AN ABSTRACT OF THE THESIS OF

Yee-Ling Ong for the degree of Bachelor of Science in Bioresource Research Biotechnology option presented on December 5, 2003.

Title: Effects of Gluten Composition and Molecular Weight Distribution on the Noodle Making Potential of Hard White Wheats

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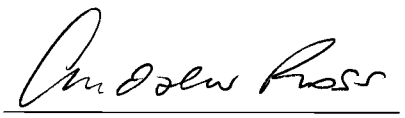

Mentor (Dr. Andrew S. Ross)

A set of flour samples with protein content ranging from 9.7 – 13.3 % was used for this study to evaluate the effectiveness of HMW-GS, SEHPLC and mixograph characteristics as screening tools for noodle texture. The sample set had a large variation in HMW-GS composition with Payne score ranging from 6 – 10. Flour protein content had a positive correlation with cooked noodle hardness ($p \leq 0.05$). Payne score showed no significant relationship with cooked noodle hardness but was strongly related to increased dough strength and mixing tolerance ($p \leq 0.001$), and SEHPLC % peak 1 ($p \leq 0.001$). Lower RVAPV or RVABD showed strong relationships with cooked noodle hardness ($p \leq 0.001$). Comparisons of flour varieties grouped by their *Glu1* loci showed that presence of *GluA1* subunit 1 was associated with noodles of equal hardness to those made from *GluA1* null lines when the *GluA1* subunit 1 lines had lower protein content. This suggests some compensatory effect of *GluA1* subunit 1 in determining noodle hardness. Higher protein content had more influence on noodle hardness than did HMW-GS composition at *GluB1* and *GluD1* loci. SEHPLC absolute peak area data suggested that there was some relationship between glutenin MWD and noodle hardness ($p \leq 0.001$). However, flour protein content was not corrected for when injecting samples onto the HPLC. Therefore, significance of the absolute peak areas may largely reflect flour protein content and not the relative proportions of the protein fractions. There was no significant relationship between % peak 1 and noodle hardness. MPT, mixograph absorption, Payne score and SEHPLC % peak 1 showed no relationship with noodle hardness, suggesting that HMW-GS, which are indicators of dough strength were not effective ways of predicting noodle hardness compared to protein content, except in the case of *GluA1*.

Effects of Gluten Composition and Molecular Weight Distribution on the Noodle Making Potential of Hard White Wheats

By
Yee-Ling Ong

A THESIS

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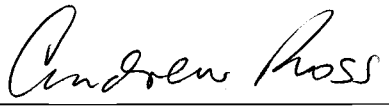

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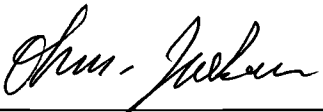

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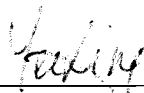

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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

Chapter 1 – Introduction

Wheat is one of the most important crops in the USA, and worldwide. Wheat production for 2002 was estimated at 562 million metric tons (mmt). Production in 2003 is predicted to be approximately 597 mmt. Of this, 429 mmt will be used solely for human consumption (International Wheat Council 2002). Such high level of consumption is due to the use of starchy foods, such as, bread, pasta and noodles, as a basic food and energy source by a large section of the world's population. For instance, noodles have been one of the most important staple foods in China for more than 2000 years. In recent years approximately 60 mmt of wheat has been used each year for noodle production in China and per capita wheat consumption increased from 20kg to 85kg between 1950 and 1985 (Huang 1996). This reflects a general trend towards increasing wheat consumption in all of eastern Asia.

Wheat is the most valuable cereal crop in Oregon. However, despite its importance, Oregon wheats lack diversity in end-use applicability. This factor, coupled with fierce international competition, has led to erosion of Oregon's export market share, and is threatening the prosperity of growers here. One way of addressing the threat is to expand the market outside the US; for example, into the Asian noodle market. The geographic location of Oregon at a major export port on the edge of the Pacific Rim gives Oregon advantages in trade. Asia's rapid population growth is another crucial factor when considering the importance of developing new wheat varieties for specific end-product targets in that market. If the US is able to increase market share in Asia, it would prove to be highly beneficial economically.

In recent years in the cereals research community, increased attention has been given to end-products other than bread. These products include noodles, steamed breads, and flat breads. Interest in these products has arisen in part due to globalization and the exporting of wheat from the western world. In addition, in Asia there has been increased consumption of wheat based foods at the expense of rice. The increased attention to noodles is also a result of the diversification of the western diets. Development of technology and mechanization allows rapid and cheap production of instant noodles, a uniquely convenient food, and this is another factor which drives the increasing popularity of noodles.

As a result of the increased attention to noodles by the US wheat industry, the development of new US hard white wheat (HWW) varieties is focused on a dual purpose role, where the HWWs

must be able to make both bread and noodle products (Peterson and Ross 2002). As such, this project is a core component of the HWW variety development activities of the Oregon State University (OSU) wheat breeding program.

The sensory quality of noodles is made up of two major components: appearance and texture. This project is focused on noodle texture. The primary determinants of noodle texture are protein and starch. As protein content of wheat flour increases, noodles generally become firmer (Park et al. 2003). However, starch attributes, such as amylose (AM) to amylopectin (AP) ratio also have a large bearing on the final texture. What is less clear is the influence of flour protein composition on noodle texture.

The aims of the project were to compare three potential methods to predict noodle making potential of hard white wheat. These methods include electrophoresis of wheat proteins, Size Exclusion (SE) HPLC separation of wheat proteins, and mixograph analyses of dough mixing characteristics. The overall aim was to determine if any of these methods can be used to determine the optimum gluten composition for noodle production and also serve as effective early generation screening tools in the breeding program. Rapid Visco Analyzer (RVA) analyses of flour pasting properties were conducted to monitor the impact of starch attributes.

Chapter 2 – Literature Review

2.1 Asian Noodle Production

Asian noodles are made from common wheat (*Triticum aestivum*) flour. Depending on the final product and its intended pH, the formulation will also include water in which is dissolved either or both table-salt or alkaline salts. The most commonly used alkaline salts are sodium and potassium carbonates. After mixing, the relatively dry crumbly dough is compressed by passing it through steel rollers to make a crude sheet. Dough sheets are then gradually reduced to the desired thickness by further rolling before being cut into strips. Depending on the presence or absence of the alkaline salts, Asian noodles can be divided into two categories based on their color. The two categories are commonly described as white salted noodles or yellow alkaline noodles. Addition of alkali gives noodles a unique yellowness, flavor and aroma, thus allowing us to differentiate them from salted noodles (Miskelly 1996). Both salted and alkaline noodles preferably have an elastic texture but alkaline noodles are generally firmer (Huang and Morrison 1988), although there is considerable overlap.

2.2 Asian Noodle Quality

The sensory quality of Asian noodles is made up of two major attributes: appearance and texture. As this project is focused on noodle texture, only the literature on noodle texture will be reviewed in detailed.

Texture is the key factor which influences white salted noodle quality (Epstein et al. 2002). Some common white salted noodles include Japanese udon, and Chinese and Korean salt noodles. Although all these noodles are made only from flour, water and salt, the preferred textural attributes are type and regional specific. Noodle texture can be determined by means of sensory tests which involve a trained panel or it can be determined instrumentally.

Texture is a complex sensory attribute made up of a number of components. These include, but are not limited to; softness, elasticity and surface smoothness (Yun et al. 1997, Konik et al. 1992). Other attributes such as springiness, surface roughness, graininess and slipperiness can also be used (Janto et al. 1998). One can also measure mechanical properties of noodles

instrumentally and obtain parameters that can be compared to their sensory counterparts. Some commonly used, instrumentally derived, textural parameters include smoothness, softness, stickiness, cohesiveness, elasticity, and chewiness (Yun et al. 1997, Baik and Lee 2003). The two types of assessment are related, for instance, Yun et al. 1997 reported strong correlations between sensory softness and instrumental softness.

Appearance can be evaluated using three parameters: brightness, yellowness and discoloration (Yun et al. 1997), although other attributes like glossiness, luster and geometry also contribute. The yellowness of noodles should "range from creamy white to yellow" (Ross 1997). Discoloration is caused by factors such as bran fragments that remain in the noodle sheet causing the surface to appear dull (Yun et al. 1997).

The physical structure of a noodle is made up of protein and starch, and these are the primary determinants of noodle texture. In addition, there is evidence that protein composition may be an important secondary determinant (Huang and Morrison 1988, Crosbie et al. 1999, Park et al. 2003). This is more fully reviewed in section 2.4, below. As protein content of flour increases, noodles generally become firmer (Park et al. 2003, Oh et al. 1985 and Crosbie et al. 1999), and as starch swelling potential increases noodles become softer (Ross 1997). In this study, we were specifically interested in the influence of the relationship between wheat protein compositions and noodle quality. However, the influences of starch attributes and protein content need to be taken into account in interpretations of the data.

2.3 Wheat starch

Wheat starch attributes were not being directly investigated in this project. However, one needs to understand the profound influence starch has on noodle texture to correctly interpret changes in texture resulting from changes in protein content or composition. Amylose (AM) is a linear polymer of α–D-glucose linked α-1,4. Amylopectin (AP) is a branched polymer with α–D-glucose chains linked α-1,4 as in AM. AP branch points are linked α-1,6 (Hoseney 1986). As AM content of wheat starch decreases, commonly as a result of absence of one or more copies of starch synthetic enzyme, granule bound starch synthase (GBSS), noodle texture generally becomes softer (Ross 1997). "Eating quality of white salted noodle is negatively correlated to starch amylose content and positively correlated to starch amylopectin content" (Black 2000). The changed textural attributes are largely a factor of the

relative increase in AP, a highly branched molecule that swells more on hydration than AM, leading to a more diffuse and softer gel structure that gives a unique soft and elastic mouthfeel.

2.4 Wheat Flour Proteins

Wheat flour is unique because it has the ability to form cohesive doughs with rheological properties suitable for making risen breads, and as a result of the ability of these doughs to retain gas. In addition, a wide variety of other foods has been developed to take advantage of the unique attributes that come from the wheat endosperm storage proteins. The wheat endosperm storage proteins go together to make gluten.

There are four categories of wheat flour proteins: glutenins, gliadins, albumins and globulins. Glutenins and gliadins make up the endosperm storage proteins and also about 80% of the gluten complex that is formed when wheat flour is mixed in the presence of water. The other 20% of the gluten complex is made of minor components such as lipids, occluded starch granules and non-starchy polysaccharides. More than 60% of gluten proteins are glutenins. These are a heterogeneous group, composed of proteins of very high molecular weights that contribute both cohesive and elastic properties. The gliadins consist of a diverse group of proteins with lower molecular weights. These are also cohesive but exhibit viscous flow. Interactions between, and within, the glutenins and gliadins provide the unique visco-elasticity of wheat flour doughs. Accordingly, most research on wheat proteins has focused on the glutenins and gliadins, but more particularly on the glutenins. The research has proceeded with the hope of understanding the roles of these proteins in the processing of flour into different end-products. However, the great bulk of this work has focused on the effects of glutenins in breads.

There are many individual glutenin subunits. These are coded for by multiple alleles at the three genetic loci responsible for glutenin formation. Glutenins are a heterogeneous mixture of proteins. Glutenin subunits, more particularly the high molecular weight glutenin subunits (HMW-GS), are crucial elements in the gluten network, as these are the major determinants of gluten elasticity. They contribute to gluten elasticity by their ability to form large disulfide linked complexes. HMW-GSs contain high levels of glutamic acid, proline, glycine and small amounts of lysine and cysteine. HMW-GS structure consists of a hydrophilic central

repetitive domain made up of short amino acid sequences and forming approximately 85% of the protein. In addition, its end is flanked by two hydrophobic non-repetitive domains, which contain most of the cysteine residues (Gianibelli et al. 2001). Cysteine residues provide the mechanism for the formation of the intermolecular disulfide bonds that lead to large polymeric aggregates of glutenins in doughs. The repetitive central domain adopts a β-reverse turn which subsequently results in elasticity as the loops elongate and compress when stress is applied or removed (Belton 1999). It is these structural features, cysteine cross-linking in concert with the natural elasticity of the repetitive domain, which are the basis of glutenin elasticity.

A study of near isogenic lines of wheat varying only in HMW-GS composition clearly showed their effects on baking performance (Payne et al. 1987). In a review, Lefebvre and colleagues (2000) indicated that lines encoded with subunit pair 5+10 have better baking performance than those with subunit pair 2+12. Changes in HMW-GS composition influenced glutenin size distributions and disulfide cross linking arrangements, which causes expression of different visco-elasticity potential. A larger polymer and aggregate thus cause an increase on visco-elasticity and "in such cases subunit related difference should be considered" (Lefebvre et al. 2000). Hence, wheat gluten is essential to the functionality of wheat flour dough and the texture of wheat based end products such as breads, pasta and Asian noodle.

Despite our understanding of the impact of flour protein content on noodle texture, there is limited information regarding how the variable composition of gluten in different wheat genotypes affects texture. With respect to flour protein content, hardness of cooked noodles has been positively related to its increase (Park et al.. 2003, Huang and Morrison, 1988). Noodles prepared from low protein wheat flour are more fragile than those made from flour with high protein content because of a weaker protein network. The literature does show some effects of the relationship between protein composition variability and effects on textural characteristics of noodles (Oh et al. 1985, Baik et al. 1994 and Lefebvre et al. 2000). For instance, "in udon noodle, a 53-kD endosperm protein appeared to be most responsible for the desirable viscoelastic texture of cooked udon-noodle. In contrast, HMW-glutenin subunit 2* appeared to be most responsible for reduced visco-elastic texture of cooked udon-noodle" (Nakamura 2002). However, it is also important to note that the expected correlation is not always true. For instance, substitution of an allele with better textural

potential may not significantly improve textural characteristics, possibly due to interactions from the low molecular weight glutenin subunits and gliadins (Wesley et al. 1999). Additionally, Huang and Morrison (1988) showed that the existence of certain gliadin components was also related to noodle texture. This then indicates that overall gluten composition, in this case gliadins, not glutenins, can affect noodle texture.

According to Lefebvre (2000), "size distribution of glutenin polymers, its aggregation and extractability" are all influenced by variations in subunit composition. In the same paper, strong correlations were seen between dough elasticity and the amount of large polymers. Studies that observed dough properties (Crosbie et al. 1999) and SDS sedimentation volumes (Huang and Morrison 1988), both of which are influenced by gluten composition, further indicate the effects of gluten composition, showing that higher dough strength or higher SDS volumes were associated with increased noodle firmness. These findings lend further weight to a hypothesis that changes in glutenin composition can affect noodle texture. If there indeed is a direct relationship between glutenin composition and noodle texture, then it may be possible that the presence of specific glutenins could be predictive of noodle making potential.

As a result, this project aimed to expand our knowledge of the influence of the relationship between wheat protein compositions on noodle quality and to investigate the possibility of predicting noodle texture attributes using indicators of glutenin or gluten composition.

Chapter 3 – Material and Methods

3.1 Plant Material

35 elite HWW breeding lines from OSU were used for this project. Samples were collected from two sites each with two replications; Corvallis, OR where growing conditions include high rainfall and leaf disease pressure, and Arlington, OR with low rainfall, deeper soils and drought stress. In the following analyses only samples from the Arlington site will be reported, as only this group had the appropriate flour protein content for noodlemaking.

3.2 Analytical Test

3.2.1 Single Kernel Analysis

AACC method 55-31 was used for single kernel characterization of wheat kernel texture on the Single Kernel Characterization System (SKCS) model 4100 (Perten Instruments, Huddinge, Sweden). Grain was hand mixed in small paper bags using a ladle. Approximately 300 grains were scooped out and placed into a plastic container. The grains were checked for defects. Broken kernels were discarded. The grains were then divided into three approximately equal sections. Grain was added to the SKCS hopper at the front of the instrument, section by section, until data from 300 grains were collected. The parameters measured included kernel hardness, diameter, size and moisture content of the kernels but only hardness was reported. Measured parameters were collected and analyzed using an IBM compatible computer with the SKCS data analysis software supplied by the manufacturer.

3.2.2 Moisture Content

Moisture content was determined using AACC method 44-15A with modification. 4.0 cm by 2.5 cm heavy gauge aluminum containers with well fitted lids were used. The containers were dried uncovered in the air oven (Fisher Isotemp Oven, model 230F) at 130^0C for 1 h. The containers were then transferred uncovered into a desiccator to be cooled for 30 min. Oven heat was maintained at 130^0C. Once cooled, the containers

with lids were measured, and weight was recorded. This is recorded as the weight of container.

3.0 ± 0.05 g of flour was measured into the aluminum containers. The container was immediately covered. Weight of container plus flour sample was recorded. This was weight "sample + container before drying". The same procedure was repeated for all samples. The containers were uncovered and the lids were placed beside their respective containers on the oven rack. Each aluminum container was placed on the oven rack approximately 2 cm apart. Then the oven rack was immediately transferred into the middle slot of the oven. The samples were heated for 60 min after the oven recovered 130^0C temperature.

Samples were removed using insulated gloves. The containers were covered and transferred into the desiccator immediately. Samples were left in the desiccator and cooled for 45 min. Then using tongs, containers plus dry flour were weighed and weight was recorded. This was weight "container + sample after drying".

Percent moisture was calculated using formula:

$$\% \text{ moisture} = \frac{\text{moisture loss in grams}}{\text{original weight of sample}} \times 100$$

3.2.3 Protein Content

Protein content was determined at the Central Analysis Lab at Oregon State University by nitrogen combustion analysis using a LECO CNS 2000 carbon, nitrogen, sulfur analyzer [Leco Corp, St. Joseph, MI]. Flour samples were weighed and placed into the LECO CNS 2000 where they were combusted in oxygen. N was directly volatilized from flour samples and detected by thermal conductivity. Protein was calculated as N x 5.7 (AACC method 46-30).

3.3 Milling

500 ± 0.05 g of grain was measured for each sample and collected in plastic containers. Grain of known moisture content was tempered to 15% by adding appropriate amounts of water. Samples were shaken 50 times using a manual rotational mixer (Bioengineering Inversina, Switzerland). Samples were left to equilibrate for 24 hours.

Grain was milled using a Quadrumat Sr. flour mill (C.W. Brabender, Instruments, NJ) with a modified AACC 26-50 procedure. Mill temperature was kept constant at 88-89°C using an external heater. Approximately 250 g of sample was measured and laid in the feeder and fed into the break rolls at a flow rate of 145-150 g/min. When about half of the sample was left on the feeder, the remaining 250 g was poured onto the feeder. This was to prevent the feeder from being too full. All wheat was considered to have passed through the break rolls when the grinding sound disappeared. At this time the break rolls were cleaned by running in reverse for 3 sec and running forward for 3 sec. This was repeated 3 times and then the hatch was cleaned and brushed. The timer was set to 15 minutes to allow sufficient time for all flour and stocks to pass through or over the sieves. At 11.5 min, a pan was left under the spout leading from the break side to the reduction side of the mill to catch remaining stock. Visual examination showed this stock to be a mixture of bran and flour. Reduction rolls were cleaned the same way as the break roll.

Bran, short, reduction and break flours were weighed and recorded. Break flour and reduction flour were mixed by shaking in a glass bottle 50 times using the manual rotational mixer. The flour was transferred into air tight bags for storage. Flour yield and break flour yield were calculated based on sum of total flour and stocks recovered.

$$\text{Flour Yield} = \frac{\text{Break flour + Reduction flour}}{\text{Break flour + Reduction flour + short + bran}} \times 100$$

$$\text{Break Flour Yield} = \frac{\text{Break flour}}{\text{Break flour + Reduction flour + short + bran}} \times 100$$

3.4 Rapid Visco-Analyzer (RVA)

3.5 ± 0.05 g of flour sample at 14% moisture basis was measured into aluminum RVA containers. 25 ml of 1 mM $AgNO_3$ was added to make a total weight of 28.5 g. $AgNO_3$ was added to inhibit and negate the effects of even the small amounts of alpha-amylase present in sound grain and that can affect relationship between RVA parameters and noodle texture (Crosbie et al. 1999). The mixture was inserted into RVA (Newport Scientific, Warriewood, New South Wales, Australia) and K-M 18 minute noodle profile described by Crosbie et al. (2002) was used. RVA parameters measured were peak viscosity (RVAPV) and breakdown (RVABD).

The RVA curve (Fig 3.1) shows starch pasting properties as starch was heated and cooled in excess water. RVAPV is the point of highest viscosity as temperature increases. RVABD is the difference between peak viscosity and holding strength. RVA setback and final viscosity were not reported in this study.

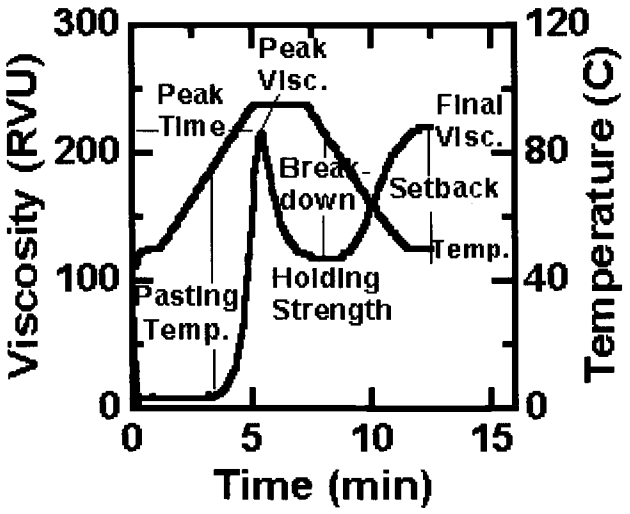

Figure 3.1: Diagram of RVA curve and common parameters (Newport Scientific 1998)

3.5 Mixograph

The mixograph (10g, National Manufacturing, Lincoln, NE) is a five pin device which uses the rotation of the pins to mix flour and water. During this process water is incorporated into flour and gluten is formed. A mixograph curve gives us information about protein quality depending on the placement and shape of the curve.

Mixograph testing was performed using a modified method based on AACC Method 54-40A. 10 ± 0.05 g of flour adjusted to 14% moisture content basis was measured and transferred into a mixograph bowl. The expected optimal requirement of the flour for deionized water was calculated using the formula below:

$$\text{Expected water} = \frac{[8.6 - 10\text{ g sample (at 14\% moisture)}] \times \text{moisture content}}{100}$$

Using expected water as a guide, deionized water was added to the flour until dough reached required appearance and consistency. The dough should look shiny, and when held should flow slightly. If the dough was too viscous, too much water was added. The mixograph curve is shown in Figure 3.2. Mixograph peak time (MPT) and optimum water absorption were recorded manually. Bandwidth at 6 min was determined by the Mixsmart program supplied by the manufacturer. Peak on the mixograph curve occurs when there is optimum dough development. MPT indicates protein strength; a longer peak time represents higher protein strength. Width and angle of descent indicates dough tolerance against over-mixing; a sharper rate of descent and increased thinning of bandwidth represents poorer mixing tolerance.

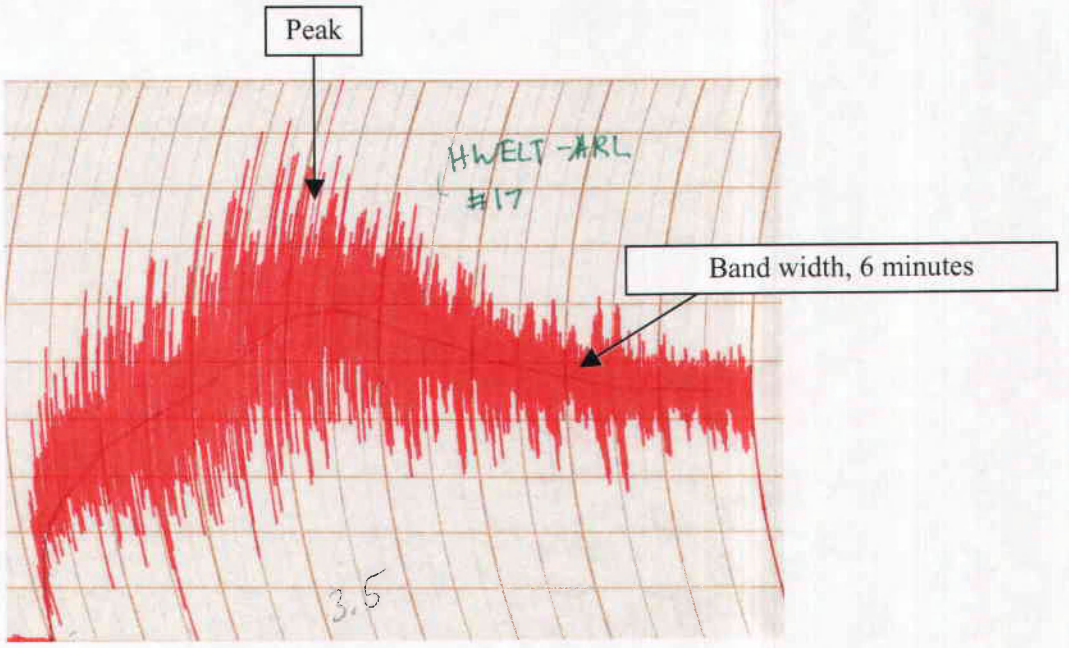

Figure 3.2: Typical Mixograph curve showing peak time and bandwidth at 6 minutes.

3.6 Electrophoresis

Acrylamide gels were made at least 4 h before protein extraction. A glass plate sandwich was made with a pair of caster screws, a 1.5 mm Spacer Mate, and two clean glass plates. The separating gel solution (Table 3.1) was degassed under vacuum, then pipetted into the sandwich from one corner carefully so as to not introduce air bubbles. The separating solution was filled to approximately 3-4 cm below the top of the glass plate. Approximately 2 cm of water was immediately pipetted into the sandwich to prevent gel exposure to oxygen and to remove any air bubbles from the separating gel surface. The gel was allowed to polymerize for 3 h.

After 3 h, the water was poured away, and the stacking gel solution was immediately pipetted carefully into the sandwich to form a top layer on the separating gel. The stacking gel was used to give a rough size order to the proteins before they enter the separating gel to make the electrophoresis more efficient. The sandwich was filled to about 1.0 cm below the top of the glass plate. A Hoefer 1.5 mm thick 15 well comb was carefully inserted into the stacking gel solution. Any air bubbles introduced were removed by gently moving the comb in the glass plate sandwich. The stacking gel was left to polymerize for 1-2 h.

Table 3.1: Gel composition.

Component	Separating Gel		Stacking Gel
	7.5%	14.5%	4%
10% v/v Acrylamide	3.56 ml	5.44 ml	0.67 ml
1.5 M TrisCl (pH 6.8)	3.75 ml	3.75 ml	0 ml
0.5 M TrisCl (pH 6.8)	0 ml	0 ml	1.25 ml
10% SDS	0.15 ml	0.15 ml	0.05 ml
Deionized water	7.49 ml	5.63 ml	3.0 ml
10% APS	50.0 µl	25.0 µl	25.0 µl
TEMED	5.0 µl	5.0 µl	2.5 µl
Final volume	15.0 ml	15.0 ml	5.0 ml

Table3.2: Components of 5x Electrophoresis buffer

Component	Concentrations	Amount
Tris	0.025 M	15.1 g
Glycine	0.192 M	72.0 g
SDS	3.5 mM	5.0 g
Deionized water		Add to total 1 L

Table 3.3: Extraction buffer composition for final volume of 10 ml.

Component	Concentrations	Amount
Tris Cl pH6.8	0.125M	2.5 ml
10% SDS	0.14M	4.0 ml
Glycerol	30% v/v	3.0 ml
Bromophenol Blue	0.03mM	0.2 mg
Deionized Water		Add to 10mL total

After the stacking gel hardened, the combs were removed by gently rocking it from side to side and then lifting slowly, making sure that the wells were not damaged. The wells were then filled with 1x electrophoresis buffer (from diluting the 5x buffer, Table 3.2) immediately. The gel was then ready for use.

Proteins (glutenins and gliadins) were fractionated by SDS-PAGE method described in Gupta and MacRitchie (1991) with modifications. 10 ± 0.05 mg of flour sample was measured into 1.5 ml Eppendorf tubes and to that 197.0 µL of sample extraction buffer was added (Table 3.3), followed by the immediate addition of 3.0 µL of 2-mercapethanol (ME). The mixture was heated in a water bath at 65°C for 1 h. After heat treatment, the samples were centrifuged using the Eppendorf Centrifuge 5413 for 15 min. Reference genotypes Cajeme 71 and Moro were treated in the same manner.

20 µl of sample was injected into each well. The gels ran at a constant temperature of 25^{0}C and constant current of 40mA/gel. The gels were stained using Neuhoff Protocol Electrophoresis which used 0.1% Coomassie Blue G-250, 2% Phosphoric acid and 10% Ammonium sulfate. Using Cajeme 71 and Moro as references, the protein fractions were identified and labeled using the Payne and Lawrence numbering system (Gianibelli et at 2001). After the experiment, the samples were disposed according to the hazard guide.

3.7 Size Exclusion High Pressure Liquid Chromatography

Molecular weight distributions (MWDs) of total protein extracts were determined using SEHPLC. 160 ± 0.05 mg of flour sample (adjusted to 14% moisture content) was placed into 50 ml centrifuge tubes. To each sample, 20 ml of 1% SDS and 0.1M sodium phosphate buffer (Table 3.4) were added using a 10 ml automatic pipette. The mixture was sonicated (Fisher Scientific, Sonic Dismembrator 100) for 3 minutes at 30% (5 W) power setting.

Table 3.4: 10% SDS Sodium phosphate buffer composition

Component	Amount
SDS	10 g
Anhydrous Sodium phosphate dibasic	14.196 g
Deionized water	Add to 1 L
Adjust pH to 6.9 with 12M HCl	

After sonication, the mixture was heated in a water bath at 65^{0}C for 30 min to inhibit protease activity and to stabilize the extract (Larroque et al. 2000). The tubes were shaken by hand to mix the solution and approximately 1.2 ml of the mixture was transferred to 1.5 ml Eppendorf tubes. The mixture was centrifuged using the Eppendorf Centrifuge 5413 for 40 minutes. The supernatant was transferred into 3 ml plastic syringes with a head (Millipore Swinnex non-sterile) and 0.45 µm HV Millipore DuraPore membrane filters inside. Then the supernatant was filtered into HPLC vials (Waters screw neck vials, 12x32 mm) and 20 µl of each sample was injected onto a Phenomenex BIOSEP SEC S4000 size-exclusion column [600 x 7.5 mm]. The samples were eluted for a duration of 30 min each. The eluting buffer was 50% acetonitrile in water with 0.1% Trifluroacetic acid (TFA). After the experiment, the samples were disposed according to the hazard guide.

SEHPLC chromatograms were collected. The curves were integrated manually using the following peak times: 12.80, 13.3, 14.50, 15.85, 18.25, 19.46, 20.85, 21.23 and 24.60. Peak area and peak height were calculated by the Empower program provided by HPLC manufacturer. Percent peak area was calculated using the following formula:

$$\% \text{ peak area} = \frac{\text{area of peak}}{\text{total area of all peaks}} \times 100$$

3.8 Noodle Making

3.8.1 Optimum Water Absorption

A modified version of the optimum water absorption procedure described in Oh et al. (1986) was used. 10 ± 0.05 g of flour was measured into a 10 g mixograph bowl. 0.50

ml of deionized water was added in the middle of the bowl using a 100 µl pipette (Gilson, Pipetman P). Flour and water were mixed for 1 min. The mixograph was turned off after 1 min and another 0.50 ml of deionized water was added. The flour was mixed for a further 1 min.

0.20 ml of deionized water was added twice to the flour at each 1 min interval using the procedure described above. Then, 0.10 ml of deionized was added to the crumble using a 20 µl pipette (Gilson, Pipetman P) at each 1 min interval as in the procedure described above, until the mixograph curve produced a constant width of at least 4 squares (Figure 3.3). The amount of water added was calculated and recorded. The amount of water added for each sample was converted to be used for 100g of flour during noodlemaking.

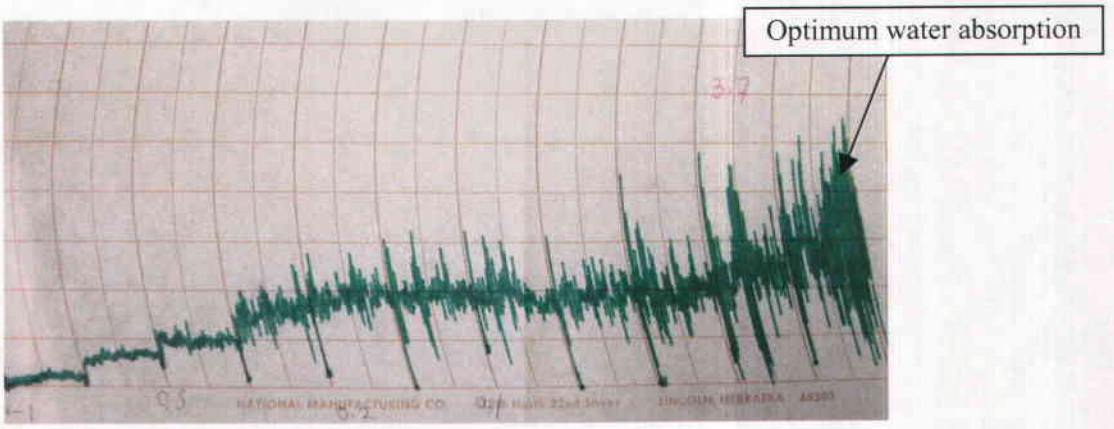

Figure 3.3: Mixograph curve used to determine optimum water absorption for noodlemaking.

The amount of water added to noodle formulations was based on results recorded from the optimum water absorption procedure described above. However, on some occasions the mixograph prediction of optimum water addition was incorrect, as a result of the impact of other flour characteristics. As a result of this, the mixograph results were initially validated on a small scale. In this confirmation test, 10 ± 0.05 g of flour was measured and mixed for 4 min in a 10 g pin mixer using the amount of water predicted from the above procedure. After mixing for 4 min the crumble was removed and placed into a plastic container for examination. If the dough crumble looked neither too wet nor too dry, then the amount of water determined by the mixograph procedure was considered optimum and was used for noodle making. If the dough crumble looked too

dry or wet, the amount of water added was adjusted accordingly until it reached acceptable appearance and hand-feel as guided by an expert noodle maker.

3.8.2 Oregon State Chinese Raw Noodle Method (White Salted, 200g Pin Mixer)

Table 3.5 Chinese raw noodle formulation.

Component	Amount	Percent (flour basis)
Flour	100 g	100%
Deionized water	32.0 g	32%
Salt	1.20 g	1.2%

The general formulation for salted noodles is shown in Table 3.5 above. Salt was dissolved in an appropriate amount of deionized water before addition to flour. Solution was stirred constantly using magnetic stirrers in 500 ml plastic screw cap containers (Nalgene) until all salt was dissolved. 100 ± 0.5 g of flour sample was measured and placed in the mixer bowl of a 200 g pin mixer (National Mfg, Lincoln NE) and the dry flour was mixed for 30 sec. Using a rubber spatula a well was made in the center of the bowl and all the salt solution was added. The mixture was mixed for 1 minute. After 1 minute, the pins were scraped of adhering dough using the rubber spatula. The mixture was mixed for a further 2 min and 30 sec. Total mixing time was 3 min and 30 sec. After mixing, the crumble was rested for 30 minutes (1^{st} resting) in closed Ziploc bags.

After 30 minutes, the crumble was compounded using the rolls of an Ohtake Noodle Machine (Ohtake Mfg, Tokyo). The gap on the rollers was set at 4.0 mm and the dough crumble was compressed between them to form the first crude sheet. The dough was resheeted 3 more times, each time being folded once and traveling through the rollers in the same direction. The dough sheet was placed loosely in a Ziploc bag which was then closed; the dough sheet rested for another 30 minutes (2^{nd} resting).

After the 2^{nd} rest, the dough was sheeted by passing it through the rollers 4 times with progressively reduced gaps of 3.5, 3.0, 2.0 and 1.5 mm. Dough thickness was measured using a Peacock thickness gauge. The roll gap was adjusted accordingly to give a final

dough thickness of 1.2 ± 0.05 mm. The final dough sheet was cut into strips with a #12 square type cutter (2.5 mm width) and the noodles were stored loosely in closed Ziploc bags at room temperature for 24 h before cooking.

3.8.3 Cooking

50 ± 0.5 g of noodles were measured and set aside. 500 ml of distilled water was boiled in stainless steel pots on an induction heater (Iwatani, US-9000, Iwatani International Corporation, Japan) until boiling rapidly. The noodles were added and boiled at high heat for 1 min, after 1 min, temperature was lowered to medium, to stop water from boiling over. Cooking then continued for another 5 minutes. The total cooking time was 6 minutes. After cooking, the noodle sample was rinsed with distilled water at room temperature (20.7 - 21.5^{0}C) for 1 minute and drained. The drained noodles were transferred into an air tight container and rested for 15 minutes before testing.

3.8.4 Texture Profile Analysis (TPA).

A Texture Analyzer (TA, TAXTPlus, Stablemicrosystems, UK) was used for TPA. The TA was calibrated with a 2 kg standard weight. Probe distance from the bottom plate was set to 5 mm. The probe was fitted with a compression blade with a contact surface 5 mm wide. Only undamaged noodles of approximately the same length were used for each test. Three noodle strands were placed side by side so than they touched along their entire length and were centered under the compression blade (Figure 3.4).

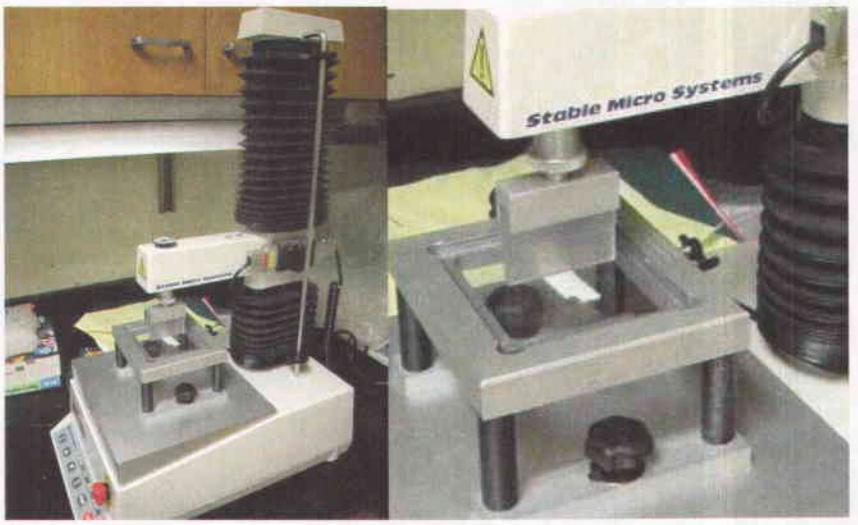

Figure 3.4: Texture analyzer showing the placement and orientation of three noodle strands under the compression tool

Table 3.6 Instrumental settings on TA.

Parameter	Setting
Pretest speed	4.0 mm/sec
Test speed	1.0 mm/sec
Posttest speed	1.0 mm/sec
Strain	70%
Time between compressions	1.0 sec
Trigger type	Auto
Trigger force	5g
Force Units	g
Distance Units	% strain

Textural profile data was collected from the TA using an IBM compatible computer using the Texture Exponent software supplied with the TA. TPA instrument settings are shown in Table 3.6 above. The bottom plate and compression blade was cleaned after each use.

TPA is a "two bite" test (Figure 3.5). There were two cycles of compression making up the first and second bite. TPA parameters used were, hardness, cohesiveness springiness, chewiness, and resilience (Bourne 1982). Definitions and calculations of the parameters are explained in Table 3.7.

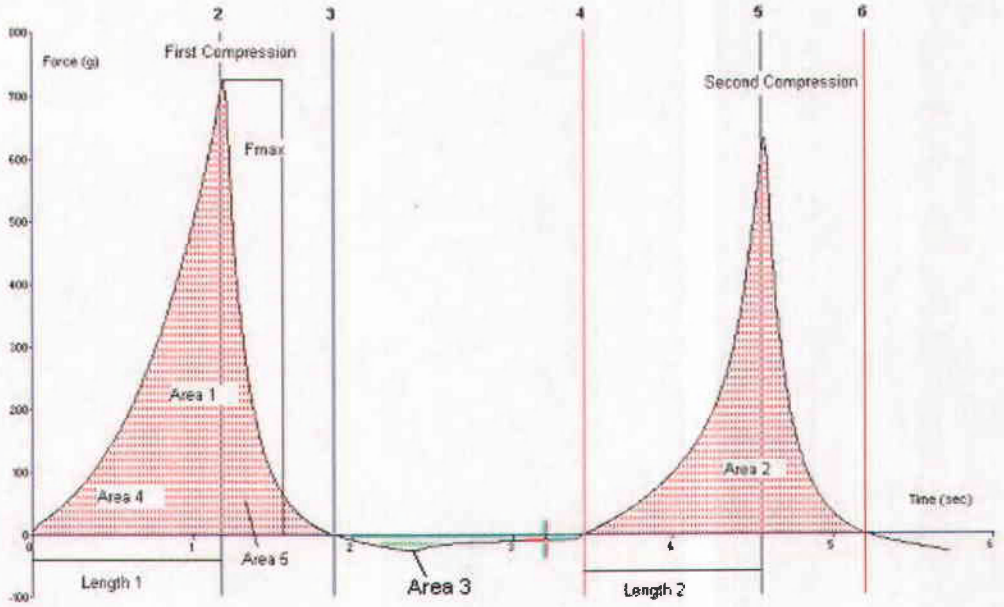

Figure 3.5: Texture profile analysis of white salted noodle

Table 3.7 Definitions and calculations of TPA parameters (Adapted from Epstein et al. 2002).

Parameters	Definitions/Calculations
Hardness	The maximum force recorded in the first compression cycle
Adhesiveness	The work done after Fmax (Area 3)
Cohesiveness	Area2/Area1
Springiness	Length2/Length1
Resilience	Area5/Area4
Chewiness	Gumminess * Springiness

Chapter 4 – Results and Discussion: Analytical, RVA, Rheology and Noodle Texture Data

Table 4.1a shows the varietal means, minima, maxima and standard deviations of the flour analytical data for the 18 genotypes used in this study. Substantial differences between HMW-GS composition were observed between the 18 cultivar varieties. All three most common *GluA1* alleles, 1, 2* and null (Gianibelli et al. 2001) were present in the studied lines.

Diversity was also observed at the *GluB1* locus. Four of the varieties have only one *GluB1* subunit (subunit 7) and the remainder had one of four other subunit pairs (e.g.; 17+18). OR942496 represented a mixed population at the *GluB1* locus, and additional selection within that variety is required to stabilize its quality attributes. Subunit pairs 2+12, 3+12, and 5+10 were present in 3, 1, and 12 of the varieties respectively. The diversity of HMW-GS compositions in this sample set resulted in "Payne scores" ranging from 6 - 10 (Table 4.1). The Payne scores take into account the combined effect of all three loci and this range indicated a range of moderate to very good baking potential (Gianibelli et al. 2001) in the samples tested here.

Flour protein content had an overall mean of 11.2%, which was considered suitable for making Chinese raw salted noodles (Hou 2001). The range of flour protein content was also enough to give a wide range of texture in the final noodle product (Table 4.1a). However, this range could make it more difficult to interpret the role of protein composition than first anticipated. Kernel hardness ranged from 40 to 99.6, the lower values indicating the inclusion of soft white check varieties Stephens, Eltan and Madsen.

RVAPV and RVABD varied from 223.8 to 311.5 and 101.7 to 173.3 respectively. The highest values obtained were partially within the range of viscosity exhibited by partial waxy wheats. However, discussion with the breeder indicated that these varieties were all wild type for GBSS and were not partial waxy. MPT ranged from 1.8 to 4.4 min, representing a difference in protein quality within the different varieties. This was also a reflection of the variation in HMW-GS and Payne scores.

Table 4.1b shows the varietal means, minima, maxima and standard deviations of cooked noodle data for the 18 genotypes used. Ranges of hardness, adhesiveness and chewiness were sufficiently varied for our needs. Springiness, cohesiveness and resilience, which were ratios of different aspects of the two bite TPA curve, had a more restricted range. This suggested that a flour protein

content range of 3.6% was insufficient to produce variability in these parameters. In addition, it also indicated that the TPA itself might not be efficient in resolving these attributes.

Table 4.2 shows the linear correlation between the cooked noodle texture parameters determined using TPA. Hardness was negatively correlated with adhesiveness, cohesiveness and resilience. Hardness was also positively correlated with chewiness, which may not be surprising since chewiness is the product of hardness, springiness, and cohesiveness, and because hardness is a very large number compared to springiness and cohesiveness. Hardness was not significantly correlated with springiness. However, this may be due to the result of a limited range of springiness as observed in Table 4.1b.

Adhesiveness was positively correlated with springiness, cohesiveness and resilience. There was no significant correlation with chewiness.

Springiness was positively related to adhesiveness and resilience.

Cohesiveness was strongly related to resilience. This suggested that the length 1 to length 2 ratio was giving very similar structural information to the area 5 to area 4 ratio with respect to the recovery of the noodles from deformation. Cohesiveness was also significantly related to hardness and adhesiveness.

Table 4.2

Correlation Coefficients Between Cooked Noodle Texture Parameters[1]

Noodle Parameter	Hardness (g)	Adhesiveness (g*sec)	Springiness	Cohesiveness	Chewiness
Adhesiveness (g*sec)	-0.723***				
Springiness	NS	0.508**			
Cohesiveness	-0.611***	0.686***	NS		
Chewiness	0.751***	NS	NS	NS	
Resilience	-0.661***	0.805***	0.386*	0.926***	NS

[1]*, **, and ***: Correlation coefficient is significant at P<0.05, 0.01 and 0.001,respectively

NS: Correlation coefficient is not significant at P<0.05.

Table 4.1a

Glutenin, Payne Score, and Mean Values of Flour Protein, Rapid Viscosity Analyzer (RVA), and Mixograph Data from 18 Wheat Genotypes

					Flour	Single	RVA		Mixograph	
	Locus			Payne	Protein	Kernel	Peak Viscosity	Breakdown	Peak Time	Absorption
Variety	*Glu A1*	*Glu B1*	*Glu D1*	Score	(%)[a]	Hardness	(RVU)[b]	(RVU)[b]	(min)	(%)[a]
Stephens	2*	7+9	2+12	7	10.1	55.7	223.8	101.7	2.0	58.4
Eltan	1	7+9	5+10	9	10.1	40.0	250.2	112.2	4.0	57.5
Nuplains	2*	13+19	5+10	NA	11.6	86.5	266.0	125.1	2.4	61.3
Madsen	2*	7	2+12	6	10.8	56.4	242.5	110.9	2.0	57.5
OR943576	2*	6+8	3+12	6	9.7	77.5	268.4	132.3	2.3	61.8
OR941048	2*	7+9	2+12	7	10.7	99.6	257.3	120.5	2.0	64.0
OR942496	1	6+8,17+18,7	5+10	NA	11.1	86.2	295.0	144.7	3.3	67.6
OR953475	2*	7+9	5+10	9	11.4	86.3	263.8	127.4	3.1	65.1
OR3971156	2*	6+8	2+12	6	12.1	86.2	273.6	133.0	2.4	64.0
OR952577	2*	7	5+10	8	12.2	82.3	259.5	122.5	2.4	62.8
Ivory-8	2*	7	2+12	6	11.7	87.5	263.1	120.7	1.8	63.8
OR9900364	2*	7	5+10	8	13.3	77.4	247.1	119.0	2.3	63.5
OR9900374	n	17+18	5+10	7	11.1	86.0	251.8	128.5	2.9	63.5
OR9900384	n	7+9	5+10	6	12.8	74.9	283.3	166.5	2.1	62.9
OR9902410	1	17+18	5+10	10	10.4	94.4	294.0	173.3	4.4	64.4
OR2020003	2*	7+9	5+10	9	11.0	91.4	274.7	135.8	3.5	66.2
OR2020006	2*	7+9	5+10	9	10.7	89.3	311.5	166.6	3.1	62.3
OR2020007	2*	7+9	5+10	9	10.1	91.4	286.0	157.5	3.0	62.3
Mean				7.6	11.2	80.5	267.3	133.2	2.7	62.7
Minimum				6	9.7	40.0	223.8	101.7	1.8	57.5
Maximum				10	13.3	99.6	311.5	173.3	4.4	67.6
Standard Deviation				1.4	1.0	15.3	21.4	20.6	0.7	2.7

[a]On a 14 % flour moisture content basis.

[b]RVU=RVA viscosity unit

Table 4.1b

Means of Noodle Texture and Color Data from 18 Wheat Genotypes

	Noodle Texture (15 min After Cooking)					
	Hardness	Adhesiveness	Springiness	Cohesiveness	Chewiness	Resilience
Variety	(g)	(g*sec)				
Stephens	847.4	-34.5	0.96	0.58	475.7	0.32
Eltan	749.2	-27.9	0.93	0.64	446.8	0.40
Nuplains	852.6	-20.3	0.94	0.64	511.9	0.38
Madsen	774.4	-28.2	0.93	0.64	461.9	0.38
OR943576	734.3	-21.7	0.94	0.65	445.0	0.39
OR941048	799.0	-21.8	0.93	0.64	475.6	0.38
OR942496	748.6	-25.2	0.94	0.64	450.7	0.37
OR953475	746.1	-22.1	0.93	0.63	438.7	0.36
OR3971156	705.8	-23.9	0.95	0.64	429.8	0.36
OR952577	786.5	-16.6	0.96	0.64	481.4	0.39
Ivory-8	766.0	-21.9	0.94	0.64	458.5	0.39
OR9900364	834.8	-33.2	0.94	0.64	502.9	0.35
OR9900374	720.3	-16.7	0.95	0.65	443.7	0.39
OR9900384	730.8	-18.0	0.95	0.64	443.2	0.38
OR9902410	628.4	-13.0	0.95	0.66	392.3	0.42
OR2020003	745.4	-16.0	0.95	0.67	474.4	0.42
OR2020006	715.1	-16.3	0.94	0.65	442.1	0.41
OR2020007	701.1	-14.0	0.97	0.66	446.0	0.41
Mean	754.8	-21.7	0.95	0.64	456.7	0.38
Minimum	628.4	-34.5	0.93	0.58	392.3	0.32
Maximum	852.6	-13.0	0.97	0.67	511.9	0.42
Standard Deviation	56.0	6.2	0.01	0.02	27.7	0.03

Similar correlations between hardness and chewiness, and cohesiveness and resilience were also observed by Epstein et al. (2002) where they were observing changes in texture resulting from large differences in starch attributes. In this study we have been observing changes in noodle properties related to wide differences in protein content and composition. The similarity of the inter-relationships between TPA parameters in the 2 different studies suggests compellingly that these internal relationships between the TPA parameters may be an artifact of the TPA procedure.

Table 4.3

Correlation Coefficient Between Cooked Noodle Texture and Flour Protein Content[1]

Noodle Characteristics	Flour Protein (%, 14 %, mb)
Hardness (g)	0.337*
Adhesiveness (g*sec)	-0.256
Springiness	-0.172
Cohesiveness	-0.198
Chewiness	0.256
Resilience	-0.409*

[1]*: Correlation coefficient is significant at P<0.05.

Table 4.3 shows linear correlation coefficients between flour protein content and cooked noodle texture. Flour protein content was positively correlated with cooked noodle hardness. This correlation has also been shown in many other studies, for example Park et al. (2003), Oh et al. (1985) and Crosbie et al. (1999). Flour protein content negatively correlated to resilience, but was not significantly related to any of the other textural parameters.

Table 4.4

Correlation Coefficients between Payne Score and Flour Protein, Mixograph Characteristics, SEHPLC[1] and Cooked Noodle Texture[2]

	Payne Score
Flour Protein Content (%)	-0.249
Computer Analyzed Mixograph Characteristic	
MidlineTime 6 width (%)	0.731***
SEHPLC	
Percent Peak 1	0.486**
Cooked Noodle Texture	
Hardness (g)	-0.277
Adhesiveness (g*sec)	0.248
Springiness	0.068
Cohesiveness	0.205
Chewiness	-0.187
Resilience	0.262

[1] SEHPLC = Size exclusion HPLC

[2] ** and ***: Correlation coefficient is significant at P<, 0.01 and 0.001 respectively.

Table 4.4 shows the linear correlations between Payne score and flour protein content, mixograph characteristics, SEHPLC, and cooked noodle texture. There was no significant relationship between Payne score and flour protein content. However, there was a negative trend, and when *GluD1* subunit 5+10 lines were assessed alone, there was a significant negative relationship between Payne score and flour protein content. A strong correlation was seen between Payne score and mixograph bandwidth at 6 min. This indicated that Payne score was strongly and positively correlated with dough strength and mixing tolerance, and was therefore related to protein quality.

% peak 1 from the SEHPLC was also positively correlated with Payne score. This then indicated that % peak 1 was made up of HMW-GS as these were the only gluten components used in its calculation. This is in agreement with the literature regarding the identity of the proteins in peak 1 of similar SEHPLC methods (Larroque et al. 2000).

In contrast to the strong correlation between Payne score and mixing tolerance (Mixograph bandwidth at 6 min), Payne score was not significantly related to any of the cooked noodle

texture parameters. This suggested that there was no strong relationship between noodle texture and protein composition, and that flour protein content was the more influential factor in determining cooked noodle texture (Table 4.3) than dough strength characteristics.

Chapter 5 – Results and Discussion: Relationships between RVA Data and Noodle Texture

Table 5.1

Correlation Coefficent of Cooked Noodle Texture and Rapid Viscosity Analyzer (RVA) Parameters[1]

RVA Parameters	Hardness (g)	Adhesiveness (g*sec)	Cohesiveness	Chewiness	Resilience	Springiness
Peak Viscosity	-0.543***	0.479**	0.414*	-0.337*	0.413*	NS
Breakdown	-0.604***	0.489**	NS	-0.489**	NS	NS

[1]*, **, and ***: Correlation coefficient is significant at P<0.05, 0.01 and 0.001,respectively.
NS: Correlation coefficient is not significant at P<0.05.

Table 5.1 shows linear correlation coefficients between flour pasting parameters and cooked noodle texture. Both RVAPV and RVABD were strongly and negatively correlated with cooked noodle hardness. Adhesiveness was positively correlated with RVAPV and RVABD. Both cohesiveness and resilience were positively correlated with RVAPV. Chewiness was negatively correlated with RVAPV and RVABD.

Negative correlations between cooked noodle hardness and RVAPV were also observed by Crosbie et al. (1999), Ross (1997) and Yun et al. (1996). This is mainly due to the influence of higher swelling of the starch granules, which leads to softer noodle texture and is related to high pasting viscosity. The relationships seen in this study between adhesiveness, RVAPV, and RVABD, may be due to using constant cooking times, allowing the softer noodles to suffer more surface erosion and therefore become more adhesive or sticky.

Cohesiveness was negatively related to hardness, so harder noodles were less cohesive, and softer noodles were more cohesive. Hence the relationship with RVAPV should be opposite to that of hardness, as was observed (Table 5.1). Flour protein content was not significantly related to RVAPV or RVABD, indicating independent effects on noodle texture from the protein and starch components.

Overall the relationships between RVA parameters and noodle texture appear to be in alignment with other observations in the literature and indicate that the samples used were not anomalous with respect to starch attributes as measured using flour pasting properties.

Chapter 6 – Results and Discussion: Effect of HMW-GS on Noodle Texture

Table 6.1 shows comparisons of flour analytical, rheology, and end-use data between varieties grouped by their *Glu1* loci. Comparison of *GluA1* null lines with *GluA1* subunit 1 lines showed that the null lines had higher flour protein and lower % peak 1 from SEHPLC. Lower % peak 1 is expected, given that the *GluA1* null lines have only four HMW-GS compared to the five possessed by the subunit 1 lines. This is also highlighted by the higher MPT of the subunit 1 lines. There were no significant differences between *GluA1* null and subunit 1 lines in RVAPV or BD, nor for noodle hardness. In this case, starch attributes had no effects on the differences between the two populations, and the extra dough strength of the subunit 1 lines appeared to somehow able to compensate for their reduced flour protein content. The final outcome was equivalent noodle hardness to the higher protein content of *GluA1* null lines. It may have been anticipated that the subunit 1 lines would have been softer as a result of their lower protein content.

In the comparisons of the *GluA1* null lines with the *GluA1* subunit 2* lines, the picture is somewhat similar. The null lines again had higher flour protein and lower % peak 1 from SEHPLC. Where they differ is that the subunit 2* lines had lower RVABD, no significant difference in MPT, and higher noodle hardness. In this case it seems that extra polymeric protein (% peak 1) in concert with lower RVABD allowed the 2* lines to have harder noodle texture despite having lower flour protein content and equivalent MPT.

It is clearer for the *GluA1* subunit 1 lines that the additional HMW-GS subunit afforded by the presence of subunit 1 or 2* does appear to somewhat compensate for lower protein content and provide noodles of equivalent or harder texture. We could speculate that at equivalent flour

protein content, presence of *GluA1* subunit 1 or 2* would give harder noodles than *GluA1* null lines.

In comparing *GluA1* subunits 1 and 2*, it again seemed that starch characteristics and protein content were the primary determinants of noodle hardness and superceded the higher % peak 1 and dough strength of the subunit 1 line when compared with the subunit 2* lines.

GluB1 subunit 7 lines when compared with *GluB1* subunit 6+8 lines had higher protein content, noodle hardness, and chewiness. *GluB1* subunit 7 lines had lower RVAPV and RVABD than *GluB1* 6+8 lines. There were no significant differences between these groups for % peak 1 and MPT. In this case, where there were no differences in dough strength that could have played a role, the lines with higher protein, lower pasting viscosity gave harder noodles in line with their normally observed roles of flour protein content and starch attributes in noodle texture.

Comparison of *GluB1* subunit 7 lines with *GluB1* subunit 7+9 lines showed that subunit 7 lines had higher flour protein content, higher % peak 1, lower RVAPV and RVABD, and lower MPT. *GluB1* subunit 7 lines, although they lacked a subunit compared to the 7+9 lines, had a higher number of polymeric proteins (% peak 1). However, this additional polymeric protein was not reflected in increased dough strength in the *GluB1* subunit 7 lines. Subunit 7 lines had harder noodle texture, primarily as a result of higher protein content and lower RVAPV and RVABD. The higher strength of the *GluB1* 7+9 lines was not effective in overcoming the primary effects.

In comparing *GluB1* subunit 7 lines and subunit 17+18 lines we saw again that the higher flour protein content and lower starch pasting properties were more important in determining noodle hardness. In this case, although subunit 7 had a lower % peak 1, mixograph water absorption and MPT was still able to product harder noodles.

Compared with subunit 6+8 lines, *GluB1* subunit 17+18 lines had higher % peak 1 and MPT. This is in agreement with Gianibelli et al. (2001), indicating that subunit 17+18 lines had higher dough mixing quality than 6+8 lines but, no significant difference was observed in noodle texture. This then indicated that at equivalent protein content, stronger dough was not effective in increasing noodle

Table 6.1

Mean Values and f-scores from ANOVA of Comparisons of Varietes Grouped by HMW-GS Composition[1]

			Flour	% Peak	RVA		Noodle Texture						Mixograph	
		n	Protein	Area 1	Peak Viscosity	Breakdown	Hardness	Adhesiveness	Springiness	Cohesiveness	Chewiness	Resilence	WaterAbs	PeakTime
GluA														
Mean values	**1**	**3**	10.6	9.5	279.7	143.4	708.7	-22.0	0.94	0.648	430.0	0.393	63.2	3.9
Mean values	**2***	**13**	11.2	9.1	262.6	126.3	775.6	-23.1	0.94	0.639	466.5	0.378	62.5	2.4
ANOVA f-scores	**1 VS 2***		6.9*	8.1*	11.6**	27.9***	17.9***	NS	NS	NS	15.2**	NS	NS	200.5***
Mean values	**1**	**3**	10.6	9.5	279.7	143.4	708.7	-22.0	0.94	0.648	430.0	0.393	63.2	3.9
Mean values	**null**	**2**	12.0	8.6	267.6	147.5	725.5	-17.3	0.95	0.645	443.4	0.382	63.2	2.5
ANOVA f-scores	**1 VS NULL**		16.3**	17.6***	NS	NS	NS	NS	NS	NS	NS	NS	NS	100.1***
Mean values	**2***	**13**	11.2	9.1	262.6	126.3	775.6	-23.1	0.94	0.639	466.5	0.378	62.5	2.4
Mean values	**null**	**2**	12.0	8.6	267.6	147.5	725.5	-17.3	0.95	0.645	443.4	0.382	63.2	2.5
ANOVA f-scores	**NULL VS 2***		6.9*	6.5*	NS	32.7***	6.7*	NS	NS	NS	NS	NS	NS	NS
***GluB*[2]**														
Mean values	**7**	**4**	12.0	9.2	253.0	118.3	790.4	-25.0	0.94	0.640	476.2	0.376	61.9	2.2
Mean values	**6+8**	**2**	10.9	8.9	271.0	132.6	720.1	-22.8	0.94	0.645	437.4	0.375	62.9	2.3
ANOVA f-scores	**7 VS 6+8**		10.6**	NS	8.7**	14.7**	13.0**	NS	NS	NS	10.2**	NS	NS	NS
Mean values	**7**	**4**	12.0	9.2	253.0	118.3	790.4	-25.0	0.94	0.640	476.2	0.376	61.9	2.2
Mean values	**7+9**	**8**	10.9	8.8	268.8	136.0	754.3	-21.3	0.95	0.639	455.3	0.386	62.3	2.9
ANOVA f-scores	**7 VS 7+9**		22.8***	8.0*	13.5**	44.7***	6.9*	NS	NS	NS	5.9*	NS	NS	43.4***
Mean values	**7**	**4**	12.0	9.2	253.0	118.3	790.4	-25.0	0.94	0.640	476.2	0.376	61.9	2.2
Mean values	**17+18**	**2**	10.8	9.6	272.9	150.9	674.3	-14.8	0.95	0.656	418.0	0.403	64.0	3.6
ANOVA f-scores	**7 VS 17+18**		13.4**	4.8*	10.7**	75.4***	35.4***	8.7**	NS	NS	23.0***	5.5*	11.3**	113.6***
Mean values	**6+8**	**2**	10.9	8.9	271.0	132.6	720.1	-22.8	0.94	0.645	437.4	0.375	62.9	2.3
Mean values	**17+18**	**2**	10.8	9.6	272.9	150.9	674.3	-14.8	0.95	0.656	418.0	0.403	64.0	3.6
ANOVA f-scores	**6+8 VS 17+18**		NS	10.5**	NS	17.8***	NS	NS	NS	NS	NS	NS	NS	76.0***
Mean values	**7+9**	**8**	10.9	8.8	268.8	136.0	754.3	-21.3	0.95	0.639	455.3	0.386	62.3	2.9
Mean values	**6+8**	**2**	10.9	8.9	271.0	132.6	720.1	-22.8	0.94	0.645	437.4	0.375	62.9	2.3
ANOVA f-scores	**7+9 VS 6+8**		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	19.9***
Mean values	**7+9**	**8**	10.9	8.8	268.8	136.0	754.3	-21.3	0.95	0.639	455.3	0.386	62.3	2.9
Mean values	**17+18**	**2**	10.8	9.6	272.9	150.9	674.3	-14.8	0.95	0.656	418.0	0.403	64.0	3.6
ANOVA f-scores	**7+9 VS 17+18**		NS	21.1***	NS	18.8***	20.1***	NS	NS	NS	11.4**	NS	8.4*	41.4***
GluD														
Mean values	**5+10**	**12**	11.3	9.3	273.6	139.9	746.6	-19.9	0.95	0.647	456.2	0.388	63.3	3.1
Mean values	**2+12**	**5**	11.1	8.7	252.1	117.3	778.5	-26.1	0.94	0.629	460.3	0.368	61.5	2.0
ANOVA f-scores	**5+10 VS 2+12**		NS	17.3***	33.1***	95.5***	7.1*	8.4**	NS	10.6**	NS	8.3*	21.6***	144.2***

[1]*, **, and ***: f score is significant at P<0.05, 0.01 and 0.001,respectively. NS: f score not significant at P<0.05

[2]Glu comparisons where one of the compared glutenin groups only has one entrant. 13+19, 3+12 ommited. GluB mixed variety omitted

hardness. However, higher RVABD in the 17+18 lines may have masked an increase in noodle hardness that might have been observed had the pasting parameters been the same.

No significant differences were seen between *GluB1* subunit 6+8 lines and subunit 7+9 lines other than MPT. Subunit 7+9 had a higher MPT. This again is in agreement with Gianibelli et al. (2001), indicating that subunit 7+9 has stronger mixing properties than subunit 6+8, but again was not translated into higher noodle hardness. In this instance, in contrast to the comparison of the 6+8 and 17+18 lines, there were no differences in starch characteristics to interfere with the results.

GluB1 subunits 17+18 had higher % peak 1, mixograph water absorption and MPT compared with subunit 7+9. The increased amount of polymeric protein in % peak 1 of subunit 17+18 appeared to be the reason for the higher mixograph water absorption and MPT. Subunit 7+9 had lower RVABD and higher noodle hardness and chewiness, suggesting that lower RVABD had more influence on noodle texture than the amount of protein in peak 1 and dough strength.

When comparing *GluD1* 2+12 and GluD1 5+10 lines we saw again the relationship between lower RVAPV and RVABD and increased noodle hardness in subunit 2+12 lines, despite the fact that subunit 5+10 had higher dough strength and higher % peak 1.

The general trend was that dough strength had either no or very little effect on noodle hardness. The exception was the case of replacing the *GluA1* null allele with the alleles coding for subunits 1 or 2*. All other noodle parameters except the related parameter chewiness were unaffected by protein effects. Where there were significant differences in cohesiveness or resilience, these could be attributed to starch characteristics.

Chapter 7 – Results and Discussion: Relationships between SEHPLC Data, Dough Rheology and Noodle Texture.

Table 7.1 shows the means, minima, maxima range and the standard deviation of the SEHPLC data. Peak area 1 has a mean value of 1703041 and standard deviation of 206611. There were

substantial differences between the maximum and minimum peak areas. There was a considerable amount of variation in the composition within the flour sample. However, flour protein was not corrected for the different varieties: therefore, means could also reflect a difference in flour protein

Table 7.1

Maximum, Mininum, Mean and Standard Deviation of SEHPLC[1] Data

SEHPLC[1] Peaks	Maximum	Minimum	Mean	Standard Deviation
Peak Area (μv*sec)				
1	1703041	1301532	2175087	206611
2	1888634	1622239	2230442	166061
3	1651733	1377709	1984011	151736
4	1572940	1332116	1892801	147637
5	1076356	914111	1307225	105600
6	942105	607179	1232374	167161
7	7160943	5462448	10057493	913477
8	2768599	2261604	3467112	316908
Total	19266487	15618433	23458050	1852122
Percent Peak Area				
1	8.8	7.7	10.2	0.62
2	9.8	7.5	10.8	0.73
3	8.6	6.6	9.6	0.61
4	8.2	6.8	9.0	0.46
5	5.6	5.1	6.1	0.22
6	4.9	3.7	6.0	0.64
7	37.1	34.5	45.3	2.18
8	14.4	13.2	16.4	0.70

[1]SEHPLC= Size exclusion high performance chromatography.

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According to Larroque et al. (2000) peak area 1 "is made up of mainly glutenins, which are the most important endosperm proteins related to quality properties of flour." Therefore, peak area 1 and % peak area 1 will be the main focus of the Table 7.2a and 7.2b when results from SEHPLC are reported.

All SEHPLC peak areas show significant positive correlation with flour protein content, which was also reported by Morel et al. (2000). However, flour protein content was not corrected when

Table 7.2a

Correlation Coefficient Between Size Exclusive HPLC (SEHPLC) Data, Flour Protein, Cooked Noodle Texture and Optimum Absorption Mixograph Data[1]

	Flour Protein	SEHPLC Peak Areas (μv*sec)			
Quality Characteristics	**(14 % mb)**	**Peak 1**	**Peak 2**	**Peak 3**	**Peak 4**
Flour Protein (%, 14 % mb)	-	0.790***	0.653***	0.592***	0.668***
Cooked Noodle Texture					
Hardness (g)	0.337*	0.511**	NS	0.619***	0.617***
Adhesiveness (g*sec)	NS	NS	NS	-0.467**	-0.522**
Springiness	NS	NS	NS	NS	NS
Cohesiveness	NS	NS	NS	NS	-0.435**
Chewiness	NS	0.495**	NS	0.504**	0.402*
Resilience	-0.409*	NS	NS	-0.478**	-0.609***
Mixograph Characteristics					
Water absorption (%, 14 % mb)	NS	0.336*	NS	NS	NS
Peak time (min)	-0.353*	NS	-0.405*	-0.432*	-0.441**
Computer analyzed mixograph characteristics					
Midline Time 6 Width (%)	NS	NS	NS	NS	NS

[1]*, **, and ***: Correlation coefficient is significant at P<0.05, 0.01 and 0.001,respectively

NS: Correlation coefficient is not significant at P<0.05.

Table 7.2b

Correlation Coefficient Between Size Exclusive HPLC (SEHPLC) Data, Flour Protein, Cooked Noodle Texture and Optimum Absorption Mixograph Data[1]

	SEHPLC Peak Areas (μv*sec)					Percent
Quality Characteristics	**Peak 5**	**Peak 6**	**Peak 7**	**Peak 8**	**Total**	**Peak Area 1**
Flour Protein (%, 14 % mb)	0.732***	0.579***	0.867***	0.716***	0.920***	NS
Cooked Noodle Texture						
Hardness (g)	0.569***	0.334*	NS	0.557***	0.451**	NS
Adhesiveness (g*sec)	-0.540***	NS	NS	-0.477**	-0.360*	NS
Springiness	-0.355*	NS	NS	NS	NS	NS
Cohesiveness	-0.496**	NS	NS	-0.478**	NS	NS
Chewiness	NS	NS	NS	NS	NS	NS
Resilience	-0.668***	-0.385*	-0.356*	-0.597***	-0.512**	NS
Mixograph Characteristics						
Water absorption (%, 14 % mb)	NS	0.352*	NS	NS	NS	0.395*
Peak time (min)	NS	NS	NS	-0.380*	-0.349*	0.454**
Computer analyzed mixograph characteristics						
Midline Time 6 Width (%)	NS	NS	NS	NS	NS	0.431**

[1]*, **, and ***: Correlation coefficient is significant at P<0.05, 0.01 and 0.001,respectively

NS: Correlation coefficient is not significant at P<0.05.

injecting samples onto the HPLC. Therefore the significance of the absolute peak areas may largely reflect flour protein content and not the relative proportions of the protein fractions.

Flour protein was significantly correlated with cooked noodle hardness as expected, and was consistent with previous studies as mentioned in Chapter 4. Flour protein was negatively correlated with MPT, but not significantly with mixograph water absorption. The negative correlation indicates that flour protein composition may be responsible for mixograph functionalities (Borneo and Khan 1999). No significant correlation was seen between flour protein content and bandwidth at 6 min. In addition, it is of interest that the strongest doughs came from, in general, flours with the lowest flour protein contents. This is an artifact of the sample set. However, it gives us an opportunity to observe that even with weak dough properties, the hardest noodles were associated with the weaker, high protein samples (Table 4.3). The low predictive capacity of flour protein content for noodle hardness is likely to be related to the superimposing of the starch properties, and the apparent relationship between HMW-GS dose and noodle hardness for the *GluA1* coded subunits (Table 6.1) over the primary effect of flour protein content.

Peak 1 is positively correlated to cooked noodle hardness, chewiness, and mixograph water absorption. The correlation with cooked noodle hardness is most likely due to cross correlation with flour protein content. The correlation seen between peak 1 and mixograph water absorption may be mainly due to the presence of a high level of large aggregates with increased ability to bind water. The correlation of peak area 1 with noodle hardness is numerically higher than that of flour protein content, hinting that there may be some underlying effects of glutenin composition on noodle texture in this sample set, as suggested by the *GluA1* results reported in Table 6.1

The SEHPLC % peak area 1, which effectively normalizes the protein content injected onto the column, showed significant correlations with mixograph water absorption and MPT, and mixograph bandwidth at 6 min. This is consistent with the literature, indicating that higher water absorption reflects higher quality of wheat protein and that a longer peak time reflects gluten strength. This is also supported by MacRitchie (1985) who showed that the higher glutenin to gliadin ratio, the stronger the dough. The correlation of percent peak area 1 with mixograph bandwidth at 6 min was another illustration of how high amounts of polymeric protein are related to different facets of dough quality – in this case, with increased tolerance to overmixing. None of the other percent peak areas (not shown) were significantly correlated with the computer analyzed

mixograph characteristics. This further indicated that glutenins present in peak 1 are the major determinant of dough strength. % peak area 1 showed no significant correlation with all the cooked noodle textures. This gives no hint, similar to that gained from the ANOVA analyses, that there were any subunit or polymer size effects on noodle texture.

Chapter 8 – Results and Discussion: Relationships between Dough Rheology and Noodle Texture.

Table 8.1 shows the linear correlations between mixograph characteristics and cooked noodle texture. Hardness was negatively correlated with mixograph water absorption and MPT. The negative relationship between MPT and noodle hardness is likely to be a reflection of the negative relationship observed between MPT and flour protein content, and the positive relationship between protein content and noodle hardness. Hardness however, was not significantly related to mixograph bandwidth at 6 min. This further reinforces the dominance of flour protein content over dough strength as a determinant of noodle texture in the set of samples.

Table 8.1

Correlation Coefficient Between Mixograph Data and Cooked Noodle Texture[1]

Mixograph characteristics	Hardness (g)	Adhesiveness (g*sec)	Springiness	Cohesiveness	Chewiness	Resilience
Manually determined mixograph characteristics						
Water absorption (%, 14 % mb)	-0.331*	0.389*	NS	NS	NS	NS
Peak time (min)	-0.505**	NS	NS	NS	-0.412*	0.339*
Computer analyzed mixograph characteristics						
MidlineTime 6 width (%)	NS	NS	NS	NS	NS	NS

[1]*, **, and ***: Correlation coefficient is significant at P<0.05, 0.01 and 0.001,respectively.

NS=correlation coefficient is not significant at P<0.05.

Adhesiveness was positively correlated with mixograph water absorption. Chewiness and resilience were both correlated with MPT, but not with mixograph water absorption. All the noodle parameters had no correlation with mixograph bandwidth at 6 min.

Chapter 9 – General Discussion and Conclusions

A diverse group of hard and soft grained wheat was available for this study. In addition, this group also had a large variation in HMW-GS composition (Table 4.1a). There were considerable interrelationships between TPA parameters measured on noodle texture (Table 4.2). This suggested that there was an element of redundancy between certain TPA parameters, especially in hardness and chewiness. Accordingly it may only be necessary to report one of these parameters in a study to adequately describe the hardness aspect of noodle texture.

Flour protein content had a positive correlation with cooked noodle hardness (Table 4.3). The same result was also seen in many other studies, for example, Park et al. (2003), Huang and Morrison (1988). In this sample set, Payne score, which is a way of combining the effects of the three HMW-GS loci, showed no significant relationship with cooked noodle hardness (Table 4.3) despite the fact that at least within the *GluD1* 5+10 population, Payne score was negatively correlated with flour protein content. However, Payne score was strongly related to increased dough strength and mixing tolerance (Table 4.3). Lower RVAPV and RVABD showed strong relationships with cooked noodle hardness (Table 5.1 and Table 6.1). This conclusion is in alignment with current knowledge (Crosbie et al. 1999, Ross 1997 and Yun et al. 1996).

The *GluA1* null lines with the higher flour protein content produced noodles in equal hardness, therefore, softer than expected, when compared to lower protein lines containing *GluA1* subunit 1. The picture was somewhat similar in comparing *GluA1*null lines with *GluA1* subunit 2* lines. However, in this care, lower RVABD, in concert with the presence of subunit 2*, led to harder noodles from the lower protein 2* lines. Presence if *GluA1* subunit 1 or 2*, rather than the null allele appeared to be able to compensate for lower flour protein content, by giving noodles of similar or higher hardness, despite significantly lower flour protein content than the *GluA1* null lines (Table 6.1). Subunit 1 lines had stronger dough characteristics than subunit 2* lines, but the differences in noodle hardness were likely to have resulted from differences in starch properties, as the stronger subunit 1 lines had higher RVAPV and RVABD. At the *GluB1* and *GluD1* loci, higher protein content had more influence on noodle hardness than did HMW-GS composition (Table 6.1).

SEHPLC absolute peak area data suggested that there was some relationship between glutenin MWD and noodle hardness (Table 7.1a). However, this was contradicted by the results from the

% peak 1 data. There was no significant relationship between % peak 1 and noodle hardness (Table 7.1b). Similarly mixograph characteristics had no relationship with noodle hardness (Table 8.1), suggesting that HMW-GS was not an effective way of predicting noodle hardness compared to protein content except in the case of *GluA1* (Table 6.1).

The results presented here are in contradiction to those of Park et al. (2003). They reported that higher Payne score was associated with harder cooked noodle texture. However, in that study Payne score and flour protein content were positively correlated, that is samples with the highest flour protein content had the highest Payne score. In this study, there was no significant correlation between Payne score, flour protein content or cooked noodle hardness. The results of this study indicated that flour protein content and starch characteristics were the primary determinants of cooked noodle texture, and that apart from a positive relationship between the presence of *GluA1* subunits 1 or 2* rather than *GluA1* null allele, HMW-GS composition had little if any effect on cooked noodle texture.

Our data from this sample set indicated that HMW-GS, SEHPLC and mixograph characteristics were not effective for screening for noodle hardness (or texture). Flour protein and starch pasting properties were the dominant factor in determining noodle hardness.

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