



QUALITY POTENTIAL OF GLUTEN PROTEINS IN HEXAPLOID WHEAT AND RELATED SPECIES

A Thesis submitted for the degree of Doctor of Philosophy

School of Agriculture, Food and Wine

The University of Adelaide

Maria-Jane Appelbee, B.Biotech (Hons.)

July 2006

TABLE OF CONTENTS

| | |
|--|-------------|
| DECLARATION..... | v |
| ACKNOWLEDGEMENTS..... | vi |
| ABSTRACT..... | vii |
| LIST OF FIGURES..... | ix |
| LIST OF TABLES..... | xiii |
| LIST OF ABBREVIATIONS..... | xiv |
| CHAPTER 1 GENERAL INTRODUCTION..... | 1-1 |
| CHAPTER 2 LITERATURE REVIEW..... | 2-4 |
| 2.1 Introduction..... | 2-4 |
| 2.2 Endosperm Storage Proteins..... | 2-5 |
| 2.2.1 Gliadins..... | 2-5 |
| 2.2.2 Glutenins..... | 2-6 |
| 2.2.3 Chromosomal location of genes encoding prolamins..... | 2-7 |
| 2.2.3.1 High molecular weight glutenin subunits..... | 2-7 |
| 2.2.3.2 Low molecular weight glutenin subunits..... | 2-8 |
| 2.2.3.3 Gliadins..... | 2-8 |
| 2.2.3.4 Additional minor loci..... | 2-9 |
| 2.2.4 Nomenclature and identification..... | 2-10 |
| 2.2.4.1 HMW-GSs of cultivated polyploid wheat..... | 2-10 |
| 2.2.4.2 HMW-GSs alleles in wild wheat progenitors..... | 2-13 |
| 2.2.4.2.1 D-genome..... | 2-15 |
| 2.2.4.2.2 A-genome..... | 2-18 |
| 2.2.4.2.3 A- and B-genomes..... | 2-20 |
| 2.2.4.3 LMW-GSs of cultivated polyploid wheat..... | 2-22 |
| 2.3 Determination of Protein Quality Characteristics..... | 2-25 |
| 2.3.1 Influence of dough rheology on bread making quality..... | 2-28 |
| 2.3.2 Physical dough testing..... | 2-29 |
| 2.3.3 Determination of polymeric protein..... | 2-32 |
| 2.4 Growth Environment and Protein Composition..... | 2-34 |
| 2.4.1 Water and nutrient availability..... | 2-34 |
| 2.4.2 Temperature stress..... | 2-36 |
| 2.5 Glutenin Genotype and Bread making Quality..... | 2-39 |
| 2.5.1 HMW-GS composition..... | 2-39 |
| 2.5.2 LMW-GS composition..... | 2-42 |
| 2.6 Strategies to Improve Bread making Potential..... | 2-47 |
| 2.6.1 Modification of molecular weight distribution..... | 2-48 |
| 2.6.1.1 Cysteine residues..... | 2-48 |
| 2.6.1.2 HMW-GS to LMW-GS ratio..... | 2-50 |
| 2.6.2 Implications of improving wheat quality..... | 2-52 |






TABLE OF CONTENTS (cont.)

| | | |
|------------------|--|-------------|
| CHAPTER 3 | GENERAL MATERIALS AND METHODS | 3-55 |
| 3.1 | <i>Grain Samples</i> | 3-55 |
| 3.1.1 | Aroona isolines | 3-55 |
| 3.1.2 | Samples used to investigate HMW-GS expression levels | 3-57 |
| 3.1.3 | Samples used to develop and validate the Turbidity assay | 3-57 |
| 3.1.4 | Novel glutenin alleles in wild wheat progenitors..... | 3-59 |
| 3.2 | <i>Statistical Analysis of Aroona Isoline Data</i> | 3-60 |
| 3.2.1 | ANOVA of isoline quality data | 3-60 |
| 3.2.2 | Estimates of variance components on quality parameters | 3-60 |
| 3.2.3 | Glutenin allele main effects and additive interactions | 3-61 |
| 3.2.3.1 | Allele main effects | 3-61 |
| 3.2.3.2 | Allele additive interactions | 3-61 |
| 3.2.4 | Effect of glutenin alleles at different protein levels | 3-63 |
| 3.3 | <i>SDS-PAGE</i> | 3-64 |
| 3.3.1 | Protein extraction..... | 3-64 |
| 3.3.1.1 | Gliadins | 3-64 |
| 3.3.1.2 | Glutenins | 3-64 |
| 3.3.2 | Electrophoresis..... | 3-65 |
| 3.3.2.1 | SDS Gradient PAGE..... | 3-65 |
| 3.3.2.2 | Uniform SDS-PAGE..... | 3-65 |
| 3.3.3 | Visualisation..... | 3-66 |
| 3.4 | <i>Quality testing</i> | 3-66 |
| 3.4.1 | Grain hardness, protein content and moisture content | 3-66 |
| 3.4.2 | Flour milling | 3-66 |
| 3.4.3 | Farinograph | 3-67 |
| 3.4.4 | Salt dough farinograph/extenograph | 3-67 |
| 3.4.4.1 | Buhler milled flour | 3-67 |
| 3.4.4.2 | Quadrumat Junior Experimental milled flour | 3-68 |
| 3.4.4.3 | Extensograph rheological data..... | 3-68 |
| 3.5 | <i>High-performance liquid chromatography</i> | 3-68 |
| 3.5.1 | Reversed-phase high-performance liquid chromatography (RP-HPLC) | 3-68 |
| 3.5.1.1 | Protein extraction | 3-68 |
| 3.5.1.2 | RP-HPLC analysis | 3-69 |
| 3.5.2 | Size-exclusion high-performance liquid chromatography (SE-HPLC) | 3-70 |
| 3.5.2.1 | Protein extraction | 3-70 |
| 3.5.2.2 | SE-HPLC analysis | 3-71 |
| CHAPTER 4 | RHEOLOGICAL DOUGH PROPERTIES OF AROONA ISOLINES DIFFERING IN GLUTENIN SUBUNIT COMPOSITION | 4-73 |
| 4.1 | <i>Introduction</i> | 4-73 |
| 4.2 | <i>Materials and Methods</i> | 4-74 |
| 4.3 | <i>Results and Discussion</i> | 4-74 |

TABLE OF CONTENTS (cont.)

| | | |
|------------------|---|--------------|
| 4.3.1 | SDS Gradient PAGE..... | 4-74 |
| 4.3.2 | Glutenin composition..... | 4-77 |
| 4.3.2.1 | Estimates of variance components for quality parameters | 4-77 |
| 4.3.2.2 | Effect of glutenin alleles on quality parameters | 4-79 |
| 4.3.2.3 | Additive effects of glutenin alleles on quality parameters..... | 4-85 |
| 4.3.3 | Flour protein content and glutenin composition | 4-90 |
| 4.3.3.1 | Response of isolines to nitrogen application | 4-90 |
| 4.3.3.2 | Effect of N treatment and glutenin alleles on dough rheology . | 4-93 |
| 4.4 | Conclusion..... | 4-96 |
| CHAPTER 5 | OVER-EXPRESSION OF HMW GLUTENIN SUBUNIT GLU-B1 7X IN HEXAPLOID WHEAT VARIETIES | 5-100 |
| 5.1 | Introduction..... | 5-100 |
| 5.2 | Materials and Methods..... | 5-101 |
| 5.3 | Results and Discussion..... | 5-101 |
| 5.3.1 | Uniform SDS-PAGE..... | 5-101 |
| 5.3.2 | RP-HPLC | 5-102 |
| 5.3.3 | Origin of the <i>Glu-B1a1</i> allele..... | 5-111 |
| 5.4 | Conclusion..... | 5-115 |
| CHAPTER 6 | DEVELOPMENT AND VALIDATION OF A SIMPLE TURBIDITY ASSAY FOR PREDICTING DOUGH STRENGTH | 6-117 |
| 6.1 | Introduction..... | 6-117 |
| 6.2 | Materials and Methods..... | 6-117 |
| 6.3 | Results and Discussion..... | 6-118 |
| 6.3.1 | Development of a Turbidity assay for percent SDS-insoluble protein . | 6-118 |
| 6.3.2 | Validation of the Turbidity assay..... | 6-120 |
| 6.4 | Conclusion..... | 6-126 |
| CHAPTER 7 | PREDICTING PROTEIN QUALITY OF WILD WHEAT RELATIVES USING SE-HPLC AND THE TURBIDITY ASSAY | 7-127 |
| 7.1 | Introduction..... | 7-127 |
| 7.2 | Materials and Methods | 7-129 |
| 7.3 | Results and Discussion | 7-129 |
| 7.3.1 | Genetic variability and protein quality screening of the A- and B-genomes | 7-129 |
| 7.3.1.1 | <i>Triticum dicoccoides</i> | 7-129 |
| 7.3.2 | Protein quality screening of the D-genome progenitor | 7-136 |
| 7.3.2.1 | <i>Triticum tauschii</i> | 7-136 |
| 7.3.2.2 | Synthetic Hexaploids | 7-141 |
| 7.4 | Conclusion..... | 7-145 |

TABLE OF CONTENTS (cont.)

| | | |
|--|---------------------------------|---|
| CHAPTER 8 | GENERAL DISCUSSION | 8-147 |
| CHAPTER 9 | REFERENCES | 9-161 |
| CHAPTER 10 | APPENDICES | 10-176 |
| Appendix I | | |
| SDS-PAGE of Aroona isolines | |  |
| Appendix II | | |
| Isoline | |  |
| Appendix III | | |
| Glutenin allele interaction tables. | |  |
| Appendix IV | | |
| Vawser M-J and Cornish G B (2004) AJAR 55, 577-588 | |  |
| Appendix V | | |
| SDS-PAGE of <i>T. dicoccoides</i> | |  |

ABSTRACT

Variation in quantity and quality of gluten proteins is largely responsible for the genotypic differences associated with the dough rheological parameters, maximum resistance (R_{\max}) and extensibility (Ext.). In the context of bread making, doughs characteristic of good quality have moderate to high extensograph maximum resistance (R_{\max}) and high extensibility (Ext.). The term usually applied to describe the balance between these two parameters is dough strength. Generally, weak doughs perform poorly in baking tests and as dough strength increases, bread making quality also increases. Important proteins that constitute the 'gluten complex' include high molecular weight glutenin subunits (HMW-GSs) and low molecular weight glutenin subunits (LMW-GSs). These proteins, which interact to produce large polymeric proteins, are coded at the *Glu-1* and *Glu-3* loci on group 1 chromosomes, respectively. Extensive allelic variation exists at each of the *Glu-1* and *Glu-3* loci. Field trials (4 years) and physical dough quality tests on harvested grain from a set of near-isogenic lines, differing in glutenin composition, were used to investigate the effect of numerous glutenin alleles on dough rheological parameters. Glutenin allele main effects were ranked as follows: *Glu-A1* $a = p = b > c$ for R_{\max} and *Glu-A1* $a = b = p > c$ for Ext.; *Glu-B1* $i \geq b = c > d = a$ for R_{\max} and *Glu-B1* $a = i = c \geq b \geq d$ for Ext.; *Glu-D1d* $> Glu-D1a = Glu-D1b \geq Glu-D1f$ for R_{\max} and *Glu-D1* $a = b = f \geq d$ for Ext.; *Glu-A3* $d = b \geq c = f \geq a > e$ for R_{\max} and *Glu-A3* $b = a = d = c = f \geq e$ for Ext.; *Glu-B3* $g \geq b = m \geq d = i = h = f \geq a \geq c$ for R_{\max} and *Glu-B3* $i = d \geq g = f = m \geq b = c = h = a$ for Ext.; *Glu-D3* $a-Gli-D1' = f \geq c = d = a \geq b$ for R_{\max} and *Glu-D3* $d \geq a-Gli-D1' \geq a \geq b = c = f$ for Ext. The influence of protein content and two-way glutenin allele interactions are also discussed.

Another aspect of this work investigated the relationship between HMW-GS expression

levels and quality. RP-HPLC was used to quantify the proportion (% area) of individual HMW-GSs relative to total HMW-GSs. Except for *Glu-B1d* (6+8*), the B-genome contributed the highest percentage of HMW-GSs and was significantly higher ($P < 0.001$) in cultivars that contained the *Glu-B1a1* allele. A high proportion of 1Bx subunits compared to 1Dx subunits (≈ 2.3 , *Glu-B1a1*) correlated with varieties reported to have extra strong dough properties, while a 1Bx:1Dx ratio of ≈ 1.3 (*Glu-B1 i, f, c, u* and *ak*) was typical of varieties with moderate to high dough strength characteristics. In varieties which contain *Glu-B1* alleles reported to produce weak doughs the 1Bx:1Dx value was ≈ 1.0 (*Glu-B1e*) and ≈ 0.6 (*Glu-B1d*). This suggests that the overall proportion of *Glu-B1* subunits has a major influence on dough strength and that the proportion of 1Bx relative to 1Dx subunits, as determined by RP-HPLC, could be used to predict dough quality. RP-HPLC analysis also enabled the identification of varieties that contained the *Glu-B1a1* allele and over-expressed subunit *Glu-B1 7x*, including the most likely source of this allele in bread wheat cultivars.

Novel HMW-GS alleles in related wheat species with good quality potential were also identified. A simple small-scale screening assay was developed to efficiently assess the protein quality attributes associated with accessions of synthetic hexaploids, *T. tauschii* and *T. dicoccoides*. Development of the Turbidity assay is described and was used in conjunction with SE-HPLC and SDS-PAGE to confirm and characterise previously undescribed HMW-GSs. The HMW-GS composition of *T. dicoccoides* is discussed in detail where there were 49 HMW-GSs which combined to produce 54 different HMW-GS banding patterns. Accordingly, allelic designations were tentatively assigned to either individual or subunit pairs and these are also reported in this manuscript.