1-16.

Greenwell, P. and Schofield, J.D. 1986. A starch granule protein associated with endosperm softness in wheat. Cereal Chemistry 63:379-380.

Gupta, R.B. and MacRitchie, F. 1991. A rapid one-step one dimensional SDS-PAGE composition of glutenin in wheat. Journal of Cereal Science 14:105-109.

Gupta, R.B. and Shepherd, K.W. 1990a. Two-step one-dimensional SDS-PAGE analysis of LMW subunits of glutenin I. Variation and genetic control of the subunits in hexaploid wheats. Theoretical and Applied Genetics 80:65-74.

Gupta, R.B. and Shepherd, K.W. 1990b. Two-step one-dimensional SDS-PAGE analysis of LMW subunits of glutenin 2. Genetic control of subunits in species related to wheat. Theoretical and Applied Genetics 80:183-187.

Henika, R.G. and Rodgers, N.E. 1965. Reactions of cysteine, bromate and whey in rapid bread making process. Cereal Chemistry 42:397-408.

Huebner, F.R. and Wall, J. S. 1976. Fractionation and quantitative differences of glutenin from wheat varieties varying in baking quality. Cereal Chemistry. 53:258-269.

Hulme, E.C. 1992. Receptor-Ligand Interactions. A practical approach. Oxford University Press, New York.

Ishida, N., Takano, H. Shigero, N., Isobe, S., Uemura, K., Haishi, T., Kose, K., Koizumi, M and Kano, H. 2001. Architecture of baked breads depicted by a magnetic resonance imaging. Magnetic Resonance Imaging 19:867-874.

Jones, I.K., Phillips, J.W., and Hird, F.R.J. 1974. The estimation of rheologically important thiol and disulfide groups in dough. Journal of Food and Agriculture 25:1-10.

Kasadra, D.D. 1989. Glutenin structure in relation to wheat quality. Pages 277-302 in: Wheat is Unique. Y. Pomeranz, ed. American Association of Cereal Chemists. St. Paul, MN.

Khan, K. and Huckle, L. 1992. Use of multistacking gels in sodium dodecyl sulfate – polyacrylamide gel electrophoresis to reveal polydispersity, aggregation, and disaggregation of the glutenin protein fraction. Cereal Chemistry 69:686-688

Khatkar, B.S. and Schofield. 2002a. Dynamic rheology of wheat flour dough. I. Non-linear viscoelastic behavior. Journal of the Science of Food and Agriculture 82:823-826.

Khatkar, B.S. and Schofield. 2002b. Dynamic rheology of wheat flour dough. II. Assessment of dough strength and bread-making quality. Journal of the Science of Food and Agriculture 82:827-829.

Khatkar, B.S. Fido1, R.J. Tatham, A.S. and Schofield, J.D. 2002c. Functional properties of Wheat Gliadins. I. Effect on mixing characteristics and bread making quality. Journal of Cereal Science 35:299-306

Kokelaar, J.J., van Vliet, T. and Prins, A. 1996. Strain hardening properties and extensibility of flour and gluten doughs in relation to breadmaking performance. Journal of Cereal Science 24:199-214.

Kraak, J.C., Busch, S. and Poppe, H. 1992. Study of protein-drug binding using capillary zone electrophoresis. Journal of Chromatography 608:257-264

Kruger, J.E., Marchylo, B.A. and Hatcher, D. 1988. Preliminary assessment of a sequential extraction scheme for evaluating quality by reversed-phase liquid chromatography and electrophoretic analysis of gliadins and glutenins. Cereal Chemistry 65:208-214.

Lagudah, E.S., Appels, R., Brown, A.H.D. and McNeil D. 1991. The molecular-genetic analysis of *Triticum tauschii*, the D-genome donor to hexaploid wheats. Genome 34:375-386.

Larroque, O.R., Gianibelli, M.C., Batey, I.L. and MacRitchie, F. 1997. Electrophoretic characterization of fractions collected from gluten protein extracts subjected to size-exclusion high performance liquid chromatography. Electrophoresis 18: 1064-1067.

Lásztity, R. 1995. The chemistry of cereal proteins. 2nd ed. CRC Press Inc. pages 4-77.

Lindsay, M.P. and Skirret, J.H. 1999. The glutenin macropolymer of wheat flour doughs: structure and function prospectives. Trends in Food Science and Technology 10:247-253.

Lookhart, G. and Bean, S. 1995. Separation and characterization of wheat protein fractions by high-performance capillary electrophoresis. Cereal Chemistry 72:527-532.

Marchylo, B.A. Kruger, J.E. and Hatcher, D.W. 1989. Quantitative Reversed phase high-performance liquid chromatographic analysis of wheat storage proteins as a potential quality prediction tool. Journal of Cereal Science 9:113-130.

Masci, S., Lafiandra, D., Porceddu, E., Lew, E.J.-L., Tao, H.P. and Kasadra, D.D. 1993. D-glutenin subunits: N- terminal sequences and evidence for the presence of cysteine. Cereal Chemistry 70:581-585.

Masci, S., Egorov, T.A., Ronchi, S., Kuzmicky, D.D. Kasadra, D.D. and Lafiandra, D. 1999. Evidence of the presence of only one cysteine residue in the D-type low molecular weight subunits of wheat subunits of wheat glutenin. Journal of Cereal Science 29:17-25.

McMaster, G.J., and Bushuk, W. 1983. Protein-carbohydrate complexes in gluten: Fractionation and proximate composition. Journal of Cereal Science 1:171.

Moonen, J.H.E., Scheepstra, A. and Graveland, A. 1985. Biochemical properties of some high-molecular weight glutenin subunits of wheat glutenin. Journal of Cereal Science 3:17-27.

National Agricultural Statistics Service. 2003. Oklahoma Wheat Varieties, Crop Year 2003. page 1.

Nakai, S. and Li-Chan, E. 1988. Hydrophobic interactions in food systems. CRC Press, Boca Raton. pp. 4-6.

Nielsen, H.C., Beckwith, A.C. and Wall, J.S. 1968. Effect of disulphide-bond cleavage on wheat gliadin fractions obtained by gel filtration. Cereal Chemistry 45:37-47.

Osborne, T. B. 1907. The protein of the wheat kernel. Publication No. 84. Carnegie Institute: Washington, DC.

Padmanabhan, M. (1995) Measurement of extensional viscosity of the viscoelastic liquid foods. Journal of Food Engineering 25:311-327.

Panozzo, J.F., Eagles, H.A., Bekes, F., and Wootton, M. 1994. Genotype and environment interactions on wheat quality in Australia. In : Proc 44th Australian Cereal Chemistry Conference. J.F. Panozzo, ed. Royal Australian Chemical Instinstitute, Melbourne, Australia.

Payne, P.I. 1987. Genetics of wheat storage protein and the effect of allelic variation on breadmaking quality. Annual Review of Plant Physiology 38:141-153.

Payne, P.J. and Corfield, K.G. 1979. Subunit composition of wheat glutenin proteins isolated by gel filtration in a dissociating medium. Planta. 145:83-88.

Payne, P.I., Corfield, K.G. and Blackman, J.A. 1981. Correlation between inheritance of certain high molecular weight subunits of glutenin and bread-making quality in progenies of six crosses of bread wheat. Journal of the Science of Food and Agriculture 32:51-60.

Payne, P.I. and Lawrence, G.J. 1983. Catalogue of alleles for the complex gene loci, Glu-A1, Glu-B1 and Glu-D1 which code for high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Research Communications 11:29-35.

Payne, P.I., Holt, L.M., Jackson, E.A. and Law C.N. 1984. Wheat storage proteins: their genetics and their potential for manipulation by plant breeding. Philosophical Transactions of the Royal Society of London, Series B 304:359-371.

Payne, P.I., Holt, L.M., Jarvis, M.G. and Jackson E.A. 1985. Two dimensional fractionation of the endosperm proteins of bread wheat (*Triticum aestivum)*: Biochemical and genetic Studies. Cereal Chemistry 62:319-326.

Payne, P.I., Nitingale, M.A. Krattiger, A.F. and Holt, L.M. 1987. The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. Journal of the Science of Food and Agriculture 40:51-65.

Pomeranz, Y., Caravahal, M.I., Hoseney, R.C., and Ward, A.B. 1970. Wheat germ in Breadmaking. I. Composition of germ lipids and germ protein fractions. Cereal Chemistry 47:373.

Popineau, Y., Comec, M., Lefebvre, J., and Marchylo, B. 1994. Influence of high M_r glutenin subunits on glutenin polymers and rheological properties of glutens and gluten sub-fractions of near isogenic lines of wheat Sicco. Journal of Cereal Science 19:231-241.

Rao, M.A. 1999. Measurement of Flow and Viscoelastic Properties. pages 105-108, 171 in : Rheology of Fluid and Semisolid Foods, Principles and Applications. M.A. Rao, ed. Aspen Publishers, Inc. Gaithersburg, MD.

Ross-Murphy, S.B. 1984. Rheological methods. Pages 128-139 in: Biophysical methods in food research. H.W.S. Chan, ed. Blackwell Scientific, London:

Rundlett, K.L. and Armstrong, D.W. 1996. Examination of origin, variation, and proper expressions for the estimation of the association constants in capillary electrophoresis. Journal of Chromatography A 712:138-200.

Rundlett, K.L. and Armstrong, D.W. 1997. Methods for the estimation of binding constants by capillary electrophoresis. Electrophoresis 18:2194-2202.

Rundlett, K.L. and Armstrong, D.W. 2001. Review: Methods for the determination of binding constants by capillary electrophoresis. Electrophoresis 22:1419-1427.

Sears, R.G., Joe Martin, T. and Shroyer, J.P. 1995. Cooperative Extension Service, Manhattan, Kansas. pp. 1-4.

Shewry P.R. and Tatham, A.S. 1990. The prolamin storage proteins of cereal seeds : structure and evolution. Biochemical Journal 267:1-12.

Shewry, P.R. Halford, N.G. and Tatham, A.S. 1989. The high molecular weight subunits of wheat, barley and rye: Genetics, molecular biology, chemistry and role in wheat gluten structure and functionality. Pages 163-219 in: Oxford Surveys of Plant and Molecular Cell Biology, Vol. 6. B.J. Miflin, ed. Oxford University Press. London.

Shewry, P.R. Halford, N.G. and Tatham, A.S. 1992. The high molecular weight subunits of wheat glutenin. Journal of Cereal Science 15:105-120.

Southan, M. and MacRitchie, F. 1999. Molecular weight distribution of wheat proteins. Cereal Chemistry 76:827-836.

Stauffer, C.E. 1998. Principles of dough formation. Pages 262-275 in: Technology of Breadmaking. S.P. Cauvain and L. Young, eds. Blackie Academic and Professional, UK.

Tamás, L., Gras, P.W., Solomon, R.G. and Morell, M.K. 2002. Chain extension and termination as a function of cysteine content and the length of the central repetitive domain in storage proteins. Journal of Cereal Science 36:313-325.

Tanaka, Y. and Terabe, S. 2002. Review: Estimation of binding constants by capillary electrophoresis. Journal of Chromatography B 768:81-92.

Tanford, C. 1961. Physical Chemistry of macromolecules. Wiley, NY. pp 130-131.

Thompson, S., Bishop, D.H.L., Tatham, A.S., and Shewry, P.R. 1994. Exploring disulphide bond formation in low molecular weight subunit of glutenin using a baculovius expression system. Pages 345-355 in : Gluten Proteins 1993. Association of Cereal Research: Detmold, Germany.

Tsiami, A.A., Bot, A., Agterof, W.G.M. and Groot, R.D. 1997. Rheological properties of glutenin subfractions in relation to molecular weight. Journal of Cereal Science 26:15-27.

Uthayakumaran, S., Tomoskozi, S., Tatham, A.S., Sewage, A.W.J., Giannibelli, M.C. Stoddard, F.L., and Bekes, F. 2000. Effects of gliadin fractions on functional properties of wheat dough depending on molecular size and hydrophobicity. Cereal Chemistry 78:138-141.

van Lonkhuijsen, H.J., Hamer, R.J., Schreuder, C. 1992. Influence of specific gliadins on breadmaking quality of wheat. Cereal Chemistry 69:174-177.

Verbruggen, I.M., Veraverbeke, W.S. and Delcour, J.A. 2001. Significance of LMW-GS and HMW-GS for dough extensibility: 'addition' versus 'incorporation' protocols. Journal of Cereal Science 33:253-260.

Verbruggen, I.M., Veraverbeke, W.S. and Delcour, J.A. 2003. In vitro polymerization of High and Low molecular weight glutenin subunits with molecular oxygen. Journal of Cereal Science 37:223-229.

Weegels, P.L., Hamer, R.J. and Schofield, J.D. 1995. RP-HPLC and capillary electrophoresis of subunits form glutenin isolated by SDS and Osborne fractionation. Journal of Cereal Science 22:211-224.

Weegels, P.L., Hamer, R.J. and Schofield, J.D. 1996. Functional properties of wheat glutenin. Journal of Cereal Science 23:1-18.

Weegels, P.L., Marseille, J.P., de Jasger, A.M. and Hamer, R.J. 1990. Structure –function relationships of gluten proteins. Pages 98-111 in Gluten Proteins 1990. W. Bushuk and R. Tkachuk eds. Amercian Association of Cereal Chemists. St. Paul, MN.

Weegels, P.L. De Groot, A.M.G., Verheok, J.A., and Hamer, R.J. 1994. Effects on gluten of heating at different moisture contents. II Changes in physico-chemical properties and secondary structure. Journal of Cereal Science 19:39-47.

Weegels, P.L., Marseille, J.P., de Jasger, A.M. and Hamer, R.J. 1990. Structure -function relationships of gluten proteins. Pages 98-111 in Gluten Proteins 1990. W. Bushuk and R. Tkachuk eds. Amercian Association of Cereal Chemists. St. Paul, MN.

Weegels, P.L., van de Pijpekamp, A.M. Graveland, A, Hamer, R.J. and Schofield, J.D. 1996. Depolymerisation and re-polymersiation of wheat glutenin during dough processing. I. Relationships between glutenin macropolymer content and quality parameters. Journal of Cereal Science 23:103-111.

Weipert, D. 1992. Review: Descriptive and Fundamental rheometry in a new light. Cereal Foods World. 31:15-24.

Wehrli, H.P., and Pomeranz, Y. 1969. Bakers Digest 43:22.

Winzor. D.J. 1995. Measurement of binding constants by capillary electrophoresis. Journal of Chromatography A 696:160-163.

Woychik J.H., Boundy, J.A., and Dimler R.J. 1961. Starch gel electrophoresis of wheat gluten proteins with concentrated urea. Archives of Biochemistry and Biophysics 94:477-482.

Wrigley, C.W. Gupta, R.B. and Bekes, F. 1993. Our obsession with high resolution in gel electrophoresis, does it necessarily give the right answer? Electrophoresis 14:1257-1258.

Wrigley, C.W., Andrews, J.L., Bekes, F., Gras, P.W., Gupta, R.B., MacRitchie, F. and Skerritt, J.H. 1998. Pages 17-40 in Interactions: The Keys to Cereal Quality. R.J. Hamer and R.C. Hoseney, eds. American Association of Cereal Chemists, St. Paul MN.

Zawistowska, U., Bekes, F. and Bushuk, W. 1985. Involvement of carbohydrates and lipids in aggregation of glutenin proteins. Cereal Chemistry 62:340-345.

APPENDIX A

SAS OUTPUT OF PERCENT PEAK AREA

Table 6. Statistical analysis of percent peak area of glutenin subfractions from hard red winter wheat Jagger and Big Dawg *cv.*[1]

Effect	Numerator df	Denominator df	F Value	Pr > F
Cultivar	1	144	0.00[2]	0.9996
Subfraction (Cultivar)	12	91	449.41	<0.0001
Contrast[3]				
1	1	91	1143.9	<0.0001
2	1	91	1821.18	<0.0001
3	1	144	71.32	<0.0001

[1] n = 14 replicates.

[2] Near zero.

[3] Contrast definitions:

1 = Jagger enriched HMW-GS vs. LMW-GS.

2 = Big Dawg enriched HMW-GS vs. LMW-GS.

3 = Difference between HMW-GS and LMW-GS (Big Dawg vs. Jagger).

APPENDIX B

SAS OUTPUT OF BINDING CONSTANTS FROM DOMINANT GLUTENIN POLYPEPTIDE

Table 7. Statistical analysis of the binding constants of dominant glutenin polypeptide from glutenin subfractions of hard red winter wheat Jagger and Big Dawg *cv.*[1]

Effect	Numerator df	Denominator df	F Value	Pr > F
Cultivar	1	28	3.25	0.0822
Subfraction(Cultivar)	12	28	1.68	0.1267
Contrast[2]				
1	1	28	4.61	0.0406
2	1	28	0.04	0.8522
3	1	28	2.73	0.1099

[1] n = 3 replicates.

[2] Contrast definitions:

1 = Jagger enriched HMW-GS vs. LMW-GS.

2 = Big Dawg enriched HMW-GS vs. LMW-GS.

3 = Difference between HMW-GS and LMW-GS (Big Dawg vs. Jagger).

APPENDIX C

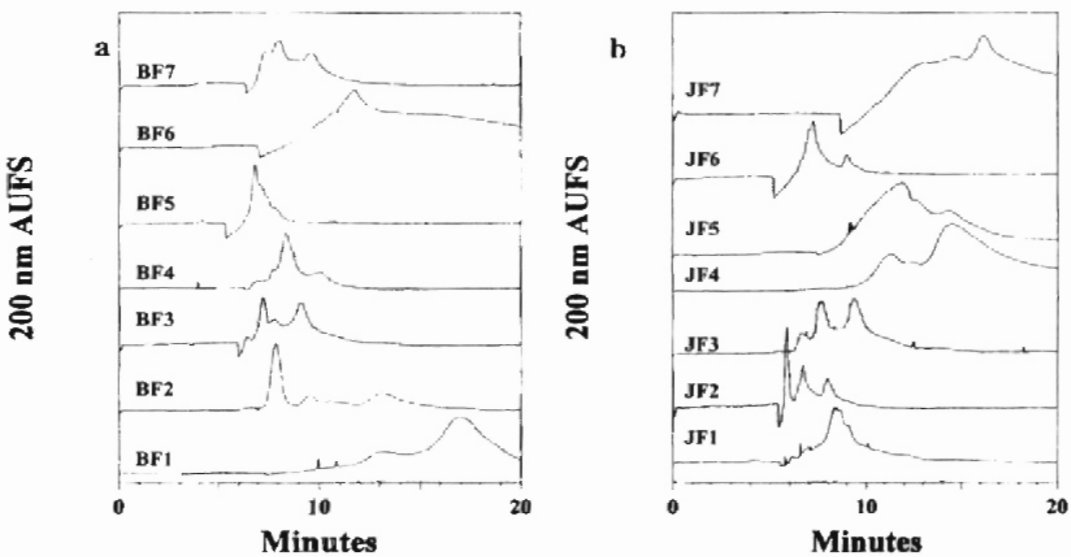

Figure 21. Electropherograms of glutenin subfractions separated by RP-HPLC from hard red winter wheat (a) Big Dawg and (b) Jagger *cv*. Conditions were as specified in Fig. 12.

APPENDIX D

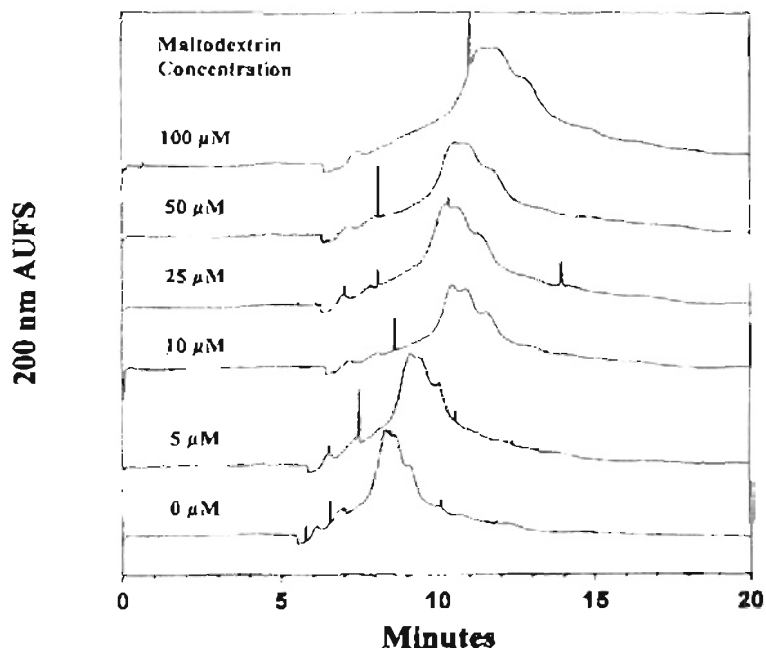

Figure 22. Electropherograms of enriched LMW-GS subfraction JF1 from hard red winter wheat Jagger *cv.* Freeze dried JF1 was solubilized in 50% n-rpopanol containing 1% DTT and 4% acetic acid (total protein 1 mg/100 µl), injected for 5 sec at 0.5 psi and separated in 100 mM phosphate buffer (pH 2.5) containing 20% acetonitrile, 0.4% glycine and maltodextrin (0, 5, 10, 25, 50 and 100 µM). Other conditions were as specified in Fig. 12.

APPENDIX E

SAS OUTPUT OF HYPTOTHETICAL BINDING CONSTANT

Table 8. Statistical analysis of the hypothetical binding constants of glutenin subfractions from hard red winter wheat Jagger and Big Dawg *cv.* [1]

Effect	Numerator df	Denominator df	F Value	Pr > F
Method	1	56	0.21	0.6452
Cultivar	1	56	2.21	0.1429
Subfraction (Cultivar)	12	56	2.58	0.0084
Cultivar*Method	1	56	0.03	0.8710
Subfraction*Method(Cultivar)	12	56	0.03	1.0000
Contrast[2]				
1	1	56	10.22	0.0023
2	1	56	0.06	0.8010
3	1	56	4.33	0.0419

[1] n = 3 replicates.

[2] Contrast definitions:

1 = Jagger enriched HMW-GS vs. LMW-GS.

2 = Big Dawg enriched HMW-GS vs. LMW-GS.

3 = Difference between HMW-GS and LMW-GS (Big Dawg vs. Jagger).

APPENDIX F

Table 9. Difference in electropheretic mobility of the peaks of interest based on Y-reciprocal method of glutenin subfractions from hard red winter wheat cultivar Big Dawg, as the maltodextrin concentration increased.

Subfractions	Difference in Electrophoretic Mobility[1] ($cm^2/V \cdot sec$)				
	5 mM	10 mM	25 mM	50 mM	100 mM
BF1					
Peak A	-6.7×10^{-7}	-1.6×10^{-6}	-2.1×10^{-6}	-3.7×10^{-6}	-3.6×10^{-6}
Peak B	-1.7×10^{-7}	-3.3×10^{-7}	-1.2×10^{-6}	-2.2×10^{-6}	-2.3×10^{-6}
BF2					
Peak A	-1.1×10^{-6}	-1.7×10^{-6}	-1.9×10^{-6}	-5.2×10^{-6}	-9.5×10^{-6}
Peak B	-1.1×10^{-6}	-1.7×10^{-6}	-1.7×10^{-6}	-5.1×10^{-6}	-9.5×10^{-6}
Peak C	-1.3×10^{-6}	-1.9×10^{-6}	-2.9×10^{-6}	-5.9×10^{-6}	-1.2×10^{-5}
BF3					
Peak A	-4.1×10^{-7}	-1.2×10^{-6}	-2.3×10^{-6}	-2.7×10^{-6}	-3.4×10^{-6}
Peak B	-3.7×10^{-7}	-1.2×10^{-6}	-1.9×10^{-6}	-3.1×10^{-6}	-4.4×10^{-6}
Peak C	-4.6×10^{-8}	-1.6×10^{-6}	-2.8×10^{-6}	-3.0×10^{-6}	-5.0×10^{-6}
BF4					
Peak A	-8.0×10^{-7}	1.0×10^{-5}	-1.4×10^{-5}	-1.1×10^{-5}	-1.3×10^{-5}
Peak B	-6.4×10^{-7}	8.3×10^{-6}	-9.9×10^{-6}	-7.1×10^{-6}	-9.2×10^{-6}
BF5					
Peak A	-1.5×10^{-6}	-2.0×10^{-6}	-2.6×10^{-6}	-2.3×10^{-6}	-2.9×10^{-6}
BF6					
Peak A	-3.0×10^{-6}	-2.7×10^{-6}	-3.5×10^{-6}	-4.2×10^{-6}	-4.3×10^{-6}
BF7					
Peak A	5.5×10^{-7}	-1.5×10^{-6}	-4.4×10^{-6}	-1.9×10^{-6}	-1.2×10^{-6}
Peak B	-1.3×10^{-6}	-1.9×10^{-6}	-4.9×10^{-6}	-3.9×10^{-6}	-3.5×10^{-6}
Peak C	-1.5×10^{-6}	-1.9×10^{-6}	-5.1×10^{-6}	-4.4×10^{-6}	-5.7×10^{-6}

[1] Based on change of electrophetic mobility from 0 mM matodextrin concentration, n=3.

Table 10. Difference in electropheretic mobility of the peaks of interest based on Y-reciprocal method of glutenin subfractions from hard red winter wheat cultivar Jagger, as the maltodextrin concentration increased.

Subfractions	Difference in Electrophoretic Mobility[1] ($cm^2/V \cdot sec$)				
	5 mM	10 mM	25 mM	50 mM	100 mM
JF1					
PeakA	-1.7×10^{-6}	-2.4×10^{-6}	-3.4×10^{-6}	-4.5×10^{-6}	-4.9×10^{-6}
JF2					
Peak A	-2.8×10^{-7}	-2.1×10^{-6}	-1.8×10^{-6}	-3.0×10^{-6}	-2.9×10^{-6}
Peak B	-4.6×10^{-7}	-1.1×10^{-6}	-1.0×10^{-6}	-2.0×10^{-6}	-2.2×10^{-6}
Peak C	-1.2×10^{-6}	3.4×10^{-6}	-1.8×10^{-6}	-3.2×10^{-6}	-1.8×10^{-6}
JF3					
Peak A	-1.7×10^{-5}	-2.2×10^{-5}	-3.0×10^{-5}	-2.6×10^{-5}	-2.9×10^{-5}
Peak B	-1.3×10^{-5}	-1.7×10^{-5}	-2.2×10^{-5}	-2.0×10^{-5}	-2.3×10^{-5}
Peak C	-8.6×10^{-6}	-1.1×10^{-5}	-1.6×10^{-5}	-1.4×10^{-5}	-1.7×10^{-5}
JF4					
Peak A	-1.2×10^{-7}	-1.2×10^{-6}	5.3×10^{-6}	-1.8×10^{-6}	-3.4×10^{-6}
Peak B	-3.3×10^{-8}	-3.3×10^{-7}	-1.9×10^{-6}	-1.6×10^{-6}	-1.1×10^{-6}
JF5					
Peak A	-1.7×10^{-5}	-2.2×10^{-5}	-1.7×10^{-5}	-2.6×10^{-5}	-2.7×10^{-5}
JF6					
Peak A	-6.7×10^{-6}	-5.1×10^{-6}	-5.7×10^{-6}	-8.3×10^{-6}	2.9×10^{-6}
Peak B	-5.6×10^{-6}	-4.7×10^{-6}	-5.3×10^{-6}	-7.6×10^{-6}	2.2×10^{-6}
Peak C	-4.0×10^{-6}	-3.7×10^{6}	-3.9×10^{-6}	-5.7×10^{-6}	1.4×10^{-6}
JF7					
Peak A	-3.0×10^{-6}	-2.7×10^{-6}	-3.1×10^{-6}	-4.2×10^{-6}	-4.3×10^{-6}

[1] Based on change of electrophetic mobility from 0 mM matodextrin concentration, n=3.

APPENDIX G

SAS OUTPUT OF VISCOELASTIC PROPERTIES

Table 11. Statistical analysis of the storage modulus (G') starch-glutenin gels containing glutenin subfractions from hard red winter wheat Jagger and Big Dawg *cv* at 9 Hz.[1]

Effect	Numerator df	Denominator df	F Value	Pr > F
Samples	15	3.21	4.83	0.0993
Contrast[2]				
1	1	401	0.18	0.6901
2	1	3.53	0.08	0.7965
3	1	7.54	0.02	0.9027

[1] n = 3 replicates.

[2] Contrast definitions:

1 = Jagger enriched HMW-GS vs. LMW-GS.

2 = Big Dawg enriched HMW-GS vs. LMW-GS.

3 = Difference between HMW-GS and LMW-GS (Big Dawg vs. Jagger).

Table 12. Statistical analysis of the loss modulus (G") starch-glutenin gels containing glutenin subfractions from hard red winter wheat Jagger and Big Dawg *cv* at 9 Hz.[1]

Effect	Numerator df	Denominator df	F Value	Pr > F
Samples	14	2.13	2.94	0.2701
Contrast[3]				
1	1	4.12	0.18	0.6920
2	1	6.33	0.00[2]	0.9628
3	1	8.09	0.10	0.7595

[1] n = 3 replicates.

[2] Near zero.

[3] Contrast definitions:

1 = Jagger enriched HMW-GS vs. LMW-GS.

2 = Big Dawg enriched HMW-GS vs. LMW-GS.

3 = Difference between HMW-GS and LMW-GS (Big Dawg vs. Jagger).

Table 13. Statistical analysis of the tan delta starch-glutenin gels containing glutenin subfractions from hard red winter wheat Jagger and Big Dawg *cv* at 9 Hz.[1]

Effect	Numerator df	Denominator df	F Value	Pr > F
Samples	14	3.74	5.27	0.0680
Contrast[2]				
1	1	4.31	1.14	0.3427
2	1	4.76	0.03	0.8725
3	1	9.06	0.74	0.4108

[1] n = 3 replicates.

[2] Contrast definitions:

1 = Jagger enriched HMW-GS vs. LMW-GS.

2 = Big Dawg enriched HMW-GS vs. LMW-GS.

3 = Difference between HMW-GS and LMW-GS (Big Dawg vs. Jagger).

VITA

Ma. Cristina Escober

Candidate for the Degree of

Master of science

Thesis: DETERMINATION OF THE BINDING PROPERTIES AND RHEOLOGICAL BEHAVIOR OF REDUCED GLUTENIN SUBFRACTIONS IN THE PRESENCE OF CARBOHYDRATES

Major Field: Food Science

Biographical:

Personal Data: Born in Cebu City, Philippines, on February 23, 1977, the daughter of Rustico and Simeonita Escober.

Education: Graduated from Colegio de las Hijas de Jesus, Iloilo City, Philippines in March 1994; received Bachelor of Science degree in Food Technology from University of the Philippine Los Baños, Philippines, in April 1998. Completed the requirements for the Master of Science degree with a major in Food Science at Oklahoma State University in December 2003.

Experience: Employed as a Research Associate in BIOTECH, Philippines and have extensive experience in the food industry in the Philippines as a Research and Development Specialist. Currently employed as a Wheat Research Specialist at Oklahoma Food and Agricultural Products Research and Technology Center.

Professional Memberships: American Association of Cereal Chemists and Institute of Food Technologist