ENVIRONMENTAL INFLUENCE ON THE EXPRESSION OF WHEAT PROTEIN FRACTIONS UNDER SOUTH AFRICAN DRYLAND CONDITIONS

By

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DECLARATION

I, Barend Smit Wentzel, declare that the thesis that I herewith submit for the Doctoral Degree in Plant Breeding at the University of the Free State, is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

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…… ……

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DEDICATION

This study is dedicated to my mother

Anna Maria Wentzel

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LIST OF ABBREVIATIONS

AG	Albumin and globulin
AGS	Sum of albumin and globulin
AlvL	Alveograph extensibility
AlvP	Alveograph tenacity
AlvP/L	Alveograph tenacity/extensibility
AlvSTR	Alveograph strength
ANOVA	Analysis of variance
ARC-SGI	Agricultural Research Council - Small Grain Institute
Bhm	Bethlehem
Bot	Bothaville
Bult	Bultfontein
Clar	Clarens
E-FS	Eastern Free State
EXP	SDS-extractable proteins
FABS	Farinograph water absorption
FLN	Falling number
FPC	Flour protein content
GLIADIN	Sum of gliadins
GLIAG	Sum of gliadins, albumin and globulin
HLM	Hectolitre mass
HMW	High molecular weight
HMW-GS	High molecular weight glutenin subunits
Lad	Ladybrand
LFV	Loaf volume
LMW	Low molecular weight
LMW-GS	Low molecular weight glutenin subunits
LUPP	Large unextractable polymeric proteins
MLR	Multiple linear regression

MPT	Mixograph peak time
MWD	Molecular weight distribution
NIL's	Near isogenic lines
NW-FS	North Western Free State
PDA	Photo diode array
POL	Sum of polymeric proteins
RIL's	Recombinant inbred lines
Rmax	Exstensigraph maximum resistance
RP-HPLC	Reversed-phase high-performance liquid-chromatography
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SDSS	SDS-sedimentation
SE-HPLC	Size-exclusion high-performance liquid-chromatography
TFA	Trifluoroacetic acid
UNP	SDS-unextractable proteins
UPP	Unextractable polymeric proteins
WGC	Wet gluten content

Chapter 1

Introduction

Wheat is adapted over a wide environmental range and regarded as the most important crop in the world regarding cultivated area. World wheat production increased between 1993 and 2013, from 564 million tons to 715 million tons (FAO, 2015). The yearly increase in wheat production of 0.9% won't be sufficient to meet the projected demands for 2050 (Ray et al., 2013). Production of wheat is the third largest after maize and rice (FAO, 2013), with the estimated demand expected to increase 60% by 2050 (Singh et al., 2011). Approximately 65% of the wheat crop is currently used for human consumption, 17% for animal fodder and 12% for industrial applications, including biofuel production (FAO, 2013).

Production areas in South Africa can be divided into three regions: winter rainfall area (Western Cape Province), summer rainfall area (Free State) and irrigation areas (cooler irrigation areas, warmer irrigation areas, Mpumalanga, Eastern Free State, Kwazulu-Natal and Eastern Cape) (SAGL, 2016).

Approximately 80% of the winter cereal crop production in South Africa consists of wheat. The other crops are malting barley and canola. The nine provinces in South Africa are divided into 36 crop production regions and wheat is produced in approximately 28 of these regions (SAGL, 2016).

Wheat production in South Africa declined with 7% during the 2014/2015 season compared to the 10-year production average, from 1 885 800 tons (2004/2005 to 2013/2014 seasons) to 1 750 000 tons (2014/2015 season). The Western Cape produced 899 000 tons, which is 51% of the total crop for 2014/2015 season. Irrigation in the Northern Cape was the second largest producer of wheat for the 2014/2015 season (285 000 tons), followed by irrigation in Limpopo and North West with 137 500 tons and 107 100 tons, respectively. Production in the Free State declined from 245 500 tons (2013/2014 season) to 24 500 tons (2014/2015 season) (SAGIS, 2016).

Wheat production area in South Africa declined almost 43% between 2004/2005 and 2013/2014 seasons, with a further 6% decline for the 2014/2015 season. Decline in the dryland Free State area is mainly due to a shift from wheat to crops like maize and soybeans. South Africa will remain a net importer of wheat to supply to local demand, due to the decline in production. During the 2013/2014 season 1 668 412 tons of wheat were imported, mainly from the Russian Federation (800 964 tons) (SAGIS, 2016)

The food industry is taking great care to keep the quality of their product as constant as possible around consumer acceptance. Flour behaviour is influenced by the amount of protein sub-fractions, rather than the total protein content, thus influencing the end product (Peña et al., 2005). Considering this, the different sub-fractions can serve as an indication of the raw material and the role in baking quality (Li Vigni et al., 2013). The quality of flour is by large influenced by the build-up process, amount and composition of grain protein, which are effected by the genetic background and environment and the interactions (Malik et al., 2013).

Bread making quality is inconsistent for winter wheat produced in the Free State and therefore detrimental to its market value. Variation in protein content is a primary factor for the inconsistency and should be taken into account during the release of new cultivars. Climatic conditions during grain filling appeared to influence protein content and mixing behaviour of dough (Van Lill et al., 1995a, b; Van Lill & Smith, 1997). A better understanding of the magnitude of environmental influence on protein composition could clarify the fluctuating bread making quality of genotypes.

The aim of the study is to establish the effect of the environment on selected wheat cultivars in the winter wheat production area, Free State in South Africa:

- To determine the effect of protein content on SDS-extractable and SDS-unextractable protein fractions
- To determine the effect of environment on SDS-extractable and SDS-unextractable protein fraction

- To investigate the contribution of size-exclusion high-performance liquid-chromatography (SE-HPLC) fractions to baking quality of wheat flour under South African dryland conditions

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Chapter 2

Wheat quality

Humans consumed wheat (*Triticum* spp.) for over 8500 years (Braun et al., 2010) and direct consumption of wheat contributes 22% of protein and 20% of calories in the human diet (Porter et al., 2007). The importance of wheat is likely to increase in future, due to its versatility of end-use products and environmental adaptability (Porter et al., 2007).

Wheat is unique because of the viscoelastic properties conferred by gluten, which serve as the main factor determining if wheat flour is suitable for the production of bread, biscuits, cakes or noodles (Li et al., 2013). Wheat quality is a complex trait and influenced by several components, of which the expression is affected by the genotype's reaction to a specific environment (Mann et al., 2009; Castillo et al., 2012). One of the main difficulties in cereal science is the understanding of bread making quality in wheat flour (MacRitchie, 2016).

2.1 Wheat protein

Protein is regarded as the most important constituent in wheat grain and the main contributor to technological and rheological properties that are related to end use quality (Zhao et al., 2010). Wheat proteins can be divided into three main groups: gluten, albumin and globulin. Gluten mainly supplies nitrogen to developing seedlings, while albumin and globulin serve specific functions for enzymes, enzyme inhibitors and structural elongation (Kucek et al., 2015).

2.1.1 Gluten

Gluten comprises almost 78 – 85% of the endosperm protein (MacRitchie, 1994). Storage proteins; glutenins and gliadins, are the main contributors to the viscoelastic properties of wheat (Souza et al., 2008), and therefore the main factor to determine end-use quality of a wheat variety (Peña et al., 2002).

2.1.2 Glutenin

Glutenin separates into four sub-groups according to electrophoretic mobility on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The A group corresponds to high molecular weight glutenin subunits (HMW-GS), with a molecular weight range of 80-130 kDa. About 60% of the glutenin fraction contains low molecular weight glutenin subunits LMW-GS, which occur in the major B and minor C groups (42 – 51 kDa and 30 – 40 kDa, respectively), with amino acid sequences in the C group similar to those of α/β- and γ-gliadins. The LMW-GS in the D group (55 – 70 kDa) are highly acidic and derived from modified ω-gliadins, with lower mobilities than the B and C groups (Payne et al., 1985; Ciaffi et al., 1999; Gianibelli et al., 2001). The LMW-GS are 4 to 5 times more abundant than HMW-GS, and their genes are located on the short arms of homoeologous group-one chromosomes at *Glu-A3*, *Glu-B3* and *Glu-D3* (D'Ovidio & Masci, 2004). LMW-GS is a complex group of proteins that consist of almost 30 different proteins and are normally classified as LMW-m, LMW-s and LMW-i types, based on their N-terminal amino acids; methionine, serine and isoleucine, respectively (D'Ovidio & Masci, 2004).

The HMW-GS are in the minority within the gluten proteins (≈ 10%) and contain an x-type subunit of higher molecular weight and a y-type subunit of lower molecular weight (Wieser, 2007). These subunits are encoded by genes present at the *Glu-1* loci, on the long arms of homoeologous group-one chromosomes at the A, B and D genomes (*Glu-A1*, *Glu-B1* and *Glu-D1* loci) (Shewry & Halford, 2002). The y-type gene, at the *Glu-A1* locus, is always silent in hexaploid and tetraploid wheat, while the x-type gene at the *Glu-A1* locus and the y-type gene at the *Glu-B1* locus are expressed only in some

cultivars. As a result, the number of subunits varies from three to five in bread wheat and from two to three in durum wheat (Shewry et al., 2006).

Payne & Lawrence (1983) developed the numbering system to identify HMW-GS. The system is currently in use and also provides a chromosomal location of the genes. Ascending numbers were initially assigned according to mobility in SDS-PAGE, with lower number indicating lower mobility. The logical order was not sustainable with the identification of new subunits. New numbers emerged with higher values and lower mobility than the initial numbers, such as subunit 21 (Anjum et al., 2007). It is customary to include the subunit and the genome from which it is derived, as well as the indication if it is an x-type or y-type subunit, for instance Dx5 + Dy10, although HMW-GS can also simply be expressed as 5 + 10 (Gao et al., 2010). For this study, HMW-GS will be expressed according to Gao et al. (2010).

Glutenin polymers are formed by disulphide bonds between HMW-GS and LMW-GS, with molecular weights exceeding one million Da, which are amongst the largest molecules found in nature (Wrigley, 1996). Glutenin proteins aggregate at two levels before the formation of the gluten polymer. At the first level, covalent polymers are formed between the HMW-GS and LMW-GS. On the second level larger aggregates are formed and stabilised by hydrogen and disulphide bonds, known as glutenin macropolymers (Weegels et al., 1996a) or unextractable polymeric protein (UPP) (Gupta et al., 1993). These essentially measure the same trait and both terms are acceptable. Naeem et al. (2012) preferred the term, UPP, because the parameter is based on an empirical measurement. The term, UPP, will be used for this study.

UPP consists of spherical glutenin particles (Don et al., 2003) and is insoluble in various solvents (SDS or acetic acid) (Weegels et al., 1997). The intensity of aggregation on the second level is highly influenced by the glutenin allelic composition (Hamer & van Vliet, 2000). The quantity of HMW-GS, LMW-GS and HMW-GS/LMW-GS ratio strongly influences the aggregation and polymerisation properties of the UPP during dough development (Wang et al., 2007). The UPP% is rather a measure of polymeric protein

above a certain critical molecular weight and may not reflect the molecular weight distribution of this fraction (Bersted & Anderson, 1990).

Gupta et al., (1993) developed one of the best methods to study gluten polymers. The extractable polymeric protein was extracted from total flour protein with SDS, and UPP was extracted with SDS after sonication. Polymeric and monomeric proteins were further separated from these extracts by means of SE-HPLC (Altenbach et al, 2016).

2.1.3 Gliadins

Gliadins are the most abundant wheat storage proteins and comprise approximately 40% wheat flour protein. Gliadins can be subdivided into three groups: α-, β-, γ-, and ω-gliadins. The α- and β-gliadins have a very similar primary structure and are therefore regarded as a single gliadin type, α/β type (Kasarda et al., 1987). Gliadins are controlled by genes located on the short arms of group one and six chromosomes, and specific loci were designated *Gli-A1*, *Gli-B1*, *Gli-D1*, *Gli-A2*, *Gli-B2* and *Gli-D2* (Payne, 1987).

The average molecular weight for α/β- and γ- gliadins ranged between 31 – 35 kDa, respectively (Shewry et al., 1986). The ω-gliadins are the largest of the gliadins with molecular weights between 46 – 74 kDa (Kasarda et al., 1983), and comprise 5 – 10% wheat flour protein and are extremely rich in proline and glutamine, with almost no methionine and cysteine (DuPont et al., 2006). Additionally, ω-gliadins can act as chain terminators because of the inclusion of modified gliadins with one cysteine residue (Gianibelli et al., 2002).

2.1.4 Albumins and globulins

The largest proportion of physiological active proteins in wheat is located in the albumins and globulins. The highest concentration of these proteins is present in the aleurone cells and the germ, with a lower concentration occurring in the mealy endosperm (Belderok et al., 2000). Albumins and globulins (AG) are regarded as soluble proteins, containing several metabolic enzymes (Dong et al., 2012). AG also occur in the

wheat endosperm and are considered to contribute to cell structure and metabolism (Hasniza et al., 2014a).

The globulin-like proteins are non-prolamines and function as storage proteins. In combination with gluten proteins their role is non-specific during the formation of the gluten network (Altenbach, 2012). Minimum research has been conducted on the non-prolamin proteins although these proteins account for 10 – 22% of total flour protein (Singh & MacRitchie, 2001).

Globulins are soluble in water or salt whereas the unextractable protein fraction is extracted with strong detergents and reducing agents. The occurrence of globulins in the unextractable protein fraction indicates a close association with proteins involved in the formation of gluten and the relationship with flour protein quality traits. Cysteine residues in the globulins could form disulphide bonds with proteins involved in the formation of the gluten network (Østergaard et al., 2000; Hasniza et al. 2014b). The six cysteine residues in globulin 3 and triticin are similar to some y-type HMW-GS and could have an effect on dough quality because of the contribution to gluten polymerisation (Hasniza et al., 2014b), through the formation of disulphide bonds (Gianibelli et al., 2001). A larger or more complex polymeric structure was observed in grain containing more globulin and triticin (Hasniza et al., 2014a).

Serpins belong to the albumins and are mainly soluble in water and may account for 4% of the endosperm protein (Cane et al., 2008). Furthermore, serpins contain glutamine-rich motifs that resemble those found in endosperm prolamins and have the ability to form intermolecular disulphide bridges. As a result, serpins may influence functional dough properties (Roberts & Hejgaard, 2008).

2.2 Baking quality

The end-use product requires a certain quality profile (Battenfield et al., 2016). Using protein content and composition as an indication of end-use quality is not simple,

therefore a combination of analysis is normally applied to evaluate flour quality (Hasniza et al., 2014a). Requirements for wheat classification and trading, such as falling number (FLN), hectolitre mass (HLM) and protein content, does not necessarily reflects the quality of the gluten, which may be extremely low (Koga et al., 2015). Differences in qualitative and quantitative protein compositions can determine the suitability of a cultivar for specific end-use quality. It is the task of wheat breeders to identify flour quality traits to satisfy processing requirements. Most of the quality traits are complex with an additive nature of inheritance. Several indirect methods were developed to screen early and advanced high yielding breeding lines with desirable quality traits (Peña et al., 2002). Some of these tests can be directly applied for selection in a breeding program while other needs to be interpreted collectively or serve only as criteria for further selection.

Laboratories across the world apply several methods to predict baking quality and the choice of instrumentation and procedures may differ between countries. Commonly used instrumentation includes mixograph, alveograph, extensigraph and farinograph. Several researchers use SDS-sedimentation (SDSS) as an indication of protein quality (Williams et al., 2008).

Some of the primary characteristics for the release of wheat cultivars in South Africa include mixograph peak time (MPT), farinograph water absorption (FABS), alveograph tenacity/extensibility (AlvP/L), alveograph strength (AlvSTR) and loaf volume (LFV) (SAGL, 2013). Wheat breeders in South Africa use SDSS to screen early generation breeding lines from F2 generations.

Farinograph and mixograph are considered as empirical dough mixing instruments, while alveograph and extensigraph are descriptive rheological instruments. Although the empirical and descriptive instruments render useful information to the processing industry, the information does not explain the fundamental viscoelastic nature of dough that could be widely applied by the processors (Edwards et al. 2003).

Correlations between rheological traits and LFV are effective over a limited range of flour properties, even with exceptions occurring within the range (Bloksma, 1990). Several dough rheological tests are not suitable to predict end-use quality because they do not consider the system under appropriate deformation conditions and not sensitive to the molecular structures involved in baking quality. The main advantage of biaxial extension is that deformation resembles the conditions experienced by cell walls, with the expansion of the gas cells during fermentation, during proofing and oven rise (Dobraszczyk & Morgenster, 2003).

Different testing procedures may result in different relationships with end-use quality (Williams et al., 2008). The Chopin alveograph measures traits similar to the extensigraph but the procedure differs somehow. The alveograph applies biaxial extension instead of uniaxial extension for the extensigraph. Furthermore, water content is constant and the dough mixing is fixed for the alveograph (Cuniberti et al., 2003). Alveograph tenacity (AlvP) measures the resistance to extension and AlvSTR represents the energy required to blow a bubble in the dough, while alveograph extensibility (AlvL) measures the extensibility of dough (Reese et al., 2007).

Alveograph parameters give an indication of dough strength and extensibility. According to Bordes et al. (2008), AlvP values, for standard bread wheat, range between 60 – 80 mm H_2O; for good quality wheat between 80 – 100 mm H_2O and higher than 100 mm H_2O for extra strong wheat. AlvL value of 100 mm is acceptable for good quality wheat. The AlvP/L value of 0.5 can be an indication of either, resistant and very extensible dough, or less resistant and moderately extensible dough. An AlvP/L value of 1.5 indicates very strong dough with moderate extensibility. AlvSTR summarises all the characteristics and is most widely used.

AlvP/L value is an indication of the balance between dough tenacity and extensibility although the prediction correlations for LFV were lower than other dough rheology traits measured with alveograph and mixograph (Battenfield et al., 2016). Increased AlvP will result in tenacious dough, which will result in porous bread. Therefore, AlvP should be compensated for by AlvL in order to achieve optimum LFV (Sanches-Garcia et

al., 2015). In a study conducted on eight wheat varieties from various localities, multiple regression analysis indicated biaxial extension viscosity and uniaxial extensibility as the best predictors for LFV. There should be a balance between AlvP and AlvL for sufficient stability of gas cells during dough expansion. If AlvP is not compensated by AlvL, dough will become overly tenacious and porous (Ktenioudaki et al., 2010).

Grain hardness showed a strong influence on dough tenacity and strength, since the alveograph test is performed at constant hydration. Alveograph results would have been different if hydration was adjusted to damaged-starch content in flour (Branlard et al., 2001). Mixograph parameters were less affected by grain hardness when hydration was adjusted according to damaged starch content, as opposed to alveograph parameters (Martinant et al., 1998). MPT is positively correlated with elasticity and mixing tolerance, while negatively correlated with dough extensibility and dough stability (Hoseney, 1994).

Direct measurements for dough rheology traits, such as mixing time, strength and extensibility, are time consuming and require large quantities of flour, compared to SDSS, an indirect measurement. The mixograph offers versatility regarding the amount of samples per day and sample sizes, compared to farinograph, alveograph and extensigraph (Peña et al., 2002). Additionally, mixograph parameters (measures of strength and extensibility) are highly correlated with alveograph and extensigraph parameters (Finney et al., 1987).

Both the mixograph and SDSS can be used as a measure of dough strength (Souza et al., 2008). The mixograph measures dough mixing time and overmixing tolerance by means of torque-recording, both measurements are indicative of gluten strength (Caffe-Treml et al., 2011), and does not provide direct information on dough extensibility (Edwards et al., 2003). SDSS gives an indication of the ability of proteins to aggregate and therefore the physico-chemical behaviour of flour (Graybosch et al., 1996). SDSS is an indirect estimation of gluten strength because it partially estimates the glutenin content in wheat flour. The method is based on the expansion of glutenins in isopropanol/lactic acid or SDS/lactic acid solution (Weegels et al., 1996a).

SDSS can serve as a simple method to predict grain quality because of the positive correlation with flour protein content (FPC) and wet gluten content (WGC) (Rozbicki et al., 2015), instead of using the alveograph, which is costlier and time consuming (Vázquez et al., 2012). Although alveograph, dough development time and dough strength, showed a weak correlation with LFV (Mladenov et al., 2001). SDSS correlated positively with FPC, MPT and AlvSTR and LFV, while SDSS did not correlate significantly with AlvP/L (Battenfield et al., 2016). Quality tests (SDSS, mixograph and alveograph traits) correlated with LFV, although correlations were not high enough. Therefore, phenotypic correlations indicated that no single quality test can substitute the baking test (Battenfield et al., 2016).

Hexaploid wheat is commonly used for bread baking, whereby the gluten forming proteins determine rheology of dough and gas retention properties as well as LFV (Delcour et al., 2012). Bread making quality is vital for the trading of wheat and therefore a priority in wheat breeding programmes. Direct determination of bread making quality through full scale analyses, including milling and baking tests, is expensive, time consuming and require large samples. It is therefore limited to advanced lines. Indirect methods, such as SDSS, alveograph and mixograph parameters are applied to screen early generation material (Groos et al., 2007).

The optimised 100 g straight dough bread making method is used to evaluate wheat breeding lines in South Africa. The method is not regarded as an indication of the baking quality of the flour, rather to establish the relationship between protein content and LFV. A factor of 40 cm^3 per 1% protein difference is used to adjust the bread volume of the line against the biological standard (SAGL, 2013).

Finney et al. (1973) associated high water absorption, a medium-long mixing time and good mixing tolerance, as desirable qualities in wheat flour for good LFV. The Chorleywood procedure is mainly used in the large scale bakeries in South Africa. Short mixing time and mixing tolerance are desirable traits for the Chorleywood procedure

(Souza et al., 2008). Tolerance to over mixing is described as the range of mix times above and below optimal dough development time (Souza et al., 2008).

2.3 Contribution of protein fractions to baking quality

Differences in wheat varieties for dough strength and extensibility could be mainly ascribed to different combinations of HMW-GS and LMW-GS. The role of LMW-GS in the gluten structure is not clearly defined, mainly due to the difficulty to identify the allelic variations associated with the LMW-GS (Gianibelli et al., 2001). LMW-GS are highly polymorphic and include proteins with gliadin-type sequences, which complicate the separation of individual proteins (Cinco-Moroyoqui & MacRitchie, 2008). Increased dough strength associated with LMW-2 subunits and a possible explanation could be the greater occurrence of LMW-2 type compared to LMW-1 type within LMW-GS (D'Ovidio et al., 1999).

The polymeric protein fraction in gluten is key to the variability in bread making quality, notwithstanding the influence of environment and genotype on end-use quality (Lagrain et al., 2012). Polymeric glutenins influenced dough stability and strength during mixing, while monomeric gliadins had a negative effect on dough development time, dough stability, resistance to extension. Glutenins and very low molecular weight monomeric proteins correlated positively with SDSS, dough development time and dough stability, and negatively with gliadin/glutenin ratio and dough weakening (Chaudary et al., 2016).

Glutenin and its sub-groups had a large influence on dough strength (Uthayakumaran et al 1999; Johansson et al., 2002; Zhang et al., 2007a; Li et al., 2013) and dough stability (Shi et al., 2005). Dough strength is negatively correlated with gliadin content and positively correlated with increased glutenin content (Li Vigni et al., 2013). A positive correlation was observed between UPP% and dough strength (Johansson et al., 2002, Hasniza et al., 2014a). The absolute amount of UPP (UPP% in the grain) showed the highest positive correlation with AlvSTR (Cuniberti et al., 2003).

The amount of UPP in flour correlates with dough strength and bread making quality (Zhang et al., 2008; Malik et al., 2013). SE-HPLC results indicated that the HMW-GS/LMW-GS ratio in the UPP fraction correlated with dough strength (Altenbach et al, 2016). The mixing behaviour of the gluten protein might be more complex than previously perceived (Johansson et al., 2013). This is further emphasised by the UPP% in flour that does not always correlate with the UPP% in dough after mixing (Hussain et al., 2012).

Soluble glutenin comprises HMW-GS, LMW-GS and approximately 7 – 10% gliadins, while insoluble glutenin contains primarily HMW-GS and LMW-GS (Suchy et al., 2003). Variation in extractable proteins and unextractable proteins was mainly influenced by the genotype (Hasniza et al., 2014a).

Glutenins are primarily responsible for viscoelastic properties in dough and gliadins are contributing to dough extensibility. However, some gliadin alleles were positively correlated to dough extensibility as well as to dough strength (Metakovsky et al., 1997). Recent studies indicated that a large component of the gliadins might participate in the formation of the gluten polymer at optimal mixing, and contribute to the extensibility properties of dough (Veraverbeke & Delcour, 2002; Johansson et al., 2013). This is due to the development of intermolecular hydrogen and hydrophobic bonds between non-polar amino acid side chains, which also interact with flour lipids (Veraverbeke & Delcour, 2002).

An increase in gliadin/glutenin ratio resulted in increased extensibility and reduced dough strength (Wieser & Kieffer, 2001). As a result, dough elasticity is negatively correlated with dough extensibility and demonstrates the difficulty to select breeding lines with high elasticity and extensibility (Caffe-Treml et al., 2011). This is in contrast to a positive correlation between dough extensibility and dough strength (Hasniza et al., 2014a). In both studies, dough properties were measured with procedures allowing the adjustment of water content and mixing time for the Kieffer dough extensibility test (Caffe-Treml et al., 2011) and the extensigraph (Hasniza et al., 2014a).

The high molecular weight glutenin subunits are key in determining the quality and end-use properties of dough (Anjum et al., 2007). Research on transgenic wheat lines indicated that an increase in HMW-GS quantity correlated positively with gluten strength, concurrently the amount of LMW-GS decreased with increased amounts of HMW-GS (León et al., 2009). Dough strength and MPT correlated positively with HMW-GS (Færgestad et al., 2000).

The various gliadin fractions affected functional properties differently, with γ-gliadin having the largest negative effect on mixing time and maximum resistance to extension, ω-gliadin showed the largest reduction in loaf height, while α- and β-gliadins had the least effect on the reduction of loaf height (Uthayakumaran et al., 2001). Gliadins correlated positively with gliadin/glutenin ratio, dough weakening and correlated negatively with SDSS, resistance/extension, dough development time and dough stability. Gliadins contributed to the extensibility of the dough and showed a weakening effect on elasticity and strength of the gluten network (Chaudary et al., 2016).

The ω-gliadins are less effective than α/β- and γ-gliadins for improving viscoelastic properties and LFV (Barak et al, 2014b). The gluten macropolymer might incorporate α/β-, and γ-gliadins with intermolecular disulphide bonds, while ω-gliadins (S-poor) would be either trapped in the polymer or unified by H- or non-covalent bonds (Kuktaite et al., 2004). The ω-gliadin fraction may contribute to changes in flour quality for wheat grown at different localities (Altenbach & Kothari, 2007). Barak et al. (2014a) suggested that gliadins may play an important role in the functional properties of wheat flour. The negative association between LFV and gliadins were reported by Ohm et al. (2010), while the positive association between gliadins and LFV were reported by Park et al. (2006).

In contrast with the findings of Uthayakumaran et al. (2001) who reported a reduction in dough resistance breakdown with the addition of gliadin sub-groups. They observed, among the gliadin fractions, γ-gliadin showed the maximum reduction in mixing time and resistance to extension. They further hypothesised that a reduction in mixing time and maximum resistance to extension associate with an increase of hydrophobicity in

gliadin fractions, in the following sequence: order ω- < α- and β- < γ-gliadins. The various gliadin fractions affected functional properties differently, with γ-gliadin having the largest negative effect on mixing time and maximum resistance to extension, ω-gliadin showed the largest reduction in loaf height, while α- and β-gliadins had the least effect on the reduction of loaf height (Uthayakumaran et al., 2001). Dough resistance breakdown increased with the addition of gliadin and gliadin sub-groups. The following sequence indicates the increase of dough resistance breakdown: ω1- < γ- < α- < β-gliadins (Khatkar et al., 2002).

Research findings for non-prolamins might be conflicting because there is no clarity regarding their structures and contribution to rheological behaviours (Song & Zengh, 2007). Processing and rheological properties were influenced by non-prolamins (Hill et al., 2008) as opposed to a limited effect reported by Singh et al. (1991). Gluten dough became more elastic and less viscous after the removal of water-soluble proteins (Dreese & Hoseney, 1990). While Hargreaves et al. (1995) did not detect a significant effect on the viscoelastic properties of gluten after the removal of non-prolamin proteins.

AG contain enzymes and enzyme inhibitors that can be beneficial for the improvement of poor quality wheat flours, for instance the addition of amylase and xylanases (Gao et al., 2009). Endoxylanases can improve dough handling properties such as oven spring and LFV by increasing the viscosity of the aqueous phase (Courtin & Delcour, 2002). Increased globulin, serpin and triticin associated with increased UPP%, dough strength and extensibility. This is an indication that the contribution of globulin proteins to functional flour properties justifies further research (Hasniza et al., 2014a; b). Relative AG values for SE-HPLC did not show a significant effect on dough rheological traits (Chaudary et al., 2016).

2.4 Effect of protein content and composition on baking quality

Grain protein composition is influenced by the genotype basically through the nitrogen-filling rate (Charmet et al., 2005). The effect of increased soil nitrogen or water stress on increased protein content did not influence the rate of changes in protein composition (Saint Pierre et al., 2007). The rate of nitrogen accumulation for specific protein fractions, rather than the duration of accumulation, appeared to be the main cause of the amount of a specific fraction in the wheat kernel. The abundance of a protein fraction correlates with the rate of nitrogen accumulation, with $r = 0.64$ for the LMW-GS and $r = 0.70$ for the α,β,γ-gliadins (Charmet et al., 2005).

Analysis of wheat proteins is difficult because of the wide range of proteins occurring in the kernel. The abundance of gluten proteins can mask the less abundant proteins in total protein extracts (Hurkman & Tanaka, 2007). Moreover, glutenins and gliadins consist of several closely related proteins with similar molecular weights in the gluten fraction (Giuliani et al., 2014), which complicates clear separation (Altenbach et al., 2011).

Triboï et al. (2000) reported a significant difference in the glutenin fraction for two genotypes, notwithstanding the same FPC and ascribed the difference to allelic composition and expression of the genotypes. Increased FPC resulted in increased gliadin to glutenin ratio. Increased glutenin content could not be attributed to increased nitrogen content but rather to the allocation of nitrogen, which favoured glutenins to the soluble fractions and AG (Triboï et al., 2000).

The relative proportion of gliadin and glutenin fractions increased linearly with FPC, proportional amounts of monomeric proteins (gliadins) increased to a larger extent than the polymeric proteins (glutenins). AG concentrations increased with increased FPC with a slight decrease in AG as a percentage of total FPC (Saint Pierre et al., 2007). Glutenins and gliadins increased with protein content although glutenins increased substantially more than gliadins with increased protein content in the wheat (Barak et al., 2014a). Total soluble protein and gliadin content increased in proportion to

increased protein content, while soluble polymeric protein, insoluble polymeric protein and albumin and globulin content did not increase in proportion to increased protein content (Park et al., 2006).

Wheat protein content varies between 8 – 17% and is affected by the genotype, genotype by environment interaction (GxE) and cultivation practices (Peña et al., 2002). Flour quality depends on the types and amounts of proteins that combine to form polymers, which are influenced by genetic and environmental factors (Altenbach et al., 2016). Grain protein content and composition are crucial for bread making quality (Johansson et al, 2003; Gao et al., 2012). Several studies indicated that flour behaviour is influenced by protein composition, rather than total protein content (Peterson et al., 1998; Uthayakumaran et al., 1999; Peña et al., 2005; Wang et al., 2007; Flagella et al., 2010; Vázquez et al., 2012).

Charmet et al. (2005) described grain protein content as the ratio of grain dry weight and grain protein quantity. Therefore, protein content is a reflection of the accumulation of both nitrogen and dry matter. Dry matter consists of 60 – 70% starch (Charmet et al., 2005). Wheat protein granules are unevenly distributed through the wheat kernel and protein content in the embryo and aleurone layer is almost 30 and 20% higher, respectively, than the protein content in the endosperm. Protein content is much higher in the tissue close to the seed coat than in the middle and centre of the endosperm (Payne & Rhodes, 1982).

FPC is reduced by 0.9 – 1.9% at 70% flour extraction level, and this requires the production of wheat with at least 12% protein before the milling process. FPC of at least 11% protein is preferred for making leavened bread (Tian, 2006). For this reason, more attention should be paid to FPC than grain protein content and studies should be directed towards molecular genetic analysis of FPC (Zhao et al., 2010).

Protein quality traits (maximum resistance to extension, mixograph properties and glutenin percentage) are mainly influenced by genotype (Carson & Edwards, 2009),

while protein quantity is largely a function of the environment (Van Lill et al., 1995; Gomez-Becerra et al. 2010).

Hurkman et al. (2013) raised the question as in how changes in gluten composition influence flour quality. The amount and size distribution of polymeric proteins influence protein composition and could be affected by genotype, environment and GxE (Johansson et al, 2005). Protein composition could be responsible for different findings regarding dough properties. Protein content correlated positively with dough extensibility and negatively with dough elasticity (Vázquez et al., 2012; Moldestad et al., 2014), although protein content did not fully explain the differences that occurred for extensibility between years (Caffe-Treml et al., 2011).

Total grain protein content correlated positively with dough strength and extensibility, while the glutenin/gliadin ratio correlated negatively with dough extensibility (Hasniza et al. 2014a). Gliadin correlated positively with dough extensibility (Johansson et al., 2002; Zhang et al., 2007a). Increased amounts of soluble LMW-GS in general correlated negatively with dough mixing properties and baking traits (Peterson et al., 1998). Increased protein content brings about changes in flour protein composition, particularly monomeric proteins increased more than polymeric proteins with increased FPC (Saint Pierre et al., 2008).

FPC correlated slightly with AlvP/L and did not correlate with AlvL and AlvSTR (Bordes et al., 2008). Soft wheat varieties may decrease AlvSTR values by lowering AlvP, while a higher degree of starch damage in very hard wheat varieties may result in non-extensible dough. Variations in AlvSTR, which are related to grain hardness, are not influenced by protein content (Bordes et al., 2008).

A negative correlation was observed between MPT and FPC (Bordes et al., 2008). MPT was influenced by genotype and increased with a decrease of extractable proteins (Martinant et al., 1998). MPT is influenced by FPC and related with the glutenin fraction in the flour (Hoseney, 1994). FPC did not correlate significantly with MPT, while the relative amount of polymeric proteins (% polymeric protein in the protein) showed the

highest correlation with MPT (Cuniberti et al., 2003). FPC correlated positively with SDSS (Zeleny) while FPC and MPT did not correlate significantly. The locality with the lowest FPC and SDSS also rendered the lowest MPT, despite of the low correlation between FPC and MPT (Bonafede et al., 2015).

Over time, plant breeders improved grain yield and selected for increased SDSS, while indirectly selecting for lower FPC. The reduction of FPC was counterbalanced by increased MPT and to a lesser extent increased SDSS, which is an indication of gluten strength (Fufa et al., 2005).

Correlations between SDSS and FPC varied between publications. SDSS is more dependent on the qualitative variation of storage proteins than on quantitative variation (Grausgruber et al., 2000). A positive correlation was observed between SDSS and FPC (Saint Pierre et al., 2008; Oelofse et al., 2010; Bonafede et al., 2015), in contrast to a negative correlation for durum wheat (Rharrabti et al., 2003a). SDSS may depend on FPC and gluten content and both traits may respond differently to climatic conditions (Rharrabti et al., 2003b). Glutenin content is positively correlated with SDSS (Shi et al., 2005). The association between SDSS and LFV could be as a result of the influence of both soluble and insoluble glutenins (Wang & Kovacs, 2002). It appeared that selection procedures based on SDSS unconsciously led to the selection of superior glutenin alleles (Zeller et al., 2007). Ng & Bushuk (1988) developed an equation where HMW-GS composition was used in combination with FPC to predict LFV.

FPC and SDSS cannot be used alone to predict bread making quality, since these traits are controlled by genetic systems that only partially overlap (Rousset et al., 2001). Additional tests are required for accurate measurement of flour quality since FPC is only one measure of flour quality (Reese et al., 2007).

LFV is one of the most important bread making traits (Chung, 2003) and protein content serves as a major predictor of LFV (Johansson et al., 2001, DuPont et al., 2006; Dowell et al., 2008). Finney & Barmore (1948) demonstrated that LFV varied between cultivars with constant protein content, and variation could be ascribed to flour composition

(Johansson et al, 2003; Wang et al., 2007; Pasha et al., 2010). Protein quality affects LFV to a lesser extent than it does loaf texture, the effect on pore size at loaf cross section (Tronsmo et al., 2003). Dough extensibility and LFV correlated positively with FPC (Færgestad et al., 2000).

Baking performance relates to the balance between dough strength and extensibility (Anderson et al., 2004). Dough must exhibit extensibility beyond a minimum value to achieve optimal LFV (Janssen et al., 1996). Excessively strong dough impairs proper enlargement of gas bubbles during fermentation and results in decreased LFV with dense bread structure. Dough should be strong enough to resist breakdown of gas cells during proofing and baking, however extensible enough to increase in response to gas pressure (Nash et al., 2006). The stability of gas cells during expansion depends on both extensibility and strength of the dough (Ktenioudaki et al., 2010).

Increased dough extensibility and decreased dough strength resulted in larger LFV, even for cultivars with low dough strength and short mixing time. Although, partial correlations, by holding the effect of FPC constant, indicated that dough strength and extensibility both correlated positively with LFV. These findings indicated that dough extensibility and resistance to extensibility should be considered for selection of cultivars with high LFV (Caffe-Treml et al., 2011).

Finney & Barmore (1948) demonstrated the positive correlation between FPC and LFV, and the linear slope of the FPC – LFV relationship differed between cultivars. Cultivars with the steepest slopes were regarded as superior quality. Differences in LFV associated with polymeric proteins, which were mainly the glutenin fractions (MacRitchie, 2016).

Specific genotypes became less stable at a higher molar mass for the polymeric protein fraction, while increased LFV associated with more stable genotypes. Molecular weight distribution seemed to be influenced by the locality, whereas monomeric/polymeric ratio seemed to be similarly influenced by genotype and locality. The study indicated

that stability for LFV associated with stability of the polymeric fraction and not stability of FPC (Lemelin et al., 2005).

A higher quantity of glutenin and a reduced gliadin/glutenin ratio had a positive effect on LFV for northern-style Chinese steamed bread (Zhang et al., 2007b). AG, glutenin and polymeric protein/monomeric protein ratio showed significant positive correlations with LFV (Wang et al., 2007). Dough extensibility showed a negative correlation for LFV while the correlation between UPP% and LFV varied between positive and not significantly, depending on the locality (Cavanagh et al., 2010). LFV and dough extensibility were highly correlated with the absolute amount of polymeric protein (% polymeric protein in the grain) (Cuniberti et al., 2003). Increased gliadin/glutenin ratio resulted in decreased LFV at constant FPC (Uthayakumaran et al., 1999).

Groos et al. (2007) analysed 194 recombinant inbred lines (RIL's) across environments. Baking score and indirect quality tests were poorly correlated. Bread making scores were poorly correlated with FPC and alveograph parameters. Multiple regression analyses can be applied to determine the contribution of predictor variables to explain variation in observed quality parameters. FPC, AlvP and AlvSTR made the largest contribution to variation in LFV, while quality analyses could only explain a small proportion of the variation observed in bread making scores across environments. Approximately 33% of the variation in LFV could be explained by quality parameters. LFV correlated positively with AlvL, AlvSTR and FPC (Groos et al., 2007).

2.5 Effect of allelic variation on baking quality

Approximately 60% of variation in the quality of bread flour could be accounted by the HMW-GS composition (Payne et al., 1987). It is therefore important to use a reliable procedure, or several procedures, to identify HMW-GS in a wheat cultivar (Lagrain et al., 2012). It should be noted that HMW-GS with molecular weights ranging from 65 – 90 kDa were unusually high on SDS-PAGE, ranging from 80 – 120 kDa (Veraverbeke & Delcour, 2002). Relative mobilities on SDS-PAGE does not always relate to their

molecular weight and can be related to structural differences, which may lead to different sensitivities to detergents and chaotropic agents (Goldsbrough et al., 1989). Further polymorphisms were identified with high resolution methods, which include reversed-phase high-performance liquid-chromatography (RP-HPLC) and mass spectrophotometry (Anjum et al., 2007).

Most of the *Glu-1* alleles comprise only two functional HMW-GS, which are relatively easy to identify in the upper region of SDS-PAGE gels. This led to the widely used GLU-1 bread making quality index, which is based on the addition of numerical scores allocated to individual *Glu-1* alleles (Payne, 1987).

The total percentage of glutenin in wheat could account for 68 – 80% of the variation in dough extensibility, dough development time and LFV (Gupta et al., 1992), despite of the difficulty to quantify the effects of HMW-GS due to the complexity of dough characteristics and the influence of the environment (Ng & Bushuk, 1988). The HMW-GS 2, 5, 7, 10 and 12 were regarded as major components, whereas HMW-GS 1, 2*, 6, 8 and 9 acted as minor components with regard to dough development time, maximum dough resistance and LFV. Within the HMW-GS, the x-type subunits (1 – 7) contributed more to dough properties than the y-type subunits (8 – 12) (Wieser & Zimmermann, 2000). According to Cinco-Moroyoqui & MacRitchie (2008), genotypes containing HMW-GS 5 + 10 (*Glu-D1d*) usually have superior quality compared to genotypes containing HMW-GS 2 + 12 (*Glu-D1a*). While Horvat et al. (2006) suggested that all the HMW-GS loci (*Glu-A1*, *Glu-B1* and *Glu-D1*) need to be considered to examine the effect of HMW-GS on quality and the interaction with the environment.

When considering the effect of allelic variation on dough strength, where two subunits are expressed at a locus, especially where both subunits contribute to dough strength, the contribution of the x-type HMW-GS seems to be dominant. For example, in the HMW-GS 5 + 10 (*Glu-D1d*) pair, the individual subunit (5) would be the major contributor to dough strength (Blechl et al., 2007).

The GLU-1 score explained less than 20% of the variation observed for baking quality in Argentinian varieties (Dubcovsky et al., 2000). Previous studies indicated that the HMW-GS in South African and Australian wheat explained less than 20% of variability in bread making quality (Gupta et al., 1991; Randall et al., 1993), because the contributions of LMW-GS and gliadins are excluded from the GLU-1 score (Bonafede et al., 2015). LMW-GS contribute to wheat bread making quality although the effect of individual *Glu-3* alleles is unclear due to the tight linkage with the *Gli-1* locus and the intricate SDS-PAGE banding patterns (Bonafede et al., 2015).

Selection of optimum HMW-GS alleles in modern wheat varieties increased the relative contribution of LMW-GS and gliadins to bread making quality (Bonafede et al., 2015). Protein quality can be improved by increasing the glutenin quantity, while considering the desirable composition of HMW-GS and LMW-GS alleles (Zhang et al., 2009). HMW-GS showed a higher association with dough strength parameters than LMW-GS, similarly a higher HMW-GS/LMW-GS ratio will result in stronger dough (Martre et al., 2006; Li Vigni et al., 2013). Dough strength was generally more influenced by the *Glu-1* alleles than the *Glu-3* alleles and from the *Glu-3* loci, *Glu-B3* made the biggest contribution, while LMW-GS were more important for dough extensibility (Cornish et al., 2006).

In contrast to the belief that bread making quality is primarily determined by variation at the *Glu-1* loci, Rousset et al. (2001) indicated that bread making quality is under complex control and the *Glu-1* loci serve only as a component in the genetic control of these traits. Weegels et al. (1996b) also reported that glutenin subunits explain a small proportion of the variation in wheat quality, and Van Bockstaele et al. (2008) concluded that acceptable quality is not guaranteed by the presence of favourable HMW-GS combinations. Some of the quantitative trait loci for LFV do not map to glutenin loci (Mann et al., 2009).

A study was conducted on 26 wheat lines with different HMW-GS and LMW-GS allelic compositions. It appeared from the study that *Glu-3* loci had a larger effect on SDSS than *Glu-1* loci. SDSS and SDSS index seemed to be mainly affected by the *Glu-B3* allele

(Figuerosa et al., 2011). Gliadin blocks related to SDSS (Zeleny) values (Barak et al., 2015). *Glu-A1*, *Glu-B1*, *Glu-D1* and *Gli-B1* showed significant associations with SDSS and did not associate significantly with protein content. Results indicated the possibility of a different genetic architecture for protein content and SDSS, although results have to be carefully interpreted because the composition of the mapping population may affect the picture (Würschum et al., 2016).

Significant contributions were made to mixograph properties by HMW-GS 17 + 18 and 5 + 10, as well as *Glu-A3b*, *Glu-A3d*, *Glu-B3g* and *Glu-D3f* (Jin et al., 2013). Branlard et al. (2001) conducted a study on 162 registered varieties from the French or European catalogues, grown at three localities in France. Phenotypic variation explained by *Glu-1* loci varied from 5% for AlvP/L up to 34% for MPT over the three localities.

AlvL was significantly influenced by variation at *Glu-B1* alleles, while *Glu-D1* alleles did not have a significant effect on AlvL. Allelic variation at *Glu-D3* did not show a significant effect on quality parameters, except for grain hardness. Variation at *Glu-A3* and *Glu-B3* had a smaller effect on wheat quality than HMW-GS, except for AlvL and AlvP/L. In general, LMW-GS had an additive effect to HMW-GS loci on wheat quality. Alleles encoded at *Glu-1*, *Glu-3* and *Gli-2* loci explained 33% of the variation for AlvP and almost 60% for MPT. Furthermore, these allelic contributions to variation were independent from FPC and grain hardness (Branlard et al., 2001). The prevalence of subunits 7 + 8 (*Glu-B1*) and the introduction of subunits HMW-GS 5 + 10 (*Glu-D1*) improved AlvSTR in Spanish wheat varieties, combined with the replacement of the null allele (*Glu-A1*) with subunits 1 and 2* (Sanches-Garcia et al., 2015).

The HMW-GS combination of *Glu-A1a* (1), *Glu-B1c* (7 + 9) and *Glu-D1d* (5 + 10) had a positive influence on LFV, while *Glu-B1d* (6 + 8) and *Glu-D1a* (2 + 12) may probable have a negative effect on LFV. Though, the positive or negative contribution of an allele to baking quality, either being present or absent, may be influenced by the combination with other alleles. Individual combination of alleles indicated that interaction between *Glu-1* and *Glu-3* as well as *Gli-1* alleles can occur. Interaction with a favourable allele

may inhibit or compensate the effects of other alleles due to partial dominance or epistatic effect (Zeller et al., 2007).

Increased grain protein was regarded as a function of yield reduction. General increases in protein content related to changes in protein composition (Saint Pierre et al., 2007). Bonafede et al. (2015) developed NIL's with fixed HMW-GS in combination with different LMW-GS. Results indicated that allelic variation at *Glu-A3* and *Glu-B3* did not have a significant difference on FPC, irrespective of the large variation in FPC between environments.

2.6 Effect of the environment and genotype on baking quality

Several studies indicated the influence of environmental factors on the amount and composition of gluten proteins, and the resulting impact on bread making quality (Shewry 2007; Johansson et al., 2013; Hasniza et al., 2014a). The degree to which growth environment and genotype affect grain quality received much attention, in an effort to develop good yielding cultivars with acceptable quality for a changing environment. The relative contributions to variability in grain quality will be determined by the genotypes and the particular environment (Hasniza et al., 2014a).

Quantitative two-dimensional gel electrophoresis was used to understand the influence of plant growth conditions on the formation of gluten polymer. Extractable polymeric protein and UPP polymers comprise many of the same proteins because of the continuum of different sizes presented by the polymers, instead of distinct classes. The overall complexity of the gluten proteins, as well as overlapping LMW-GS, α- and γ-gliadins, made it difficult to quantify specific proteins in separate fractions (Altenbach et al., 2016).

Environmental factors can shorten the grain filling period and affect protein composition, while the relative rate of accumulation of different protein fractions varied between genotypes (Charmet et al., 2005). Variation for soluble and insoluble

protein fractions, as well as UPP%, was mainly contributed to genotype (Hasniza et al., 2014a). Genotype and environment influence the amount of glutenin and gliadin in wheat flour (Branlard et al., 2001). Glutenin subunits are more dependent on genotype whereas gliadin composition depended on the environment (Panozzo & Eagles, 2000). Genotype and environment can affect the viscoelastic properties of gluten, along with polymerisation rate and build-up of large unextractable polymeric proteins (LUPP) (Johansson et al., 2005; Moldestad et al., 2014).

Carceller & Aussenac (1999) described three stages of grain developing: cell division, cell enlargement and dehydration and grain maturity. UPP started to increase towards the end of cell enlargement stage and increased more during the dehydration stage, with the formation of large glutenin polymers. AG increased during cell division and reached a plateau during cell division stage, while gluten, glutenins and gliadins, increased up to the end of cell enlargement (Carceller & Aussenac, 1999). The effect of increased soil nitrogen or water stress on increased protein content did not influence the rate of changes in protein composition (Saint Pierre et al., 2007).

Labuschagne et al. (2009) exposed bread, biscuit and durum wheat to extreme high and low temperatures. Shortened grain filling due to high temperature or drought stress reduced the duration of glutenin synthesis and resulted in reduced dough strength. Wheat types showed a much larger effect on protein composition than temperature treatments. Low temperature stress had the largest effect on the soft biscuit wheat cultivar. Protein composition in the tetraploid durum wheat was different from the hexaploid cultivars, with less polymeric proteins but more monomeric proteins. The bread wheat cultivars contained significantly less gliadins and more HMW-GS than the soft biscuit wheat. (Labuschagne et al., 2009).

Labuschagne et al. (2016) conducted a study across the three wheat production areas in South Africa, over seasons and localities. Mixsmart® software was used to determine dough mixing traits. Findings for aggregated data, per region, varied across the three production regions. Mixsmart® traits were poor predictors of baking quality in the winter rainfall area, especially for LFV, FPC and WGC. FPC and WGC were interrelated

and were the best predictors for LFV. Midline peak time was a better predictor than MPT for LFV in the summer rainfall and irrigation areas.

Frequent changes in climate may lead to a situation where a crop is exposed to several extremes during one growing season. Environmental conditions during anthesis and grain filling could impose a negative effect on wheat quality (Jiang et al., 2009). Gliadin content increased under high temperatures and led to reduced dough strength (Motzo et al., 2007).

The effect of the environment on variations for dough elasticity, extensibility and LFV could be partly explained by temperature or relative humidity. Low relative humidity and high temperatures during 20 days after anthesis could affect flour composition in a way that result in an increase in dough elasticity and a reduction in dough extensibility and LFV (Caffe-Treml et al., 2011). Dough strength increased with an increase in daily temperatures, up to 30°C, and decreased with temperature increases above 30°C (DuPont & Altenbach, 2003).

Heat stress is one of the greatest environmental effects. High temperatures during the desiccations period may affect the final quality. This is the period where glutenin polymerisation proceeds rapidly. The effect of heat stress could become more important with increased climate change and elevated temperatures (Naeem et al., 2012). High temperatures before anthesis had a negative impact on grain protein content, dough strength and extensibility, which indicates that temperature before grain development can influence grain quality. Daily maximum temperatures, during grain filling, had a large effect on the amounts of soluble and insoluble protein fractions. Especially, the number of days with temperatures exceeding 35°C and 40°C (Hasniza et al., 2014a).

The accumulation of gluten proteins continues until the end of cell enlargement phase. Lines with HMW-GS 5 + 10 (*Glu-D1d*) accumulated large polymers several days earlier than lines with HMW-GS 2 + 12 (*Glu-D1a*) and retained higher amounts till maturity. Large polymers were measured as UPP (Naeem et al., 2012). Higher concentrations of

cysteine in the x-type HMW-GS 5 + 10 (*Glu-D1d*) could be the reason for the faster polymerisation (Naeem et al., 2012). The negative effects of high temperatures on dough functionality, would be less detrimental if sufficient amounts of large polymers were formed before high temperatures were reached (Irmak et al., 2008; Naeem et al., 2012).

SDSS (Zeleny), AlvSTR and MPT were strongly influenced by genotype, while FPC and AlvL were strongly influenced by the either the locality or GxE (Branlard et al., 2001). MPT was strongly influenced by the genotype (Saint Pierre et al., 2007). SDSS and FPC were significantly influenced by the environment (Ribeiro et al., 2016). Environment contributed significantly to variability in dough strength and extensibility, while genotype was the main contributor to variation in soluble and insoluble protein fractions and UPP% (Hasniza et al., 2014a).

AlvP and AlvSTR was more influenced by the genotype than the environment, while AlvL showed the lowest stability across localities. AlvL showed the lowest stability across environments, with results indicating that AlvL was influenced by the availability of water during crop growth. FPC decreased in irrigated wheat and affected AlvL. Cultivars with HMW-GS 5 + 10 (*Glu-D1d*) were more stable under irrigated conditions for AlvL compared to cultivars with HMW-GS 2 + 12 (*Glu-D1a*) (Sanches-Garcia et al., 2015). Environment and FPC showed a significant effect on AlvL (Cornish et al., 2001).

Protein fractions in total FPC were influenced by sowing times (Singh et al., 2010). Late sowing under irrigation resulted in increased FPC, with increased monomeric proteins (AG and gliadin). Genotypes showed significant differences at late sowing for monomeric proteins and insoluble glutenin (Singh et al., 2010).

Cultivars and NIL's that correlated with strong and weak dough were used in several studies, indicating the association of HMW-GS (5 + 10) (*Glu-D1d*) with strong dough and the association of HMW-GS 2+12 (*Glu-D1a*) with weak dough. The association with strong dough was caused by a shift in molecular weight distribution (MWD) to higher molecular weight values (Naeem & MacRitchie 2005; Naeem et al., 2012). It is important

to note that there were no significant differences in the total amounts of polymeric protein between the cultivars or NIL's used in the studies, as measured with SE-HPLC (Naeem et al., 2012).

Both genetic and environmental factors influence MWD of glutenin (Naeem et al., 2012). It seemed that higher temperatures during grain development caused a shift in MWD to lower molecular weights, which resulted in decreased dough strength. It further appeared that strength related HMW-GS contributed greater tolerance to heat stress (Naeem et al., 2012).

ANOVA indicated that locality, year and environment contributed significantly to variation grain protein content, glutenin/gliadin ratio, UPP%, dough strength and extensibility (Hasniza et al., 2014a).

Environment and GxE interaction showed a larger effect on traits associated with protein content than traits associated with protein quality and rheology (Williams et al., 2008). High temperatures during grain filling resulted in stronger dough (Labuschagne et al., 2009: Moldestad et al., 2014; Labuschagne & Moloi, 2015), in contrast to a reduction in dough strength (Jarvis et al., 2008; Cavanagh et al., 2010; Li et al., 2013), especially during the later stage of grain filling (Blumenthal et al., 1991). Protein content increased with high temperatures and affected mixing tolerance negatively (DuPont et al., 2006). Temperature changes affected the proportions of some gluten proteins but did not affect the monomeric proteins/polymeric proteins and gliadin/glutenin ratios significantly (Koga et al., 2015). High temperatures during the grain filling period led to increased gliadins and decreased glutenins, which resulted in alterations of dough properties and baking quality (Jarvis et al., 2008).

Cultivars responded differently to temperature stress regarding LFV. Hours of low relative humidity had a strong negative impact on SDSS and LFV (Peterson et al., 1998). The extent of the GxE interaction variance suggests it would be almost impossible to predict LFV from genotypic knowledge alone (Peterson et al., 1998). Cultivars react differently to drought and heat stress (Labuschagne & Moloi, 2015), as a result, general

conclusions for the effect on quality parameters cannot be deducted in the event where average values for genotypes were used, especially in a study where entries showed significant differences for quality traits. The potential negative effects of both drought and heat stress can be reduced by selecting genotypes with intermediate gluten strength and good extensibility (Li et al., 2013).

LFV varied between cultivation years. Gluten strength varied between years and could not be explained by the variation in composition or relative amounts of specific storage proteins, protein groups or protein subunits (Johansson et al., 2002). Cultivation year showed a significant contribution to variation in UPP. UPP correlated positively with gluten strength (Johansson et al., 2002). UPP is the fraction in total polymeric protein that correlates positively with dough strength (Gupta et al., 1995).

Several studies indicated that environmental factors during the growth cycle can affect the amount, degree of polymerisatiion and composition of gluten proteins, and as a result, influence dough strength and bread making quality (Caffe-Treml et al., 2011; Uthayakumaran et al., 2012; Johansson et al., 2013). Grain quality can be affected by environmental factors during the early stages of plant growth. Average daily maximum temperatures before flowering correlated negatively with FPC, dough strength and extensibility (Hasniza et al., 2014a).

A cooler grain filling period in Sweden contributed to weaker dough, while a warmer grain filling period led to stronger dough. Differences in gluten strength between years, were attributed to differences in the amount and size distribution of polymeric protein. Although the total amount of HMW-GS could not explain differences in amount and size distribution of polymeric proteins (Johansson & Svensson, 1999).

Different findings were reported regarding the influence of genotype, environment and GxE interaction on SDSS and could be ascribed to different genotypes, environments and methods applied to determine SDSS (Williams et al., 2008). A high inter-environment correlation coefficient was observed for SDSS, resulting in similar rankings for genotypes at different environments (Grausgruber et al., 2000). Years contributed

more to variability in SDSS than localities under South African dryland conditions (Oelofse et al., 2008). Genotypes made the largest contribution to SDSS for durum wheat (Rharrabti et al., 2003a). A significant response to nitrogen fertiliser resulted in increased SDSS, while water stress increased SDSS at some of the localities (Saint Pierre et al., 2007).

Water stress increased protein content, SDSS and size distribution of polymeric glutenin (Flagella et al. 2010), while the glutenin/gliadin ratio did not change significantly under dryland conditions (Panozzo et al., 2001). MWD showed a significant increase under dryland conditions, while glutenin/gliadin ratio did not change significantly (Panozzo et al., 2001). Increased temperature stress resulted in decreased glutenin content (Graybosch et al., 1995).

Post-anthesis application of fertiliser doubled the protein content in flour, although the proportions of glutenin in UPP and extractable polymeric proteins did not change with the application of post-anthesis fertiliser. The application of post-anthesis fertiliser had a potentially negative effect on polymer size due to the increase of chain terminators and non-gluten proteins (Altenbach et al., 2016).

Daniel et al. (1998; 2000) reported how high temperatures increased HMW-GS in total glutenin while increased nitrogen resulted in reduced the proportion of HMW-GS. Malik et al. (2013) reported that nitrogen was one of the main contributors to protein polymerisation during grain development. The study was conducted to study the effect of temperature in combination with nitrogen application.

Bonafede et al. (2015) developed nine NIL's, which were uniform for HMW-GS and differed for LMW-GS. Three field trials were conducted. NIL's did not show significant differences in total flour protein content. GxE interaction contributed significantly to variation in measured mixograph traits and Zeleny sedimentation values, which indicate the effect of the environment on *Glu-3*/*Gli-1* alleles (Bonafede et al., 2015).

2.7 The way forward

South African wheat cultivars are classified to ensure that the wheat industry is provided with cultivars that perform agronomically, have suitable milling, rheology and baking traits. Classification norms and analytical procedures are assembled in combination with wheat breeders, millers and bakers to serve the needs of producers and processors. Selected cultivars are used as biological quality standards for classification norms and potential breeding lines are evaluated against biological standards. Only commercial cultivars with acceptable agronomical and quality traits would be considered as a biological quality standard (SAGL, 2016).

Classification norms and biological standards serve as guidelines for wheat breeding programmes. Changing these norms and standards are thus thoroughly and carefully selected for South African condiditons. Furthermore, it would be impractical to have fixed criteria or quality norms, since the effect of the environment and cultivation practices on wheat quality. Therefore, acceptable deviations from the biological quality standard were established as classification norms. These deviations, from the biological quality standards, vary between the three wheat production areas in South Africa (SAGL, 2016).

The negative correlation between grain yield and protein content (Peña et al., 2002; Shewry, 2007; Saint Pierre et al., 2008) requires careful selection of genotypes in order not to compromise bread making quality. Grain quality should be treated with the same level of importance as grain yield and disease resistance during selection (Peña et al., 2002). Grain protein content is an important trait for determining the price of grain, although FPC showed a low correlation with LFV in a study conducted in Australia (Cavanagh et al., 2010). The relationship between genotype and phenotype is masked by GxE interaction and reduces effective selection (Comstock & Moll, 1963). The interaction between genotype and environment is complex, even under conditions where water, temperature and fertiliser are controlled. The evaluation of replicated field trials in different geographical locations and years, is an opportunity to evaluate genotypes under commercially grown conditions (Hasniza et al., 2014b).

Cavanagh et al. (2010) observed differences in the relationship between genetic and phenotypic correlations and suggested the development of alternative selection procedures for wheat breeders and the baking industry, to exploit genetic gain concurrently with end-use quality. It will be essential for wheat breeders to select genotypes with a stable expression, not only for protein and starch content but also for protein and starch composition, to supply for the increased demand of wheat (Wilkes et al., 2010). Genotypes should be selected with higher stability for gluten quality to reduce variations in end-use quality (Moldestad et al., 2014). Genotypes should be selected according to performance in specific production areas, rather than aiming to breed for widely adapted cultivars across different environments (Vázquez et al., 2012).

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Chapter 3

The effect of the environment on protein composition in selected South African wheat cultivars

Abstract

Ten South African hard red wheat cultivars were planted at five localities over two years in the winter dryland wheat production area. Localities were divided into North Western Free State (NW-FS) and Eastern Free State (E-FS). Molecular weight distribution was determined to establish the relationship between protein fractions and flour protein content (FPC). The effect of the environment was also determined to identify protein fractions that show strong relationships with certain variables. Protein fractions did not increase parallel to increased FPC. The gliadin fraction in NW-FS showed the largest correlation and polymeric proteins in E-FS with FPC, while albumin and globulin showed the lowest correlation. Environment made the largest contribution to FPC in both regions. Within the gliadin group, the amount of SDS extractable α/β,γ-gliadin in total flour protein was mainly influenced by the genotype in both regions. Environment made the largest contribution to variation for unextractable polymeric proteins in NW-FS and genotype in E-FS.

3.1 Introduction

Functional properties of wheat flour are influenced by the amount of protein sub-fractions, rather than the total protein content, thus influencing the end product (Peña et al., 2005), which is determined by genetic and environmental variation and the GxE interaction (Johansson et al., 2001; Malik et al., 2013). It will increasingly be essential for plant breeders to predict genotypes that are stable over different environments, based not only on protein content but on protein composition (Wilkes et al., 2010).

Bread making quality of winter wheat is influenced by the environment, with a negative impact on market value due to inconsistent quality. A field trial conducted at eight localities over two years indicated that environment was the most influential variable for protein content in the Free State, South Africa (van Lill et al., 1995). Gomez-Becerra et al. (2010) also showed environment as the main source of variation after conducting a trial on spelt wheat, planted at six environments. Grausgruber et al. (2002) reported a greater GxE interaction effect for flour protein content and Zhang et al. (2004) reported a larger genotype influence.

Environment influences the amount of HMW-GS in the flour, although subunits respond similarly to external conditions and final proportions seem to be determined by genetic factors (DuPont et al., 2007). Drought at certain stages of development may affect HMW-GS/LMW-GS ratio differently and consequently the amount of UPP (Flagella et al., 2010). Temperature influences the rate of biochemical reactions and the timing of developing processes in grain, which varies between genotypes (DuPont & Altenbach, 2003). Increased temperature during grain filling showed significant influences on protein quantity and ratios between protein fractions (Majoul-Haddad et al., 2013). Protein content increases in water stressed environments (Flagella et al., 2010; Saint Pierre et al., 2008).

No method has been discovered for complete solubilisation of wheat glutenin, without altering the protein. Chemical reducing agents can be used to break all the disulphide bonds, while sonication breaks the disulphide bonds of the largest glutenin molecules, therefore altering the MWD. Relative measurements of MWD can be achieved by using a dilute SDS solution, although this is not an absolute method. In theory, an inverse relationship exists between molecular weight and solubility (Naeem et al., 2012).

Extensive studies were done on the MWD of unreduced wheat endosperm proteins (Ohm et al., 2010; Labuschagne et al., 2014) since the introduction of SE-HPLC (Huebner & Bietz, 1987) and sonication (Singh & MacRitchie, 1989), followed by improvement of chromatographic procedures (Batey et al., 1991; Larroque & Békés, 2000). The relative measure of MWD is based on the proportion that is unextractable with SDS-solution,

due to the relationship between the increasing molecular size and decreasing solubility of the polymer (Gupta et al., 1993). Environment may influence the quantity and size distribution of polymeric proteins in wheat kernels (Don et al., 2005; Labuschagne et al., 2006).

The objectives of this study were to investigate the influence of the environment on MWD, and the relationship with flour protein content.

3.2. Experimental

3.2.1 Materials

Ten hard red bread wheat cultivars were selected from the national cultivar adaptation trials, conducted by the Agricultural Research Council - Small Grain Institute (ARC-SGI) in South Africa. SDS-PAGE (Singh et al., 1991) was conducted as a standard procedure to determine high molecular weight glutenin composition (Table 3.1). Marquis and Sappo were used as SDS-PAGE reference samples (Manley, 1989) (Appendix, Figures 1 & 2). Material for this study was from the 2007 and 2009 trials, 2008 did not render enough seed for a complete quality analysis. Trials were planted at five localities, distributed over three regions (Appendix, Table 1). Bultfontein (Bult) and Bothaville (Bot) in the North Western Free State (NW-FS): low rainfall, high temperatures, high evaporation requirements and deep, yellow sandy loam soils. Bethlehem (Bhm) and Clarens (Clar) in the Eastern Free State (E-FS): higher rainfall, lower temperatures, lower evaporation requirement with predominantly yellow soils of average effective depth. Ladybrand (Lad) in the E-FS: moderate rainfall, moderate temperatures, a lower evaporation requirement and relatively shallow duplex soils.

Trials were planted according to a randomised complete block design with four replicates. Trial plots consisted of 5 rows of 5 m length each and an inter-row spacing of 45 cm, except for the Clar locality, where a row spacing of 50 cm was used. A spacing of 5 cm between seeds was maintained by means of a precision planter. For all five

localities 6:2:1 (31) fertiliser was applied. Total N per hectare given was 50 kg, total P: 17 kg and total K: 9 kg. Only the middle three rows per plot were harvested in an effort to avoid the side row effect. Each sample was dried to a moisture content of 12% before being cleaned with a Charter dockage tester and HLM determined per plot.

Table 3.1 List of cultivars with their high molecular weight glutenin composition

Entry	Wheat type	Origin	*Glu-A1*	*Glu-B1*	*Glu-D1*
BettaDN	Intermediate	ARC-SGI	2*	7 + 9	5 + 10
Caledon	Intermediate	ARC-SGI	1	7 + 9	2 + 12
Elands	Intermediate	ARC-SGI	1	7 + 9	5 + 10
Gariep	Intermediate	ARC-SGI	1	7 + 9	5 + 10
Komati	Intermediate	ARC-SGI	1	7 + 9	5 + 10
Limpopo	Intermediate	ARC-SGI	2*	7 + 9	5 + 10
Matlabas	Winter	ARC-SGI	Null	7 + 8	5 + 10
PAN3118	Winter	Pannar	2*	7 + 8	5 + 10
PAN3349	Intermediate	Pannar	2*	7 + 9	5 + 10
PAN3377	Intermediate	Pannar	2*	7 + 9	2 + 12

ARC-SGI = Agricultural Research Council – Small Grain Institute

3.2.2 Quality analysis

HLM was determined with AACC approved method 55-10 (AACC, 2000). Hagberg FLN was determined according to AACC approved method 56-81B (AACC, 2000). Wheat samples were conditioned to 16% moisture base (mb) according to AACC approved method 26-95 (AACC, 2000), prior to milling on a laboratory pneumatic mill, Bühler model MLU-202 (Bühler Bros., Inc., Uzwil, Switzerland) according to AACC approved method 26-21A (AACC, 2000). Flour protein content was measured with a combustion method on a LECO FP 2000 Protein/Nitrogen Analyser (St. Joseph, MI, USA) according to AACC approved method 46-30 (AACC, 2000). FPC was calculated as %N x 5.7 and expressed on a 12% mb. Moisture content was determined with a Brabender moisture oven according to AACC approved method 44-15A (AACC, 2000).

3.2.3 Analysis of protein molecular weight distribution

The procedure described by Labuschagne et al. (2014) was followed due to the collaboration between the Cereal Chemistry Laboratory at ARC-SGI and the Department of Plant Breeding, University of the Free State. The first step extracted SDS-extractable proteins (EXP), while the second step was submitted to sonication to obtain SDS-unextractable proteins (UNP).

Deionised water was used for the preparation of solvents and eluents. For EXP, white wheat flour samples (17 mg) were suspended in 1.5 ml of 0.5% (w/v) SDS-phosphate buffer (pH 6.9) and vortexed for 10 seconds at 21°C. Samples were then stirred for 5 min at 1400 rpm at 21°C in a Thermomixer® comfort (Eppendorf AG, Hamburg, Germany) followed by centrifugation for 30 min at 10 000 rpm. The supernatant was filtered through a 0.45 µm HT Tuffryn Acrodisc® Syringe Filter (Pall Corporation, Ann Arbor, MI, USA) into a glass vial.

For UNP, the pellet was resuspended in 1.5 ml SDS-phosphate buffer, vortexed for 10 s and sonicated in an ultrasonic disintegrator (Branson B12 Sonifier, Sigma, St. Louis, MO, USA) for 30 seconds at amplitude 5. The Sonifier was fitted with a 3 mm exponential tip. Samples were then centrifuged for 30 min (10 000 rpm). The supernatant was filtered through a 0.45 µm HT Tuffryn Acrodisc® Syringe Filter into a glass vial. EXP and UNP samples were heated for 2 min at 80°C, immediately after filtration to suppress protease activity (Larroque et al., 2000).

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

Separation was achieved in 15 min after injecting a 20 µl sample. Elution system (A) consisted of deionized water + trifluoroacetic acid (TFA) (99.9/0.1%, v/v); and (B) of

acetonitril (ROMIL-SpS^{TM} acetonitrile 200 far UV) + TFA (99.9/0.1%, v/v). The eluent consisted of 50% B. Flow rate was 0.4 ml/min at ambient temperature. Proteins were detected at 210 nm. Samples were extracted in duplicate and the average was used for data analysis.

3.2.3.1 Size exclusion HPLC data analysis

The following fractions were measured at specific time intervals: F1 (4.64 - 5.45 min); F2 (5.45 – 7.15 min); F3 (7.15 – 7.74 min); F4 (7.74 – 8.60) and F5 (8.50 up to where the trace cut the baseline) (Ohm et al., 2009b). Elution times varied slightly (seconds) between SDS-extractable proteins (Appendix, Figure 3) and SDS-unextractable proteins (Appendix, Figure 4). Extreme differences did not occur between elution times. The same apparatus and column were used for the duration of the study. Fractions F1 and F2, respectively, include HMW and LMW glutenin polymers; F3 includes ω-gliadin; F4 includes α/β,γ-gliadin and F5 includes AG (Larroque et al., 1997; Samson et al., 2005). Protein fractions were calculated according to Table 3.2. Percentages based on the amount of a measured fraction occurring in the protein (**relative amount**) were calculated for EXP and UNP, example for F1 (EXP) is presented in Table 3.2.

Table 3.2 Calculations for protein fractions

Relative	(F1 EXP)/((F1 EXP – F5 EXP) + (F1 UNP – F5 UNP))*100
LUPP	(F1 UNP)/(F1 EXP + F1 UNP)*100
UPP	(F1 UNP + F2 UNP)/(F1 EXP + F2 EXP + F1 UNP + F2 UNP)*100
HMW	(F1 EXP + F1 UNP)/((F1 EXP – F5 EXP) + (F1 UNP – F5 UNP))*100
LMW	(F2 EXP + F2 UNP)/((F1 EXP – F5 EXP) + (F1 UNP – F5 UNP))*100
POL	(F1 EXP + F2 EXP + F1 UNP + F2 UNP)/((F1 EXP – F5 EXP) + (F1 UNP – F5 UNP))*100
GLIADIN	(F3 EXP + F4 EXP + F3 UNP + F4 UNP)/((F1 EXP – F5 EXP) + (F1 UNP – F5 UNP))*100
AGS	(F4 EXP + F5 EXP + F4 UNP + F5 UNP)/((F1 EXP – F5 EXP) + (F1 UNP – F5 UNP))*100
GLIAG	((F3 EXP – F5 EXP) + (F3 UNP – F5 UNP))/((F1 EXP – F5 EXP) + (F1 UNP – F5 UNP))*100

LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, HMW = sum of high molecular weight proteins, LMW = sum of low molecular weight proteins, POL = sum of polymeric proteins, GLIADIN = sum of gliadins, AGS = sum of albumin and globulin, GLIAG = sum of gliadins, albumin and globulin

Sums of protein fractions were calculated for HMW, LMW, polymeric proteins (POL), gliadins (GLIADIN), albumin and globulin (AGS), gliadins and albumin and globulin (GLIAG). Percentages based on protein content were converted to the amount of protein (12% mb) in the flour (**absolute amount**) (Park et al., 2006).

3.2.4 Statistical analysis

Data was subjected to analysis of variance (ANOVA) using PROC GLM of SAS statistical software version 9.2 (SAS Institute Inc., Cary, NC, USA). A Shapiro-Wilk test for normality was performed before the results could be assumed reliable (Shapiro & Wilk, 1965). Thus the sources of variation were partitioned into years, localities, replications within years and localities, genotypes and the interactions of genotypes, years and localities.

The statistical model is given by:

$$Y_{ijkl} = \mu + Y_i + L_j + YL_{ij} + B(YL_{ijk}) + G_k + GY_{ik} + GL_{ik} + GYL_{ijk} + €_{ijkl}$$

Where:

Y_{ijkl} = observed SE-HPLC fraction or quality parameter

μ = general mean

Y_i = effect of the year

L_j = effect of the locality

YL_{ij} = interaction effect of the year and locality

$B(YL_{ijk})$ = effect of block within year and locality

G_k = effect of the genotype

GY_{ik} = interaction effect of the genotype and year effect

GL_{jk} = interaction effect of the genotype and locality

GYL_{ijk} = interaction effect of the genotype, year and locality

$€_{ijkl}$ = error or residual effect

$€_{ijkl} \sim NID(0,\sigma^2)$

The primary method used to analyse multi-environment trials is based on the ANOVA, which is a fixed effects model and requires homogenous variance-covariance of data.

Homogeneity of variances was tested by Levene's test in the PROC GLM in the SAS programme (Levene, 1960).

The Pearson's product moment correlation matrix of the pairwise correlations among the dependent variables was built to show their linear relationships. It was calculated using Proc Corr of SAS statistical software version 9.2 (SAS Institute Inc., Cary, NC, USA).

3.3 Results

Levene's test (Levene, 1960) for homogeneous variances indicated heterogeneity of variances for localities and they were therefore combined in two regions: NW-FS for Bot and Bult and E-FS for Bhm, Clar and Lad. Minimum and maximum temperatures and rainfall figures are presented in Appendix (Table 2). Low rainfall figures were recorded in all the regions preceding planting time in 2007. Although production practices are aimed at saving soil moisture, planting conditions were unfavourable during 2007. Rainfall figures of between 20 and 30 mm were recorded during June at most of the localities. July, August and September were particularly dry months, with no significant rainfall recorded until late in September. Above normal rainfall was recorded for the rest of the growing period, resulting in yields that exceeded the long-term averages.

Good rainfall figures were recorded in all the regions preceding planting time in 2009. Precipitation directly after planting caused soil crusting at Bhm. It was not a widespread problem and could be solved. Plants established well and resulted in satisfactory early growth. During the growing season, no rains occurred during spring, which was reflected in yields that were lower than the long term averages in the regions. Average or above average rainfall occurred before and during harvesting, late rains also resulted in regrowth. This had a negative effect on the coefficient of variance realised in the trials.

Proteins were divided into two main fractions, EXP and UNP. The two main fractions were further subdivided into five sub-fractions: HMW, LMW, ω-gliadin, α/β,γ-gliadin

and AG, presented as percentage in the protein (relative amount) and percentage in the flour (absolute amount). Mean values for FPC were higher in NW-FS and varied between 8.7 – 16.4% while mean values varied between 7.9 – 14.4% in the E-FS (Table 3.3). Measured means for MWD did not show large variation between the two regions, although the minimum and maximum values varied between the two regions. Maximum values for relative amount (EXP and UNP) for polymeric (Table 3.3) and monomeric (Table 3.4) fractions were higher in E-FS than in NW-FS, despite of a higher mean FPC in NW-FS. Mean values for LUPP and UPP were higher in NW-FS (Table 3.3).

Genotype contributed significantly ($p \leq 0.001$) to variation in FPC and all measured protein fractions in both regions. ANOVA showed significant contributions to FPC and measured polymeric protein fractions for year, locality and the interactions in both regions, except for absolute amounts of LMW (EXP), where year did not make a significant contribution to variation. Contributions to variation in gliadins and AG varied between significant and non-significant for environment (year, locality and the interaction) and GxE interaction (YxC, LxC and YxLxC). Environment and GxE interaction contributed significantly ($p \leq 0.001$) to variation in LUPP and UPP in both regions.

Environment was the main contributor to variation in FPC and polymeric proteins, except in NW-FS where GxE interaction made a large contribution to variation in relative amounts of HMW (EXP) and absolute amounts of HMW (EXP), and in E-FS where GxE made a large contribution to variation in relative amounts of LMW (EXP). Genotype made the largest contribution to variation in relative amounts of LMW (UNP) in E-FS (Table 3.5). GxE was mainly responsible for variation in relative amounts of ω-gliadin in E-FS for EXP and UNP, as opposed to environment and genotype in E-FS, respectively. Of all the measured protein fractions, genotype made the largest contribution for relative amounts of α/β,γ-gliadin (EXP) in both regions, GxE interaction was the main contributor to UNP variation. Environment was the main contributor to variation for absolute amounts of gliadins, except for GxE interaction in E-FS (UNP, ω-gliadin) and genotype in NW-FS (EXP, α/β,γ-gliadin) (Table 3.6).

Genotype made the largest contribution to variation for AG in NW-FS for EXP and UNP in E-FS, while environment made the largest contribution in E-FS for EXP and GxE interaction for UNP in NW-FS. Environment was the main contributor to variation for LUPP and UPP in NW-FS and genotype in E-FS (Table 3.7).

There were significant correlations between SE-HPLC protein fractions and FPC in both regions. FPC correlated in the following sequence in NW-FS for EXP from high to low, α/β,γ-gliadin, ω-gliadin, LMW, HMW and AG ; for UNP in NW-FS, ω-gliadin , α/β,γ-gliadin, HMW, LMW and AG . The correlation in E-FS showed slight changes in the sequence for absolute amounts of EXP: LMW, α/β,γ-gliadin, HMW, ω-gliadin and AG ; for UNP; α/β,γ-gliadin , LMW, HMW and AG . EXP for AG (relative amounts) showed a strong negative correlation with FPC in both regions. Correlations between unextractable polymeric proteins (UPP and LUPP) and FPC were not significant, except for UPP in E-FS (Table 3.8).

Total polymeric proteins and their ratio with GLIAG correlated negatively with FPC in NW-FS and positively in E-FS for relative amounts. GLIADIN showed a higher correlation with FPC in NW-FS than in E-FS. The LMW/HMW ratio correlated negatively with FPC in both regions. The POL/AGS ratio showed a higher correlation with FPC in E-FS compared to NW-FS. Correlations with absolute amounts were significant in both regions, the lowest correlation occurred in E-FS for LMW/HMW (Table 3.9).

Table 3.3 Simple statistics for protein content and polymeric proteins

		HMW EXP		**HMW** UNP		**LMW** EXP		**LMW** UNP		**Unextractable polymeric**	
	FPC	**%Relative**	**%Absolute**	**%Relative**	**%Absolute**	**%Relative**	**%Absolute**	**%Relative**	**%Absolute**	**LUPP**	**UPP**
Mean [a]	12.2	6.2	0.8	5.9	0.7	15.7	1.9	16.5	2.0	48.5	50.4
Range	8.7-16.4	4.2-8.0	0.4-1.1	4.0-8.0	0.3-1.1	12.5-18.8	1.3-2.7	11.8-20.1	1.3-2.8	34.2-58.6	37.4-59.5
Factor *	1.9	1.9	2.8	2.0	3.7	1.5	2.1	1.2	2.2	1.7	1.6
STD Error	0.13	0.05	0.01	0.07	0.01	0.10	0.02	0.14	0.03	0.42	0.36
Mean [b]	11.3	6.5	0.7	5.7	0.6	15.4	1.7	16.0	1.8	46.5	49.6
Range	7.9-14.4	4.9-8.9	0.5-1.1	3.4-8.6	0.4-1.1	12.8-18.8	1.0-2.4	10.4-22.0	1.0-2.9	32.4-59.5	35.5-59.6
Factor *	1.8	1.8	2.2	2.5	2.8	1.5	2.4	2.1	2.9	1.8	1.7
STD Error	0.09	0.05	0.01	0.05	0.01	0.07	0.01	0.13	0.02	0.27	0.24

[a] North Western Free State, [b] Eastern Free State, FPC = flour protein content, HMW = high molecular weight glutenin polymers, LMW = low molecular weight glutenin polymers, LUPP = large unextracatable polymeric proteins, UPP = unextractable polymeric proteins, EXP = SDS-extractable proteins, UNP = SDS-unextractable proteins, * Factor = maximum / minimum value

Table 3.4 Simple statistics for monomeric proteins

	ω-gliadin EXP		**ω-gliadin** UNP		**α/β,γ-gliadin** EXP		**α/β,γ-gliadin** UNP		**AG** EXP		**AG** UNP	
	%Relative	**%Absolute**	**%Relative**	**%Absolute**	**%Relative**	**%Absolute**	**%Relative**	**%Absolute**	**%Relative**	**%Absolute**	**%Relative**	**%Absolute**
Mean [a]	6.2	0.8	2.8	0.3	28.2	3.5	3.9	0.5	12.9	1.6	1.7	0.2
Range	4.8-8.0	0.4-1.3	2.0-3.5	0.2-0.5	22.5-32.0	2.2-4.9	2.6-5.5	0.3-0.8	10.5-17.3	1.1-2.2	1.2-2.5	0.1-0.3
Factor *	1.7	3.3	1.8	2.5	1.4	2.2	2.1	2.7	1.6	2.0	2.1	3.0
STD Error	0.06	0.01	0.03	0.01	0.16	0.05	0.04	0.01	0.11	0.02	0.02	0.00
Mean [b]	6.0	0.7	2.8	0.3	28.2	3.2	4.2	0.5	13.1	1.5	1.9	0.2
Range	5.0-9.5	0.4-1.2	1.9-4.6	0.2-0.6	22.6-33.1	2.1-4.4	3.2-6.1	0.3-0.8	10.3-18.5	1.1-2.0	1.2-2.9	0.1-0.4
Factor *	1.9	3.0	2.4	3.0	1.5	2.1	1.9	2.7	1.8	1.8	2.4	4.0
STD Error	0.05	0.01	0.03	0.00	0.15	0.03	0.03	0.01	0.11	0.01	0.02	0.00

[a] North Western Free State, [b] Eastern Free State, AG = albumin + globulin, EXP = SDS-extractable proteins, UNP = SDS-unextractable proteins, * Factor = maximum / minimum value

Table 3.5 Variance components contribution to variation (percentage of total estimate) for polymeric proteins

Variation	NW-FS	E-FS	FPC		HMW EXP		HMW UNP		LMW EXP		LMW UNP	
	d.f.	d.f.	NW-FS	Relative	NW-FS	E-FS	NW-FS	E-FS	NW-FS	E-FS	NW-FS	E-FS
Year	1	1	11.48***		4.88***	11.06***	16.99***	1.51***	32.11***	2.52***	27.54***	6.04***
Loc	1	2	33.66***		16.23***	16.22***	8.76***	36.80***	3.01***	7.88***	1.30***	19.76***
YxL	1	2	15.04***		0.63**	10.96***	25.77***	13.48***	7.15***	8.50***	11.29***	1.94***
Reps(Year*Loc)	12	18	1.91		1.40	0.69	2.47**	0.88	0.76	1.51	0.60	0.75
Cult	9	9	10.31***		12.21***	26.67***	19.66***	20.15***	30.64***	16.79***	23.30***	40.19***
YxC	9	9	3.82***		20.52***	2.84***	7.25***	0.89*	4.93***	10.63***	9.57***	2.73***
LxC	9	18	4.94***		27.41***	16.20***	5.00***	9.62***	12.96***	18.18***	15.02***	15.14***
YxLxC	9	18	6.60***		7.51***	9.10***	6.19***	8.94***	2.12***	17.51***	6.09***	6.97***
Error	108	162	12.24		9.21	6.27	7.90	7.73	6.32	16.49	5.28	6.49
R^2 (CV)			0.88 (5.68)		0.91 (4.07)	0.94 (3.66)	0.92 (2.8)	0.92 (4.64)	0.94 (2.35)	0.84 (3.38)	0.95 (3.05)	0.94 (3.79)
Variation	**d.f.**	**d.f.**	**E-FS**	**Absolute**	**NW-FS**	**E-FS**	**NW-FS**	**E-FS**	**NW-FS**	**E-FS**	**NW-FS**	**E-FS**
Year	1	1	0.86***		0.97*	2.09***	25.16***	0.31*	0.01	0.09	37.35***	4.74***
Loc	1	2	46.07***		3.22***	43.00***	35.77***	40.70***	19.78***	41.35***	31.84***	35.23***
YxL	1	2	19.87***		12.67***	19.44***	0.64**	26.29***	23.24***	7.38***	1.11***	5.72***
Reps(Year*Loc)	12	18	4.06***		2.09	1.59**	2.49*	2.89***	1.66	3.28**	1.77*	2.59***
Cult	9	9	2.28***		17.83***	13.33***	5.16***	6.58***	20.33***	8.63***	3.47***	16.48***
YxC	9	9	1.87**		15.75***	1.55***	7.50***	1.32**	5.04***	4.10***	5.60***	3.61***
LxC	9	18	5.38***		20.32***	6.76***	6.92***	6.12***	9.38***	7.22***	7.28***	14.61***
YxLxC	9	18	7.32***		9.66***	5.29***	6.64***	7.02***	5.06***	14.66***	3.37***	7.87***
Error	108	162	12.28		17.48	6.95	9.72	8.77	15.50	13.28	8.20	9.15
R^2 (CV)			0.88 (5.14)		0.83 (8.36)	0.93 (6.19)	0.90 (7.65)	0.91 (4.47)	0.84 (6.78)	0.87 (5.75)	0.92 (5.81)	0.91 (6.78)

NW-FS = North Western Free State, E-FS = Eastern Free State, FPC = flour protein content, HMW = high molecular weight glutenin polymers, LMW = low molecular weight glutenin polymers, EXP = SDS-extractable proteins, UNP = SDS-unextractable proteins, d.f. = degrees of freedom, CV = coefficient of variation, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$

Table 3.6 Variance components contribution to variation (percentage of total estimate) for gliadins

Variation	NW-FS	E-FS	ω-gliadin EXP		ω-gliadin UNP		α/β,γ-gliadin EXP		α/β,γ-gliadin UNP	
Relative amount	**d.f.**	**d.f.**	**NW-FS**	**E-FS**	**NW-FS**	**E-FS**	**NW-FS**	**E-FS**	**NW-FS**	**E-FS**
Year	1	1	0.68***	14.46***	17.63***	0.94*	0.74***	0.89***	5.59***	0.05
Loc	1	2	4.10***	4.94***	0.92**	1.47**	0.12	5.99***	0.99**	20.0***
YxL	1	2	40.79***	0.06	0.76**	1.85**	0.20*	1.02***	0.00	8.89***
Reps(Year*Loc)	12	18	0.48	1.51	1.88	1.83	0.57	0.48	2.74	2.55
Cult	9	9	15.82***	14.14***	43.91***	13.92***	78.31***	80.63***	32.55***	11.96***
YxC	9	9	9.00***	11.90***	4.10***	13.12***	4.46***	1.97***	10.10***	4.90***
LxC	9	18	18.88***	22.47***	15.29***	23.18***	4.74***	2.85***	29.09***	23.31***
YxLxC	9	18	7.34***	14.05***	5.06***	20.85***	7.41***	3.22***	3.51**	10.76***
Error	108	162	2.90	16.48	10.44	22.85	3.45	2.95	15.41	17.56
R^2 (CV)			0.97 (2.68)	0.84 (5.76)	0.9 (4.53)	0.77 (8.94)	0.97 (1.64)	0.97 (1.74)	0.85 (5.69)	0.82 (5.93)
Absolute amount	**d.f.**	**d.f.**	**NW-FS**	**E-FS**	**NW-FS**	**E-FS**	**NW-FS**	**E-FS**	**NW-FS**	**E-FS**
Year	1	1	3.42***	3.48***	25.24***	0.03	5.29***	1.90***	13.32***	0.10
Loc	1	2	22.67***	21.04***	23.90***	18.23***	19.88***	34.02***	11.57***	39.99***
YxL	1	2	36.64***	13.10***	10.83***	12.45***	8.55***	14.94***	7.12***	17.86***
Reps(Year*Loc)	12	18	1.11	1.94	2.01*	1.57	1.51	3.03***	2.56*	2.97**
Cult	9	9	6.88***	11.97***	13.47***	9.72***	37.05***	28.47***	23.66***	4.05***
YxC	9	9	4.44***	8.77***	3.82***	10.64***	4.20***	0.98	7.89***	2.60***
LxC	9	18	9.00***	15.01***	8.03***	18.76***	5.86***	2.48**	16.09***	13.89***
YxLxC	9	18	8.06***	12.74***	3.67***	15.41***	8.22***	4.59***	6.31***	6.42***
Error	108	162	7.78	11.94	9.03	13.18	9.45	9.60	11.49	12.14
R^2 (CV)			0.92 (7.51)	0.88 (6.72)	0.91 (7.03)	0.87 (9.14)	0.91 (6.19)	0.90 (5.73)	0.89 (8.22)	0.88 (8.75)

NW-FS = North Western Free State, E-FS = Eastern Free State, EXP = SDS-extractable proteins, UNP = SDS-unextractable proteins, d.f. = degrees of freedom, CV = coefficient of variation, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$

Table 3.7 Variance components contribution to variation (percentage of total estimate) for albumins and globulins and unextractable polymeric proteins

Variation	NW-FS	E-FS	AG EXP		AG UNP		Unextractable polymeric	
Relative amount	**d.f.**	**d.f.**	**NW-FS**	**E-FS**	**NW-FS**	**E-FS**	**LUPP (NW-FS)**	**UPP (NW-FS)**
Year	1	1	10.01***	0.52***	0.52	1.04**	17.34***	30.10***
Loc	1	2	0.49**	38.48***	2.12**	2.96***	18.30***	6.68***
YxL	1	2	1.52***	5.57***	2.82***	6.43***	14.81***	12.15***
Reps(Year*Loc)	12	18	2.17***	1.33*	1.00	1.41	1.46*	0.73
Cult	9	9	57.89***	30.99***	15.75***	45.10***	17.51***	16.61***
YxC	9	9	8.13***	2.55***	4.68**	2.97**	9.57***	9.02***
LxC	9	18	5.77***	10.16***	36.14***	11.05***	11.15***	15.80***
YxLxC	9	18	8.39***	3.96***	15.74***	9.54***	2.73***	2.83***
Error	108	162	5.62	6.45	21.23	19.50	7.12	6.07
R^2 (CV)			0.94 (3.04)	0.94 (3.83)	0.77 (7.7)	0.8 (8.0)	0.93 (3.54)	0.94 (2.68)
Absolute amount	**d.f.**	**d.f.**	**NW-FS**	**E-FS**	**NW-FS**	**E-FS**	**LUPP (E-FS)**	**UPP (E-FS)**
Year	1	1	1.08**	5.52***	4.43***	1.95***	2.39***	6.07***
Loc	1	2	32.60***	16.12***	27.84***	10.46***	12.98***	17.90***
YxL	1	2	22.53***	9.53***	15.00***	22.01***	7.06***	2.38***
Reps(Year*Loc)	12	18	0.79	1.84	1.88	2.21	1.11	1.06
Cult	9	9	23.89***	32.84***	6.13***	25.82***	42.91***	36.34***
YxC	9	9	3.27***	3.71***	1.71	2.33**	2.55***	3.86***
LxC	9	18	3.79***	11.21***	16.91***	11.77***	17.25***	17.87***
YxLxC	9	18	1.48	5.10***	12.54***	9.04***	4.38***	5.43***
Error	108	162	10.57	14.13	13.56	14.42	9.37	9.10
R^2 (CV)			0.89 (4.97)	0.86 (5.27)	0.86 (8.77)	0.86 (9.0)	0.91 (3.39)	0.91 (2.8)

NW-FS = North western Free State, E-FS = Eastern Free State, AG = albumin and globulin, LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, EXP = SDS-extractable proteins, UNP = SDS-unextractable proteins, d.f. = degrees of freedom, CV = coefficient of variation, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$

Table 3.8 Significant correlations between flour protein content and protein fractions

Fractions	HMW	LMW	ω-gliadin	α/β,γ-gliadin	AG	LUPP	UPP
EXP (relative) [(a)]		-0.18*	0.37***	0.27***	-0.45***		
EXP (absolute)	0.73***	0.83***	0.84***	0.91***	0.70***		
UNP (relative)				0.19*			
UNP (absolute)	0.72***	0.76***	0.81***	0.79***	0.71***		
EXP (relative) [(b)]	0.28***	-0.18**			-0.58***		0.13*
EXP (absolute)	0.80***	0.86***	0.72***	0.83***	0.49***		
UNP (relative)	0.26***	0.14*		0.53***			
UNP (absolute)	0.74***	0.74***	0.67***	0.87***	0.65***		

[a] North Western Free State (*n* = 160), [b] Eastern Free State (*n* = 240). EXP = SDS-soluble, UNP = SDS-unextractable proteins, HMW = high molecular weight glutenin polymers, LMW = low molecular weight glutenin polymers, AG = albumin and globulin, LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, *** p ≤ 0.001, ** p ≤ 0.01, * p ≤ 0.05

Table 3.9 Correlation between flour protein content and protein ratios

Ratios	Relative amount		Absolute amount	
	NW-FS	E-FS	NW-FS	E-FS
POL	-0.23**	0.30***	0.97***	0.96***
GLIADIN	0.45***	0.17**	0.96***	0.92***
GLIAG	0.23**	-0.31***	0.98***	0.96***
LMW/HMW	-0.21**	-0.24***	0.81***	0.63***
POL/GLIADIN	-0.37***		0.79***	0.81***
POL/AGS	0.31***	0.56***	0.87***	0.87***
POL/GLIAG	-0.23**	0.31***	0.89***	0.89***

NW-FS = North Western Free State (*n* = 160), E-FS = Eastern Free State (*n* = 240). POL = sum of polymeric protein, GLIADIN = sum of gliadin, GLIAG = sum of gliadin, albumin and globulin, LMW/HMW = low molecular weight protein/high molecular weight protein ratio, POL/GLIADIN = sum of polymeric protein/sum of gliadins ratio, POL/AGS = sum of polymeric protein/sum of albumin and globulin ratio, POL/GLIAG = sum of polymeric protein/sum of gliadins, albumin and globulin ratio, *** p ≤ 0.001, ** p ≤ 0.01, * p ≤ 0.05

3.4 Discussion

3.4.1 Protein composition

A wide variation occurred in FPC and the amounts of EXP and UNP protein fractions in both regions. Environment made the largest contribution to variation in FPC in both regions (Table 3.5) in agreement with van Lill et al. (1995), Gomez-Becerra et al. (2010)

and Hasniza et al. (2014), while Li et al. (2013) conducted a study on a wide range of hard and soft wheat cultivars and reported a 66% genetic contribution to variation in FPC.

Gliadin (ω-gliadin and α/β,γ-gliadin) was the main fraction comprising 41.1% and 41.2% of protein in flour and 5.1% and 4.7% of the absolute amount in flour in NW-FS and E-FS, respectively (Table 3.4). This is in agreement with Park et al. (2006) although a different extraction and separation procedure was followed, where EXP was extracted with 50% 1-propanol and UNP was determined with a combustion procedure, as opposed to the SDS-phosphate buffer for the current study. For both regions, the relative and absolute amounts for EXP were higher than the relative and absolute amounts for UNP in HMW, ω-gliadin, α/β,γ-gliadin and AG, while the relative and absolute amounts for UNP were higher in LMW than the relative and absolute amounts for EXP in LMW. For all the protein fractions in both regions, the absolute amounts varied by a larger factor compared to the factor for relative amounts. For instance, HMW in NW-FS varied for EXP (absolute amount) by a factor of 2.8 (0.4 – 1.1%) compared to a factor of 1.9 (4.2 – 8.0%) for the relative amount. There was a wider variation for EXP and UNP protein fractions in the flour than for EXP and UNP fractions in the total protein. In accordance with Park et al. (2006), variation in absolute amounts of protein fractions and the contribution to protein composition will not always remain in the same ratio.

3.4.2 Relationship between protein fractions and flour protein content

Altenbach (2012) reported, in a review, that temperature, drought and fertiliser caused several changes in the accumulation of protein fractions, but most of the changes were relatively small. FPC minimum and maximum values, were higher in NW-FS than in E-FS. The levels of protein content in the fractions increased with FPC, though not in the same ratio. Mean values for relative amounts (EXP and UNP) showed small differences between the two regions despite of a higher FPC mean value in NW-FS, while correlations with FPC varied between significant and not significant for the two regions.

The relative amount of HMW (EXP) did not correlate significantly with FPC in NW-FS but correlated significantly in E-FS. Mean values for relative amounts of HMW and LMW (UNP) did not correlate significantly with FPC in NW-FS. These findings further reflected in the negative correlation between FPC and the relative amounts of total polymeric proteins in NW-FS, as opposed to the positive correlation in E-FS. Proportions of HMW-GS are affected by the environment (Triboï et al., 2000), while DuPont et al. (2007) reported little effect of the growing conditions.

Correlations between relative amounts of ω-gliadin (EXP) and FPC were higher in NW-FS than in E-FS. The higher correlation in NW-FS could be a result of the smaller error (2.9%), compared to the larger error in E-FS (16.48%). Correlations between absolute amounts of α/β,γ-gliadin (UNP) and FPC were higher in E-FS than in NW-FS. This reflects in the higher mean value (4.2%) for absolute amounts of α/β,γ-gliadin (EXP) in E-FS compared to 3.9% in NW-FS, despite a higher mean value for FPC in NW-FS. Correlations between relative amounts of AG and FPC were higher in E-FS than in NW-FS and could be a result of higher mean values for AG in E-FS than in NW-FS, considering the lower mean FPC in E-FS compared to NW-FS. The significant negative correlation between FPC and relative amount of AG (EXP) reflects in the lower correlation between FPC and the absolute amount of AG (EXP), when compared with the correlation between FPC and polymeric proteins and gliadins.

Increased grain protein content resulted in the highest increase of gliadin content, followed by polymeric protein (Cuniberti et al., 2003; Saint Pierre et al., 2008) and with a much lower increase for AG (Cuniberti et al., 2003) and no correlation with UPP (Hajheidari et al., 2007). For the current study, FPC showed the highest correlation with the absolute amount of α/β,γ-gliadin (EXP) in NW-FS, followed by the absolute amounts of LMW (EXP), ω-gliadin (EXP) and HMW (EXP) and the lowest correlation with absolute amounts of AG (EXP). The sequence for correlations changed in E-FS, with the highest correlation between FPC and absolute amounts of LMW (EXP), followed by absolute amounts of α/β,γ-gliadin (EXP), HMW (EXP), ω-gliadin (EXP) and the lowest correlation with the absolute amounts AG. The amount of UPP and LUPP did not correlate significantly with FPC, except for a low correlation between FPC and UPP in E-FS.

Correlation between FPC and absolute amounts of α/β,γ-gliadin (UNP) in E-FS was higher than the correlation between FPC and absolute amounts of α/β,γ-gliadin (EXP) in NW-FS. The correlation between FPC and the absolute amounts α/β,γ-gliadin (UNP) was also higher in E-FS than in NW-FS. This could be related to the large environmental influence on the absolute amounts of α/β,γ-gliadin (UNP) in E-FS compared to large genotype influence in NW-FS.

This study indicated that environment, genotype and GxE interaction impacted differently on relative amounts of EXP and UNP in both environments. Genotype made a large contribution to variation for EXP in α/β,γ-gliadin in both regions. The main contributor to variation for absolute amounts in both regions was the environment, due to the influence of environment on FPC. The amounts of UPP and LUPP were strongly influenced by the environment in NW-FS and by the genotype in E-FS. Hasniza et al. (2014) reported on the significant effect of genotype on EXP, UNP and UPP.

Significant negative correlations between FPC and relative amounts of EXP in AG resulted in lower correlations with absolute amounts of AG, compared to the other fractions for absolute amounts. Gliadin and LMW showed the highest correlation with FPC in both regions for absolute amounts, with a higher correlation in EXP than for UNP.

Correlations between FPC and relative values for protein ratios were below $r = 0.50$, except for POL/AGS in E-FS ($r = 0.56$), while correlations with absolute amounts were significant from $r = 0.8$ and showed little variation between the regions, except for a higher LMW/HMW (absolute amount) ratio in NW-FS compared to E-FS.

3.5 Conclusions

Environment made the largest contribution to variation in FPC. Protein composition changed with changes in FPC but the changes were not in the same ratio. Increased FPC resulted in higher amounts of gliadin followed by polymeric protein and lower increases

in AG. Average values for protein fractions did not show large variation between the two regions in this study, although minimum and maximum values showed large variation. The effect of genotype, environment and GxE interaction on the amount of sub-fractions, especially for EXP fractions, varied between the regions. Genotype made the largest contribution to variation in α/β,γ-gliadin (EXP) in both regions. Environment had the largest influence on unextractable polymeric proteins (LUPP and UPP) in NW-FS and genotype in E-FS.

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Chapter 4

Correlations between wheat protein and baking quality of selected South African dryland wheat culivars

Abstract

Ten South African dryland winter wheat cultivars were planted at five localities over two years, divided into NW-FS and E-FS. Bread making properties were correlated with FPC and protein fractions. Significant positive correlations were observed between FPC and WGC (*r* = 86), FABS (*r* = 0.18), AlvL (*r* = 0.62), AlvSTR (*r* = 0.51) and LFV (*r* = 0.79). Significant negative correlations occurred between FPC and SDSS (*r* = -0.19), AlvP (*r* = -0.33) and AlvP/L (*r* = -0.56). MPT was mainly influenced by genotype and did not correlate significantly with FPC, while LUPP and UPP correlated significantly (*r* = 0.52) with MPT. Within the sub-fractions, absolute amounts for LMW (EXP) showed the highest correlations with AlvL and LFV. Absolute amounts of UNP made a larger contribution to AlvSTR than absolute amounts of EXP. Partial correlations were conducted to remove the quantitative effect of FPC. Polymeric and monomeric protein fractions affected bread making quality differently in NW-FS than E-FS, indicating the effect of environment on protein composition.

4.1 Introduction

Customers of bread expect a constant product and the food industry is taking great care to meet these needs, while subjected to the anomaly of wheat flour properties. Incoming flour batches vary for rheological properties after each harvest, depending on genetic and environmental conditions (Flaete et al., 2005; Zhang et al., 2007; Bordes et al., 2008). Several studies indicated that flour behaviour is influenced by protein content and the amount of certain protein sub-fractions (Peña et al., 2005; Anjum et al., 2007; Ohm et al., 2010).

Numerous publications are available on the correlation between FPC and bread making parameters. Oelofse et al. (2010) reported on the positive correlation between SDSS and FPC, in contrast with Rharrabti et al. (2003) who found a negative association between SDSS and FPC. LFV is one of the most important bread making characteristics as it represents the end product (Chung, 2003). Ohm et al. (2010) reported a significant correlation between FPC and LFV while Cavanagh et al. (2010) did not find a significant correlation.

Gluten is the main contributor to rheological properties and the balance between viscosity and elasticity determines bread making quality, although the roles of individual gluten fractions in dough functionality seem to be complex (Khatkar et al., 2002). In general, polymeric glutenin subunits are linked to increased strength and elasticity while monomeric gliadins are mainly responsible for extensibility (Gianibelli et al., 2001; Triboï & Triboï-Blondel 2001; Shewry 2003). In addition, AG are present in abundance and their role in flour quality is not clear (Gianibelli et al., 2001; D'Ovidio & Masci, 2004).

Chaudary et al. (2016) conducted SE-HPLC under different conditions than the current study: protens were extracted with 3 M Urea (pH 4.6) and the mobile phase contained 3 M Urea (pH 4.6) and 0.15 M NaCl. Proteins were detected at 280 nm. The SE-HPLC protein profiles obtained differed from the SE-HPLC protein profiles in the current study. Peak I contained glutenins, peak II (gliadins), peak III (low molecular weight gliadins), peak IV and V (albumins and globulins). Resistance to extension/extension ratio, dough development time and dough stability correlated positively with glutenins and negatively with gliadins (Chaudary et al., 2016).

The association between wheat end-use quality and molecular weight distribution of endosperm proteins has been well established (Ohm et al., 2009a; b; 2010; Labuschagne et al., 2014). SE-HPLC results indicated that UNP had a positive effect on dough strength and EXP had a negative effect on dough strength (Park et al., 2006; Tsilo et al., 2010). A strong association exists between UPP and LFV (Zhang et al., 2008; Cavanagh et al., 2010).

Wheat breeders prefer stable cultivars with consistent bread making performance over different environments (Lemelin et al., 2005). A large number of lines need to be evaluated and flour quantity is sometimes limited. LFV requires large flour samples and can only be evaluated on advanced lines. Therefore, wheat breeders rely on predictive methods for bread making quality (Caffe-Treml et al., 2011). A better understanding of the relationship between wheat protein composition and bread making quality can be achieved through the evaluation of several entries over different environments (Cuniberti et al., 2003; Li Vigni et al., 2013).

The aim of this study was to determine the correlation of protein content and protein fractions with bread making quality for South African wheat produced under winter dryland conditions.

4.2 Experimental

4.2.1 Materials

See Chapter 3.2.1.

4.2.2 Quality analysis

See Chapter 3.2.2 for milling of samples and determination of protein content. SDSS was done according to approved method 56-70 (AACC, 2000). FABS with constant flour weight procedure was followed according to the AACC approved method 54-21 method, using a Brabender® OHG Duisburg (AACC, 2000). Mixing development time was determined on a 35 g mixograph according to AACC approved method 54-40A (AACC, 2000). MPT was computed with Mixsmart® (National Manufacturing Corporation, Lincoln, NE, USA.

The resistance of dough to extension was measured according to AACC approved method 54-30A (AACC, 2000), using a Chopin Alveograph NG (AACC, 2000). Curves and

parameters were computed with Alveolink software (Tripette & Renaud, Villeneuve la Garenne, France). AlvP, AlvL and Alv P/L were computed with Alveolink software (Tripette & Renaud, Villeneuve la Garenne, France). AlvSTR was determined as deformation energy (W) of dough, and calculated as W/6.54 according to AACC approved method 54-30A (AACC, 2000). The optimised straight dough bread making method was followed according to AACC approved method 10-10B (AACC, 2000). LFV was determined by applying the rapeseed displacement procedure according to AACC approved method 10-05 (AACC, 2000).

4.2.3 Analysis of protein molecular weight distribution

As described in Chapter 3 section 3.2.3.

4.2.4 Statistical analysis

See Chapter 3.2.4. Partial correlations between SE-HPLC protein fractions and quality parameters were calculated in order to statistically remove the effect of quantitative variations in protein content (Ohm et al., 2010). Partial correlation coefficients were calculated, using Proc Corr of SAS statistical software version 9.2 (SAS Institute Inc., Cary, NC, USA).

4.3 Results

4.3.1 Simple statistics for bread making parameters

Averages for FLN and HLM values were within the limits for South African grading rules (Government Gazette, 2010). WGC values were higher in NW-FS (37.1%) than in E-FS (34.6%). SDSS values varied between 84.3 – 85.2% for NW-FS and E-FS, respectively. Average values for FABS varied between 60.9% (NW-FS) and 61.1% (E-FS). MPT varied from 1.8 – 4.4 min, with an average of 3.3 min in NW-FS, the variation in E-FS was from 2.0-5.9 min with an average of 3.5 min. AlvP was higher in E-FS with 92.5 mm H_2O and

80.5 mm H_2O in NW-FS, while AlvL was higher in NW-FS (115.4 mm) than in E-FS (104.6 mm). Minimum and maximum values for AlvP/L varied between 0.3-3.1 mm H_2O mm^{-1} in NW-FS and between 0.3 – 4.8 mm H_2O mm^{-1} in E-FS. Average values for AlvSTR varied from 48.0-49.5 J x 10^{-4} in NW-FS and E-FS, respectively and the minimum and maximum values varied from 19.3 – 80.7 J x 10^{-4} (NW-FS) and 25.4 – 95.9 J x 10^{-4} (E-FS). Average LFV was 944 cm^3 in NW-FS and 881 cm^3 in E-FS (Table 4.1).

4.3.2 ANOVA for bread making parameters

Genotype, environment and GxE interaction contributed significantly to variation for WGC, SDSS and FABS in both regions, except for GxE in E-FS for SDSS. Genotype made the largest contribution to variation for WGC (NW-FS) and SDSS (NW-FS and E-FS), while environment made the largest contribution for WGC (E-FS) and FABS (NW-FS and E-FS). A large error occurred for SDSS in E-FS, 30.06% of the variation could not be explained (Table 4.2). Genotype, environment and GxE interaction contributed significantly to variation for MPT, alveograph parameters and LFV in both regions, although GxE interaction did not contribute highly significantly to variation in AlvSTR in E-FS. Genotype made the largest contribution to MPT in both regions and AlvL in NW-FS. GxE interaction made the largest contribution for AlvP and AlvP/L in NW-FS, while environment was mainly responsible for variation in alveograph parameters and LFV in E-FS and AlvSTR and LFV in NW-FS (Table 4.3).

4.3.3 Combined Pearson's correlation

Correlations were determined on n = 400. Only significant correlations ($p \leq 0.001$) were presented and discussed. WGC correlated with FPC and not significantly with unextractable polymeric proteins. WGC correlated significantly with all the protein fractions (absolute amounts). The highest correlation was between WGC and absolute amounts of α/β,γ-gliadin (EXP) and the lowest correlation was with absolute amounts of AG. Relative amounts of AG (EXP and UNP) correlated negatively with WGC (Table 4.4).

A negative correlation occurred between SDSS and FPC. Correlations for HMW, ω-gliadin and AG were not significant with SDSS. Relative amounts of LMW (UNP) correlated positively and relative and absolute amounts of α/β,γ-gliadin (EXP) correlated negatively with SDSS. The highest correlation occurred between relative amounts of α/β,γ-gliadin (EXP) and SDSS (Table 4.4).

FABS showed a positive correlation with FPC. Relative and absolute amounts for LMW and ω-gliadin (EXP), and absolute amounts for HMW (EXP) correlated significantly with FABS, with the highest correlation between FABS and relative and absolute amounts for ω-gliadin. Correlations were significant between FABS and absolute amounts of monomeric proteins (UNP), while FABS did not correlate significantly with absolute amounts of polymeric proteins (UNP) (Table 4.4).

Positive correlations occurred between MPT and unextractable polymeric proteins (LUPP and UPP). Correlations between MPT and α/β,γ-gliadin and AG were not significant. EXP for HMW, LMW and ω-gliadin were negatively correlated with MPT, the highest correlation was between MPT and relative amounts of LMW (EXP) (Table 4.4).

AlvP correlated negatively with FPC and not significant with LUPP, UPP and ω-gliadin. Absolute amounts of HMW and LMW (EXP and UNP) correlated negatively with AlvP.

Table 4.1 Simple statistics for baking quality parameters

	FLN	HLM	WGC	SDSS	FABS	MPT	AlvP	AlvL	AlvP/L	AlvSTR	LFV
	Sec	kg hl^{-1}	%	ml	%	min	mm H_2O	mm	mm H_2O mm^{-1}	J x 10^{-4}	cm^3
Mean [a]	402	78	37.1	84.3	60.9	3.3	80.5	115.4	0.8	48.0	944
Range	232-673	71-84	23.9-50.8	70.0-97.0	54.3-65.8	1.8-4.4	51.0-145.0	39.0-186.0	0.3-3.1	19.3-80.7	710-1030
STD Error	7.06	0.21	0.43	0.50	0.18	0.05	1.19	2.59	0.03	0.77	6.08
Mean [b]	439	78	34.6	85.2	61.1	3.5	92.5	104.6	1.0	49.5	881
Range	217-744	66-83	21.3-43.5	68.-98.0	55.4-68.0	2.0-5.9	50.0-167.0	35.0-178.0	0.3-4.8	25.4-95.9	685-1030
STD Error	4.69	0.15	0.30	0.41	0.19	0.04	1.37	1.94	0.04	0.65	5.11

[a] North Western Free State, [b] Eastern Free State. FLN = Falling Number, HLM = hectolitre mass, WGC = wet gluten content, SDSS = SDS-sedimentation, FABS = farinograph water absorption, MPT = mixograph peak time, AlvP = alveograph tenacity, AlvL = alveograph extensibility, AlvP/L = alveograph tenacity/extensibility, AlvSTR = alveograph strength, LFV = loaf volume

Table 4.2 Variance component contribution to variation (percentage of total estimate) for polymeric proteins

Variation	d.f. (NW)	d.f (E)	WGC (NW)	WGC (E)	SDSS (NW)	SDSS (E)	FABS (NW)	FABS (E)
Year	1	1	3.32***	0.02	4.94***	12.12***	22.56***	52.16***
Loc	1	2	19.54***	42.69***	3.27***	5.68***	9.65***	0.62**
YxL	1	2	8.92***	7.32***	0.84*	6.46***	18.71***	11.20***
Reps (Year*Loc)	12	18	2.49	3.66**	4.07	3.63	3.15*	1.84**
Cult	9	9	37.25***	18.00***	41.86***	29.90***	21.00***	11.43***
YxC	9	9	4.82***	2.18**	4.33*	2.27	5.65***	1.99***
LxC	9	18	5.37***	5.98***	5.28**	4.54	3.06**	6.83***
YxLxC	9	18	5.47***	6.76***	13.5*	5.35	3.52**	5.96***
Error	108	162	12.82	13.38	21.84	30.06	12.70	7.95
R^2 (CV %)			0.87 (6.43)	0.87 (5.87)	0.78 (4.27)	0.70 (5.0)	0.87 (1.65)	0.92 (1.63)

d.f. = degrees of freedom, NW = North Western Free State, E = Eastern Free State, WGC = wet gluten content, SDSS = SDS-sedimentation, FABS = farinograph water absorption, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$

Table 4.3 Variance component contribution to variation (percentage of total estimate) for polymeric proteins

Variation	d.f. (NW)	d.f (E)	MPT (NW)	MPT (E)	AlvP (NW)	AlvP (E)	AlvL (NW)	AlvL (E)
Year	1	1	13.77***	20.26***	3.28***	6.72***	1.65**	0.23
Loc	1	2	5.47***	0.90***	0.94*	28.74***	28.50***	44.60***
YxL	1	2	6.05***	0.54*	7.93***	4.54***	0.09	8.70***
Reps (Year*Loc)	12	18	2.76**	1.19	2.11	3.44***	2.53	1.58
Cult	9	9	43.36***	43.14***	31.16***	31.93***	32.61***	21.29***
YxC	9	9	7.42***	12.55***	10.44***	4.79***	6.06***	3.44***
LxC	9	18	7.32***	5.57***	13.32***	5.20***	8.96***	4.19***
YxLxC	9	18	4.32***	6.56***	10.18***	4.00***	3.65**	5.23***
Error	108	162	9.54	9.30	20.64	10.63	15.95	10.74
R^2 (CV %)			0.90 (6.77)	0.91 (7.4)	0.79 (10.28)	0.89 (9.04)	0.84 (13.77)	0.89 (11.41)
Variation	**d.f. (NW)**	**d.f (E)**	**AlvP/L (NW)**	**AlvP/L (E)**	**AlvSTR (NW)**	**AlvSTR (E)**	**LFV (NW)**	**LFV (E)**
Year	1	1	0.01	3.80***	2.21**	3.14***	1.72***	5.35***
Loc	1	2	11.04***	37.94***	43.86***	18.93***	40.78***	50.76***
YxL	1	2	0.84*	8.32***	3.73**	20.19***	3.61***	4.86***
Reps (Year*Loc)	12	18	2.89	1.45	1.34	3.11	4.30***	2.24*
Cult	9	9	29.45***	17.98***	8.06**	18.02***	19.58***	13.69***
YxC	9	9	11.75***	3.14***	3.11	3.65***	8.11***	1.32
LxC	9	18	16.00***	8.77***	5.81*	8.66***	5.71***	5.63***
YxLxC	9	18	8.15***	4.13***	2.71	5.38***	5.86***	6.27***
Error	108	162	19.88	14.47	29.18	18.91	10.33	9.89
R^2 (CV %)			0.80 (26.82)	0.86 (29.51)	0.71 (13.25)	0.81 (10.71)	0.90 (3.18)	0.90 (3.41)

d.f. = degrees of freedom, NW = North Western Free State, E = Eastern Free State, MPT = mixograph peak time, AlvP = alveograph tenacity, AlvL = alveograph extensibility, AlvP/L = alveograph tenacity/extensibility, AlvSTR = alveograph strength, LFV = loaf volume, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$

Table 4.4 Combined Pearson's correlation between WGC, SDSS, FABS, MPT and protein content and protein fractions

Parameter	FPC	LUPP	UPP	Fractions	HMW	LMW	ω-gliadin	α/β,γ-gliadin	AG
WGC	0.86***			EXP (relative)	0.25***			0.32***	-0.57***
				EXP (absolute)	0.76***	0.81***	0.67***	0.84***	0.43***
				UNP (relative)				0.23***	-0.24***
				UNP (absolute)	0.55***	0.55***	0.53***	0.68***	0.41***
SDSS	-0.19***			EXP (relative)				-0.42***	
				EXP (absolute)				-0.35***	
				UNP (relative)		0.22***			
				UNP (absolute)					
FABS	0.18***			EXP (relative)		0.21***	0.30***		
				EXP (absolute)	0.25***	0.26***	0.30***		
				UNP (relative)					
				UNP (absolute)			0.21***	0.20***	0.22***
MPT		0.52***	0.53***	EXP (relative)	-0.41***	-0.51***	-0.22***		
				EXP (absolute)	-0.33***	-0.34***	-0.20***		
				UNP (relative)	0.35***	0.35***			
				UNP (absolute)		0.19***			

WGC = wet gluten content, SDSS, SDS-sedimentation, FABS = farinograph water absorption, MPT = mixograph peak time, FPC = flour protein content, LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, HMW = high molecular weight glutenin polymers, LMW = low molecular weight glutenin polymers, AG = albumin and globulin, *** $p \leq 0.001$

Correlation between AlvP and α/β,γ-gliadin was negative for EXP (relative and absolute amounts) and UNP (absolute amounts). The highest correlation with AlvP occurred for absolute amounts of α/β,γ-gliadin (EXP). AlvP showed a positive correlation with relative amounts of AG (EXP and UNP), with the higher correlation occurring between AlvP and AG (UNP) (Table 4.5).

FPC correlated significantly with AlvL and not significantly with LUPP and UPP. Absolute amounts for polymeric and monomeric proteins (EXP and UNP) correlated significantly with AlvL, with the highest correlation between AlvL and absolute amounts of LMW (EXP). Within AG sub-fractions, the highest correlation occurred between the relative amount of AG and AlvL (Table 4.5).

The correlation of AlvP/L was negative with FPC and not significant with LUPP and UPP proteins. Correlations of AlvP/L with HMW, LMW, gliadin and AG were negative and EXP showed a higher correlation than UNP for relative amounts. Similar high correlations with AlvP/L occurred for HMW, LMW and α/β,γ-gliadin. All the sub-fractions in AG correlated significantly with AlvP/L and relative amounts correlated positively with EXP, showing the highest correlation between AlvP/L and the relative amount of AG (EXP) (Table 4.5).

Correlations between AlvSTR and FPC were significant and positive and the correlation with AlvSTR was not significant with unextractable polymeric proteins. Correlations for AlvSTR were significant with polymeric and monomeric absolute values (EXP and UNP). Correlations between AlvSTR and UNP were higher than for EXP for polymeric and monomeric proteins, the highest being for absolute amounts of UNP α/β,γ-gliadin. Relative amounts of AG (EXP) showed a significant negative correlation with AlvSTR (Table 4.5).

A significant correlation was observed between LFV and FPC, while the correlation with LFV was not significant for LUPP and UPP. Correlations between LFV and absolute amounts for polymeric and monomeric proteins (EXP and UNP) were significant for all the protein fractions and higher in EXP, with the highest correlation between LFV and

absolute amounts of LMW (EXP). Correlations between LFV and relative amounts for polymeric and monomeric proteins (EXP and UNP) were not significant, except for the significant negative correlation between the relative amounts of AG (EXP) and LFV (Table 4.5).

4.3.4 Pearson's correlation between flour protein content and baking parameters for localities and years

FPC varied significantly between localities and years in NW-FS and E-FS (Table 4.6). Correlations between FPC and WGC were significant for all the localities and years in both regions. FPC did not correlate significantly with SDSS for any locality or year, except for a weak negative correlation at Clar 2009. Correlations between FABS and FPC were positive and varied between significant and non-significant (Table 4.6).

FPC did not correlate significantly with MPT, except for a negative correlation at Clar (2007 and 2009). Correlations, for AlvP and AlvP/L with FPC, were not significant in NW-FS, while significant negative correlations occurred at some localities in E-FS. Positive correlations occurred between AlvL and FPC and varied between significant and non-significant. Significant positive correlations were observed between LFV and FPC at all the localities for both years, the weakest correlation occurred at Bhm 2009 (Table 4.6).

Table 4.5 Combined Pearson's correlation between alveograph parameters, loaf volume and protein content and protein fractions

Parameter	FPC	LUPP	UPP	Fractions	HMW	LMW	ω-gliadin	α/β,γ-gliadin	AG
AlvP	-0.33***			EXP (relative)				-0.21***	0.30***
				EXP (absolute)	-0.36***	-0.35***		-0.37***	
				UNP (relative)					0.35***
				UNP (absolute)	-0.21***	-0.22***		-0.20***	
AlvL	0.62***			EXP (relative)	0.26***				-0.40***
				EXP (absolute)	0.61***	0.64***	0.44***	0.56***	0.34***
				UNP (relative)					
				UNP (absolute)	0.40***	0.42***	0.38***	0.47***	0.30***
AlvP/L	-0.56***			EXP (relative)	-0.22***			-0.20***	0.43***
				EXP (absolute)	-0.53***	-0.53***	-0.35***	-0.53***	-0.25***
				UNP (relative)					0.22***
				UNP (absolute)	-0.38***	-0.39***	-0.32***	-0.43***	-0.21***
AlvSTR	0.51***			EXP (relative)					-0.31***
				EXP (absolute)	0.40***	0.40***	0.41***	0.38***	0.27***
				UNP (relative)	0.22***			0.30***	
				UNP (absolute)	0.48***	0.45***	0.46***	0.51***	0.49***
LFV	0.75***			EXP (relative)					-0.45***
				EXP (absolute)	0.59***	0.70***	0.62***	0.64***	0.42***
				UNP (relative)					
				UNP (absolute)	0.53***	0.58***	0.56***	0.58***	0.40***

AlvP = alveograph tenacity, AlvL = alveograph extensibility, AlvP/L = alveograph tenacity/alveograph extensibility, AlvSTR = Alveograph strength, LFV = loaf volume, FPC = flour protein content, LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, HMW = high molecular weight glutenin polymers, LMW = low molecular weight glutenin polymers, AG = albumin and globulin, *** $p \leq 0.001$

Table 4.6 Pearson's correlation between protein content and baking parameters for localities and years

Parameter	North Western Free State (FPC)				Eastern Free State (FPC)					
	Bot 2007	Bot 2009	Bult 2007	Bult 2009	Bhm 2007	Bhm 2009	Clar 2007	Clar 2009	Lad 2007	Lad 2009
WGC	0.55***	0.80***	0.75***	0.70***	0.63***	0.41**	0.78***	0.94***	0.81***	0.68***
SDSS								-0.36*		
FABS		0.38*		0.32*	0.28*	0.33*	0.44**			
MPT							-0.38*	-0.48**		
AlvP								-0.62***		-0.44**
AlvL		0.43**	0.48**				0.58***	0.70***		0.49**
AlvP/L					-0.33*		-0.60***	-0.65***		-0.50**
AlvSTR		0.35*	0.39*		0.33*	0.37*	0.46**	0.34*		
LFV	0.72***	0.61***	0.67***	0.465**	0.53***	0.33*	0.59***	0.77***	0.50**	0.69***
FPC (%)	11.20c	11.36c	14.35a	11.98b	11.13c	12.42a	12.40a	11.98b	10.88c	9.19d
LSD	0.31						0.26			

WGC = wet gluten content, SDSS = SDS-Sedimentation, FABS = farinograph water absorption, MPT = mixograph peak time, AlvP = alveograph tenacity, AlvL = alveograph extensibility, AlvP/L = alveograph tenacity/alveograph extensibility, AlvSTR = alveograph strength, LFV = loaf volume, FPC = flour protein content, LSD = least significant difference at $p \leq 0.05$. *** $p \leq 0.001$, ** $p \leq 0.01$,* $p \leq 0.05$

4.3.5 Partial correlations

Quality parameters that showed significant correlations with FPC (Tables 4.4 and 4.5) were selected for partial correlation analysis and included WGC, FABS, AlvP, AlvL, AlvP/L, AlvSTR and LFV.

4.3.5.1 Polymeric proteins

WGC correlated positively and significantly with relative amounts of HMW (EXP) and LMW (EXP), and correlated negatively with relative amounts of HMW (UNP) and LMW (UNP) in NW-FS. Relative and absolute amounts of HMW (EXP) and LMW (EXP) correlated positively with WGC in E-FS, while relative and absolute amounts of LMW (UNP) correlated negatively with WGC in E-FS. FABS correlated negatively with relative amounts of HMW (UNP) and positively with relative amounts of LMW (EXP) in NW-FS. Relative and absolute amounts of HMW (EXP) correlated significantly with FABS in E-FS (Table 4.7).

AlvP correlated positively with absolute amounts of LMW (EXP) in NW-FS, whereas relative amounts of HMW (EXP) correlated negatively with AlvP in E-FS. AlvL correlated positively with relative amounts of LMW (EXP) and negatively with absolute amounts of LMW (EXP) in NW-FS. AlvL correlated positively with relative and absolute amounts of HMW (EXP) in E-FS. Polymeric proteins did not correlate significantly with AlvP/L in both regions. AlvSTR correlated positively with EXP absolute amounts of HMW (and LMW in NW-FS. AlvSTR correlated negatively with UNP absolute amounts of HMW and LMW in NW-FS. LFV correlated positively with absolute amounts of HMW (EXP) and negatively with absolute amounts of LMW (UNP) in NW-FS, while LFV correlated positively with absolute amounts of HMW (EXP) in E-FS (Table 4.7).

Table 4.7 Partial correlation coefficients between baking quality parameters and values for SE-HPLC polymeric protein fractions

	HMW (EXP)		HMW (UNP)		LMW (EXP)		LMW (UNP)	
	Relative	**Absolute**	**Relative**	**Absolute**	**Relative**	**Absolute**	**Relative**	**Absolute**
WGC [(a)]	0.39***		-0.40***		0.37***		-0.45***	
FABS [(a)]			-0.35***		0.46***			
AlvP [(a)]						0.37***		
AlvL [(a)]					0.34***	-0.27***		
AlvP/L [(a)]								
AlvSTR [(a)]		0.39***		-0.44***		0.38***		-0.45***
LFV [(a)]		0.26***						-0.43***
WGC [(b)]	0.40***	0.37***			0.23***	0.23***	-0.26***	-0.26***
FABS [(b)]	0.22***	0.22***						
AlvP [(b)]	-0.21***							
AlvL [(b)]	0.37***	0.36***						
AlvP/L [(b)]								
AlvSTR [(b)]								
LFV [(b)]	0.19***							

Partial variable, protein content; ***p ≤ 0.001, [a] North Western Free State (*n* = 160), [b] Eastern Free State (*n* = 240). EXP = extractable protein, UNP = unextractable protein, HMW= high molecular weight protein fractions, LMW = low molecular weight protein fractions, WGC = wet gluten content, FABS = farinograph water absorption, AlvP = alveograph tenacity, AlvL = alveograph extensibility, AlvP/L = alveograph tenacity/extensibility, AlvSTR = alveograph strength, LFV = loaf volume

4.3.5.2 Monomeric proteins

WGC correlated negatively with absolute amounts of ω-gliadin (EXP) and relative amounts of ω-gliadin (UNP) in NW-FS. Absolute and relative amounts of α/β,γ-gliadin (EXP) correlated positively with WGC, and absolute and relative amounts of AG (EXP and UNP) correlated negatively with WGC in NW-FS. WGC correlated positively with absolute and relative amounts of α/β,γ-gliadin (EXP) and negatively with absolute and relative amounts of AG (EXP and UNP) in E-FS. FABS correlated positively with relative amounts of ω-gliadin (EXP) and absolute amounts of AG (UNP) in NW-FS, and negatively with absolute amounts of α/β,γ-gliadin (EXP) in NW-FS. Relative and absolute amounts of ω-gliadin (EXP) and absolute amounts of α/β,γ-gliadin (UNP) correlated positively with FABS in E-FS, while relative and absolute amounts of α/β,γ-gliadin (EXP) correlated negatively with FABS in E-FS (Table 4.8).

AlvP correlated negatively with EXP relative amounts of α/β,γ-gliadin in NW-FS, and positively with relative amounts of AG (EXP and UNP) in NW-FS. AlvP correlated positively with relative and absolute amounts of AG (UNP) in E-FS. AlvL correlated positively with absolute amounts of AG (UNP) in NW-FS. AlvL did not correlate significantly with monomeric proteins in E-FS (Table 4.8).

AlvP/L correlated significantly with AG (UNP, relative) in NW-FS. AlvP/L did not correlate significantly with monomeric proteins in E-FS. AlvSTR correlated negatively with absolute amounts of ω-gliadin (UNP) and AG (UNP) in NW-FS. AlvSTR correlated positively with relative and absolute amounts of α/β,γ-gliadin (UNP) in E-FS. LFV correlated negatively with absolute amounts of ω-gliadin (EXP and UNP) and absolute amounts of AG (EXP) in NW-FS. LFV correlated positively with absolute amounts of α/β,γ-gliadin (EXP) in NW-FS. LFV did not correlate significantly with monomeric proteins in E-FS (Table 4.8).

Table 4.8 Partial correlation coefficients between quality parameters and values for SE-HPLC monomeric proteins

	ω-gliadin (EXP)		ω-gliadin (UNP)		α/β,γ-gliadin (EXP)		α/β,γ-gliadin (UNP)		AG (EXP)		AG (UNP)	
	Relative	**Absolute**	**Relative**	**Absolute**	**Relative**	**Absolute**	**Relative**	**Absolute**	**Relative**	**Absolute**	**Relative**	**Absolute**
WGC [(a)]		-0.36***	-0.34***		0.49***	0.32***			-0.26***	-0.48***	-0.36***	-0.31***
FABS [(a)]	0.33***					-0.27***						0.38***
AlvP [(a)]					-0.30***				0.27***		0.40***	
AlvL [(a)]												0.30***
AlvP/L [(a)]											0.30***	
AlvSTR [(a)]				-0.35***		0.46***						-0.36***
LFV [(a)]		-0.29***		-0.39***		0.55***				-0.26***		
WGC [(b)]					0.29***	0.26***			-0.31***	-0.28***	-0.31***	-0.30***
FABS [(b)]	0.28***	0.27***			-0.20***	-0.21***		0.21***				
AlvP [(b)]											0.25***	0.27***
AlvL [(b)]												
AlvP/L [(b)]												
AlvSTR [(b)]							0.24***	0.24***				
LFV [(b)]												

Partial variable, protein content; *** $p \leq 0.001$, [a] North Western Free State (n = 160), [b] Eastern Free State (n = 240). EXP = extractable protein, UNP = unextractable protein, AG = albumin and globulin fractions, WGC = wet gluten content, FABS = farinograph water absorption, AlvP = alveograph tenacity, AlvL = alveograph extensibility, AlvP/L = alveograph tenacity/extensibility, AlvSTR = alveograph strength, LFV = loaf volume

4.4 Discussion

4.4.1 Wet gluten content

Genotype made the largest contribution to variation in WGC in NW-FS, while environment made a large contribution in E-FS. Correlations of WGC with FPC were significant and varied between years and localities and is evident from the high combined (n = 400) correlation (r = 0.858). The combined average values for WGC were higher in NW-FS compared to E-FS and reflects the higher FPC in NW-FS (Table 3.3). The high correlation between WGC and absolute amounts of α/β,γ-gliadin and low correlation with AG, confirm the relationship between these fractions and FPC (Table 3.8).

Protein content causes variations in protein fractions and certain quality traits, which makes it difficult to estimate the influence of individual protein fractions on quality traits. Partial correlations statistically remove the effect of quantitative variation in protein content, making it possible to establish the qualitative effect of individual protein fractions at an equivalent level of protein content (Ohm et al., 2010). Partial correlations showed a significant correlation between WGC and relative amounts of polymeric proteins in NW-FS, while the partial correlation with absolute polymeric amounts were not significant ($p \leq 0.001$) in NW-FS. Furthermore, relative amounts of polymeric EXP correlated positively with WGC and UNP correlated negatively with WGC. This indicates that a higher proportion of EXP polymeric proteins had a positive effect on WGC, while a higher proportion of UNP polymeric proteins had a negative impact on WGC in the NW-FS.

Relative and absolute amounts for polymeric protein fractions (EXP and UNP) showed significant partial correlations with WGC in E-FS, except for HMW (UNP). This indicates that a higher proportion and quantity of HMW and LMW (EXP) had a positive influence on WGC, while an increased proportion and quantity of LMW (UNP) had a negative effect on WGC in E-FS. Proportional and quantitative variations of α/β,γ-gliadin (EXP)

showed a positive effect on WGC in both regions while proportional and quantitative increases in AG (EXP and UNP) had a negative effect on WGC in both regions.

4.4.2 SDS-sedimentation

Genotype showed a stronger influence on SDSS than the environment and GxE interaction. This was also confirmed by van Lill et al. (1995) who investigated released cultivars and Oelofse et al. (2010) who conducted a study on segregating populations in the Free State. SDSS and FPC did not correlate significantly, except for a negative correlation at Clar 2009, which resulted in a negative combined correlation between FPC and SDDS, in agreement with Rharrabti et al. (2003). This is in contrast with Ames et al. (2003), Clarke et al. (2004) and Oelofse et al. (2010), who reported a positive correlation between FPC and SDSS. Mean values for SDSS were lower in NW-FS than in E-FS (Table 4.1), while mean values for FPC were higher in NW-FS compared to E-FS (Table 3.3), confirming the negative association between FPC and SDSS.

Qualitative variation of storage proteins had a larger effect on SDSS than on quantitative variation (Grausgruber et al., 2000). Absolute amounts of polymeric and monomeric proteins had a negative effect on SDDS in NW-FS, while the effect for relative amounts of polymeric and monomeric proteins were not significant ($p \leq 0.001$) on SDSS in E-FS. Increased relative and absolute amounts of α/β,γ-gliadin (EXP) had a negative effect on SDSS, in agreement with Schober et al. (2006). Partial correlations with protein fractions were not determined for SDSS due to the low correlation between SDSS and FPC.

4.4.3 Farinograph water absorption

Water absorption is influenced by protein content, damaged starch, pentosans and gluten strength (Preston & Kilborn, 1984). FABS was strongly influenced by the environment and correlated significantly with FPC, although it was not high ($r = 0.18$), which is a result of the inconsistent correlations for the two years and different localities. The influence of FPC on FABS also reflects in the association with absolute amounts for EXP (HMW, LMW and ω-gliadin) and for UNP (monomeric proteins). Gluten

confers water absorption (Wieser, 2007) and FABS increases linearly with FPC although the regression slope is determined by the genotype (Eliasson & Larsson, 1993). For combined Pearson's correlation, increasing amounts of relative and absolute amounts of ω-gliadin (EXP) had a positive influence on FABS.

Pearson's correlation did not show a significant relationship between α/β,γ-gliadin and FABS. Protein content masked the effect of α/β,γ-gliadin (EXP) on FABS, while partial correlations indicated that proportional and quantitative variations of α/β,γ-gliadin (EXP) had a negative effect on FABS in E-FS, whereas quantitative variation of α/β,γ-gliadin (EXP) had a negative effect on FABS in NW-FS. Proportional and quantitative variation of EXP for HMW, LMW and ω-gliadin had a positive effect on FABS in E-FS, and proportional and quantitative variation of UNP for LMW had a negative effect on FABS in E-FS.

4.4.4 Mixograph peak time

Numerous studies indicated the positive relationship between dough development time and unextractable polymeric proteins (Cuniberti et al., 2003; Park et al., 2006; Cavanagh et al., 2010; Ohm et al., 2010; Wentzel, 2011, Hussain et al., 2012; Labuschagne et al., 2014). The same was observed for this study, with significant correlations of MPT with LUPP and UPP, while EXP showed strong negative correlations for MPT with relative and absolute amounts of HMW, LMW and ω-gliadin. Correlations between MPT with FPC, α/β,γ-gliadin and AG were not significant.

4.4.5 Alveograph parameters

4.4.5.1 Alveograph tenacity

AlvP correlated negatively with FPC and it reflects in the negative correlation with absolute amounts in HMW, LMW and α/β,γ-gliadin, which correlated positively and significant with FPC (Table 3.7). The significant positive correlation with AlvP and relative amounts of AG further demonstrates the negative association with FPC, due to

the negative correlation between FPC and relative amounts of AG (Table 3.8). UPP and LUPP did not correlate significantly with AlvP. Cavanagh et al. (2010) reported a positive correlation between UPP and extensigraph maximum resistance (Rmax). AlvP and Rmax are both measures of dough resistance but the experimental procedures differ. Alveograph uses a fixed dough mixing time with a constant water content and biaxial extension, instead of adjusted water content and mixing time and uniaxial extension for the extensigraph (Cuniberti et al., 2003).

A further explanation could be that UPP measures the amount of polymeric protein above a specific critical molecular weight and may not reflect the molecular weight distribution of the fraction (Bersted & Anderson, 1990). The absolute amounts showed a significant negative correlation with AlvP in E-FS for sums of protein classes and ratios, whereas correlations were not significant in NW-FS. This could be due to the strong environmental influence on AlvP in E-FS and the influence of GxE interaction in NW-FS.

Partial correlations indicated that proportional variation in AG (UNP) had the largest positive effect on AlvP in NW-FS. For E-FS, partial correlations indicated that proportional and quantitative variation in AG (UNP) had a positive effect on AlvP. This is in contrast with Chaudary et al. (2016) where relative AG values for SE-HPLC did not show a significant effect on dough rheological traits (Chaudary et al., 2016).

4.4.5.2 Alveograph extensibility

FPC showed a significant positive correlation with AlvL although some of the localities (Bot 2007; Bult 2009; Bhm 2007 and 2009; Clar 2007 and Lad 2007) did not show significant correlations. The strong correlation between AlvL and FPC was further confirmed by the significant correlations with absolute amounts, with a strong association between EXP for polymeric proteins, α/β,γ-gliadin and ω-gliadin, in descending order. LUPP and UPP did not correlate significantly with AlvL, in contrast with the strong negative correlations reported by Hasniza et al. (2014).

Increased relative amounts of AG (EXP) had a negative impact on AlvL and resulted in a low correlation with absolute amounts of AG. Partial correlations between protein fractions and AlvL did not show significant correlations in E-FS, while LMW (EXP) had a positive effect on AlvL for proportional variations and a negative effect for quantitative variations in LMW (EXP).

4.4.5.3 Alveograph tenacity/extensibility

AlvP/L serves as a primary criterion for the release of new cultivars in South Africa and the average values for this study fell within the ideal value of 1.0, as required by the baking industry. The correlation between AlvP/L and FPC was negative, which was reflected in the negative correlations with absolute amounts. Polymeric proteins and α/β,γ-gliadin had the largest negative impact on AlvP/L, while relative amounts in AG contributed positively.

The negative relationship between absolute amounts of polymeric proteins and α/β,γ-gliadin for AlvP, and the positive correlation with AlvL is confirmed by a previous study where RP-HPLC was employed (Wentzel, 2011). Correlations of ω-gliadin with AlvP/L were not significant, and correlations between AlvL and ω-gliadin were lower than correlations observed between α/β,γ-gliadin and AlvL. Interactions between ω-gliadin and other proteins are non-covalent because of the lack of cysteine (Tatham & Shewry, 1995) and the influence on viscoelastic properties could be less effective (Barak et al., 2013).

Partial correlations indicated that proportional and quantitative variations in protein fractions did not correlate significantly with AlvP/L in E-FS. Pearson's correlation indicated a significant correlation between FPC and AlvP/L, which most likely occurred due to interdependent associations of AlvP/L with FPC in E-FS. Proportional variations in AG had a positive effect on AlvP/L in NW-FS.

4.4.5.4 Alveograph strength

A significant correlation was observed of AlvSTR with FPC and resulted in the high correlations with absolute amounts (EXP and UNP) in polymeric and monomeric proteins. It is of interest to note that the absolute amounts of UNP showed a stronger association with AlvSTR than absolute amounts of EXP, in agreement with Bean et al. (1998) and Ohm et al. (2009a). Furthermore, the absolute amounts of monomeric proteins (UNP) showed a higher correlation than absolute amounts of polymeric proteins (UNP). This finding is in contrast with Anjum et al. (2007) and Li Vigni et al. (2013) who reported on the negative effect of gliadin on AlvSTR. The strong negative correlation with relative amounts of AG (EXP) indicates the detrimental effect of increased amounts of AG on AlvSTR.

LUPP and UPP did not correlate significantly ($p \leq 0.001$) with AlvSTR. The absolute amount of UPP (UPP% in the grain) showed the highest positive correlation with AlvSTR (Cuniberti et al., 2003). Absolute amounts were not calculated for this study.

Partial correlation analysis did not show significant correlations between AlvSTR and polymeric proteins in E-FS, whereas increased α/β,γ-gliadin (UNP) had a positive effect on AlvSTR. Quantitative increases for EXP polymeric proteins had a positive effect on AlvSTR, while quantitative increases for UNP polymeric proteins had a negative effect on AlvSTR in NW-FS. Increased quantitative amounts of α/β,γ-gliadin (EXP) had the largest positive influence on AlvSTR in NW-FS.

4.4.6 Loaf volume

An optimised straight dough baking procedure was followed and correlated positive and significantly with FPC, in accordance with Dowell et al. (2008) and Ohm et al. (2009a; 2010), while LUPP and UPP did not correlate significantly with LFV. This contradicts the findings by Zhang et al. (2008) and Cavanagh et al. (2010) where UPP correlated significantly and FPC not significantly with LFV. The different baking procedures could be a result of the different correlations. Zhang et al. (2008) baked Chinese steam bread

and Cavanagh et al. (2010) used a sponge and dough procedure. The strong association between LFV and FPC also reflects in the strong environmental influence for both, especially the large contribution made by locality in both regions.

Absolute amounts of EXP made the largest contribution to LFV for LMW and α/β,γ-gliadin. Absolute amounts of LMW and α/β,γ-gliadin (EXP) were also strongly associated with AlvL, in agreement with Nash et al. (2006) and Li et al. (2013) who suggested that a certain amount of extensibility is required for good baking performance. In order to obtain a large LFV, dough must extend beyond a minimum level (Janssen et al., 1996). HMW and ω-gliadin contributed to a lesser extent and absolute amounts of AG made the smallest contribution towards LFV.

Wang et al. (2007) reported a negative association between absolute amounts of gliadin and LFV, which was not the case for this study. All the absolute amounts of protein fractions correlated significantly, although the correlations varied, indicating that FPC alone does not contribute to LFV but also protein composition.

Pearson's combined correlation showed a significant correlation between FPC and LFV. Partial correlations for LFV with monomeric and polymeric proteins were not significant in E-FS, except for a low significant correlation between LFV and proportional amounts of HMW (EXP). This indicates the interdependent associations of LFV with FPC. Partial correlations indicated the influence of quantitative variations on LFV in NW-FS. Quantitative variation for LMW (UNP) had the largest negative effect on LFV, whereas quantitative variation for α/β,γ-gliadin (EXP) had the largest positive effect on LFV in (NW-FS).

4.5 Conclusions

FPC showed a large influence on WGC, alveograph parameters and LFV and to a lesser extent on SDSS and FABS. Increased FPC had a negative impact on AlvP and AlvP/L, while the influence was positive on AlvL and AlvSTR. The highest correlation with FPC occurred

for LFV. FPC did not correlate significantly with MPT, while LUPP and UPP correlated significantly with MPT. Absolute amounts of LMW showed the highest correlation with AlvL and LFV and α/β,γ-gliadin showed the highest correlation with WGC. Protein fractions contributed to different extents to bread making parameters, indicating that FPC alone does not account for variation but also protein composition.

The significant positive correlation between the relative amounts of AG and tenacity and negative correlation with extensibility and strength justifies further research. Partial correlations revealed how the qualitative effect of polymeric and monomeric proteins impacted differently on bread making quality in NW-FS compared to E-FS, indicating the large effect of environment on protein accumulation. Partial correlations indicated that LFV was in general more affected by quantitative variations in protein fractions, at an equivalent level of protein content, than FPC in NW-FS, while FPC had a larger effect on LFV than protein composition in E-FS.

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Chapter 5

Correlations between sums and ratios of protein fractions with baking quality for selected South African dryland wheat cultivars

Abstract

Ten South African dryland winter wheat cultivars were planted at five localities over two years, divided into NW-FS and E-FS. Proportional amounts of POL correlated negatively with WGC in NW-FS and positively in E-FS. Quantitative amounts of POL/AGS and POL/GLIAG had a positive effect on FABS in E-FS, which indicate the interdependent association with FPC. UPP showed the highest correlation with MPT in both regions. Increased amounts of AGS in the protein correlated positively with AlvP in both regions, while increased amounts of POL/AGS correlated negatively with AlvP. Increased amounts of POL, GLIADIN and AGS in the flour had a significant positive influence on AlvL in both regions. Increased amounts of sums and ratios of protein fractions in the flour correlated positively with AlvSTR in both regions. Absolute amounts for AGS, in combination with absolute amounts for GLIADIN, showed the highest correlation with LFV in NW-FS, while absolute amounts in POL showed the highest correlation in E-FS. Partial correlations were conducted to eliminate the effect of quantitative variation in FPC. At an equivalent level of protein content, GLIADIN had a negative effect on SDSS in both regions. LMW/HMW ratio had the largest positive effect on FABS in NW-FS. At an equivalent level of protein content, increased amounts of POL had a positive effect on MPT while increased amounts of GLIAG had a negative effect on MPT. The positive effect of increased amounts of AGS in the flour on LFV justifies further research.

5.1 Introduction

Inter cultivar variation has been observed in the gliadin/glutenin ratio for physical dough properties and end-use quality (Khatkar et al., 2002a). MWD showed a significant increase under dryland conditions, while glutenin/gliadin ratio did not change

significantly (Panozzo et al., 2001). MWD seemed to be influenced by the locality, whereas monomeric/polymeric ratio seemed to be similarly influenced by genotype and locality (Lemelin et al., 2005).

Glutenins influenced dough stability and strength during mixing, while gliadins had a negative effect on dough development time, dough stability and resistance to extension (Chaudary et al., 2016). The glutenin/gliadin ratio is related to the balance between dough extensibility and strength. Glutenin is mainly responsible for changes in dough strength (Sapirstein & Fu, 2000). Changes in the glutenin/gliadin ratio may alter the viscoelastic properties of dough. An increased gliadin/glutenin ratio correlated positively with dough extensibility and negatively with dough strength (Johansson et al., 2002; Khatkar et al., 2002; Zhang et al., 2007; Wieser & Kieffer, 2011; Hasniza et al., 2014).

Glutenins and very low molecular weight monomeric proteins correlated positively with SDSS, dough development time, and negatively with gliadin/glutenin ratio. Gliadins correlated negatively with SDSS (Chaudary et al., 2016).

Strong dough with reduced extensibility impairs bread making quality compared to dough with an appropriate balance between extensibility and elasticity (Khatkar et al., 2002a). Increased gliadin/glutenin ratio resulted in decreased LFV at constant FPC (Uthayakumaran et al., 1999). AG, glutenin and polymeric protein/monomeric protein ratio showed significant positive correlations with LFV (Wang et al., 2007). Relative amounts of AG did not correlate significantly with dough rheological traits (Chaudary et al., 2016). A higher quantity of glutenin and a reduced gliadin/glutenin ratio had a positive effect on LFV for northern-style Chinese steamed bread (Zhang et al., 2007).

The HMW-GS/LMW-GS ratio increased with increased molecular weight of glutenin (Larroque et al., 1997). HMW-GS showed a higher association with dough strength parameters than LMW-GS, similarly a higher HMW-GS/LMW-GS ratio will result in stronger dough (Martre et al., 2006; Li Vigni et al., 2013). The quantity of HMW-GS, LMW-GS and HMW-GS/LMW-GS ratio strongly influences the aggregation and

polymerisation properties of the UPP during dough development (Wang et al., 2007). A close relationship was observed between HMW-GS/LMW-GS ratio and UPP (MacRitchie & Gupta, 1993). SE-HPLC results indicated that the HMW-GS/LMW-GS ratio in the UPP fraction correlated with dough strength (Altenbach et al., 2016).

The aim of this study was to establish the association between sums and ratios of protein classes with quality parameters.

5.2 Experimental

5.2.1 Materials

See Chapter 3.2.1.

5.2.2 Quality analysis

As described in Chapter 4 section 4.2.2.

5.2.3 Analysis of protein molecular weight distribution

As described in Chapter 3 section 3.2.3. Sums of protein fractions were calculated as described in Table 3.2.

5.2.4 Statistical analysis

As described in Chapter 3 section 3.2.4 for Pearson's correlations and Chapter 4 section 4.2.4 for partial correlations.

5.3 Results

5.3.1 Correlations between sums and ratios of protein classes with quality parameters

Only significant correlations ($p \leq 0.001$) will be mentioned in this chapter. Correlations between UPP and sums and ratios of protein fractions varied between NW-FS and E-FS. For NW-FS, UPP did not correlate significantly with sum of gliadins (GLIADIN) and sum of low molecular weight protein/sum of high molecular weight protein ratio (LMW/HMW), as opposed to E-FS where UPP did not correlate significantly ($p \leq 0.001$) with sum of albumin and globulin (AGS). In NW-FS, UPP mainly correlated with relative amounts, except for relative and absolute amounts for sum of polymeric proteins/sum of gliadins, albumin and globulin ratio (POL/GLIAG) (Tale 5.1).

In E-FS, UPP correlated significantly with absolute and relative amounts of sum of polymeric proteins (POL), LMW/HMW, sum of polymeric proteins/sum of gliadins ratio (POL/GLIADIN), sum of polymeric proteins/sum of albumin and globulin ratio (POL/AG) and sum of polymeric proteins/sum of gliadins, albumin and globulin ratio (POL/GLIAG). The highest correlation (negative) in both regions occurred between UPP and sum of gliadins, albumin and globulin (GLIAG) (Table 5.1).

WGC correlated with relative and absolute amounts of fractions in both regions except for POL/GLIADIN in E-FS. Absolute amounts for sums of protein classes and ratios showed a higher correlation with WGC, compared to relative amounts. The highest correlation, in both regions, was between WGC and GLIADIN (Table 5.1).

Correlations with SDSS were below $r = 0.45$ for sums of protein classes (POL, GLIADIN and GLIAG) and protein ratios in both regions. In NW-FS, relative and absolute amounts (POL, GLIADIN, GLIAG, POL/GLIAG) correlated significantly ($p \leq 0.001$) with SDSS. In E-FS, absolute amounts did not correlate significantly ($p \leq 0.001$) with SDSS, except for POL/GLIAG. Relative amounts for POL, POL/GLIADIN and POL/GLIAG correlated

positively with SDSS in both regions, while relative amounts for GLIADIN, GLIAG correlated negatively with SDDS in both regions (Table 5.1).

Correlations were not significant between FABS and relative amounts of protein fractions for sums of protein and ratios in both regions. The highest correlation for FABS occurred with LMW/HMW (relative amount) in NW-FS. Correlations with FABS were not high in E-FS, except for the correlation with POL/AGS (absolute amounts) and POL/GLIAG (absolute amounts) (Table 5.1).

MPT showed the highest correlation with UPP in both regions. Sums of protein classes and ratios did not correlate significantly ($p \leq 0.001$) with MPT in E-FS. Absolute amounts did not correlate significantly ($p \leq 0.001$) with MPT in NW-FS, while POL (relative amounts), GLIAG (relative amounts) and POL/GLIAG (relative amounts) correlated significantly ($p \leq 0.001$) (Table 5.1).

Table 5.1 Pearson's correlation between sums and ratios of protein classes with quality parameters

Ratios	UPP		WGC		SDSS		FABS		MPT	
	NW-FS	E-FS	NW-FS	E-FS	NW-FS	E-FS	NW-FS	E-FS	NW-FS	E-FS
UPP							-0.29***		0.71***	0.46***
POL [(a)]	0.45***	0.55***	-0.34***	0.23***	0.27***	0.40***			0.29***	
POL [(b)]		0.29***	0.81***	0.78***	-0.31***		0.26***			
GLIADIN [(a)]		-0.33***	0.58***	0.29***	-0.41***	-0.40***				
GLIADIN [(b)]			0.89***	0.82***	-0.44***					
AGS [(a)]	-0.21***		-0.52***	-0.61***	0.34***					
AGS [(b)]			0.56***	0.31***			0.32***			
GLIAG [(a)]	-0.72***	-0.71***	0.34***	-0.23***	-0.27***	-0.40***			-0.29***	
GLIAG [(b)]			0.88***	0.80***	-0.40***		0.28***			
LMW/HMW [(a)]		0.27***	-0.22***	-0.32***						
LMW/HMW [(b)]		0.32***	0.69***	0.43***	-0.34***		0.39***			
POL/GLI [(a)]	0.30***	0.47***	-0.51***		0.37***	0.44***				
POL/GLI [(b)]		0.38***	0.57***	0.62***		0.23***				
POL/AGS [(a)]	0.39***	0.35***	0.35***	0.58***						
POL/AGS [(b)]		0.28***	0.79***	0.78***	-0.37***			0.22***		
POL/GLIAG [(a)]	0.61***	0.65***	-0.35***	0.24***	0.27***	0.40***			0.31***	
POL/GLIAG [(b)]	0.32***	0.44***	0.71***	0.72***				0.22***		

[a] relative amounts, [b] absolute amounts. NW-FS = North Western Free State (*n* = 160), E-FS = Eastern Free State (*n* = 240). WGC = wet gluten content, SDSS = SDS-sedimentation, FABS = farinograph water absorption, MPT = mixograph peak time, UPP = unextractable polymeric proteins, POL = sum of polymeric proteins, GLIADIN = sum of gliadins, AGS = sum of albumin and globulin, GLIAG = sum of gliadins and albumin and globulin, LMW/HMW = sum of low molecular weight protein/sum of high molecular weight protein ratio, POL/GLI = sum of polymeric proteins/sum of gliadins ratio, POL/AGS = sum of polymeric proteins/sum of albumin and globulin ratio, POL/GLIAG = sum of polymeric proteins/sum of gliadins and albumin and globulin ratio, *** $p \leq 0.001$

5.3.2 Correlations between sums and ratios of protein classes with alveograph parameters and loaf volume

Sums and ratios of protein classes did not show significant correlations ($p \leq 0.001$) with AlvP in NW-FS, except for a positive correlation with AGS (relative amount) and a negative correlation with POL/AGS (relative amount). Significant negative correlations were observed in E-FS for AlvP with POL (absolute amounts), GLIADIN (absolute amounts), GLIAG (absolute amounts), POL/GLIADIN (absolute amounts), POL/AGS (relative and absolute amounts) and POL/GLIAG (absolute amounts). Relative amounts of AGS correlated positively with AlvP in E-FS (Table 5.2).

AlvL correlated significantly with absolute amounts for sums and ratios of protein classes in both regions. Correlations between AlvL and relative amounts were not significant ($p \leq 0.001$) in NW-FS, as opposed to E-FS. Relative and absolute amounts correlated significantly in E-FS, except for the correlation between AlvL and relative amounts for GLIADIN and POL/GLIADIN (Table 5.2).

A significant ($p \leq 0.001$) negative correlation occurred between AlvP/L with absolute amounts for sums of protein classes and ratios in both regions. Relative amounts of AGS correlated positively with AlvP/L. UPP correlated positively with AlvSTR in E-FS but not significantly ($p \leq 0.001$) in NW-FS. Absolute amounts for sums and ratios of protein classes correlated positively with AlvSTR in both regions (Table 5.2).

Absolute amounts of sums and ratios of protein classes correlated positively with LFV in both regions. Absolute amounts of POL showed the highest correlation with LFV in E-FS while absolute amounts of GLIAG correlated the highest with LFV in NW-FS. Relative amounts of AGS correlated negatively with LFV in both regions. Relative amounts of POL and POL/GLIAG correlated negatively in NW-FS with LFV, while the correlation for POL and POL/GLIAG was positive in E-FS. Relative amounts of GLIAG correlated positively with LFV in NW-FS while it correlated negatively in E-FS (Table 5.2).

Table 5.2 Pearson's correlation between sums and ratios of protein classes with alveograph parameters and loaf volume

Ratios	AlvP		AlvL		AlvP/L		AlvSTR		LFV	
	NW-FS	E-FS	NW-FS	E-FS	NW-FS	E-FS	NW-FS	E-FS	NW-FS	E-FS
UPP								0.22***		
POL [(a)]				0.28***				0.26***	-0.29***	0.38***
POL [(b)]		-0.36***	0.49***	0.69***	-0.44***	-0.58***	0.53***	0.60***	0.64***	0.77***
GLIADIN [(a)]					-0.30***				0.40***	
GLIADIN [(b)]		-0.37***	0.46***	0.66***	-0.45***	-0.59***	0.43***	0.54***	0.69***	0.67***
AGS [(a)]	0.35***	0.32***		-0.49***	0.35***	0.46***		-0.38***	-0.30***	-0.52***
AGS [(b)]			0.39***	0.29***	-0.26***	-0.23***	0.49***	0.27***	0.54***	0.31***
GLIAG [(a)]				-0.28***				-0.27***	0.29***	-0.38***
GLIAG [(b)]		-0.35***	0.48***	0.66***	-0.44***	-0.58***	0.48***	0.54***	0.71***	0.67***
LMW/HMW[(a)]				-0.26***		0.22***				
LMW/HMW[(b)]			0.44***	0.37***	-0.36***	-0.33***	0.43***	0.363***	0.60***	0.49***
POL/GLI [(a)]									-0.39***	
POL/GLI [(b)]		-0.28***	0.41***	0.58***	-0.33***	-0.47***	0.51***	0.52***	0.48***	0.67***
POL/AGS [(a)]	-0.28***	-0.30***		0.50***		-0.44***		0.42***		0.56***
POL/AGS [(b)]		-0.36***	0.41***	0.67***	-0.42***	-0.57***	0.40***	0.57***	0.57***	0.73***
POL/GLIAG [(a)]				0.28***				0.26***	-0.30***	0.38***
POL/GLIAG [(b)]		-0.33***	0.45***	0.66***	-0.41***	-0.54***	0.51***	0.57***	0.57***	0.74***

[a] relative amounts, [b] absolute amounts. NW-FS = North Western Free State (*n* = 160), E-FS = Eastern Free State (*n* = 240). AlvP = alveograph tenacity, AlvL = alveograph extensibility, AlvP/L = alveograph tenacity/extensibility, AlvSTR = alveograph strength, LFV = loaf volume, UPP = unextractable polymeric proteins, POL = sum of polymeric proteins, GLIADIN = sum of gliadins, AGS = sum of albumin and globulin, GLIAG = sum of gliadins and albumin and globulin, LMW/HMW = sum of low molecular weight protein/sum of high molecular weight protein ratio, POL/GLI = sum of polymeric proteins/sum of gliadins ratio, POL/AGS = sum of polymeric proteins/sum of albumin and globulin ratio, POL/GLIAG = sum of polymeric proteins/sum of gliadins and albumin and globulin ratio, *** $p \leq 0.001$

5.3.3 Partial correlation coefficients between sums and ratios of protein classes with quality parameters

Relative and absolute amounts of sums and ratios of protein classes correlated significantly with WGC in NW-FS, except for LMW/HMW and POL/AGS, which did not correlate significantly ($p \leq 0.001$). Absolute and relative amounts for GLIADIN, AGS and POL/AGS correlated significantly with WGC in E-FS. Correlations were slightly higher between WGC and relative amounts than between absolute amounts in both regions, for the mentioned sums and protein ratios. Absolute and relative amounts of GLIADIN correlated negatively with SDSS in both regions, while POL/GLIADIN correlated

positively in both regions. POL, GLIAG and the ratio correlated significantly in E-FS with SDSS, while the correlation was not significant ($p \leq 0.001$) in NW-FS (Table 5.3).

FABS correlated significantly with relative and absolute amounts of LMW/HMW ratio in NW-FS, whereas absolute amounts of GLIAG and POL/GLIAG ratio correlated with FABS in E-FS. Absolute and relative amounts for POL and POL/GLIAG ratio correlated positively with MPT in NW-FS, while GLIAG correlated negatively. Sums and ratios of protein classes did not correlate significantly ($p \leq 0.001$) with MPT in E-FS.

Table 5.3 Partial correlation coefficients between sums and ratios of protein classes with quality parameters

Ratios	WGC		SDSS		FABS		MPT	
	NW-FS	E-FS	NW-FS	E-FS	NW-FS	E-FS	NW-FS	E-FS
POL [(a)]	-0.30***			0.43***			0.31***	
POL [(b)]	-0.27***			0.42***			0.27***	
GLIADIN [(a)]	0.43***	0.26***	-0.29***	-0.40***				
GLIADIN [(b)]	0.40***	0.24***	-0.30***	-0.39***				
AGS [(a)]	-0.32***	-0.34***						
AGS [(b)]	-0.29***	-0.31***						
GLIAG [(a)]	0.38***			-0.44***			-0.43***	
GLIAG [(b)]	0.37***			-0.43***		-0.24***	-0.43***	
LMW/HMW [(a)]		-0.22***			0.32***			
LMW/HMW [(b)]					0.30***			
POL/GLI [(a)]	-0.42***		0.27***	0.44***			0.27***	
POL/GLI [(b)]	-0.38***		0.28***	0.43***				
POL/AGS [(a)]		0.25***						
POL/AGS [(b)]		0.22***						
POL/GLIAG [(a)]	-0.36***			0.44***			0.38***	
POL/GLIAG [(b)]	-0.34***			0.43***		0.22***	0.37***	

Partial variable, protein content; [a] relative amounts, [b] absolute amounts. NW-FS = North Western Free State (*n* = 160), E-FS = Eastern Free State (*n* = 240). WGC = wet gluten content, SDSS = SDS-sedimentation, FABS = farinograph water absorption, MPT = mixograph peak time, POL = sum of polymeric proteins, GLIADIN = sum of gliadins, AGS = sum of albumin and globulin, GLIAG = sum of gliadins and albumin and globulin, LMW/HMW = sum of low molecular weight protein/sum of high molecular weight protein ratio, POL/GLI = sum of polymeric proteins/sum of gliadins ratio, POL/AGS = sum of polymeric proteins/sum of albumin and globulin ratio, POL/GLIAG = sum of polymeric proteins/sum of gliadins and albumin and globulin ratio, *** $p \leq 0.001$

5.3.4 Partial correlation coefficients between sums and ratios of protein classes with alveograph parameters and loaf volume

Relative and absolute amounts of AGS, and relative amounts for POL/AGS correlated significantly with AlvP in NW-FS, while sums and ratios of protein classes did not correlate significantly ($p \leq 0.001$) in E-FS. Partial correlations between sums and ratios of protein classes with AlvL and AlvP/L were not significant ($p \leq 0.001$) in both regions. Absolute amounts of GLIAG correlated significantly with AlvSTR in E-FS, while sums and ratios of protein classes did not correlate significantly ($p \leq 0.001$) in NW-FS. Sums and ratios of protein classes did not correlate significantly ($p \leq 0.001$) with LFV in NW-FS, while relative and absolute amounts of POL, GLIAG, POL/AGS and POL/GLIAG correlated significantly with LFV in E-FS (Table 5.4).

Table 5.4 Partial correlation coefficients between sums and ratios of protein classes with alveograph parameters and loaf volume

Ratios	AlvP		AlvL		AlvP/L		AlvSTR		LFV	
	NW-FS	E-FS	NW-FS	E-FS	NW-FS	E-FS	NW-FS	E-FS	NW-FS	E-FS
POL [a]										0.24***
POL [b]										0.26***
GLIADIN [a]										
GLIADIN [b]										
AGS [a]	0.33***									
AGS [b]	0.28***									
GLIAG [a]										-0.24***
GLIAG [b]								-0.22***		-0.25***
LMW/HMW [a]										
LMW/HMW [b]										
POL/GLI [a]										
POL/GLI [b]										
POL/AGS [a]	-0.26***									0.25***
POL/AGS [b]										0.25***
POL/GLIAG [a]										0.24***
POL/GLIAG [b]										0.25***

Partial variable, protein content; [a] relative amounts, [b] absolute amounts. NW-FS = North Western Free State (*n* = 160), E-FS = Eastern Free State (*n* = 240). AlvP = alveograph tenacity, AlvL = alveograph extensibility, AlvP/L = alveograph tenacity/extensibility, AlvSTR = alveograph strength, LFV = loaf volume, POL = sum of polymeric proteins, GLIADIN = sum of gliadins, AGS = sum of albumin and globulin, GLIAG = sum of gliadins and albumin and globulin, LMW/HMW = sum of low molecular weight protein/sum of high molecular weight protein ratio, POL/GLI = sum of polymeric proteins/sum of gliadins ratio, POL/AGS = sum of polymeric proteins/albumin and globulin ratio, POL/GLIAG = sum of polymeric proteins/sum of gliadins and albumin and globulin ratio, *** $p \leq 0.001$

5.4 Discussion

5.4.1 Unextractable polymeric proteins

Increased amounts of POL had a positive effect on UPP while GLIADIN and AGS had a negative influence. The quantity of HMW-GS, LMW-GS and HMW/LMW-GS strongly associates with UPP (Wang et al., 2007). Increased amounts of GLIAG had the largest effect on the amount of UPP.

5.4.2 Wet gluten content

Absolute amounts for sums and ratios of protein classes correlated positively with WGC. This indicates that quantitative increases in sums and ratios of protein classes had a positive influence on WGC, simultaneously with increased FPC. Quantitative variation in GLIADIN had the largest positive influence on WGC in both regions. Partial correlations were conducted to remove quantitative variation in FPC, to establish the qualitative effect of sums and ratios of protein classes (Ohm et al., 2010). Partial correlations confirm the positive effect of proportional and quantitative increase in GLIADIN on WGC, in both regions. At an equivalent level of protein content, proportional and quantitative increase in POL and POL/GLIAG ratio had a detrimental effect on WGC in NW-FS, while the effect was not significant ($p \leq 0.001$) in E-FS. Pearson's correlation confirmed the negative effect of proportional amounts of POL on WGC in NW-FS. In E-FS, proportional amounts of POL had a positive effect on WGC. It should be noted that genotype made the largest contribution to variation in NW-FS for WGC, while locality made the largest contribution to variation in E-FS. Partial correlations indicate that proportional and quantitative increase in AGS had a negative influence on WGC in both regions.

5.4.3 SDS-sedimentation

Pearson's correlation and partial correlations varied between the two regions for SDSS. Relative amounts for POL, GLIADIN, GLIAG, POL/GLIADIN and POL/GLIAG showed higher

correlations in E-FS (*n* = 240) with SDSS than in NW-FS (*n* = 160). Absolute amounts did not correlate significantly ($p \leq 0.001$) with SDSS in E-FS, except for POL/GLIADIN. Absolute amounts for POL, GLIADIN and GLIAG showed a higher correlation with SDSS than relative amounts in NW-FS. At an equivalent level of protein content, GLIADIN had a detrimental effect on SDSS, while POL/GLIADIN had a positive effect in both regions.

Partial correlations indicated that POL, GLIAG and the ratio did not correlate significantly ($p \leq 0.001$) in NW-FS, although Pearson's correlation was significant in NW-FS. This indicated the interdependent association with FPC for SDSS. This association with FPC did not occur prominently in E-FS, where POL, GLIADIN, GLIAG and the ratios showed stronger associations with SDSS at an equivalent level of protein content.

GLIADIN and GLIAG correlated negatively with SDSS in both regions, with a higher correlation between absolute amounts for GLIADIN and GLIAG than relative amounts in NW-FS. The negative correlation between GLIADIN and SDSS is consistent with the negative correlation between FPC and SDSS (Table 4.4) and the positive correlation between FPC and gliadin fractions (Table 3.8). Polymeric proteins had a positive effect on SDSS. These findings are in agreement with Chaudary et al. (2016) who reported the positive correlation between glutenins proteins with SDSS, while gliadins correlated negatively.

POL had a positive effect on SDSS, while GLIADIN had a negative effect. In theory, the sediment in SDSS results from swelling of glutenin (Eckert et al., 1993; Sapirstein & Suchy, 1999) and the interaction of starch and gluten (Carver & Rauburn, 1995). Furthermore, investigation of glutenin and gliadin showed that only glutenin is capable of swelling while gliadin completely dissolves in the dilute lactic acid solution (Eckert et al., 1993).

5.4.4 Farinograph water absorption

Correlations with sums and ratios of protein classes were inconsistent across the two regions and could be explained by a larger genotype influence in NW-FS, and the large

contribution (52.16%) that year made to variation in E-FS (Table 4.2). Increased amounts of UPP had a negative effect on FABS in NW-FS, while quantitative amounts of POL, AGS, GLIAG and LMW/HMW had a positive influence on FABS. Quantitative amounts of POL/AG and POL/GLIAG had a positive effect on FABS in E-FS, which indicate the interdependent association with FPC. Pearson's correlation and partial correlation indicate that LMW/HMW ratio had the largest positive effect on FABS in NW-FS.

5.4.5 Mixograph peak time

Sums and ratios for protein classes did not correlate significantly ($p \leq 0.001$) with MPT in E-FS. UPP showed the highest correlation with MPT in both regions. In NW-FS, partial correlations indicated that increased proportional and quantitative amounts of POL had a positive influence on MPT, while proportional and quantitative amounts of GLIAG had a negative effect on MPT. Glutenins showed a positive effect on dough development time while gliadins showed a negative effect (Chaudary et al., 2016).

5.4.6 Alveograph parameters

5.4.6.1 Alveograph tenacity

The absolute amounts of sums and ratios for protein classes showed a significant negative correlation with AlvP in E-FS for sums of protein classes and ratios, whereas correlations were not significant in NW-FS for absolute amounts. This indicated the negative effect of increased FPC on AlvP for these sums (POL, GLIADIN and GLIAG) and ratios (POL/GLIADIN, POL/AGS and POL/GLIAG) of protein classes in E-FS. Pearson's correlation showed that relative amounts of AGS had a positive effect on AlvP in both regions, especially in NW-FS where partial correlations confirmed the positive effect. The POL/AGS ratio had a negative effect on AlvP in both regions. Relative amounts for POL and GLIADIN did not correlate significantly ($p \leq 0.001$) with AlvP in both regions, while researchers reported the significant positive effect of glutenins on dough strength parameters and the negative effect of gliadins (Zhang et al., 2007; Wieser & Kieffer, 2011; Hasniza et al. 2014).

5.4.6.2 Alveograph extensibility

Pearson's correlation indicated that absolute amounts for sums and ratios of protein classes correlated significantly with AlvL. Partial correlations, where quantitative variation in protein content was removed, did not correlate significantly ($p \leq 0.001$) with AlvL in both regions, which confirms the strong association between FPC and AlvL. Combined Pearson's correlation also confirmed the high correlation between FPC and AlvL (Table 4.5). Absolute amounts of POL had the largest positive influence on AlvL in both regions. Proportional amounts of AGS had a larger and negative effect, than absolute amounts of AGS on AlvL in E-FS. FPC had a larger influence on AlvL in E-FS, where absolute amounts for polymeric (POL) and monomeric proteins (GLIADIN) showed a higher correlation with AlvL than in NW-FS.

Increased absolute amounts of sums and ratios for POL, GLIADIN and AGS had a significant positive influence on AlvL in both regions. Relative amounts of AGS, GLIAG and LMW/HMW had a negative effect on AlvL in E-FS, and increased relative amounts of POL had a positive effect in E-FS. The positive influence of POL and negative influence of GLIADIN is in contrast with the findings of Wieser & Kieffer (2011). It should be noted that the alveograph was used in this study to determine dough extensibility, while Wieser & Kieffer (2011) used the extensigraph. The Chopin alveograph measures traits similar to the extensigraph but the procedure differs somehow. The alveograph applies biaxial extension instead of uniaxial extension for the extensigraph. Furthermore, water content is constant and the dough mixing is fixed for the alveograph (Cuniberti et al., 2003).

5.4.6.3 Alveograph tenacity/extensibility

Absolute amounts for sums and ratios of protein classes correlated negatively with AlvP/L in both regions. This is also evident from the significant negative correlation between AlvP/L and FPC (Table 4.5). Higher correlations for absolute amounts in E-FS indicated the larger influence of FPC on AlvP/L than in NW-FS. Partial correlations were not significant ($p \leq 0.001$) with AlvP/L in both regions, which confirms the

interdependent association with FPC. Proportional increases in AGS had a positive effect on AlvP/L in both regions.

5.4.6.4 Alveograph strength

Absolute amounts for polymeric and monomeric sums of proteins and ratios showed a significant positive correlation with AlvSTR in both regions. The higher correlations in E-FS indicated a stronger association between AlvSTR and FPC than in NW-FS. Partial correlations did not correlate significantly ($p \leq 0.001$) with AlvSTR in both regions, except for a negative correlation with GLIAG (absolute amounts) in E-FS. Combined Pearson's correlation also confirmed the significant association between FPC and AlvSTR (Table 4.5). Quantitative increases in POL had the largest positive influence on AlvSTR in both regions.

5.4.7 Loaf volume

The amount of protein in the flour has a positive influence on LFV (Table 4.5). This was also demonstrated by the significant positive correlations between absolute amounts of sums and ratios of protein classes, although the correlations differed between the two regions in this study. Absolute amounts for AGS in combination with absolute amounts for GLIADIN showed the highest correlation with LFV in NW-FS, while absolute amounts in POL showed the highest correlation in E-FS. Increased relative amounts of AGS had a negative effect on LFV in both regions.

Protein composition affected LFV differently across the two regions. Considering relative amounts, increased amounts of POL in the protein (proportional variation) correlated negatively with LFV in NW-FS, while it correlated positively in E-FS. Increased amounts of GLIADIN in the protein had a positive effect on LFV in NW-FS, while the effect will not be significant in the E-FS. Increased amounts of AGS in the protein had a negative effect on LFV in both regions, while increased amounts of GLIAG (GLIADIN + AGS) in the protein had a positive effect on LFV in NW-FS and a negative effect in E-FS. Partial correlations also indicated that the amount of GLIADIN in the protein (relative

amount) and the amount in the flour (absolute amount), at an equivalent level of protein content, had a significant negative effect on LFV in E-FS, whereas POL had a positive effect. The effect was not significant in NW-FS.

5.5 Conclusions

Associations between sums and ratios of protein classes with quality parameters varied between NW-FS and E-FS. Proportional amounts of POL correlated negatively with WGC in NW-FS and positively in E-FS. At an equivalent level of protein content, proportional and quantitative increase in AGS had a negative influence on WGC in both regions. At an equivalent level of protein content, GLIADIN had a negative effect on SDSS in both regions. SDSS correlated positively with POL and negatively with GLIAG in NW-FS, due to interdependent associations with FPC, while these correlations were not affected by the association with FPC in E-FS.

Correlations between sums and ratios of protein fractions were inconsistent for FABS across the two regions. Quantitative amounts of POL/AGS and POL/GLIAG had a positive effect on FABS in E-FS, which indicate the interdependent association with FPC. Pearson's correlation and partial correlation indicate that LMW/HMW ratio had the largest positive effect on FABS in NW-FS. UPP showed the highest correlation with MPT in both regions. At an equivalent level of protein content, increased amounts of POL had a positive effect on MPT while increased amounts of GLIAG had a negative effect on MPT.

Increased amounts of POL, GLIADIN and GLIAG in the flour correlated negatively with AlvP in E-FS. Increased amounts of AGS in the protein correlated positively with AlvP in both regions, while increased amounts of POL/AGS correlated negatively. Increased amounts of POL, GLIADIN and AGS in the flour had a significant positive influence on AlvL in both regions. Increased amounts of sums and ratios of protein fractions in the flour correlated positively with AlvSTR in both regions. Increased amounts of AGS and GLIAG in the protein had a negative effect on AlvSTR.

Absolute amounts for AGS, in combination with absolute amounts for GLIADIN, showed the highest correlation with LFV in NW-FS, while absolute amounts in POL showed the highest correlation in E-FS. Increased amounts of AGS in the protein had a negative effect on LFV in both regions. Genotypes, with a high correlation between AGS and FPC, had a positive effect on LFV. The positive effect of increased amounts of AGS in the flour on LFV justifies further research.

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Chapter 6

Protein content versus protein composition

Abstract

The effect of protein content and protein composition was determined for ten selected South African dryland wheat cultivars: BettaDN, Caledon, Elands, Gariep, Komati, Limpopo, Matlabas, PAN3118, PAN3349 and PAN3377. Trials were conducted over two years at five localities, divided into two regions, NW-FS and E-FS. Locality made the largest contribution to variation in FPC in both regions. The largest percentage increases between the two regions for FPC were for BettaDN, Caledon, Elands, Gariep, Komati and Limpo. Pearson's correlation showed that FPC correlated between significantly and not significantly with AlvP, AlvL, MPT for individual cultivars. The stepwise multiple linear regression indicated that FPC could only explain variation in AlvL and LFV for two cultivars in E-FS. The regression model indicated that protein fractions contributed in different degrees to variation for AlvP, AlvL, MPT and LFV for individual cultivars and between the two regions. The model explained a larger percentage of variation in AlvL for BettaDN, Caledon, Elands, Gariep, Komati and Limpopo in E-FS than in NW-FS, while the model explained a larger proportion of variation in MPT for Matlabas and the Pannar cultivars in NW-FS than in E-FS. AG fractions contributed more to variation for AlvP, AlvL and MPT in NW-FS than in E-FS. The effect of AG justifies further research.

6.1 Introduction

Wheat quality is a complex trait and is influenced by several components, of which the expression is affected by the genotype's reaction to specific environments (Mann et al., 2009; Castillo et al., 2012). Stability of flour quality and composition is a desirable trait, notwithstanding environmental variation (DuPont et al., 2007). Grain protein content and composition are crucial for bread making quality (Gao et al., 2012).

Grain protein content is an important trait for determining the price of grain (Cavanagh et al., 2014), although FPC is not the only trait affecting processing quality of wheat flour (Chope et al., 2014). Environmental factors change protein content and composition and showed a larger influence on dough rheology and baking quality than changes in protein content alone (Fowler, 2003; Mann et al., 2009; Caffe-Treml et al., 2011). Protein content and composition in wheat flour are the main factors influencing the bread making quality and are affected by genetics, environment and crop management (Fowler, 2003). Total protein content alone could not account for the variation observed in end-use quality in a study conducted on hard red winter wheat. The magnitude of environmental influences on end-use quality should be considered when developing wheat cultivars (Peterson et al., 1992).

Genotypes with high protein content did not necessarily render better rheological quality. FPC is positively correlated with dough extensibility and negatively correlated with dough elasticity. Genotypes should be selected according to performance in specific production areas, rather than aiming to breed for widely adapted cultivars across different environments (Vázquez et al., 2012).

Improving protein content is difficult because environment accounts for two thirds of the variation in the trait (Shewry, 2009). The effect of the environment on protein composition varied between genotypes (Yahata et al., 2005). The accumulation of protein fractions does not take place simultaniously, assuming that protein composition changes throughout grain filling. As a result, conditions that shorten the grain filling period, such as drought or high temperatures, will have an effect on the ratio of protein fractions (Jamieson et al., 2001).

Bread making quality traits and LFV are not only influenced by FPC but also by protein composition (Johansson et al., 2003). The viscoelastic properties of gluten can be affected by the genotype and the environment (Moldestad et al., 2014). Johansson et al. (2005) suggested that environmental factors may influence the accumulation of gliadin and glutenin subunits during grain development, along with polymerisation and

build-up of large unextractable polymeric proteins. Variations in flour quality are attributed to genetic differences in glutenin and gliadin composition and the influence of the environment on their relative amounts (Altenbach et al., 2012).

Stability of LFV is associated with stability of the polymeric fraction and not stability of FPC (Lemelin et al., 2005). Groos et al. (2007) analysed 194 RIL's across environments. Baking score and indirect quality tests were poorly correlated. Bread making scores were poorly correlated with FPC and alveograph parameters. LFV correlated positively with AlvL, AlvSTR and FPC. Multiple regression analyses can be applied to determine the contribution of predictor variables to explain variation in observed quality parameters. FPC, AlvP and AlvSTR made the largest contribution to variation in LFV, while quality analyses could only explain a small proportion of the variation observed in bread making scores across environments. Approximately 33% of the variation in LFV could be explained by quality parameters (Groos et al., 2007).

Associations between environmental factors and gluten quality are complex, therefore genotypes need to be evaluated for stability of gluten quality over diverse environments. Contrasting findings may be a reflection of the complex relationships between growth temperature and gluten quality (Uhlen et al., 2015).

Wheat quality is highly affected by environmental conditions, although the mechanism of this is poorly understood (Triboï et al., 2000). General conclusions cannot be deducted from average values, where significant differences occurred for quality traits (Li et al., 2013). FPC alone cannot be used to predict bread making quality (Rousset et al., 2001). It is vital to understand how flour components will react to environmental conditions in order to develop varieties with improved environmental stability (DuPont et al., 2007). The aim of this study was:

- To establish whether FPC or protein fractions explain the larger proportion of variation for AlvP, AlvL, MPT and LFV.
- To establish if the expression of genotypes for FPC and protein fractions varied between regions to explain variation in baking.

6.2 Experimental

6.2.1 Materials

As described in Chapter 3 section 3.2.1.

6.2.2 Protein and quality analysis

See section 3.2.2 for protein measurements, 3.2.2 for determination of molecular weight distribution and 4.4.2 for quality analysis.

6.2.3 Statistical analysis

This was described in section 3.2.4.

Multiple linear regression (MLR) mathematically calculates the relationship between a dependent variable and one or several independent variables, especially for stepwise MLR, which entails a MLR with a variable selection scheme. The procedure begins by selecting the variable that shows the highest correlation with Y. A regression coefficient is then obtained for a selected variable Xi. The F-test is used to determine if the coefficient is significant before the variable, Xi, is retained and an additional variable is selected according to its partial correlation coefficient. The inclusion of new variables in the model may reduce the contribution of a variable that was previously included. Consequently, the significance of regression terms, that were already included in the model, is tested after each new inclusion. Non-significant terms will then be removed from the model. The process of selection and elimination is continued until the addition of new variables does not improve the model, and all the variables remaining in the model are significant (Zahn et al., 2013). Stepwise multiple regression was performed using the PROC REG of SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA).

Stepwise MLR was applied according to the procedure described by Draper & Smith (1966) to determine the variables accounting for the majority of total variability of the

dependent variable. The stepwise procedure computed a sequence of MLR in a stepwise manner. One variable was added to the regression equation at each step. The stepwise procedure introduced variables in the model only if they contributed to a significant improvement of the coefficient of determination (R^2) at $p \leq 0.05$ in the estimation of the dependent variable. The stepwise multiple regression was performed using PROC REG of SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA).

6.3 Results

6.3.1 Percentage increases for flour protein content and selected baking quality parameters

Caledon ranked amongst the highest of the cultivars for FPC, AlvL and LFV, and the lowest for AlvP and MPT in NW-FS. The ranking of Caledon for FPC, AlvP, AlvL, MPT and LFV did not change significantly in E-FS from the ranking in NW-FS.

Average values for individual cultivars for FPC, AlvL and LFV were higher in NW-FS than in E-FS, except in the case of Gariep and PAN3349 for AlvL, where the average values were higher in E-FS. Individual average values were higher in E-FS for AlvP and MPT than in NW-FS, except for PAN3349 for MPT (Table 6.1).

FPC increased between 8.72 – 12.53% in NW-FS compared to E-FS for BettaDN, Caledon, Elands, Gariep, Komati and Limpopo, while FPC increased between 1.66 – 3.95% for Matlabas, PAN3118, PAN3349 and PAN3377 (Table 6.1). The largest increases in AlvP and MPT occurred for PAN3118 (26.09% and 19.37%, respectively) (Table 6.1).

6.3.2 Pearson's correlation

Correlations of FPC with AlvP, AlvL, MPT and LFV varied between significant ($p \leq 0.001$) and not significant for individual cultivars in NW-FS and E-FS (Table 6.2). BettaDN, Elands, Gariep and Komati showed significant ($p \leq 0.001$) positive correlations between

FPC and AlvP in NW-FS, while the same cultivars showed a negative correlation in E-FS. Matlabas and PAN3118 also showed negative correlation between FPC and AlvP in NW-FS. BettaDN, Caledon, Elands, Gariep, Komati and Limpopo did not show significant correlations for FPC with AlvL in NW-FS. Cultivars correlated significantly for FPC and AlvP in E-FS, except for PAN3377 (Table 6.2).

Correlations between FPC and MPT were mainly not significant and the highest correlation was for PAN3377 in NW-FS. FPC showed significant positive correlations with LFV in both regions, except for Caledon and Elands in NW-FS, where the correlations were not significant (Table 6.2).

Table 6.1 Percentage increases between NW-FS and E-FS for flour protein content and selected baking quality parameters

Cultivar	**FPC** (%)			**AlvP** (mm H_2O)			**AlvL** (mm)			**MPT** (min)			**LFV** (cm^3)		
	NW-FS	**E-FS**	**% Increase**	**E-FS**	**NW-FS**	**% Increase**	**NW-FS**	**E-FS**	**% Increase**	**E-FS**	**NW-FS**	**% Increase**	**NW-FS**	**E-FS**	**% Increase**
BettaDN	12.70 ab	11.39 abc	11.50	86 ef	76 cd	13.16	127 b	111 bc	14.41	3.24 d	3.15 cd	2.86	970 ab	888 bc	9.23
Caledon	12.93 a	11.51 ab	12.34	76 g	66 e	15.15	155 a	134 a	15.67	2.52 e	2.40 e	5.00	990 a	921 a	7.49
Elands	12.20 c	10.88 d	12.13	105 b	90 ab	16.67	94 d	87 e	8.05	3.92 b	3.71 a	5.66	917 d	828 e	10.75
Gariep	12.34 bc	11.35 bc	8.72	83 f	78 c	6.41	99 cd	108 cd	9.09*	3.81 b	3.76 a	1.33	951 bc	900 b	5.67
Komati	12.66 abc	11.25 bc	12.53	81 f	71 de	14.08	123 b	103 d	19.42	3.39 c	3.15 cd	7.62	962 bc	849 d	13.31
Limpopo	12.85 a	11.70 a	9.83	89 de	77 cd	15.58	124 b	107 cd	15.89	3.4 c	3.19 c	6.58	959 bc	888 bc	8.00
Matlabas	11.59 d	11.15 cd	3.95	103 b	87 b	18.39	106 c	92 e	15.22	3.9 b	3.62 ab	7.73	945 c	887 bc	6.54
PAN3118	11.62 d	11.43 abc	1.66	116 a	92 a	26.09	99 c	87 e	13.79	4.19 a	3.51 b	19.37	921 d	877 c	5.02
PAN3349	11.64 d	11.30 bc	3.01	94 c	90 ab	4.44	97 cd	102 d	5.15*	3.21 d	3.23 c	0.62*	863 e	856 d	0.82
PAN3377	11.68 d	11.43 abc	2.19	92 cd	78 c	17.95	129 b	116 b	11.21	3.34 cd	3 d	11.33	960 bc	923 a	4.01
LSD	0.49	0.33		4.78	5.80		11.13	6.83		0.15	0.16		21.01	17.23	

NW-FS = North Western Free State, E-FS = Eastern Free State, FPC = flour protein content, AlvP = alveograph tenacity, AlvL = alveograph extensibility, MPT = mixograph peak time, LFV = loaf volume, * = % increase is in opposite direction

Table 6.2 Pearson's correlation for flour protein content and selected baking parameters

	BettaDN		Caledon		Elands		Gariep		Komati	
	NW-FS	**E-FS**	**NW-FS**	**E-FS**	**NW-FS**	**E-FS**	**NW-FS**	**E-FS**	**NW-FS**	**E-FS**
AlvP	0.57*	-0.47*		-0.65***	0.50*	-0.42*	0.64**	-0.49*	0.51*	-0.55**
AlvL		0.87***		0.77***		0.81***		0.78***		0.71***
MPT			0.55*	0.51*						
LFV	0.62**	0.75***		0.70***		0.794***	0.764***	0.74***	0.55*	0.80***
	Limpopo		**Matlabas**		**PAN3118**		**PAN3349**		**PAN3377**	
	NW-FS	**E-FS**	**NW-FS**	**E-FS**	**NW-FS**	**E-FS**	**NW-FS**	**E-FS**	**NW-FS**	**E-FS**
AlvP	0.67**		-0.58*		-0.68**			-0.55**		
AlvL		0.65***	0.88***	0.80***	0.65**	0.63***	0.79***	0.88***	0.65**	
MPT				-0.49*			-0.54*		0.73**	
LFV	0.56*	0.43*	0.78***	0.77***	0.85***	0.83***	0.77***	0.89***	0.91***	0.72***

NW-FS = North Western Free State, E-FS = Eastern Free State, AlvP = alveograph tenacity, AlvL = alveograph extensibility, MPT = mixograph peak time, LFV = loaf volume, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$

6.3.3 Stepwise multiple linear regression

6.3.3.1 Alveograph tenacity

Data presented in Table 6.3 show regression coefficients and the probability of the estimated variables predicting AlvP. The prediction equation was calculated according to Leilah & Al-Khateeb (2005). For example: AlvP ($\hat{Y}$) for BettaDN in E-FS is formulated using protein fraction variables as follows:

$\hat{Y}$ = 157.80 – 90.70 (LMW UNP, absolute amounts) + 27.35 (α/β,γ-gliadin EXP, relative amounts).

The model explained 53.09% of the total variation in AlvP for BettaDN. The F-value indicated that LMW UNP (absolute amounts) and α/β,γ-gliadin EXP (relative amounts) contributed significantly towards AlvP for BettaDN in E-FS. No variable correlated significantly with AlvP for Limpopo in E-FS and PAN3377 in NW-FS, while 89.4% of the total variation could be explained for PAN3118 in NW-FS. AG was the main contributor to variations in AlvP for BettaDN, Caledon, Elands and Komati in NW-FS, while LMW was the main contributor to variation for the same cultivars in E-FS for AlvP.

Relative and absolute amounts of AG correlated positively with AlvP and appeared more frequently in the model in NW-FS than in E-FS. Relative amounts for AG (UNP) explained a larger percentage of variation for AlvP than absolute amounts AG (UNP), and AG (EXP) appeared only in E-FS for PAN3118. Absolute and relative amounts of LMW (UNP and EXP) correlated significantly with AlvP and explained variation for AlvP, with the highest percentage for Caledon (65.79%) in E-FS. Relative amounts of gliadins (EXP) correlated positively with AlvP and explained most of the variation in AlvP that occurred for PAN3349, PAN3377 in E-FS, while α/β,γ-gliadin EXP correlated negatively with AlvP in NW-FS and explained most of variation in Limpopo (NW-FS).

Table 6.3 Multiple linear regression analysis for alveograph tenacity

Variable	Par Estimate	Partial R^2	Model R^2	F-Value	Correlation [(a)]
BettaDN	**NW-FS**	**AlvP**			
Intercept	10.55^{NS}				
AG UNP abs	317.32**	0.43	0.43	10.59**	0.65**
BettaDn	**E-FS**	**AlvP**			
Intercept	157.80***				
LMW UNP abs	-90.70***	0.40	0.40	15.09***	-0.63**
α/β,γ-gliadin EXP abs	27.35*	0.12	0.53	5.55*	-0.41*
Caledon	**NW-FS**	**AlvP**			
Intercept	71.70***				
AG UNP abs	272.09***	0.14	0.14	2.47^{NS}	0.38^{NS}
ω-gliadin UNP abs	-183.41***	0.51	0.66	20.17***	-0.06^{NS}
Caledon	**E-FS**	**AlvP**			
Intercept	372.29***				
LMW UNP rel	-11.05***	0.65	0.65	42.30***	-0.81***
α/β,γ-gliadin EXP abs	-4.62*	0.07	0.73	5.64*	0.26^{NS}
Elands	**NW-FS**	**AlvP**			
Intercept	8.90^{NS}				
AG UNP rel	45.56***	0.57	0.57	18.98***	0.75***
Elands	**E-FS**	**AlvP**			
Intercept	-246.15**				
LMW EXP rel	23.52***	0.56	0.56	28.83***	0.75***
Gariep	**NW-FS**	**AlvP**			
Intercept	79.91***				
α/β,γ-gliadin EXP abs	16.33***	0.44	0.44	11.35**	0.66**
AG UNP rel	-35.00**	0.23	0.67	9.30**	0.13^{NS}
Gariep	**E-FS**	**AlvP**			
Intercept	114.37***				
ω-gliadin EXP rel	8.53***	0.34	0.34	11.56**	0.58**
HMW EXP rel	-12.31**	0.22	0.57	11.03**	-0.31^{NS}

NW-FS = North Western Free State, E-FS = Eastern Free State, AlvP = alveograph tenacity, EXP = SDS-extractable proteins, UNP = SDS-unextractable proteins, rel = relative amounts, abs = absolute amounts, HMW = high molecular weight glutenin polymers, LMW = low molecular weight glutenin polymers, AG = albumin and globulin, [a] Pearson's correlation for alveograph tenacity and variables, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, NS = not significant

Table 6.3 Multiple linear regression analysis for alveograph tenacity (Continued)

Variable	Par Estimate	Partial R^2	Model R^2	F-Value	Correlation [(a)]
Komati	**NW-FS**	**AlvP**			
Intercept	-9.70^{NS}				
AG UNP rel	35.17***	0.76	0.76	44.52***	0.87***
LMW EXP abs	11.02*	0.06	0.83	5.36*	0.41^{NS}
Komati	**E-FS**	**AlvP**			
Intercept	117.28***				
LMW UNP abs	-51.40***	0.44	0.44	17.80***	-0.66**
AG UNP abs	273.49**	0.21	0.65	13.03**	0.21^{NS}
Limpopo	**NW-FS**	**AlvP**			
Intercept	187.15***				
α/β,γ-gliadin EXP rel	-4.87***	0.53	0.53	16.10**	-0.73**
LMW EXP abs	16.61**	0.25	0.79	16.13**	0.61*
Limpopo	**E-FS**	No variable correlated significantly at p ≤ 0.05			
Matlabas	**NW-FS**	**AlvP**			
Intercept	332.98***				
LMW UNP rel	-14.41**	0.52	0.52	15.19**	-0.72**
Matlabas	**E-FS**	**AlvP**			
Intercept	-55.89^{NS}				
ω-gliadin EXP rel	57.15***	0.21	0.21	12.63***	0.46*
LMW EXP abs	-277.66***	0.15	0.37	11.16**	-0.23^{NS}
FPC	36.42***	0.04	0.41	3.37^{NS}	-0.25^{NS}
LUPP	-3.77**	0.28	0.69	39.86***	-0.24^{NS}
AG EXP rel	5.03*	0.08	0.77	15.54***	0.27^{NS}

NW-FS = North Western Free State, E-FS = Eastern Free State, AlvP = alveograph tenacity, EXP = SDS-extractable proteins, UNP = SDS-unextractable proteins, rel = relative amounts, abs = absolute amounts, FPC = flour protein content, LUPP = large unextractable polymeric proteins, LMW = low molecular weight glutenin polymers, AG = albumin and globulin, [a] Pearson's correlation for alveograph tenacity and variables, *** p ≤ 0.001, ** p ≤ 0.01, * p ≤ 0.05, NS = not significant

Table 6.3 Multiple linear regression analysis for alveograph tenacity (Continued)

Variable	Par Estimate	Partial R^2	Model R^2	F-Value	Correlation [(a)]
Pan3118	**NW-FS**	**AlvP**			
Intercept	95.66***				
HMW UNP abs	-166.29***	0.67	0.67	29.50***	-0.82***
LMW UNP abs	57.36***	0.21	0.89	26.49***	-0.64**
PAN3118	**E-FS**	**AlvP**			
Intercept	-255.78**				
AG EXP rel	22.36***	0.34	0.34	11.50**	0.58**
HMW UNP abs	139.79**	0.21	0.56	10.40**	-0.23^{NS}
PAN3349	**NW-FS**	**AlvP**			
Intercept	-9.53^{NS}				
LUPP	1.98**	0.42	0.42	10.28**	0.65**
PAN3349	**E-FS**	**AlvP**			
Intercept	-365.50**				
ω-gliadin EXP rel	58.84***	0.46	0.46	19.24***	0.68***
ω-gliadin UNP rel	26.55*	0.09	0.55	4.36*	-0.17^{NS}
PAN3377	**NW-FS**	No variable correlated significantly at $p \leq 0.05$			
PAN3377	**E-FS**	**AlvP**			
Intercept	-138.53^{NS}				
α/β,γ-gliadin EXP rel	11.18***	0.47	0.47	20.11***	0.69***
ω-gliadin UNP rel	-20.07*	0.09	0.56	4.47*	-0.35*

NW-FS = North Western Free State, E-FS = Eastern Free State, AlvP = alveograph tenacity, EXP = SDS-extractable proteins, UNP = SDS-unextractable proteins, rel = relative amounts, abs = absolute amounts, LUPP = large unextractable polymeric proteins, HMW = high molecular weight glutenin polymers, LMW = low molecular weight glutenin polymers, AG = albumin and globulin, [a] Pearson's correlation for alveograph tenacity and variables, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, NS = not significant

6.3.3.2 Alveograph extensibility

Polymeric proteins and gliadins were the main contributors to variation for AlvL in both regions (Table 6.4). FPC was the only significant contributor to variation for AlvL in Matlabas in E-FS and no variable correlated significantly for Limpopo in NW-FS. AG was the only contributor to variation for AlvL in Elands, Gariep and Komati in NW-FS. The model explained more than 90% of variation for AlvL in Elands (E-FS), Matlabas and PAN3118, both in NW-FS.

Table 6.4 Multiple linear regression analysis for alveograph extensibility

Variable	Par Estimate	Partial R^2	Model R^2	F-Value	Correlation [(a)]
BettaDN	**NW-FS**	**AlvL**			
Intercept	-57.031^{NS}				
ω-gliadin UNP rel	-73.07*	0.53	0.53	16.31**	-0.73***
LMW EXP rel	24.65*	0.17	0.71	8.06*	0.72***
BettaDN	**E-FS**	**AlvL**			
Intercept	-131.09***				
LMW UNP rel	94.626***	0.77	0.77	77.80***	0.88***
HMW UNP rel	12.69**	0.07	0.85	11.03**	0.59**
Caledon	**NW-FS**	**AlvL**			
Intercept	285.61***				
α/β,γ-gliadin UNP rel	-33.40**	0.47	0.47	12.42**	-0.68**
Caledon	**E-FS**	**AlvL**			
Intercept	-60.11*				
LMW UNP abs	59.77***	0.66	0.66	44.19***	0.81***
HMW EXP abs	111.84*	0.08	0.75	7.47*	0.74***
Elands	**NW-FS**	**AlvL**			
Intercept	-19.31^{NS}				
AG EXP abs	77.23**	0.39	0.39	9.12**	0.62**
Elands	**E-FS**	**AlvL**			
Intercept	196.72**				
HMW EXP abs	130.48***	0.83	0.83	113.04***	0.91***
LMW EXP rel	-10.57*	0.05	0.88	9.53**	-0.63***
AG EXP rel	-3.62*	0.03	0.91	6.05*	-0.72***
Gariep	**NW-FS**	**AlvL**			
Intercept	-86.05^{NS}				
AG UNP rel	104.19**	0.41	0.41	9.73**	0.64**
Gariep	**E-FS**	**AlvL**			
Intercept	-101.25**				
HMW EXP abs	122.948**	0.69	0.69	49.26***	0.83***
α/β,γ-gliadin UNP rel	28.55**	0.08	0.77	8.17**	0.79***

NW-FS = North Western Free State, E-FS = Eastern Free State, EXP = SDS-extractable proteins, UNP = SDS-unextractable proteins, rel = relative amounts, abs = absolute amounts, HMW = high molecular weight glutenin polymers, LMW = low molecular weight glutenin polymers, AG = albumin and globulin, [a] Pearson's correlation for alveograph extensibility and variables, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, NS = not significant

Table 6.4 Multiple linear regression analysis for alveograph extensibility (Continued)

Variable	Par Estimate	Partial R^2	Model R^2	F-Value	Correlation [(a)]
Komati	**NW-FS**	**AlvL**			
Intercept	54.59^{NS}				
AG UNP rel	40.72*	0.25	0.25	4.89*	0.50*
Komati	**E-FS**	**AlvL**			
Intercept	-95.91*				
LMW EXP abs	65.72***	0.51	0.51	23.81***	0.72***
LMW UNP rel	5.56*	0.09	0.61	5.07*	0.32^{NS}
Limpopo	**NW-FS**	No variable correlated significantly at $p \leq 0.05$			
Limpopo	**E-FS**	**AlvL**			
Intercept	-56.52^{NS}				
α/β,γ-gliadin EXP abs	45.17***	0.49	0.49	21.97***	0.70***
Matlabas	**NW-FS**	**AlvL**			
Intercept	-212.48***				
ω-gliadin EXP rel	83.00***	0.87	0.87	99.25***	0.93***
ω-gliadin UNP rel	-62.56**	0.06	0.93	13.05**	0.42^{NS}
Matlabas	**E-FS**	**AlvL**			
Intercept	-88.68**				
FPC	16.20***	0.64	0.64	40.55***	0.80***
PAN3118	**NW-FS**	**AlvL**			
Intercept	-684.91***				
α/β,γ-gliadin EXP rel	24.95***	0.84	0.84	78.16***	0.92***
AG EXP abs	90.91***	0.08	0.93	16.67**	0.89***
ω-gliadin EXP rel	-8.24*	0.02	0.95	6.14*	0.11^{NS}
PAN3118	**E-FS**	**AlvL**			
Intercept	-209.34***				
α/β,γ-gliadin UNP abs	459.64***	0.47	0.47	19.72***	0.68***
LMW EXP rel	12.98***	0.25	0.72	19.56***	0.06^{NS}
AG UNP abs	-907.12***	0.07	0.80	8.19**	0.56**
ω-gliadin EXP abs	107.67*	0.04	0.84	5.40*	0.56**

NW-FS = North Western Free State, E-FS = Eastern Free State, AlvL = alveograph extensibility, EXP = SDS-extractable proteins, UNP = SDS-unextractable proteins, rel = relative amounts, abs = absolute amounts, FPC = flour protein content, LMW = low molecular weight glutenin polymers, AG = albumin and globulin, [a] Pearson's correlation for alveograph extensibility and variables, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, NS = not significant

Table 6.4 Multiple linear regression analysis for alveograph extensibility (Continued)

Variable	Par Estimate	Partial R^2	Model R^2	F-Value	Correlation [(a)]
PAN3349	**NW-FS**	**AlvL**			
Intercept	114.59**				
α/β,γ-gliadin EXP abs	37.42***	0.65	0.65	26.10***	0.80***
HMW EXP rel	-28.57***	0.15	0.81	10.93**	0.01^{NS}
α/β,γ-gliadin UNP abs	144.52*	0.05	0.86	4.85*	0.43^{NS}
PAN3349	**E-FS**	**AlvL**			
Intercept	179.56^{NS}				
α/β,γ-gliadin UNP abs	108.90*	0.80	0.80	92.54***	0.8***
HMW EXP abs	88.64**	0.04	0.85	7.05*	0.82***
ω-gliadin EXP rel	-30.09*	0.03	0.88	5.53*	-0.76***
PAN3377	**NW-FS**	**AlvL**			
Intercept	581.06***				
LMW UNP rel	-28.91***	0.61	0.61	21.99***	-0.78***
LMW UNP abs	36.55*	0.10	0.71	4.81*	0.53*
PAN3377	**E-FS**	**AlvL**			
Intercept	-250.89***				
ω-gliadin EXP rel	58.11***	0.63	0.63	38.14***	0.79***

NW-FS = North Western Free State, E-FS = Eastern Free State, AlvL = alveograph extensibility, EXP = SDS-extractable proteins, UNP = SDS-unextractable proteins, rel = relative amounts, abs = absolute amounts, HMW = high molecular weight glutenin polymers, LMW = low molecular weight glutenin polymers, [a] Pearson's correlation for alveograph extensibility and variables, *** $p \leq 0.001$, ** $p \leq 0.01$,* $p \leq 0.05$, NS = not significant

6.3.3.3 Mixograph peak time

No variable correlated significantly with MPT for Limpopo (E-FS) and Matlabas (NW-FS) (Table 6.5). UPP was the only variable explaining variation in MPT for Caledon in E-FS, Elands, Gariep and Komati in NW-FS. UPP explained 88.95% of the variation for MPT in Elands (NW-FS) and HMW (UNP, relative amounts) explained 86.61% in Limpopo (NW-FS). The model explained 90.48% of the variation for MPT in PAN3118 (E-FS).

Table 6.5 Multiple linear regression analysis for mixograph peak time

Variable	Par Estimate	Partial R^2	Model R^2	F-Value	Correlation [(a)]
BettaDN	**NW-FS**	**MPT**			
Intercept	-0.41^{NS}				
AG EXP rel	0.38**	0.56	0.56	18.47***	0.75***
HMW EXP abs	-1.61*	0.13	0.70	6.19*	-0.62**
BettaDN	**E-FS**	**MPT**			
Intercept	0.96^{NS}				
LMW EXP abs	-0.91**	0.29	0.29	9.14**	-0.54**
UPP	0.07**	0.19	0.48	8.08**	0.44*
Caledon	**NW-FS**	**MPT**			
Intercept	15.70***				
α/β,γ-gliadin EXP rel	-0.38***	0.52	0.52	15.63**	-0.72**
AG UNP rel	-1.11***	0.30	0.83	23.60***	0.20^{NS}
Caledon	**E-FS**	**MPT**			
Intercept	-0.10^{NS}				
UPP	0.05**	0.33	0.33	11.01**	0.57**
Elands NW-FS	**NW-FS**	**MPT**			
Intercept	-0.17^{NS}				
UPP	0.07***	0.88	0.88	112.72***	0.93***
Elands	**E-FS**	**MPT**			
Intercept	2.01^{NS}				
LUPP	0.08**	0.1725	0.17	4.59*	0.41*
α/β,γ-gliadin UNP rel	-0.50*	0.197	0.36	6.56*	-0.23^{NS}
Gariep	**NW-FS**	**MPT**			
Intercept	-0.41^{NS}				
UPP	0.08***	0.70	0.70	32.95***	0.83***
Gariep	**E-FS**	**MPT**			
Intercept	-5.87^{NS}				
α/β,γ-gliadin UNP rel	-1.18***	0.21	0.21	5.92*	-0.46*
α/β,γ-gliadin EXP rel	0.42**	0.24	0.45	9.52**	0.41*
LMW UNP abs	0.95*	0.10	0.56	4.80*	-0.23^{NS}

NW-FS = North Western Free State, E-FS = Eastern Free State, MPT = mixograph peak time, EXP = SDS-extractable proteins, UNP = SDS-unextractable proteins, rel = relative amounts, abs = absolute amounts, LUPP = large unextractable polymeric proteins, UPP = unextractable polymeric proteins, HMW = high molecular weight glutenin polymers, LMW = low molecular weight glutenin polymers, AG = albumin and globulin, [a] Pearson's correlation for mixograph peak time and variables, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, NS = not significant

Table 6.5 Multiple linear regression analysis for mixograph peak time (Continued)

Variable	Par Estimate	Partial R^2	Model R^2	F-Value	Correlation [(a)]
Komati	**NW-FS**	**MPT**			
Intercept	-0.94^{NS}				
UPP	0.08***	0.86	0.86	89.06***	0.93***
Komati	**E-FS**	**MPT**			
Intercept	2.95^{NS}				
UPP	0.06*	0.27	0.27	8.55**	0.52**
AG EXP abs	-2.00*	0.16	0.44	6.30*	-0.48*
Limpopo NW-FS	**NW-FS**	**MPT**			
Intercept	-0.39^{NS}				
HMW UNP rel	0.64***	0.86	0.86	90.57***	0.93***
Limpopo	**E-FS**	No variable correlated significantly at $p \leq 0.05$			
Matlabas	**NW-FS**	No variable correlated significantly at $p \leq 0.05$			
Matlabas	**E-FS**	**MPT**			
Intercept	10.36***				
LMW EXP abs	-1.50***	0.47	0.47	19.84***	-0.68***
ω-gliadin UNP rel	-0.63*	0.13	0.60	7.00*	-0.55**

NW-FS = North Western Free State, E-FS = Eastern Free State, MPT = mixograph peak time, EXP = SDS-extractable proteins, UNP = SDS-unextractable proteins, rel = relative amounts, abs = absolute amounts, UPP = unextractable polymeric proteins, HMW = high molecular weight glutenin polymers, LMW = low molecular weight glutenin polymers, AG = albumin and globulin, [a] Pearson's correlation for mixograph peak time and variables, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, NS = not significant

Table 6.5 Multiple linear regression analysis for mixograph peak time (Continued)

Variable	Par Estimate	Partial R^2	Model R^2	F-Value	Correlation [(a)]
PAN3118	**NW-FS**	**MPT**			
Intercept	-1.03^{NS}				
LMW UNP rel	0.26***	0.77	0.77	48.09***	0.88***
PAN3118	**E-FS**	**MPT**			
Intercept	46.89***				
ω-gliadin EXP rel	-1.92***	0.41	0.41	31.98***	-0.64***
α/β,γ-gliadin UNP rel	-1.08**	0.31	0.72	51.88***	-0.07^{NS}
LMW EXP rel	-1.33***	0.12	0.84	35.37***	-0.27^{NS}
HMW UNP rel	-1.43**	0.03	0.87	10.97**	0.00^{NS}
AG EXP abs	1.20*	0.02	0.90	11.38**	0.10^{NS}
PAN3349	**NW-FS**	**MPT**			
Intercept	0.26^{NS}				
HMW UNP rel	0.46***	0.62	0.62	22.90***	0.78***
PAN3349	**E-FS**	**MPT**			
Intercept	-5.74***				
AG EXP rel	0.43***	0.40	0.40	15.22***	0.63***
LMW UNP abs	1.37***	0.29	0.70	21.05***	-0.31^{NS}
PAN3377	**NW-FS**	**MPT**			
Intercept	0.47^{NS}				
LMW UNP abs	1.17***	0.59	0.59	20.59***	0.77***
PAN3377	**E-FS**	**MPT**			
Intercept	4.30*				
LMW EXP rel	-0.57***	0.47	0.47	20.14***	-0.69***
α/β,γ-gliadin EXP rel	0.30***	0.27	0.75	23.83***	0.38^{NS}

NW-FS = North Western Free State, E-FS = Eastern Free State, MPT = mixograph peak time, EXP = SDS-extractable proteins, UNP = SDS-unextractable proteins, rel = relative amounts, abs = absolute amounts, HMW = high molecular weight glutenin polymers, LMW = low molecular weight glutenin polymers, AG = albumin and globulin, [a] Pearson's correlation for mixograph peak time and variables, *** $p \leq 0.001$, ** $p \leq 0.01$,* $p \leq 0.05$, NS = not significant

6.3.3.4 Loaf volume

Protein fractions contributed significantly to variation for LFV in all the cultivars in both regions, except for PAN3118 (E-FS) where FPC contributed 69.24% to variation in LFV (Table 6.6). Gliadins were the main contributors to variation, followed by polymeric proteins and AG made the smallest contribution. The model explained more than 90% of variation in LFV for Gariep (NW-FS), PAN3349 and PAN3377 in both regions. AG was the only contributor to variation in LFV for Komati and Limpopo in NW-FS.

Table 6.6 Multiple linear regression analysis for loaf volume

Variable	Par Estimate	Partial R^2	Model R^2	F-Value	Correlation [(a)]
BettaDN	**NW-FS**	**LFV**			
Intercept	573.35***				
LMW EXP abs	325.56***	0.58	0.58	19.86***	0.76***
α/β,γ-gliadin UNP abs	-487.11*	0.16	0.74	8.44*	0.18^{NS}
BettaDN E	**E-FS**	**LFV**			
Intercept	2917.56***				
AG EXP rel	-90.08***	0.57	0.57	30.35***	-0.76***
ω-gliadin EXP rel	162.38***	0.21	0.79	21.82***	0.32^{NS}
α/β,γ-gliadin EXP rel	-51.80***	0.04	0.83	5.01*	0.05^{NS}
HMW EXP rel	-49.55**	0.06	0.89	11.78**	0.44*
Caledon	**NW-FS**	**LFV**			
Intercept	670.55***				
α/β,γ-gliadin EXP abs	83.22**	0.51	0.51	14.60**	0.71***
Caledon E-FS	**E-FS**	**LFV**			
Intercept	623.98***				
LMW UNP abs	176.59***	0.55	0.55	27.97***	0.74***
Elands	**NW-FS**	**LFV**			
Intercept	736.31***				
AG EXP abs	244.47***	0.39	0.39	9.14**	0.62**
ω-gliadin EXP rel	-29.54**	0.28	0.68	11.76**	-0.17^{NS}
Elands E-FS	**E-FS**	**LFV**			
Intercept	487.00***				
α/β,γ-gliadin UNP abs	711.89***	0.68	0.68	48.35***	0.82***
Gariep	**NW-FS**	**LFV**			
Intercept	2716.87***				
AG UNP abs	1184.71***	0.65	0.65	26.05***	0.80***
LMW UNP rel	-73.89***	0.16	0.81	12.03**	-0.34^{NS}
HMW UNP rel	53.24*	0.09	0.91	14.67**	-0.16^{NS}
ω-gliadin UNP rel	-101.42**	0.03	0.95	10.01**	0.42^{NS}
AG EXP rel	-42.09*	0.01	0.97	8.36*	-0.68**
Gariep	**E-FS**	**LFV**			
Intercept	572.37***				
HMW EXP abs	426.94***	0.62	0.62	36.06***	0.78***

NW-FS = North Western Free State, E-FS = Eastern Free State, LFV = loaf volume, EXP = SDS-extractable proteins, UNP = SDS-unextractable proteins, rel = relative amounts, abs = absolute amounts, HMW = high molecular weight glutenin polymers, LMW = low molecular weight glutenin polymers, AG = albumin and globulin, [a] Pearson's correlation for loaf volume and variables, *** $p \leq 0.001$, ** $p \leq 0.01$,* $p \leq 0.05$, NS = not significant

Table 6.6 Multiple linear regression analysis for loaf volume (Continued)

Variable	Par Estimate	Partial R^2	Model R^2	F-Value	Correlation [(a)]
Komati	**NW-FS**	**LFV**			
Intercept	592.58***				
AG UNP rel	218.83***	0.60	0.60	21.75***	0.78***
Komati	**E-FS**	**LFV**			
Intercept	484.31***				
HMW EXP abs	322.44***	0.73	0.73	60.69***	0.85***
α/β,γ-gliadin UNP rel	251.70**	0.08	0.81	9.45**	0.69***
Limpopo	**NW-FS**	**LFV**			
Intercept	343.07*				
AG EXP abs	390.17***	0.61	0.61	22.06***	0.78***
Limpopo	**E-FS**	**LFV**			
Intercept	1086.68***				
HMW EXP rel	34.38*	0.39	0.39	14.23***	0.62***
LMW EXP rel	-26.65***	0.37	0.76	34.12***	-0.50*
AG UNP abs	-154.29***	0.05	0.81	5.59*	-0.55**
α/β,γ-gliadin UNP abs	439.21**	0.06	0.88	9.75**	0.39^{NS}
Matlabas	**NW-FS**	**LFV**			
Intercept	379.09***				
α/β,γ-gliadin EXP abs	190.28***	0.74	0.74	40.46***	0.86***
Matlabas	**E-FS**	**LFV**			
Intercept	1093.06***				
AG EXP rel	-30.37***	0.64	0.64	40.03***	-0.80***
α/β,γ-gliadin EXP abs	91.98**	0.13	0.77	12.34**	0.68***
PAN3118	**NW-FS**	**LFV**			
Intercept	-67.29^{NS}				
α/β,γ-gliadin EXP abs	99.15***	0.78	0.78	52.64***	0.88***
HMW EXP rel	106.17*	0.05	0.84	5.09*	0.74***
PAN3118	**E-FS**	**LFV**			
Intercept	415.53***				
FPC	40.34***	0.69	0.69	49.52***	0.832***

NW-FS = North Western Free State, E-FS = Eastern Free State, LFV = loaf volume, EXP = SDS-extractable proteins, UNP = SDS-unextractable proteins, rel = relative amounts, abs = absolute amounts, FPC = flour protein content, HMW = high molecular weight glutenin polymers, LMW = low molecular weight glutenin polymers, AG = albumin and globulin, [a] Pearson's correlation for loaf volume and variables, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, NS = not significant

Table 6.6 Multiple linear regression analysis for loaf volume (Continued)

Variable	Par Estimate	Partial R^2	Model R^2	F-Value	Correlation [(a)]
PAN3349	**NW-FS**	**LFV**			
Intercept	-196.16^{NS}				
ω-gliadin EXP rel	114.04***	0.89	0.89	118.65***	0.94***
AG UNP rel	156.73**	0.05	0.94	13.34**	-0.69**
PAN3349	**E-FS**	**LFV**			
Intercept	529.00***				
α/β,γ-gliadin UNP abs	645.00***	0.84	0.84	115.59***	0.91***
HMW EXP abs	343.61***	0.07	0.91	16.94***	0.86***
AG UNP abs	-957.79*	0.02	0.93	7.88*	0.82***
PAN3377	**NW-FS**	**LFV**			
Intercept	850.67***				
α/β,γ-gliadin EXP abs	122.38***	0.83	0.83	72.33***	0.91***
α/β,γ-gliadin UNP rel	-66.22***	0.09	0.93	19.1***	-0.36^{NS}
PAN3377	**E-FS**	**LFV**			
Intercept	-291.66^{NS}				
LMW EXP abs	195.27***	0.71	0.71	115.18***	0.84***
α/β,γ-gliadin UNP rel	66.05***	0.07	0.78	15.15***	-0.59**
HMW UNP abs	-166.55***	0.04	0.82	10.48**	0.27^{NS}
ω-gliadin EXP rel	107.86***	0.09	0.92	51.45***	0.58**

NW-FS = North Western Free State, E-FS = Eastern Free State, LFV = loaf volume, EXP = SDS-extractable proteins, UNP = SDS-unextractable proteins, rel = relative amounts, abs = absolute amounts, HMW = high molecular weight glutenin polymers, LMW = low molecular weight glutenin polymers, AG = albumin and globulin, [a] Pearson's correlation for loaf volume and variables, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, NS = not significant

6.4 Discussion

In order to have a better understanding of the GxE interaction, the performance of each genotype was conducted for selected quality traits in different regions (Bonafede et al., 2015).

FPC for individual cultivars was, in general, higher in NW-FS than E-FS, especially for BettaDN, Caledon, Elands, Gariep, Komati and Limpopo, This is confirmed by the genotype's contribution to variation in FPC in NW-FS (10.31%) as opposed to 4.06% in E-FS (Table 3.5).

6.4.1 Alveograph tenacity

The significant negative combined correlation for AlvP with FPC (Table 4.5) corresponded with the significant negative correlation for individual cultivars in E-FS, and for Matlabas, PAN3118 and PAN3349 in NW-FS (Table 6.2). However, significant positive correlations occurred between AlvP and FPC for BettaDN, Elands, Gariep, Komati and Limpopo (Table 6.2).

Ranking of the cultivars for AlvP showed slight variations between the two regions, with PAN3118 at the top and Caledon at the bottom of the ranking order, despite the large difference in protein content for the mentioned cultivars in the two regions. Values for AlvP were higher in E-FS than in NW-FS (Table 6.2).

Discussion for stepwise MLR was considered for variables (measured protein fractions), which correlated significantly with selected quality parameters. Variables which did not correlate significantly with the selected quality parameters were excluded from the discussion, due to interdependent associations with the preceding variables in the model (https://www.uvm.edu/~dhowell/gradstat/psych341/lectures/MultipleRegression/multreg3.html).

AG contributed positively to variation in AlvP and occurred more frequently in the model for cultivars in NW-FS, while AG appeared only for PAN3118 in E-FS. Proportional variation in AG made a larger contribution to AlvP than quantitative variation and UNP made a larger contribution than EXP.

Proportional variation in LMW explained a larger amount of the variation for AlvP than quantitative variation in LMW, with EXP contributing positively to AlvP and UNP contributing negatively. LMW contributed more to AlvP in E-FS than in NW-FS. Proportional variation in gliadins made a larger contribution to variation for AlvP in E-FS than in NW-FS, where α/β,γ-gliadin (EXP) explained variation for Limpopo and gliadins did not contribute significantly to variation in any of the remaining cultivars in

NW-FS. Proportional amounts in gliadins (EXP) made a positive contribution to AlvP in E-FS and a negative contribution NW-FS.

6.4.2 Alveograph extensibility

The combined correlation indicated a significant association of FPC with AlvL (Table 4.5) although BettaDN, Caledon, Elands, Gariep, Komati and Limpopo did not show the same significant correlation in NW-FS and PAN3377 in E-FS. Additionally, the stepwise MLR model explained less variation in AlvL for the cultivars that showed no significant correlation between FPC and AlvL, compared to the cultivars with a significant correlation for FPC with AlvL. Ranking of the cultivars showed variation between the regions, although Caledon ranked the highest and Elands the lowest in both regions. Values for AlvL were higher in NW-FS than in E-FS, except for Gariep and PAN3349 (Table 6.1).

Variation for AlvL in Matlabas was explained by FPC. Quantitative amounts in HMW (EXP) explained variation for AlvL in E-FS, while HMW did not explain variation in NW-FS. Proportional and quantitative variation in LMW (EXP and UNP) explained variation for AlvL in both regions. Gliadins were the main contributors to variation for AlvL in both regions. Proportional and quantitative amounts of α/β,γ-gliadin (EXP and UNP) made a larger contribution to variation in AlvL than ω-gliadin. Quantitative amounts of ω-gliadin did not associate significantly with AlvL. AG explained more variation in AlvL in NW-FS than in E-FS.

6.4.3 Mixograph peak time

Individual cultivar FPC values did not correlate significantly ($p \leq 0.001$) with MPT. The highest positive correlation ($p \leq 0.01$) occurred for PAN3377 in NW-FS. The low correlation for FPC with MPT was confirmed in the combined correlations, while LUPP and UPP showed the highest positive correlation with MPT (Table 4.4). Values for MPT were higher in E-FS than in NW-FS, except for Gariep and PAN3349. Cultivars showed a

slight variation in the ranking for MPT, with Caledon the lowest in both regions and Gariep the highest in NW-FS, and PAN3118 the highest in E-FS (Table 6.2).

The stepwise MLR model explained a larger percentage of the variation for MPT in NW-FS than in E-FS for BettaDN, Caledon, Elands, Gariep, Komati and Limpopo. The model explained a larger percentage of variation in MPT for Matlabas and the Pannar cultivars in E-FS than in NW-FS. UPP explained a larger percentage of variation in NW-FS than in E-FS and did not explain variation in MPT for Matlabas and the Pannar cultivars. For polymeric proteins, UNP explained a larger percentage of variation than EXP. Polymeric proteins and ω-gliadin associated with MPT for Matlabas and the Pannar cultivars, with LMW explaining most of the variation. Proportional amounts of monomeric proteins explained the variation in MPT for certain cultivars, although combined correlations did not show a significant ($p \leq 0.001$) correlation for MPT with α/β,γ-gliadin and AG (Table 4.4).

6.4.4 Loaf volume

Pearson's correlation for FPC with LFV was significant for individual cultivars, except for Caledon and Elands in NW-FS. The combined correlation indicated a significant positive correlation ($r = 0.749$) between FPC and LFV (Table 4.5). Caledon ranked the highest for LFV and Elands the lowest (Table 6.2). FPC explained all the variation (69.24%) in LFV for PAN3118 in E-FS and variation for the rest of the cultivars could be explained by protein fractions. LFV varied between cultivars with constant protein content (Finney & Barmore, 1948) and variation could be ascribed to flour composition (Johansson et al, 2003; Wang et al., 2007; Pasha et al., 2010).

The regression model indicated that the largest single contribution (89.45%) to variation in LFV was ω-gliadin (EXP, proportional amount) for PAN3349 in NW-FS. This could relate to the high correlation between FPC and proportional variation in ω-gliadin (Table 3.8). Quantitative variation in α/β,γ-gliadin and polymeric proteins explained a larger percentage of variation in LFV than proportional variation, which is in agreement with the significant correlation for LFV and absolute amounts (Table 4.5). In NW-FS, EXP

explained most of the variation in LFV for α/β,γ-gliadin, as opposed to UNP for α/β,γ-gliadin in E-FS.

6.5 Conclusions

Pearson's correlation for FPC with individual cultivars varied between cultivars and regions, despite the significant combined correlation for FPC with AlvP and AlvL. Furthermore, cultivars with a significant correlation for FPC with AlvP showed a negative correlation in E-FS while the correlation was positive in NW-FS. FPC of BettaDN, Caledon, Elands, Gariep, Komati and Limpopo did not correlate significantly with AlvL in NW-FS while Matlabas and the Pannar cultivars' FPC correlated significantly in NW-FS with AlvL. Caledon and Elands did not show a significant correlation for FPC with LFV in NW-FS. It is worthy to note that Elands serves as the quality standard in the winter dry land production area.

FPC cannot be used alone to predict bread making quality (Rousset et al., 2001). The stepwise MLR model indicated the contribution of FPC and protein fractions to explain the variation that occurred for variables (AlvP, AlvL, MPT and LFV). FPC explained variation in LFV for PAN3118 and AlvL for Matlabas in E-FS while FPC did not contribute significantly to variables in NW-FS.

The regression model indicated that AG (UNP) and LMW made the largest contribution to variation for AlvP in NW-FS, as opposed to LMW and ω-gliadin in E-FS. Gliadins, LMW and AG explained most of the variation for AlvL in NW-FS, while polymeric proteins and α/β,γ-gliadin explained most of the variation in E-FS for AlvL. UPP and polymeric proteins explained a larger proportion of variation in MPT in NW-FS than in E-FS. AG and α/β,γ-gliadin (EXP) explained the largest proportion of variation for LFV in NW-FS, while α/β,γ-gliadin and polymeric proteins explained the largest proportion of variation for LFV in E-FS.

The regression model explained a larger percentage of variation for AlvL in E-FS than in NW-FS for BettaDN, Caledon, Elands, Gariep, Komati and Limpopo, while the regression model explained a larger percentage for Matlabas and the Pannar cultivars in NW-FS than in E-FS. The opposite happened for MPT where the regression model explained a larger percentage for variation in MPT in NW-FS than in E-FS for BettaDN, Caledon, Elands, Gariep, Komati and Limpopo, whereas the regression model explained a larger percentage of variation in MPT for Matlabas and Pannar cultivars in E-FS than in NW-FS. Cultivars reacted different between the two regions for protein content. The largest percentage increases between the two regions for FPC were for BettaDN, Caledon, Elands, Gariep, Komati and Limpo. Locality made the largest contribution to variation in NW-FS and E-FS, 33.66% and 46.07%, respectively (Table 3.5).

AG fractions contributed more to variation in NW-FS than in E-FS for AlvP, AlvL and LFV. Furthermore, these contributions were larger for BettaDN, Caledon, Elands, Gariep, Komati and Limpopo than for Matlabas and the Pannar cultivars. AG fractions correlated positively with AlvL, AlvP and LFV in NW-FS, while the correlations with AlvL and LFV were mainly negative in E-FS. Proportional amounts of AG (EXP) correlated positively with MPT and explained a significant percentage of variation for two cultivars, one per region. The positive contribution of specific AG fractions to strength related parameters (AlvP and MPT) and LFV need to be further investigated. Conflicting results exist for non-prolamins, there is no clarity regarding the structures and contribution to rheological behaviours of AG (Song & Zengh, 2007).

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Chapter 7

Conclusions

This study was conducted on 10 South African commercial cultivars (BettaDN, Caledon, Elands, Gariep, Komati, Limpopo, Matlabas, PAN3118, PAN3349 and PAN3377), over two years, at five localities in the Free State. Levene's test (Levene, 1960) indicated heterogeneity of variances for localities and the five localities were therefore divided into two regions: NW-FS for Bot and Bult, and E-FS for Bhm, Clar and Lad.

SE-HPLC was applied to determine MWD and divided into HMW, LMW, ω-gliadin, α/β,γ-gliadin and AG. Protein fractions were calculated for EXP and UNP. Results were expressed as relative values (the proportional amount of a protein fraction in the protein) and absolute values (the quantitative amount of a protein fraction in the flour, taking into account FPC on a 12% mb). Flour quality analyses were conducted according to approved AACC methods to determine baking quality.

Significant differences were measured for all the protein fractions and quality parameters. The overall ANOVA model explained between 77 – 97% of the variation in protein fractions (R^2 = 0.77 – 0.97), which suggest that the selected model provided adequate description of the observed variability.

Environment (locality) made the largest contribution to variation in FPC. Protein composition changed with changes in FPC but the changes were not in the same ratio. Gliadins showed the highest correlation with FPC, followed by polymeric proteins and AG showed the lowest correlation. Increased FPC resulted in higher amounts of gliadin followed by polymeric protein and lower increases in AG. The effect of genotype, environment and GxE interaction varied between the two regions for the amount of EXP protein fractions. Genotype made the largest contribution to variation in α/β,γ-gliadin (EXP) in both regions. Environment had the largest influence on unextractable polymeric proteins (LUPP and UPP) in NW-FS and genotype in E-FS.

ANOVA for quality parameters indicated that genotype contribution to variation was different between the two regions. Genotype made the largest contribution to variation for MPT (43%) and AlvP (31%) in both regions. Genotype contributed more to variation in NW-FS for all the quality parameters, except for AlvSTR.

Pearson's correlations indicated that increased FPC had a negative impact on AlvP and AlvP/L, while the influence was positive on AlvL and AlvSTR. The highest correlation with FPC occurred for LFV. FPC correlated negatively with SDSS. LUPP and UPP showed the highest correlation with MPT. FPC did not correlate significantly with MPT, while LUPP and UPP correlated significantly with MPT. Correlations of quality parameters with relative and absolute amounts of protein fractions, indicated that FPC alone does not account for variation in bread making quality but also protein composition.

Partial correlations revealed how the qualitative effect of polymeric and monomeric proteins impacted differently on bread making quality in NW-FS than in E-FS, indicating the large effect of environment on protein accumulation. LFV was in general more affected by quantitative variation in protein fractions, at an equivalent level of protein content, than FPC in NW-FS, while FPC had a larger effect on LFV than protein composition in E-FS.

Percentage increases for FPC between the two regions indicated that larger increases occurred for BettaDN, Caledon, Elands, Gariep, Komati and Limpopo than for Matlabas, PAN3118, PAN3349 and PAN3377. It seems that Matlabas and the Pannar cultivars would be more stable for FPC across the two regions than the other mentioned cultivars. Stability for FPC was not tested in this study. Wheat quality is affected by favourable or unfavourable environmental conditions, genotypes with a low GxE interaction are therefore considered to be economically stable (Grausgruber et al., 2000). Efficient selection by wheat breeders is affected if ranking of cultivars changes between environments for quality attributes, while millers and bakers are more concerned about consistency of cultivars, regardless of changes in cultivar ranking (Rozbicki et al., 2015).

Previous studies indicated that the HMW-GS in South African wheat explained less than 20% of variability in bread making quality (Randall et al., 1993). The effect of HMW-GS composition was not the aim of this study and the variation in HMW-GS composition was also too small for the ten cultivars to investigate the effect on quality parameters. It should be noted that Caledon, with HMW-GS 2 + 12 (*Glu-D1a*), ranked the highest for AlvL and LFV and ranked the lowest for AlvP and MPT. The two cultivars with the strongest dough were PAN3118 and Elands, both with HMW-GS 5 + 10 (*Glu-D1d*). PAN3377 was the other cultivar in the study with HMW-GS 2 + 12 (*Glu-D1a*). PAN3347 ranked second, after Caledon (HMW-GS 2 + 12, *Glu-D1a*), for AlvL in both regions and ranked second for LFV in NW-FS.

Stepwise MLR was used to establish the contribution of protein fractions to selected variables (AlvP, AlvL, MPT and LFV) for individual cultivars. FPC explained variation in LFV for PAN3118 and AlvL for Matlabas in E-FS while FPC did not contribute significantly to variables in NW-FS.

The regression model explained a larger percentage of variation for AlvL in E-FS than in NW-FS for BettaDN, Caledon, Elands, Gariep, Komati and Limpopo, while the regression model explained a larger percentage of variation for Matlabas and the Pannar cultivars in NW-FS than in E-FS. The opposite happened for MPT where the regression model explained a larger percentage of variation in MPT in NW-FS than in E-FS for BettaDN, Caledon, Elands, Gariep, Komati and Limpopo, whereas the regression model explained a larger percentage of variation in MPT for Matlabas and Pannar cultivars in E-FS than in NW-FS.

AG fractions contributed more to variation in NW-FS than in E-FS for AlvP, AlvL and LFV. Furthermore, these contributions were larger for BettaDN, Caledon, Elands, Gariep, Komati and Limpopo than for Matlabas and the Pannar cultivars. The positive contribution of specific AG fractions to strength related parameters (AlvP and MPT) and LFV need to be further investigated.

Protein fractions were differently expressed across the two regions and impacted differently on quality parameters. The statistical procedures used in this study gave an indication of the association between protein fractions and baking quality. Combined Pearson's correlation gave a rough indication of the associations between protein fractions and quality parameters. General conclusions for the effect of protein fractions and FPC on quality parameters cannot be deducted in the event where average values for genotypes were used, especially in a study where entries showed significant differences for quality traits (Li et al., 2013). Stepwise MLR made it possible to look at the effect of protein content and protein composition on selected quality parameters for individual cultivars, although MLR could not explain variation for all the cultivars. Uncontrolled variation occurs under complex environmental conditions. As a result, ANOVA and regression models do not provide accurate results (Hazen et al., 1997).

Findings apply to results for this study. It would be interesting to find out whether similar genomic and environmental effects on the specific protein parameters would also be found for other cultivars, or the same cultivars in envrionments other than the ones studied here.

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Summary

Ten South African hard red wheat cultivars (BettaDN, Caledon, Elands, Gariep, Komati, Limpopo, Matlabas, PAN3118, PAN3349 and PAN3377) were planted at five localities over two seasons in the winter dryland wheat production area of South Africa. Localities were divided into North Western Free State (NW-FS) and Eastern Free State (E-FS). Size-exclusion high-performance liquid-chromatography was applied to determine the molecular weight distribution of SDS-extractable protein and SDS-unextractable protein fractions. Relative and absolute amounts were measured for glutenin, gliadin, albumin and globulin.

Protein fractions did not increase parallel to increased flour protein content. The gliadin fraction showed the highest correlation in NW-FS, while polymeric proteins showed the highest correlation in E-FS with flour protein content. Albumin and globulin showed the lowest correlation with flour protein content in both regions. Environment made the largest contribution to variation in flour protein content in both regions.

Bread making properties were correlated with flour protein content and protein fractions. Significant positive correlations were observed between flour protein content and wet gluten content, farinograph water absorption, alveograph extensibility, alveograph strength and loaf volume. Significant negative correlations occurred between flour protein content and SDS-sedimentation and alveograph tenacity. Mixograph peak time was mainly influenced by genotype and did not correlate significantly with flour protein content, while unextractable polymeric proteins showed a significant positive correlation with mixograph peak time. Partial correlations were conducted to remove the quantitative effect of flour protein content. Polymeric and monomeric protein fractions affected bread making quality differently in NW-FS than E-FS, indicating the effect of environment on protein composition.

Pearson's correlation showed that flour protein content correlated between significantly and not significantly with alveograph tenacity, alveograph extensibility and

mixogrpah peak time MPT for individual cultivars. The stepwise multiple linear regression indicated that flour protein content could only explain variation in alveograph extensibility and loaf volume for two cultivars in E-FS. Protein fractions contributed in different degrees to variation for alveograph tenacity, alveograph extensibility, mixograph peak time and loaf volume for individual cultivars and between the two regions. The model explained a larger percentage of variation in alveograph extensibility for BettaDN, Caledon, Elands, Gariep, Komati and Limpopo in E-FS than in NW-FS, while the model explained a larger proportion of variation in mixograph peak time for Matlabas and the Pannar cultivars in NW-FS than in E-FS. Albumin and globulin fractions contributed more to variation for alveograph tenacity, alveograph extensibility and mixograph peak time in NW-FS than in E-FS. The effect of albumin and globulin on baking quality justifies further research.

Environment had a large influence on flour protein content and percentages of protein fractions. Protein fractions affected baking quality differently between environments and genotypes.

Key words: Hard red wheat cultivars, environment, molecular weight distribution, flour protein content, protein fractions, baking quality, stepwise multiple linear regression

Opsomming

Tien Suid Afrikaanse harde rooi koringkultivars (BettaDN, Caledon, Elands, Gariep, Komati, Limpopo, Matlabas, PAN3118, PAN3349 en PAN3377) is aangeplant oor vyf lokaliteite en twee seisoene, in die winter droëland koringproduksie streek van Suid Afrika. Lokaliteite was verdeel in Noordwes-Vrystaat (NW-VS) en Oos-Vrystaat (O-VS). Molekulêre gewig verspreiding van SDS-oplosbare en SDS-nie-oplosbare proteïenfraksies is bepaal met hoë uitset vloeistofchromatografie. Relatiewe en absolute hoeveelhede is bepaal vir glutenien, gliadien, albumien en globulien.

Proteïenfraksies het nie parallel met meel proteïeninhoud vermeerder nie. Gliadiene het die hoogste korrelasie met meel proteïeninhoud getoon in NW-VS, terwyl polimeriese proteïene die hoogste korrelasie getoon het in O-VS. Albumien en globulien het die laagste korrelasie getoon met meel proteïeninhoud. Omgewing het die grootste bydrae gemaak tot variasie in meel proteïeninhoud in beide streke.

Korrelasies is bepaal tussen meel proteïeninhoud en proteïenfraksies met bakkwaliteit eienskappe. Betekenisvolle positiewe korrelasies is waargeneem tussen meel proteïeninhoud en nat gluten inhoud, farinograaf waterabsorpsie, alveograaf rekbaarheid, alveograaf sterkte en broodvolume. Betekenisvolle negatiewe korrelasies het voorgekom tussen meel proteïeninhoud en SDS-sedimentasie en alveograaf weerstand teen uitrekking. Miksograaf mengtyd was hoofsaaklik deur die genotipe beïnvloed en het nie ‘n betekenisvolle korrelasie met meel proteïeninhoud getoon nie, terwyl nie-oplosbare polimeriese proteïene ‘n betekenisvolle positiewe korrelasie met miksograaf mengtyd getoon het. Gedeeltelike korrelasies is ook uitgevoer, waar die kwantitatiewe effek van meel proteïeninhoud verwyder word. Polimeriese en monomeriese proteïenfraksies beïnvloed kwaliteitseienskappe verskillend in NW-VS teenoor die O-VS, wat dui op die invloed van die omgewing op proteïensamestelling.

Meel proteïeninhoud vir individuele kultivars se korrelasies het gewissel tussen betekenisvol en nie-betekenisvol met alveograaf weerstand teen uitrekking, alveograaf

rekbaarheid en miksograaf mengtyd. Stapsgewyse veelvuldige liniêre regressie het aangedui dat meel proteïeninhoud alleen, variasie in alveograaf rekbaarheid en broodvolume slegs vir twee kultivars in O-VS kon verklaar. Proteïenfraksies het verskillend bygedra tot variasie in alveograaf weerstand tot uitrekking, miksograaf mengtyd en broodvolume vir individuele kultivars en tussen die twee streke. Die model kon ‘n groter persentasie van variasie verklaar in alveograaf rekbaarheid vir BettaDN, Caledon, Elands, Gariep, Komati en Limpopo in O-VS teenoor NW-VS, terwyl die model ‘n groter persentasie van variasie in miksograaf mengtyd in Matlabas en die Pannar kultivars verklaar in NW-VS teenoor O-VS. Albumien en globulien fraksies het ‘n groter bydrae gemaak tot variasie in alveograaf weerstand tot uitrekking, alveograaf rekbaarheid en miksograaf mengtyd in NW-VS teenoor O-VS. Die effek van albumien en globulien op eienskappe vir bakkwaliteit regverdig verdere navorsing.

Omgewing het ‘n groot invloed op meel proteïeninhoud en hoeveelhede van proteïenfraksies gehad. Die invloed van proteïenfraksies op eienskappe vir bakkwaliteit het verskil tussen genotipes en omgewings. Verskillende genotipes en omgewings mag verskillende resultate oplewer.

Sleutelwoorde: Harde rooi koring, molekulêre gewig verspreiding, meel proteïeninhoud, proteïenfraksies, eienskappe vir bakkwaliteit, stapsgewyse veelvuldige liniêre regressie

Appendix

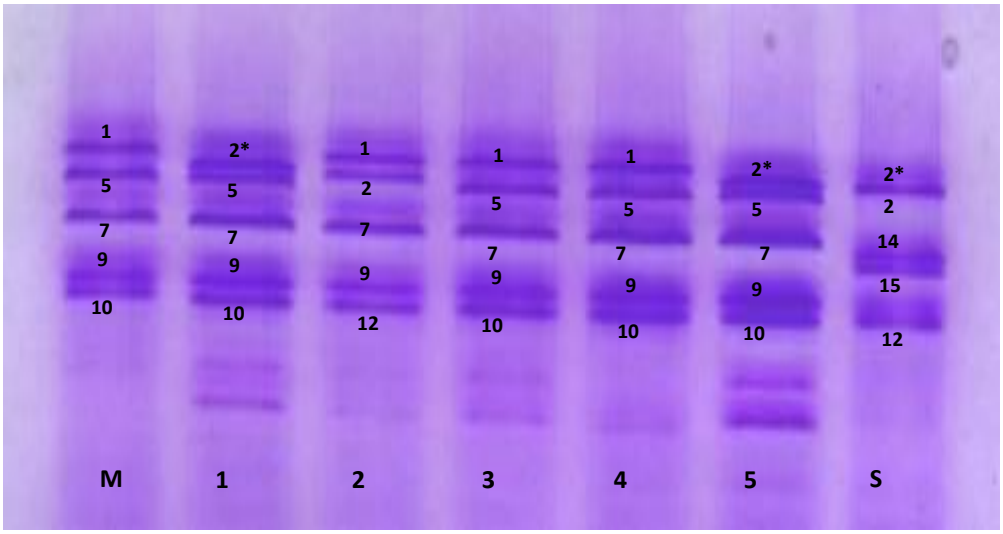

Figure 1 **SDS-PAGE profile.** M = Marquis; 1 = Betta-DN; 2 = Caledon; 3 = Elands; 4 = Gariep; 5 = Komati; S = Sappo

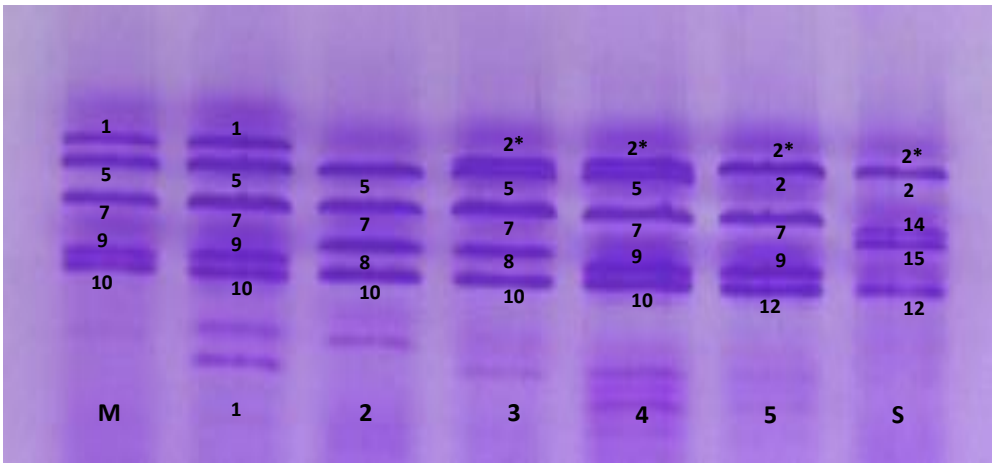

Figure 2 **SDS-PAGE profile.** M = Marquis; 1 = Limpopo; 2 = Matlabas; 3 = PAN3118; 4 = PAN3349; 5 = PAN3377; S = Sappo

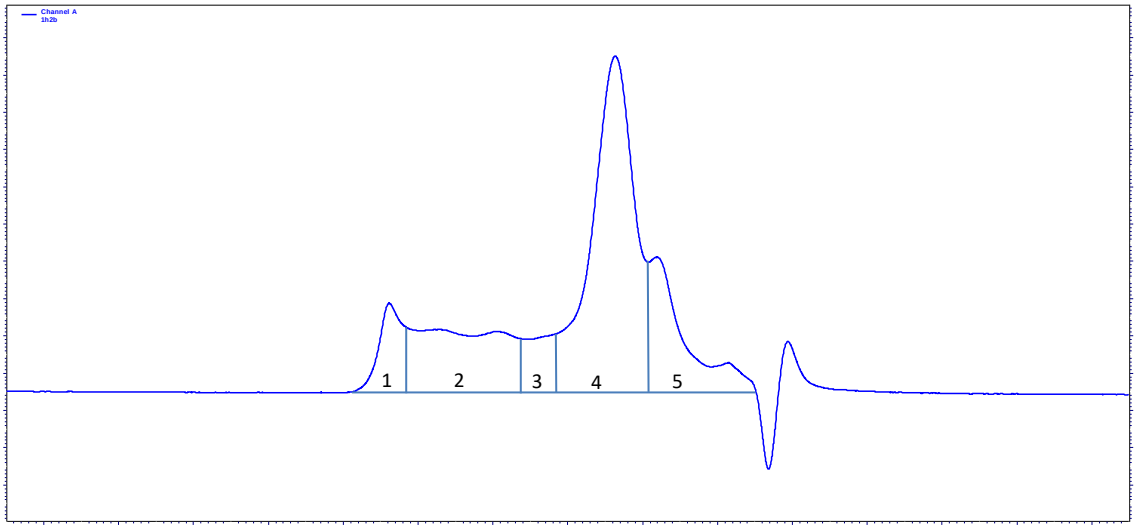

Figure 3 **SE-HPLC profile for SDS-extractable proteins.** 1 = HMW glutenin polymers; 2 = LMW glutenin polymers; 3 = ω-gliadin; 4 = α/β,γ-gliadin; 5 = albumin and globulin

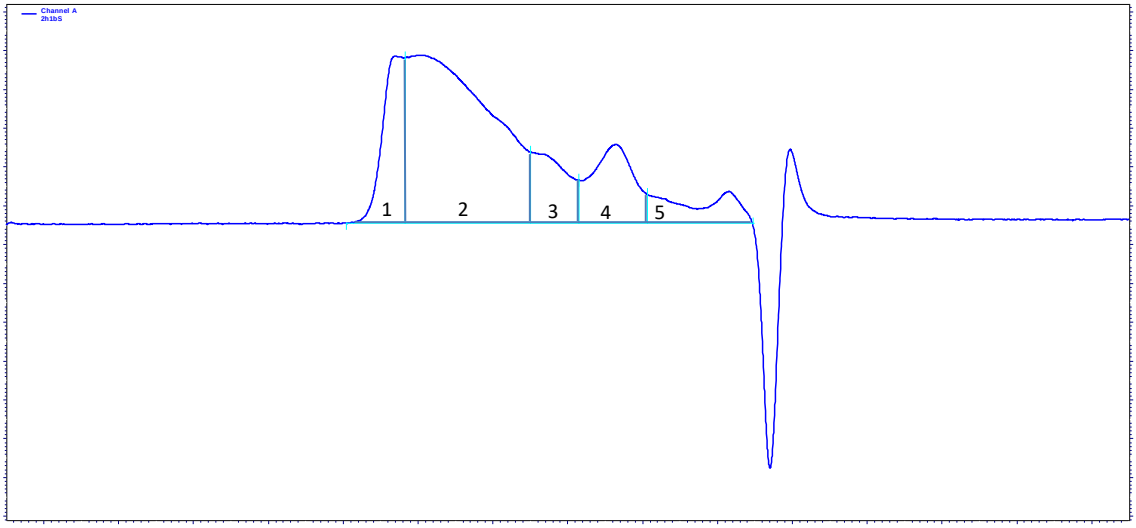

Figure 4 **SE-HPLC profile for SDS-unextractable proteins.** 1 = HMW glutenin polymers; 2 = LMW glutenin polymers; 3 = ω-gliadin; 4 = α/β,γ-gliadin; 5 = albumin and globulin

Table 1 List of localities, planting and harvesting dates

Trial site	GPS Co-ordinates	Altitude	Planting 2007	Harvest 2007	Planting 2009	Harvest 2009
North Western Free State						
Bothaville	27° 31′ 51 47″ S 26° 30′ 50 56″ E	1316	26-04-2007	28-11-2007	11-05-2009	06-12-2009
Bultfontein	28° 17′ 25 92″ S 26° 28′ 19 50″ E	1302	24-04-2007	27-11-2007	29-04-2009	26-11-2009
Eastern Free State						
Bethlehem	28° 09′ 17 25″ S 27° 31′ 45 15″ E	1721	05-06-2007	08-01-2008	22-06-2009	14-12-2009
Clarens	28° 24′ 35 37″ S 27° 23′ 49 77″ E	1714	20-06-2007	11-01-2008	25-06-2009	31-12-2009
Ladybrand	29° 14′ 30 02″ S 27° 19′ 27 89″ E	1500	13-07-2007	03-01-2008	17-06-2009	29-12-2009

Table 2 Weather data

Bothaville	Min	Max	Days > 30°C	Rainfall	Bothaville	Min	Max	Days > 30°C	Rainfall
2007	**°C**	**°C**		97.5 [a]	**2009**	**°C**	**°C**		304.3 [a]
April [b]	9.41	25.22	0	22.5	May [b]	4.96	22.02	0	18.5
May	1.97	22.21	0	2.3	June	3.55	18.56	0	35.3
June	0.71	17.92	0	0.0	July	0.64	16.97	0	5.3
July	-1.42	18.40	0	20.8	August	3.26	21.42	0	8.1
August	0.88	22.17	0	2.7	September	7.36	27.20	5	30.0
September	9.00	28.52	13	65.2	October	11.95	26.28	6	56.7
October	11.73	24.34	0	103.2	November	12.53	26.94	9	86.9
28-Nov-07 [c]	12.92	27.60	7	78.6	06-Dec-09 [c]	13.87	31.80	5	2.3
				295.3 [d]					243.1 [d]
Bultfontein	**Min**	**Max**	**Days > 30°C**	**Rainfall**	**Bultfontein**	**Min**	**Max**	**Days > 30°C**	**Rainfall**
2007	**°C**	**°C**		67.9 [a]	**2009**	**°C**	**°C**		168.1 [a]
April [b]	10.37	25.54	0	37.6	April [b]	9.75	26.12	0	13.7
May	1.64	22.10	0	0.0	May	5.73	21.19	0	26.9
June	1.33	17.33	0	21.2	June	3.48	17.77	0	47.0
July	-1.58	18.41	0	0.0	July	-0.56	16.49	0	7.1
August	0.89	21.90	0	0.0	August	3.24	21.29	0	1.8
September	7.67	28.60	14	63.6	September	6.08	26.38	3	6.1
October	11.75	24.67	2	95.8	October	11.89	26.80	6	88.9
27-Nov-07 [c]	12.12	28.76	11	44.3	26-Nov-09 [c]	12.27	26.93	7	31.8
				262.5 [d]					223.2 [d]

Min = average minimum temperature, Max = average maximum temperature, [a] measured rainfall (mm) from January till planting month, [b] planting month, [c] harvesting date, [d] measured rainfall from planting month till harvesting date

Table 2 **Weather data** (continued)

Bethlehem	Min	Max	Days > 30°C	Rainfall	Bethlehem	Min	Max	Days > 30°C	Rainfall
2007	**°C**	**°C**		130.2 [a]	**2009**	**°C**	**°C**		315.4 [a]
June [b]	-0.85	16.01	0	27.6	June [b]	2.49	14.90	0	58.7
July	-2.23	16.89	0	0.0	July	0.43	15.06	0	0.0
August	-0.06	19.87	0	0.0	August	1.74	18.41	0	20.1
September	6.89	25.59	0	0.0	September	6.61	23.29	0	8.4
October	9.87	20.05	0	158.7	October	9.13	22.58	0	42.4
November	10.57	23.08	0	96.7	November	9.95	23.39	0	74.8
December	12.29	24.02	0	86.3	14-Dec-09 [c]	12.51	26.26	0	37.1
08-Jan-08 [c]	10.23	26.89	0	16.0					241.5 [d]
				385.3 [d]					
Clarens	**Min**	**Max**	**Days > 30°C**	**Rainfall**	**Clarens**	**Min**	**Max**	**Days > 30°C**	**Rainfall**
2007	**°C**	**°C**		Error	**2009**	**°C**	**°C**		522.4 [a]
June [b]	0.72	14.91	0	33.5	June[b]	2.49	14.53	0	57.2
July	-0.73	16.23	0	3.0	July	0.43	15.05	0	0.0
August	1.68	18.72	0	0.1	August	1.74	18.36	0	0.8
September	8.67	24.18	0	47.9	September	6.61	22.70	0	3.8
October	8.92	19.23	0	208.0	October	9.13	22.61	0	65.4
November	10.40	22.42	0	129.5	November	9.95	23.54	0	55.8
December	11.78	21.92	0	99.5	31-Dec-09[c]	12.51	27.60	2	46.6
11-Jan-08 [c]	12.19	24.12	0	23.9					229.6 [d]
				545.4 [d]					

Min = average minimum temperature, Max = average maximum temperature, [a] measured rainfall (mm) from January till planting month, [b] planting month, [c] harvesting date, [d] measured rainfall from planting month till harvesting date

Table 2 **Weather data** (continued)

Ladybrand	Min	Max	Days > 30°C	Rainfall	Ladybrand	Min	Max	Days > 30°C	Rainfall
2007	**°C**	**°C**		151.0 [a]	**2009**	**°C**	**°C**		256.7 [a]
July [b]	-2.58	17.04	0	2.4	June[b]	1.77	15.56	0	28.0
August	0.58	20.22	0	3.4	July	-3.15	15.34	0	2.3
September	7.31	26.40	6	47.3	August	2.04	19.60	0	15.7
October	9.88	22.37	0	80.2	September	6.07	24.13	0	0.3
November	10.52	25.96	4	118.6	October	10.10	23.97	0	109.0
December	13.20	25.61	3	81.3	November	10.73	24.95	4	64.3
03-Jan-08 [c]	16.13	31.67	3	4.1	29-Dec-09[c]	14.05	30.67	23	15.2
				337.3 [d]					234.7 [d]

Min = average minimum temperature, Max = average maximum temperature, [a] measured rainfall (mm) from January till planting month, [b] planting month, [c] harvesting date, [d] measured rainfall from planting month till harvesting date