PROTEIN COMPOSITION-FUNCTIONALITY RELATIONSHIPS USING NOVEL GENETIC LINES

by

RAMAKANTH S. JONNALA

M.S., A.N.G. R. Agricultural University, India, 2000
M.S., Oklahoma State University, USA, 2004

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2008

Abstract

Novel genetic materials were used to deduce gluten protein composition-functionality relationships. The Pegaso bread wheat near-isogenic lines (NILs) included addition, variation and/or deletion of major loci coding for HMW-GS, LMW-GS and gliadins. The waxy wheat lines (Svevo and N11 set) included wild, partial and complete waxy lines. Triticale translocations include 1R.1D and 1A.1D lines (GDS7, Trim, Rhino and Rigel sets) with HMW-GS 5+10 and 2+12. The main goal of the study was to establish the usefulness of NILs as appropriate materials to investigate the structure-function relationships of wheat proteins and to evaluate the performance of unique triticale translocations and waxy wheat lines. Effect of genetic variation on phytochemical (phenolic acid and policosanol) contents was also studied. Innovative methods like MALLS, Lab-on-a-chip and micro (10 g) baking were utilized along with traditional analytical methods.

Results confirmed the potential of using NILs in understanding the effects of certain proteins coded at specific loci that might often be targeted in breeding programs. Removal of expected chain terminators at *Gli-1*/*Gli-2* loci causes a shift in MWD to higher values, reflected in higher UPP and dough strength. Lines with HMW-GS 5+10 were clearly separated from 2+12 lines in terms of dough strength and UPP. The present study obtained evidence that modified ω-gliadins acts as chain terminators and cause reduction of protein polymer size and thus shifts in MWD. Marked differences in terms of milling characteristics, protein composition and ultimately in end-use functionality were observed with various waxy wheat null lines. Loaf volumes with waxy wheat flour alone were higher than a 50% blend with commercial wheat; however, breads were unacceptable to consumers in all aspects. Poor milling quality, very low mixing times with low bread loaf volumes were typical of all the triticales studied. However, translocation of the HMW-GS from wheat chromosome 1D increased dough strength, particularly the HMW-GS 5+10. Among the phytochemicals studied, double nulls at *Gli*-1 loci of Pegaso NILs had the highest total policosanols and total phenolic acid contents.Slight variation to wheat phenolic acid composition and contents were observed with waxy wheat and triticale lines.

PROTEIN COMPOSITION-FUNCTIONALITY RELATIONSHIPS USING NOVEL GENETIC LINES

by

RAMAKANTH S. JONNALA

M.S., A.N.G. R. Agricultural University, India, 2000
M.S., Oklahoma State University, USA, 2004

A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas
2008

Approved by:
Major Professor
Dr. Finlay MacRitchie

Copyright

Abstract

Novel genetic materials were used to deduce gluten protein composition-functionality relationships. Pegaso bread wheat near-isogenic lines (NILs) included addition, variation and/or deletion of major loci coding for HMW-GS, LMW-GS and gliadins. The waxy wheat lines (Svevo and N11 set) included wild, partial and complete waxy lines. Triticale translocations include 1R.1D and 1A.1D lines (GDS7, Trim, Rhino and Rigel sets) with HMW-GS 5+10 and 2+12. The main goal of the study was to establish the usefulness of NILs as appropriate materials to investigate the structure-function relationships of wheat proteins and to evaluate the performance of unique triticale translocations and waxy wheat lines. Effect of genetic variation on phytochemical (phenolic acid and policosanol) contents was also studied. Innovative methods like MALLS, Lab-on-a-chip and micro (10 g) baking were utilized along with traditional analytical methods.

Results confirmed the potential of using NILs in understanding the effects of certain proteins coded at specific loci that might often be targeted in breeding programs. Removal of expected chain terminators at *Gli-1*/*Gli-2* loci causes a shift in MWD to higher values, reflected in higher UPP and dough strength. Lines with HMW-GS 5+10 were clearly separated from 2+12 lines in terms of dough strength and UPP. The present study obtained evidence that modified ω-gliadins acts as chain terminators and cause reduction of protein polymer size and thus shifts in MWD. Marked differences in terms of milling characteristics, protein composition and ultimately in end-use functionality were observed with various waxy wheat null lines. Loaf volumes with waxy wheat flour alone were higher than a 50% blend with commercial wheat; however, breads were unacceptable to consumers in all aspects. Poor milling quality, very low mixing times with low bread loaf volumes were typical of all the triticales studied. However, translocation of the HMW-GS from wheat chromosome 1D increased dough strength, particularly the HMW-GS 5+10. Among the phytochemicals studied, double nulls at *Gli*-1 loci of Pegaso NILs had the highest total policosanols and total phenolic acid contents.Slight variation to wheat phenolic acid composition and contents were observed with waxy wheat and triticale lines.

Table of Contents

List of Figures x
List of Tables xii
Acknowledgements xiv
Dedication xv
Preface xvi
Abbreviations xvii
CHAPTER 1 - INTRODUCTION 1
Objectives 3
References 5
CHAPTER 2 - LITERATURE REVIEW 8
2.1 Monomeric and Polymeric Wheat Proteins 8
2.1.1 Monomeric Proteins 9
2.1.2 Polymeric Proteins 10
2.2 Genetics of Wheat Proteins 11
2.3 Functionality Studies Using Near –isogenic Lines 13
2.4 Molecular Weight Distribution (MWD), Chain Propagators and Terminators 15
2.5 Quality and/or Physical Dough Testing 18
2.6 Waxy Wheat 20
2.7 Triticales 24
2.8 Wheat Bran Phenolics and Policosanol 26
References 29
CHAPTER 3 - MATERIALS AND METHODS 45
3.1 Initial Physical Tests and Milling Yield 45
3.1.1 Single Kernel Characterization System (SKCS) 45
3.1.2 Experimental Milling 45
3.2 Analytical Determinations on Flour 46
3.2.1 Flour Protein Content 46

3.2.2 Flour Moisture Content 46
3.3 Protein Characterization and Compositional Analysis 46
3.3.1 Lab-On-a-Chip 46
3.3.1.1 Subunit Identification 46
3.3.3 Size Exclusion-High Performance Liquid Chromatography (SE-HPLC) 47
3.3.4 High Performance Capillary Electrophoresis (HPCE) 48
3.3.5 Size Exclusion Chromatography-Multi Angle Laser Light Scattering (SEC-MALLS) 49
3.4 Quality Tests 50
3.4.1 Dough Mixing Properties 50
3.4.2 Dough Extensibility Tests 50
3.5 Test Baking 51
3.6 Phenolics of Bran 52
3.6.1 Extraction of Phenolic acids 52
3.6.1.1 Pegaso Wheat Bran 52
3.6.1.2 Triticale and Waxy Wheat Bran 52
3.6.2 Determination of Total Phenolic acid Content (TPC) 53
3.6.3 Determination of Individual Phenolic acid Content 53
3.7 Policosanol Content in Pegaso Wheat Bran 54
3.7.1 Extraction and Determination of Policosanol from Wheat Bran 54
3.8 Statistical Analysis 55
References 56
CHAPTER 4 - CHAIN TERMINATORS 57
4.1 Introduction: 57
Hypothesis 58
Objectives 58
4.2 Methodology 59
4.3 Results and Discussion 61
4.4 Conclusion 68
References: 69
CHAPTER 5 - PEGASO LINES 71

5.1 Introduction 71
5.2 Methodology 74
5.3 Results and Discussion 74
5.3.1 Initial Physical Tests 74
5.3.2 Electrophoretic Characterization of HMW-GS using Lab-On-a-Chip Method 76
5.3.3 Effect of Allelic Variation on Quality using Physical Dough Tests and SE-HPLC 80
5.3.3.1 Comparison of Subunits 5+10 and 2+12 80
5.3.3.2 Effect of Deletions at Gli-1 and Gli-2 loci 84
5.3.4 Test Baking Results 86
5.4 Conclusion 87
References 89
CHAPTER 6 - WAXY WHEAT 93
6.1 Introduction 93
Objectives 94
6.2 Methodology 94
6.3 Results and Discussion 95
6.3.1 Initial Physical Tests 95
6.3.2 Electrophoretic Characterization of HMW-GS using Lab-On-a-Chip Method 96
6.3.3 Protein Composition and Physical Dough Tests 99
6.3.4 Test Baking Results 102
6.4 Conclusions 105
References 106
CHAPTER 7 - TRITICALE 109
7.1 Introduction 109
7.2 Methodology 111
7.3 Results and Discussion 112
7.3.1 Initial Physical Tests 112
7.3.2 Electrophoretic Characterization of HMW-GS using the Lab-On-a-Chip Method 113
7.3.3 Protein Composition and Physical Dough Tests 116
7.3.4 Test Baking Results 118
7.4 Conclusions 120

References 122
CHAPTER 8 - PHENOLICS IN BRAN 125
8.1 Introduction 125
Objectives 126
8.2 Methodology 126
8.3 Results and Discussion: 127
8.3.1 Pegaso Wheat Bran 127
8.3.1.1 Phenolics 127
8.3.1.1.1 Phenolic acid Composition 127
8.3.1.1.2 Total Phenolic acid Content (TPC) 129
8.3.1.2 Policosanol 130
8.3.2 Waxy Wheat Bran 132
8.3.2.1 Phenolics 132
8.3.2.1.1 Phenolic acid Composition 132
8.3.2.1.2 Total Phenolic acid Content (TPC) 133
8.3.3 Triticale Bran 134
8.3.3.1 Phenolics 134
8.3.3.1.1 Phenolic acid Composition 135
8.3.3.1.2 Total Phenolic acid Content (TPC) 136
8.4 Conclusions 138
References 140
CHAPTER 9 - CONCLUSIONS AND FUTURE WORK 143
9.1 General Conclusions 143
9.2 Future Scope 144
Appendix A - Correlation matrices including C-cell data 145
Appendix B - Mixograms and bread pictures 149

List of Figures

Figure 2-1: Comparison of the traditional (functional) and new (molecular) classifications of gluten proteins* 9
Figure 2-2: Structure of the low molecular weight glutenin subunits (LMW-GS)* 10
Figure 2-3: Structure of the high molecular weight glutenin subunits (HMW-GS)*..... 11
Figure 2-4: Chromosomal location of genes coding for the major gluten proteins* 13
Figure 2-5: Schematic representation of the total and unextractable polymeric protein and plots of extensigraph maximum resistance (Rmax) vs. those for 74 recombinant inbred lines* .. 17
Figure 2-6: Organization of bread wheat chromosomes and location of waxy wheat loci *..... 22
Figure 3-1: Classic SE-HPLC chromatogram showing the fractions peak 1 (polymeric protein, mainly HMW-GS), peak 2(LMW-GS and monomeric proteins) and peak 3 (albumins + globulins and other minor protein groups present in wheat). 48
Figure 4-1: Schematic diagram showing the extraction procedure employed for SPP and IPP fractions run in CE. 60
Figure 4-2: Electrophoregrams from CE for (A) SPP (Reduced) (B) SPP (Unreduced) (C) Gliadins and (D) IPP fraction for Pegaso (parent line), Pegaso 170 (Line with highest UPP) and Pegaso 168b (Lowest UPP). 62
Figure 4-3: Correlation graphs for (A). Peak 1 (ω1) area (B). Peak 2 (ω2) area (C). Peak1 +Peak 2 area of reduced SPP (SPP-RA) fraction analyzed by CE and (D) Peak1+Peak 2 area from IPP fraction. 63
Figure 4.4: Schematic depiction of the peak identification in SEC-MALLS. 66
Figure 5-1: The HMW-GS composition for Pegaso NILs identified by Lab-on-a-chip method. (A) Control chip and (B), (C) and (D) are for Pegaso samples. 76
Figure 5-2: Percent Polymeric Proteins (PPP), %gliadins and %albumins/globulins fractions from SE-HPLC for Pegaso NILs. The vertical bars represent the SD (n = 2), and values denoted by the same letter are not significantly different ($P < 0.05$). 82

Figure 5-3: Loaf volume (cc) and crumb scores from micro-breads baked for Pegaso NILs. The vertical bars represent the SD (n = 2), and values denoted by the same letter are not significantly different (P < 0.05). 87
Figure 6-1: The HMW-GS composition identified by Lab-on-a-chip method. (A) Svevo set (B) N11 set. Notice that all waxy lines have the same subunit composition as the parent line. 98
Figure 6-2: Loaf volumes and crumb scores from micro-breads made with 100% waxy wheat and 50% blend with commercial wheat for Svevo and N11 waxy wheats (A). Svevo set (B). N11 set 104
Figure 7-1: The HMW-GS composition of triticale samples identified by Lab-on-a-chip method. 114
Figure 7-2: Loaf volume (cc) and crumb scores from micro-breads baked for different triticale sets..... 120
Figure 8-1: Total bran phenolic contents of Pegaso wheat genotypes analyzed by the Folin-Ciocalteau assay. Results are expressed as μg Gallic Acid Equivalents (GAE) per gram of Pegaso wheat bran samples. The vertical bars represent the SD (n = 2), and values denoted by the same letter are not significantly different (P < 0.05)..... 129
Figure 8-2: Comparison of total bran phenolic contents of waxy wheat genotypes analyzed by the Folin-Ciocalteau assay (UV) and HPLC. (A) Svevo set and (B) N11 set. The vertical bars represent the SD (n = 2), and values denoted by the same letter are not significantly different (P < 0.05). 133
Figure 8-3: Comparison of total bran phenolic contents of triticale genotypes analyzed by the Folin-Ciocalteau assay (UV) and HPLC. (A) GDS7 set (B) Trim set (C) Rhino set and (D) Rigel set. The vertical bars represent the SD (n = 2), and values denoted by the same letter are not significantly different (P < 0.05). 137

List of Tables

Table 2.1: Solubility fractions of the proteins of wheat kernel (%) reported by different authors* ... 9

Table 2.2: Quality scores assigned to individual (or pairs of) high molecular weight glutenin subunits [a]. ... 19

Table 2.3: Percentage of variation in quality parameters that can be explained by protein (FP= flour protein), glutenin (GP=glutenin content in protein; GF= glutenin content in flour) and glutenin macropolymer contents (GMP)[1]. ... 21

Table 3.1: Formulation used in the micro-bake test ... 51

Table 4.1: Mean peak areas for two omega gliadin fractions analyzed by CE. Percent UPP values are from SE-HPLC and the table is arranged according to descending order for UPP. ... 65

Table 4.2: Molecular weights in total polymeric protein (TPP) fraction for Pegaso NILs analyzed by SEC-MALLS. ... 67

Table 5.1: Description of Pegaso near-isogenic lines used in the study ('+' and '-' represents presence and absence of subunits at corresponding loci, respectively) ... 73

Table 5.2: Results from initial physical tests- Hardness Index (HI) from SKCS; Milling yield from Quadrumat Junior mill and Flour protein content analyzed by LECO protein analyzer. ... 75

Table 5.3: Apparent sizes (kDa) of HMW-GS determined using the Lab-on-a-Chip method ... 78

Table 5.4: Percent unextractable protein (%UPP) analyzed by SE-HPLC arranged in its descending order. ... 81

Table 5.5: Results from physical dough tests (MDDT & Peak width at 8 min. from Mixograph; R_{max} and Extensibility from Micro-extensibility tests) arranged in descending order of MDDT ... 83

Table 5.6: Correlation matrix for various quality parameters and protein composition*. ... 85

Table 6.1: Description of Svevo durum waxy wheat and N11 bread waxy wheat lines used in the study. ... 95

Table 6.2 : Results from initial physical tests- Hardness Index (HI) from SKCS; Milling yield from Quadrumat Junior mill and Flour protein content analyzed by LECO protein analyzer. ... 97

Table 6.3: Apparent sizes (kDa) of HMW-GS determined using the Lab-on-a-Chip method ... 98

Table 6.4: Results from SE-HPLC for near-isogenic lines of Svevo and N11 waxy wheats 100
Table 6.5: Results from quality tests (MDDT & Peak width at 8 min. from Mixograph; R_{max} & Extensibility from micro-extensibility tests) for near-isogenic lines of Svevo and N11 waxy wheats 101
Table 6.6: Correlation matrix for various quality parameters and protein composition*. 102
Table 7.1: Description of different triticale sets used in the study. 111
Table7.2: Results from initial physical tests- Hardness Index (HI) from SKCS; Milling yield from Quadrumat Junior mill and Flour protein content analyzed by LECO protein analyzer*. 113
Table 7.3: Apparent sizes (kDa) of HMW-GS determined using the Lab-on-a-Chip method..... 114
Table 7.4: Results from SE-HPLC for different triticale sets. 116
Table 7.5: Results from quality tests (MDDT & Peak width at 8 min. from Mixograph; R_{max} & Extensibility from micro-extensibility tests) for different sets of triticale. 118
Table 7.6: Correlation matrix for various quality parameters and protein composition*. 119
Table 8.1: Phenolic acid composition (μg/g bran) of Pegaso wheat bran genotypes analyzed by HPLC arranged in its descending order of total phenolic acids*. 128
Table 8.2: Policosanol composition (μg/g bran) of bran from Pegaso wheat genotypes arranged in descending order of total policosanols* 131
Table 8.3: Phenolic acid composition (μg/g bran) of Svevo and N11 waxy wheat bran analyzed by HPLC *. 132
Table 8.4: Phenolic acid composition (μg/g bran) of triticale bran analyzed by HPLC. 135

Acknowledgements

I wish to express my sincere and profound sense of gratitude to my major advisor Dr. Finlay MacRitchie, for his constant guidance, encouragement, affection and incessant support, which instilled in me the confidence to successfully complete the study.

Distinctive words and thanks are due to Dr. Scott Bean for his constant inspiration, judicious guidance, patience towards my technical questions, and unending help during the project. My sincere thanks to Dr. Virgil Smail and Dr. Tom Herald for their valuable suggestions, critical comments and for being in my committee in spite of their busy schedules. My sincere thanks and appreciation to Dr. Domenico Lafiandra (University of Viterbo, Italy) for providing the breeding samples to the study and for his technical help and advice throughout the project.

Special thanks to Dr. Kelly Getty, Dr. Mike Tilley, Brian Ioeger, Sushma Prakash, Jeff Milligan, Margo Caley, Zina Haden, Dr. Ram, Dr. Brad Seabourn, Susan and other USDA-GMPRC, Manhattan personnel who contributed, constantly encouraged and helped me during this study. Appreciation also goes to my KSU friends Hicran, Murali, Mohan,Virendra; lab mates Shuping, Sasi, Julien; colleagues Sue, Karolyn, Camila and all others in the Dept. of Grain Science who supported me during my study that made my stay memorable, pleasant and successful. My heartfelt thanks to my friends Dr. Rajeev, Ratna, Madhu, Anjna, (KSU) Dimple and Dharmendra (OSU) for their consistent, valuable, professional and moral support.

My loving and deep gratitude to all my family members, especially to Buchi and Anitha for having made me what I am today.

My loving and deep gratitude also goes to Sibel Irmak for her infinite support in all the aspects.

Last but not least, I wish to thank the Dept. of Grain Science for their financial support and KSU for giving me the opportunity to advance my career goals.

Dedication

To my beloved Parents.

Preface

This dissertation describes the investigation of a range of near-isogenic lines from the Italian bread wheat Pegaso, partial (single and double nulls) waxy lines of Svevo (durum) and N11 (bread wheat), complete waxy (triple null) of N11 and translocation lines at 1R.1D. and 1A.1D. with HMW-GS 5+10 and 2+12 for four different sets of triticales, GDS7, Trim, Rhino and Rigel. On each set of these samples, initial physical tests (SKCS, Milling and Protein analyzer), protein characterization (SE-HPLC), HMW-GS identification (Lab-on-a-chip), quality (Mixograph, Micro-extensibility) and micro-baking tests were performed. Additionally, the evidence for the role of chain terminators (HPCE) in modifying the MWD of wheat proteins (MALLS) was described using Pegaso lines. Phytochemical (Phenolic acid and policosanols) composition was also performed for most of the Pegaso lines and all of the waxy and triticale sets already mentioned.

All the sample sets were evaluated using similar procedures and thus methodology is the same for all samples and is described in Chapter 3. However, if it is necessary for any additional information on materials, it is included in that specific chapter dealing with those samples. Moreover, a brief introduction about development of the breeding samples and background information for the objective and/or hypothesis were also included in each chapter for Pegaso, Waxy, and Triticale samples.

Phytochemical composition, one of the objectives of our study, was performed in collaboration with Dr. Sibel Irmak, then Post-Doctoral Fellow in our lab.

All breeding samples used in this study were provided generously by Dr. Domenico Lafiandra, Department of Agrobiology &Agrochemistry, University of Tuscia, Viterbo, Italy.

Abbreviations

CE	Capillary Electrophoresis
Cys	Cysteine
EPP	Extractable Polymeric Protein
GBSS	Granule Bound Starch Synthase
GGR	Gliadin to Glutenin Ratio
HI	Hardness Index
HMW-GS	High Molecular Weight- Glutenin Subunits
HPCE	High Performance Capillary Electrophoresis
IPP	Insoluble Polymeric Protein
LMW-GS	Low Molecular Weight- Glutenin Subunits
MDDT	Mixograph Dough Development Time
M_r	Relative Molecular Weight
MWD	Molecular Weight Distribution
NIL	Near-Isogenic Lines
PPP	Percent Polymeric Protein
R_{max}	Maximum Resistance to Extension
SDS-PAGE	Sodium Dodecyl Sulphate- Polyacrylamide Gel Electrophoresis
SEC-MALLS	Size Exclusion Chromatography- Multi Angle Laser Light Scattering
SE-HPLC	Size-Exclusion High Performance Chromatography
SKCS	Single Kernel Characterization System
SPP	Soluble Polymeric Protein
TPC	Total Phenolic acid Content
TPP	Total Polymeric Protein
UPP	Unextractable Polymeric Protein

CHAPTER 1 - INTRODUCTION

Wheat (*Triticum aestivum L.*) is the most important food crop of the world, with an approximate current production of 606 million tons (FAO, 2006). The success of the crop's cultivation is due to its adaptation to a diversified environment, being grown in almost all continents except Antarctica, ranging from upland sites in the tropics to the great plains of North America and steppes of Russia and the Ukraine. The foremost reason for wheat's success is the ability of its gluten protein to be formed into cohesive dough which can be baked into breads or formed into many other consumer-attractive products. Consequently, gluten proteins have become the most widely and intensively researched group of plant proteins in the world (Shewry *et al.,* 2001).

Gluten proteins, that comprise gliadin and glutenin, represent about 85% of total protein in the wheat grain and are considered to contribute to the viscosity and extensibility (gliadin), and elasticity (glutenin) of the gluten mass (Bietz *et al.*, 1973; Flavell *et al.*, 1984). Based on their functionality, gluten proteins can be classified as monomeric gliadins or polymeric glutenins (MacRitchie and Lafiandra, 1997). Polymeric glutenins are further comprised of high molecular weight (HMW) and low molecular weight glutenin subunits (LMW-GS) bonded by disulphide links.

Gluten proteins are encoded by genes located at nine major loci: three *Glu*-1 loci on the long arms of chromosomes 1A, 1B, and 1D respectively, coding for HMW-GS; three complex *Gli-1/Glu-3* loci on the short arms of chromosomes 1A, 1B and 1D with tightly linked genes coding for ω– and γ–gliadins and LMW-GS; and three *Gli*-2 loci coding for α– and β–gliadins (MacRitchie and Lafiandra, 2001). All nine loci exhibit allelic variation and thus lines with deletions, additions and/or combinations of different allelic expressions are possible. Conducting research using near-isogenic lines (NILs) differing at one or more loci may help in understanding the complex relationships between protein composition and parameters that measure end-use functionality of wheat flours. The main advantage of using

such materials is to deduce the effects of specific protein alleles without the confounding effects of different genetic backgrounds (MacRitchie and Lafiandra, 2001).

It is well established that different HMW-GS are associated with bread-making quality (Payne *et al.*, 1981) and they differ at each locus. For instance, 5+10 (good quality) and 2+12, 4+12 subunits (lack of dough strength) all are expressed at the *Glu-D1* locus but they are given the quality scores of 4, 2 and 1 respectively. Several studies reported the effects of individual subunits on quality parameters located at three major loci using null lines i.e. lines in which genes are absent or not expressed. The effect of allelic variation at *Glu-1* loci (Gupta *et al.*, 1991; Lawrence *et al.*, 1988; Gupta and MacRitchie, 1994), *Gli-1/Glu-3* loci (Gianibelli *et al.*, 1998; Redaelli *et al.*, 1997; Gupta and Shepherd, 1993) and *Gli-2* loci (Rogers *et al.*, 1990; Mansur *et al.*, 1990) has been studied extensively but individually. There is no comprehensive study with variation exhibiting a combination of all these alleles using NILs. By careful selection of deletion (null) lines at nine major gluten protein loci, it should be possible to manipulate the dough properties either to increase or decrease dough strength in breeding programs (MacRitchie and Lafiandra, 2001).

To participate in a growing glutenin polymer, glutenin subunits should have at least two cysteine (Cys) residues. These would act as chain extenders (having two or more Cys residues that form intermolecular disulphide bonds) or otherwise it would be a potential chain terminator (having only one Cys available for intermolecular disulphide bond formation) (Kasarda, 1989). Numerous studies reported that chain terminators would shift the MWD to lower values and the modified gliadins with an odd number of cysteine residues would act as chain terminators (Kasarda, 1989; Shewry *et al.*, 1989; Lew *et al.*, 1992; Masci *et al.*, 1993, 1999; Gianibelli *et al.*, 1996). Chain extenders would increase the dough strength (Bekes *et al.*, 1998; Lee *et al.*, 1999) whereas chain terminators would drastically decrease the strength (Greenfield *et al.*, 1998; Tamas *et al.*, 1998). However, there is no sound evidence how these modified gliadins influence the polymer size reduction in wheat proteins.

Starch related characteristics of waxy wheats have been studied extensively. The detailed protein composition and quality parameters of waxy wheats have not been studied.

There is a need to study composition and quality of protein characteristics of waxy wheats and their effects on dough and end-use product quality (Hung *et al.*, 2006).

Compared to bread wheat, hexaploid triticale has its D genome replaced by a rye (*Secale cereale*) genome due to which triticale has only two-thirds of the loci coding for gluten proteins. Among the missing gluten loci is *Glu-D1*, with its most important allele *d*. Instead, triticale has rye proteins, secalins, which do not belong to the gluten fraction and thus have no value in bread making technology. The value of A and B genome encoded gluten fractions in triticale is also suspect because triticale's gene pool has not been created with bread making in mind. Among all the substitution lines, 1D (1R) would seem the most beneficial in terms of quality but it is agronomically unacceptable. Chromosome translocations 1R.1D, which replace *Sec-3* on 1RL with the *Glu-D1* locus (with allele *d* encoding for 5+10 subunits) (Lukaszewski, 1998) and 1A.1D that replace the A genome with the *Glu-D1* locus (with alleles *a* and *d* encoding subunits 2+12 and 5+10 respectively) (Lukaszewski and Curtis, 1994) have been produced. However, no clear effects of these translocation lines on flour quality and functionality have been reported.

Though the breeding program for the genetic lines used in this study was targeted mainly to changes in composition of proteins, we cannot ignore the unintentional changes that might occur in bioactive components of wheat bran during the development of new genetic lines.

Objectives

The important goal of the study was to establish the utilization of near-isogenic lines as appropriate materials to investigate the composition-functionality relationships of cereal proteins.

The specific objectives were as follows:

- To determine the effect of allelic variation at *Glu-1, Gli-1/Glu-3* and *Gli-2* loci on flour functionality estimated by quality and physical dough tests using Pegaso wheat NILs

- To investigate and seek evidence for the 'presence of chain terminators that reduce polymeric protein size (in terms of measuring UPP)' using Pegaso wheat NILs
- To examine the effect of partial and complete waxy null lines on flour functionality estimated by quality and physical dough tests using Svevo and N11 waxy wheat NILs
- To evaluate the advantage for flour functionality of 1R.1D or 1A.1D translocation lines that have two HMW-GS 5+10 and 2+12 using GDS7, Trim, Rhino and Rigel triticale sets
- To investigate if there are any unintentional changes in phytochemical (phenolic acids and policosanol) composition as a consequence of allelic variation at different gluten protein loci using NILs of Pegaso, waxy wheat and triticale.

References

http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567

Bekes, F., Tamas, L., Gras, P. W., Shewry, P. R., Anderson, O., and Appels, R. 1998. Functional domain within the glutenin subunits of wheat. Pages 136-138 in: Proc. 9th International Wheat Genetics Sym. Vol. 4. Grain Quality. A.E.Slinkard, ed. University of Saskatchewan: Saskatoon, Canada.

Bietz, J. A., Huebner, F. R., and Wall, J. S. 1973. Glutenin, the strength protein of wheat flour. Baker's Dig. 47:26-31, 34-35, 67.

Flavell, R. B., Payne, P. I., Thompson, R. D., and Law, C. N. 1984. Strategies for the improvement of wheat-grain quality using molecular genetics. Biotech. Genetic Eng. Reviews. 2:157-173.

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

Gianibelli, M. C., Larroque, O. R., Chan, P., Margiotta, B., De Ambrogio, E., MacRitchie, F., and Lafiandra, D. 1998. Effects of the allelic variants at the *Gli-1/Glu-3* and *Gli-2* loci on mixing properties. Pages 154-156 in: Proc. Int. Wheat Genetics Symposium, 9th Vol. 4. A. E. Slinkard, ed. University of Saskatchewan: Saskatchewan, SK, Canada.

Greenfield, J. J. A., Ross-Murphy, S. B., Tamas, L., Bekes, F., Halford, M. G., Tatham, A. S., and Shewry, P. R. 1998. Rheological properties of monomeric and polymeric forms of C hordeins, a sulphur-poor prolamins of barley. J. Cereal Sci. 27:233-236.

Gupta, R. B., MacRitchie, F., Shepherd, K. W., and Ellison, F. 1991. Relative contributions of LMW and HMW glutenin subunits to dough strength and dough stickiness of bread wheat. Pages 71-80 in: Gluten Proteins 1991. W. Bushuk and R. Tkachuk, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.

Gupta, R. B., and Shepherd, K. 1993. Production of multiple wheat-rye translocation stocks and genetic analysis of LMW subunits of glutenin and gliadins in wheats using these stocks. Theor. Appl. Genet. 85:719-728.

Gupta, R. B., and MacRitchie, F. 1994. Allelic variation at glutenin subunit and gliadin loci, Glu-1, Glu-3, and Gli-1 of common wheats. II. Biochemical basis of the allelic effects on dough properties. J. Cereal Sci. 19:19-29.

Hung, P. V., Maeda, T., and Morita, N. 2006. Waxy and high-amylose wheat starches and flours-characteristics, functionality and application. Trends Food Sci. Technol. 17:448-456.

Kasarda, D. D. 1989. Glutenin structure in relation to wheat quality. Pages 277-302 in: Wheat is Unique. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul., MN.

Lawrence, G. J., MacRitchie, F., and Wrigley, C. W. 1988. Dough and baking quality of wheat lines deficient in glutenin subunits controlled by the Glu-A1, Glu-B1 and Glu-D1 loci. J. Cereal Sci. 7:109-112.

Lee, Y.-K., Bekes, F., Gras, P., Ciaffi, M., Morell, M. K. and Appels, R. 1999. The low-molecular-weight glutenin subunit proteins of primitive wheats. IV. Functional properties of products from individual genes. Theor. Appl. Genet. 98:149-155.

Lew, E. J. L., Kuzmicky, D. D., and Kasarda, D. D. 1992. Characterization of low molecular weight glutenin subunits by reversed-phase high-performance liquid chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and N-terminal amino acid sequencing. Cereal Chem. 69:508-515.

Lukaszewski, A. J. and Curtis, A. C. 1994. Transfer of the *Glu-D*1 gene from chromosome 1D to chromosome 1A in hexaploid triticale. Plant Breed. 112:177-182.

Lukaszewski, A. J. 1998. Improvement of bread making quality of triticale through chromosome translocations, pp. 102-110. In:P. Juskiw ed. Proc. 4th Int. Triticale Symp. Red Deer, Alberta, Canada. July 26-31, 1990, International Triticale Assoc. Vol. I.

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

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

MacRitchie, F., and Lafiandra, D. 1997. Structure-function relationships of wheat proteins. Pages 293-323 in: Food Proteins and Their Applications. S. Damodaran and A. Paraf, eds. Marcel Dekker Inc: New York.

MacRitchie, F., and Lafiandra, D. 2001. Use of near-isogenic wheat lines to determine protein composition-functionality relationships. Cereal Chem. 78(5):501-506.

Mansur, L. M., Qualset, C. O., Kasarda, D. D., and Morris, R. 1990. Effects of 'Cheyenne' chromosomes on milling and baking quality in 'Chinese spring' wheat in relation to glutenin and gliadin storage proteins. Crop Sci. 30:593-602.

Payne, P. I., Holt, L. M., and Law, C. M. 1981. Structural and genetic studies on high-molecular-weight subunits of glutenin. I. Allelic variation in subunits amongst varieties of wheat (Triticu aestiaum). Theor. Appl. Genet. 60:229-236.

Redaelli, R., Pogna, N. E., Ng, P. K. W. 1997. Effects of prolamins encoded by chromosomes 1B and 1D on the rheological properties of dough in near-isogenic lines of bread wheat. Cereal Chem. 74:102-107.

Rogers, W. J., Rickatson, J. M., Sayers, E. J., and Law, C. N. 1990. Dosage effects of chromosomes of homeologous groups 1 and 6 upon bread-making quality in hexaploid wheat. Theor. Appl. Genet. 80:281-287.

Shewry, P. R., Popineau, Y., Lafiandra, D. and Belton, P. 2001. Wheat glutenin subunits and dough elasticity: findings of the EUROWHEAT project. Trends Food Sci. Technol. 11:433-441.

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

Tamas, L., Bekes, F., Greenfield, J., Tatham, A. S., Gras, P. W., Shewry, P. R., and Appels, R. 1998. Heterologous expression and dough mixing studies of wild-type and mutant C hordeins. J. Cereal Sci. 27:15-22.

.

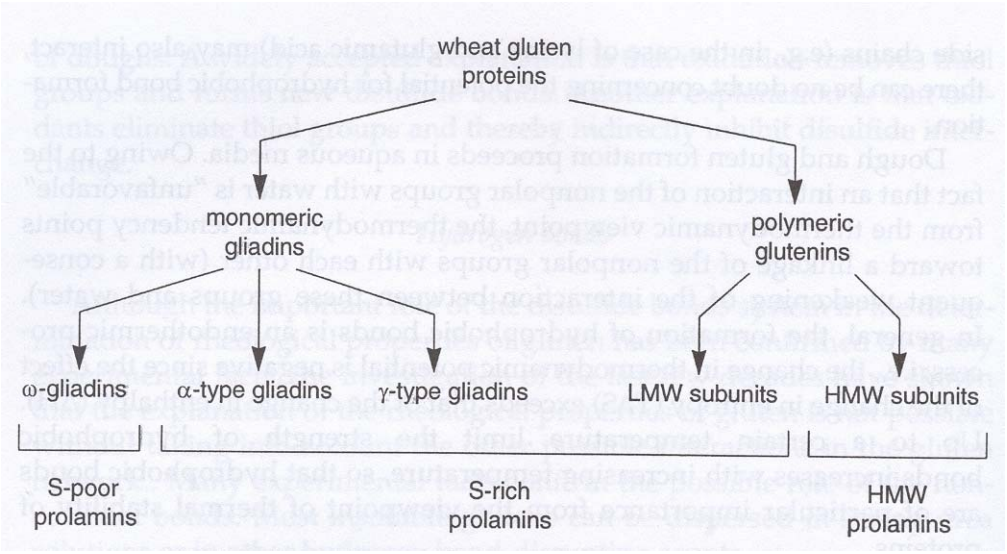
CHAPTER 2 - LITERATURE REVIEW

This chapter deals with a review of literature that is related to all other chapters in this thesis. However, any additional literature that is specific to each chapter will be described individually, if necessary.

2.1 Monomeric and Polymeric Wheat Proteins

Wheat gluten proteins were among the first to be studied by an Italian biochemist Becarri in 1748 by washing out starch and solubles from dough (Bailey, 1941). Later, in 1810, it was Taddei, who introduced the term "gliadin" to refer to the soluble portion of gluten and "glutenin" to the portion remaining after extraction of gliadin. However, the 'modern era' was started with Osborne (1907) who classified wheat proteins into four groups based on solubility: water soluble albumins, salt soluble globulins, prolamins that were soluble in 70% ethanol (gliadins) and glutelins (glutenins) soluble (partially) in dilute acids and bases. Distribution of wheat endosperm proteins based on their solubility is summarized in Table 2.1. The storage proteins gliadin and glutenin represents about 80% of the total protein in the wheat grain and are considered to contribute to the viscosity and extensibility (gliadin), and elasticity (glutenin) of the gluten mass (Bietz *et al.*, 1973; Flavell *et al.*, 1984). Lasztity (1999) suggested that approaching the problems on the basis of solubility alone is not enough to understand the molecular complexities and relationships of different protein fractions to end-use quality such as baking. From a functionality point of view, it is convenient to divide the wheat protein fractions into two main classes, monomeric and polymeric, based on whether they consist of single or multiple-polypeptide chains (MacRitchie and Lafiandra, 1997). However, current researchers broadly divide the protein groups into three (Shewry *et al.*, 1986; Wieser 1995) as shown in Fig.2.1: low molecular weight S-rich (α, β and γ-gliadins), low molecular weight S-poor (ω-gliadins) and high molecular weight glutenin subunits.

Figure 2-1: Comparison of the traditional (functional) and new (molecular) classifications of gluten proteins*



***Adapted from Shewry *et al.*, 1986**

Table 2.1: Solubility fractions of the proteins of wheat kernel (%) reported by different authors*

Fraction	Simmonds	Lasztity#	Belitz and Grosch	Konarev
Albumins	5-10	6-12	14.7	7-10
Globulins	5-10	4-7	7.0	4-6
Gliadins	40-50	30-56	32.6	40-45
Glutenins and residue	30-40	28-56	45.3	40-55

#Unpublished data

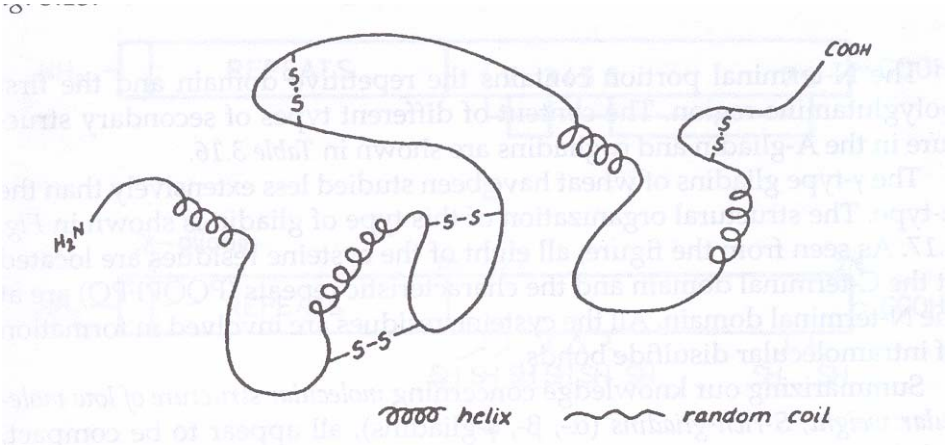
*****Modified from Lasztity, 1999

2.1.1 Monomeric Proteins

The monomeric (or single-chain) proteins comprise mainly gliadins and albumins/globulins. These albumins/globulins represent heterogeneous families; abundant low molecular mass (α-amylase) and high molecular mass (β-amylase) (Singh *et al.*, 2001). Based on electrophoretic mobility at low pH (3.1) they are usually divided into α, β and γ-gliadins that have molecular weights between 30 and 45 kDa (faster mobility groups) and ω-gliadins of M_r 50-70 kDa (slowest mobility group) (Bietz and Wall, 1980; Lookhart and

Albers, 1988). The latter are clearly identified in 10% SDS-PAGE as they do not overlap with other storage proteins (Bushuk and Sapirstein, 1990). Unlike the other gliadins which contain an even number of cysteine residues that form intramolecular disulphide bonds, ω-gliadins have no cysteine residues and have low/poor sulphur content (Shewry *et al.*, 1997). Most of the albumins/globulins have M_r with a range between 20 and 30 kDa and compared to gliadins, they show different amino acid compositions with high proportions of lysine (MacRitchie and Lafiandra, 1997).

Figure 2-2: Structure of the low molecular weight glutenin subunits (LMW-GS)*



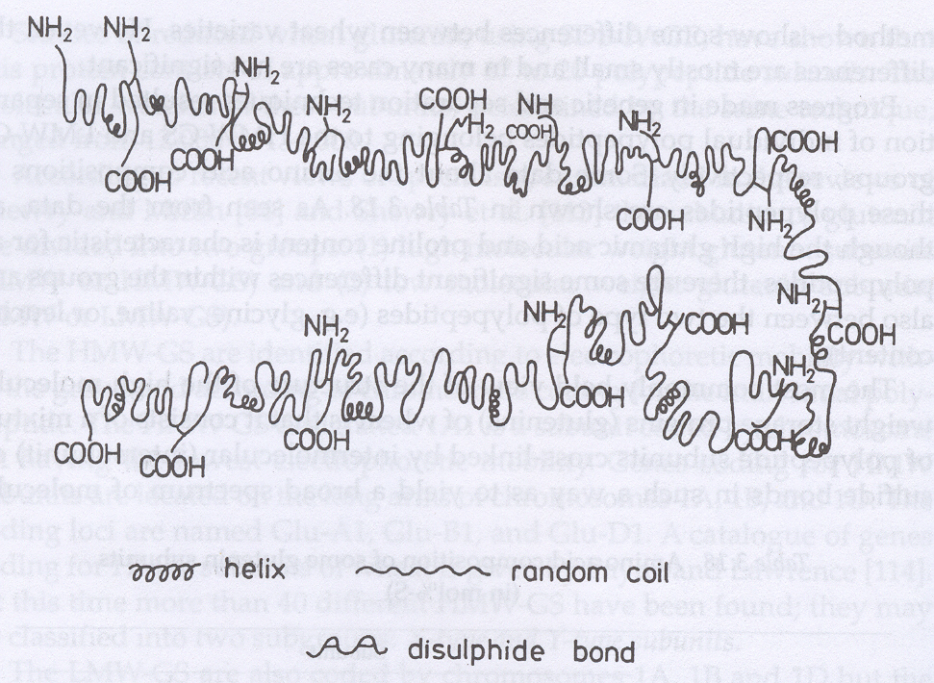
***Adapted from Lasztity, 1999**

2.1.2 Polymeric Proteins

The major groups of polymeric (multichain) protein are glutenins, HMW albumins and triticins (MacRitchie and Lafiandra, 1997). Approximately 85% of the polymeric fraction is glutenin which is similar in chemical composition to gliadin, indicating the similarities in genetic ancestry. Glutenins are mainly comprised of low molecular weight (LMW) and high molecular weight (HMW) subunits, which can be grouped according to their mobilities in SDS-PAGE. The possible structures of the LMW and HMW-GS are shown in Fig. 2.2 and 2.3 respectively. Glutenin proteins are heterogeneous mixtures of polymers formed by disulphide linkages of polypeptides. Their electrophoretic mobility in SDS-PAGE after

reduction of S-S bonds is the criterion to divide them into A, B,C, and D groups (A-, B-, C- and D- regions of the electrophoresis) (Gianibelli *et al.*, 2001). The HMW or A group fall roughly in the size range 80,000-120,000 (Payne and Corfield, 1979). The LMW subunits fall into B- (MW=40,000-55,000) and C- (MW=30,000-40,000) groups and are distantly related to α, and γ- gliadins (Payne *et al.,* 1985; Thompson *et al.*, 1994). D-group subunits, slightly lower mobility than B-group, are highly acidic and mostly related to ω-gliadins (Jackson *et al.*, 1983; Masci *et al.*, 1993).

Figure 2-3: Structure of the high molecular weight glutenin subunits (HMW-GS)*



***Adapted from Lasztity, 1999**

2.2 Genetics of Wheat Proteins

Hexaploid wheats are commonly used for bread making and they contain 42 chromosomes made up of three genomes (A, B and D) each having seven pairs of chromosomes numbered 1-7(Payne, 1987). The important endosperm storage proteins that contribute to functionality are coded by genes located on chromosomes 1 and 6 only. Thus, there are only nine major loci coding for gluten proteins in bread (hexaploid) wheats (MacRitchie and Lafiandra, 2001). A simplified form of location of genes is shown in Fig.

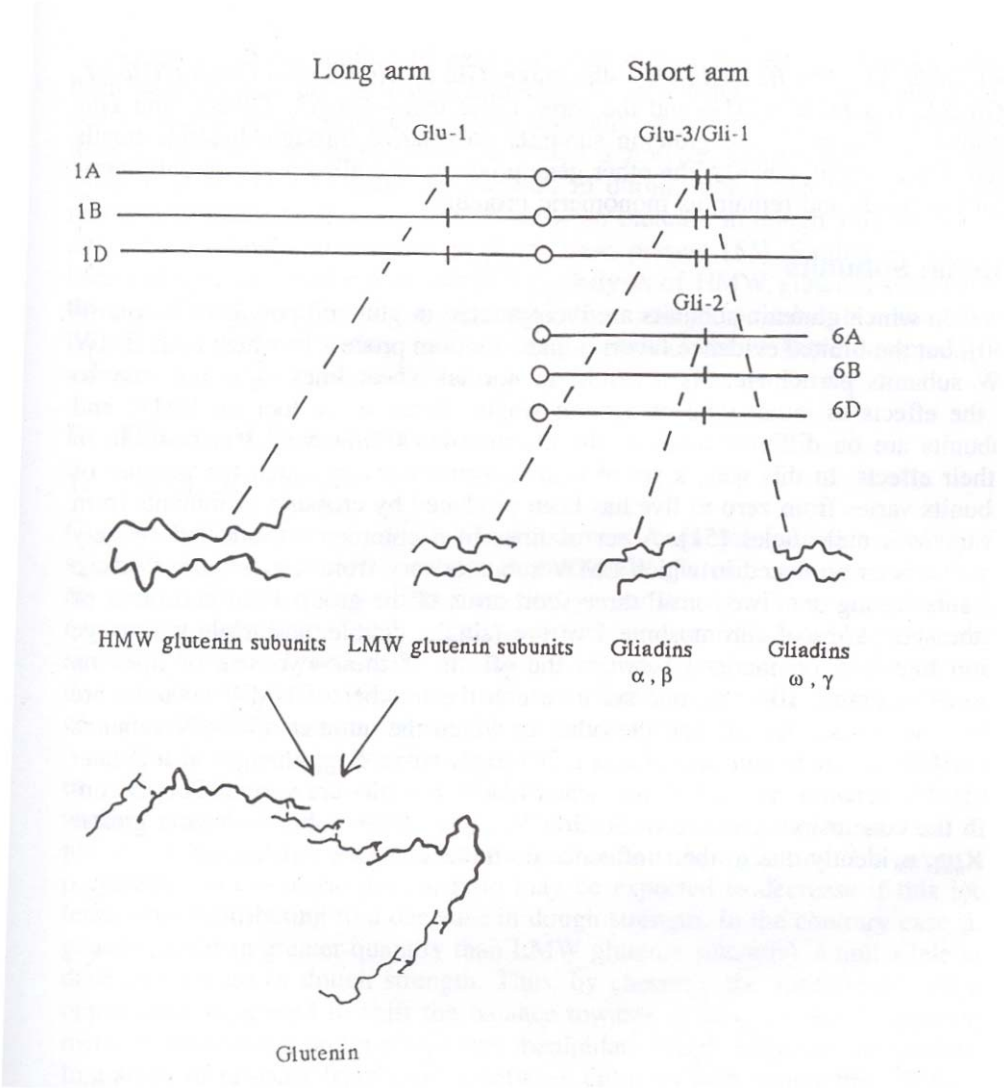
2.4. The genes coding for HMW-GS are located on the long arms of chromosomes 1A, 1B and 1D at the *Glu-A1, Glu-B1* and *Glu-D1* loci respectively (Payne, 1987). Each locus contains two tightly linked genes encoding for a high and a low M_r subunit denoted as x- and y-types, respectively (Shewry *et al.*, 1992). Theoretically, bread wheats should contain six different HMW-GS but, due to silencing of some of these genes, most common wheats possess three to five subunits (Payne 1987). Usually, both x- and y-types are expressed at the *Glu-D1* locus, two or one at the *Glu-B1* locus and one or none at the *Glu-A1* locus. Thus, all hexaploid wheats contain at least the 1Bx, 1Dx and 1Dy, while some cultivars possess 1By and 1Ax as well, which indicates that when one subunit is present at *Glu-A1* or *Glu-B1* loci, it is invariably an x-type. However, recently, some bread wheats containing six HMW-GS have been reported (Johansson *et al.*, 1993; Margiotta *et al.*, 1996).

Genes coding for LMW-GS occur on the short arms of group 1 chromosomes at the *Glu-A3, Glu-B3* and *Glu-D3* loci (Singh and Shepherd, 1988) which are tightly linked to *Gli-1* loci (Singh and Shepherd, 1988; Pogna *et al.*, 1990; Gupta and Shepherd, 1990). Chromosome 1A encodes relatively few LMW-GS and some cultivars do not exhibit any LMW-GS encoded by *Glu-A3*. Extensive polymorphism was observed for LMW-GS encoded by chromosome 1B (Pogna *et al.*, 1994). There is evidence that some LMW-GS are controlled by genes on group-6 chromosomes (Lew *et al.*, 1992; Gupta and Shepherd, 1993). The genes controlling LMW-GS are closely linked and form clusters as they are inherited together (Gupta and Shepherd, 1990; Laguadah *et al.*, 1991).

Gliadins are coded by genes located on the short arms of chromosomes 1 and 6. Specifically, the ω– and γ–gliadins are encoded by tightly clustered complexes at *Gli-1* loci (Payne, 1987) of chromosome 1, whereas the α– and β–gliadins are coded by genes at *Gli-2* loci of chromosome 6 (MacRitchie and Lafiandra, 1997). However, there are reports that minor ω–gliadins are encoded by additional, dispersed genes at *Gli-A3* (Metakovsky *et al.*, 1986; Payne *et al.*, 1988), *Gli-B3* (Jackson *et al.*, 1985), *Gli-A4* (Redaelli *et al.*, 1992) and *Gli-5 loci* (Pogna *et al.*, 1993) on the short arms of chromosome 1. Multiple allelism for gliadin clusters at *Gli-1* and *Gli-2* loci was reported by Metakovsky *et al.*, (1984). The location of the

genes controlling triticins (*Tri-A1* and *Tri-D1*) (Singh and Shepherd, 1991) and HMW albumins (Gupta *et al*., 1991) has also been reported.

Figure 2-4: Chromosomal location of genes coding for the major gluten proteins*



*** Adapted from MacRitchie and Lafiandra, 1997**.

2.3 Functionality Studies Using Near –isogenic Lines

The contribution of specific genes to any trait of interest could be assessed by using specially developed materials called 'near-isogenic lines' (NILs). Usually, such lines are developed through 10 or more backcrosses (Watanabe, 1994). The resulting NILs vary

primarily in the alleles introduced, while the remainder of the genotype is left the same (Specht *et al*., 1985). The NILs enable determination of the genetic effects attributable to a single gene, or accurately, to a short arm part of a chromosome bearing the gene in question, thus avoiding the compound effects of multiple genetic backgrounds (Amadou *et al*., 2003). Consequently, the major advantage of employing NILs in research is to minimize the differences due to epistatic interactions of the particular gene with other genes while emphasizing the major single-gene differences between lines (Boiteux *et al*., 1995).

Near-isogenic lines differing at one or more genetic loci coding for wheat storage proteins helps in elucidating the complex relationships between composition and end-use functionality without the confounding effects of differing genetic backgrounds (MacRitchie and Lafiandra, 2001). These lines can be used to manipulate functional properties predictably in breeding situations or to successfully elucidate structure-composition-functionality relationships. Pioneering work of Sears (1954, 1966) with creation of monosomic, nullisomic-tetrasomic and ditelosomic lines laid the foundation and made lines available with deletions, additions, or differences in allelic expression at specific loci. All nine major loci coding for storage proteins exhibit allelic variation and are designated as *Glu-1* (Long arms of 1A/1B/1D chromosomes coding for HMW-GS), *Gli-1*/*Glu-3* (short arms of 1A/1B/1D chromosomes coding for LMW-GS and γ and ω-gliadins) and *Gli-2* loci (short arms of 6A/6B/6D chromosomes coding for α and β-glidins).

Studies on HMW-GS null lines (*Glu-1* locus) (Lawrence *et al*., 1988; Payne *et al*., 1987a) confirmed that dough mixing strength and bread making quality decreased dramatically as HMW-GS are deleted. The quantity of HMW-GS decreases linearly with decreasing number of subunits. Translocation lines (hexaploid to 1A of a durum line) resulted in dramatic increase in MDDT (from 5 to 15min.) as a result of introduction of the extra HMW-GS (5+10) that shifted the molecular weights to higher values with higher UPP (Lafiandra *et al*., 2000). Depending on the relative amounts of LMW-GS and gliadins expressed at *Gli-1*/*Glu-3* loci, the dough strength of different lines could be altered (Gianibelli *et al*., 1998). For instance, if a greater quantity of LMW-GS than gliadins is expressed at this locus, then glutenin-to-gliadin ratio would shift to lower values leading to

reduced dough strength. Allelic variation at *Gli-D1/Glu-D3* loci in a Chinese Spring cultivar that carries two D-type subunits resulted in lower UPP and MDDT values (Gianibelli *et al.*, 1998). This might be due to D-subunits acting as chain terminators and thus shifting the MWD to lower values (Masci *et al.*, 1999). Deletion lines at *Gli-2* loci (*Gli-A2/D2*) exhibit increased MDDT and increased PPP because of glutenin-to-gliadin ratio being manipulated to higher values (Gianibelli *et al.*, 1998).

Near-isogenic 1B/1R translocation lines had significantly declined dough strength and increased stickiness compared to controls but had no differences in loaf volume (Hussain *et al.*, 1997). Both the quantity and subunit composition of HMW-GS affects the gluten viscoelasticity by modifying the polymer size distribution and aggregative properties of gluten proteins (Popineau *et al.*, 1994). Both the *Gli-1* and *Gli-2* loci may code for modified gliadins that act as chain terminators and the HMW-GS with one less Cys residue in near-isogenic lines shifted the molecular weights to lower values (Pirozi, 2003). Beneficial effects of increased subunit numbers (6 subunits) on dough properties was observed with a series of near-isogenic Pegaso wheat lines containing subunits with longer repetitive domains such as 1Dx2.2, 1Dx2.2* and $1Dy12_1$ (Shewry *et al.*, 2001).

2.4 Molecular Weight Distribution (MWD), Chain Propagators and Terminators

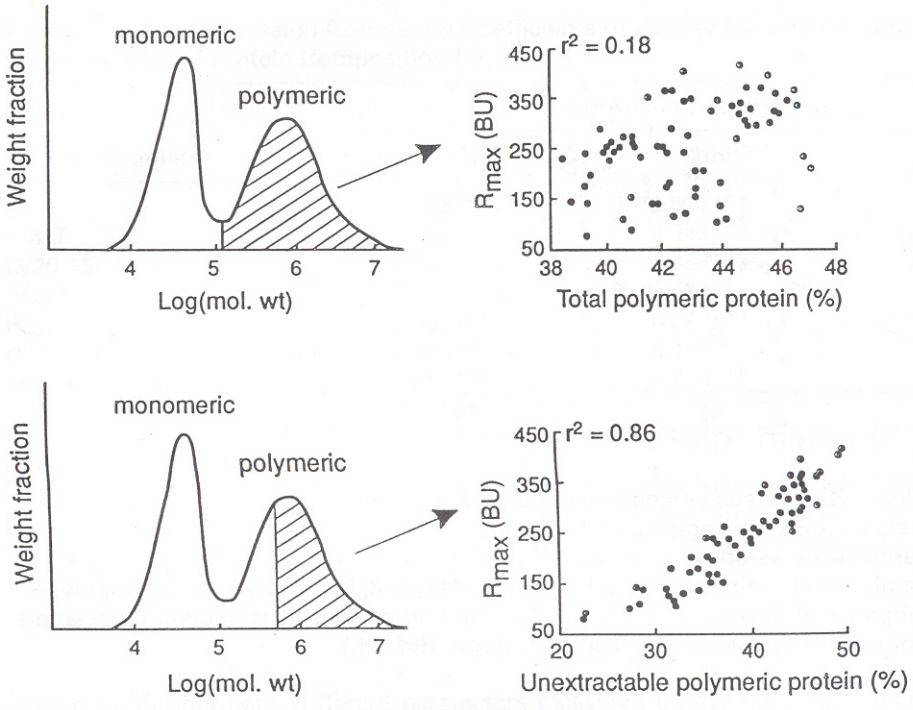
It is often stated that the molecular weight distribution (MWD) of glutenin polymers is correlated with their functionality (Wrigley and Bietz, 1988; MacRitchie, 1992; Southan and MacRitchie, 1999).The MWD of wheat proteins is mainly manipulated by two variables; monomeric-to-polymeric ratio and MWD of the polymeric proteins. The MWD of polymeric proteins in turn depends on the ratio of HMW-GS/LMW-GS, the specific HMW-GS that result from allelic variation and the presence of modified gliadins that can act as chain terminators (Southan and MacRitchie, 1999). Both the monomeric to polymeric protein ratio and MWD of polymeric protein are genetically controlled and also influenced by the environmental conditions.

Several attempts have been made to measure the molecular weights of wheat proteins. Though it is difficult to determine the size of larger protein molecules accurately,

Jones *et al.*, (1961) reported the weight average molecular weight of wheat proteins as $2x10^6$. Gel-permeation chromatography estimates the molecular weight of large size glutenin fractions in the range of $5x10^6$ (Schofield *et al.*, 1983) to $>20x10^6$ (Huebner and Wall, 1975). Recent measurements on molecular weights of large glutenin polymer reports it approaches the millions (Stevenson and Preston, 1996; Wahlund *et al.*, 1996). Nevertheless, measurement of the true MWD of glutenin polymers remains a stumbling block mainly because no solvent has been found for solubilizing the total wheat protein without any chemical alteration. However, multiangle laser light scattering (MALLS), one of the promising methods for measuring molecular weights, has been employed to characterize glutenin proteins in conjunction with RP-HPLC (Lookhart, 1997). Bean and Lookhart (2001) were the first to successfully report the MWD of SDS-insoluble protein complexes using MALLS and was found to be approximately $8x10^7$ Da.

It was well established that the MWD variable is correlated with various dough property parameters (Gupta *et al.,* 1992). Extensigraph maximum resistance (R_{max}) and mixograph dough development time (MDDT) were moderately correlated (r=0.665) with percent polymeric protein in the total protein (PPP). However, R_{max} is well correlated with UPP (Weegels *et al.*, 1996) which is also termed glutenin macro polymer (GMP), as shown in Fig. 2.5. This suggests that only a fraction of the highest molecular weight glutenins contributes to dough strength (MacRitchie and Lafiandra, 1997; Bangur *et al.*, 1997). The higher values for the HMW/LMW-GS ratio results in a shift of the MWD to higher molecular weights (Gupta *et al.*, 1991, 1993, 1995) and vice versa i.e. decreased ratio causes a shift to lower molecular weights (Larroque *et al.*, 1997). The B/C LMW-GS ratio was also positively correlated with MWD and dough strength (Gupta and MacRitchie, 1994; Larroque *et al.*, 1997). Unlike R_{max}, extensibility appears to relate positively to the total proportion of flour polymeric protein (FPP) (Gupta *et al.*, 1992; Larroque *et al.*, 1999) and larger size glutenin polymers contribute to lower extensibility (Gupta and MacRitchie, 1994). Although the differences in dough strength (measured by MDDT) between two HMW-GS 5+10 and 2+12 was well correlated with UPP , the origin of the allelic effects on MWD has not been resolved.

Figure 2-5: Schematic representation of the total and unextractable polymeric protein and plots of extensigraph maximum resistance (Rmax) vs. those for 74 recombinant inbred lines*



***(Adapted from MacRitchie and Lafiandra, 1997)**

The number and positions of cysteine residues in glutenin subunits play a key role in determining the native glutenin properties (Lew *et al.*, 1992) and thus affects the mixing and baking quality. To participate in a growing glutenin polymer, glutenin subunits should have at least two cysteine (Cys) residues. These would act as chain extenders (having two or more Cys residues that form intermolecular disulphide bonds) or otherwise it would be a potential chain terminator (having only one Cys available for intermolecular disulphide bond formation) (Kasarda, 1989). Subunits with only one Cys residue can only serve as chain terminators and thus cause the shift in size distribution of polymers to lower M_r values (Tao and Kasarda, 1989; Kasarda, 1989; Lew *et al.*, 1992). A predominance of the chain-extenders in glutenin should lead to strong gluten with good dough properties, whereas too much of chain terminator proteins would have the opposite effect on dough (Masci *et al.*, 1998). Any

change in the ratio of chain extenders/chain terminators (Ch.E/Ch. T) could shift the molecular weight distribution of polymers and thus change the properties of the dough (Gianibelli *et al*., 2002).

Increasing evidence showed that gliadin- like proteins are incorporated in glutenins and these are mainly the result of single-gene mutations that can change the number of Cys residues compared to those of monomeric forms, and these gliadin isoforms could behave as chain terminators (Lew *et al*., 1992; Masci *et al*., 1993, 1995; D'Ovidio *et al*., 1995; Gianibelli *et al*., 1996). D-subunits are a type of LMW-GS that have lower electrophoretic mobility than B subunits (Payne and Corfield, 1979). They have a M_r of around 70000 (Gianibelli *et al*., 2002) and are encoded by genes located on the short arms of chromosomes 1B and 1D (Jackson *et al*., 1983). The presence of Cys residues in these D-subunits has allowed this gliadin-type protein to be incorporated into the polymeric glutenin structure and possibly act as a chain terminator (Gianibelli *et al*., 2002). The negative effect of D-subunits on dough quality was mainly attributed to decreased average molecular weights of glutenin polymers (Masci *et al*., 1993) which is similar to that proposed for certain α- and γ-type glutenin subunits that have apparently originated from mutation of a serine codon to a cysteine codon in ancestral α- and γ-gliadin genes (Tao and Kasarda, 1989; Kasarda, 1989; Lew *et al*., 1992). Based on N-terminal amino acid sequences, two types of LMW-GS namely, LMW-m and LMW-s are identified which fall in the MW range of 35000-45000 (Kasarda, 1989; Tao and Kasarda, 1989; Lew *et al*., 1992). Both types are coded by genes located at the complex *Glu*-3 loci. The differences in quality between two wheat cultivars (Lira 42 and Lira 45) which had different amounts of LMW-m and LMW-s type glutenin subunits and α- and γ-type gliadins were reported (Masci *et al*., 1995). Relative predominance of LMW-m and LMW-s type glutenin subunits in Lira 45 resulted in good quality. Probably these subunits act as chain extenders, whereas, Lira 42 which had higher (43%) proportions of α- and γ-type gliadins cause poor quality mainly because these subunits act as chain terminators and thus contribute to a lower size range of glutenin polymers.

2.5 Quality and/or Physical Dough Testing

The baking quality of wheat flour is primarily dependent on the quantity and quality of the flour proteins (Finney, 1943). As most of the proteins in flour are gluten, at least one gluten component should relate to breadmaking quality. In fact, two of the more important flour quality factors, mixing time and loaf volume, are related to one or more gluten components (Finney *et al.,* 1982). The fractionation-reconstitution approach and correlation studies are two kinds of studies that have helped in understanding the effect of flour functionality on quality parameters. Fractionation-reconstitution studies are useful in evaluating the contribution of specific flour protein fractions by either varying their quantities in the native flour or by interchanging equivalent components between flours of contrasting functionality (MacRitchie *et al.*, 1990; MacRitchie, 1992). These approaches showed that the dough strength and baking potential of flour can be manipulated by varying the ratio of polymeric to monomeric proteins (MacRitchie, 1989). The specific role of each protein fraction i.e. glutenin, gliadin and other fractions on bread making quality was evaluated using these approaches (MacRitchie, 1987a, 1987b). It was observed that flours with poorer bread quality had a lower proportion of glutenins than flours with good quality (Dupuis *et al.*, 1996; MacRitchie, 1987a, 1987b). The HMW-GS contribute more to increased MDDT and loaf volume than the LMW-GS (MacRitchie *et al.*, 1991; Weiser and Kieffer, 2001). Individual gliadin groups cause dough weakening effects but to different degree (Uthayakumaran *et al.*, 2001).

Table 2.2: Quality scores assigned to individual (or pairs of) high molecular weight glutenin subunits [a].

Score	Chromosome 1A	Chromosome 1B	Chromosome 1D
4	-	-	5+10
3	1;2*	17+18;7+8;7+9[b] ;13+16;14+15	-
2	-	-	2+12;3+12
1	Null	7;6+8;20	4+12;2.2+12[c]

[a] Modified from Cornish *et al.*, (2006)

[b] Deduced by genetic analysis and quoted by Payne et al., 1987

[c] Takata *et al.*, (2003)

Statistical relationships between the presence or absence of specific HMW-GS and bread making quality have been studied extensively (Payne *et al.*, 1984; Campbell *et al.*, 1987; Cressey *et al.*, 1987; Lawrence *et al.*, 1988). Since genes encoding two HMW-GS can be present at the same locus, these studies indirectly correlate the effect of different alleles to quality. HMW-GS located at three *Glu-1* loci were given the quality scores shown in Table 2.2. It was clear that HMW-GS at *Glu-D1* have more effect followed by *GluA1* and *Glu-B1* loci (Lawrence *et al.*, 1988; Rogers *et al.*, 1991; Gupta *et al.*, 1994; Payne *et al.*, 1987). Specifically, *Glu-1* alleles make a greater contribution to R_{max} than *Glu-3* and, of the *Glu-3* loci, it was *Glu-B3* that contributes the most. To simplify, glutenin loci could be ranked as *Glu-D1>Glu-B1>Glu-B3>Glu-A3>Glu-D3=Glu-A1* with respect to R_{max} (Gupta *et al.*, 1994) and together (*Glu-1* and *Glu-3* loci) accounts for 80% of the variation in R_{max}. In contrast, only 25% of the variation in extensibility could be accounted for by glutenin alleles. NILs with either x-(5) or y-(10) type subunits deleted at *Glu-D1* showed that each subunit is equally important for dough properties and bread making quality (Rogers *et al.*, 1991). Other studies on NILs concluded that subunit 12 is related to poorer bread making quality compared to 10 (y-type) or 2 and 5 (x-type) (Lafiandra *et al.*, 1993; Rogers *et al.*, 1991). The quantity of large sized polymers containing glutenin was strongly correlated with R_{max} (Gupta *et al.*, 1994). R_{max} showed high correlation with loaf volume in micro-extensibility studies (Suchy *et al.*, 2000; Kieffer *et al.*, 1998; Nash *et al.*, 2006). Extensibility is mainly dependent on ratios of gliadin to glutenin subunits (Wieser and Kieffer, 2001). As reviewed by Weegels *et al.*, (1996), the contribution of different flour protein fractions to quality parameters reported by different authors is summarized in Table 2.3.

2.6 Waxy Wheat

The term 'waxy' was first introduced for amylose–free mutants of maize, and refers to the waxy appearance of endosperm in dried kernels as opposed to the flinty or translucence of normal (wild) kernels (Boyer and Hannah,1994). Granule Bound Starch Synthase (GBSS, EC 2.4.1.2.1) which is commonly called 'waxy protein' is responsible for synthesis of amylose (Tsai, 1974; Echt and Schwartz, 1981). The differences in

amylose/amylopectin ratio can cause variations in granular structure, physicochemical properties and ultimately the quality of end-use products.

Table 2.3: Percentage of variation in quality parameters that can be explained by protein (FP= flour protein), glutenin (GP=glutenin content in protein; GF= glutenin content in flour) and glutenin macropolymer contents (GMP)[1].

	Variation in quality parameter explained by (%)				
Quality parameter	FP	GP	GF	GMP[3]	References[2]
Loaf volume	12-52	---	---	2-50	Gupta *et al.*, 1993
	9	38	19	---	Gupta *et al.*, 1992
	1	---	---	37-46	Hamer *et al.*, 1992
	45	---	24-31	10	Cressey *et al.*, 1987
	79	---	---	97	Ewart, 1985
	37	---	---	75-76	Moonen *et al.*, 1982
	19	---	---	7-24	Orth *et al.*, 1976
	44-86	52	85-93	42-73	Maes, 1962
Extensibility[4]	7-45	---	---	6-76	Gupta *et al.*, 1993
	55	15	69	---	Gupta *et al.*, 1992
	7-74	8-71	---	---	Singh *et al.*, 1990
	66	---	---	37-53	Orth *et al.*, 1976
R_{max}	21-61	---	---	37-79	Gupta *et al.*, 1993
	6	44	15	---	Gupta *et al.*, 1992
	0	34-71	---	---	Singh *et al.*, 1990
	34	---	---	44-70	Orth *et al.*, 1976

[1] Modified from Weegels *et al.,* (1996)

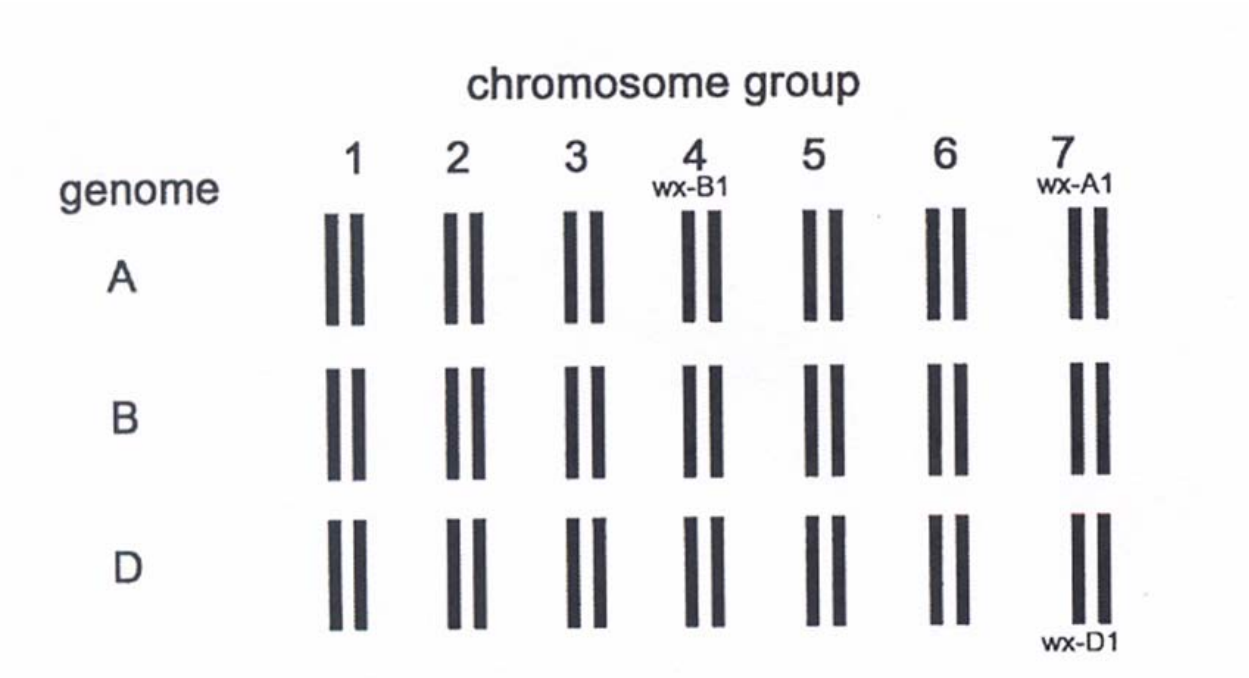
[2] All citations from Weegels *et al.*, (1996)

[3] Determination methods vary between different references

[4] Determined by Brabender Extensiograph

The world's first completely waxy wheat (amylose-free) was developed by traditional hybridizations between the *wx-D*1 single null line 'BaiHuo' and the *wx-A*1/*wx-B*1 double null line 'Kanto 107'. This resulted in progeny that lacked all isoforms of GBSS (Nakamura *et al.*, 1995). Currently these are being developed by classical breeding and genetics. Bread wheat (*Triticum aestivum* L.) is a hexaploid and contains three genomes (A, B and D) and each genome has seven homologous pairs of chromosomes. Durum wheats (*Triticum turgidum* L. *var. durum*) are tetraploids and contain only the A and B genomes. The genetic loci (*wx*) containing the genes encoding for bread wheat GBSS are located on chromosomes 7A (*wx-A*1), 4A (*wx-B*1) and 7D (*wx-D*1) as shown in Fig. 2.6. Though 4A was originally located on chromosome 7B, because of reciprocal translocation occurring, it resulted in exchange of genetic material between 7B and 4A chromosomes (Graybosch, 1998).

Figure 2-6: Organization of bread wheat chromosomes and location of waxy wheat loci *



***Adapted from Graybosch, 1998**

Waxy mutations occur spontaneously in cereals including maize, rice and barley (Eriksson 1970). Natural mutations are very useful in the production of waxy wheat lines

with low amylose content such as durum and bread wheats (Nakamura *et al.*, 2002; Miura *et al.*, 1999). The most commonly used natural variants to produce new waxy wheat lines are the Kanto 107 (Null at *wx-A*1/*B*1) and BaiHuo (*wx-D*1 null). Extensive electrophoretic studies on bread wheat have led to the identification of partial *waxy* mutant lines, characterized by the lack of one or two waxy proteins (Yamamori *et al.*, 1994; Graybosch, 1998). The molecular weights of three waxy proteins *wx-A*1, *wx-B*1, and *wx-D*1 were 60.1, 59.2 and 59.0 KDa, respectively as analyzed by conventional electrophoresis (Fujita et al., 1996). However, Nakamura *et al.*, (1992) first developed a modified SDS-PAGE system that allowed one-dimensional separation of three GBSS isoforms. Null alleles at the *wx-D*1 locus are found very rarely and, at present, they have been identified only in BaiHuo , a Chinese cultivar (Yamamori *et al.*, 1994). Null alleles in bread wheats at the *wx-A*1 locus (Japan, Korea and Turkey), *wx-B*1 locus (Australia and India) and the double *wx-A*1/*B*1 loci (Asian, European and North American) were found to be fairly common (Yamamori *et al.*, 1994). Null alleles only at *wx-A*1/*B*1 loci are found in USA cultivars (Graybosch, 1998). Waxy lines with one (single null) or two null alleles (double null) have been termed 'partial waxy lines' (Nakamura *et al.*, 1993), whereas complete waxy carry null alleles at all three waxy loci. Fairly good correlation was reported between the presence of *wx* null alleles and amylose content for various cultivars (Miura *et al.*, 1994; Yamamori *et al.*, 1992). The effect of GBSS isoforms on amylose content decreased in the order of *wx-B*1 > *wx-D*1 > *wx-A*1 (Miura *et al.*, 1994, 1999). Amylose content of non-waxy (presence of three waxy protein alleles) was 25-28% (Araki *et al.*, 2000; Graybosch, 1998), partial waxy (single or double null) 16-22% (Nakamura *et al.*, 1995) and complete waxy (triple null) 0-3% (Yasui et al., 1997; Nakamura *et al.*, 1995).

Modified food starches, blend flours for superior noodles, and shelf-life extension of baked foods are some of the applications of waxy wheat flours in the present day food industry (Graybosch, 1998). Waxy wheat is associated with low flour yield, decrease in flour color brightness (Yamaguchi *et al.*, 2003; Takata *et al.*, 2005) and an increase in the arabinoxylan and polyphenol contents of flour (Takata *et al.*, 2005). Similar findings were reported for polyphenols and flour yield contents with near-isogenic lines of waxy wheat (Takata *et al.*, 2007). Breads and noodles made with 100% waxy wheat flour resulted in poor

quality (Morita *et al*., 2000b; Baik and Lee, 2003; Park and Baik, 2004). On the other hand, breads made from 10-20% waxy wheat flour were found to improve bread quality (Yamaguchi *et al*., 2003). Blends of waxy and non-waxy wheat resulted in high loaf volume and less staling of bread crumb (Lee *et al*., 2001; Morita *et al*., 2002b).However, breads made with waxy wheat would not be accepted by consumers. Morita *et al*., (2000b) reported that waxy wheat flour can be used to substitute for up to 40% of non-waxy wheat in bakery product applications. Lee *et al*., (2001) also reported that bread crumb structure became more porous as the waxy wheat starch ratio increased. Incorporation of waxy wheat starch resulted in retention of more moisture in breadcrumb, thus helping to retard staling and extend the shelf-life of breads (Hayakawa *et al*., 2004). Partial substitution of waxy wheat flour (WWF) for common wheat flour improved the softness and glutinous texture of bread crumb (Morita *et al*., 2002a). Weak dough with less stability, and bread that could be effectively refreshed by reheating was also reported (Morita *et al*., 2002b). Higher farinograph water absorption with higher development time for waxy wheat flour were observed (Morita *et al*., 2002b; Takata *et al*., 2005). However there were no significant differences observed with waxy wheat flour compared to non-waxy for mixograph mixing time and peak height. Positive effects of null waxy alleles on bread making quality have been reported (Martin *et al*., 2004).

The detailed protein composition and quality as well as characteristics of dietary fiber of waxy and high-amylose wheats have not been studied. Hence, there is a need to study composition and quality of protein characteristics of waxy wheats and their effects on dough and end-use product quality (Hung *et al*., 2006).

2.7 Triticales

Triticale (X *Triticosecale Wittmack*), the first man–made cereal, is a hexaploid that combines the A and B genomes from wheat and the R genome of rye. It combines, to a certain extent, the high yielding capacity of wheat with the hardiness, tolerance and disease resistance of rye. It is adapted to a wide range of environmental conditions, and is grown in almost all geographical regions where the parental species are cultivated (Naeem *et al*., 2002). Triticale area under cultivation is increasing steadily all over the world (Varughese *et al*.,

1996). Triticale is bred and produced mainly for animal feed and forage. Its usage as a crop for human consumption has been limited by its inferior bread making quality. Good quality baked products from triticale can be obtained by using appropriate baking methods (Rakowska and Haber, 1991).

Triticale has higher protein content with a more favorable profile than wheat. However, gluten had inferior quality and milling properties as well as flour yield were lower due to unfavorable grain texture (Oettler, 2005; Weipert, 1986). Triticale has soft endosperm, which is responsible for poor flow properties that would negatively affect the flour sifting during milling and thus flour yield (MacRitchie, 1980). Wheat/triticale grain co-milling significantly improved the milling performance (Pena and Amaya, 1992). Villegas (1973) reported that triticale has potential to produce bread and other cereal food products such as pasta, tortillas and breakfast cereals. Hexaploid triticale has inferior bread making properties (Zeller and Hsam, 1984). This presents difficulties during the baking process and may be the result of absence of the D genome. The presence of chromosome 1D had a significant and positive effect on the Zeleny sedimentation value, which is a parameter proportional to bread making quality, but the difference between two glutenin alleles 5+10 and 2+12 was not as obvious as in wheat (Lafferty and Lelly, 2001). However, some exceptions have been reported; triticale had considerably improved bread making properties (Amaya *et al.*, 1986). Extensive research has been done on wheat-triticale flour blends as the blends have promising potential in bread making quality. Addition of triticale flour to bread wheat flour up to 30% resulted in satisfactory pan-type bread (Rooney *et al.*, 1969; Unrau and Jenkins, 1964). Even higher proportions of triticale flour could be used in specialty breads (Beaux and Martin, 1985). When 18.3% of triticale flour was blended with wheat flour, the greatest bread height (mm) and specific volume (cm^{-3} g^{-1}) were obtained (Varughese *et al.*, 1996). Furthermore, depending on the quality of wheat flour used, triticale can be added up to 50% (Doxastakis *et al.*, 2002; Pena and Amaya (1992), and up to 70 % (Tohver *et al.*, 2005), with quality similar to breads made from wheat flours only. All the loaves were of acceptable quality with good sensory properties such as fresh and pleasant smell and taste (Tohver *et al.*, 2000; Taht *et al.*, 1998). Though flour protein contents were similar, mixograph peak times,

sedimentation values and falling numbers were considerably lower for triticales compared to wheat flours (Pena and Balance, 1987). A lower mixograph time with higher extensibility was observed with triticale flours (Serna-Saldivar *et al*., 2004). Nonlinear relationships between blend composition and mixograph parameters were observed by Naeem *et al*. (2002).

Several workers have performed pioneering research to genetically improve the bread making quality of triticale either through substitution (replacement of an entire genome such as 1A/1B/1R in triticale by the 1D genome of wheat) or translocation (replacement of an entire or part of a chromosome such as 1DL long arm/1DL fragment of wheat to the long arm of 1A/1B/1R in triticale) (Lukaszewski and Curtis, 1992). Triticale lacks the D genome of bread wheat (instead it has R from rye) that carries the *Glu-1/Gli-3* locus, important for bread making (Payne *et al*., 1984, Payne, 1987).Correspondingly, the total amount of gluten in triticale is low and bread making quality is poor (Pena, 1996). Transformation of known storage protein loci from 1D of wheat and silencing of detrimental secalin loci of rye would be an option to improve quality (Shewry *et al*., 1995). Not all secalins are detrimental for bread making quality: it is valid only to the *Sec*-1 locus, and replacement of some *Glu*-1 wheat loci by the *Sec*-3 locus produced a positive effect on dough properties of bread wheat (Kumlay *et al*., 2003). No effect of *Sec-2* loci on any bread parameters has been detected so far (Gupta *et al*., 1989).Among relative contributions of individual group-1 chromosomes to bread making quality of triticale, rye chromosome 1R has always been placed higher than wheat chromosome 1A (Lukaszewski, 1996, 1998; Wos *et al*., 2002;Kumlay *et al*., 2003). Substitution of 1D for 1A appears to be most desirable in triticale breeding in spite of its cytological stability combined with minimal effect on agronomic performance (Lafferty and Lelley, 2001).Replacement of 1B with 1D would be unproductive in terms of quality (Rogers *et al*., 1990). Translocations involving transfer of *Glu-D1* genes from chromosome 1D of bread wheat to chromosome 1R (Lukaszewski and Curtis, 1992) and 1A (Lukaszewski and Curtis, 1994) of rye were produced as viable options to check the bread making quality.

2.8 Wheat Bran Phenolics and Policosanol

Cereal grains provide significant amounts of protein, energy and selected micronutrients, essential to human and animal diet. Phytochemicals in cereal grains have

significant implications in the improvement of food quality, specifically through applications in functional foods and nutraceuticals (Abdul-Hamid and Luan, 2000; Truswell, 2003). Wheat is an important agricultural commodity across the world that offers unique benefits to human health. The health beneficial phytochemicals in wheat are found in free, soluble-conjugated and bound forms in the fractions of endosperm, germ and bran (Adom and Liu, 2002; Adom *et al.*, 2003; Onyeneho and Hettiarachchy, 1992).However, the majority of health beneficial phytochemicals of whole wheat are concentrated in the bran/germ parts of the grain(Adom *et al.*, 2005).

Increasing evidence indicates that regular consumption of whole wheat (grain) food products that contain significant amounts of natural antioxidants offers numerous health benefits such as reduced total mortality (Jacobs *et al.*, 2001), reduced risk of certain types of cancer and cardiovascular diseases (Liu *et al.*, 1999; Thompson, 1994; Jacobs *et al.*, 1998), ischemic stroke (Liu *et al.*, 2000a) and type2 diabetes (Liu *et al.*, 2000b; Meyer *et al.*, 2000).

Phenolic acids are a group of natural products commonly found in the many cereal grains, higher quantities of which are found in outer layers of kernels which constitute the bran (Baublis *et al.*, 2000; Onyeneho and Hettiarachchy, 1992). Cereal grains are rich in phenolic acids and the total quantities may approach 500 mg/Kg of edible cereal (Senter *et al.*, 1983). Wheat bran contains significant levels of antioxidants, among which phenolic acids seem to have the greatest potential of being beneficial to health (Baublis *et al.*, 2000).Wheat varieties significantly differ in their antioxidant properties, total phenolic contents (TPCs) and phenolic acid compositions. These are influenced by genotype and /or environmental conditions where they are grown (Yu *et al.*, 2002; Onyeneho and Hettiarachchy, 1992; Zielinski and Kozlowska, 2000; Zhou and Yu, 2004). Mpofu *et al.*, (2006) reported as both genotype and environmental effects were significantly ($p < 0.0001$) higher on TPC, antioxidant activities and phenolic acid compositions analyzed in the study. However, genotype effects were comparatively lower (17%-63%) than environment (30%-71%), depending on the component analyzed. They also suggest that, because of highly significant differences of genotype, it would be possible to select for these quantitative traits in a breeding program.

Ferulic, vanillic and *p*-coumaric acids were the major phenolics found in wheat bran extract (Onyeneho and Hettiarachchy, 1992; Kahkonen *et al.*, 1999) along with minor phenolics caffeic, syringic, chlorogenic, genestic and *p*-hydroxy benzoic acids. Ferulic acid was the predominant phenolic acid in wheat and accounts for between 46-70% of total phenolics measured (Zhou *et al.*, 2004a; Onyeneho and Hettiarachchy, 1992; Adom *et al.*, 2003; Mpofu *et al.*, 2006). The average percentage contributions to the total ferulic acid by free, soluble-conjugated and bound ferulic contents were approximately 0.2, 1.2 and 98% respectively (Abdel-Aal *et al.*, 2001; Adoms and Liu, 2002; Adom *et al.*, 2003). The amount and activity of antioxidants such as phenolic acids were significantly influenced by the extraction procedures (Zielinski and Kozlowska, 2000), hydrolysis method (Kader *et al.*, 1996; Nuutila *et al.*, 2002) and sample preparation techniques (Onyeneho and Hettiarachchy, 1992). Ferulic acid ranged from 90-230 μg/g bran (Zhou *et al.*, 2004b) whereas it approached 650 μg/g of grain in soft wheats (Moore *et al.*, 2005).

Policosanols (PC) are mixtures of long chain aliphatic primary alcohols that contain 20-36 carbon atoms. Important policosanols are docosanol (C22), tetracosanol (C24), hexacosanol (C26), octacosanol (C28) and triacontanol (C28). Although the beeswax and sugar cane extracts are the major sources of PCs, wheat could also be a viable PC source that offers health benefits (Irmak *et al.*, 2006). Consumption of wheat germ oil with its particularly high octacosanol level was found to lead to better physical fitness (Consolazio *et al.*, 1964; Cureton, 1963). Several studies indicate that consumption of PCs cause a reduction in cholesterol levels by inhibiting cholesterol synthesis and also by increasing the LDL processing (Castano et al., 1996; Gouni-Berthold and Berthold, 2002). In addition, PCs also show effects in reduced platelet aggregation (Arruzabala *et al.*, 1993; Valdes *et al.*, 1996) and LDL peroxidation (Fraga *et al.*, 1997). However, recent studies questioned the effect of policosanol in reducing blood cholesterol levels (Dulin *et al.*, 2006; Greyling, 2006).

Irmak *et al.*, (2006) reported significant variation of PCs between the different sources; bees wax had 20-fold higher total policosanol contents than wheat germ oil and 45 times higher compared to sugar cane peel. Wheat straw had the lowest total PCs (164 mg/Kg). They also reported that the PC compositions of the commercial dietary samples

analyzed, contained less amounts than actually claimed on the product labels. The PC contents and compositions varied significantly in different milling fractions of wheat (Irmak *et al.*, 2005). The total PC contents (mg/Kg) of wheat bran (29.97) were higher followed by germ (10.1), shorts (3.29) and flour (0.17). Tetracosanol (C24), hexacosanol (C26) and octacosanol (C28) were the major PCs found in all wheat milling fractions in descending order of quantities.

References

Abdel-Aal, E. -S. M., Huci, P., Sosulski, F. W., Graf, R., Gilliott, C., and Pietrzak, L. 2001.Screening spring wheat for midge resistance in relation to ferulic acid content. J. Agric. Food Chem. 49:3559-3566.

Abdul-Hamid, A., and Luan, Y. S. 2000. Functional properties of dietary fiber prepared from defatted rice bran. Food Chem. 68:15-19.

Adom, K. K., and Liu, R. H. 2002. Antioxidant activity of grains. J. Agric. Food Chem. 50:6182-6187.

Adom, K. K., Sorrels, M. E. and Liu, R. H. 2003. Phytochemical profiles and antioxidant activity of wheat varieties. J. Agric. Food Chem. 51:7825-7834.

Adom, K. K., Sorrells, M. E., and Liu, R. H. 2005. Phytochemicals and antioxidant activity of milled fractions of different wheat varieties. J. Agric. Food Chem. 53:2297-2306.

Amadou, H. I., Kaltsikes, P. J., and Bebeli, P. J. 2003. Agronomic characters of near-isogenic lines of Durum wheat (*Triticum durum Desf.*). Breed. Sci. 53:319-324.

Amaya, A., Pena, R. J, and Varughese, G. 1986. Influence of grain hardness on the milling and baking performance of recently developed triticales. Pages 511-524 in: Proc. Int. Triticale Symp. Darvey, N. L, ed. Australian Institute of Agricultural Science, occasional publication no.24, Sydney, Australia.

Arruzazabala, M. L, Carbajal, D. R. M, Garcia, M., and Fraga, V. 1993. Effects of policosanol on platelet aggregation in rats. Thrombosis Res. 69:321-327.

Araki, E., Miura, H., and Sawada, S. 2000. Differential effects of the null alleles at the three *Wx* loci on the starch-pasting properties of wheat. Theor. Appl. Genet. 100:1113-1120.

Baik, B.-K., and Lee, M.-R. 2003. Effects of starch amylase content of wheat on textural properties of white salted noodles. Cereal. Chem. 80:304-309.

Bailey, C. H. 1941. A translation of Becaari's lecture "concerning grain" (1721). Cereal Chem. 18:555.

Baublis, A. J., Clydesdale, F. M., and Decker, E. A. 2000. Antioxidants in wheat-based breakfast cereals. Cereal Foods World. 45:71-74.

Bean, S. R., and Lookhart, G. 2001. Factors influencing the characterization of gluten proteins by size-exclusion chromatography and multiangle laser light scattering (SEC-MALLS). Cereal Chem. 78:608-618.

Beaux, Y., and Martin, G. 1985. Bread making aptitude in hexaploid triticale. Pages 651-655 in: Proc. Genetics and breeding in triticale, Eucarpia meeting, M. Bernard and S. Bernard, eds. INRA, Paris, France.

Bietz, J. A., Huebner, F. R., and Wall, J. S. 1973. Glutenin, the strength protein of wheat flour. Baker's Dig. 47:26-31, 34-35, 67.

Boiteux, L. S., Giordano, L. De. B., Furumoto, O., Arago, F. A. S. 1995. Estimating the pleotropic effect of the *jointless-2* gene on the processing and agronomic traits of tomato by using near-isogenic lines. Plant Breed. 114:457-459.

Boyer, C. D., and Hannah, L. C. 1994. Kernel mutants of Corn. Pages 2-8 in Specialty of Corns. A. Hallauer, ed. CRC Press, Inc., Boca Raton.

Bushuk, W. and Sapirstein, H. D. 1990. Modified nomenclature for gliadins. Pages 454-458 in: Gluten Proteins. W. Bushuk and R. Tkachuk, eds. American Association of Cereal Chemists: St. Paul, MN.

Campbell, W. P., Wrigley, C. W., Cressey, P. J., and Slack, C. R. 1987. Statistical correlations between quality attributes and grain-protein composition for 71 hexaploid wheats used as breeding parents. Cereal Chem. 64:293-299.

Castano, G., Tula, L., Canetti, M., Morera, M., Mas, R., Illnait, J., Fernandez, L., and Fernandez, J.C. 1996. Effects of policosanol on hypersensitive patients with type II hypercholesterolemia. Curr. Ther. Res. 57:691-699.

Consolazio, C. F., Matous, L. O., Nelson, R. A., Issac, G. J., and Hursh, L. M. 1964. Effect of octacosanol, wheat germ oil and vitamin E on performance of swimming rats. J. Appl. Physiol. 19:265-267.

Cressey, P. J., Campbell, W. P., Wrigley, C. W., and Griffin, W. B. 1987. Statistical correlations between quality attributes and grain-protein composition for 60 advanced lines of crossbred wheat. Cereal Chem. 64:299-301.

Cureton, T. K. 1963. Improvements in physical fitness associated with a course of U.S. Navy underwater trainees, with and without dietary supplements. Res. Q. 34:440-453.

D'Ovido, R., Simeone, M., Masci, S., Porceddu, E., and Kasarda, D. D. 1995. Nucleotide sequence of a γ–gliadin type gene from a durum wheat: correlation with a γ–type glutenin subunit from the same biotype. Cereal Chem. 72:443-449.

Doxastakis, G., Zafiriadis, I., Irakli, M., Marlani, H., and Tananaki, C. 2002. Lupin, soya and triticale addition to wheat flour doughs and their effect on rheological properties. Food Chem. 77: 219-227.

Dulin, M. F., Hatcher, L. F., Sasser, H. C., Barringer, T. A., 2006. Policosanol is ineffective in the treatment of hypercholesterolemia: a randomized controlled trial. Am. J. Clin. Nutr. 84: 1543-1548.

Dupis, B., Bushuk, W., and Sapirstein, H. D. 1996. Characterization of acetic acid soluble and insoluble fractions of glutenin of bread wheat. Cereal Chem. 73:131-135.

Echt, C. S., and Schwartz, D., 1981. Evidence for the inclusion of controlling elements within the structural gene at the *waxy* locus in Maize. Genetics. 99:275-284.

Eriksson, G. 1970. The waxy character. Hereditas. 63:180-204.

Finney, K. F. 1943. Fractionating and reconstituting techniques as tools in wheat flours research. Cereal Chem. 20:381-396.

Finney, K. F., Jones, B. L., and Shogren, M. D. 1982. Functional (bread-making) properties of wheat protein and fractions obtained by ultracentrifugation. Cereal Chem. 59:449-453.

Flavell, R. B., Payne, P. I., Thompson, R. D., and Law, C. N. 1984. Strategies for the improvement of wheat-grain quality using molecular genetics. Biotechnol. Genetic Eng. Reviews. 2:157-173.

Fraga, V., Menendez, R., Amor, A. M., Gonalez, R. M., Jimenez, S., and Mas, R. 1997. Effect of policosanol on in vitro and in vivo rat liver microsomal lipid peroxidation. Arch. Med. Res. 28:355-360.

Fujita, N., Wadano, A., Kozaki, S., Okabe, S., and Taira, T. 1996. Comparison of the primary structure of *Waxy* proteins (Granule-Bound Starch Synthase) between polyploidy wheats and related diploid species. Biochem. Genet. 34:403-413.

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

Gianibelli, M. C., Larroque, O. R., Chan, P., Margiotta, B., De Ambrogio, E., MacRitchie, F., and Lafiandra, D. 1998. Effects of the allelic variants at the *Gli*-1/*Glu*-3 and *Gli*-2 loci on mixing properties. Pages 154-156 in: Proc. Int. Wheat Genetics Symposium, 9th Vol. 4. A. E. Slinkard, ed. University of Saskatchewan: Saskatchewan, SK, Canada.

Gianibelli, M. C., Larroque, O. R., MacRitchie, F., and Wrigley, C. W. 2001. Biochemical, genetic and molecular characterization of wheat glutenin and its component subunits. Cereal Chem. 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.). J. Cereal Sci. 35:265-272

Gouni-Berthold, I., and Berthold, H. K. 2002. Policosanol: clinical pharmacology and therapeutic significance of a new lipid-lowering agent. Am. Heart J. 143:356-365.

Graybosch, R. A. 1998. Waxy wheats: Origin, properties, and prospects. Trends Food Sci. Technol. 9:135-142.

Greyling, A., DeWitt, C., Oosthuizen, W., Jerling, J. C., 2006. Effects of a policosanol supplement on serum lipid concentrations in hypercholesterolaemic and heterozygous familial hypercholesterolaemic subjects. Brit. J. Nutr. 95: 968-975.

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

Gupta, R. B., MacRitchie, F., Shepherd, K. W., and Ellison, F. 1991. Relative contributions of LMW and HMW glutenin subunits to dough strength and dough stickiness of bread wheat. Pages 71-80 in: Gluten Proteins 1991. W. Bushuk and R. Tkachuk, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.

Gupta, R. B., Shepherd, K. W., and MacRitchie, F. 1991. Genetic control and biochemical properties of some high molecular weight albumins in bread wheat. J. Cereal Sci. 13; 221-235.

Gupta, R. B., Batey, I. L., and MacRitchie, F. 1992. Relationships between protein composition and functional properties of wheat flours. Cereal Chem. 69:125-131.

Gupta, R. B., Khan, K., and MacRitchie, F. 1993. Biochemical basis of flour properties in bread wheats. I. Effects of variation in quantity and size distribution of polymeric proteins. J. Cereal Sci. 18:23-44.

Gupta, R. B., and Shepherd, K. W. 1993. Production of multiple wheat-rye IRS translocation stocks and genetic analysis of LMW subunits of glutenin and gliadins in wheat using these stocks. Theor. Appl. Genet. 85:719-728.

Gupta, R. B., Paul, J. G., Cornish, G. B., Palmer, G. A., Bekes, F., and Rathjen, A. J. 1994. Allelic variation at glutenin subunit and gliadin loci, *Glu-1*, *Glu-3*, and *Gli-1* of common wheats. II. Its additive and interaction effects on dough properties. J. Cereal Sci. 19:9-17.

Gupta, R. B., and MacRitchie, F. 1994. Allelic variation at glutenin subunit and gliadin loci, *Glu-1*, *Glu-3*, and *Gli-1* of common wheats. II. Biochemical basis of the allelic effects on dough properties. J. Cereal Sci. 19:19-29.

Gupta, R. B., Popineau, Y., Lefebre, J., Cornec, M., and MacRitchie, F. 1995. Biochemical basis of flour properties in bread wheats. II. Changes in polymeric protein formation and dough/gluten properties associated with the loss of low M_r or high M_r glutenin subunits. J. Cereal Sci. 21:103-116.

Hayakawa, K., Tanaka, K., Nakamura, T., Endo, S., and Hoshino, T. 2004. End use quality of waxy wheat flour in various grain based foods. Cereal Chem. 79:102-107.

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

Hung, P. V., Maeda, T., and Morita, N. 2006. Waxy and high-amylose wheat starches and flours-characteristics, functionality and application. Trends Food Sci. Technol. 17:448-456.

Hussain, A., Lukow, O. M., Watts, B. M., and McKenzie, R. I. H. 1997. Rheological properties of full-formula doughs derived from near-isogenic 1BL/1RS translocation lines. Cereal Chem. 74:242-248.

Irmak, S., Dunford, N. T., and Milligan, J. 2006. Policosanol contents of beeswax, sugar cane and wheat extracts. Food Chem. 95:312-318.

Irmak, S., and Dunford, N. T. 2005. Policosanol contents and compositions of wheat varieties. J. Agric. Food Chem. 53:5583-5586.

Jackson, E. A., Holt, L. M., and Payne, P. I. 1983. Characterization of high molecular weight gliadin and low molecular weight glutenin subunits of wheat endosperm by two-dimensional electrophoresis and chromosomal localisation of their controlling genes. Theor. Appl.Genet. 66:29-37.

Jackson, E. A., Holt, L. M., and Payne, P. I. 1985. *Glu-B2*, a storage protein locus controlling the D group of LMW subunits in bread wheat (*Triticum aestivum*).Genet. Res. Cambridge 47:11-17.

Jacobs, D. R. Jr., Meyer, K. A., Kushi, L. H., and Folsom, A. R. 1998. Whole grain intake may reduce risk of coronary heart disease death in postmenopausal women: The Iowa Women's health study. Am. J. Ckin. Nutr. 68:248-257.

Jacobs, D. R., Meyer, H. E., and Solvoll, K. 2001. Reduced mortality among whole grain bread eaters in men and women in the Norwegian county study. Eur. J. Clin. Nutr. 55:137-143.

Johansson, E., Henriksson, P., Svensson, G., and Heneen, W. K. 1993. Detection, chromosomal location and evaluation of functional value of a novel high M_r glutenin subunit found in Swedish wheats. J. Cereal Sci. 17:237-245.

Jones, R. W., Babcock, G. E., Taylor, N. W., and Senti, F. R. 1961. Molecular weights of wheat gluten fractions. Arch. Biochem. Biophys. 94:483-488.

Kader, F., Rovel, B., Giradin, M., and Metche, M. 1996. Fractionation and identification of the phenolic compounds of high bush blueberries (*Vacinium corymbosum*, L.). Food Chem. 55:35-40.

Kahkonen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J.-P., Pihlaja, K., Kujala, T. S. et al. (1999). Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. 47:3954-3962.

Kasarda, D. D. 1989. Glutenin structure in relation to wheat quality. Pages 277-302 in: Wheat is Unique. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

Kieffer, R., Wieser, H., Henderson, M. H., and Graveland, A. 1998. Correlations of the bread making performance of wheat flour with rheological measurements on a micro-scale. J. Cereal Sci. 27:53-60.

Kumlay, A. M., Baenziger, P. S., Gill, K. S., Shelton, D. R., Graybosch, R. A., Lukaszewski, A. J., and Wesenberg, D. W. 2003. Understanding the effect of rye chromatin in bread wheat. Crop Sci. 43:1643-1651.

Lafferty, J., and Lelley, T. 2001. Introduction of high molecular weight glutenin subunits 5+10 for the improvement of bread making quality of hexaploid triticale. Plant Breed. 120:33-38.

Lafiandra, D., D'Ovidio, R., Porceddu, E., Margiotta, B., and Colaprico, G. 1993. New data supporting high molecular glutenin subunit 5 as the determinant of quality differences among the pairs 5+10 vs. 2+12. J. Cereal Sci. 18:197-205.

Lafiandra, D., Margiotta, B., Colaprico, G., Masci, S., Roth, M. R., and MacRitchie, F. 2000. Introduction of the D-genome related high- and low-M_r glutenin subunits into durum wheat

and their effect on technological properties. Pages 51-54 in: Proceedings of the Gluten 2000 Workshop. P.R. Shewry and A.S.Tatham, eds. R. Soc. Chem.: London.

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 wheat. Genome. 34:375-386.

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

Larroque, O. R., Gianibelli, M. C., and MacRitchie, F. 1999. Protein composition for pairs of wheat lines with contrasting dough extensibility. J. Cereal Sci. 9:27-31.

Lasztity, R. 1999. Cereal Chemistry in Pages 52-125. Akademial Kiado, Budapest, Hungary.

Lawrence, G. J., MacRitchie, F., and Wrigley, C. W. 1988. Dough and baking quality of wheat lines deficient in glutenin subunits controlled by the *Glu-A1*, *Glu-B1* and *Glu-D1* loci. J. Cereal Sci. 7:109-112.

Lee, M.-R., Swanson, B. G., and Baik, B.-K. 2001. Influence of amylose content on properties of wheat starch and bread making quality of starch and gluten blends. Cereal Chem. 78:701-706.

Lew, E. J. L., Kuzmicky, D. D., and Kasarda, D. D. 1992. Characterization of low molecular weight glutenin subunits by reversed-phase high-performance liquid chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and N-terminal amino acid sequencing. Cereal Chem. 69:508-515.

Liu, S., Stampfer, H. J., Hu, F. B., Giovannucci, E., Rimm, E., Manson, J. E., Hennekens, C. H., and Willett, W. C. 1999. Whole grain consumption and risk of coronary heart disease: Results from the Nurse's Health Study. Am. J. Clin. Nutr. 70:412-419.

Liu, S., Manson, J. E., Stampfer, H. J., Rexrode, K. M., Hu, F. B., Rimm, E. B., and Willett, W. C. 2000a. Whole grain consumption and risk of ischemic stroke in women: A Prospective Study. Am. J. Med. Assoc. 284:1534-1540.

Liu, S., Manson, J. E., Stampfer, H. J., Hu, F. B., Giovannucci, E., and Willett, W. C. 2000b. A prospective study of whole grain intake and risk of type 2 diabetes mellitus in US women. Am. J. Public Health. 90:1409-1415.

Lookhart, G. L. 1997. New methods helping to solve the gluten puzzle. Cereal Foods World. 42:16-19.

Lukaszewski, A. J., and Curtis, A. C. 1992. Transfer of the *Glu-D*1 gene from chromosome 1D of bread wheat to chromosome 1R in hexaploid triticale. Plant Breed. 109:203-210.

Lukaszewski, A. J. and Curtis, A. C. 1994. Transfer of the *Glu-D*1 gene from chromosome 1D to chromosome 1A in hexaploid triticale. Plant Breed. 112:177-182.

Lukaszewski, A. J. 1996. Cytogenetic manipulation of the endosperm storage protein loci in the improvement of bread making quality of triticale. Genet. Pol. 37 A: 27-35.

Lukaszewski, A. J. 1998. Improvement of bread making quality of triticale through chromosome translocations. Pages 102-110 in: Proc. 4th Int. Triticale Symp., P. Juskiw ed. Red Deer, Alberta, Canada. July 26-31, 1990, International Triticale Assoc. Vol. I.

MacRitchie, F. 1980. Physicochemical aspects of some problems in wheat research. Pages 271-326 in: Advances in Cereal Science and Technology, Vol. III, Y. Pomeranz, ed. American Association of Cereal Chemists, St. Paul., MN.

MacRitchie, F. 1987a. Evaluation of contributions from wheat protein fractions to dough mixing and bread making. J. Cereal Sci. 6:259-268.

MacRitchie, F. 1987b. Identification of quality components of gluten proteins using fractionation and reconstitution methods. Pages 247-253 in: Proc. of the 3rd International workshop on Gluten Proteins, R. Lasztity and F. Bekes, eds. Budapest, Hungary.

MacRitchie, F. 1989. Identifying the baking quality related components of wheat flours. Cereal Foods World. 34:548-552.

MacRitchie, F., Du Cros, D. L., and Wrigley, C. W. 1990. Flour polypeptides related to wheat quality. Pages 79-145 in: Advances in Cereal Science and Technology. X.Y. Pomeranz, ed. AACC: St. Paul, MN.

MacRitchie, F., Kasarda, D. D., and Kuzmicky, D. D. 1991. Characterization of wheat protein fractions differing in contributions to breadmaking quality. Cereal Chem. 68:122-130.

MacRitchie, F. 1992. Physicochemical properties of wheat proteins in relation to functionality. Adv. Food Nutr. Res. 36:1-87

MacRitchie, F., and Lafiandra, D. 1997. Structure-function relationships of wheat proteins. Pages 293-323 in: Food Proteins and Their Applications. S. Damodaran and A. Paraf, eds. Marcel Dekker Inc: New York.

MacRitchie, F., and Lafiandra, D. 2001. Use of near-isogenic wheat lines to determine protein composition-functionality relationships. Cereal Chem. 78:501-506.

Margiotta, B., Urbano, M., Colaprico, G., Johansson, E., Buonocore, F., D'Ovidio, R., and Lafiandra, D. 1996. Detection of y-type subunit at the *Glu-A1* locus in some Swedish bread wheat lines. J. Cereal Sci. 23:203-211.

Martin, J. M., Talbert, L. E., Habernicht, D. K., Lanning, S. P., Sherman, J. D., Carlson, G., and Giroux, M. J. 2004. Reduced amylase effects on bread and white salted noodle quality. Cereal Chem. 81:188-193.

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

Masci, S., Lew, E. J. L., Lafiandra, D., Porceddu, E., and Kasarda, D. D. 1995. Characterization of low molecular weight glutenins type 1 and type 2 by RP-HPLC and N-terminal sequencing. Cereal Chem. 72:100-104.

Masci, S., Ovido, R. D., Lafiandra, D., and Kasarda, D. D. 1998. Characterization of a low-molecular weight glutenin subunit gene from bread wheat and the corresponding protein that represents a major subunit of the glutenin polymer. Plant Physiol. 118:1147-1158.

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

Metakovsky, E. V., Akhmedov, M. G. and Sozinov, A. A. 1986. Genetic analysis of gliadin-coding genes reveals gene clusters as well as single remote gene. Theor. Appl. Genet. 73:278-285.

Meyer, K. A, Kushi, L. H., Jacob, D. R Jr., Slavin, J., Sellers, T. A., and Folsom, A. R. 2000. Carbohydrates, dietary fiber, incident type 2 diabetes mellitus in older women. Am. J. Clin. Nutr. 71:921-930.

Miura, H., Tanii, S., Nakamura, T., and Watanabe, N. 1994. Genetic control of amylose content in wheat endosperm starch and differential effects of three *Wx* genes. Theor. Appl. Genet. 89:276-280.

Miura, H., Araki, E., and Tarui, S. 1999. Amylose synthesis capacity of the three *Wx* genes of wheat cv. Chinese spring. Euphytica. 108:91-95.

Moore, J., Hao, Z., Zhou, K., Luther, M., Costa, J., and Yu, L. 2005. Carotenoid, tocopherol, phenolic acid, and antioxidant properties of Maryland-grown soft wheat. J. Agric. Food Chem.53:6649-6657.

Morita, N., Maeda, T., Miyazaki, M. Yamamori, M., Miura, H. And Ohtsuka, I. 2002a. Effect of substitution of waxy-wheat flour for common flour on dough and baking properties. Food Sci. Technol. Res. 8:110-124.

Morita, N., Maeda, M., Miyazaki, M., Yamamori, M., Miura, H., and Ohtsuka, I. 2002b. Dough and baking properties of high amylose and waxy wheat flours. Cereal Chem. 79:491-495.

Mpofu, A., Sapirstein, H. D., and Beta, T. 2006. Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. J. Agric. Food Chem. 54:1265-1270.

Naeem, H. A., Darvey, N. L., Gras, P. W, and MacRitchie, F. 2002. Mixing properties, baking potential, and functionality changes in storage proteins during dough development of triticale-wheat flour blends. Cereal Chem. 79:332-339.

Nakamura, T., Yamamori, M., Hidaka, S., and Hoshino, T. 1992. Expression of HMW waxy protein in Japanese common wheat (*Triticum aestivum* L.) cultivars in Japan. J. Breed. 42:681-685.

Nakamura, T., Yamamori, M., Hirano, H., and Hidaka, S. 1993.Decrease of waxy (*wx*) protein in two common wheat cultivars with low amylase content. Plant Breed. 111:99-105.

Nakamura, T., Yamamori, M., Hirano, H., Hidaka, S., and Nagamine, T. 1995. Production of waxy (amylose-free) wheats. Mol. Gen. Genet. 248:253-259.

Nakamura, T., Vrinten, P., Saito, M., and Konda, M. 2002. Rapid classification of partial waxy wheats using PCR-based markers. Genome. 45:1150-1156.

Nash, D., Lanning, S. P., Fox, P., Martin, J. M., Blake, N. K., Souza, E., Graybosch, R. A., Giroux, M. J., and Talbert, L. E. 2006. Relationship of dough extensibility and dough strength in a spring wheat cross. Cereal Chem. 83:255-258.

Ng, P. K. W., and Bushuk, W. 1988. Statistical relationships between high molecular weight subunits of glutenin and bread-making quality of Canadian-grown wheats. Cereal Chem. 65:408-413.

Nuutila, A. M., Kammiovirta, K., and Oksman-Caldentey, K.-M. 2002. Comparison of methods for the hydrolysis of flavonoids and phenolic acids from onion and spinach for HPLC analysis. Food Chem. 76:519-525.

Oettler, G. (2005). Centenary review: The fortune of a botanical curiosity- Triticale: Past, present and future. J. Agri. Sci. 143: 329-346.

Onyeneho, S. N., and Hettiarchchy, N. S. 1992. Antioxidant activity of durum wheat bran. J. Agric. Food Chem. 40:1496-1500.

Osborne, T. B. 1907. The proteins of the wheat kernel. Carnegie Institution of Washington. Publication No.84.

Park, S. C., and Baik, B.-K. 2004. Significance of amylase content of wheat starch on processing and textural properties of instant noodles. Cereal Chem. 81:165-171.

Payne, P. I., 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. Holt, L. M., Jackson, E. A., and Law, C. N. 1984. Wheat storage proteins: Their genetics and their potential for manipulation by plant breeding. Philos. Trans. R. Soc. London B Biol. Sci. 304:359-371.

Payne, P. I., Jackson, E. A., and Holt, L. M. 1984. The association between γ–gliadin 45 and gluten strength in durum wheat varieties: A direct casual effect or the result of genetic linkage? J. Cereal Sci. 2:73-81.

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 Chem.62:319-326.

Payne, P. I. 1987. Genetics of wheat storage proteins and the effect of allelic variation on bread making quality. Annu. Rev. Plant. Physiol. 38:141-153.

Payne, P. I., Holt, L. M., Harinder, K., Macartney, D. P., and Lawrence, G. J. 1987a. The use of near-isogenic lines with different HMW-glutenin subunits in studying bread making quality an glutenin structure. Pages 216-221 in: Proc. 3rd Int. Workshop on Gluten Proteins. R. Lasztity and F. Bekes, eds. World Scientific Publishing: Singapore.

Pena, R. J., and Balance, G. M. 1987. Comparison of gluten quality in triticale: A fractionation-reconstitution study. Cereal Chem. 64:128-132.

Payne, P. I., Seekings, J. A., Worland, A. J., Jarvis, M. G. and Holt, L. M. Allelic variation of glutenin subunits and gliadins and its effect on breadmaking quality in wheat: analysis of F5 progeny from Chinese Spring´(Hope 1A). J. Cereal Sci. 6: 103-118.

Payne, P. I., Holt, L. M., and Lister, P. G. 1988. *Gli-A3* and *Gli-B3*, two newly designated loci coding for omega-type gliadin and D subunits of glutenin .Pages 999-1002 in: Proc. 7th Int. Wheat Genetics Symp. T.E. Miller and R.M.D. Koebner, eds. Institute of Plant Science Research: Cambridge, UK.

Pena, R. J., and Amaya, A. 1992. Milling and bread baking properties of wheat-triticale grain blends. Cereal Res. Commun. 60:483-487.

Pena, R. J. 1996. Factors affecting triticale as a food crop. Pages 753-761 in Triticale today and tomorrow. H. Guedes-Pinto et al., eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Pirozi, M. 2003. Isogenic wheat lines in determining composition-functionality relationships. Ph.D. thesis submitted to Kansas State University.

Pogna, N. E., Autran, J. C., Mellini, F., Lafiandra, D., and Feillet, P. 1990. Chromosome 1B-encoded gliadins and glutenin subunits in durum wheat: Genetics and relationship to gluten strength. J. Cereal Sci. 11:15-34.

Pogna, N. E., Metakovsky, E. V., Redaelli, R., Raineri, F., and Dachkevitch, T. 1993. Recombination mapping of Gli-5, a new gliadin-coding locus on chromosomes 1A and 1B in common wheat. Theor. Appli. Genet. 87:113-121.

Pogna, N. E., Redaelli, R., Dachkevitch, T., Curioni, A., and Dal Belin Peruffo, A. 1994.Genetics of wheat quality and its improvement by conventional and biotechnological breeding. Pages 205-224 in: Wheat-Production, Properties and Quality. W. Bushuk and V.F. Rasper, eds. Blackie Academic and Professional.

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

Rakowska, M., and Haber, T. 1991. Baking quality of winter triticale. Paes 428-434 in: Proc 2nd Int. Triticale Symp. Passo Fundo, Brazil, 1-5 Oct., 1990, CIMMYT, Mexico D.F., Mexico.

Redaelli, R., Pogna, N. E., Dachkevitch, T., Cacciatori, P., Biancardi, A. M., Metakovsky, E. V. 1992. Inheritance studies of the 1AS/1DS chromosome translocation in the bread-wheat variety Perzivan-1. J. Genet. Breed. 46:253-262.

Rogers, W. J., Rickatson, J. M., Sayers, E. J., and Law, C. N. 1990. Dosage effects of chromosomes of homeologous grou 1 and 6 upo bread making quality in hexaploid wheat. Theor. Appl. Genet. 80:281-287.

Rogers, W. J., Payne, P. I., Seekings, J. A., and Sayers, E. J. 1991. Effect on bread-making quality of x- and y-type high molecular subunits of glutenin. J. Cereal Sci. 14:209-221.

Rooney, L. W., Gustafson, C. B., Perez, N., and Porter, K. B.1969. Agronomic performance and quality characteristics of triticale grown on the Texas high plains. Progress report Texas A&M, Texas Agricultural Experimental Station, TX, USA.

Schofield, J. D., Bottomley, R. C., Timms, M. F., and Booth, M. R. 1983. The effect of heat on wheat gluten and the involvement of sulfhydryl-disulphide interchange reactions. J. Cereal. Sci. 1:241-253.

Sears, E. R. 1954. The aneuploids of common wheat. Res. Bull. Missouri Agric. Exp. Stn. 572.

Sears, E. R. 1966. Nullisomic-tetrasomic combinations in hexaploid wheat. Pages 29-5 in: Chromosome Manipulation and Plant Genetics R. Riley and K.R. Lewis, eds. Oliver and Boyd: Edinburg.

Senter, S. D., Horvat, R. J., and Forbus, W. R. 1983. Comparitive GLC-MS analysis of phenolic acids of selected tree nuts. J. Food. Sci. 48:798-803.

Serna-Saldivar, S. O., Guajardo-Flores, S., and Viesca-Rios, R. 2004. Potential of triticale as a substitute for wheat in flour tortilla production. Cereal Chem. 81:220-225.

Specht, J. E., Williams, J. H., and Pearson, D. R. 1985. Near-isogenic analysis of soybean pubescence genes. Crop Sci. 25:92-96

Shewry, P. R., Tatham, A. S., Forde, J., Kries, M., and Miflin, B. J. 1986. The classification and nomenclature of wheat gluten proteins:A reassessment. J. Cereal Sci. 4:97-106.

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

Shewry, P. R., Tatham, A. S., Barro, F., Barcelo, P., Lazzeri, P. 1995. Biotechnology of bread making: Unraveling and manipulating the multi-protein gluten complex. Biotech. 13:1185-1190.

Shewry, P. R., and Tatham, A. S. 1997. Disulphide bonds in wheat gluten proteins. J. Cereal Sci. 25:207-227.

Shewry, P. R., Popineau, Y., Lafiandra, D. and Belton, P. 2001. Wheat glutenin subunits and dough elasticity: findings of the EURO WHEAT project. Trends Food Sci. Technol. 11:433-441.

Singh, J., Blundell, M. J., Tanner, G., and Skerritt, J. H. Albumin and Globulin proteins of wheat flour:immunological and N-terminal sequence characterization. J.Cereal Sci. 34:85-103.

Singh, N. K., and Shepherd, K. W. 1988. Linkage mapping of the genes controlling endosperm proteins in wheat. 1. Genes on the short arms of group 1 chromosomes. Theor. Appl. Genet. 66:628-641.

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

Stevenson, S. G., and Preston, K. R. 1996. Flow-filed flow fractionation of wheat proteins. J. Cereal Sci. 23:121-131.

Suchy, J., Lukow, O. M., Ingelin, M. E. 2000. Dough microextensibility method using a 2-g Mixograph and Texture Analyzer. Cereal Chemistry 77:39-43.

Taht, R., Kann, A., Kasearu, P., Jaama, E., and Vijand, M. 1998. Baking qualities of winter triticales. In:Food and nutrition Vol.V. pp.76-83. Talinn Technical University, Estonia.

Takata, K., Yamauchi, H., Nishio, Z., and Kuwabara, T. 2001. Bread-making quality of a near-isogenic line with specific low molecular weight glutenin components. Breed. Sci. 51:143-146.

Takata, K., Nishio, Z., Iriki, N., Tabiki, T., Funatsuki, W., and Yamauchi, H. 2005. Comparison of quality characteristics of waxy wheat using near-isogenic line. Breed. Sci. 55:87-92.

Takata, K., Yanaka, M., Fujita, Y., and Ishikawa, N. 2007. Evaluation of the grain and flour quality in near-isogenic wheat lines with waxy and double-null *Wx* proteins. Breed. Sci. 57:79-83.

Tao, H. P., and Kasarda, D. D. 1989. Two-dimensional gel mapping and N-terminal sequencing of LMW-glutenin subunits. J. Exp. Bot. 40:1015-1020.

Thompson, L. U. 1994. Antioxidant and hormone-mediated health benefits of whole grains. Crit. Rev. Food Sci. Nutr. 34:473-497.

Thompson, S., Bishop, D. H. L., Tatham, A. S., and Shewry, P. R. 1994. Exploring di-sulfide bond formation in a lower molecular weight subunit of glutenin using a baculovirus expression system. Pages 345-355 in: Gluten proteins 1993. Assoc. Cereal Res.: Detmold, Germany.

Tohver, M., That, R., Kann, A., and Rahnu, I. 2000. Investigation of seed storage protein and bread-making quality of triticale. Acta Angron. Hungarica. 48:41-49.

Tohver, M., Kann, A., That, R., Mihhalevski, A., and Hakman, J. 2005. Quality of triticale cultivars suitable for growing and bread-making in northern conditions. Food Chem. 89:125-132.

Truswell, A. S. 2003. Cereal grains and coronary heart disease. Eur. J. Clin. Nutr. 56:1-14

Tsai, C. Y. 1974. The function of the waxy locus in starch synthesis in maize endosperm. Biochem. Genet. 1:83-96.

Uthayakumaran, S., Tomoskozi, S., Tatham, A. S., Savage, A. W. J., Gianibelli, M. C., Stoddard, F. L., and Bekes, F. 2001. Effects of gliadin fractions on functional properties of wheat dough depending on molecular size and hydrophobicity. Cereal Chem. 78:138-141.

Valdes, S., Arruzazabala, M. L., and Fernandez, E. 1996. Effect of policosanol on platelet aggregation in healthy volunteers. Int. J. Clin. Pharm. Res. 16:67-72.

Varughese, G., Pfeiffer, W. H., and Pena, R. J. 1996. Triticale: A successful alternative crop (Part 1&2). Cereal Foods World. 41:474-482,635-645.

Villegas, E. (1973). Improving nutritional quality of triticale. In triticale breeding and research at CIMMYT: A progress report, pp-55-63. CIMMYT Research Bulletin, No: 24. Mexico, CIMMYT.

Wahlund, K.-G., Gustavsson, M., MacRitchie, F., Nylander, T., and Wannerberger, L. 1996. Size characterization of wheat proteins, particularly glutenins, by assymetrical flow field-flow fractionation. J. Cereal. Sci. 23-113-119.

Watanabe, N. 1994. Near-isogenic lines of Durum wheat: their development and characteristics. Euphytica. 72:143-147.

Weegels, P. L., Hamer, R. J., and Schofield, J. D. 1996. Critical review: Functional properties of wheat gluten. J. Cereal Sci. 23:1-18.

Weipert, D. 1986. Triticale processing in milling and baking. Pages 42-411 in: Proc.. Int. Triticale Symp. N.L. Darvey, ed. Australian Institute of Agricultural Science, occasional publication No.24, Sydney, Australia.

Weiser, H., and Kieffer, R. 2001. Correlations of the amount of gluten protein types to the technological properties of wheat flours determined on a micro-scale. J. Cereal Sci. 34:19-27.

Wos, H. R., Meztger, J., Lukaszewski, A.J., and Cygankiewicz, A. 2002. The effect of D-genome chromosome substitutions and of translocations of chromosome 1D on some quality and agronomic parameters of winter triticale. Pages 59-69 in: Proc. 5th Int. Triticale Symp. June30-July5, 2002. E. Arseniuk, ed. IHAR, Radzikow, Poland.

Wrigley, C. W., and Bietz, J. A. 1988. Proteins and amino acids. Pages 159-275 in: Wheat Chemistry and Technology. Y. Pomeronz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

Yamaguchi, I., Kiribuchi, O.-C., Yanagisava, T., Nagamine, T., Ushiyama, T., and Yoshida, H. 2003. Breeding of two waxy wheat cultivars "Akebono-mochi" and "Ibuki-mochi", and their main features. Bull.Natl.Inst. Crop Sci. 3:21-23.

Yamamori, M., Nakamura, T., and Kuroda, A. 1992.Variation in the content of starch-granule bound protein among several Japanese cultivars of common wheat (Triticum aestivum L.) Euphytica.64:215-219.

Yamamori, M., Nakamura, T., Endo, T.R., and Nagamine, T. 1994. Waxy protein deficiency and chromosomal location of coding genes in common wheat. Theor. Appl. Genet. 89:179-184.

Yamamori, M., Nakamura, T., and Kiribuchi-Octobe, C. 1998. Waxy protein alleles in common wheat and emmer wheat germplasm. Misc. Publ. Natl. Inst. Agrobiol. Resour. 12:57-104.

Yasui, T., Matsuki, J., Sasaki, T., and Yamamori, M. 1997. Waxy endosperm mutants of bread wheat (Triticum aestivum L.) and their starch properties. Breed. Sci. 47:161-163.

Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J., and Qian, M. 2002. Free radical scavenging properties of wheat extracts. J. Agric. Food Chem. 50:1619-1624.

Zeller, F. J., and Hsam, S. L. K. 1984.Broadening the genetic variability of cultivated wheat by utilizing rye chromatin. Pages 161-173 in: Proceedings of the sixth International wheat genetics symposium. S. Sakamoto, ed. Kyoto, Japan.

Zhou, K., and Yu, L. 2004. Antioxidant properties of bran extracts from Trego wheat grown at different locations. J. Agric. Food Chem. 52:1112-1117.

Zhou, K., Laux, J. J., and Yu, L. 2004a. Comparison of Swiss red wheat grain, and fractions for their antioxidant properties. J. Agric. Food Chem. 52:1118-1123.

Zhou, K., Su, L., and Yu, L. 2004b. Phytochemicals and antioxidant properties in wheat bran. J. Agric. Food Chem. 52:6108-6114.

Zielinski, H., and Kozlowska, H. 2000. Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. J. Agric. Food Chem. 48:2008-2016.

CHAPTER 3 - MATERIALS AND METHODS

This chapter deals with the description of methodology followed for all the sample sets used in the study. However, specific details on development of different genotypes are described individually in each sample section.

3.1 Initial Physical Tests and Milling Yield

All the samples were received by the Department of Grain Science and Industry, KSU in the form of whole kernels. Approximately 200-300 g of grain sample was provided for each genotype. Samples were subjected to similar handling, storage and milling procedures. The goal was to avoid any post harvest external effects on samples that would adversely influence the sample performance during analysis. All grain samples received were aspirated to remove foreign material from grains. Cleaned grains were weighed and stored at -20°C in clearly labeled polythene bags until used for milling. Approximately 10-12 kernels of each sample were stored separately for future breeding needs, if necessary.

3.1.1 Single Kernel Characterization System (SKCS)

Samples were thawed and healthy kernels were run through the single kernel characterization system (SKCS) 4100 apparatus (Perten Instruments, Huddinge, Sweden) to get the preliminary information on grains such as moisture content and hardness index values. All measurements were made in duplicate. Observed grain moisture content was used to calculate the amount of moisture needed for tempering before milling.

3.1.2 Experimental Milling

Grain samples were tempered to 14% moisture, approximately 16 hours prior to milling. A Brabender Quadrumat Junior Mill (C.W. Brabender, Duisburg, Germany) was used to mill the samples following AACC method 26-50 (2000). Low ash flour, bran and high ash flour fractions were collected separately and labeled. Bran and low ash flour

fractions were stored at -20°C until needed. High ash flour was discarded after milling yield was determined.

3.2 Analytical Determinations on Flour

3.2.1 Flour Protein Content

Flour protein content (FP) was determined by the nitrogen combustion method (AACC 46-30) using a LECO FP-2000 Nitrogen/Protein analyzer (LECO Corporation, St. Joseph, MI). A factor of 5.7 was used to convert the total nitrogen into protein content. Final protein content was reported on an as is moisture basis.

3.2.2 Flour Moisture Content

Flour moisture content was determined following the air-oven method developed by AACC 44-15A (2000). Final moisture content was calculated as described in method AACC 44-01.

3.3 Protein Characterization and Compositional Analysis

3.3.1 Lab-On-a-Chip

Identification of HMW-GS composition of all flour samples was performed using the lab-on-a-chip method as described by Uthayakumaran *et al.*, (2006). The sample extraction procedure was slightly different from the above method. The lab-on-a-chip procedure, a micro-fluidic analysis of cereal proteins, works on the principle of size-based capillary electrophoresis that allows faster identification of subunit composition and quick quantification of specific glutenin subunits with computerized interpretation.

3.3.1.1 Subunit Identification

Approximately 150mg of flour sample was used to perform sequential extraction of albumins/globulins (two 5 min. extractions using Tris-HCl buffer containing KCl+EDTA, PH 7.8), gliadins (two 5 min. extractions using 50% isopropanol) and glutenins (single extraction for 30 min. using 50% isopropanol+2% DTT+2% SDS buffer, PH 7.0). Each extraction was performed with vortexing followed by centrifugation at 12000 rpm for 2 min.

except for glutenin for which centrifugation was for 10 min. Residue from removal of albumins/globulins and gliadins was used as initial material for glutenin extraction.

Each clarified glutenin extract (4 μL) was mixed with 2 μL of Agilent sample buffer and 84 μL of deionized water. This mixture (6 μL) was applied to one of 10 sample wells on the Agilent protein labchip. Proteins thus extracted were analyzed in an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) with a Protein 230 chip. Each sample contained an internal standard comprising an upper marker of 240 kDa and a lower marker of 4.5 kDa. Each chip included a ladder comprising reference proteins of 7, 15, 28, 46, 63, 95, 150 kDa, plus the lower and the upper markers (4.5 and 240 kDa), against which protein mobilities were compared for each analysis. Samples of well known subunit composition having closely related subunits were selected as controls and run on 2 of the 10 sample wells on each chip to facilitate the identification and comparison of HMW-GS. Those control samples included Chinese Spring, Jagger and Karl-92.

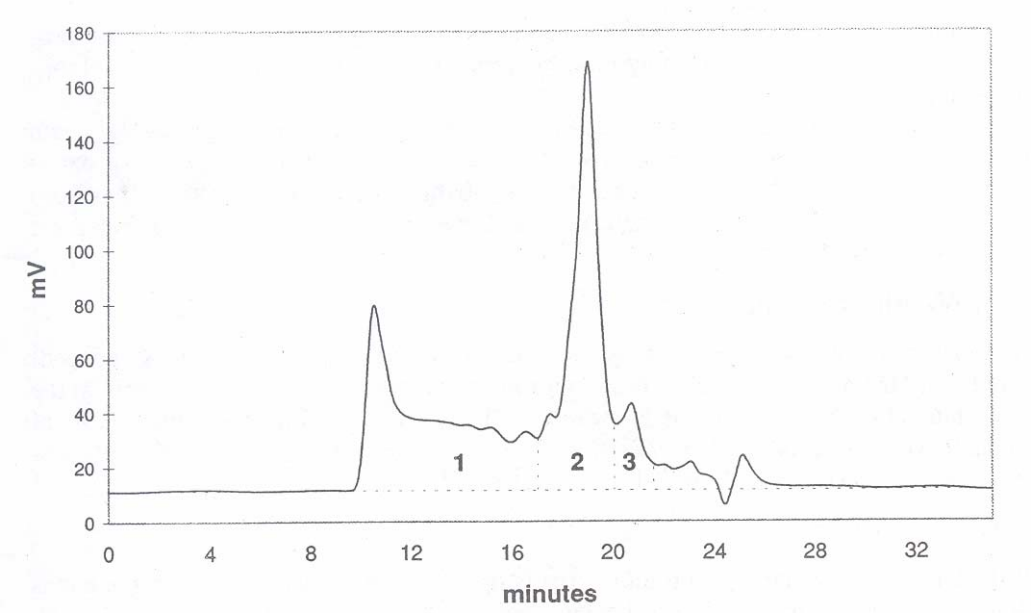
3.3.3 Size Exclusion-High Performance Liquid Chromatography (SE-HPLC)

Flour protein compositions were determined quantitatively using the extraction procedure described by Gupta *et al.*, (1993). Briefly, a 10 mg sample was used to extract total (TPP), extractable (EPP) and unextractable polymeric protein (UPP) fractions. Total polymeric protein (TPP) was extracted with 2% SDS buffer for 5 min by vortexing, sonicated for 15 sec (6 W output) and centrifuged (Eppendorf 1514, Westbury, NY) for 20 min at 12000 rpm. A similar step was followed for the extractable protein fraction (EPP) except the sonication and the residue was used for the unextractable fraction. Unextractable polymeric protein (UPP) was extracted for 10 min. and centrifuged for 20 min. after sonication for 25 sec. Protein samples were stabilized before analysis by heating the samples in a water bath at 85°C for 5 min. and cooled by keeping in ice.

Stabilized protein extracts were injected (20 μL) on to a Phenomenex Biosep-SEC-S4000 (300 x 7.8 mm) size-exclusion column (Phenomenex, Torrance, CA) connected to an 1100 Hewlett-Packard chromatography station (Hewlett-Packard, Waldbronn, Germany). A variable wavelength detector set to 214 nm was used. The column temperature was 24°C. A 1:1 solvent mixture of A (0.05% trifluoroacetic acid (TFA) in acetonitrile) and B (0.05%

TFA in deionized water) was used. All the solvents used were of HPLC grade (Fisher Scientific, Pittsburgh, PA). A flow rate of 1.0 mL/min was employed with a total run time of 28 min. The classic elution profile of polymeric fraction (peak1), gliadins (peak2) and albumins/globulins (peak3) are shown in Fig.3.1.

Figure 3-1: Classic SE-HPLC chromatogram showing the fractions peak 1 (polymeric protein, mainly HMW-GS), peak 2(LMW-GS and monomeric proteins) and peak 3 (albumins + globulins and other minor protein groups present in wheat).



The percentage of UPP in the total polymeric protein (TPP) was calculated as [peak 1 (unextractable) area/peak 1 (total) area] x100, where peak 1 (total) refers to the sum of peak 1 (extractable) and peak 1 (unextractable). The percentage of total extractable protein that was present in polymeric form (PPP) was calculated as (peak 1 area/total area) x100. Percentage of same in the flour was calculated as (percent of peak 1x percent of flour protein)/100. Similar calculations were performed for peaks 2 and 3 from total fraction (TPP) chromatograms.

3.3.4 High Performance Capillary Electrophoresis (HPCE)

The ω-gliadins were detected and quantified using the HPCE following the method of Bean and Lookhart (2000). However, a completely new sample extraction procedure was

followed as shown in Fig. 4.1. A Beckman P/ACE 2100 instrument was used for HPCE analysis. Both reduced and unreduced fractions of soluble (SPP) and insoluble (IPP) polymeric protein fractions were run to check the presence of ω-gliadins (see Fig. 4.1). Injection time was 6 sec. All separations were performed at 30 kV at 45°C in 50-μm i.d. uncoated fused silica capillaries (Polymicro Technologies) that were 27 cm in length. The sample buffer used was 50 mM IDA (pH 2.0) +20% acetonitrile+0.05% hydroxyl propyl methyl cellulose (HPMC). Proteins were detected by UV absorbance at 200 nm. The ω-gliadins were eluted in two separate peaks at the end of the run time (5-7 min.) and were well separated from the rest of the HMW-GS. Two peaks of ω-gliadins were quantified using PACE station and further data analysis was performed with ORIGIN graphics software (MicroCal software, Northampton, MA).

3.3.5 Size Exclusion Chromatography-Multi Angle Laser Light Scattering (SEC-MALLS)

The MWD of Pegaso near-isogenic lines was assessed by running SEC-MALLS as described by Bean & Lookhart (2001) with slight modifications. The total polymeric protein fraction (TPP) was extracted using 100 mg flour dispersed in 1 mL of phosphate/SDS buffer (pH 6.9). The sample was vortexed for 5min.and sonicated for 15 sec at 6 W output followed by centrifugation at 12000 rpm for 20 min. Collected supernatant was stabilized by heating to 85°C for 5 min. and cooled immediately in ice. Because of the high viscosity of the sample, all sample extracts were filtered through 0.45 μm syringe filters (Gelman Acrodisc, Ann Arbor, MI) before injecting on to a HPLC column. A Hewlett-Packard 1090 HPLC instrument was used and samples were run through a Biosep SEC 4000 column (Phenomenex, Torrance, CA). An isocratic run (30 min.) with mobile phase consisting of solvent A (DI water+0.1%TFA) and solvent B (100% acetonitrile+0.1%TFA) were employed. Duplicate runs were performed maintaining column temperature at 40°C with an injection volume of 40 μL and a flow rate of 1.0 mL/min. MALLS data were collected with a multiangle laser light scattering detector (DAWN EOS, Wyatt Technology Corp. Santa Barbara, CA) with 18 detection angles and a DRI (Optilab DSP). MALLS data was analyzed with software program ASTRA 4.50 (Wyatt Technology Corp.) using a dn/dc value of 0.31.

Only the unextractable (first peak in HPLC chromatogram) portion was quantified. The Berry processing method was employed to determine the MWD from light scattering data.

3.4 Quality Tests

3.4.1 Dough Mixing Properties

Dough mixing characteristics were determined with a computerized 10-g mixograph (National Mfg. Co., Lincoln, NE). Data analysis was performed using the Mixsmart software program. Flour samples were mixed using the method AACC 54-40A (2000) with slight modifications according to Gupta *et al.,* (1993). A 2% salt solution by flour weight on a 14% moisture basis was used at constant water addition (65%). Mixing properties such as mixograph dough development time (MDDT) in min., peak height, peak width, width at 8 min. and peak slope were measured. Mixing was done at least in duplicate for all samples with a run time of 10 min.

3.4.2 Dough Extensibility Tests

Extensibility characteristics of dough were evaluated using a micro-extensibility test described by Suchy *et al*., (2000), modified for use with a 10-g mixograph. A TA-XT2 plus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, SMS, Godalming, Surrey, UK) was equipped with a Kieffer dough and gluten extensibility rig as a probe. Water absorption results along with MDDT, analyzed by using a mixograph, were utilized to develop dough suitable for the extensibility test. A 2% NaCl solution (flour weight basis) was also employed as in the mixograph. Dough collected from the mixograph was rolled gently into a ball and placed in a plastic container, covered with a Ziploc plastic bag and kept in the proofing chamber for 20 min. After the resting period, dough was made into sausage shapes with as little manipulation to the dough as possible, and placed over 8-10 channels of a Teflon coated block, that had been prepared by placing thin Teflon strips coated in mineral oil in the channels. Mineral oil was used to avoid the dough sticking to Teflon strips and Teflon block during the experiments. The upper half of the block was placed and clamped tightly by removing the excess dough on both sides of the block. The

dough thus clamped was rested at 30±2°C and 95±1% RH for 40min. Developed dough was immediately used for the extensibility test without any equilibration time. Testing was done as rapidly as possible to avoid temperature variations. Test conditions employed were as follows: pre-test speed of 2.0 mm/sec, test speed of 3.3 mm/sec, post-test speed of 10.0 mm/sec, and trigger force of 5 g. Distance was adjusted according to sample and varied from 75-120 mm. The closest three replications were selected for data analysis. R_{max} (mN), extensibility (mm) and area under the curve (g-mm) was recorded for different dough samples.

3.5 Test Baking

Table 3.1: Formulation used in the micro-bake test

Ingredient	Amount of ingredient	
	(g)	Baker's %
Flour	10.0*	100.0
Sugar	0.6	6.0
Shortening	0.3	3.0
Salt	0.15	1.5
Yeast, Instant Dry	0.113	1.13
Malt	0.025	0.25
Ascorbic acid	50ppm	
Water	As needed**	

* 14% moisture basis

** Water added according to mixograph calculations

Bread loves were baked according to Bread-making test for 10 g of flour described by Shogren and Finney (1984). Bread formulation (shown in Table 3.2) includes mainly; flour (100%), sugar (6%), shortening (3%), Yeast (2%), salt (1.5%) and water as needed. All ingredients were mixed together to optimum gluten development and the mixing times were according to mixograph calculations done earlier. Desired dough temperature was 27°C. Developed doughs were sheeted, folded twice and placed in an open container and fermented for 120 min. in a proofing chamber maintained at 30±1°C and 95±1% RH.

Punch times were 69, 103 and 120 min during the fermentation. The doughs were then re-sheeted and molded using a 10 g molder. Doughs were proofed for 40 min at 30°C. The baking was performed at 232°C for 13 min. Loaf volumes were assessed using the rape seed displacement method (AACC 10-05, 2000) after cooling the baked bread for 2 hours. All bread loaves of developed lines were compared against the bread made from parent lines.

3.6 Phenolics of Bran

3.6.1 Extraction of Phenolic acids

3.6.1.1 Pegaso Wheat Bran

The free phenolic acids were extracted using the method described by Kim *et al.* (2006). Briefly, wheat bran samples were mixed twice with hexane at a 1:4 ratio (w/v) for 1 h at room temperature to remove lipids. Defatted samples were filtered through Whatman #1 filter paper and dried at room temperature. Free phenolic acids in defatted bran samples were extracted with 80% methanol at a 1:5 ratio (w/v) for 3 h at room temperature. The mixture was filtered and the clear solution was concentrated to 3 ml for 1 g bran sample.

The bran residue after extraction of free phenolic acids was dried and hydrolyzed by mixing in 2 M NaOH for 3 h at room temperature to determine the bound phenolic acids. The mixture was acidified to pH=2.0±0.1 with HCl and extracted three times with diethyl ether. Combined ether fractions were dried over anhydrous sodium sulfate. The ether extract was evaporated to dryness and the final residue was reconstituted with 80% methanol to a final volume of 5 ml for 1 g bran.

3.6.1.2 Triticale and Waxy Wheat Bran

The method used to extract phenolic acids was based on the method described by Kim *et al.*, (2006). Triticale and waxy wheat bran samples were defatted twice by stirring in hexane at a 1:4 ratio (w/v) for 1 h at room temperature. The mixture was filtered through Whatman #1 filter paper. Defatted bran samples were dried at room temperature and hydrolyzed by mixing 2 M NaOH in 80% methanol at a 1:5 ratio (w/v) for 3 h at room

temperature to release bound and free phenolic acids in one step. The mixture was acidified to pH=2.0±0.1 with HCl and filtered through glass wool. The clear mixture was extracted three times with diethyl ether. Combined ether fractions were dried over anhydrous sodium sulfate. The ether extract was evaporated to dryness and the final residue was reconstituted with 80% methanol to a final volume of 5 ml for 1 g bran.

3.6.2 Determination of Total Phenolic acid Content (TPC)

The total polyphenolic content of each methanol extract was assayed by the Folin-Ciocalteau assay (Folin and Ciocalteau, 1927; Singleton and Rossi, 1965) with slight modifications. The 0.3 ml methanol extract was mixed with 2 ml diluted Folin-Ciocalteau reagent and 1.6 ml of 7.5% Na_2CO_3. The mixture was stirred and kept at ambient temperature for 2 h. Absorbance at 765 nm was recorded using a spectrophotometer (Philips PU 8625, UK). A calibration curve was prepared using gallic acid at 0.03-0.20 mg/4 ml assay solution. Total phenolic acids in the methanol extracts were expressed as gallic acid equivalents.

3.6.3 Determination of Individual Phenolic acid Content

Individual phenolic acids in the bran extracts were analysed by a Hewlett Packard 1100 Series high performance liquid chromatograph equipped with UV detector (Hewlett-Packard, Palo Alto, CA) and Phenomenex Jupiter C18 column (250 x 4.60 mm, 10 µ, 300 A; Phenomenex, Torrance, CA). The mobile phases of water with 0.05% trifluoroacetic acid (solvent A) and 30% acetonitrile, 10% methanol, 59.95% water and 0.05% trifluoroacetic acid (solvent B) were used at a flow rate of 1.0 ml/min. Total run time was 50 min and the gradient program was as follows: 10% B to 12%B for 16 min, 12% to 38% for 9 min, 38% B to 70% B for 7 min, 70% B to 85% B for 8 min and 85% B to 10% B for 10 min. There was 5 min of post-run for reconditioning. The injection volume was 20 µl. Detection was at 280 nm via a UV detector. Identification and quantification of phenolic acids in samples were performed comparing to chromatographic retention times and areas of external standards. Phenolic acid standards used for peak identification, ferulic acid, vanillic acid, syringic acid, gallic acid, p-hydroxybenzoic acid, chlorogenic acid, caffeic acid, *p*-coumaric

acid, trans-cinnamic acid, were purchased from Sigma and Aldrich (Sigma-Aldrich Corporation, St. Louis, MO) and used without further purification (97% and higher purity). All samples were prepared and analyzed at least in duplicate.

3.7 Policosanol Content in Pegaso Wheat Bran

3.7.1 Extraction and Determination of Policosanol from Wheat Bran

Approximately 8 g of wheat bran sample and 150 ml of petroleum ether were refluxed for 6 h by using a Soxhlet apparatus. The total extracts from three Soxhlet extractions were combined and considered as one replicate. The extracts were filtered and solvent was evaporated from the extract/solvent mixtures using a rotary evaporator (R-114, Buchi Corporation, New Castle, DE). Extraction of policosanol from the extracted oil was performed as described by Irmak *et al.* (2006). PC compounds in the samples were silylated with MSTFA (N-Methyl-N-(trimethylsilyl)trifluoroacetamide, from Pierce, Rockford, USA) in chloroform solvent at 60°C for 15 min. Silylated samples were analyzed by a HP 5890 Series II GC Chromatograph coupled with HP 5972 Series Mass Selective Detector (Agilent Technologies, Palo Alto, USA). The column was Rtx-65 crossbond 65% diphenyl-35% dimethyl polysiloxane column (30 m x 0.25 mm x 0.25 μm film thickness) from Restek (Bellefonte, PA, USA). Oven temperature was as follows: 2 min at 200°C; from 200°C to 285°C with 4°C/min heating rate and maintained at this temperature for 18 min. Inlet temperature was 250°C. MS transfer line temperature was 280°C; the ionization energy was 70 eV and mass range was 100-550 amu. Duplicate samples (2 μl) were injected into GC-MS with 1:30 split ratio. Policosanol compositions of the samples were determined by comparison of their chromatographic retention times with those of the standards and confirmed with an MS spectral library. All policosanol standards (eicosanol (C20), heneicosanol (C21), docosanol (C22), tricosanol (C23), tetracosanol (C24), hexacosanol (C26), and octacosanol (C28), having 97% or higher purity) were purchased from Sigma (Sigma- Aldrich Corportion, St. Louis, MO).

3.8 Statistical Analysis

All extraction runs and analyses were carried out at least in duplicate and in randomized order with mean values being reported. All developed lines in each set were compared statistically with the parent line considered as the control. Analysis of variance (ANOVA) of the results was performed using the General Linear Model procedure of SAS (Software Version 9.1. SAS Institute Inc., Cary, NC). Statistical significance was declared at $P < 0.05$. Linear regression and correlation were also performed using SAS.

References

American Association of Cereal Chemists. 2000. Approved Methods. 10th ed. AACCI: St. Paul, MN. Vols. I and II.

Bean, S. R., and Lookhart, G. L. 2000. Ultrafast capillary electrophoretic analysis of cereal storage proteins and its applications to protein characterization and cultivar differentiation. J. Agric. Food Chem. 48: 344-353.

Bean, S.R., Lookhart, G.L. 2001. Factors influencing the characterization of gluten proteins by size-exclusion chromatography and multiangle laser light scattering (SEC-MALLS). Cereal Chemistry 78, 608-618.

Folin, O., Ciocalteau, V., 1927. On tyrosine and tryptophane determination in proteins. Journal of Biological Chemistry 73, 627–650.

Gupta, R. B., Khan, K., MacRitchie, M. 1993. Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quality and size distribution of polymeric protein. Journal of Cereal Science 18, 23-41.

Irmak, S., Dunford, N.T., Milligan, J., 2006. Policosanol contents of beeswax, sugar cane and wheat extracts. Food Chemistry 95, 312–318.

Kim, K.-H., Tsao, R., Yang, R., Cui, S.W., 2006. Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. Food Chemistry 95, 466–473.

Shogren, M.D., Finney, K.F. 1984. Bread-making test for 10grams of flour. Cereal Chemistry 61 (5):418-423

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybic–phosphotungstic acid reagents. American Journal of Enology and Viticulture 16, 144–158.

Suchy, J.Lukow, O.M., Ingelin, M.E. 2000. Dough microextensibility method using a 2-g Mixograph and Texture Analyzer. Cereal Chemistry 77(1):39-43.

Uthayakumaran, S., Listiohadi, M., Baratta, M., Batey, I.L., and Wrigley, C.W. 2006. Rapid identification and quantitation of high-molecular-weight glutenin subunits. Journal of Cereal Science. 44:34-39.

CHAPTER 4 - CHAIN TERMINATORS

4.1 Introduction:

Wheat gluten proteins are comprised of monomeric (mainly gliadins) proteins having only intramolecular disulphide bonds and glutenins, which are polymers of protein subunits linked by intermolecular disulphide bonds (Kasarda, 1989). The glutenin fraction of wheat plays a key role in producing the unique viscoelastic properties of dough and also in differentiating wheat cultivars based on variations in their bread making qualities (Bietz *et al.*, 1973, Payne *et al.*, 1984). Though the complete mechanisms at a molecular level have not been established for the role of glutenin in forming viscoelastic dough, development of a polymer by means of joining the protein subunits by forming intermolecular disulphide cross-linkages is definitely an essential feature (Ewart, 1990).

Glutenin polymers undergo extension by incorporating the HMW and LMW-GS in a random fashion until the growing polymer chains are terminated by proteins with single and unreacted cysteine residues or low molecular weight thiols (Kasarda, 1989). In other words, for any glutenin subunit to participate in a growing polymer, it has to have at least two cysteine residues (Southan and MacRitchie, 1999). Extensive structural studies on HMW-GS (Shewry et al., 1989) and LMW-GS (Masci et al., 1998) revealed that the requirement of the presence of two or more Cys residues that form intermolecular disulphide bonds is usually fulfilled; thereby they can act as potential chain extenders. A predominance of the chain-extender types in a glutenin polymer should lead to strong gluten with good viscoelastic properties, whereas too much of the chain terminator should have an opposite effect (Masci et al., 1998). Any change in the ratio of chain extenders/chain terminators (Ch.E/Ch.T) could shift the molecular distribution of polymers to lower values and thus change the properties of the dough (Gianibelli *et al.*, 2002).

Based on their mobility in A-PAGE, gliadins are commonly divided into α, β, γ and ω-gliadins (Gianibelli *et al.*, 2001). The latter are sulphur poor whereas the rest of the gliadins contain an even number of Cys residues which form intramolecular disulphide bonds

(Shewry and Tatham, 1997). The molecular weight of ω-gliadins ranges from 60-75 kDa. Studies have reported that there are certain α and γ types of gliadins which are different from normal ones, in that a serine residue has been mutated to a Cys residue (Okita *et al.*, 1985, Scheets and Hedgcoth 1988). These mutations result in proteins with an odd number of Cys residues and the extra Cys will almost certainly have to form an intermolecular disulphide bond, resulting in incorporation of the polypeptide chain into the glutenin fraction. Gianibelli (1996) suggested that the presence of higher concentrations of a specific 71 kDa protein decreases the polymer size and the trend was consistent with subunits that form only one inter-chain disulphide bond. This would thus act as a chain terminator and prevent the growth of glutenin polymer. Based on N-terminal amino acid sequencing, Gianibelli (1996) further concluded that the 71 kDa protein was analogous to ω-gliadins. However, the ω-gliadins are sulphur-poor proteins without any Cys residues. Therefore it was more likely that the 71 kDa polymeric protein would be a mutated ω-gliadin with at least one cysteine codon and would act as a chain terminator.

Hypothesis

Modified (mutated) gliadins or LMW-GS having an odd number of cysteines or LMW compounds having one thiol group can act as chain terminators. This should shift the MWD towards lower values that in turn would be reflected in lower UPP values. Thus a higher number of ω-gliadins cross-linked (bound) to glutenins (SPP in CE) should correlate with UPP (SE-HPLC). A set of near-isogenic lines with a combination of variation at *Glu-1, Gli-1/Glu-3* and *Gli-2* loci is thought to be valuable to investigate this idea.

Objectives

- To seek evidence for the 'role of chain terminators in decreasing UPP values' using Pegaso near-isogenic wheat lines having variation at *Glu-1*, *Gli-1/Glu-3* and /or *Gli-2* deletions
- To examine the influence of chain terminators on the MWD of gluten proteins (in terms of decreasing UPP) using Pegaso near-isogenic wheat lines

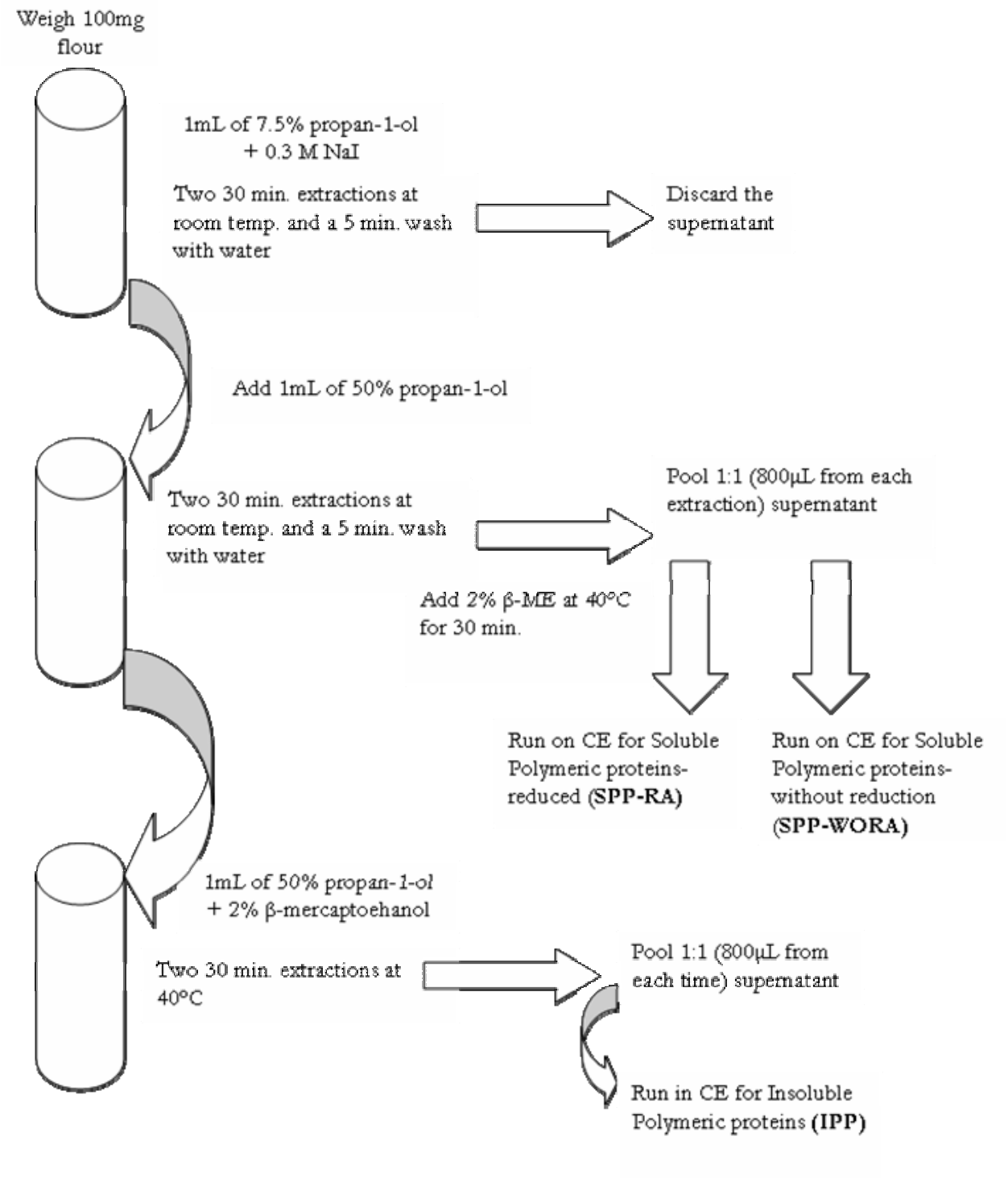
4.2 Methodology

A novel extraction method was developed based on Suchy *et al.,* (2003) and Fu and Sapirstein (1996), to retain the ω-gliadins in the extract. The detailed extraction scheme is shown in Fig. 4.1. Briefly, monomeric proteins were extracted and discarded using a 100 mg flour sample dissolved in 1 mL of 7.5% propan-1-ol +0.3 M NaI. Each extraction continued for 30 min followed by centrifugation (Eppendorf 1514, Westbury, NY) at 10000 rpm for 5 min. The residue was washed with DI water twice for 5 min followed by centrifugation. Two 30 min. extractions for soluble polymeric protein (SPP) fraction using 50% propan-1-ol were performed followed by 5 min centrifugation. Equal amounts of supernatant from each extraction (800 μL) were collected and pooled. Half of the collected supernatant (800 μL) was used to run in CE to check the unreduced fraction of SPP. The other half of the supernatant was mixed with 20 μL of BME to a final concentration of 2% and reduced at 40°C for 30 min. using a shaker. This fraction was labeled as the reduced SPP (SPP-RA). Residues were then extracted with water and centrifuged. The residue was extracted twice with 50% propan-1-ol+2%-BME (30 min. each) at 40°C for insoluble polymeric protein (IPP) followed by 5 min. centrifugation. Equal amounts of supernatant from these steps were pooled and run in CE for the IPP fraction.

The SEC-MALLS analysis was performed to determine the MWD only for the TPP fraction. The extraction procedure was similar to that for SE-HPLC except that the initial flour sample used was 100 mg instead of 10 mg. Briefly, 100 mg of flour sample was extracted with 2% SDS buffer for 5 min by vortexing, sonicated for 15 sec (6 W output) and centrifuged (Eppendorf 1514, Westbury, NY) for 20 min. at 12000 rpm.

Detailed description of the analysis conditions using MALLS, SE-HPLC and HPCE instruments are provided in Chapter 3 of this dissertation.

Figure 4-1: Schematic diagram showing the extraction procedure employed for SPP and IPP fractions run in CE.



4.3 Results and Discussion

The results from CE are shown in Table 4.1. However, for convenience, electrophoregrams are shown only for the samples with highest (Pegaso 170) and lowest (Pegaso 168b) UPP values along with parent line Pegaso. The UPP represents the unextractable polymeric protein. It is assumed that, the higher the UPP, the MWD is shifted to the larger polymers when compared to samples with lower UPP. Two ω-gliadin peaks were identified at 5.3 and 7 min. The elution times of ω-gliadins are in accordance with Bean and Lookhart (2001). The area of these two peaks identified in the SPP-reduced (SPP-RA) fraction is shown in Table 4.1. As described in the hypothesis, we would expect to find more quantities of modified ω-gliadins in the sample with lowest UPP (Pegaso 168b). These would be expected to act as chain terminators and thus result in smaller polymers. Fig. 4.2 A and B shows electrophoregrams of the reduced and non-reduced SPP fractions of the samples respectively. No ω-gliadin peaks were found in the non-reduced (SPP-WORA) fraction as shown in Fig.4.2 B, indicating that the ω-gliadins present in the SPP-RA fraction are cross linked to glutenin and thus appear along with glutenin subunits when extracted with reducing agents. As shown in Fig. 4.3.C a moderate negative correlation (R^2=0.65) exists with 24 NILs of Pegaso, between the %UPP from SE-HPLC and the peak areas from the SPP-RA fraction analyzed by CE. Similar correlations were found with peak 1(R^2=0.64) and peak 2 (R^2=0.59) separately (Fig. 4.3 A and B respectively). On the other hand, almost no correlation (R^2=0.008) is found between %UPP and the ω-gliadin peak areas from the IPP fraction. Reduced UPP values would be caused by ω-gliadins which are known to terminate the further polymerization of glutenin protein subunits which in turn would end up with smaller size proteins rather than high molecular weight polymers. These findings were in support of studies (Lew *et al.*, 1992; Masci *et al.*, 1998 and Kasarda 1989) reporting that ω-gliadins (or any modified/mutated gliadins with an odd number of cysteine residues) would act as chain terminators and negatively affect the polymerization, resulting in lower size protein molecules.

Figure 4-2: Electrophoregrams from CE for (A) SPP (Reduced) (B) SPP (Unreduced) (C) Gliadins and (D) IPP fraction for Pegaso (parent line), Pegaso 170 (Line with highest UPP) and Pegaso 168b (Lowest UPP).

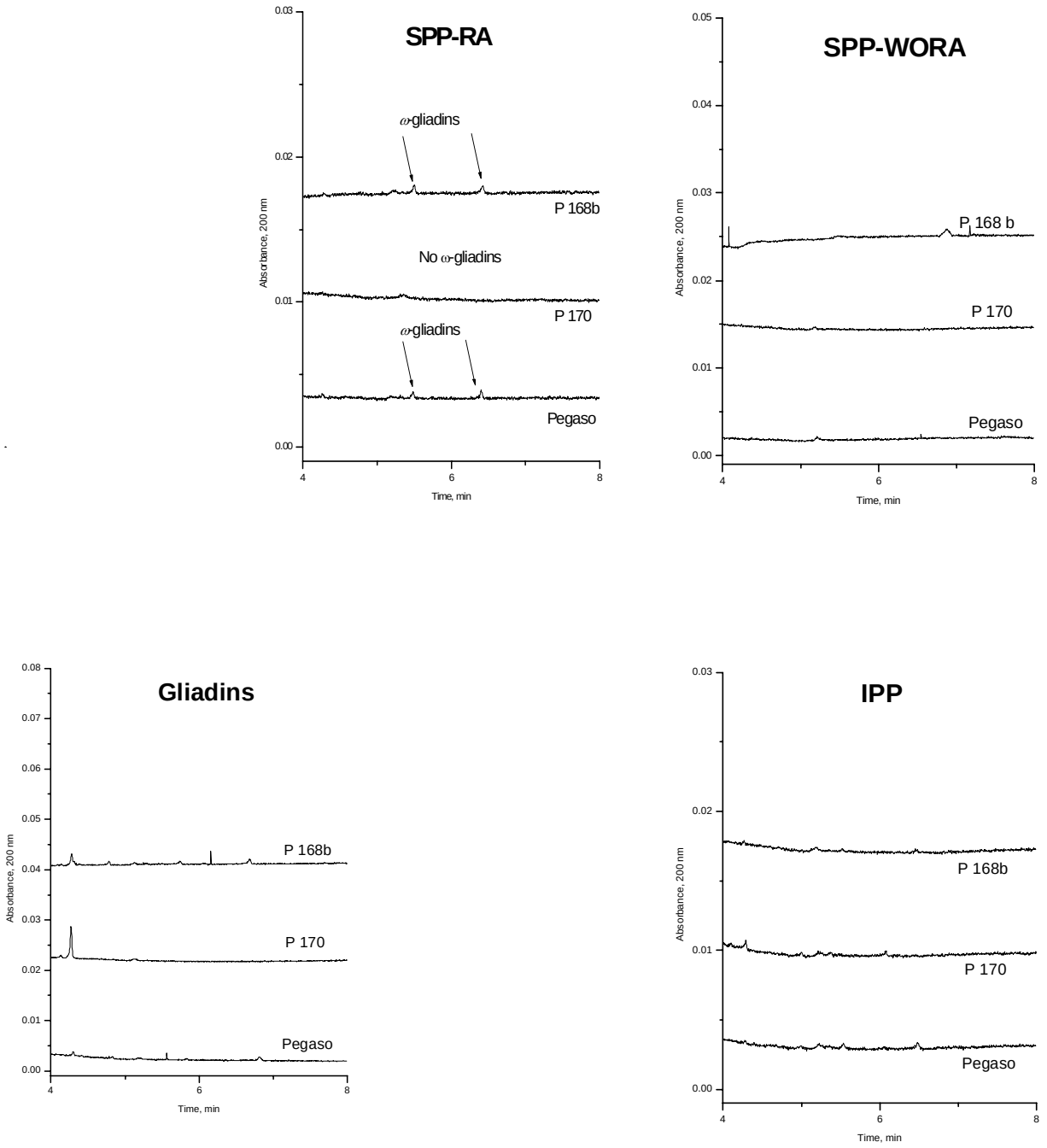
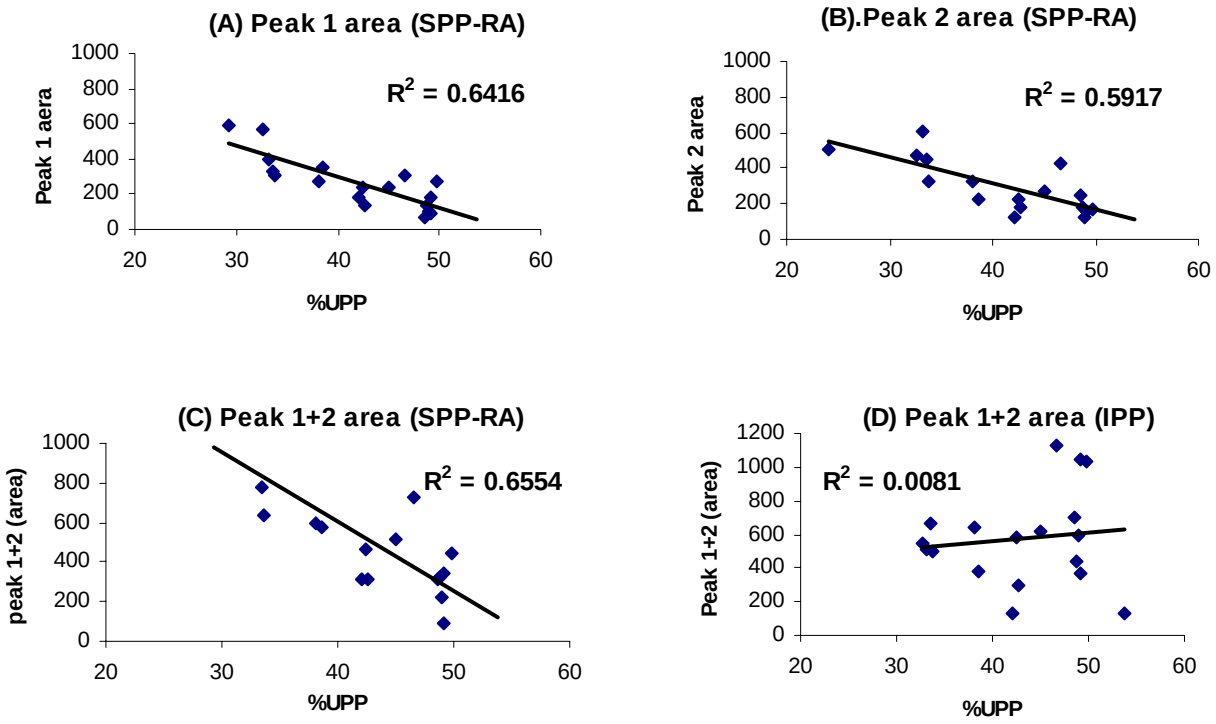


Figure 4-3: Correlation graphs for (A). Peak 1 (ω1) area (B). Peak 2 (ω2) area (C). Peak1 +Peak 2 area of reduced SPP (SPP-RA) fraction analyzed by CE and (D) Peak1+Peak 2 area from IPP fraction.



Glutenin polymers undergo extension by incorporating HMW and LMW-GS in a random fashion until the growing polymer chains are terminated by proteins with single and unreacted cysteine residues or low molecular weight thiols (Kasarda, 1989). In other words, for any glutenin subunit to participate in a growing polymer, it has to have at least two cysteine residues (Southan and MacRitchie, 1999). Studies on amino acid sequence comparison, biochemical analyses and molecular modeling shows that certain typical LMW-GS contain a specific number and distribution of Cys residues that confer the ability to form inter-molecular disulphide bonds with other glutenin subunits and thus contribute to forming the glutenin polymer (D' Ovidio, 2004). On the other hand, mutational events that affect the number of Cys residues in gliadins allow formation of inter-molecular disulphide bonds and ultimately participation in glutenin polymer formation. Most of the C-and D- type

LMW-GS and some of the B-type are α, γ and ω-gliadins that have acquired the ability to participate in polymer formation (Lew *et al.*, 1992; Tao and Kasarda, 1989) and been termed later as modified gliadins, gliadin-like or α, γ and ω-glutenins (D' Ovidio *et al.*, 2000; Masci *et al.*, 2002; Southan and MacRitchie, 1999).

A novel protein with M_r of 71 kDa was characterized by Gianibelli *et al.*, 2002.This protein, assigned to be an ω-gliadin, most likely results from a single-gene mutation. This D-type subunit with only one Cys residue can only serve as a chain terminator to block the further growth of glutenin polymers and thus shift the size distribution to lower M_rs (Tao and Kasarda, 1989; Lew *et al.*, 1992; Kasarda, 1989). The amounts of these D-type subunits collected from SE-HPLC fractions were increased with smaller size polymers. This behavior was consistent with subunits that form only one interchain disulphide bond, and would thus act as chain terminators and reduce the polymer size.

UPP is a parameter that gives a relative measure of the molecular weight distribution of the polymeric protein based on solubility (Gupta *et al.*, 1993). A relatively greater amount of UPP would suggest a shift in molecular weights to higher values. In the present study, a moderate negative correlation (R^2=0.65) exists with 24 NILs of Pegaso, between the %UPP from SE-HPLC and the ω-gliadin peak areas from the SPP-RA fraction analyzed by CE. This indicates that, presence of modified ω-gliadins reduced the glutenin polymer size distribution as reflected in lower UPP values. In other words, these ω-gliadins act as chain terminators and block the further propagation of a glutenin polymer, causing reduction in polymer size distribution.

Table 4.1: Mean peak areas for two omega gliadin fractions analyzed by CE. Percent UPP values are from SE-HPLC and the table is arranged according to descending order for UPP.

Sample Name	%UPP	Peak 1 area	Peak 2 area	Total (Peak1+2) area
Pegaso 170	53.79[a]	ND*	ND*	ND*
Pegaso 236	51.39[b]	ND*	ND*	ND*
Pegaso 235a	49.77[bc]	269.31	174.07	443.38
Pegaso 238	49.17[cd]	86.01	0.00	86.01
Pegaso 106	49.13[cd]	185.87	159.51	345.38
Pegaso 212	48.98[cd]	107.23	119.63	226.86
Pegaso 235b	48.79[cd]	140.66	178.33	318.99
Pegaso 102	48.55[cd]	66.17	244.55	310.71
Pegaso 236a	48.54[cd]	ND*	ND*	ND*
Pegaso 79	47.65[de]	ND*	ND*	ND*
Pegaso 217	46.62[ef]	310.20	421.90	732.10
Pegaso 30	45.05[f]	240.60	271.47	512.07
Pegaso 168c	45.03[f]	ND*	ND*	ND*
Pegaso 184	42.63[g]	136.06	178.85	314.91
Pegaso 170a	42.61[g]	ND*	ND*	ND*
Pegaso 167	42.45[g]	242.42	222.86	465.28
Pegaso (Parent)	42.07[g]	183.52	128.56	312.07
Pegaso 166b	38.61[h]	351.83	227.53	579.36
Pegaso 2.2*	38.13[h]	277.76	321.92	599.67
Pegaso 29	33.70[i]	303.42	327.94	631.36
Pegaso 186	33.52[i]	325.45	452.25	777.70
Pegaso 168a	33.21[i]	402.39	606.70	1009.08
Pegaso 166a	32.63[i]	565.22	474.97	1040.18
Pegaso 168b	29.35[j]	592.18	504.67	1096.84

***ND=Not Detected**

Results from SEC-MALLS are presented in Table 4.2. MWD of the SDS-insoluble portion in total polymeric protein (TPP) was measured. To keep the comparison simple, the same extraction procedure was followed as in SE-HPLC analysis to get the TPP fraction. As shown in Fig. 4.4, the SDS-insoluble portion of TPP was considered to observe the pattern of MWD. This was divided into two equal portions designated as peak1 (first half) and peak2 (remaining half) (Fig. 4.4). These peak identifications were in accord with Bean and Lookhart (2001), who divided the SDS-insoluble portion into S_E (excluded) and S1 and S2 in MALLS analysis

Figure 4.4: Schematic depiction of the peak identification in SEC-MALLS.

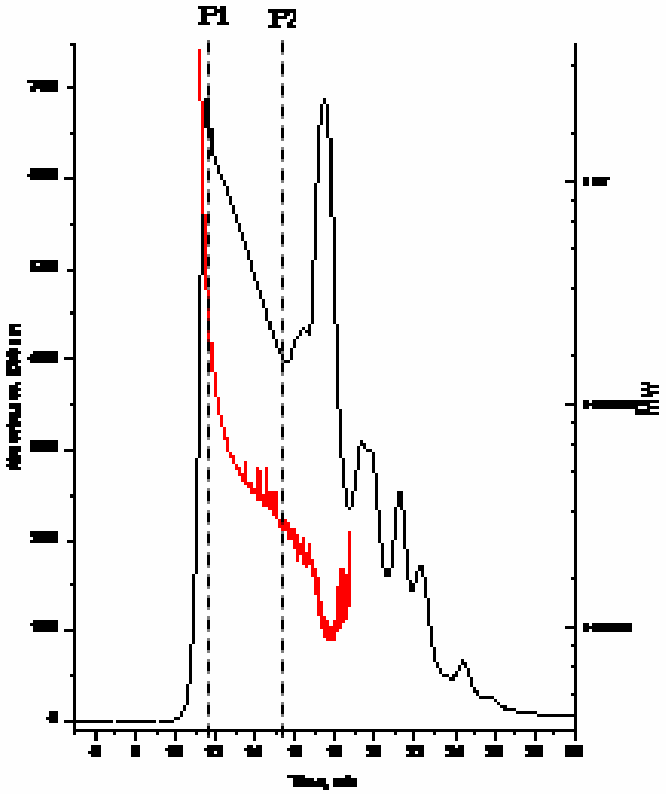


Table 4.2: Molecular weights in total polymeric protein (TPP) fraction for Pegaso NILs analyzed by SEC-MALLS.

	Glu-1			*Gli-1* /*Glu-3*			*Gli-2*			SE-HPLC	SEC-MALLS		
Sample Name	A	B	D	A	B	D	A	B	D	%UPP	Peak 1 MW(10^6)	Peak 2 MW(10^6)	Peak 1+2 MW(10^6)
P.170	-	7+9	5+10	+	-	+	+	+	-	53.79^{a}	5.90^{f}	2.67^{cd}	4.03^{ijk}
P.236	-	7+9	5+10	+	+	-	-	+	+	51.39^{b}	8.75^{e}	2.11^{fg}	4.93^{fgh}
P.235a	21*	7+9	5+10	+	-	+	+	+	+	49.77^{bc}	8.31^{e}	2.71^{cd}	5.09^{fg}
P.238	21*	7+9	5+10	+	+	+	-	+	+	49.17^{cd}	8.47^{e}	1.71^{gh}	4.27^{hij}
P.106	-	7+9	5+10	+	-	+	+	+	+	49.13^{cd}	12.50^{a}	2.70^{cd}	6.53^{b}
P.212	-	7+9	5+10	+	+	+	-	+	+	48.98^{cd}	11.10^{bc}	2.20^{defg}	5.57^{cdef}
P.235b	21*	7+9	5+10	+	+	+	+	+	+	48.79^{cd}	10.70^{bcd}	2.15^{efg}	5.48^{cdef}
P.102	1	7+9	5+10	+	+	+	+	+	+	48.55^{cd}	10.85^{bcd}	3.81^{b}	8.03^{a}
P.236a	-	7+9	12*	+	+	-	-	+	+	48.54^{cd}	8.43^{e}	2.05^{fgh}	4.53^{ghi}
P.79	-	7+9	5+10	+	+	-	+	+	+	47.65^{de}	11.75^{ab}	2.30^{def}	5.93^{bcde}
P.217	-	7+9	5+10	+	+	+	+	+	-	46.62^{ef}	7.85^{e}	4.77^{a}	6.03^{bcd}
P.30	-	7+9	5+10	-	+	+	+	+	+	45.05^{f}	10.65^{cd}	2.06^{fg}	5.19^{efg}
P.168c	21*	7+9	5+10	+	+	+	+	+	+	45.03^{f}	10.55^{cd}	1.76^{gh}	6.66^{b}
P.184	-	7+9	2+Dy	+	+	+	+	+	+	42.63^{g}	10.60^{cd}	1.82^{fgh}	5.03^{fgh}
P.170a	-	7+9	12*	+	-	+	+	+	-	42.61^{g}	5.45^{f}	2.02^{fgh}	3.44^{k}
P.167	-	7	5+10	+	+	+	+	+	+	42.45^{g}	10.65^{cd}	2.65^{cde}	6.21^{bc}
Pegaso	-	7+9	5+10	+	+	+	+	+	+	42.07^{g}	10.02^{d}	1.54^{h}	4.53^{ghi}
P.166b	-	7+9	2+12	+	+	+	+	+	+	38.61^{h}	10.80^{bcd}	1.83^{fgh}	5.18^{efg}
P.2.2*	-	7+9	2.2*+12	+	+	+	+	+	+	38.13^{h}	5.51^{f}	1.95^{fgh}	3.42^{k}
P.29	-	7+9	2.2+12	+	+	+	+	+	+	33.70^{i}	11.15^{bc}	2.13^{fg}	5.41^{def}
P.186	-	7+9	4+12	+	+	+	+	+	+	33.52^{i}	10.00^{d}	2.82^{c}	6.18^{bcd}
P.168a	21*	7+9	12*	+	+	+	+	+	+	33.21^{i}	6.25^{f}	2.03^{fgh}	3.68^{jk}
P.166a	-	7	2+12	+	+	+	+	+	+	32.63^{i}	11.60^{abc}	2.03^{fgh}	6.49^{b}
P.168b	-	7+9	12*	+	+	+	+	+	+	29.35^{j}	10.55^{cd}	2.01^{fgh}	5.18^{efg}

abcdefghijk Means with same letter in same column are not significantly different at $P<0.05$

As shown in Table 4.2. peak 1 has higher average molecular weights compared to peak 2 for all the samples analyzed. Bean and Lookhart (2001) also reported similar results in which the S_E (excluded) portion contained larger size molecules than S1 and S2. Furthermore, the upper range of Mw for the SDS-insoluble fraction from our study was $8.03x10^6$ Da compared to the upper range of $8.1x10^6$ Da by the same authors. Though there were significant differences observed in Mw among Pegaso NILs, the data could not support the expected correlations between the amounts of UPP and Mw in samples where lower UPP values were observed. However, comparatively better correlation was observed with peak 2 areas and UPP values than peak1 area alone or peak1+2 areas combined (data not shown). This indicates that, the modified (or cross-linked to HMW-GS) ω-gliadins which would elute in the second half of the SDS-insoluble portion, may cause this correlation and thus shift the MWD to lower values by reducing the polymer size. The increased UPP values of Pegaso NILs in this study could be attributed to either variation in gliadin- to-glutenin (GGR) ratio or changes in MWs shifted to lower values as described by Southan and MacRitchie (1999). Exceptionally high correlation was observed between UPP and GGR (R^2=0.93).

4.4 Conclusion

The moderately high negative correlation (R^2=0.65) between reduced (SDS- RA) polymeric protein and modified ω–gliadins suggests that these ω–gliadins act as chain terminators, resulting in smaller polymers, thus causing a reduction of UPP values. Significant differences were observed among Pegaso NILs for MWD of the SDS-insoluble fraction. However, there is no correlation found between MWD and UPP values. Nevertheless, increased UPP values are attributed more to increase in GGR than shifts in MWD of Pegaso NILs.

.

References:

Bean, S. R., and Lookhart, G. L. 1995. Separation and characterization of wheat protein fractions by high-performance capillary electrophoresis. Cereal Chem.72 (6):527-532.

Bean, S. R., and Lookhart, G. L. 2001. Factors influencing the characterization of gluten proteins by size-exclusion chromatography and multiangle laser light scattering detector (SEC-MALLS). Cereal Chem.78 (5):608-618.

Bietz, J. A., Huebner, F. R., and Wall, J. S. 1973. Glutenin, the strength protein of wheat flour. Baker's Dig. 47(1):26-31, 34-35,67.

D'Ovidio, R., Masci, S., Mattei, C., Tosi, P., Lafiandra, D., and Porceddu, E. 2000. Characterization of the LMW-GS gene family in Durum wheat. Pages 113-116 in: Wheat Gluten. Vol. 261. Royal Society of Chemistry: UK.

D'Ovidio, R., and Masci, S. 2004. The low-molecular weight glutenin subunits of wheat gluten. J. Cereal Sci. 39:321-329.

Ewart, J. A. D. 1990. Comments on recent hypothesis for glutenin. Food Chem. 38:159-169.

Fu, B.X., and Sapirstein, H.D. 1996. Procedure for isolating monomeric proteins and polymeric glutenin of wheat flour. Cereal Chem. 73(1): 143-152.

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., 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.). J. Cereal Sci. 35:265-272.

Jeng, L., and Blake, S.T. 1993. Evaluation of light scattering detectors for size exclusion chromatography. II. Light scattering equation selection. J. Appl. Polym. Sci. 49:1135-1174.

Kasarda, D. D.1989. Glutenin structure in relation to wheat quality. Pages 277-302 in: Wheat is unique. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

Lew, E. J.-L., Kuzmicky, D. D., and Kasarda, D. D. 1992. Characterization of low molecular weight glutenin subunits by reverse-phase high-performance liquid chromatography sodium dodecyl sulfate-polyacrylamide gel electrophoresis and N-terminal amino acid sequencing. Cereal Chem. 69:508-515.

Masci, S., D´Ovidio, R., Lafiandra, D. and Kasarda, D. 1998. Characterization of a low-molecular-weight glutenin subunit gene from bread wheat and the corresponding protein that represents a major subunit of the glutenin polymer. Plant Physiol. 118:1147-1158.

Masci, S., Rovelli, L., Kasarda, D.D., Vensel, W. H., and Lafiandra, D. 2002. Characterization and chromosomal localization of C-type low-molecular weight glutenin subunits in the bread wheat cultivar Chinese Spring. Theor. Appl. Genet. 104:422-428.

Okita, T. W., Cheesebrough, V., and Reeves, C. D. 1985. Evolution and heterogeneity of the α,β–type and γ–type gliadin DNA sequences. J. Biol. Chem. 260:8203-8213.

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. Philos. Trans. R. Soc. Lond. B 304:359-371.

Scheets, K., and Hedgcoth, C. 1988. Nucleotide sequence of a gliadin gene: Comparison with other gliadin sequences show the structure of gliadin genes and the general primary structure of gliadins. Plant. Sci.57:141-

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

Southan, M., and MacRitchie, F. 1999. Molecular weight distribution of wheat proteins. Cereal Chem. 76(6):827-836.

Suchy, J., Lukow, O.M., and Fu, B.X. 2003. Quantification of monomeric and polymeric wheat proteins and the relationship of protein fractions to wheat quality. J. Sci. Food Agric. 83:1083-1090.

Tao, H. P., and Kasarda, D. D. 1989. Two-dimensional gel mapping and N-terminal sequencing of LMW-glutenin subunits. J. Exp. Bot. 40:1015-1020.

CHAPTER 5 - PEGASO LINES

5.1 Introduction

Gluten proteins are the major components of wheat flour and are responsible for the formation of cohesive dough to make into bread (Finney, 1943). It is this unique property of wheat flour in nature that attracted researchers and many research programs have focused today on correlating measurements of wheat protein fractions to bread and dough quality. Quality is determined by molecular structure of wheat storage proteins that in turn governs the interactions of proteins during the bread making process (Bushuk, 1998; Shewry *et al.*, 1999). As the technology has progressed in wheat genetics, use of novel genetic lines such as near-isogenic lines to deduce the composition-functionality relationships has also been increased. Near-isogenic lines can be successfully used to manipulate the functional properties predictably in breeding situations (MacRitchie and Lafiandra, 2001).

Although it is well known that gluten proteins control the properties of both the dough and finished bread, the precise mechanism has still not been completely understood (Bean *et al.*, 1998). However, relationships between different gluten protein fractions such as the high molecular weight glutenin subunits (HMW-GS), low molecular weight subunits (LMW-GS) and bread making properties have been established. Likewise, the relationships between these protein fractions and dough and mixing strength are becoming well documented.

There are roughly nine major loci that code for gluten proteins, the main endosperm storage proteins that contribute to flour functionality. They include: three *Glu-1* loci (*Glu-A1, Glu-B1 and Glu-D1* on 1A, 1B and 1D chromosomes respectively) coding for HMW-GS; three complex *Gli-1/Glu-3* loci (*Gli-A1/Glu-A3, Gli-B1/Glu-B3* and *Gli-D1/Glu-D3*) coding for LMW-GS and ω– and γ–gliadins; and the three *Gli-2* loci (*Gli-A2/Gli-B2* and *Gli-D2*) coding for α– and β–gliadins (MacRitchie and Lafiandra, 2001). All these loci exhibit allelic variation and are thus available with a large number of allelic combinations for systematic studies to be conducted.

Studies on HMW-GS null lines (*Glu-1* locus) (Lawrence *et al*., 1988; Payne *et al*., 1987a) confirmed that dough mixing strength and bread making quality decreased dramatically as HMW-GS were deleted. Depending on the relative amounts of LMW-GS and gliadins expressed at *Gli-1/Glu-3* loci, the dough strength of different lines could be altered (Gianibelli *et al*., 1998). Allelic variation at *Gli-D1/Glu-D3* loci in a cultivar that carries two D-type subunits resulted in lower UPP and MDDT values (Gianibelli *et al*., 1998). This might be due to D-subunits acting as chain terminators and thus shifting the MWD to lower values (Masci *et al*., 1999). The impact of the null allele at *Gli-D1/Glu-D3* on gluten strength was highly positive in NILs possessing HMW-GS 2 + 12 and negligible or negative in NILs containing HMW-GS 5+10 (Redaelli *et al*., 1997). The locus *Gli-B1* is significantly related to gluten strength (Metakovsky *et al*., 1997). Deletion lines at *Gli-2* loci (*Gli-A2/Gli-D2*) exhibit increased MDDT and increased PPP because the glutenin-to-gliadin ratio has been manipulated to higher values (Gianibelli *et al*., 1998). Both the *Gli-1* and *Gli-2* loci may code for modified gliadins that act as chain terminators and the HMW-GS with one less Cys residue in near-isogenic lines shifted the molecular weights to lower values (Pirozi, 2003).

It has been well established that the *Glu-D1* encoded HMW-GS pair 5+10 is associated with greater strength while the allelic pair 2+12 is associated with lesser strength (Gianibelli *et al*., 2001). These differences in dough strength might be attributed to differences in molecular size of glutenin polymers (Gupta and MacRitchie, 1994), perhaps because of an extra cysteine residue in the Dx5 subunit (Kasarda, 1999).

The present study explores a set (24 lines) of near-isogenic lines (NILs) with background of an Italian bread wheat cultivar Pegaso. These lines include deletions (at *Gli-1/Glu-3* and *Gli-2* loci) and variations at *Glu-1* loci and/or combinations of deletions and additions. Thus, one important goal is to deduce the effects of changes in protein subunit composition on functionality of wheat proteins and thus, to establish composition-functionality relationships using NILs. The specific objective was

- To determine the effect of allelic variation at *Glu-1, Gli-1/Glu-3* and *Gli-2* loci on flour functionality estimated by quality and physical dough tests using Pegaso wheat NILs

Table 5.1: Description of Pegaso near-isogenic lines used in the study ('+' and '-' represents presence and absence of subunits at corresponding loci, respectively)

	Glu-1 loci			*Gli-1/Glu-3* loci			*Gli-2* loci		
Sample Name	A	B	D	A	B	D	A	B	D
Pegaso 102	1	7+9	5+10	+	+	+	+	+	+
Pegaso 235b	21*	7+9	5+10	+	+	+	+	+	+
Pegaso 167	-	7	5+10	+	+	+	+	+	+
Pegaso 186	-	7+9	4+12	+	+	+	+	+	+
Pegaso 166b	-	7+9	2+12	+	+	+	+	+	+
Pegaso 184	-	7+9	2+Dy	+	+	+	+	+	+
Pegaso 166a	-	7	2+12	+	+	+	+	+	+
Pegaso 29	-	7+9	2.2+12	+	+	+	+	+	+
Pegaso 2.2*	-	7+9	2.2*+12	+	+	+	+	+	+
Pegaso 168b	-	7+9	12*	+	+	+	+	+	+
Pegaso 168a	21*	7+9	12*	+	+	+	+	+	+
Pegaso 30	-	7+9	5+10	-	+	+	+	+	+
Pegaso 106	-	7+9	5+10	+	-	+	+	+	+
Pegaso 79	-	7+9	5+10	+	+	-	+	+	+
Pegaso 212	-	7+9	5+10	+	+	+	-	+	+
Pegaso 217	-	7+9	5+10	+	+	+	+	+	-
Pegaso 235a	21*	7+9	5+10	+	-	+	+	+	+
Pegaso 238	21*	7+9	5+10	+	+	+	-	+	+
Pegaso 236a	-	7+9	12*	+	+	-	-	+	+
Pegaso 236	-	7+9	5+10	+	+	-	-	+	+
Pegaso 170	-	7+9	5+10	+	-	+	+	+	-
Pegaso 170a	-	7+9	12*	+	-	+	+	+	-
Pegaso (Parent)	-	7+9	5+10	+	+	+	+	+	+
Pegaso 168c	21*	7+9	5+10	+	+	+	+	+	+

5.2 Methodology

A set of NILs with Italian bread wheat Pegaso background was developed at the University of Tuscia, Italy. It was achieved by back crossing donor lines with Pegaso as recipient (parent line) up to six generations. Description of variation in these NILs is shown in Table 5.1. All the samples were grown under green house conditions and harvested during 2004.

Details of methods used for physical dough tests and analytical methods were described elsewhere (Chapter 3) in this dissertation.

5.3 Results and Discussion

5.3.1 Initial Physical Tests

Results from initial physical tests are shown in Table 5.2. All the Pegaso NILs fell in the 'hard' category for hardness index (HI). The HI values ranged from 51% (Pegaso 167) to 84% (Pegaso170). Although a specific mechanism of grain hardness in wheat has not been established, Greenwell and Schofield (1989) stated that the hardness of wheat grains could be related to the presence or absence of proteins predominantly encoded on genes located on chromosome 5D. However, the NILs used in the present study focused on variation in protein composition at 1 and 6 chromosomes and thus we may not expect drastic changes in hardness characteristics.

Flour yield ranged from 44% (Pegaso 167) to 56% (Pegaso 2.2*) (Table 5.2). Conventionally, hard wheat grains will produce 52-58% total flour yield in the Brabender Quadrumat Jr. mill (Pirozi, 2003). Redaelli *et al.*, (1997) reported no significant differences among NILs in flour yield and other grain characteristics.

Flour protein (%) content varied significantly among the NILs (Table 5.2). Protein content ranged from 5.98% (Pegaso 236) to 9.89% (Pegaso 217) compared to the Pegaso parent line (6.50%). Although Pegaso is known as a good bread wheat variety with average protein content of around 10%, the growing season for the present samples might have affected the lower protein content. This lower protein content is reflected negatively in functional properties such as MDDT,

Table 5.2: Results from initial physical tests- Hardness Index (HI) from SKCS; Milling yield from Quadrumat Junior mill and Flour protein content analyzed by LECO protein analyzer.

Sample Name	HI	Milling Yield (%)			% Flour Protein (FP)	% Flour Moisture
		Low ash flour	Total flour	Bran		
Pegaso 102	75.50	47.11	53.03	46.97	8.22^{de}	13.78^{efgh}
Pegaso 235b	75.70	43.79	49.34	50.66	6.80^{k}	13.58^{gh}
Pegaso 167	51.07	45.14	48.00	48.93	8.01^{efg}	14.06^{bcdefg}
Pegaso 186	80.10	45.23	50.06	49.40	9.37^{b}	14.22^{abcdef}
Pegaso 166b	68.00	47.36	53.07	46.93	7.85^{ghi}	13.82^{defgh}
Pegaso 184	64.70	44.71	50.72	49.28	6.3l^{l}	14.48^{abc}
Pegaso 166a	68.60	47.34	53.48	46.52	8.71^{c}	14.58^{ab}
Pegaso 29	67.70	45.51	52.96	47.04	7.90^{fgh}	14.01^{cdefg}
Pegaso 2.2*	72.00	44.11	55.89	49.39	8.44^{d}	13.60^{gh}
Pegaso 168b	67.50	43.58	49.89	50.11	7.85^{ghi}	13.82^{defgh}
Pegaso 168a	75.60	46.38	52.73	47.26	9.22^{b}	14.17^{abcdef}
Pegaso 30	68.50	47.65	53.62	46.38	7.43^{j}	14.47^{abc}
Pegaso 106	69.30	47.74	54.53	45.46	7.74^{hi}	14.17^{abcdef}
Pegaso 79	71.20	44.49	51.48	48.52	6.83^{k}	14.51^{abc}
Pegaso 212	70.70	43.02	49.30	50.70	6.93^{k}	13.76^{fgh}
Pegaso 217	72.80	44.86	51.33	48.67	9.89^{a}	14.28^{abcde}
Pegaso 235a	81.80	43.10	48.58	51.42	8.20^{de}	14.41^{abc}
Pegaso 238	64.80	47.50	52.58	47.43	6.30^{l}	13.75^{fgh}
Pegaso 236a	70.10	45.12	50.52	49.47	6.84^{k}	14.00^{cdefg}
Pegaso 236	61.40	42.08	49.09	50.90	5.98^{m}	14.08^{abcdefg}
Pegaso 170	83.80	45.73	50.79	49.12	8.73^{c}	13.88^{defgh}
Pegaso 170a	74.80	46.82	52.27	47.73	8.14^{ef}	14.59^{a}
Pegaso(Parent)	61.70	46.33	52.56	47.74	6.50^{l}	13.45^{h}
Pegaso 168c	64.70	41.96	48.61	51.38	7.73^{hi}	14.32^{abcd}

^{abcdefghijklm} Means with same letter in same column are not significantly different at $P<0.05$

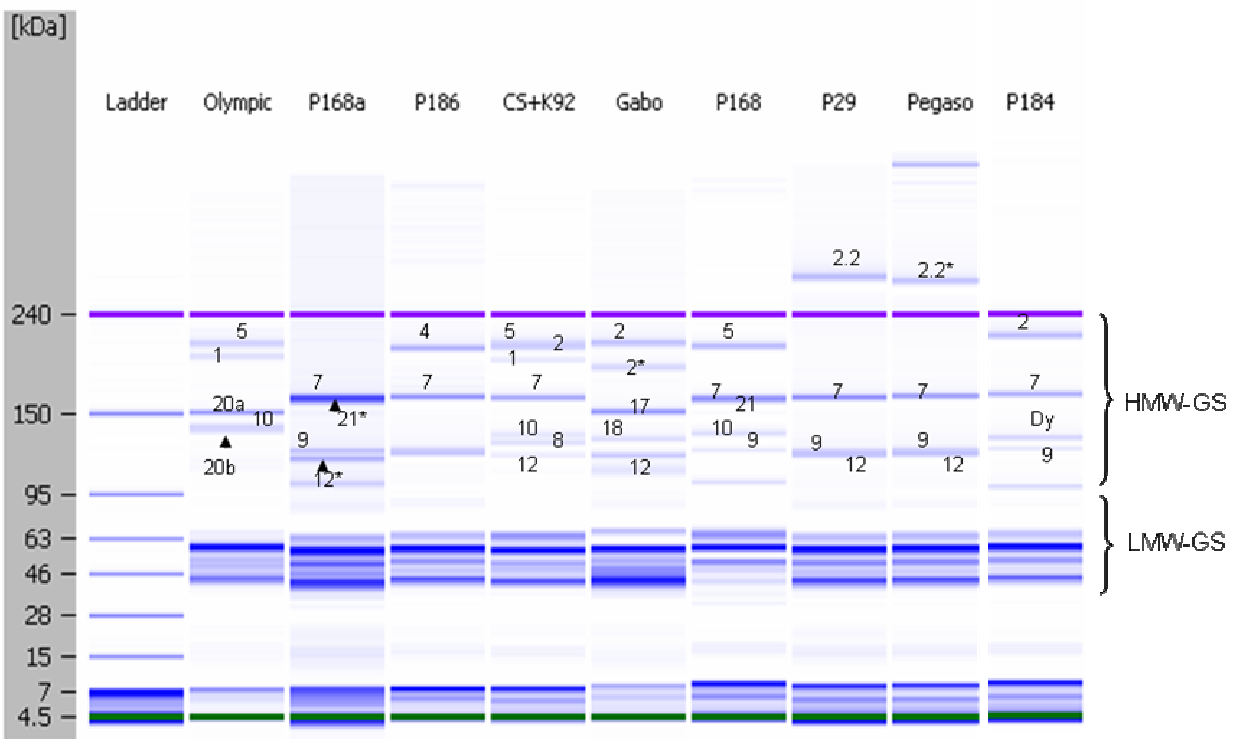
R_{max} and loaf volume. In contrast to the present results, Redaelli *et al.*, (1997) reported no significant differences among 14 NILs for protein content.

5.3.2 Electrophoretic Characterization of HMW-GS using Lab-On-a-Chip Method

In the present study, the Lab-on-a-chip (Agilent technologies, Palo Alto, CA) system was used to identify the HMW-GS as well as comparisons to previous SDS-PAGE results. The Lab-on-a-chip method is a micro-fluidic analysis of cereal proteins which works on the same principle as size-based capillary electrophoresis (Uthayakumaran *et al.*, 2006).

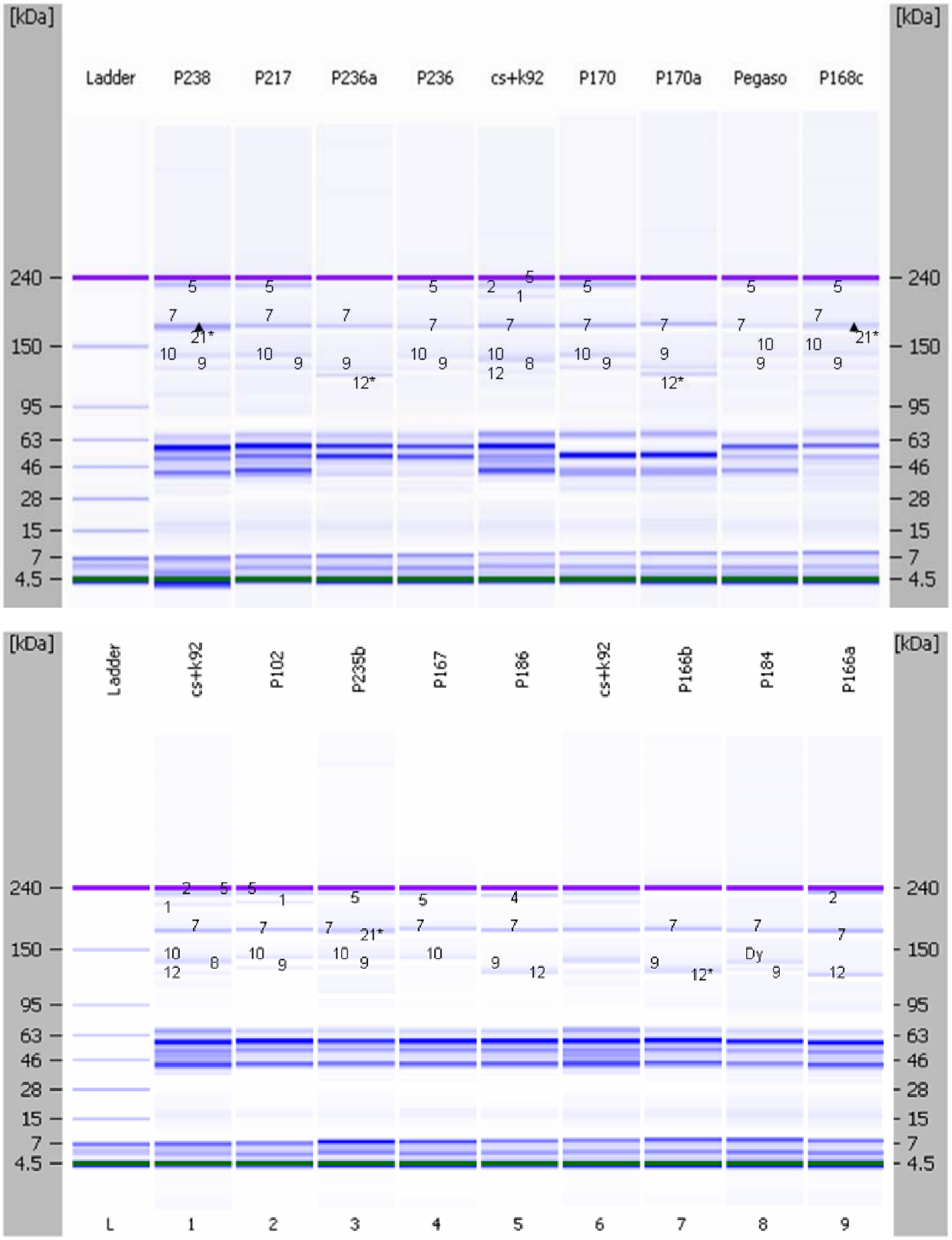
Figure 5-1: The HMW-GS composition for Pegaso NILs identified by Lab-on-a-chip method. (A) Control chip and (B), (C) and (D) are for Pegaso samples.

(A) Control chip



Lab-on-a-chip results are shown in Fig. 5.1. For the convenience of identification of HMW-GS composition in Pegaso samples, a control chip was performed (Fig. 5.1. A) with samples of known HMW-GS composition such as Olympic (1, 5+10, 20a, b), Chinese Spring (Null, 7+8, 2+12), Karl-92 (1, 7+8, 5+10), Gabo (2*, 17+18, 2+12) along with selected Pegaso samples that contain rare HMW-GS such as 2.2 (Pegaso 29), 2.2* (Pegaso 2.2*), 21*(Pegaso 168a) and Dy subunit (Pegaso 184). HMW-GS composition of Pegaso NILs is shown in Fig 5.1 B, C and D. Results were shown for different chips as each chip has the facility to run only 10 samples.

(B) Pegaso samples



(C) Pegaso samples

Lab-on-a-chip results are shown in Fig. 5.1. For the convenience of identification of HMW-GS composition in Pegaso samples, a control chip was performed (Fig. 5.1. A) with samples of known HMW-GS composition such as Olympic (1, 5+10, 20a, b), Chinese Spring (Null, 7+8, 2+12), Karl-92 (1, 7+8, 5+10), Gabo (2*, 17+18, 2+12) along with selected Pegaso samples that contain rare HMW-GS such as 2.2 (Pegaso 29), 2.2* (Pegaso 2.2*), 21*(Pegaso 168a) and Dy subunit (Pegaso 184). HMW-GS composition of Pegaso NILs is shown in Fig 5.1 B, C and D. Results were shown for different chips as each chip has the facility to run only 10 samples.

Table 5.3: **Apparent sizes (kDa) of HMW-GS determined using the Lab-on-a-Chip method**

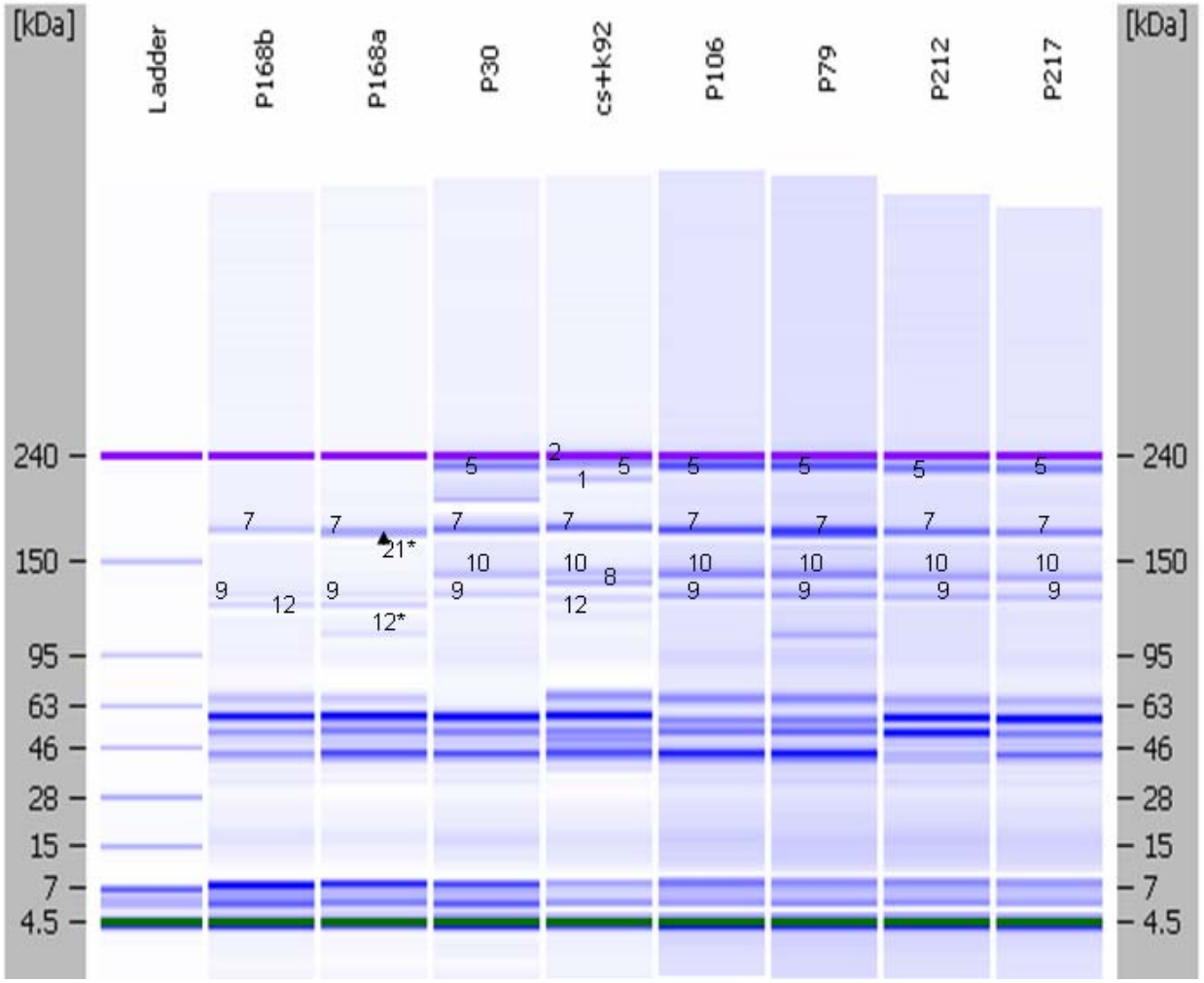
	Glu-A1 loci					*Glu-B1* loci					
Subunit	1	2*	21	21*		7	8	9	17	18	20 a&b
Size(kDa)	202	192	164.5	164.4		165.6	130.6	125.6	152.6	133.1	152&141
Glu-D1 loci											
Subunit	2	2.2	2.2*	4	5	10	12	12*			
Size(kDa)	214.4	273.5	269.9	209.2	214	135.7	121.6	119.9			

The apparent sizes of the HMW-GS obtained on the Lab-on-a-chip are listed in Table 5.6. These may not correspond exactly to the previous results by Uthayakumaran *et al* (2006) since the Protein chip used in the present study has a higher molecular range (230+ kit) against the 210+ kit that these workers used as well as different internal standards . This difference in apparent size is more pronounced with subunits such as 1, 2, 2* and 5 with higher Mw, whereas it is decreased with other subunits. This might be due to different extraction procedures from Uthayakumaran *et al* (2006); also due to different sample matrix.

Migration order of HMW-GS based on size based capillary electrophoresis doesn't strictly follow according to their molecular size or initial allocation of subunit numbers based on SDS-PAGE (Bean and Lookhart, 2001; Bietz and Sutton; Uthayakumaran *et al.,* 2006). The elution sequence of HMW-GS in size based capillary electrophoresis was reported as 12, 10, 9, 8, 18, 17, 6, 7, 2*, 1, 5, and 2 by Sutton and Bietz (1997) whereas Bean and Lookhart

(1999) reported the order as 8, 10, 7, 1, and 5. The elution sequence for the present sample fractionation in chip was 12*, 12, 9, 8, 18, 10, 20b, 20a, 17, 21*, 21, 7, 2*, 1, 4, 5, and 2 which is exactly as reported by Uthayakumaran *et al.,* 2006. However, we have identified presumably the 2.2* and 2.2 subunits above the upper limit of the protein chip used. Mr of 131.4 kDa (2.2) and 133.5 kDa (2.2*) were reported by D'Ovidio *et al.*, (1994) using SDS-PAGE. This apparent size difference of subunits between Lab-on-a-chip and SDS-PAGE presumably may arise from compounding effects of molecular conformation and shape in conjunction with interaction of specific proteins with the capillary walls (Uthayakumaran *et al.,* 2006) or the polymer used in the separation (Bean and Lookhart,).

(D) Pegaso samples



Nevertheless, HMW-GS identification using the Lab-on-a-chip offers a rapid, convenient method and gives good resolution of these protein bands. This method has been

used successfully in identification of rice protein subunits (Siriamornpun *et al.*, 2004) and wheat grains (Uthayakumaran *et al.,* 2005).

5.3.3 Effect of Allelic Variation on Quality using Physical Dough Tests and SE-HPLC

Results of SE-HPLC are presented in Table 5.3 and Fig. 5.2. Two important pieces of information arise from this study. First, a very clear separation of NILs containing HMW-GS 5+10 from 2+12 was observed for UPP. The lowest UPP was 29.35% (Pegaso 168b), whereas the highest was 53.79% (Pegaso 170). However, there were a few exceptions where *Gli-1*or *Gli-2* nulls appeared along with the 12* subunit at the *Glu-D1* locus, resulting in moderately high UPP values. The subunit 12* is known for lack of dough strength and when it appears with no nulls at *Gli-1* or *Gli-2*, it resulted in the lowest UPP of 29.35% in Pegaso 168b. Also, lines null at the *Gli-1* loci, specifically at *Gli-1B* loci gave higher UPP values. On the other hand, the lack of gliadins encoded at *Gli-A2 and Gli-D2* loci also caused a marked increase in UPP. This might be attributed to removal of expected chain terminators at *Gli-1* or *Gli-2* loci, the absence of which would shift the molecular weights to higher values.

5.3.3.1 Comparison of Subunits 5+10 and 2+12

Highly significant differences were observed for physical dough test parameters such as MDDT (min.), R_{max} (mN) and Extensibility (mm) (Table 5.4) among the Pegaso NILs. MDDT values ranged from 0.96 to 7.37 min., whereas it was 104 to 486 mN and 23.91 to 61.27 mm for R_{max} and extensibility. Correlation between different protein composition and physical dough test parameters are presented in Table 5.5. Mixograms for all Pegaso NILs studied are presented in Appendix C (Fig. 1) for reference.

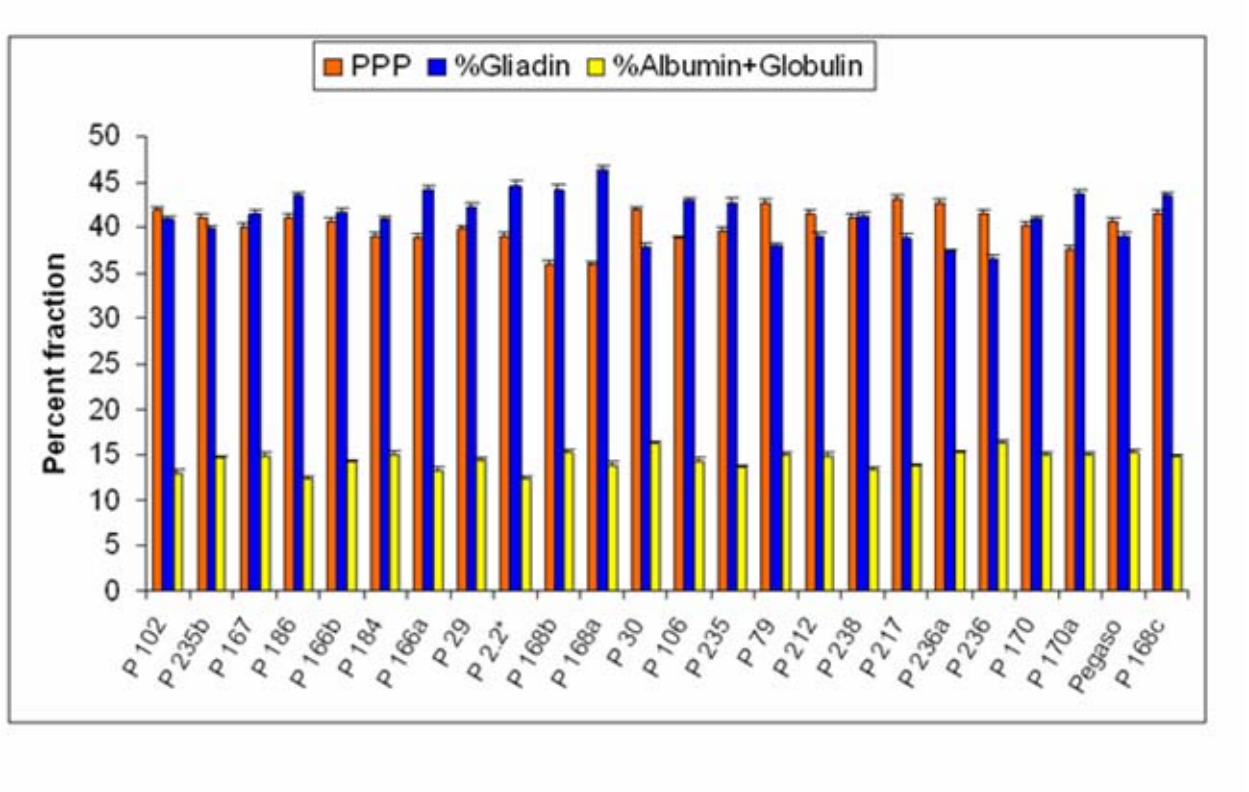
Dough strength and bread making quality of different HMW-GS arising from allelic variation at *Glu-1* loci was first reported by Payne (1987). A scoring system was developed for HMW-GS based on their performance and two pairs of subunits in particular had contrasting effects. Subunits 5+10 were associated with strength (scoring of 4) and 2+12 were associated with lack of strength (score of 1). In the present study, both MDDT (R^2=0.25) and R_{max} (R^2=0.77) were positively correlated with UPP values. UPP is a

Table 5.4: Percent unextractable protein (%UPP) analyzed by SE-HPLC arranged in its descending order.

	Glu-1 loci			*Gli-1*/*Glu-3* loci			*Gli-2* loci			%UPP	Gliadin/
Sample Name	A	B	D	A	B	D	A	B	D		Glutenin ratio
Pegaso 170	-	7+9	5+10	+	-	+	+	+	-	53.79[a]	0.76
Pegaso 236	-	7+9	5+10	+	+	-	-	+	+	51.39[b]	0.71
Pegaso 235a	21*	7+9	5+10	+	-	+	+	+	+	49.77[bc]	0.86
Pegaso 238	21*	7+9	5+10	+	+	+	-	+	+	49.17[cd]	0.84
Pegaso 106	-	7+9	5+10	+	-	+	+	+	+	49.13[cd]	0.87
Pegaso 212	-	7+9	5+10	+	+	+	-	+	+	48.98[cd]	0.80
Pegaso 235b	21*	7+9	5+10	+	+	+	+	+	+	48.79[cd]	0.82
Pegaso 102	1	7+9	5+10	+	+	+	+	+	+	48.55[cd]	0.84
Pegaso 236a	-	7+9	12*	+	+	-	-	+	+	48.54[cd]	0.77
Pegaso 79	-	7+9	5+10	+	+	-	+	+	+	47.65[de]	0.80
Pegaso 217	-	7+9	5+10	+	+	+	+	+	-	46.62[ef]	0.83
Pegaso 30	-	7+9	5+10	-	+	+	+	+	+	45.05[f]	0.84
Pegaso 168c	21*	7+9	5+10	+	+	+	+	+	+	45.03[f]	0.97
Pegaso 184	-	7+9	2+Dy	+	+	+	+	+	+	42.63[g]	0.96
Pegaso 170a	-	7+9	12*	+	-	+	+	+	-	42.61[g]	1.02
Pegaso 167	-	7	5+10	+	+	+	+	+	+	42.45[g]	0.98
Pegaso(Parent)	-	7+9	5+10	+	+	+	+	+	+	42.07[g]	0.93
Pegaso 166b	-	7+9	2+12	+	+	+	+	+	+	38.61[h]	1.08
Pegaso 2.2*	-	7+9	2.2*+12	+	+	+	+	+	+	38.13[h]	1.17
Pegaso 29	-	7+9	2.2+12	+	+	+	+	+	+	33.70[i]	1.25
Pegaso 186	-	7+9	4+12	+	+	+	+	+	+	33.52[i]	1.30
Pegaso 168a	21*	7+9	12*	+	+	+	+	+	+	33.21[i]	1.40
Pegaso 166a	-	7	2+12	+	+	+	+	+	+	32.63[i]	1.35
Pegaso 168b	-	7+9	12*	+	+	+	+	+	+	29.35[j]	1.51

[abcdefghij] Means with same letter in same column are not significantly different at $P<0.05$

Figure 5-2: Percent Polymeric Proteins (PPP), %gliadins and %albumins/globulins fractions from SE-HPLC for Pegaso NILs. The vertical bars represent the SD (n = 2), and values denoted by the same letter are not significantly different (P < 0.05).



parameter that gives a relative measure of the molecular weight distribution of the polymeric protein, based on solubility measurements (Gupta *et al.*, 1993) and the differences in dough strength are due to differences in the molecular size of glutenin polymers (Gupta and MacRitchie, 1994). A greater amount of UPP would shift the molecular weight distribution to higher values and thus appears to be associated with greater dough strength with subunits 5+10 over the lines with HMW-GS 2+12 (MacRitchie and Lafiandra, 2001). Although the mechanism for the allelic differences has not been established, the presence of an extra cysteine residue in Dx5 may be a possible explanation in the case of 5+10 vs. 2+12 (Anderson and Green, 1989; Kasarda, 1999; Shewry *et al.*, 1992). Some regulatory mechanism may be responsible for the allelic effect rather than structural differences between subunits (Gupta *et al.*, 1996).

Table 5.5: Results from physical dough tests (MDDT & Peak width at 8 min. from Mixograph; R_{max} and Extensibility from Micro-extensibility tests) arranged in descending order of MDDT

	Mixograph test		Micro-Extensibility results	
Sample Name	MDDT(min.)	Peak width at 8 min.	R_{max}(mN)	Extensibility(mm)
Pegaso 212	7.37^{a}	16.7^{bcde}	486.4^{a}	23.91^{k}
Pegaso 106	6.29^{b}	19.1^{abcd}	303.0^{f}	30.11^{ghij}
Pegaso 102	5.87^{b}	19.6^{abc}	438.0^{b}	33.58^{f}
Pegaso 235a	4.76^{c}	22.4^{a}	437.1^{b}	28.92^{ij}
Pegaso (Parent)	4.67^{c}	16.7^{bcde}	299.4^{f}	29.21^{hij}
Pegaso 217	4.62^{c}	20.35^{abc}	374.9^{d}	41.59^{d}
Pegaso 167	4.42^{c}	16.5^{cde}	360.9^{d}	30.92^{fghi}
Pegaso 30	4.18^{c}	18.7^{abcd}	399.1^{c}	27.56^{j}
Pegaso 168c	4.15^{c}	15.3^{de}f	274.6^{g}	32.61^{fg}
Pegaso 2.2*	2.46^{d}	14.9^{def}	169.3^{l}	57.26^{b}
Pegaso 235b	2.38^{de}	18.6^{abcd}	275.5^{g}	32.64^{fg}
Pegaso 168b	2.28^{def}	7.1^{g}	104.6^{o}	42.26^{d}
Pegaso 186	2.17^{def}	14.9^{def}	142.8^{m}	61.27^{a}
Pegaso 166b	2.15^{def}	16.2^{cdef}	197.1^{k}	48.44^{c}
Pegaso 166a	1.91^{defg}	12.0^{f}	126.8^{mn}	59.63^{ab}
Pegaso 29	1.87^{defg}	13.8ef	221.3^{j}	41.48^{d}
Pegaso 79	1.53^{efgh}	18.6^{abcd}	262.8^{gh}	38.84^{f}
Pegaso 168a	1.43^{fgh}	13.5^{ef}	119.6^{no}	60.61^{a}
Pegaso 238	1.26^{gh}	20.8^{ab}	367.1^{d}	32.16^{fgh}
Pegaso 184	1.24^{gh}	16.7^{bcde}	241.2^{i}	29.36^{hij}
Pegaso 236a	1.16^{gh}	16.2^{cdef}	251.0^{hi}	42.10^{d}
Pegaso 170	1.04^{gh}	15.1^{def}	295.8^{f}	49.97^{c}
Pegaso 236	1.03^{gh}	16.2^{cdef}	340.4^{e}	30.33^{ghij}
Pegaso 170a	0.96^{h}	13.8^{ef}	200.3^{k}	37.58^{e}

^{abcdefghijklmno} Means with same letter in same column are not significantly different at $P<0.05$

Although the dough strength measured by R_{max} in the present study is correlated with PPP (R^2=0.59), it correlates well with UPP (R^2=0.77). This high correlation with UPP not with PPP suggests that, not all the polymeric protein, but only a fraction of the highest molecular weight, contributes to strength (R_{max}) (Bangur *et al.*, 1997; Southan and MacRitchie, 1999; Bean *et al.*, 1998). This protein fraction has been termed the glutenin macro polymer in some studies (Weegels *et al.*, 1996).

On the other hand, extensibility measured by micro-extensibility tests, appears to correlate well (R^2=0.53) with total proportion of flour polymeric protein (FPP). This is in agreement with the previous studies by Gupta *et al.*, (1992) and Larroque *et al.*, (1999). Negative correlation of UPP with extensibility (R^2 = -0.62) indicated that the increase in polymer size decreased the extensibility. This is supported by the findings from Gupta and MacRitchie (1994) who stated that larger size glutenins contributed to lower extensibility of gluten.

Wieser and Kieffer, (2001) reported that dough extensibility was mainly influenced by the ratio of gliadin to glutenin subunits (R^2= 0.74). The present results from Pegaso NILs also supported the findings in which dough extensibility was correlated well (R^2=0.67) with gliadin to glutenin ratio. On the other hand MDDT (R^2= - 0.25^{ns}) and R_{max} (R^2= -0.79) were negatively correlated with gliadin to glutenin ratio, which was also in accord with the earlier findings.

5.3.3.2 Effect of Deletions at Gli-1 and Gli-2 loci

As mentioned earlier in this chapter, deletion of *Gli-B1* had resulted in higher UPP (Table 5.4). On the other hand, the lack of gliadins encoded at *Gli-A2 and Gli-D2* loci were correlated with a marked increase in UPP. It is evident from Table 5.4 that all the *Gli-1* or *Gli-2* null lines had higher UPP (upper part of table) than the lines with these loci.

The increased UPP with *Gli-B1* nulls could be attributed to more expression of gliadins than LMW-GS at this locus, the deletion of which would shift the gliadin to glutenin ratio to lower values and thus lead to increased dough strength. This lower gliadin to glutenin ratio of null *Gli-B1* can be seen in Table 5.3 and positive correlation (Table 5.5) of R_{max} (R^2=0.77) and MDDT (R^2=0.25) with UPP is evidence for the increased dough

strength. Gianibelli *et al.*, (1998) reported the lower glutenin-to-gliadin ratio with deletion of *Gli-B1/Glu-3* loci caused a reduction in PPP and MDDT. This is due to expression of more LMW-GS than gliadins at these loci (MacRitchie and Lafiandra, 2001). Polypeptides containing a single cysteine can act as chain terminators during formation of glutenin polymers, decreasing dough strength and stability (Greenfield *et al.*, 1998; Tamas *et al.*, 1998). The *Gli-1* loci, encoding the ω-gliadins, are known to contain gliadin like LMW-GS that could act as chain terminators (Masci *et al.*, 1993, Gianibelli *et al.*, 2002). These proteins behave like glutenin subunits but act as chain terminators, which shifts the MWD to lower values (Southan and MacRitchie, 1999). The behavior of Pegaso NILs with deletion of *Gli-1* loci is consistent with that expected from removal of chain terminators, causing in shifts of MWD to higher values.

Table 5.6: Correlation matrix for various quality parameters and protein composition*.

	MDDT	R_{max}	Ext.	LV	PPP	FPP	UPP	GGR	PAG	PG	FP
MDDT	1.000	0.644	-0.476	0.355	0.222	0.185	0.255 (0.274)	-0.258	-0.075	-0.101	0.091
R_{max}		1.000	-0.754	-0.101	0.596	-0.041	0.774 (0.738)	-0.791	0.201	-0.590	-0.265
Ext.			1.000	0.416	-0.357	0.534 (0.377)	-0.622	0.676	-0.567	0.574	0.671
LV				1.000	-0.032	0.656 (0.624)	-0.428	0.421	-0.811	0.465	0.656
PPP					1.000	0.147	0.599	-0.719	0.108	-0.790	-0.224
FPP						1.000	-0.157	0.184	-0.545	0.267	0.929
UPP							1.000	-0.967	0.300	-0.648	-0.377
GGR								1.000	-0.374	0.784	0.450
PAG									1.000	-0.570	-0.579
PG										1.000	0.557
FP											1.000

* MDDT=Mixograph dough development time; R_{max}=Maximum resistance in micro-extensibility test; Ext.=Extensibility; LV=Loaf volume; PPP= Percent polymeric protein; FPP= Flour polymeric protein; UPP=Unextractable polymeric protein; GGR=Gliadin/Glutenin ratio; PAG=Percent Albumin and Globulins; PG=Percent gliadins; FP=Flour protein

Values in parenthesis were after normalized to flour protein content

Nulls at *Gli-2* loci, particularly *Gli-A2* and *Gli-D2* also caused a marked increase in UPP values. The Russian cultivar Saratovskaja having mutant lines with deletions of *Gli-A2* and *Gli-D2* exhibited increased dough strength (MDDT) and PPP (Gianibelli *et al.*, 1998). Proportion of glutenins was increased in this case by deleting the gliadins. Lew *et al.*, (1992) provided evidence that mutated α– and γ-gliadins, encoded at group 6 chromosomes, could act as chain terminators. The changes of UPP observed could be attributed to the presence of chain terminators in *Gli-A2*, so that their detrimental effect would be suppressed in those null lines (Pirozi, 2003).

5.3.4 Test Baking Results

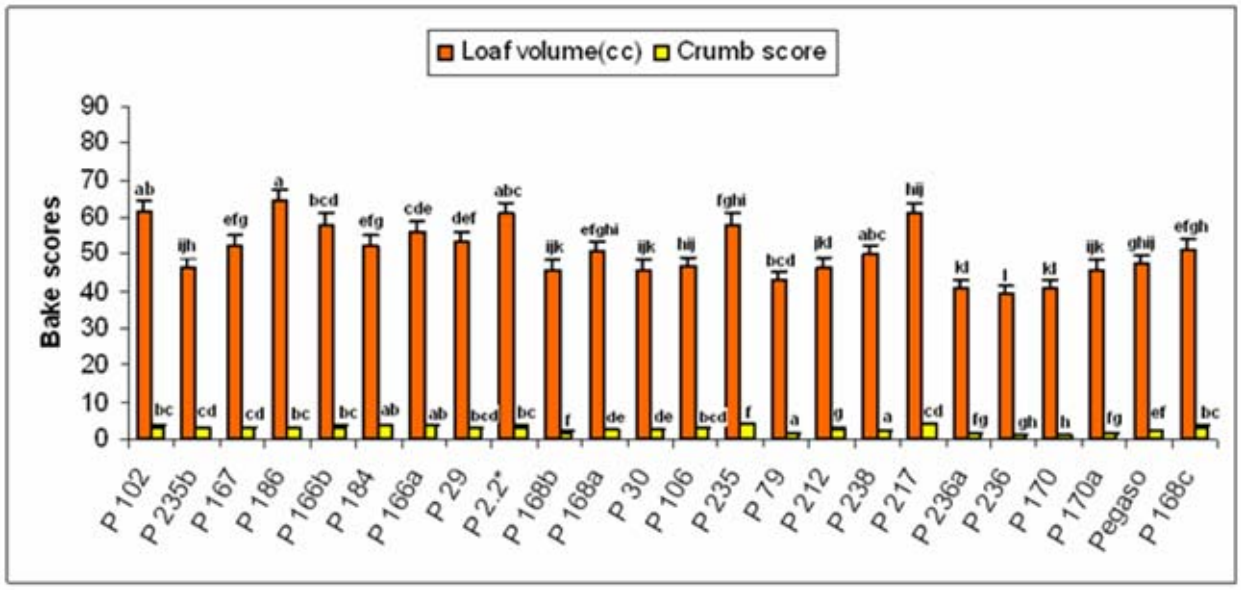
Micro-baking (10 g breads) was performed on Pegaso NILs. Loaf volume (rape seed displacement) and crumb scores were assigned visually and shown in Fig. 5.3. C-cell, a digital image analysis instrument, was used to analyze the crumb grain features such as loaf height, number of cells, and cell thickness. A correlation matrix among various protein composition, physical dough test and bread loaf characteristics were developed (Appendix A- Table 1). Pictures of whole bread and bread slices from C-cell analysis were also presented in Appendix B (Fig.B-1a, b, c, and d).

Significant differences were observed among NILs for loaf volume which ranged from 39.5 cm^3 (Pegaso 236) to 64.5 cm^3 (Pegaso 186). Loaf volume is correlated well (R^2=0.92) with total proportion of flour polymeric protein (FPP) (R^2=0.65) and MDDT (R^2=0.35) but negatively with other important protein fractions such as UPP, PPP or R_{max}. This was opposite to previous studies in which Rmax was correlated positively with loaf volume (Nash *et al.*, 2006) with micro loaves. Crumb scores also correlated well with FPP. Other crumb grain parameters measured by C-cell, such as number of cells (NC), slice area (SA), loaf height (HT), wall thickness (WT) and cell diameter (CD) were correlated well with each other.

The important quality attributes such as MDDT, R_{max}, extensibility and loaf volume were normalized to flour protein content to eliminate the effect of variation in protein

amounts. Similar trend of correlations were observed after normalization. These correlation values are indicated in parenthesis in Table 5.6.

Figure 5-3: Loaf volume (cc) and crumb scores from micro-breads baked for Pegaso NILs. The vertical bars represent the SD (n = 2), and values denoted by the same letter are not significantly different (P < 0.05).



5.4 Conclusion

Two important pieces of information arise from this study. One is that, null lines at *Gli-1* loci, specifically at *Gli-1B* loci resulted in higher UPP values. On the other hand, the lack of gliadins encoded at *Gli-A2 and Gli-D2* loci also caused a marked increase in UPP. This might be attributed to removal of expected chain terminators at *Gli-1* or *Gli-2* loci, absence of which would shift the molecular weights to higher values. The behavior of Pegaso NILs with deletion of the *Gli-1* loci is consistent with that expected from removal of chain terminators, caused in shifts of MWD to higher values.

Secondly, a very clear separation of NILs containing subunits 5+10 from 2+12 was observed for UPP, having lower values for later ones. Strength measurements like MDDT (R^2=0.25) and R_{max} (R^2=0.77) were positively correlated with UPP values. A greater amount of UPP would shift the molecular weight distribution to higher values and thus appears to be

associated with greater dough strength with subunits 5+10 over the lines with HMW-GS 2+12. This high correlation of R_{max} with UPP not with PPP suggests that, not all the polymeric protein, but only a fraction of the highest molecular weight, contributes to strength (as measured by R_{max}). Negative correlation of UPP with extensibility (R^2= -0.62) indicates that the increase in polymer size decreases the extensibility.

References

Anderson, O.D., and Green, F.C. 1989. The characterization and comparative analysis ofhigh-molecular-weight glutenin genes from genomes A and B of a hexaploid bread wheat. Theor. Appl. Genet. 77:689-700.

Bangur, R., Batey, I.L., McKenzie, E., and MacRitchie, F. 1997. Dependence of extensigraph parameters on wheat protein composition measured by SE-HPLC. J. Cereal Sci. 25:237-241.

Bean, S.R., Lyne, R.K., Tilley, K.A., Chung, O.K., and Lookhart, G.A. 1998. A rapid method for quantitation of insoluble polymeric proteins in flour. Cereal Chem. 75:374-379.

Bean, S.R., and Lookhart, G.L. 1999. Sodium dodecyl sulfate capillary electrophoresis of wheat proteins. I. Uncoated capillaries. J. Agric. Food Chem. 47:4246-4255.

D'Ovidio, R., Porceddu, E., and Lafiandra, D. 1994. PCR analysis of genes encoding allelic variants of high-molecular-weight glutenin subunits at the *Glu-D1* locus. Theor. Appl. Genet. 88:175-180.

Finney, K.F. 1943. Fractionating and reconstituting techniques as tools in wheat flour research. Cereal Chem. 20:381-396.

Gianibelli, M.C., Larroque, O.R., Chan, P., Margiotta, B., De Ambrogio, E., MacRitchie, F., and Lafiandra, D. 1998. Effects of the allelic variants at the *Gli*-1/*Glu*-3 and *Gli*-2 loci on mixing propertie. Pages 154-156 in:Proc. Int. Wheat Genetics Symposium, 9th Vol. 4. A. E. Slinkard, ed. University of Saskatchewan: Saskatchewan, SK, Canada.

Gianibelli, M.C., Larroque, O.R., MacRitchie, F., and Wrigley, C.W. 2001. Biochemical, genetic and molecular characterization of wheat glutenin and its component subunits. Cereal Chem. 78(6):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.). J. Cereal Sci. 35:265-272.

Greenfield,J.J.A., Ross-Murphy, S.B., Tamas, L., Bekes, F., Halford, N.G., Tatham, A.S., and Shewry, P.R. 1998. Rheological properties of monomeric and polymeric forms of C hordeins, a sulphur-poor prolamins of barley. J. Cereal Sci. 27:233-236.

Greenwell, P., and Schofield, J.D. 1989. The chemical basis of grain hardness and softness. Proc. of the ICC '89 symposium Wheat-end use properties: Wheat and flour characterization of specific end-uses. H. Salovaara, ed. June 13-15. Lathi, Finland.

Gupta, R.B., Khan, K., and MacRitchie, F. 1993. Biochemical basis of flour properties in bread wheats. I. Effects of variation in quantity and size distribution of polymeric proteins. J. Cereal Sci. 18:23-44.

Gupta, R.B., and MacRitchie, F. 1994. Allelic variation at glutenin subunit and gliadin loci, *Glu-1*, *Glu-3*, and *Gli-1* of common wheats. II. Biochemical basis of the allelic effects on dough properties. J. Cereal Sci. 19:19-29.

Gupta, R.B., Masci, S., Lafiandra, D., Bariana, H.S., and MacRitchie, F. 1996. Accumulation of protein subunits and their polymers in developing grains of hexaploid wheats. J. Exper. Bot. 47:1377-1385.

Kasarda, D.D. 1999. Glutenin polymers: The in vitro to in vivo transition. Cereal Foods World. 44:566-571.

Lawrence, G.J., MacRitchie, F., and Wrigley, C.W. 1988. Dough and baking quality of wheat lines deficient in glutenin subunits controlled by the *Glu-A1, Glu-B1* and *Glu-D1* loci. J. Cereal Sci. 7:109-112.

Lew, E.J.L., Kuzmicky, D.D., and Kasarda, D.D. 1992. Characterization of low molecular weight glutenin subunits by reversed-phase high-performance liquid chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and N-terminal amino acid sequencing. Cereal Chem. 69:508-515.

MacRitchie, F., and Lafiandra, D. 2001. Use of near-isogenic wheat lines to determine protein composition-functionality relationships. Cereal Chem. 78(5):501-506.

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

Masci, S., D' Ovidio, R.D., Lafiandra, D., and Kasarda, D.D. 1998. Characterization of a low-molecular weight glutenin subunit gene from bread wheat and the corresponding protein that represents a major subunit of the glutenin polymer. Plant Physiol. 118:1147-1158.

Metakovsky, E.V., Branlard, G., Chernakov, V.M., Upelniek, V.P., Redaelli, R., and Pogna, N.E. 1997.Recombination mapping of some chromosome 1A-, 1B-, 1D- and 6B-cpntrolled gliadins and low-molecular-weight glutenin subunits in common wheat. Theor. Appl. Genet. 94(6-7):788-795.

Nash, D., Lanning, S.P., Fox, P., Martin, J.M., Blake, N.K., Souza, E., Graybosch, R.A., Giroux, M.J., and Talbert, L.E. 2006. Relationship of dough extensibility and dough strength in a spring wheat cross. Cereal Chem. 83(3):255-258.

Payne, P.I. 1987. Genetics of wheat storage proteins and the effect of allelic variation on bread making quality. Annu. Rev. Plant. Physiol. 38:141-153

Payne, P.I., Holt, L.M., Harinder, K., Macartney, D.P., and Lawrence, G.J. 1987a. The use of near-isogenic lines with different HMW-glutenin subunits in studying bread making quality an glutenin structure. Pages 216-221 in: Proc. 3rd Int. Workshop on Gluten Proteins. R. Lasztity and F. Bekes, eds. World Scientific Publishing: Singapore.

Pirozi, M. 2003. Isogenic wheat lines in determining composition-functionality relationships. Ph.D. thesis submitted to Kansas State University.

Redaelli, R., Pogna, N.E., and Ng, P.K.W. 1997. Effects of prolamins encoded by chromosomes 1B and 1D on the rheological properties of dough in near-isogenic lines of bread wheat. Cereal Chem. 74(2): 102-107.

Shewry, P.R., Halford, N.G., and Tatham, A.S. 1992. The high molecular weight subunits of wheat glutenin. J. Cereal Sci. 15(2): 105-120.

Siriamornpun, S., Suttajit, M., Uthayakumaran, S., and Wrigley, C.W. 2004. Identification of Thai rice varieties using capillary electrophoresis. Pages 181-184 In: Proc. of the 54th Australia Cereal Chemistry Conference and the 11th Wheat Breeder's Assembly. Eds. Black, C.K., Panozzo, J.F., Rebetzke, G.J. Cereals 2004. Royal Australian Chemical Institute, Melbourne.

Southan, M., and MacRitchie, F. 1999. Molecular weight distribution of wheat proteins. Cereal Chem. 76(6): 827-836.

Sutton, K.H., and Bietz, J.A. 1997. Variation among high molecular weight subunits of glutenin detected by capillary electrophoresis. J. Cereal Sci. 25:9-16.

Tamas, L., Bekes, F., Grrenfield, J., Tatham, A.S., Gras, P.W., Shewry, P.R., and Appels, R. 1998. Heterologous expression and dough mixing studies of wild-type and mutant C hordeins. J. Cereal Sci. 27:15-22.

Uthayakumaran, S., Batey, I.L., and Wrigley, C.W. 2005. On-the-spot identification of grain variety and wheat-quality type by Lab-on-a-chip capillary electrophoresis. J. Cereal Sci. 41:371-374.

Uthayakumaran, S., Listiohadi, M., Baratta, M., Batey, I.L., and Wrigley, C.W. 2006. Rapid identification and quantitation of high-molecular-weight glutenin subunits. J. Cereal Sci. 44:34-39.

Weegels, P.L., Hamer, R.J., and Schofield, J.D. 1996. Critical review: Functional properties of wheat gluten. J. Cereal Sci. 23:1-18.

Weiser, H., and Kieffer, R. 2001. Correlations of the amount of gluten protein types to the technological properties of wheat flours determined on a micro-scale. J. Cereal Sci. 34:19-27.

CHAPTER 6 - WAXY WHEAT

6.1 Introduction

Granule-bound starch synthase (GBSS), well known as 'waxy protein', is responsible for amylose synthesis in the wheat endosperm (Sivak and Preiss, 1995). Hexaploid (bread) wheat (*Triticum aestivum* L.) has base chromosome number 2n=6x=42, that contains three nearly identical sets (A, B, and D genomes) of chromosomes. Durum wheat, on the other hand, has only A and B genomes. The genes coding for waxy protein (*Wx*) in hexaploid wheat are located on chromosomes 7AS (*Wx-A1*), 4AL (*Wx-B1*) and 7DS (*Wx-D1*). Durum contains the waxy alleles only *Wx-A1* and *Wx-B1*. The molecular weights of *Wx-A1, Wx-B1*, and *Wx-D1* are 60.1, 59.2 and 59.0 KDa respectively (Fujita *et al.*, 1996). Depending on the number of waxy loci carried, waxy lines with one (single null) or two null alleles (double null) have been termed 'partial waxy lines' (Nakamura *et al.*, 1993), whereas complete waxy carries null alleles at all three waxy loci (null at *Wx-A1/B1/D1*). The lines that carry all these protein loci are called 'wild types'.

The world's first waxy wheats were produced by Nakamura *et al.*, (1995).Null or non-functional alleles at loci *Wx-A1* and *Wx-B1* are common in wheat lines; however, no null alles at *Wx-D1* loci have been found in US wheats (Graybosch, 1998) . Different null alleles affect the amylose content and other functional properties differently. For instance, the reduction of amylose content due to these null alleles was as follows; *Wx-B1*>*Wx-D1*>*Wx-A1* (Miura *et al.*, 1994, 1999). Wild types (presence of all three *Wx* proteins) contain 25-28% amylose (Graybosch, 1998; Yamamori *et al.*, 2000), whereas, lack of all three *Wx* loci (waxy) resulted in 0-3% amylose (Nakamura *et al.*, 1995; Yasui *et al.*, 1997). Variation in amylose/amylopectin ratios led to differences in physicochemical properties ultimately affecting the quality of end-use products (Hung *et al.*, 2006).

Waxy wheats may find application in the production of modified starches, blending flour in superior noodle quality and bread making performance (Graybosch, 1998). Effect of waxy wheat starch on retarding bread staling has been studied widely (Lee *et al.*, 2001; Hayakawa *et al.*, 2004, Morita *et al.*, 2002). These studies reported that incorporation of waxy

starch resulted in retention of more moisture in bread crumb and extention of the shelf-life of baked products. Nevertheless, the optimum amylose/amylopectin ratio for production of good quality end-use products is still debatable (Hung *et al.*, 2006). Substitution of 50% waxy wheat flour caused lower loaf volume with high level starch retrogradation (Lee *et al.*, 2001; Hayakawa *et al.*, 2004) whereas, 40% substitution resulted in larger loaves with improved glutinous structure (Morita *et al.*, 2002).

Starch based properties such as pasting and gelatinization differ between waxy, partial waxy and non-waxy wheats (Yasui *et al.*, 1996; Kiribuchi-Otobe *et al.*, 1997; Baik *et al.*, 2003). Effects of seven kinds of *Wx* protein null types on starch properties were studied using near-isogenic lines (Miura *et al.*, 2002; Wickramasinghe *et al.*, 2003). However, except for starch properties, there is limited information about the quality characteristics of partial waxy and waxy wheats (Takata *et al.*, 2007). There is a necessity to study the protein composition and its effects on dough and end-use product quality (Hung *et al.*, 2006) using near-isogenic lines (Graybosch *et al.*, 2003).

Objectives

- To evaluate the effect of waxy and partial (single and double null) waxy proteins on flour quality and physical dough testing parameters using Svevo (durum) and N11 (bread) waxy wheat NILs.

6.2 Methodology

Two waxy wheat lines (Svevo-a durum wheat and N11-bread wheat) were used in this study. Partial waxy lines of the Svevo set contain two single nulls at *WX-A1* and *B1* loci along with one complete waxy and the parent line. The N11 set has a complete waxy, a normal parent line and a partial waxy (three lines as single nulls at *Wx-A1, B1* and *D1* and three double null combinations at *Wx-A1B1/A1D1/B1D1*). All these lines were developed for at least 6 generations, backcrossed to the same parent and thus are near-isogenic lines. Samples were run on SDS-PAGE to identify the HMW-GS composition. These waxy wheats were developed at the Department of Agrobiology & Agrochemistry, University of Tuscia, Viterbo, Italy. They were grown under green house conditions during 2005-06. A

brief description of the lines used in this study is provided in Table 6.1. The complete description of the methods followed is presented in Chapter 3.

Table 6.1: Description of Svevo durum waxy wheat and N11 bread waxy wheat lines used in the study.

Sample Name	Type of wheat	Variation
	Svevo set (Null, 7+8)	
Svevo wheat	Durum wheat	Parent line
Svevo waxy	Complete waxy	Null at $A1^-/B1^-/D1^-$
Svevo waxy $A1^-$	Partial waxy	Null at $A1^-$
Svevo waxy $B1^-$	Partial waxy	Null at $B1^-$
	N11 set (1, 7+8, 2+12)	
N11 wheat	Bread wheat	Parent line
N11 waxy	Complete waxy	Null at $A1^-/B1^-/D1^-$
N11 waxy $A1^-$	Partial waxy	Null at $A1^-$
N11 waxy $B1^-$	Partial waxy	Null at $B1^-$
N11 waxy $D1^-$	Partial waxy	Null at $D1^-$
N11 waxy $A1^-/B1^-$	Partial waxy	Null at $A1^-/B1^-$
N11 waxy $A1^-/D1^-$	Partial waxy	Null at $A1^-/D1^-$
N11 waxy $B1^-/D1^-$	Partial waxy	Null at $B1^-/D1^-$

6.3 Results and Discussion

6.3.1 Initial Physical Tests

Results from initial physical tests are presented in Table 6.2. Mean hardness index (HI) values are around 90 which indicates that these samples are very hard. The majority of samples had higher bran % than flour yield. Average flour protein contents of Svevo (14.11) were higher than the N11 set (11.78). However, in both sets, complete waxy had a higher protein content than other lines. Flour moisture contents of Svevo were also higher than the N11 set.

Complete waxy lines had the lowest HI compared to partial waxy and parent lines in both the waxy sets studied. No variation for HI was observed among partial waxy lines. Mean hardness values of above 90 indicate that hardness character is independent of starch amylose concentrations as reported by Morris and Konzak (2001).

In both the waxy wheat sets studied, parent lines had higher flour yield over waxy lines which indicate that flour extraction might be related to the waxy nature of the samples. Waxy wheats tended to produce higher starch damage during milling (Bettge *et al.*, 2000) and significantly lower flour yields (Graybosch *et al.*, 2003). This can be attributed to the nature of the endosperm in which higher crystallinity of starch granules that lack amylose influences the milling properties of waxy samples (Graybosch *et al.*, 2003). However, this may not completely hold for present samples, as lowest flour yield was not found in the lowest amylose (complete waxy) samples in both the sets studied.

Takata *et al.*, (2005, 2007) also reported lower flour yields with near-isogenic waxy wheat lines, but the double null waxy lines (AB and BD null) did not show any variation. On the contrary, double waxy nulls (AB/AD/BD nulls) had lowest flour yields followed by waxy and single nulls in the present study. The increase in non-starch polysaccharide content such as arabinoxylans (Kao *et al.*, 1997) and β-glucan (Yasui *et al.*, 1999) may cause the corresponding decrease in flour yield levels in waxy wheat (Takata *et al.*, 2007).

6.3.2 Electrophoretic Characterization of HMW-GS using Lab-On-a-Chip Method

HMW-GS composition of waxy wheat samples were analysed using the Lab-on-a-chip method. In addition, subunits were originally identified by traditional SDS-PAGE to confirm the composition. Results are shown in Figure 6.1. Samples with known subunit composition were used as control to identify the apparent sizes. The standards were Chinese Spring (Null, 7+8, 2+12), Karl-92 (1, 7+8, 5+10) and Jagger (1, 5+10, 17+18).

Table 6.2 : Results from initial physical tests- Hardness Index (HI) from SKCS; Milling yield from Quadrumat Junior mill and Flour protein content analyzed by LECO protein analyzer.

Sample Name	HI	Milling Yield (%)			% Flour Protein	% Flour Moisture
		Low ash flour	Total flour	Bran		
		Svevo Set				
Svevo wheat	88.77	43.54	49.35	50.65	13.97^{c}	15.32^{a}
Svevo waxy	81.22	4137	47.03	52.97	15.15^{a}	15.25^{a}
Svevo waxy A1^{-}	85.15	42.13	47.99	52.01	14.37^{b}	14.60^{c}
Svevo waxy B1^{-}	96.02	33.77	38.73	61.27	12.95^{d}	14.88^{b}
		N11 Set				
N11 wheat	97.78	46.85	53.08	46.92	11.40^{d}	13.59^{e}
N11 waxy	87.12	41.30	47.00	53.00	12.35^{a}	14.76^{ab}
N11 waxy A1^{-}	96.89	43.86	50.00	50.00	12.32^{a}	14.69^{abc}
N11 waxy B1^{-}	90.83	43.74	50.03	49.97	12.33^{a}	14.28^{bcd}
N11 waxy D1^{-}	95.00	40.96	46.90	53.10	11.41^{d}	15.06^{a}
N11 waxy A1^{-}/B1^{-}	94.68	40.73	46.60	53.40	11.54^{b}	15.12^{a}
N11 waxy A1^{-}/D1^{-}	94.08	37.36	43.30	56.70	11.44^{cd}	13.97^{de}
N11 waxy B1^{-}/D1^{-}	94.08	39.16	44.81	55.19	11.49^{bc}	14.15^{cde}

^{abcde} Means with same letter in same column of same group are not significantly different at $P<0.05$.

Svevo is a durum wheat and has only A and B genomes. All the Svevo series contain Null, 7+8 HMW-GS respectively at A and B genomes. N11, which is a bread wheat variety, had a HMW-GS composition of 1, 7+8, 2+12. Waxy proteins in these samples can not be identified using the Lab-on-a-chip method as they originally appear at molecular weights of ~60 kDa in SDS-PAGE. Usually, LMW-GS will appear below 95 kDa in the Lab-on-a-chip method, as we can see in Figure 6.1. Running a chip with Protein kit 80+ may help in identifying the waxy proteins efficiently.

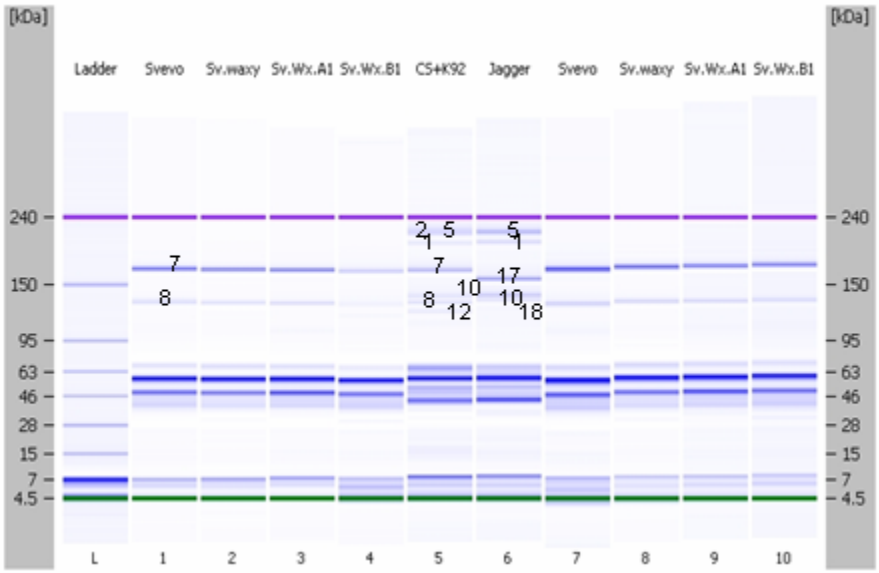
The elution sequence of HMW-GS in the present sample fractionation on the chip was 12, 8, 18, 10, 17, 7, 1, 5, and 2 which is exactly as reported by Uthayakumaran *et al.,* 2006. However, the apparent sizes of corresponding subunits may not match exactly the

previous results (Uthayakumaran *et al.,* 2006), since the Protein chip used in the present study has a higher molecular range (230+ kit) than the 210+ kit they used. The apparent molecular weights of samples used are shown in Table 6.3.

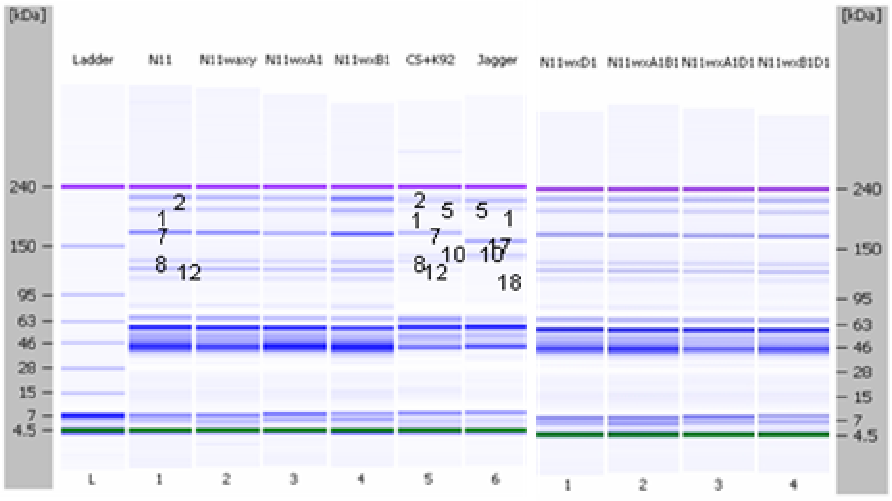
Table 6.3: Apparent sizes (kDa) of HMW-GS determined using the Lab-on-a-Chip method

	Glu-A1	*Glu-B1*				*Glu-D1*			
Subunit	1	7	8	17	18	2	5	10	12
Size(KDa)	202.0	165.6	130.6	152.6	133.1	214.4	214.0	135.7	121.6

Figure 6-1: The HMW-GS composition identified by Lab-on-a-chip method. (A) Svevo set (B) N11 set. Notice that all waxy lines have the same subunit composition as the parent line.



(A) Svevo set



(B) N11 set

6.3.3 Protein Composition and Physical Dough Tests

HPLC results are presented in Table 6.4. Significant differences were observed for UPP in which complete waxy had either lower (Svevo waxy) or higher (N11 waxy) values than other lines. Albumin and globulin composition was relatively lower than regular wheats. All the Svevo lines had gliadin to glutenin ratios above 1.

Physical dough test results are shown in Table 6.5. No large variations were found in MDDT among the waxy lines. In contrast, extensibility parameters R_{max} and extensibility differed significantly. However, there was no specific trend observed for any of the quality parameters studied.

Relatively lower mixograph peak times and tolerance scores were reported for waxy lines (Graybosch *et al.*, 2003). In contrast, moderately high peak development times with high peak width at 8 min. were observed with waxy wheat lines. High mixograph water absorption levels coupled with high protein content in complete waxy lines of both the sets suggests that lack of amylose in these lines may cause a corresponding increase of endosperm protein content and other non-polysaccharides in the flour. Significantly higher water absorption levels with waxy wheat doughs were reported which was attributed to high protein and dietary fiber content (Morita *et al.*, 2002). High starch damage levels (Bettge *et al.*, 2000; Guo *et al.*, 2003) or higher arabinoxylan contents (Michniewicz *et al.*, 1991) could also cause the increase in water absorption levels of waxy wheat.

Table 6.4: Results from SE-HPLC for near-isogenic lines of Svevo and N11 waxy wheats

Sample Name	Gliadin/Glutenin ratio	%UPP	PPP	%Gliadin	%Albumin+Globulin
		Svevo wheat			
Svevo wheat	1.18	41.92[a]	42.75[a]	49.65[ab]	5.20[b]
Svevo waxy	1.31	38.60[b]	42.75[a]	50.60[a]	5.00[b]
Svevo waxy A1$^-$	1.13	41.55[a]	44.00[a]	47.00[c]	6.61[a]
Svevo waxy B1$^-$	1.19	41.40[ab]	43.30[a]	49.20[b]	6.10[ab]
		N11 wheat			
N11 wheat	0.96	49.96[bc]	41.45[abc]	47.90[ab]	9.00[b]
N11 waxy	0.91	52.05[a]	42.45[ab]	47.50[b]	8.87[b]
N11 waxy A1$^-$	1.03	47.31[de]	40.65[c]	48.90[a]	8.65[b]
N11 waxy B1$^-$	1.06	46.44[e]	41.05[bc]	49.05[a]	8.50[b]
N11 waxy D1$^-$	0.91	51.69[ab]	42.90[a]	47.00[b]	8.55[b]
N11 waxy A1$^-$/B1$^-$	0.97	49.19[cd]	42.25[ab]	47.50[b]	8.47[b]
N11 waxy A1$^-$/D1$^-$	0.88	51.45[ab]	42.85[a]	45.50[c]	9.75[a]
N11 waxy B1$^-$/D1$^-$	0.90	52.43[a]	42.50[ab]	47.20[b]	8.96[b]

[abcde] Means with the same letter in same column of same group are not significantly different at $P<0.05$

The correlation matrix between protein composition and various quality parameters and loaf volume are presented in Table 6.6. Dough strength as measured by MDDT ($R^2=0.29$) and R_{max} ($R^2=0.38$) was correlated moderately with UPP. On the other hand, extensibility ($R^2=0.33$) and loaf volume ($R^2=0.87$) were well correlated with flour polymeric protein (FPP). Interestingly loaf volume, an important bread making quality parameter, was negatively correlated with UPP ($R^2=-0.04$) but highly and positively correlated with PPP ($R^2=0.76$) and FPP ($R^2=0.87$).

UPP is a parameter that gives a relative measure of molecular weight distribution of the polymeric protein based on solubility (Gupta *et al.*, 1993). Dough strength measurements such as R_{max} and MDDT correlate well with UPP indicating that only a portion of highest molecular weight contributes to dough strength (Southan and MacRitchie, 1999) and thus a greater amount of UPP signifies shifts in molecular weights to higher values. In the present

study, though positive correlation exists between UPP and MDDT, correlation of UPP with R_{max} for waxy lines, is weak. This may be due to a lack of dough strength contributing subunits such as 5+10 and the appearance of 2+12. In addition, the presence of 7+8 in the N11 set may also contribute strength. HMW-GS 7+8 were given a score of 'three' in terms of bread making quality (Payne *et al.*, 1987). On the other hand, extensibility correlates well with FPP in certain wheat samples (Larroque *et al.*, 1999; Gupta *et al.*, 1992). In the present study, not only extensibility but also the loaf volume correlates well with FPP and PPP. FPP depends on flour protein content which is largely determined by environment and PPP on the other hand is genetically controlled (Southan and MacRitchie, 1999). As all the waxy wheat NILs were grown under the same conditions, it is presumed that the waxy wheat nature is contributing largely to PPP.

Table 6.5: Results from quality tests (MDDT & Peak width at 8 min. from Mixograph; R_{max} & Extensibility from micro-extensibility tests) for near-isogenic lines of Svevo and N11 waxy wheats

Sample Name	Mixograph tests		Extensibility results	
	MDDT (min.)	Width at 8 min.	R_{max} (mN)	Extensibility (mm)
		Svevo set		
Svevo wheat	4.0^{ab}	26.2^{a}	486^{a}	32.0^{c}
Svevo waxy	4.7^{b}	19.6^{b}	342^{c}	40.0^{b}
Svevo waxy A1^{-}	3.6^{b}	23.7^{ab}	317^{d}	43.4^{a}
Svevo waxy B1^{-}	3.9^{ab}	23.5^{ab}	459^{b}	30.1^{c}
		N11 set		
N11 wheat	3.7^{c}	21.0^{bcd}	368^{e}	51.4^{c}
N11 waxy	3.6^{c}	21.8^{bc}	463^{bc}	61.3^{b}
N11 waxy A1^{-}	3.5^{c}	18.9^{cd}	437^{d}	44.0^{d}
N11 waxy B1^{-}	2.9^{d}	18.1^{d}	326^{f}	68.5^{a}
N11 waxy D1^{-}	4.8^{a}	27.3^{a}	554^{a}	35.6^{f}
N11 waxy A1^{-}/B1^{-}	3.3^{c}	21.7^{bc}	390^{e}	54.2^{c}
N11 waxy A1^{-}/D1^{-}	4.2^{b}	27.4^{a}	442^{cd}	39.0^{ef}
N11 waxy B1^{-}/D1^{-}	4.1^{b}	23.2^{b}	469^{b}	41.0^{de}

^{abcde} Means with same letter in same column of same group are not significantly different at $P<0.05$

The important quality attributes such as MDDT, R_{max}, extensibility and loaf volume were normalized to flour protein content to eliminate the effect of variation in protein amounts. These correlation values are indicated in parenthesis in Table 6.6. However, there is no differences in correlation trends were observed after normalization.

Table 6.6: Correlation matrix for various quality parameters and protein composition*.

	MDDT	R_{max}	Ext.	LV	PPP	UPP	FPP	GGR	PAG	PG	FP
MDDT	1.000	0.124	-0.697	-0.331	0.124	0.291 (0.275)	-0.052	0.095	-0.264	-0.025	0164
R_{max}		1.000	-0.177	-0.255	-0.205	0.389 (0.295)	-0.302	0.303	-0.467	0.215	0.505
Ext.			1.000	0.661	0.178	-0.091	0.337 (0.560)	-0.311	0471	-0.069	-0.261
LV				1.000	0.766	-0.047	0.870 (0.870)	-0.841	0.867	-0.514	-0.726
PPP					1.000	0.305	0.794	-0.785	0.691	-0.661	-0.621
UPP						1.000	-0.257	0.255	-0.346	0.072	-0.909
FPP							1.000	-0.984	0.926	-0.725	0.555
GGR								1.000	-0.938	0.822	0.900
PAG									1.000	-0.730	-0.898
PG										1.000	0.634
FP											1.000

* MDDT=Mixograph dough development time; R_{max}=Maximum resistance in micro-extensibility test; Ext.=Extensibility; LV=Loaf volume; PPP= Percent polymeric protein; FPP= Flour polymeric protein; UPP=Unextractable polymeric protein; GGR=Gliadin/Glutenin ratio; PAG=Percent Albumin and Globulins; PG=Percent gliadins; FP=Flour protein

Values in parenthesis were after normalized to flour protein content

6.3.4 Test Baking Results

Micro bread loaves (10 g flour) were baked with both durum (Svevo) and bread (N11) wheat waxy lines (Figure 6.2). Baking was performed with 100% waxy and 50% waxy

and 50% commercial bread wheat as a blend. A correlation matrix among bread quality parameters, protein composition and baking measurements using the C-cell are presented in Appendix A-2.

The two different sets of waxy lines responded differently with respect to baking parameters. However, in similarity, all the waxy lines in both sets had higher or at least equal loaf volumes as parent lines. Interestingly, except for two double nulls (A1D1/B1D1) in the N11 set, all other waxy lines show improved loaf volume for waxy flour alone over those blended with commercial bread wheat flour (Figure 6.2 B). In contrast, the Svevo set, which has durum background, the waxy lines with 50% blend showed higher loaf volumes. In both sets, under both the treatments (100% waxy or 50% blend), crumb scores were not influenced by blending.

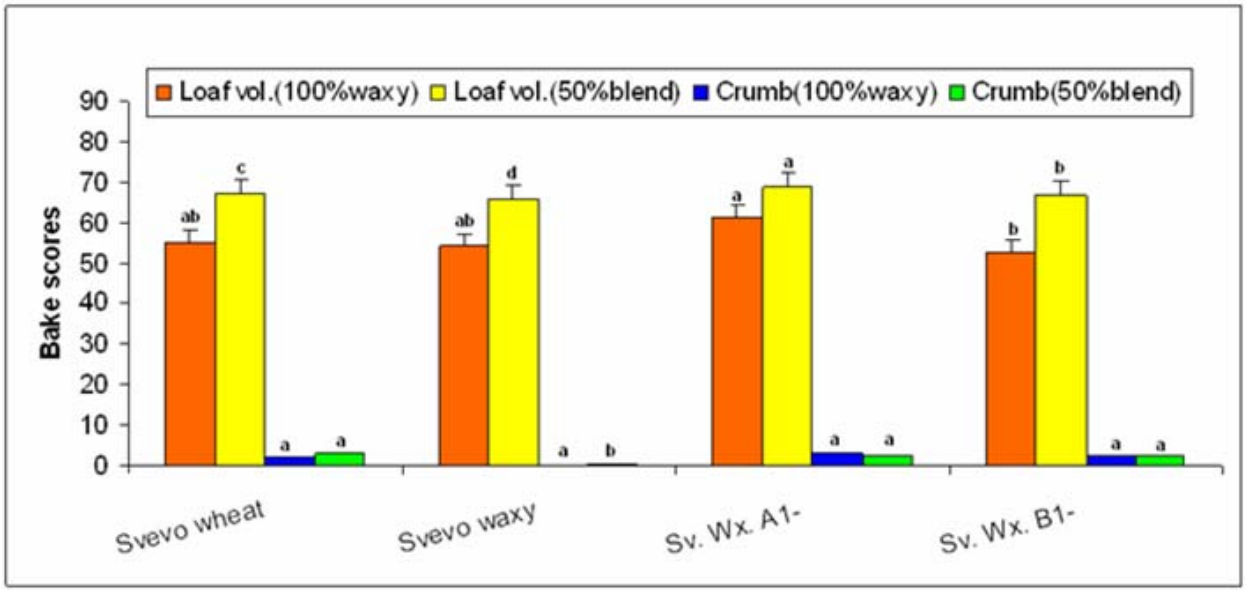
All the measurements conducted by the C-cell had good correlation among each other. As was mentioned earlier, dough strength measurements like MDDT, R_{max} and UPP were poorly correlated with loaf volume whereas loaf volume was highly correlated with PPP and FPP.

As shown in bread loaves of control (N11 wheat) and waxy wheat, crumb made with waxy has more porous, open crumb grain with large bubbles inside the crumb (Appendix Fig.B-2c and 2b). A similar trend has also been observed with Svevo waxy (Appendix Fig.B-2a and 2b). However, blending with 50% commercial flour improved the crumb structure with less porosity and smaller bubbles inside the grain. Nevertheless, breads made with 100% waxy look darker in color with dull appearance that is unacceptable to consumers. Acceptable change in color with improved appearance such as crumb grain can be achieved by blending up to 50% with any commercial bread wheat flour.

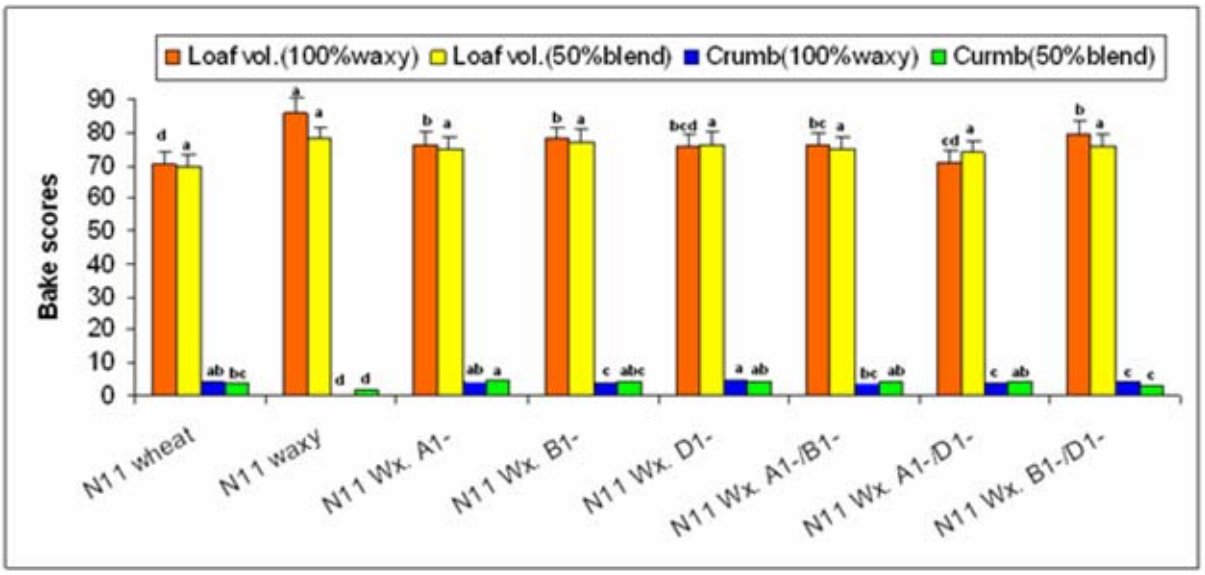
Results are in accordance with previous studies. Higher loaf volume with improved glutinous structure was observed with substitution of 40% waxy wheat flour (Morita *et al.*, 2002) whereas 50% substitution resulted in slightly decreased loaf volume with more porous crumb structure and higher retrogradation (Lee *et al.*, 2001; Haykawa *et al.*, 2004). Though there was significant difference between samples for loaf volume, highest volume was with the waxy line in the N11 set whereas single nulls had the highest volume in the Svevo set.

Figure 6-2: Loaf volumes and crumb scores from micro-breads made with 100% waxy wheat and 50% blend with commercial wheat for Svevo and N11 waxy wheats (A). Svevo set (B). N11 set

(A). Svevo set



(B). N11 set



Though shelf-life studies have not been conducted on these waxy samples, waxy lines are expected to increase the storage period as reported earlier (Lee *et al.*, 2001; Haykawa *et al.*, 2004; Morita *et al.*, 2002).

6.4 Conclusions

Loaf volume, which is an important bread making quality, was highest with 100% waxy wheat flour and was not improved with 50% blending with commercial bread wheat flour. However, dark color and poor appearance with large bubbles was observed with 100% waxy flour; this is unacceptable to consumers. In terms of protein composition, waxy wheats have relatively lower albumins/globulins than regular wheat. Dough strength parameters such as MDDT and R_{max} were poorly correlated with UPP whereas loaf volume and extensibility were highly and positively correlated with FPP and PPP.

References

Baik, B-K., and Lee, M-R. 2003. Effects of starch amylase content of wheat on textural properties of white salted noodles. Cereal. Chem. 80:304-309.

Bettge, A.D., Giroux, M.J., and Morris, C.F. 2000. Susceptibility of waxy starch granules to mechanical damage. Cereal Chem. 77:750-753.

Fujita, N., Wadano, A., Kozaki, S., Okabe, S., and Taira, T. 1996. Comparison of the primary structure of *Waxy* proteins (Granule-Bound Starch Synthase)between polyploidy wheats and related diploid species. Biochem. Genet. 34:403-413.

Graybosch, R.A. 1998. Waxy wheats: Origin, properties, and prospects. Trends Food Sci. Technol. 9:135-142.

Graybosch, R.A., Souza, E., Berzonsky, W., Baenziger, P.S., and Chung, O. 2003. Functional properties of waxy wheat flours: genotypic and environmental effects. J. Cereal Sci. 38:69-76.

Gupta, R.B., Batey, I.L., and MacRitchie, F. 1992.Relationships between protein composition and functional properties of wheat flours. Cereal Chem. 69:125-131.

Gupta, R.B., Khan, K., and MacRitchie, F. 1993. Biochemical basis of flour properties in bread wheats. I. Effects of variation in quantity and size distribution of polymeric proteins. J. Cereal Sci. 18:23-44.

Hayakawa, K., Tanaka, K., Nakamura, T., Endo, S., and Hoshino, T. 2004. End use quality of waxy wheat flour in various grain based foods. Cereal Chem. 79:102-107.

Hung, P.V., Maeda, T., and Morita, N. 2006. Waxy and high-amylose wheat starches and flours-characteristics, functionality and application. Trends Food Sci. Technol. 17:448-456.

Kiribuchi-Otobe, C., Nagamine,T., Yanagisawa, T., Ohnish, M., and Yamaguchi, I. 1997. Production of hexaploid wheats with waxy endosperm character. Cereal Chem. 74:72-74.

Larroque, O.R., Gianibelli, M.C., and MacRitchie, F. 1999. Protein composition for pairs of wheat lines with contrasting dough extensibility. J. Cereal Sci. 9:27-31.

Lee, M-R., Swanson, B.G., and Baik, B-K. 2001. Influence of amylose content on properties of wheat starch and bread making quality of starch and gluten blends. Cereal Chem. 78:701-706.

Miura, H., Tanii, S., Nakamura, T., and Watanabe, N. 1994. Genetic control of amylose content in wheat endosperm starch and differential effects of three *Wx* genes. Theor. Appl. Genet. 89:276-280.

Miura, H., Araki, E., and Tarui, S. 1999. Amylose synthesis capacity of the three *Wx* genes of wheat cv Chinese spring. Euphytica. 108:91-95.

Miura, H., Wickramasinghe, M.H.A., Subhasinghe, R.M., Araki, E., and Komae, K. 2002. Development of near-isogenic lines of wheat carrying different null *Wx* alleles and their starch properties. Euphytica. 123:353-359.

Morita, N., Maeda, M., Miyazaki, M., Yamamori, M., Miura, H., and Ohtsuka, I. 2002. Dough and baking properties of high amylose and waxy wheat flours. Cereal Chem. 79:491-495.

Morris, C.F,. and Konzak, C.F. 2001. Registration of hard and soft homozygous waxy wheat endosperm. Crop Sci. 41:934-935.

Nakamura, T., Yamamori, M., Hirano, H., and Hidaka, S. 1993.Decrease of waxy (*wx*) protein in two common wheat cultivars with low amylase content. Plant Breed. 111:99-105.

Nakamura, T., Yamamori, M., Hirano, H., Hidaka, S., and Nagamine, T. 1995. Production of Waxy (Amylose-free) Wheats. Mol. Gen. Genet. 248:253-259.

Nakamura, T., Vrinten, P., Saito, M., and Konda, M. 2002. Rapid classification of partial waxy wheats using PCR-based markers. Genome. 45:1150-1156.

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

Sivak, M.N., and Preiss, J. 1995. Starch synthesis in foods.Pages 139-168 In: Seed development and germination, Kigel, J. and Galili, G. eds. Marcel Dekker.

Southan, M., and MacRitchie, F. 1999. Molecular weight distribution of wheat proteins. Cereal Chem. 76(6): 827-836.

Takata, K., Nishio, Z., Iriki, N., Tabiki, T., Funatsuki, W., and Yamauchi, H. 2005. Comparison of quality characteristics of waxy wheat using near-isogenic line. Breed. Sci. 55:87-92.

Takata, K., Yanaka, M., Fujita, Y., and Ishikawa, N. 2007. Evaluation of the grain and flour quality in near-isogenic wheat lines with waxy and double-null *Wx* proteins. Breed. Sci. 57:79-83.

Uthayakumaran, S., Listiohadi, M., Baratta, M., Batey, I.L., and Wrigley, C.W. 2006. Rapid identification and quantitation of high-molecular-weight glutenin subunits. J. Cereal Sci. 44:34-39.

Wickramasinghe, M.H.A., Miura, H., Yamauchi, H., and Noda, T. 2003. Properties of starches from near-isogenic wheat lines with different Wx protein deficiencies. Cereal Chem. 80:662-666.

Yamamori, M., Fujita, S., Hayakawa, K., Matsuki, J., and Yasui, T. 2000.Ggenetic elimination of starch granule protein, SGP-1, of wheat generates and altered starch with apparent high amylase. Theor. Appl. Genet. 101:21-29.

Yasui, T., Matsuki, J., Sasaki, T., and Yamamori, M. 1996. Amylose and lipid contents, amylopectin structure, and gelatinization properties of waxy wheat (Triticum aestivum L.) starch. J. Cereal Sci. 24:131-137.

Yasui, T., Matsuki, J., Sasaki, T., and Yamamori, M. 1997. Waxy endosperm mutants of bread wheat (*Triticum aestivum* L.) and their starch properties. Breed. Sci. 47:161-163.

CHAPTER 7 - TRITICALE

7.1 Introduction

Triticale (X *Triticosecale Wittmack*), the first man-made cereal, is a hexaploid that combines the A and B genomes from wheat and the R genome of rye. Critical area under cultivation has been increasing very slowly (Arseniuk and Oleksiak, 2002) but steadily (Varughese et al., 1996) all over the world, with its current cultivation of 3.6 million ha wih a production of 11.3 million tons (FAO, 2006). This might be due to its limited end-uses as a human food (Briggs, 1991; Pena, 1996). Triticale is bred and produced primarily as a forage/feed crop. Its usage as a crop for human consumption has been limited by its inferior bread making quality. However, good quality baked products from triticale can be obtained possibly by using appropriate baking methods (Rakowska and Haber, 1991).

Cultivated triticales are secondary hexaploids (AABBRR) and they differ from bread wheat (AABBDD) by replacing the D genome with the R genome from rye (*Secale cereale* L.). Consequently, triticale has one third fewer loci coding for important gluten storage proteins and contains less gluten (Lukaszewski, 1998). The genes conferring bread making properties of wheat storage proteins are located on group 1 and 6 chromosomes. HMW-GS are encoded by genes (*Glu-1)* on the long arms of group 1 chromosomes, and on the short arms-LMW-GS (*Glu-3*) and gliadins (*Gli-1*) are encoded, whereas the short arms of group 6 chromosomes code for other gliadin types (*Gli-2*). The R genome of rye has brought the loci *Sec-3* (on 1RL), *Sec-1* (on 1RS) and *Sec-2* (on 2 RL) into triticale (Lukaszewski, 2001; Shewry *et al*., 1984). Thus the removal of the D genome in triticale excluded the *Glu-D1* (on 1DL), *Gli-D1* and *Glu-D3* (on 1DS) and *Gli-D2* (on 6DS) (Martinek *et al*., 2008). These differences resulted in the poor bread making quality of triticale (Zeller and Hsam, 1984). However, not all the secalins are detrimental to bread making quality; *Sec-3* had some positive effects on dough properties (Kumlay *et al*., 2003) and no effect of *Sec-2* on any dough properties has so far been reported (Gupta *et al*., 1989). Nevertheless, in all rankings of the relative contribution of individual group-1 chromosomes to bread making quality of

triticale, rye chromosome 1R has always been placed higher than wheat chromosome 1A (Kazman and Lelly, 1996; Lukaszweski, 1996, 1998; Kumaly *et al*., 2003).

If triticale is to become a bread cereal, its genetic constitution of storage protein loci has to be restored to that of wheat, especially by the introduction of the *Glu-D1d* gene. This can be achieved in several different ways; transformation with known storage protein loci from the D-genome of wheat and concomitant silencing of the detrimental secalin loci (Shewry *et al*., 1995), chromosome substitution, or cytogenetic engineering of specific segments of chromosomes that carry important loci (Lukaszewski, 2006).

Three possible substitutions, 1D (1A), 1D (1B), and 1D (1R) lead to significant increase in the Zeleny sedimentation value. The difference between two gluten alleles *Glu-D1a* (HMW-GS 2+12) and *Glu-D1d* (HMW-GS 5+10) was not as obvious as in wheat. Besides its high cytological stability and minimal effect on agronomic performance, substitution with 1D (1A) appears to be the most desirable in triticale breeding while other two substitutions (1D (1B) and 1D (1R)show considerable yield loss (Lafferty and Lelley, 2001, Lukaszewski, 1990). Although the baking quality of substitutions 1D (1R) and 1D (1A) was comparable to a wheat check cultivar and improved over previous triticale lines, replacement of 1A or 1B by 1D does not eliminate the effect of chromosome 1R and rye secalins in triticale (Brezinski and Lukaszewski, 1998).

With both substitutions 1D (1R) and 1A (1R) being agronomically unacceptable, the only option to improve the breadmaking quality of triticale is to use translocation lines involving the D genome of wheat (Lukaszewski, 1998). Introduction into the rye genome would be the most beneficial as they would not only restore genetic composition of bread wheat but also remove rye protein loci. Translocations of 1A.1D and 1R.1D were developed to introduce the *Glu-D1a* and *Glu-D1d* alleles into triticale to improve the bread making performance.

The present study was conducted with the objective of evaluation and comparing the performance of translocations 1A.1D and 1R.1D with HMW-GS 5+10 and 2+12 in terms of physical dough tests and baking quality using four different sets of triticale lines.

7.2 Methodology

Four sets of triticale translocation lines, GDS7, Trim, Rhino, and Rigel were used in the study. These translocations include 1R.1D (HMW-GS 5+10) and 1A.1D (HMW-GS 5+10 and 2+12) with an exception only in the GDS7 set where we have 1R.1D (2+12) instead of 1A.1D (2+12). These lines were developed to evaluate the effect of the specific

Table 7.1: Description of different triticale sets used in the study.

Sample ID	Translocation	HMW Glutenin Subunits on chromosome		
		1A	1B	1R
	GDS7 set			
GDS7	Parent line	Null	13+16	
GDS7 1R.1D 5+10	1R.1D	Null	13+16	5+10
GDS7 1R.1D 2+12	1R.1D	Null	13+16	2+12
GDS7 1A.1D 5+10	1A.1D	5+10	13+16	
	Trim set			
Trim	Parent line	1	13+16	
Trim 1R.1D 5+10	1R.1D	1	13+16	5+10
Trim 1A.1D 2+12	1A.1D	2+12	13+16	
Trim 1A.1D 5+10	1A.1D	5+10	13+16	
	Rhino set			
Rhino	Parent line	Null	13+16	*Sec-3*
Rhino 1R.1D 5+10	1R.1D	Null	13+16	5+10
Rhino 1A.1D 2+12	1A.1D	2+12	13+16	*Sec-3*
Rhino 1A.1D 5+10	1A.1D	5+10	13+16	*Sec-3*
	Rigel set			
Rigel	Parent line	Null	13+16	
Rigel 1R.1D 5+10	1R.1D	Null	13+16	5+10
Rigel 1A.1D 2+12	1A.1D	2+12	13+16	
Rigel 1A.1D 5+10	1A.1D	5+10	13+16	

translocation types on bread making performance. Brief description of the lines used along with HMW-GS composition is presented in Table 7.1. All the triticale sets have 13+16 subunits in common at the *Glu-B1* loci.

Protein composition analyzed using SE-HPLC, physical dough tests and other analytical measurements were described in Chapter 3.

7.3 Results and Discussion

7.3.1 Initial Physical Tests

Results from the initial physical tests such as milling yield and flour protein content are presented in Table 7.2. All the triticale samples appear to be on the low end of hard wheats with an average hardness index value of 57.73. Milling quality of all triticales studied was poor with lower flour yield (34-46%) and higher bran (53-67%). Although there is no specific trend observed for flour protein content, 1R.1D with 5+10 subunits are highest in two sets of triticales (Trim and Rigel) compared to other lines.

Higher bran yields and poor flour yields obtained with triticale samples in the present study are in accordance with previous studies. Typical grain morphological characteristics in triticale are less favorable for the production of flour than wheats (Weipert, 1986). Triticale cultivars usually will have grains with soft endosperm, a characteristic responsible for the poor flour flow properties that affect negatively both flour sifting during milling and flour yield (MacRitchie, 1980). Semi-hard triticales showed poorer milling properties than the soft wheat samples (Pena and Amaya, 1992). However, the defects in grain morphology of triticale predominated over grain hardness in defining the milling performance. Co-milling of wheat/triticale would be a good practice to improve the milling performance of triticale.

Flour protein content is extremely important because almost all flour properties (gluten content, water absorption, MDDT and loaf volume) are highly correlated with protein content (Pomeranz, 1985). Flour protein content of triticales ranged from 6.86 to 10.27 in all the different groups studied.

Table7.2: Results from initial physical tests- Hardness Index (HI) from SKCS; Milling yield from Quadrumat Junior mill and Flour protein content analyzed by LECO protein analyzer*.

Sample Name	HI	Milling Yield (%)			% Flour Protein	% Flour Moisture
		Low ash flour	Total flour	Bran		
		GDS7 Set				
GDS7	52.50	34.69	40.15	59.85	9.03[a]	14.88[a]
GDS7 1R.1D 5+10	58.12	32.60	37.80	62.20	8.34[bc]	14.07[a]
GDS7 1R.1D 2+12	59.77	27.60	32.64	67.36	8.38[b]	14.61[a]
GDS7 1A.1D 5+10	58.40	34.69	41.02	58.98	8.33[c]	14.53[a]
		Trim set				
Trim	56.98	31.80	37.83	62.17	7.99[c]	13.71[a]
Trim 1R.1D 5+10	68.11	33.90	38.81	61.19	9.69[a]	13.88[a]
Trim 1A.1D 2+12	54.59	30.07	35.94	64.06	8.97[b]	14.33[a]
Trim 1A.1D 5+10	56.37	27.35	32.55	67.45	8.07[c]	14.62[a]
		Rhino set				
Rhino	63.20	37.92	43.60	56.40	7.41[a]	13.96[b]
Rhino 1R.1D 5+10	61.96	35.36	40.91	59.09	6.86[c]	14.58[a]
Rhino 1A.1D 2+12	52.74	34.83	42.29	57.71	6.86[c]	14.14[ab]
Rhino 1A.1D 5+10	52.71	39.55	46.23	53.77	6.92[b]	14.26[ab]
		Rigel set				
Rigel	59.00	40.41	46.19	53.81	7.24[c]	13.47[c]
Rigel 1R.1D 5+10	54.45	29.73	34.10	65.90	10.27[a]	14.26[ab]
Rigel 1A.1D 2+12	51.23	39.50	45.46	54.54	7.39[c]	13.79[bc]
Rigel 1A.1D 5+10	63.58	31.76	36.85	63.15	9.13[b]	14.87[a]

[abcd] Means with the same letter in the same column of the same group are not significantly different at $P<0.05$.

7.3.2 Electrophoretic Characterization of HMW-GS using the Lab-On-a-Chip Method

HMW-GS composition of triticale samples was analyzed using Lab-on-a-chip method (Fig.7.1). Apparent molecular sizes of subunits are shown in Table 7.3. In common, all the

triticale lines have 13+16 subunits at the *Glu-B1* loci. Samples with known subunit composition were used as a control to identify specific HMW-GS. These were Chinese Spring (Null, 7+8, 2+12) and Karl-92 (1, 7+8, 5+10). In addition, SDS-PAGE analysis was also performed to confirm the subunit identification.

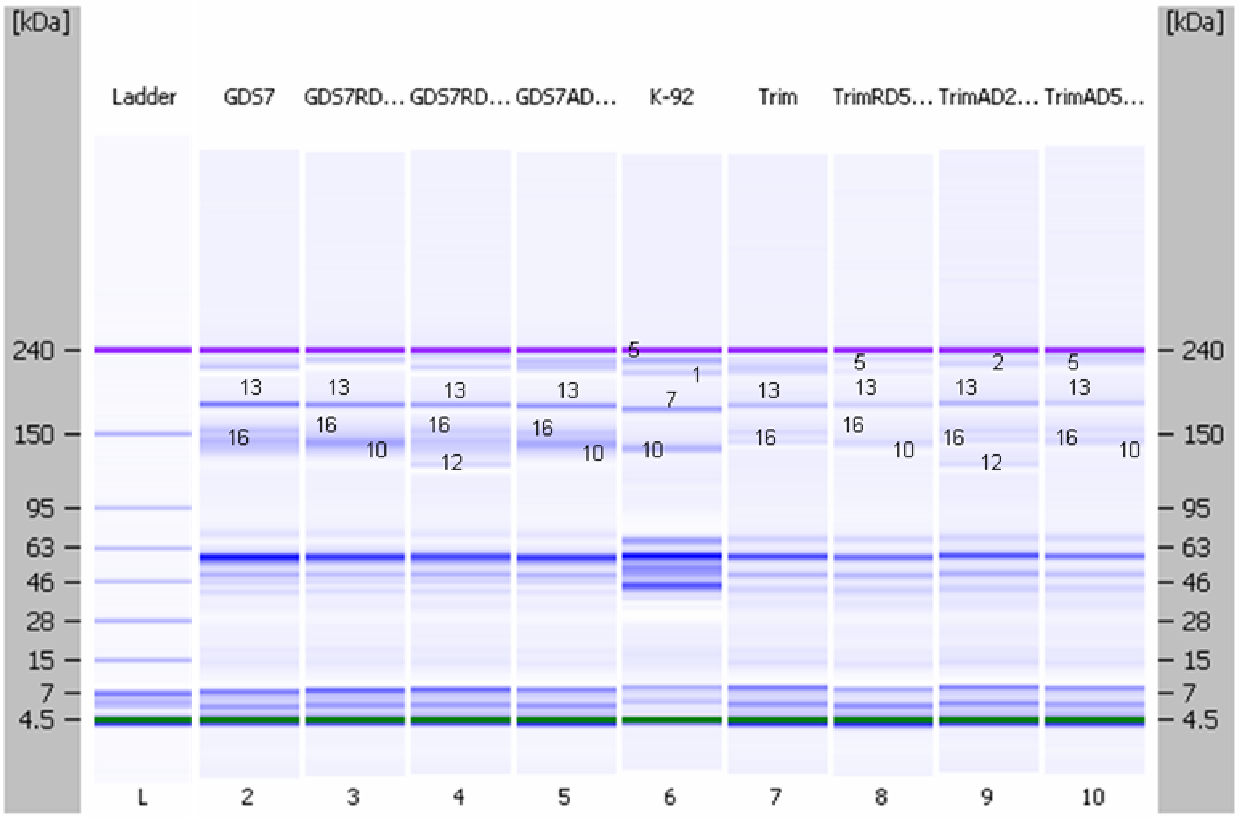
Electrophoregram patterns of triticale sets were similar to rye and wheat samples, in which HMW-GS from wheat and HMW secalins from rye have been observed (Fig.7.1). Tohver *et al*., (2005) reported glutenin patterns of most of the triticale cultivars were similar to the glutenin pattern of rye, whereas some were closer to that of wheat using SDS-PAGE. The migration sequence of HMW-GS on the chip system was 12, 8, 10, 7, 13, 1, 4, 5, and 2 which is the same as reported by Uthayakumaran *et al.,* 2006. However, apparent sizes of corresponding subunits may not match exactly the previous results (Uthayakumaran *et al.,* 2006), since the Protein chip used in the present study has a higher molecular range (230+ kit) against the 210+ kit that they used.

Table 7.3: Apparent sizes (kDa) of HMW-GS determined using the Lab-on-a-Chip method

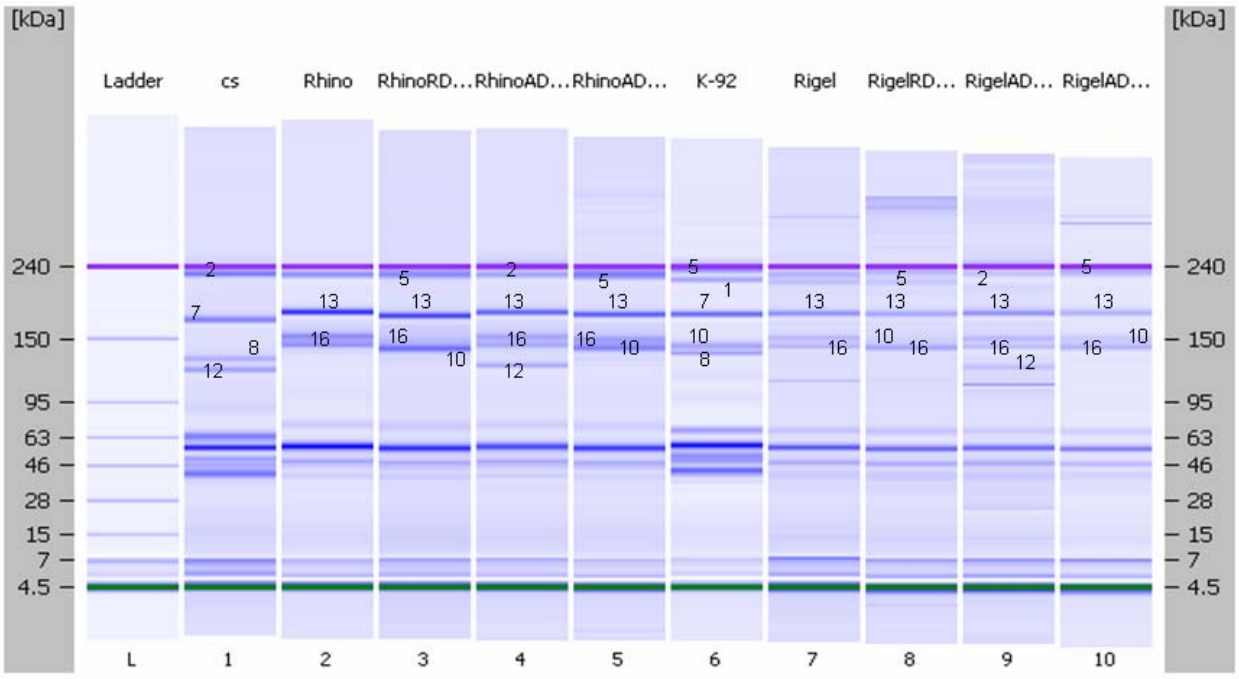
	Glu-A1	A/R genome				*Glu-B1*				R genome
Subunit	1	2	5	10	12	7	8	13	16	*HMW Secalin*
Size(kDa)	202	214.4	214	135.7	121.6	165.6	130.6	181.9	144.5	Varies

Figure 7-1: The HMW-GS composition of triticale samples identified by Lab-on-a-chip method.

(A) GDS7 and Trim sets



(B) Rhino and Rigel sets



7.3.3 Protein Composition and Physical Dough Tests

Triticale protein compositional data analyzed by SE-HPLC are presented in Table 7.4. Although there were significant differences found among triticale lines in each set, there was no specific pattern observed for any of the parameters measured. However, except for the Rigel set, 1A.1D 5+10 had the highest UPP compared to the other lines. There was not much difference found for protein fractions in triticale compared to regular wheat samples.

Table 7.4: Results from SE-HPLC for different triticale sets.

Sample Name	Gliadin/ Glutenin ratio	LMW/ HMW ratio #	%UPP	PPP	%Gliadin	%Albumin+ Globulin
		GDS7 set				
GDS7	0.88	1.28	40.57^{b}	46.24^{a}	35.74^{a}	16.61^{ab}
GDS7 1R.1D 5+10	0.92	1.05	41.64^{b}	44.76^{a}	38.17^{a}	14.09^{b}
GDS7 1R.1D 2+12	0.90	1.61	42.86^{b}	44.48^{a}	38.39^{a}	17.11^{a}
GDS7 1A.1D 5+10	0.81	1.04	48.55^{a}	42.30^{a}	39.30^{a}	18.38^{a}
		Trim set				
Trim	1.25	1.96	36.94^{bc}	35.19^{c}	46.30^{ab}	18.49^{a}
Trim 1R.1D 5+10	1.43	3.61	33.26^{c}	33.06^{d}	47.53^{a}	15.56^{b}
Trim 1A.1D 2+12	1.05	1.31	42.34^{ab}	36.43^{a}	44.51^{b}	19.05^{a}
Trim 1A.1D 5+10	0.99	2.02	45.30^{a}	35.43^{b}	44.89^{b}	15.26^{b}
		Rhino set				
Rhino	1.71	1.36	24.14^{b}	41.87^{a}	41.23^{a}	13.40^{b}
Rhino 1R.1D 5+10	1.08	1.54	35.62^{a}	42.61^{a}	38.59^{ab}	14.78^{a}
Rhino 1A.1D 2+12	1.08	1.69	35.12^{a}	43.63^{a}	37.94^{b}	14.49^{a}
Rhino 1A.1D 5+10	1.06	1.39	37.02^{a}	45.57^{a}	39.23^{ab}	14.83^{a}
		Rigel set				
Rigel	0.88	1.13	40.59^{bc}	47.40^{a}	35.68^{b}	13.32^{b}
Rigel 1R.1D 5+10	0.89	1.09	38.34^{c}	42.87^{c}	41.92^{a}	12.21^{c}
Rigel 1A.1D 2+12	0.87	1.19	46.44^{a}	44.25^{bc}	36.61^{b}	14.77^{a}
Rigel 1A.1D 5+10	0.79	1.16	42.42^{b}	46.19^{ab}	36.71^{b}	13.47^{b}

^{abcd} Means with same letter in same column of same group are not significantly different at $P<0.05$.

LMW/HMW ratio was calculated from Lab-on-a-chip analysis

Higher amounts of albumins and globulins were found with GDS7 and Trim sets which were similar to what has been previously reported (Naeem *et al.*, 2002).

Dough quality measurements like MDDT, R_{max} and extensibility are presented in Table 7.5. Micro-extensibility tests were performed to measure R_{max} and extensibility. Generally, MDDT measured by the mixograph were low compared to regular wheat with the exception that GDS7 and Rigel set in which 1A.1D 5+10 had higher MDDT. 1A.1D 5+10 samples in all triticale sets were higher in MDDT over other lines. Both R_{max} and extensibility values were low compared to regular wheat, ranging from 195-393 mN and 18.4-28.9 mm respectively. Both MDDT and R_{max} followed a similar trend in which 1A.1D 5+10 had the highest values among all the samples. Triticale doughs developed readily with low stability and fast break down, indicating they were deficient in gluten protein quantity and quality. Weaker gluten, lower water absorption and lower dough mixing tolerance with significantly lower mixing times with triticales have been reported (Serna-Saldivar *et al.*, 2004; Seguchi *et al.*, 1999).

The correlation matrix between various protein composition and physical dough test parameters are shown in Table 7.6. Dough strength as measured by MDDT (R^2=0.42) and R_{max} (R^2=0.61) was correlated well with UPP. On the other hand, extensibility (R^2=0.36) and loaf volume (R^2=0.78) were correlated positively with FPP. Naeem *et al.*, (2002) reported similar results in which both the extensibility (R^2=0.96) and R_{max} (R^2=0.66) were highly correlated with FPP. Gupta *et al.*, (1993) observed a similar trend with wheat flour.

Higher UPP, R_{max} and MDDT values along with a lower gliadin to glutenin ratio in the case of 1A.1D 5+10 of GDS7 and Rigel sets indicate that the molecular weight distribution is shifted to higher molecular weights, thus resulting in greater dough strength associated with 5+10 subunits. Similar findings were reported by MacRitchie and Lafiandra (2001).

The important quality attributes such as MDDT, R_{max}, extensibility and loaf volume were normalized to flour protein content to eliminate the effect of variation in protein amounts. These correlation values are indicated in parenthesis in Table 7.6. However, there were no differences in correlation trends observed after normalization.

Table 7.5: Results from quality tests (MDDT & Peak width at 8 min. from Mixograph; R_{max} & Extensibility from micro-extensibility tests) for different sets of triticale.

Sample Name	Mixograph tests		Extensibility results	
	MDDT (min.)	Width at 8 min.	R_{max} (mN)	Extensibility (mm)
		GDS7 set		
GDS7	1.49^{c}	12.5^{b}	232^{d}	26.8^{a}
GDS7 1R.1D 5+10	4.07^{b}	18.6^{ab}	269^{c}	19.9^{bc}
GDS7 1R.1D 2+12	1.74^{c}	16.4^{ab}	296^{b}	18.4^{c}
GDS7 1A.1D 5+10	7.36^{a}	22.5^{a}	393^{a}	20.8^{b}
		Trim set		
Trim	1.19^{ab}	9.4^{ab}	195^{d}	22.6^{a}
Trim 1R.1D 5+10	1.24^{ab}	8.9^{b}	217^{c}	20.6^{b}
Trim 1A.1D 2+12	1.03^{b}	14.0^{a}	253^{b}	21.7^{ab}
Trim 1A.1D 5+10	1.61^{a}	11.5^{ab}	289^{a}	22.1^{ab}
		Rhino set		
Rhino	1.61^{ab}	9.4^{a}	128^{c}	28.9^{a}
Rhino 1R.1D 5+10	2.19^{a}	15.5^{a}	248^{a}	18.6^{c}
Rhino 1A.1D 2+12	1.03^{b}	10.0^{a}	216^{b}	19.4^{bc}
Rhino 1A.1D 5+10	2.25^{a}	12.4^{a}	251^{a}	21.0^{b}
		Rigel set		
Rigel	1.23^{b}	13.9^{ab}	247^{b}	23.4^{b}
Rigel 1R.1D 5+10	1.66^{b}	17.3^{a}	202^{c}	26.2^{ab}
Rigel 1A.1D 2+12	1.50^{b}	12.3^{b}	197^{c}	26.5^{a}
Rigel 1A.1D 5+10	7.75^{a}	16.4^{ab}	295^{a}	24.2^{ab}

^{abcd} Means with same letter in same column of same group are not significantly different at $P<0.05$.

7.3.4 Test Baking Results

Micro bread loaves (10 g flour) were baked from only three triticale sets and the results are shown in Fig. 7.2. The correlation matrix among various protein composition,

physical dough test characteristics, and C-cell parameters are presented in Table 3 (Appendix A).

Table 7.6: Correlation matrix for various quality parameters and protein composition*.

	MDDT	R_{max}	Ext.	LV	PPP	FPP	UPP	GGR	PAG	PG	FP
MDDT	1.000	0.728	-0.226	0.140	0.264	-0.189	0.425 (0.478)	-0.208	-0.144	0.093	0.145
R_{max}		1.000	-0.568	0.159	0.195	-0.162	0.609 (0.574)	-0.203	-0.064	0.095	0.077
Ext.			1.000	0.307	-0.344	0.369 (0.263)	-0.517	0.386	0.332	-0.337	-0.061
LV				1.000	-0.798	0.787 (0.272)	-0.484	0.773	0.857	-0.709	-0.476
PPP					1.000	-0.955	0.808	-0.958	-0.950	0.835	0.555
FPP						1.000	-0.835	0.997	0.975	-0.958	-0.615
UPP							1.000	-0.935	-0.745	0.788	0.616
GGR								1.000	0.966	-0.952	-0.637
PAG									1.000	-0.916	-0.557
PG										1.000	0.663
FP											1.000

* MDDT=Mixograph dough development time; R_{max}=Maximum resistance in micro-extensibility test; Ext.=Extensibility; LV=Loaf volume; PPP= Percent polymeric protein; FPP= Flour polymeric protein; UPP=Unextractable polymeric protein; GGR=Gliadin/Glutenin ratio; PAG=Percent Albumin and Globulins; PG=Percent gliadins; FP=Flour protein
# Values in parenthesis were after normalized to flour protein content

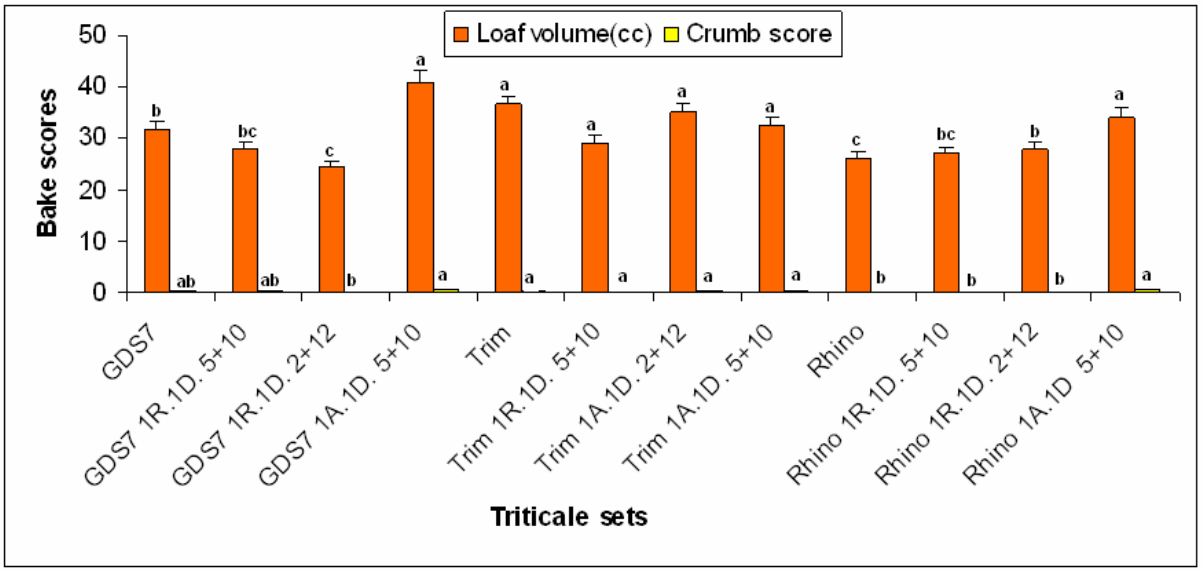
As shown in Fig. 7.2, crumb scores indicate that bread made from none of these triticale samples are acceptable. However, in supporting the results from physical dough tests, loaf volume of 1A.1D 5+10 was highest among all the lines. Average loaf volume and crumb score of 31 and 0.3 were observed with triticale samples against 60 and 3.0 respectively for control wheat (data not shown).

Mean flour absorption values of 56.4% were observed with triticales in this study. Water absorption is considered to be good if it remains in the range of 55-65% (Kasearu *et al.*, 1997). Water absorption values are negatively correlated (R^2=-0.47) with loaf volume which is in accordance with previous results (Kasearu *et al.*, 1997).

Loaf volume is negatively correlated with important dough strength parameters such as MDDT (R^2=-0.44), R_{max} (R^2=-0.81) and flour protein content (R^2=-0.31) whereas it is positively correlated only with extensibility (R^2=0.48). Most of the crumb grain characteristics measured by the C-cell such as loaf height (HT), slice area (SA), number of cells (NC) and cell diameter (CD) were positively correlated with each other.

Bread loaf and C-cell measurement pictures are presented in Appendix B, Fig-3a, b and c. All the triticale breads were round in shape with improper shred and break. Crumb structures appear to be poor (with open grain and unevenly distributed gas cells). Similar results were reported by Pena and Amaya (1992) with triticale loaves.

Figure 7-2: Loaf volume (cc) and crumb scores from micro-breads baked for different triticale sets.



7.4 Conclusions

Poor milling quality (flour yield), very low mixing times (MDDT) with lower loaf volume were typical of all the triticales studied except 1A.1D 5+10 lines. However, high loaf

volume with MDDT and R_{max} were observed with 1A.1D 5+10 translocation lines in all the triticale sets indicating that translocation of *Glu-1 d* allele with HMW-GS 5+10 is beneficial in terms of improving the quality attributes. Dough strength measurements such as MDDT, and R_{max} were correlated well with loaf volume.

References

http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567

Arseniuk, E., and Oleksiak, T. 2002. Production and breeding of cereals in Poland. Pages 11-20 in: Proc. 5th Int. Triticale Symp. E. Arseniuk, ed. IHAR, Radzikow, Poland.

Briggs, K. G.1991. Triticale for human food. Pages 435-437 in: Proc. 2nd Int. Triticale Symp. Passo Fundo, Brazil, CIMMYT, Mexico D. F., Mexico.

Brezinski, W. J., and Lukaszewski, A. J. 1998. Allelic variation at the Glu-1, Sec-2 and Sec-3 loci in winter triticale. Pages 12-13 in: Proc. 4th Int. Triticale Symp. Red Deer, Alberta, Canada.

Gupta, R. B., Shepherd, K. W., and MacRitchie, F. 1989. Effect of rye chromosome arm 2RS on flour protein and physical dough properties in bread wheat. J. Cereal Sci.10:169-173.

Gupta, R. B., Batey, I. L., and MacRitchie, F. 1992. Relationships between protein composition and functional properties of wheat flours. Cereal Chem. 69:125-131.

Gupta, R. B., Khan, K., and MacRitchie, F. 1993. Biochemical basis of flour properties in bread wheats. I. Effects of variation in quantity and size distribution of polymeric proteins. J. Cereal Sci. 18:23-44.

Kasearu, P., Laur, U., Vooremae, A., Jaama, E., and Kann, A. 1997. Critical and its fields of use.Pages 69-79 in: Food and Nutrition. Vol. IV. Tallinn Technical University, Estonia.

Kazman, E. M., and Lilley, T. 1996. Can bread making quality be introduced into hexaploid triticale by whole chromosome manipulation? Pages 141-148 in: Triticale Today and Tomorrow. H. Guedes-Pinto *et al.*, eds. Kluwer Academic Publishers, Dordrecht, Netherlands.

Kumlay, A. M., Baenziger, P. S., Gill, K. S., Shelton, D. R., Graybosch, R. A., Lukaszewski, A. J., and Wesenberg, D. W. 2003. Understanding the effect of rye chromatin in bread wheat. Crop Sci. 43:1643-1651.

Lafferty, J., and Lelley, T. 2001. Introduction of high molecular weight glutenin subunits 5+10 for the improvement of bread making quality of hexaploid triticale. Plant Breed. 120:33-38.

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

Lukaszewski, A. J. 1990. The development of aneuploid series in hexaploid triticale. Pages 397-400. in: Proc. 2nd Int. Triticale Symp.

Lukaszewski, A. J. 1996. Cytogenetic manipulation of the endosperm storage protein loci in the improvement of bread making quality of triticale. Genet. Pol. 37 A: 27-35.

Lukaszewski, A. J. 1998. Improvement of bread making quality of triticale through chromosome translocations. Pages 102-110 in: Proc. 4th Int. Triticale Symp. P. Juskiw, ed. Red Deer, Alberta, Canada.

Lukaszewski, A. J. 2001. Breeding behavior of the cytogenetically engineered wheat-rye translocation chromosome 1RS. 1BL. Crop Sci. 41:1062-1065.

Lukaszewski, A. J. 2006. Cytogenetically engineered rye chromosomes 1RS to improve bread making quality of hexaploid triticale. Crop Sci. 46:2183-2194.

MacRitchie, F. 1980. Physicochemical aspects of some problems in wheat research. Pages 271-326 in: Advances in Cereal Science and Technology, Vol. III. Y. Pomeranz, ed. American Association of Cereal Chemists, St. Paul., MN, USA.

MacRitchie, F., and Lafiandra, D. 2001. Use of near-isogenic wheat lines to determine protein composition-functionality relationships. Cereal Chem. 78:501-506.

Martinek, P., Vinterova, M., Buresova, I., and Vyhnanek, T. 2008. Agronomic and quality characteristics of triticale (X *Tritiosecale* Wittmack) with HMW glutenin subunits 5+10. J. Cereal Sci. 47:67-78.

Naeem, H. A., Darvey, N. L., Gras, P. W, and MacRitchie, F. 2002. Mixing properties, baking potential, and functionality changes in storage proteins during dough development of triticale-wheat flour blends. Cereal Chem. 79:332-339.

Pena, R. J., and Amaya, A. 1992. Milling and bread baking properties of wheat-triticale grain blends. Cereal Res. Commun. 60:483-487.

Pena, R. J. 1996. Factors affecting triticale as a food crop. Pages. 753-761 in: Triticale Today and Tomorrow. H. Guedes-Pinto *et al.*, eds. Kluwer Academic Publishers, Dordrecht, Netherlands.

Pomeranz, Y. 1985. Functional properties of food components. Pages 155-158. Academic press, New York.

Rakowska, M., and Haber, T. 1991. Baking quality of winter triticale. Pages 428-434 in: Proc 2nd Int. Triticale Symp. Passo Fundo, Brazil, 1-5 Oct., 1990, CIMMYT, Mexico D.F., Mexico.

Serna-Saldivar, O. S., Guajardo-Flores, S., and Viesca-Rios, R. 2004. Potential of triticale as a substitute for wheat in flour tortilla production. Cereal Chem. 81:220-225.

Seguchi, M., Ishihara, C., Yoshino, Y., Nakatsuka, K., and Yoshihira, T. 1999. Bread making properties of critical flour with wheat flour and relationship to amylase activity. J. Food Sci. 64:582-586.

Shewry, P. R., Bradberry, D., Franklin, J., and White, R. P. 1984. The chromosomal location and linkage relationships of the structural genes for the prolamin storage proteins (secalins) of rye. Theor. Appl. Genet. 69:63-69.

Shewry, P. R., Tatham, A. S., Barro, F., Barcelo, P., Lazzeri, P. 1995. Biotechnology of bread making: Unraveling and manipulating the multi-protein gluten complex. Biotech. 13:1185-1190.

Varughese, G., Pfeiffer, W. H., and Pena, R. J. 1996. Triticale: A successful alternative crop (Part 1&2). Cereal Foods World. 41:474-482,635-645.

Weipert, D. 1986. Triticale processing in milling and baking. Pages 402-411 in: Proc. Int. Triticale Symp. N. L. Darvey, ed. Australian Institute of Agricultural Science, occasional publication No.24, Sydney, Australia.

Zeller, F. J., and Hsam, S. L. K. 1984. Broadening the genetic variability of cultivated wheat by utilizing rye chromatin. Pages 161-173 in: Proceedings of the Sixth International Wheat Genetics Symposium. S. Sakamoto, ed. Kyoto, Japan.

CHAPTER 8 - PHENOLICS IN BRAN

8.1 Introduction

The increasing demand for natural food antioxidants hastened research to extract the biologically active substances from a variety of raw materials (Diaz-Reinoso *et al.*, 2006). Increased consumption of phenolic compounds has been correlated with a reduced risk of cardiovascular diseases and certain cancers (Arts and Hollman, 2005; Kris-Etherton *et al.*, 2002, Jacobs *et al.*, 1998). Significant levels of antioxidant activities were detected in wheat and wheat-based food products (Yu *et al.*, 2002; Adom and Liu, 2002; Baublis *et al.*, 2000). Phenolic acids in wheat grain are concentrated in the bran fraction of the kernels (Onyeneho and Hettiarachchy, 1992). Wheat phenolic compounds exist in free, esterified and insoluble bound forms (Liyana and Shahidi, 2006). Most phenolic acids exist in the bound form with other grain components such as starch, cellulose, β-glucan, and pentosans (Yu *et al.*, 2001). Insoluble-bound phenolics may be released by base, acid, or enzymatic treatment of samples prior to extraction (Kim *et al.*, 2006; Bartolome and Gomez, 1999). Phenolic acids present in the cell wall are thought to play an important part in the cross-linking of polysaccharides with other cell wall components, including lignin through ester and ether bonds, and also in the cross-linking of polysaccharide chains (Brett and Waldron, 1996; Parker *et al.*, 2005). Ferulic acid is esterified to arabinose units of cell wall arabinoxylans (Fulcher *et al.*, 1987).

Policosanols (PC) are mixtures of long chain aliphatic primary alcohols. There are numerous studies indicating that policosanol consumption is effective in lowering cholesterol levels and low-density lipoprotein (LDL) levels, and increasing high-density lipoprotein (HDL) (Mas *et al.*, 1999; Menendez *et al.*, 1994; Castano *et al.*, 1996). However, there is still debate continuing about the effect of policosanol. For instance, recent publications indicated policosanol is ineffective in reducing blood cholesterol (Dulin *et al.*, 2006; Greyling, 2006) while others showed it inhibits cholesterol synthesis in hepatoma cells by activation of AMP-kinase and persistently lowered serum total cholesterol in patients with ischemic stroke (Singh *et al.*, 2006; Ortega *et al.*, 2006). Wheat has significant levels of policosanol and they are concentrated in the bran fraction of wheat grain (Irmak *et al.*, 2006).

Thus wheat bran represents a good source of dietary fiber with health beneficial phytochemicals.

Near-isogenic wheat lines used in this study differ at one or more genetic loci coding for storage proteins. The lines with deletions, additions and/or variations in allelic expression at specific loci are sometimes available by natural mutation, while other times have been created by chromosomal engineering (MacRitchie and Lafiandra, 2001). These lines have been used for functionality studies, and the information can be successfully used to manipulate functional properties predictably in a breeding situation. Though the breeding program for the genetic lines used in this study was targeted mainly to changes in composition of proteins, we cannot ignore the unintentional changes that might occur in bioactive components of wheat bran during the development of a new genetic line.

Previous studies showed that phenolic acid and policosanol concentrations varied according to environment and wheat genotype (Gelinas and McKinnon, 2006; Moore *et al.*, 2006; Irmak and Dunford, 2005). Although antioxidant activity and phenolic acid profiles of cereals have been reported extensively, literature on the genotype effect and correlation with health-beneficial phytochemicals is scarce. To the best of our knowledge, this is the first study that attempts to determine the effect of genetic variation on phenolic acids and policosanol contents and compositions of newly developed near-isogenic wheat and waxy wheat lines and triticale translocation lines.

Objectives

- To investigate the effect of genetic variation on phenolic acid and policosanol compounds in wheat bran from the NILs of Italian bread wheat cultivar Pegaso
- To determine and compare the phenolics in waxy wheat bran using two sets of NILs, Svevo and N11
- To examine and compare the phenolic acid composition from four different sets of triticale translocation lines

8.2 Methodology

The complete extraction procedures and analysis method for phenolics (section 3.6) and policosanol (section 3.7) were described elsewhere in this dissertation. A difference in extraction procedure was employed for phenolics in Pegaso and waxy and triticale samples. Policosanol contents were analyzed only in Pegaso samples. Also, only the best represented Pegaso samples were selected for phenolics and policosanol analysis.

8.3 Results and Discussion:

8.3.1 Pegaso Wheat Bran

Phenolic acid composition was analyzed by HPLC and total phenolic content (TPC) analyzed with the Folin- Ciocalteau assay (UV spectrophotometer) as reported below.

8.3.1.1 Phenolics

8.3.1.1.1 Phenolic acid Composition

There were significant differences among all genotypes for individual phenolic acid contents which were determined by HPLC ($P < 0.05$). Results were reported in decreasing order. As observed in TPC (Fig. 8.1), total individual phenolic acid contents (Table 8.1) in all the genetic lines were below the level present in the parent line Pegaso. Phenolic acid composition in free (extractable) form showed ranges of 16.68-42.22 and 16.42-20.35 μg/g for vanillic and ferulic acids, respectively (Table 2). The bound phenolics were released by alkali hydrolysis; therefore, more phenolic acids were detected in bound extracts. However, vanillic acid concentrations were lower in bound form than in those of the free form.

Ferulic acid was the major bound phenolic acid in the wheat brans, representing about 95% of the total phenolic acids and was in accordance with previous studies (Parker *et al.*, 2005; Moore *et al.*, 2006; Beta *et al.*, 2005). However, ferulic acid content did not appear to vary much among the wheat lines which had high TPC (Pegaso 184, Pegaso 30, Pegaso 217, Pegaso 238) with one exception (Pegaso 170). Ferulic acid is esterified to arabinose units of cell wall arabinoxylans (Fulcher *et al.*, 1987) and hydrolyzed in alkali medium. *p*-coumaric, vanillic and syringic acids were only 5% of total individual phenolics and *p*-coumaric alone contributed to 4% of the total. There was no specific pattern observed for

total phenolic acid composition with different sets of samples. However, Pegaso, Pegaso 184 (variation at the *Glu-D1* locus with 2+Dy HMW-GS) and Pegaso 30 (single null at *Glu-A1*) lines had the highest ferulic acid content while Pegaso 168b and Pegaso 79 had the lowest. Previous studies showed that the total antioxidant activity correlated highly with ferulic acid content (Adom and Liu, 2002). The increased concentration of ferulates in the outer layers may provide a physical barrier to invasive disease development and consumption by insects (Zupfer *et al*., 1998). Therefore, it might be possible that Pegaso, Pegaso 184 and Pegaso 30 samples may have more resistance to disease and insects compared to other Pegaso lines.

Table 8.1: Phenolic acid composition (µg/g bran) of Pegaso wheat bran genotypes analyzed by HPLC arranged in its descending order of total phenolic acids*.

	Free Phenolic acids		Bound phenolic acids				Free+bound
Sample Name	VA	FA	VA	SA	PCM	FA	(Total)
Pegaso (Parent)	27.71^{d}	17.52^{c}	15.86^{d}	8.56^{c}	94.56^{a}	2197^{a}	2361^{a}
Pegaso 184	27.57^{d}	16.60^{de}	18.58^{b}	11.78^{a}	63.29^{e}	2136^{b}	2274^{a}
Pegaso 30	21.98^{e}	20.13^{a}	14.58^{e}	5.15^{de}	87.09^{b}	2054^{c}	2203^{b}
Pegaso 217	31.20^{bc}	18.65^{b}	22.54^{a}	11.79^{a}	90.75^{ab}	1819^{e}	1994^{c}
Pegaso 238	21.17^{e}	16.42^{e}	17.22^{c}	10.18^{b}	78.10^{c}	1846^{d}	1989^{c}
Pegaso 236	29.67^{cd}	16.65^{de}	11.23^{ij}	4.34^{ef}	64.39^{e}	1598^{f}	1723^{d}
Pegaso 166a	32.55^{b}	19.53^{a}	13.16^{fg}	8.03^{c}	53.09^{hi}	1574^{g}	1700^{d}
Pegaso 106	29.21^{cd}	20.35^{a}	11.85^{hi}	4.86^{def}	52.47^{i}	1574^{g}	1693^{d}
Pegaso 170	17.97^{f}	19.54^{a}	13.80^{ef}	5.73^{d}	68.92^{d}	1491^{h}	1617^{e}
Pegaso 235b	42.22^{a}	18.05^{bc}	10.43^{j}	4.24^{ef}	60.68^{ef}	1454^{i}	1589^{e}
Pegaso 79	16.68^{f}	19.56^{a}	12.59^{gh}	4.53^{ef}	58.08^{fg}	1312^{j}	1423^{f}
Pegaso 168b	23.38^{e}	17.45^{cd}	11.16^{ij}	3.95^{f}	56.56^{gh}	1221^{k}	1334^{g}

^{abcdefghijk} Means with same letter in same column are not significantly different at $P<0.05$.

* FA=Ferulic acid; VA=Vanillic acid; SA=Syringic acid; PCMA=*p*-coumaric acid

8.3.1.1.2 Total Phenolic acid Content (TPC)

The results showed that free, bound and total phenolic acid contents of wheat lines varied among genotypes studied. The total phenolic contents (TPC) of free and bound phenolics of wheat bran samples were expressed as micrograms of gallic acid equivalent per gram bran; µg GAE/g (Fig. 8.1). The contents of bound phenolics were 2.5-4.4-fold higher than those of free phenolics, indicating that the major phenolic acids in wheat bran were not released by methanol but extractable by alkaline hydrolysis since they were bound to the cell wall.

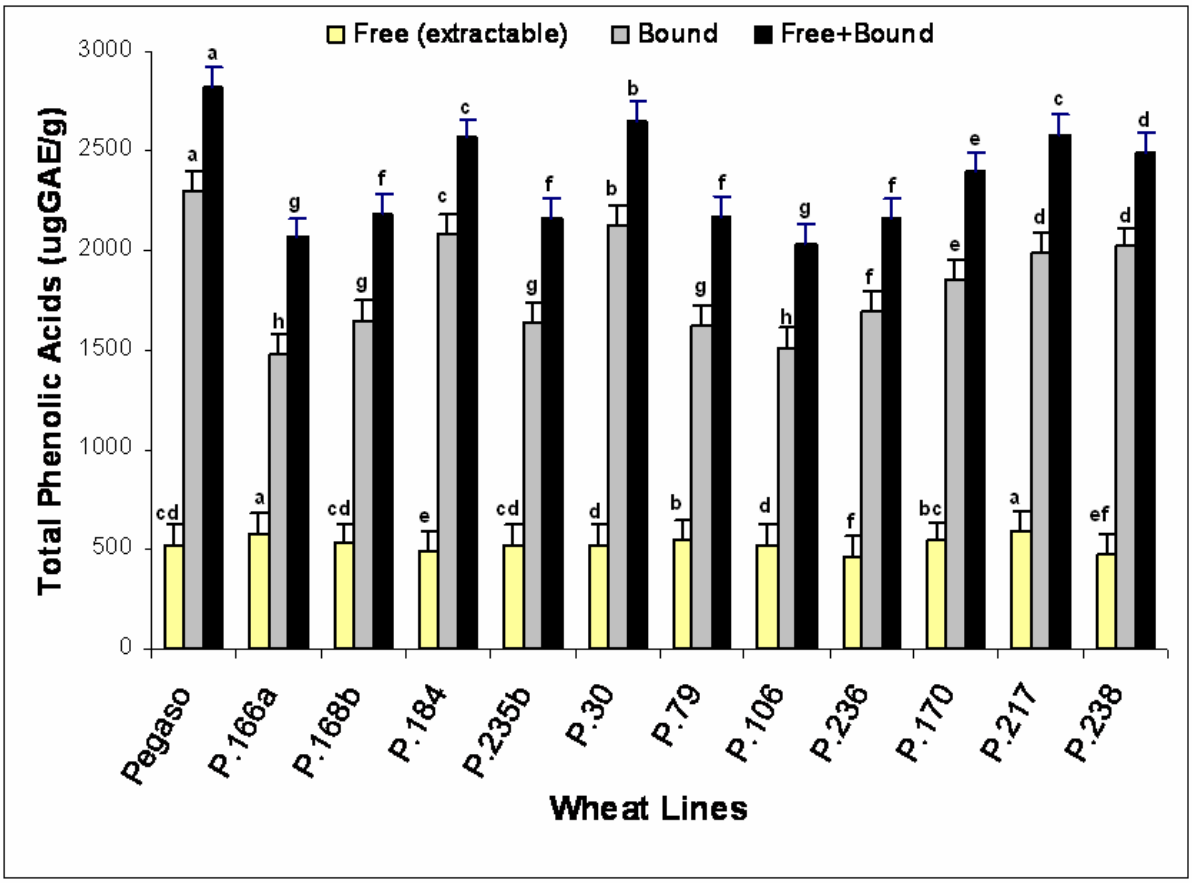
Total phenolic acid contents in all genetic lines studied were below the level present in the parent line Pegaso. Statistical analyses showed that TPC (free +bound) of Pegaso 168b, Pegaso 235b, Pegaso 79 and Pegaso 236 were the same ($P < 0.05$). TPC of five different sets of genetic variations resulted in mixed responses. In general, TPC of genetic lines with a single null at *Glu-A1* (Pegaso 30), variation at *Gli-D2* loci (Pegaso 217), variation at *Glu-1* loci with 2+Dy HMW-GS (Pegaso 184), double null at *Gli-B1/D1* (Pegaso 170) and combination of variations at *Glu-1* and *Gli-A2* loci (Pegaso 238) had higher values than other samples within the same set.

The mean free phenolic acid content was around 500 µg GAE/g; the highest being with variation at *Gli-D2* loci (Pegaso 217). It was interesting to see that the double null at *Gli-A1/D1* (Pegaso 236) had the lowest free phenolics whereas the double null at *Gli-B1/D1* (Pegaso 170) was one of the samples with above average free phenolic content. All the single nulls studied (variation at *Gli-1/Glu-3* loci) showed average free phenolic acid values.

Bound phenolics were highest in parent line Pegaso (2295 µg GAE/g); Pegaso 106 (1509 µg GAE/g) and Pegaso 166a (1481 µg GAE/g) was the least concentrated wheat line in bound phenolics among the genotypes studied. Single and double nulls studied did not show any specific trend for bound phenolics. But single null B1 (variation at *Gli-1/Glu-3* loci; Pegaso 106) resulted in the lowest values for both free and bound phenolic values among single nulls studied.

Figure 8-1: Total bran phenolic contents of Pegaso wheat genotypes analyzed by the Folin-Ciocalteau assay. Results are expressed as µg Gallic Acid Equivalents (GAE) per gram of

Pegaso wheat bran samples. The vertical bars represent the SD (n = 2), and values denoted by the same letter are not significantly different (P < 0.05).



8.3.1.2 Policosanol

Tetracosanol (C24), docosanol (C22), hexacosanol (C26), octacosanol (C28), tricosanol (C23) and heneicosanol (C21) were major policosanol compounds found in wheat bran samples (C24>C22>C26>C28>C23>C21) (Table 8.2). It was found that significant variations existed in PC contents among genotypes (P < 0.05) studied. Unlike phenolic acid contents, in which the parent line had the highest concentrations, the Pegaso 236 line had the highest policosanol content (3.29 μg/g) whereas the Pegaso parent line was slightly above the average value (average of total PC is 1.94 μg/g). Increased tetracosanol levels (~1/4 of total policosanol) appear to relate to higher total policosanol quantities in all the lines studied, except Pegaso 238 and Pegaso 79, in which docosanol was present in higher amounts than other policosanols. Octacosanol, which is the main active component of

policosanol (Gonzalez *et al*., 1996), was significantly different ($P < 0.05$) and was highest in the Pegaso 235b genotype (0.44 μg/g). Therefore, this wheat line could be a good source for both active octacosanol and total policosanol compounds. Pegaso 238, which has variation at *Glu-1* and *Gli-A2* loci, had the lowest policosanol content (1.12 μg/g) among genotypes studied.

Table 8.2: Policosanol composition (μg/g bran) of bran from Pegaso wheat genotypes arranged in descending order of total policosanols*

	C21	C22	C23	C24	C26	C28	Total PC
Pegaso 236	0.28^{a}	0.82^{a}	0.21^{b}	1.14^{a}	0.61^{a}	0.23^{c}	3.29^{a}
Pegaso 235b	0.24^{b}	0.68^{b}	0.21^{b}	1.07^{a}	0.44^{bc}	0.44^{a}	3.08^{a}
Pegaso 170	0.20^{bcde}	0.46^{cd}	0.20^{b}	0.75^{b}	0.50^{ab}	0.40^{ab}	2.51^{b}
Pegaso (Parent)	0.21^{bcd}	0.40^{cd}	0.37^{a}	0.63^{bc}	0.41^{bcd}	0.33^{b}	2.35^{bc}
Pegaso 166a	0.21^{bc}	0.48^{c}	0.19^{b}	0.60^{bcd}	0.34^{cde}	0.22^{c}	2.04^{cd}
Pegaso 30	0.23^{b}	0.46^{c}	0.19^{b}	0.55^{cde}	0.33^{de}	0.22^{c}	1.98^{d}
Pegaso 184	0.18^{cde}	0.30^{ef}	0.18^{b}	0.40^{def}	0.33^{de}	0.23^{c}	1.62^{e}
Pegaso 106	0.20^{bcde}	0.36^{de}	0.16^{b}	0.35^{efg}	0.21^{f}	0.18^{c}	1.46^{ef}
Pegaso 217	0.17^{cde}	0.28^{ef}	0.17^{b}	0.29^{fg}	0.23^{ef}	0.19^{c}	1.33^{ef}
Pegaso 168b	0.17^{de}	0.25^{f}	0.18^{b}	0.26^{fg}	0.21^{f}	0.20^{c}	1.27^{f}
Pegaso 79	0.17^{cde}	0.26^{f}	0.17^{b}	0.24^{fg}	0.19^{f}	0.17^{c}	1.20^{f}
Pegaso 238	0.16^{e}	0.24^{f}	0.16^{b}	0.20^{g}	0.18^{f}	0.18^{c}	1.12^{f}

^{abcdefg} Means with same letter in same column are not significantly different at $P<0.05$.

* Heneicosanol (C21), Docosanol (C22), Tricosanol (C23), Tetracosanol (24), Hexacosanol (C26), Octacosanol (C28) and Policosanol (PC).

In general, higher total policosanol levels were observed with the lines having double nulls. Within the double nulls studied, *Gli-A1/D1* loci (Pegaso 236; 3.29μg/g) resulted in higher policosanol amount than *Gli-B1/D1* loci (Pegaso 170; 2.51μg/g). A similar trend with double nulls was also observed for phenolic acid composition (Table 8.1). Among the single nulls studied, null A1 had higher policosanol content, which has also been the same trend observed for total phenolics (TPC), but the null D1 had lower policosanol unlike phenolics in which null B1 had lower values.

8.3.2 Waxy Wheat Bran

8.3.2.1 Phenolics

8.3.2.1.1 Phenolic acid Composition

Ferulic acid was the major phenolic acid (~82%) found in waxy wheats similar to regular wheat lines followed by *t*-cinnamic and *p*-coumaric acids (Table 8.3). Vanillic, caffeic, syringic and *p*-hydroxy benzoic acids were also present in minor quantities which is slightly different from the composition of regular wheat. Significant differences were observed among samples for different phenolic acids. However, dissimilar trends were noted for two

Table 8.3: Phenolic acid composition (µg/g bran) of Svevo and N11 waxy wheat bran analyzed by HPLC *.

Sample Name	FA	TCN	PCM	VA	CA	SA	PHBA	Total
			Svevo set					
Svevo wheat	716^{a}	54^{a}	45^{a}	16^{a}	4.4^{a}	4.8^{a}	5.8^{a}	846^{a}
Svevo waxy	548^{b}	47^{b}	40^{b}	14^{ab}	nd	4.3^{a}	5.3^{ab}	658^{b}
Svevo waxy A1^{-}	499^{c}	48^{b}	28^{c}	13^{b}	2.9^{a}	4.1^{a}	5.2^{ab}	600^{c}
Svevo waxy B1^{-}	491^{c}	54^{a}	27^{c}	13^{b}	2.8^{a}	4.2^{a}	5.1^{b}	598^{c}
			N11 set					
N11 wheat	606^{h}	57^{c}	29^{e}	28^{ab}	10.2^{ab}	5.4^{e}	5.6^{c}	736^{f}
N11 waxy	750^{d}	53^{bc}	31^{e}	29^{a}	11.5^{a}	5.7^{e}	3.1^{e}	891^{d}
N11 waxy A1^{-}	853^{a}	59^{bc}	75^{a}	26^{b}	9.7^{ab}	9.6^{ab}	7.8^{a}	1040^{a}
N11 waxy B1^{-}	799^{c}	65^{a}	45^{b}	26^{b}	8.4^{b}	9.9^{a}	7.8^{a}	961^{c}
N11 waxy D1^{-}	839^{b}	65^{a}	47^{b}	20^{cd}	9.9^{ab}	9.5^{ab}	6.4^{b}	997^{b}
N11 waxy A1^{-}/B1^{-}	742^{e}	62^{b}	45^{b}	19^{d}	9.0^{ab}	6.6^{de}	5.7^{c}	889^{d}
N11 waxy A1^{-}/D1^{-}	726^{f}	62^{b}	38^{d}	22^{c}	7.7^{b}	8.1^{bc}	5.6^{c}	870^{e}
N11 waxy B1^{-}/D1^{-}	713^{g}	61^{b}	42^{c}	18^{d}	9.8^{ab}	7.4^{cd}	4.9^{d}	856^{e}

^{abcdefgh} Means with the same letter in same column of same group are not significantly different at $P<0.05$.

* FA=Ferulic acid; TCN=*t*-cinnamic acid; PCM=*p*-coumaric acid; VA=Vanillic acid; CA= Caffeic acid; SA=Syringic acid; PHBA=*p*-hydroxybenzoic acid; nd=not detected.

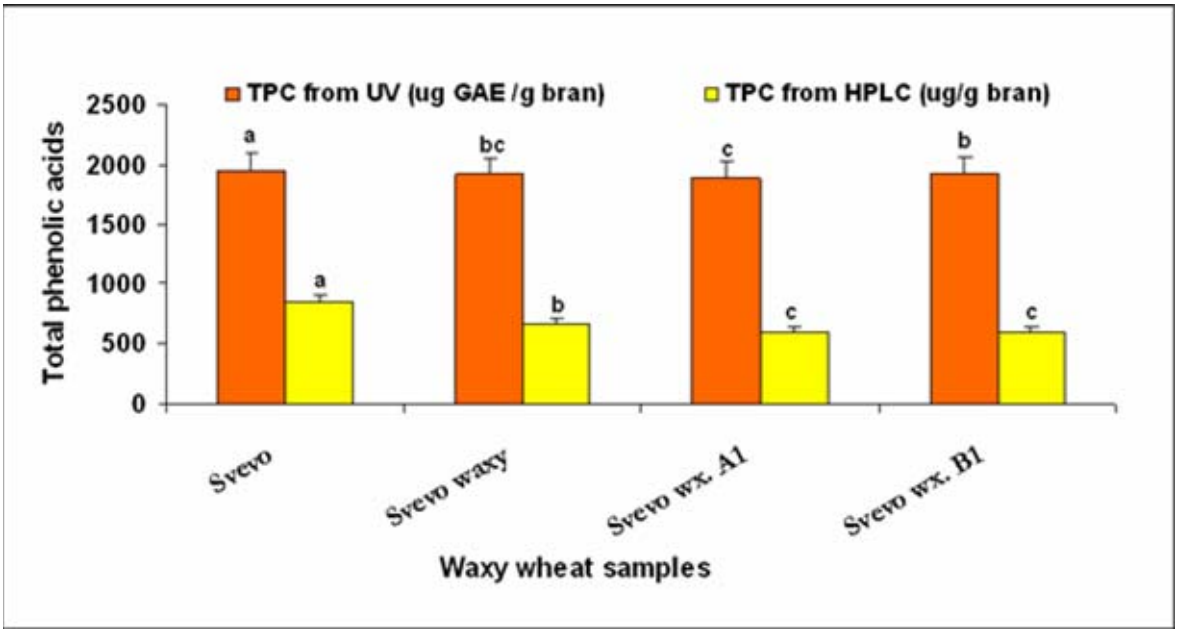
waxy sets in the results. All the developed lines have higher phenolics in the case of the N11 set, whereas the parent line has highest phenolics in the case of the Svevo set. Within the developed lines of the N11 set, single nulls (partial waxy) have the highest total phenolics (null A1-1.0 mg/g bran) followed by the triple null (complete waxy) and double nulls. Complete waxy has the highest total phenolics among all the developed lines of the Svevo set.

8.3.2.1.2 Total Phenolic acid Content (TPC)

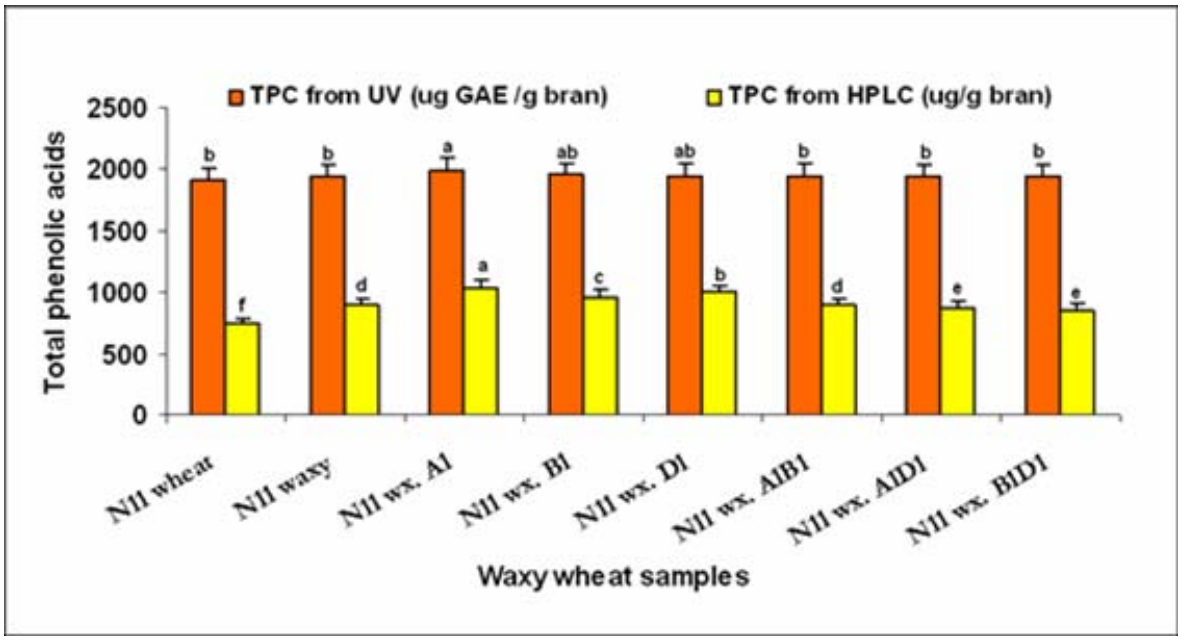
The TPC analyzed from the Folin assay using a UV spectrophotometer was compared with total phenolics from HPLC (Fig. 8.2). In general, TPC values from the Folin assay were higher than HPLC due to the use of standards restricted only to major phenolics in HPLC. No highly significant differences were found among samples for TPC analyzed by UV. However, the trend that the highest total phenolics of parent lines (Svevo set) and single null A1 (N11 set) with HPLC was also similar to the UV results. This indicates that though the quantities found with HPLC were less than UV analysis, the trend observed with results was same. Although there is no information available on phenolic acid contents of waxy wheat bran to date, polyphenol contents of grain and flour of NILs of waxy wheat have been reported. Both the grain and flour polyphenol contents of complete waxy (null at ABD) were highest (~700 and 200 μg/g grain and flour polyphenols respectively) for two sets of waxy NILs studied (Takata *et al.*, 2007). A similar trend with the single waxy wheat NIL was reported in another study (Takata *et al.*, 2005).

Figure 8-2: Comparison of total bran phenolic contents of waxy wheat genotypes analyzed by the Folin-Ciocalteau assay (UV) and HPLC. (A) Svevo set and (B) N11 set. The vertical bars represent the SD (n = 2), and values denoted by the same letter are not significantly different ($P < 0.05$).

(A) Svevo set



(B) N11 set



8.3.3 Triticale Bran

8.3.3.1 Phenolics

8.3.3.1.1 Phenolic acid Composition

More than 90% of the phenolic acids were in the form of ferulic acid (0.45-1.1 mg/g bran) in all the triticale lines. The highest ferulic acid was found in Rigel 1R.1D 5+10 sample (1101µg/g bran). Other phenolics found are *p*-coumaric, caffeic, syringic and *t*-cinnamic acids. *p*-coumaric acid is the second highest phenolic acid found in the majority of samples and syringic and t-cinnamic acids are the lowest. Among individual phenolic acids, ferulic acid, which is a major one, was lowest with 2+12 subunits in all the sets analyzed except in

Table 8.4: Phenolic acid composition (µg/g bran) of triticale bran analyzed by HPLC.

Sample Name	Ferulic	*p*-coumaric	Caffeic	Syringic	*t*-cinnamic	Total
			GDS7 set			
GDS7	653.3^{b}	28.82^{b}	11.45^{b}	4.40^{c}	48.13^{b}	746.1^{b}
GDS7 1R.1D. 5+10	556.4^{c}	21.70^{c}	9.94^{b}	4.80^{c}	52.45^{a}	645.2^{c}
GDS7 1R.1D. 2+12	511.4^{d}	23.75^{c}	14.80^{ab}	6.45^{b}	51.62^{a}	608.0^{d}
GDS7 1A.1D. 5+10	810.0^{a}	37.30^{a}	20.50^{a}	8.20^{a}	52.84^{a}	929.0^{a}
			Trim set			
Trim	687.4^{ab}	29.70^{c}	16.94^{a}	6.70^{b}	11.20^{a}	797.4^{b}
Trim 1R.1D. 5+10	520.2^{b}	21.10^{d}	14.20^{a}	7.41^{ab}	5.13^{b}	568.1^{c}
Trim 1A.1D. 2+12	886.1^{a}	42.54^{a}	20.54^{a}	9.12^{a}	9.12^{a}	967.5^{a}
Trim 1A.1D. 5+10	639.0^{b}	33.10^{b}	17.10^{a}	4.41^{c}	7.80^{ab}	701.4^{bc}
			Rhino set			
Rhino	826.2^{a}	28.20^{a}	17.42^{a}	6.24^{a}	6.13^{a}	884.2^{a}
Rhino 1R.1D. 5+10	785.7^{ab}	31.80^{a}	15.40^{b}	5.71^{a}	7.30^{a}	845.8^{a}
Rhino 1A.1D. 2+12	445.0^{c}	17.02^{b}	16.20^{ab}	5.40^{a}	4.58^{a}	481.7^{c}
Rhino 1A.1D. 5+10	662.6^{b}	17.00^{b}	17.10^{a}	5.50^{a}	6.30^{a}	708.4^{b}
			Rigel set			
Rigel	780.1^{b}	30.20^{b}	16.73^{a}	5.10^{b}	7.94^{a}	840.3^{b}
Rigel 1R.1D. 5+10	1101.1^{a}	41.70^{a}	10.20^{b}	7.20^{a}	8.51^{a}	1165.0^{a}
Rigel 1A.1D. 2+12	584.3^{b}	33.73^{ab}	nd	nd	5.94^{a}	624.0^{b}
Rigel 1A.1D. 5+10	720.1^{b}	18.40^{c}	17.83^{a}	4.94^{b}	7.10^{a}	768.0^{b}

^{abcd} Means with same letter in same column of same group are not significantly different at $P<0.05$

Trim, in which it was highest (0.86 mg/g bran). Within 5+10 HMW-GS lines, 1R.1D of Rigel and Rhino sets showed higher ferulic acid contents than 1A.1D subunit, whereas the opposite trend was observed in Trim and GDS7 lines. *p*-coumaric acid showed exactly the same trend in Trim and GDS7 sets, however, there was no specific pattern observed in the other two sets of samples.

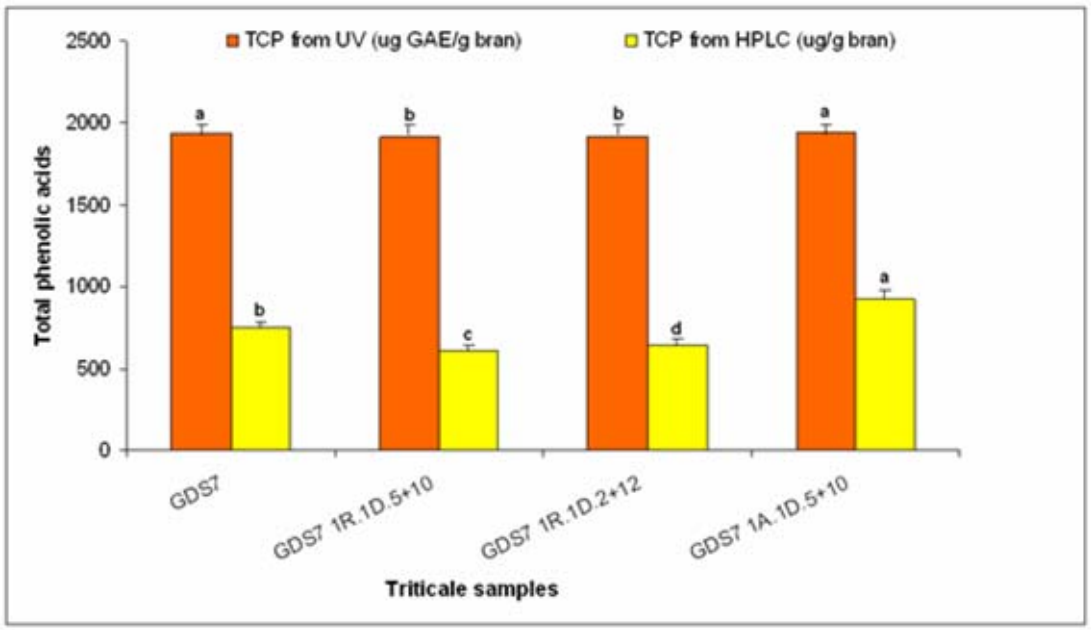
In general, at least one of the developed lines showed lower values for corresponding phenolic acids than all other developed lines and the parent line. Ferulic acid (1000 mg/g dry matter) is the most abundant phenolic acid followed by sinapic (130mg/g) and p-coumaric (60mg/g) acids in whole rye crop, which is one of the parents for triticale (Andreasen *et al.*, 1999). The other parent line, wheat bran, has ferulic, vanillic, and *p*-coumaric acids as major phenolics. Wheat bran also contains caffeic, chlorogenic, genistic, syringic and *p*-hydroxybenzoic acids in minor quantities (Onyeneho, 1992).

8.3.3.1.2 Total Phenolic acid Content (TPC)

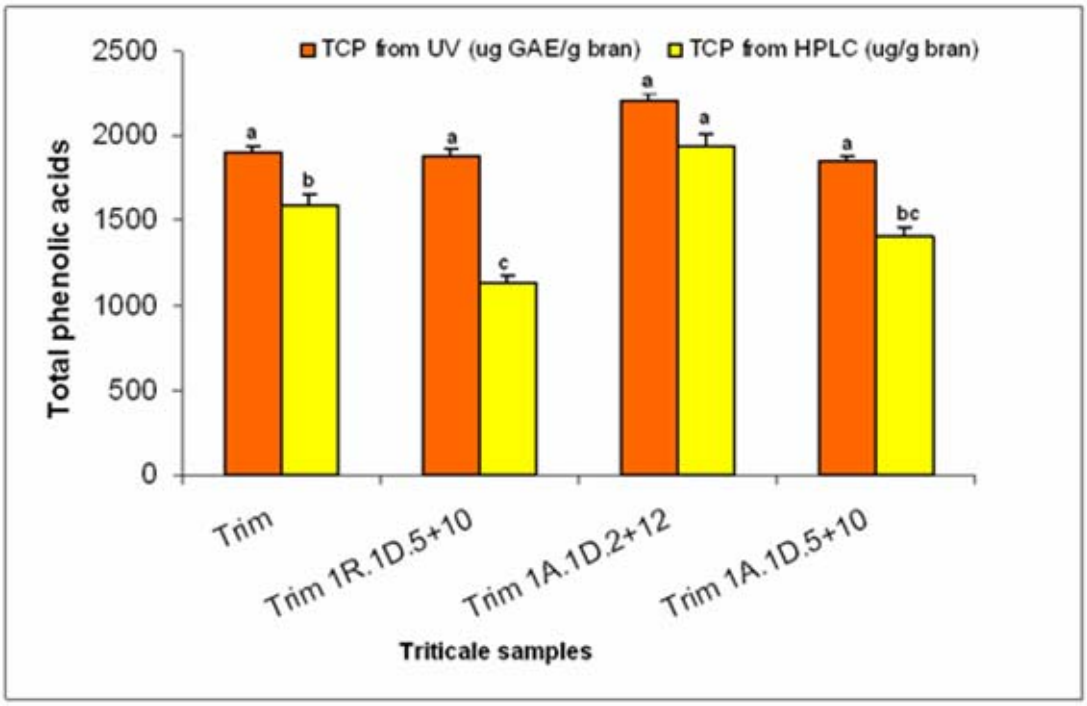
Total phenolic contents (TPC) of GDS7, Trim, Rhino and Rigel triticale sets are shown in Fig 8.3a, b, c and d respectively. Rigel and Trim lines with 2+12 subunits had higher TPC values than other samples. This is a very interesting result since the HMW-GS pair 2+12 is well known for its contribution to weak dough properties and inferior bread baking potential. However, it has the higher TPC in triticales. The TPC of triticale was found to be 0.94 mg/g of grain by Zdunczyk *et al* (2006). The mean TPC of our study (~1.9 mg/g of bran) was 2-fold higher than the earlier study. This might be attributed to more phenolic acid contents in the bran part and also the extraction method we employed releases more of these compounds. Total of individual phenolics measured by HPLC did not match the TPC values obtained from UV measurement. This is because we employed standards only for the reported individual phenolic acids and there might be more of those compounds that increased the TPC values in UV analysis. It is interesting to note that triticale bran had higher TPC and individual ferulic acid content (0.4-1.1 mg ferulic /g of bran) than either of the parent wheat lines (0.09-0.23mg ferulic/g of bran; Zhou *et al.*, 2004) or rye (0.9-1.1mg ferulic/g of grain; Andreasen *et al.*, 2000). Thus, triticale bran would be a promising potential phenolic acid source for the food industry.

Figure 8-3: Comparison of total bran phenolic contents of triticale genotypes analyzed by the Folin-Ciocalteau assay (UV) and HPLC. (A) GDS7 set (B) Trim set (C) Rhino set and (D) Rigel set. The vertical bars represent the SD (n = 2), and values denoted by the same letter are not significantly different ($P < 0.05$).

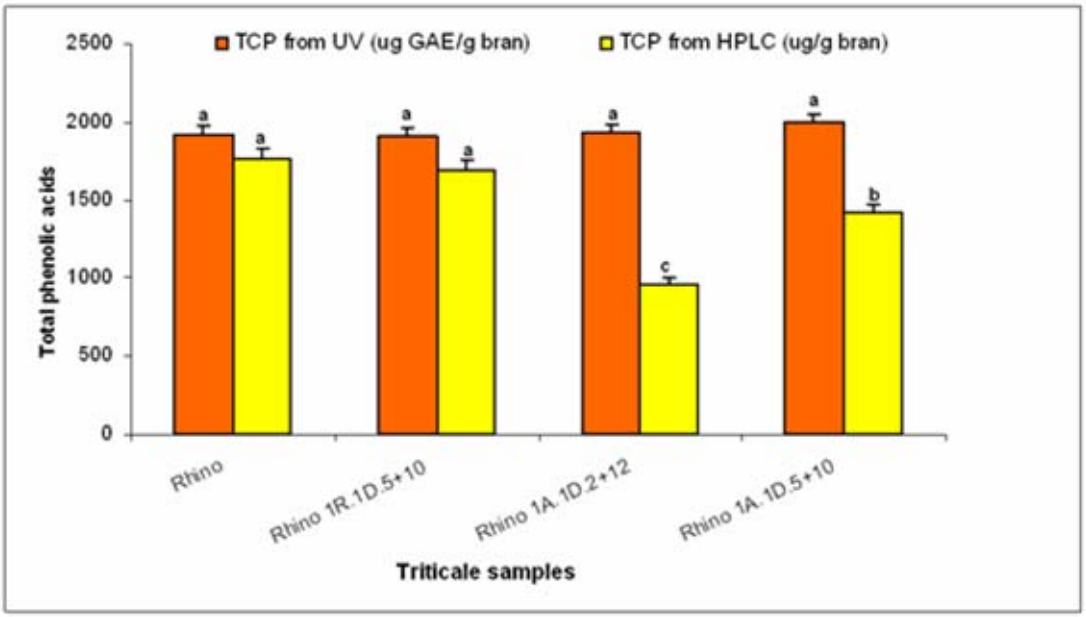
(A) GDS7 set



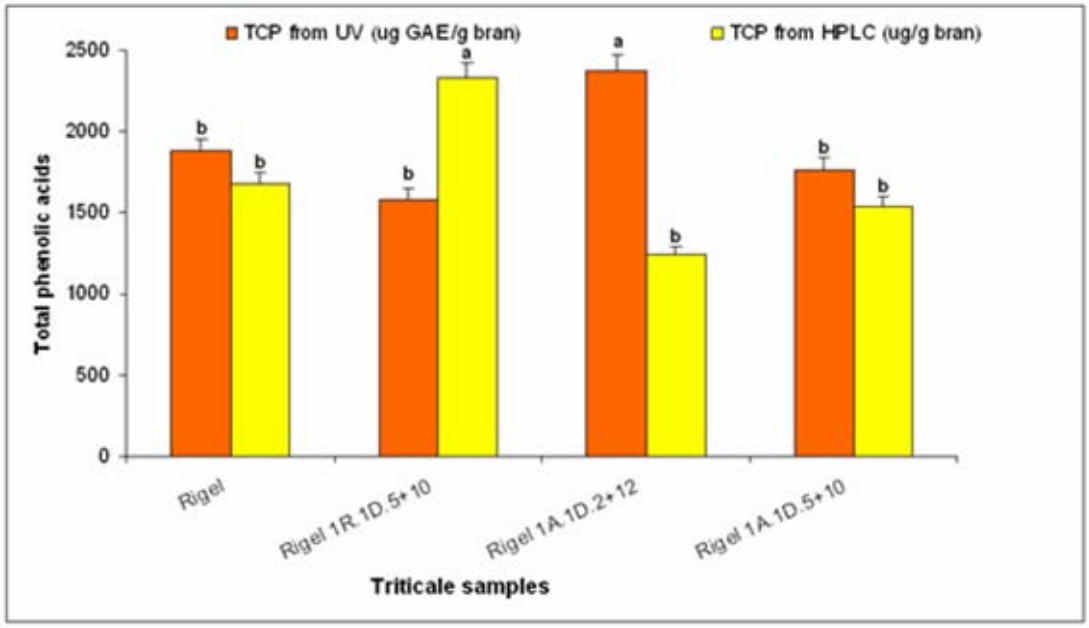
(B) Trim set



(C) Rhino set



(D) Rigel set



8.4 Conclusions

There were significant differences observed among Pegaso NILs for policosanol, phenolic acid contents and compositions. Both TPC and total individual phenolic acid content in all the genetic lines were below the levels present in the parent line Pegaso. Ferulic acid was the major bound phenolic (~95%) present in the wheat brans as reported

before, followed by *p*-coumaric, vanillic and syringic acids. Pegaso 235 genotype had the highest octacosanol and total policosanol levels among all genetic lines including the parent line Pegaso. Double nulls had higher policosanol amounts. Among the double nulls, *Gli-A1/D1* loci had higher policosanol than *Gli-B1/D1* loci. A similar trend for double nulls has been observed for total phenolic acid composition.

Although ferulic acid (~80%) was the predominant phenolic acid found in waxy wheats similar to regular wheat lines, other individual compositions were different from regular wheat. Minor quantities of vanillic, caffeic, syringic and *p*-hydroxy benzoic acids were present along with two other major phenolics *t*-cinnamic and *p*-coumaric acids. Waxy lines have higher total phenolics than the parent line in the case of the N11 set whereas the Svevo has higher phenolics than any waxy lines developed.

There is no specific pattern observed with triticale translocation lines for phenolics. However, at least one of the developed lines showed lower values for corresponding phenolic acids than all other developed lines and the parent line. Lines with HMW-GS 2+12 have lower total phenolics than other lines (except Trim set). Lines with 1R.1D HMW-GS 5+10 have higher total phenolics in two sets (Rhino and Rigel) whereas it has lower than 1A.1D in the other two sets (Trim and GDS7).

Although the genetic control of these compounds has not been established clearly, the information could be successfully used in wheat breeding situations that ultimately help in producing lines with enhanced levels of these health beneficial compounds. Further, an elaborate study on understanding of biochemical pathways for accumulation and or disappearance of such biologically active, health beneficial wheat components is required. This would enable genotypes with a good source of natural bioactive compounds to be incorporated into functional foods and nutraceuticals.

References

Adom, K.K., and Liu, R. H. 2002. Antioxidant activity of grains. J. Agric. Food Chem. 50: 6185-6187.

Andreasen, M.F., Christensen, L.P., Meyer, A.S., and Hansen, A. 1999. Release of hydroxyinnamic and hydroxybenzoic acids in rye by commercial plant cell wall degrading enzyme preparations. J. Sci. Food Agric. 79: 411-413.

Arts, I., and Hollman, P. 2005. Polyphenols and disease risk in epidemiologic studies. Am. J. Clin. Nutri. 81: 317S-325S.

Bartolome, B., and Gomez-Cordoves, C. 1999. Barley spent grain: release of hdroxycinnamic acids (ferulic and p-coumaric acids) by commercial enzyme preparations. J. Sci. Food Agric. 79: 435-439.

Baublis, A., Decker, E.A., and Clydesdale, F.M. 2000. Antioxidant effects of aqueous extracts from wheat based ready-to-eat breakfast cereals. Food Chem. 68: 1-6.

Beta, T., Nam, S., Dexter, J.E., and Sapirstein, H.D. 2005. Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions. Cereal Chem. 82: 390-393.

Brett, C.T., and Waldron, K.W. 1996. Physiology and Biochemistry of Plant Cell Walls. Pages 46-48. Chapman and Hall, London.

Castano, G., Tula, L., Canetti, M., Morera, M., Mas, R., Illnait, J., Fernandez, L., and Fernandez, J.C. 1996. Effects of policosanol in hypertensive patients with type II hypercholesterolemia. Cur. Therap. Res. 57: 691-699.

Diaz-Reinoso, B., Moure, A., Dominguez, H., and Parajo, J.C. 2006. Supercritical CO_2 extraction and purification of compounds with antioxidant activity. J. Agric. Food Chem. 54: 2441-2469.

Dulin, M.F., Hatcher, L.F., Sasser, H.C., and Barringer, T.A. 2006. Policosanol is ineffective in the treatment of hypercholesterolemia: a randomized controlled trial. Am. J. Clin. Nutri. 84: 1543-1548.

Fulcher, R.G., Pussayanawin, V., and Wetzel, D. 1987. Detection and localization of phenolic compounds in cereal grains using computerized microspectrophotometry. Pages 69-72 in: Proceedings of the 37th Australian Cereal Chemistry Conference, edited by Murray L. Royal Australian Chemical Institute, Melbourne.

Gelinas, P., and McKinnon, C.M. 2006. Effect of wheat variety, farming site, and bread-baking on total phenolics. Int. J. Food Sci.Techn. 41: 329-332.

Greyling, A., De Witt, C., Oosthuizen, W., and Jerling, J.C. 2006. Effects of a policosanol supplement on serum lipid concentrations in hypercholesterolaemic and heterozygous familial hypercholesterolaemic subjects. Brit. J. Nutri. 95: 968-975.

Irmak, S., and Dunford, N.T. 2005. Policosanol contents of wheat bran varieties. J. Agric. Food Chem. 53: 5583-5586.

Irmak, S., Dunford, N.T., and Milligan, J. 2006. Policosanol contents of beeswax, sugar cane and wheat extracts. Food Chem. 95: 312-318.

Jacobs, D.R., Meyer, K.A., Kushi, L.H., and Folsom, A.R. 1998. Whole grain intake may reduce risk of coronary heart disease death in postmenopausal women: The Iowa Women's Health Study. Am. J. Clin. Nutri. 68: 248-257.

Kim, K.-H., Tsao, R., Yang, R., and Cui, S.W. 2006. Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. Food Chem. 95: 466-473.

Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E., and Etherton, T.D. 2002. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. Am. J. Med. 113: 71S-88S.

Liyana-Pathirana, C.M., and Shahidi, F. 2006. Importance of insoluble-bound phenolics to antioxidant properties of wheat. J. Agric. Food Chem.54: 1256-1264.

MacRitchie, F., and Lafiandra, D. 2001. Use of near-isogenic wheat lines to determine protein composition-functionality relationships. Cereal Chem. 78: 501-506.

Mas, R., Rivas, P., Izquierdo, J.E., Hernandez, R., Fernandez, J., Orta, S.D., Illnait, J., and Ricardo, Y. 1999. Pharmacoepidemiologic study of polycosanol. Cur. Therap. Res. 60: 458-467.

Menendez, R., Fernandez, S.I, Del Rio, A., Gonzalez, R.M, Fraga, V., Amor, A.M, and Mas, R.M. 1994. Policosanol inhibits cholesterol biosynthesis and enhances LDL processing in cultured human fibroblasts. Biol. Res. 27: 199-203.

Moore, J., Liu, J.-G., Zhou, K., and Yu, L. 2006. Effects of genotype and environment on the antioxidant properties of hard winter wheat bran. J. Agric Food Chem. 54: 5313-5322.

Onyeneho, S.N., and Hettiarachchy, N.S. 1992. Antioxidant activity of durum wheat bran. J. Agric. Food Chem. 45: 1644-1648.

Ortega, L.L., Sanchez, J., Mas, R., Fernandez, L., Mendoza, S., Gamez, R., Fernandez, J.C., Illnait, J., and Alvarez, E. 2006. Effects of policosanol on patients with ischemic stroke: A pilot open study. J. Med. Food. 9: 378-385.

Parker, M.L., Ng, A., and Waldron, K.W. 2005. The phenolic acid and polysaccharide composition of cell walls bran layers of mature wheat (*Triticum aestivum* L. cv. Avalon) grains. J. Sci. Food Agric. 85: 2539-2547.

Singh, D.K., Li, L., and Porter, T.D. 2006. Policosanol inhibits cholesterol synthesis in hepatoma cells by activation of AMP-kinase. J. Pharm. Exp. Therap. 318: 1020-1026.

Takata, K., Nishio, Z., Iriki, N., Tabiki, T., Funatsuki, W., and Yamauchi, H. 2005. Comparison of quality characteristics of waxy wheat using near-isogenic line. Breed. Sci. 55:87-92.

Takata, K., Yanaka, M., Fujita, Y., and Ishikawa, N. 2007. Evaluation of the grain and flour quality in near-isogenic wheat lines with waxy and double-null *Wx* proteins. Breed. Sci. 57:79-83.

Yu, J., Vasanthan, T., and Temelli, F. 2001. Analysis of phenolic acids in barley by high-performance liquid chromatography. J. Agric. Food Chem. 49: 4352-4360.

Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J., and Qian, M. 2002. Free radical scavenging properties of wheat extracts. J. Agric. Food Chem. 50: 1619-1624.

Zdunczyk, Z., Flis, M., Zielinski, H., Wroblewska, M., Antoszkiewicz, Z., and Juskiewicz, J. 2006. In vitro antioxidant activities of barley, husked oat, naked oat, triticale, and buckwheat wastes and their influence on the growth and biomarkers of antioxidant status in rats. J. Agric Food Chem. 54: 4168-4175.

Zhou, K., Su, L., and Yu, L.L. 2004. Phytochemicals and antioxidant properties in wheat bran. J. Agric. Food Chem. 52: 6108-6114.

Zupfer, J.M., Churchill, K.E., Rasmusson, D.C., and Fulcher, R.G. 1998. Variation in ferulic acid concentration among diverse barley cultivars measured by HPLC and microspectrophotometry. J. Agric. Food Chem. 46: 1350-1354.

CHAPTER 9 - CONCLUSIONS AND FUTURE WORK

9.1 General Conclusions

- A set of 24 NILs of Pegaso bread wheat clearly separated lines having the *Glu-D1d* allele (HMW-GS 5+10) from lines having the *Glu-D1a* allele (HMW-GS 2+12) in terms of dough strength and UPP.
- Pegaso NILs having deletions at *Gli-1/Glu3* and *Gli-2* loci showed higher UPP and dough strengths than controls, suggesting the elimination of modified gliadins acting as chain terminators.
- Evidence for the presence of ω-gliadins that act as chain terminators was obtained by measurements of HPCE on Pegaso lines.
- Waxy wheats have relatively lower proportions of albumin/globulin fraction than regular wheat along with lower flour yields. Highest bread loaf volume was obtained with waxy flour alone, but the dark color, poor appearance with large bubbles in the crumb grain makes this bread consumer unacceptable. On the other hand, loaf volume was reduced when waxy flour was blended with 50% commercial wheat flour.
- Triticale lines showed poor milling quality, very low dough mixing times along with lower bake-test loaf volumes than bread wheats. However, lines in which the HMW-GS from the D genome of wheat were translocated into triticale lines showed increased dough strength, particularly for HMW-GS 5+10.
- Preliminary results on health beneficial phytochemicals such as phenolic acids and polycosanols in NILs of Pegaso , waxy wheat and triticale translocation lines, showed variations in their amounts which suggests that genetic modification of wheat lines could provide a potential source of these compounds.

9.2 Future Scope

Support of the present ω–gliadin data on the role of chain terminators using other analytical methods especially RP-HPLC would be beneficial. This should include the collection of fractions that correspond to specific ω–gliadin peaks from HPLC and analyze for amino acid sequence to determine if these gliadins have single cysteine residues. This would be a confirmation that they are likely chain terminators.

There is a need to further investigate the genetic basis of the health beneficial phytochemicals analyzed in this study. Such information could be successfully used in wheat breeding situations that ultimately help in producing the lines with enhanced levels of these health beneficial compounds. Further, an elaborative study to understand the biochemical pathways for accumulation and or disappearance of such biologically active, health beneficial wheat components is required.

Appendix A - Correlation matrices including C-cell data

Table A-1: Correlation matrix for various quality parameters, protein composition and baking measurements –Pegaso set *.

	MDDT	R_{max}	Ext.	PPP	FPP	UPP	GGR	FP	LV	FA	LW	Crumb	SA	HT	NC	WT	CD
MDDT	1.000	0.644	-0.476	0.222	0.185	0.255	-0.258	0.090	0.238	0.161	-0.029	0.459	0.297	0.283	0.304	-0.016	0.115
R_{max}		1.000	-0.754	0.596	-0.041	0.774	-0.791	-0.265	-0.090	-0.118	0.275	0.080	-0.119	-0.118	-0.025	-0.100	-0.198
Ext.			1.000	-0.357	0.534	-0.622	0.676	0.671	0.399	0.582	-0.333	0.077	0.342	0.319	0.222	0.063	0.288
PPP				1.000	0.147	0.599	-0.719	-0.224	-0.020	-0.093	0.214	-0.000	-0.066	-0.072	0.030	-0.070	-0.168
FPP					1.000	-0.157	0.184	0.929	0.622	0.921	-0.177	0.444	0.280	0.208	0.174	-0.349	-0.021
UPP						1.000	-0.967	-0.377	-0.406	-0.243	0.284	-0.247	-0.419	-0.394	-0.301	-0.089	-0.286
GGR							1.000	0.450	0.392	0.292	-0.341	0.220	0.429	0.407	0.284	0.134	0.353
FP								1.000	0.611	0.944	-0.276	0.424	0.308	0.240	0.168	-0.305	0.050
LV									1.000	0.591	-0.304	0.813	0.701	0.645	0.664	-0.121	0.221
FA										1.000	-0.260	0.421	0.288	0.215	0.188	-0.343	-0.009
LW											1.000	-0.269	-0.461	-0.473	-0.484	-0.125	-0.197
Crumb												1.000	0.612	0.582	0.645	-0.114	0.135
SA													1.000	0.990	0.945	0.527	0.745
HT														1.000	0.952	0.607	0.789
NC															1.000	0.482	0.623
WT																1.000	0.901
CD																	1.000

* MDDT=Mixograph dough development time; Rmax=Maximum resistance in micro-extensibility test; Ext.=Extensibility; LV=Loaf volume; PPP= Percent polymeric protein; UPP=Unextractable polymeric protein; FP=Flour protein ;GGR= Gliadin/Glutenin ratio;FA=Flour absorption in baking;LW=Loaf weight; Crumb=Crumb score (manual);SA=Slice area; HT= Loaf height; NC=Number of cells; WT=Wall thickness; CD=Cell diameter.

Table A-2: Correlation matrix for various quality parameters, protein composition and baking measurements- Waxy wheats *.

	MDDT	R_{max}	Ext.	PPP	FPP	UPP	GGR	FP	LV	FA	LW	Crumb	SA	HT	NC	WT	CD
MDDT	1.000	0.124	-0.697	0.123	0.291	-0.052	0.164	-0.331	0.095	0.132	-0.434	-0.191	-0.306	-0.308	-0.279	0.150	-0.070
R_{max}		1.000	-0.177	-0.205	0.389	-0.302	0.505	-0.255	0.303	0.530	-0.144	-0.314	-0.304	-0.329	-0.246	0.045	0.029
Ext.			1.000	0.178	-0.091	0.337	-0.261	0.661	-0.311	-0.219	0.181	-0.057	0.346	0.420	0.101	0.307	0.465
PPP				1.000	0.305	0.794	-0.621	0.765	-0.785	-0.535	-0.602	0.163	0.559	0.597	0.302	0.227	0.379
FPP					1.000	-0.257	0.555	-0.047	0.255	0.631	-0.008	-0.647	-0.406	-0.367	-0.589	0.632	0.460
UPP						1.000	-0.909	0.870	-0.984	-0.879	-0.571	0.435	0.772	0.793	0.599	-0.066	0.272
GGR							1.000	-0.726	0.900	0.988	0.500	-0.665	-0.824	-0.824	-0.744	0.308	0.019
FP								1.000	-0.841	-0.677	-0.426	0.276	0.755	0.803	0.473	0.177	0.455
LV									1.000	0.871	0.532	-0.492	-0.794	-0.798	-0.656	0.169	-0.217
FA										1.000	0.491	-0.700	-0.807	-0.797	-0.760	0.360	0.059
LW											1.000	-0.453	-0.538	-0.521	-0.449	0.116	0.118
Crumb												1.000	0.816	0.744	0.951	-0.819	-0.673
SA													1.000	0.985	0.920	-0.454	-0.227
HT														1.000	0.858	-0.336	-0.158
NC															1.000	-0.761	-0.506
WT																1.000	0.807
CD																	1.000

* MDDT=Mixograph dough development time; Rmax=Maximum resistance in micro-extensibility test; Ext.=Extensibility; LV=Loaf volume; PPP= Percent polymeric protein; UPP=Unextractable polymeric protein; FP=Flour protein ;GGR= Gliadin/Glutenin ratio;FA=Flour absorption in baking;LW=Loaf weight; Crumb=Crumb score (manual);SA=Slice area; HT= Loaf height; NC=Number of cells; WT=Wall thickness; CD=Cell diameter.

Table A-3: Correlation matrix for various quality parameters, protein composition and baking measurements-Triticale set *.

	MDDT	R_{max}	Ext.	PPP	FPP	UPP	GGR	FP	LV	FA	LW	Crumb	SA	HT	NC	WT	CD
MDDT	1.000	0.735	-0.183	0.295	-0.193	0.505	0.017	0.472	-0.443	-.399	0.158	0.528	0.537	0.508	0.481	-0.127	0.360
R_{max}		1.000	-0.539	0.144	0.119	0.900	0.178	0.465	-0.816	0.608	0.228	0.463	0.519	0.531	0.615	-0.525	-0.082
Ext.			1.000	0.000	0.153	-0.432	0.126	0.053	0.489	-0.148	-0.461	0.086	-0.033	-0.043	-0.146	0.410	0.37
PPP				1.000	-0.340	0.041	-0.425	-0.265	-0.405	0.019	0.335	0.091	-0.076	-0.058	0.001	-0.116	-0.145
FPP					1.000	0.309	0.704	0.270	-0.167	0.516	0.046	0.164	0.129	0.159	0.221	-0.227	-.0236
UPP						1.000	0.315	0.522	-0.898	0.570	0.125	0.498	0.591	0.617	0.789	-0.773	-0.375
GGR							1.000	0.169	-0.086	0.646	0.075	0.052	0.014	0.008	0.146	-0.268	-0.239
FP								1.000	-0.318	0.307	-0.037	0.902	0.960	0.953	0.817	-0.153	0.332
LV									1.000	-0.476	-0.304	-0.415	-0.461	-0.493	-0.693	0.822	0.512
FA										1.000	0.574	0.362	0.229	0.219	0.334	-0.334	-0.131
LW											1.000	0.160	0.010	-0.011	0.087	-0.200	-0.227
Crumb												1.000	0.926	0.927	0.800	-0.135	0.293
SA													1.000	0.996	0.909	-0.288	0.213
HT														1.000	0.914	-0.306	0.183
NC															1.000	-0.643	-0.185
WT																1.000	0.820
CD																	1.000

* MDDT=Mixograph dough development time; Rmax=Maximum resistance in micro-extensibility test; Ext.=Extensibility; LV=Loaf volume; PPP= Percent polymeric protein; UPP=Unextractable polymeric protein; FP=Flour protein ;GGR= Gliadin/Glutenin ratio;FA=Flour absorption in baking;LW=Loaf weight; Crumb=Crumb score (manual);SA=Slice area; HT= Loaf height; NC=Number of cells; WT=Wall thickness; CD=Cell diameter.

Appendix B - Mixograms and bread pictures

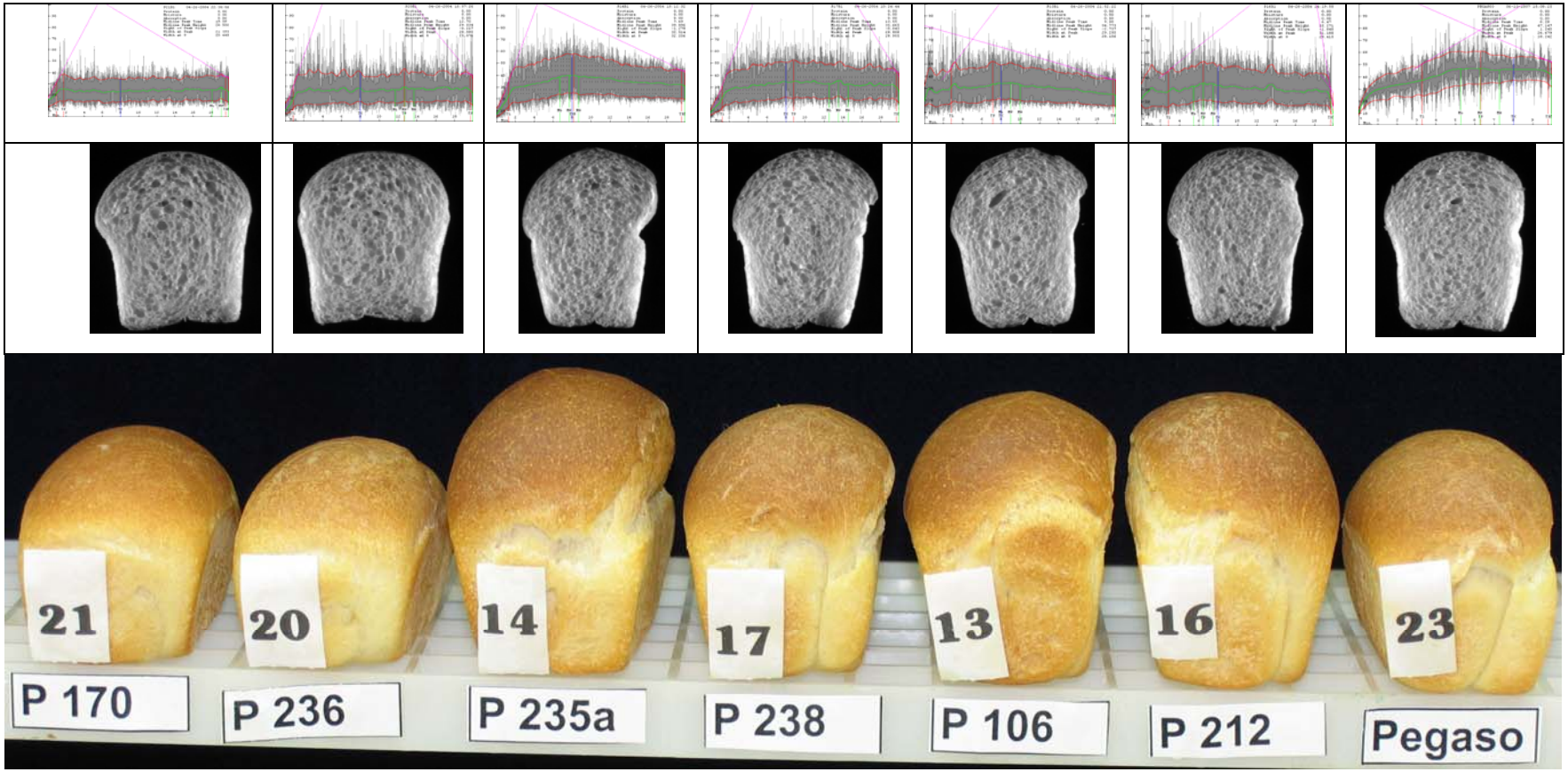


Figure.B-1a: Pictures from mixograms (top row), C-cell (middle row), and whole bread (bottom row) for a set of Pegaso sample. Read the sample names below the each bread.

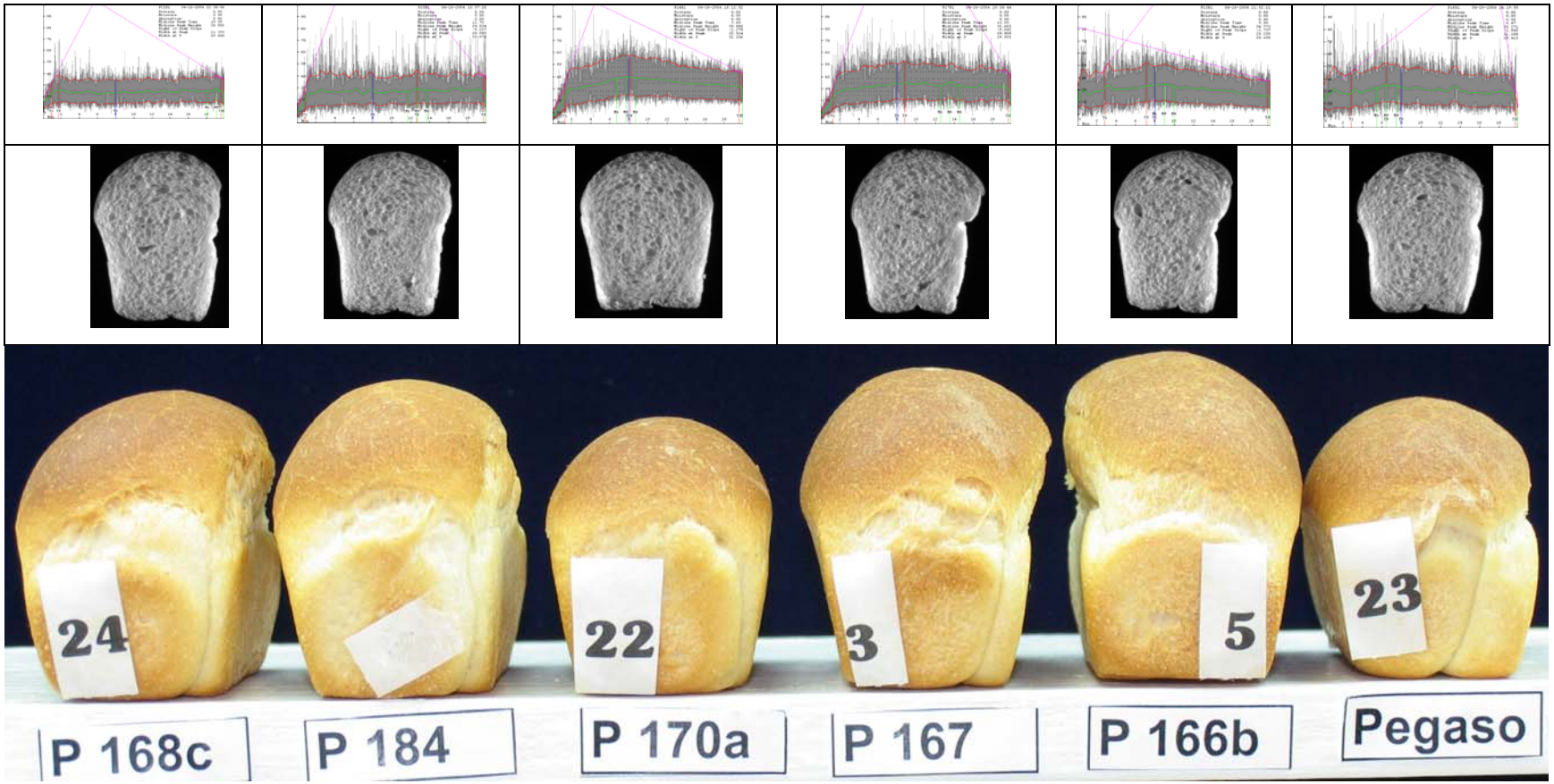


Figure.B-1b: Pictures from mixograms (top row), C-cell (middle row), and whole bread (bottom row) for a set of Pegaso sample. Read the sample names below the each bread.

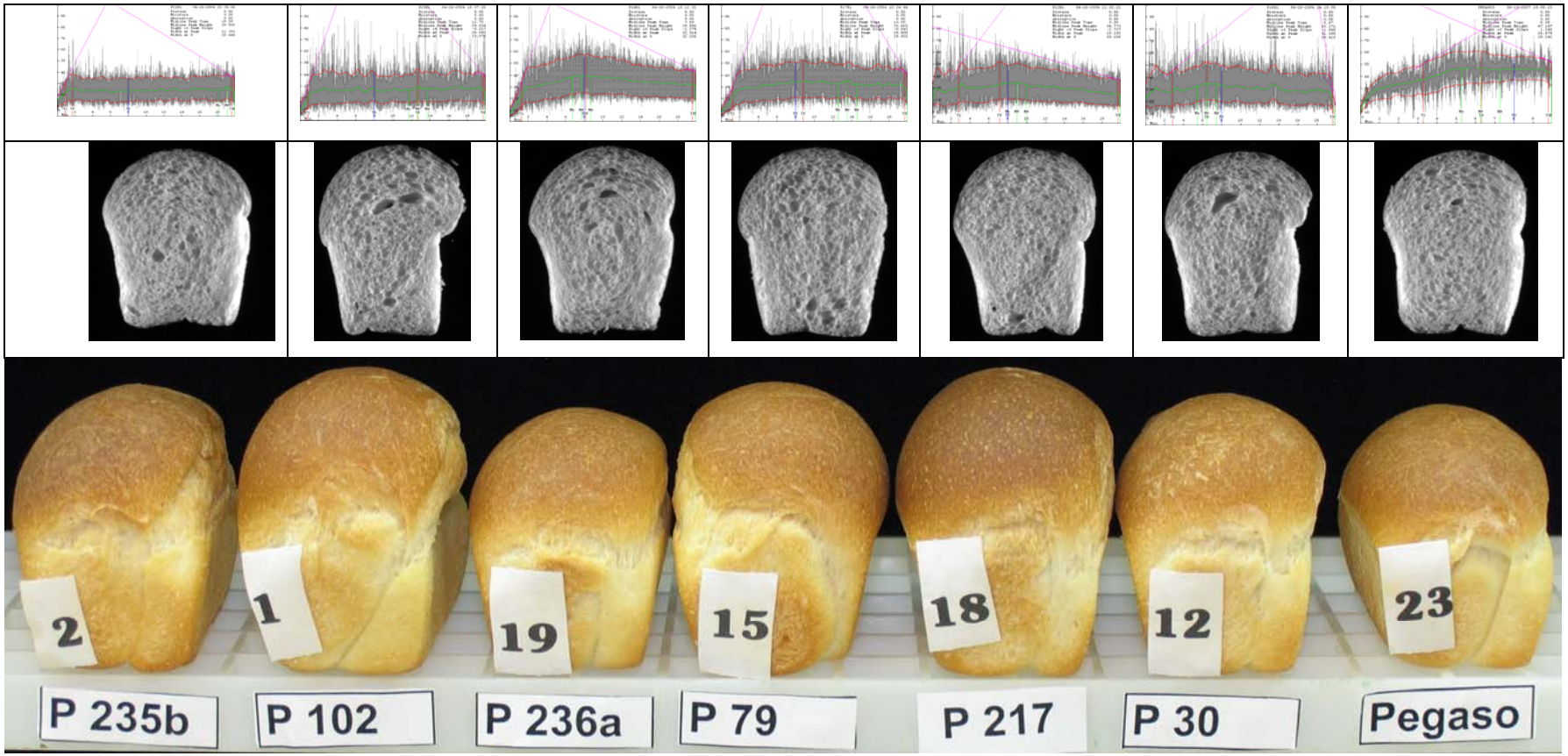


Figure.B-1c: Pictures from mixograms (top row), C-cell (middle row), and whole bread (bottom row) for a set of Pegaso sample. Read the sample names below the each bread.

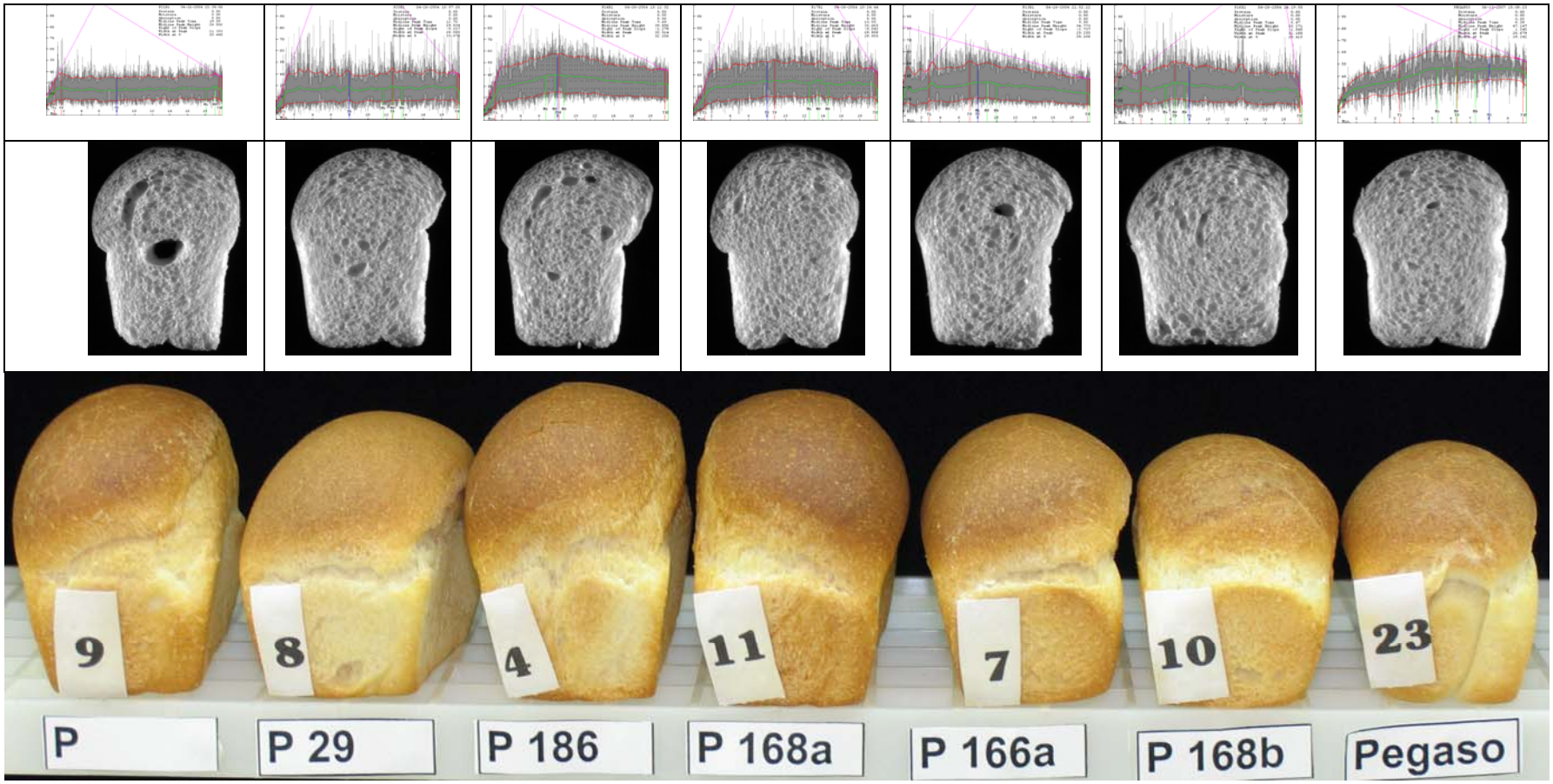


Figure.B-1d: Pictures from mixograms (top row), C-cell (middle row), and whole bread (bottom row) for a set of Pegaso sample. Read the sample names below the each bread.

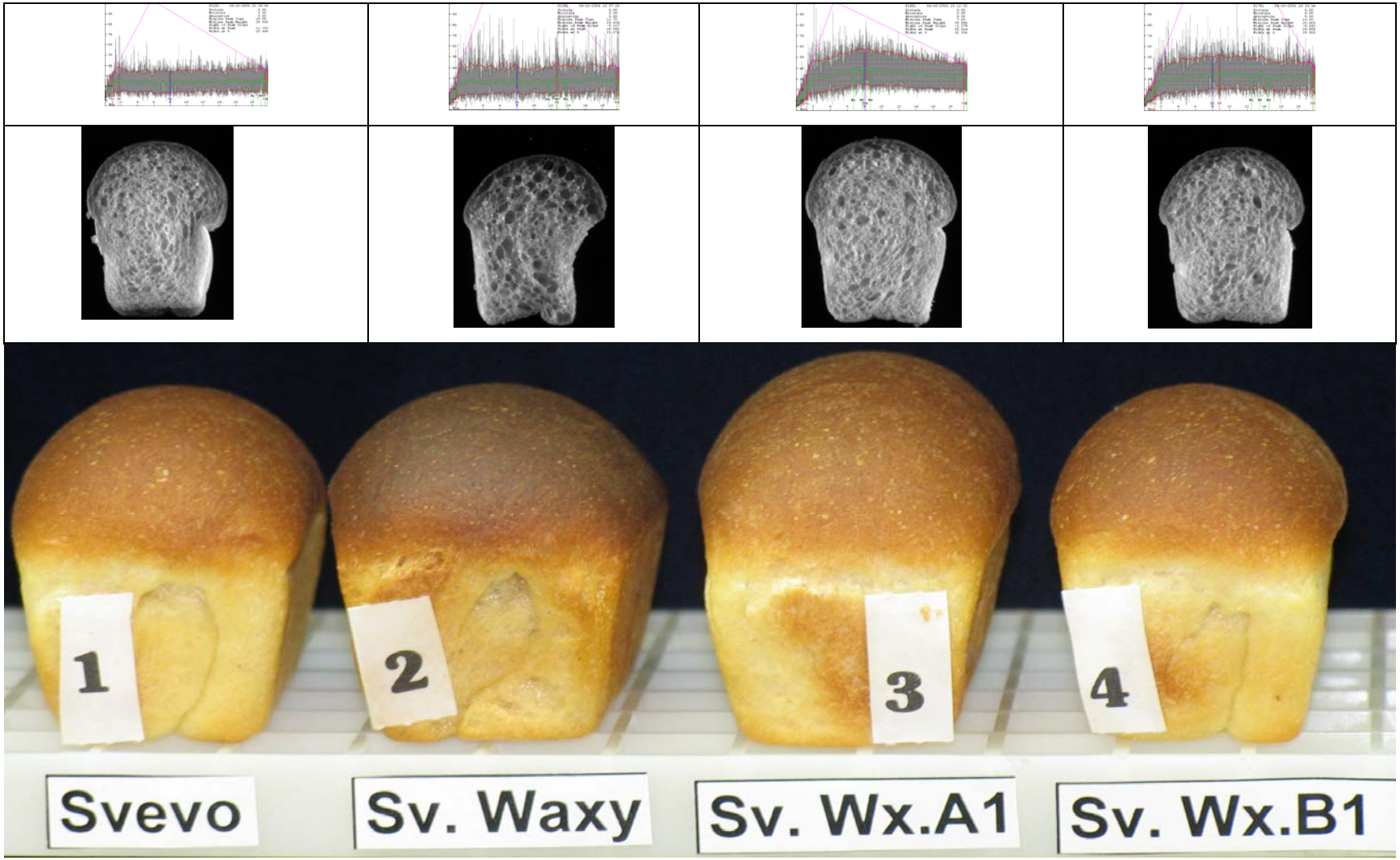


Figure.B-2a: Pictures from mixograms (top row), C-cell (middle row), and whole bread (bottom row) for Svevo set. Read the sample names below the each bread.

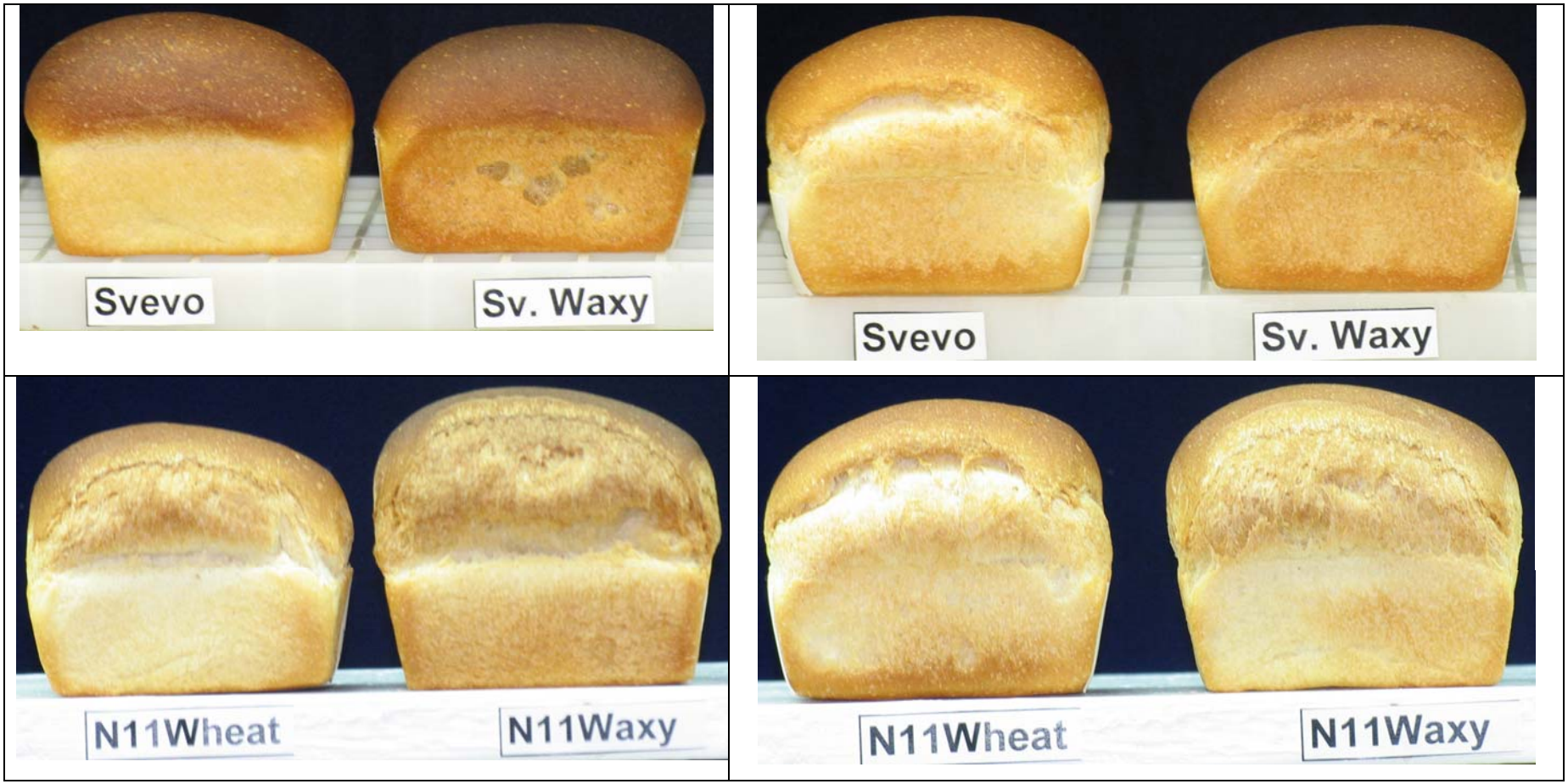


Figure.B-2b: Comparison of whole breads from waxy and regular wheats of Svevo and N11 sets. Read the sample names below the each bread. Breads on first column were made with 100% waxy where as other ones were made with 50% waxy blended with commercial flour

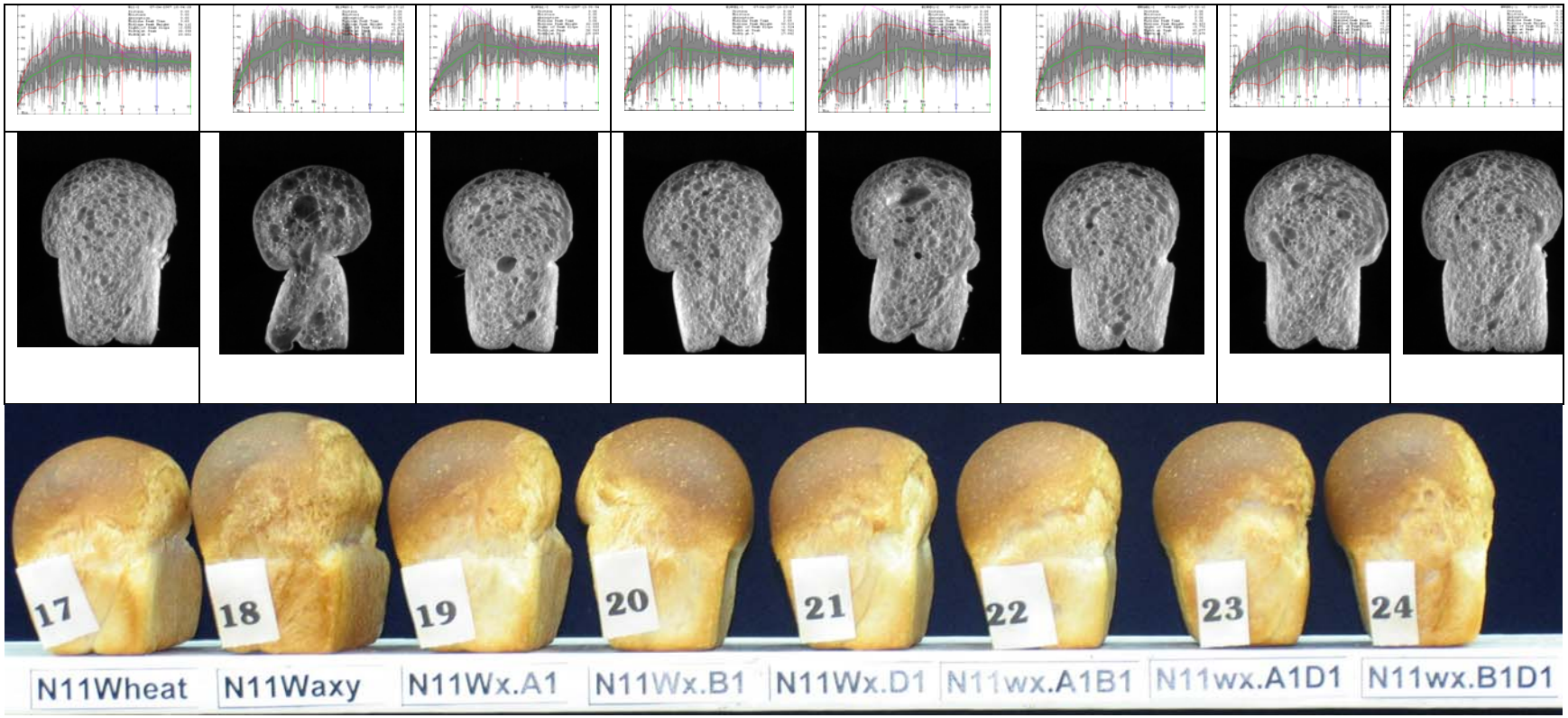


Figure.B-2c: Pictures from mixograms (top row), C-cell (middle row), and whole bread (bottom row) for N11 set. Read the sample names below the each bread.

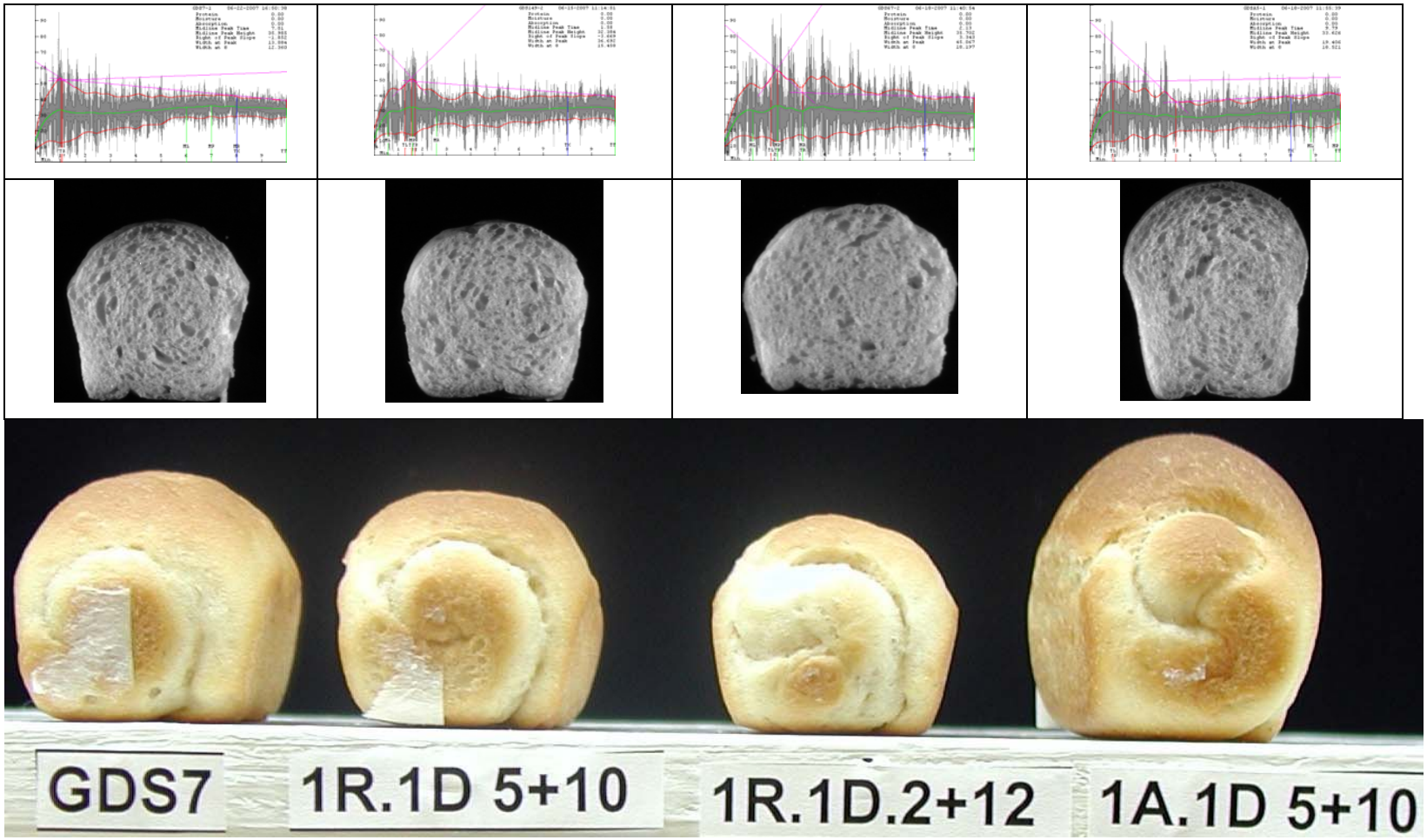


Figure.B-3a: Pictures from mixograms (top row), C-cell (middle row), and whole bread (bottom row) for GDS7 set. Read the sample names below the each bread.

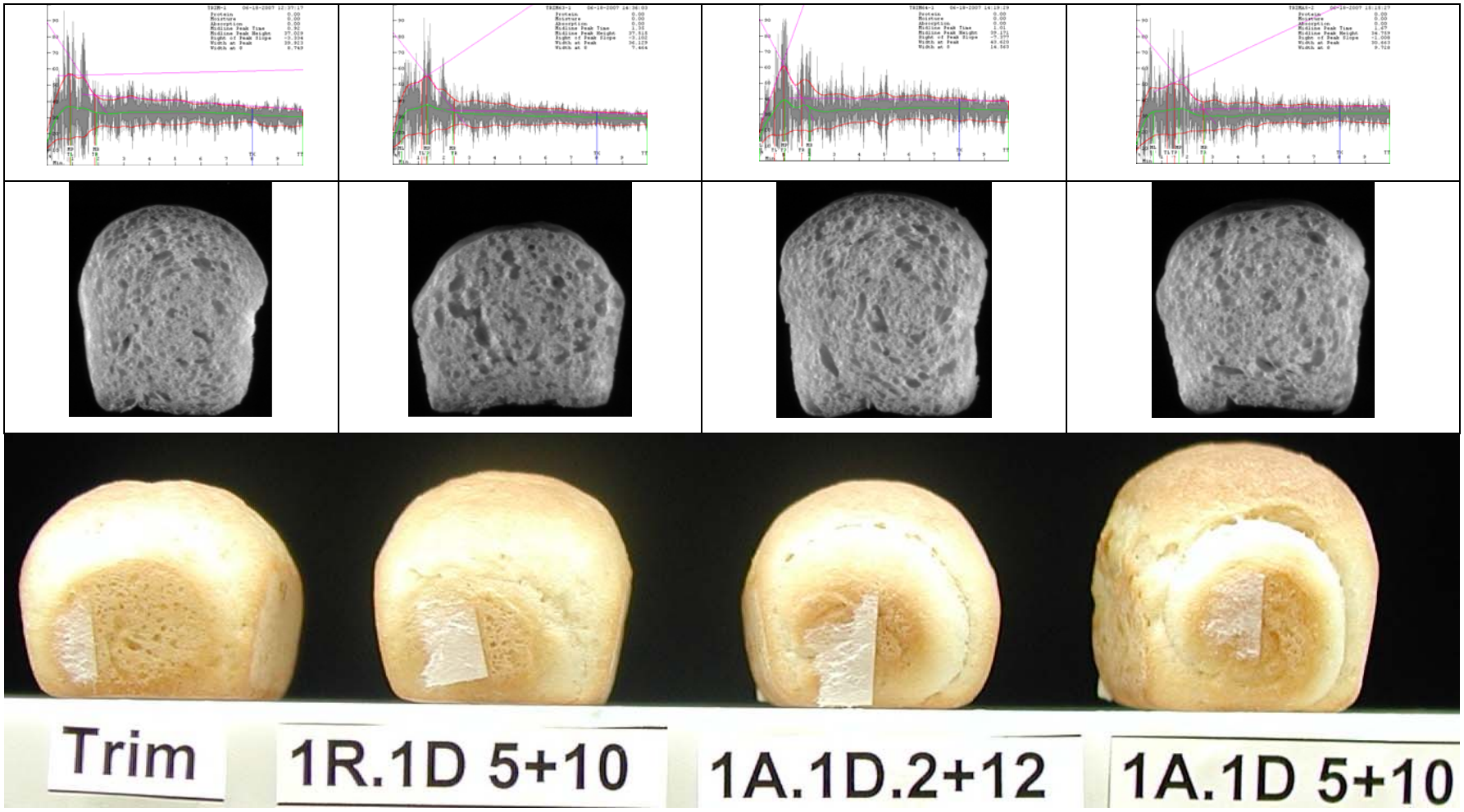


Figure.B-3b: Pictures from mixograms (top row), C-cell (middle row), and whole bread (bottom row) for Trim set. Read the sample names below the each bread.

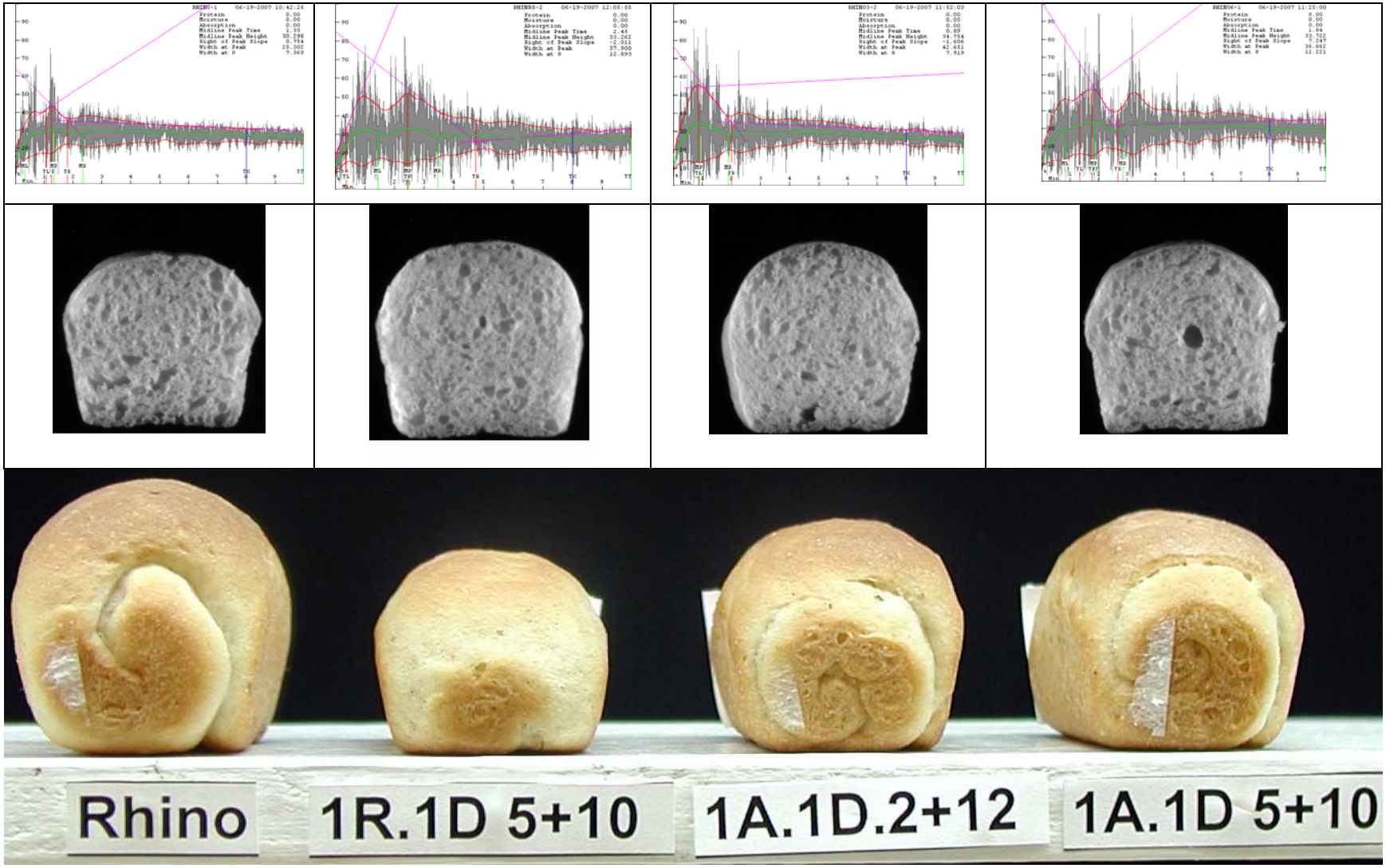
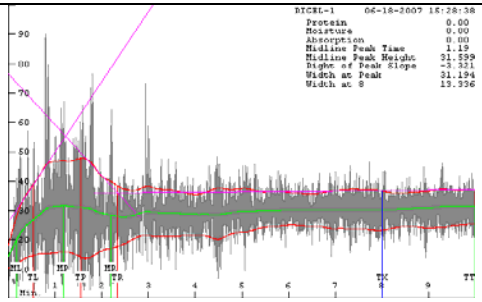
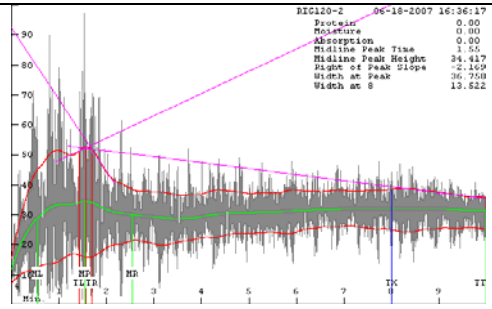


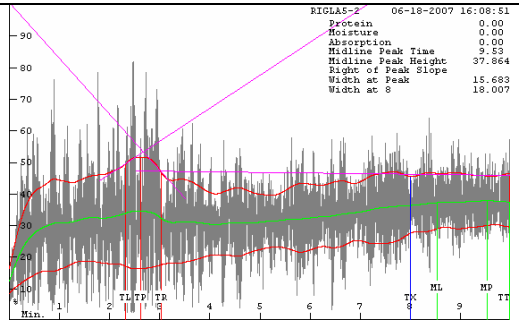
Figure.B-3c: Pictures from mixograms (top row), C-cell (middle row), and whole bread (bottom row) for Rhino set. Read the sample names below the each bread.



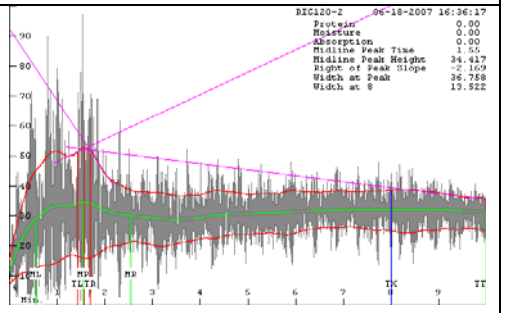
Rigel



Rigel 1R.1D 5+10



Rigel 1A.1D 5+10



Rigel 1R.1D 2+12

Figure.B-3d: Pictures from mixograms for Rigel set. Read the sample names below the each bread.