

**IMPROVED SEPARATION OF POLYMERIC AND
MONOMERIC PROTEIN FRACTIONS USING A HIGH
RESOLUTION COLUMN**

by

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DECLARATION

I Keneuoe Phakela, declare that the Master's degree dissertation that I herewith submit for the **Magister Scientiae Agriculturae** degree, at the University of the Free State, is my own independent work and that I have not previously submitted it for a qualification at another institution of higher education.

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K. Phakela..... Date.....

DEDICATION

This study is dedicated to my late daughter, Reithabetse Phakela Maleke, she will forever be remembered for the short time she lived.

Death leaves a heartache which is healed by no one,
People die but real love is forever in the hearts,
Unknown

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Abbreviations

µl	Microliter
A	Alpha
B	Beta
Υ	Gamma
Ω	Omega
°C	Degree Celsius
AACC	American association of cereal chemist
alvL	Alveograph extensibility
alvP	Alveograph stability
alvP/L	Alveograph stability to extensibility ratio
alvW	Alveograph strength
BFY	Break flour yield
BU	Brabender Units
Cm	Centimetre
cm ³	Cubic centimetre
CV	Co-efficient of variation
Da	Daltons
DDT	Dough development time
FAO	Food and Agricultural Organization
FPC	Flour protein content
FY	Flour yield
G	Gram
GGA	Gliadins and albumins-globulins
GLI	Gliadins
GLU/GLI	Glutenin to gliadin ratio
g/L	grams per litre
GV	Genetic variance
GS	Glutenin subunits
HCl	Hydrochloric acid
HMW	High molecular weight
HPLC	High performance liquid chromatography
LMW	Low molecular weight

LUPP	Large unextractable polymeric proteins
LV	Loaf volume
LV12	Loaf volume expressed on 12% protein basis
M	Meter
Mg	Milligram
%mg	Percentage of monomeric gliadins
Mm	Millimetre
Min	Minutes
ml/L	Millitre per litre
MPT	Mixograph peak time
MPV	Midline peak value
N	Nitrogen
NBC	Narrow bore column
Nm	Nanometre
PC	Protein content
PCA	Principal component analysis
PC1	First principle component
POL	Total polymeric proteins
%p	Percentage absolute proteins
%fp	Percentage relative proteins
%PP	Percentage polymeric proteins
PPF	Polymeric proteins in the flour
QTL	Quantitative trait loci
R ²	Co-efficient of determination
RP-HPLC	Reverse phase-high performance liquid chromatography
Rpm	Revolutions per minute
SAGL	South African Grain Laboratory
Sec	Seconds
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SDSVOL	Sodium dodecyl sulphate sedimentation volume
SE-HPLC	Size exclusion-high performance liquid chromatography
S-S	Disulphide bond
TFA	Trifluoroacetic acid
TKW	Thousand kernel weight

UPP	Unextractable polymeric proteins
UV	Ultraviolet
VK	Vitreous kernelss
v/v	Volume per volume
v/w	Volume per weight
WGC	Wet gluten content

Summary

The main objective of this study was to test the Yarra-SEC 4000 analytical column (300 x 7.80 mm) against the BioSep-SEC s4000 narrow bore column (300 x 4.60 mm) that was used by most researchers up to now, for separation of wheat gluten proteins, using SE-HPLC. The relationship between protein fractions and a range of quality characteristics were then determined. The trials were conducted in the three production areas of South Africa representing the dryland summer rainfall, dryland winter rainfall and irrigated regions. These regions each have specific cultivars developed for their specific conditions. The Yarra-SEC 4000 analytical column yielded nine profile peaks compared to the five of the BioSep-SEC s4000 narrow bore column. Highly significant differences were found between the protein fractions for the irrigated region and the winter rainfall cultivars. Very few significant differences were seen for protein fractions between cultivars in the dryland summer rainfall region. The GLI%p correlated positively with dough rheological characteristics in cultivars of the irrigated region. The UPP positively correlated with dough rheological parameters for cultivars of the irrigated region. No significant correlations were observed between UPP and dough rheological characteristics in the dryland winter rainfall cultivars. The irrigated region cultivars presented more significant correlations between protein fractions and quality characteristics. Significant correlations were obtained between SDSVOL and protein fractions for the winter rainfall region cultivars. No significant correlations were obtained between SDSVOL and protein fractions for the irrigated and dryland summer rainfall cultivars. In this study it was observed that Yarra-SEC 4000 analytical column can be employed successfully in the separation of wheat proteins.

Key words: BioSep-SEC s4000 narrow bore column; Quality characteristics; SE-HPLC; Yarra-SEC 4000 analytical column.

Chapter 1

General Introduction

Wheat (*Triticum aestivum L*) is one of the most important cereal crop in the world. It is consumed by nearly half of the world population (40%) (Gupta et al. 2008; FAOSTAT 2014). Wheat is a good source of proteins and energy for humans and can also be used to feed livestock (Rakszegi et al. 2005; Gupta et al. 2008). Other uses of wheat are starch and fermented drinks (Pomeranz 1988). Proteins found in mature wheat grains make up about 10-12% of the total grain. Wheat storage proteins make up about 80-90% of the total proteins (Shewry and Halford 2002; Branlard et al. 2001; Veraverbeke and Delcour 2002). The wheat storage proteins are termed gluten. Gluten is made up of two main groups, glutenins and gliadins (Branlard et al. 2001; Gianibelli et al. 2001). These two groups have unique properties and unusual structures, which allows wheat flour to be processed into a wide range of products such as bread, noodles, cake and biscuits (Gilbert et al. 2000; Rakszegi et al. 2005). Gluten gives dough its visco-elastic properties.

The gliadins are monomeric proteins while glutenins are polymeric proteins (Gianibelli et al. 2001). Glutenins are further grouped into high molecular weight (HMW) and low molecular weight (LMW) subunits. The HMW glutenin subunits (GS) have been reported to be a great source of variation (45%-70%) in bread-making performance despite forming the minor group of flour proteins (Branlard and Dardevet 1985; Payne 1987; Shewry et al. 2001). The grain protein content (PC) is a vital quality parameter of wheat. The balance between protein components and other flour components such as starch, lipids, pentosans and water are important in bread-making. The PC is largely affected by environment while the quality of proteins is affected by the genotype and environment (Panozzo and Eagles 2000; DuPont and Altenbach 2003). Gliadins have been reported to be more affected by environment while glutenins have been reported to be non-responsive to the environment (Panozzo and Eagles 2000). According to Park et al. (2006) high protein flour has high amounts of specific protein fractions compared to flours with low PC.

Bread wheat quality is a result of genetic and environment interaction (Tlanu et al. 1996) and PC as well have a large influence on bread-making (Weegels et al. 1996). The

functional end-use quality of wheat is largely influenced by variations in molecular size distribution of proteins (Singh et al. 1990; Larroque and Békés 2000; Labuschagne et al. 2004; Ohm et al. 2009; Tsilo et al. 2010).

Various methods have been employed to separate wheat proteins and determine their contribution to wheat quality. These include reverse phase-high performance liquid chromatography (RP-HPLC) (Burnouf and Bietz 1984; Marchylo et al. 1992; Gao et al. 2010), asymmetrical flow field flow fractionation, multi angle laser light scattering (Lemelin et al. 2005), matrix assisted laser desorption/ionization time off flight and size exclusion-high performance liquid chromatography (SE-HPLC) (Dachkevitch and Autran 1989; Singh et al. 1990; Ciaffi et al. 1996; Morel et al. 2000; Schober et al. 2006; Ohm et al. 2009).

SE-HPLC has been extensively used to separate proteins and determine their correlation with bread-making characteristics (Dachkevitch and Autran 1989; Gupta et al. 1993; Ciaffi et al. 1996; Labuschagne et al. 2004; Edwards et al. 2007). The technique provides information on molecular size distribution without causing changes in the chemical structure of proteins (Bietz 1986). The technique is powerful, reproducible and has good resolution (Bietz 1986), however, it is time consuming (Larroque and Békés 2000). Effective identification of protein subunits with large effects on bread-making quality can lead to improved wheat quality (Primard et al., 1991). The ability to improve wheat quality through better knowledge and understanding of its association with chemical composition still remains a challenge (Békés et al. 2006).

This research was carried out to separate polymeric and monomeric proteins using SE-HPLC and evaluate their relationship with baking quality characteristics. Specific objectives were:

- to characterise HMW-GS in South African bread wheat cultivars and assess their influence on wheat quality
- to separate wheat proteins by SE-HPLC using a narrow bore BioSep-SEC s4000 column
- to assess the potential of the Yarra-SEC 4000 analytical column for improved wheat protein separation

- to evaluate the interdependence of wheat quality parameters with molecular size distribution
- to establish the major difference in the BioSep-SEC s4000 and Yarra-SEC 4000 analytical column in quantifying protein fractions in bread wheat

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Chapter 2

Literature review

2.1 Storage proteins

2.1.1 Composition of wheat proteins

Wheat grains contain low amounts of protein ranging between 10-20% of dry weight compared to legumes with an average of 20-40% protein (Branlard et al 2001; Veraverbeke and Delcour 2002). However, in terms of world production wheat provides large amounts of proteins; 200 million tonnes (mt), which is three times more than the amount of proteins produced by legumes used for food (Shewry and Halford 2002). Wheat proteins are of great importance and confer unique properties of elasticity and extensibility, which primarily lies in the seed storage proteins of the endosperm. These unique properties play a major role in food processing and result in end-use products, which are a good source of energy (Veraverbeke and Delcour 2002; DuPont and Altenbach 2003).

Wheat endosperm proteins are primarily made up of gluten and form 80-85% of the total wheat grain proteins (Branlard et al. 2001; Veraverbeke and Delcour 2002; Song and Zheng 2007; Wieser, 2007). Gluten influences the unique visco-elastic properties of dough. It contributes towards elasticity, extensibility and water absorption capacity which influence flour quality (Branlard et al. 2001; Rasheed et al. 2014). Gliadins and glutenin together make up gluten, which determines the unique bread-making qualities. The molecular structures and interaction of proteins confer visco-elastic properties to dough (Wieser et al. 2006; Song and Zheng 2007; Rasheed et al. 2014).

First studies on separation of wheat proteins were reported by Beccarii in 1728 using a water washing technique. They gave the name “gluten” to the water insoluble fractions of grain. The alcohol extraction of proteins was first demonstrated by Einbof as stated by Osborne in 1907 (Wrigley et al. 2006). Since then, Osborne performed more extensive studies on the separation of wheat proteins. Wheat proteins were distinguished based on their solubility in various solvents. Four major groups of wheat proteins were established: albumins soluble in water, globulins in dilute saline solutions, and prolamins soluble in alcohol based mixtures and glutenins soluble in basic solutions (Owusu-Apentene 2005).

2.1.2 Polymeric proteins (glutenin)

Glutenins are formed from a polymorphic mixture of polymers bound by disulphide (S-S) bonds. Glutenin is responsible for dough strength and elasticity that traps gases during fermentation (Gianibelli et al. 2001; Wieser et al. 2006). Glutenin can be divided into two types: HMW- and LMW-GS based on their fractionation by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Gianibelli et al. 2001; Wieser 2007). Glutenin can further be divided into four subgroups based on their sizes (A-, B-, C- and D-) after S-S bond reduction and according to electrophoretic mobility on a SDS-PAGE gel. The A group consists of HMW-GS with molecular weight between 80 000-120 000 Dalton (Da). The groups B, C and D are LMW-GS, where groups B and C have molecular weights of 42 000-51 000 Da and 30 000-40 000 Da respectively. Group D, is closely related to ω -gliadins (Payne et al. 1984; Thompson et al. 1994; Ciaffi et al. 1999; Gianibelli et al. 2001).

The HMW-GS are encoded by genes situated on the long arms of the group 1 chromosomes of the A, B and D genome. These genes occur at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci (Jones et al. 1982; Payne et al. 1984). Each locus consists of two genes which are linked to HMW-GS x and y types. The x type corresponds with the larger subunits while the y type corresponds with the smaller subunits (Gupta and MacRitchie 1994; Wrigley et al. 2006; Gao et al. 2010). The LMW-GS are encoded by genes situated on the short arms of the group 1 chromosomes. These genes are positioned at *Glu-A3*, *Glu-B3* and *Glu-D3* loci (Payne et al. 1984; Brown et al. 1979, Gianibelli et al. 2001). Three to five HMW-GS subunits are expressed in common wheat cultivars. The hexaploid wheat contains a minimum of 1Bx, 1Dx and 1Dy subunits. The HMW-GS form a smaller quantity compared to other glutenin components, but they play a unique role in determining the elasticity (strength) of dough (Gianibelli et al. 2001).

Dough strength is associated with HMW-GS and the molecular weight distribution of polymeric proteins. Absence of certain HMW-GS results in weaker dough. Alterations in LMW-GS and gliadins also affect dough extensibility (Uthayakumaran et al. 2002). The *Glu-D1* subunits 5+10 was reported to have a larger influence on dough strength than *Glu-D1* subunits 2+12 (Gupta et al. 1996). Dong et al. (1992) reported significant correlations of subunits 5+10 with most quality attributes.

Some quality parameters have been associated with certain HMW-GS. *Glu-B1* and *Glu-D1* affect mixograph parameters and loaf volume (LV) and *Glu-D1* have a more noticeable effect on the midline peak value (MPV) (Branlard et al. 2001). Payne et al. (1987) reported cultivars with 1 and 2* had higher SDS-VOL.

The amino acid sequence of HMW-GS comprises of three clear domains, unrepetitive domains at the N terminus, a central domain that is repetitive and C terminal domain. Much variation is found in the repetitive domain, which is responsible for variation in the size of the whole protein. The differences in the functionality of glutenins are attributed to differences in structural domains. The N terminal and central domain are distinguished by the presence of most cysteins with 80-105 and 480-700 residues for the N terminal and central terminal respectively. Minor residues occur at the C terminal (42 residues). Variation in the N terminal can be attributed to substitutions, and deletions/insertions. Variation in the repetitive domain is based on three motifs, hexapeptides and nonapeptide present both in x and y type subunits and tripeptides. Structural predictions reveal an α -helix in the C terminal and repeated β -turns in the central domains (Bushuk and Rasper 1994; Wieser 2007), which contains high levels of proline associated with gluten elasticity (Tatham and Shewry 1995).

2.1.3 Monomeric proteins (gliadins)

Gliadins are heterogeneous in nature. They are single chained polypeptides, extractable in 70% alcohol (Gianibelli et al. 2001). Gliadins can be fractionated into four classes: alpha (α), beta (β), gamma (γ) and omega (ω) at low pH with SDS-PAGE (Gianibelli et al. 2001; Wrigley et al. 2006). Wieser (2007) proposed a new classification of gliadins; ω 5-, ω 1-, 2-, α/β - and γ -gliadins. The difference between γ -gliadins and α - and β -gliadins lies in aspartic acid, proline, methionine, trypsin, phenylalanine and tryptophan quantities. The ω gliadins are deficient in cysteine and contain amino acids absent in other gliadins. The ω -gliadins are distinguished by greater amounts of glutamate, proline and phenylalanine (Tatham and Shewry 1995; Gianibelli et al. 2001; Wrigley et al. 2006), which contribute about 80% of total amino acid residue (Wieser, 2007).

Gliadin molecular weights vary between 30 000-70 000 Da (Woychik et al. 1961; Gianibelli et al. 2001). Gliadins are controlled by genes on the short arms of group 1 and 6 chromosomes. The genes on the group 1 chromosomes occur at *Gli-A1*, *Gli-B1* and

Gli-D1 loci and on group 6 chromosomes at *Gli-A2*, *Gli-B2* and *Gli-D2* (Jones et al. 1982; Brown et al. 1979; Wrigley et al. 2006). Various studies revealed that the minority of β gliadins, the majority of γ -gliadins and all of the ω -gliadins are controlled by group 1 chromosomes while the minority of γ -gliadins, majority of β gliadins and all of the α gliadins are controlled by group 6 chromosomes (Payne et al. 1984). Gliadins constitute the majority of glutenin proteins (Gianibelli et al. 2001). Gliadins polypeptides exist in blocks and this makes it difficult to establish the contribution of individual gliadins. It also has similar molecular weight than many LMW-GS subunits (Gianibelli et al. 2001; Wrigley et al. 2006).

The amino acid sequences of gliadins are made up of three structural domains; the central domain flanked by the N terminal and C terminal region. The α/β - and γ - gliadins have high amounts of sulphur. The α/β gliadins have six cysteine remains, while the γ -gliadins have eight cysteine remains linked by intramolecular S-S bonds. The α/β and γ -gliadins are classified by low amounts of glutamine and proline compared to ω -gliadins. The α/β repetitive units are based on pentapeptides. The C terminal, which is unrepetitive, contains lower amounts of proline and glutamine than the N terminal and cysteine residues are conserved (Shewry and Tatham 1990; Wieser 2007). Gliadins have three different structures: α/β with a globular structure, γ - gliadins with extended structures and ω -gliadins with rod like structures (Bushuk and Rasper 1994). Recent studies on secondary structure of gliadins revealed β -turns and α -helixes. The C terminal contains considerable amounts of α -helixes and β sheets (Shewry and Tatham 1990; Gianibelli et al. 2001; Wieser 2007). Gupta et al. (1992) in studying the relationships between protein composition and functional properties of wheat found that wheat flour increase in gliadin concentration with an increase in PC. The ω -gliadins increased with nitrogen applications, while γ -gliadins decreased (Wieser and Seilmeier 1998). Gliadins have been reported to reduce mixing time, dough strength and lower peak resistance (Fido et al. 1997; Uthayakumaran et al. 2002).

2.1.4 Albumins-globulins

Albumins and globulins are referred to as non-gluten proteins, which make up about 10-20% of total wheat flour proteins (Singh et al. 2001; Singh and MacRitchie 2001). They are found in the aleurone layer and embryo. In terms of solubility, albumins are soluble in water while globulins are soluble in salt (Horvat et al. 2015). Albumins and

globulins have not been reported to have a great influence on flour quality (Schofield and Booth 1983; Gao et al. 2009). They have, however, been reported to have nutritional value because they are high in required amino acids such as lysine and methionine. The major parts of these proteins are enzymes involved in metabolic activities (Singh et al. 2001; Tomic et al. 2016). The HMW albumins-globulins have a storage function. Trypsin/alpha amylase serpins and methionine are the major components and they have an inhibitory effect on insects and fungi on seed during germination. In addition, they act as food reserves for the embryo during germination (Morris 2002; DuPont and Altenbach 2003).

2.2 Grain characteristics

2.2.1 Vitreous kernels

Vitreousness is a quality characteristic related to PC and hardness of kernels (Eliasson and Larsson 1993; Symons et al. 2003). Vitreous kernels are glassy, high in PC and more compact. Non-vitreous kernels are dull or floury and are considerably finer textured (Gaines 1985; Bass 1988; Dowell 2000; Dowell et al. 2006). Vitreousness is one of the vital quality parameters used in the grading of red wheat (Dexter and Matsuo 1981; Dowell et al. 2006). Vitreousness is a heritable trait, although it is also affected by the environment. Pomeranz and Williams (1990) reported high temperature and nitrogen to contribute to vitreousness. Appropriate agronomic practices are essential, as they influence PC, which is associated with vitreousness. Environment has been reported to have more pronounced effects on vitreousness than genetic factors (Greffeuille et al. 2006).

2.2.2 Kernel hardness

Kernel hardness is a physical characteristic associated with texture of the endosperm (Bettge et al. 1995), and is often measured by determining the resistance of the grain to break when a force is applied to the kernel (Yamazaki and Donelson 1983). The kernel hardness is used to distinguish between wheat cultivars and plays an important role in the flour industry as it affects the milling, baking and quality of wheat. The physical properties of wheat such as flour yield, flour density and starch damage, and water absorption are affected by the texture of the endosperm. Hard wheats require more energy to crush and break into large particles with immense starch damage. Damaged starch has

high water absorption capacity. In contrast, soft wheat is easy to crush and produces flour with a fine texture with less starch damage (Yamazaki and Donelson 1983; Bass 1988; Bettge et al. 1995).

Kernel hardness is controlled by one or two major genes located on chromosome 5DS. The genes associated with kernel hardness are closely linked to the Ha locus coding for puriondoline proteins (Yamazaki and Donelson 1983; Pasha et al. 2010). The Ha locus contains genes the puriondoline a (Pin a) and puriondoline b (Pin b). Hard wheat has deletions or mutations present in either Pin a or Pin b while soft wheat has the wild alleles with both puriondolines present (Mattern 1988; Lesage et al. 2012; Salmanowicz et al. 2012).

Positive correlation between starch damage and grain hardness was reported and depended on genotype. Environment did not have any significant effects on grain hardness (Kwasniewska-Karolak et al. 2011; Surma et al. 2012). Bergman et al. (1998) reported genetic correlation between kernel hardness and flour yield that may be due to close gene association of protein and softness genes. Marshall et al. (1986) and Ohm (1998) also reported positive correlation between kernel hardness and flour yield.

2.2.3 Thousand kernel weight

TKW determines the weight of a thousand counted kernels at 12% moisture content (Blackman and Payne 1987; Posner and Hibbs 1997), and is a result of kernel size and density (Koppel and Ingver 2008). Other factors such as climate, infections and shape contribute to TKW (Dziki and Laskowki 2005). Harvesting time also has an impact on TKW (Farrer et al. 2006), as kernels harvested late were reported to have lower TKW and kernels with lower density are inclined to have lower test weight (Czarnecki and Evans 1986). Seeds showing high TKW usually have high flour extraction and good packing efficiency (Dexter and Matsuo 1981). Broken and chipped kernels are excluded when determining TKW (SAGL 2010).

Shefazadeh et al. (2012) reported a strong significant correlation between TKW and grain yield. They further stated that TKW and the number of spikes per unit area are a good indicator of heat and drought tolerance because these traits could identify tolerant genotypes. Chinnusamy and Khanna-Chopra (2003) reported significant correlation

between grain weight and yield. Negative correlations between PC and TKW have been reported by Pomeranz et al. (1985) and Dowell et al. (2008).

2.2.4 Hectolitre mass

Hectolitre mass or test weight is the weight per given volume of wheat, expressed as kilogram per hectolitre. It is used for grading of wheat and provides a measure of density and soundness of wheat (Fowler and de la Roche 1975; Czarnecki and Evans 1986; SAGL 2010). Kernels should be found without any form of damage (Berman et al. 1996). Hectolitre mass has a large influence on the transportation costs of wheat and is affected by various factors such as agronomic practices, weather and climate, fungal disease, insect damage, and kernel shape (Dziki and Laskowski 2005). Test weight can also be influenced by season and kernel hardness. Sound and plump kernels have higher test weight and high flour yield. Lower test weights are associated with lower density and lower kernel mass (Czarnecki and Evans 1986). Marshall et al. (1986) found significant positive correlation between test weight and milling yield, however weaker correlations were reported for varieties at a particular site. Troccoli and di Fonzo (1999) reported positive correlation between kernel shape and test weight.

2.3 Milling characteristics

2.3.1 Break flour yield

Break flour yield (BFY) is obtained when wheat grain is passed through a series of roller mills and sieves (Prabhasankar et al. 2000). The roller mills begin by opening up the wheat grain. The endosperm and germ are then separated from the bran (Campbell and Webb 2001, Fang and Campbell 2003). Break flour yield is recorded as a percentage of the total wheat flour (Bass 1988). Break flour is associated with kernel texture. Soft textured kernels produce greater BFY (Gaines 1985; Wang 2010; Pasha et al. 2010). PC negatively correlated with BFY (Gaines 1991). Van Lill and Smith (1997) found wheat grains with high PC to be harder and to produce less BFY.

2.3.2 Flour extraction rate/flour yield

Flour yield can be defined as the quantity of flour that can be derived from a certain amount of wheat and is expressed as percentage. (Kent and Evers 1994; Bass 1988). The quantity of flour extracted is of considerable importance to markets, millers and consumers. Consumers' main priority is flour of a white colour, which does not contain

non-endosperm materials. Flour yield relies on the efficiency of the reduction stage during milling (Shuey et al. 1977). Genetic and environmental factors also affect flour yield (Marshall et al. 1984). Parker et al. (1998) found three quantitative trait loci (QTL) on chromosomes 3A, 5A and 7A to have a large influence on flour yield. Ohm et al. (1998) reported significant correlations between flour yield, kernel hardness, kernel density and hectolitre mass.

2.4 Rheological characteristics

2.4.1 Mixograph characteristics

The mixograph is widely used for testing the mixing properties of dough, which are used to determine end-use quality (Wikström and Bohlin 1996). The mixograph has also been used to study the influence of additives and flour constituents on dough behaviour during the bread-making process. The technique requires small flour samples and is less time consuming. However, it cannot be used to measure water absorption due to its principle to use some water (Shogren and Finney 1984; Wikström and Bohlin 1996).

The mixograph is a mixer with four moving pins that stretch the dough between fixed pins and the resistance is recorded as a curve, the mixogram. Mixogram properties are dependent on plasticity, elasticity and visco-elasticity of dough mixing. The mixogram provides information on the following parameters: peak time (dough development time, DDT), ascending slope (dough development) and the descending slope (tolerance to over mixing and stability) (Walker and Hazelton 1996).

Neacșu et al. (2009) reported five parameters suitable for use in breeding programmes and predicting end-use quality. They are peak time (mixing requirement), peak height (dough strength), end width (extensibility) and breakdown (stability). These characteristics accounted for 91% of variation in LV. They further stated that other parameters are a function of more than one mixing property. Initial slope is affected by mixing requirements and dough strength. The end width measures dough extensibility and stability. The areas below and within the curve indicate mixing properties. Wikström and Bohlin (1996) reported that the following five parameters could be used to predict LV: build up (initial build up to maximum height of the top of the curve), peak time and initial width, area below the mixogram curve and peak height and proteins. Peak time is associated with build-up and water absorption. These parameters contributed 92.8% of

variation in loaf volume while build up alone accounted for 77% of variation in bread volume. The mixograph mixing properties are used in the industry to assess the bread-making quality (Peña 2000; Neacșu et al. 2009). In South Africa, peak time is one of the important mixograph parameter used by the industry (Miles et al. 2013).

Dong et al. (1992) found a significant positive relationship between mixing time and mixing tolerance. Protein concentration was highly correlated with LV and water absorption was significantly correlated with LV, mixing time and mixing tolerance. The *Glu-1* score was significantly correlated with LV and mixing time. Cultivars with *Glu-D1* subunits 3+12 and 2+12 had significantly shorter mixing times than cultivars with subunits 5+10. Subunits 5+10 had the most consistently positive influence on quality characteristics.

The mixograph parameters; peak height, ascending angle and total area under the curve revealed highly significant positive correlations with flour protein content (FPC). Peak time showed positive correlation with descending angle, the width parameters as well as area parameters (Miles et al. 2013). Peak height was correlated with grain hardness. A strong positive correlation was also observed between midline parameters and top envelope parameters. Protein content was negatively correlated with peak time (Martinant et al. 1998). Mixograph bandwidth at 6 min correlated with mixing tolerance (Chung et al. 2001). Wikström and Bohlin (1996) reported midline peak height, midline peak width, midline time X height, and midline time X width, to be related to grain hardness. Peña (2000) reported significant high correlations between mixograph peak height and LV. Flours with weak gluten are characterised by less mixing time and short peak time compared to flours with strong gluten (Figure 2.1)

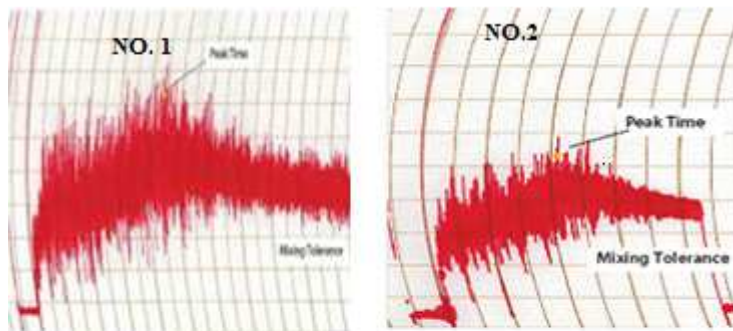


Figure 2.1. Mixograph for strong gluten flour, NO.1 and for weak gluten flour, NO. 2. (Wheat and flour testing methods, 2008)

2.4.2 Farinograph

The farinograph is a dough mixing instrument that measures and records resistance of dough over time, developed by the mixing action of blades. The resulting curve is called a farinogram. The farinograph allows measurement of parameters such as water absorption, dough stability, DDT and the weakening point. Measurements are done on dough that has not been fermented (Walker and Hazelton 1996; Koppel and Ingver 2010).

Water absorption is an important parameter as it shows the potential of the protein network to absorb water and is directly associated with the finished products. Water absorption shows the amount of water needed for dough to reach a desired consistency (500 Brabender units (BU) line), at the peak of the curve or point of optimum dough development. The curve height is influenced by PC and genotypes with high PC have increased water absorption. Hard wheats were observed to have high PC (Finney and Shogren 1972; Van Lill and Smith 1997; Constantin et al. 2011). Farinograph water absorption is also used in evaluating dough strength. Starch and gluten also affect water absorption. In South Africa water absorption is important in the release of the cultivars. Water absorption values between 62-64% are acceptable (SAGL 2010).

Dough development time is a point at which dough has reached maximum consistency and is able to retain gas, and is often referred to as peak time (Atwell 2001). Dough stability indicates tolerance of dough to mechanical mixing. Stability relates to the time during which dough reaches maximum consistency, that is the time difference in minutes during which the top of the curve is above the 500 BU line (arrival time) and the curve leaves the 500 BU line (departure time) (Atwell 2001; Dowell et al. 2008). A peak of 2.5-3.5 min is acceptable for South African wheat.

The ω -gliadins and LMW albumins and globulins were reported to have positive correlations with water absorption and softening of dough (Farooq et al. 2014). Significant correlations were reported between HMW-GS and rheological characteristics. Subunits 5+10, 13+16 and 7+9 were reported to have the largest effect on rheological characteristics including farinograph. (Randall et al. 1993). The presence of subunits 2 and 9 shortened dough mixing time, but increased farinograph absorption (Khan et al. 1989). Significant positive correlations were reported between HMW-GS and farinograph water absorption (Wentzel 2010). Farinograph water absorption

increases as PC increases (Finney et al. 1987; Fowler and Kovacs 2004). Generally strong gluten flour has higher water absorption, longer mixing and more tolerant than weak gluten flour (Figure 2.2) (Wheat and flour testing methods 2008)

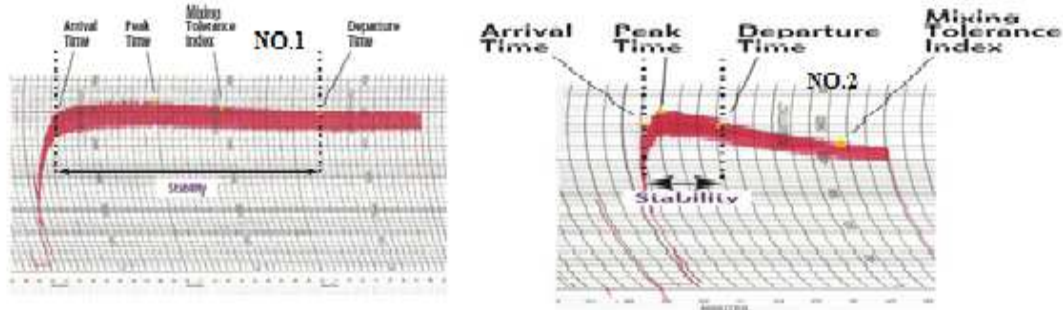


Figure 2.2 Farinograph for strong gluten, NO.1 and for weak gluten flour, NO.2 (Wheat quality and carbohydrate research, 2015)

2.4.3 Alveograph

The instrument blows a thin sheet of dough into a bubble until it bursts. The dough sample is prepared by mixing flour with a standardised salt solution. The resulting pressure is recorded until the dough bursts. The graph obtained is called an alveogram (Figure 2.3). From the graph, information on parameters such as dough extensibility (L-value), dough stability and tenacity (P-value), dough strength (W-value) and the relation between P and L, expressed as a ratio (P/L-value), can be obtained. The L-value measures the distance from the beginning of the curve to a point where the dough bubble bursts. This value represents the ability of dough to rise. The W-value, which is the area under the curve, is associated with energy required to form dough. The W-values range from 45 for very soft to 400 for hard red wheat. The P-value is related to resistance of dough to deformation. A high P-value is related to high gluten strength and high water absorption (Khattak et al. 1974; Walker and Hazelton 1996; Atwell 2001; Rees et al. 2007).

Certain HMW-GS have been reported to have a large effect on alveograph parameters. Subunit 1 correlated significantly with the L-value, subunit 2 correlated with P-value and P/L- value and subunit pair 5+10 correlated with P- and W-value (Blackman and Payne 1987; Hou et al. 1996). Flours with low α -amylase activity have high resistance and the L-value is low. Falling number significantly correlated with L- and P/L-value. Gluten deformation index significantly correlated with L-, P- and P/L-value. The rate of gluten deformation is related to extension that occurs during fermentation (Codina et al. 2011).

Codina and Paslaru (2011) reported that additional increase of vital gluten results in increase in P-value and decrease in extensibility index. The balance between dough viscosity and strength result into bread of a good quality flours with strong gluten are have P-value, whereas those with weak gluten have low P-value (Figure 2.3)

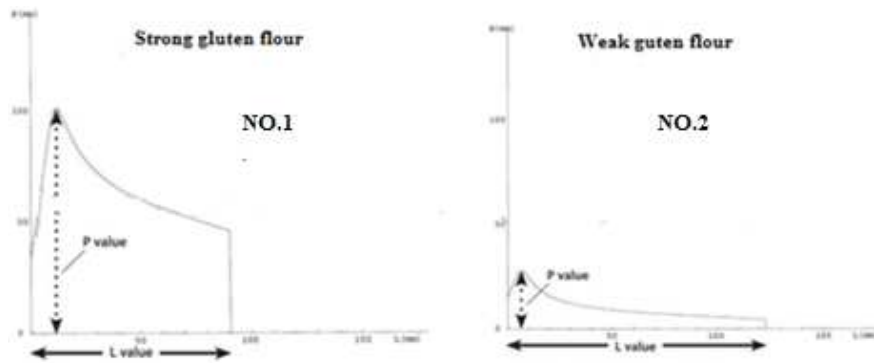


Figure 2.3 Alveograph for strong gluten flour, NO.1 and for weak gluten flour, NO.2 (Wheat flour testing methods, 2008)

2.5 Baking related characteristics

2.5.1 Wet gluten content

Wet gluten content (WGC) is obtained after treating a flour sample with sodium chloride solution. Starch and other soluble components are removed (Neufeld and Walker 1990). Wet gluten is a rubbery material made of gliadins and glutenins (Baslar and Ertugay 2011). It is used as a primary quality flour test in most countries due to its simplicity (Atwell 2001). Differences in flour quality have been associated with gluten quantity and quality. Significant correlations were reported between WGC and PC (Simic 2006; Surma et al. 2012). Ponte and Ingelin (1997) pointed out that wheat exhibiting strong gluten is resistant to over mixing and extensibility.

2.5.2 Loaf volume

Loaf volume is the final test used to determine bread-making quality. LV indicates the ability of dough to retain gas during fermentation (Shogren and Finney 1984). Water absorption and mixing time affect LV. In order to obtain good LV, mixing time and baking absorption must be optimised. LV increases with prolonged mixing time and high water absorption (Roels et al. 1993). Hard wheats are suitable for good bread-making because of their high water absorption potential, this result into bread with increased

volume, (Blacman and Payne 1987). Bakers prefer flour which produces bread with large LV (Rozylo and Laskowski 2011).

A linear association between PC and LV exists (Koppel and Ingver 2010) and several studies confirm this significant correlation (Simmonds 1989; Dong et al. 1992; Roels et al. 1993). In a study by Rozylo and Laskowski (2011) they reported sedimentation index, falling number and dough strength as the best predictors of LV. Gluten proteins influences bread-making quality and variation in gluten proteins resulted into most of the differences in LV (Khatkar et al. 1996). Millar (2003) indicated that PC and the ratio of HMW- to LMW-glutenin affected LV. Positive correlation between gliadins and LV were reported by Dong et al. (1992) and a considerable amount of variation in LV could be attributed to gliadins.

2.5.3 Sodium dodecyl sulphate sedimentation volume

Sodium dodecyl sulphate sedimentation volume (SDSVOL) includes the acidification of the flour water suspension with lactic acid containing SDS, a detergent. The lactic acid causes the flour/water suspension to sink in the form of sediment; the levels indicate the gluten strength (Moonen et al. 1982; Krattiger and Law 1991; Eckert et al. 1993).

Moonen et al. (1982) as well as Huang and Khan (1997) reported SDSVOL as a good technique in determining dough strength and bread-making performance of wheat flours. Dexter and Matsuo (1977) reported SDSVOL to be adequate for comparing durum wheat cultivars. Gupta and Shepherd (1990) reported SDSVOL as a good technique in characterising endosperm proteins. Dhaka et al. (2012) reported significant correlations between SDSVOL and PC. SDSVOL is widely accepted as a good tool in determining the difference between PC and gluten quality (Axford et al. 1979; Carter et al. 1999). Preston et al. (1982) reported a high correlation co-efficient between SDSVOL and LV. Varieties with PC less than 13% have shown high correlations between SDSVOL and farinograph DDT and extensigraph area (Preston et al. 1982).

SDSVOL has the inability to differentiate between strong and medium quality wheat with PC higher than 13% (Ayoub et al. 1993; Carter et al. 1999). Higher SDSVOL are associated with strong gluten strength while low SDSVOL is associated with weaker gluten (Carter et al. 1999; Eckert et al. 1993).

2.6. Separation techniques

2.6.1 Size exclusion-high performance liquid chromatography

SE-HPLC is a valuable technique widely used for protein analysis. Proteins are separated on the basis of their molecular weight distribution. SE-HPLC separates and evaluates various characteristics related to molecular size distribution of proteins. Proteins in their native and reduced state can be analysed using this technique. SE-HPLC can also be used to characterise and compare isolated protein fractions. Other biochemical analyses of proteins are based on the reduction of S-S bonds, which results in loss of information regarding structure, interaction and stability of protein complexes. SE-HPLC has the ability to keep large aggregates in their original state. The technique is sensitive, reproducible and easily automated (Bietz and Kruger 1994). The major drawback of the technique is poor resolution after hundreds of injections, although columns and procedures are being improved all the time. Ohm et al. (2009) described SE-HPLC as a complex procedure requiring a long time.

SE-HPLC can also be used to determine flour characteristics such as percentage of unextractable polymeric proteins (%UPP), percentage of polymeric proteins (%PP), percentage of monomeric gliadins (%MG), polymeric protein in the flour (PPF), and gluten to gliadin ratio (GLU/GLI). These parameters can be used as markers for predicting bread quality. Studies reported significant positive correlations between %UPP and end-use quality (Kuktaite et al. 2004). Percentage SDS extractable protein positively correlated with dough extensibility (Zhang et al. 2008). Extraction of proteins was reported to be variable, being proteins from strong wheat flours proteins are less extractable than those from weak flours. Singh et al. (1990) introduced extraction of proteins using 2% SDS at pH 6.9 by sonication. After sonication, extractability of proteins increased up to 100% within 30 sec, and also, small wheat flour samples are required (Singh et al. 1990).

Earlier, the extraction procedures yielded unstable protein extracts which resulted in reduction in percentage of excluded peaks during the first hour (Autran 1994). This could be associated with the reducing effect of SDS resulting in slow disruption of non-covalently bond large aggregates until more stable S-S bonds are obtained. Extraction of proteins at higher temperatures overcame the problem of instability, and resulted in more

stable elution curves (Dachkevitch and Autran 1989). The use of 50% acetonitrile lead to better resolution of proteins (Batey et al. 1991).

SE-HPLC correctly distinguishes the three major groups of wheat proteins namely, glutenins, gliadins and albumins-globulins. The technique has been used for evaluation of baking quality. Ahmad et al. (2001) found significant positive associations between monomeric glutenin (insoluble fractions) and gliadin (soluble fractions). Polymeric glutenin (insoluble fractions) was positively correlated with mixograph tail width, area under the mixograph curve and extensibility. The ratio of insoluble to soluble polymeric glutenin was positively correlated with all quality attributes and highly positively correlated with mixograph peak development time and area under the mixograph curve. Extensibility was significantly positively correlated with monomeric glutenin. Labuschagne and Aucamp (2004) reported significant positive correlation between SDS soluble gliadins and quality, especially LV irrespective of the environment. However Labuschagne et al. (2004) observed significant negative correlation between gliadins and bread-making quality, which indicated that both genotype and environment influence these relationships. The sonicated large polymeric proteins significantly positively affected grain protein, flour protein and LV across the localities, however sonicated small monomeric proteins negatively associated with grain and flour protein (Labuschagne et al. 2004).

Across localities both, non-sonicated and sonicated fractions, highly correlated with SDSVOL, vitreous kernels and FPC and mixograph development time. Gliadins significantly negatively correlated with quality attributes (Labuschagne et al. 2004). Tsilo et al. (2010) found positive correlations between HMW polymeric unextractable proteins and dough mixing, strength and LV. The ratio of HMW unextractable to extractable polymeric proteins correlated with dough mixing properties. The HMW polymeric protein fraction significantly and positively correlated with kernel hardness, mixograph water absorption and tolerance (Ohm et al. 2009). Percentage insoluble polymeric proteins positively correlated with mixing time, and percentage soluble polymeric proteins negatively correlated with mixing time (Park et al. 2006).

2.6.2 Reversed phase-high performance liquid chromatography

RP-HPLC is a useful analytical technique used for separation and characterisation of proteins. The technique involves fractionation of proteins on the basis of their hydrophobicity. Proteins with high hydrophobicity elute faster than proteins with lower hydrophobicity (Bietz 1986; Marchylo and Kruger 1988). The technique offers a wide range of advantages, such as automation, high-resolution power, reproducibility, quantification and computerisation of different protein subunits. It also complements other chromatographic and electrophoretic methods as it distinguishes samples incorrectly identified using other techniques (Bietz 1986; Cinco-Moroyoqui and MacRitchie 2008), however, it cannot be used to differentiate between subunits with similar hydrophobicity (Gao et al. 2010). The cost of running samples and the apparatus is very high. The technique was first used to identify proteins associated with end-use quality (Dong et al. 2009) and RP-HPLC is a useful tool to identify variety (Marchylo and Kruger 1988).

RP-HPLC was not an ideal technique for the separation of proteins until the introduction of wide pore end capped spherical silica supports in the 1980's. Superior resolution, improved stability and reproducibility were obtained from wide bore columns (Bietz 1986; Wieser and Seilmeier 1998). The availability of columns packed with silica enabled separation of proteins by RP-HPLC (Bietz and Kruger 1994).

RP-HPLC has been used in various studies to predict quality of wheat because of specific correlations of some specific peaks with quality parameters. Sutton et al. (1989) used RP-HPLC and found two HMW-GS correlated with LV. Horvat et al. (2012) reported significant correlations between α -gliadins and dough water absorption. HMW-GS were significantly and positively correlated with DDT and water absorption was negatively correlated with albumin-globulin.

2.6.3 Sodium dodecyl sulphate–polyacrylamide gel electrophoresis

SDS-PAGE fractionates proteins based on their mobility under an electric current. When proteins are fractionated by SDS-PAGE, various parameters such as molecular weight and protein distributions among fractions can be determined. SDS denatures protein, breaks down S-S bonds causing proteins to have a linear arrangement and also imparts a negative charge to proteins (Singh et al. 1990).

SDS-PAGE separation of proteins can be a useful tool in determining genetic polymorphism and identifying wheat cultivars. Allelic variants vary in terms of mobility, number and intensity and can be characterised by SDS-PAGE (Benmoussa et al. 2000; Nemati et al. 2012). Various studies on electrophoresis have shown variation in number and mobility both in bread wheat and pasta wheat (Branlard et al. 1989; Lawrence and Shepherd 1980). Zilic et al. (2011) used SDS-PAGE to characterise proteins from grain of different bread wheat genotypes.

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Chapter 3

High molecular weight glutenin subunits in South African bread wheat cultivars and their influence on bread-making quality characteristics

3.1 Abstract

This study investigated the influence of high molecular weight glutenin subunits (HMW-GS) on quality parameters of South African medium hard to hard red wheat. This was done as a background for the following chapters where high performance liquid chromatography analysis was used. The trial was conducted in three different locations representing dryland winter rainfall (Moorreesburg), irrigated (Vaalharts) and dryland summer rainfall (Bethlehem) regions. Allelic variations of HMW-GS of 51 genotypes were investigated using sodium dodecyl sulphate polyacrylamide-gel electrophoresis. A total of 17 allelic variations were detected in cultivars from all three production regions with three alleles on *GLU-1A*, ten on *GLU-1B* and four on *GLU-1D*. A total of 22 banding patterns were identified. Analysis of variance for the dryland summer rainfall region showed significant differences between genotypes for all characteristics except for protein content. The cultivar Matlabas ranked highest for most quality characteristics. Subunits combination 5+10 as the most prevalent for HMW-GS present. For the irrigated region, significant differences were observed among genotypes for all quality characteristics and the largest contributor to variation was the genotypes for all quality characteristics except for SDSVOL. Cultivar SST043 ranked the highest for most quality characteristics and subunits 1 and 2+12 were the most prevalent HMW-GS found in cultivars cultivated in this region. Significant differences were observed among genotype for all quality characteristics for the dryland winter rainfall region. Genotype contributed the largest amount of variation for most characteristics except for dough strength and FPC. Cultivar Kwartel ranked the highest for most quality characteristics. A variety of HMW-GS were seen among the genotypes in this environment. The HMW-GS were found to have inconsistent effects on quality characteristics and will probably not be useful for quality selection in South African wheat cultivars.

3.2 Introduction

During the evaluation of new bread wheat cultivars in South Africa, the South African Grain Laboratory (SAGL) has set strict quality standards. These are divided into primary and secondary standards. The primary standards are fixed and include characteristics such as FPC, mixograph peak time (MPT), falling number, LV, farinograph water absorption, alveograph strength (alvW) and alveograph stability to extensibility ratio (alvP/L). All new lines are evaluated based on the primary standards. A new cultivar can only be accepted if it has better yield, agronomical and quality characteristics than existing commercial cultivars in that area (SAGL, 2013).

Bakers and millers are interested in flour quality, while growers are only interested in yield. Quality is the suitability of flour for the end product that millers provide to the processors and consumers (Maihot and Patton 1988; Jones and Kosina 2007). Wheat quality is very complex and depends on various factors such as rheological, baking, milling and chemical composition (Pomeranz 1988; Finney et al. 1987; Gianibelli et al. 2001). The end-use quality depends on protein quantity and quality, which is a function of environment and genetic factors (Kent and Evers 1994; DuPont and Altenbach 2003). Wheat storage proteins form gluten, which has a large influence on bread-making properties (Branlard et al. 2001; Gianibelli et al. 2001). Certain protein subunits have been correlated with quality of dough. Subunits 5+10 have been associated with dough strength (Dong et al. 1992; Gupta et al. 1996; Edwards et al., 2007), while Khatkar et al. (1996) reported subunits 2+12 to have pronounced negative effects on dough strength. Subunits 7+8 in combination with 2* or 1 result in better MPTs and LV (Khatkar et al., 1996).

The objective of this study was to identify HMW-GS separated by SDS-PAGE, and study their contribution to wheat quality characteristics in cultivars from three different production regions in South Africa.

3.3 Materials and methods

3.3.1 Cultivars used

Medium hard to hard red wheat cultivars from the National Cultivar Adaptation Trials conducted by the Agricultural Research Council - Small Grain Institute, Bethlehem in

South Africa were used in this study. The trials were conducted in three different locations, which fall in three different production areas:

- Dryland winter rainfall region (Moorreesburg): 12 cultivars were used which included Kwartel, PAN3408, PAN3471, Ratel, SST015, SST027, SST047, SST056, SST087, SST096, SST088 and Tankwa.
- Dryland summer rainfall region (Bethlehem): 17 cultivars were used which included Elands, Gariep, Koonap, Matlabas, PAN3118, PAN3120, PAN3161, PAN3195, PAN3368, PAN3379, Senqu, SST316, SST317, SST347, SST356, SST387 and SST398.
- Irrigation region (Vaalharts): 22 cultivars were used which included Buffels, Duzi, Krokodil, PAN3471, PAN3478, PAN3489, PAN3497, Sabie, SST806, SST822, SST835, SST843, SST866, SST867, SST875, SST876, SST877, SST884, SST895, Tamboti, Timbavati and Umlazi.

3.3.2 Trial design and locations

A randomised complete block design with three replicates was used. Each plot consisted of five rows 5 m in length and spacing between the rows was 45 cm. For the dryland summer and dryland winter regions a compound fertiliser 4:2:1 (28) was applied at planting time together with the seeds at a rate of 60 kg nitrogen (N) ha⁻¹. The seeding rate was 45-60 plants per m². The irrigated region (Vaalharts) received a compound fertiliser 2:3:4 (28) and LAN (28) at a rate of 280 kg N. ha⁻¹. For the winter rainfall region a commercial fertilizer 4:1:1 was applied at 130 kg N. ha⁻¹. A seeding rate of 250-300 plants per m² was used.

Global positioning system coordinates for each location, rainfall, planting and harvesting dates are listed in Table 3.1. Pests and diseases were controlled when needed according to good agricultural practices. Only the middle three rows per plot were harvested in an effort to avoid the side row effect.

Table 3.1 Global positioning system coordinates, rainfall, planting- and harvesting time of the three production areas

Production regions Location	Coordinates	Rainfall (Growing season)	Planting time	Harvesting time
Dryland summer rainfall Bethlehem	28°09'18.67" S 28°17'59.99" E	362 mm	Early July 2012	Early January 2012
Dryland winter rainfall Moorreesburg	33°8'60" S 18°40'0" E	313 mm	Mid May 2012	End November 2012
Irrigated Vaalharts	27°56'47.96" S 24°48'12.001" E	Optimum Irrigation	Early May 2012	Mid November 2012

3.3.3 Quality measurements

All quality analyses were done at the Agricultural Research Council-Small Grain Institute's quality laboratory in Bethlehem.

Break flour yield

BFY was determined according to American Association of Cereal Chemists (AACC) method 26-95 (AACC, 2000). Wheat samples were conditioned 18 hours prior to milling and milled on a laboratory pneumatic Buhler mill (MLU 202). The percentage of BFY was determined according to Bass (1988).

Flour protein content

FPC was determined according to AACC method 46-30 (AACC, 2000). Crude protein was determined by the combustion method using a LECO FP-2000. PC was calculated as $N \times 5.7$ expressed on 12% moisture basis.

Sodium dodecyl sulphate sedimentation volume

The AACC 56-70 approved method (AACC, 2000) was used to determine SDSVOL. Lactic acid was added to the flour sample and the SDSVOL values were recorded after 30 min.

Wet gluten content

AACC method 38-12A (AACC, 2000) was used, where a 10 g flour sample was washed with 2% NaCl and centrifuged.

$$\text{WGC (14\% moisture basis)} = \frac{\text{Total wet gluten (g)} \times 860}{100 - \% \text{ sample moisture}}$$

Mixograph

Mixograph development time was determined according to the AACC 50-40A method (AACC, 2000). The PC (AACC method 46-30, 2000) and moisture (AACC method 44-15A, 2000) of flour samples were determined before a mixograph was performed as they determine the amount of water to be added. A 35 g flour sample was weighed on a 12% moisture basis and placed in the mixograph bowl. Water was added to the flour to form dough. As the dough was mixed, the mixograph recorded a curve on the graph paper. The MPT was determined as time in minutes from the origin of the curve to the highest point on the centre of the curve.

Alveograph

The approved AACC 54-30A method (AACC, 2000) was used. Moisture content was first determined according to method AACC 44-15A. Alveograph analysis was done using 250 g white flour and 2.5% NaCl solution was added. The amount added was according to the flour's moisture content. Characteristics used for this study were dough extensibility (L-value) and dough stability (P-value). This was obtained by multiplying the height of the curve with a correction factor of 1.1. The ratio between stability and extensibility (P/L ratio) was also calculated. Dough strength (W-value) was determined by dividing the W value by 6.54.

Loaf volume

The rapeseed displacement procedure was used to determine LV according to AACC 10-05 method (AACC, 2000).

Loaf volume adjusted to 12% protein basis

LV was determined on the corrected protein level of the cultivar. A correction factor of 40 cm³ is used per 1% protein level differences to adjust the bread volume to 12% protein basis, for example if a cultivar has a PC of 11%, 40 cm³ will be added to the line's bread volumes and vice versa (SAGL, 2010)

Sodium dodecyl sulphate polyacrylamide gel electrophoresis

One dimensional separation of protein subunits was done according to Singh et al. (1990). Electrophoresis was done on the Mighty Small II SE 250 (Hoefer instrument). Wheat seeds were crushed to powder. Gliadins were extracted for 30 min in 1 ml of 50% (v/v) propanol. Extracts were vortexed every 10 min and after 30 min it was centrifuged for 1 min. The supernatant was then discarded.

Glutenin was extracted from the residue in 0.1 ml solution containing 50% (v/v) propanol, 0.08 M Tris-HCl at pH 8.0 and 1% (w/v) dithiothreitol was added. The samples were then vortexed and placed in a water bath (65°C) for 30 min. The samples were centrifuged for 5 min before the addition of 0.1 ml solution of 50% (v/v) propanol, 0.08 M Tris-HCl (pH 8.0) containing 1.4% (v/v) 4-vinylpyridine and incubated for 15 min for protein alkylation. The samples were centrifuged for 2 min before 0.1 ml aliquot from the supernatant was transferred to a new tube containing 0.1 ml, 2% (w/v) SDS, 40% (w/v) glycerol, 0.02% (w/v) bromo-phenol blue and 0.08 M Tris-HCl, pH 8.0.

Polyacrylamide gels were prepared as described by Laemmli (1970). The concentration of the gel (T) and the cross linker (C) were determined as $\%C = [(g \text{ Bis-Acrylamide} / (g \text{ Acrylamide} + g \text{ Bis-Acrylamide})) * 100]$, $\%T = [(g \text{ Acrylamide} + g \text{ Bis-Acrylamide}) / \text{Total volume}] * 100$. The polyacrylamide gel comprised of 17.33% T, 1.5 Tris-HCl (pH8.8), 10% SDS (w/v) and 0.45% C while the stacking gel comprised of 0.5 M Tris-HCl (pH 6.8), 10% SDS (w/v) 1.42% C and 3.04% T).

After running the gels they were stained with 6% (w/v) trichloroacetic acid and 0.01% (w/v) Coomassie brilliant blue R250 and methanol (5% v/v).

3.3.4 Statistical analysis

The data was subjected to analysis of variance (ANOVA) using Agrobase Generation II software (Agrobase 2015). The contribution of genotype to total variation was calculated from the sum of squares for genotype as a percentage of the total sum of squares.

3.4 Results

3.4.1 Quality results of the dryland summer rainfall cultivars

Break flour yield

Genotypes revealed significant differences ($p \leq 0.01$) for BFY (Table 3.2). Gariep had the highest BFY value followed by PAN3195 and PAN3368. PAN3379 had the lowest BFY value. The grand mean was 22.20%. The average values for BFY ranged between 18.67% (PAN3379) and 25.97% (Gariep) (Table 3.3). Genotype variation contributed 92.31% of total sum of squares (Table 3.2).

Flour protein content

No significant differences were found in FPC between the genotypes tested in this region (Table 3.2). Matlabas ranked the highest followed by Senqu and PAN3118. SST347 ranked the lowest. FPC varied between 13.33% (SST347) to 15.67% (Matlabas). The mean was 14.59% (Table 3.3).

Wet gluten content

Significant differences ($p \leq 0.01$) were found in WGC among genotypes tested (Table 3.2). PAN3120 had the highest WGC value, followed by SST398 and Koonap. PAN3161 had the lowest WGC value (Table 3.3). Genotype contributed 80.93% of total variation (Table 3.2). WGC varied between 38.90% (PAN3161) and 48.73% (PAN3120) and the mean was 43.29% (Table 3.3).

Sodium dodecyl sulphate sedimentation volume

Significant differences ($p \leq 0.01$) were observed in SDSVOL between genotypes (Table 3.2). SST347 ranked the highest, followed by SST317 and Gariep. PAN3379 ranked the lowest. SDSVOL values varied between 74.00 ml (PAN3379) and 93.67 ml (SST347) (Table 3.3). Genotype contribution to total sum of squares was 76.35% (Table 3.2).

Mixograph peak time

There were significant differences ($p \leq 0.01$) for genotypes for MPT among the cultivars (Table 3.2). Senqu ranked the highest followed by Elands and Gariep, PAN3120 ranked the lowest (Table 3.3). Genotypes contributed 86.32% to total variation (Table 3.2). MPT varied between 2.16 min (PAN3120) and 4.20 min (Senqu). The grand mean was 3.08 min (Table 3.3).

Table 3.2 Analysis of variance for quality characteristics for the three production areas cultivars

Characteristics	Dryland summer rainfall				Irrigated region				Dryland winter rainfall			
	Mean squares		Contribution of genotype SS to total		Mean squares		Contribution of genotype SS to total		Mean squares		Contribution of genotype SS to total	
	Rep	Genotype	SS (%)	R ²	Rep	Genotype	SS (%)	R ²	Rep	Genotype	SS (%)	R ²
BFY	1.74	13.85**	92.31	0.94	0.2	9.05***	92.89	0.93	0.63	8.013***	95.61	0.97
FY	0.31	4.66**	91.11	0.92	0.57	3.59***	82.79	0.84	1.34	2.99***	68.22	0.74
FPC	1.65	1.147 ^{NS}	36.45	0.43	0.29	3.35***	79.02	0.80	3.25	2.42*	51.28	0.64
WGC	7.86	18.69**	80.93	0.75	3.22	15.45***	68.19	0.70	4.57	19.61***	77.94	0.81
SDSVOL	32.88	132.91**	76.35	0.79	13.88	71.29*	57.69	0.59	6.19	32.26***	77.94	0.75
MPT	0.23	0.849**	86.32	0.84	0.02	0.473***	88.96	0.90	0.02	0.57***	89.16	0.90
alvP	103.61	545.17**	72.85	0.88	126.4	589.85***	92.98	0.95	18.08	264.43***	84.88	0.84
alvL	0.43	5.51**	78.61	0.74	261.1	1387.85***	82.19	0.84	9.75	732.61**	68.94	0.69
alvP/L	0.04	0.18**	70.25	0.81	0.02	0.13***	93.85	0.95	0.00	0.09***	82.12	0.83
alvW	18.58	159.03**	77.12	0.71	29.65	192.10***	88.29	0.90	5.35	46.58*	58.72	0.60
LV	194.61	1264.03**	77.12	0.79	66.29	7337.54***	77.28	0.77	2031.25	3654.74***	71.00	0.78
LV12	1263.08	2924.56*	49.12	0.52	501.2	4220.84***	26.63	0.73	342.25	3605.95*	54.66	0.56

BFY = break flour yield in %, FY = flour yield in %, FPC = flour protein content in %, SDSVOL = sodium dodecyl sulphate-volume in ml, WGC = wet gluten content in %, MPT = mixograph peak time in min, alvP=alveograph stability in mm, alvL = alveograph extensibility in mm, alvP/L = alveograph stability to extensibility ratio, alvW = alveograph strength 10⁻⁴J, LV = loaf volume, LV12 = loaf volume adjusted to 12% protein basis, Rep = replications, R² = coefficient of determination, *** p≤ 0.001, ** p≤ 0.01, * p≤ 0.05, NS = p>0.05

Table 3.3 Measured quality characteristics for the dryland summer rainfall region cultivars

Cultivar	BFY	FY	FPC	WGC	SDSVOL	MPT	alvP	alvL	alvP/L	alvW	LV	LV12
Elands	23.99	74.69	14.00	42.00	90.67	3.74	101.33	94.67	1.10	53.87	1025.00	943.67
Gariep	25.97	75.56	14.33	40.36	92.33	3.74	73.00	114.33	0.64	46.64	1025.00	938.33
Koonap	21.35	73.94	15.00	45.97	91.33	3.52	114.33	89.00	1.29	58.72	1023.33	904.67
Matlabas	22.48	74.59	15.67	43.21	92.00	3.24	104.67	110.67	0.95	65.49	1028.33	887.00
PAN3118	24.33	75.94	15.33	43.41	90.00	3.09	85.67	122.00	0.71	58.05	1013.33	888.00
PAN3120	21.07	73.18	14.67	48.73	77.33	2.16	103.33	89.67	1.16	48.67	1025.00	915.67
PAN3161	23.51	76.65	14.00	38.90	92.00	3.31	110.67	102.00	1.10	63.76	1010.00	938.00
PAN3195	24.36	77.29	15.00	45.18	87.33	2.82	73.33	124.67	0.61	46.38	943.33	816.67
PAN3368	24.35	76.58	14.67	43.86	83.33	3.39	81.33	141.67	0.58	65.09	1008.33	895.00
PAN3379	18.67	75.36	14.00	41.39	74.00	2.65	93.33	130.00	0.73	56.88	1010.00	931.33
Senqu	23.74	74.62	15.33	42.67	88.33	4.20	105.33	84.33	1.28	50.92	1025.00	894.33
SST316	19.70	73.34	14.33	41.93	90.67	2.98	96.33	103.67	0.93	52.95	1016.67	919.33
SST317	19.43	74.25	14.00	44.89	92.33	2.49	77.33	101.67	0.77	40.67	995.00	915.00
SST347	20.74	75.69	13.33	43.65	93.67	2.55	79.33	118.00	0.68	46.69	991.67	931.67
SST356	19.41	73.20	14.33	40.95	91.67	2.99	93.33	99.00	0.95	49.23	1001.67	912.33
SST387	22.35	73.87	15.00	41.83	75.67	3.04	78.00	123.00	0.63	47.45	995.00	868.33
SST398	21.95	74.88	15.00	46.93	78.33	2.54	84.67	114.33	0.74	49.54	1006.67	897.33
Mean	22.20	74.92	14.59	43.29	87.12	3.08	91.49	109.57	0.87	53.00	1008.43	905.69
LSD	0.95	0.63	1.31	2.48	5.95	0.40	8.38	16.28	0.20	7.89	18.31	52.40
CV	3.08	0.61	6.49	4.15	4.94	9.49	6.62	10.74	16.78	10.76	1.31	4.18

BFY = break flour yield in %, FY = flour yield in %, FPC = flour protein content in %, WGC = wet gluten content in %, SDSVOL = Sodium dodecyl sulphate sedimentation volume in ml, MPT = mixograph peak time in min, alvP = alveograph stability in mm, alvL = alveograph extensibility in mm, alvP/L = alveograph stability to extensibility ratio in mm, alvW = alveograph strength in 10⁻⁴J, LV = loaf volume, LV12= loaf volume to 12% protein basis. The largest value in each column is indicated in bold.

Alveogram stability

There were significant genotype differences ($p \leq 0.01$) for alvP values (Table 3.2). Koonap had the highest alvP value, followed by PAN3161 and Senqu, and Gariep ranked the lowest. Values ranged from 73 mm (Gariep) to 114.33 mm (Koonap) and the mean was 91.49 mm (Table 3.3). The genotypes contributed 72.85% to the total variation (Table 3.2).

Alveogram extensibility

There were significant differences ($p \leq 0.01$) in alvL values among the cultivars (Table 3.2). Cultivar PAN3368 ranked the highest followed by PAN3379 and PAN3195, while Senqu ranked the lowest. AlvL values ranged between 84.33 mm (Senqu) and 141.67 mm (PAN3368). The mean was 109.57 mm (Table 3.3). Much variation (78.61%), was due to the genotypes (Table 3.2).

Alveogram stability to extensibility ratio

Significant differences ($p \leq 0.01$) were found in alvP/L values among genotypes (Table 3.2). Values ranged between 0.58 (PAN3368) and 1.29 (Koonap), the mean value was 0.87 (Table 3.3). Cultivar Koonap ranked the highest, followed by Senqu and PAN3120. PAN3368 ranked the lowest (Table 3.3). The genotypes contributed 70.25% to the total variation (Table 3.2).

Alveogram strength

There were significant differences ($p \leq 0.01$) in alvW values between genotypes (Table 3.2). Matlabas ranked the highest, followed by PAN3368 and PAN3161. SST317 ranked the lowest (Table 3.3). Genotypes contributed 77.12% of total variation (Table 3.2). The alvW values ranged between 40.67×10^{-4} J (SST317) and 65.49×10^{-4} J (Matlabas). The grand mean was 53.00×10^{-4} J (Table 3.3).

Loaf volume

Significant differences ($p \leq 0.01$) were observed among genotypes for LV (Table 3.2). Matlabas had the highest LV followed by Elands, Gariep, PAN3120 and Senqu (1025.00). PAN3195 ranked the lowest. LV ranged between 1028.33 cm^3 (Matlabas) to 943.33 cm^3 (PAN3195). The grand mean was 1008.43 cm^3 (Table 3.3). Much variation (77.12%) was contributed by the genotypes (Table 3.2).

Loaf volume adjusted to 12% protein basis

Differences ($p \leq 0.05$) were observed between genotypes (Table 3.2). Elands had the highest LV12 value (943.67 cm^3) followed by Gariep (938.33 cm^3) and PAN3161 (938.00 cm^3). PAN3195 (816.67 cm^3) ranked the lowest and an average of 905.69 cm^3 was measured for all cultivars tested (Table 3.3). The genotypes contributed 49.12% to the total variation (Table 3.2).

3.4.2 HMW-GS for the dryland summer rainfall region cultivars

SDS-PAGE analysis revealed HMW-GS 1, 2* and null on *GLU-1A*, 7+9, 7+8 and 17+18 on *GLU-1B* and 5+10 and 2+12 on the *GLU-1D*. HMW-GS 5+10 were the most frequently occurring subunits (Table 3.4). Elands, Gariep, Senqu and SST356 had the same subunit combination (1, 7+9 and 5+10). PAN3120, PAN3195, SST347 and SST387 had the same subunit combination (2*, 7+9, 5+10). PAN3118, PAN3161 had the same subunit combination (2*, 7+8, 5+10). Matlabas, SST317 and SST398 had a null expression at *GLU-1A*. SST317 and SST398 had the same subunit combination (null, 7+9 and 5+10). Koonap differed at *GLU-1B* (17+18) with Elands, Gariep, Senqu and SST356 but had corresponding subunits at *GLU-1A* and *GLU-1D* (Table 3.4).

Table 3.4 HMW-GS of the dryland summer rainfall region cultivars

ENTRY	<i>GLU-1A</i>	<i>GLU-1B</i>	<i>GLU-1D</i>
Elands	1	7+9	5+10
Gariep	1	7+9	5+10
Koonap	1	17+18	5+10
Matlabas	Null	7+8	5+10
PAN3118	2*	7+8	5+10
PAN3120	2*	7+9	5+10
PAN3161	2*	7+8	5+10
PAN3195	2*	7+9	5+10
PAN3368	2*	7+8	2+12
PAN3379	2*	7+8	2+12
Senqu	1	7+9	5+10
SST316	1	7+9	2+12
SST317	Null	7+9	5+10
SST347	2*	7+9	5+10
SST356	1	7+9	5+10
SST387	2*	7+9	5+10
SST398	Null	7+9	5+10

3.4.3 Quality results measured for the irrigated region cultivars

Break flour yield

Genotypes showed highly significant differences ($p \leq 0.001$) for BFY (Table 3.2). The cultivar means for BFY varied between 18.04% (SST895) and 23.94% (Buffels), the grand mean was 21.10% (Table 3.5). Buffels ranked the highest followed by Timbavati and Umlazi. SST895 had the lowest BFY content. The most variation was contributed by genotypes (92.89%) (Table 3.2).

Flour protein content

There were highly significant differences ($p \leq 0.001$) in FPC among cultivars and the contribution of genotype to the total variation was 79.02% (Table 3.2). FPC varied between 10.33% (Timbavati and Umlazi) and 15% (Buffels) (Table 3.5). The genotype with the second highest FPC was Duzi (13.67%) followed by Krokodil, PAN3471 and PAN3478 (11.67%).

Wet gluten content

There were highly significant differences ($p \leq 0.001$) between genotypes for WGC (Table 3.2). The averages for WGC varied between 29.86% (SST867) and 40.21% (SST822), the grand mean was 34.19% (Table 3.5). SST822 ranked the highest followed by SST843 and Duzi. Genotypes contributed 68.19% (Table 3.2) to the total variation.

Sodium dodecyl sulphate sedimentation volume

There were differences ($p \leq 0.05$) for SDSVOL among the cultivars. The SDSVOL varied between 75.67 ml (SST877) and 94 ml (Umlazi) (Table 3.5). Umlazi ranked the highest followed by Tamboti and Krokodil. SST877 ranked the lowest and PAN3471 (78.67) was second lowest. Genotypes only contributed 57.69% to total variation for this characteristic (Table 3.2).

Mixograph peak time

Highly significant differences ($p \leq 0.001$) were observed for MPT among cultivars (Table 3.2). MPT varied between 1.61 min (SST876) and 3.69 (SST843) (Table 3.5). The grand mean was 2.23 min. SST843 had the highest MPT value followed by SST895 and SST866. SST876 had the lowest MPT value and was followed by Timbavati (1.97 min; Table 3.5). Genotypes contributed 88.96% to total variation (Table 3.2).

Table 3.5 Measured quality characteristics for cultivars used in the irrigated region

Cultivar	BFY	FY	FPC	WGC	SDSVOL	MPT	alvP	alvL	alvP/L	alvW	LV	LV12
Buffels	23.94	78.95	15.00	33.08	89.33	2.06	52.00	163.67	0.32	31.04	878.33	1009.00
Duzi	23.22	78.37	13.67	37.00	88.33	2.12	50.33	177.33	0.29	31.55	870.00	978.67
Krokodil	22.66	77.31	11.67	33.66	93.33	2.15	60.33	155.00	0.39	39.20	863.33	970.00
PAN3471	20.40	77.72	11.67	32.77	78.67	2.12	63.00	142.33	0.44	35.58	833.33	964.00
PAN3478	20.18	77.94	11.67	35.75	82.00	2.00	58.67	136.33	0.43	30.89	853.33	963.00
PAN3489	20.78	78.37	11.33	31.97	85.00	2.50	69.33	119.00	0.59	37.31	840.00	950.67
PAN3497	20.95	79.81	11.33	33.80	90.00	2.06	64.67	148.00	0.44	38.48	915.00	947.33
Sabie	22.25	77.16	11.33	34.46	90.33	1.99	66.00	158.00	0.42	41.54	998.33	947.00
SST806	19.39	78.73	11.33	32.85	90.33	2.37	62.67	164.67	0.39	39.60	920.00	945.33
SST822	20.47	74.53	11.00	40.21	84.67	2.11	63.33	170.33	0.38	40.27	983.33	933.33
SST835	19.69	79.15	11.00	32.83	83.67	2.01	58.67	157.00	0.38	33.74	881.67	931.67
SST843	18.68	76.32	11.00	38.28	89.33	3.69	113.67	90.67	1.27	63.71	1020.00	922.00
SST866	19.89	78.20	11.00	33.03	84.33	2.53	59.33	120.67	0.50	31.85	860.00	921.67
SST867	22.83	78.68	11.00	29.86	82.67	2.28	54.67	135.00	0.41	29.87	881.67	910.67
SST875	19.24	78.16	11.00	34.36	89.67	2.29	71.00	151.33	0.47	43.37	923.33	908.67
SST876	19.61	78.30	11.00	36.98	88.00	1.61	55.33	146.67	0.38	28.14	868.33	890.33
SST877	21.90	78.19	11.00	33.38	75.67	2.01	49.33	150.00	0.33	28.18	930.00	889.33
SST884	20.47	77.68	10.67	33.85	81.33	2.47	71.33	117.67	0.61	36.59	893.33	888.00
SST895	18.04	76.77	10.67	33.29	85.00	2.57	87.33	115.33	0.76	48.88	878.33	887.00
Tamboti	22.87	78.46	10.67	33.51	93.67	2.05	61.33	144.00	0.43	33.89	900.00	886.67
Timbavati	23.59	77.83	10.33	32.62	90.00	1.97	51.00	172.33	0.30	30.53	900.00	876.00
Umlazi	23.25	78.48	10.33	34.57	94.00	2.11	62.33	139.00	0.46	34.81	940.00	871.33
Mean	21.10	77.96	11.35	34.19	86.79	2.23	63.89	144.29	0.47	36.77	901.44	926.89
LSD	0.80	0.81	0.90	2.55	6.93	0.23	5.54	16.13	0.08	4.62	45.04	38.23
CV	2.75	0.75	5.78	5.43	5.82	7.55	6.31	8.14	12.04	9.15	3.64	3.00

BFY = break flour yield in %, FY = flour yield in %, FPC = flour protein content in %, WGC = wet gluten content in %, SDSVOL = sodium dodecyl sulphate sedimentation volume in ml, MPT = mixograph peak time in min, alvP = alveograph stability, alvL = alveograph extensibility, alvP/L = alveograph stability to extensibility ratio, alvW = alveograph strength, LV = loaf volume, LV12 = loaf volume adjusted to 12% protein content The largest value in each column is indicated in bold

Alveogram stability

There were highly significant differences ($p \leq 0.001$) between cultivars for this characteristic. SST843 ranked the highest followed by SST985 and SST884. SST877 ranked the lowest. The alvP values ranged between 49.33 mm (SST 877) and 113.67 mm (SST843). The grand mean was 63.89 mm (Table 3.5). Genotypes contributed the most (92.98%) to the total variation (Table 3.2).

Alveogram extensibility

There were highly significant differences ($p \leq 0.001$) in alvL values among genotypes (Table 3.2). The alvL values ranged between 90.67 mm (SST843) and 177.33 mm (Duzi). The mean was 144.29 mm. Duzi ranked the highest followed by Timbavati (172.33 mm) and SST822 (170.33 mm). SST843 had the lowest alvL value followed by SST895 (115.33 mm; Table 3.5). Genotype contributed 82.19% to the total variation (Table 3.2).

Alveogram stability to extensibility ratio

There were highly significant differences ($p \leq 0.001$) between cultivars for alvP/L values. Genotypes explained most of the total variation (93.85%; Table 3.2). The values varied between 0.29 for Duzi to 1.27 (SST843). The mean was 0.47 and SST843 had the highest alvP/L value followed by SST895 (0.76) and SST884 (0.61). Duzi was followed by Timbavati that had the second lowest alvP/L value (Table 3.5).

Alveogram strength

Highly significant differences ($p \leq 0.001$) were observed in alvW between cultivars (Table 3.2). The alvW values ranged between 28.14×10^{-4} J (SST876) and 63.71×10^{-4} J (SST843) with a grand mean of 36.77×10^{-4} J (Table 3.5). SST843 ranked the highest followed by SST895 and SST875. The most variation was contributed by genotypes (88.29%) (Table 3.2).

Loaf volume

Highly significant differences ($p \leq 0.001$) were observed in LV between cultivars. Much of the total variation was due to genotypes (77.28%) (Table 3.2). LV varied between 833.33 cm^3 and 1020.00 cm^3 (SST843). The grand mean was 901.44 cm^3 . SST843 had the highest LV value followed by Sabie and SST822. PAN3471 had the smallest LV value (Table 3.5).

Loaf volume adjusted to 12% protein basis

Average values ranged between 1009.00 cm³ (Buffels) and 871.33 cm³ (Umlazi) with a grand mean of 926.89 cm³ (Table 3.5). Genotypes differed highly significantly ($p \leq 0.001$) in LV12. Genotypes contributed 26.63% (Table 3.2) to the total variation.

3.4.4 HMW-GS of the irrigated region cultivars

The most frequently occurring subunit was 1 (*GLU-1A*) followed by 2+12 and 5+10 (*GLU-1D*). Buffels, Duzi, Sabie, Tamboti, Timbavati and Umlazi showed the same subunit combinations (2*, 17+18, 2+12) (Table 3.6). Krokodil differed at *GLU-1B* (7+9) with Buffels, Duzi, Sabie, Tamboti, Timbavati and Umlazi but had corresponding subunit combinations at *GLU-1A* and *GLU-1D*. SST866, SST867, SST875, SST876, SST877, SST884 and SST895 had the same subunit combination (1, 7+8, 5+10) and PAN3471, PAN3489 and SST822 had corresponding subunits at *GLU-1A* and *GLU-1D* but differed at *GLU-1B*. SST835 and SST843 had corresponding subunit combinations at *GLU-1B* and *GLU-1D* but differed at *GLU-1A* where SST843 expressed 2*. PAN3478 and PAN3489 had corresponding subunit combinations at *GLU-1A* and *GLU-1B* but differed at *GLU-1D* (Table 3.6).

Table 3.6 HMW-GS of the irrigated region cultivars

ENTRY	<i>GLU-1A</i>	<i>GLU-1B</i>	<i>GLU-1D</i>
Buffels	2*	17+18	2+12
Duzi	2*	17+18	2+12
Crocodile	2*	7+9	2+12
PAN3471	1	7+9 (13+16) §	5+10
PAN3478	1	13+16	2+12
PAN3489	1	13+16	5+10
PAN3497	1	7+8 (17+18) §	2+12
Sabie	2*	17+18	2+12
SST806	1	7+5	2+12
SST822	1	13+16 (7+8) §	5+10
SST835	1	7+8	2+12
SST843	2*	7+8	2+12
SST866	1	7+8	5+10
SST867	1	7+8	5+10
SST875	1	7+8	5+10
SST876	1	7+8	5+10
SST877	1	7+8	5+10
SST884	1	7+8	5+10
SST895	1	7+8	5+10
Tamboti	2*	17+18	2+12
Timbavati	2*	17+18	2+12
Umlazi	2*	17+18	2+12

§Cultivars with segregating patterns on *GLU-1B*, primary allele in parenthesis

3.4.5 Quality results for the dryland winter rainfall region cultivars

Break flour yield

There were highly significant differences ($p \leq 0.001$) for BFY between genotypes (Table 3.2). SST047 ranked the highest followed by Ratel and SST027. SST087 had the lowest BFY value. BFY ranged between 17.47% (SST087) and 22.98% (SST047). The average was 20.32% (Table 3.7). Genotypes contributed most to the total variation (95.61%) (Table 3.2).

Flour protein content

Differences were observed for FPC among cultivars ($p \leq 0.05$; Table 3.2). The average for this characteristic was 13.00%. The lowest FPC value was 12.00% (Ratel and SST096) and the highest 14.67% (SST047). SST047 ranked the highest followed by Kwartel, Tankwa and SST027 (Table 3.7). Genotypes contributed 68.22% to the total variation (Table 3.2).

Wet gluten content

Highly significant differences ($p \leq 0.001$) were observed in WGC between cultivars. The genotypes contributed 77.94% to the total variation (Table 3.2). SST047 ranked the highest followed by Kwartel and PAN3408. SST088 had the lowest WGC value. Values ranged from 35.12% (SST088) to 45.59% (SST047) and the mean was 39.48% (Table 3.7).

Sodium dodecyl sulphate sedimentation volume

There were highly significant differences in SDSVOL ($p \leq 0.001$) among the cultivars tested. Most of the variation was due to genotypes (77.94%) (Table 3.2). SDSVOL ranged between 83.33 ml and 94.00 ml. SST096 and SST088 ranked the highest followed by Ratel and SST015 (93.33 ml). SST047 had the lowest SDSVOL value. The grand mean was 90.89 ml (Table 3.7).

Mixograph peak time

MPT ranged between 1.88 min (Tankwa) and 3.49 min (Kwartel). The mean was 2.65 min (Table 3.7). There were highly significant ($p \leq 0.001$) differences in MPT among the genotypes and genotype contributed 89.16% to the total variation (Table 3.2). Kwartel

had the highest MPT followed by PAN3408 and PAN3471. The lowest ranked cultivar, Tankwa, was followed by SST088 (2.26 min).

Alveogram stability

Highly significant differences ($p \leq 0.001$) were observed in alvP among genotypes and variation was due to genotypes (84.88%) (Table 3.2). The alvP ranged between 58.67 mm (PAN3408) and 91.33 mm (SST096). The mean was 75.75 mm. The highest ranking cultivar for alvP value was followed by Kwartel and PAN3471 (83.00; Table 3.7).

Alveograph extensibility

There were significant differences ($p \leq 0.01$) in alvL values among cultivars (Table 3.2). An average alvL value was 120.33 mm and values ranged between 86.00 mm (Tankwa) and 134.67 mm (PAN3408). The highest ranked cultivar was followed by SST015 (132.33 mm) and SST027 (131.00 mm; Table 3.7). Genotype contributed 68.94% (Table 3.2) to the total variation.

Alveogram stability to extensibility ratio

Genotype showed highly significant differences ($p \leq 0.001$) for alvP/L values (Table 3.2). The ratio ranged between 0.44 (PAN3408) and 0.98 (SST096). This highest ranked cultivar was followed by Tankwa (0.95) and PAN3471 (0.77; Table 3.7). The largest variation for the measured alvP/L values was contributed by genotype (82.12%) (Table 3.2).

Alveogram strength

There were differences ($p \leq 0.05$) in alvW values among the genotypes and 58.72% of the total variation was contributed by genotypes (Table 3.2). The alvW values ranged between 38.43×10^{-4} J (PAN 3408) and 52.14×10^{-4} J (Kwartel) and the mean was 45.69×10^{-4} J. Kwartel had the highest alvW value followed by SST047 and SST087 (Table 3.7).

Loaf volume

There were highly significant differences ($p \leq 0.001$) for LV among the cultivars (Table 3.2). LV varied between 926.67 cm^3 (Tankwa) and 1030.00 cm^3 (Kwartel). The mean value was 977.08 cm^3 . The highest ranked cultivar was followed by PAN3408 and PAN3471 (Table 3.7). Much variation was due to genotype (71.00%) (Table 3.2).

Table 3.7 Analysis of variance for quality characteristics for the dryland winter rainfall cultivars

Cultivar	BFY	FY	FPC	WGC	SDSVOL	MPT	alvP	alvL	alvP/L	alvW	LV	LV12
Kwartel	21.51	72.72	14.33	41.89	93.00	3.49	83.00	125.33	0.67	52.14	1030.00	925.33
PAN3408	19.28	74.83	12.33	40.67	88.67	3.02	58.67	134.67	0.44	38.43	1021.67	988.00
PAN3471	18.86	75.79	13.00	39.44	91.67	2.96	83.00	110.00	0.77	47.35	1020.00	914.67
Ratel	22.66	73.82	12.00	38.25	93.33	2.87	74.00	125.67	0.59	45.06	1006.67	966.33
SST015	19.73	75.37	13.00	39.03	93.33	2.84	81.00	132.33	0.62	47.25	980.00	882.67
SST027	21.88	75.82	13.67	39.49	92.00	2.83	66.33	131.00	0.50	46.99	968.33	957.67
SST047	22.98	75.20	14.67	45.59	83.33	2.59	80.67	118.00	0.70	49.13	965.00	928.67
SST056	20.02	73.60	12.33	39.47	90.00	2.36	73.00	128.33	0.57	44.65	963.33	951.00
SST087	17.47	74.37	12.33	39.40	90.67	2.33	72.67	130.67	0.56	47.86	961.67	968.00
SST096	19.76	74.34	12.00	37.82	94.00	2.31	91.33	94.67	0.98	46.69	946.67	955.33
SST088	19.39	75.00	12.67	35.12	94.00	2.26	64.00	127.33	0.51	38.79	935.00	939.00
Tankwa	20.35	73.28	13.67	37.53	86.67	1.88	81.33	86.00	0.95	43.99	926.67	872.33
Mean	20.32	74.51	13.00	39.48	90.89	2.65	75.75	120.33	0.66	45.69	977.08	937.42
LSD	0.50	1.06	1.30	2.15	3.28	0.25	6.56	17.96	0.13	5.59	33.22	53.65
CV	1.75	1.02	7.12	3.89	2.57	6.84	6.18	10.65	14.65	8.72	2.43	4.08

BFY = break flour yield in %, FY = flour yield, FPC in %, = flour protein conten in %, t, WGC = wet gluten content in %, , SDSVOL = sodium dodecyl sulphate sedimentation volume in ml, MPT = mixograph peak time in min, alvP = alveograph stability, alvL = alveograph extensibility, alvP/L = alveograph stability to extensibility ratio, alvW = alveograph strength, LV = loaf volume, LV12 = loaf volume adjusted to 12% protein content The largest value in each column is indicated in bold

Loaf volume adjusted to 12% protein basis

Differences ($p \leq 0.05$) were observed between genotypes for LV12 (Table 3.2). PAN3408 ranked the highest followed by SST087 and Ratel. Tankwa had the lowest LV12. The grand mean was 937.42 cm³ and values between 988 cm³ (PAN3408) and 872.33 cm³ (Tankwa) (Table 3.7). Genotypes contributed 54.66% to the total variation (Table 3.2).

3.4.6 HMW-GS for the dryland winter rainfall region

The most frequently occurring subunit was 1 for the *GLU-1A*. SST096, SST088, SST047 and Ratel had the same subunit combinations (1, 7+8, 2+12). Kwartel, SST027, SST056 had corresponding subunits at *GLU-1A* and *GLU-1D* but differed at *GLU-1B*. SST087 and Tankwa have null expression at *GLU-1A*. PAN3471, SST015, SST087 and Tankwa had different subunit combinations and differed from the rest of the genotypes (Table 3.8).

Table 3.8 HMW-GS for dryland winter rainfall region cultivars

ENTRY	<i>GLU-1A</i>	<i>GLU-1B</i>	<i>GLU-1D</i>
Kwartel	1	7+9	5+10
PAN3408	2*	17+18	2+12
PAN3471	2*	17+18 (7+9) [§]	2+12 (5+10) [§]
Ratel	1	7+8	2+12
SST015	1	7+8 (13+16) [§]	5+10 (2+12) [§]
SST027	1	13+16	5+10
SST047	1	7+8	2+12
SST056	1	13+16	5+10
SST087	Null	13+16	5+10
SST096	1	7+8	2+12
SST088	1	7+8	2+12
Tankwa	Null	7+8	5+10 (2+12) [§]

[§]Cultivars with segregating patterns on *GLU-1B*, primary allele in parenthesis

3.5 Discussion

Dryland summer rainfall region

There were highly significant differences between genotypes for all characteristics except for FPC. A large percentage of variation was contributed by genotypes in all characteristics except for FPC (Table 3.2). This is in agreement with previous studies (Mamuya 2000; Marchylo et al. 2001; Edwards et al. 2007; Miles 2010) where FPC showed little variation contributed by genotypes. However, it is worth noting that multi

location testing was done in the other studies, which was not done in this study. The genotypes contained high FPC but variation in FPC was relatively small, ranging between 13.33% and 15.67%. (Table 3.3).

Matlabas ranked the highest for alvW values, LV and FPC. It also had high alvP and alvL values. Edwards et al. (2007) indicated that alvL values and alvW values are related to FPC, this suggests that cultivars high in FPC will have high alvW and alvL values. The genotypes in this location exhibited high alvL values, which might be attributed to high FPC as mentioned by Edwards et al. (2007). Koonap ranked the highest in alvP/L and alvP values.

The results obtained in this location demonstrated genotypes having HMW-GS 5+10 to be high in most quality attributes as observed with cultivars Matlabas, Koonap, Elands, Gariep, PAN312, Senqu, and SST347. SST317 and SST398 ranked the lowest in most quality attributes, which might be due to the null allele at the *GLU-1A* locus. Carrillo et al. (1990) and Weegels et al. (1996) indicated that the null allele has a negative influence on quality characteristics.

PAN3368, PAN3379 and SST316 were the only cultivars with subunits 2+12 on the *GLU-1D* genome while all other cultivars had subunits 5+10 (Table 3.4). Matlabas had subunits null, 7+8 and 5+10 (Table 3.4) and had the highest alvW value. Subunits 5+10 and 7+8 have been reported to have a pronounced effect on alvP and alvW values (Gupta and MacRitchie 1994; Hou et al. 1996; Dumur et al. 2010). Cultivar PAN3368 ranked the second after Matlabas in terms of alvW and had subunits 2*, 7+8, 2+12 and this corroborates the findings of Khatkar et al. (1996) and Koen (2006). They indicated cultivars with 2*, 2+12 to have high alvW values. Cultivars with low alvW values had subunits 5+10.

Irrigated region

The largest variation of the measured characteristics was contributed by the genotypes for all the characteristics except for LV12 (Table 3.2). The greatest variation attributed to the genotypes for FPC in this study is contradictory to previous studies (Mamuya 2000; Edwards et al. 2007; Miles 2010). But it must be taken into account that multiple locations were used in the other studies, which was not the case in this study. FPC content

varied between 10.33% and 15.00% and this indicated high variability in PC. SST843 ranked the highest for many quality characteristics (MPT, alvP value, alvP/L value, alvW value and LV,) but this cultivar ranked the lowest for alvL (Table 3.5).

SST843 contained subunits 2*, 7+8 and 2+12 (Table 3.6). Khatkar et al. (1996) reported that cultivars containing subunits 2+12 in combination with subunit 2* produced stronger doughs, and higher LVs. Gupta and MacRitchie (1994) also reported that genotypes possessing 7+8, and 1 or 2* to produce stronger dough and acceptable peak times. Dumur et al. (2010) also reported that 2+12 had pronounced effects on alvW values and it increased alvP/L values. SST895 ranked second and SST875 third after SST843 for alvW value, alvP value and MPT. These two genotypes subunit pattern was 1, 7+8 and 5+10. Most cultivars with high means for measured quality attributes had subunits 5+10 (cultivars SST884, SST843 and SST895).

Dryland winter rainfall region

Genotypes contributed the largest part of variation for all the characteristics except FPC, alvW values and LV12 (Table 3.2). FPC content varied between 12.00% and 14.67%. FPC has been reported to be greatly affected by the environment (Miles 2010; Edwards et al. 2007). SST096 ranked the highest for alvP value and alvP/L value. Kwartel ranked the highest for MPT, alvW value and LV. Kwartel also had a high FPC of 14.33%. Graybosch et al. (1993) reported that FPC had a major impact on alvW values and LV as was observed with Kwartel. PAN3408 ranked the highest for alvL but ranked the lowest for alvP/L, alvW and alvP values (Table 3.7).

Kwartel ranked the highest in most quality attributes and had subunits 1, 7+9 and 5+10 (Table 3.8). Branlard and Dardevet (1985), Gupta and MacRitchie (1994) and Sadouki et al. (2005) reported that genotypes with subunits 5+10 had stronger dough than those containing subunits 2+12. SST047 ranked second after Kwartel for alvW values and had subunits 1, 7+8, 2+12. SST096 ranked the highest for alvP and alvP/L values and comprised of subunits 1, 7+8, and 2+12. Dumur et al. (2010) reported *GLU-1D* alleles 2+12 to have a large influence on dough strength, resulting in increased alvP/L values, as was observed with SST047 and SST096. Cultivars having subunits 13+16 had greater alvL (SST056, SST027, SST015 and SST087; Table 3.8), which was in agreement with what was reported by Branlard and Dardevet (1985). Cultivars with subunit 2*

(PAN3408 and PAN3471) had higher LV, which was in agreement with what was reported by Dong et al. (1992) and Khatkar et al. (1996). SST088 and Tankwa performed the poorest in quality attributes.

It is worth noting in this study that cultivars which had subunits that have been reported to negatively affect technological characteristics ranked the highest in most technological characteristics. This was evident with Matlabas with the null allele on *GLU-1A*, however, cultivars like Tankwa and SST317 showed inferior performance. This might be due to the null allele they carried on *GLU-1A* (Payne 1987; Weegels et al. 1996). For cultivars with the same HMW-GS combination, different quality results were observed in one location. Contrarily cultivars with different subunits pairs showed similar quality results. This indicates that quality is a result of many factors. The bread-making quality is affected by rheology, baking, the quantity and quality of proteins. The genotype, environment and GxE alters the composition of protein and these makes dough quality a complex phenomenon (Finney et al. 1987; Gianebelli et al. 2001).

In the irrigated region SST843, SST895 and SST875 ranked the highest in most quality characteristics, though they comprised of different banding patterns, also for the dryland winter rainfall region, Kwartel and SST047 ranked highest in most quality characteristics but differed in terms of subunit patterns. Randall et al. (1993) reported significant negative correlations between the HMW-GS and quality in South African wheats. Gupta et al. (1991) indicated that HMW-GS were not able to fully account for variation in quality in Australian wheats. The HW-GS accounted for less than 20% variation in bread quality characteristics.

A total of 17 allelic variations were detected in all assessed genotypes in the three production areas, three alleles for the *GLU-A1* locus, 8 alleles for the *GLU-B1* locus and four alleles for the *GLU-D1* locus. For the dryland and winter rainfall locations the *GLU-1A* expressed null, 1 and 2*. Yan et al. (2007), studied 229 hexaploid Tibetan landraces and found the same three HMW-GS at the *GLU-A1* locus, as did Giraldo et al. (2010) in Spanish wheat cultivars and Dessalegn et al. (2011) in Ethiopian wheat cultivars, Rodriguez-Quijano et al. (1998) in Portuguese landraces of *Triticum aestivum spp vulgare*, and Payne and Lawrence (1983) in a set of wheat genotypes from Europe.

Although the null allele was found in the dryland summer and winter rainfall regions cultivars, it occurred at low frequencies. From a total of 29 genotypes, it was only found in five genotypes. The irrigated region cultivars had subunits 1 and 2*, which were reported to have a more positive influence on quality than the null subunit (Cornish et al. 2006).

For *GLU-1B*, subunits 7+8 and 7+9 were the most common subunits in the three production regions. This was also observed in other studies (Sontag-Strohm, 1997; Igrejas et al. 1999; Tarekegne and Labuschagne 2005). The *GLU-B1* expressed the most variation compared to the other two loci. This is in accordance with the findings by Rodriguez-Quijano et al. (1998); Tarekegne and Labuschagne (2005) and Yan et al. (2007).

For the *GLU-1D* locus the most prominent subunits were 5+10 for all three production regions. This is in agreement with reports of Galova et al. (2002) in Slovak wheat varieties and Wentzel (2010) in South African wheat cultivars. Subunits 5+10 have been associated with good bread-making quality and have been used for selection of good bread-making performance (Lukow 1989; MacRitchie et al. 1990; Rodriguez-Quijano et al. 1998; Kasarda 1999). For the irrigated region, subunits 2+12 occurred in higher frequencies. High occurrences of subunits 2+12 were also reported by Rodriguez-Quijano et al. (1998); Yan et al. (2007) and Fang et al. (2009). Subunits 1, 2, 7+8, 7+9, 5+10 and 2+12 were present in all cultivars used in the three different production areas.

In all three production areas a total of 22 banding patterns were observed. The most frequently occurring banding patterns were: dryland summer rainfall 1, 7+9, 5+10 and 2, 7+9, 5+10 while for the irrigated region it was 2*, 17+18, 2+12; 1, 7+8, 2+12 and 1, 7+8, 5+10. For the dryland winter rainfall region it was 1, 7+8, 2+12. This suggests low variability in HMW-GS combinations in South African bread wheat cultivars. This might be due to repeated use of good parental lines over a period of time, causing a narrowing of the genetic base. Subunits 1, 2, 7+8, 7+9, 5+10 and 2+12 were present in all three production areas.

3.6 Conclusions

Low allelic variation was observed in the HMW banding patterns in the three production areas. The lowest allelic variation in the HMW-GS was seen for the *GLU-1A*, and *GLU-1D* locus for the three production areas. The *GLU-1B* locus exhibited much variation across the cultivars. The traditional tendency of breeding and selecting for traits contributing to good bread quality might be a source of reduced variation in the HMW banding patterns. For cultivars with the same subunit pairs, different quality results were observed, this confirms that dough quality is a result of many factors and it is a complex phenomenon. The concentration and molecular size distribution affects dough quality. The HMW-GS were not consistent predictors of good quality. For the dryland summer rainfall Matlabas ranked high for most quality attributes, while for the irrigated region SST843 showed superior performance. For the dryland winter rainfall region Kwartel ranked high in rheological parameters. Therefore these cultivars can be selected for and included in the breeding programmes.

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Chapter 4

Protein fraction separation by a narrow bore size exclusion-high performance liquid chromatography column and the fractions relationship with quality characteristics in South African bread wheat cultivars

4.1 Abstract

Size exclusion-high performance liquid chromatography (SE-HPLC) is a valuable technique used in the separation of wheat proteins according to their molecular weight distribution. Associations between protein subunits as determined by SE-HPLC using a narrow bore column, and flour quality characteristics of various bread wheat genotypes, representing medium to hard wheat, in three different production areas in South Africa, were studied. Significant associations between both absolute and relative protein fractions and between these fractions and quality parameters were seen. However, more significant correlations were seen between absolute values and quality characteristics. The total gliadins showed significant relationships with flour protein content and wet gluten content in all the locations. No significant relationships were observed between relative values of proteins and quality data for the dryland winter rainfall area. The irrigated region showed more significant correlations than the other two regions. The total gliadins and globulins-albumins in proteins in flour showed significant relationships with dough strength. In the dryland summer region, the first principle component (PC1) accounted for 66.43% of variation, with large positive loadings from alveograph P-value and loaf value, and negative loadings from alveograph extensibility. In the dryland winter rainfall region PC1 accounted for 75.44% of the total variation and alveograph stability was the only characteristic that contributed positively to PC1 and alveograph extensibility had a negative value. In the irrigated region PC1 contributed 76.91% to total variation, with loaf volume contributing the most to this component.

4.2 Introduction

In many parts of the world wheat is among the most important cereal crops (FAO, 2014). Wheat can be used for a wide range of products. The wheat grain constitutes about 8-20% protein, which is divided into four major groups based on their solubility in various solvents: glutenin, gliadin and albumins-globulins. Glutenin and gliadin together are

termed gluten. Gluten makes up about 80% of total wheat proteins (Gianibelli et al. 2001; Horvat et al. 2015), while globulins-albumins makes up about 15-25% (Hurkman and Tanaka 2007). Gluten is responsible for visco-elastic properties of dough and is further divided into monomeric gliadins and polymeric glutenins. The polymeric glutenins are of two forms namely HMW and LMW (Branlard et al. 2001; Gianibelli et al. 2001). The molecular size distribution of proteins has a direct effect on wheat end-use quality (Gianibelli et al. 2001; Hurkman and Tanaka 2007). Albumins and globulins play a major role in enzymatic activities and contribute to the breaking down of starch (Horvat et al. 2015). The contribution made by albumins and globulins to end-use quality is poorly understood.

Protein quality and quantity are the major determinants of flour characteristics for functional end-use quality (Gianibelli et al. 2001; Bushuk and Békés 2002). Various studies have established significant correlations between protein quality and flour quality (Dachkevitch and Autran 1989; Gupta et al. 1993; Labuschagne and Aucamp 2004; Labuschagne et al. 2004; Edwards et al. 2007). Dough extensibility and strength are regarded as the major components affecting the suitability of flour for making good quality bread (Bushuk and Békés 2002).

SE-HPLC is an important tool to study proteins in their native state, allowing the separation of proteins based on their molecular weight distribution (Dachkevitch and Autran 1989; Gupta et al. 1993). Previous studies using SE-HPLC indicated that molecular weight distribution has a large influence on bread-making quality (Gupta et al. 1993; Ciaffi et al. 1996; Larroque and Békés 2000). The technique is simple, reproducible and has good resolution (Bietz 1986). The SE-HPLC analysis of wheat proteins was first demonstrated by Bietz (1986) using the TSK columns: TSK-4000SW, TSK-3000SW and TSK 2000SW. These columns sizes were 7.5 x 500-600 mm. The results obtained were reproducible, had good resolution and the columns could analyse proteins of high molecular size. The columns could also withstand high pressure and differentiate between cultivars. However the columns were less stable (Bietz 1986; Bietz and Simpson 1992).

Complete extraction of proteins also remained a challenge due to complex solubility of wheat proteins. Singh et al. (1990) described the extraction of wheat protein after

sonication. The wheat proteins became highly extractable after sonication but couldn't quantitate proteins well (Bietz and Simpson 1992). Since then major developments have taken place in columns used in the separation of wheat proteins (Larroque et al. 1997; Bean et al. 1998). A narrow bore column (BioSep-SEC s4000) was introduced by Larroque and Békés (2000). Protein separation with this column is fast, highly reproducible, and large numbers of samples can be analysed before replacement is needed. It is sensitive and results in reduced use of toxic organic solvents (Larroque and Békés 2000; Ohm et al. 2009). Ohm et al. (2009) further stated that highly significant correlations and repeatability between protein fractions and quality were obtained with the narrow bore column.

The objective of this study was to relate molecular weight distribution of proteins, separated using a BioSep-SEC s4000 narrow bore column by SE-HPLC, with quality characteristics of South African medium hard to hard red wheat varieties.

4.3 Materials and methods

4.3.1 Cultivars used

See Chapter 3 (3.3.1).

4.3.2 Quality measurements

See Chapter 3 (3.3.3).

4.3.3 Size exclusion-high performance liquid chromatography

Proteins from wheat flour samples were extracted using a two-step procedure according to Gupta et al. (1993). The first step involves extraction of proteins soluble in SDS buffer while the second step extracts proteins soluble after sonication in SDS buffer. For the first extraction, 17 mg white flour sample was weighed in triplicate; each sample was suspended in 1.5 ml of 0.05 M SDS phosphate buffer (pH 6.9). Samples were vortexed briefly and then shaken for 10 min at 21⁰C. The samples were then centrifuged at 10 000 rpm for 30 min to obtain a supernatant. The supernatant for each sample was filtered through a 0.45 µm filter (Millipore Durapore membrane filters) into a glass vial. These samples were placed in a water bath for 2 min at 80⁰C to stop protease activity. It was kept for routine HPLC analysis.

For the second extraction the remaining pellet was resuspended in 1.5 ml of 0.05 M SDS phosphate buffer (pH 6.9), vortexed briefly and sonicated using an ultrasonic disintegrator for 30 sec, at amplitude 5, fitted with a 3 mm exponential microtip. The samples were centrifuged as indicated above and the supernatant was filtered through 0.45 µm filters (Millipore Durapore membrane filters). These samples were again placed in a water bath for 2 min at 80°C to stop protease activity.

Routine analysis was done using a BioSep-SEC s4000 Phenomenex narrow bore column (300 x 4.6 mm). Separation of the proteins was obtained in 30 min by loading 20 µl of sample into an elution solution of 50% (v/v) acetonitrile and water containing 0.1% (v/v) trifluoroacetic acid (TFA) at a flow rate of 0.2 ml min⁻¹. Protein fractions were detected at 210 nm using a UV/VIS photodiode array detector.

4.3.4 Protein fractions

The SE-HPLC fractions were partitioned into five fractions measured at F1 (4.64-5.45 min), F2 (5.45-7.15 min), F3 (7.15-7.74 min), F4 (7.74-8.60 min) and F5 (8.60 min). Fraction F1 constituted HMW, F2 LMW, F3 ω gliadins and F4 α/β and γ gliadins. Fraction F5 contained albumins and globulins (Larroque et al. 1997; Samson et al. 2005).

The protein fractions were calculated based on percentage of the respective areas relative to the total HPLC area. In all cases the percentages of protein fractions were determined as percentage of total proteins based on flour weight basis (absolute/%p) and percentage proteins in the flour (relative/%fp) (Schober et al. 2006; Ohm et al. 2010).

Absolute values were calculated using the following formulas:

Percentage of large unextractable polymeric protein

$$\text{LUPP} = [(\text{F1 insoluble}) / (\text{F1 soluble} + \text{F1 insoluble})] * 100$$

Percentage total unextractable polymeric proteins

$$\text{UPP} = [(\text{F1 insoluble} + \text{F2 insoluble}) / (\text{F1 soluble} + \text{F2 soluble} + \text{F1 insoluble} + \text{F2 insoluble})] * 100$$

Percentage high molecular weight proteins

$$\text{HMW} = [(F1 \text{ soluble} + F1 \text{ insoluble}) / (F1 \text{ soluble} + F2 \text{ soluble} + F3 \text{ soluble} + F4 \text{ soluble} + F5 \text{ soluble} + F1 \text{ insoluble} + F2 \text{ insoluble} + F3 \text{ insoluble} + F4 \text{ insoluble} + F5 \text{ insoluble})] * 100$$

Percentage low molecular weight proteins

$$\text{LMW} = [(F2 \text{ soluble} + F2 \text{ insoluble}) / (F1 \text{ soluble} + F2 \text{ soluble} + F3 \text{ soluble} + F4 \text{ soluble} + F5 \text{ soluble} + F1 \text{ insoluble} + F2 \text{ insoluble} + F3 \text{ insoluble} + F4 \text{ insoluble} + F5 \text{ insoluble})] * 100$$

Percentage total polymeric proteins

$$\text{POL} = [(F1 \text{ soluble} + F2 \text{ soluble} + F1 \text{ insoluble} + F2 \text{ insoluble}) / (F1 \text{ soluble} + F2 \text{ soluble} + F3 \text{ soluble} + F4 \text{ soluble} + F5 \text{ soluble} + F1 \text{ insoluble} + F2 \text{ insoluble} + F3 \text{ insoluble} + F4 \text{ insoluble} + F5 \text{ insoluble})] * 100$$

Percentage total gliadins

$$\text{GLI} = [(F3 \text{ soluble} + F4 \text{ soluble} + F3 \text{ insoluble} + F4 \text{ insoluble}) / (F1 \text{ soluble} + F2 \text{ soluble} + F3 \text{ soluble} + F4 \text{ soluble} + F5 \text{ soluble} + F1 \text{ insoluble} + F2 \text{ insoluble} + F3 \text{ insoluble} + F4 \text{ insoluble} + F5 \text{ insoluble})] * 100$$

Percentage total gliadins and globulins-albumins

$$\text{GGA} = [(F3 \text{ soluble} + F4 \text{ soluble} + F5 \text{ soluble}) + (F3 \text{ insoluble} + F4 \text{ insoluble} + F5 \text{ insoluble}) / (F1 \text{ soluble} + F2 \text{ soluble} + F3 \text{ soluble} + F4 \text{ soluble} + F5 \text{ soluble} + F1 \text{ insoluble} + F2 \text{ insoluble} + F3 \text{ insoluble} + F4 \text{ insoluble} + F5 \text{ insoluble})] * 100$$

Relative values were calculated as percentage of the absolute protein fractions of the flour, multiplied by FPC, divided by 100

4.4 Statistical analysis

Agrobase Generation II (Agrobase, 2015) was used to perform ANOVA, correlations and GenStat (Payne et al. 2008) was used to perform and principal component analysis (PCA) on measured fractions and quality characteristics.

4.5 Results

4.5.1 Size exclusion-high liquid performance chromatography

Figures 4.1 and 4.2 are examples of SE-HPLC profiles in the study.

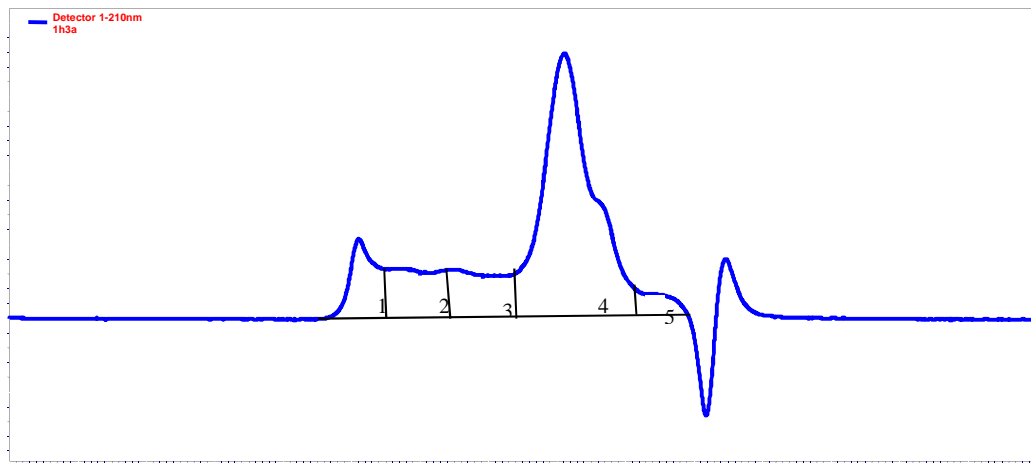


Figure 4.1 SE-HPLC profiles for the non-sonicated protein fractions.

1 = HMW, 2 = LMW, 3 = ω -gliadins, 4 = α - β - and γ gliadins, 5 = albumins-globulins

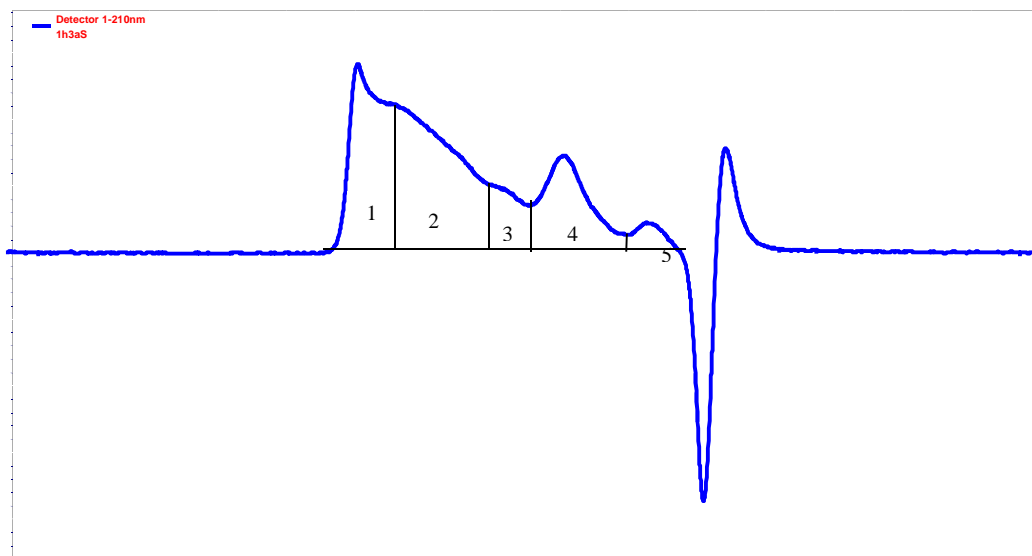


Figure 4.2 SE-HPLC profiles for the sonicated protein fractions.

1 = HMW, 2 = LMW, 3 = ω -gliadins, 4 = α - β and γ , gliadins, 5 = albumins-globulin

4.5.2 Analysis of variance for absolute and relative protein fractions in the cultivars of the dryland summer rainfall region

Large unextractable polymeric proteins

Genotype contributed 27.65% to the total variation in LUPP (Table 4.1). The means ranged between 44.21% (PAN3161) and 52.52% (PAN3120) (Table 4.2). No significant differences were observed in LUPP among the cultivars tested (Table 4.1). PAN3120 was the cultivar with the highest LUPP followed by SST347 and PAN3118. PAN3161 had the smallest LUPP value.

Unextractable polymeric proteins

Genotype contributed 56.29% to the total variation in UPP. ANOVA revealed significant differences ($p \leq 0.01$) among the cultivars for UPP (Table 4.1). Senqu was the cultivar that ranked highest followed by PAN3120 and PAN3195. PAN3161 ranked the lowest. The average UPP value was 46.42%, ranging between 42.38% (PAN3161) and 48.70% (Senqu) (Table 4.2).

Total polymeric proteins

There were significant differences ($p \leq 0.01$) between the cultivars for POL%fp, but no significant differences were seen for POL%p (Table 4.1). PAN3195 had the highest POL%p value followed by Matlabas and PAN3118. SST356 had the lowest POL%p value. PAN3379 (46.99%) was the cultivar that ranked the highest in POL%fp followed by PAN3161 and PAN3195. SST356 had the smallest POL%fp value (Table 4.2). Genotype contributed 45.94% in POL%p and 55.26% in POL%fp to the total variation (Table 4.1). Values ranged from 6.97% (PAN3195) to 5.61% (SST356) for POL%p, with a grand mean of 6.38%. The grand mean for POL%fp was 43.77%. The POL%fp ranged from 39.37% (SST356) to 46.99% (PAN3379) (Table 4.2).

High molecular weight proteins

There were no significant differences in HMW%p and HMW%fp among cultivars (Table 4.1). SST387 had the highest HMW%p and HMW%fp value followed by Matlabas and SST398 for both fractions. Gariiep had the lowest HMW%p value and Senqu had the lowest HMW%fp value. HMW%fp ranged between 9.33% (Senqu) and 13.33% (SST387), the grand mean was 10.90%.

Table 4.1 Analysis of variance for protein fractions in the protein and the flour for three different production areas

Regions	Absolute (fractions in total protein)					Relative (flour proteins)				
	Characteristics	Rep	Cultivar	Contribution of genotype SS to total SS (%)	R ²	Characteristics	Rep	Cultivar	Contribution of genotype SS to total SS (%)	R ²
Dryland	LUPP	14.46	14.45NS	27.65	0.31					
summer	UPP	1.21	10.587**	56.29	0.57					
rainfall	POL%p	0.09	0.48NS	45.94	0.47	POL%fp	4.35	12.487**	55.26	0.58
Region	HMW%p	0.001	0.09NS	42.95	0.43	HMW%fp	2.49	2.74NS	41.95	0.47
	LMW%p	0.15	0.33*	54.42	0.56	LMW%fp	1.33	11.83***	68.66	0.70
	GLI%p	0.58	0.38NS	43.18	0.51	GLI%fp	3.47	13.42***	63.79	0.66
	GGA%p	0.92	0.61NS	45.16	0.54	GGA%fp	4.34	12.485**	55.26	0.58
Dryland	LUPP	7.01	35.02*	57.39	0.60					
winter	UPP	2.71	17.12*	66.52	0.68					
rainfall	POL%p	1.32	1.09NS	45.12	0.55	POL%fp	23.25	15.83NS	79.42	0.28
region	HMW%p	0.02	0.14*	52.87	0.54	HMW%fp	1.361	7.36NS	48.49	0.50
	LMW%p	1.03	0.80NS	46.00	0.57	LMW%fp	23.51	16.82NS	28.27	0.35
	GLI%p	0.29	0.33NS	32.74	0.38	GLI%fp	18.84	13.32NS	33.42	0.42
	GGA%p	0.66	0.45NS	22.91	0.29	GGA%fp	23.26	15.83NS	22.09	0.28
Irrigated	LUPP	33.15	181.486***	70.57	0.77					
region	UPP	8.06	52.712***	70.57	0.72					
	POL%p	0.08	0.73***	78.75	0.80	POL%fp	0.11	11.697***	93.80	0.94
	HMW%p	0.03	0.19***	67.77	0.67	HMW%fp	3.65	8.333*	50.14	0.52
	LMW%p	0.08	0.41*	52.86	0.54	LMW%fp	10.23	19.274NS	44.91	0.47
	GLI%p	0.14	1.08***	77.24	0.78	GLI%fp	6.75	37.589***	82.60	0.84
	GGA%p	0.09	1.07***	79.91	0.81	GGA%fp	0.11	11.701***	93.80	0.94

LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, POL%p = absolute total polymeric proteins, HMW%p = absolute high molecular weight, LMW%p = absolute low molecular weight, GLI%p = absolute total gliadins, GGA%p = absolute total gliadins and albumen-globulins, POL%fp = relative total polymeric proteins, HMW%fp = relative high molecular weight, LMW%fp = relative low molecular weight, GLI%fp = relative total gliadins, GGA%fp = relative total gliadins and albumins-globulins, Rep = replications. SS= sum of squares *** p≤ 0.001, ** p≤ 0.01, * p≤ 0.05, NS = p>0.05.

Table 4.2 Means for absolute and relative protein fractions of the dryland summer rainfall region cultivars

Cultivar	LUPP (%)	UPP (%)	POL%p	POL%fp	HMW%p	HMW%fp	LMW%p	LMW%fp	GLI%p	GLI%fp	GGA%p	GGA%fp
Elands	48.66	46.56	6.01	42.77	1.53	11.00	4.47	31.87	6.19	44.04	8.04	57.23
Gariep	47.66	45.49	6.09	42.98	1.41	10.00	4.68	33.01	5.99	42.30	8.07	57.01
Koonap	44.82	44.77	6.60	44.10	1.52	10.33	5.08	33.95	6.36	42.49	8.36	55.90
Matlabas	45.85	46.24	6.95	44.68	1.83	12.00	5.12	32.93	6.19	39.83	8.60	55.32
PAN3118	49.93	45.24	6.88	45.50	1.67	11.00	5.21	34.47	6.08	40.23	8.24	54.50
PAN3120	52.52	48.64	6.50	44.12	1.63	11.00	4.87	33.04	6.15	41.74	8.24	55.87
PAN3161	44.21	42.38	6.38	46.33	1.50	10.67	4.88	35.45	5.51	40.04	7.40	53.67
PAN3195	49.42	48.21	6.97	45.82	1.71	11.00	5.25	34.56	6.04	39.80	8.23	54.18
PAN3368	47.71	46.27	6.49	43.81	1.57	10.67	4.92	33.24	6.43	43.23	8.34	56.18
PAN3379	49.57	47.54	6.56	46.99	1.45	10.00	5.11	36.61	5.58	40.00	7.40	53.01
Senqu	49.20	48.70	6.35	41.55	1.45	9.33	4.90	32.06	6.90	45.22	8.92	58.45
SST316	46.97	43.39	5.87	40.60	1.49	10.33	4.38	30.26	6.47	44.73	8.59	59.40
SST317	49.39	47.52	6.24	44.67	1.63	11.67	4.61	32.99	6.29	44.95	7.74	55.33
SST347	50.57	48.11	5.95	44.15	1.49	11.00	4.46	33.09	5.87	43.54	7.52	55.85
SST356	49.25	44.51	5.61	39.37	1.47	10.00	4.14	29.09	6.47	45.41	8.64	60.63
SST387	45.41	47.78	6.82	44.75	2.06	13.33	4.77	31.42	6.32	41.70	8.38	55.25
SST398	48.23	47.75	6.18	41.89	1.81	12.00	4.36	29.58	6.68	45.31	8.57	58.11
Means	48.20	46.42	6.38	43.77	1.60	10.90	4.78	32.80	6.21	42.62	8.19	56.23
LSD	5.87	2.78	0.72	3.02	0.33	10.90	0.50	2.24	0.64	2.62	0.77	3.02
CV	8.80	4.33	8.20	5.00	15.06	12.10	7.51	4.93	7.43	4.45	6.80	3.89

LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, POL%p = absolute total polymeric proteins, POL%fp = relative total polymeric proteins, HMW%p = absolute high molecular weight, HMW%fp = relative high molecular weight, LMW%p = absolute low molecular weight, LMW%fp = relative low molecular weight, GLI%p = absolute total gliadins, GLI%fp = relative total gliadins, GGA%p = absolute total gliadins and albumen-globulins, GGA%fp = relative total gliadins and albumins-globulins, LSD = least significant difference, CV = coefficient of variation.

The largest value in each column is indicated in bold

For the HMW%p, the values obtained ranged between 2.06% (SST387) and 1.41% (Gariép), the grand mean was 1.60% (Table 4.2). Genotype contributed 41.95% for HMW%fp and 42.95% for HMW%p to variation in these characteristics (Table 4.1).

Low molecular weight proteins

The LMW%p ranged between 4.14% (SST356) and 5.25% (PAN3195) with a grand mean of 4.78%. LMW%fp varied from 29.09% (SST356) to 36.61% (PAN3379), the grand mean was 32.80% (Table 4.2). ANOVA (Table 4.1) showed highly significant differences ($p \leq 0.001$) for LMW%fp and ($p \leq 0.05$) for LMW%p among genotypes. PAN3195 had the highest value for LMW%p followed by PAN3118 and Matlabas. SST356 had the lowest LMW%p value. PAN3379 ranked the highest, followed by PAN3161 and PAN3195 for LMW%fp. SST356 had the smallest LMW%fp value (Table 4.2). The variation contributed by genotypes was 68.66% for LMW%fp and 54.42% for LMW%p (Table 4.1).

Total gliadins

No significant differences were observed for GLI%p. Genotype showed highly significant ($p \leq 0.001$) differences for GLI%fp. Genotypes contributed 63.79% for GLI%fp and 43.18% for GLI%p to the total variation (Table 4.1). The average GLI%fp was 42.62%, and ranged between 39.80% (PAN3195) and 45.41% (SST356). GLI%p varied between 5.51% (PAN3161) and 6.90% (Senqu) with a grand mean of 6.21% (Table 4.2). SST356 was the cultivar with the highest GLI%fp content followed by SST398 and Senqu. PAN3195 had the lowest GLI%fp content. Senqu had the highest GLI%p value followed by SST398, and SST316 and SST356 (6.47). PAN3161 had the lowest GLI%p value.

Total gliadins and globulins-albumins

No significant differences were observed in GGA%p. There were significant differences ($p \leq 0.01$) in GGA%fp among the cultivars evaluated. (Table 4.1). SST356 was the cultivar with the highest GGA%fp content followed by SST316 and Senqu. PAN3379 had the lowest GGA%fp content. Senqu had the highest GGA%p value followed by SST356 and Matlabas. PAN3161 and PAN3379 had the lowest GGA%p value (Table 4.2). Genotype contributed 55.26% for GGA%fp and 45.16% for GGA%p to the total variation for these characteristics (Table 4.1). The GGA%fp values ranged between

60.63% (SST 356) and 53.01% (PAN 3379), with the grand mean of 56.23%. The grand mean was 8.19% for GGA%p and varied between 8.92% (Senqu) and 7.40% (PAN3161 and PAN3379) (Table 4.2).

4.5.3 Analysis of variance for absolute and relative proteins fractions in the cultivars of the dryland winter rainfall region

Large unextractable polymeric proteins

ANOVA revealed significant differences ($p \leq 0.05$) in LUPP among the cultivars (Table 4.1). SST056 had the highest LUPP value followed by SST087 and Kwartel. Ratel (46.09%) had the smallest LUPP value (Table 4.3). A large amount of variation was due to genotypes (57.39%) (Table 4.1). The LUPP ranged from 46.09% (Ratel) to 59.27% (SST056) with a grand mean of 52.63% (Table 4.3).

Unextractable polymeric proteins

There were significant differences ($p \leq 0.05$) in UPP among cultivars. Genotypes contributed 66.52% to the total variation (Table 4.1). SST087 was the cultivar with the highest UPP content followed by SST056 and Kwartel. Ratel had the lowest UPP content. The mean values ranged between 48.74% (Ratel) and 58.17% (SST087) and grand mean was 53.33% (Table 4.3).

Total polymeric proteins

There were no significant differences in POL%p and POL%fp among cultivars (Table 4.1). Tankwa was the cultivar with the highest POL%fp content followed by SST047 and SST088. The average was 44.00% for POL%fp and varied between 39.71% (SST087) and 48.85% (Tankwa). SST047 had the highest POL%p value followed by Tankwa and Kwartel. POL%p varied between 6.73% (SST047) and 4.88% (SST087). The grand mean was 5.73% (Table 4.3). Genotypes contributed 79.42% for POL%fp and 45.12% for POL%p to the total variation (Table 4.1).

Table 4.3. Means for absolute and relative protein fractions of the dryland winter rainfall region cultivars

Cultivar	LUPP (%)	UPP (%)	POL%p	POL%fp	HMW%p	HMW%fp	LMW%p	LMW%fp	GLI%p	GLI%fp	GGA%p	GGA%fp
Kwartel	54.80	54.69	6.38	44.42	1.77	12.33	4.61	32.12	5.93	41.33	7.98	55.58
PAN3408	54.19	54.50	5.49	43.99	1.45	11.67	4.04	32.35	5.11	40.96	6.99	56.01
PAN3471	50.09	54.17	5.68	44.36	1.61	13.00	4.07	31.76	5.39	42.08	7.13	55.64
Ratel	46.09	48.74	4.94	41.25	1.31	11.00	3.64	30.35	5.46	45.60	7.04	58.75
SST015	49.33	50.74	5.81	44.34	1.61	12.33	4.20	32.03	5.42	41.35	7.29	55.66
SST027	53.40	53.61	5.85	42.93	1.57	11.67	4.28	31.41	6.05	44.45	7.78	57.07
SST047	52.63	53.08	6.73	46.27	1.90	13.00	4.83	33.23	5.83	40.10	7.80	53.73
SST056	59.27	55.01	5.33	43.33	1.78	15.00	3.55	28.60	4.92	40.31	6.95	56.67
SST087	56.31	58.17	4.88	39.71	1.55	12.67	3.33	27.13	5.43	44.16	7.42	60.29
SST096	52.14	52.05	5.31	43.53	1.67	14.00	3.64	29.84	5.37	44.11	6.88	56.47
SST088	52.30	51.61	5.74	45.05	2.12	16.67	3.62	28.36	5.36	42.03	7.01	54.95
Tankwa	51.03	53.60	6.62	48.85	1.78	13.00	4.84	35.73	5.24	38.47	6.96	51.15
Mean	52.63	53.33	5.73	44.00	1.68	13.03	4.05	31.08	5.46	42.08	7.27	56.00
LSD	4.93	2.83	1.03	7.12	0.34	2.73	0.86	6.14	0.78	4.77	1.16	7.12
CV	6.68	3.78	12.84	11.54	14.55	14.93	15.07	14.10	10.19	8.08	11.41	9.07

LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, POL%p = absolute total polymeric proteins, POL%fp = relative total polymeric proteins, HMW%p = absolute high molecular weight, HMW%fp = relative high molecular weight, LMW%p = absolute low molecular weight, LMW%fp = relative low molecular weight, GLI%p = absolute total gliadins, GLI%fp = relative total gliadins, GGA%p = absolute total gliadins and albumen-globulins, GGA%fp = relative total gliadins and albumins-globulins, LSD = least significant difference, CV = coefficient of variation.

The largest value in each column is indicated in bold

High molecular weight proteins

Genotypes contributed 52.87% for HMW%p and 48.49% for HMW%fp to total variation. No significant differences were observed in HMW%fp. For the HMW%p significant differences ($p \leq 0.05$) were seen between genotypes (Table 4.1). SST088 was the cultivar with the highest HMW%fp content followed by SST056 and SST096. Ratel had the lowest HMW%fp content. For the HMW%p SST088 ranked the highest followed by SST047, Tankwa and SST056. Ratel ranked the lowest (Table 4.3). The grand mean was 13.03% and varied between 11.00% (Ratel) and 16.67% (SST088) for the HMW%fp. The HMW%p varied between 2.12% (SST088) and 1.31% (Ratel) with a mean value of 1.68% (Table 4.3).

Low molecular weight proteins

LMW%p varied between 3.33% (SST087) and 4.84% (Tankwa). The grand mean was 4.05%. The average for LMW%fp was 31.08% and ranged between 27.13% (SST087) and 35.73% (Tankwa). Tankwa ranked the highest for both fractions and was followed by SST047 as second highest for both LMW%fp and LMW%p fractions. SST087 had the lowest LMW%fp and LMW%p values. (Table 4.3) There were no significant differences in LMW%fp and LMW%p between cultivars. Genotypes contributed 28.27% for LMW%fp and LMW%p 46.00% to the total variation (Table 4.1).

Total gliadins

Cultivar SST027 ranked the highest followed by Kwartel and SST047 for GLI%p. SST056 had the lowest GLI%p value. Ratel was the cultivar with the highest GLI%fp content followed by SST027 and SST087. Tankwa was the cultivar with the lowest GLI%fp content (Table 4.3). Genotypes contributed 33.42% for GLI%fp and GLI%p 32.74% to the total variation. There were no significant differences between cultivars for GLI%fp and GLI%p (Table 4.1). The average GLI%fp was 42.08% and ranged between 38.47% (Tankwa) and 45.60% (Ratel) while the GLI%p varied between 4.92% (SST056) and 6.05% (SST027), with a grand mean of 5.46% (Table 4.3).

Total gliadins and albumins-globulins

The GGA%p ranged from 6.88% (SST096) to 7.98% (Kwartel) with a grand mean of 7.27%. The GGA%fp content ranged between 51.15% (Tankwa) and 60.29% (SST087). The grand mean was 56.00%. (Table 4.3). No significant differences were seen in

GGA%fp and GGA%p among cultivars. Genotypes contributed 22.09% for GGA%fp and 22.91% for GGA%p to total variation (Table 4.1). SST087 was the cultivar with the highest GGA%fp content followed by Ratel and SST027. Tankwa had the lowest GGA%fp content. Kwartel had the highest GGA%p value followed by SST047 and SST027, and SST096 ranked the lowest (Table 4.3).

4.5.4 Analysis of variance for absolute and relative proteins fractions in the cultivars of the irrigated rainfall region

Large unextractable polymeric proteins

ANOVA revealed highly significant differences ($p \leq 0.001$) in LUPP content among cultivars. Variation due to the genotypes was 70.57% (Table 4.1). PAN3497 had the highest amount of LUPP followed by SST843 and PAN3489. SST876 had the smallest amount of LUPP. The grand mean was 44.65% with values ranging between 29.07% (SST876) and 54.70% (PAN3497) (Table 4.4).

Unextractable polymeric proteins

There were highly significant differences in UPP ($p \leq 0.001$) between cultivars (Table 4.1). The UPP content ranged between 40.86% (SST876) and 57.77% (PAN3497), the grand mean was 50.36%. PAN3497 had the highest amount of UPP followed by SST843 and Buffels. SST876 had the lowest UPP value (Table 4.4). A large amount of variation was due to genotypes (70.57%) (Table 4.1).

Total polymeric proteins

There were highly significant differences ($p \leq 0.001$) for POL%fp and POL%p between cultivars (Table 4.1). SST867 had the highest POL%fp followed by SST875 and Umlazi. PAN3497 had the lowest POL%fp content. For POL%p, SST843, ranked the highest followed by SST822 and Duzi. PAN3497 had the lowest POL%p value (Table 4.4). Variation was largely due to the genotypes for both POL%fp (93.80%) and POL%p (78.75%) (Table 4.1). POL%fp ranged between 48.85% (SST867) and 41.35% (PAN3489), the grand mean was 44.59%. The grand mean for POL%p was 5.07%. The POL%p value varied between 6.73% (SST 843) and 4.44% (PAN3497) (Table 4.4).

Table 4.4 Means for absolute and relative protein fractions of the irrigated region cultivars

Cultivar	LUPP	UPP	POL%p	POL%fp	HMW%p	HMW%fp	LMW%p	LMW%fp	GLI%p	GLI%fp	GGA%p	GGA%fp
Buffels	51.40	54.55	4.53	42.49	1.01	9.67	3.57	33.52	4.52	45.05	6.13	57.51
Duzi	39.42	46.58	5.39	45.02	1.82	15.33	3.67	30.50	3.85	44.77	6.59	54.98
Krokodil	45.38	48.49	4.57	42.15	1.06	10.00	3.48	32.10	4.72	43.87	6.28	57.85
PAN3471	51.79	53.39	4.76	43.58	0.99	9.00	3.74	34.19	4.67	43.52	6.16	56.43
PAN3478	50.60	52.30	5.13	46.20	1.25	11.00	3.87	34.94	4.39	43.39	5.97	53.80
PAN3489	53.63	53.00	4.48	41.35	1.16	10.67	3.29	30.35	4.86	42.77	6.36	58.65
PAN3497	54.70	57.77	4.44	40.95	1.05	10.00	3.45	31.84	4.70	42.37	6.39	59.05
Sabie	50.75	52.19	5.07	43.17	1.27	11.00	3.82	32.56	5.28	40.70	6.67	56.83
SST806	49.91	51.27	5.22	46.09	1.34	11.67	3.93	34.67	3.91	40.44	6.11	53.91
SST822	51.70	52.71	5.78	42.80	1.52	11.00	4.15	30.72	5.92	40.24	7.73	57.20
SST835	39.51	47.10	5.05	45.76	1.10	10.00	3.95	35.77	4.27	40.17	5.99	54.23
SST843	54.11	57.50	6.73	45.66	1.67	11.67	4.97	33.74	5.93	39.57	8.01	54.34
SST866	42.90	49.53	5.04	44.61	1.13	10.00	4.16	36.79	4.47	39.54	6.26	55.39
SST867	38.22	45.10	5.07	48.85	0.94	9.00	3.97	38.31	3.67	39.26	5.30	51.15
SST875	36.88	49.11	5.34	46.77	0.95	8.00	4.49	39.32	4.2	38.67	6.08	53.23
SST876	29.07	40.86	5.17	44.70	1.04	9.00	4.09	35.44	4.54	37.45	6.39	55.30
SST877	29.32	43.30	4.74	43.06	0.86	8.00	3.75	34.07	4.43	36.85	6.27	56.94
SST884	36.18	49.28	4.89	44.50	0.89	8.00	3.93	35.83	4.45	36.72	6.11	55.50
SST895	48.78	53.56	5.22	44.66	1.20	10.00	4.12	35.19	4.76	36.47	6.47	55.34
Tamboti	44.62	49.90	4.89	45.48	1.11	10.00	3.82	35.55	4.03	35.38	5.86	54.51
Timbavati	39.50	48.57	4.85	46.51	0.86	8.00	4.00	38.41	3.80	34.48	5.57	53.49
Umlazi	43.99	51.97	5.15	46.64	0.95	8.67	3.53	32.25	4.07	31.64	5.89	53.35
Mean	44.65	50.36	5.07	44.59	1.14	9.99	4.4	34.37	4.52	39.7	6.3	55.41
LSD	7.27	4.47	0.42	0.85	0.29	2.74	0.58	4.62	0.54	2.62	0.50	0.85
CV	11.85	6.47	6.07	1.38	18.48	19.95	10.82	9.80	8.63	4.80	5.73	1.12

LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, POL%p = absolute total polymeric proteins, POL%fp = relative total polymeric proteins, HMW%p = absolute high molecular weight, HMW%fp = relative high molecular weight, LMW%p = absolute low molecular weight, LMW%fp = relative low molecular weight, GLI%p = absolute total gliadins, GLI%fp = relative total gliadins, GGA%p = absolute total gliadins and albumen-globulins, GGA%fp = relative total gliadins and albumins-globulins, LSD = least significant difference, CV = coefficient of variation.

The largest value in each column is indicated in bold

High molecular weight proteins

There were highly significant differences for HMW%p ($p \leq 0.001$) and significant differences ($p \leq 0.05$) for HMW%fp (Table 4.1). Duzi ranked the highest followed by SST843 for both HMW%fp and HMW%p. SST806 ranked third for HMW%fp, while SST822 ranked third for HMW%p (Table 4.4). Genotypes contributed 50.14% for HMW%fp and 62.77% for HMW%p to the total variation in these characteristics (Table 4.1). For HMW%fp, the grand mean was 9.99%, average values ranged between 8.00% and 15.33%. The HMW%p ranged from 1.82% (Duzi) to 0.86% (Timbavati and SST877), the grand mean was 1.14% (Table 4.4).

Low molecular weight proteins

Significant differences between genotypes were observed ($p \leq 0.05$) in LMW%p. No significant differences were observed in LMW%fp between cultivars. (Table 4.1). SST843 had the highest LMW%p value followed by SST875 and SST866. PAN3489 had the lowest LMW%p value. For the LMW%fp, SST875 ranked the highest followed by Timbavati and SST867. PAN3489 ranked the lowest (Table 4.4). Genotypes contributed 44.91% for LMW%fp and 52.86% for LMW%p to the total variation (Table 4.1) The LMW%fp content varied between 30.35% (PAN3489) to 39.32% (SST875) with a grand mean of 34.37%. The average values for LMW%p varied between 3.29% (PAN3489) and 4.97% (SST843). The grand mean was 4.40%. (Table 4.4).

Total gliadins

There were highly significant differences ($p \leq 0.001$) between genotypes for GLI%p and GLI%fp. (Table 4.1). Buffels had the highest amount of GLI%fp followed by Duzi and Krokodil. Umlazi had the lowest GLI%fp value. For GLI%p, SST843 ranked the highest followed by SST822 and Sabie. SST867 had the lowest GLI%p value (Table 4.4). Genotypes contributed 82.60% for GLI%fp and 77.24% for GLI%p to the total variation (Table 4.1). The average values for GLI%fp ranged between 31.64% (Umlazi) and 45.05% (Buffels), the grand mean was 39.70%. The GLI%p had a grand mean of 4.52%, with the average values ranging between 3.67% (SST867) and 5.93% (SST843) (Table 4.4).

Total gliadins and albumins-globulins

Highly significant differences ($p \leq 0.001$) were observed in GGA%p and GGA%fp among genotypes (Table 4.1). The grand mean was 6.30% for GGA%p, with the means ranging from 5.30% (SST867) to 8.01% (SST843). The GGA%fp content ranged from 51.15% (SST867) to 59.05% (PAN3497), the mean was 55.41%. PAN3497 was the cultivar with highest GGA%fp content, followed by PAN3489 and Krokodil. SST867 had the smallest value. SST843 had the highest GGA%p value followed by SST822 and Sabie. SST867 had the lowest GGA%p value (Table 4.4). Much variation was due to the effect of genotypes (93.80%) for GGA%fp and 79.91% for GGA%p. (Table 4.1).

4.5.5 Significant correlations between protein fractions and wheat quality characteristics of the dryland summer rainfall, dryland winter rainfall and irrigated regions' cultivars

Dryland summer rainfall cultivars

Many of the protein fractions significantly negatively correlated with quality characteristics for the dryland summer rainfall cultivars (Table 4.5). LUPP correlated significantly negatively with alvW. UPP negatively correlated with alvW and alvP. UPP positively correlated with WGC. POL%fp was negatively associated with FY and POL%p positively correlated with FPC, alvW, alvL and WGC. POL%p negatively correlated with LV12.

HMW%fp negatively correlated with SDSVOL and MPT, HMW%p correlated positively with FPC and WGC, and negatively correlated with LV12. Highly significant positive correlations were found between LMW%fp and FY as well as LMW%p and FPC. Significant positive correlations were found between LMW%p and BFY and highly significant negative association was found with LV12.

GLI%fp negatively correlated with FY. GLI%p showed significant positive correlations with FPC and WGC, and correlated negatively with FY and LV12. GGA%fp negatively correlated with FY, GGA%p correlated positively with FPC and MPT and negatively with FY and LV12.

Table 4.5 Significant correlations between protein fractions and quality characteristics in the three production areas' cultivars

Dryland summer rainfall			Irrigated region			Dryland winter rainfall		
Character1	Character2	Correlations	Character1	Character2	Correlations	Character1	Character2	Correlations
LUPP	alvW	-0.279*	LUPP	alvW	0.458***	LUPP	LV12	0.329**
UPP	alvW	-0.404**		alvP	0.384**	UPP	BFY	-0.471*
	alvP	-0.309*		alvP/L	0.298*	POL%p	FPC	0.702***
	WGC	0.293*		SDSVOL	0.240*		BFY	0.361*
POL%fp	FY	-0.418*		MPT	0.315*		LV12	-0.434**
POL%p	FPC	0.741***	UPP	alvW	0.532***		WGC	0.344*
	alvW	0.336*		alvP	0.489**		MPT	0.421**
	alvL	0.294*		alvP/L	0.409***	HMW%p	FPC	0.375*
	LV12	-0.688***		MPT	0.411***	LMW%p	FPC	0.683***
	WGC	0.308*	POL%p	FPC	0.913***		BFY	0.416**
HMW%fp	SDSVOL	-0.286*		BFY	-0.448***		WGC	0.393*
	MPT	-0.323*		FY	-0.583***		MPT	0.381*
HMW%p	FPC	0.552***		alvW	0.647***	GLI%p	FPC	0.598***
	LV12	-0.553***		alvP	0.599***		BFY	0.354*
	WGC	0.280*		LV	0.617***		WGC	0.341*
LMW%fp	FY	0.483***		WGC	0.671***	GGA%p	FPC	0.608***
LMW%p	FPC	0.647***		MPT	0.523***		LV12	-0.344*
	BFY	0.330*	HMW%p	FPC	0.583***		WGC	0.341*
	LV12	-0.577***		FY	-0.304*			
GLI%fp	FY	-0.396**		alvW	0.355**			
GLI%p	FPC	0.700***		alvP	0.290*			
	FY	-0.410**		LV	0.276*			
	LV12	-0.557***		LV12	-0.251*			
	WGC	0.319*		WGC	0.456***			
GGA%fp	FY	-0.418**		MPT	0.31**			
GGA%p	FPC	0.807***	LMW%fp	WGC	-0.249**			
	FY	-0.410**	LMW%p	FPC	0.572***			
	LV12	-0.640***		BFY	-0.453***			
	MPT	0.296*						

LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, POL%fp = relative total polymeric proteins, POL%p = absolute total polymeric proteins, HMW%fp = relative high molecular weight, HMW%p = absolute high molecular weight, LMW%fp = relative low molecular weight, LMW%p = absolute low molecular weight, GLI%fp = relative total gliadins, GLI%p = absolute total gliadins, GGA%fp = relative total gliadins and albumins-globulins, GGA%p = absolute total gliadins and albumen-globulins, BFY=break flour yield, FY=flour yield, FPC=flour protein content, WGC= wet gluten content, SDSVOL=sodium dodecyl sulphate sedimentation volume, MPT = mixograph peak time, alvP = alveograph stability, alvL= alveograph extensibility, alvP/L = alveograph extensibility to stability ratio, alvW = alveograph strength, LV = loaf volume, LV12 = loaf volume adjusted to 12% protein basis, *= $p \leq 0.05$, **= $p \leq 0.01$, ***= $p \leq 0.001$

Table 4.5 (continued)

Dryland summer rainfall			Irrigated region			Dryland Summer rainfall		
Character1	Character2	Correlations	Character1	Character2	Correlations	Character1	Character2	Correlations
			LMW%p	FY	-0.417***			
				alvW	0.512***			
				alvP	0.484***			
				LV	0.401**			
				WGC	0.370**			
				MPT	0.384*			
			GLI%fp	FY	-0.029*			
			GLI%p	FPC	0.772***			
				BFY	-0.393***			
				FY	-0.654***			
				alvW	0.626***			
				alvP	0.551***			
				LV	0.499***			
				WGC	0.596***			
				MPT	0.349***			
			GGA%p	FPC	0.940***			
				BFY	-0.419***			
				alvW	0.666***			
				alvP	0.557***			
				LV	0.582***			
				WGC	0.750***			
				MPT	0.423***			

LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, POL%fp = relative total polymeric proteins, POL%p = absolute total polymeric proteins, HMW%fp = relative high molecular weight, HMW%p = absolute high molecular weight, LMW%fp = relative low molecular weight, LMW%p = absolute low molecular weight, GLI%fp = relative total gliadins, GLI%p = absolute total gliadins, GGA%fp = relative total gliadins and albumins-globulins, GGA%p = absolute total gliadins and albumen-globulins, BFY=break flour yield, FY=fLOUR yield, FPC=fLOUR protein content, WGC= wet gluten content, SDSVOL=sodium dodecyl sulphate sedimentation volume, MPT = mixograph peak time, alvP = alveograph stability, alvL= alveograph extensibility, alvP/L = alveograph extensibility to stability ratio, alvW = alveograph strength, LV = loaf volume, LV12 = loaf volume adjusted to 12% protein basis, *= $p \leq 0.05$, **= $p \leq 0.01$, ***= $p \leq 0.00$

Dryland winter rainfall cultivars

Significant correlations between protein fractions and quality characteristics were limited for the dryland winter rainfall region cultivars (Table 4.5). LUPP significantly positively correlated with LV12 and UPP significantly negatively correlated with BFY. No significant correlations were found between protein subunits in POL%fp and quality characteristics. POL%p correlated positively with FPC, BFY, WGC and MPT, and correlated negatively with LV12.

HMW%p correlated positively with FPC. LMW%p positively correlated with FPC, BFY, WGC and MPT. GLI%p correlated positively with FPC, BFY and WGC. GGA%p positively correlated with FPC and WGC, and negatively correlated LV12.

Irrigated region cultivars

Highly significant positive correlations were seen between most characteristics in the irrigated region (Table 4.5). LUPP correlated positively with alvW, alvP, alvP/L SDSVOL and MPT. UPP correlations were highly positive with alvW, alvP, alvP/L and MPT. POL%p correlated positively with FPC, alvW, alvP, LV, WGC and MPT, and negatively correlated with BFY and FY.

The HMW%p correlated positively with FPC, alvW, alvP, LV, WGC and MPT but correlated negatively with FY and LV12. LMW%fp correlated negatively with WGC. The LMW%p correlated positively with FPC, alvW, alvP, LV, WGC and MPT but correlated negatively with BFY and FY. GLI%fp correlated negatively with FY. The GLI%p correlated positively with FPC, alvW, alvP, LV, WGC and MPT. The GLI%p correlated negatively with BFY and FY (Table 4.5). The GGA%p positively correlated with FPC, alvW, alvP, LV, WGC and MPT, and negatively with BFY.

4.5.6 Principal component analysis

Dryland summer rainfall region cultivars

Principle components with eigenvalues greater than 1 were considered significant. The first four principle components had eigenvalues greater than 1 (Table 4.6). The cumulative variation explained by these four principle components was 98.60%. The cut off point was set for 0.3 for loading values. PC1 accounted for 66.43% of the variation. Eigenvectors for PC1 carried large positive weights for alvP and LV and negative

weights for alvL. PC2 explained 20.85% of the total variation. Eigenvectors for PC2 carried large positive weights for LV, alvW and alvL. PC3 accounted for 10.17% of the total variation. In PC3 alvP and alvW carried large positive weights while LV carried a large negative weight. PC4 accounted for 1.15% of the total variation and eigenvectors for PC4 carried positive weights for POL%fp and LMW%fp, while GGA%fp, GLI%fp and alvW carried large negative weights.

Table 4.6 Loadings of principal component analysis for the measured characteristics for the dryland summer rainfall region cultivars

Characteristics	PC1	PC2	PC3	PC4
FPC	0.00	0.00	0.01	-0.02
GGA%p	0.00	0.00	-0.01	-0.08
GGA%fp	0.03	-0.03	-0.09	-0.48
GLI%fp	0.02	-0.04	-0.12	-0.38
GLI%p	0.00	0.00	-0.01	-0.06
HMW%p	0.00	0.00	0.00	0.00
HMW%fp	-0.01	0.01	-0.01	0.02
alvL	-0.47	0.76	0.20	0.00
LMW%p	0.00	0.00	0.02	0.06
LMW%fp	-0.01	0.03	0.10	0.45
LUPP	-0.02	-0.03	-0.09	0.20
LV	0.75	0.55	-0.37	0.07
alvP/L	0.01	-0.01	0.01	0.00
MPT	0.01	0.00	0.00	-0.04
alvP	0.45	-0.18	0.70	0.04
POL%p	0.00	0.01	0.02	0.06
POL%fp	-0.03	0.03	0.09	0.48
UPP	-0.02	-0.02	-0.06	0.23
alvW	0.11	0.29	0.53	-0.28
Eigenvalue	33.50	18.30	12.10	3.00
Percentage variation	66.43	20.85	10.17	1.15
Cumulative variation	66.43	87.28	97.45	98.60

LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, POL%fp = relative total polymeric proteins, POL%p = absolute total polymeric proteins, HMW%fp = relative high molecular weight, HMW%p = absolute high molecular weight, LMW%fp = relative low molecular weight, LMW%p = absolute low molecular weight, GLI%fp = relative total gliadins, GLI%p = absolute total gliadins, GGA%fp = relative total gliadins and albumins-globulins, GGA%p = absolute total gliadins and albumen-globulins, FPC=flour protein content, MPT = mixograph peak time, alvP = alveograph stability, alvL= alveograph extensibility, alvP/L = alveograph extensibility to stability ratio, alvW = alveograph strength, LV = loaf volume

The clustering (Figure 4.3) of wheat cultivars can be defined as an indication of cultivars with similar characteristics. The biplot of PC1 against PC2 depicted a close relationship between alvW, FPC, MPT and LV. LMW%p and LMW%fp, POL%fp and POL%p were also closely associated. Another close relationship was between GGA%p and alvL/P. GLI%p, GLI%fp and GGA%p were also closely associated. HMW%p was closely associated with HMW%fp.

The cultivars grouped as follows; PAN3379, PAN3118 and Gariep; SST316, PAN3161, Elands; PAN3161, Koonap and Senqu. The HMW%p correlated positively with FPC, alvW, alvP, MPT, FPC, WGC and LV but correlated negatively with LV and LV12. Cultivar Matlabas was closely associated with MPT, LV and FPC. Cultivar PAN3195 was on the extreme negative end due to its low alvP/L value. The alvW was placed at the top of PC1 while on the downside is LUPP on the extreme negative end, these two characteristics were found to be negatively correlated.

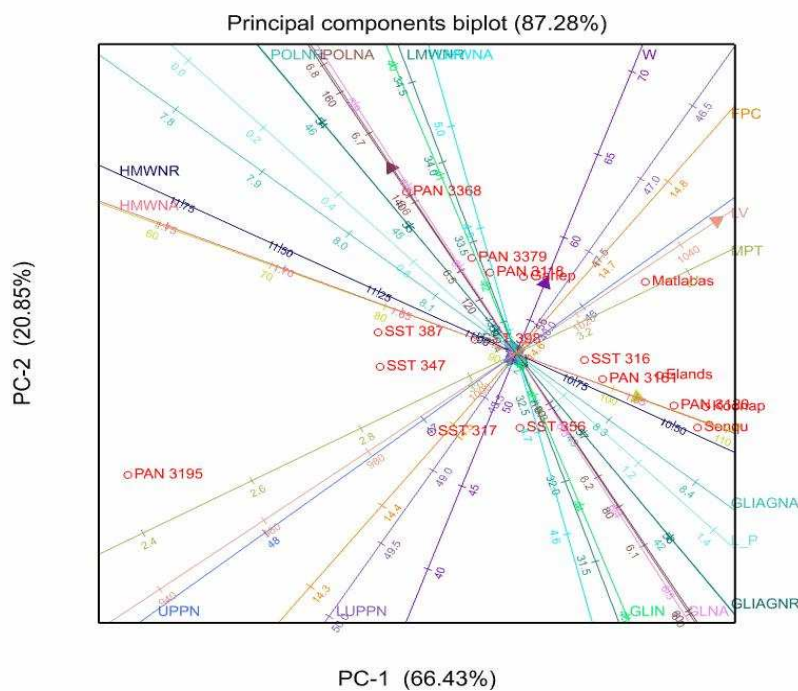


Figure 4.3 Principal component biplot for protein fractions and quality characteristics for the dryland summer rainfall region

Dryland winter rainfall region cultivars

AlvP was the characteristic that contributed positively to PC1 while alvL contributed negatively to PC1. PC1 accounted for 75.44% of the total variation. PC2 accounted for 14.32% of the total variation. Characteristics alvW, alvP and alvL carried large positive weights for PC2 while POL%fp, LMW%fp and HMW%fp carried negative weights for PC2 (Table 4.7).

Table 4.7 Loadings of principal component analysis of measured characteristics for the dryland winter rainfall cultivars

Characteristics	PC1	PC2
FPC	0.01	0.03
GGA%p	-0.01	0.03
GGA%fp	-0.08	0.11
GLI%fp	-0.03	0.07
GLI%p	0.00	0.02
HMW%p	0.00	0.00
HMW%fp	0.01	-0.05
alvL	-0.90	0.37
LMW%p	0.01	0.00
LMW%fp	0.06	-0.05
LUPP	-0.05	0.00
alvP/L	0.01	0.00
MPT	-0.01	0.03
alvP	0.42	0.78
POL%p	0.01	0.00
POL%fp	0.08	-0.11
UPP	-0.02	0.00
alvW	0.05	0.47
Eigenvalue	7.00	2.50
Percentage Variation	75.44	14.32
Cumulative variation	75.44	81.76

LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, POL%fp = relative total polymeric proteins, POL%p = absolute total polymeric proteins, HMW%fp = relative high molecular weight, HMW%p = absolute high molecular weight, LMW%fp = relative low molecular weight, LMW%p = absolute low molecular weight, GLI%fp = relative total gliadins, GLI%p = absolute total gliadins, GGA%fp = relative total gliadins and albumins-globulins, GGA%p = absolute total gliadins and albumen-globulins, FPC=flour protein content, MPT = mixograph peak time, alvP = alveograph stability, alvL= alveograph extensibility, alvP/L = alveograph extensibility to stability ratio, alvW = alveograph strength.

Three major distinct groups of cultivars could be identified on the biplot (Figure 4.4). Group 1 (Kwartel and SST015), group 2 (SST087, Ratel, SST056 and SST027) and group 3 (PAN3408 and SST088). The characteristics separated into six major groups. The first group contained GGA%fp and GLI%p, the second group comprised of GGA%p, alvW and GLI%p, the third group included FPC and P. LMW%p and alvL/P formed the fourth group. LUPP and UPP grouped together in the five groups. HMW%p, HMW%fp and POL%fp also grouped together. The characteristic GGA%p and GLI%p showed high association with alvW. SST096 was closely associated with LMW%p and alvL/P. This cultivar also had the highest alvL/P value. Cultivar Tankwa was closely related to POL%p.

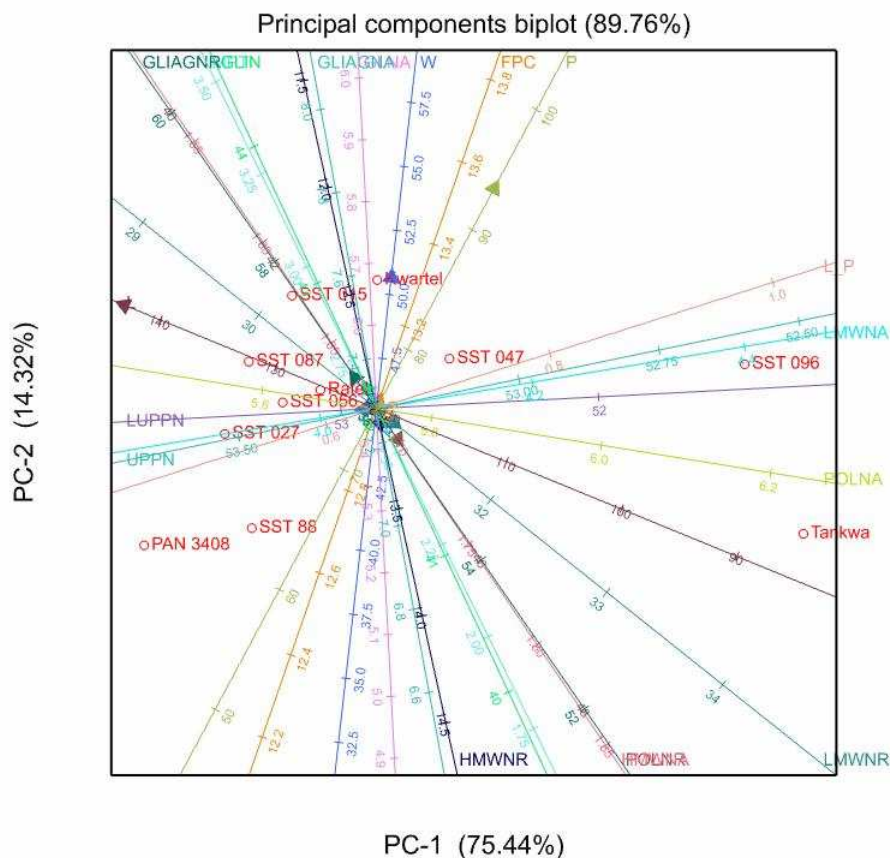


Figure 4.4 Principal components biplot for protein fractions and quality characteristics for cultivars of the dryland winter rainfall region

Irrigated region

The characteristic that contributed most to PC1 was LV, while alvP contributed positively to PC2 and alvL contributed negatively to PC2. AlvL, LUPP, alvP, UPP and alvW contributed positively to PC3. AlvL, alvP and alvW made a positive contribution towards PC4 while LUPP contributed negatively towards PC4. PC1, PC2, PC3 and PC4 contributed 76.91%, 18.25%, 3.05% and 1.09% to the total variation respectively.

Table 4.8 Loadings of principal component analysis of the measured characteristics for the irrigated region cultivars

Characteristics	PC1	PC2	PC3	PC4
FPC	-0.01	-0.01	0.05	-0.02
GGA%p	0.01	0.01	0.02	0.01
GGA%fp	0.00	0.00	0.08	-0.11
GLI%fp	-0.02	-0.01	0.22	-0.08
GLI%p	0.01	0.01	0.02	-0.01
HMW%fp	0.00	-0.01	0.09	0.01
alvL	-0.03	-0.86	0.36	0.33
LMW%p	0.00	0.01	-0.01	0.03
LMW%fp\	-0.01	0.01	-0.12	0.15
LUPP	0.03	0.08	0.65	-0.53
LV	0.98	-0.12	-0.10	-0.09
alvP/L	0.00	0.01	0.00	0.01
alvP	0.14	0.45	0.37	0.57
POL%fp	0.00	0.00	-0.08	0.11
UPP	0.03	0.06	0.31	-0.24
POL%p	0.01	0.01	0.00	0.03
alvW	0.10	0.19	0.34	0.40
HMW%p	0.00	0.00	0.01	0.01
Eigenvalue	7.00	8.00	9.00	10.00
Percentage variation	76.91	18.25	3.05	1.09
Cumulative variation	76.91	95.16	98.29	99.38

LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, POL%fp = relative total polymeric proteins, POL%p = absolute total polymeric proteins, HMW%fp = relative high molecular weight, HMW%p = absolute high molecular weight, LMW%fp = relative low molecular weight, LMW%p = absolute low molecular weight, GLI%fp = relative total gliadins, GLI%p = absolute total gliadins, GGA%fp = relative total gliadins and albumins-globulins, GGA%p = absolute total gliadins and albumen-globulins, FPC=fLOUR protein content, alvP = alveograph stability, alvL= alveograph extensibility, alvP/L = alveograph extensibility to stability ratio, alvW = alveograph strength, LV = loaf volume.

The first two PCs explained 95.16% of the total variation. The biplot (Figure 4.5) of PC1 against PC2 loadings revealed that UPP, LUPP, alvP, alvL/P and alvW grouped together. The rheological characteristics in this group correlated positively with UPP and LUPP. LMW%p closely associated with GLI%p. HMW%p grouped together with LV. HMW%p positively correlated with LV. GGA%fp was closely associated with FPC. The cultivars formed six distinct groups, Sabie and SST822; SST806, Timbavati and SST877; SST875, Umlazi, Tamboti and PAN3497; Buffels, SST855, Krokodil SST867 and SST876; PAN3489, STT866, PAN3479 and PAN3471, and SST895 and SST884.

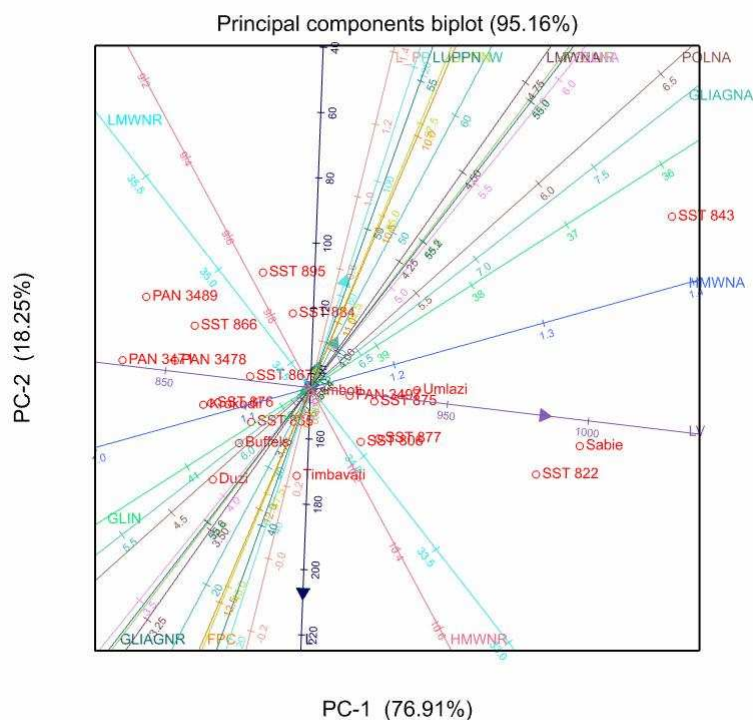


Figure 4.5 Principal component bi-plot for quality characteristics and protein fractions of the irrigated region

4.6 Discussion

The values for various rheological characteristics and protein fractions varied between the cultivars of different production areas. In the irrigated region UPP correlated positively with alvW, alvP, alvP/L and MPT. In contrast to this, in the dryland summer rainfall region, UPP showed negative correlations with alvW and alvP. No significant correlations were found between MPT, alvP/L and UPP. For the dryland winter rainfall region cultivars no significant correlations were shown between UPP and rheological characteristics. Significant positive correlations obtained in the irrigated region cultivars

between UPP and rheological characteristics are in agreement with the findings by Gupta et al. (1993), where they found positive correlations between UPP and alvW in different wheat sets and this led to the conclusion that UPP can be a reliable biochemical indicator for predicting alvW of wheat with different genetic backgrounds. Southan and MacRitchie (1999) and Edwards et al. (2007) reported similar findings that UPP positively affected rheological characteristics. This suggests that UPP have a large influence on alveogram attributes and can thus be used as a good predictor of dough strength in the irrigated region breeding programme. The POL%p correlated with alvW for the dryland summer rainfall and irrigated regions. Morel et al. (2000) reported that POL in the flour was a poorer predictor for alvW than POL in the total PC.

For the irrigated region LUPP mostly correlated with the quality characteristics alvW, alvP, alvL/P, SDSVOL and MPT. For the dryland summer rainfall location LUPP correlated negatively with alvW and alvP. Significant positive correlations between LUPP and SDSVOL were observed in this study and were also reported by Labuschagne et al. (2004). Weegels et al. (1996) stated that during the determination of SDSVOL, the unextractable glutenin adds to sedimentation and this causes the strong correlations between SDSVOL and unextractable glutenin. LUPP positively correlated with MPT, which was in agreement with findings of Koen (2006).

Significant positive associations were found between POL%p, HMW%p, LMW%p, GLI%p, GGA%p and LV in the irrigated region. Mkhadywa (2014) reported significant correlations between HMW, both in total protein and in the flour, with LV. Dong et al. (1992), Labuschagne and Aucamp (2004), and Wentzel (2010), reported significant correlations between GLI and LV, which corroborates the findings in this study. Dachkevitch and Autran (1989) reported significant correlations between F2 and LV. Uthayakumaran et al. (2001) and Khatkar et al. (2002) reported that addition of total gliadins caused increased LV. Huebner et al. (1997) reported significant positive associations between Υ - gliadins while ω , α and β gliadins correlated positively with LV. The relationship between protein fractions and LV is largely dependent on the bread-making procedure or solvents used (Hamada et al. 1982; Gupta et al. 1992). Significant correlations between protein fractions and LV was only found in the irrigated region. The dryland summer and dryland winter rainfall regions did not present any significant correlations between protein fractions and LV.

The irrigated region showed significant correlations between GLI and dough rheological characteristics. GLI%p correlated positively with alvW, alvP, and MPT. Sozinov et al. (1980) and Metakovsky et al. (1997) reported significant correlations between GLI and alvW. However Khatkar et al. (2002) reported that addition of total gliadins resulted in lower dough strength and shorter mixing times. Positive correlations between GLI%p and dough strength might be due to the LMW, as Metakovsky et al. (1997) and Gupta et al. (1989) pointed out that there is a close genetic linkage between *GLU-3* and *GLI-1*. No significant correlations were seen between GLI and dough rheological characteristics in the dryland summer rainfall and dryland winter rainfall region.

Negative correlations between HMW%fp and MPT for the dryland summer rainfall location is in agreement with Koen (2006), however Labuschagne et al. (2004) and Wentzel (2010) reported positive correlations between HMW and MPT. No link was found between POL%fp and quality characteristics in the irrigated region and dryland summer rainfall regions, indicating that POL%fp did not contribute towards dough quality.

More significant correlations were found between protein fractions in the flour and quality characteristics than protein fractions in total proteins. Flour yield did not show any significant correlations with protein fractions in the dryland winter rainfall region cultivars. In the irrigated region, FY correlated negatively with absolute values of POL%p, HMW%p, GLI%fp, GGA%p and relative amounts of GLI%fp. In the dryland summer rainfall region FY negatively associated with GLI and GGA. Significant positive correlations between GLI%p and WGC, corroborates the findings by Schober et al. (2006). Flour protein content highly positively correlated with all the absolute protein fractions. Highly significant positive correlations between FPC and absolute amount of HMW%p, LMW%p and GLI%p were also reported by Morel et al. (2000). However weak correlation existed between HMW%fp, LMW%fp and FPC. This corroborates the findings by Morel et al. (2000) where they found weak correlations between relative proteins and FPC. Correlations between FPC and absolute GLI%p and GGA%p were very high. This suggests that genotypes having high FPC will not produce high amounts of HMW and LMW. The FPC rather increased with GLI and GGA (Bean et al. 1998). The protein fractions that correlated positively with FPC also correlated positively with WGC. Similar results were found by Mkhadywa (2014). The significant correlations

between GGA%p and FPC and WGC contradicts findings of Mkhattywa (2014) but is in agreement with Morel et al. (2000). They found strong positive correlations between F5 and FPC. The HMW%p correlated with FPC. Park et al. (2006) observed significant correlations between insoluble polymeric proteins and FPC. When the polymeric proteins increased, the monomeric proteins decreased due to the build up of storage proteins (Labuschagne and Aucamp 2004).

The HMW%p in the irrigated cultivars correlated positively with alvW. This suggests that increased dough strength is due to increased HMW%p. This is in accordance with Mkhattywa (2014) where HMW polymeric proteins in proteins and in flour correlated with alvW. Morel et al. (2000) reported significant correlation between absolute insoluble F1 fractions and alvW. The HMW for both dryland summer rainfall and dryland winter rainfall region cultivars did not show any significant correlation with alveogram characteristics. LMW%p correlated positively with alvW. This is in agreement with the findings of Mkhattywa (2014). AlvW correlated with low polymeric protein for both extractable and unextractable protein in flour, indicating that LMW affects dough strength. Gupta et al. (1993) and Ciaffi et al. (1996) observed significant positive correlations between SDS-insoluble polymeric proteins and dough strength.

LMW%p correlated negatively with BFY. This corroborates findings of Mkhattywa (2014). GLI%p and GGA%p correlated positively with BFY, this corroborates findings of Mkhattywa (2014), however Wentzel (2010) reported negative association between albumin-globulins and BFY. Significant positive correlations between GLI%p and WGC were observed in all the regions, this is in agreement with Schober et al. (2006). Morel et al. (2000) indicated total gliadins (F4) to be a good indicator of FPC and this corroborates with results found in this study where GLI%p positively correlated with FPC and WGC in all three regions.

PCA reduces redundancy in data by projecting original data of interrelated variables into a small number of variables called principal components with Eigenvalues > 1 (Ahmad et al. 2014; Janmohammadi et al. 2014). The cumulative variance amounted to 98.6%, 81.76% and 89.30% for the dryland summer rainfall, dryland winter rainfall and irrigated region cultivars respectively. The PCA simplifies data and makes it easy to ascertain

what principal components represent (Martens and Martens 2001; Janmohammadi et al. 2014).

The biplot of PCs gives an overview of data. It shows which characteristics are related and which characteristics are most important in differentiating between samples (Tronsmo et al. 2003). Characteristics which are placed close to each other in the loading plot show a relationship between them in the data set (Tronsmo et al. 2003; Martens and Martens, 2001). For the irrigated region, LUPP, UPP, alvW, alvP and alvL/P grouped close to each other and these characteristics correlated positively. This indicates the influence of LUPP and UPP on dough rheological characteristics. For the winter rainfall region, FPC and alvP grouped close together. Other characteristics grouping close together were GGA%p and GLI%fp and alvW, showing that these protein fractions were closely related to these alveograph characteristics. For the dryland summer rainfall region MPT, alvW, LV and FPC grouped together, which suggested that FPC influenced dough rheological characteristics, as expected. GGA%p and alvP grouped together showing positive association.

The PCA biplots can also indicate which cultivars associate with which characteristics and also the relationship between the cultivars being tested. Cultivars exhibiting large variation in the right direction can be selected and used for the breeding programmes (Yan and Rajcan 2002). For the dryland summer rainfall region PAN3368 was closely associated with POL%p and POL%fp, while Matlabas was closely associated with MPT, LV and FPC. Matlabas contained high values for these characteristics. This confirms findings from previous studies that cultivars with higher FPC have longer mixing times (Curic et al. 2001; Mastilovic et al. 2014). PAN3195 showed an inverse relationship with LV. SST356 inversely associated with LMW%p. For the dryland winter rainfall region Kwartel showed close association with alvW, while SST096 was closely associated with LMW%p and alvL/P. Tankwa was closely associated with POL%p while Ratel and SST056 showed an inverse association with POL%p. Characteristics close to the centre of the biplot are insignificant and do not contribute much to variation.

4.7 Conclusions

The objective of this study was to relate molecular weight distribution of proteins separated using a BioSep-SEC s4000 narrow bore column by SE-HPLC, with quality

characteristics of South African medium hard to hard red wheat varieties. The absolute protein fractions highly significantly correlated with FPC in cultivars of all three production areas. The GLI%p significantly correlated with alveograph parameters. The irrigated region cultivars presented more significant correlations between protein fractions and quality data. The protein fractions separated by the narrow bore column significantly correlated with quality characteristics. The protein fractions separated by the narrow bore column had specific relationships with quality characteristics and can be used to assess quality characteristics. The dryland winter rainfall region indicated little differences between genotypes for the characteristics tested, while the irrigated programme presented more significant differences between genotypes for the characteristics tested. Genotypes contributed much to total variation of the measured characteristics in all three locations, showing that selection for most of these characteristics should be successful. It is, however, difficult to make good conclusions from single trials, and these results should be verified across locations and seasons. It will also shed light on genotype by environment interaction for measured characteristics.

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Chapter 5

Evaluation of an ultra-high resolution analytical column for its potential to improve separation of wheat protein fractions and the relationship of these fractions with wheat quality characteristics in South African bread wheat cultivars

5.1 Abstract

Wheat storage proteins play an important role in bread-making quality characteristics. This study investigated the relationship between protein fractions, measured by size exclusion-high liquid performance chromatography, using a newly released analytical Yarra-SEC 4000 column and rheological characteristics determined by alveograph and mixograph, in various bread wheat cultivars. The trials consisted of cultivars developed and grown in three different production areas of the country: dryland winter rainfall, dryland summer rainfall and irrigated regions. Absolute (%p) and relative (%fp) protein fractions showed association with dough rheology. The unextractable polymeric proteins (UPP) positively correlated with mixograph peak time (MPT) and alveograph strength (alvW) in the dryland summer region, however in the irrigated region, UPP correlated negatively with MPT and alvW. No significant correlations for these characteristics were observed in the dryland winter rainfall region. The absolute protein fraction percentages for total gliadins and total polymeric proteins showed significant correlations with rheological characteristics in the irrigated regions. The dryland winter rainfall region presented mostly negative relationships between protein fractions and quality characteristics. The absolute low molecular weight (LMW%p) and the relative low molecular weight (LMW%fp) fractions negatively associated with alvW in the dryland winter rainfall region, while in the dryland summer rainfall region LMW%p positively correlated with alvW. For both the dryland winter rainfall and irrigated region, genotype effects were significant for all protein subunits. The Yarra-SEC 4000 analytical column produced nine profile peaks. The significant differences obtained between protein fractions and their relationship with quality characteristics indicated that the Yarra-SEC 4000 analytical column is useful in the separation of wheat proteins.

5.2 Introduction

Wheat is one of the most important crops consumed by humans and can survive under a broad range of environments such as rain fed, dry and warm conditions. Production and quality of wheat vary due to environmental conditions that occur especially during the grain filling stage (DuPont and Altenbach 2003). The end-use quality of wheat flours is mainly determined by endosperm proteins. (Weegels et al. 1996). When water is added to wheat flour, flour proteins interact with water, forming a gluten network, comprises of polymeric glutenin (HMW and LMW) and monomeric gliadins that provides the visco-elastic properties needed for bread-making (Tatham and Shewry 1995; Branlard et al. 2001; Gianibelli et al. 2001).

Other factors that have been reported to influence bread-making quality include enzymatic activities and damaged starch, together with wheat proteins. They have a large influence on LV (Kuchel et al. 2006; Rozylo and Laskowski 2011). Loaf volume is considered as the final procedure in assessing bread quality in many parts of the world (Lever et al. 2005).

Efforts have been made to assess bread-making potential of wheat by investigating percentages of UPP, HMW, LMW, GLI, GGA and their ratios, determined by SE-HPLC (Dachkevitch and Autran 1989; Gupta et al. 1993; Ciaffi et al. 1996; Labuschagne and Aucamp 2004; Edwards et al. 2007). These and other studies found significant correlations between SE-HPLC protein fractions and bread-making quality (Dachkevitch and Autran 1989; Gupta et al. 1993; Ciaffi et al. 1996; Kuktaite et al. 2004; Labuschagne et al. 2004; Schober et al. 2006; Edwards et al. 2007; Ohm et al. 2009).

The use of SE-HPLC in wheat quality is difficult, time consuming and very complex (Ohm et al. 2009). However, a new wider bore, Yarra-SEC 4000 analytical column from Phenomenex® has recently been released. The anticipated benefits of using this column are distinct separation of wheat protein fractions. The Yarra-SEC 4000 column has never been used to determine protein fractions of South African commercial wheat cultivars. Therefore, the objectives of this study were to test this ultra-high resolution analytical column for its potential for improved separation of wheat protein fractions and to correlate the molecular weight distribution of these fractions with wheat quality characteristics.

5.3 Materials and methods

5.3.1 Cultivars used

See Chapter 3, Section 3.3.1.

5.3.2 Quality measurement

See Chapter 3, Section 3.3.3.

5.3.3 Size exclusion-high liquid performance liquid chromatography

An ultra-high resolution size exclusion 3 µm particle ultra-pure silica column that allows for very high efficiency and resolution of proteins was used as stationary phase in this experiment. The Yarra-SEC 4000 analytical column, operating at 30 µl 27 min⁻¹ was used with the Thermo FinniganTM Surveyor Plus (Thermo Electron, San Jose, CA) HPLC system with PDA detector, equipped with ChromQuestTM 4.2 chromatography data system for integration events.

The elution system consisted of A) deionised water + TFA (0.1%, v/v); and B) acetonitrile (ACN) (ROMIL-SpSTM acetonitrile 200 far UV) + TFA (99.9/0.1%, v/v). The linear elution gradient consisted of: 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.

Protein extraction

Proteins were extracted following a two-step procedure by Gupta et al. (1993) described in Chapter 4 (Section 4.3.3). The profiles obtained from SE-HPLC were partitioned into nine peaks (as seen in Figure 5.1), F1 contains HMW, F2-F4 contains LMW, F5-F7 GLI and F8-F9 contains GGA. These were used to calculate the different protein fractions based on percentage protein in the grain (relative/%p) and percentage proteins in the flour (absolute/%fp).

Protein fractions in total proteins (relative proteins)

Percentage of large unextractable polymeric protein

$$\text{LUPP} = [(F1 \text{ insoluble}) / (F1 \text{ soluble} + F1 \text{ insoluble})] * 100$$

Percentage unextractable polymeric proteins

UPP= [(F1 insoluble + F2 insoluble + F3 insoluble + F4 insoluble) / (F1 insoluble + F2 insoluble + F3 insoluble + F4 insoluble + F1 soluble + F2 soluble + F3 soluble + F4 soluble)]*100

Percentage high molecular weight

HMW= [(F1 insoluble + F1 soluble) / (F1 insoluble + F2 insoluble + F3 insoluble + F4 insoluble + F5 insoluble + F6 insoluble + F7 insoluble + F8 insoluble + F9 insoluble + F1 soluble + F2 soluble + F3 soluble + F4 soluble + F5 soluble + F6 soluble + F7 soluble + F8 soluble + F9 soluble)]*100

Percentage low molecular weight

LMW= [(F2 insoluble + F3 insoluble + F4 insoluble) + (F2 soluble + F3 soluble + F4 soluble) / (F1 insoluble + F2 insoluble + F3 insoluble + F4 insoluble + F5 insoluble + F6 insoluble + F7 insoluble + F8 insoluble + F9 insoluble + F1 soluble + F2 soluble + F3 soluble + F4 soluble + F5 soluble + F6 soluble + F7 soluble + F8 soluble + F9 soluble)]*100

Percentage total polymeric proteins

POL= [(F1 insoluble + F2 insoluble + F3 insoluble + F4 insoluble) + (F1 soluble + F2 soluble + F3 soluble + F4 soluble) / (F1 insoluble + F2 insoluble + F3 insoluble + F4 insoluble + F5 insoluble + F6 insoluble + F7 insoluble + F8 insoluble + F9 insoluble + F1 soluble + F2 soluble + F3 soluble + F4 soluble + F5 soluble + F6 soluble + F7 soluble + F8 soluble + F9 soluble)]*100

Percentage total gliadins

GLI= [(F5 insoluble + F6 insoluble + F7 insoluble) + (F5 soluble + F6 soluble + F7 soluble) / (F1 insoluble + F2 insoluble + F3 insoluble + F4 insoluble + F5 insoluble + F6 insoluble + F7 insoluble + F8 insoluble + F9 insoluble + F1 soluble + F2 soluble + F3 soluble + F4 soluble + F5 soluble + F6 soluble + F7 soluble + F8 soluble + F9 soluble)]*100

Percentage total gliadins and globulins-albumins

GGA= [(F5 soluble + F6 soluble + F7 soluble + F8 soluble + F9 soluble) + (F5 insoluble + F6 insoluble + F7 insoluble + F8 insoluble + F9 insoluble) / (F1 insoluble + F2 insoluble + F3 insoluble + F4 insoluble + F5 insoluble + F6 insoluble + F7 insoluble + F8 insoluble + F9 insoluble + F1soluble + F2 soluble + F3 soluble + F4 soluble + F5 soluble + F6 soluble + F7 soluble + F8 soluble + F9 soluble)]*100

Protein fractions in flour proteins

Absolute values were calculated as percentage of protein subunits multiplied by percentage flour protein divided by 100.

5.4 Statistical analysis

Agrobase Generation II (Agrobase 2015) was used for analysis of variance, correlations and GenStat (Payne et al. 2008) was used to perform principal component analysis.

5.5 Results

5.5.1 Size exclusion-high performance liquid chromatography

Figure 5.1 and 5.2 indicate examples of sonicated and non-sonicated SE-HPLC profiles

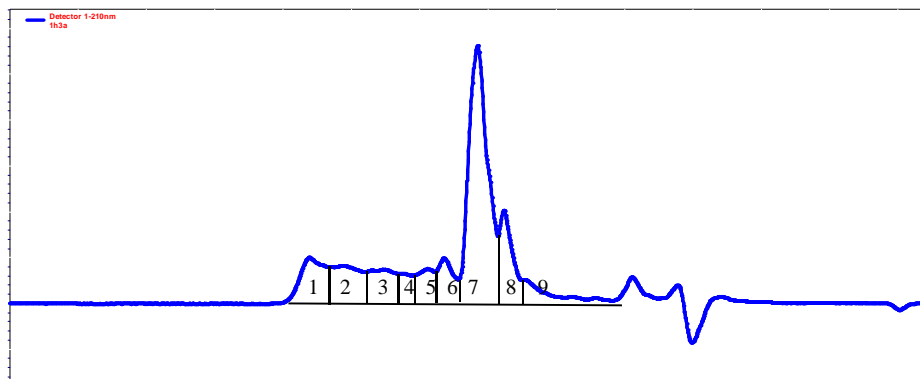


Figure 5.1. SE-HPLC profile for the non-sonicated protein fractions

1=HMW, 2-4=LMW, 5-7=GLI, 8-9=globulins-albumins

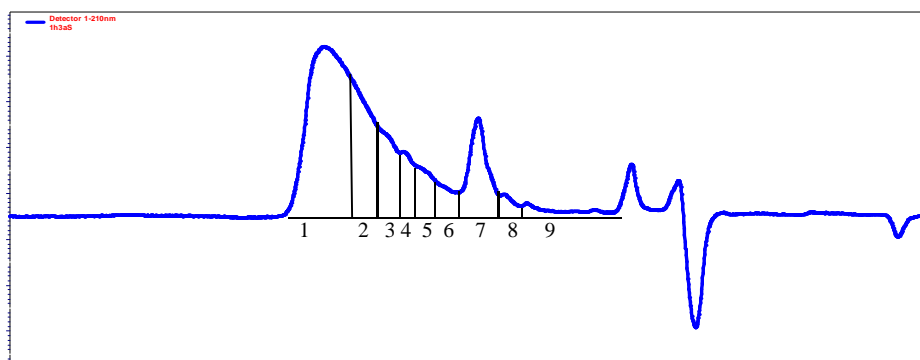


Figure 5.2. SE-HPLC profile for the sonicated protein fractions

1=HMW, 2-4=LMW, 5-7=GLI, 8-9=globulins-albumins

5.5.2 Analysis of variance for absolute and relative protein fractions in the cultivars of the dryland summer rainfall region

Large unextractable polymeric proteins

No significant difference was found in LUPP among the cultivars (Table 5.1). The average value for LUPP was 68.68% and varied between 51.39% (SST317) and 81.28% (SST316). SST316 ranked the highest for LUPP, followed by Senqu and Koonap. SST317 had the lowest LUPP value (Table 5.2). Genotypes contributed 42.99% (Table 5.1) to the variation of this fraction.

Unextractable polymeric proteins

Genotypes revealed highly significant ($p \leq 0.001$) differences for UPP (Table 5.1). SST316 had the highest UPP value followed by SST356 and SST347 and SST317 ranked the lowest (Table 5.2). Genotypes contributed 63.74% (Table 5.1) of the total variation for this fraction. The UPP varied between 50.82% (SST317) to 63.12% (SST316). The mean was 57.94% (Table 5.2).

Total polymeric proteins

There was no significant difference in POL%p and POL%fp among the genotypes. Genotype contributed 45.82% to POL%fp and 42.64% to POL%p, respectively (Table 5.1). The POL%p ranged from 7.08% (PAN3118) to 5.39% (PAN3379), the mean was 6.47%. The POL%fp ranged from 38.52% (PAN3379) to 50.36% (PAN3161).

Table 5.1 Analysis of variance for protein fractions in flour for all three production areas

Regions	Absolute (proteins in total proteins)				Relative (proteins in flour)					
	Characteristics	Rep	Cultivar	Contribution of genotype SS to total SS	R ²	Characteristics	Rep	Cultivar	Contribution of genotype SS to total SS	R ²
Dryland summer rainfall region	LUPP	49.53	125.15 ^{NS}	42.99	0.45					
	UPP	3.16	30.20 ^{***}	63.74	0.65					
	POL%p	0.8	0.69 ^{NS}	42.64	0.49	POL%fp	7.78	23.17 ^{NS}	45.82	0.48
	HMW%p	0.05	0.09 ^{NS}	35.84	0.38	HMW%fp	2.61	5.25 ^{NS}	38.76	0.41
	LMW%p	0.73	0.66 [*]	48.66	0.55	LMW%fp	11.92	21.19 [*]	51.76	0.55
	GLI%p	0.37	4.17 ^{NS}	82.44	0.83	GLI%fp	22.23	187.76 ^{***}	86.29	0.88
	GGA%p	0.137	0.77 ^{NS}	43.69	0.45	GGA%fp	7.78	23.17 ^{NS}	45.82	0.48
Dryland winter rainfall region	LUPP	170.94	93.65 [*]	46.21	0.62					
	UPP	71.95	79.07 [*]	49.5	0.56					
	POL%p	0.80	1.70 ^{***}	68.73	0.75	POL%fp	7.57	76.70 ^{***}	22.09	0.81
	HMW%p	0.01	0.08 ^{***}	87.54	0.90	HMW%fp	1.00	2.94 ^{***}	82.91	0.88
	LMW%p	0.64	1.16 ^{**}	63.96	0.70	LMW%fp	5.55	56.59 ^{**}	75.99	0.77
	GLI%p	0.51	1.76 ^{***}	86.11	0.91	GLI%fp	1.32	98.27 ^{***}	91.5	0.92
	GGA%p	0.75	2.18 ^{***}	72.61	0.29	GGA%fp	7.57	76.70 ^{***}	79.42	0.81
Irrigated region	LUPP	7.36	53.34 ^{***}	62.23	0.63					
	UPP	1.23	24.10 ^{***}	66.89	0.63					
	POL%p	0.22	1.73 ^{***}	84.94	0.86	POL%fp	2.8	88.88 ^{***}	90.93	0.91
	HMW%p	0.01	0.06 ^{***}	86.14	0.87	HMW%fp	0.05	3.66 ^{***}	89.86	0.9
	LMW%p	0.17	1.25 ^{***}	84.82	0.86	LMW%fp	2.55	67.37 ^{***}	90.28	0.91
	GLI%p	0.01	1.59 ^{***}	87.79	0.88	GLI%fp	0.63	84.68 ^{***}	96.27	0.96
	GGA%p	0.01	2.00 ^{***}	15.40	0.85	GGA%fp	2.8	88.88 ^{***}	90.93	0.91

Rep=replications, LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, POL%p = absolute total polymeric proteins, HMW%p = absolute high molecular weight, LMW%p = absolute low molecular weight, GLI%p = absolute total gliadins, GGA%p = absolute total gliadins and albumen-globulins, POL%fp = relative total polymeric proteins, HMW%fp = relative high molecular weight, LMW%fp = relative low molecular weight, GLI%fp = relative total gliadins, GGA%fp = relative total gliadins and albumins-globulins. SS= sum of squares. *** p≤ 0.001, ** p≤ 0.01, * p≤ 0.05, NS = p>0.05.

Table 5.2. Means for absolute and relative protein fractions of the dryland summer rainfall region cultivars

Cultivar	LUPP (%)	UPP (%)	POL%p	POL%fp	HMW%p	HMW%fp	LMW%p	LMW%fp	GLI%p	GLI%fp	GGA%p	GGA%fp
Elands	65.45	58.03	6.13	43.66	0.73	5.00	5.40	38.48	6.13	43.68	7.91	56.34
Gariep	70.85	58.95	5.86	41.30	0.68	4.67	5.19	36.53	6.32	44.66	8.30	58.70
Koonap	72.46	60.36	6.84	45.79	0.72	4.67	6.12	40.98	1.98	13.18	8.12	54.21
Matlabas	67.32	56.72	7.02	45.12	0.77	5.00	6.25	40.15	6.38	41.04	8.53	54.88
PAN3118	64.48	59.07	7.08	46.83	0.81	5.33	6.27	41.50	6.14	40.64	8.04	53.17
PAN3120	61.29	52.99	6.49	44.01	0.85	6.00	5.64	38.26	6.56	44.52	8.26	55.99
PAN3161	67.70	56.60	6.95	50.36	0.69	5.33	6.26	45.37	5.23	38.00	6.83	49.64
PAN3195	67.35	53.89	6.95	45.78	0.82	5.00	6.13	40.38	6.22	40.93	8.24	54.22
PAN3368	70.37	59.98	6.49	43.77	0.87	6.00	5.62	37.89	6.53	44.03	8.34	56.23
PAN3379	72.19	58.59	5.39	38.52	0.64	4.33	4.75	33.94	4.08	29.14	8.57	61.48
Senqu	77.04	59.67	6.71	43.94	0.68	4.33	6.03	39.49	6.65	43.54	8.56	56.06
SST316	81.28	63.12	5.95	41.15	0.64	4.33	5.32	36.78	6.53	45.18	8.51	58.85
SST317	51.39	50.82	6.53	46.73	1.38	10.00	5.16	36.87	5.84	41.73	7.45	53.27
SST347	71.67	60.62	6.34	47.05	0.77	5.67	5.57	41.31	5.82	43.23	7.14	52.95
SST356	68.10	61.68	5.90	41.41	0.79	5.67	5.11	35.83	6.33	44.40	8.34	58.59
SST387	67.27	56.50	6.72	44.18	0.80	5.00	5.92	38.97	6.54	43.02	8.48	55.82
SST398	71.38	57.33	6.62	44.92	0.96	6.33	5.66	38.40	6.23	42.26	8.12	55.08
Mean	68.68	57.94	6.47	44.38	0.80	5.45	5.67	38.89	5.85	40.19	8.10	55.62
LSD	12.36	4.01	0.89	5.03	0.80	2.76	0.76	4.18	0.90	5.09	0.96	5.03
CV	13.01	5.00	9.97	8.19	34.56	36.61	9.71	7.77	11.08	9.15	8.63	6.54

LUPP=large unextractable polymeric proteins, UPP=unextractable polymeric proteins, POL%p= absolute total polymeric proteins, POL%fp=relative total polymeric proteins, HMW%p=absolute high molecular weight, HMW%fp=relative high molecular weight, LMW%=absolute low molecular weight, LMW%fp=relative low molecular weight, GLI%p=absolute total gliadins, GLI%fp=relative total gliadins GGA%p=absolute total gliadins albumen-globulins, GGA%fp=relative total gliadins and albumins-globulins, LSD= least significant differences, CV=coefficient of variation. The largest value in each column is indicated in bold

The mean was 44.38%. (Table 5.2). PAN3118 had the highest POL%p followed by Matlabas and PAN3161 and PAN3195. PAN3161 (50.36%) had the highest POL%fp followed by SST347 (47.05%) and PAN3118 (46.83%). PAN3379 had the lowest POL%fp (Table 5.2).

High molecular weight proteins

The genotypes contributed 35.84% for HMW%p and 38.76% for HMW%fp to the total variation. There was no significant difference in HMW%p and HMW%fp among genotypes tested (Table 5.1). For the HMW%p SST317 ranked the highest followed by SST398 and PAN3368. SST316 and PAN3379 ranked the lowest. SST317 was the cultivar with the highest HMW%fp content followed by SST398 and PAN3120 and PAN3368. SST316 Senqu and PAN3379 (4.33) had the lowest HMW%fp content. The HMW%fp content varied from 4.33% to 10.00% (SST317). The mean was 5.45%, and for HMW%p the values ranged between 0.64% (SST316 and PAN3379) to 1.38% (SST317), the grand mean was 0.80% (Table 5.2).

Low molecular weight proteins

There were significant differences ($p \leq 0.05$) (Table 5.1) in LMW%p and LMW%fp among genotypes. For the LMW%p the mean was 5.67%, the values LMW%p varied from 4.75% (PAN3379) to 6.27% (PAN3118). LMW%fp content ranged from 33.94% (PAN3379) to 45.37% (PAN3161) and the mean was 38.89%. (Table 5.2). PAN3161 was the cultivar with the highest LMW%fp content followed by PAN3118 and SST347. While for the LMW%p, PAN3118 ranked the highest, followed by PAN3161 and Matlabas (Table 5.2). Genotypes contributed 51.76% for LMW%fp and 48.66% for LMW%p to total variation (Table 5.1).

Total gliadins

Highly significant differences ($p \leq 0.001$) were found in GLI%fp among genotypes tested and for GLI%p no significant differences were found among genotypes tested (Table 5.1). Senqu ranked the highest followed by PAN3120 and SST387 for the GLI%p. Koonap had the lowest GLI%p value. Cultivar SST316 had the highest GLI%fp value followed by Gariep and PAN3120. Koonap had the lowest GLI%fp value (Table 5.2). Much variation was due to genotypes (86.29%) for GLI%fp and 82.44% for GLI%p (Table 5.1). GLI%p ranged between 1.98% (Koonap) and 6.65% (Senqu) with a mean of

5.85%. The GLI%fp content ranged from 13.18% (Koonap) to 45.18% (SST316) with the mean of 40.19%. (Table 5.2).

Total gliadins and albumins-globulins

There were no significant differences in GGA%p and GGA%fp among cultivars tested (Table 5.1). For the GGA%p PAN3379 ranked highest, followed by Senqu and Matlabas. PAN3379 had the highest GGA%fp followed by SST316 and Gariep. (Table 5.2). Genotypes contributed 45.82% and 43.69% to the total variation for GLI%fp and GLI%p respectively. PAN3161 had the lowest GGA%p and GGA%fp values. The GGA%p ranged between 6.83% (PAN3161) and 8.57% (PAN3379), with a mean of 8.10%. For the GGA%fp the average was 55.62%, and ranged between 49.64% (PAN3161) and 61.48% (PAN3379) (Table 5.2).

5.5.3 Analysis of variance for relative and absolute protein fractions of the dryland winter rainfall region

Large unextractable polymeric proteins

The average value was 51.36% and varied between 45.52% (PAN3408) to 64.89% (SST056) (Table 5.3). Genotypes were significantly different ($p \leq 0.05$) for LUPP. SST056 was the cultivar with the highest LUPP followed by Ratel and SST015. PAN3408 had the lowest LUPP value. Much variation was due to genotype (46.21%) (Table 5.1).

Unextractable polymeric proteins

There were significant differences ($p \leq 0.05$) in UPP among genotypes (Table 5.1). SST056 had the highest UPP value followed by Ratel and SST027. Tankwa had the smallest UPP content (Table 5.3). Genotypes contributed 49.50 % to the total variation for this trait. (Table 5.1). The values varied from 40.95% (Tankwa) to 60.34% (SST056) and the mean was 45.79% (Table 5.3).

Table 5.3 Means for absolute and relative protein fractions of the dryland winter rainfall region cultivars

Cultivar	LUPP (%)	UPP (%)	POL%p	POL%fp	HMW%p	HMW%fp	LMW%p	LMW%fp	GLI%p	GLI%fp	GGA%p	GGA%fp
Kwartel	50.02	44.86	5.80	42.65	0.91	6.67	4.89	35.95	5.41	39.84	7.79	57.35
PAN3408	45.52	44.68	7.08	49.05	1.14	8.00	5.93	41.06	5.03	35.08	7.28	50.95
PAN3471	52.79	44.97	5.53	46.14	0.66	5.67	4.87	40.65	4.68	39.03	6.45	53.86
Ratel	59.06	49.35	5.53	44.34	0.97	7.33	4.57	36.61	5.11	40.97	6.94	55.66
SST015	52.84	46.42	5.65	44.09	0.81	6.33	4.84	37.77	5.25	40.97	7.17	55.91
SST027	49.92	46.88	5.77	39.72	0.93	6.33	4.84	33.29	6.18	42.89	8.76	60.28
SST047	49.20	43.14	3.71	28.27	0.53	4.00	3.17	24.18	3.08	23.48	9.40	71.73
SST056	64.89	60.34	5.53	40.56	0.73	5.33	4.79	35.20	5.81	42.60	8.10	59.44
SST087	49.24	42.09	5.58	43.81	0.75	6.00	4.83	37.94	5.29	41.45	7.17	56.19
SST096	47.50	42.66	4.99	40.69	0.71	6.00	4.28	34.90	5.68	46.31	7.28	59.31
SST88	49.59	43.10	5.38	43.67	0.70	6.00	4.67	37.97	5.03	40.86	6.93	56.33
Tankwa	45.74	40.95	5.23	42.88	0.79	6.33	4.44	36.44	5.12	42.04	6.96	57.12
Mean	51.36	45.79	5.48	42.16	0.80	6.17	4.68	36.00	5.14	39.63	7.52	57.84
LSD	8.75	8.15	0.78	4.26	0.10	0.65	0.73	4.07	0.43	2.96	0.82	4.26
CV	12.15	12.70	10.23	7.21	8.50	7.47	11.09	8.07	6.01	5.32	7.78	5.26

LUPP=large unextractable polymeric proteins, UPP=unextractable polymeric proteins, POL%p= absolute total polymeric proteins, POL%fp=relative total polymeric proteins, HMW%p=absolute high molecular weight, HMW%fp=relative high molecular weight LMW%p=absolute low molecular weight, LMW%fp=relative low molecular weight, GLI%p=absolute total gliadins, GLI%fp=relative total gliadins GGA%p=absolute total gliadins albumen-globulins, GGA%fp=relative total gliadins and albumins-globulins, LSD= least significant differences, CV=coefficient of variation. The largest value in each column is indicated in bold.

Total polymeric proteins

Genotypes showed highly significant ($p \leq 0.001$) differences for POL%p and POL%fp (Table 5.1). PAN3408 had the highest POL%p value followed by Kwartel and SST027. SST047 had the lowest POL%p value. PAN3408 was the cultivar with the highest POL%fp followed by PAN3471 and Ratel. SST047 had the lowest POL%fp value. (Table 5.3). The genotypes contributed 79.42% for POL%fp and 68.73% for POL%p to total variation (Table 5.1). For POL%fp ranged from 28.27% (SST 047) to 49.05% (PAN3408). The mean was 42.16% while POL%p means varied from 3.72% (SST047) and 7.08% (PAN3408), with a grand mean of 5.45% (Table 5.3).

High molecular weight proteins

Genotypes showed highly significant ($p \leq 0.001$) differences for HMW%p and HMW%fp (Table 5.1). The means for HMW%p ranged between 0.53% (SST047) to 1.14% (PAN3408). The mean value was 0.80%. PAN3408 had the highest HMW%fp value followed by Ratel and Kwartel. SST047 had the lowest HMW%fp value. The HMW%fp content ranged from 4% (SST047) to 8% (PAN3408) (Table 5.3). Much variation was due to the genotype for both HMW%fp (82.91%) and HMW%p (87.54%) (Table 5.1).

Low molecular weight proteins

LMW%p varied between 3.17% (SST047) and 5.93% (PAN3408). The LMW%fp ranged from 24.18% (SST047) to 41.06% (PAN3408). The mean was 36.00% (Table 5.3). There were highly significant differences ($p \leq 0.01$) observed in LMW%p and LMW%fp between genotypes (Table 5.1). PAN3408 had the highest LMW%fp content followed by PAN3471 and SST088. SST047 had the lowest LMW%fp content. PAN3408 ranked the highest followed by Kwartel and PAN3471 for the LMW%p. SST047 had the lowest LMW%p value (Table 5.3). The genotypes contributed 75.99% and 63.96% of total variation for LMW%fp and LMW%p respectively (Table 5.1).

Total gliadins

There were highly significant ($p \leq 0.001$) differences in GLI%p and GLI%fp between the genotypes (Table 5.1). Cultivar SST027 ranked the highest, followed by SST056 and SST096 for GLI%p. SST047 had the lowest GLI%p value. SST096 had the highest GLI%fp content followed by SST027 and SST056. SST047 had the lowest GLI%fp content. The GLI%fp average was 39.63% and ranged from 23.48% (SST047) to 46.31%

(SST096) and GLI%p ranged between 3.08% (SST047) and 6.41% (SST027), with a mean of 5.14% (Table 5.3). The contribution made by the genotypes was 91.50% and 86.11% for GLI%fp and GLI%p respectively (Table 5.1).

Total gliadins and albumins-globulins

Highly significant ($p \leq 0.001$) differences were found in GGA%p and GGA%fp among genotypes (Table 5.1). For mean values varied from 6.45% (PAN3471) to 9.40% (SST047) for GGA%p, with the mean of 7.52%. The average for GGA%fp was 57.84% and ranged between 50.95% (PAN3408) to 71.73% (SST047) (Table 5.3). The genotypes contributed 79.42% for GGA%fp and 72.61% for GGA%p. (Table 5.1). SST047 had the highest GGA%fp followed by SST027 and SST056. PAN3408 had the lowest GGA%fp. SST047 had the highest GGA%p value followed by SST027 and SST056. PAN3471 rendered the lowest GGA%p value (Table 5.3).

5.5.4 Analysis of variance for absolute and relative protein fractions in the irrigated region cultivars

Large unextractable polymeric proteins

SST877 had the highest LUPP value, followed by SST835 and Buffels. SST843 was the cultivar with the lowest LUPP value (Table 5.4). There were highly significant differences ($p \leq 0.001$) in LUPP between cultivars and genotypes contributed 62.23% (Table 5.1) to the total variation. The LUPP varied between 38.72% (SST 843) and 54.35% (SST 877). The mean was 48.66% (Table 5.4).

Unextractable polymeric proteins

There were highly significant ($p \leq 0.001$) differences in UPP between cultivars (Table 5.1). SST877 had the highest UPP value, followed by SST876 and SST867. SST843 had the lowest UPP value (Table 5.4). Much variation was due to genotype (66.89%) (Table 5.1). The UPP content ranged from 39.83% (SST843) to 49.91% (SST877) with a mean of 45.35% (Table 5.4).

Table 5.4 Means for absolute and relative protein fractions of irrigated region cultivars

Cultivar	LUPP (%)	UPP (%)	POL%p	POL%fp	HMW%p	HMW%fp	LMW%p	LMW%fp	GLI%p	GLI%fp	GGA%p	GGA%fp
Buffels	53.10	43.45	4.03	37.82	0.66	6.00	3.37	42.98	5.32	49.89	6.63	62.18
Duzi	47.17	46.67	4.89	40.58	0.72	6.00	4.18	42.45	5.12	42.82	7.09	59.42
Krokodil	47.21	43.39	4.71	43.45	0.78	7.33	3.94	42.40	4.80	44.23	6.13	56.55
PAN3471	52.15	46.13	5.29	48.46	0.70	6.33	4.59	42.08	4.32	39.58	5.63	51.54
PAN3478	48.13	45.65	5.20	47.10	0.79	7.00	4.41	42.07	4.60	41.19	5.90	52.90
PAN3489	50.93	46.67	5.29	48.73	0.62	6.00	4.66	41.62	4.30	39.59	5.56	51.27
PAN3497	52.55	48.41	5.40	49.91	0.81	7.33	4.60	41.31	4.14	38.22	5.42	50.09
Sabie	48.30	46.38	5.68	48.39	0.83	7.00	4.85	41.29	4.57	38.93	6.06	51.61
SST806	52.12	46.36	5.62	49.54	0.85	7.67	4.78	41.03	4.20	37.05	5.72	50.46
SST822	46.48	47.23	6.37	47.15	1.01	7.33	5.36	40.48	5.42	40.12	7.14	52.85
SST835	53.92	47.53	5.28	47.86	0.99	9.00	4.28	40.01	4.31	39.01	5.75	52.14
SST843	38.72	39.83	6.70	45.43	1.10	7.67	5.60	39.93	6.14	41.67	8.04	54.57
SST866	48.09	46.25	2.93	25.95	0.46	4.00	2.47	39.75	2.35	20.79	8.37	74.05
SST867	51.66	48.82	4.96	47.82	0.81	8.00	4.15	39.65	3.92	37.79	5.41	52.18
SST875	48.21	41.93	5.61	49.13	0.99	9.00	4.62	38.82	4.27	37.31	5.81	50.87
SST876	52.63	49.40	5.36	46.40	0.91	8.00	4.45	38.53	4.68	40.48	6.20	53.60
SST877	54.35	49.91	5.25	47.60	0.87	8.00	4.38	37.98	4.30	39.02	5.77	52.40
SST884	48.72	44.51	5.30	48.15	0.76	7.00	4.54	36.33	4.39	39.90	5.70	51.85
SST895	42.61	41.17	5.74	49.15	0.79	7.00	4.95	35.33	4.58	39.10	5.95	50.85
Tamboti	43.19	43.64	4.44	41.27	0.64	6.00	3.80	34.63	5.07	47.19	6.32	58.73
Timbavati	48.66	43.76	5.01	48.06	0.73	7.33	4.28	31.64	3.81	36.56	5.42	51.94
Umlazi	41.66	40.72	5.39	48.88	0.80	7.00	4.59	21.88	4.04	36.63	5.64	51.12
Mean	48.66	45.35	5.20	45.77	0.80	7.09	3.90	38.74	4.48	39.41	6.17	54.24
LSD	5.46	3.34	0.52	2.85	0.10	0.62	0.44	2.57	0.45	1.74	0.59	2.85
CV	8.18	5.36	7.27	4.53	8.74	6.37	7.32	4.83	7.40	3.22	6.93	3.82

LUPP=large unextractable polymeric proteins, UPP=unextractable polymeric proteins, POL%p= relative total polymeric proteins, POL%fp=absolute total polymeric proteins HMW%p=relative high molecular weight, HMW%fp=absolute high molecular weight LMW%=relative low molecular weight, LMW%fp=absolute low molecular weight, GLI%p=relative total gliadins, GLI%fp=absolute total gliadins GGA%p=relative total gliadins albumen-globulins, GGA%fp=absolute total gliadins and albumins-globulins, CV=coefficient of variation, LSD= least significant differences

Total polymeric proteins

Genotypes contributed 84.94% to POL%p and 90.93% to POL%fp for total variation. Genotypes showed highly significant ($p \leq 0.001$) differences in POL%p and POL%fp (Table 5.1). For the POL%p, SST843 ranked the highest followed by SST822 and SST895. SST866 ranked the lowest. PAN3497 had the highest amount of POL%fp, followed by SST806 and SST895. SST866 had the lowest amount of POL%fp. The POL%fp ranged between 25.95% (SST 866) and 49.54% (PAN3497), the mean was 45.77%. The POL%p mean was 5.20%. The POL%p varied between 6.70% (SST843) to 2.93% (SST866) (Table 5.4).

High molecular weight proteins

There were highly significant ($p \leq 0.001$) differences in HMW%p and HMW%fp among the genotypes (Table 5.1). The HMW%p ranged between 0.46% (SST866) and 1.10% (SST843). The mean was 0.80%. The average was 7.10% for the HMW%fp and values ranged between 4.00% (SST866) and 9.00% (SST835). SST835 was the cultivar with the highest HMW%fp followed by SST875 and SST876. SST866 had the lowest HMW%fp value. For the HMW%p, SST843 ranked the highest, followed by SST822 and SST835. SST 866 had the lowest HMW%p value (Table 5.4). Genotypes explained 84.9% (HMW%fp) and 86.14% (HMW%p) of total variation (Table 5.1).

Low molecular weight proteins

Highly significant differences ($p \leq 0.001$) were found between cultivars for LMW%p and LMW%fp (Table 5.1). The LMW%p varied between 2.47% (SST866) and 5.60% (SST 843) The LMW%fp average values ranged between 21.88% (SST 866) and 42.98% (PAN3489). The LMW%fp mean was 38.74% and LMW%p mean was 4.40%. PAN3489 ranked the highest for LMW%fp followed by PAN3497 and SST895. SST866 had the lowest LMW%fp. For the LMW%p, SST843 ranked the highest followed by SST822 and SST895. SST866 ranked the lowest (Table 5.4). Much variation was due to genotypes for LMW%fp and LMW%p (90.28% and 84.82% respectively) (Table 5.1).

Total gliadins

There were highly significant differences ($p \leq 0.001$) in GLI%p and GLI%fp among the cultivars (Table 5.1). Cultivar SST843 ranked the highest followed by SST822 and Buffels for the GLI%p. Buffels had the highest GLI%fp followed by Tamboti and

Krokodil. SST866 had the lowest GLI%p and GLI%fp values. The average value was 39.41% and values varied from 20.79% (SST866) to 49.89% (Buffels) for GLI%fp. The GLI%p varied between 2.35% (SST866) and 6.14% (SST843), the mean was 4.48% (Table 5.4). Much variation was due to the genotypes (96.27% for GLI%fp and 87.79% for GLI%p) (Table 5.1).

Total gliadins and albumins-globulins

The GGA%p mean was 6.17% and the values varied between 5.41% (SST867) and 8.37% (SST866). The GGA%fp ranged between 50.09% (PAN3497) and 74.05% (SST866). The GGA%fp mean was 54.24%. SST866 had the highest amount of GGA%fp followed by Buffels and Duzi. PAN3497 ranked the lowest (Table 5.4). Highly significant differences ($p \leq 0.001$) were found in GGA%p and GGA%fp among the genotypes. The genotypes contributed 15.40% for GGA%p and 90.93% for GGA%fp to total variation. (Table 5.1).

5.5.5 Significant correlations between protein fractions and quality wheat characteristics of dryland summer rainfall, dryland winter rainfall and the irrigation areas

Dryland summer rainfall cultivars

UPP correlated highly significantly with MPT but negatively with WGC. POL%p correlated positively with FPC, but negatively with LV12. HMW%fp correlated negatively with alvW and alvP. HMW%p correlated positively with WGC but negatively with alvP. LMW%fp correlated positively with BFY and FY. LMW%p correlated positively with alvW but negatively with LV12. GLI%fp correlated negatively with alvP but positively with alvP/L. GGA%p correlated positively with FPC but negatively with LV12.

Dryland winter rainfall cultivars

POL%fp correlated negatively with BFY and WGC but positively with SDSVOL. POL%p had a negative correlation with alvP but showed a positive correlation with alvP/L. HMW%p correlated negatively with alvP. LMW%fp had a negative correlation with BFY and WGC, but correlated positively with SDSVOL. LMW%p showed a negative correlation with alvP and alvP/L. GLI%fp correlated positively with SDSVOL but negatively with WGC. GLI%p correlated positively with the SDSVOL. GGA%fp

correlated negatively with BFY, WGC, FPC and alvW. GGA%p correlated positively with WGC, LV, BFY, FPC and FY.

Irrigated region

LUPP correlated negatively with alvW, FPC, alvP, alvP/L, LV, and MPT. UPP had negative correlations with alvW, alvP, alvP/L and MPT. POL%p correlated positively with alvW, alvP, LV, WGC and MPT, but negatively with BFY and FY. HMW%fp correlated negatively with FPC, BFY and FY but positively with WGC, MPT, LV, alvP/L, alvP and LV12. HMW%fp correlated positively with alvP, LV, WGC, alvP/L and negatively with FPC, BFY and FY. HMW%p correlated positively with alvW. The LMW%p showed positive correlations with alvP, LV, WGC and alvP/L. GLI%p correlated positively with FY but negatively with alvW, alvP, LV, WGC, and alvP/L. GGA%p correlated negatively with FY, and LV12 but positively with alvW, alvP/L, MPT, alvP, LV, WGC, FPC, and MPT (Table 5.5).

Table 5.5 Significant correlations between protein fractions and quality characteristics in the three production areas

Character1	Dryland summer rainfall		Character 1	Dryland winter rainfall		Character 1	Irrigated region	
	Character 2	Correlations		Character 2	Correlations		Character 2	Correlations
UPP	alvW	0.279*	POL%fp	BFY	-0.518**	LUPP	FY	0.398*
	MPT	0.360***		alvW	-0.347*		alvW	-0.492***
	WGC	-0.339*		LV	-0.363*		FPC	-0.371**
POL%fp	FY	0.286*	POL%p	WGC	-0.449**	UPP	alvP	-0.506***
POL%p	FPC	0.629***		FPC	-0.407*		alvL	0.304*
	BFY	0.377*		SDSVOL	0.468**		alvP/L	-0.477***
	LV12	-0.561***	alvP	-0.455**	LV	-0.331**		
HMW%fp	WGC	0.303*	HMW%p	SDSVOL	0.359*	UPP	WGC	-0.241*
	alvW	-0.288*		alvP/L	0.388*		MPT	-0.456***
	alvP	-0.301*		alvP/L	-0.384**		FY	0.244*
HMW%p	WGC	0.282*	LMW%fp	alvP	-0.444**	POL%fp	alvW	-0.513***
	alvP	-0.286*		BFY	-0.585***		alvP	-0.490***
	BFY	0.271*		alvW	-0.344*		alvP/L	-0.429***
LMW%fp	FY	0.312*	LMW%p	LV	-0.402*	POL%p	MPT	-0.450***
	LV12	-0.563***		WGC	-0.445**		LV12	0.244*
	alvW	0.388**		FPC	-0.407*		FPC	-0.248*
GLI%fp	alvP	-0.395**	GLI%p	SDSVOL	0.469**	POL%p	BFY	-0.411***
	alvP/L	0.028*		BFY	-0.381*		FY	-0.485***
	alvP	-0.290*		alvP	-0.432**		alvW	0.602***
GGA%fp	FPC	0.284*	GLI%fp	SDSVOL	0.372*	HMW%fp	alvP	0.523***
	LV12	-0.296*		alvP/L	-0.367**		LV	0.559***
	alvP/L	-0.290*		alvW	-0.333*		WGC	0.492***
GGA%p	FY	-0.284*	GLI%fp	LV	-0.370*	HMW%fp	MPT	0.253**
	LV12	-0.546**		WGC	-0.664**		FPC	-0.630***
	FY	-0.340*		SDSVOL	0.602***		LV12	0.268*
	FPC	0.667***		FPC	-0.347*		alvP	0.391***

LUPP=large unextractable polymeric proteins, UPP=unextractable polymeric proteins, POL%p=absolute total polymeric proteins, HMW%p=absolute high molecular weight, LMW%p=absolute low molecular weight, GLI%p= absolute total gliadins, GGA%p=absolute total gliadins and albumins-globulins, POL%fp=relative total polymeric proteins, HMW%fp=relative high molecular weight, LMW%fp=relative low molecular weight, GLI%fp= relative total gliadins, GGA%fp=relative total gliadins and albumin-globulins, alvW=alveograph strength, LV=loaf volume, LV12=loaf volume adjusted to 12% protein, MPT=mixograph peak time, alvP=alveograph stability, alvP/L=alveograph extensibility to stability ratio, alvL=extensibility, WGC=gluten wet content, SDSVOL=sodium dodecyl sulphate sedimentation volume, FY=flour yield, BFY=break flour yield, FPC=flour protein content *= $p \leq 0.05$, **= $p \leq 0.01$, ***= $p \leq 0.001$

Table 5.5 continued

Dryland summer rainfall			Dryland winter rainfall			Irrigated region		
Character 1	Character 2	Correlations	Character 1	Character 2	Correlations	Character 1	Character 2	Correlations
			GLI%fp	BFY	-0.358*	HMW%fp	LV	0.584***
			GLI%p	SDSVOL	0.548**		WGC	0.513***
				WGC	-0.422*		FPC	-0.617***
			GGA%fp	BFY	0.517**		alvP/L	0.293*
				LV	0.363*		BFY	-0.395***
				WGC	0.449**		FY	-0.486***
				alvW	0.347*	HMW%p	alvW	0.600***
				FPC	0.412*	LMW%p	alvP	0.528***
			GGA%p	SDSVOL	0.362*		LV	0.527***
				WGC	0.576***		WGC	0.463***
				LV	0.610***		MPT	0.260*
				BFY	0.526**		alvP/L	0.398**
				FPC	0.526**	GLI%fp	BFY	0.301*
				FY	0.610***	GLI%p	FPC	0.630*
							FY	-0.439***
							alvW	0.480***
							alvP	0.387**
							LV	0.434
							WGC	0.581***
							alvP/L	0.312**
						GGA%fp	LV12	-0.244*
							FPC	0.248*
						GGA%p	FY	-0.410***
							alvW	0.347**
							alvP/L	0.346*

HMW%p=absolute high molecular weight, HMW%fp=relative high molecular weight, LMW%p=absolute low molecular weight, LMW%fp=absolute low molecular weight GLI%fp= relative total gliadins, GGA%p=absolute total gliadins and albumins-globulins, GLI%p= absolute total gliadins, GLI%fp= relative total gliadins, GGA%fp=relative total gliadins and albumin-globulins, alvW=alveograph strength, LV=loaf volume, LV12=loaf volume adjusted to 12% protein, MPT=mixograph peak time, alvP=alveograph stability, alvP/L=alveograph extensibility to stability ratio, alvL=alveograph extensibility, WGC=gluten wet content, SDSVOL=sodium dodecyl sulphate sedimentation volume, FY=fLOUR yield, BFY=break flour yield, FPC=fLOUR protein content *= $p \leq 0.05$, **= $p \leq 0.01$, ***= $p \leq 0.001$

Table 5.5 continued

Dryland summer rainfall			Dryland winter rainfall			Irrigated region		
Character 1	Character 2	Correlations	Character 1	Character 2	Correlations	Character 1	Character 2	Correlations
						GGA%p	alvP	0.308*
							LV	0.305**
							LV12	-0.329**
							WGC	0.527***
							FPC	0.693***
							MPT	0.410***
							alvP/L	0.346**

GGA%p=absolute total gliadins and albumin-globulins, LV=loaf volume, LV12=loaf volume adjusted at 12% protein basis, MPT=mixograph peak time, alvP=stability, alvP/L=alveograph extensibility to stability ratio, alvL=alveograph extensibility, WGC=gluten wet content, FPC=flour protein content *=p≤ 0.05, **=p≤ 0.01, ***= p≤ 0.001.

5.5.6 Principal component analysis

Dryland summer rainfall cultivars

The principal components with Eigenvalues greater than 1 were used (Table 5.6). A cut-off of 0.3 on the loadings was used to consider characteristics of importance. The first two principal components explained 78.64% of the total variation in the dataset. PC1 accounted for 59.63% of the total variation and carried large positive loadings for LV and alvP, but carried large negative loading for alvL. PC2 accounted for 19.01% of the total variation and carried large positive loadings for alvL and LV.

Table 5.6 Loadings of the measured characteristics on the first two principal components in the dryland summer rainfall cultivars

Characteristics	PC1	PC2
FPC	0.00	0.00
GGA%p	0.00	0.01
GGA%fp	0.01	0.07
GLI%fp	-0.08	0.05
GLI%p	-0.01	0.01
HMW%p	0.00	0.00
HMW%fp	-0.01	-0.02
alvL	-0.46	0.75
LMW%p	0.00	-0.01
LMW%fp	0.01	-0.05
LUPP	0.05	0.12
LV	0.75	0.54
alvP/L	0.01	-0.01
MPT	0.01	0.01
alvP	0.46	-0.20
POL%p	0.00	-0.01
POL%fp	-0.01	-0.07
UPP	0.03	0.08
alvW	0.11	0.26
Eigenvalue	3.52	2.05
Percentage variation	59.63	19.01
Cumulative variation	59.63	78.64

LUPP=large unextractable polymeric proteins, UPP=unextractable polymeric proteins, POL%p=absolute total polymeric proteins, HMW%p =absolute high molecular weight, LMW%p=absolute low molecular weight, GLI%p=absolute total gliadins, GGA%p=absolute total gliadins, POL%fp =relative total polymeric proteins, HMW%fp=relative high molecular weight, LMW%fp=relative low molecular weight, GLI%fp=relative total gliadins, GGA%fp=relative total gliadins albumin-globulins, LV=loaf volume MPT=mixograph peak time, alvP=alveograph stability, alvP/L=alveograph extensibility to stability ratio, alvL=alveograph extensibility alvW= alveograph strength

Dryland winter rainfall cultivars

A cut-off point of 0.3 was used to identify the most important characteristics. The cumulative variation amounted to 78.64% (Table 5.7). PC1 accounted for 59.63% of the total variation. LV and alvP contributed positively to PC1. PC2 accounted for 19.01% of the total variation and alvL and LV contributed positively to this principal component.

Table 5.7 Loadings of measured characteristics on the first two principal components of the dryland winter rainfall cultivars

Characteristics	PC1	PC2
FPC	0.00	0.00
GGA%p	0.00	0.01
GGA%fp	0.01	0.07
GLI%fp	-0.08	0.05
GLI%p	-0.01	0.01
HMW%p	0.00	0.00
HMW%fp	-0.01	-0.02
alvL	-0.46	0.75
LMW%p	0.00	-0.01
LMW%fp	0.01	-0.05
LUPP	0.05	0.12
LV	0.75	0.54
alvP/L	0.01	-0.01
MPT	0.01	0.01
alvP	0.46	-0.20
POL%p	0.00	-0.01
POL%fp	-0.01	-0.07
UPP	0.03	0.08
alvW	0.11	0.26
Eigenvalue	3.52	2.05
Percentage Variation	59.63	19.01
Cumulative variation	59.63	78.64

LUPP=large unextractable polymeric proteins, UPP=unextractable polymeric proteins, POL%p=absolute total polymeric proteins, HMW%p =absolute high molecular weight, LMW%p=absolute low molecular weight, GLI%p= absolute total gliadins, GGA%p=absolute total gliadin and albumins -globulins, POL%fp=relative total polymeric proteins, HMW%fp=relative high molecular weight, LMW%fp=relative low molecular weight, GLI%fp=relative total gliadins, GGA%fp=relative total gliadins albumin-globulins, LV=loaf volume MPT=mixograph peak time, alvP=alveograph stability, alvP/L=alveograph extensibility to stability ratio, alvW=alveograph strength, alvL=alveograph extensibility

Irrigation region

The first principal components accounted for 76.31% of the total variation and carried large positive loadings for LV. PC2 had a large positive loading for alvL and large negative loading for alvP, and it accounted for 18.07% of the total variation. The cumulative variation explained by these two principal components was 94.38%.

Table 5.8 Loadings of the measured characteristics on the first two principal components for the irrigated cultivars

Characteristics	PC1	PC2
FPC	-0.01	0.01
GGA%p	0.00	-0.01
GGA%fp	-0.03	-0.01
GLI%fp	0.00	0.04
GLI%p	0.01	0.00
HMW%p	0.00	0.00
HMW%fp	0.01	0.01
alvL	-0.03	0.86
LMW%p	0.01	0.00
LMW%fp	-0.02	0.01
LUPP	-0.04	0.08
LV	0.98	0.11
alvP/L	0.00	-0.01
MPT	0.00	-0.01
alvP	0.14	-0.44
POL%p	0.01	0.00
POL%fp	0.03	0.01
UPP	-0.02	0.05
alvW	0.10	-0.18
Eigenvalue	2.90	1.50
Percentage Variation	76.31	18.07
Cumulative variation	76.31	94.38

LUPP=large unextractable polymeric proteins, UPP=unextractable polymeric proteins, POL%p=absolute total polymeric proteins, HMW%p=absolute high molecular weight, LMW%p=absolute low molecular weight, GLI%p=absolute total gliadins, GGA%p=absolute total gliadins, POL%fp=relative total polymeric proteins, HMW%fp=relative high molecular weight, LMW%fp=relative low molecular weight, GLI%fp=relative total gliadins, GGA%fp=relative total gliadins albumin-globulins, LV=loaf volume MPT=mixograph peak time, alvP=alveograph stability, alvP/L=alveograph extensibility to stability ratio, alvW=alveograph strength

5.5.7 Principle component biplots

Dryland summer rainfall cultivars

The biplot (Figure 5.3) assesses relationships between characteristics and shows cultivars with similar characteristics. Characteristics which cluster together, are related in the data set. The first two principal components accounted for 78.64% of variation in this location. The biplot of PC1 against PC2 showed groupings for FPC, LV and MPT; GGA%fp and GGA%p; GLI%fp and GLI%p; LUPP and UPP; HMW%fp and HMW%p; POL%fp and POL%p, LMW%fp and LMW%p. The cultivars formed four distinct groups: SST316, Elands, PAN3161, PAN3120, Senqu and Koonap. SST317 and SST356 grouped together, SST347 and SST387, PAN3379, PAN3118 and Gariep. Matlabas could be associated with MPT, LV, and FPC. PAN3368 could be associated with alvL. LMW%fp and LMW%p showed an inverse relationship with PAN3118.

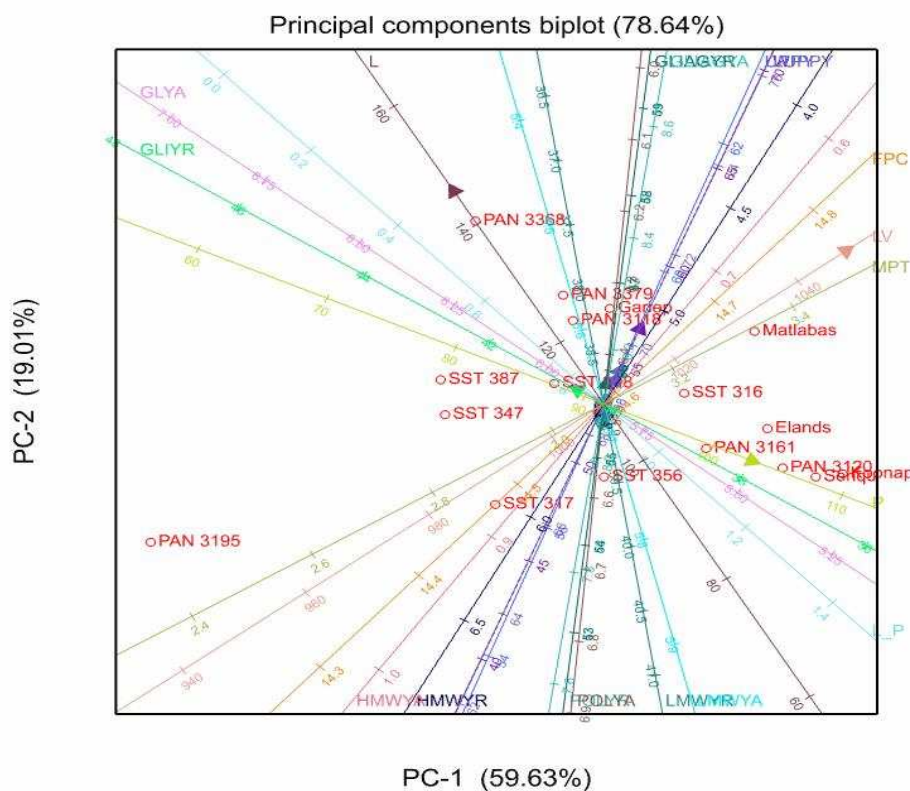


Figure 5.3 Principal component biplot for protein fractions and quality characteristics for the dryland summer rainfall cultivars

Dryland winter rainfall cultivars

The first two principal components accounted for a large percentage (87.64%) of the total variation. Cultivars that grouped together have similar characteristics (Figure 5.4). The biplot of PC1 against PC2 showed that Kwartel associated with MPT, Ratel closely associated with HMW%p and PAN3408 closely related with HMW%fp. GLI%p inversely related with Ratel while GGA%fp inversely related with SST096 and SST047. The characteristics separated into five distinct groups, the first group comprised of FPC and alvW, the second group of alvP/L and alvP, the third group of LUPP, UPP, LMW%p and GLI%fp, the fourth group of LMW%fp, POL%fp, POL%p and GGA%fp while MPT, HMW%fp and LMW%fp formed the fifth group. The cultivars formed two distinct groups. One group comprised of SST027, SST087 and SST056, the second group comprised of PAN3408 and Kwartel (Figure 5.4).

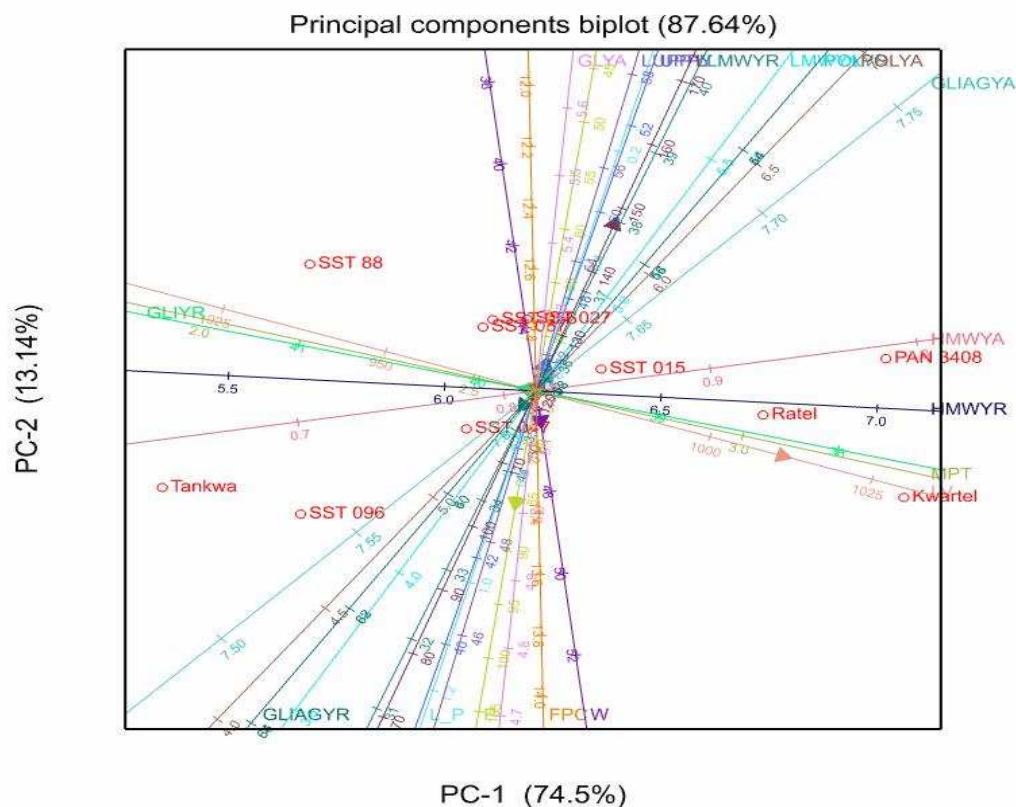


Figure 5.4 Principal component biplot for protein fractions and quality characteristics of the dryland winter rainfall cultivars

Irrigated region cultivars

The biplot (Figure 5.5) of PC1 against PC2 revealed that Timbavati closely associated with alvL, Sabie closely associated with LMW%fp and that Umlazi closely associated with GLI%fp. GLI%fp inversely related with SST 884 and SST895. PAN3489 closely associated with GGA%p, and SST822 closely associated with POL%fp. The characteristics grouped as follows: LUPP, UPP and alvP; alvL and GLI%p; alvW and GGA%fp; LMW%fp and POL%fp and MPT and alvP/L.

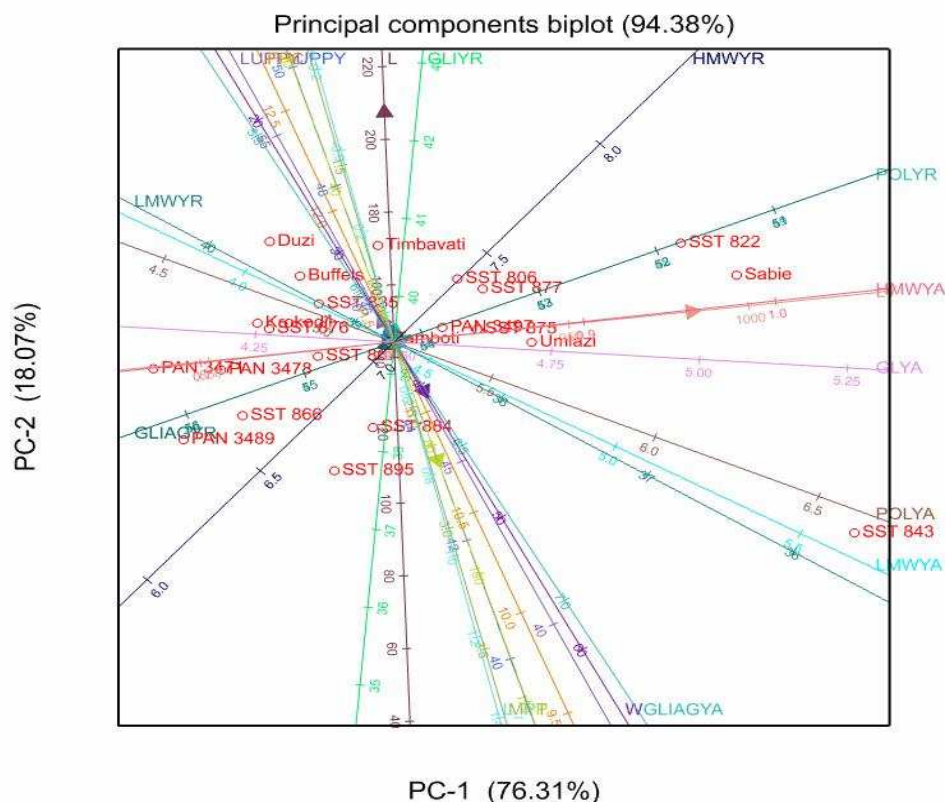


Figure 5.5 Principal component biplot for protein fractions and quality characteristics of the irrigated region cultivars

5.6 Discussion

In the dryland summer rainfall region, UPP positively correlated with alvW and MPT, which is in agreement with the findings of previous authors (Gupta et al. 1993; Bean et al. 1998; Sadouki et al. 2005; Edwards et al. 2007). This confirms the contribution of UPP to dough rheology and quality. However, at the irrigated region UPP correlated negatively with rheological characteristics, which disagree with these findings.

In the irrigated region cultivars, GLI%p significantly correlated with most quality characteristics. The significant correlation reported between GLI%p and dough quality may be due to the LMW, as it has been reported that there is a close genetic linkage between *Glu-3* loci of LMW and *Gli-1* of gliadins and have similar molecular weight. This makes it difficult to determine whether the influence is due to LMW or gliadins (Gupta et al. 1989; Payne et al. 1984; Gianibelli et al. 2001). Ahmad et al. (2001) did not report any significant correlations between gliadins and quality characteristics. Fido et al. (1997) reported that addition of all groups of gliadins yielded a decrease in dough strength. Wang et al. (2008) confirmed that ω -gliadins have an influence on dough strength. Metakovsky et al. (1997) reported positive relationships between gliadin alleles and dough strength in French and Italian wheat cultivars. Wentzel (2010) reported significant correlations between large monomeric proteins and alvW in South African bread wheat and Mkhatywa (2014) reported significant correlations between ω -gliadins in total proteins and alvW in South African wheat cultivars. The findings from this research suggest that gliadins might contribute to dough strength. However further investigations are necessary to establish whether GLI contributes to rheological characteristics.

HMW%p positively correlated with alvW in the irrigated region, this corroborates the findings by several authors (Morel et al. 2000; Sadouki et al. 2005; Mkhatywa 2014). Dachkevitch and Autran (1989) reported significant correlations between the insoluble F1 fraction and alvW. However in the dryland summer rainfall region negative correlations between HMW and alvW were found, this is contradictory to these findings. The GLI%p positively correlated with FPC in the dryland summer rainfall region and irrigation region cultivars. This suggests absolute F4 fractions as a good indicator of FPC, which was also reported by Morel et al. (2000). In dryland winter rainfall cultivars, GLI%fp negatively correlated with FPC which is contradictory to these findings. More significantly positive correlations were found between GGA and FPC. Bean et al. (1998) also reported that FPC increased with GGA. Morel et al. (2000) found significant association between F5 and GGA. Many protein fractions that correlated positively with FPC showed positive correlations with WGC, this indicates that variation of these protein fractions has a direct influence on FPC and WGC.

SDSVOL correlated significantly with POL, LMW and GLI%p for the dryland winter rainfall cultivars. Positive correlations between LMW and SDSVOL are in agreement with reports by Gallia et al. (1996) and Wentzel (2010). Weegels et al. (1996) indicated that there is a good correlation between unextractable glutenin and SDSVOL. Positive correlations found between GLI%p and SDSVOL are contradictory to a previous study by Schober et al. (2006) where they reported that SDSVOL negatively correlated with relative amounts of gliadins, the absolute amounts did not reveal any correlations.

At the dryland winter rainfall region LMW, both in total proteins and in the flour, were negatively associated with alvW. This is contradictory to the findings from previous studies (Zhang et al. 2009; Wentzel 2010), however Sadouki et al. (2005) reported significant negative association between the absolute amounts of LMW and alvW. The POL correlated with some quality characteristics in this study and this disagrees with other authors (Gupta et al. 1993; Sadouki et al. 2005) who reported no significant correlations between relative total polymeric proteins and quality attributes. However, Wentzel (2010) found significant correlations between POL and rheological characteristics such as SDSVOL and alvP. This corroborates the findings in this study. The HMW%fp positively correlated with alvP for the irrigated region cultivars, and similar results were reported by Wentzel (2010) but it was contradictory to what Mkhatywa (2014) found that HMW in the flour and proteins negatively correlated with alvP. Ciaffi et al. (1996) reported significant correlations between absolute insoluble polymeric proteins and alvP. The HMW%fp for the irrigated region and HMW%p for the dryland winter rainfall region positively correlated with alvP/L and similar findings were reported by Peña et al. (2005) who found significant correlations between HMW and alvP/L. Significant negative correlations between GLI and alvP/L for the dryland summer cultivars were also reported by Labuschagne and Aucamp (2004) and Mkhatywa (2014).

For the dryland winter rainfall cultivars the fractions POL%p, LMW%p and GLI%p were negatively associated with LV, these protein subunits also correlated negatively with FPC, however, both GGA in the protein and in the flour correlated positively with LV. For the irrigation region cultivars, POL%fp correlated positively with LV. Similar results were reported by Gupta et al. (1992). The LMW%p positively correlated with LV, similar findings were reported by Dachkevitch and Autran (1989). Weegels et al. (1996)

indicated that glutenins in the flour associated better with LV. The HMW%fp positively correlated with LV. This agrees with the findings by Tronsmo et al. (2003) where relative HMW (sonicated) correlated with LV. Chaudhary et al. (2016) reported significant correlations between glutenin and LV. GLI has been found to positively correlate with LV for the irrigated region cultivars, this is in agreement with Labuschagne and Aucamp (2004); Park et al. (2006) and Chaudhary et al. (2016) but contradictory to previous findings (Khatkar et al. 2002; Ohm et al. 2010). Correlations between protein subunits and LV vary, depending on solvents or extraction procedures used (Gupta et al. 1992; Weegels et al. 1996). No significant correlations were found between protein fractions and LV in the dryland summer rainfall region cultivars in this study.

The PCA is an important data reduction tool which gives information on trait association, and the relationship between characteristics and specific cultivars, which makes it very useful for breeding programmes (Tronsmo et al. 2003; Leilah and AI-Khateeb 2005; Ahmad et al. 2014; Janmohammadi et al. 2014). The PCA also presents the importance of the largest contributors to the total variation (Martens and Martens 2001). Characteristics that cluster together are related, while those appearing on the opposite sides of the biplot are negatively correlated (Tronsmo et al. 2003).

In the dryland summer region FPC, MPT and LV clustered together, while alvL was placed on the upper left side of the biplot. AlvP/L and alvP were plotted on the lower right side of the biplot. For the dryland winter rainfall region HMW%fp and MPT clustered together, FPC and alvW clustered together, and alvP/L and alvP grouped together.

For the irrigated region alvW and GGA%p grouped together while alvL and GLI%fp grouped together. LUPP was plotted opposite alvW and GGA%p, while UPP plotted opposite to MPT. Cultivars and characteristics which are closely related suggest that the cultivar expresses high levels of those characteristics and such cultivars can be selected as parents to improve such characteristics (Yan and Racjan 2002).

For the dryland summer rainfall region, cultivar Matlabas closely associated with MPT, LV and FPC, while for the dryland winter rainfall region Kwartel was closely associated with MPT and HMW%fp, while Ratel closely associated with HMW%fp, and PAN3408

closely associated with HMW%p. For the irrigation region SST843 closely associated with POL%p and LMW%p, while PAN3489 closely associated with SST866.

The first two principal components accounted for 78.64%, 87.64% and 94.38% of total variation for the dryland summer rainfall region, dryland winter rainfall region and irrigation region, respectively. In all the localities the largest contributor to the total variation was LV for PC1 while for PC2 it was alvL.

5.7 Conclusions

This study was carried out to assess the Yarra-SEC 4000 analytical column as an improved protein fraction separation tool. The results indicated this column to be efficient and stable in the separation of wheat proteins. Highly significant differences were found among the genotypes tested for the dryland winter rainfall and irrigated region for the relative and absolute protein fractions. The UPP contributed most to dough rheological characteristics in the dryland summer rainfall region cultivars, however, in the irrigation cultivars UPP contributed negatively to dough quality characteristics. The GLI%p contributed significantly to dough rheology characteristics. This might be due to some of the gliadins and LMW having the same molecular weights. Genotype effect was significant for all protein subunits for the dryland winter rainfall region and irrigated region cultivars. Significant differences between protein fractions and their relationship with quality characteristics indicate that the Yarra-SEC 4000 analytical column can be used to separate and then assess the influence of the protein fractions on bread-making quality. This analytical column produced nine profile peaks. It is therefore important to determine the relationship of each profile peak with quality characteristics to assess which peak contributes to quality, instead of using total areas from the chromatograms.

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Chapter 6

A comparison of a narrow bore and analytical column, used in size exclusion-high performance liquid chromatography, for the analysis of protein fractions of South African bread wheat cultivars

6.1 Abstract

The narrow bore column (300 x 4.60 mm id, BioSep-SEC s4000) was used up to now as a standard column which separates gluten proteins at 20 μ l 30 min⁻¹. The objective of this study was to assess the Yarra-SEC 4000 analytical column (300 x 7.80 mm, id) for its ability to distinctly separate wheat proteins in small flour samples, and its potential for the evaluation of South African bread wheat quality in the early generations of the breeding programmes. Precise early generation selection of cross progenies is of great importance to breeders, as this will cut down the costs for quality breeding and the lines with inferior traits can then be discarded early in the breeding programmes. When the F values were considered, the Yarra-SEC 4000 analytical column was shown to be superior to the narrow bore column. Highly significant differences were found between protein fractions for the irrigated region and winter rainfall region cultivars, indicating the Yarra-SEC 4000 analytical column to be more discriminative than the narrow bore BioSep-SEC s4000 column. Absolute protein fractions separated by the narrow bore column were highly significantly correlated with FPC with higher correlation coefficients than the Yarra-SEC 4000 analytical column. The Yarra-SEC 4000 analytical column yielded nine profile peaks while the BioSep-SEC s4000 narrow bore column yielded five profile peaks. This indicates that the analytical column distinctly separated wheat proteins. Multi location trial testing over years is required to fully assess the potential of the Yarra-SEC 4000 analytical column for quality analysis.

6.2 Introduction

Wheat is recognised as an important crop because of the desirable and unique dough forming characteristics which allows the crop to be processed into a variety of products which includes bread, biscuits, cakes, pizza, chapatti and doughnuts (Rakszegi et al. 2005; Dewettinck et al. 2008). It provides essential nutrients such as energy, protein and vitamins. Globally it provides about a quarter of energy and proteins to the human diet

(Branlard et al. 2001). In terms of magnitude of production among cereals, wheat is one of the most important crops both globally (FAO, 2014; Labuschagne et al. 2014) and in South Africa (SAGL, 2013).

Wheat storage proteins reside in the endosperm. The storage proteins are referred to as gluteins. The monomeric gliadins and polymeric glutenins form gluten (Shewry et al. 1995; Gianibelli et al. 2001). The gluten protein makes up about 80% of wheat proteins and gives dough visco-elastic properties (Branlard et al. 2001). Based on electrophoretic mobility, the polymeric glutenin can be separated into HMW-GS and LMW-GS. The HMW-GS have molecular weights between 70 000-140 000 Da, held together by disulphide bond. Genes coding for the HMW-GS are found on the long arm of group 1 chromosomes. The LMW-GS have molecular weights between 30 000-50 000 Da and the genes coding them are found on the short arm of group 1 chromosomes (Payne et al. 1984; Gianibelli et al. 2001; Gao et al. 2010). The HMW-GS and LMW-GS have been reported to be important for their influence on strength and elasticity of wheat dough (Gianibelli et al. 2001; Gao et al. 2010). The gliadins affect viscous properties of dough (DuPont and Altenbach 2003).

The bread wheat quality in South African cultivars is very good due to very strict quality standards set by the Wheat Technical Committee before commercial release (SAGL 2013.) The quality of wheat is the result of genetic factors, however the environment and the genetic and environment interaction also contributes towards wheat quality (Kent and Evers 1994; DuPont and Altenbach 2003). The inheritance of wheat quality traits is polygenic in nature and very complex. This makes it difficult to select desired traits suitable for bread-making quality (Gras and O'Brien 1992; DuPont and Altenbach 2003). This led to the development of various quality tests that can be used to assess the ability of cultivars to meet specific requirements for breeders, producers, millers and bakers (Atkins et al. 1965)

SE-HPLC has been used to evaluate bread-making quality. Variations in molecular weight distribution of gluten proteins have been related with wheat end-use quality (Singh et al. 1990; Ciaffi et al. 1996; Bean et al. 1998; Larroque and Békés 2000; Park et al. 2006; Labuschagne and Aucamp 2004; Sadouki et al. 2005; Ohm et al. 2009; Singh and Singh 2013; Vensel et al. 2014). However the application of this technique has been

difficult due to the time it takes to run the samples. The technique is not amenable to analysis of large samples in breeding programmes and in the industry (Ohm et al. 2009).

Due to the fact that bread-making quality is polygenic in nature, it is necessary to provide plant breeders with precise information about end-use quality potential of single plants in the early generations of the breeding programmes. The precise and accurate selection of plant materials in early generations will decrease the costs of quality breeding as lines with undesirable traits can be discarded in the early generations; however, if a desirable trait is lost or discarded, it cannot be recovered in the following generations (Shebeski 1967; Gras and O'Brien, 1992).

The objective of this study was to test the Yarra-SEC 4000 analytical column for its potential for more distinct separation of gluten proteins compared to the BioSep-SEC s4000 narrow bore column used up to now.

6.3 Materials and methods

6.3.1 Cultivars used

See Chapter 3 (Section 3.3.1).

6.3.2 Quality measurements

See Chapter 3 (Section 3.3.3).

6.3.3 Size exclusion-high liquid performance liquid chromatography

See Chapter 4 (Section 4.3.3) and Chapter 5 (Section 5.3.3) for details

6.4 Statistical analysis

See Chapter 4 (Section 4.4) and Chapter 5 (Section 5.4) for details

The differences between the two columns used in this study are listed in Table 6.1.

Table 6.1 Differences between the two columns used

	BioSep-SEC s4000 Narrow bore	Yarra SEC-4000 Analytical
Column hardware	Standard 316 stainless steel column with stainless steel frits	Standard 316 stainless steel column with stainless steel frits
Dimensions	300 x 4.6 mm	300 x 7.8mm
Resin type	Silica	Silica
Particle size (µm)	5	3
Pore size Å	500	500
pH range	2.5-7.5	2.5-7.5
MW Range in native conditions (Da)	15K-1500K	15K-1500K
Maximum backpressure (psi)	1500	1700
Typical efficiency (min no theoretical plates)	25000 (for a 300 x 7.8 mm column)	38000 (for a 300 x 7.8 mm column)
Maximum temperature (°C)	50	50
According to Phenomenex®	Capable of resolving large MW proteins	Column allow for very high efficiency and resolution
Price	R23,634 (bought 12-11-15)	R14,606 (bought 30-10-12)
Running conditions		
Mobile phase	Isocratic 50/50 (ACN/H ₂ O)	Isocratic 50/50 (ACN/H ₂ O)
Maximum flow rate	1.5 ml/min	1.5 ml/min
Maximum temperature	50°C	50°C
Detection	210 nm	210 nm
Run time	15 min	30 min
Guard cartridge	Yes	Yes

Source Phenomenex

6.5 Results

6.5.1 Size exclusion-high performance liquid chromatography profiles

The BioSep-SEC s4000 narrow bore column yielded five peaks in the profiles. Peak1 (F1) corresponded with the HMW, while the F2 corresponded with LMW, F3-F4 corresponded with gliadins, while F5 corresponded with globulin-albumins (Figure 4.1 and Figure 4.2). The Yarra-SEC 4000 analytical column yielded nine peak profiles; F1 corresponded with HMW, F2-F4 corresponded with LMW, the F5-F7 corresponded with gliadins while F8-F9 corresponded with globulins-albumins (Figure 5.1 and Figure 5.2). With the Yarra-SEC 4000 analytical column more peaks were obtained when compared to the narrow bore column. When the narrow bore column was used for LMW-GS separation, only one profile peak was produced, while with the analytical column three profile peaks were produced (F2-F4). In the case of the gliadins, the narrow bore column yielded two profile peaks (F3-F4), while the analytical column yielded three profile peaks (F5-F7). For the globulins –albumins, the narrow bore column yielded only one profile peak, while the analytical column yielded two profile peaks.

6.5.2 Analysis of variance for absolute proteins of the dryland summer, dryland winter rainfall and irrigated region cultivars

The dryland summer rainfall region

When the SE-HPLC of protein fractions were separated using the narrow bore and analytical columns for the dryland summer rainfall area cultivars, the LUPP did not show significant differences for either. The UPP was significant at $p \leq 0.01$ for the narrow bore and non-significant for the analytical column. For the dryland summer rainfall region the F ratios were significant at $p \leq 0.01$ for LMW%p and GGA%p and significant at $p \leq 0.05$ for the narrow bore column. For the analytical column the F ratios were only significant for LMW%p at $p \leq 0.05$ and GLI%p at $p \leq 0.001$ (Table 6.2)

The dryland winter rainfall

When the narrow bore column was used for the winter rainfall region cultivars, the F ratios were significant for only the LUPP and UPP ($p \leq 0.01$ respectively). When the analytical column was used the F ratios were significant at $p \leq 0.05$ for LUPP and UPP. When the analytical column was used the F ratios were significant for all the protein fractions. POL%p, HMW%p, GLI%p and GGA%p were significant at $p \leq 0.001$. LMW%p was significant at $p \leq 0.01$. While for the narrow bore column HMW%p was the only significant parameter at $p \leq 0.05$ (Table 6.2).

The irrigated region

The LUPP and UPP showed significant differences for both columns at $p \leq 0.001$. The Yarra-SEC 4000 analytical column showed significant differences for all protein fractions at $p \leq 0.001$. F ratios for the BioSep-SEC s4000 narrow bore column were significant for all the protein fractions; LMW%p was significant at $p \leq 0.01$, while other protein fractions were highly significant at $p \leq 0.001$ (Table 6.2).

Table 6.2 Analysis of variance for absolute and relative protein fractions in the dryland summer, winter rainfall and irrigated region cultivars

Regions	Characteristics	Absolute proteins		Relative proteins		
		Narrow bore column	Analytical column	Narrow bore column	Analytical column	
		F-value	F-value	F-value	F-value	F-value
Dryland summer rainfall region	LUPP	0.80 ^{NS}	1.57 ^{NS}			
	UPP	2.62 ^{**}	3.60 ^{NS}			
	POL%p	1.74 ^{NS}	1.66 ^{NS}	POL%fp	2.61 ^{**}	2.61 ^{**}
	HMW%p	1.52 ^{NS}	1.16 ^{NS}	HMW%fp	1.57 ^{NS}	1.32 ^{NS}
	LMW%p	2.57 ^{**}	2.18 [*]	LMW%fp	4.52 ^{***}	2.32 [*]
	GLI%p	1.78 ^{NS}	9.90 ^{***}	GLI%fp	3.71 ^{***}	13.88 ^{***}
	GGA%p	0.61 [*]	1.58 ^{NS}	GGA%fp	2.61 ^{**}	1.75 ^{NS}
Dryland winter rainfall region	LUPP	2.83 [*]	2.40 [*]			
	UPP	4.22 ^{**}	2.43 [*]			
	POL%p	2.01 ^{NS}	5.42 ^{***}	POL%fp	0.61 ^{NS}	8.29 ^{***}
	HMW%p	2.31 [*]	17.00 ^{***}	HMW%fp	1.94 ^{NS}	13.86 ^{***}
	LMW%p	2.13 ^{NS}	4.31 ^{**}	LMW%fp	0.88 ^{NS}	6.71 ^{***}
	GLI%p	1.06 ^{NS}	18.40 ^{***}	GLI%fp	1.15 ^{NS}	22.12 ^{***}
	GGA%p	0.65 ^{NS}	6.36 ^{***}	GGA%fp	0.61 ^{NS}	8.29 ^{***}
Irrigated Region	LUPP	6.48 ^{***}	3.37 ^{***}			
	UPP	4.97 ^{***}	4.08 ^{***}			
	POL%p	7.70 ^{***}	20.67 ^{***}	POL%fp	30.67 ^{***}	12.12 ^{***}
	HMW%p	4.34 ^{***}	17.91 ^{***}	HMW%fp	2.10 [*]	13.07 ^{***}
	LMW%p	2.29 ^{**}	19.22 ^{***}	LMW%fp	1.70 ^{NS}	12.03 ^{***}
	GLI%p	7.07 ^{***}	52.53 ^{***}	GLI%fp	10.33 ^{***}	14.46 ^{***}
	GGA%p	8.21 ^{***}	20.67 ^{***}	GGA%fp	30.63 ^{***}	10.98 ^{***}

LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, POL%p = relative total polymeric proteins, POL%fp = absolute total polymeric proteins, HMW%p = relative high molecular weight, HMW%fp = relative high molecular weight LMW%p = relative low molecular weight, LMW%fp = low molecular weight GLI%p = relative total gliadins, GLI%fp = absolute total gliadins, GGA%p = relative total gliadins albumen-globulins, GGA%fp = relative total gliadins and albumins-globulins*** p≤ 0.001, ** p≤ 0.01, * p≤ 0.05, NS = p>0.05.

6.5.3 The analysis of variance for relative proteins of the dryland summer, dryland winter rainfall and irrigated region cultivars

The dryland summer rainfall cultivars

The POL%fp was significant at p≤ 0.01 for the BioSep-SEC s4000 narrow bore column, while POL%fp was not significant for the Yarra-SEC 4000 analytical column. HMW%p was not significant for both columns. LMW%fp was significant at p≤ 0.001 for the narrow bore column and significant (p ≤ 0.05) when the analytical column was used. The GLI%fp was significant at p ≤ 0.001 for both columns. The GGA%fp was significant at p ≤ (0.01) for the narrow bore column and non-significant for the analytical column (Table 6.2).

The dryland winter rainfall cultivars

When the BioSep-SEC s4000 narrow bore column was used for the winter rainfall region cultivars, the F ratios were non-significant. When the Yarra-SEC 4000 analytical column was used, the F ratios for POL%fp, HMW%fp, LMW%fp, GLI%fp and GGA%fp were highly significant at $p \leq 0.001$ (Table 6.2).

The irrigated region cultivars

For the irrigated region cultivars, the F ratios were highly significant for all the protein fractions at $p \leq 0.001$ (Table 6.2). When the narrow bore column was used, the F ratios were significant for all the protein fractions except for the LMW%fp. The POL%fp, GLI%fp and GGA%fp were significant at $p \leq 0.001$ while HMW%fp was significant at $p \leq 0.05$.

6.5.4. Comparing the relationships between SE-HPLC protein fractions separated by the narrow bore and analytical columns for bread-making quality characteristics in the three production areas

The irrigated region cultivars

Flour protein content

When the Yarra-SEC 4000 analytical column was used, significant but low negative correlation was found between LUPP and FPC, however, no correlations were found when the BioSep-SEC s4000 narrow bore column was used (Table 6.3). The POL%p showed significant correlations with FPC for both columns, the narrow bore column revealed highly significant positive correlations at $p \leq 0.001$, while the analytical column showed negative correlations at $p \leq 0.001$. When the Yarra-SEC 4000 analytical column was used, POL%fp was negatively associated with FPC at $p \leq 0.05$ with low r values. The BioSep-SEC s4000 narrow bore column demonstrated positive correlation between HMW%p and FPC; however, no significant correlations existed with the Yarra-SEC 4000 analytical column. The HMW%fp correlated negatively with FPC for the analytical column at $p \leq 0.001$. The narrow bore column fractions did not show any significant correlations with quality characteristics. The LMW%p correlated with FPC for the narrow bore column. The GLI%p showed positive correlation with FPC for both columns, the narrow bore column values were highly significant at $p \leq 0.001$ with a larger r value while the analytical column's values were significant at $p \leq 0.05$. The GGA%fp showed significant correlation with FPC at $p \leq 0.05$ for the Yarra-SEC 4000 analytical

column. The GGA%p demonstrated significant correlations for both columns at $p \leq 0.001$ (Table 6.3).

Alveograph strength

Highly significant ($p \leq 0.001$) correlations were seen between LUPP, UPP and alvW when using the BioSep-SEC s4000 narrow bore column and Yarra-SEC 4000 analytical column, however for the analytical column, LUPP and UPP negatively correlated with alvW. The POL%p correlated positively with alvW with both columns. The LMW%p correlated significantly ($p \leq 0.001$) with alvW when the narrow bore column was used. No significant correlations were obtained when the analytical column was used. The GLI%p correlated positively at $p \leq 0.001$ with alvW when using both the BioSep-SEC s4000 narrow bore column and the Yarra-SEC 4000 analytical column. An r value of 0.626 was found with the narrow bore column ($p \leq 0.001$), while an r value of 0.480 was obtained for the analytical column. The GGA%p correlated positively with alvW when both columns were used (Table 6.3).

Mixograph peak time

LUPP ($p \leq 0.01$) and UPP ($p \leq 0.001$) correlated positively with MPT for the BioSep-SEC s4000 narrow bore column. However when the Yarra-SEC 4000 analytical column was used, negative correlations at $p \leq 0.001$ between LUPP, UPP and MPT were seen. The POL%p correlated positively ($p \leq 0.001$) with MPT for both columns ($p \leq 0.01$). The HMW%p, LMW%p, GLI%p ($p \leq 0.001$) and GGA%p ($p \leq 0.001$) were positively correlated with MPT with low r values for the BioSep-SEC s4000 narrow bore column. For the Yarra-SEC 4000 analytical column the MPT correlated positively with LMW%p ($p \leq 0.05$) and GGA%p ($p \leq 0.001$) although the values were quite low (Table 6.3).

Loaf volume

Significant negative correlations were seen between LUPP and LV when the Yarra-SEC 4000 analytical column was used. The BioSep-SEC s4000 narrow bore column did not show any significant correlations between the LUPP and LV. Highly significant ($p \leq 0.001$) correlations were seen between POL%p and LV when the narrow bore column and analytical column were used. The HMW%p correlated positively with LV but the r value was low when the narrow bore column was used. The HMW%fp correlated positively with LV at $p \leq 0.001$. When the Yarra-SEC 4000 analytical column was used,

HMW%fp positively correlated with LV. When the BioSep-SEC s4000 narrow bore column were used no significant correlations was observed between HMW%fp and LV. The LMW%p correlated positively with LV at $p \leq 0.01$ when the narrow bore column was used and at $p \leq 0.001$ when the analytical column was used. With the analytical column, LUPP was negatively correlated ($p \leq 0.001$) with LV. The GLI%p and GGA%p correlated positively with LV when the narrow bore column and analytical columns were used. The r values obtained when using the BioSep-SEC s4000 narrow bore column were greater than those obtained when using the Yarra-SEC 4000 analytical column. They were also highly significant at $p \leq 0.001$ (Table 6.3).

The dryland summer rainfall cultivars

Flour protein content

The POL%p correlated positively with FPC for both columns at $p \leq 0.001$ (Table 6.3). The HMW%p and LMW%p significantly correlated with FPC for the BioSep-SEC s4000 narrow bore column ($p \leq 0.001$). Both columns presented significant correlations between GLI%p and FPC, the narrow bore column value was highly significant ($p \leq 0.001$) with greater r values, while the Yarra-SEC 4000 analytical column was significant at $p \leq 0.05$. The GGA%p showed significant correlations with FPC at $p \leq 0.001$ for both columns

Alveograph strength

Negative correlations were obtained between alvW and LUPP and UPP with low r values for the BioSep-SEC s4000 narrow bore column. However the Yarra-SEC 4000 analytical column presented positive correlation between UPP and alvW at $p \leq 0.05$. The POL%p correlated positively with alvW for the narrow bore column. For the analytical column HMW%fp correlated negatively with alvW, although the r value obtained was low, while the LMW%p correlated positively at $p \leq 0.01$ with alvW (Table 6.3)

Table 6.3 Significant correlations between protein fractions and selected quality parameters

	Parameters	FPC		alvW		MPT		LV	
		Narrow bore column	Analytical column	Narrow bore column	Analytical column	Narrow bore column	Analytical column	Narrow bore column	Analytical column
Irrigated region	LUPP	NS	-0.371**	0.458***	-0.492***	0.315*	-0.456**	NS	-0.331**
	UPP	NS	NS	0.532***	-0.513***	0.411***	-0.450***	NS	NS
	POL%p	0.913***	-0.630***	0.647***	0.602***	0.523***	0.253**	0.617*	0.559***
	POL%fp	NS	-0.248*	NS	NS	NS	NS	NS	NS
	HMW%p	0.583***	NS	0.355**	0.600***	0.310**	NS	0.276*	NS
	HMW%fp	NS	-0.617*	NS	NS	NS	NS	NS	0.584***
	LMW%p	0.572***	NS	0.512***	NS	0.384*	0.260*	0.401**	0.527***
	LMW%fp	NS	NS	NS	NS	NS	NS	NS	NS
	GLI%p	0.772***	0.630*	0.626***	0.480***	0.349***	NS	0.499***	0.434
	GLI%fp	NS	NS	NS	NS	NS	NS	NS	NS
Dryland summer rainfall	GGA%p	0.940***	0.693***	0.693***	0.347*	0.423***	0.410***	0.582***	0.305**
	GGA%fp	NS	0.248*	NS	NS	NS	NS	NS	NS
	LUPP	NS	NS	-0.279*	NS	NS	NS	NS	NS
	UPP	NS	NS	-0.404**	0.279*	NS	0.360***	NS	NS
	POL%p	0.741***	0.629***	0.336*	NS	NS	NS	NS	NS
	POL%fp	NS	NS	NS	NS	NS	NS	NS	NS
	HMW%p	0.552***	NS	NS	NS	NS	NS	NS	NS
	HMW%fp	NS	NS	NS	-0.288*	-0.323*	NS	NS	NS
	LMW%p	0.647***	NS	NS	0.388**	NS	NS	NS	NS
	LMW%fp	NS	NS	NS	NS	NS	NS	NS	NS
	GLI%p	0.700***	0.284*	NS	NS	NS	NS	NS	NS
	GLI%fp	NS	NS	NS	NS	NS	NS	NS	NS
	GGA%p	0.807***	0.667***	NS	NS	0.296*	NS	NS	NS
	GGA%fp	NS	NS	NS	NS	NS	NS	NS	NS

LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, POL%p = absolute total polymeric proteins, HMW% p= absolute high molecular weight, LMW%p = absolute low molecular weight, GLI%p = absolute total gliadins, GGA%p = absolute total gliadins, POL%fp = relative total polymeric proteins, HMW%fp = relative high molecular weight, LMW%fp = relative low molecular weight, GLI%fp = relative total gliadins, GGA%fp = relative total gliadins albumin-globulins, , alvW = alveograph strength, LV = loaf volume MPT = mixograph peak time FPC = flour protein content *p≤0.05.** p≤0.01, *** p≤0.001

Table 6.3 continued

	Parameters	FPC		alvW		MPT		LV	
		Narrow bore column	Analytical column	Narrow bore column	Analytical column	Narrow bore column	Analytical column	Narrow bore column	Analytical column
Dryland	POL%p	0.702***	NS	NS	NS	0.421***	NS	NS	NS
winter	POL%fp	NS	-0.407*	NS	-0.347	NS	NS	NS	-0.363*
rainfall	HMW%p	0.375*	NS	NS	NS	NS	NS	NS	NS
	HMW%fp	NS	NS	NS	NS	NS	NS	NS	NS
	LMW%p	0.683***	NS	NS	-0.333*	0.381*	NS	NS	NS
	LMW%fp	NS	-0.407*	NS	-0.344*	NS	NS	NS	-0.402*
	GLI%p	0.598***	NS	NS	NS	NS	NS	NS	NS
	GLI%fp	NS	-0.347*	NS	NS	NS	NS	NS	NS
	GGA%p	0.608***	0.526**	NS	NS	NS	NS	NS	0.610***
	GGA%fp	NS	0.412*	NS	0.347*	NS	NS	NS	0.363*

POL%p=absolute total polymeric proteins, HMW%p=absolute high molecular weight, LMW%p=absolute low molecular weight, GLI%p= absolute total gliadins, GGA%p=absolute total gliadins, POL%fp=relative total polymeric proteins, HMW%fp=relative high molecular weight, LMW%fp=relative low molecular weight, GLI%fp=relative total gliadins, GGA%fp=relative total gliadins albumin-globulins, , alvW=alveograph strength, LV=loaf volume MPT=mixograph peak time, FPC=flour protein content *p≤ 0.05, **p≤0.01, *** p≤0.001

Mixograph peak time

MPT correlated negatively with the HMW%fp, but positive with GGA%fp for the BioSep-SEC s4000 narrow bore column. For the Yarra-SEC 4000 analytical column, the MPT only correlated with UPP at $p \leq 0.001$.

Loaf volume

LV did not present any significant correlations with protein fractions for both columns used.

The dryland winter rainfall region cultivars

Flour protein content

When the BioSep-SEC s4000 narrow bore column was used, POL%p significantly correlated with FPC at $p \leq 0.001$. No correlation was found when the Yarra-SEC 4000 analytical column was used. The POL%fp negatively correlated with FPC for the analytical column. The LMW%p and GLI%p correlated positively with FPC for the narrow bore column at $p \leq 0.001$. The GGA%p correlated positively with FPC for both columns. For the narrow bore column correlations were highly significant at $p \leq 0.001$, while for the analytical column it was significant at $p \leq 0.05$. The GGA%fp correlated with FPC for the Yarra-SEC 4000 analytical column only at $p \leq 0.05$ (Table 6.3).

Alveograph strength

No significant correlation was observed between protein fractions and alvW for the narrow bore column. The POL%fp, LMW%fp and LMW%p negatively correlated with alvW at $p \leq 0.05$ for the analytical column. GGA%fp correlated positively with alvW at $p \leq 0.05$ for the analytical column.

Mixograph peak time

The MPT correlated positively with POL%p and LMW%p with low r values of 0.421 and 0.381 for the narrow bore column. No significant correlations were observed between protein fractions and MPT when the analytical column was used.

Loaf volume

No significant correlations occurred between the LV and protein fractions when the BioSep-SEC s4000 narrow bore column was used. When the Yarra-SEC 4000 analytical

column was used POL%_{fp} and LMW%_{fp} negatively correlated with the LV with low *r* values. The GGA%_p positively correlated ($p \leq 0.001$) with LV with a larger *r* value 0.610. Also a significant positive correlation was seen between GGA%_{fp} and LV (Table 6.3).

6.6 Discussion

All the absolute protein fractions correlated positively with FPC ($p \leq 0.001$) in all three production area cultivars with the BioSep-SEC s4000 narrow bore column. The FPC had high correlations (0.70-0.94) with POL%_p, GLI%_p and GGA%_p in all three production area cultivars, except for GGA%_p in the winter rainfall region. The results indicated that the quantitative variation in FPC was due to GLI, GGA and POL. However Ohm et al. (2009) found both HMW and GLI to have a major influence on quantitative variation in FPC. The results reported in this study are in agreement with Park et al. (2006), who reported significant positive correlations between albumins-globulins and gliadins in the flour, with FPC. Gupta et al. (1993) reported significant positive correlations between gliadins in the flour and FPC. Labuschagne and Aucamp (2004) reported significant correlations between soluble gliadins and FPC. Bean et al. (1998) reported significant correlations between FPC and insoluble polymeric proteins in the flour.

When the Yarra-SEC 4000 analytical column was used, protein fractions both in the flour and in the flour protein correlated with FPC. For the winter rainfall cultivars the GLI%_p negatively correlated with FPC, which is in agreement with findings of Singh et al. (1990). The GGA%_{fp} and GGA%_p for both irrigated and winter rainfall cultivars showed significant associations with FPC. This was also reported by Gupta et al. (1992) where the albumins-globulins in the flour and protein correlated with FPC. The results obtained in this study indicated that absolute protein fractions are a good indicator of FPC, as high correlations were obtained between the absolute protein fractions and FPC when both columns were used. These findings corroborate with those of Ciaffi et al. (1996), where they found that total protein was highly correlated with the absolute areas of individual peaks. They further pointed out that peak 1, which corresponds with the HMW, showed lower correlation coefficients than peaks 2 and 3, which correspond with monomeric proteins. This is in agreement with what was found in this study. The correlations obtained with the BioSep-SEC s4000 narrow bore column were higher than those for the

Yarra-SEC 4000 analytical column. The POL%_{fp} correlated with FPC when both columns were used, which has never been reported from previous studies.

When the narrow bore column was used, no significant correlations were seen between LV and protein fractions for the dryland summer rainfall and dryland winter rainfall cultivars. The absolute protein fractions correlated with LV in these location cultivars when both narrow bore and analytical columns were used. Gupta et al. (1992) reported higher significant correlations between LV and protein fractions in protein than protein fractions in flour proteins in the flour, especially for the HMW. A significant correlation between GLI%_p and LV was also reported by Park et al. (2006). Ohm et al. (2010) reported positive correlations between LV and GLI. Mkhatywa (2014) reported significant correlations between LV and ω -gliadins in the flour. The LUPP negatively correlated with FPC. This might be due to the negative correlation between LUPP and FPC. Labuschagne and Aucamp (2004) observed a negative correlation between LUPP and LV. HMW%_{fp}, LMW%_p, GGA%_p also correlated with LV. The correlations obtained between LMW%_p and POL%_p were highly significant ($p \leq 0.001$), however, the correlations values were moderate. Gupta et al. (1992) indicated that LV correlated well with proteins in the flour when a long fermentation cycle applied. The HMW%_p and LMW%_p correlated positively with LV. They also reported significant correlations between glutenins in the flour and LV (Gupta et al. 1992). Park et al. (2006) also reported significant correlations between insoluble protein fractions in the flour and LV. Chaudhary et al. (2016) reported significant correlations between HMW and LV.

The results obtained indicated that LUPP, UPP and HMW%_p significantly ($p \leq 0.001$) affected alvW when the BioSep-SEC s4000 narrow bore column was used for the irrigated region. This suggests that these protein fractions obtained from the narrow bore column can be used to assess flour strength. The significant correlations between these protein fractions and alvW have been reported by other authors. MacRitchie et al. (1991) observed significant correlations between alvW and insoluble polymeric proteins. Gupta et al. (1993) observed significant correlations between UPP and alvW. Bean et al. (1998) reported that the unextractable proteins left in the flour pellet correlated with dough strength. Edwards et al. (2007) and Mkhatywa (2014), observed significant correlations between the UPP in the flour and alvW. Cornish et al. (2006) indicated that *GLU-1* alleles had a greater effect on dough strength. Kuktaite et al. (2004) and Peña et al. (2005)

indicated the contribution of HMW in dough strength. Johansson et al. (2001) observed significant correlations between large UPP and dough strength. The HMW%p correlated highly significantly with alvW using the Yarra-SEC 4000 analytical column, indicating that this column separated fractions which were superior for predicting dough strength. However, when the Yarra-SEC 4000 analytical column was used, LUPP and UPP negatively correlated with alvW. This was contradictory to previous reports by Gupta et al. (1993), MacRitchie et al. (1991) and Morel et al. (2000). The LMW%p correlated positively with alvW when both columns were used. GLI%p correlated positively with alvW when the BioSep-SEC s4000 narrow bore column was used, however, with the Yarra-SEC 4000 analytical column, no significant correlations were observed. This suggests that the narrow bore column was more efficient in separating GLI. Previous studies indicated that the contribution of GLI towards alvW is due to close genetic linkage between *GLI-1* and *GLU-3*. Fido et al. (1997) pointed out that increased gliadin concentration results in decreased dough strength. For the dryland summer rainfall area the narrow bore column presented negative correlation between LUPP, UPP and alvW. However, UPP positively correlated with alvW when the analytical column was used, although the correlation coefficient was weak. The dryland winter rainfall area cultivars presented negative correlations between LMW%p, LMW%fp and alvW which is not in agreement with previous studies (Dachkevitch and Autran 1989; Weegels et al. 1996).

For the irrigated region UPP, LUPP, POL%p, HMW%p significantly correlated with MPT when the BioSep-SEC s4000 narrow bore column was used, with moderate *r* values. Koen (2006) reported significant correlation between LUPP and MPT. Ohm et al. (2008) and Zhang et al. (2009) reported significant correlations between MPT and HMW, which was also found in this study. Park et al. (2006) reported significant correlations between MPT and UPP, which indicates the contribution of large polymeric proteins in MPT. Significant correlation between HMW%p and MPT is in agreement with Gupta et al. (1992) who reported positive correlations between MPT and glutenins in the flour, which corroborates the findings in this study for the winter rainfall region cultivars when the narrow bore column was used. GLI%p positively correlated with MPT. This is contradictory to what was found by Park et al. (2006), who indicated that gliadins negatively correlated with MPT. When the Yarra-SEC 4000 analytical column was used, LUPP and UPP negatively correlated with MPT, while the LMW%p correlated positively with MPT. This corroborates findings of Zhang et al. (2009). Ohm et al. (2009)

indicated correlations between F2 and farinograph peak time. For the dryland summer rainfall region there were very few significant correlations between MPT and protein fractions for both columns. HMW%fp negatively correlated with MPT for the narrow bore column, which is contradictory to what was reported by Ohm et al. (2008; 2009).

When the ANOVA was performed, the F ratios for the Yarra-SEC 4000 analytical column were comparable and superior to those found using the BioSep-SEC s4000 narrow bore column. This suggests that the analytical column was more discriminative in the separation of proteins, with reduced errors. This column showed superiority for both the winter rainfall and irrigated region; cultivars with larger F ratios which were significant at $p \leq 0.001$ for most characteristics. For the dryland location's cultivars, neither column demonstrated much variation, as for most parameters there were no significant differences. For the absolute proteins, both columns have shown superiority for the irrigated region's cultivars, but the analytical column was superior for the winter rainfall region cultivars. Ohm et al. (2009), in his study of SE-HPLC of wheat proteins using a narrow bore column for evaluating bread-making quality of hard spring wheat, found the BioSep-SEC s4000 narrow bore column (300 x 4.5) to be more superior in the separation of wheat proteins than the regular BioSep-SEC s4000 (300 x 7.8) column.

6.7 Conclusions

The study assessed whether the Yarra-SEC 4000 analytical column can distinctly separate wheat proteins. More profile peaks were seen when the Yarra-SEC 4000 analytical column was used which suggests that this column separated protein fractions accurately. The Yarra-SEC 4000 demonstrated superiority over the BioSep-SEC s4000 column. Highly significant differences were observed in protein fractions separated by the analytical column. In order to have a better understanding about the correlations between protein fractions and quality traits, another study should be done to include more localities for testing over the number of years. Quality is an end result of environmental as well as genetic factors and their interaction, hence it is important to determine the contribution made by environment, genetic and environment interaction which was not done in this study because only single location testings were available.

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Chapter 7

General conclusions

The Wheat Technical Committee regulates the wheat quality standards in South Africa. Wheat quality is a complex of different characteristics. It is determined by environment, genetic factors and the interaction between the two. Selection for good bread-making characteristics is very difficult due to the polygenic nature of wheat quality. Gluten proteins have been reported to significantly affect the baking quality of wheat. In this study little allelic variation was found among the HMW banding patterns for *GLU-A1* and *GLU-D1* loci in all the cultivars of the three different regions. The *GLU-B1* locus exhibited the most variation. The HMW-GS as such were found to be poor predictors of quality characteristics, which lead to the following investigations of polymeric and monomeric fractions as predictors of baking quality, using a narrow bore, and a wide bore column with SE-HPLC.

SE-HPLC analysis performed using a narrow bore column showed less significant differences in the protein fractions among the cultivars of the three production regions than when the analytic column was used. Significant differences were observed for all the absolute protein fraction values for the irrigated region cultivars. More significant correlations were observed between absolute than relative protein values and quality data in cultivars in all three production regions. No significant correlations were found between relative proteins and quality characteristics for the dryland summer rainfall cultivars. More significant correlations were observed between protein fractions and quality characteristics in the irrigated region cultivars compared to cultivars of the other regions. The UPP correlated significantly with rheological parameters. The GLI correlated with dough rheological parameters, which might be due to some of the LMW which have similar molecular weights with some GLI. It is difficult to establish whether the contribution was due to GLI or LMW. The GLI%p correlated with FPC and GWC in all the locations. The cumulative variances obtained from PCA were 98.60%, 81.76% and 89.30% for the dryland summer rainfall, dryland winter rainfall and irrigated regions respectively.

The results obtained from the Yarra-SEC 4000 analytical column showed significant differences in relative and absolute protein fractions between the genotypes for the irrigated and dryland winter rainfall regions. The dryland summer rainfall region presented little significant differences between the genotypes for both absolute and relative protein fractions. Much variation was contributed by genotypes for the measured traits for the irrigated region except for GGA%p. For dryland winter rainfall, genotypes contributed much for the total variation for the absolute proteins, this indicates that selection for these characteristics would be successful. The UPP correlated positively with dough rheology for the dryland summer rainfall region cultivars. The irrigated region cultivars showed negative relationships between UPP and dough rheological characteristics. The GLI%p positively correlated with alvW for the irrigated region cultivars. The cumulative variance attributed by PCA was 78.64%, 87.64% and 94.38% for the dryland summer rainfall, dryland winter rainfall and irrigated regions respectively.

The results indicated that the Yarra-SEC 4000 analytical column was better than the narrow bore column in the separation of wheat proteins. The analytical column produced nine profile peaks compared to the five produced by the narrow bore column. Due to single location testing, it is difficult to make a lot of conclusions from this study. Multi location testing is necessary to determine location effect within regions, and to study the effect of season. This will also help to determine the genotype by environment interaction for the measured characteristics.

Appendix

Table 1 Descriptive statistics for the measured quality characteristics in the cultivars of dryland summer rainfall, dryland winter rainfall and irrigation regions

Characteristics	Dryland summer rainfall			Dryland winter rainfall			Irrigated region		
	MIN	mean	MAX	MIN	mean	MAX	MIN	mean	MAX
BFY	18.17	22.20	26.75	16.80	20.32	23.36	17.86	21.10	24.68
FY	72.43	74.92	77.57	71.64	74.51	76.35	73.98	77.96	80.15
FPC	12.62	14.57	16.30	11.34	13.00	16.78	9.60	11.37	14.91
WGC	37.27	43.29	51.15	31.29	39.48	46.25	29.05	34.19	41.69
SDSVOL	70.00	87.12	95.00	80.00	90.89	95.00	72.00	86.79	96.00
MPT	1.93	3.08	4.50	1.81	2.65	3.59	1.44	2.23	3.92
alvP	66.00	91.49	118.00	57.00	75.75	96.00	46.00	63.89	120.00
alvL	70.00	109.57	157.00	84.00	120.33	144.00	85.00	144.29	197.00
alvP/L	0.45	0.87	1.62	0.41	0.66	1.13	0.26	0.47	1.38
alvW	37.31	53.00	75.23	35.78	45.69	60.70	24.31	36.77	66.06
LV	925.00	1008.43	1030.00	895.00	977.08	1030.00	780.00	901.44	1030.00
LV12	778.00	905.69	996.00	836.00	937.42	1000.00	803.00	926.89	1036.00

BFY = break flour yield, FY = flour yield, FPC = flour protein content, WGC = wet gluten content, SDSVOL = Sodium dodecyl sulphate sedimentation-volume, alvL = alveograph extensibility in mm, alvP/L = alveograph stability to extensibility ratio in mm, alvP = alveograph stability in mm, alvW = alveograph strength in 10^{-4} J, LV12 = loaf volume on 12% flour protein basis, LV = loaf volume, MPT = mixograph peak time, MIN = minimum, MAX = maximum.

Table 2 Descriptive statistics for measured protein fractions in the cultivars of dryland summer, dryland winter rainfall and irrigation regions separated by the BioSep-SEC s4000 narrow bore column

Characteristics	Dryland summer rainfall			Dryland winter rainfall			Irrigated region		
	MIN	Mean	MAX	MIN	Mean	MAX	MIN	Mean	MAX
LUPP	27.21	48.20	55.55	45.22	52.63	70.91	12.78	44.65	57.95
UPP	40.85	46.42	52.35	48.48	53.33	61.03	30.88	50.36	60.61
POL%p	5.24	6.38	7.85	4.67	5.73	9.24	4.34	5.07	6.98
POL%fp	38.08	43.77	53.61	38.87	44.00	68.72	40.83	44.59	49.18
HMW%p	1.26	1.60	3.04	1.24	1.68	2.35	0.75	1.14	2.89
HMW%fp	9.26	10.97	18.73	10.56	12.93	20.73	7.53	10.02	25.52
LMW%p	3.86	4.78	5.76	2.35	4.05	6.92	2.30	3.90	5.11
LMW%fp	28.04	32.80	39.80	20.74	31.08	51.47	20.08	34.37	41.10
GLI%p	5.17	6.21	7.32	3.45	5.46	6.75	2.59	4.52	6.01
GLI%fp	37.21	42.62	50.23	25.65	42.08	46.16	22.86	39.70	46.38
GGA%p	6.44	8.19	9.58	4.20	7.27	8.95	5.16	6.30	8.30
GGA%fp	46.39	56.23	61.92	31.28	56.00	61.13	50.82	55.41	59.17

MIN = minimum, MAX = maximum, LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, POL%p = absolute total polymeric proteins, POL%fp = relative total polymeric proteins, HMW%p = absolute high molecular weight, HMW%fp = relative high molecular weight, LMW%p = absolute low molecular weight, LMW%fp = relative low molecular weight, GLI%p = absolute total gliadins, GLI%fp = relative total gliadins, GGA%p = absolute total gliadins and albumen-globulins, GGA%fp = relative total gliadins and albumins-globulins.

Table 3 Descriptive statistics for protein fractions in the cultivars of dryland summer, dryland winter rainfall and irrigation regions measured by Yarra-SEC 4000 analytical column

Characteristics	Dryland summer rainfall			Dryland winter rainfall			Irrigated region		
	MIN	Mean	MAX	MIN	Mean	MAX	MIN	Mean	MAX
LUPP	17.09	68.68	99.84	31.71	51.36	84.16	32.90	48.66	62.11
UPP	42.02	57.94	64.49	37.76	45.79	80.81	37.49	45.35	51.19
POL%p	3.59	6.47	8.12	3.56	5.48	8.61	2.79	5.20	7.09
POL%fp	26.18	44.38	57.98	28.00	42.16	56.79	25.89	45.77	50.52
HMW%p	0.42	0.80	2.61	0.50	0.80	1.23	0.44	0.80	1.19
HMW%fp	3.07	5.50	18.82	4.03	6.16	8.70	3.90	7.03	9.15
LMW%p	0.42	0.80	2.61	3.05	4.68	7.38	2.33	4.40	5.90
LMW%fp	23.11	38.89	52.17	23.93	36.00	48.67	21.68	38.74	43.49
GLI%p	1.83	5.85	7.26	2.91	5.14	6.57	2.25	4.48	6.34
GLI%fp	12.91	40.19	49.04	23.31	39.63	46.92	20.54	39.41	50.20
GGA%p	5.88	8.10	10.14	6.08	7.52	10.38	4.91	6.17	8.81
GGA%fp	42.02	55.62	73.82	43.21	57.84	72.00	49.48	54.24	74.11

MIN = minimum, MAX = maximum, LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, POL%p = absolute total polymeric proteins, POL%fp = relative total polymeric proteins, HMW%p = absolute high molecular weight, HMW%fp = relative high molecular weight, LMW%p = absolute low molecular weight, LMW%fp = relative low molecular weight, GLI%p = absolute total gliadins, GLI%fp = relative total gliadins, GGA%p = absolute total gliadins and albumen-globulins, GGA%fp = relative total gliadins and albumins-globulins.