The use of gluten proteins to predict bread and durum wheat quality

By

Elizma Koen

Submitted in fulfilment of the requirements of the degree of ***Philosophiae Doctor****, in the Department of Plant Sciences (Plant Breeding), Faculty of Natural and Agricultural Sciences*

University of the Free State
Bloemfontein
Republic of South Africa
June 2006

Promoter: **Professor M.T. Labuschagne**
Co-promoter: **Professor C.D. Viljoen**

Life is girt all round with a zodiac of sciences, the contributions of men who have perished to add their point of light to our sky…
These road-makers on every hand enrich us.
We must extend the area of life and multiply our relations.
We are as much gainers by finding a property in the old earth as by acquireing a new planet.
- Emerson –

Acknowledgements

I wish to extend my sincere appreciation to the following people and organisations for their contribution towards the success of this study:

- Professor Maryke Labuschagne as my mentor, without her support, continued encouragement, enthusiasm and dedication, this study would not have been possible.
- My mother and late father, for their constant support, love and unfaltering faith, throughout this study. Thank you for allowing me the opportunity to chase after my dreams. Letasha, going above and beyond the call of sisterly-duty. I would have been lost without you.
- Heinrich for his support, love, patience and especially, understanding, in the difficult times, when spending long hours in front the books.
- My friends, whom I have severely neglected, for their constant support and encouragement, especially Sadie, Liezel, Rouxlene and Lukeshni, for being there and listening.
- The Venter-family for believing in me.
- Professor C.D. Viljoen for his co-supervision and inspiration.
- Mr. Piet Botes (Microbiol., Biochem. And Food Science) for his vital theoretical and practical input.
- Dr. Tadesse Dessalegn Woldegiorgis for the provision of the experimental material.
- The NRF for financial support.
- Ultimately, our heavenly Father for giving me perseverance and insight to complete this study.

In loving memory of my father
"It was worth the stretch"

Table of Contents

List of Figures

List of Tables

Abbreviations

µg	Microgram
µl	Microlitre
µm	Micrometre
µM	Micromolar
AACC	American Association of Cereal Chemists
ACN	Acetonitrile
ANOVA	Analysis of variance
A-PAGE	Acidic Polyacrylamide Gel Electrophoresis
BFY	Break Flour Yield
ºC	Degrees Celsius
cm	Centimetre
cm^2	Centimetre Squared
cm^3	Cubic Centimetre
CV	Coefficient of Variation
Da	Dalton
DDT	Dithiothreitol
DF	Degree of Freedom
FABS	Farinograph Water Absorption
FCL	Flour Colour
FLN	Falling number
FLY	Flour yield
FPC	Flour Protein Content
g	gram
g	Gravitational Force
Gli	Gliadin
Glu	Glutenin
h	Hour
ha	Hectar
hl	Hectolitre
HML/W	Hectolitre Mass/Weight
HMW	High Molecular Weight

HMW-GS	High Molecular Weight Glutenin Subunits
HPLC	High-Performance Liquid Chromatography
IEF	Isoelectric Focusing
IE-HPLC	Ion-Exchange High-Performance Liquid Chromatography
kDa	Kilodalton
kg	Kilogram
LFV	Loaf Volume
LMP	Large Monomeric Protein
LMW	Low Molecular Weight
LMW-GS	Low Molecular Weight-Glutenin Subunits
LPP	Large Polymeric Protein
LSD	Least Significant Difference
LUPP	Large Unextractable Polymeric Protein
M	Mole
mAU	Milli Absorption Units
MDT	Mixograph Development Time
mg	Miligram
min	Minute
ml	Millilitre
mM	Milli Molar
mm	Millimetre
MS	Mean Square
MSE	Mean Square Error
NIR	Near-Infrared Reflectance
nm	Nanometre
P	Probability
P/L	Alveograph P/L Ratio
pH	Power of Hydrogen
PR	Predictability Ratio
PVDF	Polyvinylidene Difluoride
R^2	Coefficient of Multiple Determination
RCB	Randomised Complete Block Design
RP-HPLC	Reversed-Phase High-Performance Liquid Chromatography
Rpm	Revolutions Per Minute

SD	Standard Deviation
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate- Polyacrylamide Gel Electrophoresis
SDSS	Sodium Dodecyl Sulphate Sedimentation Volume
SE-HLPC	Size-Exclusion – High-Performance Liquid Chromatography
SKCS/SK	Single Kernel Characterization System
SKCSD	Single Kernel Characterization System Diameter
SKCSH	Single Kernel Characterization System Hardness
SKCSW	Single Kernel Characterization System weight
SMP	Small Monomeric Protein
SPP	Small Polymeric Protein
TFA	Trifluoroacetic Acid
TKM	Thousand Kernel Mass
TUPP	Total Unextractable Polymeric Protein
VK	Vitreous Kernels
v/v	Volume per Volume
W	Alveograph Strength
w/v	Weight per Volume

Chapter 1

Introduction

Wheat is one of the three major cereals dominating world agriculture to date. The importance of wheat is attributed to the gluten storage proteins present in the endosperm, conferring unique viscoelastic properties to dough (Shewry *et al*., 1997).

In breeding programmes across the world more emphasis is being placed on breeding for improved quality and maintaining improved agronomical performance. Improving quality is heavily dependent on understanding the complexities of endosperm storage proteins and extensive research has been done on this in the last few years. These studies have revealed that storage proteins can be divided into two major classes: gliadins that confer extensibility and glutenins that bestow elasticity (Khatkar *et al*., 1994). It is the unique combination of these two properties that determines the functional properties of dough, ultimately determining the end-use quality (Payne *et al*., 1984).

Within the glutenins, the high molecular weight glutenin subunits (HMW-GS) contribute the most to variation in baking quality (Tatham *et al*., 1985). HMW-GS have been identified that are closely associated with bread making quality. In many countries breeding programmes use these HMW-GS as indicators of baking quality at early stages of selection. MacRitchie *et al.* (1990) reported that the HMW-gluten score is more representative of quality in some populations than in others. These proteins are genetically determined, though the relative amount and size distribution of the proteins vary as a result of environmental factors (Payne *et al*., 1987). Quality characteristics have been found to largely be influenced by an interaction between the quality and quantity of the different protein subunits (Wrigley *et al*., 1998)

Quality assessment is still heavily dependent on a number of tests that needs to be performed. These tests are specifically developed to evaluate the different rheological, elastic and extensible properties of the dough. Unfortunately quality tests require large amounts of flour, are time consuming, expensive and require expertise. Alternative indications of baking potential and a sound understanding of the important interactions would have an enormous positive impact on breeding programmes, especially in developing countries, where it is too expensive to set up quality laboratories.

In South Africa, and Africa, the use of high-performance liquid chromatography (HPLC), size-exclusion as well as reversed-phase, has never been used in the study of wheat quality. This technique holds the advantage of a small sample size required and the possiblity of quantifying the expression of protein (Marchylo *et al.,* 1989). By establishing correlations between specific subunits, amounts and size distribution and quality parameters, HPLC could be used as a tool in wheat quality research and in breeding programmes.

The objectives of this study were to:

- examine the influence and contribution of different protein fractions determined by size-exclusion high-performance liquid chromatography (SE-HPLC), on baking quality, across two diverse environments in bread and durum wheat.
- determine whether significant correlations exist, across environments, which can be used to predict baking quality.
- identify specific protein subunits and their correlation to bread making quality, using reversed-phase high-performance liquid chromatography (RP-HPLC), across diverse environments.
- determine individual protein subunits' interaction and contribution to quality.
- in so doing, assess the potential of HPLC to predict baking quality in diverse genotypes across environments.

References

Khathar, B.S., Bell, A.E. and Schofield, J.D., 1994. The dynamic rheological properties of gluten and gluten sub-fractions from wheat of good and poor bread-making quality. *J. Cereal Science.* 22: 29-44.

MacRitchie, F., DuCros, D.L., and Wrigley, C.W., 1990. Flour polypeptides related to wheat quality. In: Y. Pomeranz (Ed.), Advances in Cereal Science and Technology, Vol. 10. American Association of Cereal Chemists, St. Paul, MN, pp 79-145.

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

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. *Phil. Trans. R. Soc. Lond. B*. 304: 359-371.

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

Shewry, P.R., Tatham, A.S., Lazzeri, P., 1997. Biothechnology of Wheat Quality. *J. Sci. Food Agric.* 73:397-406.

Tatham, A.S., Miflin B.J. and Shewry, P.R., 1985. The beta-turn conformation in wheat gluten proteins: Relationship to gluten elasticity. *Cereal Chemistry* 62(5): 405-412.

Wrigley, C.W., Andrews, J.L., Bekes, F., Gras, P.W., Gupta, R.B., MacRitchie, F. and Skerrit, J.H., 1998. In: Interactions: The Keys to Cereal Quality. R.J. Hamer and R.C. Hoseney (Eds.), American Association of Cereal Chemists, St. Paul, MN, pp 17-48.

Chapter 2

Literature review

Wheat, both durum and bread wheat, is one of the most commonly grown cereals in the world. This is mainly due to wheat's versatility, not only in its adaptability in terms of geographical distribution but also in regard to its end use products (Poehlman and Sleper, 1995). Wheat is the only grain cereal with the exceptional ability to form leavened bread, a property used in other food products. The bread-making quality of wheat is the product of the interaction between different flour components, such as proteins, starch, lipids and pentosans. Most of the differences in quality are conferred by the gluten–forming storage proteins of the endosperm (Gianibelli *et al.*, 2001). It is thus necessary to study the endosperm storage protein structure and compositions to understand its functionality and ultimately its quality attributes.

2.1 Storage proteins

Eighty percent of the total protein in wheat grain is constituted by the endosperm storage proteins. With the original Osborne fractional extraction procedure, five protein fractions were obtained: albumins (soluble in water), globulins (soluble in salt solutions), gliadins (soluble in aqueous ethanol), glutenins (soluble, or rather dispersible, in dilute acid or alkali) and an insoluble residue (Osborne, 1907). Gliadins and glutenins are often described as gluten proteins. Gluten is formed when wheat flour dough is washed to remove all soluble components and starch. Glutens constitute up to 50% of the total protein in wheat flour (Eliasson and Larsson, 1993). Gluten is a large complex constituting of mainly glutenin (polymeric) and gliadin (monomeric) proteins (MacRitchie, 1994).

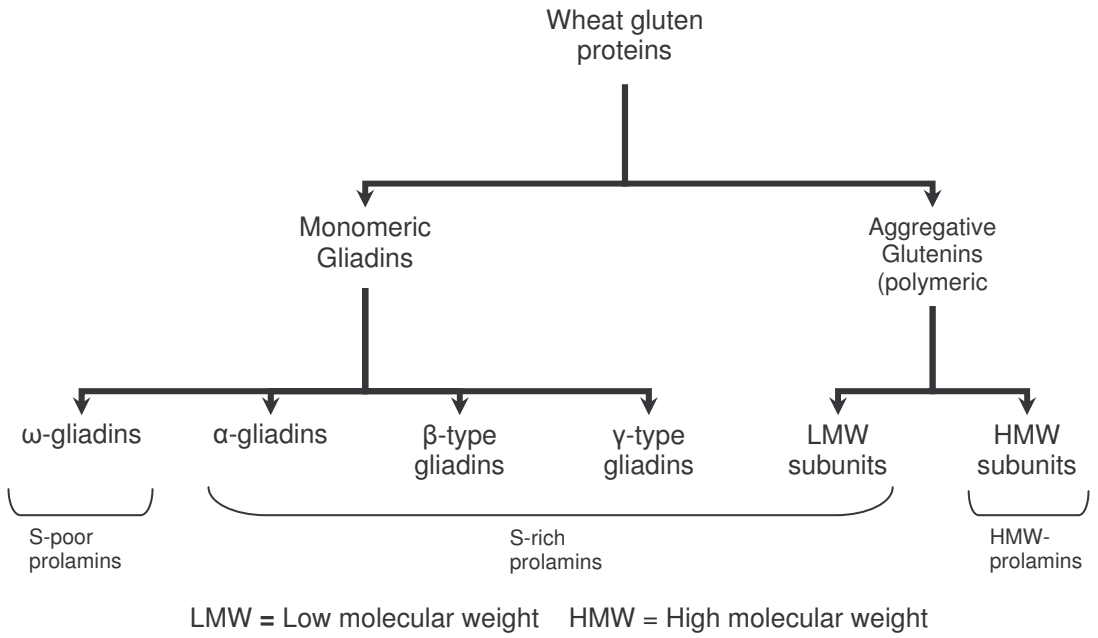


Figure 2.1 Separation of flour components and the definition of gluten (Shewry *et al.*, 1986; Shewry and Tatham, 1990).

The introduction of better protein fractionation procedures, especially those separating in two dimensions, has made the identification of proteins determining good bread-making quality possible (Shewry *et al.*, 1986; Shewry and Tatham, 1990) (Figure 2.1). The major contributors to quality are: glutenin, which confers elasticity and gliadin, which confers extensibility to dough (Gupta and Shepherd, 1990). Wheat quality is therefore dependent on the structures and interactions of the different proteins with each other and other grain components (Shewry and Tatham, 1997).

The composition of albumins and globulins does not vary between wheat varieties and no correlation exists between the amount of albumins or globulins and baking performance (Eliasson and Larsson, 1993).

2.1.1 Glutenin

Glutenins are multiple polypeptide chains held together by disulphide bonds. Glutenins partial insolubility is due to the high molecular weights of these polymeric structures (Gianibelli *et al*. 2001). Glutenin has a much lower solubility than gliadins. It is virtually insoluble in 70% ethanol and only a portion dissolves in dilute acid solutions. It is built up from subunits into protein aggregates of high molecular weights between 200 000 and 20 million Da. When glutenin is treated with reagents that dissociate disulphide bonds, the subunits are released and fractionated by sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE) into two groups on the basis of molecular mass: the HMW-GS (80-120 kDa) and the low molecular weight glutenin subunits (LMW-GS) (10-70 kDa) (Bietz and Wall, 1972). Glutenins affect baking performance of wheat in at least three ways: through the molecular weight distribution, the presence of certain HMW–GS and the gliadin/glutenin ratio (Schepers *et al.*, 1993).

2.1.1.1 HMW-GS

Glutenins are polymers belonging to the polymeric prolamines (Shewry *et al.*, 1986). The molecular weight of glutenin can extend into millions as it is the product of polymerisation of polypeptides through intermolecular disulphide bonds (Hamauzu *et al.*,1972). HMW-GS have unusually high glutamic acid content, mostly in the amidated form glutamine and high contents of proline and glycine, but low lysine (Gianibelli *et al.*, 2001).

HMW-GS consist of three structural domains: a non-repetative sequence containing 3-5 cysteine residues at the N terminus, another non-repetative sequence containing only one cysteine at the C terminus and a central region of repetitive sequences of between 490-700 residues. The central domain is thought to be hydrophilic as opposed to the N and C terminal domains that are hydrophobic. This hydrophobic characteristic makes separation by reversed-

phase HPLC (RP-HPLC) possible, with HMW-GS being less hydrophobic than LMW-GS. The structures of HMW-GS are similar to that of ω-gliadins. However, HMW-GS differ from gliadins in their higher glycine and lower proline contents (Shewry *et al.*, 1986). The conformation is characterised by a large proportion of β-turns, which has been associated with the elasticity of glutenins, in the central domain (Tatham *et al.*, 1985). Studies by Belton (1999) have shown that high glutamine residue levels have a high capacity to form both inter- and intramolecular hydrogen bonds. This allows loops and trains to form, giving rise to the elastic restoring force, as the loops stretch and reform.

The genes coding for the HMW-GS are found on the long arms of chromosomes 1A, 1B and 1D with their loci indicated as *Glu-A1, Glu-B1* and *Glu-D1* respectively. Each of these loci contains two linked genes, encoding two subtypes of HMW-GS, the *x*-type and the *y*-type (Payne *et al.*, 1981). The *x*–type subunits have a higher molecular weight than the *y*–type subunits (Lafiandra *et al.*, 1994).

Initially HMW-GS were identified according to their electropheric mobility in SDS-PAGE, where separation occurred according to molecular weight. Numbers were assigned based on the subunit mobility, lower numbers indicating lower mobility (Payne and Lawrence, 1983). This also provided chromosomal location of the genes and this system is currently still being used. Payne *et al*. (1987) related dough strength and baking performance to allelic variation in HMW-GS of wheat cultivars. This resulted in a quality score, assigning numbers based on quality evaluations. By adding the numbers, the *Glu-1* score is obtained for each variety; this score is positively correlated to baking quality in the case of bread wheat, and negatively in the case of biscuits wheat. Unfortunately this score does not always explain the variation in quality for all wheat, because it does not make allowance for the complex interaction that exists between protein components (MacRitchie *et al*., 1990).

The most significant HMW-GS bands are *Glu* 5+10 and *Glu* 2+12, both of which are coded for by genes on the D-genome**.** The HMW subunits 5+10 are said to be present in varieties of good baking performance, strength and high sedimentation volume in the SDS sedimentation test. The inverse is true for subunits 2+12 (Lukow *et al.*, 1989). Similar results were obtained for other allelic variants. *Glu-B1* subunits 17+18 were associated with strong dough (Gupta and MacRitchie, 1994).

The consistent prominence of Glu 5+10 and Glu 2+12 among HMW glutenin subunits is most striking and is consistent with studies on several other sets of wheat. It is significant that these proteins are associated with the D-genome, which distinguishes bread wheat from durum wheat. This explains why HMW-GS have not been found to be associated with dough properties in durum wheat (DuCros, 1987).

In some countries e.g. Australia the correlation between the 5+10 subunits and baking quality seem lower (Campbell *et al*., 1987). In South African wheat, bands 13+16 and 17+18 were found to be more prevalent than what was published for American, British, and Canadian wheat (Randall *et al*., 1993).

Sutton (1991) found that differences exist between subunits with the same electrophoretic mobility on SDS-PAGE. When these subunits (7 and 8) were subjected to RP-HPLC, differences in retention times were observed. This indicated different protein sequences and surface hydrophobicities.

2.1.1.2 LMW-GS

LMW-GS, unlike HMW-GS and gliadins, are not easily separated and analysed by one-dimensional SDS-PAGE or isoelectric focusing (IEF), since many of the LMW-GS overlap with gliadins (Zhen and Mares, 1991). This is not unexpected, seeing that LMW-GS are controlled by genes found on the short arms of group 1

chromosomes, which are closely linked to genes controlling gliadins found on the same chromosomes (Rodriguez-Quijano and Carrillo, 1996).

This caused some confusion and Bietz and Rothus (1970) considered that some polypeptides may be common to both gliadins and glutenins, since α, β,and γ-gliadins and LMW-GS have similar electrophoretic mobilities and both are soluble in aqueous ethanol. This problem was resolved by the use of a two-dimensional electrophoresis, since LMW-GS had different positions to α, β, ω-gliadins which indicated that they indeed were distinct proteins (Jackson *et al.,* 1983).

Despite the limitations of the one-dimensional SDS-PAGE system, Payne *et al.* (1984) were able to map the genes coding for the b subunits. It has further been proved that each of the *Gli-1* loci, *Gli-A1*, *Gli-B1* and *Gli-D1* located on the short arm of chromosomes 1A, 1B and 1D, respectively, are closely linked to a locus coding for LMW-GS (*Glu-3*). Examination of the banding patterns revealed that some bands were inherited simultaneously and formed combinations whilst others occurred as alternatives to each other, in the same cultivar (Gupta and Shepherd, 1988).

LMW-GS have been divided into two subunit groups, B (higher molecular weight, slower moving) and C (lower molecular weight, faster moving), subdivided into three groups (1-3). These subdivisions were further divided into patterns, indicated by letters. Group one consists of six combinations indicated by letters a-f. Genes on chromosome 1A control the few bands represented in these patterns. Group 2 was divided into nine pattern combinations (a-i). These patterns consist of a lot more bands, with at least two or more B subunit bands. Combinations in group 2 are mainly controlled by genes on chromosome 1BS. Group 3 consists of five different combinations (a-e), controlled by genes on the short arms of chromosomes 1D. In this group (3) banding patterns mostly constitute two bands from each subunit (Konarev *et al*., 1979).

Despite the amount of information already available on LMW-GS, a few questions remain unanswered. This is due to difficulties analysing the LMW subunit combinations. Bands in group 2, for example, represent a wide range of mobilities, which overlaps with bands in group 1 (*Glu-A3*) and group 3 (*Glu-D3*), and *visa versa*. Although this nomenclature is still used, Lew *et al*. (1992) proposed a system based on sequence similarities, rather than mobilities. LMW-GS can be divided based on the N-terminal sequences. Leading to the identification of two groups. The first group, LMW-m and LMW-s indicates the first amino acid in the sequence (s=serine and m=methionine) and the second group have sequences similar to α- and γ-gliadins.

Within the two groups, sequencing revealed seven types of LMW-GS, based on the N-terminal sequences (Gianibelli *et al*., 2001). Although the LMW-s are the most abundant (only one type), three types of LMW-m was identified. The LMW-GS with the N-teminal sequence of METSH showed improved mixing properties. This was confirmed by other studies (Sissons *et al*, 1998; Lee *et al*., 1999a).

The last three types resembling α-, γ- and ω- gliadins, have odd numbers of cysteine residues that allow formation of intermolecular disulfide bonds (Kasarda, 1989). LMW-GS secondary structure, except for the D-subunits, is similar to that of the S-rich gliadins. The D-subunits are similar to the S-poor ω-gliadins in terms of mobility and N-terminal sequences (Masci *et al*., 1991). Apart from the sequence similarities, a close linkage between the *Glu-3* (encoding LMW-GS) and *Gli-1* (encoding gliadins) have been reported by Pogna and colleagues (1990). This close linkage and the ease of screening for gliadins makes this potentially useful as markers for LMW-GS (Singh *et al*., 1991b).

2.1.2 Gliadins

Gliadins are more polymorphic than glutenins, they are inherited at the more complex *Gli-1* and *Gli-2* loci (Metakovsky, 1991). Gliadins are readily soluble in

aqueous ethanol and consist of a complex mixture of polypeptides whose molecular weights range from about 30 000-70 000 Da as determined by SDS-PAGE (Bietz and Wall, 1972). Shewry *et al*. (1986) defined gliadins as monomeric proteins with intramolecular disulphide bonds, and that the conformations are thus stabilised by hydrogen bonds and hydrophobic interactions.

When fractioned by A-PAGE (acid polyacrylamide gel electrophoresis) they are subgrouped into α-, β-, γ- and ω- gliadins (Woychik *et al.* 1961; Mosleth and Uhlen, 1990). The molecular weight of most gliadins are in the range 30 000-40 000 Da, with the ω- gliadins being larger with a molecular weight around 60 000-80 000 Da. There is considerable variation in gliadin-banding patterns between varieties, making it possible to use A-PAGE to identify varieties and varietal mixtures of grains (Wrigley, 1992).

Gliadins are inherited codominantly, with certain gliadins inherited as a block (Sozinov and Poperellya, 1980). This might be an indication that the gliadins inherited as a block are a cluster of structural genes (Wrigley, 1982).

The genes that synthesize gliadins are found on the short arms of chromosomes 1 and 6 respectively (Khelifi *et al.*, 1992). Genes found at the *Gli-A1, Gli-B1*, and *Gli-D1* loci on chromosome 1A, 1B and 1D respectively are referred to as the *Gli-1* genes. Those found at the *Gli-A2, Gli-B2* and *Gli-D2* loci of chromosomes 6A, 6B and 6D respectively are referred to as the *Gli-2* genes (Jackson *et al.*, 1983, Rodriguez-Quijano and Carrillo, 1996).

To utilize variations in gliadin banding patterns, as means of identifying biotypes and cultivars or as indication of possible influence on baking quality, a standard nomenclature system is needed. The system most commonly used to analyse banding patterns, is a combination of the nomenclature used by Woychik *et al.* (1961) and Konarev *et al.* (1979). Gliadin zones are designated by a Greek letter

as α, β, γ and ω (Woychik *et al.,* 1961). Bands within these zones are identified by numbers, making this method more accurate (Konarev *et al*., 1979). Additional adjustments were allowed to indicate deviations from the standard e.g. greater mobility (subscript 1), lower mobility (subscript 2), higher intensity bands (underlined number), and lower intensity bands (overlined number). Table 2.1 shows this system in use.

Table 2.1 A summary of the nomenclature system developed by Konarev *et al.* (1979)

Gliadin zones and bands		**Chromosome and its arm**
α	2	6A
	4	6A
	6	6D
	7	1B(S)
β	3	6B(S)
	4	6B(S)
	5	6B(S)
γ	2	1B(S)+6B(S)+1D(S)
	3	1D(S) +1A+1A(S)
	5	1A+1A(S)
ω	3	1B(S)
	4	1B(S)
	5	1B(S)
	7	1D(S)
	8	1D(S)
	9	1D(S)

Gliadins do not seem to be crucial to baking performance. When interchanged between wheat flours of different baking performances, the effect compared to

that of glutenin is very minor, although groups of gliadins have been indicated to be related to endosperm hardness, dough strength, Chopin values, or Zeleny tests (Branlard et al., 2001; Branlard and Metakovsky, 2006).

Gliadins indicated to be involved in flour quality, are coded for by genes on chromosomes 1D and 1B. The gliadin bands most strongly associated with dough resistance in the study have not previously been studied, but they probably correspond to components of the compound gliadin 34 (Wrigley, 1982). These gliadins are presumably coded for by genes on the homologous group 6 chromosomes.

2.2 Baking quality

The criteria of wheat quality for baking are as varied as its uses (Halverson and Zeleny, 1988). Protein quality and quantity are considered primary factors in measuring the potential of flour in relation to its end use (Mailhot and Patton, 1988). Wheat proteins contribute to the functionality of flour in the breadmaking process in two distinct ways: the bread flour must have relatively high protein content; secondly, the protein must have the right quality (Graybosch *et al.*, 1996).

2.2.1 Grain protein

The protein content of wheat grain can vary from 6% to as much as 25%, depending on the growing conditions. Grain protein is a major contributor to the nutritional quality of wheat. In South Africa grain protein of 12% and higher is preferable. The availability of nitrogen is the major determining factor for the protein content of grain (Blackman and Payne, 1987).

There is a strong negative relationship between the grain protein percentage and the grain yield. The rare varieties, which have high grain protein without a yield

penalty, may achieve this by more efficient relocation of nitrogen from senescing tissues to grains, or by a more efficient uptake of nitrate and ammonia from the soil.

The nutritional quality of grain protein becomes very important where wheat is the major protein source for people. The first limiting essential amino acid is lysine, so in breeding programmes the major aim is to increase the amount of this amino acid. Unfortunately a negative correlation exists between lysine content and the protein content of grain. As the protein increases from 7-15% the lysine content falls from 4-3%. Increasing the protein concentration causes a significant increase in the ratio of storage protein to metabolic and structural proteins in the grain, the former being lysine deficient and the latter two relatively lysine rich. However, storage proteins are more digestible than structural proteins. For practical purposes it may therefore be better to simply opt for increased protein content when seeking to improve the lysine content (Blackman and Payne, 1987).

2.2.2 Flour protein

Higher protein percentage are often associated with better quality for a given sample. Flour protein plays a major role in the functionality of wheat. It influences parameters such as mixograph, alveograph, farinograph, extensograph, SDS-sedimentation and loaf volume (Koekemoer *et al*., 1999) In South Africa wheat with a protein content of about 12% and above is preferable (Koekemoer, 1997). Near Infrared Reflectance Analysis (NIR) is used to measure protein and moisture contents, but can also be used to measure grain texture and to predict potential starch damage. The reflectance energies of the different wavelengths are related to the physical and chemical nature of each sample. Multiple regression analysis is used to determine the relationship between reflectance energies of a test sample with known standards. Once

calibrated the test samples can be analysed for several characters simultaneously in a 20 s period (Blackman and Payne, 1987).

2.2.3 Flour extraction

Milling properties are complex and may be divided, in relation to the breeding objectives, into percentage extraction of white flour, endosperm texture and water absorption. Judging milling texture by the appearance of the grain is often misleading because grains appearing flinty may actually be soft textured. Texture appears to be simply inherited and there are a number of tests to measure this characteristic (Blackman and Payne, 1987).

Traditionally, vitreousness is associated with high-protein hard wheat, whereas opaque or mealy kernels are associated with softness and low protein content. The proportion of vitreous kernels has been used as an indication of kernel hardness (Eliasson and Larsson, 1993).

Hardness is highly heritable and wheat cultivars are specified either to be hard or soft. The harder durum wheat are used for pasta production, and the softest wheat for biscuits, whereas the wheat most suitable for bread-making have an intermediate hardness. The milling capacity as well as the flour yield will be higher with harder wheat than with softer wheat (Stenvert and Kingswood, 1977). Flour yield is related to kernel hardness. Van Lill *et al.* (1995) reported that grains containing higher protein content were inclined to be harder, which in turn, increased flour yield. Extraction is a function of hardness and the endosperm of hard firm wheat grains tend to separate more easily from the bran during the milling processes. More starch granules are damaged when hard wheat is milled, thereby improving water absorption (Bass, 1988).

Wheat conditioning is necessary to improve the physical state of the grain for milling and sometimes to improve the baking quality of milled flour. Conditioning

involves adjustment of the average moisture content. This causes bran to toughen and become less brittle thus leading to better separation of the endosperm from the bran and making the endosperm more friable. Reducing the power required for grinding. All the above-mentioned are related to the grain texture and wheat type (Eliasson and Larsson, 1993).

2.2.4 Breakflour yield (BFY)

Breakflour is the flour produced when wheat is broken open in the first break system (Bass, 1988). Bran has a detrimental effect on loaf volume. However, the effect is related to the composition of the bran and the mill it comes from, as the method of separating the bran and the endosperm differs among mills. The coarser the bran fraction, the more detrimental its effect will be. The detrimental effect is attributed to a decrease in the gas retention capacity (Pomeranz, 1988).

2.2.5 Falling number (FLN)

The FN value represents the time, in seconds, required to stir a hot aqueous flour gel undergoing liquefaction in a viscometer and then allowing the viscometer stirrer to fall a measured distance through the gel (Kaldy and Rubenthaler, 1987).

Falling number is the effect of α-amylase activity resulting in the degradation of starch into simple sugars. Screening for this activity has a high priority in most breeding programmes, because the majority of wheat products are adversely affected by this enzyme. Selection for offspring with genetically controlled low levels of resistance to premature germination is difficult because of the large environmental component in sprouting and α-amylase production.

Several methods exist for measuring α-amylase activity, including those of Farrand and Phadebas or determination of the Hagberg Falling number (Hagberg, 1960). An amylograph or Visco analyser (RVA) can also be used to

evaluate the the effects of α-amylase on a flour water system (Atwell, 2001). The Falling Number (FN) method is widely used commercially. Although it does not reflect the enzyme levels directly, it is sufficiently accurate for most purposes (Blackman and Payne, 1987).

Germinating wheat undergoes morphological and chemical changes whereby carbohydrates are converted into complex sugar compounds by enzymatic activity. The α-amylase hydrolyses of starch reduces the viscosity of the suspension and thus increases the falling rate of the stirrer during FN tests. This starch can be turned into a dextrin-like substance during baking. This reduces water holding capacity, the crumb weakened and made sticky (Blackman and Payne, 1987).. Flour with normal α-amylase activity and good baking quality has a FN value of 250 seconds or higher. Wheat with high α-amylase activity has a value of 65 seconds and produces sticky breads. High FN values in the range of 400 seconds indicate too low α -amylase activity for bread baking.

2.2.6 SDS-sedimentation (SDSS)

SDSS is a simplified water retention capacity test in the presence of lactic acid. Baking quality largely depends on the gluten proteins and the latter are caused to hydrate and swell by the lactic acid. Flour, water, and lactic acid are shaken together in a glass cylinder under specified conditions and the height of the sediment subsequently read. It has been shown that the sedimentation value is related to the granularity of the flour and that the sediment is an agglomeration of the course particles rather than the swollen protein. The sedimentation value is thus an indicator of hardness rather than of strength of the wheat (Lorenzo and Kronstad, 1987). This method is used for measuring relative gluten strength. Sedimentation values can range from 20 or less for low protein wheat of inferior bread-baking strength to as high as 70 or more for high protein wheat of superior baking strength. The high-protein helps to retain gas during fermentation, which results in higher loaf volumes (AACC, 1995).

2.2.7 Hectolitre mass (HLM)

The hectolitre mass (HLM) is the mass per volume of wheat and depends on kernel density and its packing efficiency. HLM and 1000 kernel mass are the two parameters used as an indication of the flour yield after milling and are therefore an important selection criterion (Fowler and De la Roche, 1975). In South Africa a hectolitre mass of more than 77 kg/hl is preferable (Francois Koekemoer, personal communication).

2.3 Yield

Yield remains one of the most important factors in wheat production (Jalaluddin and Harrison, 1989). Yield of cereals is composed of three components, namely the amount of spikes per unit area, number of kernels per spike, and the individual kernel weight (Bulman and Hunt, 1988). Yield is affected by both the environment and the genotype, making it difficult to predict the harvest outcome (Fowler and De la Roche, 1975).

2.3.1 Thousand kernel mass (TKM)

In South Africa a thousand kernel mass (TKM) of more than 32 g is preferable (Francois Koekemoer - personal communication). The weight of 1000 counted kernels is determined, or the number of kernels is counted in a preweighed sample and the weight of the 1000 kernels is calculated from it. The weight of 1000 kernels can be corrected to a dry basis or any moisture basis. TKM can give the miller important information about the wheat' milling potential. TKM is one of the wheat quality parameters highly correlated with flour yield (Blackman and Payne, 1987).

2.4 Rheological characteristics

When bread ingredients are mixed in the correct proportions to make a dough, two processes commence. Firstly, the protein in the flour begins to hydrate, i.e. to combine with some of the water to form a cohesive mass called gluten, which has particular extensible properties. It can be stretched like an elastic band, and possesses a certain degree of recoil or spring. Secondly, evolution of the gas carbon dioxide by the action of the enzyme in the yeast upon the sugars commences (Eliasson and Larsson, 1993).

2.4.1 Mixograph development time (MDT)

The quality of the final loaf of bread is strongly dependent on the mixing of each combination of flour and water. It is possible to find an optimum stage of dough development. The mixograph mixer measures the power used to mix the dough or the resistance to mixing is recorded. The resulting mixing curve is described with such terms as dough development and breakdown. Higher amounts of glutenins combined with higher molecular weights will lead to longer development times. Breakdown starts after a decrease in the mixing curve is recorded. The rate of breakdown shows the stability of the dough and its sensitivity to mechanical treatment. Flour with the best baking performance has medium to medium-long mixing times. The aim of many rheological measurements is to find a way to differentiate between wheat varieties according to their baking performance without actually performing the baking test (Eliasson and Larsson, 1993).

Molecular weight distribution of proteins, differs among wheat varieties, and strong wheat with medium-long mixing time contains more of the high molecular weight material. Moreover, these wheat varieties contain more residual protein. It was found that fractions rich in LMW proteins decrease the mixograph developing time as well as the loaf volume in test baking (Tanaka and Bushuk,

1973). Fractions with a high proportion of HMW proteins, on the other hand, increased the mixograph developing time as well as the loaf volume in test baking. Such a relationship seems promising in the case of HMW glutenin subunits. These subunits are of greater importance for dough strength and dough stickiness than LMW glutenin subunits (Eliasson and Larsson, 1993).
Flour protein was reported to be negatively correlated to mixograph tolerance. Mixograph tolerance was independent of corrected or uncorrected loaf volume. Dough type is phenotypically correlated to all other characters except mixing tolerance (Souza *et al.*, 1993). Flours with medium to medium-long mixing times usually have good mixing tolerance, good dough handling properties, and good loaf volume (Finney *et al.*, 1987)

The suggested mixing time in South Africa is 2 to 3 minutes, with 2.5 minutes as optimum (Francois Koekemoer – personal communication). A higher mixing time is not desirable, as apart from spending more time, the energy consumption is also higher.

2.4.2 Farinograph

It is not possible to make bread without water. Water is necessary for gluten formation, and water is the medium for all types of interactions and reactions that occur during the breadmaking process. The water content of standard bread dough is about 40%. However, the ingredients in the formula are usually expressed as a percentage of the flour by weight, and the water content in bread dough will then be around 65%. The optimum level of water addition is related to the composition of the flour. Both quantity and quality of protein influences water absorption (MacRitchie, 1984). Therefore it is necessary to determine this optimum level for each flour. This may, of course, be done in test baking, but it is more common to determine water absorption by the use of the Brabender farinograph, although it needs larger size samples than for most other tests and is a relative expensive apparatus (Finney *et al.*, 1987).

The farinograph measures and records resistance of a dough to mixing. It is used to evaluate water absorption of flours and to determine stability and other characteristics of doughs during mixing. The important factors are the absorption capacity, peak time, and stability. In South Africa the absorption is suggested to be 60 as optimum but it can go up to 63 (Francois Koekemoer – personal communication). The water absorption of a flour is described as the amount of water necessary to bring the dough to a specified consistency at the point of optimum development. Absorption increases linearly with the amount of protein, but the slope of the regression line depends on the wheat variety. The rheological properties of a wheat flour dough are extremely sensitive to water content. It is evident that a decrease in the amount of water added has a greater effect than an increase, at least within the range of water content (Eliasson and Larsson, 1993).

Flour from large wheat kernels have higher water absorption and a longer peak time than flour from small and medium sized wheat kernels. Smaller wheat kernels showed greater mixing stability than flours obtained from large and medium sized wheat kernels. The rheological variation among flours from different sized wheat indicates the potential differences in baking qualities. Uniformity of wheat kernel size plays an important role in milling stability (Blackman and Payne, 1987).

2.4.3 Alveograph

The alveograph was one of the first machines used to predict baking quality. It measures the resistance to biaxial extension obtained from a thin sheet of flour-water-salt dough (Bettge *et al.*, 1989). The dough prepared for use in the alveograph test needs to be stiff and have a low water concentration. The dough undergoes treatment similar to that of the baking process, by being sheeted, rolled, and moulded. It is moulded into a patty, which is then exposed to air

pressure, forming a bubble. The alveograph records the pressure and time needed for the bubble to burst.

The interpretation of the alveograph results is similar to that of the extensograph. The maximum curve height is an indication of the resistance and the length of the curve measures the elasticity. The resistance is influenced by the water absorption of the dough and the dough is developed with a constant increase of water added.

Randall *et al*. (1993) found the values of the alveograph (P, L and W) to be correlated with values obtained from the extensograph, but that only the P-value showed a negative correlation with flour protein content, wet gluten and loaf volume.The P-value indicates the dough's ability to retain gas, the L-value is related to the dough's handling properties and its extensibility, while the W-value indicates the energy input needed to deform the dough. As with all the other rheological characteristics, protein content and composition have an influence on the alveograph.

2.5 Baking characteristics

2.5.1 Loaf volume (LFV)

Baking is the final test of wheat quality as it indicates what the final product looks like. The desired higher loaf volume and good texture is a result of high protein content especially gluten in wheat grains. High protein flours with good quality are required for long fermentation baking methods, but low protein levels are tolerated for mechanically developed bread processes (Blackman and Payne, 1987). Higher loaf volumes also indicates that there was no sprouting damage, as flour from sprouted wheat grains results in low loaf volumes and poor texture regardless of a cultivar being of good quality.

Strong flours must be used which develop an extensive viscoelastic matrix during dough formation, to retain the gas produced by fermentation. The dough expands and, after baking, a large well-aerated loaf is formed. If weak flours are used, loaves of small volume are produced which have poor crumb structure, being too firm and lacking resilience. Hard wheat are also preferred to soft wheat because their high water-absorption properties increase bread yield and resistance to staling (Blackman and Payne, 1987).

The loaf volume method provides a basic baking test for evaluating bread-wheat flour quality by a straight-dough process that employs short fermentation and in which all ingredients are incorporated in the initial mixing step. It is intended primarily for laboratory assessment of bread-wheat flour quality under vigorous fermentation conditions. Effects of ingredients and processing conditions, and particularly oxidation response, can also be assessed (Mamuya, 2000).

2.5.2 Baking strength index

Strong dough requires a high energy input to mix it to a consistency, which is optimal for breadmaking, whereas a weak dough requires little mixing. The difference is mainly caused by the protein quality and quantity. Stronger dough has higher quality glutenin content, the protein complex that imparts elasticity. Whereas weaker dough, deficient in glutenin, may exhibit extensibility imparted by the gliadin proteins (Blackman and Payne, 1987).

2.6 Protein quality

The quantity and the quality of flour protein largely determine bread quality. Quality is mainly controlled genetically while quantity is largely influenced by environmental factors (Peterson *et al*., 1992). Protein quality is a major factor in determining whether a sample of wheat meets the required standard for potential dough development. Protein quantity is determined through assessing the

nitrogen in wheat or flours. The nitrogen level is multiplied by 5.7 to approximate the protein content in flour. Near-infrared reflectance analysis of wheat has been developed as a means for fast protein quantification (Eliasson and Larsson, 1993).

2.7 Proteomics

Proteomics is the study of the full compliment of expressed polypeptides in specific tissue, at a particular developmental stage, under specific growth conditions (Dunn, 2000).

Our understanding of the role of wheat proteins on baking quality is still incomplete, and two reasons for that are undoubtedly the complexity of their composition and their physical properties. The fractionation and characterisation of plant storage proteins are difficult to work with: these proteins have unusual solubility, atypical amino acid composition, are heterogenous, and have the tendency to aggregate (Bietz, 1985a). Due to these difficulties, many of the exciting techniques are unsuccessful or unsatisfactory.

A constant need for improved methods is required for the complete study of the proteome. The ability to identify the multitude of polypeptides synthesised, as result of gene expression, will help us to utilize the genetic information (Skylas *et al.*, 2005). Proteins can serve as markers for particular genes since it is the product of structural genes. Thus, from the proteins considerable information can be obtained about the chromosomes and the genome as a whole (Cánovas *et al.*, 2004).

2.7.1 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

One of the techniques used to determine protein composition is gel electrophoresis, which separates proteins in a polymer matrix on the basis of their apparent size, charge, and pH. The SDS surfactant in SDS-PAGE binds to the denatured proteins, countermanding any intrinsic charges that exsists, providing a uniform charge. This allows the proteins to separate based on their mass (Görg *et al.,* 2000). The replacement of starch with polyacrylamide has made the formation of more reproducible gels with a wider variation in molecular sieving of proteins possible (Lookhart and Wrigley, 1995). However, some of the HMW-GS, with distinct functionality, appear to have the same mobility. This results in the incorrect identification of fragments, e.g. 7 and 7*, and 8 and 8* have the same electrophoretic mobility (Butow *et al*., 2004).

Another restriction of SDS-PAGE is that it can only be used on grain material, consequently, breeding selections can only be made after harvest (Lei *et al*., 2006). The banding patterns of proteins, as obtained from the electropherograms show only genotypic variations, so the environmental factors can be excluded to a large extent.

2.7.2 Size-exclusion high-performance liquid chromatography (SE-HPLC)

SE-HPLC is based on the principle of restricted molecular diffusion in the gel depending on the porosity. The larger aggregates are not able to diffuse into the pores and thus are eluted at void volume. The smaller molecules penetrate differentially into the porous stationary phase and get retarded. Proteins are fractionated based on their Stokes radii or hydrodynamic volumes, making it possible to determine the molecular sizes (Bietz, 1984a). This technique is also ideal for quantitation of protein fractions.

SE-HPLC separates proteins in four major classes or fractions namely, the high molecular weight glutenins, low molecular weight glutenins, gliadins and albumins/globulins (Larroque *et al*., 1997). This separation can be achieved within 20-30 minutes and analysis of the resulting curve is simple (Autran, 1994).

Another advantage of SE-HPLC is that it has the potential of keeping large aggregates in a quasi-native state (no disruption of S-S bonds), which allows the examination of stability of protein complexes, interactive aspects, and structure of unreduced aggregates (Autran, 1994). Due to this complexity and thus insolubility of endosperm proteins, one of the major problems in the past has been accomplishing complete protein extraction, without altering their chemical state.

This problem was resolved by the introduction of sonication. Singh *et al*. (1990a) found that using an ultrasonic probe solubilizes total protein from small flour samples. This method allows complete extraction of proteins with loss of only the very large glutenin polymers, because they require very little energy to degrade. They found that after sonication, a strong correlation existed between the proportion of main peaks and absolute areas and the percentage of protein recovered as determined by Kjeldahl N.

SE-HPLC was not used for quality prediction, until recent years when the technology became more equipped. An increase in the concentration of high molecular weight proteins are correlated with improved quality in wheat. Some results showed that a correlation existed between dough mixing time and the amount of HMW-GS present, or the ratio of polymeric to monomeric proteins, indicating possible use in breeding (Huebner and Bietz, 1985).

Initially the instability and poor control of extractions rendered inconclusive results of correlations between different fractions and quality characteristics. Dachkevitch and Autran (1989) did an extended study and attempted to

overcome the instability. Results obtained from this study showed a negative relationship between the proportion of peak 1 and baking strength. It was further demonstrated that the percentage of peak 1 and the ratio of peak1/peak 2 had good discriminative value, and may be a more reliable method used in breeding programmes. MacRitchie *et al*. (1989) found that dough mixing time was correlated with the amounts of HMW-glutenin and that correlations existed between the ratio of polymeric to monomeric proteins and mixing time.

SE-HPLC also furthermore to be a useful tool in studying the influences of changes in agro-climatic conditions on quality. Scheromm *et al*. (1992) found that the amount of protein aggregates remained stable, even though nitrogen levels changed. The opposite was apparent for other cultivars, indicating that SE-HPLC has the potential to evaluate the stability quality in response to environmental changes.

2.7.3 Reversed-phase high-performance liquid chromatography (RP-HPLC)

Unlike SE-HPLC and SDS-PAGE, which separates proteins based on molecular weight and charge, reversed-phase HPLC fractionates based on the protein hydrophobicities. This technique has proven to be a highly efficient tool for qualitative and quantitative studies (Wieser *et al*., 1994). The sensitivity of the technique makes it suitable for use on single kernels, giving it the potential of non-destructive analysis. It has high reproducibility and has the additional advantage of being automated (Bietz, 1990). Vast amounts of data can be generated, making visual analysis very difficult.

Fractionation occurs due to differences in protein surface hydrophobicity. Wide pore columns are necessary and a gradient of water and acetonitrile are usually required. Eluted proteins are usually detected at 210 nm, since this gives good detection sensitivity (Burke *et al*., 1991)

The technique holds the potential for both varietal identification and quality prediction (Marchylo *et al*., 1988). Unfortunately it requires prefractination of proteins into glutenin and gliadins (Heubner and Bietz, 1985). Initially it was thought that environmental influences could affect and thus complicate the use for varietal identification, but a study done by Kruger and Marchylo (1985) indicated only quantitative changes due to environment. Blumenthal *et al*. (1990) found that environmental conditions, especially severe stresses could influence the gene expression of gliadins, resulting in different proteins being produced. However, normally environmental influences appear to be small and do not influence RP-HPLC, severely.

Many peaks identified by RP-HPLC have been correlated to baking quality. Studies by Sutton *et al*. (1989) found two HMW-GS peaks correlating to loaf volume. One of the major contributions to protein studies by RP-HPLC was the fractionation of subunits with similar SDS-PAGE mobilities, as was the case with subunits 7 (7 and 7*) and subunit 8 (8 and 8*) (Sutton, 1991, Butow *et al*., 2004). The amount of HMW-GS was related to dough extensibility and bread and pastry water absorption (Hay, 1993).

Some low molecular weight subunit peaks have been identified and linked to bread-making quality (Gupta *et al*., 1991). LMW-GS have higher surface hydrophobicity than HMW-GS, but similar hydrophobicity to that of gliadins. Unfortunately the application of RP-HPLC in studying LMW-GS was inhibited by the homologous nature and the number of these subunits. Improving separation of both HMW and LMW gluten subunits will allow the use of this technique in predicting and breeding for quality. Not only in determining specific composition of proteins, but also the quantitative ratio of LMW and HMW gluten subunits can be a useful predictor. Increased HMW/LMW and glutenin/gliadin ratio led to a decrease in wet gluten content SDS sedimentation and extensibility, and an increase in mixing time and dough strength (Rakszegi *et al*., 2005).

Research not only confirmed the importance of the HMW/LMW ratio but results also indicated the importance of the glutenin/gliadin ratio. Gliadins have most extensively been used for cultivar identification. Van Lonkhuijsen *et al*. (1992) found that differences in loaf volume of bread varieties with the same HMW-GS composition could be explained by differences in gliadins, indicating a possible use of gliadins in quality prediction.

Determining of definite roles of each constituent is complicated by the linkage that exists between certain gliadin and LMW-glutenin subunits (Lafiandra *et al*., 1994). RP-HPLC has proven itself as an invaluable tool in studying the interactions between the different proteins with each other and with other constituents of wheat grain. It can monitor changes in structure during processing, or environmental changes.

RP-HPLC possess the potential of identifying specific proteins related to quality. These proteins can then act as markers. This is an invaluable tool in a breeding programme and can be used to screen early generations.

Although progress has been made, our understanding of the endosperm proteins still needs to be improved. There remains a need for the continuous improvement of methods of separation and analysis.

One of the major challenges of wheat breeding today is to improve not only agronomical traits, but also to improve quality. In the past the selection of breeding lines relied on direct measurements of the quality traits of interest. SE- and RP-HPLC can be used to establish correlations between protein subunits and quality parameters, resulting in an tool of indirect quality prediction that can be used in breeding programmes.

2.8 References

American Association of Cereal Chemists (AACC), 1995. Approved methods of the American Association of Cereal Chemists Inc., St Paul, Minnesota, USA.

Autran, J.-C., 1994. Size-exclusion high-performance liquid chromatography for rapid examination of size differences of cereal proteins. In: High-Performance Liquid Chromatography of Cereal and Legume Proteins. J.E. Kruger and J.A. Bietz (Eds.), American Association of Cereal Chemists, St. Paul, Minnesota, USA, pp 326-372.

Atwell, W.A., 2001. Wheat Flour. Eagan Press Handbook Series. St. Paul, Minnesota, USA.

Bass, E.J., 1988. Wheat flour milling; In: Wheat Chemistry and Technology. Y. Pomeranz (Ed.), Second edition, Minnesota, USA, pp 1-68.

Belton, P.S., 1999. On the elasticity of wheat gluten. *Journal of Cereal Science* 29:103-107.

Bettge, A., Rubenthaler, G.L. and Pomeranz, Y., 1989. Alveograph algorithms to predict functional properties of wheat in bread and cookie baking. *Cereal Chemistry* 66:81-86.

Bietz, J.A., 1984a. Analysis of wheat gluten proteins by reversed-phase high-performance liquid chromatography, part I. *Bakers Dig*est 58(1):15-32.

Bietz, J.A., 1985a. Analysis of wheat gluten proteins by reversed-phase high-performance liquid chromatography: techniques and applications. In: Gluten Proteins. A. Graveland and J.H.E. Moonen (Eds.), TNO, Wageningen, p 1.

Bietz, J.A., 1990. HPLC of cereal endosperm storage proteins. In: HPLC of Biological Macromolecules-Methods and Applications. K.M. Gooding and F.E. Regnier (Eds.), Marcel Dekker, New York, pp 429-455.

Bietz, J.A. and Rothus, J.A., 1970. Comparison of peptides from wheat gliadin and glutenin. *Cereal Chemistry* 47:381-392.

Bietz, J.A. and Wall, J.S., 1972. Wheat glutenin subunits: molecular weights determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis. *Cereal Chemistry* 49:416-430.

Blackman, J.A. and Payne, P.I., 1987. Grain quality. In: Wheat Breeding, Its Scientific Basis. F. G. H. Lupton (Ed.), Chapman and Hall Ltd, University Press, Cambridge, Great Britain, pp 455-485.

Blumenthal, C.S., Batey, I.L., Bekes, F., Wrigley, C.W. and Barlow, E.W.R., 1990. Gliadin genes contain heat-shock elements: Possible relevance to heat-induced changes in grain quality. *Journal of Cereal Science*, 11:185-187.

Branlard, G.P., Dardevet, M., Saccomano, R., Lagoutte, F., and Gourdon, J., 2001. Genetic diversity of wheat storage proteins and bread wheat quality. *Euphytica* 119:59-67.

Branlard, G.P., and Metakovsky, E.V., 2006. Some Gli alleles related to common wheat dough quality. In: Gliadin and Glutenin: The unique balance of wheat quality. C. Wrigley, F. Békés and W. Bushuk (Eds.), American Association of Cereal Chemists, St. Paul, Minnesota, USA, pp115-139.

Bulman, P. and Hunt, I.A., 1988. Relationships among tillering, spike number and grain yield in winter wheat (*Triticum aestivum* L) in Ontario. *Canadian Journal of Plant Science* 68:583-596.

Burke, T.W.L., Mant, C.T. and Hodges, R.S., 1991. The effect of varying flow-rate, gradient-rate, and detection wavelength on peptide elution profiles in reversed-phase chromatography. In: C.T. Mant and R.S. Hodges (Eds.), High-performance Liquid Chromatography of Peptides and Proteins: Separation, Analysis, and Conformation. CRC Press, Boca Raton, FL, pp 307-317.

Butow, B.J., Gale, K.R., Ikea, J., Juhász, A., Bedö, Z., Tamás, L. and Gianibelli, M.C., 2004. Dissemination of the highly expressed Bx7 glutenin subunit (*Glu-B1al* allele) in what as revealed by novel PCR markers and RP-HPLC. *Theoretical and Applied Genetics* 109:1525-1535.

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 Chemistry* 64:293-299.

Cánovas, F.M., Dumas-Gaudot, E., Recorbet, G., Jorrin, J., Mock, H.-P. and Rossignol, M., 2004.

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

DuCros, D.L. 1987. Glutenin proteins and gluten strength in durum wheat. *Journal of Cereal Science* 5:3

Dunn, M.J., 2000. From Genome to Proteome. Wiley, London.

Eliasson, A. and Larsson, K., 1993. Cereals in breadmaking, a molecular colloidal approach. Marcal Dekker, Inc. New York.

Finney, K.F., Yamazaki, W.T., Young, V.L. and Rubenthaler, 1987. Quality of hard, soft and durum wheats. In: Wheat and wheat improvement. E.G. Heyne (Ed.), Madison, Wisconsin, USA.

Fowler, D.B. and De la Roche, I.A.,1975. Wheat quality evaluation. 3. Influence of genotype and environment. *Canadian Journal of Plant Science* 55:263 – 269.

Gianibelli, M.C., Larroque, O.R., MacRitchie, F. and Wrigley, C.W., 2001. Biochemical, genetic, and molecular characterization of wheat endosperm proteins. Online review American Association of Cereal Chemists, pp1-20.

Görg, A., Obermaier, C., Boguth, G., Harder, A., Scheibe, B., Wildgruber, R and Weiss, W., 2000. The current state of two-dimensional electrophoresis with immobilised pH gradients. *Electrophoresis* 21:1037-1053.

Gupta, R.B. and Shepherd, K.W., 1988. Low molecular weight glutenin subunits in wheat: their variation, inheritance and association with bread-making quality. In: Proceedings of the Seventh International wheat Genetic Symposium, Volume 2, pp 943-949.

Gupta, R.B. and Shepherd, K.W., 1990. Two-step one-dimensional SDS-PAGE analysis of LMW Subunits of glutelin. 1. Variation and genetic control of the subunits in hexaploid wheats. *Theoretical and Applied Genetics* 80:65-74.

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

Gupta, R.B., Bekes, F. and Wrigley, C.W., 1991. Predicting values of LMW glutenin alleles for dough quality of bread wheat. In: Gluten Proteins 1990. W. Bushuk and R. Tkachuk (Eds.), 4th ed. American Association of Cereal Chemists, St. Paul, MN, pp 615-621.

Graybosch, R.A, Peterson, C.J., Shelton, R.D. and Baenziger, P.S., 1996. Genotypic and environmental modification of wheat flour protein composition in relation to end-use quality. *Crop Science* 36:296-300.

Hagberg, S., 1960. A rapid method for determining *alpha-amylase* activity. *Cereal Chemistry* 37:218-222.

Halverson, J. and Zeleny, L., 1988. Criteria of wheat quality. In: Wheat Chemistry and Technology. Y. Pomeranz (Ed.), Second edition, Minnesota, USA, pp 15-45.

Hamauzu, Z.I., Arakawa, T. and Yonezawa, D., 1972. Molecular weights of glutenin and gliadin polypeptides estimated by SDS polyacrylamide gel electrophoresis. *Agricultural and Biological Chemistry* 10:1829-1830.

Hay, R.L., 1993. Effect of flour quality characteristics on puff pastry baking performance. *Cereal Chemistry* 70:392

Huebner, F.R. and Bietz, J.A., 1985. Detection of quality differences among wheats by high-performance liquid chromatography. *Journal of Chromatography* 327:333-342.

Jackson, E.A., Holt, L.M. and Payne, P.I., 1983. Characterisation of high molecular weight gliadin and low molecular weight glutenin subunits of wheat endosperm by two-dimensional electrophoresis and the chromosomal localisation of their controlling genes. *Theoretical and Applied Genetics* 66:29-37.

Jalaluddin, M.D. and Harrison, S.A., 1989. Heritability, genetic correlation and genotype × environment Interactions of soft red winter wheat yield and test weight. *Cereal Research Communications* 17:43-49.

Kaldy, M.S. and Rubenthaler, G.L., 1987. Milling, baking and physical-chemical properties of selected soft white winter and spring wheats. *Cereal Chemistry* 64:302-307.

Kasarda, D.D., 1989. Glutenin structure in relation to wheat quality. In: Wheat is Unique. Y. Pomeranz (Ed.), AACC, St Paul, MN, pp 277-302.

Khelifi, D., Branlard, G. and Bourgoin-Greneche, M., 1992. Division of some D-zone omega gliadins of bread wheats as revealed by two-step A-PAGE/SDS-PAGE technique. *Journal of Genetics and Breeding* 46:351-358.

Koekemoer, F.P., 1997. Important bread wheat quality characteristics affecting the producer and processing industry. Wheat Farmer's day 1997, ARC-Small Grain Institute, Bethlehem.

Koekemoer, F.P., Labuschagne, M.T. and Van Deventer, C.S., 1999. A selection strategy for combining high grain yield and high protein content in South African wheat cultivars. *Cereal Research Communication* 27:107-114.

Konarev, V.G., Gavrilyuk, I.P., Gubaeva, N.K. and Peneva, T.I., 1979. Seed proteins in genome analysis, cultivar identification and documentation of cereal genetic resources: A review. *Cereal Chemistry* 56:272-278.

Kruger J.E. and Marchylo, B.A., 1985. Selection of column and operating conditions for reversed-phase high-performance liquid chromatography of poteins in Canadian wheat. *Canadian Journal of Plant Science* 65: 285-298.

Lafiandra, D., Porceddu, E., Colaprico, G. and Margiotta, B., 1994. Combined reversed-phase high-performance liquid chromatography (RP-HPLC) and electrophoretic techniques in genetics and breeding of wheat storage proteins. In: High-Performance Liquid Chromatography of Cereal and Legume Proteins. J.E. Kruger and J.A. Bietz (Eds.), American Association of Cereal Chemists, St.Paul, MN, pp 273-325.

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

Lee, Y.-K., Bekes, F., Gras, P., Ciaffi, M., Morrel, M.K. and Appels, R., 1999a. The low-molecular weight glutenin subunit proteins of primitive wheats IV. Functional properties of products from individual genes. *Theoretical and Applied Genetics* 98:149-155.

Lei, Z.S., Gale, K.R., He, Z.H., Gianibelli, C., Larroque, O., Xia, X.C., Butow, B.J. and Ma, W., 2006. Y-type gene specific markers for enhanced discrimination of high-molecular weight glutenin alleles at the *Glu-B1* Locus in hexaploid wheat. *Journal of Cereal Science* 43:94-101.

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 Chemistry* 69: 508-515.

Lookhart, G.L. and Wrigley, C.W., 1995. Variety identification by electrophoretic analysis. In: Identification of food grain varieties. C.W. Wrigley (Ed.), American Association of Cereal Chemists., pp 55-71.

Lorenzo, A. and Kronstad, W.E., 1987. Reliability of two laboratory techniques to predict wheat protein quality in non-traditional growing areas. *Crop Science* 27:247-252.

Lukow, O.M., Payne, P.I. and Tkachuk, R., 1989. The HMW glutenin subunit composition of Canadian wheat cultivars and their association with bread-making quality. *Journal of the Science Food and Agriculture* 46:451-460.

MacRitchie, F., 1984. Baking quality of wheat flours. *Advances in Food Research* 29:201-277.

MacRitchie, F. 1994. Role of polymeric proteins in flour functionality. In: Wheat kernel proteins: molecular and functional aspects, Bitervo, Italy, Universita degli studi della Tuscia, pp 145-150.

MacRitchie, F., Du Cros, D.L., and Wrigley, C.W., 1990. Flour polypeptides related to wheat quality. In: Advances in Cereal Science and Technology, Vol. 10. Y. Pomeranz (Ed.), American Association of Cereal Chemists, St. Paul, MN, pp 79-145.

MacRitchie, F., Gupta, R.B. and Wrigley, C.W., 1989. Chemical aspects of wheat proteins related to breadmaking potential. In: Wheat End-Use Properties, Proceedings from ICC' 89. H. Salovaara (Ed.), University of Helsinkie, Lahti, Finland

Mailhot, W.C. and Patton, J.C., 1988. Criteria of flour quality. In: Wheat Chemistry and Technology. Y. Pomeranz (Ed.), Second edition, Minnesota, USA, pp 69-90.

Mamuya, I.N., 2000. Genotype x environment interaction for quality parameters of irrigated spring wheat. MSc. Agricultural Thesis, University of the Free State.

Marchylo, B.A., Hatcher, D.W. and Kruger, J.E., 1988. Identification of wheat cultivars by reversed-phase high-performance liquid chromatography of storage proteins. *Cereal Chemistry* 65: 28-40.

Masci, S.M., Porceddu, E., Colaprico, G. and Lafiandra, D., 1991. Comparison of the B and D subunits of glutenin encoded at the *Glu-D3* locus in two biotypes of the common wheat cultivar Newton with different technological characteristics. *Journal of Cereal Science* 14:35-46.

Metakovski, E.V., 1991. Gliadin allele identification in common wheat II. Cataloque of gliadin alleles in common wheat. *Journal Genetical Breeding* 45:325-344.

Mosleth, E. and Uhlen, A.K., 1990. Associations between the composition of gliadin and HMW glutenin subunits and the gluen quality in wheat (*T. aestivum* L.). In: Gluten Proteins. W. Bushuk and R. Tkachuk (Eds.), AACC. St. Paul, Minnesota, pp112-128.

Osborne, T.B., 1907. The proteins of the wheat kernel. Carnegie Institute. Washington D.C. Publication no. 84.

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

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

Payne, P.I., Jackson, E.A., Holt, L.M. and Law, C.N., 1984. Genetic linkage between endosperm storage protein genes on each of the short arms of chromosome 1A and 1B in wheat. *Theoretical and Applied Genetics* 67: 235-243.

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

Peterson, C.J., Graybosch, R.A., Baenziger, P.S. and Grombacher, A.W., 1992. Genotype and environmental effects on quality characteristics of hard winter wheat. *Crop Science* 32:98-103.

Poehlman, J.M. and Sleper, D.A., 1995. Breeding Field Crops. 4th ed. Iowa State University Press. pp. 259–262, 274-277.

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. *Journal of Cereal Science* 11:15-34.

Pomeranz, Y., 1988. Composition and functionality of wheat flour components. In: Wheat Chemistry and Technology. Y. Pomeranz (Ed.), second edition, Minnesota USA, pp 219-370.

Rakszegi, M., Békés, F., Láng, L., Tamás, L., Shewry, P.R. and Bedö, Z., 2005. Technological quality of transgenic wheat expressing an increased amount of a HMW glutenin subunit. *Journal of Cereal Science* 42:15-23.

Randall, P.G., Manley, M., McGill, A.E.J. and Taylor, J.R.N., 1993. Relationships between the high Mr subunits of glutenin of South African wheats and end-use quality. *Journal of Cereal Science* 18:251-258.

Rodriguez-Quijano, M. and Carrillo, J.M., 1996. Linkage maps of prolamin loci Gli-D4 and Gli-D5 in hexaploid wheat. *Plant Breeding* 115:189-191.

Schepers, J., Keizer, L.C.P. and Kolster, P., 1993. The relation between high molecular weight glutenin subunits, bread-making quality and agronomic properties of winter wheat. *Cereal Research Communication* 21:289-237

Scheromm, P., Martin, G., Bergoin, A. and Autran, J.C. 1992. Influence of nitrogen fertilization on the potential bread-baking quality of two wheat cultivars differing in their responses to increasing nitrogen supplies. *Cereal Chemistry* 69:664-670.

Shewry, P.R. and Tatham, A.S., 1990. Review article: The prolamin storage proteins of cereal seeds: Structure and evolution. *Biochemical Journal* 167:1-12.

Shewry, P.R. and Tatham, A.S, 1997. Disulphide bonds in wheat gluten proteins. *Journal of Cereal Science.* 25:207-227.

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

Singh, N.K., Donovan, G.R., Batey, I.L. and MaxRitchie, F., 1990a. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. I. Dissolution of total proteins in the absence of reducing agents. *Cereal Chemistry* 67:150-161.

Singh, N.K., Shepherd, K.W., Gupta, R.B., Moss, H.J. and MacRitchie, F., 1991b. Proportion of glutenin in the total flour protein as a measure of dough strength. In: W. Bushuk and R. Tkachuk (Eds.), Gluten proteins, American Association of Cereal Chemists, St. Paul, Minnesota, USA, pp 129-144.

Sissons, M.J., Bekes, F. and Skerrit, J.H., 1998. Isolation and functionality testing of low molecular weight glutenin subunits. *Cereal Chemistry* 75:30-36.

Skylas, D.J., Van Dyk, D. and Wrigley, C.W., 2005. Review: Proteomics of wheat grain. Journal of Cereal Science 41:165-179.

Souza, E., Tyler J.M., Kephart, K.D. and Kruk, M., 1993. Genetic improvement of milling and baking quality of hard red spring wheat cultivars. *Cereal Chemistry* 70:280-285.

Sozinov, A.A. and Poperellya, F.A., 1980. Genetic classification of prolamines and its use for plant breeding. *Ann. Technol. Agric*. 29(2):229-245.

Stenvert, N.L. and Kingswood, K., 1977. The influence of the physical structure of the protein matrix on wheat hardness. *Journal of the Science of Food and Agricultural* 28:11-19

Sutton, K.H., 1991. Qualitative and quantitative variation among high molecular weight subunits of glutenin detected by reverse-phase high-performance liquid chromatography. *Journal of Cereal Scienc* 14:25-34.

Sutton, K.H., Hay, R.L. and Griffin, W.B., 1989. Assessment of the potential bread baking quality of New Zealand wheats by RP-HPLC of glutenins. *Journal of Cereal Science* 10:113-121.

Tanaka, K. and Bushuk, W., 1973. Effect of protein content and wheat variety on solubility and electrophoretic properties of flour proteins. *Cereal Chemistry* 49:247.

Tatham, A.S., Miflin, B.J. and Shewry, P.R., 1985. The beta-turn conformation in wheat gluten proteins: Relationship to gluten elasticity. *Cereal Chemistry* 62:405-412.

Van Lill, D., Purchase, J.L., Smith, M.F., Agenbag, G.A and De Villiers, O.T., 1995. Multivariate assessment of environmental effects on hard red winter wheat. 1. Principal components analysis on yield and breadmaking characteristics. *South African Journal of Plant and Soil* 12:158-163.

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

Wieser, H., Seilmeier, W. and Belitz, H.-D., 1994. Use of RP-HPLC for a better understanding of the structure and functionality of wheat gluten proteins. In:

High-Performance Liquid Chromatography of Cereal and Legume Proteins. J.E. Kruger and J.A. Bietz (Eds.), American Association of Cereal Chemists, St.Paul, MN, pp 235-272.

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

Wrigley, C.W., 1982. The use of genetics in understanding protein composition and grain quality in wheat. *Qualitas Plant for Foods Human Nutrition* 31:205-227.

Wrigley, C.W., 1992. Identification of cereal varieties by gel electrophoresis of the grain proteins. In: Modern methods of plant analysis new series, volume 14. Seed analysis. H.F. Linskens and J.F. Jackson (Eds.), Springer-Verlag, Berlin Heidelberg, pp 17-41.

Zhen, Z. and Mares, D.J., 1991. A simple extraction and one step SDS-PAGE system for separating HMW and LMW glutenin subunits of wheat and high molecular weight proteins of rye. In: Cereals International. D.J. Martin and C.W. Wrigley (Eds.), Royal Parade, Parkville, Australia, pp 324-325.

Chapter 3

Prediction of baking quality in Ethiopian bread wheat by size-exclusion high-performance liquid chromatography

3.1 Abstract

It has long since been accepted that breadmaking quality of wheat flour is primarily determined by its proteins. Hence, considerable effort has been made to elucidate which protein constituents are responsible for quality differences. SE-HPLC is a powerful tool to study native protein aggregates and physicochemical basis of baking strength, and allows the rapid assessment of baking quality of wheat genotypes in breeding programmes. In this study 13 Ethiopian cultivars/lines and two South African bread wheat cultivars were compared in two diverse environments. This was done to assess the effect of SE-HPLC determined storage proteins on the breadmaking quality. It was found that across environments, protein fractions had a major influence on quality. The amount of polymeric proteins in bread wheat was significantly higher in the high protein environment. Both the SDS soluble and insoluble polymeric proteins significantly influenced important quality characteristics. An increase in the polymeric-to-monomeric protein ratio led to improvement of quality characteristics. Despite a large environmental effect on all fractions, a large polymeric-to-monomeric protein ratio can be an effective measure of baking quality tests, especially in developing countries where quality-testing facilities are often not available.

3.2 Introduction

Wheat is one of the world's leading cereal crops: It is grown on over 200 million hectares and yields almost 600 million tons annually (Marshall *et al*., 2001). Ethiopia is one of the largest producers of wheat in sub-Saharan Africa today (Hailu, 1991).

The reason for the importance of wheat is the wide diversity of end-use products. Wheat alone has the unique rheological properties required for dough formation. This characteristic is primarily influenced by the storage proteins of the grain (Gianibelli *et al*., 2001). Although the protein composition and subunits are genetically determined, the environment influences the concentration and size distribution of these proteins (Zhu and Khan, 2001). Protein content and distribution has been correlated with rheological properties of wheat (Gupta *et al*., 1993). Increasing the protein or improving the protein quality will help to improve the baking quality.

The effect of different protein fractions on quality created the need to understand and quantify the contributions. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and reversed-phase high-performance liquid chromatography (RP-HPLC) became widely used. However, it caused a reduction of S-S bonds and resulted in a loss of information (Autran, 1994).

Size-exclusion HPLC (SE-HPLC) is a valuable tool for measuring the proportion of main endosperm proteins. The methodology accurately separates the three main classes of endosperm proteins: glutenins (polymeric proteins), gliadins, and albumins and globulins (monomeric proteins). This is particularly important because the relationship between protein classes (e.g. glutenin-to-gliadin ratio) and the molecular size distribution of polymeric proteins affect quality attributes.

The aim of this study was to determine the effect of SDS soluble and SDS insoluble proteins from SE-HPLC on quality characteristics in wheat types in two environments in Ethiopia.

3.3 Material and Methods

3.3.1 Plant materials

Thirteen popularly grown Ethiopia cultivars/lines and two South African cultivars of known quality were used in this study (Table 3.1). The main criterion of selection for the Ethiopian material was agronomic performance. Trials were grown at two environments in Ethiopia in 2001, namely: Adet Research Center, which is a higher protein potential area, and Motta, which is a low protein potential area. A randomised complete block (RCB) design with three replications was used. The plot size was 2.5 m^2 (six rows of 2.5 m length and 20 cm spacing between the rows) at both localities. All recommended wheat management practices were exercised.

Table 3.1 Entries of bread wheat cultivars/lines included in this study

Entry	Cultivar	Country
1	HAR2457	Ethiopia
2	HAR2348	Ethiopia
3	HAR2807	Ethiopia
4	HAR2096	Ethiopia
5	HAR2562	Ethiopia
6	ET13A2	Ethiopia
7	HAR1709	Ethiopia
8	HAR1685	Ethiopia
9	HAR604	Ethiopia
10	HAR1522	Ethiopia
11	HAR1775	Ethiopia
12	HAR1868	Ethiopia
13	HAR2505	Ethiopia
14	Kariega	South Africa
15	SST825	South Africa

After harvesting, the material was transported to South Africa and yield and quality trait data was measured in triplicate at the laboratories of the Agriculteral Research Centre-Small Grains Institute (ARC-SGI), Bethlehem, South Africa (Table 3.2).

Table 3.2 Methods, units and abbreviations of quality traits measurements

Traits	**Abbreviations**	**Unit**	**Method of measurements**
Hectoliter mass	HLW	$kg.hl^{-1}$	
Breakflour yield	BFY	%	AACC 26-21A
Flour yield	FLY	%	AACC 26-21A
Flour colour	FCL		Kent Jones,C76
Flour protein content	FPC	%	AACC 39-11
SDS-sedimentation test	SDSS	ml	AACC 56-70
Vitreous kernels	VK	%	
SKCS-hardness index	SKHI		AACC 53-31
SKCS-seed diameter	SKDM	mm	AACC 53-31
SKCS-seed weight	SKWT	mg	AACC 53-31
Mixograph development time	MDT	min	AACC 54-40A
Farinograph water absorption	FABS	%	AACC 54-21
Alveograph P/L ratio	P/L		AACC 54-30A
Alveograph strength	W	cm^2	AACC 54-30A
Loaf volume	LFV	cm^3	AACC 10-09

SKCS, Single Kernel Characteristic System; AACC, American Association of Cereal Chemists (2000)

3.3.2 Electrophoresis

SDS-PAGE was performed using the adapted procedure of Singh *et al*. (1991). The advantage of this method is that HMW-GS and LMW-GS can be read from the same gel. Six seeds were sampled for each genotype and crushed to a fine powder. Gliadins were extracted with 70% ethanol, and subsequently removed by adding 50% 1-n-propanol. This avoids gliadin contamination of the glutenins. Glutenins were extracted and reduced in extraction buffer containing [1.25% (w/v) DTT (80 mM Tris-HCl, 90ml 50% n-propanol pH 8.0)] and the protein was alkylated by adding 16.8 µl/ml 4-vinyl pyridine. After centrifugation (2 min at 10 000 rpm) the supernatant was transferred to a new tube containing sample buffer (80 mM Tris-HCl (pH8), 40% glycerol, 2% SDS and 0.02% Bromophenol blue) and incubated at 60°C for 15 min. Electrophoresis was performed with a 10% polyacrylamide separating gel (30:1 acrylamide:bis-acrylamide, 10x running buffer (0.25M Tris, 2M glysine,1% (w/v) SDS). The gel was fixed in fixing solution (40% methanol, 10% glacial acetic acid) and then stained (15% (w/v) trichloroacetic acid, 0.01% (w/v) coomassie blue and 5% methanol).

3.3.3 Protein extraction and SE-HPLC

Proteins were extracted from wheat flour with a two-step extraction procedure developed by Gupta and colleagues (1993). The first step extracts the proteins soluble in dilute SDS, while the second extract contains proteins soluble only after sonication.

For the first extraction 17 mg of white flour was suspended in 1.5 ml of 0.5% (w/v) SDS-phosphate buffer (pH 6.9) and vortexed for 10 s. Samples were stirred for 5 min at 2 000 rpm and centrifuged for 30 min at 10 000 rpm to obtain the supernatant protein. The supernatant was filtered through a 0.45 µm filter (Millipore, Durapore Membrane Filters) and submitted to SE-HPLC fractionation.

The pellet was subsequently resuspended in SDS buffer as above and sonicated in an ultrasonic desintegrator (Branson B12 sonifier) for 30 s, amplitude 5, fitted with a 3 mm exponential microtip. Samples were centrifuged as above, to obtain a supernatant of "unextractable" proteins. Extracts were filtered through 0.45 µm filters (Millipore, Durapore Membrane Filters) before running on HPLC.

Size-exclusion HPLC analyses were performed on a System Gold HPLC system (Beckman Instruments Inc., Fullerton, CA, USA) using a BIOSEP SEC-4000 Phenomenex column. Separation was achieved in 30 min by loading 20 µl of sample into an eluant of 50% (v/v) acetonitrile and water containing 0.1% (v/v) trifluoroacetic acid at a flow rate of 0.2 ml/min. Proteins were detected by UV absorbance at 210 nm.

Areas of the different peaks were calculated. The percentage of total unextractable polymeric protein in the total polymeric protein [(SDS-insoluble large and smaller protein polymers)/SDS-soluble and insoluble large and smaller protein polymers)] and the percentage of large unextractable polymeric protein in the total large polymeric protein [(SDS-insoluble large protein polymers)/(SDS-soluble and SDS-insoluble large protein polymers)] were calculated according to the method of Gupta *et al*. (1993).

The measured HPLC fractions were: (a) SDS-soluble (b) SDS-insoluble, where A = large polymeric proteins (LPP), B = smaller polymeric proteins (SPP), C = large monomeric proteins (LMP) mainly gliadins and D = smaller monomeric proteins (SMP) mainly albumins and globulins (Appendix A, Figures 1 and 2).

3.3.4 Statistical analysis

All statistical analyses were done with Agrobase (2000) software. Relationships between the SE-HPLC protein fractions and quality characteristics were

investigated by carrying out analysis of variance (ANOVA) and linear correlation coefficients.

SE-HPLC was performed in triplicate for each of the three replicates. The mean of the nine replicates was calculated and used in statistical analysis. This was done to obtain more accurate representations of profiles for each replication.

3.4 Results and discussion

3.4.1 Results

SDS-PAGE HMW-GS banding patterns observed for 15 cultivars/lines at Adet and Motta, Ethiopia

Results are given in Table 3.3.

Different HMW subunit combinations were observed. Please refer to Appendix A (Figure 1.) for SDS-PAGE-results.

HAR2457 and HAR1522 had the same subunit combinations (1, 7+9, 5+10). HAR2096, HAR1709 and HAR1775 had similar subunit combinations (2*, 7+9, 5+10). HAR1685 and HAR1868 had similar combinations with the exception of the absence of the band 9. HAR2562 and HAR604 shared the same banding pattern (2*, 17+18, 5+10) and HAR2348 and ET13A2 had a similar combination (0/2*, 7+8, 2+12). Unique combinations were observed for two Ethiopian entries, (HAR2807, HAR2505) and for the two South African cultivars (Kariega and SST825)

Table 3.3 SDS-PAGEHMW-GS banding patterns observed for 15 cultivars/lines at Adet and Motta, Ethiopia

Entry	Cultivar	HMW-GS
1	HAR2457	1, 7+9, 5+10
2	HAR2348	0/2*, 7+8, 2+12
3	HAR2807	1, 13+16, 2+12
4	HAR2096	2*, 7+9, 5+10
5	HAR2562	2*, 17+18, 5+10
6	ET13A2	0/2*, 7+8, 2+12
7	HAR1709	2*, 7+9, 5+10
8	HAR1685	2*, 7, 5+10
9	HAR604	2*, 17+18, 5+10
10	HAR1522	1, 7+9, 5+10
11	HAR1775	2*, 7+9, 5+10
12	HAR1868	2*, 7, 5+10
13	HAR2505	1, 7, 5+10
14	Kariega	0/2*, 17+18, 2+12
15	SST825	1, 7+8, 5+10

Quality characteristics for 15 cultivars/lines at Adet and Motta, Ethiopia

Results are given in Table 3.4.

SDSS: The SDSS of Kariega was significantly higher than all other entries at both localities. HAR2348 had the lowest SDSS value at both localities.

VK: The VK of SST825, at Adet, was the highest with HAR2562 and HAR1775 ranking second and third, respectively. The VK of HAR2562 was significantly higher than the other entries planted at Motta, except HAR2807. Kariega had the lowest VK value at both localities.

FPC: There was a strong genotype effect at Adet and Motta for FPC. At Adet, HAR2562 had the highest FPC value but not significantly higher than HAR1709, SST825, Kariega and ET13A2. At Motta, HAR2348 ranked first but was not significantly higher than the FPC of HAR2096, HAR2562 and ET13A2.

FLY: The FLY value for ET13A2, at Adet was the highest, with Kariega ranking second. The reverse was true for entries planted at Motta, where Kariega ranked first. Values for the two environments were similar but genotype values differed.

MDT: The MDT for HAR1522 was significantly higher than all other entries. There was a strong genotype effect, and it seems that MDT was largely influenced by the environment. The MDT for SST825, at Motta was significantly higher than that of HAR2505, HAR1685 and Kariega.

SKCS-weight: The SK weight of HAR1522 was significantly lower than for all other entries at both localities, with the exception of HAR1775, at Adet and HAR1775, ET13A2 and HAR1709 at Motta.

SKCS-diameter: There were significant differences among entries for SKCS-diameter. HAR1775 had the lowest value at both localities.

SKCS-hardness index: HAR2348 had significantly the lowest hardness index at Adet and Kariega at Motta.

Table 3.4 Means of measured quality characteristics for 15 cultivars/lines at Adet and Motta, Ethiopia

ENTRY	NAME	LOC	SDSS	VK	FPC	FLY	MDT	WGHT	DIAM	HI
			ml	%	%	%	min	mg	mm	%
1	HAR2457	A	41	69	9.10	59.50	2.60	34.94	2.34	49.84
		M	32	43	7.40	60.60	2.80	34.24	2.37	41.96
2	HAR2348	A	38	63	9.60	61.30	2.00	33.21	2.29	13.67
		M	28	57	8.40	58.90	2.70	33.54	2.37	20.68
3	HAR2807	A	39	75	9.80	61.90	2.00	32.54	2.12	57.37
		M	28	75	7.60	59.80	2.10	34.20	2.23	57.36
4	HAR2096	A	42	66	9.50	60.20	2.50	30.91	2.16	58.66
		M	33	67	8.20	62.00	2.90	30.99	2.20	49.25
5	HAR2562	A	45	76	10.60	62.10	2.90	34.54	2.30	59.30
		M	32	78	7.90	60.30	3.20	35.81	2.44	61.37
6	ET13A2	A	51	69	9.90	63.80	1.80	31.66	2.31	20.56
		M	30	56	7.80	63.40	2.30	29.59	2.18	24.66
7	HAR1709	A	57	75	10.10	62.50	2.40	30.78	2.15	51.73
		M	35	57	6.70	60.40	3.30	29.73	2.17	42.57
8	HAR1685	A	55	62	8.90	59.90	2.70	27.83	2.01	48.11
		M	42	35	6.70	62.20	3.50	32.48	2.25	29.80
9	HAR604	A	70	67	8.90	60.90	3.60	34.51	2.33	49.74
		M	46	51	6.80	59.30	4.90	38.18	2.55	44.51
10	HAR1522	A	52	71	9.90	59.60	4.80	24.52	1.95	64.46
		M	33	62	6.80	58.90	5.50	26.87	2.11	52.70
11	HAR1775	A	47	75	9.60	60.50	4.00	25.50	1.83	63.27
		M	32	58	7.00	60.80	5.30	27.37	2.00	56.40
12	HAR1868	A	58	61	8.70	59.00	2.90	28.43	2.07	51.22
		M	45	40	6.60	61.00	5.40	30.58	2.14	27.80
13	HAR2505	A	55	56	9.60	61.70	2.70	28.57	2.09	46.50
		M	38	42	6.60	61.00	3.70	32.47	2.33	35.66
14	KARIEGA	A	77	53	10.00	63.50	2.50	31.92	2.21	34.59
		M	52	26	7.40	63.80	3.40	34.68	2.40	19.02
15	SST825	A	57	77	10.00	60.60	3.90	28.75	2.18	61.37
		M	42	56	7.10	60.40	5.50	31.93	2.41	45.39
	Average	A	52.30	67.70	9.60	61.10	2.90	30.60	2.20	48.70
		M	36.50	53.50	7.30	60.90	3.80	32.20	2.30	40.60
	LSD (0.05)	A	6.00	11.75	0.73	2.35	0.23	2.38	0.15	5.90
		M	4.61	10.58	0.63	1.74	0.74	3.30	0.18	7.27

LOC = locality SDSS = SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, Wght = SKCS single kernel weight, Diam = SKCS single kernel diameter, HI = SKCS-hardness index

A = Adet, M = Motta

LSD = Least significant difference

Combined averages of quality characteristics for 15 cultivars/lines at Adet and Motta, Ethiopia

Results are given in Table 3.5.

SDSS: The SDSS of Kariega was significantly higher than all other entries. HAR2348 had the lowest SDSS value. This reflects data observed at Motta.

VK: The VK of HAR2562 was significantly higher than other entries, except HAR2807. Kariega had the lowest VK value. This is similar to results obtained for the trial at Motta.

FPC: There was a strong genotype effect for FPC. HAR2562 had the highest FPC value but not significantly higher than HAR2348, HAR2096 and ET13A2.

FLY: The FLY value of Kariega was the highest, with ET13A2 ranking second. The two environments had similar values.

MDT: The MDT of HAR1522 was significantly higher than other entries.

SKCS-weight: The SK weight of HAR1522 was significantly lower than the other entries, with the exception of HAR1775.

SKCS-diameter: There were significant differences among entries for SKCS-diameter. HAR1775 had the lowest value.

SKCS-hardness index: HAR2348 had the lowest hardness index.

Table 3.5 Combined averages of quality characteristics for 15 cultivars/lines at two different localities

ENTRY	NAME	SDSS	VK	FPC	FLY	MDT	WGHT	DIAM	HI
1	HAR2457	37	56	8.30	60.10	2.70	34.59	2.36	45.90
2	HAR2348	33	60	9.00	60.10	2.40	33.37	2.33	17.17
3	HAR2807	34	75	8.70	60.90	2.00	33.37	2.18	57.37
4	HAR2096	38	66	8.90	61.10	2.70	30.95	2.18	53.96
5	HAR2562	39	77	9.20	61.20	3.00	35.18	2.37	60.34
6	ET13A2	41	63	8.90	63.60	2.00	30.63	2.25	22.61
7	HAR1709	46	66	8.40	61.50	2.90	30.26	2.16	47.15
8	HAR1685	49	49	7.80	61.10	3.10	30.16	2.13	38.96
9	HAR604	58	59	7.80	60.10	4.30	36.35	2.44	47.12
10	HAR1522	42	66	8.30	59.30	5.20	25.70	2.03	58.58
11	HAR1775	40	67	8.30	60.70	4.70	26.43	1.92	59.83
12	HAR1868	52	51	7.70	60.00	4.20	29.51	2.11	39.51
13	HAR2505	47	49	8.10	61.40	3.20	30.52	2.21	41.08
14	KARIEGA	65	40	8.70	63.70	3.00	33.30	2.31	26.81
15	SST825	50	66	8.60	60.50	4.70	30.34	2.30	53.38
	Average	44.73	60.67	8.45	61.02	3.34	31.38	2.22	44.65
	LSD (0.05)	2.88	6.02	0.37	1.11	0.30	1.55	0.09	3.57

SDSS=SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, Wght = SKCS single kernel weight, Diam = SKCS single kernel diameter, HI = SKCS-hardness index

LSD = least significant difference

Protein fractions for 15 cultivars/lines at Adet and Motta, Ethiopia

Results are given in Table 3.6.

Please refer to Appendix B for SE-HPLC chromatograms of SDS-soluble proteins (Figure 1.) and SDS-insoluble proteins (Figure 2).

SDS soluble LPP: There were significant differences between entries tested. HAR2457 had the highest fraction of LPP, with HAR1522 and HAR2807 ranking second and third, respectively, at Adet. HAR1775 had the lowest LPP at both Adet and Motta. HAR1709 was significantly higher than HAR2457, HAR2348, HAR2562, HAR1522 and HAR1775, at Motta.

SDS soluble SPP: HAR1775 had the lowest SPP at Adet, significantly lower than the five highest ranking entries. HAR2807 had the highest SPP at Adet, significantly higher than all the other entries except HAR2348. The South African entry SST825, at Motta had the highest SPP content, followed by HAR1685 and HAR1709, with no significant difference. The SPP of SST825 at Motta, was significantly higher than that of HAR1522, HAR2096, and HAR2562.

SDS soluble LMP: There was a strong genotype effect at Adet. The LMP for all the entries was significantly lower than HAR1775 (64.24), except for SST825 and Kariega. HAR1775 at Motta had the highest and HAR604 the lowest LMP values, respectively.

SDS soluble SMP: A strong genotype effect was visible. The South African entries, Kariega and SST825 had the lowest SMP at Adet. SST825 was significantly lower than all the entries except Kariega. HAR1709 had the highest SMP, significantly higher than HAR604, HAR2505 and HAR1775. At Motta HAR2457 had the highest SMP, followed by ET13A2 and HAR2807, respectively. HAR1775 had the lowest SMP.

SDS insoluble LPP: There were significant differences between entries at Adet. SST825 ranked third and Kariega fifth. HAR2562 had the highest LPP, significantly higher than the LPP of HAR1709, HAR1685, HAR1868 and HAR2348. HAR1522 had a significantly lower LPP than these

entries. At Motta HAR2457 and HAR2096 were significantly lower than HAR2505, HAR1775, HAR1868, HAR1685 and Kariega.

SDS insoluble SPP: HAR604 had the highest amount of SPP at both localities. SST825 at Adet had the lowest SPP fraction.

SDS insoluble LMP: HAR2807 had the highest proportion of LMP and HAR604 the lowest at Adet. ET13A2, SST825 and HAR604 had significantly lower values than HAR2807, at Adet. HAR2348 had a significantly higher LMP than ET13A2, Kariega and SST825, at Motta.

SDS insoluble SMP: Kariega had the highest amount of SMP and HAR604 the lowest at Adet. The differences were not significant. HAR1775 had the lowest SMP at Motta.

TUPP: HAR1775, planted at Adet and Motta had the highest TUPP value, but the differences were not significant.

LUPP: HAR1775 had the highest LUPP value, at Motta and the third highest at Adet. The South African cultivars, Kariega and SST825, ranked first and second, respectively.

Table 3.6 Means of measured protein fractions for 15 cultivars/lines at Adet and Motta, Ethiopia

			SDS Soluble				SDS Insoluble					
ENTRY	NAME	LOC	LPP	SPP	LMP	SMP	LPP	SPP	LMP	SMP	TUPP	LUPP
1	HAR2457	A	7.34	16.63	49.00	22.81	21.37	34.33	30.78	6.01	69.80	74.20
		M	3.43	9.10	68.49	15.42	10.28	27.64	37.96	16.86	74.94	74.55
2	HAR2348	A	6.95	18.29	44.97	24.62	18.04	32.74	31.26	12.61	66.84	72.41
		M	3.41	8.87	69.59	13.57	13.23	25.48	42.23	13.47	75.62	77.07
3	HAR2807	A	7.04	23.56	43.58	19.91	16.26	33.08	33.50	10.73	62.02	69.60
		M	4.46	11.69	66.21	14.52	15.76	27.80	37.88	11.94	72.94	77.09
4	HAR2096	A	6.63	15.39	50.95	23.15	20.66	35.16	31.20	6.61	71.73	75.79
		M	3.54	10.07	71.04	12.24	11.31	28.61	38.72	15.23	74.57	75.74
5	HAR2562	A	6.49	15.61	50.86	21.68	22.75	35.50	29.04	8.73	72.58	77.83
		M	3.39	9.75	71.14	12.86	13.35	30.15	36.35	12.06	76.89	78.99
6	ET13A2	A	6.20	16.33	51.74	21.24	17.53	35.19	28.25	10.28	70.04	73.79
		M	4.20	10.87	66.56	14.76	14.70	28.50	35.72	14.19	74.40	76.54
7	HAR1709	A	6.94	16.30	47.45	25.02	19.32	35.83	29.56	12.30	70.35	73.56
		M	4.48	11.87	66.80	13.49	14.06	29.97	34.68	16.10	72.65	73.27
8	HAR1685	A	6.39	16.42	49.60	21.82	18.49	36.77	30.78	8.83	71.09	74.62
		M	4.11	12.22	68.44	10.97	16.67	31.07	35.80	10.16	74.50	79.20

Table 3.6 Continued

ENTRY	NAME	LOC	SDS Soluble				SDS Insoluble				TUPP	LUPP
			LPP	SPP	LMP	SMP	LPP	SPP	LMP	SMP		
9	HAR604	A	6.91	17.88	50.52	19.07	19.73	39.17	26.56	5.32	70.33	73.86
		M	3.71	11.55	65.60	13.99	15.35	33.06	34.70	12.50	76.01	79.73
10	HAR1522	A	7.33	17.03	48.25	24.57	14.66	34.58	33.34	11.37	67.19	67.24
		M	3.32	10.32	70.51	12.96	13.06	30.45	38.77	12.80	76.00	78.71
11	HAR1775	A	3.98	11.75	64.24	18.12	16.95	33.16	33.32	13.15	76.18	81.05
		M	3.31	9.01	72.19	11.73	17.12	29.25	36.83	9.66	79.34	83.80
12	HAR1868	A	5.52	15.58	55.33	20.38	18.24	35.87	30.61	10.90	71.95	76.85
		M	3.63	11.07	68.57	11.18	16.72	31.54	29.89	9.99	76.94	82.48
13	HAR2505	A	5.50	15.64	56.28	18.27	19.87	35.78	30.98	8.67	72.46	78.21
		M	4.04	11.68	71.07	9.13	17.70	27.63	35.76	11.49	74.48	81.12
14	KARIEGA	A	4.56	13.98	61.17	13.09	19.90	34.63	29.04	13.42	74.68	81.28
		M	3.94	11.46	69.55	12.12	16.60	30.37	34.85	12.05	75.32	80.49
15	SST825	A	4.83	13.91	64.07	12.23	20.75	30.38	27.95	6.34	72.94	81.15
		M	3.76	12.39	68.1	12.39	13.32	32.70	34.84	16.11	74.12	77.88
	Average	A	6.2	16.3	52.5	20.4	19.0	34.8	30.4	9.7	70.7	75.4
		M	3.8	10.8	68.9	12.8	14.6	29.6	36.3	13.0	75.2	78.4
	LSD (0.05)	A	1.64	4.59	7.23	5.82	3.33	4.41	4.55	5.44	5.45	5.09
		M	0.94	1.53	3.04	3.09	5.04	3.92	6.45	4.28	3.69	7.44

LPP = large polymeric proteins, SPP = small polymeric proteins, LMP = large monomeric proteins, SMP = small monomeric proteins, TUPP = % total unextractable polymeric proteins, LUPP = % large unextractable polymeric proteins, LSD = least significant difference,

Loc = locality, A = Adet, M = Motta

Combined averages of protein fractions for 15 cultivars/lines at two different localities

Results are given in Table 3.7.

SDS soluble LPP: The value for HAR2807 was significantly higher than HAR2562, HAR2505, HAR1868, SST825, Kariega and HAR1775.

SDS soluble SPP: HAR2807 had the highest SPP content, significantly higher than all other entries, followed by HAR604 and HAR1685. HAR1775 was significantly lower than the other entries.

SDS soluble LMP: HAR1775 had the highest LMP fraction followed by SST825 and Kariega. The LMP value for HAR2807 was the lowest for all entries.

SDS soluble SMP: The two South African cultivars, SST825 and Kariega, had the lowest SMP values.

SDS insoluble LPP: The SDS insoluble LPP of HAR2505 was the highest, followed by Kariega and HAR2562.

SDS insoluble SPP: The SPP of HAR604 was higher than HAR1685, but not significantly. HAR1685 was significantly higher than SST825, HAR1775, HAR2457, HAR2807 and HAR2348.

SDS insoluble LMP: HAR2348 had a significantly higher LMP than ET13A2, Kariega and SST825. The LMP of HAR1868 and HAR604 were the lowest.

SDS insoluble SMP: The SMP of HAR604 was significantly lower than HAR1522, ET13A2, Kariega, HAR2348 and HAR1709.

TUPP: HAR1775 was significantly higher than Kariega and HAR2562 ranking second and third, respectively.

LUPP: HAR1775 had the highest LUPP value, but not significantly higher than that of Kariega, HAR2505, HAR1868 and SST825.

Table 3.7 Combined averages of protein fractions for 15 cultivars/lines at two different localities

		SDS Soluble				SDS Insoluble					
ENTRY	**NAME**	**LPP**	**SPP**	**LMP**	**SMP**	**LPP**	**SPP**	**LMP**	**SMP**	**TUPP**	**LUPP**
1	HAR2457	5.38	12.87	58.74	19.11	15.83	30.99	34.37	11.44	71.95	74.63
2	HAR2348	5.18	13.58	57.28	19.09	15.64	29.11	36.74	13.04	70.46	75.12
3	HAR2807	5.75	17.62	54.90	17.22	16.01	30.44	35.69	11.34	66.53	73.58
4	HAR2096	5.09	12.73	61.00	17.69	15.99	31.88	34.96	10.92	72.87	75.85
5	HAR2562	4.94	12.68	61.00	17.27	18.05	32.82	32.70	10.39	74.27	78.51
6	ET13A2	5.20	13.60	59.15	18.00	16.11	31.85	31.98	12.24	71.84	75.60
7	HAR1709	5.71	14.09	57.13	19.25	16.69	32.90	32.12	14.20	71.47	74.51
8	HAR1685	5.25	14.32	59.02	16.39	17.58	33.92	33.29	9.50	72.46	77.00
9	HAR604	5.31	14.72	58.06	16.53	17.54	36.12	30.63	8.91	72.82	76.76
10	HAR1522	5.33	13.68	59.38	18.77	13.86	32.51	36.05	12.08	70.92	72.23
11	HAR1775	3.65	10.38	68.21	14.93	17.04	31.21	35.08	11.40	77.47	82.36
12	HAR1868	4.58	13.32	61.95	15.78	17.48	33.71	30.25	10.44	74.09	79.24
13	HAR2505	4.77	13.66	63.68	13.70	18.79	31.71	33.37	10.08	73.26	79.75
14	Kariega	4.25	12.72	65.36	12.61	18.25	32.50	31.94	12.74	74.94	81.11
15	SST825	4.30	13.15	66.09	12.31	17.04	31.54	31.39	11.22	73.57	79.85
	Average	4.98	13.54	60.73	16.58	16.79	32.21	33.37	11.33	72.60	77.07
	LSD (0.05)	0.72	1.85	2.99	2.51	2.30	2.25	3.00	2.64	2.51	3.44

SDS = sodium dodecyl sulphate, LPP = large polymeric proteins, SPP = small polymeric proteins, LMP = large monomeric proteins, SMP = small monomeric proteins, TUPP = % total unextractable polymeric proteins, LUPP = % large unextractable polymeric proteins

LSD = Least significant difference

Mean squares of specific protein fractions at the two locations

Results are given in Table 3.8.

There were no significant differences for the eight protein fractions measured at Adet, but at Motta a replication effect was visible for SDS soluble LPP, SPP, LMP and SDS insoluble LPP and LMP. There was a strong genotype effect at Motta for SPP and LMP and at Adet for LPP, LMP, SMP and SDS insoluble LPP.

The genotype effect was seen only for the four SDS soluble fractions, across the two localities. There was a significant locality effect for all eight fractions. The replication x locality effect was significant for SDS soluble LPP, SPP, LMP and for SDS insoluble LPP and LMP. There was also a significant locality x entry effect for SDS soluble LPP and LMP and SDS insoluble LPP and SMP. This indicated that the environment largely influenced the fractions.

Table 3.8 Mean squares of protein fractions at separate locations and across locations

		SDS soluble proteins				SDS insoluble proteins			
Source	**Loc**	**LPP**	**SPP**	**LMP**	**SMP**	**LPP**	**SPP**	**LMP**	**SMP**
Rep	A	3.93	1.65	42.43	15.48	5.13	3.31	1.63	5.98
	M	9.53**	40.65**	112.74**	1.40	318.11**	7.40	391.75**	15.41
Entry	A	3.35*	20.10	124.13**	44.18*	13.32*	12.13	12.57	22.06
	M	0.487	4.28**	12.38*	8.06	14.55	12.76	22.29	15.99
	A+M	2.01*	13.57*	83.04**	31.94**	9.45	16.14	24.67	11.64
Loc	A+M	128.76**	678.59**	6044.88**	1314.84**	426.32**	607.52**	788.66**	243.28**
Rep x Loc	A+M	6.73**	21.15*	77.58**	8.44	161.62**	5.35	196.69**	10.69
Loc x Entry	A+M	1.82*	10.82	53.48**	20.30	18.41*	8.75	10.19	26.40*

SDS = sodium dodecyl sulphate, LPP = large polymeric proteins, SPP = small polymeric proteins, LMP = large monomeric proteins, SMP = small monomeric proteins, TUPP = % total unextractable polymeric proteins, LUPP = % large unextractable polymeric proteins, LSD = Least significant difference

LOC = locality, Rep = replication, A = Adet, M = Motta

* $p \leq 0.05$ ** $p \leq 0.01$

Significant correlations between specific protein fractions and quality characteristics for Adet and Motta

Results are given in Table 3.9.

Correlations between specific subunits and quality characteristics were limited for Adet. SDS soluble LMP and SMP correlated positively with SDS sedimentation, and LMP also with MDT. The reverse was true for the SDS insoluble LMP fraction that correlated negatively with SDS sedimentation. There was a significantly positive correlation between SDS insoluble SPP, SKCS-weight and diameter.

At Motta, there were more correlations between SDS insoluble fractions and quality than soluble fractions. The SDS soluble SPP correlated negatively with FPC. Similar negative results were obtained for SDS insoluble LPP, SPP and positive with LMP. The SDS insoluble SPP also correlated positively with SDSS.

Table 3.9 Significant correlations between specific protein fractions and quality characteristics for Adet and Motta

Location	Fraction	Characteristic	Correlation
Adet	SDS soluble LMP	SDSS	0.344*
		MDT	0.322*
	SDS soluble SMP	SDSS	-0.426**
	SDS insoluble LPP	SKCS-weight	0.391**
		SKCS-diameter	0.343*
	SDS insoluble LMP	SDSS	-0.354*
Motta	SDS soluble SPP	FPC	-0.425**
	SDS insoluble LPP	FPC	-0.376*
	SDS insoluble SPP	SDSS	0.340*
		FPC	-0.405**
	SDS insoluble LMP	FPC	0.464**

SDS = sodium dodecyl sulphate, LPP = large polymeric proteins, SPP = small polymeric proteins, LMP = large monomeric proteins, SMP = small monomeric proteins, TUPP = % total unextractable polymeric proteins, LUPP = % large unextractable polymeric proteins, LSD = Least significant difference, SDSS = SDS sedimentation, MDT = mixograph development time, FPC = flour protein content, SKCS = single kernel characterization system

* $p \leq 0.05$ ** $p \leq 0.01$

Significant correlations between specific protein fractions and quality characteristics for combined localities

Results are given in Table 3.10.

SDS soluble LPP was highly significantly correlated with SDS sedimentation, vitreous kernels, flour protein content, and negatively with mixograph development time. SPP were correlated with the same characteristics as LPP. The SDS soluble LMP were negatively correlated with SDS sedimentation, vitreous kernels, and flour protein content and positively with mixogram development time. The SMP correlations were similar to that of LPP, with the addition of a negative correlation with SKCS-diameter.

The combined data for the SDS insoluble protein fractions showed that LPP and SPP were highly significantly correlated with SDS sedimentation and flour protein content. SDS insoluble LMP and SMP were negatively correlated with SDS sedimentation and flour protein content. The LMP was also negatively correlated with vitreous kernels.

The total unextractable protein (TUPP) was negatively correlated with SDS sedimentation, vitreous kernels and flour protein content and positively with mixogram development time. LUPP was negatively correlated with vitreous kernels, flour protein content and positively with mixograph development time.

Table 3.10 Significant correlations between specific protein fractions and quality characteristics for combined localities

SDS Soluble proteins			SDS Insoluble proteins		
LPP	SDSS	0.376**	LPP	SDSS	0.364**
	VK	0.364**		FPC	0.303**
	FPC	0.582**			
	MDT	-0.359**	SPP	SDSS	0.532**
				FPC	0.420**
SPP	SDSS	0.411**			
	VK	0.326*	LMP	SDSS	-0.492**
	FPC	0.530**		VK	-0.225*
	MDT	-0.312**		FPC	-0.324**
LMP	SDSS	-0.401*	SMP	SDSS	-0.254*
	VK	-0.401**		FPC	-0.288**
	FPC	-0.674**			
	MDT	0.412**	TUPP	SDSS	-0.222**
				VK	-0.305**
SMP	SDSS	0.218*		FPC	-0.465**
	VK	0.546**		MDT	0.386*
	FPC	0.675**			
	MDT	-0.369**	LUPP	VK	-0.210*
	Diam	-0.236*		FPC	-0.313**
				MDT	0.325**

SDS = sodium dodecyl sulphate, LPP = large polymeric proteins, SPP = small polymeric proteins, LMP = large monomeric proteins, SMP = small monomeric proteins, TUPP = % total unextractable polymeric proteins, LUPP = % large unextractable polymeric proteins, SDSS = SDS sedimentation, MDT = mixograph development time, FPC = flour protein content, SKCS = single kernel characterization system, VK = vitreous kernels, Diam = SKCS single kernel diameter

* $p \leq 0.05$ ** $p \leq 0.01$

Significant correlations between ratios of mean protein fractions and measured quality characteristics

Results are given in Table 3.11.

The ratio between SDS soluble LPP and LMP significantly influenced SDS sedimentation, vitreous kernels and flour protein content. The ratio between SDS soluble SPP and LMP influenced the same characteristics, with the exception of SDS sedimentation.

The ratio between the SDS insoluble LPP and LMP and between SPP and LMP significantly influenced SDS sedimentation, vitreous kernels and flour protein content. The ratio between SDS insoluble LPP and SMP significantly influenced SDS sedimentation and flour protein content. The SDS insoluble SPP and SMP and LPP and SPP also significantly influenced SDS sedimentation and flour protein content respectively.

Table 3.11 Significant correlations between ratios of mean protein fractions and measured quality characteristics

Ratio	Characteristic	Correlation
SDS soluble LPP:LMP	SDS sedimentation	0.406*
	Vitreous kernels	0.507**
	Flour protein content	0.653**
SDS soluble SPP:LMP	Vitreous kernels	0.428*
	Flour protein content	0.621**
SDS insol LPP:LMP	SDS sedimention	0.650**
	Vitreous kernels	0.469*
	Flour protein content	0.598**
SDS insol LPP:SMP	SDS sedimentation	0.487**
	Flour protein content	0.430*
SDS insol SPP:LMP	SDS sedimention	0.751**
	Vitreous kernels	0.387*
	Flour protein content	0.505**
SDS insol SPP:SMP	SDS sedimention	0.526**
SDS insol LPP:SPP	Flour protein content	0.395*

SDS = sodium dodecyl sulphate, LPP = large polymeric proteins, SPP = small polymeric proteins, LMP = large monomeric proteins, SMP = small monomeric proteins,

* $p \leq 0.05$ ** $p \leq 0.01$

3.4.2 Discussion and conclusions

The HMW subunit composition of thirteen Ethiopian and two South African lines were determined. Similar HMW-GS compositions were observed for some of the entries tested. Two cultivars expressed the 1, 7+9, 5+10 composition. Three others had a similar combination except for the *Glu-A1* (2* replaced by 1). Two entries had a 2*, 7, 5+10 combination, and entries HAR2562 and HAR604 had a *Glu-B1* combination of 17+18. Kariega, SST825, HAR2807 and HAR2505 had unique compositions differeing from each other and the rest.

Commonly, HMW-GS *Glu-D1* (5+10) is associated with a larger contribution to dough properties than those encoded by Glu-B1 (17+18) (Uthayakumaran *et al*., 2001). In this study most of the entries (11 of the 15) had the 5+10 combination. Despite the similarity, differences in quality were still observed between the cultivars/lines. Cultivars exhibiting subunit 2+12 in their patterns, gave better results than the cultivars/lines with 5+10 present. This confirms the complexity of the interactions between the factors that define wheat quality.

None of the cultivars complied with the South African preferred 12% flour protein content, which is preferable for good baking quality. Adet had higher values for most of the quality characteristics, with the exception of mixograph development time, SKCS-weight, diameter and hardness index. The SDS sedimentation for all cultivars, except for HAR604 and Kariega at Adet, were lower than 70 ml, which is indicative of superior baking strength (De Villiers and Laubscher, 1995). The MDT for HAR1522 and HAR1775 was longer, at both environments, than the preferred 2.6 min for South African conditions.

The mixograph development time seemed to show a decrease with an increase in flour protein content. The reverse was seen for vitreous kernels, HI and SDS sedimentation that increased with flour protein content.

High molecular weight glutenin subunit composition has been proven to be useful in the prediction of baking quality, due to the high correlations excisting between certain subunits and quality parameters (Payne *et al*., 1987). In this study, different quality results were obtained for the entries with similar HMW-GS within a single environment and between the same entries planted at different environments. This confirmed the findings of MacRitchie *et al*. (1990) that the HMW-GS have more influence in certain wheat sets than in others. This indicated that baking quality is more complex than just the sum of the contribution of the HMW-GS. Baking quality is influenced by both the concentration and the size distribution of the proteins (Southan and MacRitchie, 1999).

There were significant differences between the eight SE-HPLC extracted protein fractions across different environments, indicating that the environment largely influenced the fractions (Table 3.8).

Bread wheat planted at Adet showed higher average concentrations for all fractions extracted, except for soluble and insoluble LMP (Table 3.6). The Adet trial also had higher protein content and consequently improved breadmaking quality. This confirms that Adet is indeed an area with high protein potential.

Across the two diverse environments the different protein fractions had a major effect on quality. Both the soluble and insoluble large polymeric proteins significantly influenced the most important quality characteristics. The environment had a significant influence on all the fractions, but the genotype effect was only visible for the SDS soluble fraction (Table 3.8). This confirmed findings of Dachkevitch and Autran (1989) where the insoluble polymers were not taken into consideration, and positive correlation was found between glutenins and rheological parameters.

At Adet, the SDS soluble and insoluble large monomeric proteins influenced SDS sedimentation positively and negatively, respectively. The SDS soluble SMP had a positive correlation with mixograph development time. This

contradicted the influence of polymeric proteins on quality and the direct correlation between glutenin polymers and quality characteristics (Dachkevitch and Autran, 1989). This is possibly as a result of the tight linkage that exists between LMW-GS and gliadins (Metakovsky *et al.,* 1990). The SDS insoluble SPP correlated positively with SDS sedimentation.

The SDS soluble large (gliadins), had a positive, and the small monomeric proteins (albumins and globulins) a negative correlation with mixograph development time (Table 3.10). This confirmed the contribution of gliadins to the viscosity and extensibility properties of dough (Gianibelli *et al*., 2001, Uthayakumaran *et al*., 2001). The reverse was true for the SDS soluble large and small polymeric proteins, which had a negative relationship with mixograph development time.

The total unextractable proteins as well as the large unextractable proteins both had a high positive influence on the mixograph development time. TUPP also had a significant positive correlation with SDS sedimentation. The individual SDS insoluble fractions did not have an influence on mixograph development time, but expressed as a ratio (TUPP), there was a correlation. It is evident that bread making quality is thus a result of the interaction of the different protein fractions as well as the concentration of the individual fractions.

The importance of the glutenin (polymeric proteins) to gliadin (monomeric proteins) ratio was confirmed by an increase in the polymeric proteins in the ratio. Increasing either small or large or SDS soluble or insoluble polymeric proteins in the ratio, led to an improvement in all the most important quality characteristics (Table 3.11). This confirmed worked done by Lafiandra *et al.* (2000) who suggested that an increase in the polymeric proteins will lead to stronger dough.

In this study, both SDS soluble and insoluble large and small polymeric proteins, TUPP and LUPP were good predictors of quality characteristics. It was found that a high polymeric to monomeric protein ratio resulted in better

breadmaking quality, which confirmed that bread making quality is an interaction between the concentrations of the different components of gluten proteins and can be used with success as predictors of quality.

3.5 References

Agrobase, 2000. Agronomix Software, Inc., Agrobase™, 71 Waterloo St. Winnipeg, Manitoba R3N0S4, Canada.

American Association of Cereal Chemists (AACC), 2000: Approved methods of the American Association of Cereal Chemists. Tenth Edition. American Association of Cereal Chemists Inc., St. Paul, Minnesota, USA.

Autran, J.C., 1994. Size-exclusion high-performance liquid chromatography for rapid examination of size differences of cereal proteins. In: High-Performance Liquid Chromatography of Cereal and Legume Proteins. J.E. Kruger and J.A. Bietz (Eds.), American Association of Cereal Chemists, St. Paul, Minnersota, pp 326-372.

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

De Villiers, O.T. and Laubscher, E.W., 1995. Use of the SDS test to predict the protein content and bread volume of wheat cultivars. *South African Journal of Plant and Soil* 12:140-142.

Gianibelli, M.C., Larroque, O.R., Macritchie, F. and Wrigley, C.W., 2001. Biochemical, genetic, and molecular characterization of wheat endosperm proteins. Online review: American Association of Cereal Chemists, pp1-20.

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

Hailu G.-M., 1991. Bread wheat breeding and genetics in Ethiopia. In: Wheat research in Ethiopia: A historical perspective. Hailu G.-M., Tanner, D.G., and Mengistu Hulluka (Eds.), Addis Ababa. IAR/CIMMYT, pp 73-93.

Lafiandra, D., Masci, S., D'Ovidio R. and Margiotta, B., 2000. The genetics of wheat gluten proteins: An overview. In: P.R. Shewry and A.S. Tatham (Eds.), Wheat Gluten Royal Society of Chemistry, pp 3-10.

MacRitchie, F., DuCros, D.L., and Wrigley, C.W., 1990. Flour polypeptides related to wheat quality. In: Y. Pomeranz (Ed.), Advances in Cereal Science and Technology, Vol. 10. American Association of Cereal Chemists, St. Paul, MN, pp 79-145.

Marshall, D.R., Langridge, P. and Appels, R., 2001. Wheat breeding in the new century- Preface. *Australian Journal of Agricultural Research* i-iv.

Metakovsky, E.V., Wrigley, C.W., Bekes, F. and Gupta, R.B., 1990. Gluten polypeptides as useful genetic markers of dough quality in Australian wheats. *Australian Journal of Agricultural* 41:289-306.

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

Singh, N.K., Shepherd, K.W. and Cornish, G.B., 1991. A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. *Journal of Cereal Science* 14: 203-208.

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

Uthayakumaran, S., Tömösközi, S., Tatham, A.S., Savage, A.W.J., Gianibelli, M.C., Stoppard, F.L. and Békés, F., 2001. Effects of gliadin fractions on functional proporties of dough depending on molecular size and hydrophobicity. *Cereal Chemistry* 78:138-141.

Zhu, J., and Khan, K., 2001. Effects of genotype and environment on glutenin polymers and breadmaking quality. *Cereal Chemistry* 78:125-130.

Chapter 4

Prediction of baking quality in Ethiopian durum wheat by size-exclusion high-performance liquid chromatography

4.1 Abstract

Durum wheat (*Triticum turgidum* L. (Thell.) *spp. durum* (Desf) Husn.) is an ancient crop in Ethiopia. In Ethiopia, durum wheat is largely used for production of local fermented and flat bread. Bread making quality is based on the gluten composition of wheat. The aim of this study was to determine the effect of SDS soluble and SDS insoluble proteins on quality characteristics, and the potential alternative end-use of durum wheat. Fifteen advanced lines and three commercial lines were tested at two environments, Adet and Motta, in Ethiopia. SE-HPLC was used to measure the SDS soluble and insoluble protein fractions. Correlations were found between specific fractions and quality characteristics. The influence of the glutenin/gliadin ratio's were also studied. Increasing the SDS insoluble fractions, polymeric to monomeric proteins, either small or large, led to an improvement of the most important quality characteristics. This study indicated that durum wheat is suitable for bread making and that it is possible to improve the bread making quality of durum wheat. SE-HPLC proved to be a useful tool in determining the protein composition and their influence on quality characteristics.

4.2 Introduction

Durum is one of the major wheat species cultivated today (Peña *et al*., 2002). Ethiopia is considered as one of the centres of genetic diversity of durum wheat, with important sources of rust and drought resistance, waterlogging tolerance and early ripening (Payne *et al*., 2001). In Ethiopia, consumption of durum wheat is largely in the form of whole wheat fermented or leavened local bread. The fermented bread making process from whole-wheat flour is traditionally similar for both durum and bread wheat. There is an increased demand in Ethiopia for raw product of improved quality (Woldegiorgis, 2003).

Unlike bread wheat, which is hexaploid (genome constitution ABD), durum wheat is tetraploid (genome constitution AB). Due to the fact that genes coding for proteins are present on all three genomes, bread wheat is more complex than pasta or durum wheat (Feldman *et al*., 1995).

The absence of the D genome in durum leads to a reduction in gluten strength and baking quality. However, breeding for the improvement of these traits is made possible by the existence of variability found for gluten strength and baking quality (Peña *et al*., 1994).

Subunits LMW-1 and LMW-2 were related to poor and good gluten strength, respectively (Kosmolak *et al*., 1980). The dough quality of durum wheat lines have been associated with the presence of a LMW-GS (LMW-2) and with some HMW-GS encoded at the *Glu-B1* locus based on fractionation by SDS-PAGE (Pogna *et al*., 1990).

Although SDS-PAGE can be used to separate proteins based on molecular size, the required reduction in disulfide bonds leads to a loss of information on the formation of the large glutenin polymers. Size-exclusion high-performance liquid chromatography proved to be effective in determining protein aggregates and interactions between components (Autran, 1994).

The aim of this study was to determine the effect of SDS soluble and SDS insoluble proteins fractionated by SE-HPLC on quality characteristics in durum wheat types in two environments in Ethiopia.

4.3 Material and Methods

4.3.1 Plant materials

Fifteen Ethiopian durum genotypes (Table 4.1) were used in this study. The lines were selected based on agronomic performance. Trials were grown at two environments in Ethiopia in 2001 namely at Adet Research Center, which is a higher protein potential area, and Motta, which is a low protein potential area. A RCB design with three replications was used. The plot size was 2.5 m^2 consisting of six rows, 2.5 m length and 20 cm spaced apart. Standard management practices were exercised.

Table 4.1 Entries of durum wheat lines included in this study

Entry	Line	Country
1	CD96486	Ethiopia
2	CD6630	Ethiopia
3	CD95294-1y	Ethiopia
4	DZ2023	Ethiopia
5	Yilma	Ethiopia
6	DZ1721	Ethiopia
7	DZ2212	Ethiopia
8	DZ1924	Ethiopia
9	Bichena	Ethiopia
10	LD357	Ethiopia
11	DZ1640	Ethiopia
12	DZ1691	Ethiopia
13	DZ1652	Ethiopia
14	DZ1748	Ethiopia
15	DZ900	Ethiopia

After harvesting, seeds of the three replicates of each lines were transported to South Africa. Yield and quality trait data was measured in triplicate (Chapter 3 Table 3.2) at the laboratories of the ARC-SGI, Bethlehem, South Africa.

4.3.2 Electrophoresis

Fifteen genotypes were analysed using the one-step one-dimensional SDS-PAGE separation as described by Singh *et al.* (1991), on a 10% separation polyacrylamide gel (Chapter 3). Six repeats of each line were compared with standards of known bands from Canada and Spain. The standards were Langdon (LMW1, γ-42), Mexicali (LMW-2, γ-45), Alaga (γ-44) and Marquis (R50).

4.3.3 Protein extraction and SE-HPLC

Proteins were extracted from wheat flour with a two-step extraction procedure developed by Gupta and colleagues (1993). The first step, as described in Chapter 3, extracts the proteins soluble in dilute SDS, while the second extract contains proteins soluble only after sonication. SE-HPLC was performed as described in section 3.3.3.

4.3.4 Statistical analysis

All statistical analyses were done with Agrobase (2000) as described in section 3.3.4.

4.4 Results and discussion

4.4.1 Results

SDS-PAGE banding patterns observed for 15 lines at Adet and Motta, Ethiopia

Results are given in Table 4.2.

Please refer to Appendix A (figures 2 and 3) for SDS-PAGE-results. Lines CD96486, CD6630, CD95294-1y, DZ1652 and DZ1748 had similar banding patterns for HMW, LMW-GS and gliadin. Similar patterns were observed for DZ2023 and DZ900 (N, 6+8, LMW-2 and γ-45). DZ1924 and DZ1691 were heterogeneous at the *Glu-B1* locus. The banding patterns of DZ1721, DZ2212 and Bichena were similar. High molecular weight glutenin subunits, N and 7+8, LMW-1 and γ-42 were observed for lines LD357 and DZ1640.

Table 4.2 SDS-PAGE banding patterns observed for 15 lines at Adet and Motta, Ethiopia

Entry	Cultivar	HMW-GS	LMW-GS	Gliadin
1	CD96486	N, 7+8	2	45
2	CD6630	N, 7+8	2	45
3	CD95294-1y	N, 7+8	2	45
4	DZ2023	N, 6+8	2	45
5	Yilma	N, 20	1	42
6	DZ1721	N, 20	2	45
7	DZ2212	N, 20	2	45
8	DZ1924[a]	N, 6+8, 14+15		
9	Bichena	N, 20	2	45
10	LD357	N, 7+8	1	42
11	DZ1640	N, 7+8,	1	42
12	DZ1691[a]	N, 6+8, 7+9, 17+18		
13	DZ1652	N, 7+8	2	45
14	DZ1748	N, 7+8	2	45
15	DZ900	N, 6+8	2	45

[a] –Heterogeneous at *Glu-B1* locus

HMW-GS = High molecular weight glutenin subunits, LMW-GS = low molecular weight subunits, SDS-PAGE = sodium dodecyl sulphate- polyacrylamide gel electrophoresis

N = null

Quality characteristics for 15 lines at Adet and Motta, Ethiopia

Results are given in Table 4.3.

SDSS: The SDSS of CD96486 was significantly higher than that of DZ1640, DZ900 and LD357, at Adet. Yilma had the highest SDSS value at Motta. DZ1721 was significantly lower than all the entries, except for CD6630, DZ1691, LD357 and DZ1924 at Motta.

VK: The VK of DZ2212 was the highest at Adet, with DZ1924 and DZ1652 ranking second and third, respectively. The VK of DZ1748, planted at Motta, was the highest and that of DZ1640 the lowest. The average VK at Motta was significantly higher than at Adet.

FPC: At Adet, line CD6630 had the highest FPC value but not significantly higher than that of DZ1721, Bichena, DZ1640, LD357 and DZ2023. DZ1748 ranked first, at Motta, with CD6630 and DZ2212 ranking second and third, respectively.

FLY: The FLY value for LD357, at Adet was the highest, but only significantly higher than that of DZ2212, DZ1652 and CD6630. There was an environmental effect at Adet. DZ1640 had the highest FLY value at Motta and DZ1652 the lowest.

MDT: At Adet the MDT for DZ1721 was significantly higher than that of DZ1652, DZ2212 and DZ900. The MDT for CD96486 and DZ1652, at Motta was significantly higher than that of DZ1691, DZ1924 and DZ1721.

SKCS-weight: The SK weight of Bichena was the highest, at Adet, but it was only significantly higher than DZ1748 and CD96486. DZ1748 had a significantly higher SK weight than all the others at Motta, except for CD6630, DZ2023, Bichena and DZ1721.

SKCS-diameter: There were no significant differences among lines for SKCS-diameter at Adet. There were significant differences at Motta: DZ1748 had the highest and LD357 the lowest SKCS-diameter.

SKCS-hardness index: The only significant difference found at Adet, was between Yilma, the highest and DZ1640, lowest ranking lines. CD6630 planted at Motta was significantly higher than the SKCS-hardness index of DZ1640, DZ2023 and Bichena.

Table 4.3 Means of measured quality characteristics for 15 lines at Adet and Motta, Ethiopia

ENTRY	NAME	Locality	SDSS	VK	FPC	FLY	MDT	WGHT	DIAM	HI
1	CD96486	A	32	76	10.8	54.6	3.3	34.46	2.45	65.59
		M	21	43	7.2	60.1	5.3	34.22	2.47	48.34
2	CD6630	A	24	89	12.5	52.4	2.8	36.02	2.51	72.20
		M	19	63	8.1	60.8	3.4	37.12	2.62	58.04
3	CD95294-1y	A	23	87	11.4	54.1	3.0	37.30	2.64	73.87
		M	20	47	7.2	60.9	4.1	34.42	2.42	50.22
4	DZ2023	A	24	81	10.0	55.3	2.7	37.92	2.63	67.54
		M	22	48	7.8	61.8	3.6	36.68	2.55	48.84
5	Yilma	A	23	85	12.5	54.8	2.7	35.86	2.54	74.17
		M	28	54	7.4	62.4	4.7	36.13	2.54	53.41
6	DZ1721	A	27	81	10.7	54.0	3.8	36.19	2.51	69.25
		M	11	49	7.5	61.9	2.6	36.24	2.59	49.14
7	DZ2212	A	24	97	11.9	53.4	2.4	37.23	2.57	73.37
		M	20	59	7.6	60.1	3.6	35.74	2.49	50.81
8	DZ1924	A	27	91	11.9	54.0	3.0	36.90	2.59	73.86
		M	15	54	7.4	61.9	3.0	35.76	2.55	53.73
9	Bichena	A	23	87	10.5	54.6	2.7	41.74	2.80	68.75
		M	22	51	7.2	61.9	4.1	36.49	2.54	44.62
10	LD357	A	18	67	10.1	56.1	2.7	36.77	2.61	65.84
		M	15	38	7.1	61.8	3.7	30.61	2.28	46.87
11	DZ1640	A	20	79	10.1	56.0	2.8	35.86	2.57	65.06
		M	21	28	6.7	62.7	4.8	33.91	2.41	35.91
12	DZ1691	A	24	85	11.4	55.9	2.6	35.54	2.55	70.00
		M	18	45	7.8	61.2	3.1	36.23	2.53	51.68
13	DZ1652	A	21	90	11.2	52.8	2.6	36.18	2.54	71.45
		M	24	43	7.4	59.9	5.3	32.19	2.33	46.84
14	DZ1748	A	28	87	10.9	55.5	2.7	34.97	2.46	69.37
		M	24	65	8.1	60.5	3.7	40.41	2.78	56.97
15	DZ900	A	18	73	11.0	55.4	2.3	38.20	2.65	69.47
		M	21	35	7.0	61.2	4.7	32.72	2.41	51.41
16	**Average**	A	23.73	83.67	11.13	54.59	2.81	36.74	2.57	69.99
		M	20.07	48.13	7.43	61.27	3.98	35.26	2.50	49.79
17	**LSD** (0.05)	A	12.01	17.29	1.68	2.36	1.14	6.90	0.38	8.82
		M	6.90	15.84	0.66	2.23	1.57	3.20	0.18	8.73

SDS sodium dodecyl suphate, SDSS =SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, Wght = SKCS single kernel weight, Diam = SKCS single kernel diameter, HI = SKCS-hardness index, A = Adet, M = Motta, LSD = least significant difference

Combined averages of quality characteristics for 15 lines at Adet and Motta, Ethiopia

Results are given in Table 4.4.

SDSS: The SDSS of CD96486 was the highest, with DZ1748 and Yilma ranking second and third, respectively. The top three top ranking entries had a significantly higher SDSS value than LD357, the lowest ranking entry.

VK: There were significant differences between the lines tested. DZ2212 had the highest VK. DZ2212, CD6630 and DZ1748 were significantly higher than the VK of CD96486, DZ2023, DZ900, DZ1640 and LD357.

FPC: There was a strong environmental effect for FPC. DZ1640, LD357 and DZ2023 were significantly lower than CD6630, Yilma and DZ2212.

FLY: DZ2023 had the highest flour yield, with DZ1640 and LD357 ranking second and third, respectively. DZ1652, CD6630 and DZ2212 were significantly lower than the highest yielding lines.

MDT: The only significant differences were between CD96486, with the longest mixing time, and lines DZ1691, DZ1924, DZ2212 and CD6630.

SKCS-weight: The SK weight of LD357 was significantly lower than Bichena and DZ1748.

SKCS-diameter: Bichena was significantly higher than DZ1652 and LD357.

SKCS-hardness index: DZ1640 had the lowest hardness index, and was significantly lower than all the lines tested, with the exception of DZ2023, LD357, Bichena and CD96486.

Table 4.4 Combined averages of quality characteristics for 15 lines at two different localities

ENTRY	NAME	SDSS	VK	FPC	FLY	MDT	WGHT	DIAM	HI
1	CD96486	27	60	9.0	57.4	4.3	34.34	2.46	56.96
2	CD6630	22	76	10.3	56.6	3.1	36.57	2.56	65.12
3	CD95294-1y	22	67	9.3	57.5	3.6	35.86	2.53	62.04
4	DZ2023	22	58	8.6	59.6	3.3	37.25	2.60	54.88
5	Yilma	25	70	9.9	58.6	3.7	35.99	2.54	63.79
6	DZ1721	19	65	9.1	58.0	3.2	36.22	2.55	59.19
7	DZ2212	22	78	9.8	56.7	3.0	36.49	2.53	62.09
8	DZ1924	21	72	9.7	58.0	3.0	36.33	2.57	63.79
9	Bichena	22	69	8.9	58.2	3.4	39.11	2.67	56.68
10	LD357	17	52	8.6	59.0	3.2	33.69	2.44	56.36
11	DZ1640	20	53	8.4	59.4	3.8	34.88	2.49	50.49
12	DZ1691	21	65	9.6	58.5	2.9	35.89	2.54	60.84
13	DZ1652	22	67	9.3	56.4	4.0	34.19	2.43	59.15
14	DZ1748	26	76	9.5	58.0	3.2	37.69	2.62	63.17
15	DZ900	20	54	9.0	58.3	3.5	35.46	2.53	60.44
	Average	21.87	65.47	9.30	58.01	3.41	36.00	2.54	59.67
	LSD (0.05)	5.71	10.23	0.72	1.42	0.89	3.07	0.17	5.47

SDS = sodium dodecyl sulphate, SDSS = SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, Wght = SKCS single kernel weight, Diam = SKCS single kernel diameter, HI = SKCS-hardness index

LSD = least significant difference

Protein fractions for 15 lines at Adet and Motta, Ethiopia

Results are given in Table 4.5.

SDS soluble LPP: There was a strong environmental effect at Adet. DZ1652 (8.36) had the highest fraction of LPP at Adet. The difference was not significantly higher than DZ2212 or CD96486 ranking second and third, respectively. DZ2023 had the lowest SDS soluble LPP value at Adet. The reverse for these two entries was true at Motta, with DZ2023 with the highest LPP fraction and DZ1652 the lowest. This was seen for most entries, except for Yilma that consistently had a low LPP fraction at both environments. There was also a strong genotype effect for lines planted at Motta.

SDS soluble SPP: DZ2023 was significantly lower than DZ2212 (highest), DZ1652 and CD95294-1y at Adet. Bichena was higher than all lines at Motta, but only significantly higher than DZ1652. DZ1652 had the lowest SPP fraction at Motta.

SDS soluble LMP: There was a strong genotype effect at Adet. The LMP for all the entries was significantly higher than DZ900, except for DZ2023, DZ1721 and DZ1691. DZ1924 at Motta had the highest and DZ1652 the lowest LMP values, respectively. DZ1652 was significantly lower than all the lines, except for DZ2023 and DZ1721, at Motta.

SDS soluble SMP: CD95294-1y (highest) and CD96486, ranking second were the only entries with a significantly higher SMP fraction than DZ2023 (lowest) at Adet. The SMP for DZ1652, planted at Motta, was significantly higher than all the other lines, except for CD95294-1y, DZ1721, DZ2023 and CD6630.

SDS insoluble LPP: CD95294-1y had the highest LPP fraction at Adet. It was only significantly higher than the LPP of DZ900, DZ1640 and DZ1691. At Motta, DZ1640 was the highest, but was only significantly higher than DZ1924. CD95294-1y ranked second and CD96486 third, at Motta.

SDS insoluble SPP: Bichena, DZ2212, DZ900 and Yilma were significantly lower than DZ2023, the highest ranking entry at Adet. Yilma had the

highest fraction of SPP at Motta, with DZ1691 and DZ2023, ranking second and third, respectively. The highest ranking three entries were significantly higher than CD95294-1y, DZ900, DZ1721 and LD357.

SDS insoluble LMP: DZ900 had the highest LMP fraction at Adet, but not significantly higher than Yilma, DZ1748, DZ1691 or DZ1640. There were no significant differences between entries at Motta, except between DZ1924 (highest) and LD357 (lowest).

SDS insoluble SMP: There were no significant differences between the entries planted at Adet. Yilma had the highest SMP proportion and DZ2212 the lowest. At Motta the SMP for DZ1721 was significantly higher than DZ1640, LD357, CD6630, DZ1652, CD96486 and DZ1691.

TUPP: At Adet, DZ2023 and DZ1721 had significantly higher TUPP than DZ900 and DZ1640 (lowest). The TUPP of DZ1652 and Yilma at Motta were significantly higher than DZ1721 and LD357.

LUPP: The LUPP for most of the lines were not significantly lower than DZ1640, except for DZ1924, DZ2023, CD6630 and DZ1721, at Adet. There was a strong genotype effect at Motta. DZ2212 had the highest LUPP value, but it was only significantly higher than CD95294-1y, Yilma, DZ1640 and DZ1652.

Table 4.5 Means of measured protein fractions for 15 lines at Adet and Motta, Ethiopia

			SDS soluble				SDS insoluble					
ENTRY	NAME	Locality	LPP	SPP	LMP	SMP	LPP	SPP	LMP	SMP	TUPP	LUPP
1	CD96486	A	8.02	17.09	40.28	30.59	25.75	23.91	29.47	16.18	66.43	23.67
		M	6.79	20.39	45.49	24.34	31.18	26.10	24.81	11.59	67.86	18.04
2	CD6630	A	6.69	15.89	44.57	29.08	25.41	23.80	29.11	18.74	68.68	20.85
		M	6.96	20.41	44.10	25.99	31.00	23.40	25.73	12.89	66.13	19.35
3	CD95294-1y	A	7.79	17.41	40.00	32.10	26.66	23.76	28.22	17.03	66.65	22.63
		M	6.67	18.40	43.70	28.65	31.61	22.15	23.88	16.04	68.94	17.38
4	DZ2023	A	6.16	14.78	48.13	25.53	22.95	26.91	27.41	17.19	70.61	21.40
		M	7.53	19.18	44.48	26.47	25.80	27.28	25.62	17.84	66.77	22.26
5	Yilma	A	6.69	15.82	44.49	29.25	22.74	21.97	31.97	18.89	66.26	23.14
		M	6.04	18.42	47.53	23.88	29.96	29.13	22.88	15.33	70.96	16.66
6	DZ1721	A	6.35	15.25	47.26	26.89	25.73	24.65	29.14	17.60	70.02	19.69
		M	8.16	21.05	42.07	27.01	27.02	20.09	22.57	24.10	61.26	24.17
7	DZ2212	A	8.27	17.61	41.47	29.15	25.96	22.39	29.40	14.74	65.13	24.12
		M	7.77	20.29	45.26	24.00	27.32	23.37	25.46	20.53	64.38	22.24
8	DZ1924	A	6.95	15.95	43.32	29.41	25.20	23.15	28.14	18.55	67.87	21.59
		M	6.92	19.43	49.50	20.20	24.39	24.70	26.39	19.99	65.12	21.85

Table 4.5 Continued

			SDS soluble				SDS insoluble					
ENTRY	**NAME**	**Locality**	**LPP**	**SPP**	**LMP**	**SMP**	**LPP**	**SPP**	**LMP**	**SMP**	**TUPP**	**LUPP**
9	Bichena	A	7.39	16.77	42.01	28.78	25.05	22.74	29.24	16.58	66.37	22.79
		M	8.10	21.66	43.51	24.29	29.57	23.94	22.57	19.46	64.14	21.92
10	LD357	A	7.78	17.34	41.01	29.16	25.80	23.21	29.57	15.87	66.13	23.06
		M	6.42	21.09	45.68	24.76	28.54	19.92	20.40	13.31	62.88	20.02
11	DZ1640	A	7.94	16.97	43.58	26.77	20.98	24.33	30.32	16.20	64.51	28.35
		M	6.20	20.51	48.43	20.42	32.27	26.18	23.03	13.63	68.40	16.26
12	DZ1691	A	6.83	16.00	45.26	28.18	20.07	26.30	31.85	15.09	66.29	26.20
		M	8.26	21.08	42.74	24.61	28.76	27.76	21.81	10.49	65.84	22.56
13	DZ1652	A	8.36	17.43	44.05	25.67	25.58	23.83	29.74	16.37	65.74	24.49
		M	5.94	17.11	36.26	32.57	30.88	24.81	22.29	12.34	71.57	15.64
14	DZ1748	A	7.05	16.16	44.11	28.47	24.78	23.19	31.89	16.36	67.36	22.32
		M	7.90	21.14	46.11	18.69	28.69	23.85	24.94	18.21	64.40	21.58
15	DZ900	A	6.90	16.48	39.74	27.07	21.45	22.13	33.79	17.50	64.94	24.82
		M	7.08	21.39	43.88	24.55	29.67	21.17	23.28	19.92	63.93	19.35
	Average	A	7.28	16.46	43.29	28.41	24.27	23.75	29.95	16.86	66.87	23.27
		M	7.12	20.10	44.58	24.70	29.11	24.26	23.71	16.38	66.17	19.95
	LSD (0.05)	A	1.26	1.52	4.91	4.37	4.84	3.86	3.89	4.72	6.61	4.95
		M	1.73	2.80	4.48	5.49	4.76	4.13	4.13	7.39	5.30	5.13

SDS = sodium dodecyl, suphate, LPP = large polymeric proteins, SPP = small polymeric proteins, LMP = large monomeric proteins, SMP = small monomeric proteins, TUPP = % total unextractable polymeric proteins, LUPP = % large unextractable polymeric proteins

A = Adet, M = Motta, LSD = least significant difference

Combined averages of protein fractions for 15 lines at two different localities

Results are given in Table 4.6.

Please refer to Appendix B for SE-HPLC chromatograms of SDS-soluble proteins (Figure 1.) and SDS-insoluble proteins (Figure 2).

SDS soluble LPP: The only significant difference was between highest ranking entry DZ2212 and lowest ranking Yilma.

SDS soluble SPP: Bichena and LD357 had the highest SPP content, but was only significantly higher than DZ1652 and Yilma.

SDS soluble LMP: The LMP values for DZ1652, DZ900 and CD95294-1y were significantly lower than the highest yielding entry, DZ1924.

SDS soluble SMP: The SMP of CD95294-1y was significantly higher than DZ2023, DZ900, DZ1924, DZ1640 and DZ1748.

SDS insoluble LPP: The SDS insoluble LPP of CD95294-1y was the highest, significantly higher than that of DZ2023, DZ1924 and DZ1691.

SDS insoluble SPP: DZ2023 had the highest SPP value, but not significantly higher than DZ1691, Yilma, DZ1640, CD96486 and DZ1652.

SDS insoluble LMP: DZ900 and DZ1748 ranking first and second, respectively, were significantly higher than LD357 (lowest rank).

SDS insoluble SMP: The SMP of DZ1691 and CD96486 were significantly lower than DZ1721 and DZ1924.

TUPP: DZ1652 was significantly higher than the DZ900, the lowest ranked line.

LUPP: DZ1691 had the highest LUPP value, but it was not significantly higher than any of the other entries.

Table 4.6 Combined averages of protein fractions for 15 lines at two different localities

		SDS soluble				SDS insoluble					
ENTRY	**NAME**	**LPP**	**SPP**	**LMP**	**SMP**	**LPP**	**SPP**	**LMP**	**SMP**	**TUPP**	**LUPP**
1	CD96486	7.40	18.74	42.89	27.46	28.47	25.00	27.14	13.88	67.15	20.86
2	CD6630	6.83	18.15	44.33	27.54	28.21	23.60	27.42	15.82	67.41	20.10
3	CD95294-1y	7.23	17.91	41.85	30.37	29.13	22.95	26.05	16.54	67.80	20.01
4	DZ2023	7.42	17.92	44.84	26.25	24.87	27.29	25.72	17.32	67.57	22.98
5	Yilma	6.36	17.12	46.01	26.56	26.35	25.55	27.43	17.11	68.61	19.90
6	DZ1721	7.26	18.15	44.67	26.95	26.37	22.37	25.85	20.85	65.64	21.93
7	DZ2212	8.02	18.95	43.36	26.57	26.64	22.88	27.43	17.63	64.76	23.18
8	DZ1924	6.94	17.69	46.41	24.81	24.80	23.92	27.26	19.27	66.50	21.72
9	Bichena	7.75	19.22	42.76	26.53	27.31	23.34	25.90	18.02	65.26	22.35
10	LD357	7.10	19.22	43.35	26.96	27.17	21.57	24.99	14.59	64.51	21.54
11	DZ1640	7.07	18.74	46.01	23.60	26.63	25.25	26.67	14.92	66.46	22.30
12	DZ1691	7.55	18.54	44.00	26.40	24.42	27.03	26.83	12.79	66.06	24.38
13	DZ1652	7.15	17.27	40.15	29.12	28.23	24.32	26.02	14.35	68.65	20.06
14	DZ1748	7.48	18.65	45.11	23.58	26.73	23.52	28.42	17.29	65.88	21.95
15	DZ900	6.99	18.94	41.81	25.81	25.56	21.65	28.54	18.71	64.44	22.09
	Average	7.24	18.35	43.84	26.57	26.73	24.02	26.78	16.61	66.45	21.69
	LSD (0.05)	0.98	1.50	2.90	3.18	2.99	2.52	2.52	4.07	3.23	3.57

SDS = Sodium dodecyl sulphate, LPP = large polymeric proteins, SPP = small polymeric proteins, LMP = large monomeric proteins, SMP = small monomeric proteins, TUPP = % total unextractable polymeric proteins, LUPP = % large unextractable polymeric proteins

LSD = least significant difference

Mean squares of specific protein fractions at the two locations

Results are given in Table 4.7.

There was a replication effect for SDS soluble LPP, SPP and LMP, at both localities, which indicated that the technique was not as repeatable across replications as would be desired. For SDS insoluble fractions the replication effect was only for SPP and LMP at Adet and LPP at Motta. There was no genotype effect at Motta and at Adet, only for SDS soluble LPP.

There was no genotype effect for any of the fractions tested, across the two localities. A significant locality effect was observed for SDS soluble SPP and SMP and SDS insoluble LPP and LMP. The replication x locality effect was significant for SDS soluble LPP, SPP and LMP and for SDS insoluble LPP and LMP. There was also a significant locality x entry effect for SDS soluble LMP. This indicated that the environment largely influenced the fractions.

Table 4.7 Mean squares of protein fractions at separate locations and across locations

Source	Loc	SDS soluble proteins				SDS insoluble proteins			
		LPP	SPP	LMP	SMP	LPP	SPP	LMP	SMP
Rep	A	17.26**	5.90*	117.46**	19.53	33.07	40.07*	32.75*	16.91
	M	24.28**	50.66**	70.81*	26.44	291.17**	2.95	46.98	67.16
Entry	A	1.49*	2.20	19.30	9.51	13.00	5.84	8.94	4.62
	M	2.35	5.18	30.59	35.55	13.77	22.99	8.05	47.27
	A+M	0.95	2.66	18.77	19.13	11.98	18.17	6.20	29.32
Loc	A+M	0.17	318.85**	27.41	304.26**	540.862**	6.373	906.37**	5.80
Rep x Loc	A+M	20.77**	28.28**	94.14**	22.99	162.12**	21.51	39.87*	42.03
Loc x Entry	A+M	2.88	4.72	31.12*	25.93	14.75	10.67	10.79	22.60

SDS = sodium dodecyl sulphate, LPP = large polymeric proteins, SPP = small polymeric proteins, LMP = large monomeric proteins, SMP = small monomeric proteins, Rep = replication, Loc = Locality
$p \leq 0.05$, ** $p \leq 0.01$, A = Adet, M = Motta

Significant correlations between specific protein fractions and quality characteristics for Adet and Motta

Results are given in Table 4.8.

Correlations were found between all four SDS soluble fractions and quality characteristics, at Adet. The polymeric proteins of the SDS soluble fraction (LPP and SPP) were negatively correlated with MDT. SPP had a negative correlation with SDSS. The reverse was true for the SDS soluble small monomeric protein fraction that was positively correlated with SDS sedimentation and MDT. The large monomeric fractions were negatively correlated with flour protein content. Results for the SDS insoluble LPP and TUPP showed positive correlations with SDSS and MDT, while LUPP were negatively correlated with the same characteristics. The SDS insoluble LPP had an additional negative correlation with FLY.

Correlations between specific subunits and quality characteristics were limited for Motta. There were more correlations between the SDS insoluble fractions and quality than the soluble fractions. The SDS soluble LPP correlated positively with single kernel weight. The SDS insoluble polymeric proteins correlated positively with SDSS and MDT, except for the LUPP fraction that had a negative correlation. SDS insoluble LPP correlated negatively and LUPP positively with flour yield. LUPP correlated positively with SKCS-weight and diameter.

Table 4.8 Significant correlations between specific protein fractions and quality characteristics for Adet and Motta

Location	SDS soluble Fraction	Characteristic	Correlation	SDS insoluble Fraction	Characteristic	Correlation
Adet	LPP	MDT	-0.326*	LPP	SDSS	0.482**
					FLY	-0.381*
	SPP	SDSS	-0.384*		MDT	0.416**
		MDT	-0.400**	TUPP	SDSS	0.560**
	LMP	FPC	-0.304*		MDT	0.612**
	SMP	SDSS	0.441**	LUPP	SDSS	-0.538**
		MDT	0.312*		MDT	-0.534**
Motta	LPP	SKCS-wght	0.348*	LPP	SDSS	0.362*
					FLY	-0.417**
					MDT	0.388*
				SPP	SDSS	0.472**
					MDT	0.362*
				TUPP	SDSs	0.367*
					MDT	0.350*
				LUPP	SDSS	-0.316*
					FLY	0.356*
					MDT	-0.393**
					SKCS-wght	0.392**
					SKCS-diam	0.352*

SDS = sodium dodecyl sulphate, LPP = large polymeric proteins, SPP = small polymeric proteins, LMP = large monomeric proteins, SMP = small monomeric proteins, TUPP = % total unextractable polymeric proteins, LUPP = % large unextractable polymeric proteins, SDSS = SDS sedimentation, MDT = mixograph development time, FPC = flour protein content, SKCS = single kernel characterization system, Wght = weight, diam = diameterVK = vitreous kernels
* $p \leq 0.05$, ** $p \leq 0.01$

Significant correlations between specific protein fractions and quality characteristics for combined localities

Results are given in Table 4.9.

SDS soluble LPP was negatively correlated with MDT. The small polymeric fractions were negatively correlated with VK, FPC, HI, SDSS and positively correlated with FLY and SKCS-weight. The reverse was true for SMP except that no correlations were found with SDSS and SKCS-weight.

There were no significant correlations between the SDS insoluble small monomeric protein fraction and any of the quality characteristics. The large, small and total unextractable polymeric proteins correlated positively with SDSS and MDT. The large unextractable polymeric proteins correlated negatively with SDSS and MDT, but positively with HI. The large monomeric proteins correlated positively with VK, FPC, HI and negatively with FLY and MDT.

Table 4.9 Significant correlations between specific protein fractions and quality characteristics for combined localities

SDS Soluble proteins			SDS Insoluble proteins		
LPP	MDT	-0.239*	LPP	SDS	0.220*
SPP	SDSS	-0.250*		VK	-0.287**
	VK	-0.444***		FPC	-0.403***
	FPC	-0.554***		MDT	0.525***
	FLY	0.542***		SKCS-HI	-0.395***
	SKCS-HI	-0.453***	SPP	SDSS	0.286**
	SKCS-wght	0.265*		MDT	0.337**
SMP	FLY	-0.400***	LMP	VK	0.537***
	FPC	0.389***		FPC	0.629***
	VK	0.321**		FLY	-0.552***
	SKCS-HI	0.274**		MDT	-0.464***
				HI	0.589***
			TUPP	SDSS	0.437***
				MDT	0.322**
			LUPP	SDSS	-0.3081**
				MDT	-0.4997***
				SKCS-HI	0.346***

SDS = sodium dodecyl sulphate, LPP = large polymeric proteins, SPP = small polymeric proteins, LMP = large monomeric proteins, SMP = small monomeric proteins, TUPP = % total unextractable polymeric proteins, LUPP = % large unextractable polymeric proteins, SDS = sodium dodecyl sulphate, SDSS = SDS sedimentation, MDT = mixograph development time, FPC = flour protein content, FLY = flour yield, SKCS = single kernel characterization system, wght = weight, HI = hardness index VK = vitreous kernels

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Significant correlations between ratios of mean protein fractions and measured quality characteristics

Results are given in Table 4.10.

The ratio between SDS soluble LPP and SPP significantly influenced vitreous kernels, flour protein content, flour yield, mixograph development time, and SK weight, diameter and hardness index. The SPP:SMP ratio correlated with the same characteristics, with the exception of MDT, SKCS-weight and SKCS-diameter. The ratio between LPP and SMP influenced SDS sedimentation and flour yield. SPP:LMP significantly influenced FPC and FLY.

The ratio between the SDS insoluble LPP and LMP and between SPP and LMP significantly influenced VK, FPC, FLY, MDT and SKCS-hardness index. The ratio between LPP and SPP significantly influenced MDT

Table 4.10 Significant correlations between ratios of mean protein fractions and measured quality characteristics

Ratio	Characteristics	Correlations
SDS soluble LPP:SPP	VK	0.622***
	FPC	0.643***
	FLY	-0.483***
	MDT	-0.492***
	SKCS-wght	0.277**
	SKCS-diam	0.262*
	SKCS-HI	0.611***
SDS soluble LPP:SMP	SDSS	-0.218*
	FLY	0.280**
SDS soluble SPP:LMP	FPC	-0.338**
	FLY	0.342**
SDS soluble SPP:SMP	VK	-0.360***
	FPC	-0.460***
	FLY	0.495***
	SKCS-HI	-0.349***
SDS insoluble LPP:SPP	MDT	0.238*
SDS insoluble LPP:LMP	VK	-0.436***
	FPC	-0.550***
	FLY	0.401***
	MDT	0.545***
	SKCS-HI	-0.535***
SDS insoluble SPP:LMP	VK	-0.408***
	FPC	-0.521***
	FLY	0.455***
	MDT	0.518***
	SKCS-HI	-0.510***

LPP = large polymeric proteins, SPP = small polymeric proteins, LMP = large monomeric proteins, SMP = small monomeric proteins, SDS = sodium dodecyl sulphate, SDSS = SDS sedimentation, MDT = mixograph development time, FPC = flour protein content, FLY = flour yield, SKCS = single kernel characterization system, wght = weight, HI = hardness index VK = vitreous kernels

* $p \leq 0.05$, ** $p \leq 0.01$, ***$p \leq 0.001$

4.4.2 Discussion and conclusions

Low polymorphism was detected at the *Glu-B3* locus (LMW-2/LMW-1). The presence of subunit LMW-2/γ45 was dominant to LMW-1/γ42 in the lines tested. The null allele was present in all lines. The lines could be divided into three groups based on the *Glu-B1* subunit composition. Seven had subunits 7+8 present, two of which contained LMW-1/γ42. Four lines had subunit 20, with only one containing LMW-1/γ42. The last group consisted of two individuals with subunits 6+8. Lines DZ1721 and DZ1691 were heterogeneous for the HMW-GS.

Across environments, line CD96486 (N, 7+8, LMW-2/γ45) had the highest SDSS value and longest mixing time. This might be as result of the direct influence of the presence of the subunit or due to markers tightly linked with the LMW-GS (Ruiz and Carrillo, 1993).

Two of the cultivars at Adet, namely CD6630 and Yilma, complied with the South African preferred 12% flour protein content, accepted for good baking quality. Adet gave higher values than Motta for most of the quality characteristics, with the exception of flour yield and MDT. The lower flour protein content at Motta resulted in lower SDSS, longer MDT and lower vitreousness and SK hardness index. Unfortunately baking tests could not be performed due to low flour yield.

The average vitreousness and hardness index at Adet was significantly higher than at Motta. This confirms the association generally found between vitreousness, high protein content and hardness (Hoseney, 1986).

The lower protein content and lower vitreousness or hardness index resulted in longer mixograph development times at Motta (2.6-5.3 min). The average mixograph development time at Adet was 2.8 min, which is slightly higher than the preferred 2.6 min for South African conditions. The SDS

sedimentation for all cultivars were lower than 70 ml, which is indicative of superior baking strength (De Villiers and Laubscher, 1995).

The differences in SDSS and MDT of cultivars with the same protein content can be attributed to the protein composition (Sabine *et al*., 1997). The compositional aspects of gluten, namely the glutenins and gliadins, are mostly genetically determined, though the protein concentration can be influenced by the environment.

The presence of LMW-1 did not necessarily result in poor quality. No distinct association was visible between the different subunits and individual baking quality tests. The baking quality of lines differed across environments, which indicated that quality was not influenced by the presence of subunits alone.

Durum wheat planted at Adet showed on average higher concentrations for SDS soluble LPP, SMP and insoluble LMP, SMP, TUPP and LUPP (Table 4.5). More significant differences between the protein fractions of the two environments would have been expected, as Adet is thought to be an area with higher protein potential. The average protein content at Adet was 3.7% higher than at Motta, confirming that Adet is an area with high protein potential (Table 4.3).

Across the two diverse environments in this study the different protein fractions had a major effect on quality. Both the soluble and insoluble large polymeric proteins highly significantly influenced the most important quality characteristics.

At Adet the SDS soluble polymeric protein (large and small) fractions had a significantly negative correlation with MDT and SDSS (Table 4.8). The opposite was true for SDS soluble SMP that had a positive correlation with MDT and SDSS. With the exception of LUPP, most of the SDS insoluble polymeric proteins correlated significantly positively with SDSS and MDT for both environments. This contradicts the findings of Dachkevitch and Autran

(1989) where the insoluble polymers were not taken into consideration and a positive correlation was found between glutenins and rheological parameters.

Across the two environments, the SDS soluble large and small polymeric protein fractions had significantly negative correlations with both MDT and SDSS. The small polymeric protein (LMW glutenins) correlated negatively and the small monomeric proteins (albumins and globulins) positively with flour protein content (Table 4.9).

The SDS soluble small monomeric proteins lacked correlation with the important characteristics such as MDT or SDSS, and the SDS insoluble SMP had no significant correlations with any of the quality traits. The relatively few correlations between SMP and quality characteristics is similar to results reported by MacRitchie (1984) who stated that there are very few correlations between the amount of albumins and globulins and baking quality.

The SDS insoluble LPP and SPP had significant positive correlations with SDSS and MDT. The insoluble large monomeric fraction had a significant negative correlation with MDT and FLY, but correlated positively with VK, FPC and SKCS-HI. This indicated that an increase in the gliadin concentration would detrimentally influence the mixing time of the flour. Glutenins are responsible for the dough elasticity while the extensibility and viscosity are determined by the gliadins (Colt, 1990; Shewry *et al*., 1995).

The total unextractable proteins positively influenced the mixograph development time and SDS sedimentation. The reverse was true for LUPP that had a negative influence on SDSS and MDT. This indicated that the effect of an increase of SDS insoluble LPP which will lead to a decrease in SDS sedimentation volume and mixing time. It is evident that bread making quality is thus a result of the interaction of the different protein fractions as well as the concentration of the individual fractions.

The consistently negative correlation (Table 4.10) found between MDT and SDS soluble polymeric fractions at and across both environments can be

attributed to the absence of the D genome in durum. Studies by Dong *et al.* (1992) showed a consistently positive correlation between mixograph development time and HMW-GS 5+10. The ratio of SDS soluble LPP:SPP confirms findings by Tanaka and Bushuk (1973). They found that varieties rich in higher molecular weight fractions usually had medium to medium long mixing times, and those rich in lower molecular weight fractions had decreased mixing times.

The importance of the absence of the D-genome was seen in the influence of the glutenin (polymeric proteins) to gliadin (monomeric proteins) ratio. An increase in the SDS soluble polymeric proteins in the ratio, either small or large led to a significantly negative correlation with flour protein content and SDS sedimentation (SPP:SMP). This contradicted findings by Lafiandra *et al.* (2000) that suggested that an increase in the polymeric proteins will lead to stronger dough.

The opposite was true for ratios between the SDS insoluble fractions. An increase of polymeric to monomeric proteins, either small or large, led to an improvement of all the most important quality characteristics (Table 4.10). This confirmed research done by Lafiandra *et al*. (2000) that suggested that an increase in the polymeric proteins will lead to stronger dough. This also indicates the importance of the SDS insoluble fractions' influence on quality.

Therefore this study confirmed the alternative end-use of durum for bread baking and that it is possible to improve the protein quality and quantity through breeding and consequently improve the bread making quality. It confirmed that bread making quality is an interaction between the concentrations of the different components of gluten proteins and can be used with success as predictor of quality. It also indicateed that SDS soluble and insoluble large and small polymeric proteins and percentage total and large unextractable proteins can be used as good predictors of quality characteristics, not only bread making, but also for pasta-quality.

4.5 References

Agrobase, 2000. Agronomix Software, Inc., Agrobase™, 71 Waterloo St. Winnipeg, Manitoba R3N0S4, Canada.

Autran, J., 1994. Size-exclusion high-performance liquid chromatography for rapid examination of size differences of cereal proteins. In: J.E. Kruger and J.A. Bietz (Eds.), HPLC: High-performance liquid chromatography of cereal and legume proteins. American Association of Cereal Chemists, St. Paul, Minnesota, USA, pp 326-372.

Colt, V., 1990. The genes encoding wheat storage proteins: towards a molecular understanding of breadmaking quality and its genetic manipulation. In: J.K. Setlow (Ed.), Genetic engineering: Principles and methods, Vol. 12. Plenum Press, New York, pp 225-304.

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

De Villiers, O.T. and Laubscher, E.W., 1995. Use of the SDS test to predict the protein content and bread volume of wheat cultivars. *South African Journal of Plant and Soil* 12:140-142.

Dong, H., Sears, R.G., Cox, T.S., Hoseney, R.C., Lookhart, G.L. and Shogren, M.D., 1992. Relationship between protein composition and Mixograph and loaf characteristics in wheat. *Cereal Chemistry* 69:132-136.

Feldman, M., Lupton, F.G.H. and Miller, T.E., 1995. Wheats *Triticum* spp. (Gramineae-Triticinae). In: J. Smart and N.W. Simmonds (Eds.), Evolution of Crop Plants. Longman Scientific and Technical, Harlow, UK, pp 184-192.

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

Hoseney, R.C., 1986. Principles of Cereal Science and Technology. American Association of Cereal Chemistry, St Paul, Minnesota, USA.

Kosmolak, F.G., Dexter, J.E., Matsuo, R.R., Leslie, D. and Marchylo, B.A., A relationship between durum wheat quality and gliadin electrophoregrams. *Canadian Journal Plant Science* 60:427-432.

Lafiandra, D., Masci, S., D'Ovidio, R. and Margiotta, B., 2000. The genetics of wheat gluten proteins: An overview. In: P.R. Shewry and A.S. Tatham (Eds.), Wheat Gluten Royal Society of Chemistry, pp 3-10.

MacRitchie, F., 1984. Baking quality of wheat flours. *Advances in Food Research* 29:201-277.

Payne, T.S., Wanjama J.K. and Girma, B., 2001. Eastern Africa wheat pool. In: A.P. Bonjean and W.J. Angus (Eds.), The wheat world book: A brief history of wheat breeding. Lavoisier publishing, France, pp 902-911.

Peña, R.J., Trethowan, R. Pfeiffer W.H. and Van Ginkel, M., 2002. Quality (end-use) improvement in wheat: compositional, genetic, and environmental factors. In: A.S. Basra and L.S. Randhawa (Eds.), Quality improvement in field crops. Food products press, Binghampton, USA, pp 1-39.

Peña R.J., Zarco-Hernandez, J., Amaya-Celis, A. and Majeeb- Kazi, A., 1994. Relationship between chromosome 1B-encoded glutenin subunits compositions and bread making quality characteristics of some durum wheat (*Triticum turgidum*) cultivars. *Journal of Cereal Science.* 19:243-249.

Pogna, N.E., Autran, J.-C., Mellini, F., Lafiandra, D. and Feillet, P., 1990. Chromosome 1B-encoded gliadin and glutenin subunits in durum wheat: genetics and relationship to gluten strength. *Journal of Cereal Science.* 11:15-34.

Ruiz, M. and Carrillo, J.M., 1993. Linkage relationships between prolamin genes on chromosomes 1A and 1B of durum wheat. *Theoretical and Applied Genetics* 87:353-360.

Sabine, G., Oberfoster, M., Werteker, M., Grausgruber, H. and Lelly, T., 1997. HMW glutenin subunit composition and bread-making quality of Austrian grown wheats. *Cereral Research Communication* 25:955-962.

Shewry, P.R., Tatham, A.S., Barro, F., Barcelo P. and Lazzeri, P., 1995. Biothechnology of bread making: Unravelling and manipulating the multi-protein gluten complex. *Bio/Technology* 13:1185-1190.

Singh, N.K., Shepherd, K.W. and Cornish, G.B., 1991. A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. *Journal of Cereal Science* 14:203-208.

Tanaka, K. and Bushuk, W. 1973. Effect of protein content and wheat variety on solubility and electrophoretic properties of flour proteins. *Cereal Chemistry* 49:247.

Woldegiorgis, T.D., 2003. Genetic variability and combininb ability for quality parameters in Ethiopian wheat cultivars. PhD Agricultural Thesis, University of the Free State.

Chapter 5

Comparison of baking quality in Ethiopian bread and durum wheat by size-exclusion high-performance liquid chromatography

5.1 Abstract

SE-HPLC is a powerful tool to study native protein aggregates and the physicochemical basis of baking strength, and allows the rapid assessment of baking quality of wheat genotypes in breeding programmes. The methodology accurately separates the three main classes of endosperm proteins, namely: glutenins (polymeric proteins), gliadins, and albumins and globulins (monomeric proteins). This is particularly important because the relationship between protein classes (e.g., glutenin-to-gliadin ratio) and the molecular size distribution of polymeric proteins affect quality attributes. In this study 13 Ethiopian and two South African bread wheat cultivars were compared to 15 Ethiopian durum wheat cultivars in two diverse environments. This was done to assess the effect of SE-HPLC determined storage proteins on the bread making quality. It was found that across environments, protein fractions had a major influence on quality. The amount of polymeric proteins in bread wheat was significantly higher in the high protein environment, but in durum wheat the opposite was found. Durum wheat had higher percentages of large polymeric and small monomeric protein fractions, compared to bread wheat. Both the SDS soluble and insoluble polymeric proteins significantly influenced important quality characteristics. In bread wheat, the large monomeric proteins, mainly gliadins, consistently had a significantly negative effect on quality. An increase in the polymeric-to-monomeric protein ratio, led to an improvement of quality characteristics. Despite a large environmental effect on all fractions, a large polymeric-to-monomeric protein ratio can be an

effective measure of baking quality tests, especially in developing countries where quality-testing facilities are often not available.

5.2 Introduction

Bread and durum wheats are allopolyploids, belonging to the the genus *Triticum*. Bread wheat (*T. aestivum* L. *ssp. aestivum*) is a hexaploid (2n = 6x = 42) and durum wheat (*T. turgidum* L. *(Thell.)* ssp. *durum* (Desf.) Husn.) a tetraploid (2n = 4x = 24) with three (AABBDD) and two (AABB) genomes, respectively (Baenziger *et al*., 1994).

The accepted theory of McFadden and Sears (1946) is that bread wheats originated from a cross between a cultivated tetraploid (possibly *T. turgidum*) and a diploid (DD, *Aegilops squarrosa*). Durum and bread wheat can be considered related, due to the genomes derived from related species, with genes for similar, not necessarily identical proteins encoded on the different chromosomes (Tatham *et al*., 1990).

It is this gluten composition, conferred by different proteins, of durum and bread wheat that determines the quality of end-use products. The absence of the D-genome in durum is responsible for the lack of gluten strength required for good baking quality (Peña *et al*., 1994). Despite this, durum wheat is extensively used in Ethiopia for breadmaking.

Pogna *et al*. (1990) found that some favourable dough properties of durum were associated with the presence of a subunit group, LMW-2 (low molecular weight subunits), encoded at *Glu-B1*, as well as some high molecular weight glutenin subunits at the same locus (Boggini *et al*., 1995). The LMW-2 subunit was related to good gluten strength and LMW-1 to poor gluten strength. To improve durum wheat for bread making, the gluten elasticity needs to decrease and become more extensible.

The increased demand and use of durum wheat, in addition to bread wheat, for bread making, necessitates a study to compare and evaluate durum wheat quality to that of existing commercial bread wheat cultivars/lines.

Variation in flour quality is not only attributed to the protein constituents, but is highly influenced by the relative amounts and interactions of the different fractions (Singh *et al*., 1990b). SE-HPLC is a valuable method able to retain information on the protein structure, size-distribution and interactions between protein components, without denaturing the protein polymers (Singh and MacRitchie, 1989). This enables us to better understand the effect of the absence of the D-genome.

The aim of this study was to determine and compare the effect of SDS soluble and SDS insoluble proteins from SE-HPLC on quality characteristics in two wheat types in two environments in Ethiopia.

5.3 Material and Methods

5.3.1 Material

In this study 13 Ethiopian and two South African bread wheat cultivars were compared to 15 Ethiopian durum wheat cultivars. Trials were grown at two environments in Ethiopia in 2001: Adet Research Centre, which is a higher protein potential area, and Motta, which is a low protein potential area. A RCB design with three replications was used. The plot size was 2.5 m^2 at both localities. The bread and durum wheat trials were planted as separate trials adjacent to each other. The environmental effects were therefore similar enough to make comparisons of the protein fractions and quality characteristics. As trials could not be compared statistically in one locality, trends in the averages of the trials were compared.

After harvesting, the material was transported to South Africa and yield and quality trait data was measured in triplicate at the laboratories of the ARC-SGI, Bethlehem, South Africa (Chapter 3, Table 3.2).

5.3.2 Methods

Proteins were extracted from the wheat flour with a two-step extraction procedure developed by Gupta and colleagues (1993). The first step, as described in Chapter 3, extracts the proteins soluble in dilute SDS, while the second extract contains proteins soluble only after sonication.

5.3.3 SE-HPLC

SE-HPLC was performed as described in section 3.3.3.

5.3.4 Statistical analysis

All statistical analyses were done with Agrobase (2000) as described in section 3.3.4.

5.4 Results and discussion

5.4.1 Results

Protein fractions for bread and durum cultivars/lines at Adet and Motta, Ethiopia

Results are given in Table 5.1.

SDS soluble LPP: Durum wheat had a higher average for LPP at both locations than the bread wheat. At Motta the difference was very large. The average of the bread wheat genotypes for the two environments differed. The average obtained for durum seemed stable.

SDS soluble SPP: Similar averages were obtained for both durum and bread wheats at Adet. The opposite was true at Motta where the bread wheat's averages were almost half, compared to durum wheat. A significant reduction in this fraction was seen for bread wheat across environments.

SDS soluble LMP: Consistently lower averages were obtainded for the durum wheat, compared to bread wheat, across localities. The bread wheat genotypes showed a higher average for Motta than Adet.

SDS soluble SMP: The average SMP fraction for bread wheat was lower than that obtained for durum wheat genotypes. The bread wheat genotypes averages differed across environments, with the average obtained at Motta the lowest.

SDS insoluble LPP: Durum had higher averages for this fraction at both environments. The difference between averages at Motta was distinct. Durum wheat had a slightly higher average at Motta while the reverse was true for bread wheat.

SDS insoluble SPP: The highest average was that of bread wheat at Adet. The durum wheat averages were lower at both environments. No distinct differences were observed across environments.

SDS insoluble LMP: This was higher for bread wheat genotypes at both environments while, the highest averages were obtained at Motta. Durum wheat had a slightly lower average at Motta, compared to Adet. The reverse was true for the bread wheat averages.

SDS insoluble SMP: This was higher for durum wheat genotypes at both environments. The bread wheat average at Motta was slightly higher than that obtained at Adet.

LUPP: The average for LUPP was higher for bread wheat genotypes at both environments. It was slightly higher at Motta. The durum wheat averages showed no differences across environments with the averages obtained 66.87 (Adet) and 66.17 (Motta).

TUPP: Bread wheat had a distinctly higher average TUPP at both environments, with averages of 75.43 and 78.44, respectively. The averages of durum wheat genotypes ranged from 23.27 (Adet) to 19.95 (Motta).

Table 5.1 Comparison of average protein fractions for bread and durum wheat for two localities

Protein fraction	Adet		Motta	
	Bread	Durum	Bread	Durum
LPP	6.17	7.28	3.78	7.12
SPP	16.29	16.46	10.79	20.10
LMP	52.53	43.29	68.92	44.58
SMP	20.40	28.41	12.76	24.70
LPP	18.97	24.27	14.62	29.11
SPP	34.81	23.75	29.61	24.26
LMP	30.41	29.95	36.33	23.71
SMP	9.68	16.86	12.97	16.38
LUPP	70.68	66.87	75.25	66.17
TUPP	75.43	23.27	78.44	19.95

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble) mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins.

Comparison of quality characteristics for bread and durum wheat at Adet and Motta, Ethiopia

Results are given inTable 5.2.

SDSS: The highest average was that of bread wheat at Adet. This value was much higher than the average obtained at Motta and the averages of durum wheat at both environments.

VK: Both bread and durum wheat's averages at Adet was higher than at Motta. The durum average at Adet was much higher than the other averages.

FPC: Similar average flour protein content was seen for durum and bread wheat at Motta. The FPC average was the highest for durum wheat at Adet.

FLY: The flour yield was almost similar for durum and bread wheat at Motta, and for bread wheat at Adet. The average flour yield for durum wheat at Adet was the lowest.

MDT: The mixograph development times obtained for both durum and bread wheat were shorter at Adet when compared to the averages obtained for Motta. The MDT for durum at Adet was slightly shorter, the reverse was true at Motta.

SKCS-weight: The average SKCS-weight was slightly lower for bread wheat at both environments when compared to durum wheat. The average SKCS-weight for durum wheat at Adet was the highest.

SKCS-diameter: The average for durum wheat was slightly higher at both environments than bread wheat. The bread wheat average (2.16) at Adet was the lowest.

SKCS-hardness index: The average SKCS-hardness obtained for durum wheat at Adet was distinctly higher compared to the rest. The lowest average was that of bread wheat genotypes at Motta.

Table 5.2 Comparison of averages of bread making results obtained for durum and bread wheat in two localities

	Adet		Motta	
	Bread	**Durum**	**Bread**	**Durum**
SDSS	52.27	23.73	36.53	19.93
VK	67.67	83.67	53.53	47.33
FPC	9.61	11.13	7.27	7.39
FLY	61.13	54.59	60.85	61.41
MDT	2.89	2.81	3.77	4.00
WEIGHT	30.57	36.74	32.18	35.25
DIAM	2.16	2.57	2.28	2.50
HI	48.69	69.99	40.61	49.35

SDSS = SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, Weight = SKCS single kernel weight, Diam = SKCS single kernel diameter, HI = SKCS-hardness index

Comparison of combined averages of protein fractions for bread and durum wheat at two environments

Results are given in Table 5.3.

SDS soluble LPP: The durum wheat entries had averages higher than the bread wheat entries, across both environments. Durum wheat entry DZ2212 had the highest LPP fraction. Yilma had the lowest LPP fraction. HAR2807 was the highest ranking bread wheat entry. SST825, Kariega and HAR1775 had the lowest LPP fractions.

SDS soluble SPP: The durum entries had the highest mean averages across both environments. There were no significant differences between any of the durum entries. The SPP for Yilma, the lowest ranking durum wheat, was higher than the SPP of all the bread wheat entries. HAR1775 had the lowest SDS soluble SPP average over both localities.

SDS soluble LMP: The bread wheat entries had the highest LMP values. Bread wheat entry HAR1775 had the highest LMP value, followed by SST825 and Kariega ranking second and third respectively. The highest ranking durum wheat DZ1924 had a LMP fraction lower than the lowest ranking bread wheat HAR2807. DZ1652 had the lowest LMP average across both environments.

SDS soluble SMP: Durum CD95294-1 was higher than all the durum and bread entries. The lowest ranking durum wheat DZ1748 was higher than the highest ranking bread wheat HAR1709. The South African entries, Kariega and SST825 had the lowest SMP fraction of all the entries.

SDS insoluble LPP: The bread wheat entries had averages lower than that of the durum wheat, across both environments. HAR2505, the bread wheat entry with the highest LPP value, was lower than the lowest durum entry, DZ1691. HAR1522 had the lowest LPP value.

SDS insoluble SPP: The bread wheat had the highest averages across both environments. HAR604 was higher than all the entries tested, with the

exception of HAR1685 and HAR1868. All durum wheat had lower averages than that of the lowest ranking bread wheat, HAR2348.

SDS insoluble LMP: The durum wheat had lower averages compared to that of the bread wheat entries tested across two environments. Durum wheat DZ1721, DZ2023 and LD357 had lower values than all the bread wheat. HAR2348 had the highest LMP fraction, followed by HAR1522 and HAR2807, ranking second and third, respectively.

SDS insoluble SMP: Bread wheat mostly had lower averages for SMP than durum wheat. The values for HAR604 was lower than all durum wheat.

TUPP: Bread wheat had higher TUPP averages than the durum wheat. Bread wheat HAR2348 had the lowest TUPP average, but it was still higher than that of most of the durum wheat.

LUPP: The average LUPP values of the durum wheat were lower than the averages of the bread wheat. Bread wheat HAR1775 had the highest LUPP value, followed by Kariega, and HAR2505. Durum wheat Yilma and CD95294-1 had the lowest LUPP.

Table 5.3 Combined averages of protein fractions for 15 bread wheat and 15 durum wheat cultivars/lines at two different localities

		SDS Soluble				SDS Insoluble					
TYPE	**NAME**	**LPP**	**SPP**	**LMP**	**SMP**	**LPP**	**SPP**	**LMP**	**SMP**	**TUPP**	**LUPP**
Bread	HAR2457	5.38	12.87	58.74	19.11	15.83	30.99	34.37	11.44	71.95	74.63
	HAR2348	5.18	13.58	57.28	19.09	15.64	29.11	36.74	13.04	70.46	75.12
	HAR2807	5.75	17.62	54.90	17.22	16.01	30.44	35.69	11.34	66.53	73.58
	HAR2096	5.09	12.73	61.00	17.69	15.99	31.88	34.96	10.92	72.87	75.85
	HAR2562	4.94	12.68	61.00	17.27	18.05	32.82	32.70	10.39	74.27	78.51
	ET13A2	5.20	13.60	59.15	18.00	16.11	31.85	31.98	12.24	71.84	75.60
	HAR1709	5.71	14.09	57.13	19.25	16.69	32.90	32.12	14.20	71.47	74.51
	HAR1685	5.25	14.32	59.02	16.39	17.58	33.92	33.29	9.50	72.46	77.00
	HAR604	5.31	14.72	58.06	16.53	17.54	36.12	30.63	8.91	72.82	76.76
	HAR1522	5.33	13.68	59.38	18.77	13.86	32.51	36.05	12.08	70.92	72.23
	HAR1775	3.65	10.38	68.21	14.93	17.04	31.21	35.08	11.40	77.47	82.36
	HAR1868	4.58	13.32	61.95	15.78	17.48	33.71	30.25	10.44	74.09	79.24
	HAR2505	4.77	13.66	63.68	13.70	18.79	31.71	33.37	10.08	73.26	79.75
	KARIEGA	4.25	12.72	65.36	12.61	18.25	32.50	31.94	12.74	74.94	81.11
	SST825	4.30	13.15	66.09	12.31	17.04	31.54	31.39	11.22	73.57	79.85

Table 5.3 Continued

		SDS Soluble				SDS Insoluble					
TYPE	**NAME**	**LPP**	**SPP**	**LMP**	**SMP**	**LPP**	**SPP**	**LMP**	**SMP**	**TUPP**	**LUPP**
Durum	CD96486	7.40	18.74	42.89	27.46	28.47	25.00	27.14	13.88	67.15	20.86
	CD6630	6.83	18.15	44.33	27.54	28.21	23.60	27.42	15.82	67.41	20.10
	CD95294-1y	7.23	17.91	41.85	30.37	29.13	22.95	26.05	16.54	67.80	20.01
	DZ2023	7.42	17.92	44.84	26.25	24.87	27.29	25.72	17.32	67.57	22.98
	YILMA	6.36	17.12	46.01	26.56	26.35	25.55	27.43	17.11	68.61	19.90
	DZ1721	7.26	18.15	44.67	26.95	26.37	22.37	25.85	20.85	65.64	21.93
	DZ2212	8.02	18.95	43.36	26.57	26.64	22.88	27.43	17.63	64.76	23.18
	DZ1924	6.94	17.69	46.41	24.81	24.80	23.92	27.26	19.27	66.50	21.72
	Bichena	7.75	19.22	42.76	26.53	27.31	23.34	25.90	18.02	65.26	22.35
	LD357	7.10	19.22	43.35	26.96	27.17	21.57	24.99	14.59	64.51	21.54
	DZ1640	7.07	18.74	46.01	23.60	26.63	25.25	26.67	14.92	66.46	22.30
	DZ1691	7.55	18.54	44.00	26.40	24.42	27.03	26.83	12.79	66.06	24.38
	DZ1652	7.15	17.27	40.15	29.12	28.23	24.32	26.02	14.35	68.65	20.06
	DZ1748	7.48	18.65	45.11	23.58	26.73	23.52	28.42	17.29	65.88	21.95
	DZ900	6.99	18.94	41.81	25.81	25.56	21.65	28.54	18.71	64.44	22.09
Bread	**Average**	4.98	13.54	60.73	16.58	16.79	32.21	33.37	11.33	72.60	77.07
Durum		7.24	18.35	43.84	26.57	26.73	24.02	26.78	16.61	66.45	21.69

LPP = larger polymeric proteins (SDS-soluble), SPP = smaller polymeric proteins (SDS-soluble), LMP = larger monomeric proteins (SDS-soluble) mainly gliadins (SDS-soluble), SMP = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP = larger polymeric proteins (SDS-insoluble), SPP = smaller polymeric proteins (SDS-insoluble), LMP = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins, SDS = sodium dodecyl sulphate.

Comparison of combined averages of quality characteristics for bread and durum wheat at two environments

Results are given in Table 5.4.

SDSS: Averages for the bread wheat were higher than durum wheat. Kariega had a higher SDS sedimentation than all other entries. HAR2348 had the lowest SDS sedimentation of all the bread wheat, but it was higher than that of the highest ranking durum wheat, CD96486. Durum wheat LD357 had the lowest value.

VK: There were no distinct groupings between the different types of wheat for vitreousness. Durum wheat DZ2212 had the highest percentage vitreousness, followed by HAR2562 (bread wheat) and CD6630 (durum wheat). Entry DZ1640 and Kariega had the lowest VK value of the durum and bread wheat entries, respectively.

FPC: Durum wheat had higher average flour protein content than bread wheat. Durum wheat CD6630 had a much higher protein content than all bread wheat.

FLY: Bread wheat had the highest percentage flour yield. Kariega and ET13A2 had the highest flour yield. Durum wheat DZ1652 had the lowest flour yield percentage.

MDT: Most of the bread wheat had mixing times lower than that of the durum wheat. CD96486 had the longest mixing time of all the durum wheat. Bread wheat HAR2807 and ET13A2 had the shortest mixing times. In South Africa a mixing time of between 2 and 2.5 min is usually required for bread making.

SKCS-weight: Almost all durum wheat had the highest weight values. Durum wheat Bichena had a higher SKCS-weight than all other entries. Bread wheat HAR1522 had the lowest weight.

SKCS-diameter: Durum wheat had higher SKCS-diameters than bread wheat. Bichena had the largest diameter, followed by DZ1748 and DZ2023. Bread wheat HAR604 had a diameter larger than the lowest durum wheat, DZ1652. HAR1775 had the smallest diameter.

SKCS-Hardness: The distinction between the two wheat types was less obvious based on hardness index. The durum wheat had an average hardness index higher than that of bread wheat. Bread wheat HAR2348 had the lowest hardness index across both environments.

Table 5.4 Combined averages of quality characteristics for 15 bread wheat and 15 durum wheat cultivars/lines at two different localities

TYPE	NAME	SDSS	VK	FPC	FLY	MDT	WEIGHT	DIAM	HI
Bread	HAR2457	37.00	56.00	8.30	60.10	2.70	34.59	2.36	45.90
	HAR2348	33.00	60.00	9.00	60.10	2.40	33.37	2.33	17.17
	HAR2807	34.00	75.00	8.70	60.90	2.00	33.37	2.18	57.37
	HAR2096	38.00	66.00	8.90	61.10	2.70	30.95	2.18	53.96
	HAR2562	39.00	77.00	9.20	61.20	3.00	35.18	2.37	60.34
	ET13A2	41.00	63.00	8.90	63.60	2.00	30.63	2.25	22.61
	HAR1709	46.00	66.00	8.40	61.50	2.90	30.26	2.16	47.15
	HAR1685	49.00	49.00	7.80	61.10	3.10	30.16	2.13	38.96
	HAR604	58.00	59.00	7.80	60.10	4.30	36.35	2.44	47.12
	HAR1522	42.00	66.00	8.30	59.30	5.20	25.70	2.03	58.58
	HAR1775	40.00	67.00	8.30	60.70	4.70	26.43	1.92	59.83
	HAR1868	52.00	51.00	7.70	60.00	4.20	29.51	2.11	39.51
	HAR2505	47.00	49.00	8.10	61.40	3.20	30.52	2.21	41.08
	KARIEGA	65.00	40.00	8.70	63.70	3.00	33.30	2.31	26.81
	SST825	50.00	66.00	8.60	60.50	4.70	30.34	2.30	53.38

Table 5.4 Continued

TYPE	NAME	SDSS	VK	FPC	FLY	MDT	WGHT	DIAM	HI
Durum	CD96486	27.00	60.00	9.00	57.40	4.30	34.34	2.46	56.96
	CD6630	22.00	76.00	10.30	56.60	3.10	36.57	2.56	65.12
	CD95294-1y	22.00	67.00	9.30	57.50	3.60	35.86	2.53	62.04
	DZ2023	22.00	58.00	8.60	59.60	3.30	37.25	2.60	54.88
	Yilma	25.00	70.00	9.90	58.60	3.70	35.99	2.54	63.79
	DZ1721	19.00	65.00	9.10	58.00	3.20	36.22	2.55	59.19
	DZ2212	22.00	78.00	9.80	56.70	3.00	36.49	2.53	62.09
	DZ1924	21.00	72.00	9.70	58.00	3.00	36.33	2.57	63.79
	Bichena	22.00	69.00	8.90	58.20	3.40	39.11	2.67	56.68
	LD357	17.00	52.00	8.60	59.00	3.20	33.69	2.44	56.36
	DZ1640	20.00	53.00	8.40	59.40	3.80	34.88	2.49	50.49
	DZ1691	21.00	65.00	9.60	58.50	2.90	35.89	2.54	60.84
	DZ1652	22.00	67.00	9.30	56.40	4.00	34.19	2.43	59.15
	DZ1748	26.00	76.00	9.50	58.00	3.20	37.69	2.62	63.17
	DZ900	20.00	54.00	9.00	58.30	3.50	35.46	2.53	60.44
Bread	**Average**	44.73	60.67	8.45	61.02	3.34	31.38	2.22	44.65
Durum		21.87	65.47	9.27	58.01	3.41	36.00	2.54	59.67

SDSS = SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, Wght = SKCS single kernel weight, Diam = SKCS single kernel diameter, HI = SKCS-hardness index

Significant correlations between specific protein fractions and quality characteristics for Adet

Results are given in Table 5.5.

Correlations were determined from the combined data of both wheat types.

Highly significant correlations were found between all four SDS soluble fractions and quality characteristics. The same was true for the SDS insoluble fractions with the exception of the large monomeric proteins that did not correlate significantly with any of the quality traits. Both SDS soluble and insoluble LPP and SMP correlated negatively with SDS sedimentation, and positively with SKCS-wght, -diam and HI. The reverse was true for the SDS soluble LMP fraction that correlated positively with SDSS and negatively with SKCS-wght, SKCS-diam and SKCS-HI.

The SDS insoluble SPP had a high and significantly positive correlation with SDS sedimentation and flour yield, and negative correlations with SKCS-weight, diameter and hardness. Mixograph development time correlated negatively with both the large and small SDS-soluble polymeric proteins.

Both the total and large unextractable polymeric proteins had a significantly positive correlation with SDS sedimentation, and negative correlations with the single kernel characteristics.

Table 5.5 Significant correlations between specific protein fractions and quality characteristics for Adet

SDS soluble fractions	Characteristic	Correlation	SDS insoluble Fractions	Characteristic	Correlation
LPP	SDSS	-0.422***	LPP	SDSS	-0.412***
	VK	0.362***		VK	0.415***
	FPC	0.326**		FPC	0.439***
	FLY	-0.344**		FLY	-0.613***
	MDT	-0.241*		SKCS-weight	0.561***
	SKCS-weight	0.328**		SKCS-diameter	0.568***
	SKCS-diameter	0.360***		SKCS-hardness	0.440***
	SKCS-hardness	0.245*	SPP	SDSS	0.763***
SPP	MDT	-0.283**		VK	-0.598***
LMP	SDSS	0.583***		FPC	-0.616***
	VK	-0.389***		FLY	0.796***
	FPC	-0.430***		SKCS-weight	-0.545***
	FLY	0.517***		SKCS-diameter	-0.626***
	SKCS-weight	-0.479***		SKCS-hardness	-0.636***
	SKCS-diameter	-0.491***	SMP	SDSS	-0.567***
	SKCS-hardness	-0.331**		VK	0.460***
SMP	SDSS	-0.634***		FPC	0.502***
	VK	0.430***		FLY	-0.588***
	FPC	0.463***		SKCS-weight	0.421***
	FLY	-0.596***		SKCS-diameter	0.469***
	SKCS-weight	0.432***		SKCS-hardness	0.454***
	SKCS-diameter	0.479***	TUPP	SDSS	0.527***
	SKCS-hardness	0.432***		VK	-0.346**
				FPC	-0.330**
				FLY	0.359***
				MDT	0.366***
				SKCS-weight	-0.307**
				SKCS-diameter	-0.335**
				SKCS-hardness	-0.257*
			LUPP	SDSS	0.804***
				VK	-0.567***
				FPC	-0.573***
				FLY	0.861***
				SKCS-weight	-0.621***
				SKCS-diameter	-0.705***
				SKCS-hardness	-0.661***

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Significant correlations between specific protein fractions and quality characteristics for Motta

Results are given in Table 5.6.

Correlations were determined from the combined data of both wheat types.

At Motta none of the fractions correlated significantly with vitreous kernels, flour protein content, and flour yield. SDS soluble and insoluble LPP and SMP fractions correlated negatively with SDS sedimentation and positively with the single kernel characteristics. This was similar for SDS soluble SPP, but the reverse was true for the SDS insoluble SPP fraction. Both the SDS soluble and insoluble large monomeric protein fraction, TUPP and LUPP, correlated positively with SDS sedimentation and negatively with the single kernel characteristics. Mixograph development time correlated negatively with SDS insoluble large monomeric protein and total unextractable polymeric protein.

Table 5.6 Significant correlations between specific protein fractions and quality characteristics for Motta

SDS soluble fractions	Characteristic	Correlation	SDS insoluble Fractions	Characteristic	Correlation
LPP	SDSS	-0.595***	LPP	SDSS	-0.523***
	SKCS-weight	0.448***		MDT	0.242*
	SKCS-diameter	0.486***		SKCS-weight	0.254*
	SKCS-hardness	0.325**		SKCS-diameter	0.336**
SPP	SDSS	-0.641***		SKCS-hardness	0.247*
	SKCS-weight	0.399***	SPP	SDSS	0.664***
	SKCS-diameter	0.485***		SKCS-weight	-0.266*
	SKCS-hardness	0.329**		SKCS-diameter	-0.351***
LMP	SDSS	0.710***		SKCS-hardness	-0.260*
	SKCS-weight	-0.395***	LMP	SDSS	0.520***
	SKCS-diameter	-0.492***		MDT	-0.223*
	SKCS-hardness	-0.319**		SKCS-weight	-0.225*
SMP	SDSS	-0.641***		SKCS-diameter	-0.319**
	SKCS-weight	0.249*		SKCS-hardness	-0.210*
	SKCS-diameter	0.351***	SMP	SDSS	-0.284**
	SKCS-hardness	0.274**		SKCS-weight	0.213*
				SKCS-diameter	0.309***
				SKCS-hardness	0.228*
			TUPP	SDSS	0.654***
				MDT	-0.235*
				SKCS-weight	-0.429**
				SKCS-diameter	-0.495***
				SKCS-hardness	-0.318**
			LUPP	SDSS	0.746***
				SKCS-weight	-0.372***
				SKCS-diameter	-0.484***
				SKCS-hardness	-0.333**

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Significant correlations between specific protein fractions and quality characteristics for combined localities

Results are shown in Table 5.7.

Correlations were determined from the combined data of both wheat types across both environments.

SDS soluble LPP was negatively correlated with SDSS, FLY and MDT and positively with VK, FPC, SKCS-weight, SKCS-diameter and SKCS-hardness. SMP were correlated with the same characteristics as the LPP with the exception of MDT. SDS soluble LMP was negatively correlated with VK, FPC, SKCS-weight, SKCS-diameter and SKCS-hardness. SDSS and FLY were positively correlated with LMP. The SPP was negatively correlated with SDS sedimentation, SKCS-weight and SKCS-diameter.

The combined data for the SDS insoluble protein fractions showed that LPP and SMP were highly significantly correlated with single kernel characteristics, and negatively with SDSS. The LPP correlated negatively and positively with flour yield and MDT respectively. The SDS insoluble LMP and SMP were negatively correlated with SDSS and FPC. The LMP was negatively correlated with vitreous kernels.

The total unextractable protein (TUPP) was negatively correlated with SDSS, VK and FPC and positively with MDT. LUPP was positively correlated with VK, FPC and positively with MDT.

Table 5.7 Significant correlations between specific protein fractions and quality characteristics for the combined localities

SDS Soluble fractions	Characteristic	Correlation	SDS Insoluble Fractions	Characteristic	Correlation
LPP	SDSS	-0.319***	LPP	SDSS	-0.386***
	VK	0.271***		FLY	-0.201**
	FPC	0.364***		MDT	0.198**
	FLY	-0.210**		SKCS-weight	0.328***
	MDT	-0.227**		SKCS-diameter	0.374***
	SKCS-weight	0.351***		SKCS-hardness	0.244**
	SKCS-diameter	0.367***	SPP	SDSS	0.745***
	SKCS-hardness	0.373***		FLY	0.381***
SPP	SDSS	-0.306***		SKCS-weight	-0.438***
	SKCS-weight	0.267***		SKCS-diameter	-0.528***
	SKCS-diameter	0.283***		SKCS-hardness	-0.341***
LMP	SDSS	0.388***	LMP	SDSS	0.221**
	VK	-0.264***		SKCS-diameter	-0.224**
	FPC	-0.430***	SMP	SDSS	-0.448***
	FLY	0.320***		SKCS-weight	0.318***
	SKCS-weight	-0.369***		SKCS-diameter	0.391***
	SKCS-diameter	-0.402***		SKCS-hardness	0.245**
	SKCS-hardness	-0.422***	TUPP	SDSS	0.454***
SMP	SDSS	-0.414***		MDT	0.265***
	VK	0.320***		SKCS-weight	-0.347***
	FPC	0.477***		SKCS-diameter	-0.382***
	FLY	-0.443***		SKCS-hardness	-0.320***
	SKCS-weight	0.303***	LUPP	SDSS	0.712***
	SKCS-diameter	0.352***		FPC	-0.208**
	SKCS-hardness	0.461***		FLY	0.405***
				SKCS-weight	-0.497***
				SKCS-diameter	-0.591***
				SKCS-hardness	-0.444***

LPP = large polymeric proteins, SPP = small polymeric proteins, LMP = large monomeric proteins, SMP = small monomeric proteins, TUPP = % total unextractable polymeric proteins, LUPP = % large unextractable polymeric proteins, SDS = sodium dodecyl sulphate, SDSS = SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, SKCS = single kernel characterization system, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Significant correlations between ratios of mean protein fractions and measured quality characteristics

Results are given in Table 5.8.

The ratio between SDS soluble LPP and LMP had a positive influence on almost all quality characteristics tested, except on SDSS, FLY and MDT. The large polymeric to small monomeric ratio only had an influence on flour yield. The small polymeric protein to large monomeric protein ratio had a significant negative influence on SDSS, but was positively correlated to FPC and single kernel characteristics. The reverse was true for the ratio between the SPP and SMP, with positive correlations with SDSS and FLY, and negative influence on FPC, SKCS-hardness and VK.

The ratio between the SDS insoluble LPP and LMP significantly influenced SDSS, SKCS-weight and SKCS-diameter. The SPP:LMP ratio significantly influenced all quality characteristics tested negatively, except for SDSS, FLY and MDT. The SDS insoluble SPP to SMP significantly influenced SDSS and SKCS-diameter**.**

Table 5.8 Significant correlations between ratios of protein fractions and measured quality characteristics

SDS soluble			SDS insoluble		
Ratio	**Characteristic**	**Correlation**	**Ratio**	**Characteristic**	**Correlation**
LPP:LMP	SDS sedimentation	-0.382***	LPP:LMP	SDS sedimentation	-0.361***
	Vitreous kernels	0.278***		MDT	0.275***
	Flour protein content	0.385***		SKCS-weight	0.242**
	Flour yield	-0.279***		SKCS-diameter	0.300***
	MDT	-0.190*	SPP:LMP	SDS sedimentation	0.363***
	SKCS-weight	0.387***		Vitreous kernels	-0.172*
	SKCS-diameter	0.411***		Flour protein content	-0.190*
	SKCS-hardness	0.393***		Flour yield	0.369***
LPP:SMP	FLY	0.265***		MDT	0.243**
SPP:LMP	SDS sedimentation	-0.382***		SKCS-weight	-0.199**
	Flour protein content	0.208***		SKCS-diameter	-0.224**
	SKCS-weight	0.327***		SKCS-hardness	-0.245**
	SKCS-diameter	0.347***	SPP:SMP	SDS sedimentation	0.191*
	SKCS-Hardness	0.287***		SKCS-diameter	-0.150*
SPP:SMP	SDS sedimentation	0.216**			
	Vitreous kernels	-0.280***			
	Flour protein content	-0.348***			
	Flour yield	0.420***			
	SKCS-hardness	-0.292***			

* $p \leq 0.05$ ** $p \leq 0.01$

5.4.2. Discussion and conclusions

The absence of the D-genome in durum apparently did not influence the protein concentrations much when compared to bread wheat. Durum wheat had higher average SDS soluble and insoluble LPP and SMP fractions, at both environments. Despite this, the averages obtained for large unextractable and total unextractable polymeric proteins of bread wheat were much higher than the averages of durum wheat lines.

The average SDS soluble SPP fraction of durum wheat was higher than that bread wheat at Motta. This might be due to favourable conditions for the expression of LMW-1 and especially LMW-2, present in the durum population. Further studies are needed to confirm this.

The effect of differences in quantity of protein fractions were clear in results obtained from the quality test. Bread wheat lines had the highest SDS sedimentation volumes. This may be related to the higher SDS soluble SPP fraction detected in durum. This confirmed results by Masci and colleques (2003), who found that an over-expression of certain LMW-GS lead to a reduction in SDS sedimentation volumes. Low SDS sedimentation values are indicative of poorer gluten strength.

Kariega had the least number of vitreous kernels. No conclusive bread wheat or durum wheat groups could be identified at Motta, the protein poor environment, though at Adet most of the durum lines had more vitreous kernels than the bread lines. Bread wheat lines with a high vitreousness percentage confirmed the correlation between hardness, vitreousness and flour protein content (Eliasson and Larsson, 1993). The average hardness was higher for durum wheat types than for bread wheat types, except at Motta where no distinction between the two wheat types could be made. This indicates the environmental influence on grain filling and kernel hardness.

Mixograph development time is considered to be a direct measure of quality of flour. The mixograph development time average obtained for durum wheat

was slightly longer than that of bread wheat lines. This contradicted the findings of Lee *et al.* (1999c) who found that an increase in LMW-GS reduced dough mixing time and peak resistance. The small differences between the mixing times obtained for the two wheat types may be an indication of the importance of LPP:SPP (HMW-GS to LMW-GS) ratio. This agreed with the findings of MacRitchie and Gupta (1993) who found that increasing the relative HMW-GS to LMW-GS quantity relates positively to dough mixing time.

SDS soluble and insoluble LMP correlated positively with SDS sedimentation across environments, individually and combined. Only the large and total unextractable proteins and SDS insoluble SPP had the same positive correlation with SDS sedimentation, continuously. The small polymeric proteins and the large unextractable polymeric protein had an exceptionally high correlation value with SDS sedimentation, across localities, compared to the rest.

The correlation indicated the importance of the large monomeric proteins' contribution, direct or indirect, to baking quality and SDS sedimentation specifically. The correlation found between SDS sedimentation and large monomeric proteins, might be due to the strong linkage that exists between certain gliadins and low molecular weight subunits (Porceddu *et al.*, 1998). The other protein fractions correlated negatively with SDS sedimentation.

At Adet, the high protein environment, the large and small polymeric proteins both correlated negatively with mixograph development time. The only positive correlation with MDT was with TUPP. At the low protein environment, Motta, only the SDS insoluble proteins influenced MDT significantly. Mixograph development time was positively correlated with LPP and negatively with LMP and TUPP. Across environments, SDS soluble LPP correlated positively and SDS insoluble LPP negatively with MDT, respectively. The large unextractable proteins had a positive influence on MDT. This shows that an increase in the ratio of SDS insoluble to SDS soluble proteins will lead to an increase in mixing time.

A negative correlation existed between SDS soluble and insoluble LPP:LMP and SDS sedimentation. With the exception of SDS soluble SPP:LMP, the SDS soluble and insoluble ratio's of SPP:LMP and SPP:SMP positively influenced the SDS sedimentation. This confirmed the importance of the low molecular weight glutenin subunits' influence on this quality trait.

SDS soluble LPP:LMP negatively influenced mixograph development time. The reverse was true for the SDS insoluble ratios of LPP:LMP and SPP:LMP that were positively correlated to mixograph development time. The positive correlation between the SPP:LMP and MDT contradicted literature. This confirmed the importance of SDS insoluble fractions, especially the ratio between extractable and unextractable proteins. This also points to the need of reassessing the importance of the small polymeric proteins (LMW-GS) as well as the monomeric proteins (gliadins).

Durum wheat yielded comparable results to that of bread wheat, despite the absence of the D-genome. Some of the durum lines outperformed the bread wheat lines for quality characteristics.

This study confirmed the importance of quantifying the different protein constituents in the quest to better understand the interactions and functionality of protein components. Comparing bread wheat with durum wheat re-affirmed the importance of not only the presence of proteins, but also the quantity of specific protein fractions.

The significant correlations observed between specific proteins and protein ratios, and quality traits created the possibility of predicting quality. Incorporation of this knowledge in breeding programmes will allow us to utilise more of the diversity existing in breeding populations.

Though it is impossible to cross durum and bread wheat, the comparison indicated the possibility of using durum wheat as successful alternative for bread making. The diversity found among durum wheat has the potential to be used in a breeding programme to help improve the general baking quality

of durum flour. Even the over stable lines, as indicated by a long mixing time may be useful commercially, to fortify material of poorer quality.

5.5 References

Agrobase, 2000. Agronomix Software, Inc., Agrobase™, 71 Waterloo St. Winnipeg, Manitoba R3N0S4, Canada.

Baenziger, P.S., Moreno-Sevilla, B., Yen, Y., Oberthur L. and Gustavson, V., 1994. Wheat Breeding and Genetics. In: Encyclopedia of Agricultural Science, Vol. 4. Academic Press, Inc. California, pp 515-524.

Boggini, G., Tusa P. and Pogna, N.E., 1995. Bread making quality of durum wheat genotypes with some novel glutenin subunit compositions. *Journal of Cereal Science* 22:105-113.

Eliasson, A. and Larsson, K., 1993. Cereals in breadmaking, a molecular colloidal approach. Marcal Dekker, Inc., New York.

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

Lee, Y.-K., Ciaffi, M., Morrel, M.K. and Appels R., 1999a. The low molecular-weight glutenin subunit proteins of primitive wheats. III. The genes from the the A-genome species. *Theoretical and Applied Genetics* 98:119-125.

MacRitchie, F. and Gupta, R.B., 1993. Functionality composition relationships of wheat flour as a result of variation in sulphur availability. *Australian Journal of Agricultural Research* 44:1767-1774.

Masci, S., D'Ovidio, R., Scossa, F., Patacchini, C., Lafiandra, D., Anderson, O.D. and Blechl, A.E., 2003. Production and characterization of a transgenic bread wheat line over-expressing a low-molecular-weight glutenin subunit gene. *Molecular Breeding* 12:209-222.

McFadden, E.S. and. Sears, E.R., 1946. The origin of *Triticum* spelta and its free threshing hexaploid relatives. *Journal of Heredity* 37:81-89, 107-116.

Peña, R.J., Zarco-Hernandez, J., Amaya-Celis, A. and Majeeb-Kazi, A., 1994. Relationship between chromosome 1B-encoded glutenin subunits

compositions and bread making quality characteristics of some durum wheat (*Triticum turgidum*) cultivars. *Journal of Cereal Science* 19:243-249.

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. *Journal of Cereal Science* 11:15-34.

Porceddu, E., Turchetta, T., Masci, S., D'Ovidio, R., Lafiandra, D., Kasarda, D.D., Impiglia, A. and Nachit, M.M., 1998. Variation in endosperm protein composition and technological quality properties in durum wheat. *Euphytica* 100:197-205.

Singh, N.K. and MacRitchie, F., 1989. Controlled degradation as a tool for probing wheat protein structure. In: Wheat end-use properties: Wheat and flour characterization for specific end-uses. H. Slovaara (Ed.), University of Helsinki, Helsinki, pp 321-326.

Singh, N.K., Donovan, R. and MacRitchie, F., 1990a. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. II. Relative quantity of glutenin as a measure of bread-making quality. *Cereal Chemistry* 67:161-170.

Tatham, A.S., Shewry, P.R. and Belton, P.S., 1990. Structural studies of cereal prolamins including wheat gluten. In: Advances in Cereal Science and Technology, Vol. 10. Y. Pomeranz (Ed.), American Association of Cereal Chemists, Inc., St. Paul, Minnesota, pp 1-78.

Chapter 6

Prediction of baking quality in Ethiopian bread wheat by reversed-phase high-performance liquid chromatography

6.1 Abstract

Gluten proteins play a key role in determining the functionality and quality of dough. The complexity of the role of gluten proteins in bread-making quality is due to the interaction between high and low molecular weight glutenins and gliadins. Reversed-phase high-performance liquid chromatography has become the tool of choice in studying protein interactions. In this study, reversed-phase high-performance liquid chromatography (RP-HPLC) was used to compare the glutenin and gliadin composition of 13 Ethiopian and two South African bread wheat cultivars/lines planted in two diverse environments. This was done to determine the influence of the proteins individually and their interactions, on breadmaking quality. It was found that the environment had a major effect on the protein constituents, especially quantitatively. Significant correlations were found between the different HMW-GS, LMW-GS and gliadin proteins and quality parameters and between different protein components. Stepwise multiple regression analysis was used to identify specific optimal combinations that could best account for the variation. Stepwise regression models were obtained indicating the contribution and possible interaction between protein subunits. Specific proteins were identified that significantly influenced quality parameters across the environments.

6.2 Introduction

Baking quality of wheat is influenced by the quality and quantity of the gluten proteins present in the flour. Quality characteristics of dough can thus be seen as the interaction between the different protein components. The different subunits contribute to different functionalities of the dough and gluten. Monomeric gliadins interact with lipids during fermentation and help trap gas bubbles, adding to viscosity of the dough. Polymeric glutenin subunits impart strength and elasticity to the dough through the formation of large aggregates (Primard *et al.*, 1991).

To improve baking quality one must attain the optimal combinations and amounts of gliadins and glutenins (Gianibelli *et al.*, 2001). This necessitates a better understanding of the correlations and interactions between the different protein subunits.

The characterization of proteins has been inhibited due to their heterogeneity, tendency to polymerise and poor solubility. RP-HPLC has been found capable of overcoming these problems giving high-resolution separation of these proteins (Bietz, 1983).

Separation is based on the hydrophobicity of the proteins, eluting in order of increasing hydrophobicity. According to Gianibelli *et al.* (2001), LMW-GS has a higher surface hydrophobicity than HMW-GS. Lookhart and Albers (1988) found that ω-gliadins were the least hydrophobic and that the hydrophobicity increased from ω-, β-, α-, to γ gliadins. This causes the LMW-GS to elute after the HMW-GS and the gliadins in the order of ω-,β-, α-, and γ gliadins.

The aim of this study was to identify and quantify individual polymeric and monomeric proteins by RP-HPLC and to determine the effect of the individual proteins on quality characteristics in bread wheat types in two environments in Ethiopia.

6.3 Material and Methods

6.3.1 Material

Thirteen popularly grown Ethiopian and two South African wheat cultivars/lines of known quality (Table 6.1) were used in this study. The main criterion of selection for the Ethiopian material was agronomic performance. Trials were grown at two environments in Ethiopia in 2001 namely at: Adet Research Center, which is a higher protein potential area, and Motta, which is a low protein potential area. A RCB design with three replications was used. The plot size was 2.5 m^2 (six rows of 2.5 m length and 20 cm spacing between the rows) at both localities. Fertilizer was applied at the recommended rate for high yield, i.e., 92/46 kg N/P_2O_5 ha^{-1}. All recommended wheat management practices were exercised.

Table 6.1 Entries of wheat cultivars/lines included in this study

Entry	Cultivar	Country
1	HAR2457	Ethiopia
2	HAR2348	Ethiopia
3	HAR2807	Ethiopia
4	HAR2096	Ethiopia
5	HAR2562	Ethiopia
6	ET13A2	Ethiopia
7	HAR1709	Ethiopia
8	HAR1685	Ethiopia
9	HAR604	Ethiopia
10	HAR1522	Ethiopia
11	HAR1775	Ethiopia
12	HAR1868	Ethiopia
13	HAR2505	Ethiopia
14	Kariega	South Africa
15	SST825	South Africa

After harvesting, the material was transported to South Africa and yield and quality trait data were measured in triplicate at the laboratories of the ARC-SGI, Bethlehem, South Africa (Chapter 3, Table 3.2).

6.3.2 Methods

Proteins were extracted using a modified method of Marchylo *et al.* (1989). Flour samples (100 mg) were extracted with 1 ml 70% (v/v) ethanol for 30 min at room temperature with continuous shaking. Following centrifugation at 17 000 g for 4 min, the supernatant, consisting of monomeric protein was transferred to a new vial and stored at 4°C. The remaining pellet was washed twice with 1 ml 50% (v/v) propan-1-ol with continuous shaking for 30 min at room temperature. Following centrifugation at 17 000 g for 4 min, the supernatant was discarded and the pellet re-suspended in 1 ml buffer (pH 6.6) containing 50% (v/v) propan-1-ol, 2 M urea, 0.2 M Tris and 1% (w/v) DTT. Samples were vortexed and placed in a waterbath at 60°C for 1 h. The samples were alkylated with the addition of 10 µl of 4-vinylpyridine at 60°C for 15 min. The supernatant recovered after centrifugation at 17 000 g for 4 min, was filtered through PVDF syringe filters (0.45 µm) into glass vials.

6.3.3 RP-HPLC

Reversed-phase HPLC analysis was performed on a Thermo Surveyor^{TM} LC System using a Supelcosil LC-308 column (C8, 300 Å pore size, 5 µm particle size, 250 x 4.6 mm). Samples (80 µl) were injected and run at a solvent flow rate of 0.800 ml/min using a column temperature of 50°C. The two eluants used were: (A) 95% acetonitrile (ACN) containing 0.1% (v/v) trifluoroacetic acid (TFA); (B) water containing 5% (v/v) ACN and 0.11% (v/v) TFA.

The following linear solvent gradient was used for gliadin analysis: 20-26% (A), 0-15 min; 26-46% (A), 15-30 min; 46-60% (A), 30-35 min; 60-90% (A), 35-40 min; 90% (A), 40-45 min; 90-20% (A), 45-55 min; 20% (A), 55-60 min.

Effluent was detected at 210 nm, and gliadin components were quantified by integration of the chromatogram areas using ChromQuest™ software.

The following linear solvent gradient was used for glutenin analysis: 20-40% (A), 0-40 min; 40-56% (A), 40-42 min; 56-90% (A), 42-60 min; 90-20% (A), 70-80 min. Effluent was detected at 210 nm, and glutenin components were quantified by integration of the chromatogram areas using ChromQuest™ software.

6.3.4 Statistical analysis

The chromatographic peaks (Appendix A, Figure 3 and 4) were labeled according to their retention times. Subsequent relationships between single and groups of RP-HPLC protein peaks and quality characteristics were investigated by determining linear correlation coefficients. Minor and major peaks were summed to form groups. Statistical analyses were done with Agrobase (2000) software. The best combinations of gliadins and glutenins for each quality parameter were selected based on the highest amount of variation (R^2 value) using the stepwise multiple regression procedure (SAS, 2004). Only fractions meeting the statistical significance level of 0.05 were included in the model. Multiple regression models were developed independently for the two localities and the combined data set.

6.4 Results and discussion

6.4.1 Results

Significant correlations between specific glutenin protein fractions and quality characteristics for Adet and Motta

Results are given in Table 6.2.

Please refer to Appendix B for RP-HPLC chromatogram of glutenin proteins (Figure 3.).

At Adet, peaks 27, 39 and 48 correlated positively with mixograph development time. Peaks 27 and 39 correlated negatively with SKCS-weight. Peak 33 and peak 41 respectively, correlated positively with vitreous kernels and SKCS-hardness index. Proteins eluting at 14, 20, 23, and 25 min correlated with SDSS. Only peak 23 correlated positively with SDSS.

Peaks 20 and 45, at Motta had similar negative correlations with SDSS, than at Adet. Mixograph development time was positively correlated with the protein eluting at 36 min. Peaks 14, 20, 23, 25 and 45 were positively correlated with vitreous kernels. Peaks 20 and 23 had additional negative correlations with flour yield and positive correlations with SKCS-hardness index. Proteins eluting at 29 and 42 min correlated positively with flour protein content.

Table 6.2 Significant correlations between specific glutenin protein fractions and quality characteristics for Adet and Motta

Location	Peak	Characteristic	Correlation
Adet	P14	SDSS	-0.303*
	P20	SDSS	-0.334*
	P23	SDSS	0.308*
	P25	SDSS	-0.418**
	P26	SKCS-Diameter	0.402**
	P27	MDT	0.405**
		SKCS-Weight	-0.424**
	P33	VK	0.400**
	P39	MDT	0.414**
		SKCS-Weight	-0.475**
		SKCS-Diameter	-0.434**
	P41	SKCS-Hardness Index	0.418**
	P47	SKCS-Diameter	0.398**
	P48	MDT	0.476**
Motta	P12	FLY	-0.413**
	P14	VK	0.400**
	P16	FLY	0.494**
	P20	SDSS	-0.471**
		VK	0.489**
		FLY	-0.422**
		SKCS-Hardness index	0.478**
	P23	VK	0.526**
		FLY	-0.422**
		SKCS-Hardness index	0.430**
	P25	VK	0.480**
		SKCS-Hardness index	0.465**
	P29	FPC	0.442**
	P34	FLY	0.418**
	P36	MDT	0.401**
	P40	FLY	0.406**
		SKCS-Hardness index	-0.476**
	P42	FPC	0.404**
	P45	SDSS	-0.446**
		VK	0.515**

SDS = sodium dodecyl sulphate, SDSS=SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, SKCS = single kernel characterization system

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Significant correlations between specific gliadin protein fractions and quality characteristics for Adet and Motta

Results are given in Table 6.3.

Please refer to Appendix B for RP-HPLC chromatogram gliadin proteins (Figure 4).

Gliadin peaks 17 and 20.9 correlated positively and peaks 24.9, 25.5 and 36.2 negatively with SDSS, at Adet. Flour protein content correlated negatively with peaks 42.2, 42.6, 42.8, 43.2, 43.5 and 46, and positively with peak 33.5. Peak 27.5 was negatively correlated with MDT. Vitreous kernels were negatively correlated with peaks 39.5, 40.9, 42.2 and positively with peak 33.5.

At Motta, peaks 24.5, 33.2, 33.5 (negative) and peak 50 (positive) correlated with SDSS. Vitreous kernels were negatively correlated with peaks 17, 28.5, 34.6 and 46 and positively correlated with peak 25.5. Peak 17 correlated negatively with flour protein content and peaks 24.5, 25.5 and 33.2 positively. SKCS-hardness index correlated with peaks 24.9 and 28.5.

Table 6.3 Significant correlations between specific gliadin protein fractions and quality characteristics for Adet and Motta

Location	Gliadin Peak	Characteristic	Correlation
Adet	P17	SDSS	0.5452***
	P20.9		0.5141***
	P24.9		-0.4937***
	P25.5		-0.4452***
	P36.2		0.5321***
	P33.5	VK	0.3918**
	P39.5		-0.4338**
	P40.9		-0.4163**
	P42.2		-0.4725**
	P33.5	FPC	0.4397**
	P42.2		-0.4534**
	P42.6		-0.4214**
	P42.8		-0.4087**
	P43.2		-0.5921***
	P43.5		-0.5444***
	P46		-0.4197**
	P29.2	FLY	0.4190**
	P27.5	MDT	-0.4469**
	P21.5	SKCS-Weight	0.3979**
	P31.2		-0.5235***
Motta	P24.5	SDSS	-0.4773**
	P33.2		-0.4387**
	P33.5		-0.4156**
	P50		0.5190***
	P17	VK	-0.4879***
	P25.5		0.4502**
	P28.5		-0.4097**
	P34.6		-0.4081**
	P46		-0.4103**
	P17	FPC	-0.4750**
	P24.5		0.4351**
	P25.5		0.4147**
	P33.2		0.3947**
	P24.9	SKCS-Hardness index	0.4111**
	P28.5		-0.4648**

SDS = sodium dodecyl sulphate, SDSS=SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, SKCS = single kernel characterization system

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Significant correlations between specific glutenin protein fractions and quality characteristics for both localities

Results are given in Table 6.4.

SDS sedimentation correlated negatively with peaks 20, 42, 45, 46, 47, 48, and 49. Peaks 47 and 48 correlated negatively with vitreous kernels and flour protein content. The reverse was true for peaks 14, 25, 29, 37, 52 and 56 which correlated positively with vitreous kernels and flour protein content. Mixograph development time was positively influenced by peaks 39, 45, 46, 48 and negatively by peaks 31 and 37. Peaks 25, 41 (positive) and 47 (negative) correlated significantly with SKCS-diameter. SKCS-hardness index was positively influenced by peaks 25, 29 and 41.

Table 6.4 Significant correlations between specific glutenin protein fractions and quality characteristics for both localities

Glutenin peak	Characteristic	Correlation
P20	SDSS	-0.5005***
P42		-0.4992***
P45		-0.6620***
P46		-0.6262***
P47		-0.5688***
P48		-0.5310***
P49		-0.4234***
P14	VK	0.3401**
P25		0.3717***
P29		0.3342**
P37		0.4738***
P47		-0.4120***
P48		-0.4695***
P52		0.3392**
P56		0.3226**
P14	FPC	0.4798***
P16		0.3688***
P24		0.3261***
P25		0.4368***
P29		0.4777***
P31		0.4294****
P37		0.4738***
P45		-0.7726***
P46		-0.7183***
P47		-0.7136***
P48		-0.6696***
P49		-0.3218***
P52		0.5003***
P56		0.4473***
P59		0.4829***
P39	FLY	-0.3262**
P31	MDT	-0.3228**
P37		-0.3195**
P39		0.3620***
P45		0.3431**
P46		0.3632***
P48		0.3182**
P39	SKCS-Weight	-0.3148**
P25	SKCS-Diameter	-0.3358**
P41		-0.3064**
P47		0.3483***
P25	SKCS-Hardness index	0.3392***
P29		0.3377**
P41		0.4008***

SDS = sodium dodecyl sulphate, SDSS=SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, SKCS = single kernel characterization system

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Significant correlations between specific gliadin protein fractions and quality characteristics at both locations

Results are given in Table 6.5.

Peaks 24.5, 24.9, 26.6 and 33.5 correlated negatively with SDSS. The reverse was true for peaks 17, 20.9, 32.2, 35.8, 42.6 and 42.8, which correlated positively with SDSS. Vitreous kernels were positively correlated with peaks 25.5, 42.8 and negatively with peaks 32.6, 34.6 and 50. Flour yield was negatively correlated with peak 25.2. Peak 31.2 (positive) and peak 33.2 (negative) were correlated with mixograph development time. SKCS-hardness index was positively correlated with peak 26.2 and negatively with peaks 28.5 and 34.6. Flour protein content was positively correlated with peaks 17, 26.2, 29.2, 32.2, 33.2 and 35.8 and negatively with peaks 22.5, 23, 26.6, 31.2, 32.6, 34.6 and 39.2.

Table 6.5 Significant correlations between specific gliadin protein fractions and quality characteristics at both locations

Gliadin Peak	Characteristic	Correlation
P17	SDSS	0.7059***
P20.9		0.5005***
P24.5		-0.3599***
P24.9		-0.3366**
P26.6		-0.3474**
P32.2		0.4185***
P33.5		-0.4246***
P35.8		0.4653***
P42.6		0.3447**
P42.8		0.3368**
P25.5	VK	0.3439**
P32.6		-0.3671***
P34.6		-0.4694***
P42.8		0.3754***
P50		-0.3419**
P17	FPC	0.4786***
P22.5		-0.3863***
P23		-0.3413**
P26.2		0.3980***
P26.6		-0.3400**
P29.2		0.4339***
P31.2		-0.3195**
P32.2		0.4065***
P32.6		-0.3361**
P33.2		0.3384**
P34.6		-0.4302***
P35.8		0.4464***
P39.2		-0.3018**
P25.2	FLY	-0.3055**
P31.2	MDT	0.3028**
P33.2		-0.3056**
P26.2	SKCS-hardness	0.3095**
P28.5		-0.3298**
P34.6		-0.2842**

SDS = sodium dodecyl sulphate, SDSS=SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, SKCS = single kernel characterization system

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Significant correlations between specific glutenin-glutenin and gliadin-gliadin proteins

Results are given in Table 6.6.

Significant correlations existed between the presence of certain peaks with the presence of others.

Glutenin peak 20 was positively correlated with peak 49, and peak 47 with peaks 42, 46 and 49. Peak 42 was also positively correlated with peak 45.

Strong correlations were observed between glutenin peaks and groups of peaks. The strongest correlations were between peaks 49 and 49A (r= 0.92), as well as 46 and 45A (r=0.9). Only one negative correlation was observed between glutenin and groups of peaks, namely peak 45A and peak 52

A higher number of correlations were observed between single gliadin and summed gliadin peaks. Peaks correlated significantly positively with some and strongly negatively with others. The strongest correlations were between peaks at 26.2 and 26.6 (r=-0.706), 43.2 and 44.5 (r=0.691), 29.2 and 29.5 (r=-0.63).

The highest correlations between single and summed gliadin peaks were between 24.9 and 24A (r=0.853), and 39.2 and 39A (r=0.852).

Table 6.6 Significant correlations between glutenin-glutenin and gliadin-gliadin fractions

Glutenin			Gliadin		
Peak 1	**Peak 2**	**Correlation**	**Peak 1**	**Peak 2**	**Correlation**
P14	P16	0.5039***	P17	P20.9	0.4748***
P20	P49	0.5093***		P34.6	-0.4515***
	P49A	0.5189***		P42.6	0.4061***
P25	P26A	0.6587***		42.8	0.4999***
P26	P26A	0.5476***	P20.9	P32A	0.4261***
P27	P26A	0.5197***	P21.5	P22.2	0.4106***
P29	P30A	0.7420***	P22.5	P23	0.6840***
P32	P33A	0.6964***		P26.6	0.4023***
P33	P33A	0.7952***		P39.2	0.5055***
P34	P35A	0.5845***		P39A	0.6181***
P40	P40A	0.6499***		P42.2	-0.5011***
P42	P42A	0.7947***		P42.5A	-0.5327***
	P45	0.5876***		P43.5	-0.4132***
	P47	0.6727***	P24.5	P24A	0.6551***
	P49A	0.6123***		P27.5	0.4155***
P46	P45A	0.9000***	P24.9	P24A	0.8527***
	P47	0.7613***		P32A	-0.4104***
	P49A	0.6246***		P42.2	-0.5991***
P45A	P47	0.7363***	P24A	P32A	-0.4188***
	P49A	0.5676***		P39.2	0.4230***
	P52	-0.5066***		P39A	0.5310***
P47	P49	0.6228***		P42.6	0.4090***
	P49A	0.7998***		P42.5A	-0.6000***
P49	P49A	0.9245***	P25.5	P28.5	-0.4258***
			P26.2	P26.6	-0.7061***
			P27.5	P39A	0.4149***
				P46	-0.4481**
			P28.5	P29A	-0.4074***
			P29.2	P29.5	-0.6318***
				P29A	0.5210***
				P43.2	-0.4811***
			P29A	P49	-0.5036***
				P50	-0.5000***
			P31.2	P32.2	-0.5737***
			P32.2	P32A	0.7449***
			P32.6	P32A	0.6584***
			P33.5	P42.6	-0.4855***
			P34.6	P35.8	-0.5060***
				P35A	0.6421***
			P35A	P42.2	0.4901***
			P39.2	P39A	0.8521***
				P42.2	-0.6322***
			P39A	P42.2	-0.7010***
				P46	-0.5102***
			P40.5	P40.9	-0.6064***
			P42.2	P43.5	0.5585***
				P44.5	0.5026***
				P46	0.5313***
				P47	0.5767***
			P42.6	P43.2	0.5103***
			P43.2	P43.5	0.5160***
				P44.5	0.6908***
				P47	0.5517***
				P49	0.5355***
			P44.5	P47	0.5238***
				P49	0.5042***
			P45.5	P47	0.5838***
			P46	P47	0.5028***
			P49	P50	0.5728***

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, A = Summed minor and major fractions

Significant correlations between specific glutenin-gliadin fractions

Results are given in Table 6.7.

The strongest negative correlations were between peaks 46 (glutenin) and 17 (gliadin) (r=-0.665) and 45 (glutenin) and 17 (gliadin) (r=-0.661). The strongest positive correlations were between glutenin peaks 45 and 46 and gliadin peak 23 with r= 0.550 and r=0.591, respectively.

The highest correlation between a single peak and a summed group was between glutenin peak 46 and gliadin summed group 39A (r=0.575).

Table 6.7 Significant correlations between glutenin and gliadin fractions

Glutenin	Gliadin	Correlation
P14	P42.8	0.3568***
P20	P17	-0.3960***
	P25.5	0.4206***
P20A	P25.5	0.3818***
P25	P29A	0.3933***
P26A	P25.5	0.3737***
P37	P17	0.3564***
	P32.2	0.4646***
	P35.8	0.4428***
P35A	P35.8	0.3669***
P39	P25.2	0.3994***
P42	P17	-0.4600***
P45	P17	-0.6608***
	P20.9	-0.4011***
	P22.5	0.4519***
	P23	0.5496***
	P26.2	-0.4254***
	P26.6	0.4350***
	P32.2	-0.4798
	P39A	0.4773***
	P42.8	-0.4141***
P46	P17	-0.6654***
	P20.9	-0.3976***
	P22.5	0.4902***
	P23	0.5910***
	P26.2	-0.3866***
	P26.6	0.4089***
	P29A	-0.3539***
	P32.2	-0.4506***
	P35.8	-0.3799***
	P39.2	0.4571***
	P39A	0.5745***
	P42.8	-0.4686***
P47	P34.6	0.4360***
	P42.6	-0.3619***
	P42.8	-0.5361***
P49A	P34.6	0.4492***
	P42.8	-0.4141***

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, A = Summed minor and major fractions

Stepwise multiple regression analysis for quality traits at Adet

Results are given in Table 6.8.

Models for each quality characteristic were obtained. Table 6.8 is a summary of the derived equations. Only the variables that met the 0.05 significance level were listed.

SDSS: Seven gliadin and two glutenin subunits influenced this parameter significantly. The highest contributor is the gliadin peak eluted at 17 minutes, with a partial contribution of R^2= 0.2932, and the lowest contributor was gliadin peak 41.2. The overall contribution of the nine protein fractions were R^2= 0.8504.

VK: The most important contributors to vitreous kernels were five gliadin and two glutenin subunits. The highest contributor was the summed glutenin fractions of P20A (R^2= 0.2443). Gliadin peak 26.2 had the lowest partial contribution (R^2= 0.0308). The complete contribution of the seven peaks were R^2= 0.7331.

FPC: The overall contribution of the four gliadin and two glutenin peaks were R^2= 0.7423, with gliadin peaks 43.2 and 29A contributing the most. The lowest contribution was glutenin peak 34 (R^2= 0.0381).

FLY: Flour yield was explained by the contribution of three peaks. Gliadin peaks 29.2 and 39.5 had the highest contribution and glutenin peak 36 the lowest. The overall contribution was R^2= 0.3858.

MDT: Two glutenin and one gliadin peak contributed to an overall R^2-value of 0.5149. Glutenin peak 48 had the highest partial contribution, followed by gliadin peak 17 and glutenin peak 27.

SKCS-weight: The highest contributors to SKCS-weight were gliadin peaks 31.2 (R^2= 0.2741), 29.5 and glutenin peak 39. Glutenin peak 14 had the lowest value, R^2= 0.0415, and the overall value for the model was R^2= 0.7108.

SKCS-diameter: SKCS-diameter was influenced by four glutenin and two gliadin subunits. The highest contributors were glutenin peaks 39 and 47 and gliadin peak 47. Gliadin peak 30.2 had the lowest contribution.

SKCS-hardness index: This parameter was influenced by six glutenin and one gliadin peak. The highest contributors were glutenin peaks 41, 24 and 21, followed by the summed gliadin fraction 35A.

Table 6.8 Results of stepwise multiple regression analyses for quality traits at Adet

Variable	Number of steps	Partial R^2	Model R^2	Pr>F
SDSS (Full model R^2 = 0.8504)				
G17	1	0.2932	0.2932	0.0001
P45A	2	0.1393	0.4326	0.0025
G35A	3	0.1016	0.5341	0.0047
G24.9	4	0.0782	0.6124	0.0070
P20	5	0.0686	0.6810	0.0062
G25.2	6	0.0460	0.7269	0.0157
G49	7	0.0457	0.7727	0.0097
G21.5	8	0.0384	0.8110	0.0104
G41.2	9	0.0394	0.8504	0.0045
VK (Full model R^2 = 0.7331)				
P20A	1	0.2443	0.2443	0.0006
G40A	2	0.1453	0.3895	0.0029
G49	3	0.1292	0.5187	0.0019
P36	4	0.0584	0.5771	0.0238
G34.6	5	0.0690	0.6461	0.0088
G42.5A	6	0.0563	0.7024	0.0108
G26.2	7	0.0308	0.7331	0.0459
FPC (Full model R^2 = 0.7423)				
G43.2	1	0.3253	0.3523	0.0010
G29A	2	0.1164	0.4688	0.0041
G33.5	3	0.0920	0.5607	0.0055
P25	4	0.0832	0.6440	0.0040
G46	5	0.0602	0.7042	0.0075
P34	6	0.0381	0.7423	0.0230
FLY (Full model R^2 = 0.3858)				
G29.2	1	0.1757	0.1757	0.0042
G39.5	2	0.1190	0.2947	0.0110
P36	3	0.0911	0.3858	0.0180
MDT (Full model R^2 = 0.5149)				
P48	1	0.2264	0.2264	0.0010
G17	2	0.1735	0.3999	0.0012
P27	3	0.1150	0.5149	0.0033
SKCS-weight (Full model R^2 = 0.7108)				
G31.2	1	0.2741	0.2741	0.0002
P39	2	0.1340	0.4081	0.0036
G21.5	3	0.1040	0.5113	0.0054
G29.5	4	0.0795	0.5907	0.0081
P41	5	0.0786	0.6693	0.0042
P14	6	0.0415	0.7108	0.0249
SKCS-diameter (Full model R^2 = 0.6708)				
P39	1	0.1880	0.1880	0.0029
P47	2	0.1710	0.3590	0.0017
G47	3	0.1058	0.4648	0.0069
P26	4	0.0893	0.5541	0.0072
P27	5	0.0716	0.6256	0.0094
G30.2	6	0.0452	0.6708	0.0280
SKCS-hardness index (Full model R^2 = 0.6789)				
P41	1	0.1749	0.1749	0.0043
P24	2	0.1571	0.3320	0.0031
P21	3	0.1021	0.4341	0.0095
G35A	4	0.0821	0.5162	0.0128
P27	5	0.0717	0.5879	0.0129
P32	6	0.0437	0.6316	0.0403
P51	7	0.0473	0.6789	0.0251

All variables left in the model were significant at the 0.05 level. P = Glutenin, G = Gliadin, A = Summed minor and major peaks

SDS = sodium dodecyl sulphate, SDSS=SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, SKCS = single kernel characterization system, Pr = probability determination, F = F-test, R^2 = coefficient of multiple determination

Stepwise multiple regression analysis for quality traits at Motta

Results are given in Table 6.9.

Models for each quality characteristic were obtained. Table 6.9 is a summary the derived equations, not all the variables are presented.

SDSS: A total of 12 peaks contributed to a R^2 = 0.9006. Ten gliadin and two glutenin subunits influenced this parameter significantly. The highest contributor was the gliadin peak eluted at 50 minutes, with a partial contribution of R^2= 0.2657, followed by the gliadin summed peak 32A. The lowest contributor was the glutenin summed fraction 40A (R^2 = 0.0168).

VK: The most important contributors to vitreous kernels were four glutenin and three gliadin subunits. The highest contributor was the glutenin peak P23 (R^2= 0.2840). Gliadin peak 25 had the lowest partial contribution (R^2= 0.0303). The total contribution of the seven peaks was R^2= 0.7424.

FPC: Flour protein content was explained by the contribution of four gliadin and a single glutenin peak. The glutenin peak had the highest partial contribution (R^2 = 0.2418), followed by gliadin peaks 17, 35.8, 25.5 and 24.5.

FLY: The overall contribution of the four gliadin and three glutenin peaks were R^2= 0.6132, with glutenin peak 23 contributing the most.

MDT: At Motta the mixograph development time was influenced by seven protein subunits. The highest contributor was the glutenin peak 36 (R^2= 0.1606). The two gliadin peaks contributing were 49 and 22.5. The lowest contribution was made by the summed glutenin fraction 30A.

SKCS-weight: Only glutenin peak 28 contributed significantly to this model. The partial and overall contribution was R^2= 0.1487.

SKCS-hardness index: Twelve peaks contributed to an overall R^2= 0.6789. The highest contributor was the summed gliadin fraction 29A, followed by glutenin peaks 14, 27 and 16. Glutenin peak 22 had the lowest partial contribution to the total variation.

Table 6.9 Results of stepwise multiple regression analyses for quality traits at Motta

Variable	Number of steps	Partial R^2	Model R^2	Pr>F
SDSS (Full model R^2 = 0.9006)				
G50	1	0.2657	0.2657	0.0003
G32A	2	0.1842	0.4498	0.0006
G31.2	3	0.0844	0.5343	0.0103
G20.9	4	0.0537	0.5880	0.0298
P30	5	0.0706	0.6586	0.0079
G40.9	6	0.0507	0.7092	0.0155
G30.2	7	0.0405	0.7497	0.0210
G25.2	8	0.0313	0.7810	0.0319
G26.6	9	0.0390	0.8200	0.0103
G42.5A	10	0.0338	0.8538	0.0093
G36A	11	0.0301	0.8838	0.0071
P40A	12	0.0168	0.9006	0.0292
VK (Full model R^2 = 0.7424)				
P23	1	0.2840	0.2840	0.0002
G17	2	0.1777	0.4616	0.0007
G46	3	0.0895	0.5512	0.0073
P40A	4	0.0550	0.6062	0.0249
G50	5	0.0673	0.6735	0.0080
P46	6	0.0386	0.7121	0.0320
P25	7	0.0303	0.7424	0.0469
FPC (Full model R^2 = 0.6132)				
P16	1	0.2418	0.2418	0.0007
G17	2	0.2004	0.4422	0.0004
G35.8	3	0.0631	0.5054	0.0294
G25.5	4	0.0651	0.5705	0.0198
G24.5	5	0.0427	0.6132	0.0475
FLY (Full model R^2 = 0.6132)				
P23	1	0.2064	0.2064	0.0019
G40A	2	0.1138	0.3202	0.0123
P40	3	0.0867	0.4070	0.0202
P37	4	0.0576	0.4645	0.0473
G30.2	5	0.0592	0.5237	0.0361
G43.5	6	0.0909	0.5815	0.0066
G26.2	7	0.0580	0.6395	0.0196
MDT (Full model R^2 = 0.5149)				
P36	1	0.1606	0.1606	0.0070
P39	2	0.1221	0.2828	0.0116
G49	3	0.0862	0.3690	0.0245
P14	4	0.0896	0.4585	0.0152
G22.5	5	0.0833	0.5418	0.0123
P31	6	0.0626	0.6045	0.0205
P30A	7	0.0452	0.6497	0.0378
SKCS-weight (Full model R^2 = 0.1487)				
P28	1	0.1487	0.1487	0.0097
SKCS-hardness index (Full model R^2 = 0.6789)				
G29A	1	0.2276	0.2276	0.0011
P14	2	0.1977	0.4253	0.0005
P27	3	0.0871	0.5124	0.0108
P16	4	0.0627	0.5750	0.0213
P40	5	0.0757	0.6508	0.0067
P32	6	0.0430	0.6938	0.0286
G21.5	7	0.0474	0.7412	0.0145
G32.6	8	0.0352	0.7764	0.0247
G45.5	9	0.0345	0.8109	0.0179
G47	10	0.0265	0.8374	0.0266
P30A	11	0.0239	0.8613	0.0251
P22	12	0.0212	0.8825	0.0245

All variables left in the model are significant at the 0.05 level; P = Glutenin, G = Gliadin, A = Summed minor and major peaks, SDS = sodium dodecyl sulphate, SDSS=SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, SKCS = single kernel characterization system, Pr = probability determination, F = F-test, R^2 = coefficient of multiple determination

Stepwise multiple regression analysis for quality traits at both localities

Results are given in Table 6.10.

Models for each quality characteristic were obtained. Table 6.10 is a summary of the derived equations, not all the variables are presented.

SDSS: Five gliadin and six glutenin subunits contributed (R^2 = 0.8126) to this parameter. The highest contributor was the gliadin peak eluted at 17 minutes, with a partial contribution of R^2= 0.4935, followed by the glutenin summed peak 35A.

VK: Vitreous kernels were explained by the contribution of five glutenin and four gliadin peaks. The highest contributing factors were glutenin peak 48 (R^2 = 0.2238) and summed fraction 20A.

FPC: The most important contributors to flour protein content were six gliadin and four glutenin subunits. The highest contributor was the glutenin peak 45 (R^2= 0.5945). Glutenin peak 32 had the lowest partial contribution (R^2= 0.00082). The complete contribution of the seven peaks was R^2= 0.8599.

FLY: The overall contribution of the two glutenin peaks was R^2= 0.1381, with glutenin peak 39 contributing the most.

MDT: Mixograph development time was influenced by six protein subunits. The highest contributor was the summed glutenin fraction 45A (R^2= 0.1474). The three contributing gliadin peaks were 46, 39.2 and summed 24A. The overall contributions were R^2 = 0.4115.

SKCS-weight: Only glutenin peak 39 (R^2= 0.0944) and summed 49A (R^2= 0.1154) contributed significantly to this model. Four gliadin peaks further contributed to an overall R^2= 0.4383.

SKCS-diameter: Three glutenin and one gliadin peak contributed to the overall R^2= 0.3219. The highest partial contribution was made by peak 47, followed by peak 39, gliadin peak 21.5 and glutenin peak 26.

SKCS-hardness index: Ten peaks contributed to an overall R^2= 0.6789. The highest contributor was the summed gliadin fraction 29A, followed by glutenin peak 27.

Table 6.10 Results of stepwise multiple regression analyses for quality traits for two localities

Variable	Number of steps	Partial R^2	Model R^2	Pr>F
SDSS (Full model R^2 = 0.8126)				
G17	1	0.4935	0.4935	<0.0001
P35A	2	0.0828	0.5764	<0.0001
P45A	3	0.0665	0.6428	0.0001
P20	4	0.0385	0.6813	0.0020
P52	5	0.0298	0.7111	0.0044
P31	6	0.0232	0.7343	0.0090
G27.5	7	0.0176	0.7520	0.0186
G24.5	8	0.0202	0.7722	0.0093
G33.5	9	0.0156	0.7878	0.0182
P23	10	0.0139	0.8017	0.0222
G42.6	11	0.0109	0.8126	0.0376
VK (Full model R^2 = 0.6473)				
P48	1	0.2238	0.2238	<0.0001
P20A	2	0.1594	0.3831	<0.0001
G28.5	3	0.0713	0.4544	0.0013
G34.6	4	0.0623	0.5168	0.0015
P16	5	0.0364	0.5532	0.0110
G39.5	6	0.0349	0.5882	0.0100
G30.2	7	0.0200	0.6081	0.0454
P27	8	0.0214	0.6296	0.0344
P44	9	0.0178	0.6473	0.0495
FPC (Full model R^2 = 0.8599)				
P45	1	0.5945	0.5945	<0.0001
G46	2	0.0810	0.6747	<0.0001
G32.6	3	0.3260	0.7425	0.0016
G35.8	4	0.0333	0.7758	0.0007
G28.5	5	0.0222	0.7980	0.0036
P16	6	0.0161	0.8141	0.0097
G25.2	7	0.0185	0.8403	0.0043
G33.2	8	0.0093	0.8497	0.0296
P27	9	0.0081	0.8578	0.0382
P32	10	0.0082	0.8599	0.0332
FLY (Full model R^2 = 0.1381)				
P39	1	0.0963	0.0963	0.0031
P23.5	2	0.0419	0.1381	0.0440

Table 6.10 Continued

Variable	Number of steps	Partial R^2	Model R^2	Pr>F
		MDT (Full model R^2 = 0.4115)		
P45A	1	0.1474	0.1474	0.0002
P27	2	0.0587	0.2842	0.0098
P39	3	0.0402	0.3244	0.280
G46	4	0.0369	0.3613	0.0313
G39.2	5	0.0367	0.3763	0.0298
G24A	6	0.0352	0.4115	0.0296
		SKCS-weight (Full model R^2 = 0.4383)		
P39	1	0.0944	0.0944	0.0034
P49A	2	0.1154	0.2098	0.0006
G21.5	3	0.0498	0.2595	0.0190
G29.5	4	0.0599	0.3195	0.0079
G25.2	5	0.0510	0.3706	0.0112
G50	6	0.0677	0.4383	0.0023
		SKCS-diameter (Full model R^2 = 0.3219)		
P47	1	0.1283	0.1283	0.0006
P39	2	0.0892	0.2176	0.0024
G21.5	3	0.0634	0.2809	0.0075
P26	4	0.0409	0.3219	0.0269
		SKCS-hardness index (Full model R^2 = 0.6789)		
G29A	1	0.1919	0.1919	<0.0001
P27	2	0.1049	0.2969	0.0006
G35A	3	0.0884	0.3852	0.0008
P21	4	0.0398	0.4250	0.0181
G26.2	5	0.0365	0.4615	0.0199
P58	6	0.0364	0.4979	0.0170
G40.9	7	0.0241	0.5220	0.0465
G45.5	8	0.0248	0.5468	0.0397
P35	9	0.0221	0.5689	0.0475
G21.5	10	0.0209	0.5898	0.0498

All variables left in the model are significant at the 0.05 level. P = Glutenin, G = Gliadin

SDS = sodium dodecyl sulphate, SDSS=SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, SKCS = single kernel characterization system, Pr = probability determination, F = F-test, R^2 = coefficient of multiple determination

6.4.2 Discussion and conclusions

A total of 38 glutenin (including minor peaks and unresolved shoulders) were resolved in the glutenin extract, after gliadin extraction with 70% ethanol. A total of 42 gliadin peaks were differentiated. Peaks not only varied in concentration but also in presence between cultivars, across the two environments.

For simplicity and due to the objectives of this study, the discussion will not focus on individual differences between entries, but on correlations between different peaks and quality parameters and on the interactions between different protein subunits.

At Adet, only 11 of the possible 38 glutenin peaks correlated significantly with quality traits. In most cases single correlations were found between individual peaks and quality parameters. The higher molecular weight fractions (eluting first) correlated with SDS sedimentation. The lower molecular weight proteins had a significant influence on the mixograph development time and single kernel characteristics such as weight and diameter.

The same trend was visible at Motta, with the lower molecular weight proteins correlating with mixograph development time (peak 36) and SKCS-hardness index, with the exception of peak 45 that correlated with SDS sedimentation. Flour yield had a positive relationship with the lower molecular range and a negative relationship with the higher molecular range. Only peak 20 correlated with the same quality characteristic (SDSS) at both environments.

The combined analysis across the two environments indicated that glutenins, especially the lower molecular weight fractions, had a negative relationship with SDS sedimentation. Most of the peaks influencing SDS sedimentation eluted between 40 and 50 minutes. The only higher molecular peak influencing SDSS was the peak eluting at 20 minutes. However, it is difficult to determine whether this is a direct correlation, due to possible linkage

suggested by the significant inter-protein correlations existing between peaks 20 and 49, 47 and 42, and 46 and 49 (Table 6.6).

Mixograph development time was mostly positively influenced by the later eluting proteins and negatively by the earlier eluting peaks.

The higher and lower molecular weight proteins had a positive relationship with flour protein content, except for the intermediate group (45-49 minutes) which had a negative influence on flour protein content.

Similar to the glutenin results, highly significant correlations were found between individual gliadin proteins and quality characteristics. In contrast with correlations between glutenins and quality parameters, gliadins mostly had a negative relationship with the different quality characteristics. The only exception was the mostly positive relationship between individual gliadin proteins and flour protein content, at Motta.

The influence of different environments became apparent when the same individual proteins, across the environments, influenced different traits. Peak 17 at Adet positively influenced SDS sedimentation, while at Motta it was negatively correlated with vitreous kernels and flour protein content.

Across both environments, individual proteins showed both positive and negative relationships with the quality parameters. Peaks 17 and 20.9 had the highest positive correlation with SDS sedimentation. However, a highly significant correlation also existed between peaks 17 and 20.9.

Apart from the correlation between gliadin peaks 17 and 20.9, a correlation between peak 17 and glutenin peak 20 was also observed. Glutenin peak 20 also correlated with SDSS. This occurrence of linkage or concurrent relationship makes it difficult to assign specific roles to the different gliadin and low molecular weight glutenin subunits, in determining quality traits (Lafiandra *et al*., 1994). The high number of correlations between gliadin peaks and quality parameters might be due to low molecular weight subunit

contamination in the extracts. Graybosch and Morris (1990) found that some LMW-glutenin subunits are partially soluble in 70% ethanol.

Stepwise multiple regression analysis helps to distinguish between direct and indirect protein influences. It is a tool that helps find relationships between variables.

Diverse results were obtained across the two environments. Some of the peaks consistently influenced the same quality parameters across environments and in the combined analysis, though most proteins influencing a specific quality in the regression model were environment-specific. The models mostly included proteins indicated by the correlation studies for that specific protein parameter.

It is apparent from the regression models that there is a distinct interaction between glutenin and gliadin proteins present in the flour. This is in agreement with Hoseney *et al*. (1987), who hypothesised that quality is largely influenced by the interaction of all polypeptides in the flour. Although, if the partial contribution of individual proteins are considered, it appears that the highest contribution of a single protein can account for up to 59% of the variation. This was the case with glutenin peak 45 and its contribution to flour protein content (Table 6.10) across both environments. This agreed with the argument of Lukow and colleagues (1989) who suggested that a small number of important proteins are responsible for gluten quality.

Glutenin peak 20 and gliadin peak 17 appear to be specifically linked to SDS sedimentation according to both correlation and regression data. Glutenin peak 39 significantly contributed, in and across both environments, to mixograph development time, even though at Motta it did not significantly correlate with mixograph development time. It thus appears that these peaks are very specifically linked to certain functionalities.

Despite the contribution of gliadins to most of the quality parameters, if the partial contribution of each protein is considered individually, it is apparent that

the major contributor to quality was the glutenins, both HMW and LMW fractions. The only exception is where gliadins contributed to SDS sedimentation.

In conclusion, this study confirmed that baking quality is an interaction between different protein components, and that the components differ significantly in their individual contribution to quality parameters. It is, however, possible to identify single proteins with significant contributions, which are stable across environments in diverse populations (e.g. glutenin peak 20, influencing SDSS and peak 27 influencing SKCS-HI). These proteins have the potential of development into possible markers related to quality with possible use in a breeding programme.

Future work will include the confirmation of the correlation of these proteins in different genetic backgrounds, the isolation of the specific proteins identified, sequencing and development of markers.

6.5 References

Agrobase, 2000. Agronomix Software, Inc., Agrobase^{TM}, 71 Waterloo St. Winnipeg, Manitoba R3N0S4, Canada.

Bietz, J.A., 1983. Separation of cereal proteins by reversed-phase high-performance liquid chromatography. *Journal of. Chromatography* 255:219-238.

Gianibelli, M.C., Larroque, O.R., MacRitchie F. and Wrigley, C.W., 2001. Biochemical, genetic, and molecular characterization of wheat endosperm proteins. Online review: American Association of Cereal Chemists, pp1-20.

Graybosch, R.A. and Morris, R., 1990. An improved SDS-PAGE method for the analysis of wheat endosperm storage proteins. *Journal of Cereal Science* 11:201.

Hoseney R.C., Dreese, P.C., Doescher, L.C. and Faubion, J.M., 1987. Thermal properties of gluten. In: Proc. 3rd Int. Workshop on Gluten Protein. T.N. Teaneck, (Ed.), World Scientific Publishing Co.

Lafiandra, D., Porceddu, E., Colaprico, G. and Margiotta, B., 1994. Combined reversed-phase high-performance liquid chromatography (RP-HPLC) and electrophoretic techniques in genetics and breeding of wheat storage proteins. In: High-Performance Liquid Chromatography of Cereal and Legume Proteins. J.E. Kruger and J.A. Bietz (Eds.), American Association of Cereal Chemists, St.Paul, MN, pp 273-325.

Lookhart , G.L. and Albers, L.D., 1988. Correlations between reversed-phase high-preformance liquid chromatography and acid- and sodium dodecyl sulphate-polyacrylamide gel electrophoretic data on prolamins from wheat sister lines differing in baking quality. *Cereal Chemistry* 65:222-227.

Lukow, O.M., Payne, P.I. and Tkachuk, R., 1989. The HMW glutenin subunit composition of Canadian wheat cultivars and their association with bread-making quality. *Journal of Science of Food and Agriculture* 46:451-460.

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

Primard, S., Graybosch, R., Peterson, C.J. and Lee, J., 1991. Relationships between gluten protein composition and quality characteristics in four populations of high-protein, hard red winter wheat. *Cereal Chemistry* 68:305-312.

SAS Institute Inc., 2004. SAS OnlineDoc® 9.1.3. Cary, NC: SAS Institute Inc.

Chapter 7

Prediction of baking quality in Ethiopian durum wheat by reversed-phase high-performance liquid chromatography

7.1 Abstract

In Ethiopia, durum wheat is extensively used for bread making. Most of the breeding programmes are currently focused on agronomic superiority and not good quality. An increasing demand for improved nutrition and quality necessitates the assessment of quality of the durum lines/cultivars. Reversed-phase high-performance liquid chromatography (RP-HPLC) offers an excellent method to study different protein components and their relationship to baking quality. Fifteen advanced lines were tested at two environments, Adet and Motta, in Ethiopia. RP-HPLC was used to analyze the glutenins and gliadins from wheat flour and their influence on quality determined. RP-HPLC patterns differed between lines across environments. Proteins were identified that had a significant influence on quality independent of environmental influences.

7.2 Introduction

Grain quality is generally assessed as the grain protein concentration and processing quality of the grain with a specific end use product in mind. The higher elasticity and extensibility of durum wheat makes it highly suitable for pasta making (Liu *et al*., 1996).

This suitability of durum wheat for pasta production has made it the second most cultivated crop in the world (Peña *et al*., 2002). However, there is an increasing demand for the use of durum wheat for breadmaking (He *et al*.,

1999). In countries like Italy and Sicily as well as parts of West Asia and Northern Africa large amounts of durum is already being used for the production of leavened breads. In Ethiopia, durum is commonly used for bread baking.

Extensive research has been done on factors contributing to good pasta-making quality (Liu *et al*., 1996), but very little to determine durum wheat's breadmaking potential. Increasing our knowledge of this potential might increase the commercial value of this crop, and open up the potential for alternative uses (Boggini *et al*., 1995).

Bakhshi and Bains (1987) found some durum cultivars which had increased elasticity, making it more suitable for bread making, however, bread wheat doughs are still stronger. This indicates that the potential exists to improve durum wheat cultivars through breeding for better baking quality.

The aim of this study was to identify and quantify individual polymeric and monomeric proteins, by RP-HPLC and to determine the effect of these proteins on quality characteristics in durum wheat types in two environments in Ethiopia.

7.3 Material and Methods

7.3.1 Material

Fifteen advanced lines, popularly grown Ethiopian were used in this study. The main criterion of selection for the Ethiopian material was agronomic performance. Trials were grown at two environments in Ethiopia in 2001: Adet Research Center, which is a higher protein potential area, and Motta, which is a low protein potential area. A RCB design with three replications was used. The plot size was 2.5 m^2 (six rows of 2.5 m length and 20 cm spacing between the rows) at both localities. All recommended wheat management practices were exercised.

Table 7.1 Entries of wheat lines included in this study.

Entry	Cultivar	Country
1	CD96486	Ethiopia
2	CD6630	Ethiopia
3	CD95294-1y	Ethiopia
4	DZ2023	Ethiopia
5	Yilma	Ethiopia
6	DZ1721	Ethiopia
7	DZ2212	Ethiopia
8	DZ1924	Ethiopia
9	Bichena	Ethiopia
10	LD357	Ethiopia
11	DZ1640	Ethiopia
12	DZ1691	Ethiopia
13	DZ1652	Ethiopia
14	DZ1748	Ethiopia
15	DZ900	Ethiopia

After harvesting, the material was transported to South Africa and the data on yield and quality traits were measured in triplicate at the laboratories of the ARC-SGI, Bethlehem, South Africa (Chapter 3, Table 3.2).

7.3.2 Methods

Proteins were extracted as in section 6.3.2.

7.3.3 RP-HPLC

Reversed-phase HPLC analysis was performed as described in section 6.3.3.

7.3.4 Statistical analysis

Statistical analyses were performed as described in section 6.3.4.

7.4 Results and discussion

7.4.1 Results

Significant correlations between specific glutenin protein fractions and quality characteristics for Adet and Motta

Results are given in Table 7.2.

Please refer to Appendix B for RP-HPLC chromatogram of glutenin proteins (Figure 3.).

At Adet, peaks 16, 23, 30.5, 42.5 and 49 correlated negatively with SDS sedimentation. Peak 23 also correlated negatively with MDT (highest correlation), but positively with vitreous kernels and SKCS-hardness.

Only peaks 12 and 48.2 correlated positively with MDT, while peaks 16 and 42.5 had a negative correlation. Flour yield was influenced by peaks 19, 42.5 (positively) and 20 (negatively). SKCS-weight was only correlated with peak 35.5 (negatively). Peaks 21, 28 and 38 correlated negatively with SKCS-hardness.

Peaks 20 and 45, at Motta, had similar negative correlations with SDSS than at Adet. MDT was positively correlated with the protein eluting at 36 minutes. Peaks 14, 20, 23, 25 and 45 were positively correlated with vitreous kernels. Peaks 20 and 23 had additional negative correlations with flour yield and positive correlation with SKCS-hardness index. The proteins eluting at 29 and 42 minutes correlated positively with flour protein content.

Table 7.2 Significant correlations between specific glutenin protein fractions and quality characteristics for Adet and Motta

Location	Peak 1	Characteristic	Correlation
Adet	P16	SDSS	-0.3191*
	P23		-0.5121***
	P30.5		-0.3198*
	P42.5		-0.5486***
	P49		-0.3115
	P23	VK	0.3551*
	P49		-0.3251*
	P19	FLY	0.3128*
	P20		-0.3494*
	P42.5		0.3538*
	P12	MDT	0.3245*
	P16		-0.4113***
	P23		-0.5134***
	P42.5		-0.4574
	P48.2		0.3352*
	P35.5	SKCS-Weight	-0.3063*
	P21	SKCS-Hardness	-0.3260
	P23		0.3418*
	P28		-0.3678*
	P38		-0.3357*
Motta			
	P15	SDSS	-0.3893***
	P33.2	VK	0.3207*
	P44.5		-0.3200*
	P33.2	FPC	0.3199*
	P44.5		-0.3219*
	P24	SKCS-Weight	0.3108*
	P35		-0.5363*** *
	P17.5	SKCS-Diameter	0.3149*
	P26.5		0.3202*
	P31.4		0.3761*
	P34.5		-0.3306*
	P35		-0.4493***
	P41		-0.3277*
	P28	SKCS-Hardness	0.3342*

SDS = sodium dodecyl sulphate, SDSS=SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, SKCS = single kernel characterization system

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Significant correlations between specific gliadin protein fractions and quality characteristics for Adet and Motta

Results are given in Table 7.3.

Please refer to Appendix B for RP-HPLC chromatogram of gliadin proteins (Figure 4.).

Gliadin peaks 26.4 and 34.8 correlated negatively with SDSS at Adet. Peak 38.5 correlated negatively with vitreous kernels, SKCS-hardness and positively with FLY. Similar negative correlations were found between SKCS-weight, SKCS-diameter and peak 32.2, and a positive correlation with MDT. Peak 27.5 was negatively correlated with SKCS-weight and SKCS-diameter.

At Motta, peaks 24.2 and 33.2 were negatively correlated with SDSS and MDT and peak 31.6 was positively correlated with SDSS and vitreous kernels. Flour yield was negatively correlated with peaks 20.9, 21.5, 22.6, 26.4, 37.6, and 40.2 and positively with peaks 38.5, 42.4, 47.8 and 50. Peaks 15, 38.5, 43, 44.9 and 47 were positively correlated with both SKCS-weight and diameter.

Table 7.3 Significant correlations between specific gliadin protein fractions and quality characteristics for Adet and Motta

Gliadin Peak	Characteristic	Correlation	Gliadin Peak	Characteristic	Correlation
	Adet			**Motta**	
P26.4	SDSS	-0.3930***	P24.2	SDSS	-0.3722*
P34.8		-0.3201*	P31.6		0.3623*
			P33.2		-0.3327*
P17	VK	-0.3410*			
P28		0.3917***	P22.6	VK	0.4423***
P30.2		-0.4148***	P31.6		0.3713*
P38.5		-0.3953***			
P42.4		-0.3776*	P24.9	FPC	0.3050*
P45.5		-0.4383			
			P20.9	FLY	-0.4516***
P15	FPC	-0.3450*	P21.5		-0.5041***
P30.2		-0.3188*	P22.6		-0.4190***
			P26.4		-0.4064***
P28.5	FLY	-0.3785*	P28.5		-0.3981***
P38.5		0.3059*	P37.6		-0.4686***
			P38.5		0.4457***
P26.4	MDT	-0.4737***	P40.2		-0.5276***
P32.2		0.3179*	P42.4		0.5359***
P34.8		-0.3339*	P47.8		0.4381***
			P50		0.4274***
P27.5	SKCS-Weight	-0.3672*			
P32.2		-0.3258*	P15	MDT	-0.2639
P33.2		0.3069*	P17		-0.3687*
			P24.2		-0.3095*
P27.5	SKCS-Diameter	-0.3857*	P33.2		-0.3709*
P32.2		-0.3415*			
			P15	SKCS-Weight	0.3220*
P29.5	SKCS-Hardness	0.3409*	P38.5		0.3701*
P38.5		-0.3872*	P43		0.4707***
			P44.9		0.3698*
			P46		0.3167*
			P47		0.4359***
			P15	SKCS-diameter	0.3194*
			P34.8		0.3646*
			P38.5		0.3576*
			P42.4		0.3175*
			P43		0.4448***
			P44.9		0.3461*
			P47		0.3587*

SDS = sodium dodecyl sulphate, SDSS=SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, SKCS = single kernel characterization system

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Significant correlations between specific glutenin protein subunits and quality characteristics for both localities

Results are given in Table 7.4.

Across the two environments, eight subunits correlated with SDS sedimentation. Peaks 16, 23, 35, 42.5 and 45.6 correlated negatively and peaks 17.5, 33.7 and 41 positively with SDSS. Peaks 16 and 23 also correlated negatively with mixograph development time and peaks 19 and 34.5 positively.

Peaks 35, 42.5 and 45.6 correlated negatively with vitreous kernels. Flour yield was positively influenced by subunits 32.6, 34.5, 42.3 and 45.6. Peaks 45.6, 42.3, 34.5 (negative) and 32.6 (positive) were correlated with flour protein content. SKCS-hardness index was positively correlated with peak 44.5 and negatively with peaks 45.6 and 48.7.

Table 7.4 Significant correlations between specific glutenin protein fractions and quality characteristics for both localities

Glutenin peak	Characteristic	Correlation
P16	SDSS	-0.3556****
P17.5		0.2999***
P23		-0.4636***
P33.7		0.2923***
P35		-0.3387***
P41		0.3958***
P42.5		-0.5885***
P45.6		-0.3014***
P35	VK	-0.2834***
P42.5		-0.3455***
P45.6		-0.3649***
P32.6	FPC	0.2800***
P34.5		-0.2820***
P42.3		-0.3479***
P45.6		-0.3669***
P35	FLY	0.2744***
P42.3		0.2971***
P45.6		0.3270***
P16	MDT	-0.3508***
P19		0.3767***
P23		-0.4222***
P34.5		0.4678***
P44.5	SKCS-hardness index	0.4116***
P45.6		-0.3913***
P48.7		-0.2848***

SDS = sodium dodecyl sulphate, SDSS=SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, SKCS = single kernel characterization system
* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Significant correlations between specific gliadin protein fractions and quality characteristics

Results are given in Table 7.5.

Vitreous kernels were positively correlated with peaks 24.2, 35.8, 36.2, and 37, and negatively with peaks 25, 42.2, 45.5, 46, 47.8 and 49. Similar correlations, with the exception of peak 45.5, were observed for flour protein content. Fourteen subunits were both positively and negatively correlated with flour protein content.

Mixograph development time was negatively correlated with peaks 24.2, 33.2, 36.2 and positively with peaks 42.4, 49 and 51. Peak 33.2 correlated positively with SKCS-weight. SKCS-hardness was positively correlated with peaks 24.2, 30.6, 35.8, 36.2, and 37.6 and negatively with peaks 25, 42.4, 46, 47.8 and 49.

Table 7.5 Significant correlations between specific gliadin protein fractions and quality characteristics

Gliadin Peak	Characteristic	Correlation	Gliadin Peak	Characteristic	Correlation
P24.2	VK	0.4619***	P24.2	MDT	-0.3442***
P25		-0.4843***	P33.2		-0.3435***
P35.8		0.4496***	P36.2		-0.3349***
P36.2		0.6035***	P42.4		0.3253***
P37		0.4996***	P49		0.3385***
P42.2		-0.5486***	P51		0.3487***
P45.5		-0.4389***			
P46		-0.5470***	P33.2	SKCS-Weight	0.3003***
P47.8		-0.4749***			
P49		-0.5745***	P24.2	SKCS-Hardness	0.4331***
			P25		-0.4597***
P24.2	FPC	0.4501***	P30.6		0.4501***
P25		-0.4975***	P35.8		0.4239***
P35.8		0.4975***	P36.2		0.6379***
P36.2		0.7032***	P37.6		0.4627***
P37		0.5086***	P42.4		-0.5450***
P42.4		-0.5852***	P46		-0.4977***
P46		-0.5664***	P47.8		-0.5357***
P47.8		-0.5275***	P49		-0.5637***
P49		-0.6375***			
P24.2	FLY	-0.4777***			
P25		0.5347***			
P28.5		-0.5213***			
P29.5		0.5404***			
P30.6		-0.4915***			
P35.8		-0.5151***			
P36.2		-0.6929***			
P37		-0.205***			
P42.4		0.7216***			
P43		0.4797***			
P44.9		0.4723***			
P46		0.5984***			
P47.8		0.6595***			
P49		0.6884***			

SDS = sodium dodecyl sulphate, SDSS=SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, SKCS = single kernel characterization system

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Significant correlations between specific glutenin-glutenin and gliadin-gliadin proteins

Results are given in Table 7.6.

Significant correlations existed between the presences of certain peaks with the presence of others.

Glutenin peak 15 correlated with peaks 18 and 33.7. Peak 21 was negatively correlated with peak 40 and positively with peak 32.4. Peak 24 correlated positively with peaks 25.5, 38, 39.5 and 42.3. Additionally peak 25.5 correlated with peaks 38, 39.5 (positive) and negatively with peak 34.5. Single correlations between peaks occurred with the highest correlation between peak 47.5 and peak 48.7 ($r = 0.7899$)

Gliadin peak 25 correlated with peaks 17, 49 (positive) and 36.2 (negative). Peak 29.5 and 36.2 correlated with five other gliadin subunits, individually. The highest correlations were found between peak 42.4 and peaks 46 ($r = 0.8328$) and 47.8 ($r = 0.8189$), respectively.

Table 7.6 Significant correlations between glutenin-glutenin and gliadin-gliadin fractions

Glutenin			Gliadin		
Peak 1	**Peak 2**	**Correlation**	**Peak 1**	**Peak 2**	**Correlation**
P15	P18	0.4479***	P17	P25	0.6128***
	P33.7	0.4864***	P20.9	P21.5	0.7484***
				P22.2	0.6192***
P21	P32.4	0.4528***	P24.2	P24.5	0.6383***
	P40	-0.5062***		P35.8	0.6101***
				P36.2	0.6403***
P24	P25.5	0.5940***		P37	0.8090***
	P38	0.4924***	P24.3	P36.2	0.5022***
	P39.5	0.5287***		P37	0.5563***
	P42.3	0.4895***	P25	P36.2	-0.5340***
				P49	0.5000***
P25.5	P34.5	-0.4582***	P29.5	P36.2	-0.5171***
	P38	0.5032***		P42.4	0.5644***
	P39.5	0.4722***		P46	0.5164***
				P47.8	0.5557***
P29.5	P30.5	-0.4622***		P49	0.5238***
			P30.6	P42.4	-0.5288***
P32.4	P32.6	-0.4680***	P35.8	P36.2	0.6445***
				P37	0.5626***
P33.7	P35	-0.5573***		P37.6	0.5102***
P34.5	P42.5	-0.4561***		P42.4	-0.5123***
			P36.2	P37	0.5643***
P39.5	P40	-0.6717***		P42.4	-0.6736***
P44.5	P45.6	-0.5440***		P46	-0.6040***
P46.6	P47.5	0.6654***		P47.8	-0.6520***
P47.5	P48.2	0.6200***		P49	-0.5906***
	P48.7	0.7899***	P37.6	P39.5	0.7773***
				P40.2	0.6033***
				P47.8	-0.5467***
			P38.5	P39.5	-0.7223***
			P42.4	P43	0.6156***
				P46	0.8328***
				P47.8	0.8189***
				P49	0.7605***
			P44.9	P45.5	0.6133***
			P45.5	P45.7	0.6259***
				P46	0.6983***
				P49	0.7126***
			P45.7	P49	0.7273***
			P46	P47.8	0.6344***
				P49	0.8002***
			P47.8	P49	0.6952***
				P51	0.6306***
			P49	P51	0.6446***

SDS = sodium dodecyl sulphate, SDSS=SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, SKCS = single kernel characterization system

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Significant correlations between specific glutenin-gliadin fractions

Results are given in Table 7.7.

Significant positive and negative correlations were observed between glutenin and gliadin peaks.

The strongest negative correlations were between peaks 44.5 (glutenin) and 36.2 (gliadin) ($r = -0.4713$), and the strongest positive correlations were between glutenin peaks 42.3 (glutenin) and 20.9 (gliadin) ($r = 0.4583$).

Most of the correlations observed were positive.

Table 7.7 Significant correlations between glutenin and gliadin fractions

Glutenin	Gliadin	Correlation
P12	P35.4	0.3332***
P14	P35.4	0.3332***
P15	P24.2	0.3998***
	P35.8	0.3546***
	P37	0.4065***
P21	P28	-0.3165***
P24	P20.9	0.3806***
	P21.5	0.3379***
P25.5	P21.9	0.3385***
P30.5	P29.2	0.3024***
P32.4	P34.8	-0.3505***
P33.7	P24.2	0.3389***
	P34.8	-0.3187***
	P44.9	-0.3164***
P34.5	P24.2	-0.4254***
	P33.2	-0.3658***
	P35.8	-0.3457***
	P36.2	-0.3293***
	P37	-0.3452***
	P42.4	0.3230***
	P44.9	0.4349***
	P45.5	0.3733***
P38	P46	0.3861***
	P21.5	0.3712***
	P40.2	0.4173***
P42.3	P20.9	0.4583***
P42.5	P17	0.3192***
	P32.6	-0.3413***
	P33.5	0.3353***
P44.5	P20.9	-0.3209***
	P37	0.3315***
	P35.8	-0.3124***
	P36.2	-0.4745***
	P37	-0.3760***
	P42.4	0.3027***
	P43	0.3736***
	P50	0.3000***

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Stepwise multiple regression analysis for quality traits at Adet

Results are given in Table 7.8.

Models for each quality characteristic were obtained. Table 7.8 is a summary of the derived equations. Not all the variables are presented.

SDSS: A total of seven peaks contributed to a R^2 = 0.7372. Five gliadin and two glutenin subunits influenced this parameter significantly. The highest contributor is the glutenin peak eluted at 42.5 minutes, with a partial contribution of R^2= 0.3009, followed by peak 23.

VK: Vitreous kernels were influenced by 18 peaks with 96.6% of the variation explained. The most important contributors to vitreous kernels were three gliadin subunits. The highest contributor was the gliadin peak 45.5 (R^2= 0.1921).

FPC: Flour protein content was explained by the contribution of five gliadin and a single glutenin peak. The glutenin peak had the lowest partial contribution (R^2 = 0.0587), and gliadin peaks 45.5, 44.9 and 22.2 the highest. The total contribution of the six peaks were R^2= 0.6641.

FLY: Flour yield was explained by the contribution of four peaks. Gliadin peak 28.5 and glutenin peak 20 had the highest contribution and glutenin peak 49 the lowest. The overall contribution was R^2= 0.4577.

MDT: Six glutenin peaks contributed to an overall R^2-value of 0.7068. Glutenin peak 23 had the highest partial contribution, followed by peaks 14 and 41.

SKCS-weight: The highest contributor to SKCS-weight was gliadin peaks 27.5 (R^2= 0.1348) and glutenin peak 39.5. The overall value for the model was R^2= 0.6261.

SKCS-diameter: SKCS-diameter was influenced by three gliadin and one glutenin subunit. The highest contributors were gliadin peaks 27.5 and 50 and glutenin peak 39.5. Gliadin peak 48 had the lowest contribution.

SKCS-hardness index: This parameter was influenced by four glutenin and two gliadin peaks. The highest contributors were glutenin peaks 16, 19 and 22, followed by gliadin fraction 15.

Table 7.8 Results of stepwise multiple regression analyses for quality traits at Adet

Variable	Number of steps	Partial R^2	Model R^2	Pr>F
SDSS (Full model R^2 = 07372)				
P42.5	1	0.3009	0.3009	<0.0001
P23	2	0.1477	0.4486	0.0017
G45.5	3	0.0890	0.5375	0.0076
G24.2	4	0.0541	0.5917	0.0266
G29.5	5	0.0441	0.6358	0.0359
G37.6	6	0.0664	0.7022	0.0600
G22.6	7	0.0350	0.7372	0.0325
VK (Full model R^2 = 0.9666)				
G45.5	1	0.1921	0.1921	0.0026
G38.5	2	0.1703	0.3624	0.0017
G22.2	3	0.0970	0.4594	0.0097
P16	4	0.1006	0.5600	0.0043
P35	5	0.0887	0.6487	0.0032
P33.2	6	0.0492	0.7535	0.0099
P39.5	7	0.0292	0.7827	0.0344
P40	8	0.0365	0.8192	0.0118
G20.9	9	0.0347	0.8538	0.0076
G44.9	10	0.0183	0.8721	0.0371
P46.6	11	0.0245	0.8806	0.0137
G45.7	12	0.0160	0.8966	0.0332
P34.5	13	0.0185	0.9151	0.0142
P17.5	14	0.0129	0.9280	0.0274
G33.5	15	0.0135	0.9115	0.0151
G51	16	0.0119	0.9534	0.0123
G41.6	17	0.0073	0.9607	0.0336
P49	18	0.0059	0.9666	0.0410
FPC (Full model R^2 = 0.6641)				
G45.5	1	0.1211	0.2402	0.0132
G44.9	2	0.0819	0.3220	0.0317
G22.2	3	0.1314	0.4534	0.0035
G45.7	4	0.0508	0.5300	0.0021
G23.6	5	0.0754	0.6054	0.0095
P19	6	0.0587	0.6641	0.0140
FLY (Full model R^2 = 0.4577)				
G28.5	1	0.1433	0.1433	0.0103
P20	2	0.1240	0.2673	0.0108
G43.7	3	0.0810	0.3483	0.0294
P49	4	0.1094	0.4577	0.0071
MDT (Full model R^2 = 0.7068)				
P23	1	0.2636	0.2636	0.0003
P14	2	0.1327	0.3963	0.0041
P41	3	0.0947	0.4911	0.0085
P48.2	4	0.0910	0.5811	0.0053
P19	5	0.0476	0.6296	0.0310
P22	6	0.0772	0.7068	0.0031

Table 7.8 Continued

Variable	Number of steps	Partial R^2	Model R^2	Pr>F
SKCS-weight (Full model R^2 = 0.6261)				
G27.5	1	0.1348	0.1348	0.0131
P39.5	2	0.1316	0.2665	0.0089
G50	3	0.1358	0.4033	0.0040
P17.5	4	0.0675	0.4698	0.0295
G49	5	0.0553	0.5251	0.0394
G45.5	6	0.0559	0.5810	0.0302
G28.5	7	0.0451	0.6261	0.0415
SKCS-diameter (Full model R^2 = 0.4910)				
G27.5	1	0.1488	0.1488	0.0089
G50	2	0.1363	0.2851	0.0071
P39.5	3	0.1170	0.4027	0.0070
G48	4	0.0883	0.4910	0.0119
SKCS-hardness index (Full model R^2 = 0.6900)				
P16	1	0.1136	0.1136	0.0147
P19	2	0.1061	0.3697	0.0121
P22	3	0.1527	0.5223	0.0009
G15	4	0.0860	0.6083	0.0057
G51	5	0.0409	0.6492	0.0420
P32.6	6	0.0602	0.6900	0.0099

All variables left in the model were significant at the 0.05 level. A = Summed major and minor fractions

P = Glutenin, G = Gliadin, SDS = sodium dodecyl sulphate, SDSS=SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, SKCS = single kernel characterization system, Pr = probability determination, F = F-test, R^2 = coefficient of multiple determination

Stepwise multiple regression analysis for quality traits at Motta

Results are given in Table 7.9.

Models for each quality characteristic were obtained. Table 7.9 is a summary of the derived equations, not all the variables are presented.

SDSS: A total of 10 peaks contributed to a R^2 = 0.9239. An equal number of gliadin and glutenin subunits influenced this parameter. The highest contributor was the glutenin peak eluted at 42.5 minutes, with a partial contribution of R^2= 0.3665, followed by peak 23.

VK: A total of 13 subunits contributed to vitreous kernels, 10 gliadin and three glutenin peaks. The most important contributors to vitreous kernels were two gliadin peaks, 22.6 and 32.6.

FPC: Flour protein content was explained by the contribution of four gliadin and two glutenin peaks. Gliadin peak 49 (R^2= 0.0.1251) had the highest partial contribution followed by glutenin peaks 21 and 34.5. The total contribution of the six peaks were R^2= 0.5678.

FLY: The overall contribution of the nine gliadin and five glutenin peaks were R^2= 0.8649, with gliadin peak 42.4 contributing the most (R^2= 0.3030).

MDT: At Motta the mixograph development time was influenced by 14 protein subunits. The highest contributor was the glutenin peak 23 (R^2= 0.2685).

SKCS-weight: Two glutenin and two gliadin peaks contributed to this parameter. Gliadin peaks 47 and 43 contributed the most. The overall contribution was R^2= 0.4824.

SKCS-diameter: Four subunits contributed to an overall of R^2= 0.4332. Glutenin peak 46.6 had the highest partial contribution (R^2= 0.1582), followed by gliadin peak 43.

SKCS-hardness index: Twelve peaks contributed to an overall R^2= 0.6789. The highest contributor was the glutenin peak 38, followed by glutenin peak 25.5, and gliadin peak 29.5.

Table 7.9 Results of stepwise multiple regression analyses for quality traits at Motta

Variable	Number of steps	Partial R^2	Model R^2	Pr>F
SDSS (Full model R^2 = 0.9239)				
P42.5	1	0.3665	0.3665	<0.0001
P23	2	0.1958	0.5623	<0.0001
G30.2	3	0.1151	0.6774	0.0004
G25	4	0.0601	0.7374	0.0043
P22	5	0.0512	0.7886	0.0039
P21	6	0.0437	0.8322	0.0032
G41.9	7	0.0349	0.8672	0.0035
G28	8	0.0299	0.8971	0.0026
P17.5	9	0.0144	0.9115	0.0225
G50	10	0.0124	0.9239	0.0246
VK (Full model R^2 = 0.8461)				
G22.6	1	0.1754	0.1754	0.0042
G32.6	2	0.0670	0.4008	0.0382
P15	3	0.0648	0.4656	0.0334
G45.5	4	0.0746	0.5402	0.0161
G37.6	5	0.0452	0.5854	0.0488
G31.6	6	0.0418	0.6772	0.0490
P48.7	7	0.0558	0.6495	0.0202
P33.2	8	0.0363	0.6858	0.0488
G51	9	0.0379	0.7237	0.0353
G24.5	10	0.0316	0.7553	0.0438
G48	11	0.0353	0.7906	0.0243
G24.9	12	0.0338	0.8244	0.0185
G41	13	0.0217	0.8461	0.0448
FPC (Full model R^2 = 0.5678)				
G49	1	0.1251	0.1251	0.0171
P21	2	0.1418	0.2669	0.0067
P34.5	3	0.0803	0.3472	0.0301
G47	4	0.1050	0.4523	0.0085
G26.4	5	0.0624	0.5147	0.0309
G31.6	6	0.0532	0.5678	0.0370
FLY (Full model R^2 = 0.8649)				
G42.4	1	0.3030	0.3030	<0.0001
P36.5	2	0.0898	0.3928	0.0167
G22.6	3	0.0876	0.4804	0.0120
P15.	4	0.0730	0.5534	0.0144
G41.6	5	0.0586	0.6120	0.0200
P44	6	0.0464	0.6584	0.0288
P37	7	0.0484	0.7068	0.0181
P22	8	0.0378	0.7446	0.0268
G47	9	0.0303	0.7750	0.0367
G20.9	10	0.0266	0.8016	0.0401
G32.6	11	0.0253	0.8269	0.0351
G33.2	12	0.0206	0.8475	0.0457
G42.4	13	0.0184	0.8291	0.0578
G38.5	14	0.0358	0.8649	0.0065

Table7.9 Continued

Variable	Number of steps	Partial R^2	Model R^2	Pr>F
		MDT (Full model R^2 = 0.8921)		
P23	1	0.2685	0.2685	0.0003
P34.5	2	0.2316	0.5002	<0.0001
P22	3	0.0781	0.5783	0.0087
P48.2	4	0.0433	0.6216	0.0386
P28	5	0.0668	0.6884	0.0062
G41.6	6	0.0464	0.7348	0.0139
G38.5	7	0.0305	0.7654	0.0345
P22	8	0.0230	0.7423	0.0645
P16	9	0.0387	0.7810	0.0148
G37	10	0.0317	0.8127	0.0185
G15	11	0.0296	0.8423	0.0149
G41.9	12	0.0237	0.8660	0.0195
P24	13	0.0316	0.8876	0.0167
P46.6	14	0.0154	0.9030	0.0312
G41.6	15	0.0109	0.8921	0.0669
		SKCS-weight (Full model R^2 = 0.4824)		
G47	1	0.1664	0.1664	0.0054
G43	2	0.1420	0.3084	0.0054
P46.6	3	0.1182	0.4266	0.0059
P48.2	4	0.0558	0.4824	0.0443
		SKCS-diameter (Full model R^2 = 0.4332)		
P46.6	1	0.1582	0.1582	0.0068
G43	2	0.1162	0.2744	0.0130
P48.2	3	0.0971	0.3715	0.0159
G47	4	0.0617	0.4332	0.0434
		SKCS-hardness index (Full model R^2 = 0.6789)		
P38	1	0.1028	0.1028	0.0317
P25.5	2	0.2058	0.2058	0.0245
G29.5	3	0.2984	0.2984	0.0250
P34.5	4	0.3646	0.3646	0.0478
G31.6	5	0.4441	0.4441	0.0233
G21.5	6	0.5064	0.5064	0.0346
G15	7	0.5631	0.5631	0.0349
G32.6	8	0.6224	0.6224	0.0229
P28	9	0.0478	0.6701	0.0307

All variables left in the model are significant at the 0.05 level; P = Glutenin, G = Gliadin, SDS = sodium dodecyl sulphate, SDSS=SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, SKCS = single kernel characterization system, Pr = probability determination, F = F-test, R^2 = coefficient of multiple determination

Stepwise multiple regression analysis for quality traits at both localities

Results are given in Table 7.10.

Models for each quality characteristic were obtained. Table 7.10 is a summary of the derived equations. Not all the variables are presented.

SDSS: A total of 15 subunits contributed to this parameter, four gliadin and nine glutenin subunits, with a total contribution of R^2 = 0.8158. The highest contributor was glutenin peak 42.5, with a partial contribution of R^2= 0.3455, followed by the glutenin peak 23.

VK: An equal number of glutenin and gliadin suabunits contributed to vitreous kernels. The highest contributing factors were gliadin peak 36.2 (R^2= 0.3681) followed by glutenin peak 41.

FPC: The most important contributors to flour protein content were six gliadin and two glutenin subunits. The highest contributor was the glutenin peak 36.2 (R^2= 0.4896). Gliadin peak 26.4 had the lowest partial contribution (R^2= 0.0205). The complete contribution of the seven peaks were R^2= 0.7150.

FLY: The overall contribution of the 18 gluten peaks were R^2= 0.8130, with gliadin peaks 42.4, 36.2 and 49 contributing the most.

MDT: The highest contributor was the glutenin subunit 34.5 (R^2= 0.2230). The three gliadin peaks contributing were peaks 33.5, 32.2 and 26.4. The lowest contribution was made by glutenin fraction 44. The overall contributions were R^2 = 0.6412.

SKCS-weight: Only glutenin peaks 39.5 (R^2= 0.0629) and 28 (R^2= 0.0760) contributed significantly to this model. Gliadin peaks 33.2 and 47 further contributed to an overall R^2= 0.2794.

SKCS-diameter: Two glutenin and one gliadin peak contributed to the overall R^2= 0.1931. The highest partial contribution was made by peak 39.5, followed by peak 28 and gliadin peak 33.2.

SKCS-hardness index: Five peaks contributed to an overall R^2= 0.5958. The highest contributor was gliadin fraction 36.2, followed by glutenin peak 21.

Table 7.10 Results of stepwise multiple regression analyses for quality traits for two localities

Variable	Number of steps	Partial R^2	Model R^2	Pr>F
SDSS (Full model R^2 = 0.8158)				
P42.5	1	0.3455	0.3455	<0.0001
P23	2	0.1647	0.5103	<0.0001
G30.2	3	0.0355	0.5457	0.0117
G46	4	0.0282	0.5739	0.0208
P22	5	0.0241	0.5980	0.0283
P21	6	0.0606	0.6587	0.0003
P25.5	7	0.0206	0.6793	0.0251
P24	8	0.0227	0.7019	0.0158
P45.6	9	0.0178	0.7198	0.0277
P19	10	0.0170	0.7368	0.0276
P30.5	11	0.0151	0.7519	0.0337
P29.5	12	0.0235	0.7754	0.0061
P44	13	0.0144	0.7898	0.0261
G41.6	14	0.0131	0.8029	0.0295
P48.2	15	0.0129	0.8158	0.0269
VK (Full model R^2 = 0.6496)				
G36.2	1	0.3681	0.3681	<0.0001
P41	2	0.8240	0.4505	0.0005
G45.5	3	0.6300	0.5134	0.0013
P21	4	0.3300	0.5464	0.0155
G26.4	5	0.0303	0.5767	0.0170
G20.9	6	0.0294	0.6061	0.0153
P35	7	0.0260	0.6321	0.0191
P17.5	8	0.0175	0.6496	0.0488
FPC (Full model R^2 = 0.7150)				
G36.2	1	0.4896	0.4896	<0.0001
G49	2	0.0776	0.5672	0.0002
P21	3	0.0442	0.6115	0.0025
P18	4	0.0237	0.6352	0.0220
G33.2	5	0.0186	0.6537	0.0378
G28.5	6	0.0174	0.6711	0.0404
G21.5	7	0.0234	0.6945	0.0149
G26.4	8	0.0205	0.7150	0.0189

Table 7.10 Continued

Variable	Number of steps	Partial R^2	Model R^2	Pr>F
FLY (Full model R^2 = 0.8130)				
G42.4	1	0.5199	0.5199	<0.0001
G36.2	2	0.0757	0.5956	0.0001
G49	3	0.0321	0.6276	0.0082
G28.5	4	0.0279	0.6556	0.0107
G29.2	5	0.0217	0.6773	0.0204
G51	6	0.0218	0.6990	0.0171
G25	7	0.0142	0.7132	0.0489
G36.2	8	0.0137	0.6996	0.0530
G23.6	9	0.0171	0.7166	0.0300
P20	10	0.0180	0.7347	0.0223
P17.5	11	0.0156	0.7502	0.0292
P33.7	12	0.0168	0.7671	0.0200
P35	13	0.0151	0.7822	0.0236
G39.5	14	0.0160	0.7982	0.0164
G42.4	15	0.0093	0.7888	0.0647
P20	16	0.0105	0.7783	0.0535
P42.3	17	0.0111	0.7894	0.0470
G25	18	0.0054	0.7841	0.1657
G23.6	19	0.0065	0.7776	0.1286
G36.2	20	0.0125	0.7900	0.0345
G45.7	21	0.0118	0.8018	0.0353
P26.5	22	0.0111	0.8130	0.0368
MDT (Full model R^2 = 0.6412**)**				
P34.5	1	0.2230	0.2230	<0.0001
P23	2	0.1952	0.4182	<0.0001
G33.5	3	0.0636	0.4818	0.0018
P22	4	0.0396	0.5214	0.0100
G32.2	5	0.0334	0.5548	0.0146
P19	6	0.0331	0.5878	0.0122
G26.4	7	0.0323	0.6201	0.0104
P44	8	0.0212	0.6412	0.0328
SKCS-weight (Full model R^2 = 0.2794)				
G33.2	1	0.0871	0.0871	0.0050
P39.5	2	0.0629	0.1500	0.0135
P28	3	0.0760	0.2260	0.0049
G47	4	0.0534	0.2794	0.0145
SKCS-diameter (Full model R^2 = 0.1931)				
P39.5	1	0.0752	0.0752	0.0093
P28	2	0.0659	0.1411	0.0119
G33.2	3	0.0520	0.1931	0.0216
SKCS-hardness index (Full model R^2 = 0.5958)				
G36.2	1	0.4005	0.4005	<0.0001
P21	2	0.0821	0.4826	0.0004
G47.8	3	0.0538	0.5364	0.0023
P44.5	4	0.0390	0.5754	0.0068
P41	5	0.0204	0.5958	0.0438

All variables left in the model are significant at the 0.05 level.

P = Glutenin, G = Gliadin, SDS = sodium dodecyl sulphate, SDSS=SDS sedimentation value, VK = vitreous kernels, FPC = flour protein content, FLY = flour yield, MDT = mixograph mixing time, SKCS = single kernel characterization system, Pr = probability determination, F = F-test, R^2 = coefficient of multiple determination

7.4.2 Discussion and conclusions

A total of 42 glutenin (including minor peaks and unresolved shoulders) were resolved in the glutenin extract, after gliadin extraction with 70% ethanol. A total of 51 gliadin peaks were differentiated. Peaks not only varied in concentration but also in presence between cultivars across the two environments.

For simplicity and due to the objectives of this study, the discussion will not focus on individual differences between lines, but on correlations between different peaks and quality parameters and also on the interactions between different protein subunits.

The negative correlation found between the subunits and SDS sedimentation contributes to SDSS values when compared to bread wheat (Boyacioglu and D'Appolonia, 1994b).

At Adet and Motta it was seen from the correlations that some of the glutenin peaks related to more than one quality trait, simultaneously. At Adet, the higher protein environment, more protein subunits correlated significantly with SDS sedimentation and mixograph development time, than at Motta. In fact no significant correlation between any subunit and MDT could be determined at Motta. The only peak correlating with the same trait across both localities was peak 28, correlating with SKCS-hardness.

Similar results were obtained for the gliadin subunits, with some subunits correlating with more than one baking trait simultaneously. At Motta more subunits correlated with each trait than at Adet. Despite the higher number of correlations per quality characteristic, averages obtained for quality tests at Motta were lower than that at Adet (Chapter 3, Table 3.4). This should be an indication that again it is not necessarily the number of subunits associated with the characteristic that determines quality, but also the identity and quantity that plays a role.

Gliadins had a distinct negative relationship with most of the quality characteristics, even though different subunits affected the trait at each locality. The only peaks influencing the same traits at both environments were peaks 28.5 and 35.5 correlating with flour yield.

Different subunits affecting the same quality characteristic at different environments is not unexpected, as protein concentration are polygenic and influenced by the environment. The diversity of the environments clearly played a role, with Adet being considered a protein rich and Motta a protein poor environment. Stewart (2002) studied the effect of site specific wheat quality in a single field. Significant differences in quality were observed in the quality of wheat samples from different sites. This indicated sensitivity of quality to environmental effects.

The combined analysis across the two environments indicated that proteins influenced more than one characteristic concurrently. Peaks 16 and 23, for example, had a negative relationship with both SDS sedimentation and mixograph development time.

There was no distinct pattern to the relationships of the higher and lower molecular weights and specific quality traits, since both the large and smaller polymeric proteins influenced SDS sedimentation. The most significant negative correlation was between SDSS and peak 42.5.

Results obtained for the gliadin correlations, across environments, indicated distinct negative correlations between the quality parameters of the more hydrophobic proteins. Vitreous kernels, flour protein content and SK hardness showed significant negative relationships with peaks eluting at 40 minutes. This confirmed the negative correlation found by Heubner and Gaines (1992), between quantity of gliadins and hardness. The opposite was found for flour yield and mixograph development time. There was no significant correlation observed between gliadins and SDS sedimentation.

A vast number of correlations were observed between the glutenin-glutenin and gliadin-gliadin subunits. These individual correlations are already an indication of the interaction exsiting between the protein fractions. Most of the correlations between the glutenin and gliadin proteins were significantly positive and between glutenin peaks eluting between 33 and 45 minutes. This confirmed the strong similarities and tight linkage between these two groups of proteins (Pogna *et al.*, 1990). These interactions create difficulty in the association of specific subunits to functionality.

Stepwise multiple regression offers a tool to help understand the interactions and more specifically the individual role of the contributors.

The included peaks, contributing to each parameter, differed between and across environments. However, even though the main regression model of quality parameters differed, some proteins were found to contribute to the same parameter in and across different environments.

Glutenin peaks 42.5 and 23 contributing to SDS sedimentation are good examples of these environmentally stable contributors. There was also a significant link between correlations existing between protein fractions and the individual contribution. It is evident that the regression model is not reliant on the correlations, but does give an indication of the interaction. A correlation is not required for the inclusion of a subunit in the regression model.

From the regression models it is also possible to deduce which protein fraction (polymeric or monomeric) are the most important contributors to a specific quality parameter. Most of the variation in mixograph development time is explained by the glutenin fraction, with more glutenin peaks contributing to this trait, than gliadin peaks. The opposite is true for flour protein content, with gliadins being the highest contributors.

Apart from the contribution of groups of proteins, the significant influence of individual proteins are evident from the high partial R-squared values. Individually some proteins accounted for 30-50% of the variation. As in the

case of the combined regression model for flour protein content (Table 7.10) across two localities, gliadin peak 36.2 had a partial contribution of R^2 = 0.4896 for FPC. The contribution of gliadins cannot be accepted without consideration of the potential linkage or correlation with LMW-GS (Payne *et al.,* 1984).

This study confirmed the complex interaction between the different flour components and the effect on quality. It is clear that although quality parameters were influenced by more than one protein subunit simultaneously, some subunits were more important in their contribution to a specific trait.

RP-HPLC offers the optimal tool for predicting the functional effect of different protein subunits with particular quality parameters.

Predicting the main protein contributors for specific quality traits offer the advantage of the development of markers, useful for predicting quality, especially if the expression is stable across environments.

Knowledge of specific protein subunits contribution to and correlation with quality parameters holds the added advantage of incorporation in a breeding programme.

Currently more research is invested in improving durum wheat through the development of substitution and transformed lines. This however, is not an economical approach for developing countries, lacking funds, facilities and expertise. Understanding the interactions and relationships between different proteins and finding improved methods of identifying and characterising subunits can provide the necessary criteria for use in a breeding programme.

7.5 References

Agrobase, 2000. Agronomix Software, Inc., Agrobase™, 71 Waterloo St. Winnipeg, Manitoba R3N0S4, Canada.

Bakhshi, A.K. and Bains, G.S., 1987. Study of the physio-chemical, rheological, baking and noodle quality of improved durum and bread wheat cultivars. *Journal off Food Science and Technology* 24:217-221.

Boggini, G., Tusa, P. and Pogna, N.E., 1995. Bread making quality of durum wheat genotypes with some novel glutenin subunit compositions. *Journal of Cereal Science* 22:105-113.

Boyacioglu, M.H. and D'Appolonia, B.L., 1994a. Durum wheat and bread products. *Cereal Foods World* 39:168-174.

He, G.Y., Rooke, L., Steele, S., Békés, F., Gras, P., Tatham, A.S., Fido, R., Barcelo, P., Shewry, P.R. and Lazzeri, P.A., 1999. Transformation of pasta wheat (*Triticum turgidum* L. var. *durum*) with high-molecular-weight glutenin genes and modification of dough functionality. *Molecular Breeding* 5:377-386.

Heubner, F.R. and Gaines, C.S., 1992. Relationship between wheat kernel hardness, environment and protein composition. *Cereal Chemistry* 69:148-151.

Liu, C.Y., Shepherd, K.W. and Rathjen, A.J., 1996. Improvement of durum wheat pastamaking and bread making qualities. *Cereal Chemistry* 73:155-166

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

Payne, P.I., Jackson, E.A., Holt, L.M. and Law, C.N., 1984. Genetic linkage between endosperm storage protein genes on each of the short arms of chromosome 1A and 1B in wheat. *Theoretical and Applied Genetics* 67: 235-243.

Peña, R.J., Trethowan, R., Pfeiffer, W.H. and Van Ginkel, M., 2002. Quality (end-use) improvement in wheat: Compositional, genetic and

environmental factors. In: A.S. Basra, and L.S. Randhawa, (Eds.), Quality improvement in field crops. Food products press, Binghampton, USA, pp 1-39.

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. *Journal of Cereal Science* 11:15-34.

SAS Institute Inc., 2004. SAS OnlineDoc® 9.1.3. Cary, NC: SAS Institute Inc.

Stewart G.M., 2002. Site-specific durum wheat quality and its relationship to soil properties in a single field in Northern South Wales. *Precision Agriculture* 3:155-168.

Chapter 8
General conclusions

Bread and durum wheat cultivars/lines having similar HMW-GS patterns had varied expressions in the quality parameters studied. Some bread wheat genotypes with subunits 2+12 (associated with poor quality) gave comparable results to cultivars/lines containing 5+10. This study therefore confirmed the findings of MacRitchie *et al.* (1990) that baking quality is not due to *Glu-1* allelic variation alone. This indicated the importance of incorporating and understanding all contributing factors in quality.

There was no deliberate selection for quality in the durum wheat lines used in this study; selection was based mainly on agronomical traits. Therefore the high frequency of presence of subunit LMW-2/γ45 might also be linked to agronomical traits, apart from its known influence on quality parameters. The influence of the same subunits differed across the diverse environments, as is evident from the variation in baking quality observed.

The SDS soluble and insoluble proteins were found to be equally important for baking quality. The importance of polymeric proteins was indicated by the significantly increased positive correlations observed between the ratios of protein fractions and quality parameters.

In durum wheat, the SDS insoluble proteins seemed to play a more important role in determining bread making quality. An increase in number and magnitude was found between insoluble proteins and quality traits.

Correlations observed, for both wheat types, between different fractions and baking quality differed significantly across the environments. This indicated the importance of gene interactions and the important role of environmental effects (Gupta *et al*., 1994).

The effect of certain SDS insoluble protein fractions, in durum, seemed to be less sensitive to environmental influences than that of bread wheat. This was indicated by the continuous correlations observed between insoluble protein fractions and quality parameters, within and across different environments. This confirmed that the SDS insoluble proteins are more genetically controlled than the SDS soluble fractions, in durum (Zhu and Khan, 2001).

In comparing the average proportions of different protein fractions in durum and bread wheat, durum had a higher content of SDS soluble and insoluble large polymeric proteins than bread wheat. This higher concentration was obtained despite the absence of the D-genome. Durum normally has two to three HMW-GS subunits compared to the three to four subunits of bread wheat (Lafiandra *et al*., 2000b). This increased concentration resulted in the expected longer mixing time. The true significance of this is that the quantity of proteins present, and not necessarily specific proteins, influences baking quality.

The combined correlations of bread and durum wheat protein fractions with baking quality, confirmed the stability of SDS insoluble large polymeric proteins, across environments.

The relatively small differences observed in quality parameters of bread wheat compared to durum wheat indicated the possibility of improving durum wheat' bread making quality through conventional breeding. This is possible due to the large genetic diversity existing in Ethiopian durum wheat, as Ethopia is seen as the centre of genetic diversity (Demissie *et al*., 1990).

A large number of polymeric and monomeric subunits were successfully resolved in both wheat types with the use of RP-HPLC. Contrary to the findings of Heubner *et al.* (1995), the environmental effect was visible in differences in protein content and composition. This was similar to the variation observed in SE-HPLC fractionation.

RP-HPLC creates the possibility to identify individual proteins correlated to baking quality. If these correlations are stable across environments and genotypes, suggesting a strong genetic control, it can be utilized by incorporation in a breeding programme. However, in agreement with Primard *et al*. (1991), due to the complexity of the proteins, predictions will have to be based on several protein components.

Unlike bread wheat proteins, it was difficult to relate specific functionality to specific protein groups in durum wheat. The absence of the D-genome and the subsequent importance of LMW-2 subunits could be a reason for this (Porceddu *et al*., 1998). In addition, the high correlation, linkage and similarity between LMW-GS and gliadins could also be a possible explanation.

Although quality is based more on the interaction of different components, especially proteins, specific subunits were identified as making a larger contribution to end product quality. Glutenin peaks 45 and 39, present in bread wheat cultivars/lines, contributing to FPC and MDT respectively. Similar results were obtained for durum lines, with glutenin peak 42.5 contributing the most to SDSS. These proteins had a varying degree of sensitivity to the environment, but some appeared to be environmentally stable. The stability might be due to a more important genetic control. This could affect the possible use of these proteins as predictors in baking quality. In the past the large environmental effect always inhibited the use of these markers in predicting quality.

Breeding and selection, could incorporate these protein markers combined with one or more direct physical (MDT or W) measurements of quality, to improve results, especially if working in diverse environments.

It is evident from this study that the importance and influence of the different protein fraction ratio cannot be denied. This suggests that breeding for quality improvement cannot rely on a single test or a single protein, but rather that effort should be made to select for the best ratio.

8.1 References

Demissie, A.H, Mariam, G. and Feyissa, R., 1990. Ethiopian wheat germplasm collection, conservation, characterization/evaluation and utilisation. In: D.G. Tanner, M. van Ginkel and W. Mwangi (Eds.), Six regional workshop for Eastern, Central and Southern Africa. Mexico, D.F. CIMMYT.

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

Heubner, F.R., Nelsen, T.C. and Bietz, J.A., 1995. Differences among gliadins from spring and Winter wheat cultivars. *Cereal Chemistry* 72:341.

Lafiandra, D., Masci, S., D'Ovidio, R. and Margiotta, B., 2000a. The genetics of wheat gluten proteins: An overview. In: P.R. Shewry and A.S. Tatham (Eds.), Wheat Gluten. Royal Society of Chemistry, pp 3-10.

MacRitchie, F., DuCros, D.L. and Wrigley, C.W., 1990. Flour polypeptides related to wheat quality. In: Y. Pomeranz (Ed.), Advances in Cereal Science and Technology, Vol. 10. American Association of Cereal Chemists, St. Paul, MN, pp 79-145.

Porceddu, E., Turchetta, T., Masci, S., D'Ovidio, R., Lafiandra, D., Kasarda, D.D., Impiglia, A. and Nachit, M.M., 1998. Variation in endosperm protein composition and technological quality properties in durum wheat. *Euphytica* 100:197-205.

Primard, S., Graybosch, R., Peterson, C.J. and Lee, J., 1991. Relationships between gluten protein composition and quality characteristics in four populations of high-protein, hard red winter wheat. *Cereal Chemistry* 68:305-312.

Zhu, J. and Khan, K., 2001. Effects of genotype and environment on glutenin polymers and breadmaking quality. *Cereal Chemistry* 78:125-130.

Chapter 9

Summary and recommendations

9.1 Summary

- Ethiopian cultivars and advanced lines of bread and durum wheat were studied for their grain quality characteristics under different environmental conditions. The influence of different protein fractions, ratios and subunits on bread making quality were determined by means of HPLC. Significant correlations across diverse genotypes and environments might indicate the potential use of these techniques in breeding programmes.

SE-HPLC

Bread wheat

- The genotypes studied expressed genetic variability in most of the important quality traits. Protein levels across the two environments were relatively low. A decrease in protein content led to an increase in mixing time. Genotypes at the lowest protein site had the longest mixing times.
- The higher protein site had higher average concentrations for all fractions extracted, except for SDS soluble and insoluble LMP.
- Across the two environments, the SDS soluble and insoluble polymeric proteins had a highly significant influence on quality.
- The number of correlations observed increased across environments compared to individual environments.
- Higher significant correlations were observed between ratios of proteins compared to that found between individual proteins.

SE-HPLC

Durum

- Similar results to bread wheat were seen for durum genotypes tested. The lower protein environment resulted in a lower protein content and increased mixing time.
- The average concentrations for SDS soluble LPP and SMP and insoluble LMP, SMP, TUPP and LUPP were higher at the high protein potential site.
- The number of correlations increased across environments, than when environments were considered individually.
- The magnitude of the correlations increased across environments.
- Higher significant correlations were observed between ratios of protein fractions and quality traits, notably the correlations between the ratio of SDS insoluble LPP:LMP and SPP:LMP with mixograph development time (0.545*** and 0.518***, respectively).
- Durum has the potential of improvement for bread making quality.

Bread and durum wheat

- The average flour protein content was slightly higher for durum wheat, at the higher protein site, but similar at Motta.
- The mixograph development time for both wheat types was longer at Motta, than at Adet, with the mixing time for durum slightly longer than that of bread wheat at Motta.
- The durum genotypes had higher average SDS soluble and insoluble LPP and SMP fractions, at both environments, compared to bread wheat.
- The opposite was true for LUPP and TUPP fractions, where the bread wheat genotypes had a higher average.
- The LUPP fraction and the SDS insoluble SPP displayed a direct relationship with SDS sedimentation, continuously across environments.
- The averages of baking quality traits for bread and durum wheat, with the exception of SDS sedimentation, did not differ extensively.

This indicated the potential application of durum wheat for comparable breadmaking utility.

RP-HPLC

Due to the large amount of data generated by RP-HPLC, emphasis was placed on the general trends across entries and differences between entries were not discussed.

Bread wheat

- A total of 38 glutenin subunits (including minor peaks and unresolved shouders) were resolved.
- Forty two bread wheat gliadin peaks were differentiated.
- The environmental effect was visible in entries in both variation in quantity observed and as presence or absence of subunits.
- Peaks/subunits correlating with baking parameters differed across different environments. This indicated sensitivity to environmental influences.
- Some correlations occurred consistently, regardless of the differences in environment.
- Significant polymeric-polymeric and monomeric-monomeric as well as polymeric-monomeric correlations were observed. These interactions need to be considered when determining functionality. Correlations might be due to indirect effects.
- Stepwise multiple regression can assist in determining direct relationships. It also gives an indication of the interactions excisting between protein components.
- The regression models indicated the individual proteins contributing to the quality trait. Some proteins were major contibutors, explaining 59% of the variation occurring in the parameter. The most important contributors to quality remained the glutenin subunits.

Durum wheat

- A total of 42 glutenin subunits (including minor peaks and unresolved shouders) were resolved.
- Fifty one durum wheat gliadin peaks were differentiated.
- Similar results to bread wheat were obtained for durum wheat entries.
- Unlike the bread wheat results, specific durum subunits influenced more than one baking parameter simultaneously.
- More interactions between the different protein subunits influencing specific traits were visible. No distinct patterns were visible between the subunit type and functional property.

9.1 Opsomming

- Etiopiese brood en durum koring kultivars en gevorderde lyne is in verskillende omgewingstoestande vir kwaliteitseienskappe bestudeer. Die invloed van verskillende proteïenfraksies, -verhoudings en subeenhede op broodbak-kwaliteit is m.b.v.. HPLC ontleed. Betekenisvolle korrelasies tussen en binne diverse omgewings en genotipes kan die moontlike gebruik van die tegniek in teelprogramme aandui.

SE-HPLC

Broodkoring

- Genotipes wat vir die studie gebruik is, het variasie in meeste van die belangrike kwaliteitskenmerke getoon. Die proteïen-inhoud in beide omgewings was relatief laag. Die lae proteïen-inhoud het die mengtyd verkort. Genotipes wat in die lae proteïen-potensiaal omgewing geplant is, het die langste mengtyd getoon.
- Die hoër proteïen-potensiaal omgewing het die hoogste gemiddelde konsentrasie vir al die proteïenfraksies wat bestudeer is, getoon, buiten vir SDS-oplosbare en onoplosbare groot monomeriese proteïene.

- Die SDS oplosbare en onoplosbare proteïene het ‘n hoogs betekenisvolle invloed op die bakkwaliteit, oor beide omgewings, uitgeoefen.
- ‘n Groter aantal korrelasies tussen proteïenfraksies en kwaliteit, oor beide omgewings as binne die verskillende omgewings, is waargeneem.
- Hoër betekenisvolle korrelasies tussen die verskillende proteïen-verhoudings en kwaliteit, as tussen kwaliteit en individuele proteïene, is waargeneem.

SE-HPLC

Durumkoring

- Die waargenome resultate vir durum koring was soortgelyk aan brood koring. Die laer proteïen-potensiaal omgewing het tot ‘n laer proteïen-inhoud en langer mengtye gelei.
- Die hoër proteïen-potensiaal-omgewing het ook die hoogste gemiddelde SDS oplosbare groot polimeriese, klein monomeriese en onoplosbare LMP, SMP, TUPP en LUPP opgelewer.
- ‘n Groter aantal korrelasies is oor die twee omgewings as binne die afsonderlike omgewings waargeneem.
- Die waargenome korrelasies was hoër oor verskillende omgewings as binne omgewings.
- Korrelasies tussen verskillende proteïen-verhoudings en die bak-kwaliteit was betekenisvol hoër as tussen die individuele proteïenfraksies, veral die verhoudings van SDS onoplosbare LPP:LMP en SPP:LMP met mengtye van 0.545*** en 0.518***, onderskeidelik.
- Durum koring besit gevolglik die potensiaal om vir broodbak-kwaliteit verbeter te word.

Brood- en durumkoring

- Die gemiddelde meel proteïen-inhoud in die hoër proteïen potensiaal-omgewing was effens hoër vir durumkoring, maar dieselfde by Motta.
- Die miksogram-ontwikkelingstyd vir beide koring-soorte was langer by Motta as by Adet. Die mengtyd van durumkoring by Motta was effe langer in vergelyking met die van brood.
- In vergelyking met die gemiddeldes van broodkoring, is hoër gemiddelde SDS oplosbare en onoplosbare LPP en SPP fraksies by beide omgewings vir durumgenotipes waargeneem.
- Die teenoorgestelde is vir die gemiddelde LUPP en TUPP van broodkoring waargeneem.
- 'n Volgehoue verwantskap tussen LUPP en SDS onoplosbare SPP met SDS sedimentasie is oor beide omgewings waargeneem.
- Die gemiddelde resultate van die bak-kwaliteitstoetse vir brood- en durumkoring, uitgesonderd SDS sedimentasie, het nie 'n groot verskil getoon nie, wat daarop dui dat durumkoring wel die potensiaal het om brood van vergelykbare gehalte te produseer.

RP-HPLC

Aangesien die RP-HPLC tegniek groot hoeveelhede data genereer het, is 'n volledige bespreking van die individuele inskrywings baie kompleks. In hierdie afdeling is daar dus op die algemene neigings en patrone wat waargeneem kan word, gefokus terwyl die individuele verskille binne die genotipes nie bespreek is nie.

Brood koring

- 'n Totaal van 38 glutenien subeenhede (insluitende kleiner pieke en onvolledige skouers) is geïdentifiseer.
- Twee-en-veertig gliadien pieke is onderskei.
- Die omgewings-effek was sigbaar in die verskillende konsentrasies en teenwoordigheid of afwesigheid van sekere fragmente.

- Verskillende korrelasies tussen pieke (subeenhede) en bak-eienskappe in verskillende omgewings is gevind, wat weereens op die omgewing se invloed gedui het.
- Sommige korrelasies is, ongeag verskille in die omgewings, konstant waargeneem.
- Betekenisvolle korrelasies is tussen polimeries-polimeries, monomeries-monomeries en polimeries-monomeriese fraksies waargeneem. Hierdie korrelasies moet in ag geneem word wanneer die funksionele aspek van proteïene bestudeer word. Korrelasies tussen kwaliteitstoetse en proteïene kan indirek ontstaan.
- Stapsgewyse veelvuldige regressie-bepaling kan tot die bepaling van direkte verwantskappe bydra. Dit kan ook ‘n aanduiding wees van die interaksies wat tussen die verskillende proteïenkomponente bestaan.
- Die regressie modelle dui die individuele bydrae van die verskillende proteïene tot kwaliteit aan. Die individuele bydrae van sommige proteïene was groter as ander, en kan tot 59% van die variasie wat voorkom, verklaar. Die gluteniene blyk steeds die belangrikste bydraers totkwaliteit te wees.

Durum koring

- Vir durumkoring is ‘n totaal van 42 glutenien subeenhede (kleiner pieke en onvolledige skouers) waargeneem.
- Die gliadien gemiddeldes was bietjie meer, met 51 pieke.
- Soortgelyke resultate as die van broodkoring is waargeneem.
- Die grootste verskil tussen durum- en broodkoring was dat een proteïen meer as een kenmerk gelyktydig affekteer.
- ‘n Groter aantal interaksies tussen spesifieke proteïene en kwaliteits-eienskappe is waargeneem, maar geen spesifieke patroon kon tussen spesifieke groepe proteïene en die funksionele kenmerke van deeg herken word nie.

9.2 Recommendations

The experiment needs to be repeated across different years to be more conclusive. Enough seeds/flour to include actual baking tests would add to the correlations and understanding of interactions.

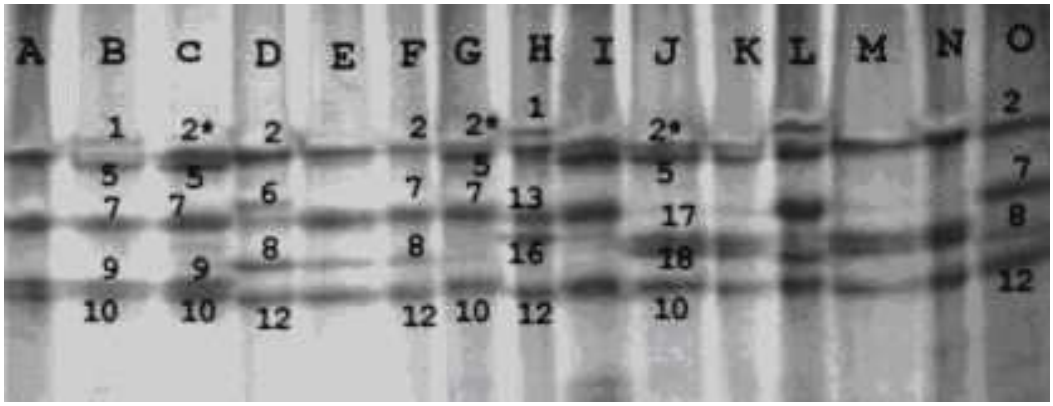
The RP-HPLC technique can be further optimized. Separate fractionation of HMW and LMW glutenin can further increase our knowledge about the functionality of individual proteins, leading to a better understanding of the interactions. A study to determine specific proteins and the environmental influences on each would necessitate the use of a homogenic population.

Correlations found between different proteins need to be confirmed in different genetic backgrounds across more environments. The confirmation of specific proteins correlated with baking quality could be utilized as markers in early generations.

Although not deliberately selected for quality, most of the cultivars/lines studied, performed reasonably well in the quality tests. The differences visible between the results obtained for the different environments, requires further investigation in relation to quality traits and protein environments. This suggests however that separate selection for high and low protein environments would benefit breeding programmes.

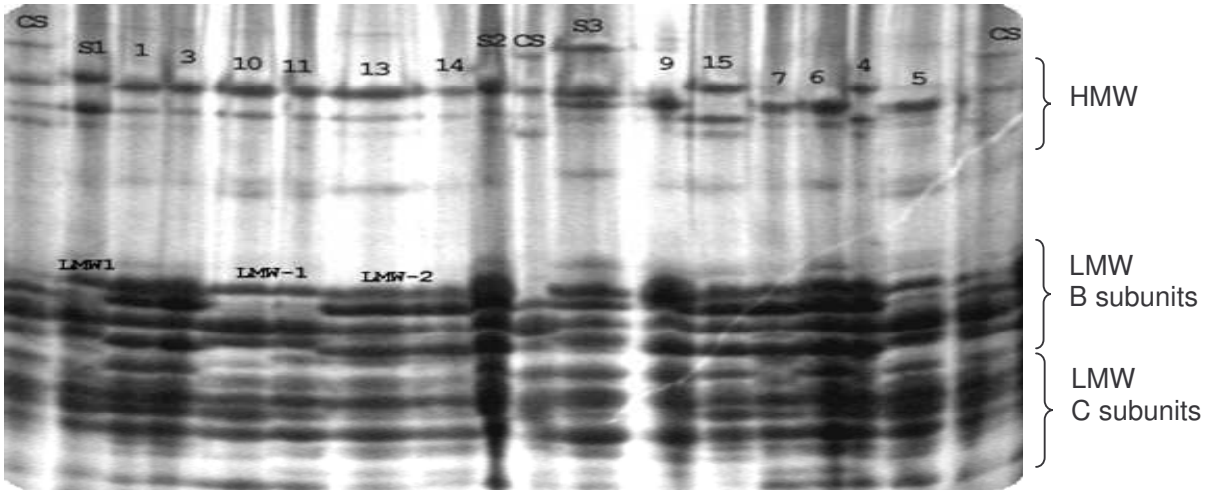
Appendices

APPENDIX A



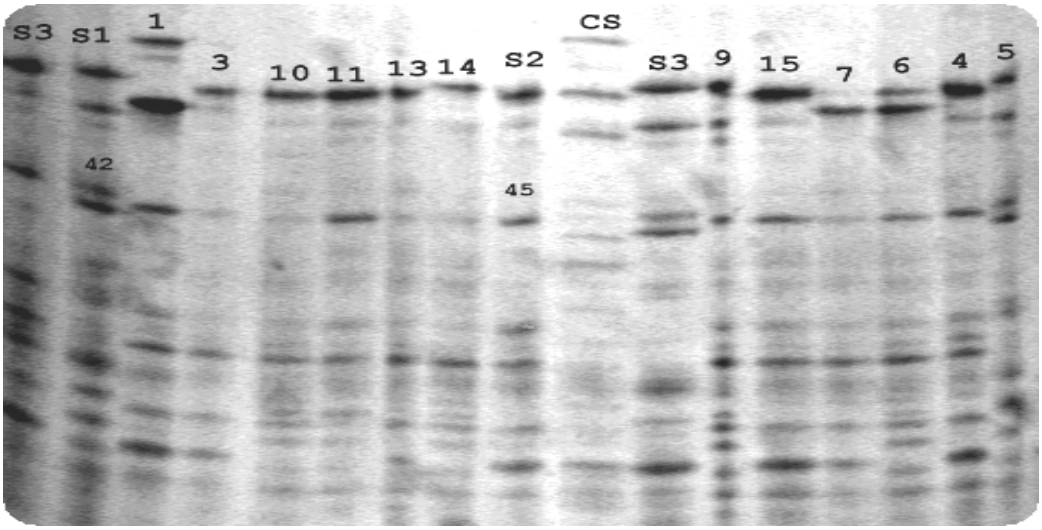
A=HAR1709; B=HAR1522; C=HAR1775; D=HAR2348; E=ET13A_2; F &O=Chinese Spring; G=HAR1868; H=HAR2807; I= HAR1685; J= HAR2562; K= HAR604; L=SST-825; M=Kariega

Figure 1. SDS-PAGE patterns of HMW-GS of some bread wheat lines/cultivars.



S1, Langdon; S2, Mexicali; S3,Alaga; CS, Chinese Spring (S =Standard)
1-15 = Entries tested.

Figure 2. SDS-PAGE patterns of HMW- and B-LMW-GS of durum lines.



S1, Langdon; S2, Mexicali; S3,Alaga; CS, Chinese Spring (S =Standard)
1-15 = Entries tested.

Figure 3. SDS-PAGE patterns of gliadin in some of the genotypes.

APPENDIX B

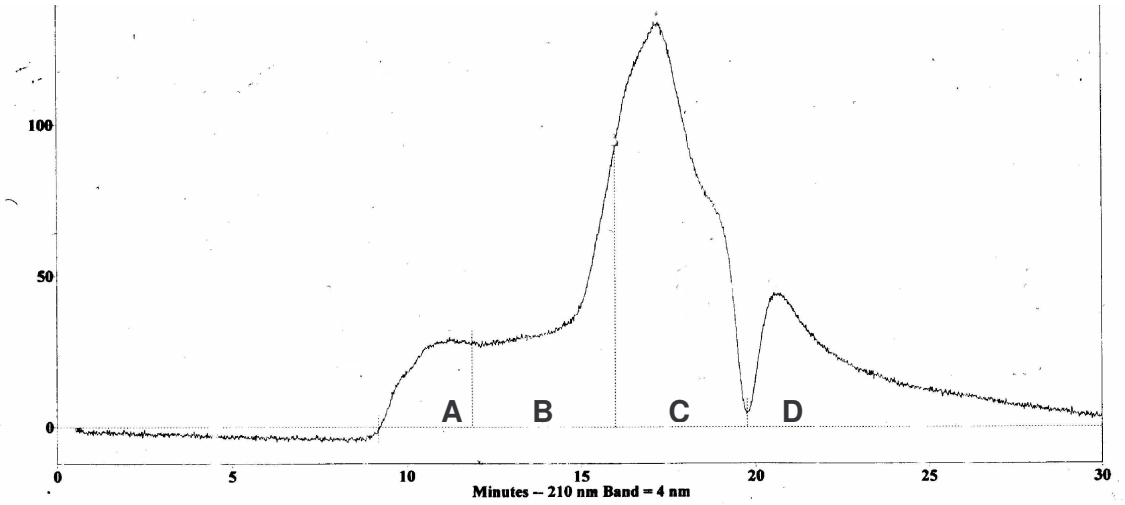


Figure 1 An example of SDS-soluble proteins as separated with SE-HPLC where A = large polymeric proteins (LPP), B=smaller polymeric proteins (SPP), C= large monomeric proteins (LMP) mainly gliadins, D=smaller monomeric proteins (SMP) mainly albumins and globulins.

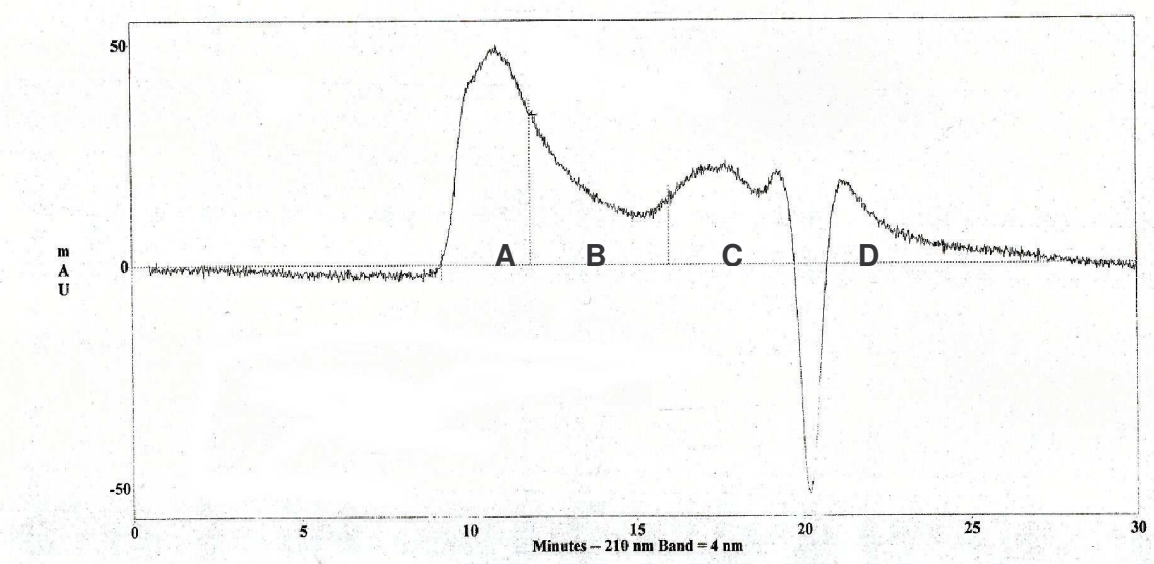


Figure 2 An example of SDS-insoluble proteins as separated with SE-HPLC where A = large polymeric proteins (LPP), B=smaller polymeric proteins (SPP), C= large monomeric proteins (LMP) mainly gliadins, D=smaller monomeric proteins (SMP) mainly albumins and globulins.

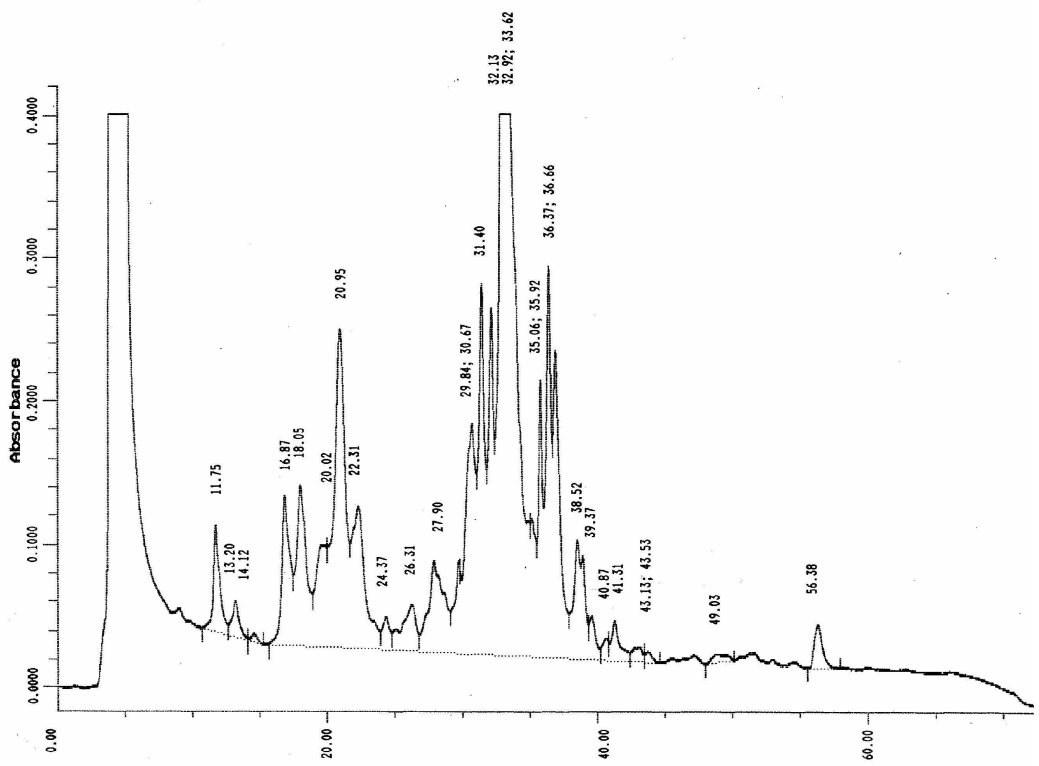


Figure 3 RP-HPLC analysis of glutenin proteins extracted with 50% propanol from flour.

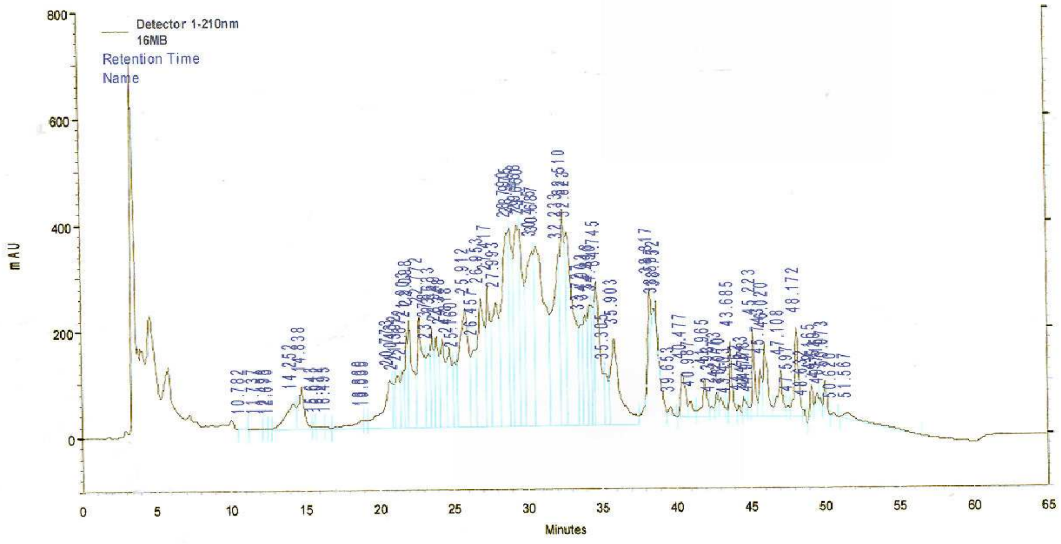


Figure 4 An example of RP-HPLC of gliadin proteins extracted with 70% ethanol from flour.