The utilisation of gluten fractions as quality parameters in selected South African wheat cultivars

B.S. Wentzel

The utilisation of gluten fractions as quality parameters in selected South African wheat cultivars

By

Barend Smit Wentzel

Thesis submitted in accordance with the requirements for the **Magister Scientiae Agriculturae** degree**,** in the Department of Plant Sciences (Plant Breeding), Faculty of Natural and Agricultural Sciences

University of the Free State
Bloemfontein
Republic of South Africa
November 2010

Promoter:	**Prof. M.T. Labuschagne**
Co-promoters:	**Dr. W.M. Otto**
	Dr. A. van Biljon

DECLARATION

I declare that the thesis hereby submitted for the **Magister Scientiae Agriculturae** degree, at the University of the Free State, is my own independent work and have not previously been submitted at another university/faculty. I further concede copyright of the thesis in favour of the University of the Free State.

B.S. Wentzel
Department of Plant Sciences (Plant Breeding)
Faculty of Natural and Agricultural Sciences
University of the Free State
South Africa

Acknowledgements

I wish to express my appreciation to the following people and organisations for their contribution to this study:

- Prof. Maryke Labuschagne as my mentor, without her enthusiasm, continued encouragement and dedication, this study would not have been possible.
- ARC – Small Grain Institute management for the opportunity to further my studies and providing the facilities.
- Pannar for granting me permission to use their cultivars.
- The NRF for financial assistance.
- Dr. Willem Otto and Dr. Angeline van Biljon, my co-promoters.
- Mrs. Sadie Geldenhuys for all the administration and motivation.
- Dr. Derek Stewart and Dr. Robert Hancock (Scottish Crop Research Institute) for their contribution to my HPLC knowledge.
- Prof. Klaus Pakendorf for his advice.
- Robbie Lindeque and Hesta Hatting for assisting with GenStat.
- Mrs. Juliette Kilian for assisting with literature searches.
- The wheat quality team: Benson Majola, Margaret Radebe, Lydia Dlamini, Christina Matla, Elizabeth Mtjale and Topsy Moloi.
- My mother for her support and patience.
- Our heavenly Father for giving me perseverance to complete the study.

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Abbreviations

µl	Microlitre
µm	Micrometre
AACC	American Association of Cereal Chemists
ABS	Mixograph water absorption
ACN	Acetonitrile
AlvG	Swelling index
AlvL	Alveograph extensibility
AlvP	Alveograph tenacity
AlvP/L	Alveograph configuration of the curve
AlvW	Flour strength
ANOVA	Analysis of variance
ARC-SGI	Agricultural Research Council – Small Grain Institute
Ar	Arlington
Be	Bethlehem
BFY	Break flour yield
Bo	Bothaville
C76	Flour colour at a 76% flour yield
°C	Degrees Celsius
cm^3	Cubic centimetre
Cult	Cultivar
CV	Coefficient of variation
DTT	Dithiothreitol
d.f.	Degrees of freedom
Env	Environmental
FABS	Farinograph water absorption
FN	Falling number
FFF	Flow field-flow fractionation
FY	Flour yield
g	gram
g	Gravitational force
$g\ m^{-2}$	gram per square metre

Gli	Gliadin
Gli/Glu	Gliadin to glutenin ratio
Gli/HMW	Gliadin to high molecular weight glutenin subunits ratio
Gli/LMW	Gliadin to low molecular weight glutenin subunits ratio
Glu	Glutenin
GMP	Glutenin macroplymer
GXE	Genotype by environmental interaction
h^{-2}	Narrow sense heritability
HI	Hardness index
hl	Hectolitre
HLM	Hectolitre mass
HMW-GS	High molecular weight glutenin subunits
HPLC	High-performance liquid chromatography
J	Joule
KD	Kernel diameter
kDa	Kilodalton
LFV	Loaf volume
LMP	Large monomeric protein
LMW-GS	Low molecular weight glutenin subunits
LMW/HMW	Low molecular weight to high molecular weight glutenin subunits
LPP	Large polymeric protein
LUMP	Large unextractable monomeric protein
LUPP	Large unextractable polymeric protein
M	Mole
MALDI-TOF	Matrix-assisted laser desorption / ionization time-of-flight
MALLS	Multi-angle laser light scattering
mAU	Milli absorbance units
MDT	Mixograph development time
mg	Milligram
min	Minutes
mM	Milli molar

mm	Millimetre
NBC	Narrow bore column
nm	Nanometre
P	Probability
Prot	Flour protein content
R^2	Coefficient of multiple determination
RP-HPLC	Reversed-phase high–performance liquid chromatography
rpm	Revolutions per minute
RT	Room temperature
SAGL	South African Grain Laboratory
SDS	Sodium dodecyl sulphate
SDSS	SDS sedimentation volume
SDS-PAGE	SDS-Polyacrylamide gel electrophoresis
SE-HPLC	Size-exclusion high-performance liquid chromatography
SKCS	Single Kernel Characterisation System
SMP	Small monomeric protein
SPP	Small polymeric protein
SUMP	Small unextractable monomeric protein
SUPP	Small unextractable polymeric protein
Temp	Temperature
TFA	Trifluoroacetic acid
ton ha^{-1}	Ton per hectare
TPP	Total polymeric protein
TUPP	Total unextractable polymeric protein
UPP	Unextractable polymeric protein
VK	Vitreous kernels
v/v	Volume per volume
WGC	Wet gluten content
w/v	Weight per volume

Chapter 1

Introduction

More than one-third of the world's population use wheat as a staple food, due to its diverse uses, nutritional value and storage traits. Wheat originated in southwestern Asia, where it has been cultivated for more than 10 000 years (Sleper & Poehlman, 2006). Wheat flour renders dough with unique visco-elastic properties, suitable for different applications, such as bread, noodles, pasta, biscuits, cakes, etc. (Branlard & Dardevet, 1985). Flour from hexaploid wheat (*Triticum aestivum* L.) is used for commercial bread making (Sleper & Poehlman, 2006).

Wheat breeding programmes strive to provide new cultivars that perform well agronomically and have suitable milling, rheological and baking properties. In South Africa, these quality norms are determined by the South African Grain Laboratory (SAGL), in conjunction with wheat breeders, millers and bakers. For bread wheat cultivars, primary quality norms include kernel characteristics, protein content, flour yield and flour colour, dough properties and loaf volume. Final classification for a new line requires a minimum of three years' data from five localities per annum (SAGL, 2010).

The most important quality traits are complex and quantitatively inherited, particularly milling yield, dough strength and extensibility. The use of multi-environment trials has led to considerable gains in some traits, although the genetic advance in key wheat quality traits has been considerably lower (Raman et al., 2009). The quality of wheat flour relates to the protein composition and as a result, to the end-use suitability (Khelifi & Branlard, 1992).

Protein quantity is strongly influenced by the environment, whereas quality is determined by the genotype and the environment (DuPont & Altenbach,

2003). Plant breeding evolved to the stage where wheat can be grown with a lower, but more focused, protein content with outstanding bread making properties (Anderssen et al., 2004). The ultimate challenge in cereal science is to improve wheat quality through a better understanding of its relationship to the chemical composition of wheat flour (Békés et al., 2006).

Dough strength and extensibility are the most important factors to define the suitability of flour to bake good bread (Bushuk & Békés, 2002). Different types of equipment are used to define dough properties in order to simulate industrial procedures, some of the equipment were developed more than 70 years ago (Swanson & Working, 1933). Procedures became more objective with computerised technology, resulting in more accurate and precise measurements, high throughput and considerably less flour sample (Wrigley et al., 2006). Comprehensive rheology tests and baking procedures require large sample sizes that are only available after six or seven generations in a breeding programme.

Several chromatographic methodologies have been employed to separate wheat proteins and study their relationship to dough properties and bread making performance. For instance, gel electrophoresis (Payne et al., 1979); reversed-phase high-performance liquid chromatography (RP-HPLC) (Huebner & Bietz, 1987); size-exclusion HPLC (SE-HPLC) (Gupta et al., 1993); asymmetrical flow field-flow fractionation (FFF) (Wahlund et al., 1996); matrix-assisted laser desorption / ionization, time-of-flight (MALDI-TOF) mass spectrometry (Dworschak et al., 1998) and multi-angle laser light scattering (MALLS) photometer on-line to a SE-HPLC system (Carceller & Aussenac, 2001). These procedures require small samples and analysis can be performed on early generation material.

The Department of Plant Breeding (University of the Free State, South Africa) initiated HPLC analyses on South African wheat cultivars. Significant correlations were reported between molecular weight distribution and quality parameters (Labuschagne & Aucamp, 2004; Labuschagne et al., 2006). Koen (2006) used SE-HPLC and RP-HPLC in a study on Ethiopian wheat cultivars.

Individual peaks in the gliadin and glutenin (RP-HPLC) chromatograms were correlated with the quality characteristics. Molecular weight distribution of South African grown wheat has never been determined with a narrow bore column, which reduces the conventional runtime with 50% (Ohm et al., 2009). The correlation between RP-HPLC gluten fractions and South African wheat quality is still uncertain.

The objectives of this study were to:

- determine the correlation between gluten fractions and wheat quality parameters
- correlate wheat quality with molecular weight distribution
- determine whether RP-HPLC or SE-HPLC is more suitable to predict quality in South African grown wheat

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Chapter 2

Literature review

2.1 Introduction

The art of bread baking has been existing for more than 12 000 years. Bread quality is affected by many factors. The importance of these factors reflects in the amount of periodicals that are devoted to cereal science and technology (Mondal & Datta, 2008). Improved yield and protein content, with acceptable milling and baking characteristics, have been the main selection criteria in wheat breeding (Cho et al., 2001). Yield components and kernel features (Larik et al., 1995), falling number, flour colour, SDS-sedimentation and loaf volume (Barnard et al., 2002) were added to the list of quality parameters.

2.2 Hectolitre mass

Hectolitre mass, also referred to as test weight, is the mass per volume of wheat and is one of the primary criteria used in wheat trading. It has a direct impact on the transportation costs because of the weight of grain that can be loaded in a fixed volume, and it gives an indication of flour yield (Fowler & De la Roche, 1975). Hectolitre mass is also an indication of sound grain because wheat grain density can be affected by wet or shrivelled kernels. The value can be influenced by the environment and genetic background (Marshall et al., 1986). The minimum value of 75 kg hl^{-1} is required for new lines to be released in South Africa (SAGL, 2010).

2.3 Kernel characteristics

Kernel hardness describes endosperm texture and vitreousness describes the structure. The latter refers to the optical states of the endosperm, whether it appears glassy or mealy, which is strongly influenced by the environment (Haddad et al., 1999). Improved kernel size and weight generally mean more endosperm, and kernel hardness allows better control of endosperm particle

size during grinding (Finney et al., 1987). Kernel texture is regarded as the most important single characteristic that influences the functionality of common wheat, except gluten strength and its associated factors. An increase in kernel hardness results in an increase in energy input during milling, flour granularity, damaged starch and water absorption properties (Pomeranz & Williams, 1990). During the first break of milling, harder wheats break to give a more even distribution of particles than soft wheats (Campbell et al., 2007). Consequently, milling performance is influenced by the uniformity of single kernel hardness (Ohm et al., 1998).

Symes (1965) reported that kernel hardness is controlled by a major gene, close to loci coding for puroindoline proteins (Sourdille et al., 1996). Dough rheological properties are influenced by the texture of the grain (Martinant et al., 1998; Branlard et al., 2001), mostly through the proportion of starch damage and as a result, the water absorption capacity of the flour (Groos et al., 2004). Grain hardness correlated with a peak within the RP-HPLC gliadin profile (Huebner & Gaines, 1992) and storage protein alleles (*Glu-B1* and *Gli-B1*) (Félix, 1996). The Single Kernel Characterisation System (SKCS) is the most well developed system to evaluate quality characteristics of individual wheat kernels (Osborne et al., 1997; Sissons et al., 2000).

2.4 Falling number

Falling number (FN) defines preharvest sprouting, which has a direct influence on baking quality. Loaf volumes decreased gradually with increased germination. Unfavourable weather and damp conditions may trigger wheat kernels to sprout (Neethirajan et al., 2007). Excessive α-amylase levels activate starch degradation and reduce dough viscosity (Rasper & Walker, 2000). Dough becomes sticky and causes handling problems. The crumb becomes darker and the structure is coarse and gummy (Moot & Every, 1990), which makes it difficult to slice (Dexter, 1993).

The Hagberg FN method is widely used, although it does not serve as a direct measurement of enzyme activity (Blackman & Payne, 1987). An increase in

preharvest sprouting is indicated by a decrease in FN value. Higher FN values means less α-amylase activity (Kosmolak & Dyck, 1981). Flour with acceptable α-amylase activity has a FN value ≥ 250 seconds (Chrissie Miles - personal communication).

2.5 Milling properties

The purpose of milling is to separate the endosperm from bran and germ. The aim is to separate as much flour as possible while maintaining high flour quality. Bran contamination increases with higher flour extraction rates. Extraction rates for commercial mills vary between 70 and 80%, although the average wheat kernel contains approximately 85% endosperm. The break roll system separates the bran from the endosperm with some flour at each stage. The endosperm fraction that is still too big to be considered as flour, is sent to the reduction system. Starch granules can be damaged by the smooth reduction rolls and therefore affect the water absorption properties of the flour (Campbell et al., 2007). The first break is regarded as a critical point in milling, since it determines the stream flows through the rest of the mill. A constant particle distribution from the first break would be essential in order to deliver even flow through the milling system (Hsieh et al., 1980; Yuan et al., 2003; Campbell et al., 2007).

2.6 Flour colour

Flour colour is influenced by two independent factors, brightness and yellowness. Brightness is controlled by the milling process, through particle size and the presence of bran. Yellowness is caused by carotenoid pigments that occur in some genotypes. Millers have used bleaching agents to control flour colour, although consumer demands for reduced additives are increasing. The production of unbleached flour is regarded as an advantage (Oliver et al., 1993).

Differences in flour colour can be influenced by genetic, environment, genotype by environmental interactions or the milling process (Bass, 1988). The flour colour of winter wheat cultivars released since 1967 in South Africa

was 46% brighter than cultivars released from 1930 – 1964 (Van Lill & Purchase, 1995).

2.7 SDS sedimentation

The SDS sedimentation (SDSS) test was developed by Zeleny (1947), and modified by Axford et al. (1978) to estimate the bread baking quality of wheat cultivars. De Villiers and Laubser (1995) reported a significant positive correlation between SDSS values and protein content, in addition to loaf volume. Significant positive correlations were also reported between SDSS and extensograph dough strength, extensibility, farinograph and alveograph parameters (Gröger et al., 1997). Khatkar et al. (1996) suggested that SDSS alone was not sufficient to consider the bread making potential of a wheat cultivar, their findings were based from a study on wheat cultivars with a wide diversity of bread making performances. A study conducted by Oelofse (2008) indicated that years contributed more to variability in SDSS than locations under South African conditions.

2.8 Mixograph

The mixograph is one the most widely used instruments for physical dough testing, providing parameters essential for classifying wheat and predict end use quality (Wikström & Bohlin, 1996). The rate of dough development is the primary measurement and mixograph peak time was selected as a measure of this factor (Fowler & De la Roche, 1975).

The resistance of a dough to mixing with pins is measured and recorded. The peak time is the dough development time. The ascending slope of a mixograph indicates the rate of dough development, the descending slope specifies the rate of dough breakdown. The angle between the developing and weakening slopes indicates mixing tolerance (Walker & Hazelton, 1996). Variation in mixing times between entries primarily correlates to protein fractions and their ratios (Bietz et al., 1973). The effect of high molecular weight glutenin subunits (HMW-GS) on mixograph parameters were reported by several researchers. A positive effect on dough development time and

dough strength was observed for HMW-GS 5+10, 17+18, 7+8, 1 and 2*. Weak dough and shorter mixing time were associated with HMW-GS 2+12, 6+8, 3+12 and 20 (Campbell et al., 1987; Cressey et al., 1987; Ng & Bushuk, 1988; Dong et al., 1992; Gupta & MacRitchie, 1994). Dough development time decreases with an increase in nitrogen fertilisation rate (Saint Pierre et al., 2008).

2.9 Farinograph

Rheological properties of wheat flour are measured while mixing and developing into a dough. The farinograph measures the energy required to mix dough as it progresses through water absorption, dough development and dough breakdown. Results did not correlate directly to baking test results or to other types of recording mixers. The measurements are useful to determine relative water absorption properties between different flours (Walker & Hazelton, 1996).

Water absorption in wheat is regarded as a function of protein content, damaged starch, pentosans and gluten strength (Preston & Kilborn, 1984). Absorption increases linearly with protein content, although the slope of the regression is determined by the genotype. Rheological properties in wheat flour are particularly sensitive to the amount of water, the effect is more visible with a decrease in the amount of water (Eliasson & Larsson, 1993).

2.10 Alveograph

The rheological behaviour is evaluated by blowing a dough sample into a bubble until it ruptures. This type of deformation is called bi-axial extension, the dough bubble extends in two directions during inflation, along the meridian and parallel of the bubble (Launay, 1987).

Bi-axial extension simulates the deformation of the dough caused by the pressure from fermentation and oven rise. The alveograph tenacity (AlvP) value measures dough tenacity as related to the maximum pressure required for the deformation of the dough, while the alveograph extensibility (AlvL)

value indicates the extensibility of the curve and AlvP/L is the configuration ratio of the curve. The swelling index (AlvG) is the square root of the essential volume of air required to rupture the bubble and is primarily a measure of dough extensibility. The AlvW value is regarded as the measure of flour strength (Faridi & Rasper, 1987).

Extensive research done on hard wheat (Chen & D'Appolonia, 1985) and soft wheat (Rasper et al., 1986) has shown that processing behaviour of wheat flour can be determined by the alveograph and suitability for specific end-use can be evaluated. Variation in AlvL and AlvW is more influenced by the environment than AlvP, due to the influence of protein content on AlvL and AlvW (Ames et al., 2003).

2.11 Loaf volume

The baking test is considered as the final measure of wheat quality and is still the only reliable method for determining bread making performance (Wikström & Bohlin, 1996). Three main methods are applied to make bread. The first method is a straight dough method where all the ingredients are mixed in one step. Sponge and dough method is a two step procedure. Leavening agent is prepared in the first stage and the remaining ingredients are added after a few hours. The Chorleywood process requires ultrahigh mixing for a few minutes where all the ingredients are added at once (Giannou et al., 2003).

Baking quality of wheat is determined by protein quantity and quality (Aussenac et al., 2001), as well as the baking process applied (Švec & Hrušková, 2010). Gluten protein is the main contributor to the viscoelastic properties in wheat flour that is suitable for the preparation of leavened bread (Bushuk, 1998). Intra and inter molecular disulphide bonds of gluten proteins are important in the formation of the gluten matrix in dough (Singh, 2005). A sufficient number of gas cells have to be incorporated in the dough to obtain bread with a light and even texture (Bloksma, 1990). High strength dough can inhibit the extensibility of dough films between gas cells and limit the expansion of gas cells during fermentation and baking, and thus reduce the

loaf volume (Sliwinski et al., 2004). Molecular weight distribution indicated that polymeric proteins decreased while low molecular weight proteins tend to increase during bread baking (Singh, 2005).

Cauvain and Young (1998) suggested that a link exists between extension testing and baking performance, while Stojceska et al. (2007) did not find a significant correlation with small and large deformation rheology measurements. Sliwinski et al. (2004) suggested that a more reliable correlation between dough rheology and loaf volume will be obtained if the same ingredients are incorporated in the measurements. Mendichi et al. (2008) reported on a highly significant correlation between baking performance and molecular weight and size distribution of glutenin polymers in wheat flour. The SAGL (2010) does not regard the 100 g baking test as an indication of baking quality, it rather refers to the relationship between protein content and loaf volume.

2.12 Storage proteins

Approximately 80% of the endosperm protein is comprised of gluten and is the main determinant of the unique baking quality in leavened bread. Gluten confers water absorption capacity, viscosity and elasticity to dough, and can be separated in two main fractions: gliadins and glutenins. Glutenins can be separated in HMW-GS and low molecular weight glutenin subunits (LMW-GS) (Wieser, 2007). The balance between gliadin and glutenin is crucial for dough with acceptable strength and extensibility formation (Sapirstein & Fu, 2000; Cornish et al., 2006; Zhang et al., 2009).

2.12.1 Glutenin

Glutenins contribute up to 12% of the total protein in the wheat endosperm (Halford et al., 1992). Polymeric glutenins can be reduced to produce two types of polymeric proteins: HMW-GS and LMW-GS. The HMW-GS are in the minority within the gluten proteins (≈ 10%) and contain an x-type subunit of higher molecular weight and a y-type subunit of lower molecular weight, with molecular weights ranging from 83-88 kDa and 67-74 kDa, respectively

(Wieser, 2007). These subunits are encoded by genes present at the *Glu-1* loci, on the long arms of homoeologous group-one chromosomes at the A, B and D genomes (*Glu-A1*, *Glu-B1* and *Glu-D1* loci) (Shewry & Halford, 2002). The y-type gene, at the *Glu-A1* locus, is always silent in hexaploid and tetraploid wheat, while the x-type gene at the *Glu-A1* locus and the y-type gene at the *Glu-B1* locus are expressed only in some cultivars. As a result, the number of subunits varies from three to five in bread wheat and from two to three in durum wheat (Shewry et al., 2006).

The introduction of recombinant inbred lines, aneuploids, isogenic lines, biotypes and doubled-haploid populations, combined with analytical methods, such as sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and RP-HPLC, made it possible to study the relationship between functional properties and genetics of HMW-GS (MacRitchie & Lafiandra, 2001; Shewry et al., 2003a;b). HMW-GS 2, 5, 7, 10 and 12 were regarded as major components, whereas HMW-GS 1, 2*, 6 ,8 and 9 acted as minor components with regards to dough development time, maximum resistance of dough and loaf volume. Within the HMW-GS, the x-type subunits (1-7) contributed more to dough properties than the y-type subunits (8-12) (Wieser & Zimmermann, 2000).

Glutenins separate into four subgroups according to electrophoretic mobility on SDS-PAGE. The A group corresponds to the HMW-GS, with a molecular weight range of 80-130 kDa on SDS-PAGE. About 60% of the glutenin fraction contains the LMW-GS, which occur in the major B and minor C groups (42-51 kDa and 30-40 kDa, respectively), with amino acid sequences in the C group similar to those of α- and γ-gliadins. LMW-GS in the D group (55-70 kDa) are highly acidic and derived from modified ω-gliadins, with lower mobilities than the B and C groups (Payne et al., 1985; Ciaffi et al., 1999; Gianibelli et al. 2001). The LMW-GS are located on the short arms of homoeologous group-one chromosomes at *Glu-A3*, *Glu-B3* and *Glu-D3* (D'Ovidio & Masci, 2004). LMW-GS are highly polymorphic and include proteins with gliadin-type sequences, which complicate the separation of

individual proteins (D'Ovidio & Masci, 2004; Cinco-Moroyoqui & MacRitchie, 2008).

HMW-GS and LMW-GS play important roles in determining dough-related properties and end use quality (Eagles et al., 2002; He et al., 2005; Liu et al., 2005; Cornish et al., 2006; Zhang et al., 2009). Dough strength was generally more influenced by the *Glu-1* alleles than the *Glu-3* alleles and from the *Glu-3* loci, *Glu-B3* made the biggest contribution, while LMW-GS were more important for dough extensibility (Cornish et al., 2006). Protein quality can be improved by increasing the glutenin quantity, while considering the desirable composition of HMW-GS and LMW-GS alleles (Zhang et al., 2009).

Gluten proteins aggregate at two levels before the formation of the gluten polymer. At the first level, covalent polymers are formed between the HMW- and LMW-GS. On the second level larger aggregates are formed and stabilised by hydrogen and disulphide bonds, known as glutenin macropolymers (GMP) (Graveland et al., 1982; Weegels et al., 1996a) or unextractable polymeric protein (UPP) (Gupta et al., 1993). The intensity of aggregation on the second level is highly influenced by the glutenin allelic composition (Hamer & van Vliet, 2000). The quantity of HMW-GS, LMW-GS and HMW/LMW-GS ratio strongly influences the aggregation and polymerisation properties of the UPP during dough development (Wang et al., 2007). UPP consists of spherical glutenin particles (Don et al., 2003) and is insoluble in various solvents (SDS or acetic acid) (Weegels et al., 1996b; 1997).

2.12.2 Gliadin

Gliadins are the most abundant wheat storage proteins. The six main loci encoding for gliadins (*Gli*) were mapped on the distal ends of the short arms of the chromosomes of the first (*Gli-1*) and sixth (*Gli-2*) homoeological groups. The specific loci were designated *Gli-A1*, *Gli-B1*, *Gli-D1*, *Gli-A2*, *Gli-B2* and *Gli-D2* (Payne et al., 1982; Payne, 1987). Numerous additional loci encoding

a few minor gliadin bands were later identified (Pogna et al., 1993; Ruiz & Carrillo, 1993; Metakovsky et al., 1997).

Gliadins are highly polymorphic, alcohol extractable proteins and were traditionally separated by means of acidic electrophoresis into α-, β-, γ- and ω-zones (Bushuk & Sapirstein, 1991). Electrophoretic mobility does not always reflect the relationship between proteins, as a result α- and β-gliadins fall into one group (α- and β-type). Gliadins can be grouped into four different types: ω5-, ω1,2-, α/β- and γ-gliadins, derived from complete or partial amino acid sequences, amino acid compositions and molecular weights (Wieser, 1996).

The possibility for disulphide cross links is not likely due to the lack of cysteine in most ω-gliadins. The molecular weights of ω5-gliadins are higher (≈ 50 kDa) than ω1,2-gliadins (≈ 40 kDa). The molecular weights of α/β- and γ-gliadins are overlapping (≈ 28-35 kDa) with much lower proportions of glutamine and proline than observed in ω-gliadins (Bunce et al., 1985).

The gliadin fraction can be divided into more than 100 components by means of RP-HPLC (Wieser, 2007). It is difficult to study the influence of gliadins on quality parameters since LMW-GS genes are tightly linked to some gliadin genes (Schofield, 1994). In general, higher gliadin concentrations were associated with higher viscosity, and had a negative effect on dough strength and sometimes resulted in lower loaf volume (Branlard & Metakovsky, 2006).

2.13 Protein separation methods

Fractionation of wheat proteins is the basis of further studies to establish the relationship between protein fractions and bread making properties. The classic fractionation procedure (Osborne, 1907) divided wheat proteins into five groups: albumins (soluble in water), globulins (soluble in salt solutions), gliadins (soluble in organic solvents), glutenins (soluble in diluted acids) and an insoluble residue.

2.13.1 Electrophoresis

Glutenin fractions were first separated by means of starch gel electrophoresis (Huebner, 1970), followed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) (Orth & Bushuk, 1973). The use of SDS-PAGE permits the separation of individual components on the basis of molecular size and shape. Consequently, this technique allows the determination of molecular weights of proteins, through denaturation of the s-s covalent bonds into individual polypeptide chains by means of sodium dodecyl sulphate (Wrigley et al., 1982).

SDS-PAGE became a widely used method for screening preferred subunits across the world, although the disadvantages sometimes resulted in wrong identification of subunits. The relatively poor resolution and low reproducibility usually makes it difficult to correctly identify certain subunits with almost similar mobilities (Shewry et al., 1984), such as 1Ax2* and 1Dx2 (Gao et al., 2010).

In the event of scanning gels to quantify subunits, SDS-PAGE poses another obstacle. The staining agent, coomassie brilliant blue, does not produce the same staining intensities, depending on the content of the basic amino acid remains in the subunits. The amino acid content increased parallel with the subunit mobility on SDS-PAGE gels. Consequently, the amounts and proportions of subunits 1-5 were underestimated whereas the subunits 10 and 12 were overestimated (Burnouf & Bietz, 1985; Wieser & Zimmermann, 2000).

2.13.2 Reversed-phase HPLC

Protein fractions separate according to surface hydrophobic properties. The sensitivity of the technique enables qualitative and quantitative analyses on small samples (Wieser et al., 1994). RP-HPLC fractions correlated significantly with wheat (Wieser & Kieffer, 2001; Peña et al., 2005) and durum (Edwards et al., 2007) quality parameters. Fractionation of the gluten fractions is a prerequisite for analyses (Huebner & Bietz, 1985). Proteins separated with RP-HPLC showed that LMW-GS have higher hydrophobic surfaces than

those from HMW-GS, while the hydrophobic surfaces are analogous to those of gliadins (Juhász & Gianibelli, 2006).

Different solvents have been used by researchers to remove monomeric proteins by solubilisation (Burnouf & Bietz, 1989; Gupta & MacRitchie, 1991; Sapirstein & Fu, 1998; Wieser et al., 1989; 1990, Hou & Ng, 1995). A sharp separation of polymeric from monomeric proteins could not be achieved, because a part of the polymeric protein is removed by the solvents. Quantitation of the solubilised monomeric proteins indicated that more LMW-GS were removed from wheat flour associated with weak dough properties. This may result in incorrect measurements of LMW-GS and the error will be related to the strength of the dough (Cinco-Moroyoqui & MacRitchie, 2008).

2.13.3 Size-exclusion HPLC

The introduction of SE-HPLC (Huebner & Bietz, 1985; Dachkevitch & Autran, 1989) and sonication (Singh et al., 1990) elucidated the role of wheat proteins and the technological properties of dough. Sonication makes the originally unextractable polymers extractable. It is not the size but the amounts of the non-sonicated, and then the sonicated extracts, that are used to calculate the amount of unextractable polymeric proteins (UPP). UPP is regarded as a simple way to characterise the relative amount of the dough strength-related large polymers without creating artifacts caused by sonication (Haraszi et al., 2008).

SE-HPLC is the most widely used procedure to measure the amount of the biggest polymers. The solubilisation of glutenin polymers depends on the pH of the solvent and fractionation efficiency is influenced by the choice of SE-HPLC columns (Mendichi et al., 2008).

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Chapter 3

Relationship between gluten proteins and wheat quality in South African cultivars

3.1 Abstract

Reversed-phased high-performance liquid chromatography (RP-HPLC) showed significant correlations with wheat flour quality in previous studies. In this chapter, correlations between wheat quality parameters and gluten fractions were studied. Correlations between yield, kernel characteristics, falling number (FN) and milling properties were low, mainly below r=0.50. Significant ($p \leq 0.001$) correlations were observed between gluten fractions and bread making properties. Stepwise multiple regression indicated that gliadins made the biggest contribution to alveograph extensibility (AlvL) and alveograph configuration of the curve (AlvP/L). Gluten made the biggest contribution to flour strength (AlvW) and loaf volume (LFV). Alveograph tenacity (AlvP) and mixograph development time (MDT) were mainly influenced by the ratio of gliadin to high molecular weight subunits. Glutenin made the biggest contribution to farinograph water absorption (FABS). This study indicated that an increase in gliadins resulted in an increase in AlvL and a decrease in AlvP, AlvP/L and MDT.

3.2 Introduction

The SAGL, in conjunction with wheat breeding companies, millers and bakers set strict quality norms for the release of new cultivars (SAGL, 2010). The objective of wheat breeding programmes is to select towards improved grain characteristics, milling properties and bread making performance in order to comply with the needs of the industry. The expression of these traits can be influenced by genetic factors, the environment or both (McGuire & McNeal, 1974; Gaines et al., 1996; Sleper & Poehlman, 2006).

A better understanding of the biochemical and genetic factors influencing wheat quality and yield will lead to improvement of these characteristics (Branlard & Dardevet, 1985).

Quality can be described as how suitable a sample is for producing a specific end product and dough properties are regarded as critical quality aspects in wheat flour (Wrigley et al., 2006). Flour quality is mainly influenced by protein quantity and quality. Protein quantity is strongly influenced by the environment and quality is influenced by the genotype and environment (DuPont & Altenbach, 2003). Glutenin polymers have the biggest effect on the bread making quality of flour, albeit the other storage proteins also contribute to the unique functional properties (Shewry, 1995; Fu & Sapirstein, 1996). Eagles et al. (2002) ascribed 50% of the genetic variation for dough extensibility to flour protein content and 50% to glutenin genes, while 50% of the genetic variation for dough strength could be explained by glutenin genes.

Different strategies have been applied to improve the prediction of quality traits in wheat. For example, HMW-GS and LMW-GS as markers in wheat breeding programmes (Payne et al., 1981; Payne, 1987; Gupta & MacRitchie, 1994; Pogna et al., 1996; Blanco et al., 1998; Eagles et al., 2006). Gluten fractions, separated by means of RP-HPLC, showed significant correlations with rheological properties and loaf volume (Wieser & Kieffer, 2001; Peña et al., 2005).

The aim of this study was to quantify gluten proteins in South African wheat cultivars, and to correlate the quantities and ratios with quality parameters that serve as norms for the release of new cultivars.

3.3 Material and methods

3.3.1 Material

Ten hard red winter wheat cultivars (Table 3.1) were selected from the national cultivar adaptation trials, conducted by the Agricultural Research Council - Small Grain Institute (ARC-SGI) in South Africa. Trials were planted under dryland conditions in 2007. The three locations represent different climate conditions:

- Bothaville (North Western Free State): low rainfall, high temperatures, high evaporation requirements and deep, yellow sandy loam soils (water table often present).
- Arlington (Central Free State): moderate rainfall, moderate temperatures, a lower evaporation requirement and relatively shallow duplex soils.
- Bethlehem (Eastern Free State): higher rainfall, lower temperatures, lower evaporation requirement, predominantly yellow soils of average effective depth.

Trials were planted according to a randomised complete block design with four replicates. Fertilising detail is presented in Appendix A, Table 1. Trial plots consisted of five rows of 5 m length each and an inter-row spacing of 5 cm. A spacing of 5 cm between seeds was maintained by means of a precision planter. Planting and harvest dates are presented in Appendix A, Table 2. Only the middle three rows were harvested in an effort to avoid the side row effect. Weather data was recorded at the three localities, monthly averages are presented in Appendix A, Table 3. Quality analyses were done at the ARC-SGI, Bethlehem, South Africa.

Table 3.1 Entries of wheat cultivars included in this study

Origin	Cultivar		Wheat type	Released
ARC-SGI	Betta-DN	Pure line	Intermediate	1993
ARC-SGI	Gariep	Pure line	Intermediate	1994
ARC-SGI	Caledon	Pure line	Intermediate	1996
ARC-SGI	Limpopo	Pure line	Intermediate	1994
ARC-SGI	Elands	Pure line	Intermediate	1998
ARC-SGI	Komati	Pure line	Intermediate	2002
ARC-SGI	Matlabas	Pure line	Winter	2004
Pannar	PAN3349	Pure line	Intermediate	1994
Pannar	PAN3377	Pure line	Intermediate	1997
Pannar	PAN3118	Pure line	Winter	2001

ARC-SGI = Agricultural Research Council – Small Grain Institute

3.3.2 Quality measurements

3.3.2.1 Grain yield

Harvesting of the three middle rows of each plot was done and the mass was converted from g m^{-2} to ton ha^{-1} to determine grain yield.

3.3.2.2 Hectolitre mass

Hectolitre mass was determined according to AACC 55-10 (AACC, 2000).

3.3.2.3 Vitreous kernels

Kernels were sliced with a farinator and vitreous kernels counted.

3.3.2.4 Flour protein content

A combustion method was used to determine protein according to AACC 46-30 (AACC, 2000). Using a Leco FP2000, protein content was expressed on a 12% moisture base.

3.3.2.5 Hardness index

Hardness index was determined using the SKCS AACC 55-31 (AACC, 2000) with the SKCS model 4100 instrument.

3.3.2.6 Kernel diameter

Kernel diameter was determined by using the SKCS AACC 55-31 (AACC, 2000) method with the SKCS model 4100 instrument.

3.3.2.7 Falling number

Hagberg falling number was determined according to AACC 56-81B (AACC, 2000).

3.3.2.8 SDS sedimentation

The approved AACC 56-70 method was followed (AACC, 2000).

3.3.2.9 Break flour yield

All the wheat samples were milled on a laboratory pneumatic mill, Bühler model MLU-202 (manufactured by Bühler Bros., Inc., Uzwil, Switzerland). The AACC 26-21A for milling hard wheat was followed (AACC, 2000).

3.3.2.10 Flour yield

This was measured according to the AACC 26-21A method (AACC, 2000).

3.3.2.11 Flour colour

Flour colour was determined according to AACC 14-30 (AACC, 2000). A Martin series III colour grader was used to determine the brightness of the flour at 540 nm.

3.3.2.12 Mixograph

Mixing development time was determined on a 35 g mixograph (National Manufacturing Corporation, Lincoln, Nebraska) according to AACC 54-40A (AACC, 2000).

3.3.2.13 Farinograph

Constant flour weight procedure was followed according to the AACC 54-21 method, using a Brabender® $_{OHG}$ Duisburg (AACC, 2000).

3.3.2.14 Alveograph

The resistance of dough to extension was measured with the approved method described in AACC 54-30A, using a Chopin Alveographe NG (AACC, 2000).

3.3.2.15 Baking procedure

The optimised straight dough bread making method was followed according to AACC 10-10B (AACC, 2000).

3.3.2.16 Loaf volume

Loaf volume was determined by applying the rapeseed displacement procedure, AACC 10-05 (AACC, 2000).

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

Analyses were done according to Wieser et al. (1998), with modifications. Samples were analysed in duplicate.

Deionised water (Millipore Direct-Q UV 3) was used to prepare solvents and eluants. White flour samples (100 mg) were extracted stepwise. Each extraction step was initiated with vortexing for 2 min at room temperature (RT) and continued with magnetic stirring. Samples were centrifuged at 6000 x *g*.

Albumins and globulins were extracted twice with 1.0 ml of 0.4 M NaCl + 0.067 M $HKNaPO_4$, pH 7.6. Samples were stirred for 10 min followed by centrifugation for 10 min at room temperature. Supernatants were discarded. Gliadins were extracted three times by adding 0.5 ml of 60% (v/v) aqueous ethanol to the pellet. Samples were stirred for 10 min and centrifuged for 15 min at room temperature.

Glutenins were extracted twice with 1.0 ml of 50% (v/v 1-propanol + 2 M urea + 0.05 M Tris-HCl, pH 7.5) + 1% dithiothreitol (DTT) under nitrogen. Samples were stirred for 20 min at 60℃, and centrifuged fo r 20 min at room temperature.

Corresponding supernatants were combined and diluted to 2.0 ml with the respective extraction solvent. Aliquots of the extracts were filtered through a 0.45 µm HT Tuffryn Acrodisc® Syringe Filter into a glass vial.

Routine analyses were performed on a Thermo Finnigan™ Surveyor Plus (Thermo Electron, San Jose, CA) HPLC system with PDA detector; equipped with ChromQuest™ 4.2 chromatography data system for integration events; YMC-Pack ODS-A 150 x 4.0 mm inner diameter C_{18} column; column temperature was 50℃; injection volume was 50 µl fo r gliadins and 100 µl for

glutenins; quantification was achieved by using a detection wavelength of 210 nm.

Elution system A) deionised water + trifluoroacetic acid (TFA) (0.1%, v/v); B) acetonitrile (ACN) (ROMIL-SpS^{TM} acetonitrile 200 far UV) + TFA (99.9/0.1%, v/v). Linear elution gradient: 0 - 2 min 100% A, 2 - 10 min 28% B, 10 - 40 min 56% B, 40 - 41 min 90% B, 41 - 45 min 90% B, 45 - 46 min 100% A, 46 - 55 min 100% A. Flow rate was 1.0 ml min^{-1}.

Absorbance units under the different peaks were calculated according to Wieser et al. (1998). Gliadins were measured from 15.60 - 38.73 min. Quantities for glutenin fractions included ωb-gliadins, HMW-GS and LMW-GS, ωb-gliadins were measured from 14.82 - 18.17 min; HMW-GS, 18.17 - 24.93 min and LMW-GS, 24.93 - 41.54 min. Protein quantities were expressed as HPLC milli-absorbance units (mAU) corresponding to 1 mg flour.

3.3.4 Statistical analyses

GenStat (Payne et al., 2008) was used for statistical analyses. Sum of squares was used to determine contribution of factors to total variation.

3.4 Results

3.4.1 Descriptive statistics and analysis of variance for quality measurements

Environment and genotype by environmental interaction (GXE) contributed significantly ($p \leq 0.001$) to the variance in yield (Appendix A, Table 5). Environment explained 34.84% of the total variance, and genotype explained 8.55% of the variance (Appendix A, Table 6). Arlington and Bothaville differed significantly from Bethlehem, the average yield over three localities was 4.11 ton ha^{-1} and differed significantly between 3.72 ton ha^{-1} (PAN3349) and 4.65 ton ha^{-1} (PAN3377) (Appendix A, Table 7). All three major components (genotype, environment and GxE) contributed to the total variance in hectolitre mass (HLM) (Appendix A, Table 5). The genotype mean was 80.53 kg hl^{-1} and ranged from 78.98 kg hl^{-1} (PAN3349) to 81.82 kg hl^{-1} (Elands). PAN3349 and Elands differed significantly. Arlington and Bothaville differed significantly from Bethlehem (Appendix A, Table 7).

The analysis of variance (ANOVA) for vitreous kernels (VK) revealed significant ($p \leq 0.001$) differences for environment and genotype (Appendix B, Table 5). Environment contributed 49.84% of the total variance (Appendix B, Table 6). Arlington and Bethlehem differed significantly from Bothaville. Cultivar means varied significantly between Elands (64.67%) and Betta-DN (82.17%) (Appendix B, Table 9). Genotype was the main contributor (63.12%) to the total variance in kernel diameter (KD) (Appendix B, Table 6). The average KD was 2.89 mm, Matlabas ranked the highest (3.11 mm) and differed significantly from all the entries accept PAN3118 (3.06) (Appendix B, Table 9). ANOVA for hardness index (HI) revealed significant ($p \leq 0.001$) differences for all three major components (Appendix B, Table 5). Environment made the biggest contribution (42.76%) to the total variance (Appendix B, Table 6). The average value for HI was 62.69% (Appendix B, Table 9).

The ANOVA for FN confirmed significant ($p \leq 0.001$) differences for genotypes and environment (Appendix B, Table 5). Environment contributed 43.72% of the total variance (Appendix B, Table 6). Values varied between 440.00 s (Gariep) and 562.70 s (PAN3349). Significant differences were observed for Caledon, Gariep and PAN3349 (Appendix B, Table 9). Genotype and GXE showed significant ($p \leq 0.01$) differences for protein content (Appendix B, Table 5). Environment explained 23.22% of the total variance. Genotype contributed 10.64% of the total variance (Appendix B, Table 6). Arlington differed significantly from Bethlehem and Bothaville. The average protein content was 10.86% and ranged from 10.38% (Elands) to 11.29% (Caledon). Elands differed significantly from Betta-DN, Caledon, Komati, PAN3118 and PAN3377 (Appendix B, Table 9).

Break flour yield (BFY) showed significant ($p \leq 0.001$) differences for genotype and GXE, while flour yield (FY) showed highly significant ($p \leq 0.001$) differences for genotype and significant ($p \leq 0.01$) differences for GXE. No significant differences were observed between the three localities for BFY and FY (Appendix B, Table 5). Genotype contributed 41.55% of the total variance for FY (Appendix B, Table 6). The average was 72.92% and varied significantly between 71.78% (Elands) and 73.71% (PAN3349) (Appendix B, Table 10). Flour colour at a 76% flour yield level (C76) showed significant ($p \leq 0.001$) differences for environment, followed by GXE (Appendix B, Table 5). Environment contributed 25.14% to the total variance (Appendix B, Table 6). Limpopo differed significantly from Elands and Matlabas. All three localities differed significantly (Appendix B, Table 10).

Genotype and GXE caused significant ($p \leq 0.001$) differences for wet gluten content (WGC) (Appendix B, Table 5). Genotype explained 36.51% of the total variance (Appendix B, Table 6). The average WGC was 33.52% and ranged from 30.07% (Matlabas) to 39.78% (Caledon). Caledon, Gariep, Komati and Matlabas differed significantly (Appendix B, Table 10). SDSS varied significantly between 93.00 ml (Matlabas) and 78.97 ml (Komati), the average was 85.05 ml (Appendix B, Table 10). Genotype was the only

component that showed significant ($p \leq 0.001$) differences (Appendix B, Table 7), and contributed 80.74% of the total variance (Appendix B, Table 8).

Mixograph water absorption (ABS) varied between 59.13 (Elands) and 60.54 (Caledon). Betta-DN, Caledon, Gariep, Komati, Limpopo, PAN3118 and PAN3377 did not differ significantly (Appendix B, Table 12). Environment contributed 20.44% of the total variance. Genotype added 40.26% to the total variance in farinograph water absorption (FABS) (Appendix B, Table 8). Genotype means varied between 56.05 (Gariep) and 59.49 (PAN3118). PAN3377 and PAN3118 did not differ significantly albeit both cultivars differed significantly from Caledon, Gariep ans Limpopo (Appendix B, Table 12). Genotype and GXE revealed significant ($p \leq 0.001$) differences for loaf volume (LFV) (Appendix B, Table 7). Genotype contributed 40.06% of the total variance and GXE contributed 23.31% (Appendix B, Table 8). Average LFV was 865.60 cm^3 and varied between 798.80 cm^3 (PAN3349) and 940.00 cm^3 (Caledon). Matlabas, Caledon and PAN3349 differed significantly (Appendix B, Table 12).

ANOVA revealed significant ($p \leq 0.001$) differences for genotype and GXE regarding mixograph development time (MDT) (Appendix B, Table 7). Genotype explained 65.12% of the total variance for MDT (Appendix B, Table 8). Arlington and Bothaville differed significantly from Bethlehem. The mean value was 3.35 min, Caledon had the lowest MDT (2.41 min) and PAN3118 the highest (4.29 min). Betta-DN, Caledon, Elands, Limpopo and PAN3118 differed significantly (Appendix B, Table 11). Genotype and GXE revealed significant ($p \leq 0.001$) differences for AlvP (Appendix B, Table 7). Genotype contributed 66.02% of the total variance (Appendix B, Table 8). Arlington differed significantly from Bethlehem and Bothaville. Average values ranged from 58.58 mm H_2O (Caledon) to 96.83 mm H_2O (PAN3118) and the mean was 73.17 mm H_2O. Betta-DN, Caledon, Matlabas, PAN3118 and PAN3349 differed significantly (Appendix B, Table 11).

Genotype showed highly significant ($p \leq 0.001$) differences for AlvL. Differences were significant ($p \leq 0.01$) for environment and GXE (Appendix B,

Table 7). Genotype explained 55.25% of the total variance (Appendix, Table 8). Caledon had the highest value (144.8 mm) for AlvL and PAN3349 the lowest (59.0 mm), the average was 99.4 mm. Caledon, Limpopo, Matlabas, PAN3349 and PAN3377 differed significantly. Bethlehem differed significantly from Arlington and Bothaville (Appendix B, Table 11). Genotype demonstrated highly significant ($p \leq 0.001$) differences for alveograph configuration of the curve (AlvP/L). Differences were also significant ($p \leq 0.01$) for environment and GXE (Appendix B, Table 7). Genotype contributed 57.53% of the total variance (Appendix B, Table 8). The mean value was 0.84 mm H_2O mm^{-1} and ranged between 0.41 mm H_2O mm^{-1} (Caledon) and 1.47 mm H_2O mm^{-1} (PAN3118). Gariep and PAN3349 did not differ significantly although both entries differed significantly from Betta-Dn, Caledon, Elands and PAN3118. Bethlehem differed significantly from Arlington and Bothaville (Appendix B, Table 11).

Genotype was the only component that revealed significant ($p \leq 0.001$) differences for flour strength (AlvW) (Appendix B, Table 7). Genotype explained 19.39% of the variance (Appendix B, Table 8). The average for AlvW was 258.7 10^{-4}J and varied between 235.8 10^{-4}J (Gariep) and 304.0 10^{-4}J (PAN3377). Bethlehem differed significantly from Arlington and Bothaville (Appendix B, Table 11).

3.4.2 RP-HPLC

Figures 3.1 and 3.2 give examples of gliadin and glutenin RP-HPLC profiles.

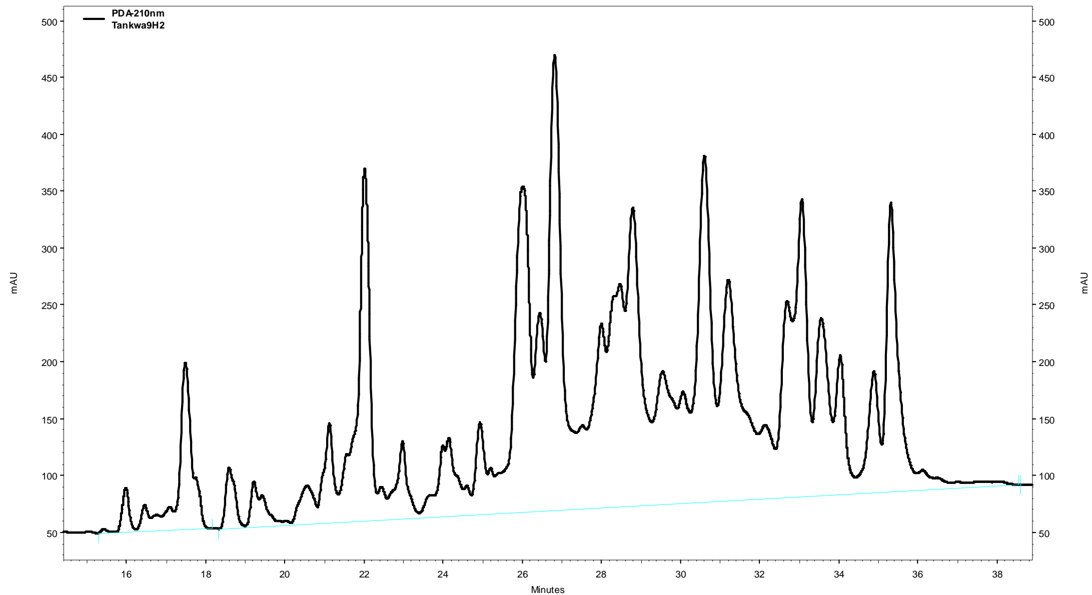

Fig. 3.1 RP-HPLC profile for gliadin extract

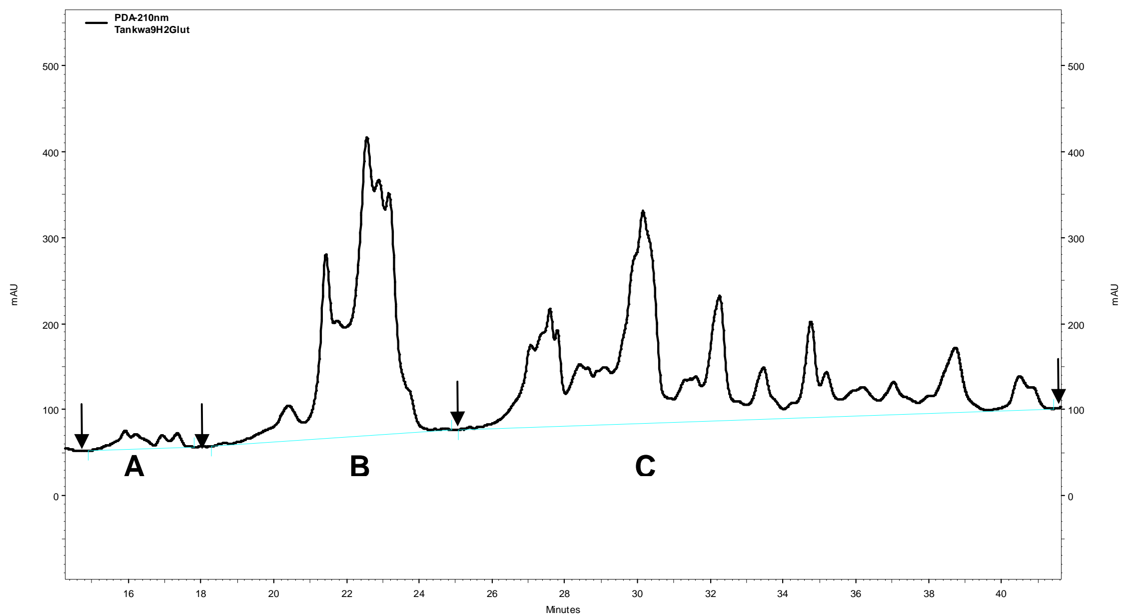

Fig. 3.2 RP-HPLC profile for glutenin extract. Elution ranges for A = ωb-gliadins, B = high molecular weight glutenin subunits (HMW-GS), C = low molecular weight glutenin subunits (LMW-GS)

Table 3.2 Descriptive statistics for gluten factions determined on three localities

Parameters	Gluten			Gliadin			Glutenin			HMW			LMW		
	Be	Ar	Bo	Be	Ar	Bo	Be	Ar	Bo	Be	Ar	Bo	Be	Ar	Bo
Mean	2056	1853	2108	1310	1157	1236	746	696	874	267	262	319	470	427	540
Minimum	1791	1420	1638	1020	841	993	565	565	645	176	158	230	376	364	399
Maximum	2290	2196	2388	1512	1382	1551	1146	847	1308	409	383	519	751	500	681
Std. deviation	123	183	176	111	134	146	114	74	125	69	50	63	71	35	60

HMW = high molecular weight glutenin subunits, LMW = low molecular weight glutenin subunits, Be = Bethlehem, Ar = Arlington, Bo = Bothaville, Std. deviation = standard deviation

Table 3.3 Descriptive statistics for gluten ratios determined on three localities

Parameters	Gli/HMW			Gli/LMW			Gli/Glu			LMW/HMW		
	Be	Ar	Bo	Be	Ar	Bo	Be	Ar	Bo	Be	Ar	Bo
Mean	5.21	4.53	4.01	2.84	2.71	2.32	1.80	1.67	1.44	1.84	1.68	1.73
Minimum	2.86	3.21	2.08	1.47	2.11	1.59	0.97	1.32	0.83	1.08	1.21	1.24
Maximum	8.59	5.91	5.37	3.55	3.29	3.12	2.42	2.00	1.85	2.61	2.45	2.17
Std. deviation	1.34	0.74	0.82	0.43	0.30	0.36	0.32	0.19	0.24	0.39	0.25	0.23

Gli/HMW = gliadin/high molecular weight glutenin subunits, Gli/LMW = gliadin/low molecular weight glutenin subunits, Gli/Glu = gliadin/glutenin, LMW/HMW = low molecular weight glutenin subunits/high molecular weight glutenin subunits, Be = Bethlehem, Ar = Arlington, Bo = Bothaville, Std. deviation = standard deviation

Table 3.4 Analysis of variance for gluten fractions and ratios

SOURCE	d.f.	MEAN SQUARES								
		Gluten	Gliadin	Glutenin	HMW	LMW	Gli/HMW	Gli/LMW	Gli/Glu	LMW/HMW
Total	119									
Reps	3	38717	7222	13761	1704	4089	0.15	0.05	0.01	0.03
Environment	2	726606**	235034**	339316***	40060**	128465***	14.56***	2.96***	1.30***	0.29*
Residual A	6	43929	17543	8403	1977	3927	0.17	0.08	0.018	0.05
Genotype	9	35173	47433***	62725***	32468***	12767***	8.38***	0.67***	0.45***	0.72***
GXE	18	45047**	38704***	17424***	2588**	6447***	1.13***	0.30***	0.12***	0.08**
Residual B	81	19746	9415	4480	960	1414	0.25	0.05	0.017	0.03
Grand mean		2006	1234	772	282	479	4.6	2.6	1.6	1.7
CV (%)		7.0	7.9	8.7	11	7.9	10.9	8.3	8.1	9.6

HMW = high molecular weight glutenin subunits, LMW = low molecular weight glutenin subunits, Gli/HMW = gliadin/high molecular weight glutenin subunits, Gli/LMW = gliadin/low molecular weight glutenin subunits, Gli/Glu = gliadin/glutenin, LMW/HMW = low molecular weight glutenin subunits/high molecular weight glutenin subunits, GXE = genotype by environmental interaction, CV = coefficient of variation, d.f. = degrees of freedom

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

Table 3.5 Percentage contribution of each variance component to the combined analysis of variance for gluten fractions and ratios

SOURCE	Gluten	Gliadin	Glutenin	HMW	LMW	Gli/HMW	Gli/LMW	Gli/Glu	LMW/HMW
Reps	2.05	0.87	2.55	1.00	1.92	0.32	0.64	0.28	0.69
Environment	33.74	18.93	31.87	15.60	40.26	19.83	27.21	25.01	5.23
Residual A	2.51	4.24	5.78	2.31	3.69	0.70	2.09	1.02	2.96
Genotype	28.07	17.19	6.94	56.89	18.00	51.38	27.83	39.09	58.33
GXE	15.59	28.06	17.78	9.07	18.18	13.89	24.68	21.03	12.03
Residual B	18.04	30.71	35.08	15.14	17.94	13.88	17.56	13.57	20.77
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

HMW = high molecular weight glutenin subunits, LMW = low molecular weight glutenin subunits, Gli/HMW = gliadin/high molecular weight glutenin subunits, Gli/LMW = gliadin/low molecular weight glutenin subunits, Gli/Glu = gliadin/glutenin, LMW/HMW = low molecular weight glutenin subunits/high molecular weight glutenin subunits, GXE = genotype by environmental interaction

Table 3.6 Means of measured gluten fractions determined on three localities for 10 selected South African wheat cultivars

	Gluten			Cult	Gliadin			Cult	Glutenin			Cult	HMW			Cult	LMW			Cult
Cultivar	**Ar**	**Be**	**Bo**	**Means**	**Ar**	**Be**	**Bo**	**Means**	**Ar**	**Be**	**Bo**	**Means**	**Ar**	**Be**	**Bo**	**Means**	**Ar**	**Be**	**Bo**	**Means**
Betta-DN	1827	1991	2249	**2022** ab	1161	1322	1406	**1296** de	666	670	843	**726** abc	233	224	292	**250** a	422	445	543	**470** abc
Caledon	1882	2136	2254	**2091** b	1215	1427	1383	**1342** e	667	709	897	**758** bc	234	216	282	**244** a	423	484	577	**495** c
Elands	1745	2039	2101	**1962** a	1087	1351	1290	**1243** bcd	658	688	811	**719** abc	239	224	292	**251** a	406	453	504	**454** ab
Gariep	1891	2094	1873	**1953** a	1229	1386	1139	**1252** bcd	662	709	734	**701** a	245	241	269	**252** a	410	460	457	**442** a
Komati	1878	1918	2167	**1988** ab	1151	1309	1368	**1276** cde	727	609	800	**712** ab	271	215	296	**260** ab	448	395	482	**442** a
Limpopo	1709	2113	2219	**2014** ab	1015	1407	1311	**1245** bcd	693	706	908	**769** c	262	248	335	**282** b	421	458	555	**478** bc
Matlabas	1855	2010	2033	**1966** a	1154	1089	1164	**1136** a	701	922	869	**831** d	242	282	321	**281** b	448	627	577	**550** d
PAN3118	1904	2150	2189	**2084** b	1144	1285	1051	**1160** a	760	865	1146	**924** e	329	406	476	**404** d	421	453	618	**497** c
PAN3349	1803	2076	1930	**1936** a	1178	1313	1112	**1201** abc	625	764	819	**736** abc	222	268	276	**255** a	399	467	531	**466** ab
PAN3377	2035	2031	2054	**2040** ab	1235	1213	1135	**1194** ab	800	819	919	**846** d	338	349	350	**346** c	476	459	554	**496** c
Env mean	**1853** a	**2056** b	**2108** b	**Grand mean= 2006**	**1157** a	**1310** c	**1236** b	**Grand mean= 1234**	**696** a	**746** a	**874** b	**Grand mean= 772**	**262** a	**267** a	**319** b	**Grand mean= 282**	**427** a	**470** b	**540** c	**Grand mean= 479**
LSD Env	**114.7**				**72.47**				**50.16**				**24.33**				**34.29**			
LSD Cult	**114.1**				**78.82**				**54.37**				**25.17**				**30.54**			

Means followed by the same letter, did not differ significantly at $p \leq 0.05$. HMW = high molecular weight glutenin subunits, LMW = low molecular weight glutenin subunits, Cult means = cultivar means for the three localities, Env mean = environmental means, LSD Env = least significant difference for environments, LSD Cult = least significant difference for cultivars, Ar = Arlington, Be = Bethlehem, Bo = Bothaville

Table 3.7 Means of measured gluten ratios determined on three localities for 10 selected South African wheat cultivars

	Gli/HMW			Cult	Gli/LMW			Cult	Gli/Glu			Cult	LMW/HMW			Cult
Cultivar	**Ar**	**Be**	**Bo**	**Means**	**Ar**	**Be**	**Bo**	**Means**	**Ar**	**Be**	**Bo**	**Means**	**Ar**	**Be**	**Bo**	**Means**
Betta-DN	5.05	6.00	4.81	**5.29** gh	2.77	2.98	2.59	**2.78** cde	1.75	1.98	1.67	**1.80** e	1.83	2.01	1.86	**1.90** de
Caledon	5.23	6.75	4.92	**5.63** h	2.88	2.96	2.40	**2.75** cde	1.83	20.27	1.59	**1.81** e	1.83	2.27	2.05	**2.05** f
Elands	4.61	6.09	4.43	**5.04** fg	2.66	2.99	2.56	**2.74** cd	1.67	1.97	1.59	**1.75** de	1.74	2.04	1.73	**1.83** cde
Gariep	5.06	5.78	4.23	**5.02** fg	2.99	3.03	2.50	**2.84** de	1.86	1.96	1.55	**1.79** e	1.69	1.91	1.69	**1.77** cd
Komati	4.23	6.16	4.68	**5.02** fg	2.61	3.30	2.84	**2.92** e	1.59	2.14	1.71	**1.81** e	1.64	1.86	1.67	**1.72** c
Limpopo	3.87	5.71	3.92	**4.50** de	2.40	3.08	2.38	**2.62** c	1.46	1.99	1.45	**1.63** c	1.62	1.87	1.66	**1.71** c
Matlabas	4.84	4.06	3.63	**4.18** c	2.62	1.78	2.03	**2.14** a	1.64	1.21	1.35	**1.40** ab	1.88	2.28	1.80	**1.99** ef
PAN3118	3.49	3.17	2.22	**2.96** a	2.71	2.84	1.71	**2.42** b	1.51	1.49	0.93	**1.31** a	1.29	1.12	1.30	**1.23** a
PAN3349	5.30	4.92	4.03	**4.75** ef	2.95	2.82	2.10	**2.62** c	1.88	1.73	1.36	**1.66** cd	1.8	1.75	1.92	**1.83** cd
PAN3377	3.66	3.49	3.25	**3.47** b	2.54	2.65	2.05	**2.41** b	1.54	1.49	1.24	**1.42** b	1.45	1.32	1.59	**1.45** b
Env mean	**4.53** b	**5.21** c	**4.01** a	**Grand mean= 4.59**	**2.71** b	**2.84** b	**2.32** a	**Grand mean= 2.63**	**1.67** b	**1.80** c	**1.44** a	**Grand mean= 1.64**	**1.68** a	**1.841** b	**1.73** ab	**Grand mean= 1.75**
LSD Env	**0.2270**				**0.1507**				**0.0727**				**0.1278**			
LSD Cult	**0.4074**				**0.1765**				**0.1071**				**0.1369**			

Means followed by the same letter, did not differ significantly at $p \leq 0.05$. Gli/HMW = gliadin/high molecular weight glutenin subunits, Gli/LMW = gliadin/low molecular weight glutenin subunits, Gli/Glu = gliadin/glutenin, LMW/HMW = low molecular weight glutenin subunits/high molecular weight glutenin subunits, Cult means = cultivar means for the three localities, Env mean = environmental means, LSD Env = least significant difference for environments, LSD Cult = least significant difference for cultivars, Ar = Arlington, Be = Bethlehem, Bo = Bothaville

3.4.3 Descriptive statistics and analysis of variance for RP-HPLC measurements

3.4.3.1 Gluten

ANOVA showed significant ($p \leq 0.01$) differences for environment and GXE. Genotypes did not differ significantly (Table 3.4). Environment contributed 33.74% of the total variance (Table 3.5). Arlington differed significantly from Bethlehem and Bothaville. Cultivar means varied between 1936 and 2091. Matlabas, PAN3349, Elands and Gariep differed significantly from Caledon and PAN3118 (Table 3.6).

3.4.3.2 Gliadin

Highly significant ($p \leq 0.001$) differences were revealed for genotype and GXE, and significant ($p \leq 0.01$) differences for environment (Table3.4). The share of GXE was 28.06% to the total variance (Table 3.5). Arlington, Bethlehem and Bothaville differed significantly. Matlabas and PAN3118 differed significantly from Beta-DN, Caledon, Elands, Gariep, Komati and Limpopo. Caledon showed the highest gliadin content, Matlabas had the lowest content (Table 3.6).

3.4.3.3 Glutenin

ANOVA revealed significant ($p \leq 0.001$) differences for all three major components (environment, genotype and GXE) (Table 3.4). Genotype made a small (6.94%) contribution to the total variance and environment contributed 31.87% (Table 3.5). Arlington and Bethlehem differed significantly from Bothaville. PAN3118 contained the highest glutenin content. Gariep, Limpopo and PAN3118 differed significantly from Matlabas and PAN3377 (Table 3.6).

3.4.3.4 High molecular weight glutenin subunits (HMW-GS)

Genotype illustrated highly significant ($p \leq 0.001$) differences (Table 3.4) and contributed 56.89% of the total variance (Table 3.5). Differences for environment and GXE were significant ($p \leq 0.01$) (Table 3.4). Betta-DN, Caledon, Elands, Gariep, Komati and PAN3349 did not differ significantly, but differed significantly from Limpopo, Matlabas, PAN3118 and PAN3377. The lowest content was measured for Caledon and the highest for PAN3118, and the difference was significant (Table 3.6).

3.4.3.5 Low molecular weight glutenin subunits (LMW-GS)

ANOVA demonstrated highly significant ($p \leq 0.001$) differences for all three major components (Table 3.4). Environment contributed 40.26% of the total variation (Table 3.4). The three localities differed significantly (Table 3.6). The lowest average value was recorded at Arlington and the highest at Bothaville (Table 3.2). Matlabas ranked the highest and differed significantly from all the cultivars (Table 3.6).

3.4.3.6 Gliadin to HMW-GS ratio (Gli/HMW)

Highly significant ($p \leq 0.001$) differences were observed for all three major components (Table 3.4). Genotype contributed 51.38% of the total variance, environment 19.83% and GXE 13.89% (Table 3.5). The three localities differed significantly. The average mean was the lowest at Bothaville and the highest at Bethlehem. Significant differences were observed between Caledon, Limpopo, Matlabas, PAN3118 and PAN3377. Caledon showed the highest Gli/HMW ratio and PAN3118 the lowest and they differed significantly (Table 3.7).

3.4.3.7 Gliadin to LMW-GS ratio (Gli/LMW)

ANOVA revealed significant ($p \leq 0.001$) differences for all three major components (Table 3.4). The contributions to the total variance were 27.83% (genotype), 27.21% (environment) and 24.68% (GXE) (Table 3.5). Bothaville differed significantly from Arlington and Bethlehem. The highest ratio was observed for Komati and the lowest for Matlabas (Table 3.7).

3.4.3.8 Gliadin to glutenin ratio (Gli/Glu)

Significant ($p \leq 0.001$) differences were illustrated for all three major components (Table 3.4). Genotype made the biggest contribution (39.09%) to the total variance, followed by environment (25.01%) and GXE (21.03%) (Table 3.5). The three localities differed significantly. The lowest average ratio was at Bothaville. Betta-DN, Caledon, Elands, Gariep and Komati did not differ significantly, but differed significantly from Limpopo, PAN3118, and PAN3377. Caledon ranked the highest and PAN3118 the lowest (Table 3.7).

3.4.3.9 LMW-GS to HMW-GS ratio (LMW/HMW)

ANOVA demonstrated significant ($p \leq 0.001$) differences for genotype, ($p \leq 0.01$) for GXE and ($p \leq 0.05$) for environment (Table 3.4). Environment explained 58.33% of the total variance (Table 3.5). Arlington and Bethlehem differed significantly. Caledon ranked the highest for LMW/HMW ratio and PAN3118 the lowest, with a significant difference (Table 3.7).

Table 3.8 Significant correlations between gluten fractions and quality characteristics

Fraction	Characteristic	Correlation	Fraction	Characteristic	Correlation
Gliadin	ABS	0.70***	Glutenin	ABS	0.52***
	AlvL	0.61***		AlvW	0.38***
	AlvP/L	-0.56***		BFY	-0.38***
	AlvP	-0.39***		LFV	0.35***
	AlvW	0.37***		C76	0.34***
	LFV	0.48***		FN	0.31***
	FY	0.27**		FABS	0.58***
	FN	0.29**		MDT	0.20*
	WGC	0.79***		Prot	0.53***
	MDT	-0.41***		SDSS	0.39***
	Prot	0.69***		KD	0.19*
	SDSS	-0.37***		HI	0.31***
	KD	-0.32***		VK	0.39***
	VK	0.18*			
	Yield	0.22*			
HMW	ABS	0.43***	LMW	ABS	0.49***
	AlvP	0.32***		AlvW	0.27**
	AlvW	0.41***		BFY	-0.20*
	BFY	-0.49***		LFV	0.34***
	LFV	0.27**		C76	0.34***
	C76	0.25**		FN	0.36***
	FABS	0.57***		FABS	0.48***
	MDT	0.35***		Prot	0.50***
	Prot	0.44***		SDSS	0.27**
	SDSS	0.43***		HI	0.30***
	KD	0.22*		VK	0.46***
	HI	0.24**			
	VK	0.23*			

HMW = high molecular weight glutenin subunits, LMW = low molecular weight glutenin subunits, ABS = mixograph water absorption, AlvL = alveograph extensibility, AlvP = alveograph tenacity, AlvP/L = alveograph configuration of the curve, AlvW = flour strength, BFY = break flour yield, LFV = loaf volume, C76 = flour colour at 76% flour yield level, FY = flour yield, FABS = farinograph water absorption, FN = falling number, WGC = wet gluten content, MDT = mixograph development time, Prot = flour protein content, SDSS = SDS sedimentation volume, KD = kernel diameter, HI = hardness index, , VK = vitreous kernels. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Table 3.8 Continued

Fraction	Characteristic	Correlation	Fraction	Characteristic	Correlation
Gluten	ABS	0.86***	Gli/HMW	AlvL	0.34***
	AlvL	0.47***		AlvP/L	-0.38***
	AlvP/L	-0.40***		AlvP	-0.43***
	AlvW	0.52***		AlvW	-0.18*
	LFV	0.59***		BFY	0.40***
	FN	0.42***		C76	-0.33***
	FABS	0.49***		FABS	-0.37***
	WGC	0.69***		WGC	0.33***
	Prot	0.85***		MDT	-0.49***
	HI	0.29**		SDSS	-0.53***
	VK	0.39***		KD	-0.28**
				Yield	0.22*
Gli/Glu	AlvL	0.33***	Gli/LMW	AlvL	0.28**
	AlvP/L	-0.36***		AlvP/L	-0.26**
	AlvP	-0.32***		AlvP	-0.24**
	BFY	0.30***		C76	-0.38***
	C76	-0.38***		FY	0.22*
	FY	0.21*		FABS	-0.30***
	FABS	-0.37***		WGC	0.32***
	WGC	0.35***		MDT	-0.29**
	MDT	-0.42***		SDSS	-0.45***
	SDSS	-0.52***		KD	-0.29**
	KD	-0.32***		VK	-0.26*
	VK	-0.20*			
LMW/HMW	AlvL	0.20*			
	AlvP/L	-0.27**			
	AlvP	-0.37***			
	AlvW	-0.26**			
	BFY	0.43***			
	FABS	-0.27**			
	MDT	-0.38***			
	SDSS	-0.26**			
	Yield	0.20*			

Gli/HMW = gliadin/high molecular weight glutenin subunits, Gli/LMW = gliadin/low molecular weight glutenin subunits, Gli/Glu = gliadin/glutenin, LMW/HMW = low molecular weight glutenin subunits/high molecular weight glutenin subunits, ABS = mixograph water absorption, AlvL = alveograph extensibility, AlvP = alveograph tenacity, AlvP/L = alveograph configuration of the curve, AlvW = flour strength, BFY = break flour yield, LFV = loaf volume, C76 = flour colour at 76% flour yield level, FY = flour yield, FABS = farinograph water absorption, FN = falling number, WGC = wet gluten content, MDT = mixograph development time, Prot = flour protein content, SDSS = SDS sedimentation volume, KD = kernel diameter, HI = hardness index, VK = vitreous kernels. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

3.4.4 Significant correlations between gluten fractions and quality characteristics

3.4.4.1 Gliadin

Highly significant ($p \leq 0.001$) positive correlations were observed with ABS, AlvL, AlvW, LFV, WGC and flour protein content. Significant ($p \leq 0.001$) negative correlations were detected with AlvP/L, AlvP, MDT, SDSS and kernel diameter (Table 3.8).

3.4.4.2 Glutenin

Highly significant ($p \leq 0.001$) positive correlations were seen with ABS, AlvW, LFV, C76, FN, FABS, flour protein content, SDSS, HI and VK. The correlation for BFY was significantly ($p \leq 0.001$) negative (Table 3.8).

3.4.4.3 High molecular weight glutenin subunits (HMW-GS)

Mixograph water absorption, AlvP, AlvW, FABS, MDT, flour protein content and SDSS showed significant ($p \leq 0.001$) positive correlations. BFY was negatively correlated (Table 3.8).

3.4.4.4 Low molecular weight glutenin subunits (LMW-GS)

Highly significant ($p \leq 0.001$) positive correlations were revealed for ABS, LFV, C76 FN, FABS, flour protein content, HI and VK (Table 3.8).

3.4.4.5 Gluten

AlvP/L showed a significant ($p \leq 0.001$) negative correlation. Flour protein content was highly significant ($p \leq 0.001$) and positively correlated. Significant ($p \leq 0.001$) positive correlations were observed for ABS, AlvL, AlvW, LFV, FN, FABS, WGC and VK (Table 3.8).

3.4.4.6 Gliadin to HMW-GS ratio (Gli/HMW)

The correlations with MDT, SDSS and AlvP were significantly ($p \leq 0.001$) negative. Correlations were also significantly negative for AlvP/L, FABS and C76. Significant positive correlations were observed for BFY, AlvL and WGC (Table 3.8).

3.4.4.7 Gliadin to glutenin ratio (Gli/Glu)

Significant ($p \leq 0.001$) negative correlations were detected for AlvP/L, AlvP, C76, FABS, MDT, SDSS and KD. Correlations were significantly ($p \leq 0.001$) positive for AlvL, BFY and WGC (Table 3.8).

3.4.4.8 Gliadin to LMW-GS ratio (Gli/LMW)

Correlations were significantly ($p \leq 0.001$) negative for C76, FABS and SDSS, and positive for WGC (Table 3.8).

3.4.4.9 LMW-GS to HMW-GS ratio (LMW/HMW)

Highly significant ($p \leq 0.001$) negative correlations were observed for MDT and AlvP. Correlations were significantly ($p \leq 0.01$) negative for AlvP/L, AlvW, FABS and SDSS. The correlation with BFY was highly significantly positive (Table 3.8).

Table 3.9 Stepwise multiple regression for quality characteristics and gluten fractions

AlvL	Constant	Gli	Gluten	Gli/HMW	Gli/Glu	R^2	Pr>F
	-56.1[a]	0.1260				0.37	<.001
	18.9[b]	*0.0152*					
	-59.6	0.1213	0.0046			0.37	<.001
	22.9	*0.0231*	*0.0171*				
	-92.3	0.0531	0.0471	6.92		0.39	<.001
	28.9	*0.0437*	*0.0287*	*3.78*			
	-18.9	0.199	-0.038	11.11	-62.2	0.40	<.001
	54.9	*0.102*	*0.0613*	*4.61*	*39.7*		
AlvP	**Constant**	**Gli/HMW**	**Gli**	**LMW/HMW**	**Gli/Glu**	**R^2**	**Pr>F**
	96.77	-5.146				0.18	<.001
	4.62	*0.980*					
	117.12	-3.85	-0.0213			0.23	<.001
	9.15	*1.08*	*0.0083*				
	135.6	-0.99	-0.0297			0.27	<.001
	12.1	*1.64*	*0.0089*				
	160.4	7.90	-0.0282	-26.2	-26.2	0.28	<.001
	22.8	*7.11*	*0.0090*	*12.1*	*20.4*		
AlvP/L	**Constant**	**Gli**	**Gluten**	**Gli/HMW**	**Gli/Glu**	**R^2**	**Pr>F**
	2.773	-0.0016				0.31	<.001
	0.265	*0.00021*					
	2.701	-0.0016	0.00009			0.31	<.001
	0.320	*0.00032*	*0.00023*				
	3.295	-0.0004	-0.0006	-0.1252		0.35	<.001
	0.400	*0.00060*	*0.00039*	*0.0524*			
	2.424	-0.0022	0.00033	-0.1749	0.737	0.36	<.001
	0.764	*0.00143*	*0.00086*	*0.0641*	*0.552*		
AlvW	**Constant**	**Gluten**	**HMW**	**Glu**		**R^2**	**Pr>F**
	2.6	0.1277				0.27	<.001
	38.9	*0.0193*					
	14.5	0.1032	0.1316			0.29	<.001
	39.0	*0.0229*	*0.0684*				
	13.9	0.1252	0.291	-0.1147		0.31	<.001
	38.6	*0.0259*	*0.112*	*0.0644*			
MDT	**Constant**	**Gli/HMW**	**Gli/Glu**	**Gli**		**R^2**	**Pr>F**
	4.658	-0.2862				0.24	<.001
	0.223	*0.0472*					
	4.554	-0.332	0.190			0.24	<.001
	0.314	*0.108*	*0.407*				
	5.542	-0.383	0.748	-0.00135		0.30	<.001
	0.441	*0.106*	*0.432*	*0.000439*			
WGC	**Constant**	**Gli**	**Gluten**	**Gli/Glu**	**Gli/HMW**	**R^2**	**Pr>F**
	2.89	0.02482				0.63	<.001
	2.19	*0.00176*					
	-1.08	0.01943	0.0052			0.65	<.001
	2.56	*0.00259*	*0.0019*				
	-4.89	0.0118	0.0098	2.54		0.65	<.001
	6.11	*0.0114*	*0.0068*	*3.69*			
	-1.48	0.0182	0.0064	-3.62	1.233	0.67	<.001
	6.15	*0.0115*	*0.0068*	*4.44*	*0.516*		

All protein fractions were significant at $p \leq 0.001$. (a) = correlation coefficient, (b) = standard error of correlation coefficient. AlvL = alveograph extensibility, AlvP = alveograph tenacity, AlvP/L = alveograph configuration of the curve, AlvW = flour strength, MDT = mixograph development time, WGC = wet gluten content, Gli = gliadin, Glu = glutenin, Gli/HMW = gliadin/high molecular weight glutenin subunits, Gli/Glu = gliadin/glutenin, LMW/HMW = low molecular weight glutenin subunits/high molecular weight glutenin subunits, Pr = probability determination, F = F-test, R^2 = coefficient of multiple determination

Table 3.9 Continued

ABS	**Constant**	**Gluten**	**Gli**	**Glu**	**LMW**	**R^2**	**Pr>F**
	48.205[(a)]	0.0058				0.74	<.001
	0.647[(b)]	*0.0003*					
	48.056	0.0051	0.0012			0.74	<.001
	0.646	*0.0004*	*0.0006*				
	48.019	0.012	-0.0057	-0.0067		0.75	<.001
	0.660	*0.0069*	*0.0068*	*0.0069*			
	48.019	0.0122	-0.0057	-0.0067	-0.0005	0.75	<.001
	0.660	*0.0069*	*0.0069*	*0.0069*	*0.0019*		
FABS	**Constant**	**Glu**	**HMW**	**Gluten**	**Gli/LMW**	**R^2**	**Pr>F**
	51.96	0.0075				0.35	<.001
	0.746	*0.00095*					
	52.298	0.00478	0.00622			0.36	<.001
	0.768	*0.00188*	*0.00372*				
	50.40	0.00290	0.00683	0.00159		0.38	<.001
	1.27	*0.00211*	*0.00369*	*0.00085*			
	54.46	0.00725	0.00972	0.00473	-5.24	0.41	<.001
	3.95	*0.00669*	*0.00662*	*0.00314*	*2.37*		
SDSS	**Constant**	**Gli/HMW**	**Gli/Glu**	**Glu**		**R^2**	**Pr>F**
	96.59	-2.517				0.27	<.001
	1.76	*0.373*					
	99.10	-1.410	-4.63			0.29	<.001
	2.46	*0.847*	*3.19*				
	106.29	1.64	-6.3	-0.0323		0.33	<.001
	6.82	*2.04*	*10.2*	*0.0125*			
Prot	**Constant**	**Gluten**	**Gli**	**Glu**		**R^2**	**Pr>F**
	3.108	0.00386				0.73	<.001
	0.437	*0.00022*					
	3.029	0.00035	0.00064			0.73	<.001
	0.438	*0.00033*	*0.00044*				
	2.988	0.00790	-0.0037	-0.0044		0.74	<.001
	0.441	*0.00468*	*0.00466*	*0.00465*			
LFV	**Constant**	**Gluten**	**Gli**	**Glu**	**LMW**	**R^2**	**Pr>F**
	470.0	0.1973				0.35	<.001
	50.2	*0.0249*					
	464.8	0.1740	0.0419			0.35	<.001
	50.7	*0.0378*	0.0512				
	458.8	0.808	-0.588	-0.632		0.36	<.001
	50.9	*0.540*	*0.537*	0.537			
	443.8	0.764	-0.551	-0.720	0.264	0.38	<.001
	51.0	*0.535*	*0.532*	*0.534*	*0.144*		

All protein fractions were significant at $p \leq 0.001$. (a) = correlation coefficient, (b) = standard error of correlation coefficient. Gli = gliadin, Glu = glutenin, LMW = low molecular weight glutenin subunits, HMW = high molecular weight glutenin subunits, Gli/Glu = gliadin/glutenin, Gli/HMW = gliadin/high molecular weight glutenin subunits, Gli/LMW = gliadin/low molecular weight glutenin subunits, ABS = mixograph water absorption, FABS = farinograph water absorption, SDSS = SDS sedimentation volume, Prot = flour protein content, LFV = loaf volume, Pr = probability determination, F = F-test, R^2 = coefficient of multiple determination

3.4.5 Stepwise multiple regression for quality characteristics and gluten fractions

The largest contributor to variability in AlvL was gliadins (37%). Protein fractions explained 28% of the total variability in AlvP, Gli/HMW explained 18% of the variation, gliadins, LMW/HMW and Gli/Glu ratio made smaller contributions. Gliadins explained 31% of the variation in AlvP/L. Gluten explained 27% of the total variation in AlvW (Table 3.9).

Protein fractions contributed 30% to the variation in MDT. Gli/HMW contributed 24% and gliadins made a smaller contribution. The largest contributor to variation in WGC was gliadins (63%). Gluten explained 74% of the variation in ABS, while glutenins explained 35% of the variation in FABS. Protein fractions contributed 33% to the total variation in SDSS, Gli/HMW contributed 27%. Gluten contributed 73% to the variation in flour protein content. Protein fractions contributed 38% to the variation in LFV, gluten explained 35% of the variation (Table 3.9).

3.4.6 Discussion

Low rainfall figures were recorded in all the regions preceding planting time, therefore planting conditions were unfavourable during 2007. Rainfall figures of between 20 and 30 mm were recorded during June at all the localities. July, August and September were particularly dry months, with no significant rainfall recorded until late in September. Arlington had no rain in September. Above normal rainfall was recorded for the rest of the growing period, resulting in yields that exceeded the long term averages over the three localities (Appendix A, Table 3). The FN values indicated that no preharvest sprouting occurred at any locality. Variation in flour protein content was relatively small and ranged between 10.38% and 11.29%.

Correlations between yield and protein fractions were low and not highly significant. Kernel characteristics correlated significantly with gluten fractions, although the values were low. Gliadin and gliadin ratios correlated negatively with KD. Glutenin and LMW-GS correlated positively with HI and VK correlated positively with gluten and glutenin. Gluten showed the highest correlation with FN and LMW/HMW showed the highest correlation with BFY. Gli/HMW and Gli/Glu revealed a significantly negative correlation with SDSS.

Correlations above r=0.50 were considered for further discussion. Within this range, quality parameters of interest were those that serve as norms for the release of South African wheat cultivars (SAGL, 2010). MDT, alveograph parameters, FABS and LFV were selected for discussion.

RP-HPLC results were mainly compared with the results of Wieser and Kieffer (2001) because the same extraction procedures were followed. HPLC conditions differed, as a C_{18} column was used as opposed to a C_8 column. Protein fractions showed significant variation, despite of the narrow range for protein content. Gluten correlated significantly with protein content and explained 73% of the variation. Gliadins correlated higher with protein content than glutenins. This is confirmed by Wieser and Kieffer (2001). Genotypes differed significantly for gliadin, glutenin, HMW-GS fractions and the ratios.

Environment showed significant influences on gliadin, LMW-GS, Gli/HMW and Gli/Glu. This was confirmed by DuPont and Altenbach (2003).

Gliadin correlated positively with AlvL and AlvW, and negatively with AlvP AlvP/L, and MDT. This is in contrast with previous reports where gliadin did not correlate with rheological properties (Wieser & Kieffer, 2001; Peña et al., 2005). The negative correlation with MDT is confirmed by several researchers (Hoseney et al., 1969a; b; MacRitchie, 1978; 1985; 1987a; b; Khatkar et al., 2002). Gliadin correlated negatively with dough development time and positively with dough strength. This supports the hypothesis that gliadin also contributes to gluten's viscoelastic properties (Hou et al., 1996; Hussain & Lukow, 1997; Xu et al., 2001; 2002).

Gluten showed the highest correlation with AlvW. These positive correlations with flour strength are in agreement with Wieser and Kieffer (2001) and Peña et al. (2005), although both reported higher correlations for HMW-GS than gluten. Significant correlations were observed between HMW-GS and dough strength and dough development time. LMW-GS showed smaller correlations with AlvW and not significant for dough development time. These results are in agreement with Wieser and Kieffer (2001) and Peña et al. (2005). HMW-GS and LMW-GS did not show significant correlations with dough properties in a study conducted by Sadouki et al. (2005).

The ratio of LMW/HMW showed negative correlations with AlvW and AlvP/L, in agreement with Peña et al. (2005) and Cornish et al. (2006). Caledon and PAN3118 did not differ significantly for gluten content, but differed significantly for gliadin, HMW-GS, Gli/HMW, Gli/Glu and LMW/HMW. These significant differences were reflected in the alveograph and mixograph parameters. Caledon ranked the highest for AlvL and the lowest for AlvP, AlvP/L and MDT. PAN3377 ranked the lowest for AlvL and PAN3118 did not differ significantly. PAN3118 ranked the highest for AlvP, AlvP/L and MDT. This indicated an increase in gliadin resulted in a decrease in dough strength and increase in extensibility, in agreement with Wieser and Kieffer (2001) and Peña et al. (2005).

Glutenin showed the highest correlation with FABS. ABS showed a much higher correlation with gluten, although this parameter is not regarded as a quality norm in South Africa (SAGL, 2010). Gluten showed the highest correlation with LFV. This is in agreement with previous research (Weegels et al., 1996; 1997; Wieser & Kieffer 2001, Sliwinski et al., 2004).

3.4.7 Conclusions

Gliadin fractions revealed the highest correlation with AlvL and AlvP/L. Gluten made the biggest contribution to AlvW and loaf volume. Gli/HMW ratio showed the highest correlation with AlvP and MDT. Glutenin made the biggest contribution to FABS. Stepwise multiple regression indicated that protein fractions could not explain more than 40% of the total variation in rheological properties and baking quality. Several factors might influence the low contribution of protein fractions to variation in dough properties and LFV. GXE contributed more to variation in gliadin than the genotype or environment. The protein range in this study was relatively narrow. A study that includes very strong and weak dough properties might render higher correlations.

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Chapter 4

Size-exclusion HPLC of South African hard red winter wheats and the association with quality characteristics

4.1 Abstract

This study investigated the relationship between flour quality characteristics of South African hard red winter wheat (*Triticum aestivum* L.) cultivars and the absorbance areas of SDS-insoluble protein fractions, separated by size-exclusion high-performance liquid chromatography (SE-HPLC). Comprehensive quality analyses were conducted on the samples. Significant correlations between quality characteristics and SE-HPLC data were observed, indicating associations of molecular weight distribution of proteins with quality characteristics. Specifically, large polymeric protein (LPP) fractions appeared to have a large effect on dough strength and extensibility. This was evident from the significant correlations with mixograph development time (MDT) and alveograph configuration of the curve (AlvP/L). The narrow sense heritability (h^{-2}) for LPP and total polymeric protein (TPP) was much higher than the values calculated for MDT and AlvP/L. This increases the possibility to use SE-HPLC for indirect selection of MDT and AlvP/L properties.

4.2 Introduction

Gluten is produced by the interaction between wheat flour proteins in the presence of water, which confers the unique viscoelastic properties essential for making bread (Shewry et al., 2002). Bread making quality is predominantly influenced by the gluten proteins, which can be separated in two major fractions, the polymeric glutenins and the monomeric gliadins (Shewry & Tatham, 1990; Singh & MacRitchie, 2001). Most of the technological properties of flour are influenced by the polymeric fraction (Jia et al., 1996), especially dough strength and extensibility (Bietz & Huebner, 1980).

The relative quantities of polymeric and monomeric proteins have been utilised to characterise the molecular weight distribution (MWD) of flour proteins. Several researchers reported on the association between wheat end-use quality and variations in MWD of endosperm proteins (Singh et al., 1990a,b; Gupta et al., 1993, Zhu & Khan, 2002, Kuktaite et al., 2004; Labuschagne et al., 2004; Ohm et al., 2006; 2008; 2009;Tsilo et al., 2010).

Environmental conditions may influence the amount and size distribution of polymeric proteins in wheat kernels (Don et al., 2005; Labuschagne et al., 2006). The size distribution of glutenin polymers is largely genetically determined and entails the composition of HMW-GS and LMW-GS (Gupta & MacRitchie, 1994). The glutenin fraction that is insoluble in sodium dodecyl sulphate (SDS) solution is called insoluble glutenin (Orth & Bushuk, 1972; Sapirstein & Fu, 1998), glutenin macropolymer (GMP) (Weegels et al., 1996a) or unextractable polymeric protein (UPP) (Gupta et al., 1993).

The quantity of UPPs is influenced by the different quaternary structures, which result from the polymers formed by disulphide bridges and aggregates that involve hydrogen bonding (Aussenac et al., 2001). Variation in dough mixing strength (MacRitchie & Lafiandra, 1997; Weegels et al., 1996b, Kuktaite et al., 2004) and LFV (Dobraszczyk & Morgenstern, 2003) were more strongly affected by unextractable LPP, than other UPPs. HMW-GS favour the formation of UPP (Vasil & Anderson, 1997), and are encoded by pairs of genes present at the *Glu-1* loci, on the long arms of homoeologous group-one chromosomes at the A, B and D genomes (*Glu-A1*, *Glu-B1* and *Glu-D1* loci) (Payne & Lawrence, 1983).

Hard wheat cultivars differ inherently for FY, kernel characteristics, water absorption, dough behaviour and baking performance (Sleper & Poehlman, 2006). Comprehensive quality analyses are performed to select wheat lines with acceptable bread making properties. These procedures require large sample sizes and would only apply to breeding lines at more advanced stages of development, such as the F_6 and onward. Falconer (1982) suggested that more rapid progress may be achieved under selection with a correlated

response, rather than selection for the observed trait itself. Certain conditions apply for indirect selection. The heritability of the secondary trait has to be substantially higher than the primary trait and the genetic correlation between the two traits needs to be high.

The relative measure of MWD is based on the proportion that is unextractable with SDS-solution, due to the relationship between the increasing molecular size and decreasing solubility of the polymer (Gupta et al., 1993). Extensive studies were done on the MWD of unreduced wheat endosperm proteins since the introduction of SE-HPLC (Bietz, 1984) and sonication (Singh & MacRitchie, 1989), followed by improvement of chromatographic procedures (Batey et al., 1991; Larroque & Békés, 2000).

The objective of this study was to establish the correlation between SDS-insoluble protein fractions and wheat quality traits in selected South African wheat cultivars. The second objective was to determine the possible use of SE-HPLC for indirect selection of highly correlated bread making quality traits.

4.3 Material and methods

4.3.1 Material

See Chapter 3.3.1.

4.3.2 Quality measurements

See Chapter 3.3.2.

4.3.3 Electrophoresis

A simplified procedure for one dimensional (1-D) separation of glutenin subunits was followed (Singh et al., 1991). The method allows rapid glutenin preparation from single kernels. Electrophoresis was performed on a Mighty Small II SE250 (Hoefer Scientific Instruments).

Three stock solutions were prepared: (A) 50% (v/v) propan-1-ol; (B) 50% (v/v) propan-1-ol, 0.08 M Tris-HCl, pH 8.0; (C) sample buffer, 2% (w/v) SDS, 40% (w/v) glycerol, 0.02% (w/v) bromophenol blue, 0.08 M Tris-HCl, pH 8.0. All the protein extractions (unreduced and reduced), alkylation of SH groups and the treatment of propanol extracts with sample buffer, were conducted in a water bath at 65℃ and centrifuged at 10 000 x *g*.

Proteins were extracted from the endosperm half of a crushed single wheat kernel. Gliadins were extracted for 30 min in 1 ml of solution A, with intermitted vortexing, followed by 1 min centrifugation, the supernatant was discarded. This extraction step was repeated and the residue was washed with 5 ml of solution A before 5 min centrifugation.

Glutenin was extracted from the residue in 0.1 ml solution B, containing 1 % (w/v) freshly added dithiothreitol (DTT). Samples were briefly vortexed and placed in the water bath for 30 min. Samples were centrifuged for 5 min before the addition of 0.1 ml of solution B, containing 1.4 % (v/v) freshly mixed 4-vinylpyridine and incubated for 15 min for protein alkylation. Samples were centrifuged for 2 min before an aliquot of 0.1 ml from the supernatant was

transferred to a new tube, containing 0.1 ml solution C. Samples were vortexed briefly and incubated for 15 min for complexing of SDS with the reduced and alkylated glutenin polypeptides, 10 µl sample was loaded per slot.

Gel preparation was done according to Laemmli (1970). Gel concentration (T) and the cross-linker (C) were calculated as: % T = [(g Acrylamide + g Bis-Acrylamide)/Total volume] x 100; % C = [g Bis-Acrylamide/(g Acrylamide + g Bis-Acrylamide)] x 100. The separating gel consisted of [(17.33% T; 0.45% C, 10% SDS (w/v) and 1.5 M Tris-HCl (pH 8.8)], and the stacking gel [(3.04% T; 1.42% C, 10% SDS (w/v) and 0.5 M Tris-HCl (pH 6.8)]. Running buffer (pH 8.3) contained 0.2 M Glycine, 24.76 mM Tris-HCl and 8.67 mM SDS.

Gels were stained in a solution containing 6% (w/v) trichloroacetic acid and 0.01% (w/v) Coomassie brilliant blue R250 and 5% (v/v) methanol. The destaining solution contained 2.5% methanol (v/v) and 3.5% (v/v) glacial acetic acid. The nomenclature by Payne and Lawrence (1983) was used for identification of HMW-GS.

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

The procedure of Gupta et al. (1993) was followed, with modifications. Flour samples were extracted twice. The first step extracted SDS-soluble proteins, while the second step was submitted to sonication to obtain SDS-insoluble proteins. White flour was used and analyses were done in duplicate.

Deionised water was used for the preparation of solvents and eluants. For SDS-soluble proteins, white wheat flour samples (17 mg) were suspended in 1.5 ml of 0.5% (w/v) SDS-phosphate buffer (pH 6.9) and vortexed for 10 seconds. Samples were then stirred for 5 min followed by centrifugation for 30 min at 10 000 rpm. The supernatant was filtered through a 0.45 µm HT Tuffryn Acrodisc® Syringe Filter into a glass vial.

For SDS-insoluble proteins, the pellet was resuspended in 1.5 ml SDS-phosphate buffer, vortexed for 10 seconds and sonicated in an ultrasonic disintegrator (Branson B12 Sonifier) for 30 seconds at amplitude 5. The Sonifier is fitted with a 3 mm exponential tip. Samples were then centrifuged for 30 min (10 000 rpm). The supernatant was filtered through a 0.45 μm HT Tuffryn Acrodisc® Syringe Filter into a glass vial.

4.3.4.1 SE-HPLC

Routine analyses were performed using a Thermo Finnigan™ Surveyor Plus (Thermo Electron, San Jose, CA) HPLC system with PDA detector, equipped with ChromQuest™ 4.2 chromatography data system for integration events. A narrow bore column (NBC) (300 x 4.6 mm BioSep-SEC-S 4000 Phenomenex®) was used in this study (Ohm et al., 2009).

Separation was achieved in 15 min after injecting a 20 μl sample. Elution system A) trifluoroacetic acid (TFA) (0.1%, v/v); B) acetonitrile (ACN) (ROMIL-SpS™ acetonitrile 200 far UV) + TFA (99.9/0.1%, v/v). The eluant consisted of 50% B. Flow rate was 0.4 ml/min at ambient temperature. Proteins were detected at 210 nm.

Absorbance areas under the different peaks were calculated according to Gupta et al. (1993). The following fractions were measured at specific time intervals: large polymeric proteins (LPP), 4.57 to 5.54 min; smaller polymeric proteins (SPP), 5.54 to 6.98 min; large monomeric proteins (LMP) mainly gliadins, 6.98 to 8.61 min; smaller monomeric proteins (SMP) mainly albumins and globulins, 8.61 up to where the trace cut the baseline.

The percentage of total unextractable polymeric protein (%TUPP) was calculated as: 100[(SDS-insoluble LPP and SDS-insoluble SPP)/SDS-soluble and SDS-insoluble LPP and SPP)]; the percentage of large unextractable polymeric protein (%LUPP), 100[(SDS-insoluble LPP)/SDS-soluble and SDS-insoluble LPP)]; the percentage of small unextractable polymeric protein (%SUPP), 100[(SDS-insoluble SPP)/SDS-soluble and SDS-insoluble SPP)].

The percentage of large unextractable monomeric protein (%LUMP) was calculated as: 100[(SDS-insoluble LMP)/SDS-soluble and SDS-insoluble LMP)], and the percentage of small unextractable monomeric protein (%SUMP), 100[(SDS-insoluble SMP)/SDS-soluble and SDS-insoluble SMP)].

4.3.5 Statistical analyses

GenStat (Payne et al., 2008) was used for statistical analyses. Narrow sense heritability (h^2) was calculated according to Falconer (1982).

4.4 Results

4.4.1 SDS-PAGE HMW-GS observed for 10 selected South African wheat cultivars

Examples of electrophoresis profiles are presented in Fig. 4.1 and Fig. 4.2. Gariep, Elands and Komati had the same subunit combination (1, 7+9, 5+10). Betta-DN, Limpopo and PAN3349 had corresponding subunit combinations on *Glu-B1* and *Glu-D1* but differed on *Glu-A1,* where they expressed 2* (Table 4.1). Matlabas was the only entry with a null expression on the *Glu-A1* genome, while *Glu-B1* and *Glu-D1* were the same as PAN3118 (2*, 7+8, 5+10). Caledon (1, 7+9, 2+12) and PAN3377 (2*, 7+9, 2+12) were similar for *Glu-B1* and *Glu-D1* but differed on *Glu-A1*.

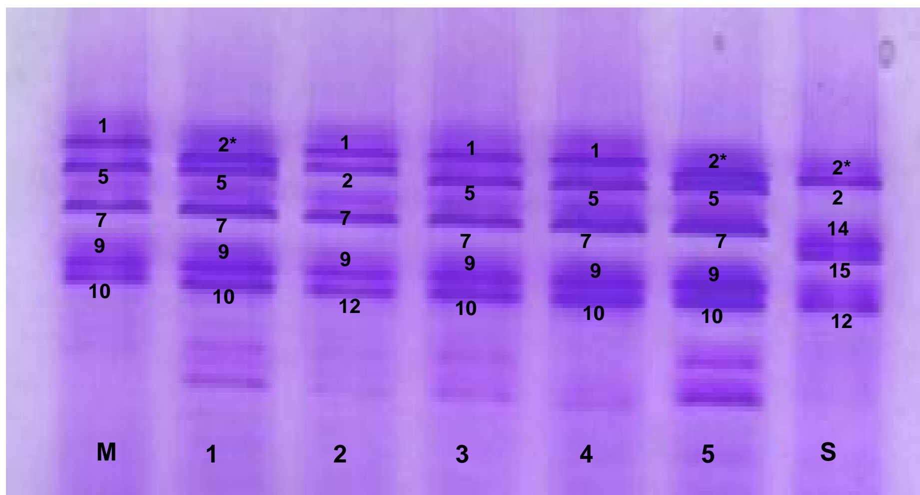

Fig. 4.1 SDS-PAGE profiles. Marquis and Sappo were used as reference samples (Manley, 1989). 1. Betta-DN; 2. Caledon; 3. Elands; 4. Gariep; 5. Limpopo; M = Marquis; S = Sappo

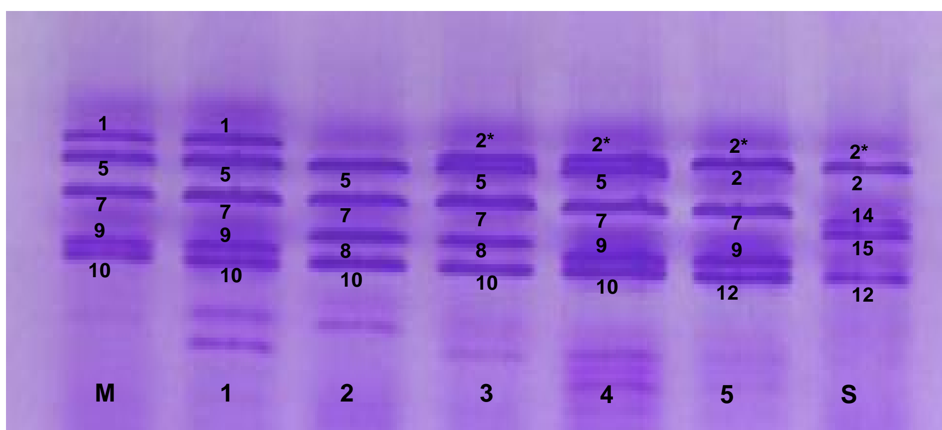

Fig. 4.2 SDS-PAGE profiles. Marquis and Sappo were used as reference samples (Manley, 1989). 1. Komati; 2. Matlabas; 3. PAN3118; 4. PAN3349; 5. PAN3377; M = Marquis; S = Sappo

Table 4.1 SDS-PAGE HMW-GS observed for 10 selected South African wheat cultivars

Entry	High Molecular Weight Glutenin Subunits		
	A	B	D
Betta – DN	2*	7+9	5+10
Gariep	1	7+9	5+10
Caledon	1	7+9	2+12
Limpopo	2*	7+9	5+10
Elands	1	7+9	5+10
Komati	1	7+9	5+10
Matlabas	Null	7+8	5+10
PAN3349	2*	7+9	5+10
PAN3377	2*	7+9	2+12
PAN3118	2*	7+8	5+10

4.4.2 SE-HPLC

Figures 4.3 and 4.4 give examples of SDS-soluble and SDS-insoluble HPLC profiles

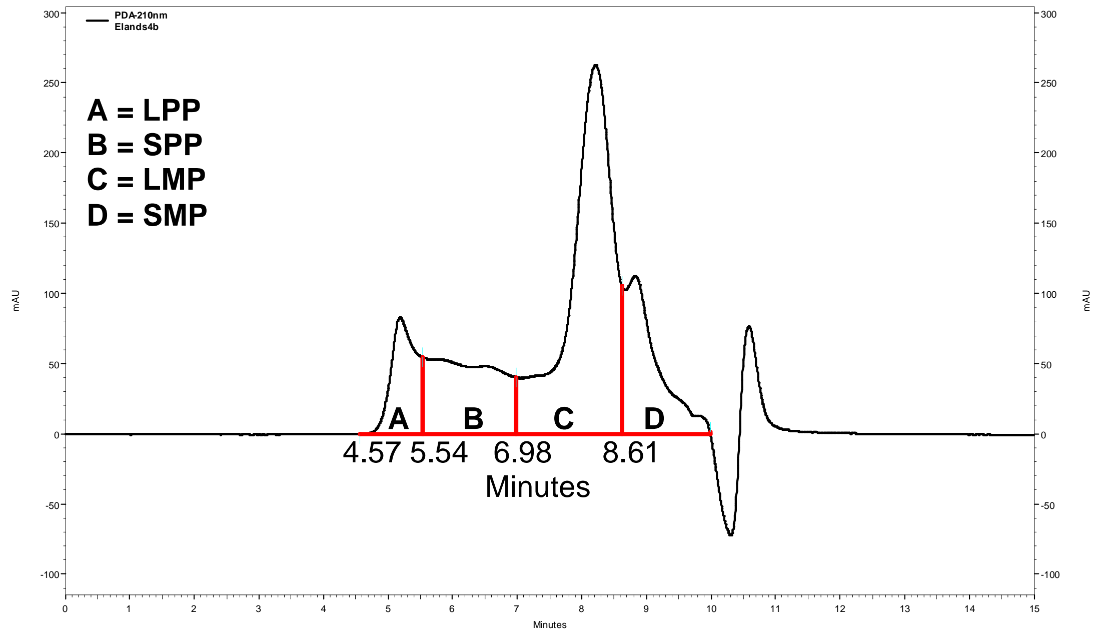

Fig. 4.3 Size-exclusion HPLC profile for SDS-soluble fractions.
LPP = large polymeric proteins; SPP = small polymeric proteins; LMP = Large monomeric proteins; SMP = Small monomeric proteins

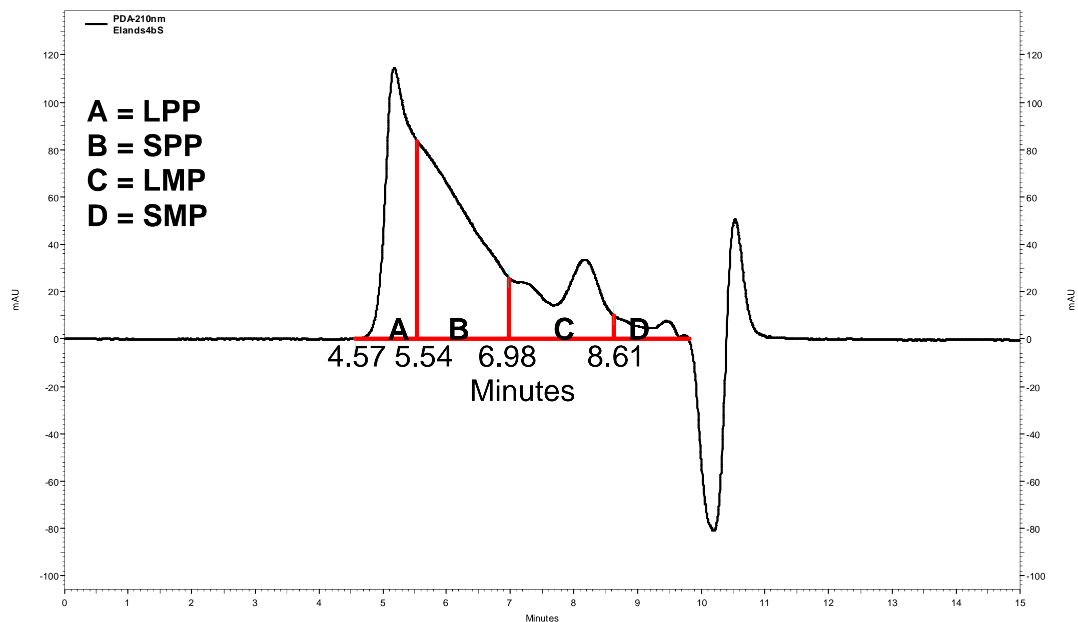

Fig. 4.4 Size-exclusion HPLC profile for SDS-insoluble fractions.
LPP = large polymeric proteins; SPP = small polymeric proteins; LMP = Large monomeric proteins; SMP = Small monomeric proteins

Table 4.2 Means of measured SDS-insoluble protein fractions determined on three localities

Parameters	LPP			SPP			LMP			SMP			TPP		
	Be	Ar	Bo	Be	Ar	Bo	Be	Ar	Bo	Be	Ar	Bo	Be	Ar	Bo
Mean	52.60	53.26	54.83	49.15	48.44	49.24	12.16	12.49	12.15	8.93	8.71	8.79	50.45	50.29	51.42
Minimum	43.93	46.30	44.87	40.21	44.01	44.79	8.51	8.88	9.58	7.07	7.34	7.41	43.19	45.25	44.82
Maximum	58.87	60.61	60.41	55.82	55.62	57.94	14.98	16.12	16.70	10.75	10.36	10.76	56.53	57.29	58.84
Std. deviation	3.81	3.44	3.60	4.03	3.49	3.38	1.42	1.62	2.07	0.81	0.68	0.76	3.72	3.24	3.23

SDS = sodium dodecyl sulphate, LPP = large polymeric protein, SPP = small polymeric protein, LMP = large monomeric protein, SMP = small monomeric protein, TPP = total polymeric protein, Be = Bethlehem, Ar = Arlington, Bo = Bothaville, Std. deviation = standard deviation

Table 4.3 Analysis of variance for SDS-insoluble protein fractions determined on three localities

SOURCE	d.f.	MEAN SQUARES				
		LPP	SPP	LMP	SMP	TPP
Total	119					
Reps	3	3.45	3.84	1.02	0.47	2.45
Environment	2	52.52**	7.78	1.49	0.52	14.98*
Residual A	6	2.66	1.79	1.18	0.16	1.70
Genotype	9	128.44***	135.69***	19.64***	3.11***	121.12***
GXE	18	11.34***	13.14***	3.91***	0.77***	9.97***
Residual B	81	1.82	0.95	1.12	0.27	0.83
Grand mean		53.56	48.94	12.27	8.81	50.72
CV (%)		2.5	2.0	8.6	5.9	1.8

SDS = sodium dodecyl sulphate, LPP = large polymeric protein, SPP = small polymeric protein, LMP = large monomeric protein, SMP = small monomeric protein, TPP = total polymeric protein, GXE = genotype by environmental interaction, CV = coefficient of variation, d.f. = degrees of freedom
* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Table 4.4 Percentage contribution to total variance for SDS-insoluble fractions

SOURCE	LPP	SPP	LMP	SMP	TPP
Reps	0.63	0.73	0.87	2.11	0.53
Environment	6.41	0.99	0.85	1.53	2.16
Residual A	0.97	0.69	2.02	1.44	0.74
Genotype	70.56	77.62	50.36	41.57	78.73
GXE	12.45	15.05	20.05	20.54	12.96
Residual B	8.98	4.87	25.83	32.81	4.88
Total	100.0	100.00	100.00	100.0	100.0

SDS = sodium dodecyl sulphate, LPP = large polymeric protein, SPP = small polymeric protein, LMP = large monomeric protein, SMP = small monomeric protein, TPP = total polymeric protein, GXE = genotype by environmental interaction

Table 4.5 Means of measured SDS-insoluble protein fractions determined on three localities for 10 selected South African wheat cultivars

		LPP		Cult		SPP		Cult		LMP		Cult		SMP		Cult		TPP		Cult
Cultivar	**Ar**	**Be**	**Bo**	**means**	**Ar**	**Be**	**Bo**	**means**	**Ar**	**Be**	**Bo**	**means**	**Ar**	**Be**	**Bo**	**means**	**Ar**	**Be**	**Bo**	**means**
Betta-DN	53.77	49.26	53.40	**52.15** b	46.64	42.19	46.30	**45.04** a	10.96	10.25	10.73	**10.65** a	8.46	7.78	8.55	**8.26** a	49.41	45.02	49.02	**47.81** b
Caledon	47.27	45.42	46.80	**46.50** a	44.97	43.93	45.67	**44.86** a	12.33	10.88	11.18	**11.46** ab	8.97	8.37	9.10	**8.81** bcd	45.82	44.47	46.10	**45.46** a
Elands	52.92	54.57	55.19	**54.22** c	47.48	50.08	51.35	**49.64** d	13.36	11.81	14.93	**13.37** cd	8.11	8.59	8.99	**8.56** ab	49.61	51.78	52.84	**51.41** e
Gariep	53.12	51.62	56.67	**53.80** c	46.99	49.50	49.49	**48.67** c	11.27	11.77	11.13	**11.39** ab	8.42	9.09	8.69	**8.73** bc	49.36	50.27	52.22	**50.62** d
Komati	52.10	51.04	52.60	**51.91** b	45.14	48.26	46.68	**46.69** b	10.73	11.73	11.88	**11.45** ab	8.47	8.91	8.45	**8.61** abc	47.90	49.27	49.07	**48.75** c
Limpopo	53.70	53.18	53.59	**53.49** c	49.87	49.03	47.00	**48.64** c	12.64	11.71	10.88	**11.75** b	9.56	9.17	8.32	**9.02** cd	51.30	50.59	49.64	**50.51** d
Matlabas	57.69	58.00	55.89	**57.19** d	52.22	51.93	47.50	**50.55** e	14.32	13.42	10.62	**12.79** c	8.84	8.60	8.66	**8.70** bc	54.24	54.17	50.82	**53.08** f
PAN3118	59.10	58.14	59.18	**58.81** e	55.11	55.25	56.39	**55.58** g	14.31	14.02	15.46	**14.60** e	9.40	10.29	10.28	**9.99** e	56.60	56.30	57.44	**56.78** g
PAN3349	51.06	52.00	56.93	**53.33** c	44.89	47.34	49.05	**47.09** b	11.70	11.61	11.11	**11.47** ab	7.92	8.73	8.03	**8.23** a	47.27	49.06	52.14	**49.49** c
PAN3377	51.90	52.73	58.02	**54.22** c	51.08	53.99	53.00	**52.69** f	13.27	14.41	13.58	**13.75** de	8.92	9.77	8.83	**9.17** d	51.37	53.56	54.90	**53.28** f
Env mean	**53.26**a	**52.60**a	**54.83**b	**Grand mean= 53.56**	**48.44**a	**49.15**b	**49.25**b	**Grand mean= 48.94**	**12.49**a	**12.16**a	**12.15**a	**Grand mean= 12.27**	**8.71**a	**8.93**b	**8.79**ab	**Grand mean= 8.81**	**50.29**a	**50.45**a	**51.42**b	**Grand mean= 50.72**
LSD Env	**0.892**				**0.7335**				**0.5952**				**0.2200**				**0.7135**			
LSD Cult	**1.094**				**0.7898**				**0.8593**				**0.4245**				**0.7420**			

Means followed by the same letter, did not differ significantly at $p \leq 0.05$. SDS = sodium dodecyl sulphate, LPP = large polymeric protein, SPP = small polymeric protein, LMP = large monomeric protein, SMP = small monomeric protein, TPP = total polymeric protein, Cult means = cultivar means for the three localities, Env mean = environmental means, LSD Env = least significant difference for environments, LSD Cult = least significant difference for cultivars, Ar = Arlington, Be = Bethlehem, Bo = Bothaville

4.4.3 Descriptive statistics and analysis of variance

4.4.3.1 SDS-insoluble large polymeric protein (LPP)

The analysis of variance (ANOVA) showed highly significant ($p \leq 0.001$) differences for two major components (genotypes and GXE), and significant ($p \leq 0.01$) differences for environment (Table 4.3). The share of the genotypes was 70.56% of the total variance, followed by 12.45% for GXE (Table 4.4). The genotype means for LPP varied from 46.50% to 58.81%. Bothaville differed significantly from Arlington and Bethlehem. PAN3118 was the cultivar with the highest LPP content and differed significantly from Caledon, which had the smallest LPP content (Table 4.5).

4.4.3.2 SDS-insoluble small polymeric protein (SPP)

Significant ($p \leq 0.001$) differences for genotypes and GXE were observed (Table 4.3). Genotypes contributed 77.62% of the total variance. Environments contributed 0.99% while GXE contributed 15.05% of the total variance (Table 4.4). The smallest amount (40.21%) of SPP was measured at Bethlehem and the largest amount (57.94%) at Bothaville (Table 4.2). PAN3118 rendered the highest amount of SPP and Caledon the smallest amount, and differed significantly. Arlington differed significantly from Bethlehem and Bothaville (Table 4.5).

4.4.3.3 SDS-insoluble large monomeric protein (LMP)

ANOVA confirmed significant ($p \leq 0.001$) differences for genotypes and GXE (Table 4.3). The genotypes added 50.36% to the total variance and GXE 20.05% (Table 4.4). Minimum and maximum values for genotypes varied between 8.51% and 16.70% and differed significantly (Table 4.2).

4.4.3.4 SDS-insoluble small monomeric protein (SMP)

Significant differences were observed for genotypes and GXE (Table 4.3). Genotypes contributed 41.57% to the total variance and GXE contributed 20.54% (Table 4.4). Arlington and Bethlehem differed significantly. PAN3118 was the cultivar with the highest SMP fraction and differed significantly from PAN3349, which had lower SMP fractions (Table 4.5).

4.4.3.5 SDS-insoluble total polymeric protein (TPP)

ANOVA demonstrated highly significant ($p \leq 0.001$) differences for genotypes and GXE, and significant ($p \leq 0.05$) differences for environments. Genotypes made the biggest contribution (78.73%) to the total variance (Table 4.4). The minimum value for TPP was recorded at Bethlehem while the highest value occurred at Bothaville (Table 4.2). PAN3118 was the genotype with the highest LPP value and Caledon the lowest, and they differed significantly. Bothaville differed significantly from Arlington and Bethlehem (Table 4.5).

Table 4.6 Significant correlations between SDS-insoluble protein fractions and quality characteristics

Fraction	Characteristic	Correlation	Fraction	Characteristic	Correlation
LPP	AlvL	-0.68***	LMP	AlvP	0.31***
	AlvP	0.65***		AlvP/L	0.27**
	AlvP/L	0.66***		AlvW	0.21*
	BFY	-0.20*		BFY	-0.27**
	LFV	-0.27**		FABS	0.37***
	FY	-0.36***		WGC	-0.20*
	WGC	-0.57***		MDT	0.35***
	MDT	0.71***		SDSS	0.41***
	SDSS	0.57***		KD	0.31***
	KD	0.40***		VK	-0.25***
SPP	AlvL	-0.38***	SMP	AlvW	0.30***
	AlvP	0.56***		BFY	-0.41***
	AlvP/L	0.49***		LFV	0.26**
	AlvW	0.24**		FABS	0.26**
	BFY	-0.31***		MDT	0.24**
	FY	-0.20*		Prot	0.28**
	FABS	0.29**			
	WGC	-0.37***	TPP	AlvL	-0.53***
	MDT	0.56***		AlvP	0.63***
	SDSS	0.56***		AlvP/L	0.59***
	KD	0.38***		BFY	-0.29**
				FY	-0.28**
				FABS	0.23*
				WGC	-0.48***
				MDT	0.67***
				SDSS	0.60***
				KD	0.41***

SDS = sodium dodecyl sulphate, LPP = large polymeric protein, SPP = small polymeric protein, LMP = large monomeric protein, SMP = small monomeric protein, TPP = total monomeric protein, AlvL = alveograph extensibility, AlvP = alveograph tenacity, AlvP/L = alveograph configuration of the curve, AlvW = flour strength, BFY = brake flour yield, LFV = loaf volume, FY = flour yield, FABS = farinograph water absorption, WGC = wet gluten content, MDT = mixograph development time, Prot = flour protein content, SDS = sodium dodecyl sulphate, SDSS = SDS sedimentation volume, KD = kernel diameter, VK = vitreous kernel

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

4.4.4 Significant correlations between SDS-insoluble protein fractions and quality characteristics

4.4.4.1 Large polymeric protein (LPP)

Significant ($p \leq 0.001$) positive correlations were observed with MDT, AlvP, AlvP/L, SDSS and KD. Significant negative correlations were expressed with AlvL, WGC and FY (Table 4.6).

4.4.4.2 Large monomeric protein (LMP)

SDSS, AlvP, FABS, MDT and KD showed a significant ($p \leq 0.001$) positive correlation. A significant ($p \leq 0.001$) negative correlation was observed for VK (Table 4.6).

4.4.4.3 Small polymeric protein (SPP)

Significant ($p \leq 0.001$) positive correlations were observed for MDT, SDSS and AlvP, AlvP/L and KD. AlvL, WGC and BFY had significant negative correlations with SPP (Table 4.6).

4.4.4.4 Small monomeric protein (SMP)

BFY had a significant negative correlation with SMP. AlvW correlated significantly positively. Protein content, LFV, FABS and MDT correlated significantly positively (Table 4.6).

4.4.4.5 Total polymeric protein (TPP)

MDT displayed a significant positive correlation and AlvP, AlvP/L, SDSS, and KD correlated significantly positively. AlvL and WGC correlated significantly negatively with TPP (Table 4.6).

Table 4.7 Stepwise multiple regression for quality characteristics and SDS-insoluble protein fractions

AlvL	**Constant**	**LPP**	**TPP**	**SPP**		**R^2**	**Pr>F**
	394.4[(a)]	-5.508				0.464	<.001
	29.2[(b)]	*0.545*					
	369	-8.83	4.00			0.502	<.001
	29.5	*1.2*	*1.34*				
	359.7	-27.4	50.9	-28.1		0.514	<.001
	29.9	*11.2*	*28.1*	*16.8*			
AlvP	**Constant**	**LPP**	**TPP**	**SPP**	**LMP**	**R^2**	**Pr>F**
	-50.7	2.313				0.427	<.001
	13.2	*0.24*					
	-56.4	1.552	0.916			0.437	<.001
	13.7	*0.574*	*0.624*				
	-53.2	7.59	-14.4	9.15		0.443	<.001
	14.0	*5.23*	*13.2*	*7.89*			
	-52.9	7.63	-14.4	9.13	0.133	0.444	<.001
	14.2	*5.26*	*13.2*	*7.92*	*0.811*		
AlvP/L	**Constant**	**LPP**	**TPP**	**SPP**	**LMP**	**R^2**	**Pr>F**
	-2.989	0.07156				0.433	<.001
	0.404	*0.00753*					
	-2.980	0.0727	-0.0014			0.433	<.001
	0.423	*0.0177*	*0.0192*				
	-2.834	0.347	-0.696	0.416		0.447	<.001
	0.428	*0.160*	*0.403*	*0.241*			
	-2.832	0.347	-0.696	0.416	0.0011	0.447	<.001
	0.435	*0.161*	*0.405*	*0.243*	*0.0248*		
MDT	**Constant**	**LPP**	**TPP**	**SPP**	**LMP**	**R^2**	**Pr>F**
	-3.345	0.1249				0.504	<.001
	0.612	*0.0114*					
	-3.507	0.1033	0.0260			0.507	<.001
	0.639	*0.0267*	*0.0290*				
	-3.538	0.045	0.174	-0.089		0.508	<.001
	0.654	*0.245*	*0.616*	*0.369*			
	-3.479	0.052	0.164	-0.093	0.0239	0.509	<.001
	0.663	*0.245*	*0.618*	*0.370*	*0.0379*		
SDSS	**Constant**	**TPP**	**SPP**	**LMP**		**R^2**	**Pr>F**
	57.53	0.937				0.363	<.001
	5.80	*0.114*					
	36.6	1.276	-0.332			0.368	<.001
	5.90	*0.394*	*0.370*				
	37.47	1.402	-0.576	0.376		0.374	<.001
	5.95	*0.411*	*0.432*	*0.345*			
FarABS	**Constant**	**LMP**	**SMP**	**SPP**	**TPP**	**R^2**	**Pr>F**
	53.55	0.3605				0.139	<.001
	1.02	*0.0827*					
	52.48	0.323	0.149			0.142	<.001
	1.67	*0.101*	*0.213*				
	52.52	0.325	0.153	-0.002		0.142	0.001
	2.11	*0.124*	*0.258*	*0.064*			
	55.76	0.249	0.042	0.177	-0.170	0.150	0.003
	2.42	*0.127*	*0.279*	*0.183*	*0.163*		
LFV	**Constant**	**LPP**	**SMP**			**R^2**	**Pr>F**
	1120	-4.76				0.073	0.003
	84.2	*1.57*					
	937.9	1.53	32.14			0.197	<.001
	89.5	*1.5*	*7.53*				

All protein fractions were significant at $p \leq 0.001$ (a) = correlation coefficient, (b) = standard error of correlation coefficient. SDS = sodium dodecyl sulphate, LPP = large polymeric protein, SPP = small polymeric protein, LMP = large monomeric protein, SMP = small monomeric protein, TPP = total polymeric protein, AlvL = alveograph extensibility, AlvP = alveograph tenacity, AlvP/L = alveograph configuration of the curve, MDT = mixograph development time, SDSS = SDS sedimentation volume, FarABS = farinograph water absorption, LFV = loaf volume, Pr = probability determination, F = F-test, R^2 = coefficient of multiple determination

4.4.5 Stepwise multiple regression for quality characteristics and SDS-insoluble protein fractions

LPP can be regarded as the most important contributor to variability in AlvL, (up to 46.4%). TPP and SPP made slight contributions. The largest contributor to variability in AlvP was LPP, while TPP, SPP and LMP made minor contributions. LPP explained 42.7% of the variation. LPP explained 43.3% of the variance in AlvP/L. LPP made a 50.4% contribution to MDT variability. LMP explained 13.9% of the variance in FABS. TPP explained up to 36.3% of the variation in SDSS. SMP contributed 19.7% of variability in LFV, while LPP made an insignificant contribution (7.3%) (Table 4.7).

Table 4.8 Narrow sense heritability values

Primary Traits	Heritability (h^2)	Secondary Traits	Heritability (h^2)
AlvL	0.07	LPP	0.22
AlvP	0.13	TPP	0.37
AlvP/L	0.08		
MDT	0.12		

Heritability was calculated for primary and secondary traits with the highest correlations at $p \leq 0.001$. AlvL = extensibility, AlvP = tenacity, AlvP/L = alveograph configuration of the curve, MDT = mixograph development time, LPP = large polymeric protein, TPP = total polymeric protein, h^2 = narrow sense heritability

4.4.6 Narrow sense heritability calculated for highly correlated primary and secondary traits

The heritability values for primary traits varied between h^2=0.07 and h^2=0.13 for AlvL and AlvP, respectively. Heritability values for secondary traits varied between h^2=0.22 and h^2=0.37 for LPP and TPP, respectively (Table 4.8).

4.5 Discussion

Results in this study showed poor separation on SDS-PAGE between subunits 2* and 2 on the D-genome. This is in agreement with Gao et al. (2010). Caledon and PAN3377 were the only cultivars with HMW-GS 2+12 on the D-genome, while the other cultivars had 5+10. Gupta et al. (1995) reported that lines with subunits 5+10 contributed more to dough strength than lines with subunits 2+12, due to a higher amount of total unextractable polymeric proteins (TPP). Lemelin et al. (2005) reported the opposite from different entries. No significant differences occurred in the amount of TPP between cultivars containing HMW-GS 5+10 and 2+12 (Tronsmo et al., 2002).

Significant differences were observed between Caledon (HMW-GS 2+12) and PAN3349 (HMW-GS 5+10) for rheology traits (Appendix B, Table 11), but the trend was not constant for PAN3377 (HMW-GS 2+12) and the other entries (HMW-GS 5+10) in this study. Previous studies indicated that the HMW-GS in South African and Australian wheats explained less than 20% of variability in bread making quality (Gupta et al., 1991; Randall et al., 1993). Weegels et al. (1996b) also reported that glutenin subunits explain a small proportion of the variation in wheat quality.

Significant correlations were observed between MWD of SDS-insoluble protein fractions and kernel characteristics, although correlations were low. Poor correlations were observed between TPP and LFV. Loaf volume was determined with the long fermentation procedure and ascorbic acid oxidizing improver was added. Caledon ranked the highest for LFV, followed by PAN3377 (Appendix B, Table 12). Both entries have HMW-GS 2+12 on the D-genome. Ascorbic acid improves dough strength of HMW-GS 2+12 during proofing, thus increasing the amount of TPP (Every et al., 2008).

Previous studies indicated that correlations varied between LFV (long fermentation method) and TPP, depending on the solvent systems used for SE-HPLC sample extraction. In this study, protein fractions for SE-HPLC were extracted with 0.5% SDS (Gupta et al., 1993). Correlations were strong for

1.5% SDS solvent (Sapirstein & Suchy, 1996), moderate for 50% 1-propanol solvent (Bean et al., 1998) and poor for 0.5% SDS (Gupta et al., 1992; 1993). The same sample set rendered a moderate correlation with the short fermentation method (Gupta et al., 1992; 1993).

LPP made the biggest contribution to mixograph and alveograph properties. The highest polymer fraction was the only contributor to variation in dough properties, as indicated by the correlations between TPP and MDT and dough strength (Weegels et al., 1996b; MacRitchie & Lafiandra, 1997; Tsilo et al., 2010). Significant positive correlations were observed for MDT, AlvP and AlvP/L, while the correlation for AlvL was negative.

No significant correlation was observed between TPP and AlvW. This is in contrast with previous reports (Dachkevitch & Autran, 1989: Sadouki et al., 2005; Labuschagne et al., 2006). AlvW correlated significantly with SMP, which is in agreement with Labuschagne et al. (2006). Sadouki et al. (2006) has shown that the crop year, under Algerian conditions, has a significant influence on alveograph paramaters, especially AlvW and to a lesser extent AlvP. Genotypes in this study did not differ significantly for AlvW, but differed significantly for the other alveograph properties (Appendix B, Table 11).

PAN3118 was the cultivar with the largest percentage of LPP and TPP, and Caledon the smallest (Table 4.5). PAN3118 ranked the highest and Caledon the lowest for MDT, AlvP and AlvP/L (Appendix B, Table 11). Caledon ranked the highest for dough extensibility as measured by AlvL and PAN3118 ranked the lowest (Appendix B, Table 11). The increase in AlvL values is caused by the decrease in UPP. The significant negative correlation between LPP and WGC confirms the decrease of extensibility caused by an increase of LPP. AlvL correlated positively with WGC (Taylor & Randall, 1994). Flour strength increases according to an increase in the polymeric protein fraction from SDS-soluble to SDS-insoluble, resulting in changes in the amount of TPP (Johansson et al., 2001; Kuktaite et al., 2004).

Primary and secondary traits, with highly significant correlations, were identified to calculate the h^2. Heritability values for LPP and TPP (secondary traits) were much higher than the heritability values for MDT, AlvP, AlvL and AlvP/L (primary traits) (Table 4.8). One of the main criteria for indirect selection is a high correlation between the traits of interest, and a much higher heritability of the secondary method (Falconer, 1982).

4.6 Conclusions

SDS-insoluble LPP and TPP fractions showed a strong correlation with dough strength and extensibility properties, as measured with the mixograph and alveograph. No correlations were observed between SDS-insoluble protein fractions and LFV. The high h^2 for LPP and TPP makes SE-HPLC an attractive choice for indirect selection for AlvL, AlvP, AlvP/L and MDT. The efficacy needs to be verified with a wider range of samples that include weak and very strong flours.

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Chapter 5

General conclusions

Plant breeding has evolved to the stage where wheat can be grown with a lower, but more focused, protein content with outstanding bread making properties. This, consequently, raises the need to understand the relationship between protein composition and dough properties (Anderssen et al., 2004). RP-HPLC and SE-HPLC have been applied in this study to determine the correlation between gluten proteins and wheat quality characteristics. Significant differences were observed between separated protein fractions and quality parameters, despite of the relatively narrow protein range that was used in this study. Variation for dough properties between cultivars could not be explained by grain protein content (Primard et al., 1991; Dong et al., 1992)

The highest correlations were observed between bread making properties and HPLC fractions. SE-HPLC showed the highest correlations for these traits, except for the correlations between RP-HPLC and AlvW and LFV. The environment had a much larger effect on the expression of RP-HPLC fractions (Table 3.4) compared to the SE-HPLC fractions. This is in agreement with data reported by Edwards et al. (2007). Genotype contributed much more to the expression of SE-HPLC fractions than the environment. The coefficient of variation was much higher for RP-HPLC fractions (Table 3.4) as opposed to SDS-insoluble polymeric proteins (Table 4.3).

Furthermore, the h^{-2} values were much higher for SDS-insoluble polymeric proteins than for mixograph and alveograph properties. As a result, SE-HPLC could be considered for indirect selection for dough strength and extensibility characteristics. The LPP fraction is the main contributor to variation in dough properties (Weegels et al, 1996; MacRitchie & Lafiandra, 1997; Hamer & van Vliet, 2000; Schober et al., 2006).

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Summary

The association between hexaploid wheat (*Triticum aestivum* L.) quality parameters and gluten protein were determined for selected South African wheat cultivars. Reversed-phase high-performance liquid chromatography (RP-HPLC) and size-exclusion HPLC (SE-HPLC) were used to separate protein fractions. The influence of the environment was much higher for RP-HPLC fractions than for SE-HPLC fractions, and genotype contributed much more to the expression of SE-HPLC. Correlations between yield, kernel characteristics and milling properties were low for both chromatography procedures. Bread making properties showed significant correlations with HPLC fractions. RP-HPLC gluten fraction showed the best correlation with alveograph strength and loaf volume. Polymeric proteins showed the highest correlation with mixograph development time, alveograph extensibility, tenacity and configuration of the curve. This confirms the effect of gluten on dough strength and extensibility. Narrow sense heritability indicated that large polymeric proteins could be considered for indirect selection of alveograph and mixograph parameters.

Keywords: RP-HPLC, SE-HPLC, wheat quality, gluten, dough strength, dough extensibility.

Opsomming

Die verwantskap tussen kwaliteitseienskappe van heksaploïede koring (*Triticum aestivum* L.) en gluten proteïene is bepaal vir geselekteerde Suid Afrikaanse koring kultivars. Twee verskillende vloeistof chromatografiese metodes is aangewend om die proteïenfraksies te skei, naamlik RP-HPLC and SE-HPLC. Die invloed van die omgewing was aansienlik hoër op RP-HPLC fraksies as op SE-HPLC fraksies, en die genotipe het meer bygedra tot die uitdrukking van die SE-HPLC fraksies. Korrelasies was laag vir die chromatografiese metodes en graanopbrengs, korrel- en maaleienskappe. Hoë korrelasies is aangeteken tussen HPLC fraksies en reologiese eienskappe. Die RP-HPLC gluten fraksie het die beste korrelasie getoon met alveograaf sterkte en broodvolume. Groot polimeriese proteïene het die hoogste korrelasie getoon met mixograaf mengtyd, alveograaf uitrekking, weerstand teen uitrekking en konfigurasie van die kurwe. Dit bevestig die invloed van gluten op deeg se sterkte en rekbaarheid. Die bepaling van oorerflikheid het aangedui dat groot polimeriese proteïene oorweeg kan word vir die indirekte seleksie van alveograaf en mixograaf eienskappe.

Sleutelwoorde: RP-HPLC, SE-HPLC, koring kwaliteit, gluten, deeg sterkte, deeg rekbaarheid.

Appendices

Appendix A

Table 1 Fertilising programme for 2007 season

Locality	Fertiliser source used	Total N (kg N ha^{-1})	Total P (kg N ha^{-1})	Total K (kg N ha^{-1})
Bothaville	6:2:1(31)	50	17	9
Arlington	6:2:1(31)	35	12	6
Bethlehem	6:2:1(31)	50	17	9

Table 2 Planting and harvest dates of trials for 2007 season

Locality	Planting date	Harvest date
Bothaville	2007-04-26	2007-11-28
Arlington	2007-06-18	2008-01-10
Bethlehem	2007-06-05	2008-01-08

Table 3 Weather data for 2007 at three localities

Locality	Latitude	Longitude	Altitude	Month	Temp (N)	Temp (X)	Rain
Bothaville	-27.30342	26.68219	1316	1	14.84	31.69	34.8
				2	13.16	32.45	25.4
				3	11.79	29.84	37.3
				4	8.93	25.72	22.5
				5	1.36	22.71	2.3
				6	0.04	18.37	20.8
				7	-2.18	18.82	0
				8	0.57	22.6	2.7
				9	8.38	29	65.2
				10	11.33	24.94	103.2
				11	12.44	28.1	121.3
				12	14.03	28.21	74.4
Arlington	-27.53229	27.56386	1601	1	14.52	31.91	19.8
				2	13.93	32.82	29.9
				3	11.18	29.72	23.3
				4	8.96	25.3	40.3
				5	0.99	21	1
				6	-0.97	17.17	27.8
				7	-2.81	17.93	0.6
				8	-0.25	20.82	0
				9	7.97	28.79	0
				10	10.6	22.81	93.3
				11	11.05	25.73	106.6
				12	13.43	26.87	78.1
Bethlehem	-28.1628	28.29733	1653	1	13.03	28.25	25.2
				2	12.68	29.31	25.1
				3	10.56	27.18	33.9
				4	7.76	23.73	44.5
				5	0.08	20.79	1.5
				6	-1.33	16.49	27.6
				7	-2.89	17.33	0
				8	-0.73	20.36	0
				9	6.76	26.14	37.8
				10	9.46	20.54	158.7
				11	10.62	23.77	96.7
				12	12.24	24.57	86.3

Temp (N) = average minimum temperature ℃, Temp (X) = average maximum temperature ℃

Table 4 Descriptive statistics for grain yield and hectolitre mass determined on three localities

Parameters	Yield			HLM		
	Be	Ar	Bo	Be	Ar	Bo
Mean	4.84	3.53	3.97	79.31	81.11	81.18
Minimum	3.36	2.65	1.78	75.40	78.70	78.30
Maximum	6.84	4.17	5.84	82.50	83.20	84.00
Std. deviation	0.76	0.37	0.99	1.96	1.20	1.46

HLM = hectolitre mass, Be = Bethlehem, Ar = Arlington, Bo = Bothaville, Std. deviation = standard deviation

Table 5 Analysis of variance for grain yield and hectolitre mass

SOURCE	d.f.	MEAN SQUARES	
		Yield	HLM
Total	119		
Reps	3	0.702	0.56
Environment	2	17.69***	44.95***
Residual A	6	0.75	0.41
Genotype	9	0.97**	13.56***
GXE	18	1.53***	4.50***
Residual B	81	0.29	1.02
Grand mean		4.11	80.53
CV (%)		13.1	1.3

HLM = hectolitre mass, GXE = genotype by environmental interaction, CV = coefficient of variation, d.f. = degrees of freedom
* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Table 6 Percentage contribution of each variance component to the combined analysis of variance for grain yield and hectolitre mass

Source	**Yield**	**HLM**
Reps	2.07	0.44
Environment	34.84	23.70
Residual A	4.41	0.65
Genotype	8.55	32.17
GXE	27.10	21.37
Residual B	23.00	21.67
Total	100.0	100.0

HLM = hectolitre mass, GXE = genotype by environmental interaction

Table 7 Means of measured grain yield and hectolitre mass determined on three localities

Cultivar	Ar	Yield Be	Bo	Cult means	Ar	HLM Be	Bo	Cult means
Betta-DN	3.73	5.25	3.60	**4.07**abcd	80.98	80.68	81.25	**80.97**de
Caledon	3.50	4.20	4.91	**4.20**cd	80.98	79.95	83.45	**81.46**ef
Elands	3.74	5.31	3.96	**4.34**de	81.25	82.03	82.20	**81.82**f
Gariep	3.39	4.14	3.78	**3.77**ab	80.58	77.83	79.85	**79.42**ab
Komati	3.51	5.15	4.25	**4.31**cde	81.33	81.10	80.80	**81.08**def
Limpopo	3.29	4.89	3.90	**4.03**abcd	82.38	80.65	82.40	**81.81**f
Matlabas	3.84	5.81	2.81	**4.15**bcd	80.90	79.00	81.75	**80.55**cd
PAN3118	4.01	4.03	3.61	**3.88**abc	81.15	77.25	81.60	**80.00**bc
PAN3349	3.43	4.28	3.43	**3.72**a	81.25	76.45	79.25	**78.98**a
PAN3377	3.23	5.31	5.41	**4.65**e	80.33	78.78	79.28	**79.26**ab
Env mean	**3.53** a	**4.84** b	**3.97** a	**Grand mean= 4.11**	**81.11** b	**79.31** a	**81.18** b	**Grand mean= 80.53**
LSD Env	**0.4730**				**0.3513**			
LSD Cult	**0.4362**				**0.8183**			

Means followed by the same letter, did not differ significantly at $p \leq 0.05$. HLM = hectolitre mass, Cult means = cultivar means for the three localities, Env mean = environmental means, LSD Env = least significant difference for environments, LSD Cult = least significant difference for cultivars, Ar = Arlington, Be = Bethlehem, Bo = Bothaville

Appendix B

Table 1 Descriptive statistics for grain characteristics determined on three localities

Parameters	VK			KD			HI			FN			Prot		
	Be	Ar	Bo	Be	Ar	Bo	Be	Ar	Bo	Be	Ar	Bo	Be	Ar	Bo
Mean	66.90	69.08	88.30	2.89	2.91	2.87	58.70	60.82	68.55	523.0	395.6	509.5	11.13	10.26	11.20
Minimum	52.00	50.00	67.00	2.58	2.56	2.62	47.48	40.72	62.87	408.0	312.0	390.0	9.40	7.60	9.30
Maximum	83.00	93.00	100.00	3.30	3.31	3.15	68.92	69.92	75.01	744.0	512.0	673.0	12.10	12.00	12.50
Std. deviation	8.54	11.99	8.35	0.15	0.17	0.17	5.05	6.13	3.27	75.1	51.6	68.0	0.63	0.93	0.76

VK = vitreous kernels, KD = kernel diameter, HI = hardness index, FN = falling number, Prot = flour protein content, Be = Bethlehem, Ar = Arlington, Bo = Bothaville, Std. deviation = standard deviation

Table 2 Descriptive statistics for milling characteristics determined on three localities

Parameters	BFY			FY			C76		
	Be	Ar	Bo	Be	Ar	Bo	Be	Ar	Bo
Mean	23.18	22.96	22.73	73.22	72.66	72.88	-1.71	-1.33	-0.52
Minimum	19.88	20.65	19.81	71.12	70.64	68.24	-3.60	-2.80	-2.20
Maximum	25.78	30.30	27.34	74.79	74.44	74.91	0.10	1.10	1.90
Std. deviation	1.33	1.65	1.79	1.02	0.98	1.11	0.87	0.81	0.92

BFY = break flour yield, FY = flour yield, C76 = flour colour at 76% flour yield level, Be = Bethlehem, Ar = Arlington, Bo = Bothaville, Std. deviation = standard deviation

Table 3 Descriptive statistics for rheological characteristics determined on three localities

Parameters	AlvP			AlvL			AlvP/L			AlvW			MDT		
	Be	Ar	Bo	Be	Ar	Bo	Be	Ar	Bo	Be	Ar	Bo	Be	Ar	Bo
Mean	71.55	75.85	72.13	112.8	90.6	94.7	0.69	0.96	0.88	278.9	246.8	250.4	3.14	3.40	3.50
Minimum	50.00	56.00	51.00	51.00	65.00	43.00	0.32	0.41	0.31	195.0	168.0	126.0	2.00	2.18	2.35
Maximum	115.0	98.00	176.0	176.0	165.0	163.0	1.44	2.28	1.85	374.0	369.0	375.0	4.76	5.05	4.43
Std. deviation	13.02	25.1	28.5	25.1	28.5	31.9	0.28	0.46	0.41	37.9	44.9	54.50	0.67	0.70	0.55

AlvP = alveograph tenacity, AlvL = alveograph extensibility, AlvP/L = alveograph configuration of the curve, AlvW = flour strength, MDT = mixograph development time, Be = Bethlehem, Ar = Arlington, Bo = Bothaville, Std. deviation = standard deviation

Table 4 Descriptive statistics for flour characteristics determined on three localities

Parameters	WGC			ABS			FABS			SDSS			LFV		
	Be	Ar	Bo	Be	Ar	Bo	Be	Ar	Bo	Be	Ar	Bo	Be	Ar	Bo
Mean	35.11	31.43	34.02	60.29	59.07	60.39	57.74	57.42	58.11	85.36	85.15	84.626	888.1	838.5	870.2
Minimum	27.14	17.88	23.94	57.70	55.00	57.55	55.35	53.60	54.30	70.00	75.00	75.00	800.0	730.0	710.0
Maximum	43.55	42.99	45.24	61.75	61.60	62.35	60.25	60.95	62.00	95.00	94.00	94.00	985.0	1010.0	1005.0
Std. deviation	3.46	4.88	4.40	0.94	1.45	1.14	1.21	1.70	1.96	5.66	4.79	5.52	46.8	69.4	69.4

WGC = wet gluten content, ABS = mixograph water absorption, FABS = farinograph water absorption, SDSS = SDS sedimentation volume, LFV = loaf volume, Be = Bethlehem, Ar = Arlington, Bo = Bothaville, Std. deviation = standard deviation

Table 5 Analysis of variance for grain and milling characteristics

SOURCE	d.f.	MEAN SQUARES								
		VK	KD	HI	FN	Prot	BFY	FY	C76	WGC
Total	119									
Reps	3	193.47	0.0053	21.26	1252	1.12	3.202	0.25	0.92	30.12
Environment	2	5548.61***	0.0216**	1074.4***	19584***	10.85*	2.033	3.19	14.78***	143.06
Residual A	6	65.24	0.0022	6.96	2672	1.76	1.51	0.90	0.47	29.99
Genotype	9	406.77***	0.2202***	176.56***	15289***	1.10**	10.23***	6.10***	1.88**	98.81***
GXE	18	84.42	0.0292***	27.34***	4862	0.93**	5.070***	1.19**	0.99	19.06**
Residual B	81	61.93	0.0069	8.52	3201	0.38	1.221	0.53	0.59	7.99
Grand mean		74.76	2.8901	62.69	476	10.86	22.96	72.92	-1.19	33.52
CV (%)		10.5	2.9	4.7	11.9	5.7	4.8	1.0	64.7	8.4

VK = vitreous kernels, KD = kernel diameter, HI = hardness index, FN = falling number, Prot = flour protein content, BFY = break flour yield, FY = flour yield, C76 = flour colour at 76% flour yield level, WGC = wet gluten content, GXE = genotype by environmental interaction, CV = coefficient of variation, d.f. = degrees of freedom
* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Table 6 Percentage contribution of each variance component to the combined analysis of variance for grain and milling characteristics

SOURCE	VK	KD	HI	FN	Prot	BFY	FY	C76	WGC
Reps	2.61	0.51	1.27	0.42	3.58	3.15	0.58	2.34	3.71
Environment	49.84	1.38	42.76	43.72	23.22	1.33	4.83	25.14	11.75
Residual A	1.76	0.43	0.83	1.79	11.33	2.97	4.09	2.38	7.39
Genotype	16.44	63.12	31.62	15.36	10.64	30.19	41.55	14.36	36.51
GXE	6.82	16.75	9.79	9.77	17.93	29.92	16.23	15.11	14.08
Residual B	22.53	17.81	13.73	28.94	33.30	32.44	32.72	40.67	26.57
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

VK = vitreous kernels, KD = kernel diameter, HI = hardness index, FN = falling number, Prot = flour protein content, BFY = break flour yield, FY = flour yield, C76 = flour colour at 76% flour yield level, WGC = wet gluten content, GXE = genotype by environmental interaction

Table 7 Analysis of variance for rheological and flour quality characteristics

SOURCE	d.f.	MEAN SQUARES								
		AlvP	AlvL	AlvP/L	AlvW	MDT	ABS	FABS	SDSS	LFV
Total	119									
Reps	3	11.73	789.9	0.11	358	0.46	2.40	3.34	14.27	1612
Environment	2	218.09*	5569.7**	0.77**	12380	1.37*	21.57*	4.67	5.79	25205*
Residual A	6	30.01	287.0	0.03	2518	0.16	4.13	2.77	5.85	4831
Genotype	9	1505.62***	6573.4***	1.23***	5929***	3.67***	2.44*	14.71***	299.89***	22779***
GXE	18	159.96***	632.4**	0.12**	1861	0.31***	2.09**	2.63*	3.53	6628***
Residual B	81	42.52	263.0	0.048	1702	0.09	0.94	1.40	6.06	1274
Grand mean		73.17	99.4	0.84	258.7	3.35	59.92	57.76	85.05	865.6
CV (%)		8.9	16.3	25.9	15.9	8.8	1.6	2.0	2.9	4.1

AlvP = alveograph tenacity, AlvL = alveograph extensibility, AlvP/L = alveograph configuration of the curve, AlvW = flour strength, MDT = mixograph development time, ABS = mixograph water absorption, FABS = farinograph water absorption, SDSS = SDS sedimentation volume, LFV = loaf volume, GXE = genotype by environmental interaction, CV = coefficient of variation, d.f. = degrees of freedom

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

Table 8 Percentage contribution of each variance component to the combined analysis of variance for rheological and flour quality characteristics

SOURCE	AlvP	AlvL	AlvP/L	AlvW	MDT	ABS	FABS	SDSS	LFV
Reps	0.17	2.21	1.77	3.85	2.74	3.41	3.05	1.28	0.95
Environment	2.13	10.40	7.92	8.99	5.41	20.44	2.84	0.35	9.85
Residual A	0.88	1.61	1.05	5.49	1.84	11.74	5.04	1.05	5.66
Genotype	66.02	55.25	57.53	19.39	65.12	10.41	40.26	80.74	40.06
GXE	14.03	10.64	11.70	12.18	11.01	17.84	14.40	1.90	23.31
Residual B	16.77	19.89	20.03	50.10	13.88	36.16	34.41	14.68	20.17
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

AlvP = alveograph tenacity, AlvL = alveograph extensibility, AlvP/L = alveograph configuration of the curve, AlvW = flour strength, MDT = mixograph development time, ABS = mixograph water absorption, FABS = farinograph water absorption, SDSS = SDS sedimentation volume, LFV = loaf volume, GXE = genotype by environmental interaction

Table 9 Means of measured grain characteristics determined on three localities

		VK		Cult		KD		Cult		HI		Cult		FN		Cult		Prot		Cult
Cultivar	**Ar**	**Be**	**Bo**	**Means**	**Ar**	**Be**	**Bo**	**means**	**Ar**	**Be**	**Bo**	**means**	**Ar**	**Be**	**Bo**	**means**	**Ar**	**Be**	**Bo**	**means**
Betta-DN	83.00	69.00	94.50	**82.17**f	2.90	2.93	2.79	**2.88**c	64.08	57.89	65.88	**62.62**cd	389.50	467.00	491.80	**449.40**ab	10.23	10.98	11.73	**10.98**bc
Caledon	72.25	68.25	94.50	**78.33**def	2.92	2.87	2.81	**2.87c**	65.14	62.11	70.35	**65.87**e	415.00	519.80	570.80	**501.80**c	10.50	11.75	11.63	**11.29**c
Elands	52.75	62.50	78.75	**64.67**a	2.85	2.98	2.76	**2.86**c	57.08	59.75	58.02	**61.62**c	356.50	471.00	544.20	**457.30**abc	9.55	10.40	11.20	**10.38**a
Gariep	64.50	68.75	86.00	**73.08**bcde	2.74	2.69	2.83	**2.75**ab	63.77	56.01	67.63	**62.47**cd	365.50	510.30	444.30	**440.00**a	10.65	11.45	10.25	**10.78**abc
Komati	73.25	78.00	93.00	**81.42**f	2.85	2.79	2.66	**2.76**b	67.61	65.67	72.48	**68.59**f	362.30	531.10	487.50	**460.30**abc	10.85	11.07	11.63	**11.18**c
Limpopo	72.50	71.75	93.50	**79.25**ef	2.68	2.76	2.64	**2.69**a	66.13	64.02	69.94	**66.70**ef	391.80	453.80	515.50	**453.70**ab	9.93	11.08	11.55	**10.85**abc
Matlabas	68.75	68.75	92.50	**76.67**cdef	3.13	3.13	3.08	**3.11**e	53.64	54.83	58.56	**59.01**b	414.30	552.50	500.00	**488.90**bc	10.13	10.53	11.08	**10.58**ab
PAN3118	69.00	57.00	88.50	**71.50**bc	3.15	2.90	3.14	**3.06**e	60.6	60.93	72.14	**64.64**de	382.80	525.50	506.80	**471.70**abc	10.15	11.60	11.53	**11.09**c
PAN3349	73.00	66.50	79.00	**72.83**bcd	2.99	2.83	3.00	**2.94**d	55.41	56.44	65.06	**58.97**b	456.50	667.50	564.00	**562.70**d	9.55	11.50	10.40	**10.48**ab
PAN3377	61.75	58.50	82.75	**67.67**ab	2.94	3.04	2.97	**2.98**d	54.52	49.37	65.43	**56.44**a	422.00	531.50	470.00	**474.50**abc	11.08	10.90	10.98	**10.98**bc
Env mean	**69.08** a	**66.90** a	**88.30** b	**Grand mean= 74.76**	**2.91** b	**2.89** ab	**2.87** a	**Grand mean= 2.89**	**60.82** b	**58.70** a	**59.55** ab	**Grand mean= 62.69**	**395.60** a	**523.00** b	**509.50** b	**Grand mean= 476.00**	**10.26** a	**11.13** b	**11.20** b	**Grand mean= 10.86**
LSD Env	**4.419**				**0.02604**				**1.443**				**28.29**				**0.7267**			
LSD Cult	**6.392**				**0.06750**				**2.370**				**45.96**				**0.5034**			

Means followed by the same letter, did not differ significantly at $p \leq 0.05$. VK = vitreous kernels, KD = kernel diameter, HI = hardness index, FN = falling number, Prot = flour protein content, Cult means = cultivar means for the three localities, Env mean = environmental means, LSD Env = least significant difference for environments, LSD Cult = least significant difference for cultivars, Ar = Arlington, Be = Bethlehem, Bo = Bothaville

Table 10 Means of measured milling characteristics and flour quality determined on three localities

Cultivar	BFY Ar	BFY Be	BFY Bo	Cult means	FY Ar	FY Be	FY Bo	Cult means	C76 Ar	C76 Be	C76 Bo	Cult means	WGC Ar	WGC Be	WGC Bo	Cult means	SDSS Ar	SDSS Be	SDSS Bo	Cult means
Betta-DN	23.27	24.75	24.81	**24.28**d	71.68	73.49	73.41	**72.86**bc	-0.78	-2.38	-0.98	**-1.38**abcd	32.39	35.70	35.63	**34.57**de	83.75	84.00	93.75	**83.83**d
Caledon	21.33	23.31	22.80	**22.48**b	72.73	73.93	73.36	**73.34**cd	-1.48	-2.25	0.75	**-1.50**abc	36.54	41.66	41.13	**39.78**f	81.50	79.00	78.25	**79.58**ab
Elands	23.57	22.09	23.20	**22.95**bc	71.23	71.73	72.37	**71.78**a	-0.58	-1.30	-0.43	**-0.77**e	27.99	33.12	36.32	**32.48**bcd	83.75	85.00	72.25	**83.67**d
Gariep	23.00	24.33	24.09	**23.81**cd	72.08	72.89	73.22	**72.73**b	-1.18	-0.65	-1.18	**-1.00**cde	31.92	36.72	30.63	**33.09**cd	82.00	81.50	79.75	**81.08**bc
Komati	22.92	23.62	23.37	**23.30**bc	73.05	74.56	73.14	**73.58**d	-2.00	-2.56	-0.33	**-1.63**ab	35.66	35.91	36.71	**36.09**e	79.25	78.90	78.75	**78.97**a
Limpopo	22.03	23.44	23.88	**23.12**bc	73.38	73.35	73.43	**73.39**cd	-2.05	-2.75	-0.73	**-1.84**a	30.98	63.81	35.55	**34.44**de	82.25	81.75	82.00	**82.00**cd
Matlabas	24.97	24.04	20.97	**23.33**bc	71.99	71.99	71.42	**71.80**a	-0.98	-1.05	0.13	**-0.63**e	27.61	30.55	32.04	**30.07**a	91.75	94.25	93.00	**93.00**g
PAN3118	21.80	20.61	20.04	**20.82**a	72.68	72.38	72.42	**72.49**b	-1.70	-1.18	-0.83	**-1.23**abcde	28.11	31.84	32.18	**30.71**ab	90.50	92.00	90.00	**90.83**f
PAN3349	23.28	22.05	23.24	**22.86**b	74.06	74.03	73.04	**73.71**d	-1.28	-1.48	0.23	**-0.84**de	30.09	36.34	27.93	**31.45**abc	86.75	87.00	87.50	**87.08**e
PAN3377	23.46	23.57	20.91	**22.64**b	73.70	73.83	72.97	**73.50**d	-1.28	-1.55	-0.38	**-1.07**bcde	33.00	32.46	32.09	**32.52**bcd	90.00	90.25	91.00	**90.42**f
Env mean	**22.96** a	**23.18** a	**22.73** a	**Grand mean= 22.96**	**72.66** a	**73.22** b	**72.88** ab	**Grand mean= 72.92**	**-1.33** b	**-1.71** a	**-0.52** c	**Grand mean= -1.19**	**31.43** b	**35.11** b	**34.02** ab	**Grand mean= 33.52**	**85.15** a	**85.35** a	**84.62** a	**Grand mean= 85.05**
LSD Env	**0.672**				**0.51936**				**0.3737**				**2.996**				**1.323**			
LSD Cult	**0.898**				**0.5933**				**0.6240**				**2.296**				**1.999**			

Means followed by the same letter, did not differ significantly at p ≤ 0.05. BFY = break flour yield, FY = flour yield, C76 = flour colour at 76% flour yield level, WGC = wet gluten content, SDSS = SDS sedimentation volume, Cult means = cultivar means for the three localities, Env mean = environmental means, LSD Env = least significant difference for environments, LSD Cult = least significant difference for cultivars, Ar = Arlington, Be = Bethlehem, Bo = Bothaville

Table 11 Means of measured rheological characteristics determined on three localities

		AlvP		Cult		AlvL		Cult		AlvP/L		Cult		AlvW		Cult		MDT		Cult
Cultivar	**Ar**	**Be**	**Bo**	**Means**	**Ar**	**Be**	**Bo**	**means**	**Ar**	**Be**	**Bo**	**means**	**Ar**	**Be**	**Bo**	**means**	**Ar**	**Be**	**Bo**	**means**
Betta-DN	73.25	64.25	62.50	**67.58**b	97.5	118.8	109.5	**108.6**f	0.75	0.55	0.60	**0.63**b	256.0	266.8	263.2	**262.0**ab	3.05	2.65	3.17	**2.96**b
Caledon	62.25	58.00	55.50	**58.58**a	122.8	156.8	154.8	**144.8**h	0.51	0.37	0.36	**0.41**a	243.0	261.0	265.2	**256.4**a	2.60	2.19	2.45	**2.41**a
Elands	80.50	88.75	76.75	**82.00**d	66.0	79.5	73.2	**72.9**bc	1.24	1.12	1.22	**1.19**e	235.2	282.0	190.2	**241.1**a	4.35	3.55	4.07	**3.99**e
Gariep	77.00	63.75	72.75	**71.17**bc	80.5	121.2	63.5	**88.4**de	1.05	0.53	1.25	**0.94**d	240.2	246.5	238.2	**235.8**a	3.89	3.07	4.02	**3.66**d
Komati	66.00	58.98	58.25	**61.08**a	101.2	123.5	110.2	**111.7**fg	0.68	0.50	0.53	**0.57**ab	227.0	259.5	273.2	**241.7**a	3.14	2.75	3.26	**3.05**bc
Limpopo	77.00	65.75	66.25	**69.67**bc	78.8	113.2	112.5	**101.5**ef	0.99	0.59	0.65	**0.74**bc	223.2	291.7	219.0	**253.2**a	3.40	2.91	3.43	**3.25**c
Matlabas	72.50	79.50	70.00	**74.00**c	77.2	96.8	79.0	**84.3**cd	1.01	0.84	0.89	**0.91**cd	272.5	334.8	265.0	**244.7**a	3.72	3.67	3.59	**3.66**d
PAN3118	107.50	94.75	88.25	**96.83**e	57.5	82.8	67.8	**69.3**ab	1.92	1.18	1.32	**1.47**f	272.5	334.8	265.0	**290.8**bc	4.02	4.61	4.24	**4.29**f
PAN3349	78.50	76.00	90.00	**81.50**d	86.0	106.5	74.5	**59.0**a	0.98	0.72	1.22	**0.97**d	242.0	273.5	256.5	**257.3**ab	3.00	2.82	3.66	**3.16**bc
PAN3377	64.00	65.75	78.25	**69.33**bc	138.8	129.0	102.2	**123.3**g	0.47	0.52	0.79	**0.59**ab	310.2	296.2	305.5	**304.0**c	2.79	3.19	3.1	**3.03**bc
Env mean	**75.85** b	**71.55** a	**72.12** a	**Grand mean= 73.17**	**90.6** a	**112.8** b	**94.7** a	**Grand mean= 99.40**	**0.96** b	**0.69** a	**0.88** b	**Grand mean= 0.84**	**246.8** a	**278.9** b	**250.4** a	**Grand mean= 258.7**	**3.40** b	**3.14** a	**3.50** b	**Grand mean= 3.35**
LSD Env	**2.998**				**9.27**				**0.1005**				**27.46**				**0.2157**			
LSD Cult	**5.297**				**13.17**				**0.1777**				**33.51**				**0.2395**			

Means followed by the same letter, did not differ significantly at $p \leq 0.05$. AlvP = alveograph tenacity, AlvL = alveograph extensibility, AlvP/L = alveograph configuration of the curve, AlvW = flour strength, MDT = mixograph development time, Cult means = cultivar means for the three localities, Env mean = environmental means, LSD Env = least significant difference for environments, LSD Cult = least significant difference for cultivars, Ar = Arlington, Be = Bethlehem, Bo = Bothaville

Table 12 Means of measured bread making characteristics determined on three localities

Cultivar	ABS Ar	ABS Be	ABS Bo	Cult means	FABS Ar	FABS Be	FABS Bo	Cult means	LFV Ar	LFV Be	LFV Bo	Cult means
Betta-DN	58.94	60.06	61.19	**60.06**cd	57.54	57.45	57.97	**57.65**bcd	780.00	971.30	897.50	**849.60**bc
Caledon	59.35	61.23	61.04	**60.54**d	58.24	58.76	57.97	**58.32**d	915.00	958.80	946.20	**940.00**e
Elands	57.80	59.20	60.40	**59.13**a	56.14	57.19	57.54	**56.95**ab	768.80	828.80	882.50	**826.70**ab
Gariep	59.58	60.78	58.98	**59.78**abcd	56.22	56.40	55.52	**56.05**a	845.00	923.80	805.00	**857.90**c
Komati	60.18	60.21	61.04	**60.47**d	56.49	57.10	57.19	**56.92**ab	810.00	848.10	890.00	**849.40**bc
Limpopo	58.49	60.21	60.93	**59.88**abcd	56.84	57.10	57.71	**57.22**b	812.50	902.50	832.50	**849.20**bc
Matlabas	58.79	59.39	60.21	**59.46**abc	56.75	58.15	57.01	**57.30**bc	911.20	885.00	902.50	**899.60**d
PAN3118	58.83	61.00	60.89	**60.24**cd	58.59	58.41	61.47	**59.49**e	817.50	877.50	885.00	**860.00**c
PAN3349	58.56	60.85	59.20	**59.54**ab	57.97	58.85	57.89	**58.24**cd	785.00	871.20	740.00	**798.80**a
PAN3377	60.21	59.95	60.06	**60.08**cd	59.16	57.98	60.77	**59.40**e	940.00	913.80	921.20	**925.00**de
Env mean	**59.07** a	**60.29** b	**60.39** b	**Grand mean= 59.92**	**57.42** a	**57.74** a	**58.11** a	**Grand mean= 57.76**	**838.50** a	**888.10** b	**870.20** ab	**Grand mean= 865.60**
LSD Env	**1.1118**				**0.910**				**38.03**			
LSD Cult	**0.7886**				**0.960**				**29.00**			

Means followed by the same letter, did not differ significantly at $p \leq 0.05$. ABS = mixograph water absorption, FABS = farinograph water absorption, LFV = loaf volume, Cult means = cultivar means for the three localities, Env mean = environmental means, LSD Env = least significant difference for environments, LSD Cult = least significant difference for cultivars, Ar = Arlington, Be = Bethlehem, Bo = Bothaville