Protein quality vs. quantity in South African commercial bread wheat cultivars

by

Robert Crowther Lindeque

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Promoter: Prof M.T. Labuschagne

Co-promoter: Dr A. Van Biljon

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ABBREVIATIONS

ANOVA	Analysis of variance
AU	Amylograph units
BU	Brabender units
CBP	Chorleywood baking process
CV%	Percentage coefficient of variation
˚C	Degrees Celcius
Da	Daltons
E	Environment
G	Genotype
GPY	Grain protein yield
G x E	Genotype by environment interaction

Ha	Hectare
IRR	Irrigation
L	Distensibility
LSD	Lowest significant difference
EFS	Eastern Free State
EU	Extensograph units
FU	Farinograph units
g	Grams
HMW	High molecular weight
ITSC	Institute for Climate, Soil and Water
LMP	Large monomeric proteins
LMW	Low molecular weight
LPP	Large polymeric proteins
Mb	Moisture basis
MPT	Minutes peak time
MS	Mean squares
MW	Molecular weight
N	Nitrogen
NWFS	North-western Free State
P	Stability
REP	Replication
SA	South-Africa
SAGL	South-African Grain Laboratories
SDS	Sodium-dodecyl sulphate
secs	Seconds
SE	Standard error
SE-HPLC	Size-Exclusion High performance liquid chromatography

SMP	Smaller monomeric proteins
SPP	Smaller polymeric proteins
SRR	Summer rainfall region
SS	Sum of squares
T ha^{-1}	Ton per hectare
TKW	Thousand kernel weight
UK	United Kingdom
Wf	Deformation energy
WRR	Winter rainfall region
ZAR	South African Rand
α	Alpha
β	Beta
γ	Gamma
ω	Omega

Chapter 1

Introduction

Wheat in the post-subsidised era of South Africa

1.1 Challenges facing wheat farmers

The modern commercial wheat farmer is pivotal in producing the essential calories of the human diet across the globe. Commercial wheat production regions in the minor wheat producing countries are shrinking due to rising production costs, increased competition from non-traditional crops such as soybeans and imports from the grain production gaints of the world. Ironically, this scenario plays off amidst the rising demand for wheat, particularly on the African continent where it is associated with modernisation and improved life styles. In 2014 South Africa (SA) only produced approximately 50% of its local wheat demands, although technology, production resources and supporting infrastructure are capable of meeting and exceeding the national demand. The final number of tons of wheat produced in 2013 was established at 1, 870 000 tons, which is slightly more than the 10-year average of 1 852 800 tons. The sharp decline in production of the summer rainfall region (SRR) from approximately 500 000 tons in 2004 to only 270 000 tons in 2013 is not a reflection of the national production trend as yield per hectare (ha) and area harvested for irrigated (IRR) and rainfed wheat in the winter rainfall regions (WRR) increased over the same period. In the 2012 production year 1 396 000 tons of wheat were imported for domestic consumption with the bulk of these imports originating from the Ukraine (24%), Russia (18%) and Brazil (17%). For the period September 2013 to beginning of July 2014 a large consignment (47%) from the Russian Federation formed part of the 1 308 562 tons imported into SA for this period (SAGL 2014). These huge bulks of imported wheat in SA harbours awaiting distribution are probably the most prominent factor shaping the SA wheat industry at present. Amid this scenario, another

frequent discussion nowadays revolves around food self-sufficiency versus food supremacy. All these factors contribute towards creating an uncertainty which also affects wheat production and a large number of farmers have either completely changed over to a more profitable summer crop rotation system or abandoned farming altogether (Bester 2014).

For a developing country facing serious social and economic challenges such as the rising incidence of HIV, unemployment coupled with large scale urbanisation and lurking effects of climate change, the loss of revenue through importation of commodities that can be produced locally is irrational. A primary stimulus is required to increase the competiveness and sustainability of wheat production in SA and rekindle production interest in wheat among grain producers.

1.2 Meeting the growing continental demand through modern technology by optimising the wheat to bread chain

Incentives drive production trends and it is not different in the wheat to bread chain. A common remark among farmers in regard to rainfed wheat production in SA is that a shortfall equalling approximately 1.5 ton ha^{-1} exists and must be attained to regain profitability and redirect current production trends into an upward curve. The responsibility of acquiring this critical 1.5 ton ha^{-1}, equating to around 4000 SA Rand (ZAR), more often lands in the lap of the breeding programmes and is, erroneously, related directly to grain yield. In SA, as around the globe, commercial production units struggle to fully exploit the high genetic yield potential in local wheat germplasm, which creates considerable yield gaps. Breeders will always have a primary responsibility in developing high yielding crops but the reality is that a collective effort is required between crop improvement (transgenic traits and hybrid wheat) and improved agronomic practises (such as conservation agriculture).

1.3 Are high yielding genotypes the true determinant of profitability?

A major misconception among wheat farmers around the world is that grain yield is the sole component determining long term sustainability of wheat production on farms. The truth is that grain yield is only one of the parameters determining the price a farmer receives, the other consisting of the grading parameters (measuring grain quality) of the respective grading scale. In SA the three primary grading parameters are hectolitre mass (kg hl^{-1}), grain protein content (%) and falling number. Of the three, hectolitre mass (test weight) is probably the most volatile, as it is affected by the same factors determining trends in grain yield.

1.3.1 Case-study 1 from trial data of the national wheat adaptation trials of 2014

An example of the inconsistency of grain yield to solely determine final wheat price is found in trial data of the national cultivar evaluation programme (NCEP) for rainfed wheat produced at two planting dates in the Eastern Free State (EFS) and North Western Free State (NWFS) in 2014. Firstly, grade of each genotype was determined and secondly an approximate price (ZAR 3500 t^{-1} for grade 1 with ZAR 250 less between each respective lower grade) for the achieved grade was multiplied with the average yield (ton ha^{-1}) of each genotype. Thirdly, a monetary value was calculated for each of the genotypes (early planting and/or late planting) in the EFS and NWFS after which they were ranked according to the respective prices they achieved (Tables 1.1 and 1.2).

When quality (as expressed through the grading parameters) is included in an equation with yield, a substantial change of ranking takes place between genotypes, particularly at the late seeding date of the EFS (Table 1.1) and early seeding date of the NWFS (Table 1.2). A higher number of environmental variables, particularly affecting hectolitre mass (test weight), occur later during the growing season and contribute towards the change in ranking.

Table 1.1 Grain yield rank compared with profit rank of rainfed wheat in the Eastern Free State

Early seeding				Late seeding			
Genotype	**Yield rank**	**ZAR rank**	**ZAR ha^{-1}**	**Genotype**	**Yield rank**	**ZAR rank**	**ZAR ha^{-1}**
SST 347	1	1	12576.00	SST 398	3	1	13024.00
PAN 3111	2	2	12480.00	PAN 3368	5	2	12704.00
PAN 3195	3	3	12192.00	SST 317	6	3	12512.00
Matlabas*	4	4	12160.00	PAN 3111	1	4	12419.50
SST 356	5	5	11680.00	Elands	8	5	12064.00
SST 316	6	6	11424.00	SST 347	4	6	11741.00
SST 317	7	7	11264.00	Senqu	11	7	11616.00
SST 387	8	8	11168.00	PAN 3195	2	8	11043.00
SST 398	9	9	11168.00	SST 316	12	9	10679.00
PAN 3120*	10	10	10976.00	PAN 3379	12	10	10620.00
PAN 3118*	11	11	10560.00	Gariep	15	11	10400.00
PAN 3379	13	12	10176.00	PAN 3161	7	12	10206.00
Gariep	14	13	9888.00	Koonap	16	13	10144.00
Senqu	15	14	9792.00	SST 356	9	14	10098.00
PAN 3368	16	15	9728.00	SST 387	14	15	9504.00
PAN 3161	12	16	9617.00	PAN 3198	16	16	8424.00
Elands	17	17	9120.00				
Koonap	19	18	8544.00				
PAN 3198	18	19	8348.50				

Genotypes marked with an asterisk* are only adapted for early seeding dates

For the later seeding date in the EFS the difference in ZAR between the number one yield ranking (PAN 3111) and number one “financial” ranking (SST 398) is ZAR 604.50 ha^{-1} which, if applied to a harvested area of 1000 ha, would have resulted in a difference in income of approximately ZAR 600 000.00.

Similar trends to the EFS occurred for genotypes adapted for production in the NWFS, except that the higher number of changes in ranking occurred in the early seeding date (Table 1.2). The financial ranking also seems to reflect the adaptability of genotypes to early and late seeding. For instance, SST 347 has a similar financial value for both seeding dates, whereas PAN 3195 appears to be better adapted for late seeding (Table 1.2).

Table 1.2 Grain yield rank compared with profit rank of rainfed wheat in the North Western Free State

Early seeding				Late seeding			
Genotype	**Yield rank**	**ZAR rank**	**ZAR ha^{-1}**	**Genotype**	**Yield rank**	**ZAR rank**	**ZAR ha^{-1}**
SST 347	5	1	9696.00	PAN 3111	1	1	10656.00
Matlabas*	6	2	8100.00	PAN 3195	2	2	10240.00
SST 387	9	3	7031.50	SST 347	3	3	9664.00
PAN 3161	2	4	6912.00	PAN 3161	4	4	8960.00
PAN 3195	1	5	6737.50	PAN 3118	6	5	8256.00
PAN 3111	11	6	6394.50	SST 317	7	6	7904.00
SST 398	18	7	6321.00	SST 387	5	7	7847.00
PAN 3198	16	8	6183.00	SST 356	8	8	7456.00
PAN 3120*	12	9	5940.00	PAN 3379	10	9	7200.00
PAN 3368	19	10	5886.00	SST 316	11	10	7008.00
SST 356	10	11	5373.00	PAN 3198	12	11	6944.00
SST 316	15	12	5265.00	PAN 3368	12	12	6944.00
PAN 3379	3	13	5049.00	SST 398	9	13	6696.50
SST 317	7	14	4994.00	Elands	14	14	6048.00
Elands	17	15	4631.50	Senqu	15	15	5984.00
Koonap	14	16	4401.00	Gariep	16	16	5856.00
Senqu	8	17	4347.00	Koonap	17	17	5504.00
PAN 3118	3	18	4239.00				
Gariep	13	19	4131.00				

Genotypes marked with an asterisk* are only adapted for early seeding dates

1.4 Is high protein content the primary determinant of baking quality?

The simple example above illustrates the weakness of basing crop performance on a single factor. Unfortunately, a myriad of similar examples exists in the evaluation of crop performance in agriculture. Focus on the milling and baking industry reveals a similar situation. As grain yield does not fully reflect the on-farm profitability of a genotype, grain protein content is often inadequate in explaining and indicating baking quality of wheat, which is critical in determining sustainability of the milling and baking industry.

Numerous research papers emphasise the strong correlation between grain yield and grains per m^2 (Slafer et al. 1996) and kernels per spikelet and kernels per spike (Bennet et al. 2012). In regard to grain quality, Seleiman et al. (2011) reported strong associations between thousand kernel weight and flour yield after milling and grain protein content with gluten

concentration, water absorption, dough stability time and weakness. Protein content is firstly one of the grading parameters determining the price a farmer receives for wheat (as seen in the previous paragraph) and secondly, is applied as indicator of the end use potential of the flour. Protein content provides millers and bakers with some indication of dough development time and water absorption of the flour. But protein content, as parameter on its own, often does not provide a realistic prediction of baking quality. In regard to Swedish wheat, Fossati et al. (2010) remarked that a better breeding strategy would be to select genotypes with lower protein content but higher bread making quality. They validate this statement by the fact that high protein quality (better bread making) in genotypes was achieved more frequently with high molecular weight glutenin subunits (HMW-GS) 5+10 (*Glu-D1d* allele) resulting in stronger gluten and often also in higher grain yield. The problem was that although more than 30% of Swedish cultivars were listed to have very good baking quality (top quality class) and approximately 50% good quality (class 1), up to 2% dry gluten had to be added to flour to attain a sufficient wet gluten content as required by the industry. Here, the problem resembles that of case study 1 and several similarities exist. Firstly, wheat genotypes with high grain yield seldom have high baking quality, secondly, grain protein, or flour protein for that matter, are incorrectly applied as main indicators of bread making quality and do not predict wet gluten content accurately and thirdly, after bulking cultivars of the same quality class in storage silos the variation in wet gluten quality between genotypes are lost. The fact is that these “quality classes” may be impractical for production requirements of specific end products by industry (Fossati et al. 2010). Reese et al. (2007) highlights an important issue that may prove to be the centre of the predicament when they ask: *“Is protein enough when assessing wheat flour quality?”* From their study they concluded that grain protein is only one of many measurements available for determining flour quality and additional tests such as farinograph and alveograph should be included for a truer prognosis of baking quality.

1.4.1 Case-study 2 from the annual report for 2014 of the South African Grain Laboratories

In order to form an opinion of variation in flour protein content, wet gluten and loaf volume in SA wheat, a second case study was conducted. Data from the annual report of the SA Grain Laboratory (SAGL) for 2013 for dryland wheat of the Free State (eastern region) and Western Cape (Rûens, western region) and irrigated wheat from the Northern Cape was summarised (Table 1.3). Bread loaf volume (obtained from the 100 g baking test) is generally regarded as the final measure of end product quality and indirectly reflects bread making quality. As grade of wheat samples reduces, several expectations are anticipated namely that; (i) flour protein, wet gluten and loaf volume of the same samples reduce accordingly, (ii) loaf volume between grades will vary with more than 10% (percentage variation allowed from that of the quality standard for release of a new cultivar).

Wheat quality from the 2013 season indicated that, firstly flour protein, wet gluten and loaf volume of dryland wheat from the Free State and Rûens was reduced with a decline in wheat grade. For irrigated wheat from Griqualand West though, flour protein and wet gluten of B4 wheat were higher than B3 and loaf volume of B4 was higher than loaf volume of B3 and B2 wheat (Table 1.2a). Secondly, loaf volume of the B1, B2 and B3 grades from the Free State and Rûens differed with less than 10% from each other and loaf volume of none of the neighbouring grades for irrigation wheat differed with more than 10% (Table 1.3).

Table 1.3 Summary of flour quality of South African wheat reported in the 2013 annual report of the South African Grain Laboratory (2014)

	Parameter	Flour protein (%) 12% mb	Wet gluten (%) 14% mb	Loaf volume (cm^3) 100 g baking test
Dryland Free State (Eastern region)	Grade B1	11.5	30.9	888
	Grade B2	11.0	29.1	867
	Grade B3	9.7	25.8	810
	Grade B4	9.0	21.8	721
	Variation	± 2.5	± 9.1	± 176
	Average	10.3	26.9	821.5
	Minimum value of a 10% variation from the B1 loaf volume			799.2
	Minimum value of a 10% variation from the B2 loaf volume			780.3
	Minimum value of a 10% variation from the B3 loaf volume			729.0
Dryland Rûens (Western region)	Grade B1	11.4	-	850
	Grade B2	10.7	30.2	829
	Grade B3	9.4	30.4	762
	Grade B4	8.8	24.9	716
	Variation	± 2.6	± 5.3	± 134
	Average	10.1	28.5	789.3
	Minimum value of a 10% variation from the B1 loaf volume			765.0
	Minimum value of a 10% variation from the B2 loaf volume			746.1
	Minimum value of a 10% variation from the B3 loaf volume			685.8
Irrigation (Griqualand West)	Grade B1	11.6	31.8	952
	Grade B2	10.5	28.6	836
	Grade B3	9.8	27.4	838
	Grade B4	10.3	28.6	886
	Variation	± 1.8	± 4.4	± 116
	Average	10.6	29.1	878.0
	Minimum value of a 10% variation from the B1 loaf volume			856.8
	Minimum value of a 10% variation from the B2 loaf volume			752.4
	Minimum value of a 10% variation from the B3 loaf volume			754.2

Considerable amounts of wheat have been imported into SA over the past decade and quality of these imports is often under suspicion. In 2013 Russia, Ukraine and Germany were the three major countries from which wheat were imported and the SAGL tested baking quality of these imports according to SA standards (SAGL 2014). Wheat from Russia, Ukraine and Germany had similar quality than local wheat and also showed similar trends to SA wheat. Loaf volume seemed to be determined by external factors other than grain protein and wet gluten and did not weaken as grades dropped (Table 1.4). Only bread loaf volumes of B2 and B3 wheat from Germany differed with more than 10% from the SA grades.

Table 1.4 Summary of flour quality of imported wheat (for the period 1 Oct 2012 to 30 Sep 2013) reported in the 2013 annual report of the South African Grain Laboratory (2014)

	Parameter	Flour protein (%) 12% mb	Wet gluten (%) 14% mb	Loaf volume (cm^3) 100g baking test
Russia (609 000 tons)	Grade B1			
	Grade B2	10.0	25.2	741
	Grade B3			
	Grade B4	10.0	25.1	775
	Variation	0	±0.1	±34
	Average	10	25.15	758
	Minimum value of a 10% variation from the B2 loaf volume			666.9
	Minimum value of a 10% variation from the B4 loaf volume			697.5
Ukraine (327 000 tons)	Grade B1	11.0	28.4	842
	Grade B2	10.5	26.4	789
	Grade B3	10.5	26.6	835
	Grade B4	10.1	25.2	800
	Variation	±0.9	±3.2	±53
	Average	10.5	26.7	816.5
	Minimum value of a 10% variation from the B1 loaf volume			757.8
	Minimum value of a 10% variation from the B2 loaf volume			710.1
	Minimum value of a 10% variation from the B3 loaf volume			751.5
Germany (114 000 tons)	Grade B1			
	Grade B2	10.1	27.5	755
	Grade B3	9.3	24.7	659
	Grade B4			
	Variation	±0.8	±2.8	±96
	Average	9.7	26.1	707.0
	Minimum value of a 10% variation from the B2 loaf volume			679.5
	Minimum value of a 10% variation from the B3 loaf volume			593.1

This trend was similar for both local and imported wheat and indicated a very important fact. Although price differences between grades in 2013 were approximately ZAR 250, no substantial variation in loaf volumes occurred which lead to the following conclusions, (i) protein content, as applied in wheat grading, cannot accurately predict bread making quality and, (ii) wheat farmers are receiving a lower price for wheat producing bread quality (loaf volume) equal to the top grade (B1).

1.5 Study objectives

Yield and quality of SA wheat will increasingly become important to prevent excessive imports resulting in unnecessary expenditure of revenue. Furthermore, as the two case studies illustrate, grain quality and bread making quality are critically important in determining the profitability of patrons in the wheat industry, although their respective roles are often misunderstood. Improvement of the quality assessment system for wheat in SA will contribute towards re-establishing confidence in the industry by ensuring that, (i) wheat producers receive a fair wheat price, (ii) wheat bulks are assessed accurately for milling and baking quality and (iii) consumers have access to high quality end products.

The primary objective of this study was to investigate the validity of protein content (protein quantity) and protein fractions and ratios (protein quality) to predict and explain loaf volume (bread baking quality). The roles of protein content (both grain and flour) and the various protein fractions, as separated by Size-Exclusion High Performance Liquid Chromatography (SE-HPLC), was assessed by determining associations of both parameters with the primary quality parameters, rheology and protein fractions of wheat from the three major production regions of SA. The study addressed these objectives by addressing the following:

Objective 1: (i) To determine the variation in the composition of gluten over the three different wheat production regions of SA. (ii) What is the relationship between the primary quality parameters and protein concentrations for each region?

Objective 2: To establish associations (correlations) between flour protein, grain protein, loaf volume and (i) bread quality (primary quality parameters) and (ii) gluten composition in the three production regions

Objective 3: To establish associations (correlations) between (i) flour protein, grain protein, loaf volume and (ii) gluten with grain yield

Objective 4: To determine if genotypes with low protein content can achieve good baking quality.

Objective 5: To determine the effects of genotype and environment on protein quality over the three production regions.

1.6 References

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Chapter 2

The quantity and quality of wheat protein

2.1 Bread: Adaptability and variation through flavour, form and function

2.1.1 Development of the western civilisation

In all of the major civilisations contributing to the development of the modern western world, bread from wheat seems to play a central role, often extending further than just a food form and helped to shape religion, traditions and the way society regards themselves. Although this study is about the relationship between protein quantity (protein content) and protein quality (protein fractions) and the influence thereof on bread quality, a part of this overview is focussed on the importance of bread in the general wellbeing of global society. The objectives of this study are directly linked with sustainability of the wheat to bread chain in modern SA and as numerous industries developed out of commonwealth backgrounds, a significant part of this literature study focuses on the Chorleywood Baking Process (CBP). The CBP is continually changing as new technologies become available, although the basic processes remain the same. Commercial bread production of numerous countries around the world are based on the CBP and its main attribute of utilising low protein wheat has since its inception in the early 1960's, influenced wheat production and trading in many countries (Cauvain 2012).

In crop production, wheat cultivation is unequalled in its range, extending from Scandinavia and Russia in the northern hemisphere to Argentina, Australasia and SA in the southern hemisphere (Feldman 1995). The domestication process of wheat initially started with landraces and two traits, probably contributing most to development of the current description

of modern wheat (Shewry 2009), are loss of shattering of the spike at maturity and the change from hulled forms to free threshing, naked forms, freeing seeds (Feldman 2001). The spread of wheat from its origin (the Fertile Crescent encompassing present day Turkey and Iran) into Europe was probably through Greece at 8000 before present (BP) and then northwards to the Balkans at around 7000 BP. From here cultivation spread across to Italy, France and Spain (7000 BP) and then finally to the UK and Scandinavia by about 5000 BP. A southward spread of these landraces through Iran entered into central Asia and reached China by about 3000 BP. Wheat found its way into Central America through the Spaniards in 1529 (Feldman 2001). Wheat was introduced into northern Africa via Egypt and much later into southern Africa by European settlers.

The progression in the processes of making bread dough is just as fascinating as the history of the bread itself. Modern archaeology traced the origin of *"*bread*"* flour preparation, through discovery of starch residues on primitive rock tools, to date back as far as 30 000 years ago. Starch was extracted from plant material through continual pounding of plant parts with rock pestles and baked on flat rocks over an open fire. But gradually the main source of starch for bread shifted to grains during the Neolithic age. As yeast spores are ever present, any dough left to remain in the open for a period before baking will become naturally leavened and soon the process and accompanying novel sources of yeasts were exploited. The Gaul's kept a certain sense of sanity during their beer drinking extravaganzas and discovered that foam skimmed from beers produced excellent leavening. The regions of the ancient world unaccustomed to this idle time, developed their own sources of yeasts through a paste formed from flour and fermented grape juice or wheat bran drenched in wine. Eventually the most popular source for leavening became a piece of dough retained from bread baking preparations of the previous day (http://www.quillshift.com/downloads).

Erdkamp (2005) writes that until around 280 BC bread from wheat was not common in the Roman diet due to few kitchens in general living apartments and wide scale use of unleavened

bread. The most common at the time was *"Polta"* or "porridge" made from wild grains, legumes and sometimes meat. Bread from wheat was probably introduced into Rome by captives from conquered territories including "professional" Egyptian bakers accustomed to the production of leavened bread making since 2000 BC. The Egyptian tradition of making refined white bread grew in popularity in Rome but remained the privilege of the upper class, due to its expensiveness. Sherwood et al. (1998) state that the most important wheat types as described by Pliny were called *far* (referring to emmer or spelt wheat, *Triticum dicoccum* with a coarse grain difficult to thresh), *triticum* (consisting of "hard or soft" wheat, *T. durum*) and common wheat (*T. vulgare). Triticum* or "hard" wheat was soon identified to produce the best flour, it threshed easily and had high yields. With the spread of Roman influences even after the demise of the Empire, the Anglo Saxons quickly became accustomed to wheat bread and the pale colour of the flour probably was one of the main features distinguishing it from other bread flours of the time. Another characteristic contributing to the instant popularity of wheat bread was that it consisted of a lighter loaf, making it more palatable than, for instance, barley bread. Archaeological botany, through pottery impressions, concluded that from the eighth and ninth centuries wheat production overtook barley in Roman Britain and by the tenth and eleventh centuries the production of bread wheat (*T. aestivum*) became twice the amount of that for barley. Dominance of *T. aestivum* indicated that the requirement of the Roman Britain society at this time was specifically for bread wheat and not wheat types used for general cooking or even beer making (Higham and Ryan 2010).

As the previous section indicates, production of wheat became a primary agricultural practise in Roman Britain and baker's guilds were founded and became the earliest associations of craftsmen formed in Europe (Adamson 2004). The first significant step towards white bread becoming more accessible to the broader segment of society occurred in Austro Hungary in the latter part of the 19th century with development of roller mills. This development spread across Europe and allowed the production of higher volumes of whiter flour compared to traditional stone milling (Jones 2007).

Figure 2.1 White bread from wheat was expensive and only for the wealthy

Not everyone approved of this new found luxury and the Bread Reform League (BRL) was founded in London in 1880 with the purpose of revitalising consumption of wholegrain bread. The BRL was particularly concerned about the nutrition of children from poor households and contributed largely towards implementation of “standard bread” in 1909. The principle behind “standard bread” was the demand for the adoption of an official, minimum extraction rate of 80%, practically meaning that 80% of the ground grain sample is retained (Davis 2012).

The outbreak of the second world war contributed largely to establishment of the inter dependency between local wheat production and bread making in Britain, which had a significant influence on commercialisation of bread and the baking industry as we perceive it today. With the outbreak of the war, reduced shipping space resulted in food imports into Britain being cut by around a quarter, particularly for commodities such as grains and vegetables that could be replaced by increased local production (Edgerton 2011). The result was that domestic wheat production doubled in two years and by 1943 local wheat production exceeded imports. The extraction rate of wheat was furthermore increased from 70% to 85% by 1941, resulting in more bran or grist included to produce a brown bread rather than the preferred white. This regulation did result in a higher nutritional value in bread but primarily freed more space on ships through reduced imports of high quality wheat needed for white bread. Improvement of social conditions in early post war Britain were strongly linked to the

growing economy of the 1950's and prompted very influential changes to take place. The first of these was that the focus in the market place shifted from seller controlled markets to consumer controlled markets, forcing companies to produce food items according to consumer demands. Another change closely linked with the first, was that general grocery shopping changed to self-service, enabling consumers to shop quicker, cheaper and with more freedom. General food items became more available and affordable to everyone and the food producing industry was under pressure to keep up with the growing demand, particularly for white bread, through research and development of new processes and machinery. This social and economic background in the early period of rebuilding post war Britain probably directly prompted the development of the Chorleywood Baking Process (CBP) which soon dominated bread making in all the commonwealth countries.

2.1.2 The Chorleywood Baking Process revolution

Historically, wheat produced in the UK (United Kingdom) had low protein content and for production of white bread, wheat of higher protein had to be imported from Canada and the United States. Shortly after World War II a major milling and baking company in Britain increased imports from both these countries. In an attempt to prevent them from losing a foothold in the local baking industry through imports, local millers and bakers consequently established many independent small bakeries that ultimately started the UK milling and baking enterprise. Intensive research by the British Baking Industries Research Association (BBIRA) at the same time discovered important deviations from traditional bread making processes resulting in superior bread (as defined by loaf volume, softness and cell structure). These innovations lead to development and launching of the CBP in the UK in 1961, changing bread baking forever (Cauvain and Young 2006). The CBP allowed use of low protein flours from the UK and after implementation it soon used between 80-100% of the UK wheat varieties. Intensive and high speed dough mixing combines flour, yeast, salt, water, CBP improver and hard fat that effectively reduce the fermentation period resulting in a flour to loaf rate of

approximately 3.5 hours. A basic list of the ingredients required for the CBP recipe involves several “non-traditional” items which became essential for large scale commercialisation of bread and particularly relates to shelf life and production of more than one bread type (Table 2.1).

Table 2.1 Ingredients list of the Chorleywood Baking Process industrial ingredients

Bakery term	Ingredient	Function in the CBP
Fat	Hard fats consisting of fractionated fats not containing or producing transfats	Improves loaf volume, crumb softness and shelf life
Bleach	Chlorine dioxide gas	Improves whiteness of white flour
Reducing Agent	L cysteine hydrochloride (E920) often derived from animal hair and feathers	Creates dough with higher stretching ability
Flour Improvers	Soybean flour (defatted)	Improves whiteness of flour, dough volume and softness of bread through increased machinability
	Emulsifier namely mono and di glycerides of fatty acids (E472a-f) and/or sodium stearoyl-2-lactylates and distilled monoglycerides and lecithins	Forms emulsion between water and oil (fat). Prolongs shelf life by controlling the size of gas bubbles enabling dough to hold more gas
	Oxidising agents such as L ascorbic acid (E300) which is added to the flour or during the baking stage	Improves loaf volume and crumb softness
	Enzymes with the most common being α-amylase (fungal and cereal), hemi cellulase and lipase	Similar function as oxidants and has grown in importance due to restrictions on use of oxidants
Preservatives	Calcium propionate or acetic acid	Prolongs shelf life

Generally, the advantages of the CBP overshadowed the disadvantages and the CBP was implemented by 30 countries, mostly commonwealth countries of which SA is one. The main advantage of the CBP, namely rapid maturing of dough during mixing time, reduced the time required for general bread production, resulting in additional major advantages (Table 2.2) directly related to increased profitability (Cauvain and Young 2006).

Table 2.2 Advantages and disadvantages of the Chorleywood Baking Process

Advantages	Disadvantages
• Reduction of total processing time by at least the time required for fermentation in bulk fermentation process	• The need to process dough at a faster rate because of higher dough temperatures
• Space saving by eliminating the need to keep bowls of dough during bulk fermentation resulting in an estimated space saving of 75%	• The need for large amounts of refrigerated water to control final dough temperature during mixing
• Energy saving through reduction of temperature controlled areas for bulk dough in the fermentation process	• A second mixing for incorporation of fruit into breads and buns
• Improved process control and reduced wastage as in the case of bulk fermentation	• Probable reduction in breadcrumb flavour because of reduced fermentation time.
• More consistent dough and consequently final product quality	
• Financial savings through higher dough yield by addition of extra water and retention of flour solids generally fermented away	

Optimum energy required for dough development in the CBP increases with increasing flour protein content and flours are blended to produce an optimal energy expenditure of approximately 42 kJ kg^{-1} that is appropriate for low flour protein. As in most bread making processes, low flour protein content reduces loaf volume in CBP, although it is not a critical factor. The addition of dry vital gluten by most bakeries compensates for too low protein content without any detrimental effects on the process (Collins and Davies 1985). Cauvain and Young (2006) stated that protein content alone is not the sole factor determining the optimum energy input level of CBP and its role is less clear due to high amounts of oxidants in the recipe. In the early 1970's Chamberlain (1970) established that the CBP had strong economic attractions resulting from higher dough and bread yield through the ability of the process to use weaker and less expensive flours. It was furthermore established that the British public was unaware that such a drastic change in production methods took place as various test panels were unable to distinguish the difference in flavour between CBP bread and traditional long bulk fermented bread.

Continuous improvement of the CBP, for instance implementation of the pressurised vacuum mixer, was furthermore supported by development of a knowledge based system which

allowed transfer of existing operational knowledge of the CBP to a wider commercial forum. Through this system, knowledge from “lessons learned” about the CBP is available for equipping bakers with a system assisting in production of high quality bread on a repeatable scale (Cauvain and Young 2006).

2.2 Grain quality, flour quality and end-use application of wheat

2.2.1 Wheat standards (grades)

All countries involved in commercial wheat production has a set of standards for the different wheat classes. The classes are determined according to official standards and include durum, hard red spring, hard red winter, soft red winter, hard white, soft white, mixed and unclassed. In SA, each class is separated into six grades as defined by hectolitre mass (test weight), grain protein content (%) and falling number (seconds) as well as the percentage of damaged kernels, broken or shrunken kernels and foreign material (Table 2.3).

Table 2.3 The South African wheat grading table

Grade	Grading parameter for class B (bread wheat)		
	Hectolitre mass (kg hl^{-1})	Falling number (secs)	Protein content (%)
Grade 1	77	220	12
Grade 2	76	220	11
Grade 3	74	220	10
Grade 4	72	200	9
Utility grade	70	150	8
Other Wheat	< 70	< 150	< 8

(Amended by Government Notice No. R. 1210 of 29 August 2003)

2.2.2 Grain and flour quality assessment

Complex processing methods are required to manufacture a vast range of products derived from wheat, varying in quality and nutritional value. For wheat genotypes (cultivars) to be

relevant in the respective industries, it is necessary that the intended use of the grain determines the appropriate traits which, in turn, determine the measurement or test to gauge whether a specific trait is present and expressed in a specific cultivar. Numerous tests exist for accurate assessment and prediction of quality and end use application (Table 2.4) and a brief description, adapted from Freund and Kim (2006), is provided in this section.

Table 2.4 Common tests for evaluating grain and flour quality of wheat

Commodity	Traits	Component
Wheat grain	Quality of grain	* Grain moisture content (%)
		* Hectolitre mass (Hl kg^{-1})
		* Thousand kernel weight (g 1000 $kernels^{-1}$)
		* Grain hardness
	Quality of grain components	* Grain wet gluten (%)
		* Grain protein content (%)
		* Grain falling number (seconds)
Wheat flour	Quantitative measurement of flour components	* Flour moisture content (%)
		* Ash (mineral) content (mL 100g)
		* Acidity (ml)
		* Gelatinisation of starch (amylograph)
		* Flour falling number as a measure of α-amylase activity (seconds)
	Quantitative measurement of protein	* Flour wet gluten (%)
		* Flour protein content (%)
		* Sedimentation value (Zeleny test)
Non-fermented dough	Qualitative measurement of protein	* Water absorption and mixing behaviour of flour (farinograph) * Stretching properties of dough (extensograph) Elasticity, extensibility and elasticity/extensibility ratio (alveograph) * Energy (W)
Fermented dough	Fermentation testing of dough (maturograph)	* Analysis is conducted on dough that includes all the ingredients
	Testing the volume increase during the baking process (Oven Rise recorder)	* Measuring baked volume increase during heating and stages of gas escape
	Measure the baked volume increase	
End product evaluation	Baking test for wheat flour in Europe	* Measures loaf volume of pan bread and rolls and wholemeal flours for pan bread
	Standard baking test for classified German wheat flours	* Measures loaf volume of pan bread
	Standard baking test for wheat flour type 550 (rapid mix test)	* Measures loaf volume of bread rolls
	Standard baking test for wholemeal flour	* Measures loaf volume of pan bread

2.3 Wheat grain

2.3.1 Quality of grain

Grain moisture content

Measurement of moisture content is a first step in determining wheat grain and flour quality, and is measured by electrical current or oven drying. Grain moisture indicates storability and wheat grain with high moisture content (> 14.5%) is subject to mould, bacteria, and insect damage, resulting in a decline of quality during storage. Quality of wheat grain with low moisture is more stable and less susceptible to quality reduction during storage. Due to the fact that grain is bought by weight and flour is sold by weight, moisture content relates strongly to milling profitability. Moisture (water) is added to grain in order to obtain the standard moisture level before milling and higher amounts of water added to grain result in higher weight and profitability from the flour, whereas wheat with too low moisture may require special equipment or processes that escalates processing costs (http://www.wheatflourbook.org/).

Hectolitre mass (test weight)

Hectoliter mass refers to the mass in kilogram per hectolitre of wheat and is a grading factor together with grain protein content and falling number. The minimum hectolitre mass for the different grades of Class B (bread wheat) are presented in Table 2.4.

Thousand kernel weight

Thousand kernel weight (TKW) is the weight, in grams, of a 1000 wheat kernels (seeds) and relates to the mass of the wheat kernel. This test provides an indication of kernel composition and flour extraction potential and complements test weight (hectolitre mass) to better describe grain quality (McFall and Fowler 2009).

Grain hardness

Hardness of the grain kernel is used in wheat classification but also as an indication of baking quality (Obuchowski and Bushuk 1980). Wheat that produces higher extraction (higher percentage of flour from the endosperm) consequently produces flour with a higher percentage of damaged starch which results in higher water absorption of the flour and ultimately, higher bread yield (profitability).

2.3.2 Quality of grain components

Grain wet gluten (wholemeal)

The only difference between grain wet gluten (wholemeal) and flour wet gluten analyses is that two different flours are used in the analyses namely wholemeal flour (low extraction rate) for grain wet gluten and white flour (high extraction rate) for flour wet gluten. Wet gluten content is the measure of the amount of swollen gluten in wheat flour based on the principle that dough, buffered with a solution of common salt (pH adjusted to 5.95), that is washed to remove starch and water-soluble remnants, results in wet gluten remaining which is washed and weighed (Freund and Kim 2006).

Grain protein content (also referred to as total protein or wholemeal protein)

The total protein content of wheat is determined by the Kjeldahl method. Organic matter of the grain sample is oxidised in the presence of a catalyst, ammonia formed is distilled and the amount of nitrogen, determined through titration, is multiplied with the prescribed factor of 5.7 (specific for wheat). Another recognised method is the automated Dumas combustion method in which grain samples are incinerated under oxygen and the resulting nitric oxide converted into nitrogen. The heat conductivity of the nitrogen and helium is then measured against that of pure helium. This method is much faster and less complicated than the Kjeldahl method (Freund and Kim 2006).

Grain falling number

An indication of α-amylase activity in grain preceded by a sprouting process. The method uses starch as the medium and analyses viscosity by the resistance of a flour and water paste to a falling stir rod (McFall and Fowler 2009).

2.4 Wheat flour

2.4.1 Quantitative measurement of flour quality

Flour moisture content

Moisture content determines the shelf life and content of solids in flours. Analyses based on drying samples at high temperatures require that the weight of the flour sample be adjusted to compensate for the moisture content. Moisture content of flour is largely determined by climatic conditions during harvesting and immediate moisture levels of storage conditions. A general guide illustrating the effect of moisture content of flour on storability is that moisture content higher than 16% cannot be stored; moisture content of about 15% has limited storage potential but moisture lower than 15% indicates good storage potential (Freund and Kim 2006).

Ash (mineral) content

Flours are often traded on the basis of their mineral content (ash content) which varies with different extraction rates. As extraction rate increases (bran segment in flour increases), protein content and mineral content increase accordingly but wet gluten and volume yield decreases sharply (Freund and Kim 2006).

Acidity

Acidity is an important indicator of flour freshness as fats and phosphatides (lecithin) are broken down through enzyme activity during storage. These changes in flour occur mainly

through increased free fatty acids and the amount of 0.1 N (mol L^{-1}) sodium hydroxide solution necessary to bring the solution (suspension of the flour sample in ethanol) to a pH of 8.5 which determines the degree of acidity (Freund and Kim 2006).

Gelatinisation properties of starch with the amylograph

The amylograph enables continuous measurement of changes in the viscosity of a flour and water suspension over time during heating. Gelatinisation of starch in wheat depends primarily on α-amylase activity and viscosity of a starch solution is determined by measuring the time a stirring rod takes to sink to the bottom of a measuring cylinder (Freund and Kim 2006).

- *Gelatinisation maximum and gelatinisation temperature*

When gelatinisation starts, the suspension reaches an optimal point for α-amylase activity and higher viscosity is the result of increased gelatinisation and liquefaction by the amylase. The curve shows maximum viscosity and temperature at the start of gelatinisation and at maximum gelatinisation. A minimum of 200 amylograph units (AU) and a maximum temperature of 63˚C is required for rye to be approved for bread baking, 350 AU and a temperature of 77°C for wheat flour whereas wholemeal wheat flour requires 400 AU and 80°C (Brabender 2009).

- *Maltose content*

Another method for measuring the breakdown of starch is to determine the maltose content in a flour. The amounts of maltose are determined primarily by β-amylase enzyme activity and higher maltose (malt sugar) can indicate a higher number of sprouted grain but can also be the result of starch damaged during milling. A maltose content value exceeding 1.83% for wheat indicates that the activity of high starch degrading enzymes is too high or too much damage of starch occurred during milling (Freund and Kim 2006).

Flour falling number as a measure of α-amylase activity

Falling number is the sum of the stirring and sinking time measured in seconds and if enzyme activity is high, starch breaks down rapidly during gelatinisation and the stirring rod falls to the bottom much quicker and falling number is low (the minimum falling number is 60 secs).

2.4.2 Quantitative measurement of protein

Quantitative measurements are analyses in which amounts of matter are measured. Important to remember in this context, is that grain protein has an important effect on the baking properties of flour. If the level of extraction results in mainly white flour of the endosperm (high level of extraction) grain protein content increases considerably in addition to ash (mineral) content (Figure 2.2) although a sharp reduction in flour volume yield occurs (bran is removed). An increase in protein content will result from an increase in extraction rate (due to the high amount of protein in the aleuron layer) but gluten does not necessarily increase. Gluten quality enhances dough and baking properties of flour (Freund and Kim 2006).

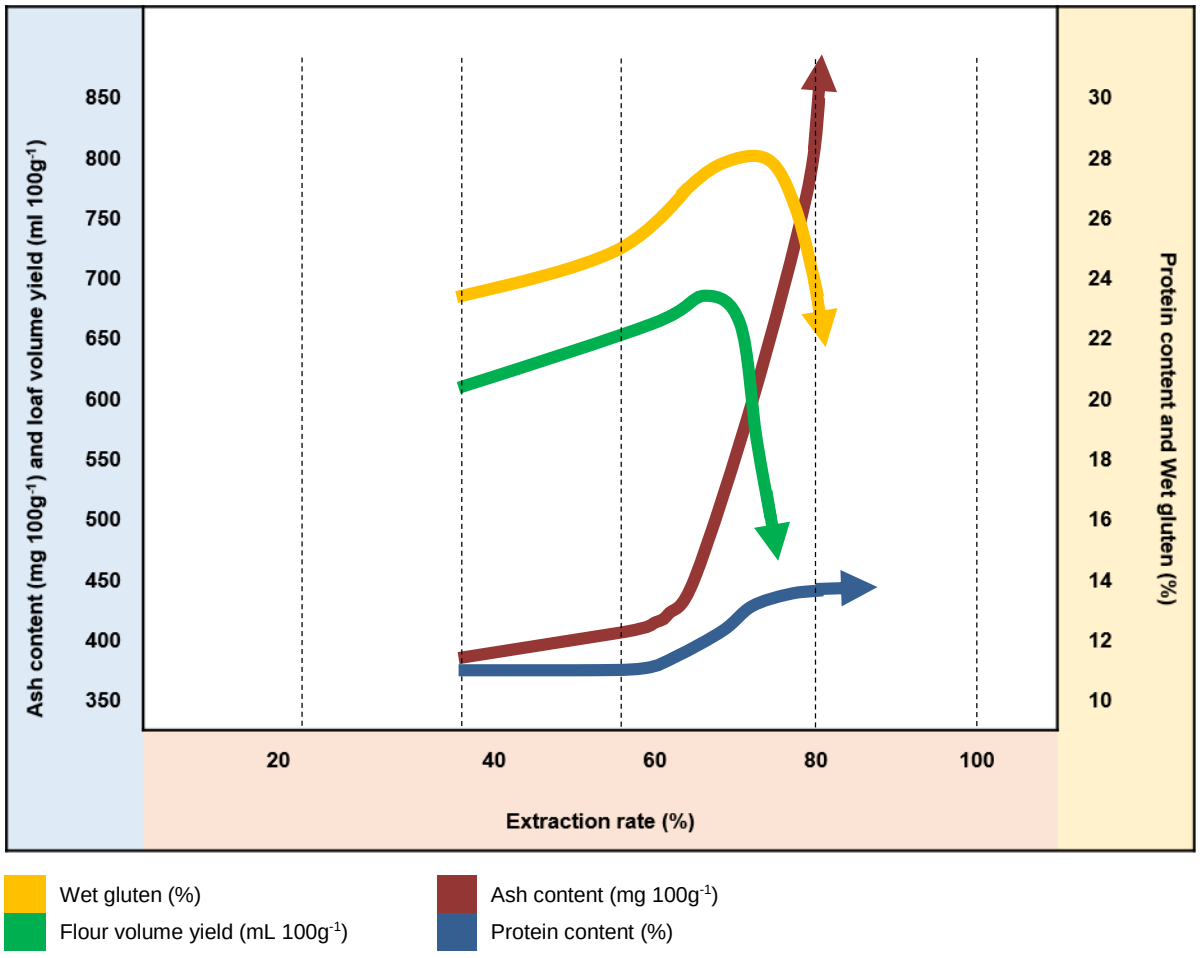


Figure 2.2 Changes in flour values according to extraction rate

Flour wet gluten

The only difference between grain wet gluten and flour wet gluten analyses is that wholemeal flour is used for grain wet gluten analysis and white flour with a high extraction rate (from endosperm) for flour wet gluten analysis (Freund and Kim 2006).

Flour protein content

Similar to grain protein content analysis, except that white flour with a high extraction rate (from endosperm) is used.

Determining the sedimentation value (Zeleny test)

Swelling properties of wheat flours are determined by calculating the sedimentation value (based on the sinking rate of a solid through a liquid) and measures the volume of swollen gluten proteins. Measurement of the volume of sediment has a two-fold function, it provides an indication of the amount of gluten but also reflects the swelling properties of the gluten. The sedimentation value combines both quantitative and qualitative properties of wheat flour during calculation. It has proved possible to predict resting time of dough, gas retention capacity and volume yield through sedimentation testing. The more millimetres that is read on the measuring cylinder the more suitable the flour is for bakery products requiring stronger protein flours (Freund and Kim 2006).

The Zeleny sedimentation test determines the amount and quality of the gluten fraction of a flour by measuring the sedimentation volume (swelling of the gluten fraction suspended in a lactic acid solution) and provides data of the expected dough properties, gas retaining capacity and baking volume of the end product.

2.4.3 Qualitative measurement of protein

2.4.3.1 Rheology

Rheology is strongly based on physics and the deformation and flow of dough in response to an applied stress or strain and categorises results as Newton or non-Newton (Steffe 1996; Schramm 2004). Rheology is particularly important for identifying the influence of flour components on dough behaviour in bread making. The numerous tests available for measuring rheological properties are commonly described as either empirical (descriptive, imitative) or fundamental (basic) (Weipert 1990). Empirical rheological tests are often criticised, for example for interpretation of results in non-SI units and requirement of large sample sizes (Weipert 1990; Dobraszczyk and Morgenstern 2003) but enables characterisation of flour properties for production of specific end products. The dough properties dependent on the gluten of flour are defined by measurements of water absorption, mixing stability and dough extensibility.

Measuring water absorption and mixing behaviour of wheat flour with the farinograph

According to the handbook of the AACCI (AACCI Method 54-21.2), dough is prepared under (1) standardised conditions and while the dough is being developed (2) resistance to the mixing paddles is measured and recorded continuously, to achieve uniform dough properties, (3) water absorption of the dough is determined by adding water to the flour sample (standardised at a moisture content of 14%) with a burette until the dough reaches a consistency of 500 farinograph units (FU). This amount of water applied to the dough is a measure of the water absorption of the flour required to reach an average dough firmness of 500 FU. Once the amount of water is determined, a second batch of dough is prepared from the same flour by adding the whole amount of water all at once and mixing the dough for 12 min. (4) A curve is drawn of the mixing process from which development time, dough stability and dough softening can be read. This farinograph curve resembles the gluten properties of a dough and indicates water absorption, which is largely dependent on gluten quality, the dough development time which indicates the rate at which dough swelling takes place and the degree of softening (reduction in consistency) indicating whether the flour is

strong or weak. Farinograph curves with a broad band (a) and only slight fall over the mixing time (b) indicate an elastic dough that does not become weaker even after exposure to intensive mechanical stress and are suitable for bread rolls (Figure 2.3a).

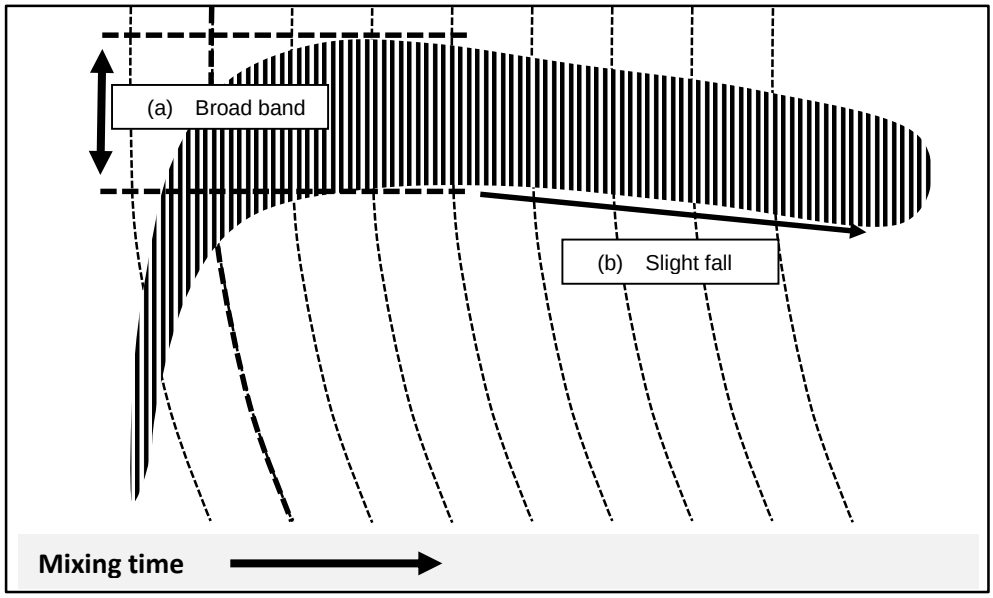


Figure 2.3a Representation of a farinograph curve from a strong flour

Narrow curves (a) with very obvious dough softening represented by a steep fall (b) result from weak flour (flour with weak gluten) which is preferable for biscuit production (Figure 2.3b).

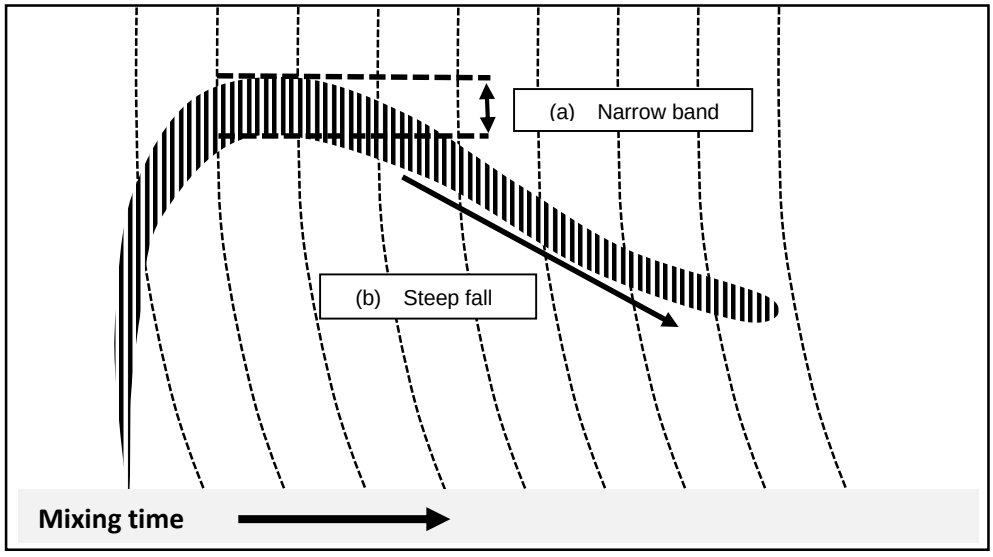
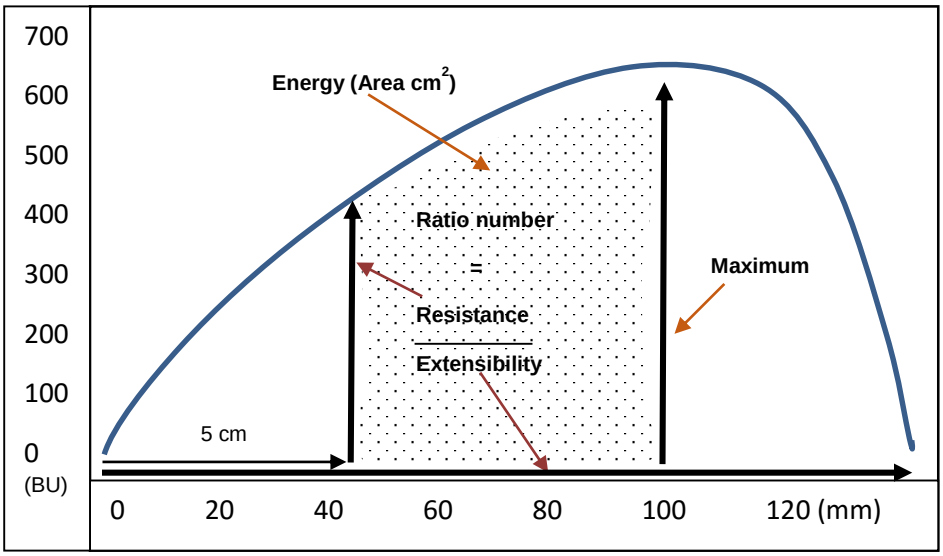


Figure 2.3b Representation of a farinograph curve from a weak flour

Measuring the stretching properties of wheat dough with an extensograph

A dough is prepared from flour, salt and water under standard conditions in the farinograph and then rolled out in the rounder and shaping unit. After 45, 90 and 135 min the piece of dough held by clips is pulled by the stretching hook until it ruptures. The force applied during stretching (resistance to extension) and the extensibility (length of the extension until rupture) are recorded on graph paper. A flat extensograph curve indicates that the resistance to extension is low and the dough has weak characteristics with resulting low loaf volume, but if the curve rises sharply and is pointed at the top, the dough may have short characteristics. With this type of dough, it is difficult to influence the structure of the dough as the gas formed by yeast is not enough to achieve sufficient leavening. Bread and rolls have an extensograph with a well balanced curve as illustrated in Figure 2.4. The extensograph also provides a means of determining the effect of additives (such as ascorbic acid, cysteine, cysteine and proteinases) on the properties of the gluten (AACCI Method 54-10.01).



BU (Brabender units) = EU (Extensograph units)

Figure 2.4 A well balanced extensograph curve showing dough with properties suitable for baking of bread rolls

Measuring the extensibility of dough with the alveograph

For testing extensibility of dough with an alveograph, a disc of dough in a holder is blown up into a bubble with the aim of investigating the stretching properties of the dough up to the point when the bubble bursts (AACCI Method 54.30A). The process of extension and the volume achieved are recorded as a curve from which baking properties of a wheat flour can be determined (Figure 2.4). The P-value indicates the resistance of the dough to deformation and is related to workability and stability and measured in millimetres (mm). Flours with high P-values mostly have a high gluten content and absorb a relatively large quantity of water. The L-value measures the distance (in mm) from the point where the curve starts, to the point where the dough bubble ruptures under conditions of the test and represents the extensibility of the dough or its ability to rise. Measuring the area under the curve and then multiplying it by another factor (6.54) determines the value "W" which is proportional to the baking strength of the dough. Values of "W" range from 45 for very soft flours to 400 for very strong, hard red wheat flours. The relationship between "P" and "L" expressed as a ratio, serves as an index of gluten behaviour. High values of "P" and "W" indicate a strong flour (Dapčević Hadnađev et al. 2011).

Mixograph for determining the water absorption and mixing properties of dough

Mixograph analyses enables prediction of baking functionality with very low amounts of flour. Water absorption, dough tolerance, gluten strength and mixing time can be determined (AACCI Method 54-40A).

2.4.3.2 Testing dough fermentation

Testing the fermentation of doughs with a maturograph

The maturograph measures the increase in volume of dough during the process of proving and enables determination of optimal proving (final proving time), proving stability, elasticity and dough level. This test provides an objective analysis and is conducted on the actual piece of dough that includes all the other ingredients (Freund and Kim 2006).

Testing increase in the volume during the baking process with the oven rise recorder

Dough is heated in an oil bath from approximately 30˚C to 100˚C at the core. The resulting curve provides information on development of baked volume by measuring baked volume increase during heating and recording stages during which gas escape.

2.4.3.3 Baking tests to determine baking properties of flour

All the previous tests are regarded as indirect testing whereas baking tests show real results of all the complex interactions of the combined ingredients and provide accurate prediction of properties of the dough and baked products. Standardisation of baking tests are crucial and very important for accurate predictions.

Determining quality of European classified wheat flours

These tests are applied in Europe for analysing bread flour for manufacturing of pan bread and rolls and wholemeal flours for pan bread.

Standard baking test of wheat flour for pan bread of German classified wheat flours

This is a baking test suitable for all wheat flours classified according to their ash content and provides information on baking volume, texture and crumb characteristics. The procedures for this test are specific, as application of results in predicting baking quality relies on standardised testing procedures.

Standardised baking test for bread rolls made with wheat flour type 550 (rapid mix test)

Bread rolls are made from flour of wheat flour type 550 (flour type 550 is the German equivalent to all-purpose flour made from a blend of hard and soft wheat with a gluten content of 9-11%). The rapid mixing test was developed to test flours for suitability for making bread rolls and employs very quick methods of mixing and production of rolls.

Standard baking test for pan bread prepared from wholemeal flour

This is suitable for testing wheat flours with a consistency set at 500 FU and flour water content of 14%. The test relies on very specific dough preparation procedures, resting and mixing sequences and evaluations are conducted 15 to 20 hours after baking. The criterion applied during evaluation is very similar to pan loaf evaluation for classified white flour (Freund and Kim 2006).

2.5 Wheat protein quantity vs wheat protein quality

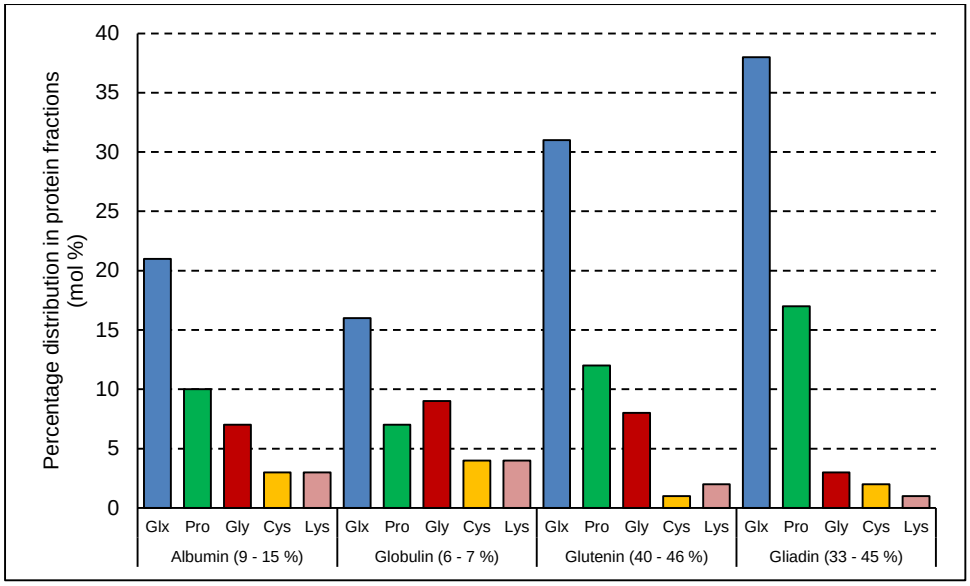
2.5.1 Functionality of protein in the plant and its nutritive value

The word “protein” originates from the Greek word *prōtos* which means *first*. Proteins are part of the macromolecules and are composed of at least 20 amino acids (monomers) joined together by peptide bonds to form a linear protein polymer (chain) that contains nitrogen. The sequence in which the amino acids are linked in the chain is determined by the DNA of the cell that produces the amino acids. This specific sequence of amino acids is important since it firstly determines the structure and secondly the function of the protein. Protein functions can contribute to the structure of the organism (fibrous protein), act as enzymes (globular protein) or serve as receptors or channels for charged (polar) molecules to pass through the cell membrane (membrane proteins). Enzymes are specifically important for the functioning of digestive systems in animals and humans, but also during production of leavened bread dough by acting as organic promoters for initiating or accelerating specific biochemical reactions.

Protein is one of the three nutrients used as energy sources (calories) by the human body and is an essential component in the everyday diet for building muscle, skin, and bones and similarly to carbohydrates, provides four calories of energy per gram (http://www.medicinenet.com). One of the major differences between plants and animals, which include humans, is that animals must access carbon compounds from external sources

whereas plants have the capacity to acquire all of their amino acids from their own cellular proteins. The diet of animals provides approximately half of their total amino acid requirement, as well as the functional nitrogen necessary for production of the other half (Young and Pellet 1985). It is furthermore estimated that approximately 50% of protein in diets of developing countries come from cereals, with a consequent increase in wheat consumption, resulting from the gluten component capable of baking western-type soft breads. Similarly, to the early years of the Roman Empire, this trend is associated with increased urbanization, convenience of wheat bread as staple food and its association with modernisation. Protein intake through food provides amino acids necessary for synthesis of new proteins vital for growth, replenishes the amounts of protein lost through digestion and strengthens immunity against infection, aiding recuperation after sickness or injury. Wheat is an excellent source of complex carbohydrates occurring in the starch component of seeds and is more important for the human diet than the simple carbohydrates. Complex carbohydrates are a more sustainable energy source as it has a low glycaemic value (releasing energy more gradually). Essential amino acids are the amino acid group that cannot be manufactured by living organisms and must be obtained through consumption of specific foods, such as hard wheat (high protein content) containing essential amino acids equivalent to other cereals (Sarani et al. 2013).

Protein in the wheat grain is an integral component of the bread making process and has been the subject of numerous research studies. Osborne (1924) classified wheat proteins into two groups, namely structural and storage proteins with albumin/globulin and gliadin/glutenin forming the respective solubility classes of each group. These proteins occur in different concentrations in the endosperm and ranges between 40 to 46% for both glutenin and gliadin and 6 to 7% for globulin (Figure 2.5). The concentrations and ratios between protein fractions also determine the bread making ability of different wheat types but also influence the nutritional value through distribution of amino acids in these fractions. Wheat contains relatively high values of glutamine/glutamic acid compared to other cereals (Figure 2.5).



Glx – glutamine/glutamic acid, Pro – prolamine, Gly – glycine, Cys – cysteine, Lys – lysine (Adapted from Eliasson and Larsson 1993)

Figure 2.5 Amino acid distribution of protein fractions in wheat (mol%)

Wheat is an excellent source of group B vitamins (niacin) and group E vitamins (α-tocopherol) and provides significant amounts of iron, zinc, calcium, chromium, potassium and selenium through the everyday diet, although in lower quantities than other food groups. During the production process of white flour, approximately 1% of protein is lost through the removal of bran and germ. Ironically, in the late 1960's investigations were conducted on blending bran and endosperm fractions with white flour to increase protein levels but retain the white flour baking characteristics (Hulse 1974). These blends between straight grade hard winter wheat flour and fine ground particle size mixtures of bran and endosperm fractions produced several baked products (not including pan bread) with acceptable quality and high nutritional value.

Proteins, to a large extent, determine the nutritive value of wheat through amino acids but are also responsible for providing wheat flour with the unique ability to produce a wide and contrasting range of baked products.

2.5.2 Protein quantity

Definition and origin of grain protein quantity (content)

Grain protein content is a measurement of grain quality expressed through unique wheat grades that is of key importance for international wheat trading and purchase of wheat flour required for the various end use applications. Grain protein content is based on the total nitrogen content of the grain which is multiplied by a factor of 5.7 to obtain an estimate of percentage protein content. In this regard, two general assumptions are made namely, (i) that protein is the highest nitrogen containing component of the grain and (ii) that most of the protein is gluten (the primary factor determining baking quality). Definitions of bakers and wheat breeders in regard to bread making quality are different but address the same factors.

Baker's definition: Bakers are more focussed on the bread production processes and define factors responsible for bread quality as: *"… complex interactions of raw materials, their qualities and quantities used in the recipe and the dough processing method".* Cauvain and Young (2009) elaborated on this view point when they explained their problem solving procedure and key solutions for low loaf volume (Table 2.5). They stated that low loaf volume is a result of low gas production (related to the fermentation process) and/or gas retention (containing the gas within the dough medium).

Table 2.5 Primary factors responsible for low loaf volume

Low gas production	Low gas retention
• Yeast activity or level too low	• Improver level too low
• Lack of yeast substrate	• Incorrect improver formulation
• Dough temperature too low	• Combination of improver and flour too weak for the bread making process to be used
• Proving temperature too low	• Enzyme activity too low
• Salt level too high	• Energy input during mixing too low
• Proving temperature/time/yeast combination incorrect	• Mixing time too short
	• Dough temperature too low

(Adapted from Cauvain and Young 2009)

Although the above example makes no mention of protein content or gluten formation, Cauvain and Young (2006) put the baker's definition in the correct perspective when they commented that both protein quantity and quality are of great importance in formation of gluten networks, determining the extent to which gas generated by yeast fermentation is trapped. They also acknowledge that protein content affects water absorption as high protein flour has higher water absorbing capacity and bran of whole wheat flour reduces gas retention and consequently loaf volume and should be produced from flour with higher protein content. A saying exists that the baking industry purchases flour and not wheat varieties, indicating that blending of wheat grades by millers and the addition of various flour enhancers for utilisation in the commercial baking process render raw grain protein content only as a rudimentary starting point for flour production. Therefore, the protein content of flour utilised at bakeries generally varies not according to varietal differences, but largely because of blends and adjustments made by millers and bakers (Cauvain 2012). Flour protein content used in the baking industry for typical sponge and dough production, will be adjusted through various means to have a protein content of more or less 12% and a falling number higher than 250 seconds.

Cereal scientists and food technologists' definition: The general opinion in food science and plant breeding regarding bread quality is summarised by Tipples et al. (1996) stating that the three factors determining wheat type, also influence bread quality: *"These are hardness, gluten strength and protein content"*. They, however, also add that: *"Even relatively poor quality wheat can produce bread that is significantly more palatable than that made from flour of other cereals"*. Abang Zaidel et al. (2010) combined both perspectives by stating that the rheological properties of dough and gluten are determined by flour composition (protein quality), processing factors (mixing time, energy and temperature) and ingredients (water, salt, yeast, fats and emulsifiers).

Results from studies conducted on the role of protein content in rheology (Janssen et al. 1996; Sliwinski et al. 2004) agree that high protein content flour (strong flour) produces a better gluten and dough quality through higher extensibility, loaf volume and height and volume expansion. Longer mixing which requires more energy and involves higher production expenditure, is however required for stronger flour and positive correlations exists between dough mixing time and percentage of glutenin protein in the flour (Sliwinski et al. 2004). As far back as the late 1940's, research by Finney and Barmore (1948) found that loaf volume of hard red winter and spring wheat varieties from different environments was determined by protein quantity. Follow up research (Finney and Yamazaki 1967; Finney 1985) however, changed the original conclusion and reported that both protein quantity and protein quality determined important aspects of bread making (mixing time, dough handling properties, water absorption, loaf volume and crumb characteristics). Grain protein content of a wheat sample does not provide a true reflection of the bread making quality of that sample, although it is widely applied as such, often to the frustration of industrial baking plants in both developed and developing countries.

Protein quantity and wheat grades

Wheat grain grades are applied to specify the protein content of grain and define it in terms of its potential processing quality that eventually determines its end use application. Grades have a double function in wheat commerce, as it firstly determines the payment a producer receives for harvested wheat and secondly, determines the payment a grain dealer receives from a local or export customer for the grade of grain being sold. Assessment of grain protein content promptly after harvesting is of primary importance, as it enables dealers to match or blend wheat grades for industrial flour requirements as quality grades cannot be separated once large scale storage has taken place (blending).

The dealer furthermore needs to ensure that the cost of conducting post-harvest protein content testing and grade separation will be justified through market demands for specific flour

types and ultimately end products from the consumer. Grading determinants all over the world include grain protein content as one of several standards. Grain protein content is determined by a genotype's ability to translocate amino acids to the grain endosperm for conversion to storage proteins (glutenin and gliadin) through efficient utilisation of plant nitrogen reserves. Protein content over a season results from the constant interaction between three factors namely genotype (G) x environment (E) x processing. In this interaction, genotype is the responsibility of the breeder, production management of the environment the responsibility of the farmer and processing the responsibility of millers and bakers (Wrigley and Batey 2012). Globally, industry requirements shaping buyer's specifications drive wheat markets and although these specifications vary between borders, grain protein content is a primary component of most grading systems. In SA the protein content requirement for Grade 1 (highest grade) is ≥ 12% compared to between 8 and 9% for Utility Grade (generally used in animal feed). Although the grain protein content difference between the two grades is only between 3 and 4%, the payment implication for a wheat farmer is substantial and approximately between 800 to 1000 ZAR per ton.

Canadian wheat is classed similarly by variation in protein content levels of individual grades. Premiums, however, are paid for protein content defined as protein price "spreads". The Canadian open market system analyses variations in flour demand of both the Canadian and International wheat markets and provides local wheat farmers access to better prices by disseminating this supply and demand trends through as wheat price "signals" on a daily basis. The benefit for the Canadian wheat farmers is substantial, as effective separation of spreads (grades) in a specific season ensures that farmers with high yield but low protein content assist in evening out high protein content from other regions.

The CBP makes economic sense for countries historically producing lower protein wheat, such as the UK. However, in SA the release requirements for new wheat varieties are determined by high norms, particularly for the primary milling and baking parameters and rheological

quality as specified by the milling and baking industry. Local wheat breeders have over many years adhered to these quality prescriptions and modern day wheat varieties express high quality with high grain yield. Implementation and wide use of the CBP in SA since the 1960's and deregulation of the wheat industry in 1997 have rendered importation of low protein wheat more profitable for bread making in SA than buying wheat from the local markets. The general opinion among wheat farmers is that high protein should be discarded as selection parameter of new varieties in order to allow quicker improvement of high yielding wheat varieties with consequent profitability of wheat production in the country.

Protein quantity in commercial milling and baking quality

Although grain protein content is one of the important factors determining baking potential of wheat flour (MacRitchie 1997; Graybosch et al. 1993; Weegels et al. 1996; Huebner et al. 1999) it often proves inadequate to accurately predict baking quality on its own. Results by Fossati et al. (2010) of baking quality in Swedish wheat varieties address a typical example of bakers acquiring a specific protein content that yields poor wet gluten content. The consequence was that additional expenditure had to be made by supplementing the flour with about 2% dry gluten to meet end product requirements. The correlation between grain protein content and loaf volume for the standard 500 g baking test was good or acceptable but for baking tests requiring longer fermentation correlations, were low (Fossati et al. 2010). Predicting loaf volume through modelling (Lee et al. 2006) with grain protein content, hardness index, mixograph water absorption and peak height and break flour extraction achieved an equation of R^2 = 0.70 with end product quality. Dowell et al. (2008) applied grain, flour, and dough quality into models for prediction of bread quality and concluded that loaf volume, dough mixing time and water absorption could be predicted with R^2 between 0.78 and 0.93.

Variations in the production and climatic environments of the semiarid wheat production regions of Israel occur in and between growing seasons, resulting in significant variation in grain yield and quality (Har Gil et al. 2011). Recently, fluctuations in baking quality of this

region became more common and are leading to similar frustration than in Sweden, with wheat grain often not meeting requirements of the milling and baking industries. Bonfil and Posner (2012) remarked that, as total protein content (quantity) alone does not adequately explain the variation in bread making quality, protein quality should also be considered. According to Ćurić et al. (2001) protein or gluten quantity is not a measure of gluten quality and should rather be characterized by dough extensibility and elasticity. They recommend that gluten index is a better and more reliable indicator of the baking quality of Croatian wheat varieties. Although strong correlations existed for dough stability time with protein content and farinograph absorption, loaf volume only correlated with protein content in one out of five years (Koppel and Ingver 2010).

2.6 Protein quality

2.6.1 Size-Exclusion High Performance Liquid Chromatography

Origin and development

Protein quality primarily refers to gluten quality, which is determined by the concentrations and ratios of protein fractions in kernels. The high speed and reproducibility of SE-HPLC (Hong et al. 2012) and improved protocols inspired by studies applying SE-HPLC, have established SE-HPLC as the preferred method for routine and confirmation analyses of proteins.

Chromatography refers to the specific process of separating molecules in a solute (substance dissolved in another substance) on the basis of their size and shape. The separation procedure consists of two inert phases (chemically unreactive phases) namely a mobile phase (consisting of a gas or liquid solvent carrying the sample molecules) which is pressurised through a solid or stationary phase (the solid or liquid bound to a support medium). Molecules in the sample with sizes larger than the pore size or the chromatographic matrix is not retained or delayed and will elute into the void volume of the column, whereas molecules smaller than

the pore size of the matrix penetrate into the pores, follow longer flow paths and eventually elute in order from large to small sized molecules.

Size based separations of molecules originated from Synge and Tiselius (1950) who noted that small molecules are excluded from the small pores of zeolites as a result of their molecular size. Zeolites have an open, three dimensional crystal structure consisting of elements such as aluminium, oxygen, and silicon with alkali metals (such as sodium, potassium and magnesium) and water molecules trapped in the fissures between them. This characteristic inspired McBain (Huo 2011) to rename the zeolites to “molecular sieves” which consequently formed the basis of SE-HPLC. Lindqvist and Storgårds (1955) and Lathe and Ruthven (1955; 1956) improved SE-HPLC methodology for effective separation of peptides from amino acids on columns packed with starch. These columns (packed with potato or maize starch) resulted in very low adsorption (bonding of atoms, ions, or molecules from a gas, liquid, or dissolved solid to a surface) and were able to separate a variety of proteins and peptides. Starch, however, had very low mechanical strength limiting the speed of separations and was also poorly defined. The challenge facing chromatographers and SE-HPLC were therefore the continual betterment of new packing materials for columns, allowing effective separation at acceptable separation speeds without interference from surfaces and chemicals used in the technique itself. Developments in this regard started with Sephadex (dextrans cross linked with epichlorohydrin) which has limited interaction with proteins but provided much more mechanical strength than starch. Porous silica became the primary chromatographic stationary phase media in the 70’s and had superior mechanical strength, a non-swelling nature and was insensitive to varying conditions. Increased performance became possible as the increased mechanical strength allowed for reduced particle size. A drawback of silicon as size-exclusion medium for separating proteins, was the presence of silanols occurring on the surfaces of medium matter and creating strong ionic interactions. If free silanol groups remain on the surface of a silica gel after bonding treatment, the retention of solutes can be reduced, since the disconnected groups will attract cations (Chongying 1992). Recent developments

are exploring porous hybrid organic and inorganic particles (Wyndham et al. 2003) as base particles of which alteration of their surfaces with diol groups (a chemical compound containing two hydroxyl groups) reduced silanol activity. Reduced silanol interference allows for inclusion of smaller amounts of salt additives (generally used for reducing ionic interactions with proteins) resulting in significant gains in chromatographic efficiency (Bouvier et al. 2010). Most SE-HPLC columns are packed with porous particles with absorbency between 35-40% which prolongs analysis time without benefitting separation efficacy. Bundles of aligned porous fibres applied in SE-HPLC (Czok and Guiochon 1990) were able to reduce absorbency to between 15 and 18% that significantly increased the intra particle pore volume. The improved pore volume did, however, not improve resolution probably because the aligned fibres limited outward spreading through the column. Li et al. (2009) prepared poly monoliths for biopolymer separations that (i) exhibited low protein binding in aqueous buffers and (ii) had chromatographic efficiency comparable to a packed bed of approximately 8 µm particles and exhibited separation of peptides and proteins across a broad molecular weight (MW) range (to 670000 Da) with most of the resolving power available for MW less than 66000 Da.

Size-Exclusion High Performance Liquid Chromatography in quality analyses of the wheat protein complex

HPLC is very important for analyses of biological and pharmacological substances, including organic acids, proteins, saccharides, vitamins and various metabolites. In the assessment of the role of wheat proteins in baking quality, SE-HPLC is a fast and accurate technique for separation of protein into the three wheat endosperm protein classes namely glutenin, gliadin and albumins/globulins (Larroque et al. 1997). This technique is used to generate information of different protein fractions (Autran 1994) and the inherent factors (physical and chemical) determining baking quality of bread wheat genotypes and results generally correlate strongly with bread making quality. Studies on wheat in SA using SE-HPLC have been conducted on a regular basis. A study by Labuschagne and Aucamp (2004) showed that insoluble polymeric proteins had no significant effect on quality in a single environment but when measured over

environments had a significant positive effect on quality. Sodium-dodecyl sulphate (SDS) insoluble small monomeric proteins furthermore correlated negatively with grain protein and flour protein. Application of SE-HPLC and RP-HPLC (reverse phase high performance liquid chromatography) by Koen (2006) confirmed that gluten proteins can be used for prediction of quality in durum and bread wheat. Glutenin peaks 45 and 39 in bread wheat genotypes contributed significantly to flour protein content and mixograph development time, whereas in durum, glutenin peak 42.5 primarily contributed to SDS-sedimentation volume. Morojele and Labuschagne (2014) used SE-HPLC data to calculate ratios of glutenin to gliadin, polymeric to monomeric, HMW subunits to LMW subunits and correlations between quality parameters to verify high bread making quality of SA genotypes.

2.6.2 Defining protein quality

Gluten is a key component of bread making quality but an artefact (manufactured article) only forming with the combination of glutenin and gliadin proteins during hydration and mechanical mixing of flour during dough preparation. These two fractions constitute between 80 to 85% of the total protein concentrations in wheat endosperm, which are largely determined by genotype (wheat variety) and environment (production conditions, soil and climate). Wheat flour proteins can be divided into two broad groups, the gluten and non-gluten proteins. Non-gluten proteins primarily include albumin and globulin, which are considered as metabolic proteins, but may have some role in bread making (Hoseney et al. 1969a, b). Similarly, to protein content, protein quality is largely influenced by genotype and environment where genotype determines the concentrations and ratios of the gluten forming proteins. These proteins are divided into two main fractions based on their solubility in aqueous alcohols: soluble gliadin fractions and insoluble glutenin fractions (Wieser 2007). Gliadin fractions are monomeric proteins with MW distribution between 28 000 to 55 000 Daltons (Da) classified into α/β (fastest migrating), γ and ω types (slowest migrating). The glutenin fractions are polypeptides (linked by both intra and intermolecular disulfide bonds) forming large polymers

with MW ranging from 500 000 to more than 10 million Da (Khan and Nygard 2006). Based on primary structure, glutenin subunits are divided into the HMW subunits (MW approximately 67 000 to 88 000 Da) and LMW subunits (MW approximately 32 000 to 35 000 Da). Supply of nitrogen primarily during advanced growth stages of the plant, determines the concentrations and ratios of gluten protein accumulating in grain. Gliadin, for instance, increased at a faster rate in response to increased nitrogen. Variations in the concentrations and ratios of gluten proteins are determined by the genotype, but Peña (1996) reported that this genotypic effect on gluten proteins, however, disappears when grain protein content decreases below 9%.

2.6.3 Protein quality: correlations with rheology and milling and baking quality parameters and measurement with Size-Exclusion High Performance Liquid Chromatography

Through rheology (the study of flow and deformation of matter) various food ingredients have been classified into groups between the solid and liquid states, meaning they contain both viscous and elastic behaviours, collectively referred to as viscoelasticity (Abang Zaidel et al. 2010). In molecular models (Figure 2.6a) designed from small deformation studies, gluten development is related to interactions between glutenin proteins through disulphide bonding. The viscoelastic (viscosity + elasticity) character of dough (gluten) is determined by the concentrations and quantities of glutenin and gliadin proteins in a flour type and also by the structural characteristics of proteins, particularly cysteine residue distribution and number.

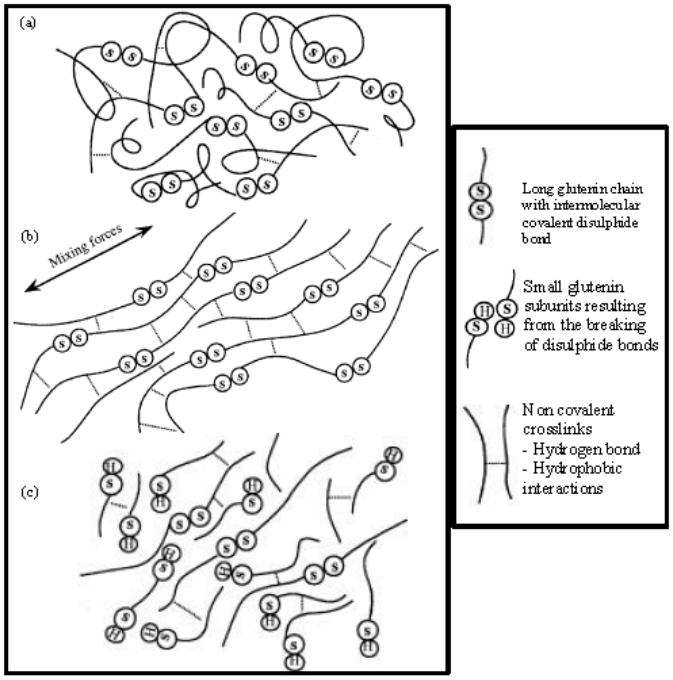


Fig. 2.6 Schematic diagram of gluten proteins during different stages of dough production: (a) gluten chain formation (b) long gluten chains linked at disulphide bonds (c) chains begin to break due to over mixing forming sticky dough (Letang et al. 1999)

Elasticity, determined by glutenin, refers to the ability of matter to return to its original form once the deforming force is removed, whereas viscosity, determined by gliadin, describes the ability to remain deformed even after removal of such a force. Both glutenin and gliadin occur in different concentrations and ratios in wheat flour types and when hydrated during dough mixing, the elastic and viscous components are combined to provide the unique viscoelastic properties discerning different baked products from one another. As dough mixing continues, glutenin proteins become hydrated and align due to shear and stretching forces occurring between the mixer blades and sides of the mixing bowl (Figure 2.6b). Gluten networks at optimum development have strong interactions between the polymer cross links, resulting in optimum dough strength with maximum resistance to extension and restoring forces (Letang et al. 1999). During this process, the monomeric gliadin proteins form an adjoining constituent within the long polymeric networks strengthening resistance to extension and deformation by adding viscosity to the dough matrix. With over mixing, glutenin depolymerises as a result of breaking cross links due to the weakening and breaking of disulphide bonds, with these ensuing shorter chains producing sticky dough (Figure 2.6c).

Conflicting results have been reported on the direct roles of gliadin and glutenin protein fractions on baking. Gliadin proteins are strongly and positively related to loaf volume (Finney et al. 1982; Weegels et al. 1994; Khatkar et al. 2002a; b) and results have indicated increases in loaf volume and peak dough resistance when the baking flour was supplemented with individual or total gliadins. MacRitchie et al. (1991) stated that although both soluble protein and insoluble polymeric protein (glutenin) of flour correlated with loaf volume, correlations of soluble protein were much stronger than correlations of insoluble polymeric protein. An increase of HMW glutenin (a major component of insoluble glutenin) resulted in higher dough strength, but loaf volume was not affected. Gupta et al. (1992) also reported significant correlations between total polymeric protein (sum of soluble and insoluble polymeric proteins) and loaf volume, with the outcome varying according to the type of baking test but found no correlation between concentrations of gliadin and loaf volume. According to Naeem and MacRitchie (2005) the two primary factors controlling the physical properties of dough are (i) the ratio of glutenin to gliadin and (ii) the molecular weight distribution of the glutenin fraction. This would imply that an optimum glutenin to gliadin ratio exists in genotypes for achieving maximum loaf volume, although this ratio may vary with changes in the molecular weight distribution of glutenin.

2.6.4 Genetic- and environmental effects determining the amounts and concentrations of protein fractions

Genetic effects

The composition of proteins and protein subunits is genetically determined (Payne et al. 1987; Johansson et al. 1993) but the relative quantity of specific proteins, protein subunits, and protein groups, as well as amount and size distribution of polymeric proteins are dependent on environmental conditions (Wieser and Seilmeier 1998) and genetic determination (MacRitchie 1999). Proteins forming gluten during dough formation originate from the endosperm and act as a source of amino acids for the germinating grain and seedling. Seed

storage protein content (gliadin and glutenin) was found to be the most important baking quality component of wheat (Dowell et al. 2008). The gliadin proteins (prolamins) are encoded by several loci on chromosome groups one and six (Shewry and Halford 2003). Branlard et al. (2001) and Eagles et al. (2002) conducted extensive research on associations between allelic variability at these loci and functional dough properties. The synergistic interaction of combinations of transcription factors (a substance such as a protein contributing to making RNA from a DNA template through RNA polymerase) characterised by Mena et al. (1998) and Dong et al. (2007) resulted in complete initiation of gliadin and glutenin fractions (Yamamoto et al. 2006). Plessis et al. (2013) studied the effects of chromosome regions on gliadin and glutenin composition and identified distinct trans-regulatory determinants (DNA sequences encoding transcription factors) of gliadin and glutenin composition operating in different ways. They also found that several transcription factors with unknown functions were involved in the genetic control of the scaling parameters of an eco physiological model predicting the effect of the environment on grain protein content as well as gliadin and glutenin composition. This is in accordance with findings by Martre et al. (2003) and Triboï et al. (2003) that gliadin and glutenin fractions in grain, scales allometrically (allo = different, metric = measure) with the total amount of nitrogen per grain and is not dependent on nitrogen availability or environmental effects. Genes related to nitrogen assimilation (forming of amino acids from inorganic nitrogen compounds present in the environment) may also affect the relative concentrations of gliadin and glutenin proteins without direct involvement in gliadin and glutenin gene regulation. These genes (related to nitrogen assimilation) may affect the quantity of nitrogen per grain (Hirel et al. 2007; Habash et al. 2009; Quraishi et al. 2011) and consequently also affect grain protein content and gliadin and glutenin protein composition.

Environmental effects

Temperature and moisture stress can directly affect gliadin and glutenin concentrations through protein polymerization (the process during which intermolecular disulphide bonds are formed) or the duration and timing of growth stages (Har Gil et al. 2011). Before anthesis,

temperature and moisture stress during vegetative growth stages affects wheat protein composition through the sink/source relationship with nitrogen that affects yield and grain protein content (Triboï et al. 2006). After anthesis high temperatures and low relative humidity firstly alters the glutenin to gliadin ratio (Blumenthal et al. 1993) with less effect on gliadin than on glutenin accumulation (Stone and Savin 1999) and secondly, accelerate onset of protein polymerization (Daniel and Triboï 2002). Genotypic variations also occur as a result of different alleles on different genes resulting in different responses to temperature change, heat shock and moisture stress (Lawrence et al. 1988). Garrido-Lestache et al. (2004) and Har Gil et al. (2011) reported that under farm conditions where moisture availability is optimal, gluten index gradually reduces if moisture levels continue increasing. However, when moisture is limited, a positive correlation develops between moisture increases and gluten index. They reported that the factors with the largest influence on wheat quality in southern Israel are variety and negative effects from cumulative temperature stress, excess water and excess nitrogen fertilization. Dough properties and baking performance of wheat are strongly dependent on both genotype and environment (Peterson et al. 1992; Johansson and Svensson 1998; 1999). Johansson et al. (2001) concluded in their study that composition of gliadin and glutenin, variation in total amount of HMW-GS, glutenin: gliadin ratio and differences in relative amounts of SDS-soluble and SDS-insoluble polymeric proteins are primary factors determining gluten strength of genotypes. Nitrogen levels influenced protein components containing glutenin and gliadin and lead to variations in protein content and loaf volume. Gluten strength of genotypes was not stable at different nitrogen levels, indicating that nitrogen levels influenced gluten strength as well as loaf volume.

2.6.5 The effect of sink/source on the amounts and concentrations of protein fractions

Accumulation of non-gluten proteins (albumin and globulin) occurs at the early stages of grain development (before 250°Cd) when endosperm cells are still dividing, whereas accumulation of gluten proteins (gliadin and glutenin) occurs later after completion of cell division (after

250°Cd) when grain growth only occurs from cell expansion (Stone and Nicolas 1996; Triboï et al. 2003). Grain protein composition depends primarily on genotype, but is significantly affected by environmental factors and their interactions (Graybosch et al. 1996; Huebner et al. 1997; Triboï et al. 2000; Zhu and Khan 2001). It is still unclear what mechanisms in genotype and environment modify the accumulation of protein fractions and if accumulation is regulated either through processes within the grains or by nitrogen supply from the vegetative organs or by both (Triboï and Triboï-Blondel 2002). In a study by Martre et al. (2003) the sink/source ratio was manipulated to determine if overall, nitrogen in the grain (sink) is controlled by nitrogen supply to the grain (source). Most crop and plant simulation models assume that accumulation of nitrogen in the grain is sink controlled (Jamieson and Semenov 2000) and have not considered that separation of nitrogen within the grain can also occur. The conclusion of Martre et al. (2003) was that nitrogen accumulation is sink limited or co-limited by both source and sink for the first 10 to 15 days after anthesis and any modification of the gliadin: glutenin ratio resulted from modification of total nitrogen content per grain.

2.6.6 Explaining the effects of proteins on baking quality

Biological phenotyping is a modern and all-inclusive approach for assessing the intricacies in living organisms, in both plants and animals, and traditionally consists of two characterisation planes (“omics”) namely genomics and proteomics (Wright et al. 2012). Genomics-transcriptomics is the study of gene expression with the transcriptome corresponding to the complete set of RNA transcripts present in particular cells, whereas proteomics refers to the study of protein expression in a specified biological system. Recently, metabolomics came into being and is growing rapidly in popularity. Metabolomics allows for an even wider approach through incorporation of inputs from both genomic and proteomic levels (Courant et al. 2014). Metabolomics is defined as the study of the complete set of metabolites/low MW intermediates that are context dependent and varies according to the physiology and developmental state of the cell (Oliver et al. 1998). Metabolomics focusses on

differences in the profiles of test samples resulting from response to external stimuli such as the effect of biochemical or environmental stresses and food processing.

Perhaps the study topic of examining the role of protein in bread making quality should be approached on a metabolomics level which will incorporate all genetic, environmental and interaction effects in final formulating of the roles the individual proteins have on the quality of the end product.

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Chapter 3

Material and methods applied for determining protein quantity, protein quality and grain yield

3.1 Wheat production and variations in geography and meteorology

The rainfall regions of SA consist of the winter rainfall regions of the western, south-western and southern Cape, transitional regions of the Eastern Cape receiving winter and summer rainfall simultaneously and the summer rainfall regions covering the Free State, Mpumalanga, Northern Cape, North West province and KwaZulu Natal (Figure 3.1). Weather systems of the SRR develop through influences of inter tropical systems originating from the north (FAO 2013), whereas WRR are characterised by frontal systems developing over the southern oceans and then sweeping in over the mainland.

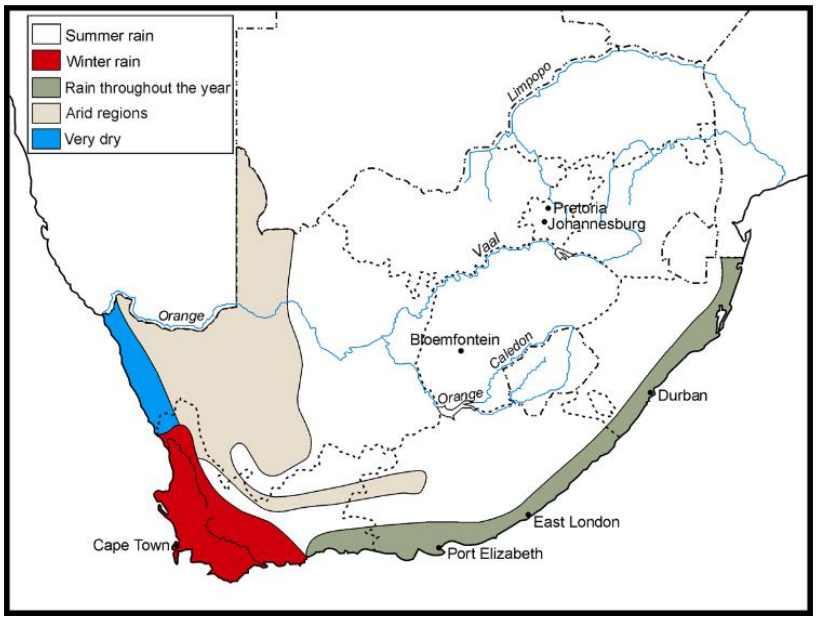

Figure 3.1 The major rainfall patterns in South Africa

3.1.1 Irrigated wheat in the cooler production regions in the summer rainfall region

Both irrigation schemes feeding the Upington and Vaalharts test sites fall within the Orange River basin, encompassing approximately 885 000 km^2. The Vaalharts irrigation scheme (Vaal River and Harts River) covers 35 302 ha and includes 31 372 ha in the Northern Cape and 3 570 ha in the North West province (Verwey and Vermeulen 2011). The annual rainfall for Jan Kempdorp (Vaalharts test site) is approximately 470 mm (from October to March) with Hutton, Kimberley, Hutton/Mispah, Dundee and Katspruit/Kroonstad soil types the most common of the region (J.H. Barnard 2008 personal communication). Hough and Rudolph (2003) described soils of the region as alluvial and Kalahari sand with the implication that water holding capacity is poor. The confluence of the Vaal River (beginning in the Mpumalanga province) and Orange River (rising in the Lesotho Highlands) is close to the town of Douglas. From here the Orange River passes Upington and eventually becomes the boundary between SA and Namibia, providing irrigation water to 884 ha of farmland of which 600 ha is on the SA side. A significant increase in evapotranspiration occurs from the origin of the Vaal in the east to the lower Orange in the west. This is the result of gradual increase of temperatures together with a significant decline in precipitation towards the west. Spring type wheat produced at high seeding densities and fertiliser rates form the base of wheat production under irrigation in this region (Conley 1996).

Trial means for grain yield in the NCEP for irrigated wheat of the cooler production regions in 2012 were 0.76 and 1.14 ton ha^{-1} respectively higher than the four-year mean (2011 to 2014) and the 2013 yield (Table 3.1). The good growing conditions of 2012 are also reflected in higher hectolitre mass (82.17 kg hl^{-1}) but also higher α-amylase activity (falling number of 371 secs) compared to the four year and 2013 means. The low percentage grain protein content of 2012 is a common trend in seasons with high grain yield. A very interesting observation is that although intensive irrigation scheduling in combination with split

applications of nitrogen is practised, climate continues to affect grain yield and quality in marginal wheat seasons (ARC-SGI 2014a).

Table 3.1 Grain yield and grain quality of irrigated wheat in the cooler production regions of the summer rainfall region (ARC-SGI 2014a)

	Season	Grain yield (ton ha^{-1})	Hectolitre mass (kg hl^{-1})	Grain protein (12% moisture base)	Falling number (secs)
NCEP mean for the cooler irrigation area	2013 (n=5)	8.74	80.29	12.17	330
	2012 (n=5)	9.88	82.17	11.73	371
1st seeding date	2011 to 2014	9.50	81.39	12.12	356

n = number of test sites used for the first seeding date in 2012 and 2013 in the irrigated trials

Higher wheat yields and quality in 2012 for irrigated wheat can be linked to lower deviation from the nine year mean for rainfall during the Post-anthesis period at both Upington and Vaalharts (Table 3.2). This period also corresponds with the highest maximum temperatures during the wheat cycle when irrigation scheduling might not keep up with the increased transpiration rate. Higher temperatures in 2013, probably resulting from the drier conditions, would also have affected maturation of wheat.

Table 3.2 Meteorological data for Upington and Vaalharts (summer rainfall region) in 2012 and 2013 with the deviations from the long term mean (2005 to 2013) of the ARC Institute for Soil, Climate and Water (ARC-ISCW 2014)

Test site	Season	Crop stage	Seasonal rainfall total (mm)	Monthly temperature (˚C)	
				Min	Max
Upington	2012	Pre-seeding (Jan to May)	133.86 (-5.97)	7.03 (-6.86)	31.72 (-0.41)
		Seeding period (Jun and Jul)	31.24 (+15.05)	3.03 (-0.13)	22.64 (+0.34)
		Early growth (August)	0.76 (-0.38)	2.43 (-0.45)	24.69 (+0.57)
		Pre-anthesis (September)	1.02 (+0.74)	4.15 (-1.73)	27.73 (-0.45)
		Post-anthesis and grain filling (Oct and Nov)	62.48 (-8.17)	12.96 (-0.23)	35.29 (+2.35)
	2013	Pre-seeding (Jan to May)	107.69 (-32.14)	6.79 (-7.10)	34.77 (+2.63)
		Seeding period (Jun and Jul)	18.29 (+2.10)	2.42 (-0.74)	23.99 (+1.69)
		Early growth (August)	0 (-1.14)	0.98 (-1.90)	23.86 (-0.26)
		Pre-anthesis (September)	0 (-0.28)	3.11 (-2.77)	27.67 (-0.51)
		Post-anthesis and grain filling (Oct and Nov)	47.25 (-23.40)	12.92 (-0.26)	34.95 (+2.01)
Vaalharts	2012	Pre-seeding (Jan to May)	170.95 (-26.95)	6.64 (-4.00)	31.52 (+0.67)
		Seeding period (Jun and Jul)	12.20 (-35.48)	-0.16 (-1.97)	21.94 (-0.16)
		Early growth (August)	1.78 (-2.23)	4.14 (+1.52)	25.23 (+1.18)
		Pre-anthesis (September)	12.45 (+3.16)	5.72 (-0.38)	26.87 (-1.67)
		Post-anthesis and grain filling (Oct and Nov)	119.88 (-2.93)	13.57 (+0.71)	33.45 (+1.33)
	2013	Pre-seeding (Jan to May)	212.15 (+14.25)	7.33 (-3.31)	31.45 (+0.59)
		Seeding period (Jun and Jul)	5.59 (-42.09)	2.83 (+1.02)	22.57 (+0.47)
		Early growth (August)	5.33 (+1.32)	1.90 (-0.72)	23.31 (-0.74)
		Pre-anthesis (September)	0.25 (-9.04)	5.98 (-0.12)	28.79 (+0.25)
		Post-anthesis and grain filling (Oct and Nov)	102.37 (-20.44)	12.96 (+0.09)	33.09 (+0.97)

Values in brackets are deviations of the year value from the nine-year mean (2005 to 2013)

3.1.2 Rainfed wheat production in the summer rainfall region

In the northern, eastern and coastal belt of South Africa summer rainfall coincides with higher temperatures and encourages production of C_4 type crops such as maize and grain sorghum. Potential arable land of the Free State is estimated at approximately 382 million ha with only 100 000 ha developed and suitable for irrigation (Directorate Agricultural Statistics 2004). In spite of being classified as semi-arid, with the exception of the South Western Free State (arid), the ten year mean for wheat production expressed as a percentage of the total for SA is 37% (Statistics South Africa 2005). Hensley et al. (2006) grouped the Bethlehem test site in the medium potential region of the Free State with plinthic catena (defined as upland-duplex-margalitic soils-common) with rainfall between 680 and 700 mm per year. The Clarens test site is also in the medium potential group but with plinthic catena (defined as upland-duplex-margalitic soils-rare). Annual rainfall of this region is very reliable, well distributed over the growing season and more than 700 mm. Both regions are suitable for production of wheat under rainfed as well as supplementary irrigation (application of irrigation only during critical growth stages of crop development). Wheat yield and quality is primarily determined by the frequency and intensity of main regional showers from late October to the beginning of November. During this period of the season wheat growth stages according to the Zadoks scale (Anderson and Garlinge 2000) vary between anthesis (GS69) and early grain filling (GS75). Rainfed wheat from the SRR has the strongest rheological quality of wheat produced in SA (SAGL 2014). Seeding density and fertiliser rates are low and winter and/or facultative wheat cultivars are cultivated. A major threat to rainfed wheat production in the SRR is poor profitability, resulting in farmers changing over to summer cropping systems with soybeans and maize.

Trial means for grain yield of the NCEP in the EFS in 2012 was 1.49 ton ha^{-1} higher than the four-year mean (2011 to 2013) and 1.98 ton ha^{-1} higher than the 2013 mean (Table 3.3). The optimal growing conditions in 2012 also resulted in higher hectolitre mass but with higher

falling number compared to the four-year mean. Percentage grain protein content in 2012 was low, which is common, as this trait is generally negatively associated with high grain yield. The 2013 season was in sharp contrast with other years and grain yield and hectolitre mass were below the four year and 2012 averages (Table 3.3). The percentage grain protein content (15.55%) was highest compared to 2012 (12.90%) and the four-year mean (14.39%) and falling number (254 secs) was lower than the four year mean and 2012 (ARC-SGI 2014a).

Table 3.3 Grain yield and grain quality of rainfed wheat in the summer production regions (ARC-SGI 2014a)

	Season	Grain yield (ton ha^{-1})	Hectolitre mass (kg hl^{-1})	Grain protein (12% moisture base)	Falling number (secs)
NCEP mean for the eastern Free State 1st seeding date	2013 (n=5)	2.74	76.8	15.55	254
	2012 (n=5)	4.72	79.0	12.90	343
	2011 to 2014	3.23	77.9	14.39	308

n = number of test sites used for the first seeding date in 2012 and 2013 in the Eastern Free State trials

The most important and significant element affecting grain yield and grain quality of rainfed wheat in 2012 and 2013 in the SRR was rainfall (Table 3.4). Although rainfall in the pre-seeding period of 2012 for Bethlehem and Clarens was respectively 132.28 mm and 68.99 mm less than the nine year mean of the EFS, the critical period determining crop performance was during pre-anthesis. In 2012 Bethlehem and Clarens respectively received 30.42 mm and 61.97 mm more than the nine year mean in this period and in contrast, this high rainfall was absent for the corresponding period in 2013. Showers occurring in September are generally considered as an important factor determining yield and high quality of rainfed wheat in the SRR. Another important rainfall period in 2012 at Bethlehem and Clarens was during the pre-seeding period between June and July. These two months are in the middle of the SA winter and coincides with seeding time of wheat. Generally, rainfall during these two months is uncommon, but when it occurs (Table 3.4) contributes significantly towards higher seed germination and seedling vigour, resulting in higher plant stand.

Table 3.4 Meteorological data for Bethlehem and Clarens (summer rainfall region) in 2012 and 2013 with the deviations from the long term mean (2005 to 2013) (ARC-ISCW 2014)

Test site	Sea-son	Crop stage	Seasonal rainfall total (mm)	Monthly temperature (˚C)	
				Min	Max
Beth	2012	Pre-seeding (Jan to May)	208.53 (-132.28)	5.23 (-4.57)	25.09 (+1.40)
		Seeding period (Jun and Jul)	35.06 (+13.28)	-2.03 (-0.55)	16.42 (-0.56)
		Early growth (Aug)	0.76 (-0.85)	0.58 (-0.10)	21.26 (+1.36)
		Pre-anthesis (Sep)	42.93 (+30.42)	4.84 (+0.02)	20.28 (-3.63)
		Post-anthesis and grain filling (Oct and Nov)	189.73 (-47.42)	11.28 (+0.39)	25.23 (-0.04)
	2013	Pre-seeding (Jan to May)	335.02 (-5.79)	5.18 (-4.62)	23.92 (+0.23)
		Seeding period (Jun and Jul)	0 (-21.78)	-1.14 (+0.34)	17.93 (+0.95)
		Early growth (Aug)	4.06 (-5.20)	0.45 (-0.23)	18.91 (-0.99)
		Pre-anthesis (Sep)	7.87 (-4.64)	4.38 (-0.44)	23.50 (-0.41)
		Post-anthesis and grain filling (Oct and Nov)	352.3 (+115.15)	10.23 (-0.67)	24.46 (-0.81)
Clar	2012	Pre-seeding (Jan to May)	286.51 (-68.99)	5.22 (-4.45)	23.54 (+0.98)
		Seeding period (Jun and Jul)	74.93 (+50.83)	-0.75 (-0.68)	15.69 (-0.51)
		Early growth (Aug)	10.16 (-6.73)	2.08 (-0.35)	19.73 (+0.37)
		Pre-anthesis (Sep)	85.09 (+61.97)	5.15 (-0.72)	18.82 (-3.79)
		Post-anthesis and grain filling (Oct and Nov)	289.56 (+13.50)	10.53 (+0.03)	23.32 (-0.53)
	2013	Pre-seeding (Jan to May)	346.46 (-9.04)	4.86 (-4.80)	22.79 (+0.22)
		Seeding period (Jun and Jul)	0 (-24.10)	1.02 (+1.08)	17.18 (+0.98)
		Early growth (Aug)	11.94 (-4.95)	1.52 (-0.91)	17.71 (-1.65)
		Pre-anthesis (Sep)	2.54 (-20.58)	5.47 (-0.40)	22.51 (-1.00)
		Post-anthesis and grain filling (Oct and Nov)	301.48 (+25.42)	9.98 (-0.52)	23.47 (-0.39)

Values in brackets are deviations of the year value from the nine-year mean (2005 to 2013), Beth: Bethlehem, Clar: Clarens

3.1.3 Rainfed wheat production in the winter rainfall region

The central and south western regions of SA are semi-arid, receive winter rainfall and have a temperate climate suitable for production of C_3 plants, including most small seeded cereal

crops such as wheat, barley and oats. The primary production regions of wheat in the WRR of SA are concentrated in the Swartland and Rûens regions of the Western Cape. Wheat is produced on residual and duplex soil types with limited depth and water holding capacity (ARC-SGI 2014b) and seasonal variation in precipitation determines grain yield of the Swartland and Rûens areas (Marais 1985). Generally, the peak of the rain season occurs during anthesis of the wheat crop, after which it declines rapidly with simultaneous increasing temperatures. Long term weather data, however, suggests that the Rûens region experiences conditions that leans towards a slightly longer growing season in comparison to the Swartland. Seeding densities and fertiliser rates are relatively high and spring type wheat cultivars are produced in these regions. Record yields were achieved over the last few seasons, as implementation of conservation agriculture has reduced the traditional risks associated with varying rainfall and high maximum temperatures.

The 2012 and 2013 wheat seasons in the Swartland and Rûens regions varied between seasons but also between test sites (Table 3.5). Grain yield (4.77 ton ha^{-1}) and grain protein content (12.82%) of the Swartland in 2013 was higher than in 2012 (4.05 ton ha^{-1} and 12.34%) and the four-year mean (4.11 ton ha^{-1} and 12.41%) respectively. Hectolitre mass (81.6 kg hl^{-1}) and α-amylase activity (falling number of 365 secs) in contrast, were higher in 2012. Although grain yields in the Rûens were higher in 2012, the differences between the two seasons were small. In 2012 hectolitre mass and falling number were high, but protein content declined below 12% in the Rûens (Table 3.5).

Table 3.5 Grain yield and grain quality of rainfed wheat in the winter rainfall region (ARC-SGI 2014b)

	Season	Grain yield (ton ha^{-1})	Hectolitre mass (kg hl^{-1})	Grain protein (12% moisture base)	Falling number (secs)
NCEP mean for middle Swartland (Moorreesburg)	2013 (n=5)	4.77	76.8	12.82	349
	2012 (n=5)	4.05	81.6	12.34	365
	2011 to 2014	4.11	78.7	12.41	361
NCEP mean for eastern Rûens (Riversdale)	2013 (n=5)	3.66	74.7	13.40	294
	2012 (n=5)	3.95	79.0	11.68	343
	2011 to 2014	3.75	77.9	12.31	341

n = number of test sites used for the first seeding date in 2012 and 2013 in the Swartland and Rûens trials

In 2013 when the highest grain yields occurred in the Swartland (Table 3.6), an increase of more than 85 mm rain was recorded between the early growth stages (July/August) and pre-anthesis (August/September). This higher rainfall followed on the pre-seeding and seeding periods (January to July) during which relatively small deviations from the nine-year mean occurred (Table 3.6).

Table 3.6 Meteorological data for Moorreesburg and Riversdale (winter rainfall region) in 2012 and 2013 with the deviations from 2005 to 2013 (ARC-ISCW 2014)

Test site	Sea-son	Crop stage	Seasonal rainfall total (mm)	Monthly temperature (˚C)	
				Min	Max
Moor (Swrl)	2012	Pre-seeding (Jan to May)	15.94 (-8.53)	15.51 (-0.29)	28.46 (-0.10)
		Seeding period (Jun and Jul)	62.35 (-23.06)	8.23 (-0.77)	17.57 (-0.67)
		Early growth (Aug)	108.50 (+29.28)	6.21 (-1.61)	16.79 (-1.31)
		Pre-anthesis (Sep)	38.00 (-0.09)	8.45 (-0.52)	19.95 (-0.54)
		Post-anthesis and grain filling (Oct and Nov)	8.33 (-11.77)	14.70 (+0.63)	28.46 (+0.92)
	2013	Pre-seeding (Jan to May)	25.50 (+1.03)	15.03 (-0.77)	28.61 (+0.05)
		Seeding period (Jun and Jul)	84.35 (-1.06)	9.07 (+0.07)	18.04 (-0.19)
		Early growth (Aug)	129.40 (+50.18)	8.14 (+0.32)	17.55 (-0.55)
		Pre-anthesis (Sep)	73.20 (+35.11)	8.09 (-0.88)	18.71 (-1.78)
		Post-anthesis and grain filling (Oct and Nov)	14.37 (-5.74)	14.50 (+0.43)	28.47 (+0.93)
Rivr (Rûe)	2012	Pre-seeding (Jan to May)	171.60 (+15.07)	7.58 (-6.33)	25.52 (-0.32)
		Seeding period (Jun and Jul)	149.4 (+50.08)	12.97 (-0.21)	16.86 (-1.56)
		Early growth (Aug)	62.60 (+13.94)	5.04 (-0.90)	17.10 (-1.62)
		Pre-anthesis (Sep)	21.40 (+1.96)	6.87 (-0.43)	20.15 (-0.59)
		Post-anthesis and grain filling (Oct and Nov)	95.60 (-23.53)	12.68 (+0.25)	24.20 (+0.04)
	2013	Pre-seeding (Jan to May)	129.00 (-27.53)	7.23 (-6.68)	25.85 (+0.02)
		Seeding period (Jun and Jul)	76.60 (-22.72)	12.59 (-0.59)	18.14 (-0.27)
		Early growth (Aug)	80.80 (+32.14)	5.24 (-0.70)	18.04 (-0.68)
		Pre-anthesis (Sep)	19.00 (-0.44)	5.18 (-2.12)	20.04 (-0.70)
		Post-anthesis and grain filling (Oct and Nov)	214.20 (+95.07)	12.93 (+0.50)	24.29 (-0.13)

Values in brackets are deviations of the year value from the nine-year mean (2005 to 2013), Moor (Swrl): Moorreesburg (Swartland), Rivr (Rûe): Riversdal (Rûens)

3.2 Experimental design, genotypes (cultivars) and environments (test sites)

Data of grain yield, grain quality and seed samples for analysis of baking quality and protein fractions from the three major wheat production regions in SA were obtained from the NCEP.

Field experiments of this programme are under the management of the Crop Production section of the ARC-SGI with the objective to compile the annual production guidelines. Field experiments were designed as randomised complete block designs with three replications. A list of the genotypes in the respective field trials are provided in Table 3.7.

Table 3.7 Test sites and genotypes used in field experiments in 2012 and 2013

Production Regions	Test sites	Cultivars	
		2012	2013
Irrigated SRR	Upington and Vaalharts	Buffels, Duzi, Krokodil, PAN 3471, PAN 3478, PAN 3489, PAN 3497, Sabie, SST 806, SST 822, SST 835, SST 843, SST 866, ***SST 867***, ***SST 875***, SST 876, SST 877, SST 884, SST 895, Tamboti, ***Timbavati***	Buffels, Duzi, Krokodil, PAN 3471, PAN 3478, PAN 3489, PAN 3497, Sabie, SST 806, SST 822, SST 835, SST 843, SST 866, SST 876, SST 877, SST 884, SST 895, Tamboti, Umlazi
Rainfed SRR	Bethlehem and Clarens	Elands, Gariep, Koonap, ***Matlabas*****,** ***PAN 3118, PAN 3120***, PAN 3161, PAN 3195, PAN 3368, PAN 3379, Senqu, SST 316, SST 317, SST 347, SST 356, ***SST 387, SST 398***	Elands, Gariep, Koonap, PAN 3161, PAN 3195, PAN 3368, PAN 3379, Senqu, SST 316, SST 317, SST 347, SST 356
Rainfed WRR	Moorreesburg and Riversdale	Kwartel, PAN 3408, PAN 3471, Ratel, SST 015, SST 027, SST 047, SST 056, SST 087, SST 096, SST 88, Tankwa	Kwartel, PAN 3408, PAN 3471, Ratel, SST 015, SST 027, SST 047, SST 056, SST 087, SST 096, SST 88, Tankwa

Genotypes in bold italics were excluded from the second year analyses

The genotype entries (cultivars) for IRR, rainfed SRR and rainfed WRR trials used in this study were different between 2012 and 2013 for two reasons. Firstly, experimental protocol of the NCEP restricts genotypes to a four-year evaluation period, resulting in replacement of some genotypes during 2012 and 2013. Secondly, for the rainfed SRR in 2013 the first seeding date at Clarens (preferred trial for this study) had to be aborted due to bird damage to plots. A third important note is that PAN 3471 is recommended for production in both IRR and rainfed WRR.

The second seeding date established three weeks later was used, but due to the trial design and objectives, excluded the winter type genotypes (indicated in italics) that were present in the first seeding date (Table 3.7). These genotypes were Matlabas, PAN 3118, PAN 3120, SST 387 and SST 398. Test sites for rainfed wheat in the SRR were established at Bethlehem

and Clarens with 17 genotypes in 2012 and 12 in 2013. These sites were planted with a precision, six row planter adapted for seeding yield plots according to standard production practises. Plot size was 11.25 m^2 although the harvested plot area consisted only of the inner four rows, resulting in a harvested area of 6.75 m^2. A commercial fertiliser mixture of 4 (N):2 (P):1 (K) (28) was applied during seeding at an amount of 60 kg N ha^{-1} (ARC-SGI 2014b).

The two test sites for IRR field experiments in 2012 and 2013 were Upington and Vaalharts. The experiments were planted with a Wintersteiger Plotman and plots contained eight rows spaced 16 cm apart in row lengths of 5 m, resulting in a plot area of 6.4 m^2 and a harvested plot area of 4 m^2. Commercial genotypes (cultivars) recommended for production under irrigation in the Upington and Vaalharts regions were 21 genotypes in 2012 and 19 in 2013. A commercial fertilizer mixture of 2 (N):3 (P):4 (K) (28) and K.A.N (28) was applied at 280 kg N ha^{-1} (ARC-SGI 2014b). Application was divided between 160 kg N ha^{-1} at seeding, 60 kg N ha^{-1} between tillering (Zadoks 20) and stem elongation (Zadoks 39) followed by 60 kg N ha^{-1} between flag leaf (Zadoks 47) to anthesis (Zadoks 61) (Anderson and Garlinge 2000).

Rainfed field experiments for the WRR were planted at Moorreesburg and Riversdale with 15 genotypes in each trial. Field experiments were planted with an adapted commercial planter designed for seeding plots under no till conditions common in the Rûens and Swartland regions. Seeding density was adequate for 250 to 300 plants per m^2 and plots consisted of seven rows of 5 m with 30 cm between row spacing providing an effective plot size of 9 m^2 and harvested area of 6 m^2. A total amount of 130 kg N ha^{-1} was supplied through a commercial mixture of 4 (N):1 (P):1 (K) (31), (ARC-SGI 2014b). Fertilizer application was split between 100 kg N ha^{-1} during seeding and the remaining 30 kg N ha^{-1} applied as K.A.N (28) between tillering (GS20) and stem elongation (GS39) as described by Zadoks (Anderson and Garlinge 2000).

3.3 Measuring grain yield, grain quality (hectolitre mass, grain protein content and falling number), primary quality characteristics and ratios of protein fractions

Following mechanical harvesting of field experiments, seed samples were sieved for removal of deformed and broken kernels and straw remnants. Grain quality and primary quality characteristics and SE-HPLC were conducted at ARC-SGI in Bethlehem. All measurements (Table 3.8) were generated from the same set of seed samples received from each locality of each region.

Table 3.8 Analyses of quality and protein fractions

Grain quality	Primary quality analyses	Size-Exclusion High Performance Liquid Chromatography	
		Protein fractions	Protein ratios
Hectolitre mass Falling number Wet gluten Grain protein content Flour protein content	Wet gluten, flour protein Water absorption, P (Dough stability), L (Dough distensibility), P/L ratio, Dough strength (cm^2) Extraction (%), Peak time (minutes), Loaf volume (cm^3)	Large polymeric proteins (LPP) Small polymeric proteins (SPP) ω Gliadin (Large monomeric) α/β, γ Gliadin (Large monomeric) Albumin and globulin (Small monomeric)	% soluble fraction of different protein fractions in total soluble protein % insoluble fraction of different protein fractions in total insoluble protein % soluble fraction of different protein fractions in total protein % insoluble fraction of different protein fractions in total protein % insoluble fraction of different protein fractions in total same fraction % insoluble glutenin in total glutenin

3.3.1 Grain yield and -quality

Grain yield was determined after mechanical harvesting of test plots. The two outside rows of plots were excluded to eliminate side row effects and grain yield per plot was converted to present yield data per ha. Methods for conducting the various quality parameters were done according to established and approved methods of either the AACCI (International Approved

methods of Analyses; http://www.methods.aaccnet.org/), ICC (International Association for Cereal Science and Technology, Standard Methods; http://www.icc.or.at/standard_methods) or ISO (International Organisation of Standards; http://www.iso.org/iso/home.html).

Hectolitre mass

Hectolitre mass (ISO 7971-3:2009) provides a measure of the bulk density of the grain which, in turn, indicates grain consistency and potential flour extraction.

Percentage grain- and flour protein content

The measurement of grain- and flour protein content was based on a calibration to a suitable standard method (AACCI Method 46-11.02, 46-16.01, or 46-30.01), whereupon an empirical model is designed relating spectral response to the reference value of the model. Near-infrared absorption, attributable to the combination or overtone vibrational frequencies of NH (functional group existing in the amino acid "proline" but without the second hydrogen H) enables the protein content to be determined.

Falling number

This test primarily measures the change in viscosity of a heated flour or ground whole wheat water suspension due to enzymatic breakdown of starch by α-amylase (ISO 3093:2009). Sound wheat flour contains ample β-amylase but very little α-amylase and the initial products resulting from α-amylase action on the starch are sugars (oligosaccharides) of 6-7 glucose units in length. Cleavage or breakdown occurs at the glycosidic linkages in the interior of the chain. This shortening of the chain length is manifested by a rapid loss in viscosity of the starch solution. The loss in viscosity is such that the viscosity of a gelatinized starch drops 50% when only 0.1% of the glycosidic linkages are broken. With this in mind, it is easy to visualise how a highly sprouted wheat sample, which has a high level of α-amylase activity, will react during the falling number test, whereas with a sound wheat sample, which has virtually no α-amylase

present, this reaction will not occur. The contents of the falling number tube will be thick or viscous.

3.3.2 Primary milling and baking parameters

Seed conditioning

Seed for flour analyses was firstly conditioned for 18 hours prior to milling after which milling in a laboratory pneumatic mill (Bühler model MLU-202, Bühler-Miag, Uzwil, Switzerland) commenced (AACCI Method 26-95.01).

Wet gluten

Wet gluten is the remnant of dough after automated washing correlating strongly with loaf volume. Wheat gluten is the water insoluble complex of protein fractions consisting of gliadin and glutenin proteins (AACCI Method 38-12.02).

Alveograph

Alveograph measures resistance to stretching and extensibility. Stretching by the alveograph is in more than one direction compared to the extensiograph which stretches dough in only one direction (ICC Standard Methods No. 121, latest edition).

Farinograph

In the Farinograph an amount of flour, determined by the size of the mixer (50 or 300g), is used to prepare dough under standardised conditions. from 50 or 300 g of flour. During mixing the resistance of dough to the mixing paddles is recorded continuously. Water absorbtion of the dough is determined by adding water throughout mixing until 500 farinograph units (FU) are obtained. A second batch of dough is then prepared by adding the amount of water to obtain the 500 FU to the flour all at once and mix it for 12 minutes. Development time, dough stability and -softening can then be read of a graph generated over this 12 min.

Loaf volume

Loaf volume (cm^3) provides a method for evaluating bread wheat flour quality referring to the relationship between protein content and bread or loaf volume. Loaf volume was determined by rapeseed displacement after following the optimised, straight dough baking procedure (AACCI Method 10-10.03 and AACCI Method 10-10B).

3.3.3 Size-Exclusion High Performance Liquid Chromatography and protein fractions

SE-HPLC is a powerful tool to assess protein aggregates and is applied for quick analyses of baking quality of bread wheat. SE-HPLC effectively separates the two main classes of wheat proteins namely storage proteins (glutenin and gliadins) and structural proteins (albumins and globulins) and results have been highly correlated with primary quality parameters.

Extraction procedure

The SE-HPLC procedure developed by Gupta et al. (1993) was followed, with modifications. The first step extracted SDS-soluble proteins, while the second step was submitted to sonication to obtain SDS-insoluble proteins. White flour was used as well as deionised water for preparation of solvents and eluents. For SDS-soluble proteins, white wheat flour samples (17 mg) were suspended in 1.5 ml of 0.5% (w/v) SDS-phosphate buffer (pH 6.9) and vortexed for 10 seconds. Samples were then stirred for 5 min followed by centrifugation for 30 min at 10 285 standard gravity or acceleration *(g)*. The supernatant was filtered through a 0.45 µm HT Tuffryn Acrodisc® Syringe Filter into a glass vial. For SDS-insoluble proteins, the pellet was re-suspended in 1.5 ml SDS-phosphate buffer, vortexed for 10 secs and sonicated in an ultrasonic disintegrator (Branson B12 Sonifier) for 30 secs at amplitude 5. The Sonifier is fitted with a 3 mm exponential tip. Samples were then centrifuged for 30 min (10 285 *g*). The supernatant was filtered through a 0.45 µm HT Tuffryn Acrodisc® Syringe Filter into a glass vial.

Size-Exclusion High Performance Liquid Chromatography analyses

Analysis was performed using a Thermo Finnigan™ Surveyor Plus (Thermo Electron, San Jose, CA) HPLC system with PDA detector, equipped with ChromQuest™ 4.2 chromatography data system for integration events. A narrow bore column (NBC) (300 x 4.6 mm BioSep SEC-S 4000 Phenomenex®) was used in this study (Ohm et al. 2009). Separation was achieved in 15 min after injecting a 20 µl sample. The elution arrangement consisted of (a) Trifluoroacetic acid (TFA) (0.1%, v/v) and (b) Acetonitrile (CAN) (ROMIL SpS™ acetonitrile 200 far UV) + TFA (99.9/0.1%, v/v) with 50% of (b) used as the isocratic eluent. Flow rate was 0.4 ml min^{-1} at ambient temperature. Absorbance areas under the different peaks were calculated according to Gupta et al. (1993). The following fractions were measured at specific time intervals: large polymeric proteins (LPP), 4.57 to 5.54 min; smaller polymeric proteins (SPP), 5.54 to 6.98 min; large monomeric proteins (LMP) mainly gliadins, 6.98 to 8.61 min; smaller monomeric proteins (SMP) mainly albumins and globulins, 8.61 up to where the trace cut the baseline. Once the different fractions were separated it was possible to calculate the various ratios between fractions and in different parts of the solution (Table 3.9). The ratios enabled more accurate comparisons as solutions were standardised through expression of concentrations as percentages.

Table 3.9 Protein ratios and calculations of respective fractions separated by SE-HPLC

Protein fraction	% Protein ratio	Calculations
Larger polymeric proteins (LPP), i.e HMW Glutenin subunits	% soluble fraction of different protein fractions in total soluble protein	<u>In soluble or insoluble protein:</u> Soluble or insoluble fraction of different protein fractions /(LPP + SPP + ω gliadin + α/β, γ gliadin + albumin and globulin)*100
Smaller polymeric proteins (SPP), i.e LMW Glutenin subunits	% insoluble fraction of different protein fractions in total insoluble protein	
ω Gliadin (Larger monomeric proteins)	% soluble fraction of different protein fractions in total protein	<u>in total protein:</u> Soluble or insoluble fraction of different protein fractions/((soluble: LPP + SPP + ω gliadin + α/β, γ gliadin + albumin and globulin)+(insoluble: lpp + spp + ω gliadin + α/β, γ gliadin + albumin/globulin)*100
α/β, γ Gliadin (Larger monomeric proteins)	% insoluble fraction of different protein fractions in total protein	
Albumin and globulin (Smaller monomeric proteins)	% insoluble fraction of different protein fractions in total same fraction	<u>In total same fraction:</u> Insoluble fraction of different protein fractions/(corresponding soluble fraction + corresponding insoluble fraction)*100
	% insoluble LPP and SPP in total glutenin	<u>In total glutenin (LPP + SPP):</u> Insoluble LPP + insoluble SPP/(Insoluble LPP + insoluble SPP + soluble LPP + soluble SPP)*100

3.4 Statistical analyses

Analysis of variance

In order to achieve the objective of the study (comparing protein quantity with protein quality) the focus had to be on the variation for both parameters and not a comparison of differences between genotypes (cultivars). Therefore, genotypes adapted for production in the three production regions were grouped according to high or low rankings for grain yield, grain protein content, flour protein content and loaf volume. Rankings were obtained from two way analyses over seasons (2012 and 2013) x test localities (two localities of each region) with the requisite that all genotypes within the highest ranking group of a trait (for example loaf volume) differed significantly from all the genotypes in the lowest ranking group of the same trait.

Simple full correlations

The highest ranking and lowest ranking groups of each trait were correlated with the primary baking parameters and with the protein fractions.

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Chapter 4

Correlations between protein content and composition in irrigated wheat

4.1 Introduction

Globally, wheat is the most widely cultivated food plant in the world (Awika 2011) and second to rice in providing calories for the human diet. In the developing countries of Asia and sub-Saharan Africa, wheat consumption is steadily rising in popularity above rice. Awika (2011) contributes this trend to the wide adaptability of wheat to most temperate production environments all over the world. Shewry (2009) endorsed the wide adaptability of wheat by adding the unique properties of wheat dough to bake a variety of products as another critical key determining wheat's global success. But higher demands of a growing world population on food production and uncertainty regarding the long term impacts of climate change on natural resources, calls for novel approaches to increase yields. In the UK, El Chami et al. (2015) reported that the expected increase in world wheat prices, impacts of climate change and availability of unused river water might justify new economic investment in irrigation schemes. Water resources available for agriculture in SA are becoming critically important as industrial extraction and consequent detrimental reduction of water quality increase. Dennis and Nell (2002) stated that groundwater in SA irrigates 24% and surface water 76% of irrigable production units respectively. They concluded that precision irrigation (differential management of inputs per production unit) can save water resources and make a substantial economic difference compared to traditional irrigation production systems (standard management practises across the complete production unit). The cooler production region for irrigated wheat is situated in the central part of SA and allows for earlier crop establishment

than irrigated wheat in the warmer production regions. Heat stress avoidance during the critical stages of grain filling combined with the need for a quick economical turnover rate, resulted in production of only spring type wheat under irrigation. Although production of fully irrigated wheat in SA is steadily increasing due to increased profitability from higher yields (SAGL 2014) this production system requires high investment inputs and managerial skills.

A commonly accepted fact is that improved grain yield is mostly negatively associated with protein content. Fischer et al. (1993) reported that single applications of supplemental nitrogen did not increase grain yield until after stem elongation, but these positive yield responses were negatively correlated with grain protein content. Grain protein content is applied worldwide for predicting the gluten fraction of a wheat sample. This measurement has been refined and the modern version is a simple and quick procedure. Wheat buyers apply protein content as an indicator of the end use application of wheat bulks available on markets whereas bakers regard protein content as the standard reference for gluten strength and dough mixing properties of flour. Protein content is highly sensitive to environmental factors. Noor Hasniza et al. (2014) reported that total grain protein and dough functional properties were significantly influenced by environment, whereas protein content of soluble and insoluble extractable protein fractions was determined by genotype.

Under fully irrigated conditions, natural moisture deficits occurring during the growing season is removed as a variable. The high amounts of prescribed irrigation and fertilisation with high temperature and consequent evapotranspiration have a substantial effect on quality and composition of proteins in genotypes adapted for production under irrigated conditions.

This study aimed to (a) validate protein content as a measurement for bread making quality of irrigated wheat, (b) identify prominent protein fractions and ratios correlated with bread baking quality and (c) define the effects of genotype and environment on variation of protein quantity and quality of wheat produced under irrigation in SA.

4.2 Material and methods

4.2.1 Genotypes (cultivars) and environments (field test sites)

Wheat samples from the NCEP for irrigated wheat in the cooler production regions in 2012 and 2013 were used for the purpose of the study. Twenty-one and 19 commercial cultivars (genotypes), released for production under irrigation (refer to Table 3.7 in Chapter 3), were seeded at Upington and Vaalharts in 2012 and 2013 respectively. In 2013 SST 867 and SST 875 were excluded and Timbavati replaced by Umlazi by the respective breeding companies. The commercial cultivar SST 806 (developed by SENSAKO) is the current biological quality standard for irrigated environments and is used by the milling and baking industry to monitor effects of climate, environment and production practises in different seasons.

4.2.2 Meteorology and climatic trends

Climatic data was obtained from records of the ARC-ISCW 2014. Data of 2012 and 2013 and a nine-year mean (2005 to 2013) was applied to determine trends in the pre-seeding (January to May), seeding (June and July), early growth (August), pre-anthesis (September) and post-anthesis and maturity (October to December) periods of the season.

4.2.3 Grain quality and primary quality parameters

Grain samples received from Upington and Vaalharts for 2012 and 2013 were analysed at the quality laboratory of the ARC-SGI (Bethlehem, SA). Hectolitre mass, protein content, alveograph (P, L and P/L), farinograph (W), wet gluten and loaf volume were determined to establish milling and baking quality of the cultivars from both test sites. For detailed information on methods used refer to Chapter 3.

Grain quality was determined by measuring the hectolitre mass (ISO 7971-3:2009), percentage grain protein content (AACCI Method 46-10) and falling number (ISO 3093:2009). The primary quality parameters were determined and served as indicators of bread making quality. The processes and tests consisted of seed conditioning (AACCI Method 26-95.01), wet gluten measurement (AACCI Method 38-12.02), alveograph analyses (ICC Standard Methods No. 121, latest edition), percentage flour protein content (AACC method 39-11), loaf volume (AACCI Method 10-10.03 and AACCI Method 10-10B) and farinograph water absorption (AACCI method 54-21.02).

4.2.4 Size-Exclusion High Performance Liquid Chromatography

SE-HPLC is applied to assess protein aggregates and is used for quick analyses of baking quality of bread wheat. SE-HPLC effectively separates the two main classes of wheat proteins (storage proteins and structural proteins) and results are highly correlated with primary quality parameters. The SE-HPLC procedure of Gupta et al. (1993) was followed, with modifications as described in 3.3.3 of chapter 3. After the different fractions were separated, the various ratios between fractions were calculated which enabled more accurate comparisons, as solutions were standardised through expression of concentrations as percentages.

4.2.5 Statistical analyses

Two-way ANOVA for genotype (G), environment (E) and G x E interactions were done over the 2012 and 2013 seasons. The results were used to divide genotypes into three (high, middle and low ranking) groups for flour content, grain protein content and loaf volume. Simple correlations were determined on combined two-year data for identification of relationships through statistical analyses with GenStats for Windows version 10 (Payne et al. 2007). The use of the terms highest or lowest ranking average for flour protein, grain protein and loaf volume in the results and discussion section, refers to the expression of the three traits by

genotypes in the highest ranking and lowest ranking groups and the consequent comparison thereof with other quality parameters of the same group of genotypes. For example, correlation of highest ranking average flour protein with loaf volume would refer to correlations of the average flour protein of the three highest ranked genotypes with loaf volume of the same genotypes.

4.3 Results and discussion

4.3.1 Genotype x environment interaction and analysis of variance of flour protein, grain protein and loaf volume

4.3.1.1 Effects of environment and genotype

The most important climatic trend in 2012 was that the amount of rainfall during the post-anthesis and grain filling periods at Upington and Vaalharts only had a small deviaton from the nine year and 2013 means. Although substantial differences in rainfall occurred during the pre-seeding period at both Upington and Vaalharts (refer to Chapter 3 Section 3.1.2.2 for detailed description of climate) any moisture deficits were probably replenished during pre-seeding irrigation. Grain yield deviations at the Upington and Vaalharts tests sites in 2012 and 2013 were less than for all the irrigated test sites of the NCEP (refer to Table 3.1 in Chapter 3) but slightly more pronounced for hectolitre mass, protein content and falling number (Table 4.1).

Table 4.1 Grain yield and grain quality of irrigated wheat at Upington and Vaalharts in 2012 and 2013

Parameters	2012				2013			
	Upington	Vaalharts	LSD	P-value	Upington	Vaalharts	LSD	P-value
Yield (ton ha^{-1})	9.03[b]	10.98[a]	0.157	< 0.001	9.10[b]	11.19[a]	0.160	< 0.001
Grain protein (%)	12.91[a]	11.48[b]	0.263	< 0.001	14.22[a]	9.24[b]	0.331	< 0.001
Hectolitre mass (kg hl^{-1})	83.8[a]	83.0[b]	0.38	< 0.001	82.8[b]	83.9[a]	0.32	< 0.001
Falling number (secs)	374[a]	315[b]	4.2	< 0.001	335[b]	360[a]	6.3	< 0.001

Same letters within a parameter for a season indicate no significant differences between the two localities

4.3.1.2 Analysis of variance of flour protein, grain protein and loaf volume

In Table 4.2 differences in flour and grain protein as well as loaf volume of G and E were highly significant ($P < 0.001$) whereas the G x E interaction was also significant for loaf volume ($P < 0.001$) and flour protein content ($P < 0.05$). E (test sites) had the largest impact on flour protein in terms of contribution of sum of squares to total sum of squares (72.08%) and grain protein (76.18%) with relatively small effects from G (10.78% and 8.22% respectively).

Table 4.2 Analysis of variance of flour and grain protein content and loaf volume and percentage contribution to total sum of squares

	Source of variation	SS	% SS	MS	P-value
% Flour protein	Genotype (G)	111.22	10.78	6.54	< 0.001
	Environment (E)	743.85	72.08	247.95	< 0.001
	G x E	60.88	5.90	1.19	< 0.05
	Residual	113.05		0.80	
	Total	1031.97			
	Stratum	**SE**	**CV%**		
	Rep	0.14	1.20		
	Rep.*Units*	0.89	7.70		
% Grain protein	Genotype (G)	77.74	8.22	4.57	< 0.001
	Environment (E)	720.61	76.18	240.20	< 0.001
	G x E	41.18	4.35	0.81	NS
	Residual	100.67		0.71	
	Total	945.98			
	Stratum	**SE**	**CV%**		
	Rep	0.20	1.70		
	Rep.*Units*	0.84	7.00		
Loaf volume (cm^3)	Genotype (G)	314407	18.25	18495	< 0.001
	Environment (E)	873864	50.73	291288	< 0.001
	G x E	276363	16.04	5419	< 0.001
	Residual	256663		1807	
	Total	1722662			
	Stratum	**SE**	**CV%**		
	Rep	3.10	0.30		
	Rep.*Units*	42.5	4.80		

NS - not significant

Loaf volume was also primarily determined by E (50.73%) although G (18.25%) and G x E interaction (16.04%) were more prominent compared to G and G x E interaction in flour and grain protein (Table 4.2).

4.3.1.3 Two way analyses of variance for ranking of genotypes into groups

The four genotypes forming the highest ranking group for flour protein content were SST 843, SST 822, SST 884 and Duzi (Table 4.3a) obtaining a mean of 12.57% flour protein content that is within the requirement of the B1 grade (B1 ≥ 12%). The genotypes of the lowest ranking

group for flour protein were PAN 3497 and Krokodil (Table 4.3a) with a mean of 10.45%, meeting specifications of protein content for grade B3 (10% ≤ B3 ≤ 11%).

Table 4.3a Ranking of irrigated genotypes for flour protein content

Genotype	% Value	Means	Rank group
SST 843	13.50[a]	12.57	High
SST 822	12.66[ab]		
SST 884	12.12[bc]		
Duzi	12.01[bc]		
Sabie	11.78[bcd]	11.34	Middle
SST 877	11.70[bcd]		
Buffels	11.61[bcde]		
SST 895	11.45[bcde]		
Tamboti	11.40[bcde]		
SST 835	11.32[bcde]		
SST 876	11.30[cde]		
SST 806 (quality standard)	11.29[cde]		
PAN 3478	11.19[cde]		
SST 866	11.14[cde]		
PAN 3489	10.99[cde]		
PAN 3471	10.96[cde]		
PAN 3497	10.58[de]	10.45	Low
Krokodil	10.31[e]		
CV%	7.7		
LSD	0.72		
P-value	< 0.001		

Values within a column with different letters are statistically different

The two genotypes SST 843 and SST 822 formed the highest ranking group for grain protein (Table 4.3b) and had an average grain protein content of 13.30% that is well above the requirement for the B1 grade (≥ 12%). The lowest ranking group consisted of six genotypes with significantly lower grain protein content than the highest ranking group. These genotypes

were PAN 3478, PAN 3489, SST 806, PAN 3471, PAN 3497 and Krokodil (Table 4.3b) with an average grain protein content of 11.42% equal to a B2 grade (11% ≤ B2 ≤ 12%).

Table 4.3b Ranking of irrigated genotypes for grain protein content

Genotype	% Value	Wheat grade (Class B wheat)	Means	Rank group
SST 843	13.67[a]	B1	13.30	High
SST 822	12.93[ab]	B1		
SST 877	12.52[abc]	B1	12.01	Middle
Duzi	12.17[bcd]	B1		
Sabie	12.09[bcd]	B1		
SST 884	12.08[bcd]	B1		
SST 895	12.05[bcd]	B1		
Buffels	11.93[bcd]	B2		
SST 876	11.91[bcd]	B2		
Tamboti	11.8[bcd]	B2		
SST 866	11.78[bcd]	B2		
SST 835	11.74[bcd]	B2		
PAN 3478	11.66[cd]	B2	11.42	Low
PAN 3489	11.64[cd]	B2		
SST 806 (quality standard)	11.56[cd]	B2		
PAN 3471	11.36[cd]	B2		
PAN 3497	11.32[cd]	B2		
Krokodil	10.97[d]	B3		
CV%	7.0			
LSD	0.68			
P-value	< 0.001			

Values within a column with different letters are statistically different, Class B grading is applicable for grading of bread wheat

The highest ranking group for loaf volume consisted of Sabie, SST 822, Buffels and PAN 3497 (average of 933.0 cm^3) and the lowest ranking group of SST 895, Krokodil, SST 866, PAN 3478, PAN 3489 and PAN 3471 (average of 839.0 cm^3) (Table 4.3c).

Table 4.3c Ranking of irrigated genotypes for loaf volume

Genotype	Cm ^{3}	Means	Rank group
Sabie	955.4^{a}	933.0	High
SST 822	941.3^{ab}		
Buffels	919.2^{ab}		
PAN 3497	915.8^{abc}		
SST 877	909.6^{abcd}	892.7	Middle
SST 884	907.1^{abcd}		
Duzi	903.3^{abcd}		
Tamboti	896.7^{abcde}		
SST 835	895.4^{abcde}		
SST 806 (quality standard)	889.2^{bcdef}		
SST 843	885.4^{bcdef}		
SST 876	855^{cdefg}		
SST 895	850.4^{defg}	839.0	Low
Krokodil	847.3^{defg}		
SST 866	847.1^{defg}		
PAN 3478	839.2^{efg}		
PAN 3489	829.6^{fg}		
PAN 3471	820.4^{g}		
CV%	4.8		
LSD	34.30		
P-value	< 0.001		

Values within a column with different letters are statistically different

Specifications of the SA milling and baking industry allow loaf volumes of cultivars to vary within a ± 10% range of the value of the quality standard of the specific production region (SST 806 for irrigated wheat in the SRR). Loaf volumes in both the highest and lowest ranking groups in Table 4.3c were within these specifications (between 978.2 and 800.3 cm^3).

4.3.2 Simple correlations of flour protein, grain protein and loaf volume with the primary quality parameters and protein fractions

4.3.2.1 Correlations with the primary quality parameters

Particular strong (Table 4.4a) correlations occurred between the lowest ranking flour protein content average and grain protein content (0.96***) and lowest ranking grain protein content average and flour protein content (0.94***). Lowest ranking loaf volume average correlated more strongly with flour protein (0.84***) and grain protein (0.80***) although a significant positive correlation occurred between the highest ranking loaf volume average and grain protein content (0.71***) and flour protein content (0.81***). Environment has a large effect on loaf volume and Koppel and Ingver (2010) only found significant correlations between loaf volume and protein content in one out of five years. Bruckner et al. (2001) studied the effects of white flour and whole wheat flour on relationships between bread quality parameters and concluded that significant correlations between final loaf volume of whole wheat and white flour occurred at only two of four test environments. The high grain protein content group only comprised two genotypes (Table 4.3b) which could have amplified the effect of environment on grain protein content. Research of prediction models for loaf volume by Dowell et al. (2008) recommended inclusion of both grain and flour protein content, although the addition of dough strength, absorption, and protein quality or viscoelastic properties strengthens the model further. Flour protein, grain protein and loaf volume furthermore had strong positive correlations with alveograph, dough strength and wet gluten (Table 4.4a). Abboud Al-Saleh and Brennan (2013) found a significant positive correlation between flour protein content and water absorption, vitreousness of the kernel, dough stability and dough extensibility. Flour protein content was shown by Graybosch et al. (1993) to be the major contributing factor to dough strength.

Table 4.4a Correlations between flour and grain protein content, and loaf volume with primary quality parameters

Para-meter	ANOVA ranking	Mean	Alveo (Wf)	Dough strength	Dough stability (P)	Dough distensibility (L)	Dough P/L value	Flour extraction	Falling number	Flour protein content	Grain protein content	Hecto-litre mass (kg hl^{-1})	Loaf volume (cm^3)	Peak time minutes (MPT)	Wet Gluten
% Flour protein content	High (n=4)	12.57	0.73***	0.70***	NS	NS	NS	NS	NS		0.91***	NS	0.67***	NS	0.85***
	Low (n=2)	10.45	0.79***	0.79***	NS	0.55**	NS	NS	NS		0.96***	NS	0.89***	NS	0.93***
% Grain protein content	High (n=2)	13.30	0.59**	0.46*	NS	NS	NS	NS	NS	0.92***		NS	NS	NS	0.89***
	Low (n=6)	11.42	0.84***	0.82***	NS	NS	NS	NS	NS	0.94***		NS	0.79***	NS	0.91***
Loaf volume (cm^3)	High (n=4)	933	0.70***	0.69***	NS	NS	NS	NS	NS	0.80***	0.71***	NS		NS	0.74***
	Low (n=6)	839	0.60***	0.56***	NS	0.53***	NS	NS	NS	0.84***	0.80***	NS		NS	0.82***

n - Number of genotypes in the high and low rank groups. Mean value is average of the rank group
*** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05, NS - not significant

Correlations were positive and strongest (0.89***) between wet gluten and highest ranking average grain protein content and wet gluten and lowest ranking average flour protein content (0.93***). Šimić et al. (2006) found that wet gluten correlated highly with grain protein content, which, in turn, is strongly influenced by the growing environment.

4.3.2.2 Correlations with the protein ratios

Correlations between flour protein, grain protein and loaf volume with protein ratios of the highest ranking group

Positive correlations occurred (Table 4.4b) between insoluble glutenin (large polymeric proteins) in total insoluble protein and the highest ranking average flour protein content (0.52***) and grain protein content (0.57**). Insoluble glutenin in total protein correlated with highest ranking average flour protein (0.54***) and grain protein (0.60**) and insoluble glutenin in total glutenin with highest ranking flour protein average (0.54***) and grain protein (0.56**). Graybosch et al. (1993) are of the opinion that the quantity of insoluble or total HMW-GS (glutenins) could replace protein content as predictor of loaf volume because of its high correlations with protein content.

Soluble α/β, γ gliadin (large monomeric proteins) in total soluble protein and in total protein correlated positively with flour protein content, grain protein content and loaf volume in the highest ranking groups for these three characteristics (Table 4.4b). Soluble α/β, γ gliadin (large monomeric proteins) in total soluble protein (0.73***) and in total protein (0.61***) correlated positively and strongest with flour protein content which is in line with findings of Gupta et al. (1993) and Meintjés (2004). Processes generating higher flour protein content changes the proportional composition of protein classes and gliadin protein numbers escalate much faster than other proteins (Gupta et al. 1993) which is then reflected in higher gliadin content.

Soluble albumin/globulin (small monomeric proteins) in soluble protein and in total protein correlated negatively with flour protein content, grain protein content and loaf volume in the

highest ranking groups for these characteristics (Table 4.4b). Soluble albumin/globulin in total soluble protein (-0.84***) and in total protein (-0.77***) correlated negatively but strongly with flour protein content. Soluble albumin/globulin in soluble protein (-0.81***) and insoluble albumin/globulin in insoluble protein (-0.73***) correlated strongest with grain protein content. Singh et al. (1990) reported strong negative correlations between albumin/globulin and flour protein from 15 cultivars. Results from this study are in agreement with previous studies on SA genotypes by Labuschagne and Aucamp (2004) and Meintjés (2004). Results from these studies reported that soluble and insoluble fractions of albumin/globulin were significantly and negatively correlated with grain and flour protein content. Park et al. (2006) reported that the albumin/globulin amounts in total protein weight had a highly negative correlation with flour protein content. They postulated that an increase in protein content resulted in increased total soluble proteins and gliadin amounts but not in levels of albumin/ globulin and soluble polymeric proteins. The increments were much less compared to increases in other protein subclass fractions. The results from a study on winter wheat in Croatia by Horvat et al. (2012) showed that albumin/globulin correlated negatively with protein content, wet gluten (flour protein) and loaf volume.

Table 4.4b Correlations between protein fractions and flour protein, grain protein and loaf volume of the highest ranking groups

Solubility classes	Protein fractions	Flour protein	Grain protein	Loaf volume
% soluble fraction of different protein fractions in total soluble protein	Large polymeric proteins (LPP)	NS	NS	NS
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	NS	NS	NS
	α/β, γ Gliadin (Large monomeric)	0.73***	0.62**	0.56***
	Albumin and globulin (Small monomeric)	-0.84***	-0.81***	-0.66***
% insoluble fraction of different protein fractions in total insoluble protein	Large polymeric proteins (LPP)	0.52***	0.57**	NS
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	NS	NS	NS
	α/β, γ Gliadin (Large monomeric)	NS	NS	NS
	Albumin and globulin (Small monomeric)	-0.64***	-0.73***	NS
% soluble fraction of different protein fractions in total protein	Large polymeric proteins (LPP)	NS	NS	NS
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	NS	NS	NS
	α/β, γ Gliadin (Large monomeric)	0.61***	0.58**	0.47***
	Albumin and globulin (Small monomeric)	-0.77***	-0.70***	-0.65***
% insoluble fraction of different protein fractions in total protein	Large polymeric proteins (LPP)	0.54***	0.60**	NS
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	NS	NS	NS
	α/β, γ Gliadin (Large monomeric)	NS	NS	0.52***
	Albumin and globulin (Small monomeric)	NS	-0.55**	NS
% insoluble fraction of different protein fractions in total similar fraction	Large polymeric proteins (LPP)	0.54***	0.56**	NS
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	NS	NS	NS
	α/β, γ Gliadin (Large monomeric)	NS	NS	NS
	Albumin and globulin (Small monomeric)	NS	NS	NS
% insoluble glutenin in total glutenin		NS	NS	NS

*** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05, NS - not significant

Correlations between flour protein, grain protein and loaf volume with protein ratios of the lowest ranking group

The mean percentage grain protein content decreased with 1.88% from 13.30% of the highest ranking group to 11.42% of the lowest ranking group adapted for IRR. Overall the number of correlations reduced from 22 for the highest ranking group to 19 for the lowest ranking group for flour and grain protein content and loaf volume (Table 4.3c).

Although no significant correlations (Table 4.4c) occurred between large or small polymeric proteins and flour protein content, grain protein content and loaf volume, significant positive correlations existed with the α/β, γ gliadin proteins in the lowest ranking groups for these characteristics. Insoluble α/β, γ gliadin in total protein correlated positively with average low flour protein (0.62**), grain protein (0.51***) and loaf volume (0.61***). Insoluble α/β, γ gliadin in total insoluble protein correlated positively with average low flour protein (0.55**) and loaf volume (0.51***) but not with average low grain protein. Soluble α/β, γ gliadin in total soluble protein had a stronger correlation with average low flour protein (0.66***) than average low grain protein (0.60***) but insoluble α/β, γ gliadin in total protein correlated strongest with average low loaf volume (0.61***). Johansson et al. (2001) report instability of components containing glutenin and gliadin when nitrogen application was varied, that lead to variations in protein concentration and loaf volume.

Albumins/globulins constitute 20% to 25% of the total grain proteins present in wheat endosperm (Merlino et al. 2008) and increases in concentration from anthesis to approximately 20 days after anthesis, after which the concentrations remain at an almost constant level (Triboï et al. 2003). Similarly, to results reported by Labuschagne and Aucamp (2004) and results for the highest ranking group, strong negative correlations ensued between soluble albumin/globulin and the average lowest flour protein content, grain protein content and loaf volume.

Table 4.4c Correlations between protein fractions and low flour protein, grain protein and loaf volume of the lowest ranking groups

Solubility classes	Protein fractions	Flour protein	Grain protein	Loaf volume
% soluble fraction of different protein fractions in total soluble protein	Large polymeric proteins (LPP)	NS	NS	NS
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	NS	NS	NS
	α/β, γ Gliadin (Large monomeric)	0.66***	0.60***	NS
	Albumin and globulin (Small monomeric)	-0.81***	-0.82***	-0.56***
% insoluble fraction of different protein fractions in total insoluble protein	Large polymeric proteins (LPP)	NS	NS	NS
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	NS	NS	NS
	α/β, γ Gliadin (Large monomeric)	0.55**	NS	0.51***
	Albumin and globulin (Small monomeric)	NS	-0.53***	-0.51***
% soluble fraction of different protein fractions in total protein	Large polymeric proteins (LPP)	NS	NS	NS
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	NS	NS	NS
	α/β, γ Gliadin (Large monomeric)	0.46*	NS	0.50***
	Albumin and globulin (Small monomeric)	-0.89***	-0.85***	-0.72***
% insoluble fraction of different protein fractions in total protein	Large polymeric proteins (LPP)	NS	NS	NS
	Small polymeric proteins (SPP)	NS	NS	0.52***
	ω Gliadin (Large monomeric)	NS	NS	NS
	α/β, γ Gliadin (Large monomeric)	0.62**	0.51***	0.61***
	Albumin and globulin (Small monomeric)	NS	NS	NS
% insoluble fraction of different protein fractions in total similar fraction (A:E)	Large polymeric proteins (LPP)	NS	NS	NS
	Small polymeric proteins (SPP)	NS	NS	0.46***
	ω Gliadin (Large monomeric)	NS	NS	NS
	α/β, γ Gliadin (Large monomeric)	NS	NS	NS
	Albumin and globulin (Small monomeric)	NS	NS	NS
% insoluble glutenin in total glutenin		NS	NS	NS

*** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05, NS - not significant

The strongest negative correlations of soluble albumin/globulin in total soluble protein were with average low grain protein content (-0.82***) and of soluble albumin/globulin in total protein with average low flour protein content (-0.89***). Labuschagne et al. (2006) found flour protein content and break flour yield of hard and soft wheat were significantly negatively correlated with albumin/globulin.

4.4 Conclusions

Positive three way correlations occurred between percentage flour protein content, percentage grain protein content and loaf volume of IRR wheat. Both values of the highest and lowest ranking flour protein groups correlated positively and strongly with loaf volume. Flour protein also correlated stronger with wet gluten and loaf volume except for the higher ranking group of grain protein correlating stronger with wet gluten than flour protein. The most unusual result was that no correlation occurred between loaf volume and grain protein content of the highest ranking group. A possible explanation could be that the high grain protein is compiled of only two genotypes, amplifying the environmental effects on grain protein content. Flour protein content, grain protein content and loaf volume of both the highest and lowest ranking groups furthermore had strong positive correlations with alveograph and dough strength. Correlations were positive and strongest between flour protein and grain protein. Flour protein content of the highest ranking group correlated strongest and positive with alveograph and dough strength but loaf volume in the lowest ranking group correlated strongest with flour protein and grain protein.

Positive correlations occurred between soluble α/β, γ gliadin (large monomeric proteins) in total soluble protein and in total protein with flour protein content, grain protein content and loaf volume of the highest ranking group. Both soluble α/β, γ gliadin in soluble protein and in total protein correlated positive and stronger with flour protein than with grain protein or loaf volume. Soluble albumin/globulin (small monomeric proteins) in soluble protein, in insoluble

protein and in total protein correlated negatively with flour and grain protein content and loaf volume. The strongest negative correlation of albumin/globulin in soluble protein and in total protein was with flour protein content. Insoluble albumin/globulin in insoluble protein correlated strongest with grain protein content. Insoluble large polymeric proteins (small monomeric protein) in total insoluble protein, in total protein and in total large polymeric proteins correlated positively with high flour protein and high grain protein.

The strongest negative correlations between soluble albumin/globulin in total soluble protein was with grain protein content and of soluble albumin/globulin in total protein with flour protein for the lowest ranking genotypes. Although no correlations occurred between glutenin and flour protein content, grain protein content and loaf volume in the lowest ranking group, some significant positive correlations existed with α/β, γ gliadin (large monomeric). The strongest significantly positive correlation of insoluble α/β, γ gliadin in total insoluble protein was with flour protein in the lowest ranking group. In total protein, the strongest positive correlation of soluble α/β, γ gliadin was with loaf volume of the lowest ranking group. In total protein, insoluble α/β, γ gliadin correlated strongest with flour protein in the lowest ranking group.

For irrigated wheat production, high grain protein content did not correlate with loaf volume. Positive correlations occurred between flour and grain protein content and loaf volume with wet gluten. Protein composition might, in the long term, prove to explain environmental and production effects on protein content more accurately. The negative correlations of flour protein, grain protein and loaf volume with albumin/globulin and positive correlations of α/β, γ gliadin with protein content and loaf volume were very prominent.

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Chapter 5

Correlations between protein content and composition of rainfed wheat produced in the summer rainfall region

5.1 Introduction

Current projections indicate that climate change will significantly contribute towards higher food prices, resulting in increased malnutrition and food shortages, particularly in developing countries. Nelson et al. (2009) predicted a decline of between 18 to 36% in wheat yields by 2050 compared to similar production conditions with no climate change. Increased pressure on natural resources, of which water is probably the most widely affected, will require that increased food production will most likely have to come from higher productivity rather than an increase in cropping area (Rosegrant et al. 2014). Rainfed wheat places substantially less pressure on natural resources compared to irrigated wheat production. In the context of the water footprint concept, Mekonnen and Hoekstra (2010) calculated that an additional 63 litres of water per kilogram wheat is necessary for irrigated wheat compared to rainfed wheat. The main reason is that the blue water footprint of rainfed wheat is zero, as no surface and groundwater are “mined” during crop production. Raised yields and improved quality of rainfed wheat are therefore of high priority, but practises to achieve this objective at regular frequencies are not simple and straightforward. Erekul et al. (2012) found that protein content, sedimentation value and gluten index at two test sites in western Turkey did not increase after supplementary irrigation. Higher grain yields often coincide with declines in protein content and baking quality and particularly protein content is highly sensitive to environmental factors. From their study, Noor Hasniza et al. (2014) reported that total grain protein and dough functional properties were significantly influenced by environment but protein content in soluble and insoluble extractable protein fractions was determined by genotype. Flagella et al.

(2008) found that high rainfall at critical growth stages (grain filling) of durum wheat resulted in higher protein and gluten content, probably due to an increased uptake of nitrogen. In the Flagella et al. (2008) study, gluten index also benefitted from the number of days with temperatures between 30 to 35˚C although Garrido-Lestache et al. (2004) found high temperatures to reduce gluten quality of bread wheat. One of the most important attributes of rainfed wheat in the SRR of SA is its excellent rheological quality. In the 2013 season the average whole wheat protein content (12.7%), alveograph strength (44.6 cm^2), extensogram strength (111 cm^2) and gluten index (89) was higher for rainfed wheat in the SRR than irrigated wheat (SAGL 2014). But increasing imports of wheat into SA has raised questions about the impacts thereof on end product quality and if acceptable loaf volumes can be produced from low protein wheat imports. Farmers are concerned that the current SA grading system is discriminating against local producers and new variety development. This particular issue seems to be a global concern, as a most relevant question in the context of this study is the one posed by Reese et al. (2007) when they ask: *"Is protein enough for assessing wheat flour quality?"* They recommend that for accurate predictions of flour quality, additional information apart from protein content is needed, possibly from farinograph and alveograph analyses.

This study aimed to (a) validate protein content as a measurement for bread making quality of rainfed wheat in the SRR, (b) identify prominent protein fractions and ratios correlated with bread baking quality and (c) define the effects of genotype and environment on variation of protein quantity and quality of wheat produced under rainfed conditions in the SRR of SA.

5.2 Material and methods

5.2.1 Genotypes (cultivars) and environments (field test sites)

Seed samples of 17 commercial cultivars included in 2012 and 12 in 2013 from the NCEP for rainfed wheat in the SRR were used for the purpose of this study. Test sites were established

at Bethlehem, ARC-Small Grain Institute and Clarens respectively. Five cultivars of 2012 were excluded from 2013 trials and only cultivars corresponding in both 2012 and 2013 were used for two-way ANOVA. Data of the early seeded trial at Clarens in 2013 was discarded due to a very high coefficient of variation and replaced with results from the second trial at this site seeded three weeks later. Cultivars not included in 2013 data were Matlabas, PAN 3118, PAN 3120, SST 387 and SST 398. The commercial cultivar Elands is the biological quality standard for rainfed wheat in the SRR and is the reference for monitoring effects of seasonal variation in rainfall, temperature and production practises on wheat quality. Experimental design at both trial sites was a complete randomized block design with three replications. A detailed explanation of procedures and equipment used for seeding, trial maintenance, harvesting and generation of data are provided in Chapter 3.

5.2.2 Meteorology and climatic trends

Climatic data was obtained from records of the ARC-ISCW 2014. Data of 2012 and 2013 and a nine-year mean (2005 to 2013) was applied to determine trends in the pre-seeding (January to May), seeding (June and July), early growth (August), pre-anthesis (September) and post-anthesis and maturity (October to December) periods of the season.

5.2.3 Grain quality and primary quality parameters

Grain samples received from Bethlehem and Clarens for 2012 and 2013 were analysed at the quality laboratory of the ARC-SGI (Bethlehem, SA). Hectolitre mass, protein content, alveograph (P, L and P/L), farinograph (W), wet gluten and loaf volume were determined to establish milling and baking quality of the cultivars from both test sites. For detailed information on methods used refer to Chapter 3.

Grain quality was determined by measuring the hectolitre mass (ISO 7971-3:2009), percentage grain protein content (AACCI Method 46-10) and falling number (ISO 3093:2009). The primary quality parameters were determined and served as indicators of bread making quality. The processes and tests consisted of seed conditioning (AACCI Method 26-95.01), wet gluten measurement (AACCI Method 38-12.02), alveograph analyses (ICC Standard Methods No. 121, latest edition), percentage flour protein content (AACC method 39–11), loaf volume (AACCI Method 10-10.03 and AACCI Method 10-10B) and farinograph water absorption (AACCI method 54-21.02).

5.2.4 Size-Exclusion High Performance Liquid Chromatography

SE-HPLC is applied to assess protein aggregates and is used for quick analyses of baking quality of bread wheat. SE-HPLC effectively separates the two main classes of wheat proteins (storage proteins and structural proteins) and results are highly correlated with primary quality parameters. The SE-HPLC procedure of Gupta et al. (1993) was followed, with modifications. After the different fractions were separated, the various ratios between fractions were calculated which allowed more accurate comparisons, as solutions were standardised through expression of concentrations as percentages.

5.2.5 Statistical analyses

Two-way ANOVA for G, E and G x E interactions were done over the 2012 and 2013 seasons. Simple correlations were determined on combined two-year data for identification of relationships through statistical analyses with GenStats for Windows version 10 (Payne et al. 2007). Two-way ANOVA for genotype (G), environment (E) and G x E interactions were done over the 2012 and 2013 seasons and results were used for dividing genotypes into three (high, middle and low ranking) groups for flour content, grain protein content and loaf volume. Simple correlations were determined on combined two-year data for identification of relationships

through statistical analyses with GenStats for Windows version 10 (Payne et al. 2007). The use of the terms highest or lowest ranking average for flour protein, grain protein and loaf volume in the results and discussion section, refers to the expression of the three traits by genotypes in the highest ranking and lowest ranking groups and the consequent comparison thereof with other quality parameters of the same group of genotypes. For example, correlation of high ranking average flour protein with loaf volume would refer to correlations of the average flour protein of the three highest ranked genotypes with loaf volume of the same genotypes.
In the results and discussion section, flour protein, grain protein and loaf volume of these three groups were compared with other quality parameters in the same group of genotypes. Simple correlations were determined on combined two-year data for identification through statistical analyses with GenStats for Windows version 10 (Payne et al. 2007).

5.3 Results and discussion

5.3.1 Genotype x environment interaction and analysis of variance of flour protein, grain protein and loaf volume

5.3.1.1 Effects of environment and genotype

Two-way ANOVA indicated two distinctly different production trends in 2012 and 2013 for the Bethlehem and Clarens test sites (Table 5.1). Higher rainfall in 2012 caused better grain yields at both Bethlehem and Clarens test sites which are in contrast to poorer yields from both sites in 2013 (see Chapter 3 for a detailed summary of climate and yields). Higher grain yield at the Clarens site compared to Bethlehem over both years was negatively associated with grain protein content. Grain yield (5.38 ton ha^{-1}) and falling number (367 secs) at Clarens in 2012 were significantly higher than at Bethlehem (3.69 ton ha^{-1} and 331 secs respectively). The Bethlehem site, however, produced higher grain protein (14.64%) and hectolitre mass (78.2 kg hl^{-1}) compared to Clarens (13.11% and 77.1 kg hl^{-1}). In 2013 grain yield (2.49 ton ha^{-1}), hectolitre mass (80 kg hl^{-1}) and falling number (244 secs) at Clarens were higher than

Bethlehem (1.99 ton ha^{-1}, 74.8 kg hl^{-1} and 233 secs respectively) but grain protein (14.78%) was lower than Bethlehem (17.49%). Frequency and intensity of rainfall contributing to grain yield and grain protein contents are clearly counterbalanced between the optimal production conditions of 2012 and sub-optimal conditions in 2013. The critical periods for rainfall influencing grain yield and quality was during the seeding (June/July) and pre-anthesis (September) periods. In the 2012 seeding period, Bethlehem and Clarens received respectively 13.28 mm and 50.83 mm more rainfall than the nine-year mean. These two months occur in the middle of the SA winter and rainfall during this period contributes significantly towards higher seed germination and seedling vigour with a consequently higher plant stand. Showers occurring in September are generally considered as an important factor determining the outcome of yield and quality of rainfed wheat in the SRR. In 2012 Bethlehem and Clarens respectively received 30.42 mm and 61.97 mm more than the nine year mean for this period.

Table 5.1 Grain yield and grain quality of rainfed wheat (summer rainfall region) in 2012 and 2013

Parameters	2012				2013			
	Beth-lehem	Clarens	LSD	P-value	Beth-lehem	Clarens	LSD	P-value
Yield (ton ha^{-1})	3.69^{b}	5.38^{a}	0.13	< 0.001	1.99^{b}	2.49^{a}	0.10	< 0.001
% Grain protein (12% mb)	14.64^{a}	13.11^{b}	0.33	< 0.001	17.49^{a}	14.78^{b}	0.30	< 0.001
Hectolitre mass (kg hl^{-1})	78.2^{a}	77.1^{b}	0.49	< 0.001	74.8^{b}	80.0^{a}	0.30	< 0.001
Falling number (secs)	331^{b}	367^{a}	10.40	< 0.001	233^{b}	244^{a}	9.30	< 0.001

Values within a row with different letters are statistically different

5.3.1.2 Analysis of variance of flour protein, grain protein and loaf volume

Differences between G, E and G x E interaction were highly significant (P < 0.001) for flour and grain protein content and loaf volume (Table 5.2). E (test sites) had the largest impact on flour protein content (55.01%) and grain protein content (74.93%).

Table 5.2 Analysis of variance of flour and grain protein content and loaf volume and percentage contribution to total sum of squares

	Source of variation	SS	% SS	MS	P-value
% Flour Protein	Genotype (G)	71.82	19.89	6.53	< 0.001
	Environment (E)	198.60	55.01	66.20	< 0.001
	G x E	40.24	11.14	1.21	0.001
	Residual	50.36		0.54	
	Total	361.04			
	Stratum	**SE**	**CV%**		
	Rep	0.02	0.10		
	Rep.*Units*	0.73	5.10		
% Grain Protein	Genotype (G)	43.58	8.15	3.96	< 0.001
	Environment (E)	400.46	74.93	133.49	< 0.001
	G x E	40.11	7.51	1.22	< 0.001
	Residual	49.58		0.53	
	Total	534.44			
	Stratum	**SE**	**CV%**		
	Rep	0.09	0.60		
	Rep.*Units*	0.72	4.90		
Loaf volume (cm^3)	Genotype (G)	79965.60	20.42	7269.60	< 0.001
	Environment (E)	182159.70	46.51	60719.90	< 0.001
	G x E	77770.50	19.86	2356.70	< 0.001
	Residual	49865.60		530.50	
	Total	391637.50			
	Stratum	**SE**	**CV%**		
	Rep	4.40	0.40		
	Rep.*Units*	23	2.30		

The effect of G was larger on variation of flour protein (19.89%) than grain protein (8.15%). General variation in loaf volume was primarily determined by E (46.51%) although contributions of G (20.42%) and G x E interaction (19.86%) were also substantial (Table 5.2).

5.3.1.3 Two way analyses of variance for ranking of genotypes into groups

Two-way ANOVA (Table 5.2) was employed to rank genotypes for flour and grain protein content and loaf volume and then these ranked groups were applied in correlations with the primary quality parameters and protein fractions.

Table 5.3a Means for percentage flour protein content of genotypes in the summer rainfall region

Genotype	% Value	Means	Rank group
PAN 3368	15.37[a]		
Koonap	15.17[ab]	15.21	High
Gariep	15.08[ab]		
Senqu	14.96[abc]		
PAN 3195	14.92[abc]	14.70	Middle
Elands (quality standard)	14.78[abcd]		
PAN 3379	14.15[bcde]		
SST 317	13.97[cde]		
PAN 3161	13.78[de]		
SST 347	13.70[e]	13.64	Low
SST 316	13.38[e]		
SST 356	13.35[e]		
CV%	5.1		
LSD	0.593		
P-value	< 0.001		

Values within a column with different letters are statistically different

High flour protein was expressed by the highest ranking group consisting of PAN 3368, Koonap and Gariep with a mean of 15.21%. Low flour protein was expressed by the lowest ranking group of genotypes; SST 317, PAN 3161, SST 347, SST 316 and SST 356 with an average flour protein content of 13.64% (Table 5.3a).

High grain protein was expressed in the highest ranking group of genotypes for grain protein content namely Koonap, PAN 3368, Senqu and Gariep with a mean of 15.54%. Only SST 316 and SST 356 formed the lowest ranking protein group which had a mean of 14.04% (Table 5.3b). Both high and low protein groups would have achieved a B1 grade (≥ 12%) according to the SA grading norms.

Table 5.3b Means for percentage grain protein content of genotypes in the summer rainfall region

Genotype	% Value	Wheat grade (Class B wheat)	Means	Rank group
Koonap	15.85[a]	B1	15.54	High
PAN 3368	15.60[ab]	B1		
Senqu	15.39[abc]	B1		
Gariep	15.30[abc]	B1		
Elands (quality standard)	15.13[abcd]	B1	14.77	Middle
PAN 3161	14.87[abcde]	B1		
PAN 3195	14.74[bcde]	B1		
SST 347	14.73[bcde]	B1		
PAN 3379	14.64[bcde]	B1		
SST 317	14.50[cde]	B1		
SST 316	14.13[de]	B1	14.04	Low
SST 356	13.95[e]	B1		
CV%	4.9			
LSD	0.589			
P-value	< 0.001			

Values within a column with different letters are statistically different

Specifications of the SA milling and baking industry allow loaf volumes of cultivars to vary within a ±10% range of the value of the quality standard of the specific production region (Elands for rainfed wheat in the SRR). Loaf volumes of genotypes in both the highest and lowest ranked groups in Table 5.3c were within these specifications. The genotypes PAN 3368, Gariep, Koonap, Senqu and Elands were in the highest ranking loaf volume group of 1012.76 cm^3. The lowest ranking loaf volume group consisted of PAN 3379, SST 317, SST 356 and PAN 3195 with a mean of 959.83 cm^3 (Table 5.3c).

Table 5.3c Means for loaf volume of genotypes in the summer rainfall region

Genotype	Cm^3	Means	Rank group
PAN 3368	1017.1[a]		
Gariep	1014.2[ab]		
Koonap	1014.2[ab]	1012.8	High
Senqu	1010.8[ab]		
Elands (quality standard)	1007.5[ab]		
SST 347	995.0[abc]		
PAN 3161	990.0[abcd]	989.0	Middle
SST 316	981.9[bcd]		
PAN 3379	968.8[cde]		
SST 317	965.8[cde]	959.8	Low
SST 356	958.5[de]		
PAN 3195	946.2[e]		
CV%	2.3		
LSD	18.7		
P-value	< 0.001		

Values within a column with different letters are statistically different

5.3.2 Simple correlations of flour protein, grain protein and loaf volume with the primary quality parameters and protein fractions

5.3.2.1 Correlations with the primary quality parameters

The summary table (Table 5.4a) illustrates positive three way correlations between flour protein content, grain protein content and loaf volume. This result is in line with other studies implicating positive correlations between protein content and loaf volume (Perez Borla et al. 2004; Wilkstrőm and Bohlin 1999). Both high and low flour protein group averages correlated more strongly with loaf volume than grain protein content and had correlations of 0.61*** and 0.71*** respectively. Wieser and Kieffer (1999) reported stronger correlations between gluten proteins (flour) and loaf volume than between whole grain protein and loaf volume. High and low flour protein and grain protein and loaf volume furthermore had strong positive correlations

with alveograph (Wf), dough strength and wet gluten. Correlations between high and low flour protein group averages (0.74*** and 0.80*** respectively) and high and low grain protein content group averages (0.84*** and 0.80*** respectively) with wet gluten and are in agreement with findings by numerous authors worldwide (Cozzolino et al. 2006; Šimić et al. 2006; Bilgin et al. 2010; Egesel et al. 2012). Kulkarni et al. (1987) found that strong correlations between protein content and wet and dry gluten applied to both hard red winter and hard red spring wheat. Significant negative correlations occurred between falling number and high and low flour protein (-0.58*** and -0.57*** respectively), high and low grain protein (-0.64*** and -0.59** respectively) and high and low loaf volume (-0.46*** and -0.52*** respectively) group averages. General high grain protein content (above 12%) over both years and high falling numbers (≥ 233 secs) at both sites over the two years (Table 5.1) probably contributed to this result.

A specific amount of α-amylase enzyme is required for breaking down starches to free sugars for fermentation in bread dough and the correct activity produces high loaf volumes (Perten 2010). In a study to diversify bread products in Romania through addition of fungal α-amylase, loaf volumes of flours with high falling number (> 390 secs) were increased with additional enzyme (Chereji et al. 2008). Loaf volumes of two flours with falling numbers below 290 seconds, however, reached a peak with addition of 56 x 10^3 SKB (after the technique developed by Sandstedt et al. 1939) per 100 kg flour, after which additional enzyme resulted in reduced loaf volume. In the modern era millers and bakers optimise dough consistency, stability and bread quality through fermentation technology (Biotech s.r.o 2011). Low dosages of α-amylase and xylanase or maltogenic α-amylase in combination with fungal α-amylase added to flour enhances crumb softness and structure and loaf volume. The α-amylase degrades the damaged starch in wheat flour into small dextrin, improving yeast activity during dough fermentation, proofing and initial stages of baking. Moderate increases of α-amylase (lower falling number) in samples without sprouted grain would, in the context of the falling number test, indicate lower falling numbers and contradict the general perception that lower falling number is negatively associated with bread quality.

Another interesting observation is the negative correlations between the highest ranking group for flour protein content and the highest ranking group for grain protein content with hectolitre mass (-0.58*** and -0.67*** respectively) (Table 5.4a). A significant negative relationship was reported to exist between protein content and hectolitre mass for hard red spring wheat in western Canada (Tkachuk and Kuzina 1979). They attributed the negative association to the fact that protein content correlated negatively with density (specific gravity or weight) and density correlated positively with hectolitre weight. This negative correlation is an indirect indication of the widely accepted negative association between grain yield (density) and protein content.

In Table 5.4a most of the primary quality parameters correlated with flour and grain protein and loaf volume (at slightly weaker r values). In a study on the effects of white flour and whole wheat flour on relationships between bread quality parameters, Bruckner et al. (2001) found significant correlations between final loaf volumes of whole wheat and white flour in only two of their four test environments, indicating possible significant environmental effects.

Table 5.4a Correlations between flour and grain protein content and loaf volume with primary quality parameters

Para-meter	ANOVA ranking	Mean	Alveo (Wf)	Dough strength	Dough stability (P)	Dough distensibility (L)	Dough P/L value	Flour extraction	Falling number	Flour protein content	Grain protein content	Hecto-litre mass (kg hl^{-1})	Loaf volume (cm^3)	Peak time minutes (MPT)	Wet Gluten
% Flour protein content	High (n=3)	15.21	0.62***	0.61***	NS	NS	NS	NS	-0.58***		0.78***	-0.58***	0.61***	NS	0.74***
	Low (n=5)	13.64	0.49***	0.47***	NS	NS	NS	NS	-0.57***		0.84***	NS	0.71***	NS	0.80***
% Grain protein content	High (n=4)	15.54	0.73***	0.72***	0.48***	NS	NS	NS	-0.64***	0.77***		-0.67***	0.52***	NS	0.84***
	Low (n=2)	14.04	0.78***	0.78***	0.46*	NS	NS	0.52**	-0.59**	0.88***		NS	0.68***	NS	0.80***
Loaf volume (cm^3)	High (n=5)	1012.8	0.49***	0.50***	NS	NS	NS	NS	-0.46***	0.60***	0.56***	NS		NS	0.61***
	Low (n=4)	959.8	0.60***	0.60***	0.45**	NS	NS	0.51***	-0.52***	0.66***	0.66***	NS		NS	0.55***

n - Number of genotypes in the highest and lowest ranking groups. Mean value is average of the rank group
*** P ≤ 0.001, ** P 0.01, * P ≤ 0.05, NS - not significant

5.3.2.2 Correlations with protein ratios

Correlations between flour protein, grain protein and loaf volume with protein ratios of the highest ranking group

There were high positive correlations between percentage insoluble glutenin (larger polymeric proteins) in total glutenin with highest flour protein content (0.63***), highest grain protein content (0.63***) and highest loaf volume (0.53***) (Table 5.4b). High amounts of glutenin molecules result in extended mixing times (Bietz and Wall 1973, Bietz and Huebner 1980) which, in turn, is strongly associated with genotypes exhibiting very good loaf volume potential (Finney and Yamazaki 1967). Uthayakumaran et al. (2002) reported that glutenin interacted significantly in determining many rheological properties of doughs. The overall contribution of glutenin was more important than the sum of contributions made by the different glutenin subunits and indicated that these proteins contribute to good baking quality. Higher flour protein resulting from increased nitrogen uptake changes the proportional composition of protein classes. The principle behind increased SDS-unextractable proteins in malting barley addressed by Johansson (2013) indirectly explain the strong positive correlations between insoluble glutenin in total glutenin (% TUPP) and high protein (Table 5.4b). Higher protein concentrations have been positively correlated with break down rates of proteins and unextractable polymeric proteins are positively linked to gluten strength (flour protein).

Negative correlations between small polymeric proteins of the SDS-soluble fraction and flour protein content (Table 5.4b) corresponded with results of Meintjés (2004).

Table 5.4b Correlations between protein fractions and highest ranking averages for flour protein, grain protein and loaf volume of the highest ranking groups

Solubility classes	Protein fractions	Flour protein	Grain protein	Loaf volume
% soluble fraction of different protein fractions in total soluble protein	Large polymeric proteins (LPP)	NS	NS	NS
	Small polymeric proteins (SPP)	-0.48**	NS	NS
	ω Gliadin (Large monomeric)	0.49**	0.48***	NS
	α/β, γ Gliadin (Large monomeric)	NS	NS	NS
	Albumin and globulin (Small monomeric)	NS	NS	NS
% insoluble fraction of different protein fractions in total insoluble protein	Large polymeric proteins (LPP)	0.51**	NS	NS
	Small polymeric proteins (SPP)	-0.50**	NS	NS
	ω Gliadin (Large monomeric)	0.50**	NS	NS
	α/β, γ Gliadin (Large monomeric)	0.46**	NS	NS
	Albumin and globulin (Small monomeric)	NS	NS	NS
% soluble fraction of different protein fractions in total protein	Large polymeric proteins (LPP)	NS	NS	NS
	Small polymeric proteins (SPP)	-0.51**	NS	NS
	ω Gliadin (Large monomeric)	0.45**	NS	NS
	α/β, γ Gliadin (Large monomeric)	NS	NS	NS
	Albumin and globulin (Small monomeric)	NS	NS	NS
% insoluble fraction of different protein fractions in total protein	Large polymeric proteins (LPP)	0.57***	0.49***	NS
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	0.58***	0.52***	NS
	α/β, γ Gliadin (Large monomeric)	0.55***	0.55***	NS
	Albumin and globulin (Small monomeric)	NS	NS	NS
% insoluble fraction of different protein fractions in total similar fraction	Large polymeric proteins (LPP)	0.63***	0.63***	0.53***
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	NS	NS	NS
	α/β, γ Gliadin (Large monomeric)	0.57***	0.53***	NS
	Albumin and globulin (Small monomeric)	NS	NS	NS
% insoluble glutenin in total glutenin		0.51**	0.45***	NS

*** P ≤ 0.001, ** P ≤ 0.01, NS - not significant

Several of the ω and α/β, γ gliadin fractions (large monomeric proteins) correlated with highest average flour- and grain protein (Table 5.4b). The most prominent result however, was the insoluble ω gliadin in total protein correlating positively with highest average flour protein (0.58**) and grain protein (0.52***) and insoluble α/β, γ gliadin in total protein correlating positively with highest average flour protein (0.55***) and grain protein (0.55***). During the processes involved in converting additional nitrogen into higher protein content, gliadin protein numbers escalates at a faster rate than other proteins (Gupta and Shepherd (1993). Similarly, to results of Gupta and Shepherd (1993) and Meintjés (2004) results from this study indicate positive correlations between flour protein content and the SDS-soluble fraction of gliadin.

Correlations between flour protein, grain protein and loaf volume with protein ratios of the lowest ranking group

The grain protein content reduced with 1.50% from 15.54% of the highest ranking group to 14.04% of the lowest ranking group (Table 5.3b). The overall number of correlations for the lowest ranking flour and grain protein content and loaf volume groups (21 correlations) was very equal to the highest ranking flour, grain and loaf volume groups (22 correlations).

A very important outcome is the negative correlations of percentage soluble albumin and globulin in total soluble protein with lowest ranking group flour protein (-0.45***), grain protein (-0.80***) and loaf volume (-0.52***) values. Both lowest ranking flour protein and grain protein correlated negatively with insoluble albumin and globulin in total insoluble protein, soluble albumin and globulin in total protein and insoluble albumin and globulin in total protein. Singh et al. (1990) reported a very strong negative correlation between the relative quantity of albumin/globulin and flour protein from 15 cultivars.

Table 5.4c Correlations between protein fractions and flour protein, grain protein and loaf volume of the lowest ranking groups

Solubility classes	Protein fractions	Flour protein	Grain protein	Loaf volume
% soluble fraction of different protein fractions in total soluble protein	Large polymeric proteins (LPP)	NS	NS	NS
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	NS	0.58**	NS
	α/β, γ Gliadin (Large monomeric)	NS	NS	NS
	Albumin and globulin (Small monomeric)	-0.45***	-0.80***	-0.52***
% insoluble fraction of different protein fractions in total insoluble protein	Large polymeric proteins (LPP)	NS	0.51*	NS
	Small polymeric proteins (SPP)	-0.48***	NS	-0.51***
	ω Gliadin (Large monomeric)	NS	NS	NS
	α/β, γ Gliadin (Large monomeric)	NS	NS	NS
	Albumin and globulin (Small monomeric)	NS	-0.73***	-0.67***
% soluble fraction of different protein fractions in total protein	Large polymeric proteins (LPP)	NS	NS	NS
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	NS	0.50*	NS
	α/β, γ Gliadin (Large monomeric)	NS	NS	NS
	Albumin and globulin (Small monomeric)	NS	-0.86***	-0.55***
% insoluble fraction of different protein fractions in total protein	Large polymeric proteins (LPP)	NS	0.62**	0.48***
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	NS	0.66***	NS
	α/β, γ Gliadin (Large monomeric)	NS	NS	NS
	Albumin and globulin (Small monomeric)	NS	-0.50**	-0.57***
% insoluble fraction of different protein fractions in total similar fraction	Large polymeric proteins (LPP)	NS	0.65***	0.67***
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	NS	NS	NS
	α/β, γ Gliadin (Large monomeric)	NS	0.50**	NS
	Albumin and globulin (Small monomeric)	NS	0.63**	NS
% insoluble glutenin in total glutenin			NS	NS

*** P ≤ 0.001, ** P ≤ 0.01, NS - not significant

The results from this study are furthermore supported by previous studies on SA wheat by Labuschagne and Aucamp (2004) and Meintjés (2004). Both studies reported that the small monomeric proteins (albumin and globulin) of the SDS-soluble and insoluble fractions were significantly negatively correlated with grain and flour protein content. Park et al. (2006) reported that amounts of albumin and globulin in relation to total protein weight had a highly negative correlation with flour protein content. They attributed their results to the fact that with a 3.7% increase of protein content the related increases of albumin and globulin are much smaller compared to other protein subclass fractions, hence the negative associations. Results from a study by Horvat et al. (2012) on winter wheat in Croatia correlated albumin and globulin negatively with protein content, wet gluten (flour protein) and loaf volume.

The lowest ranking group grain protein content and loaf volume values were positively correlated with the larger polymeric proteins. Both parameters correlated positively with percentage insoluble larger polymeric proteins in total protein and percentage insoluble larger polymeric proteins in total larger polymeric proteins (Table 5.4c).

5.4 Conclusions

The observation that flour from rainfed wheat produced in the SRR generally has high protein content with strong rheological characteristics is verified by results in this study. If only grain protein content is considered, all genotypes in both the highest and lowest ranking groups used in this chapter would have obtained a B1 grading (≥ 12% protein). Loaf volumes were also well within the 10% variation allowed by the baking industry when compared to the biological standard (Elands).

Global reports of strong and positive three way correlations between flour protein content, grain protein content and loaf volume, and between these three parameters and wet gluten were confirmed for genotypes adapted for rainfed conditions in the SRR. These correlations

were significant for both the highest ranking protein and lowest ranking protein groups and exerts confidence in the current grading system. As elsewhere, grain protein content in SA determines farmers' wheat price as the method is very simple and quick to use. Wheat buyers apply protein content as an indicator of the end use application of wheat bulks available on markets, whereas bakers regard protein content as the standard reference of gluten strength and dough mixing properties of flour. An interesting observation, although of marginal importance in this study, is the negative correlation between highest and lowest ranked flour protein content with falling number. The main conclusion from this result is verification that high starch and protein content exist in rainfed wheat from the SRR. Low α-amylase activity results in higher falling numbers in sound wheat (wheat without any sprouted seed) which frees up more sugars to increase protein content and loaf volume.

The most significant result regarding protein fractions and consequent ratios in rainfed wheat is the positive correlations between percentage insoluble glutenin in total glutenin with high flour protein content, high grain protein content and high loaf volume. Glutenins are associated with extended mixing times that, in turn, are strongly associated with very good loaf volumes. For the lowest ranked values for flour protein, grain protein and loaf volume the negative correlations with percentage soluble albumin and globulin in total soluble protein were prominent.

For rainfed wheat production in the SRR whole wheat and flour protein content were reliable indicators of loaf volume for both 2012 and 2013. Strong positive correlations occurring directly between protein content and loaf volume or indirectly through strong associations with wet gluten were seen. Protein composition might in the long term prove to explain environmental and production effects on protein content more accurately.

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Chapter 6

The relationship between protein content and composition of wheat produced in the winter rainfall region

6.1 Introduction

Researchers in Canada made a very important statement when they said: "*Drought is one of the costliest weather related natural hazards in the world*" (Mkhabela et al. 2010). They concluded that the drought index associated most strongly with grain yield and flour quality parameters, is water demand (evapotranspiration). Rainfed wheat in the western and southern Cape regions of SA is produced on residual and duplex soil types with limited depth and water holding capacity (ARC-SGI 2014). These restrictions, together with rapidly declining rainfall and steep temperature increases in early October after anthesis (Marais 1985), primarily determine grain yield and quality. Implementation of conservation agriculture in this region has contributed to significant increases in wheat yields. Conservation agriculture is less intensive than conventional systems and applies three basic principles: minimal soil disturbance, permanently implemented soil cover and crop rotations (Moreno et al. 2010). In global quests for higher yields, there is mostly a trade-off between yield and protein content as plant available reserves are shared by both. In a study to determine optimal seeding rates for organically produced spring wheat in Alberta, Beavers et al. (2008) found increasing seeding rates by 25% improved yields and did not affect grain protein. In a comparison of yield and quality of Siberian genotypes developed from 1900 to 2010 (Morgounov et al. 2013) reductions in protein and wet gluten contents occurred simultaneously with improvements of loaf volumes. These results are confirmed by other authors reporting that genetic gains in yield were associated with improved dough quality despite a decrease in protein content (Souza et

al. 1993; Gómez et al. 2009). In SA protein content is applied as determining factor of baking quality and a parameter for wheat grading. Grain protein content (crude protein) together with test weight (hectolitre mass) and falling number (α-amylase activity) are key parameters of the national grading system determining a farmers' wheat price. Protein content is furthermore used by wheat buyers as indicator of the end use application of wheat bulks whereas the baking industry regards protein content as the standard reference of gluten strength and dough mixing properties of flour. Protein content is highly sensitive to environmental factors and Noor Hasniza et al. (2014) reported significant influences of environment on total grain protein and dough functional properties. In contrast however, protein content of the soluble and insoluble extractable protein fractions was determined by genotype. Gómez et al. (2009) showed that genetic gains in grain yield are associated with increased physical dough properties in spite of reduced protein contents. A question posed by Reese et al. (2007) summarises a general query among wheat farmers when they ask: *"Is protein enough for assessing wheat flour quality?*" They recommend that for accurate predictions of flour quality, additional information supporting protein content should be obtained from farinograph and alveograph analyses.

This study aimed to (a) validate protein content as a measurement for bread making quality of rainfed wheat in the WRR, (b) identify prominent protein fractions and ratios correlated with bread baking quality and (c) define the effects of genotype and environment on variation of protein quantity and quality of rainfed wheat produced in SA.

6.2 Material and methods

6.2.1 Genotypes (cultivars) and environments (field test sites)

Rainfed field experiments at the WRR sites of Moorreesburg and Riversdale were established with 15 genotypes in each trial. The commercial cultivar SST 027 is the current biological

quality standard for rainfed wheat in the WRR with the purpose of monitoring effects of seasonal variation in rainfall, temperature and production practises on wheat quality. Experimental design of both trial sites was a complete randomized block design with three replications. A detailed explanation of procedures and equipment used for seeding, trial maintenance, harvesting and generation of data are provided in Chapter 3.

6.2.2 Meteorology and climatic trends

Meteorological data for the two test sites in 2012 and 2013 were obtained from the database of the ARC-ISCW 2014. Monthly annual rainfall and minimum and maximum temperatures from 2005 to 2013 were used for calculating a nine year mean for the three meteorological elements. The nine year means and 2012 and 2013 data were compared to identify major climatic trends influencing grain yield and quality at the two test sites over the two years. Climatic data was applied to determine trends in the pre-seeding (January to May), seeding (June and July), early growth (August), pre-anthesis (September) and post-anthesis and maturity (October to December) periods of the season.

6.2.3 Grain quality and primary quality parameters

Grain samples received from Moorreesburg and Riversdal for 2012 and 2013 were analysed at the quality laboratory of the ARC-SGI (Bethlehem, SA). Hectolitre mass, protein content, alveograph (P, L and P/L), farinograph (W), wet gluten and loaf volume were determined to establish milling and baking quality of the cultivars from both test sites. For detailed information on methods used refer to Chapter 3.

Grain quality was determined by measuring the hectolitre mass (ISO 7971-3:2009), percentage grain protein content (AACCI Method 46-10) and falling number (ISO 3093:2009). The primary quality parameters were determined and served as indicators of bread making

quality. The processes and tests consisted of seed conditioning (AACCI Method 26-95.01), wet gluten measurement (AACCI Method 38-12.02), alveograph analyses (ICC Standard Methods No. 121, latest edition), percentage flour protein content (AACC method 39–11), loaf volume (AACCI Method 10-10.03 and AACCI Method 10-10B) and farinograph water absorption (AACCI method 54-21.02).

6.2.4 Size-Exclusion High Performance Liquid Chromatography

SE-HPLC is applied to assess protein aggregates and is used for quick analyses of baking quality of bread wheat. SE-HPLC effectively separates the two main classes of wheat proteins (storage proteins and structural proteins) and results are highly correlated with primary quality parameters. The SE-HPLC procedure of Gupta et al. (1993) was followed, with modifications. After the different fractions were separated, the various ratios between fractions were calculated which enabled more accurate comparisons as solutions were standardised through expression of concentrations as percentages.

6.2.5 Statistical analyses

Two-way ANOVA for genotype (G), environment (E) and G x E interactions were done for 2012 and 2013 and results were used to divide genotypes into three (high, middle and low ranking) groups for flour content, grain protein content and loaf volume. Simple correlations were determined on combined two-year data for identification of relationships through statistical analyses with GenStats for Windows version 10 (Payne et al. 2007). The use of the terms highest or lowest ranking average for flour protein, grain protein and loaf volume in the results and discussion section, refers to the expression of the three traits by genotypes in the highest rank and lowest rank groups and the consequent comparison thereof with other quality parameters of the same group of genotypes. For example, correlation of high ranking average

flour protein with loaf volume would refer to correlations of the average flour protein of the three highest ranked genotypes with loaf volume of the same genotypes.

6.3 Results and discussion

6.3.1 Genotype x environment interaction and analysis of variance of flour protein, grain protein and loaf volume

6.3.1.1 Effects of environment and genotype

The general trend in regard to grain yield observed for Moorreesburg and Riversdale are also evident in NCEP data from the middle Swartland (Moorreesburg) and eastern Rûens (Riversdale) as described in Chapter 3. Two way analyses indicated that average grain yield (Table 6.1) at Moorreesburg in 2012 (4.99 ton ha^{-1}) was approximately half a ton less than in 2013 (5.52 ton ha^{-1}) resulting in higher grain quality (hectolitre mass and falling number but slightly lower grain protein content). Rainfall during the early growth and pre-anthesis periods at Moorreesburg in 2013 was approximately 80 mm more than the long term average and could be the primary reason for the higher yields in 2013 (refer to Table 3.6 in Chapter 3). In contrast, grain yield at Riversdale in 2012 (5.09 ton ha^{-1}) was 0.8 ton ha^{-1} more than in 2013 (4.31 ton ha^{-1}) with lower grain protein content and falling number but higher hectolitre mass in 2012 (Table 6.1). This reversed trend in comparison to Moorreesburg is a result of higher rainfall at Riversdale in 2012 for the pre-seeding, seeding, early growth and pre-anthesis periods compared to the long term and 2013 means (refer to Chapter 3).

Table 6.1 Grain yield and grain quality of rainfed wheat (winter rainfall region) in 2012 and 2013

Parameters	2012				2013			
	Moor-reesburg	Rivers-dale	LSD	P-value	Moor-reesburg	Rivers-dale	LSD	P-value
Yield (ton ha^{-1})	4.99[b]	5.09[a]	0.18	NS	5.52[a]	4.31[b]	0.19	< 0.001
% Grain protein (12% mb)	13.18[a]	11.34[b]	0.34	< 0.001	14.31[a]	12.36[b]	0.42	< 0.001
Hectolitre mass (kg hl^{-1})	80.9[a]	79.4[b]	0.50	< 0.001	76.9[a]	76.2[b]	0.40	0.002
Falling number (secs)	383.1[a]	291.5[b]	14.23	< 0.001	312.7[b]	364.6[a]	9.04	< 0.001

Values within a row with different letters are statistically different

6.3.1.2 Analysis of variance of flour protein, grain protein and loaf volume

Differences in flour and grain protein content and loaf volume (Table 6.2) of G and E were highly significant ($P < 0.001$) whereas the G x E interaction was highly significant for loaf volume ($P < 0.001$) and significant only for flour protein content ($P < 0.01$). E (test sites) had the largest impact on flour protein (43.81%) as measured as a percentage of total sum of squares, followed by the G x E interaction (16.89%) and G (15.03%). E also had the largest effect on variation of grain protein (49.70%) followed by genotype (23.23%) and G x E interaction (8.46%). Variation of loaf volume was approximately equally determined by E (29.68%), G x E interaction (24.14%) and G (20.71%).

Table 6.2 Analysis of variance of flour and grain protein content and loaf volume and percentage contribution to total sum of squares

	Source of variation	SS	% SS	MS	P-value
% Flour Protein	Genotype (G)	45.58	15.03	4.14	< 0.001
	Environment (E)	132.86	43.81	44.29	< 0.001
	G x E	51.22	16.89	1.55	< 0.01
	Residual	67.94		0.72	
	Total	303.25			
	Stratum	**SE**	**CV%**		
	Rep	0.24	2.00		
	Rep.*Units*	0.85	6.90		
% Grain Protein	Genotype (G)	80.10	23.23	7.28	< 0.001
	Environment (E)	171.35	49.70	57.12	< 0.001
	G x E	29.18	8.46	0.88	NS
	Residual	63.61		0.68	
	Total	344.78			
	Stratum	**SE**	**CV%**		
	Rep	0.08	0.60		
	Rep.*Units*	0.82	6.40		
Loaf volume (cm^3)	Genotype (G)	98814	20.71	8983	< 0.001
	Environment (E)	141599	29.68	47200	< 0.001
	G x E	115182	24.14	3490	< 0.001
	Residual	113828		1211	
	Total	477162			
	Stratum	**SE**	**CV%**		
	Rep	9	1.00		
	Rep.*Units*	34.80	3.70		

NS - not significant

6.3.1.3 Two way analyses of variance for ranking of genotypes into groups

Two-way ANOVA was conducted to identify the highest and lowest ranking group averages for flour and grain protein content and loaf volumes which were used in correlations with the primary quality parameters and protein fractions (Tables 6.3 a-c).

Table 6.3a Means for percentage flour protein content of genotypes in the winter rainfall region

Genotype	% Value	Means	Rank group
SST 047	13.82[a]	13.01	High
Kwartel	12.87[ab]		
SST 027	12.70[ab]		
Tankwa	12.66[ab]		
PAN 3408	12.32[b]	12.26	Middle
PAN 3471	12.29[b]		
SST 096	12.28[b]		
SST 015	12.14[b]		
Ratel	12.04[b]	11.84	Low
SST 056	11.82[b]		
SST 88	11.78[b]		
SST 087	11.71[b]		
CV%	6.90		
LSD	0.69		
P-value	< 0.001		

Numbers followed by the same letter were not statistically significantly different

Few of the genotypes differed significantly and ranking of genotypes in this particular instance was based on simply dividing the complete rank list into thirds. High flour protein was expressed by genotypes SST 047, Kwartel, SST 027 and Tankwa with a mean of 13.01%, achieving a B1 grade. The four genotypes forming the lowest ranking group were Ratel, SST 056, SST 88 and SST 087 with an average of 11.84% (Table 6.3a).

The two genotypes forming the highest ranking group of grain protein content were SST 047 and Kwartel with a mean of 14.03% achieving a B1 grade. The lowest ranking group consisted of SST 087 and SST 88 with a mean of 11.99% achieving a B2 grade (≥ 12%) according to the SA grading norms (Table 6.3b).

Table 6.3b Means for percentage grain protein content of genotypes in the winter rainfall region

Genotype	% Value	Wheat grade (Class B wheat)	Means	Rank group
SST 047	14.82[a]	B1	14.03	High
Kwartel	13.24[b]	B1		
SST 027	13.18[bc]	B1	12.69	Middle
Tankwa	12.95[bcd]	B1		
PAN 3408	12.84[bcd]	B1		
SST 096	12.79[bcd]	B1		
Ratel	12.76[bcd]	B1		
PAN 3471	12.76[bcd]	B1		
SST 015	12.13[bcd]	B1		
SST 056	12.12[bcd]	B1		
SST 087	12.01[cd]	B1	11.99	Low
SST 88	11.96[d]	B2		
CV%	6.40			
LSD	0.67			
P-value	< 0.001			

Values within a column with different letters are statistically different

Specifications of the SA milling and baking industry allow loaf volumes of cultivars to vary within a ± 10% range of the loaf volume obtained by SST 027 (quality standard for rainfed wheat in the WRR). Both the highest ranking average (980.8 cm^3) and lowest ranking averages for loaf volume (908.7 cm^3) for groups of rainfed wheat in the WRR (Table 6.3c) were within the acceptable range for loaf volume (874.1 cm^3 to 1068.3 cm^3).

Table 6.3c Means for loaf volume of genotypes in the winter rainfall region

Genotype	Cm^3	Means	Rank group
SST 047	990.4[a]	980.8	High
SST 027 (quality standard)	971.2[ab]		
SST 096	954.2[abc]	939.7	Middle
Kwartel	948.3[abc]		
PAN 3408	945.8[abc]		
SST 015	935.8[bcd]		
PAN 3471	931.2[bcd]		
Ratel	922.9[bcd]		
SST 087	915.8[cd]	908.7	Low
SST 056	913.8[cd]		
SST 88	910.0[cd]		
Tankwa	895.0[d]		
CV%	3.7		
LSD	28.2		
P-value	< 0.001		

Values within a column with different letters are statistically different

6.3.2 Simple correlations of flour protein, grain protein and loaf volume with the primary quality parameters and protein fractions

6.3.2.1 Correlations with the primary quality parameters

Positive three way correlations occurred between highest ranking average flour protein content, highest ranking average grain protein content and highest ranking average loaf volume (Table 6.4a). These results correspond with international studies reporting positive correlations between protein content and loaf volume (Perez Borla et al. 2004; Wilkstrőm and Bohlin 1999). Highest ranking average flour protein content correlated strongest with wet gluten (0.84***), grain protein content (0.76***) and dough strength (0.61***). Lowest ranking average flour protein correlated strongest with wet gluten (0.83***), alveograph strength (0.56***) and grain protein content (0.54***) but negatively with flour extraction (-0.49***).

Correlations of the highest and lowest ranking average flour protein and grain protein values with wet gluten follow results reported by Cozzolino et al. (2006), Šimić et al. (2006), Bilgin et al. (2010) and Egesel et al. (2012).

Highest ranking average grain protein content correlated strongest with wet gluten (0.85***), flour protein content (0.73***) and alveograph strength (0.66***). Lowest ranking average grain protein correlated strongest with wet gluten (0.60**) and falling number (0.56**) but a negative correlation occurred between lowest ranking average grain protein and flour extraction (-0.58***). Highest ranking average loaf volume correlated strongly with dough strength (0.79***), flour protein content (0.77***) and wet gluten (0.74***). Lowest ranking average loaf volume had no correlations with any of the quality parameters. As the main objective of this study was the comparison between protein quantity and protein quality, it is noteworthy that the only significant correlation between loaf volume and grain protein occurred between the highest ranking average loaf volume and grain protein content (0.50*). This correlation was weaker than correlations of highest ranking average loaf volume with flour protein (0.77***) or wet gluten (0.74***). The low number of correlations for loaf volume could probably be explained by the findings of Bruckner et al. (2001) reporting significant correlations between final loaf volumes of whole wheat and white flour at only two of their four test environments, indicating significant environmental interactions.

Table 6.4a Correlations between flour and grain protein content and loaf volume with primary quality parameters

Para-meter	ANOVA ranking	Mean	AlveoW	Dough strength	Dough stability (P)	Dough distensibility (L)	Dough P/L value	Flour extraction	Falling number	Flour protein content	Grain protein content	Hecto-litre mass (kg hl^{-1})	Loaf volume (cm^3)	Peak time minutes (MPT)	Wet Gluten
% Flour protein content	High (n=3)	13.01	0.59***	0.61***	NS	NS	NS	NS	NS		0.76***	NS	0.59***	NS	0.84***
	Low (n=5)	11.84	0.56***	NS	NS	NS	NS	-0.49***	NS		0.54***	NS	NS	NS	0.83***
% Grain protein content	High (n=4)	14.03	0.66***	0.46*	NS	NS	NS	NS	NS	0.73***		NS	0.53**	NS	0.85***
	Low (n=2)	11.99	NS	NS	NS	NS	-0.45*	-0.58**	0.56**	0.51*		NS	NS	NS	0.60**
Loaf volume (cm^3)	High (n=5)	980.8	0.46*	0.79***	NS	NS	NS	NS	NS	0.77***	0.50*	0.63***		0.49*	0.74***
	Low (n=4)	908.7	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		NS	NS

n - Number of genotypes in the high- and low rank groups. Mean value is average of the rank group
*** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05, NS - not significant

6.3.2.2 Correlations with protein ratios

Correlations between protein ratios and highest ranking groups of flour protein, grain protein and loaf volume

Positive and significant correlations occurred between insoluble glutenin (large polymeric proteins) fractions in total insoluble protein and flour protein content (0.60***), grain protein content (0.85***) and loaf volume (0.55**) (Table 6.4b). Soluble glutenin in total protein also correlated positively with grain protein (0.55**) but negatively with loaf volume (-0.48*). The insoluble glutenin fraction in total protein correlated positively with flour protein (0.46***), grain protein (0.55**) and loaf volume (0.46***). Bietz and Huebner (1980) reported that high amounts of glutenin molecules extend mixing times which correlates strongly with good loaf volume potential. Uthayakumaran et al. (2002) remarked that the overall contribution of HMW-GS seems more important for good baking quality than individual contributions by the different HMW-GS. During the processes responsible for converting supplemental nitrogen into higher protein content, glutenin protein numbers escalate faster than any other proteins (Gupta and Shepherd, 1993) and consequently reflect in higher glutenin concentrations.

Soluble percentage ω gliadin (large monomeric proteins) fractions in total soluble protein (0.49*) and in total protein (0.52**) correlated positively with grain protein. Insoluble ω gliadin in total insoluble protein (-0.63***) correlated negatively with loaf volume and in total ω gliadin (-0.59**) correlated negatively with grain protein content. Soluble α/β, γ gliadin (large monomeric proteins) in total soluble protein correlated positively with grain protein (0.62**) and loaf volume (0.50*) whereas soluble α/β, γ gliadin in total protein correlated positively with grain protein (0.64***).

Table 6.4b Correlations between protein fractions and flour protein, grain protein and loaf volume of the highest ranking groups

Solubility classes	Protein fractions	Flour protein	Grain protein	Loaf volume
% soluble fraction of different protein fractions in total soluble protein	Large polymeric proteins (LPP)	NS	NS	NS
	Small polymeric proteins (SPP)	NS	-0.57**	NS
	ω Gliadin (Large monomeric)	NS	0.49*	NS
	α/β, γ Gliadin (Large monomeric)	NS	0.62**	0.50*
	Albumin and globulin (Small monomeric)	-0.48***	-0.77***	NS
% insoluble fraction of different protein fractions in total insoluble protein	Large polymeric proteins (LPP)	0.60***	0.85***	0.55**
	Small polymeric proteins (SPP)	-0.45**	-0.74***	NS
	ω Gliadin (Large monomeric)	NS	NS	-0.63***
	α/β, γ Gliadin (Large monomeric)	NS	NS	NS
	Albumin and globulin (Small monomeric)	NS	-0.57**	NS
% soluble fraction of different protein fractions in total protein	Large polymeric proteins (LPP)	NS	0.55**	-0.48*
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	NS	0.52**	NS
	α/β, γ Gliadin (Large monomeric)	NS	0.64***	NS
	Albumin and globulin (Small monomeric)	-0.50***	-0.74***	NS
% insoluble fraction of different protein fractions in total protein	Large polymeric proteins (LPP)	0.46***	0.55**	0.46***
	Small polymeric proteins (SPP)	NS	-0.66***	NS
	ω Gliadin (Large monomeric)	NS	NS	NS
	α/β, γ Gliadin (Large monomeric)	NS	NS	NS
	Albumin and globulin (Small monomeric)	NS	-0.53**	NS
% insoluble fraction of different protein fractions in total similar fraction	Large polymeric proteins (LPP)	NS	NS	0.52**
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	NS	-0.59**	NS
	α/β, γ Gliadin (Large monomeric)	NS	NS	NS
	Albumin and globulin (Small monomeric)	NS	NS	NS
% insoluble glutenin in total glutenin			NS	NS

*** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05, NS - not significant

Soluble albumin/globulin in total soluble protein correlated negatively with flour protein content (-0.48***) and grain protein content (-0.77***) and soluble albumin/globulin in total protein with flour protein (-0.50***) and grain protein content (-0.74***) (Table 6.4b). Insoluble albumin/globulin in total insoluble protein correlated negatively with grain protein (-0.57**) and insoluble albumin/globulin in total protein also correlated negatively with grain protein (-0.53**). These negative correlations between albumin/globulin and flour and grain protein content (Table 6.4b) correspond with results from similar studies on SA wheat by Meintjés (2004) and Labuschagne et al. (2003).

Correlations between protein ratios and lowest ranking groups of flour protein, grain protein and loaf volume

The average grain protein content reduced with 2.04% from 14.03% to 11.99% between the highest and lowest ranking groups adapted to rainfed production in the WRR (Table 6.3b). Overall the number of correlations reduced from 26 for the highest ranking group (Table 6.4b) to 17 for the lowest ranking group (Table 6.4c).

The two protein fractions correlating strongest with the flour protein content, grain protein content and loaf volume were glutenin (large polymeric proteins) and albumin/globulin (small polymeric proteins) (Table 6.4c). Insoluble glutenin in total insoluble protein correlated positively with flour protein (0.47***) and grain protein (0.52**), insoluble glutenin in total protein correlated positively with flour protein (0.50***) and grain protein content (0.57**) and insoluble glutenin in total insoluble glutenin with flour protein (0.46***) and grain protein (0.49*).

Table 6.4c Correlations between protein fractions and flour protein, grain protein and loaf volume of the lowest ranking groups

Solubility classes	Protein fractions	Flour protein	Grain protein	Loaf volume
% soluble fraction of different protein fractions in total soluble protein	Large polymeric proteins (LPP)	NS	0.55**	NS
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	NS	NS	-0.45**
	α/β, γ Gliadin (Large monomeric)	NS	NS	NS
	Albumin and globulin (Small monomeric)	-0.45**	-0.62**	NS
% insoluble fraction of different protein fractions in total insoluble protein	Large polymeric proteins (LPP)	0.47***	0.52**	NS
	Small polymeric proteins (SPP)	NS	-0.53**	NS
	ω Gliadin (Large monomeric)	NS	NS	-0.47***
	α/β, γ Gliadin (Large monomeric)	NS	NS	NS
	Albumin and globulin (Small monomeric)	NS	NS	NS
% soluble fraction of different protein fractions in total protein	Large polymeric proteins (LPP)	NS	NS	NS
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	NS	NS	-0.52***
	α/β, γ Gliadin (Large monomeric)	NS	NS	NS
	Albumin and globulin (Small monomeric)	-0.53***	-0.75***	NS
% insoluble fraction of different protein fractions in total protein	Large polymeric proteins (LPP)	0.50***	0.57**	NS
	Small polymeric proteins (SPP)	NS	NS	0.52***
	ω Gliadin (Large monomeric)	NS	NS	NS
	α/β, γ Gliadin (Large monomeric)	NS	NS	NS
	Albumin and globulin (Small monomeric)	NS	NS	NS
% insoluble fraction of different protein fractions in total similar fraction	Large polymeric proteins (LPP)	0.46***	0.49*	NS
	Small polymeric proteins (SPP)	NS	NS	NS
	ω Gliadin (Large monomeric)	NS	NS	0.51***
	α/β, γ Gliadin (Large monomeric)	NS	NS	NS
	Albumin and globulin (Small monomeric)	NS	NS	NS
% insoluble glutenin in total glutenin			NS	NS

*** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05, NS - not significant

An important result is the negative correlations between soluble ω gliadin in total soluble protein (-0.45**), soluble ω gliadin in total protein (-0.52***) and insoluble ω gliadin in total insoluble protein (-0.47***) with loaf volume (Table 6.4c). Negative correlations occurred between soluble albumin/globulin in total soluble protein and lowest ranking flour protein (-0.45**) and lowest ranking grain protein (-0.62**) and soluble albumin/globulin in total protein with flour protein (-0.53***) and grain protein (-0.75***). These negative correlations between albumin/globulin and protein content were also reported by Singh et al. (1990) showing strong negative correlations between the relative quantity of albumin/globulin and flour protein content of 15 wheat cultivars.

Previous studies on SA wheat by Labuschagne and Aucamp (2004) and Meintjés (2004) have also found that albumin/globulin of the SDS-soluble and insoluble fractions were significantly negatively correlated with grain and flour protein content. Park et al. (2006) hypothesised that high negative correlations between albumin/globulin in relationship to total protein weight had a highly negative correlation with flour protein content, probably resulting from increased protein content. They stated that related increases of albumin/globulin are much smaller when compared to other protein fractions which result in the negative associations.

6.4 Conclusions

In the context of the study objectives determining the association between protein quantity (protein content) and protein quality (loaf volume through protein fractions), significant and positive three way correlations occurred between high flour protein content, grain protein content and loaf volume. The only significant correlation between grain protein content with loaf volume was for average grain protein of the highest ranking group and at lower significance levels than with flour protein. The strongest correlations for flour protein content of the highest ranking group were with wet gluten and grain protein and for lowest ranking flour protein group average with wet gluten and alveograph strength. Similarly, highest ranking

grain protein correlated strongest with wet gluten and flour protein content and lowest ranking grain protein with wet gluten and falling number. Negative correlations occurred between lowest ranking flour protein with grain protein and flour extraction. Highest ranking loaf volume correlated more strongly with dough strength and flour protein content whereas lowest ranking loaf volume had no significant correlations with any of the quality parameters.

Glutenin proteins (large polymeric proteins) were prominent in correlations with both highest and lowest ranking flour and grain protein and highest and lowest ranking loaf volume. Insoluble glutenin fractions in total insoluble protein correlated strongly with highest flour and grain protein and loaf volume. Soluble glutenin fractions in total protein was positively correlated with highest ranking grain protein but negatively with highest ranking loaf volume. Insoluble glutenin in total protein correlated positively with highest ranking flour and grain protein and highest ranking loaf volume.

Lowest ranking flour and grain protein and lowest ranking loaf volume correlated positively with glutenin and negatively with albumin/globulin. Insoluble glutenin fractions in total insoluble protein, in total protein and in total glutenin correlated positively with flour protein and grain protein content. Negative correlations occurred between soluble albumin/globulin in total soluble protein and soluble albumin/globulin in total protein with low flour protein and low grain protein.

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Chapter 7

The relationship between protein fractions and protein content and loaf volume of the three production regions of South Africa

7.1 Introduction

Improvement of quality and yield of grain crops will become increasingly important in the future in order to meet the growing world demand for food. Objective focussed crop breeding has in the past and will continue in future, to apply novel approaches for development and selection of genotypes to meet industrial and consumer demands. The gluten protein complex provides such an approach.

Gluten protein is customarily divided into gliadin and glutenin as initially described by Osborne (1924) who extracted gliadin with a 70% aqueous ethanol solution and named the insoluble remnants glutenin. More recent approaches are to separate soluble gliadin and insoluble glutenin through their respective MW. The MW; sum of the atomic weights of the atoms in a molecule; of gliadins range between 28 000 and 55 000 Da whereas MW of glutenin is between 500 000 to 10 million Da (Wieser 2007). MacRitchie (1984) compiled a summary of results from early work from the 1980's investigating whether the quality component of gluten is linked to gluten as an entity, or to individual protein fractions. He concluded that gluten quality is more attributable to specific protein fractions and that the contribution of insoluble compared to soluble fractions is negligible and may even, in some cases, have detrimental effects on quality. Application of protein fractions for defining bread baking quality in germplasm has since increased significantly. Kong et al. (2013) for example, found that the

amounts of flour protein, albumin/globulin and high and low MW glutenin were significantly and positively related with loaf volume.

An important result from a study by Triboï et al. (2003) is that amino acid and protein fraction composition of wheat grains at harvest ripeness merely reflect differences in the total quantity of nitrogen accumulated during the grain filling period. This association between nitrogen and grain protein, according to Weegels et al. (1996), gave rise to the belief that high protein content (calculated from nitrogen in the kernel) is associated with higher protein quality for bread making. The income of wheat farmers in SA, as over the world, is determined by wheat grade, which has a direct bearing on the price for wheat. Grain protein content, hectolitre mass and falling number are the three primary grading parameters currently differentiating between the grades of Class B wheat (bread wheat). Snape et al. (1993) reported that the typical difference between protein content of UK bread making wheat and feed wheat is only 2% (12 to 14%) when cultivated under similar conditions.

The aim of this study was to compare protein quantity (content) and protein quality (concentrations and ratios of protein fractions) by identifying trends and correlations between grain protein content, loaf volume and protein fractions. The aim was addressed by (a) comparison of loaf volumes and grain protein content of wheat from the three production regions in SA, (b) establishing ratios between flour protein content and grain protein content in genotypes of the three production regions, (c) comparing the correlations and concentration of protein fractions in wheat from the three production regions.

7.2 Material and methods

7.2.1 Genotypes (cultivars) and environments (field test sites)

Wheat samples from the NCEP for irrigated wheat, rainfed wheat from the SRR and rainfed from the WRR regions in 2012 and 2013 were analysed for the purpose of this study.

7.2.2 Grain quality and primary quality parameters

Grain quality (hectolitre mass; ISO 7971-3:2009, percentage grain protein content; AACCI Method 46-10 and falling number; ISO 3093:2009) and primary quality parameters (wet gluten; AACCI Method 38-12.02, alveograph analyses; ICC Standard Methods No. 121 latest edition, percentage flour protein content; AACC method 39-11, loaf volume; AACCI Method 10-10.03 and AACCI Method 10-10B and farinograph water absorption; AACCI method 54-21.02) were determined (refer to Chapter 3 for more detail).

7.2.3 Comparing wheat grades with loaf volumes

Wheat grades were assigned to the various genotypes from each of the three production regions according to the grain protein content of these respective genotypes. On a commercial level, hectolitre mass and falling number, together with protein content, form the primary parameters of the bread wheat grading table (Table 7.1).

Table 7.1 The South African wheat grading table

Grade	Grading parameter for grade B (bread wheat)		
	Hectolitre mass (kg hl^{-1})	Falling number (secs)	Protein content (%)
Grade 1	77	220	12
Grade 2	76	220	11
Grade 3	74	220	10
Grade 4	72	200	9
Utility grade	70	150	8
Other Wheat	< 70	< 150	< 8

(Amended by Government Notice No. R. 1210 of 29 August 2003)

7.2.4 Determining the variations in the ratio of grain protein to flour protein content

Flour protein was expressed as a percentage of the corresponding grain protein within each of the ranking groups (high with high, medium with medium and low with low). The result was expressed as a percentage difference.

7.2.5 Size-Exclusion High Performance Liquid Chromatography

Flour samples from all three production regions were analysed with SE-HPLC, according to the protocol developed by Gupta et al. (1993) with modifications, to determine protein fractions and ratios.

7.2.6 Determining the correlations of protein fractions with grain protein content and loaf volume

In Chapters 4, 5 and 6 strong and positive three way correlations were seen between flour protein content, grain protein content and loaf volume. Results from these Chapters also showed strong positive correlations between ratios of protein fractions with flour and grain protein content and loaf volume of all three production regions. The protein fractions showing the highest correlations with protein content and loaf volume in all three regions were: (i) soluble α/β, γ gliadin and (ii) soluble albumin/globulin in total soluble protein, (iii) insoluble glutenin and (iv) insoluble albumin/globulin in total insoluble protein, (v) soluble glutenin, (vi) soluble α/β, γ gliadin and (vii) soluble albumin/globulin in total protein, (viii) insoluble glutenin, (ix) insoluble α/β, γ gliadin, and (x) insoluble albumin/globulin in total protein and (xi) insoluble glutenin in total glutenin. These 11 fractions were used to determine correlations with grain protein and loaf volume.

7.2.7 Statistical analyses

Two-way ANOVA for G, E and G x E interactions were done over the 2012 and 2013 seasons and results were used for compiling high, medium and low ranking groups for flour content, grain protein content and loaf volume. Simple correlations were determined through statistical analyses with GenStats for Windows (version 10) (Payne et al. 2007).

7.2.8 Protein fractions in high and low ranking classes of grain protein content and loaf volume

To determine if protein fractions differed significantly and to identify the most prominent fractions in high, medium and low ranking classes of grain protein and loaf volume concentrations of soluble and insoluble glutenin, α/β, γ gliadin and albumin/globulin in total protein were determined in the three classes for grain protein content and loaf volume.

7.3 Results and discussion

7.3.1 Comparing flour and grain protein content with loaf volume

7.3.1.1 Comparing wheat grades and loaf volumes

The SA Grain Laboratory reported that for the 2013/14 season grain protein was a good indicator of loaf volume (SAGL 2014). Percentage grain protein content should consistently over seasons, provide an indication of loaf volume and end product application of wheat bulks. Grain protein should serve as incentive, promote fair compensation of farmers and not be misused to pressurise local wheat prices. Fair compensation would entail that wheat producers receive a fair price for their wheat crop in the context of the value bakers generate from the end product they manufacture. In this context, this study attempted to establish the

reliability of grain protein content in predicting loaf volume of wheat flour from the three production regions.

7.3.1.2 Analysis of variance of grain protein and loaf volume

Flour protein differed significantly for G ($P < 0.001$ for all three regions), E ($P < 0.001$ for all three regions) and G x E ($P < 0.05$, $P < 0.01$ and $P < 0.01$ for IRR, rainfed SRR and rainfed WRR respectively). E primarily determined variation in flour protein in all three production regions (Table 7.2). Grain protein only differed significantly for G and E in IRR and rainfed WRR ($P < 0.001$ for both) but significantly for G, E and G x E interaction (all significant at $P < 0.001$). Similarly, to flour protein, E was the primary factor determining variation in grain protein in all three production regions. Differences in loaf volume were highly significant for G, E and G x E interaction ($P < 0.001$ for all three regions) and were largely determined by E in IRR and rainfed SRR. In rainfed production in the WRR, G (20.71%), E (29.68%) and G x E interaction (24.14%) contributed approximately equally to the variation (Table 7.2).

Table 7.2 Analysis of variance for flour and grain protein content and loaf volume

		Irrigated				Rainfed SRR				Rainfed WRR			
	Source of variation	**SS**	**% SS**	**MS**	**P-value**	**SS**	**% SS**	**MS**	**P-value**	**SS**	**% SS**	**MS**	**P-value**
% Flour protein	Genotype (G)	111.22	10.78	6.54	< 0.001	71.82	19.89	6.53	< 0.001	45.58	15.03	4.14	< 0.001
	Environment (E)	743.85	72.08	247.95	< 0.001	198.60	55.01	66.20	< 0.001	132.86	43.81	44.29	< 0.001
	G x E	60.88	5.90	1.19	0.03	40.24	11.14	1.21	0.001	51.22	16.89	1.55	0.002
	Residual	113.05		0.80		50.36		0.54		67.94		0.72	
	Total	1031.97				361.04				303.25			
	Stratum	**s.e.**	**CV%**			**s.e.**	**CV%**			**s.e.**	**CV%**		
	Rep	0.14	1.20			0.02	0.10			0.24	2.00		
	Rep.*Units*	0.89	7.70			0.73	5.10			0.85	6.90		
% Grain protein	Genotype	77.74	8.22	4.57	< 0.001	43.58	8.15	3.96	< 0.001	80.10	23.23	7.28	< 0.001
	Environment	720.61	76.18	240.20	< 0.001	400.46	74.93	133.49	< 0.001	171.35	49.70	57.12	< 0.001
	G x E	41.18	4.35	0.81	NS	40.11	7.51	1.22	< 0.001	29.18	8.46	0.88	NS
	Residual	100.67		0.71		49.58		0.53		63.61		0.68	
	Total	945.98				534.44				344.78			
	Stratum	**s.e.**	**CV%**			**s.e.**	**CV%**			**s.e.**	**CV%**		
	Rep	0.20	1.70			0.09	0.60			0.08	0.60		
	Rep.*Units*	0.84	7.00			0.72	4.90			0.82	6.40		
Loaf volume (cm^3)	Genotype	314407	18.25	18495	< 0.001	79965.6	20.42	7269.60	< 0.001	98814	20.71	8983	< 0.001
	Environment	873864	50.73	291288	< 0.001	182159.7	46.51	60719.90	< 0.001	141599	29.68	47200	< 0.001
	G x E	276363	16.04	5419	< 0.001	77770.5	19.86	2356.70	< 0.001	115182	24.14	3490	< 0.001
	Residual	256663		1807		49865.6		530.50		113828		1211	
	Total	1722662				391637.5				477162			
	Stratum	**s.e.**	**CV%**			**s.e.**	**CV%**			**s.e.**	**CV%**		
	Rep	3.10	0.30			4.40	0.40			9	1.00		
	Rep.*Units*	42.50	4.80			23.00	2.30			34.80	3.70		

NS – not significant

The reality is that protein content often does not correlate with good bread making quality. This is clearly illustrated in Table 7.3 where genotypes that obtained Grade 2 (< 12% grain protein content) and Grade 3 (< 11% grain protein content) were capable of producing loaf volumes within the allowed 10% variation from the loaf volume of the quality standard. Reese et al. (2007) concluded that protein content does not describe the true quality of wheat flour and recommended that for accurate predictions of flour quality, additional information supporting protein content should be obtained from farinograph and alveograph analyses. Results from wheat mill streams (Yahata et al. 2006; Okrajková et al. 2007) indicate that flour from the central endosperm have better baking quality, albeit being lower in total protein content, than flour from the outer grain layers.

Table 7.3 Means for grain protein content and loaf volume of wheat from the different production regions in South Africa (2012 and 2013)

			Grain protein		Loaf volume	
Production region	**Ranking**	**Genotype**	**% Content**	**Grade**	**(cm^3)**	**10% range of acceptable variation**
Irrigation	High	SST 843	13.67^{a}	**B1**	885.4^{bcdef}	800.3 to 978.1
		SST 822	12.93^{ab}	**B1**	941.3^{ab}	
	Low	PAN 3478	11.66^{cd}	**B2**	839.2^{efg}	
		PAN 3489	11.64^{cd}	**B2**	829.6^{fg}	
		SST 806	11.56^{cd}	**B2**	889.2^{bcdef}	
		PAN 3471	11.36^{cd}	**B2**	820.4^{g}	
		PAN 3497	11.32^{cd}	**B2**	915.8^{abc}	
		Krokodil	10.97^{d}	**B3**	847.3^{defg}	
	Quality standard	SST 806	11.56^{cd}	**B2**	889.2^{bcdef}	
Rainfed SRR	High	Koonap	15.85^{a}	**B1**	1014.2^{ab}	906.8 to 1108.3
		PAN 3368	15.60^{ab}	**B1**	1017.1^{a}	
		Senqu	15.39^{abc}	**B1**	1010.8^{ab}	
		Gariep	15.30^{abc}	**B1**	1014.2^{ab}	
	Low	SST 316	14.13^{de}	**B1**	981.9^{bcd}	
		SST 356	13.95^{e}	**B1**	958.5^{de}	
	Quality standard	Elands	15.13^{abcd}	**B1**	1007.5^{ab}	
Rainfed WRR	High	SST 047	14.82^{a}	**B1**	990.4^{a}	874.1 to 1068.3
		Kwartel	13.24^{b}	**B1**	948.3^{abc}	
	Low	SST 087	12.01^{cd}	**B1**	915.8^{cd}	
		SST 88	11.96^{d}	**B2**	910.0^{cd}	
	Quality standard	SST 027	13.18^{bc}	**B1**	971.2^{ab}	

Values within a column within a region with different letters are statistically different

7.3.1.3 Variations in the ratio of grain protein to flour protein content

Hypothetically, the grain protein content to flour protein content ratio indicates the levels of protein content in the endosperm (flour protein content) in relation to total protein content from the endosperm and outer layers of the grain (grain protein content). This ratio is important for interpreting trends in ratios of different protein fractions. Variation between flour protein content and grain protein content was substantial for the three wheat production regions (Figure 7.1). Grain protein content is higher than flour protein content as the germ (embryo) and bran (pericarp, testa, nucellar layer, and aleurone layer) are removed during milling for production of white flour (Shewry and Halford 2002). White flour relates to the starchy endosperm cells in wheat grain and contains high amounts of starch and gluten (Tosi et al. 2011). The concentration of protein is low near the endosperm cavity and progressively increases outwards (Farrand 1974; Farrand and Hinton 1974; Shewry and Halford 2002) potentially allowing extraction of high protein flour from the outer layers and low protein flour from the central part of the grain endosperm (Tosi et al. 2011).

Differences between flour protein content and grain protein content of genotypes from rainfed wheat of the SRR in 2012 and 2013 were small (between 1.8% and 2.9%) (Figure 7.1). Differences between flour protein content and grain protein content of irrigated genotypes for the same period were, however, substantially larger and ranged between 5.5% and 8.5%. Of particular interest are the smaller differences between flour and grain protein content of the high and medium grain protein groups compared to the larger differences of the low grain protein group in irrigated and rainfed wheat from the SRR. In contrast to the irrigation and rainfed SRR, differences in the flour protein to grain protein ratio of rainfed wheat in the WRR were large (between 1.2% and 7.3%) and extreme between high, medium and low rank groups (Figure 7.1). In contrast to irrigation and rainfed SRR, large differences occurred for high grain protein (7.3%) reducing sharply to 1.2% for the low grain protein group. Tosi et al. (2011) suggested that a higher amount of protein in the sub aleurone cells compared with those in the inner endosperm is probably due to higher protein synthesis in the outer layers of the

endosperm and not by a longer duration of deposition. The large differences in flour protein to grain protein content in this study, particularly for irrigated and rainfed WRR, imply that the rate of protein synthesis (accumulation) of the grain protein groups from the three regions might be different. Triboi et al (2003) emphasise that protein synthesis did not depend on N-rate or timing, drought- or heat stress but on N accumulation during cell division and cell expansion shortly before and during grain filling.

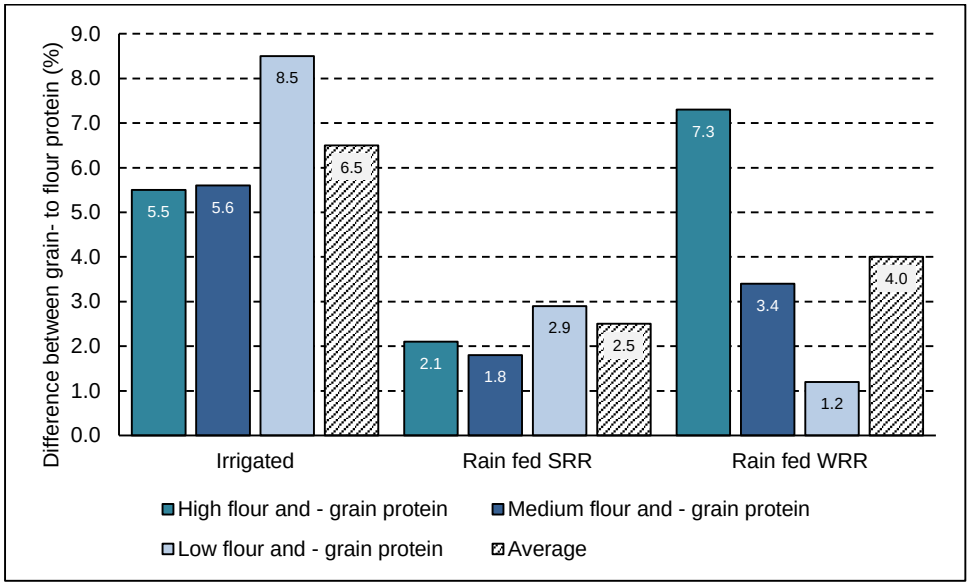


Figure 7.1 Percentage difference between grain and flour protein content of wheat from the three different wheat production regions in South Africa

These results prompted the investigation into the dynamics of protein fractions in flours (Glutenin, α/β, γ gliadin and albumin/globulin in total protein) varying in grain protein content and loaf volumes of the three production regions.

7.3.2 Comparing protein ratios with grain protein content and loaf volume

7.3.2.1 Analysis of variance of protein ratios in high, medium and low grain protein and loaf volume

Significant differences occurred between most of the rank groups (genotypes), test sites (environments) and the G x E interactions for soluble and insoluble protein ratios (Table 7.4).

Table 7.4 Analysis of variance for protein ratios in high, medium and low grain protein content and loaf volume

Para-meter	Produc-tion region	Source of varia-tion	Soluble									Insoluble								
			Glutenin in total protein			α/β, γ Gliadin in total protein			Albumin/globulin in total protein			Glutenin in total protein			α/β, γ Gliadin in total protein			Albumin/globulin in total protein		
			SS	MS	P-value	SS	MS	P-value	SS	MS	P-value	SS	MS.	P-value	SS	MS	P-value	SS	MS	P-value
Grain protein	Irrigation	Rank groups (G)	1.13	0.57	NS	55.96	27.98	< 0.001	27.56	13.78	< 0.001	21.29	10.65	<.001	7.63	3.82	< 0.001	1.38	0.69	0.024
		Environ-ment (E)	37.71	12.57	< 0.001	313.16	104.39	< 0.001	363.80	121.27	< 0.001	145.86	48.62	<.001	7.50	2.50	< 0.001	6.28	2.09	< 0.001
		G x E	6.99	1.16	0.005	29.41	4.90	0.045	7.63	1.27	NS	9.25	1.54	0.003	5.16	0.86	0.024	3.75	0.62	0.003
	Rainfed SRR	Rank groups (G)	3.61	1.81	NS	56.97	28.48	0.018	6.93	3.46	0.07	17.43	8.72	0.001	6.44	3.22	< 0.001	0.52	0.26	NS
		Environ-ment (E)	13.74	4.58	< 0.001	150.56	50.19	< 0.001	89.97	29.99	< 0.001	101.34	33.78	< 0.001	20.31	6.77	< 0.001	3.54	1.18	< 0.001
		G x E	17.4	2.90	0.002	153.74	25.62	0.002	66.24	11.04	< 0.001	35.48	5.91	< 0.001	7.56	1.26	0.006	6.36	1.06	< 0.001
	Rainfed WRR	Rank groups (G)	2.18	1.09	0.011	250.53	125.27	< 0.001	56.23	28.12	< 0.001	1.26	0.63	NS	2.09	1.05	< 0.001	0.42	0.21	0.035
		Environ-ment (E)	2.48	0.83	0.016	32.65	10.88	0.01	129.16	43.05	< 0.001	96.18	32.06	< 0.001	0.88	0.29	NS	1.72	0.57	< 0.001
		G x E	3.10	0.52	0.045	13.35	2.23	NS	12.26	2.04	NS	0.68	0.11	NS	1.70	0.28	0.03	1.18	0.20	0.005
Loaf volume	Irrigation	Rank groups (G)	2.62	1.31	0.029	57.81	28.90	< 0.001	2.92	1.46	NS	3.62	1.81	0.034	3.08	1.54	0.019	1.93	0.96	0.007
		Environ-ment (E)	37.71	12.57	< 0.001	313.16	104.39	< 0.001	363.80	121.27	< 0.001	145.86	48.62	< 0.001	7.50	2.50	< 0.001	6.28	2.09	< 0.001
		G x E	6.59	1.10	0.007	17.75	2.96	NS	10.00	1.67	NS	9.38	1.56	0.008	2.14	0.36	NS	1.90	0.32	NS
	Rainfed SRR	Rank groups (G)	3.76	1.88	NS	23.18	11.59	NS	13.46	6.73	0.013	14.61	7.30	0.003	7.48	3.74	< 0.001	1.15	0.57	0.045
		Environ-ment (E)	13.74	4.58	0.001	150.56	50.19	< 0.001	89.97	29.99	< 0.001	101.34	33.78	< 0.001	20.31	6.77	< 0.001	3.54	1.18	< 0.001
		G x E	8.82	1.47	NS	109.23	18.21	0.03	29.23	4.87	0.005	39.84	6.64	< 0.001	14.31	2.38	< 0.001	8.47	1.41	< 0.001
	Rainfed WRR	Rank groups (G)	0.84	0.42	NS	111.76	55.88	< 0.001	45.20	22.60	< 0.001	0.92	0.46	NS	0.46	0.23	NS	0.77	0.38	< 0.001
		Environ-ment (E)	2.48	0.83	0.015	32.65	10.88	0.037	129.16	43.05	< 0.001	96.18	32.06	< 0.001	0.88	0.29	NS	1.72	0.57	< 0.001
		G x E	4.85	0.81	0.003	25.20	4.20	NS	15.64	2.61	0.04	7.92	1.32	< 0.001	1.83	0.31	0.033	1.98	0.33	< 0.001

NS - not significant

7.3.2.2 Correlations of protein ratio with grain protein and loaf volume

An important fact to remember is that correlations between two traits do not imply cause. Overlapping external factors causing increases in one parameter may result in increases in the other parameter, but is not a given fact. Similarly, strong correlations between a protein ratio and grain protein content and/or loaf volume is therefore not proof that the specific ratio causes increases (or reductions in the case of negative correlations) of grain protein content and/or loaf volumes.

Glutenin protein correlated significantly with grain protein content of the highest ranking group for the rainfed WRR region and grain protein of the lowest ranking group for the rainfed SRR region (means of 14.03% and 14.04% respectively) and loaf volumes for the lowest ranking group of the rainfed WRR (mean of 980.8 cm^3) and loaf volume for the lowest ranking group of the rainfed SRR (mean of 959.8 cm^3) (Table 7.5). Although production environments of these two regions are diverse, grain protein content might be the common denominator (just over 14%) resulting in the high number of glutenin correlations. Dowell et al. (2008), similarly found that the amount of total glutenins and total glutenin composition (percentage of total proteins) were two of only four variables required to explain predictive models for hard red winter and hard red spring wheat in a standardised protein content ranging between 11.4% to 15.8%. Glutenin is the primary factor responsible for dough quality and loaf volume. Size of glutenin proteins depends on the condition of the disulphide bonds determined by G, E and redox state (Wieser et al. 2006).

Insoluble glutenin in total insoluble protein correlated strongly and positively with the high ranking groups of grain protein content (0.85***) and loaf volume (0.55**) for rainfed wheat from the WRR (Table 7.5). Soluble glutenin in total protein correlated positively with the high ranking group of grain protein content (0.55**) but negatively with the high ranking group of loaf volume (-0.48*) in rainfed wheat in the WRR. Insoluble glutenin in total protein correlated strongly and positively with the high ranking groups of grain protein content of irrigation

(0.60**), rainfed SRR (0.49***) and rainfed WRR (0.55**) and loaf volume of rainfed wheat in the WRR (0.46***). Insoluble glutenin in total protein also correlated strongly with the low ranking groups of grain protein content (0.61**) and loaf volume (0.48***) in rainfed SRR. The percentage insoluble glutenin in the total concentration of glutenin correlated strongly and positively with high ranking groups of grain protein content for irrigation (0.56**) and rainfed SRR (0.63***). Insoluble glutenin in the total concentration of glutenin also correlated strongly with the high ranking group of loaf volume for rainfed SRR (0.53***) and low ranking groups of grain protein content (0.65***) and loaf volume (0.67***) for rainfed SRR (Table 7.5).

In contrast, the α/β, γ gliadin proteins correlated more frequently with grain protein content (mean of 13.30%) and loaf volume (933.0 cm^3) of the highest ranking groups in the irrigated region (Table 7.5). The molecular size distribution (MW) of glutenin and gliadin was reported by Wieser (2007) to be largely determined by wheat variety (genotype) and growing conditions (environment). Although Blumenthal et al. (1993) reported that short periods of high temperatures (above 35°C) change dough and baking quality of certain genotypes by increasing the gliadin: glutenin ratio, Triboï et al. (2003) found that protein fraction composition depended mostly on the total quantity of nitrogen per grain. Both Upington (± 35°C) and Vaalharts (± 33°C) test sites experience very high temperatures during the post-anthesis to grain filling period of October to early December (see Chapter 3). Irrigated wheat production in the Upington and Vaalharts regions furthermore necessitate high nitrogen fertilisation (180 to 200 kg N ha^{-1} for a yield potential between 7 to 8 ton ha^{-1}) applied in split applications (ARC-SGI 2014). Both high temperatures and fertilisation could have contributed to the higher number of correlations of gliadins with the protein content and loaf volume. Soluble α/β, γ gliadin in total soluble protein correlated positively with both grain protein content (0.62**) and loaf volume (0.56***) of irrigated wheat and grain protein (0.62**) and loaf volume (0.50*) of rainfed wheat in the WRR, both in the highest ranking group. Soluble α/β, γ gliadin in total protein correlated positively and strongly with grain protein content (0.58**) and loaf volume

(0.47***) in the highest ranking groups whereas insoluble α/β, γ gliadin in total protein correlated with low grain protein content (0.51***) and low loaf volume (0.61***).

Albumin/globulin (structural proteins) were strongly but negatively correlated with grain protein content (-0.81***) and loaf volume (-0.66***) of the highest ranking groups for irrigated production and with grain protein content and loaf volume of the lowest ranking groups for irrigation and rainfed production in the SRR. In contrast to the trend for gliadins, Triboï et al. (2003) reported that the albumins/globulins decreased by 26% when the total quantity of nitrogen per grain increased from 560 to 1000 mg nitrogen grain^{-1}. Soluble albumin/globulin in total soluble protein correlated strongly but negatively with grain protein content (-0.81***) and loaf volume (-0.66***) in the highest ranking groups and with grain protein content (-0.82*** and -0.80***) and loaf volume (-0.56*** and -0.52***) in the lowest ranking groups of irrigated and rainfed wheat of the SRR respectively. Insoluble albumin/globulin in total insoluble protein correlated negatively with grain protein content (-0.53***and -0.73***) and loaf volume (-0.51***and -0.67***) of the lowest ranking groups of irrigated and rainfed wheat of the SRR respectively. Soluble albumin/globulin in total protein correlated negatively with grain protein content (-0.70***) and loaf volume (-0.65***) in the highest ranking group of irrigated wheat and with grain protein content (-0.85*** and -0.86***) and loaf volume (-0.72*** and -0.55***) in the lowest ranking groups of irrigated and rainfed wheat of the SRR respectively. Insoluble albumin/globulin in total protein correlated negatively with grain protein content (-0.50**) and loaf volume (-0.57***) in the lowest ranking groups of rainfed wheat of the SRR. Results on albumin/globulin accumulation from a study by Gupta et al. (1996) reported that these proteins reach peak concentrations between seven to 19 days after anthesis, suggesting possible threshold concentrations of monomeric subunits or initial polymers must first be achieved before polymerization into larger molecular sizes commence.

Table 7.5 Simple correlations of protein fractions with grain protein content and loaf volume in high and low ranking classes

		High						Low					
		Irrigation		Rainfed SRR		Rainfed WRR		Irrigation		Rainfed SRR		Rainfed WRR	
Solubility classes	**Protein fraction**	Grain protein (13.30%)	Loaf volume (933.0 cm^3)	Grain protein (15.54%)	Loaf volume (1012.8 cm^3)	Grain protein (14.03%)	Loaf volume (980.8 cm^3)	Grain protein (11.42%)	Loaf volume (839 cm^3)	Grain protein (14.04%)	Loaf volume (959.8 cm^3)	Grain protein (11.99%)	Loaf volume (908.7 cm^3)
Soluble fraction in total soluble protein	α/β, γ Gliadin (LMP)	0.62**	0.56***	NS	NS	0.62**	0.50*	0.60***	NS	NS	NS	NS	NS
	Albumin and globulin (SMP)	-0.81***	-0.66***	NS	NS	NS	NS	-0.82***	-0.56***	-0.80***	-0.52***	NS	NS
Insoluble fraction in total insoluble protein	Glutenin (LPP)	NS	NS	NS	NS	0.85***	0.55**	NS	NS	NS	NS	NS	NS
	Albumin and globulin (SMP)	-0.73***	NS	NS	NS	NS	NS	-0.53***	-0.51***	-0.73***	-0.67***	NS	NS
Soluble fraction in total protein	Glutenin (LPP)	NS	NS	NS	NS	0.55**	-0.48*	NS	NS	NS	NS	NS	NS
	α/β, γ Gliadin (LMP)	0.58**	0.47***	NS	NS	NS	NS	NS	0.50***	NS	NS	NS	NS
	Albumin and globulin (SMP)	-0.70***	-0.65***	NS	NS	NS	NS	-0.85***	-0.72***	-0.86***	-0.55***	NS	NS
Insoluble fraction in total protein	Glutenin (LPP)	0.60**	NS	0.49***	NS	0.55**	0.46***	NS	NS	0.61**	0.48***	NS	NS
	α/β, γ Gliadin (LMP)	NS	0.52***	0.55***	NS	NS	NS	0.51***	0.61***	NS	NS	NS	NS
	Albumin and globulin (SMP)	-0.55**	NS	NS	NS	NS	NS	NS	NS	-0.50**	-0.57***	NS	NS
Insoluble fraction in total similar fraction	Glutenin (LPP)	0.56**	NS	0.63***	0.53***	NS	NS	NS	NS	0.65***	0.67***	NS	NS

*** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05, NS - not significant

7.3.2.3 Concentrations of glutenin, α/β, γ gliadin and albumin/globulin in total protein of genotypes with varying grain protein content and loaf volume

Only the concentration of protein fractions (soluble and insoluble) in total protein was selected for statistical comparison and analyses.

Concentrations (soluble and insoluble) of glutenin, α/β, γ gliadin and albumin/globulin in total protein in high, medium and low grain protein content are shown below (Figure 7.2). The α/β, γ gliadin concentration in total protein was highest for the rainfed SRR (32.2-34.1%). In literature, gliadin concentrations in total protein content are reported to vary between 30 to 45% (Bénétrix et al. 1994; Jia et al. 1996; Stone and Nicolas 1996). Interestingly, gliadin concentrations in low grain protein of rainfed SRR wheat (34.1%) were higher than in high grain protein content (33.6%). In contrast, glutenin concentrations were highest for rainfed WRR genotypes (12.0-12.3%) whereas albumin/globulin concentrations were higher in genotypes for irrigated production (14.3-15.2%).

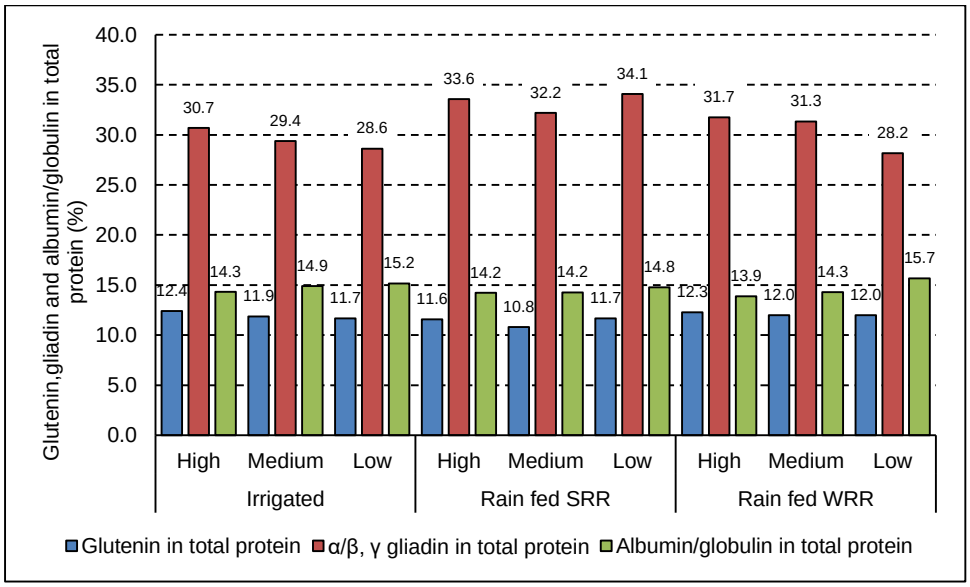
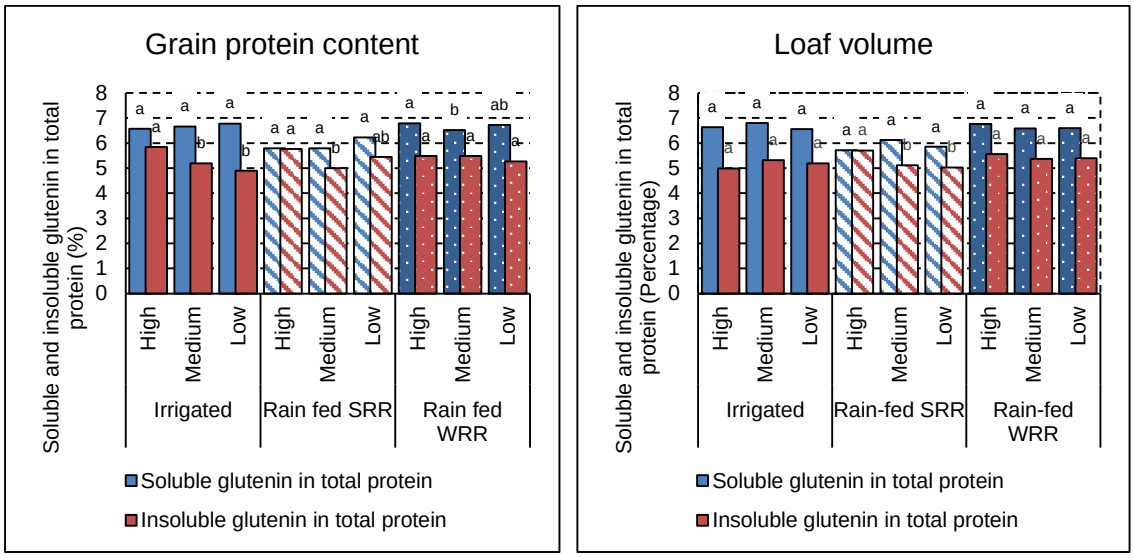


Figure 7.2 Percentage aggregate of total (insoluble + soluble) glutenin, α/β, γ gliadin and albumin/globulin in total protein of genotypes with high, medium and low grain protein content

7.3.2.4 Variations in the concentrations of soluble and insoluble glutenin, α/β, γ gliadin and albumin/globulin in total protein of genotypes with varying grain protein content and loaf volume

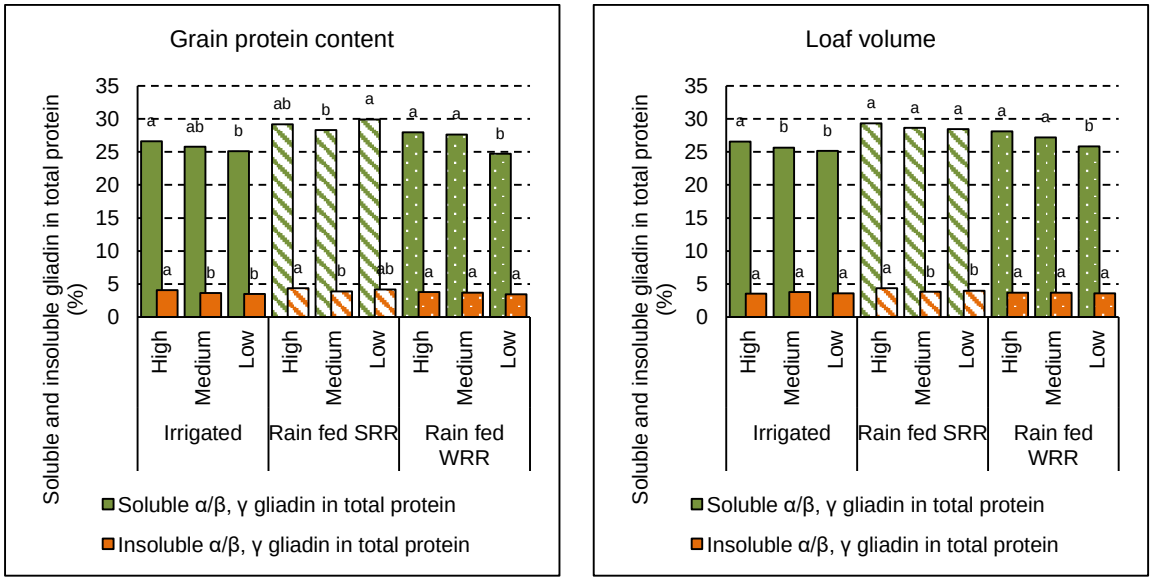
Glutenin (large polymeric proteins), gliadin (large monomeric proteins) and albumin/globulin (small monomeric proteins) correlated strongly and positively with grain protein content and loaf volume (Table 7.5). Except for rainfed WRR, concentrations of soluble glutenin were inverse to insoluble glutenin in high, medium and low grain protein (Figure 7.3). Rank groups with medium grain protein content for irrigated and rainfed SRR production had significantly lower concentrations of insoluble glutenin in total protein than rank groups with high protein content. This trend only repeated for high, medium and low loaf volume in rainfed production in the SRR. The insoluble to soluble ratio for glutenin was approximately one for grain protein content and ranged from 0.88 to 0.72 for irrigated production, 0.99 to 0.87 for rainfed SRR and 0.80 to 0.82 for rainfed WRR. The insoluble to soluble ratio of glutenin for loaf volume ranged from 0.79 to 0.75 for irrigated production, 0.99 to 0.83 for rainfed SRR and 0.82 and 0.83 for rainfed WRR (Figure 7.3).



Different letters within a production region indicate significant statistical difference only within that production region

Figure 7.3 Concentrations of soluble and insoluble glutenin in total protein of genotypes with high, medium and low grain protein and loaf volume

The concentrations of insoluble and soluble α/β, γ gliadin mirrored results of insoluble glutenin. Medium and/or low grain protein content and loaf volume had equal or lower concentrations of insoluble and soluble α/β, γ gliadin compared to high grain protein content and loaf volume (Figure 7.4). The ratio of insoluble to soluble α/β, γ gliadin were much larger compared to glutenin, indicating larger variation. Values of high, medium and low grain protein ranged from 0.15 to 0.14 for irrigated, 0.15 to 0.14 for rainfed SRR and 0.14 for rainfed WRR. Ratios of insoluble to soluble α/β, γ gliadin in high, medium and low loaf volume ranged from 0.15 to 0.13 for irrigated, 0.15 to 0.13 for rainfed SRR and 0.13 to 0.14 for rainfed WRR indicating slightly larger differences.

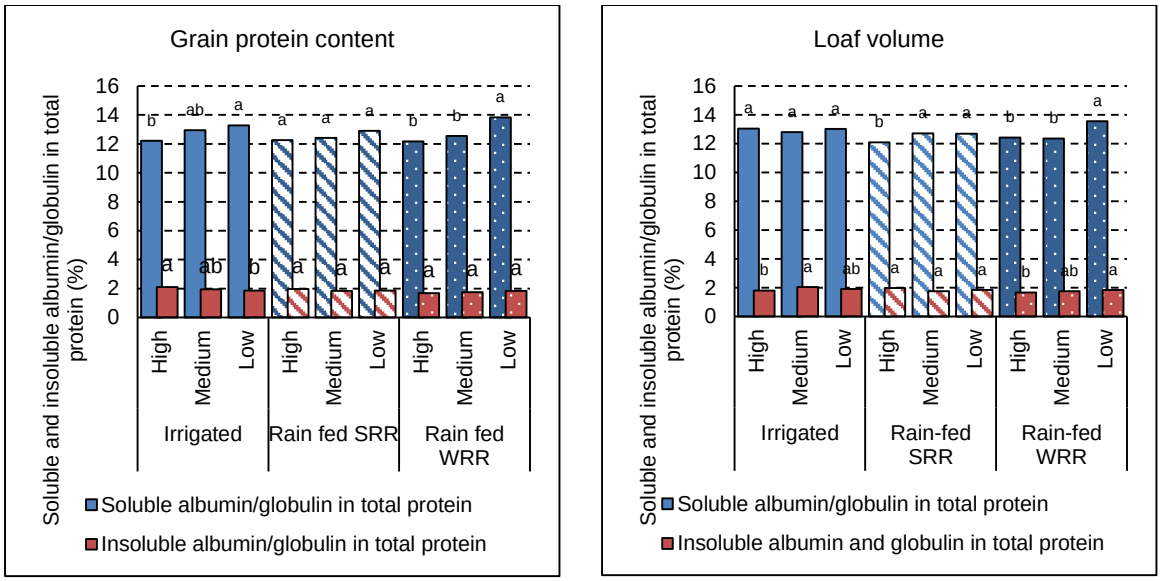


Different letters within a production region indicate significant statistical difference only within that production region

Figure 7.4 Concentrations of soluble and insoluble α/β, γ gliadin in total protein in genotypes with high, medium and low grain protein and loaf volume

The concentrations of insoluble and soluble albumin/globulin tended to incline from higher ranking- to lower ranking groups in contrast to the trend of grain protein content and loaf volume. Soluble concentrations of albumin/globulin in low grain protein content were significantly higher in the irrigated and rainfed WRR regions (Figure 7.5). In the lower ranking groups of loaf volume, soluble concentrations of albumin/globulin were significantly higher for rainfed SRR and rainfed WRR and insoluble concentrations of albumin/globulin in rainfed

WRR. The ratios of insoluble to soluble albumin/globulin indicated large variation between concentrations of soluble and insoluble fractions. For high, medium and low grain protein content the ratios ranged from 0.14 to 0.17 for irrigation, 0.14 to 0.16 for rainfed SRR and 0.13 to 0.14 for rainfed WRR. Genotypes ranked for loaf volume ranged from 0.15 to 0.16 for irrigation, 0.15 to 0.16 for rainfed SRR and 0.14 to 0.13 for rainfed WRR (Figure 7.5).



Different letters within a production region indicate significant statistical difference only within that production region

Figure 7.5 Concentrations of soluble and insoluble albumin/glutenin in total protein in genotypes with high, medium and low grain protein and loaf volume

7.4 Conclusions

Although grain protein quantity (content) is traditionally accepted as a parameter indicating bread baking quality, comparison between wheat grade (as determined by grain protein content) and loaf volume for the different production regions in this study, indicated inconsistency of grain protein content to accurately reflect potential loaf volume of a specific wheat sample.

Variations between flour protein content and grain protein content of genotypes from rainfed wheat of the SRR were small (1.8% and 2.9%) compared to irrigated genotypes with

substantially larger variation (5.5% and 8.5%). Only small variations occurred between flour protein content and grain protein content of genotypes in the high and medium protein groups. Larger differences were, however, evident for genotypes with low protein content for irrigated production and low protein genotypes for rainfed production in the SRR.

Glutenin proteins frequently correlated positively with high grain protein and high loaf volumes of the rainfed WRR region (14.03% and 980.8 cm^3 respectively) and low grain protein and low loaf volumes of the rainfed SRR region (14.04% and 959.8 cm^3 respectively). Insoluble glutenin in the total concentration of glutenin correlated strongly and positively in genotypes for production under rainfed conditions in the SRR exhibiting high grain protein content (15.54%), high loaf volume (1012.8 cm^3) as well as low grain protein content (14.04%) and low loaf volume (959.8 cm^3). Glutenin concentrations were highest in genotypes adapted for rainfed production in the WRR. The differences between concentrations of insoluble and soluble glutenin were small for both grain protein content and loaf volumes of all genotypes from the three production regions. The α/β, γ gliadin proteins correlated significantly with protein content (13.30%) and loaf volume (933.0 cm^3) in the highest ranking groups. The percentage α/β, γ gliadin concentration in total protein was highest for genotypes for rainfed production in the SRR but inversely, gliadin concentrations in genotypes with low grain protein content (14.04%) were higher than in genotypes with high protein (15.54%). The variations between concentrations of soluble and insoluble α/β, γ gliadin were large for both grain protein content and loaf volume of all genotypes from the three production regions. The albumins/globulins were strongly but negatively correlated with grain protein content and loaf volumes in genotypes for highest and lowest ranking groups of irrigated production. They also correlated strongly and negatively with grain protein content and loaf volumes in genotypes of the lowest ranking groups for rainfed production in the SRR. Albumin and globulin concentrations were highest in genotypes for irrigated production. Similarly, to α/β, γ gliadin, albumin/globulin concentrations were inverse to grain protein content increases and were highest at low grain protein content. Variation between insoluble and soluble albumin/globulin

were large (high soluble to low insoluble ratio) for both grain protein content and loaf volumes of all genotypes from the three production regions.

Applications of protein ratios for more accurate prediction of quality in the baking industry or as selection parameter for high quality germplasm in breeding programmes may become a reality in the near future. The many intricate associations between the various protein fractions and the seemingly large effects of genotype and environment on protein concentrations as reported in scientific literature may, however, still require dedicated future research to identify fractions or combinations thereof for functional and practical applications.

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Chapter 8

Relationships between grain yield and protein quantity and quality in wheat produced in South Africa

8.1 Introduction

Hollamby and Bayraktar (1996) made an important point when they stated: *"Yield is the prime objective. Despite a new cultivar's improvement, including quality capable of attracting premiums, farmers will not grow it unless they expect it to yield well on their farms".* Grain yield and baking quality have, through history, shared the accolade as primary selection parameters in wheat. Often breeding programmes are faced with conflicting interests as grain yield and grain protein content (representing baking quality) are negatively associated and new varieties need to comply with both requirements in the wheat to bread chain. The negative correlation between grain yield and grain protein content features in many reports and Lawes and Gilbert reported on it as early as 1857 (Barraclough et al. 2010). Although scientific evidence validates this negative correlation (Slafer et al. 1990; Simmonds 1995; Triboï et al. 2006; Cesevičienė et al. 2009) the cause and proper approach to achieve both higher yield and quality, remain unsure. In an overview of the genetic improvement of milling and baking quality in the United States between 1919 and 1988, Cox et al. (1989) reported that any decline in quality results from adverse climate, substandard production conditions lacking soil nitrogen or a combination of both and not from declining genetic potential in end use quality.

These conclusions have been true for environmental and soil effects and have been confirmed regarding the influence of genotype. Oury and Godin (2007) reported that the ability of some genotypes to accumulate higher grain protein concentrations than predicted by grain yield

alone, indicate that this trait is under genetic control and may be improved through breeding. Some findings even indicate that despite the general tendency of protein content reducing with genetic yield gains, dough properties often improve in the same instance (Souza et al. 1993; Gómez et al. 2009). This trend underlines the unreliability of grain protein content on its own to predict baking quality accurately.

Gluten quality is the main factor determining baking quality and consists of various protein fractions and concentrations of these fractions. Although protein structures are genetically determined (Johansson et al. 1993) the relative quantity of protein subunits and amount and MW distribution are largely determined by environment (Wieser and Seilmeier 1998) and genotype (MacRitchie 1999). The primary environmental factors influencing the quantity of protein fractions are nitrogen, water availability and temperature (Stapper and Fischer 1990; McDonald 1992). Most reports from literature acknowledges the significant role of environment (climate and production practises) in the negative correlation between yield and protein content. The physiological basis of this negative correlation has been suggested to be based in varying carbon and nitrogen metabolisms (Bogard 2010) resulting in competition between carbon and nitrogen for energy (Munier-Jolain and Salon 2005) and a dilution effect of nitrogen by carbon based compounds (Acreche and Slafer 2009). Marinciu and Săulescu (2008) summarise the two hypotheses by stating that the removal of a specific amount of nitrogen during the process of increasing carbohydrates (provide building blocks for grain yield) will result in lower amounts remaining for grain protein content (quality). Factors such as soil fertility and soil nitrogen have been indicated in literature to affect both quality and yield (Carr et al. 1992). Before anthesis genotype, environment and nitrogen fertilisation indirectly influence grain yield and grain content through planting density, root growth and number of tillers and florets per head (Bahrman et al. 2004). After anthesis though, kernel growth and grain filling is determined directly by soil and air temperature, soil water and nitrogen, as well as source sink relations in leaves and stems which may be increased by adding post-anthesis nitrogen. Heat or drought occurring after anthesis is known to increase grain protein content,

but reduce yield because of heating effects on carbohydrate (cellulose, sugar and starch) production (Fowler 2003).

In their study on the effects of a gradual increase of drought stress on agricultural production in SA, Blignaut et al. (2009) reported that a reduction of 1.0% in annual rainfall will lead to a 0.5% decline in yield of winter wheat. They concluded that if the current warming trend with consequent reduction in rainfall continues, the major maize and wheat production regions in SA will be susceptible to significant reductions in production. If these warmer conditions materialise, it may prove to be beneficial for quality of wheat, but will place additional emphasis on breeding programmes for development of high yielding wheat varieties for sustainable food production.

The objectives of this chapter were to determine, (i) the correlations of grain yield with flour protein content, grain protein content and loaf volumes for the three wheat production regions in SA, (ii) the correlations of grain yield with soluble and insoluble protein fractions in genotypes of the three production regions and (iii) the concentrations of protein fractions in genotypes with high yield, medium yield and low yield for the three production regions.

8.2 Material and methods

8.2.1 Genotypes (cultivars) and environments (field test sites)

Grain yield and grain protein yield, grain quality and baking quality were determined on wheat cultivars from the three major wheat production regions in SA from field trials of the NCEP. Test sites for rainfed wheat in the SRR were established at Bethlehem and Clarens with 17 genotypes in 2012 and 12 in 2013. Plots at these sites were seeded with a precision, six row commercial planter adapted for seeding plots at seeding densities between 15 to 40 kg ha^{-1}. Plot size was 11.25 m^2, although only the inner four rows were harvested, resulting in an

effective plot size of 6.75 m^2. Fertilisation was done with a commercial mixture of 4 (N):2 (P): 1 (K) (28) applied during seeding at an amount of 60 kg N ha^{-1} (ARC-SGI 2014a).

The two test sites selected from irrigation field experiments of 2012 and 2013 were at Upington and Vaalharts. Experiments were planted with a Wintersteiger Plotman at seeding densities adequate for 175 to 375 plants m^2 (depending on cultivar) and effective plot size harvested was 4.0 m^2. According to recommendations for commercial genotypes (cultivars) for full scale production under irrigation in the Upington and Vaalharts regions, 21 genotypes were included in 2012 and 19 in 2013. A fertiliser mixture; 2 (N):3 (P):4 (K) (28) and K.A.N (28); was applied at 280 kg N ha^{-1} (ARC-SGI 2014a). Fertilisation was spread between 160 kg N ha^{-1} at seeding, 60 kg N ha^{-1} between tillering (Zadoks 20) and stem elongation (Zadoks 39) followed by 60 kg N ha^{-1} between flag leaf (Zadoks 47) and anthesis (Zadoks 61) as described by Anderson and Garlinge (2000).

Rainfed field experiments at the WRR sites of Riversdale and Moorreesburg were established with 15 genotypes in each trial. Field experiments were planted with a commercial planter adapted for seeding plots under no till conditions common in the Rûens and Swartland regions of the WRR. Seeding density was sufficient for 250 to 300 plants per m^2 and plots consisted of seven rows of 5 m with 30 cm between row spacing resulting in an effective harvested area of 6.0 m^2. A total nitrogen amount of 130 kg N ha^{-1} was supplied through a commercial mixture of 4 (N):1 (P):1 (K) (31), (ARC-SGI 2014b). Fertiliser application was split between 100 kg N^{-1} during seeding and the remaining 30 kg N ha^{-1} applied as K.A.N (28) between tillering (GS20) and stem elongation (GS39) growth stages as described by Zadoks (Anderson and Garlinge 2000).

8.2.2 Grain yield, grain protein yield, flour protein content, grain protein content, loaf volume and protein ratios

Grain yield was determined by mechanical harvesting of test plots, after which seed samples were sieved for removal of deformed and broken kernels and straw remnants. Primary quality parameters and SE-HPLC were conducted on the same wheat samples at the ARC-SGI in Bethlehem. Grain protein yield (kg ha^{-1}) was calculated for determining the real protein achievement capability of a genotype (expressed in kg protein ha^{-1}) and refers to the amount of protein harvested from a hectare of the particular genotype. This parameter is determined by a simple calculation of multiplying grain yield with grain protein percentage.

The primary quality parameters measured were percentage flour protein content (AACC method 39–11), percentage grain protein content (AACC method 46–10) and loaf volume (100 g baking test) determined according to Industry Accepted Method 022 based on AACCI Method 10-10.03 (AACC 2000). The SE-HPLC procedure developed by Gupta et al. (1993) with modifications, was applied and flour samples were extracted in two steps. During the first step, SDS-soluble proteins were extracted, whereas samples were submitted in the second step for sonication to obtain SDS-insoluble proteins. White flour was used and analyses were done in duplicate. ANOVA was conducted on cultivar (genotype), localities x years (environment) and the interaction (G x E) between the two. Results were applied to rank genotypes into high yield, medium yield and low yield groups for each of the three production regions. Correlations were determined between grain yield of these groups and protein fractions (calculations are specified in Table 3.9 of Chapter 3).

8.3 Results and discussion

8.3.1 Grain yield and grain protein yield, flour protein, grain protein and loaf volume

8.3.1.1 Analysis of variance for grain yield, grain protein yield, flour protein content, grain protein content with loaf volume

The large effect of E on baking quality has been reported on extensively (Graybosch et al. 1995; Shewry 2009; Koppel and Ingver 2010). Grain yield is a quantitative trait and also interacts significantly with environment. The conclusion of Bryan et al. (2014) was that although fertilisation strongly increased wheat yield, the advantage was largely influenced by climatic factors and soil water availability.

Results from the three wheat production regions in SA show that under irrigated production conditions differences in grain yield, grain protein yield, flour protein content and loaf volume were significant between G, E and G x E interaction. Grain protein content varied significantly only for G and E (Table 8.1). For the irrigated production region, E had the largest influence on variability of all four parameters. Differences in the four parameters for rainfed wheat in the SRR were highly significant for G, E and G x E interaction and similar to irrigation conditions, E had the largest influence on variation. Rainfed wheat of the WRR had significant differences in grain yield, flour protein content and loaf volume for G, E and G x E interaction whereas grain protein yield and grain protein content varied significantly only for G and E. (Table 8.1). Similarly, to irrigation and rainfed SRR, E of rainfed WRR had the largest influence on variation of grain yield, grain protein yield, flour protein content, grain protein content and loaf volume. The variation in loaf volume, however, was more or less equally determined by G, E and G x E. During development of wheat before anthesis, which is the period defining grain yield, environment affects germination, photosynthesis, number of tillers and flower development that determine grain number (Herzog 1986; Egli 1998). After anthesis, environmental conditions primarily affect kernel size and composition, which primarily determine wheat quality. Results from this chapter are also in line with a study by Anderson (2010) on wheat in western Australia which reported that environmental influences on variation of grain yield outweighs that of production management, genotype or the interactions between these two factors and environment.

Table 8.1 Analysis of variance for grain yield, grain protein yield, flour protein content, grain protein content and loaf volume

Para-meters	Source of variation	Irrigation			Rainfed SRR			Rainfed WRR		
		% SS	MS	P-value	% SS	MS	P-value	% SS	MS	P-value
Grain yield	Genotype	17.12	3.90	<.0.001	2.59	0.83	<.0.001	17.74	1.11	<.0.001
	Environment	54.21	69.70	<.0.001	85.00	100.45	<.0.001	39.99	9.16	<.0.001
	G x E	21.39	1.62	<.0.001	9.84	1.06	<.0.001	19.30	0.40	<.0.001
	Residual		0.19			0.09			0.15	
	CV% Rep		0.7			2.5			2.4	
	CV% Rep.*Units*		4.3			8.5			7.9	
Grain protein yield	Genotype	2.67	67837	0.004	3.72	90777	<.0.001	9.83	115468	<.0.001
	Environment	43.61	738079	<.0.001	74.85	1219229	<.0.001	59.68	467559	<.0.001
	G x E	6.08	51497	<.0.001	4.85	39473	<.0.001	1.54	6038	NS
	Residual		11810			6133			5078	
	CV% Rep		1.3			2.4			2.3	
	CV% Rep.*Units*		9.1			15.7			11.2	
Flour protein content	Genotype	10.78	6.54	<.0.001	19.89	6.53	<.0.001	15.03	4.14	<.0.001
	Environment	72.08	247.95	<.0.001	55.01	66.20	<.0.001	43.81	44.29	<.0.001
	G x E	5.90	1.19	0.033	11.14	1.22	0.001	16.89	1.55	0.002
	Residual		0.80			0.54			0.72	
	CV% Rep		1.2			0.1			2	
	CV% Rep.*Units*		7.7			5.1			6.9	
Grain protein content	Genotype	8.22	4.57	<.0.001	8.15	3.96	<.0.001	23.23	7.28	<.0.001
	Environment	76.18	240.20	<.0.001	74.93	133.49	<.0.001	49.70	57.12	<.0.001
	G x E	4.35	0.81	NS	7.51	1.22	<.0.001	8.46	0.88	NS
	Residual		0.71			0.53			0.67	
	CV% Rep		1.7			0.6			0.6	
	CV% Rep.*Units*		7.0			4.9			6.4	
Loaf volume	Genotype	18.25	18495	<.0.001	20.42	7269.6	<.0.001	20.71	8983	<.0.001
	Environment	50.73	291288	<.0.001	46.51	60719.9	<.0.001	29.68	47200	<.0.001
	G x E	16.04	5419	<.0.001	19.86	2356.7	<.0.001	24.14	3490	<.0.001
	Residual		1807			530.5			1211	
	CV% Rep		0.3			0.4			1.0	
	CV% Rep.*Units*		4.8			2.3			3.7	

G x E = genotype environment interaction; CV = coefficient of variance; NS = not significant

8.3.1.2 Ranking of grain yield

Grain yield of genotypes (Table 8.2) for irrigated production was the highest and ranged from 9.37 ton ha^{-1} for the low yielding group to 10.63 ton ha^{-1} of the high yielding group. Variation in yield for rainfed production in the SRR was small and between 3.14 ton ha^{-1} and 3.79 ton ha^{-1} whereas rainfed wheat in the WRR varied between 4.38 ton ha^{-1} and 5.15 ton ha^{-1}. The difference between the highest yielding group and lowest yielding group was highly significant (< 0.001) for all three production regions (Table 8.2).

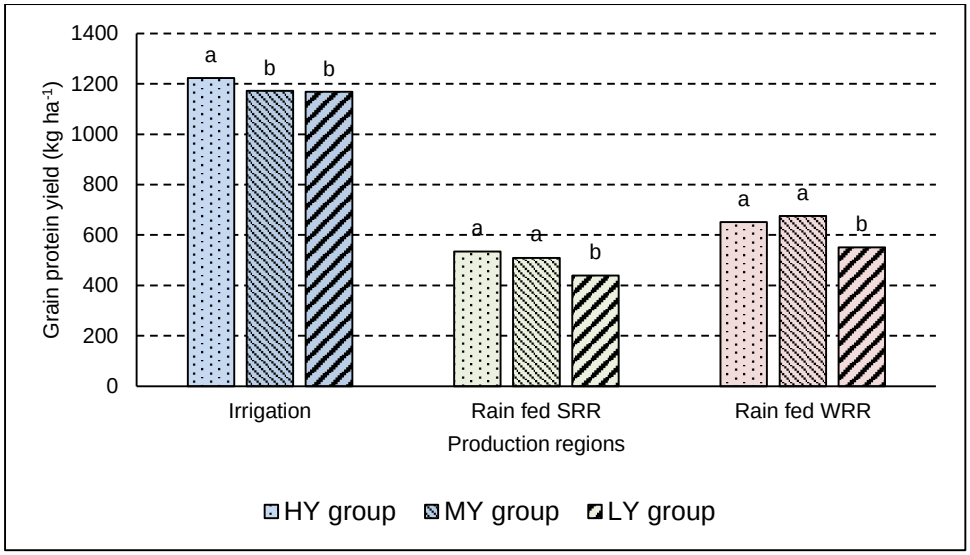
Table 8.2 Ranking of genotypes in the three production regions for grain yield (ton ha^{-1})

Irrigation				Rainfed SRR				Rainfed WRR			
Genotype	**Grain yield (ton ha^{-1})**	**Rank Group**	**Mean**	**Genotype**	**Grain yield (ton ha^{-1})**	**Rank group**	**Mean**	**Genotype**	**Grain yield (ton ha^{-1})**	**Rank group**	**Mean**
PAN 3497	10.84^{a}	High Yield	10.63	**SST 316**	3.84^{a}	High yield	3.79	**PAN 3471**	5.19^{a}	High yield	5.15
SST 866	10.83^{ab}			**SST 317**	3.77^{ab}			**PAN 3408**	5.17^{a}		
Buffels	10.58^{abc}			**SST 347**	3.75^{ab}			**Tankwa**	5.17^{a}		
Tamboti	10.53^{abc}			**PAN 3161**	3.66^{abc}	Medium yield	3.54	**SST 056**	5.15^{a}		
PAN 3471	10.50^{abc}			**Senqu**	3.63^{abc}			**SST 015**	5.14^{a}		
SST 876	10.49^{abc}			**PAN 3368**	3.61^{abc}			**SST 096**	5.14^{a}		
SST 835	10.32^{abcd}	Medium yield	10.17	**Koonap**	3.50^{abcd}			**SST 027**	5.13^{a}		
Krokodil	10.23^{abcde}			**SST 356**	3.45^{abcd}			**SST 087**	5.09^{a}		
SST 884	10.23^{abcde}			**Elands**	3.38^{bcde}			**Ratel**	5.01^{ab}	Medium yield	4.91
SST 806	10.20^{abcde}			**PAN 3195**	3.27^{cde}	Low yield	3.14	**SST 047**	4.80^{abc}		
PAN 3478	10.18^{bcde}			**Gariep**	3.17^{de}			**SST 88**	4.49^{bc}	Low yield	4.38
PAN 3489	10.04^{cde}			**PAN 3379**	2.98^{e}			**Kwartel**	4.27^{c}		
Duzi	9.97^{cde}			**CV%**	8.5			**CV%**	7.9		
SST 895	9.76^{def}	Low yield	9.37	**LSD**	0.24			**LSD**	0.32		
SST 877	9.66^{ef}			**P-value**	< 0.001			**P-value**	< 0.001		
Sabie	9.64^{ef}										
SST 822	9.16f^{g}										
SST 843	8.62^{g}										
CV%	4.3										
LSD	0.35										
P-value	< 0.001										

Values within a column with different letters are statistically different

8.3.1.3 Grain protein yield

Due to higher yields under irrigation, the grain protein yield (GPY) per ha for this production region (ranging from 1, 168 to 1, 223 kg ha^{-1}) was approximately double than for the rainfed WRR (551.3 to 675.8 kg ha^{-1}) and rainfed SRR (439.5 to 534.8 kg ha^{-1}) production regions (Figure 8.1). The differences between GPY of the high yield, medium yield and low yield groups were significant for all three production regions (Figure 8.1) and possibly reflected the fact that the groups were selected on the basis of significant differences in grain yield. Even considering the effect of current input cost ha^{-1} (data not shown) for irrigated wheat (GrainSA 2015) the production of protein under irrigation remains the most feasible and cost effective production system for wheat calories in SA. Important though, is that GPY for irrigation comes from a total of approximately 280 kg N ha^{-1} per season, rainfed WRR from approximately 130 kg N ha^{-1} per season and rainfed SRR from only approximately 60 kg N ha^{-1} per season.



HY-High yielding group, MY-Medium yielding group, LY-Low yielding group

Different letters within a production region indicate significant statistical difference only within that production region

Figure 8.1 Grain protein yield (kg ha^{-1}) of the three production regions

8.3.1.4 Correlations between grain yield and flour protein, grain protein and loaf volume

Few significant correlations (r > 0.45***) occurred between the grain yield of the highest and lowest ranking yield groups and flour protein content, grain protein content and loaf volume of wheat in the three production regions (Table 8.3). Negative correlations between grain yield and grain protein only occurred between the lowest yielding group of irrigation (mean of 9.37 ton ha^{-1}) and flour protein content (-0.53***) and grain protein content (-0.60***). These correlations are expected under high yielding conditions for irrigation and correspond with reports by Saint Pierre et al. (2008) that irrigation may decrease flour protein content through a “dilution effect” of grain nitrogen (quality) with carbohydrates (yield). In contrast, high yield of rainfed wheat in the SRR (mean of 3.79 ton ha^{-1}) correlated positively and strongly with loaf volume (0.78*). The general low number of correlations could be attributed to limited trait variation in the samples studied (Bergman et al. 1998).

Table 8.3 Correlations of flour protein, grain protein and loaf volume with high and low ranked grain yield

Quality parameter	Irrigation		Rainfed SRR		Rainfed WRR	
	High yield	Low yield	High yield	Low yield	High yield	Low yield
Flour protein (%)	NS	-0.53***	NS	NS	NS	NS
Grain protein (%)	NS	-0.60***	NS	NS	NS	NS
Loaf volume (cm^3)	NS	NS	0.78*	NS	NS	NS

*** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05, NS - not significant

8.3.2 Grain yield and protein fractions

8.3.2.1 Analysis of variance of protein fraction concentrations (%)

E of all three production regions caused significantly different soluble and insoluble glutenin, α/β, γ gliadin and albumin/globulin in total protein (Table 8.4). The sources of variation contributing most to variation in concentrations of protein fractions varied between the three production regions, although the two rainfed regions exhibited similarities (Table 8.4). E contributed most to the variation of percentage soluble glutenin in total protein for irrigation (31.36%) whereas G x E interaction resulted in the highest variation for rainfed SRR (12.26%) and rainfed WRR (7.20%). The factors with the highest contribution to variation of soluble α/β, γ gliadin in total protein were E (36.78%) for irrigated production regions and G for both rainfed SRR (13.72%) and rainfed WRR (22.77%). E contributed most to the variation of percentage soluble albumin/globulin in total protein for irrigation (59.63%), rainfed SRR (27.32%) and rainfed WRR (37.97%).

Variations in the percentage insoluble glutenin in total protein for irrigation (54.80%), rainfed SRR (32.08%) and rainfed WRR (72.24%) were largely determined by E (Table 8.4). The amounts of insoluble α/β, γ gliadin in total protein were also determined primarily by E of the irrigation (8.26%) and rainfed SRR (23.77%) regions whereas G determined variation in the rainfed WRR (7.71%). The amounts of insoluble albumin/globulin in total protein were primarily

determined by the E of the irrigation region (13.03%) and rainfed WRR (15.36%) whereas G x E interaction determined most of the variation in the rainfed SRR region (15.19%).

Table 8.4 Analysis of variance of the percentage soluble and insoluble glutenin, gliadin and albumin/globulin fractions in total protein of wheat from the three production regions of South Africa

		Irrigation			Rainfed SRR			Rainfed WRR		
Protein fraction	**Source of variation**	**% SS**	**MS**	**P-value**	**% SS**	**MS**	**P-value**	**% SS**	**MS**	**P-value**
% soluble glutenin (LPP) in total protein	Genotype	3.14	1.89	0.006	2.57	1.74	NS	1.28	0.24	NS
	Environment	31.36	12.57	< 0.001	10.17	4.58	< 0.001	6.50	0.83	0.022
	G x E	4.18	0.84	0.036	12.26	2.76	0.003	7.20	0.46	NS
	Residual		0.36			0.78			0.25	
	CV% Rep	0.6			0.8			0.7		
	CV% Rep.*Units*	9.0			15.0			7.5		
% soluble α/β, γ-gliadin (LMP) in total protein	Genotype	9.18	39.09	< 0.001	13.72	86.55	< 0.001	22.77	74.64	< 0.001
	Environment	36.78	104.39	< 0.001	11.94	50.19	< 0.001	4.98	10.88	0.027
	G x E	2.16	3.07	NS	7.42	15.61	0.031	4.04	4.41	NS
	Residual		2.18			6.48			3.43	
	CV% Rep	0.3			0.4			0.4		
	CV% Rep.*Units*	5.7			8.8			6.9		
% soluble albumin and globulin (SMP) in total protein	Genotype	6.81	20.78	< 0.001	0.27	0.45	NS	15.11	25.69	< 0.001
	Environment	59.63	121.27	< 0.001	27.32	29.99	< 0.001	37.97	43.05	< 0.001
	G x E	1.31	1.34	NS	6.95	3.81	0.038	2.20	1.25	NS
	Residual		0.97			1.65			1.16	
	CV% Rep	0.4			0.6			1		
	CV% Rep.*Units*	7.6			10.3			8.4		
% insoluble glutenin (LPP) in total protein	Genotype	5.01	6.67	< 0.001	5.96	9.41	< 0.001	0.43	0.29	NS
	Environment	54.80	48.62	< 0.001	32.08	33.78	< 0.001	72.24	32.06	< 0.001
	G x E	3.03	1.35	0.013	12.54	6.60	< 0.001	4.84	1.07	< 0.001
	Residual		0.49			1.20			0.23	
	CV% Rep	1.5			0.5			0.7		
	CV% Rep.*Units*	13.4			20.6			8.8		
% insoluble α/β, γ-gliadin (LMP) in total protein	Genotype	6.87	3.12	< 0.001	5.28	2.26	0.005	7.71	0.77	0.003
	Environment	8.26	2.50	< 0.001	23.77	6.77	< 0.001	4.40	0.29	NS
	G x E	3.89	0.59	NS	9.46	1.35	0.004	3.14	0.10	NS
	Residual		0.36			0.40			0.13	
	CV% Rep	2.1			0.7			0.5		
	CV% Rep.*Units*	16.4			15.5			9.9		
% insoluble albumin and globulin (SMP) in total protein	Genotype	0.27	0.06	NS	5.94	1.09	0.005	2.78	0.16	NS
	Environment	13.03	2.09	< 0.001	9.63	1.18	< 0.001	15.36	0.57	< 0.001
	G x E	4.29	0.34	NS	15.19	0.93	< 0.001	8.46	0.16	0.026
	Residual		0.20			0.20			0.06	
	CV% Rep	0.9			1.0			0.2		
	CV% Rep.*Units*	22.7			23.5			14.2		

CV = coefficient of variance; NS = not significant

Saint Pierre et al. (2008) reported that on average for the three test localities in their study, irrigation treatment had less impact on protein composition than the nitrogen fertilisation, although flour protein, monomeric protein fraction and the ratio of monomeric to polymeric

proteins did increase as water stress increased. The percentage of polymeric protein was not affected by water stress.

8.3.2.2 Correlations between grain yield and protein fractions

Significant correlations between grain yield and protein fractions (Table 8.5) were more frequent for genotypes adapted for production under irrigation and rainfed WRR production regions and average grain yield for rainfed SRR (3.49 ton ha^{-1}) was lower than for rainfed WRR (4.81 ton ha^{-1}) and irrigation (10.06 ton ha^{-1}). Interestingly, very few correlations occurred between protein fractions and grain yield of rainfed SRR. Probable explanations may come from the fact that only a single positive significant correlation occurred between grain yield of rainfed SRR and loaf volume and few protein fractions correlated with loaf volume of rainfed SRR (Chapter 5). The frequency and intensity of glutenin and gliadin proteins in wheat grain are more often influenced by water deficits or excesses and nitrogen management (Daniel and Triboï 2002; Przednowek et al. 2002; Dai et al. 2013).

The larger fraction of insoluble glutenin in total protein (-0.62***), insoluble glutenin in total glutenin (-0.61***) and insoluble glutenin in total glutenin (-0.56***) correlated negatively with the low yield group of the irrigation region. Frequent negative correlations of insoluble glutenin with yield for irrigation may result from negative correlations between yield and flour protein content and yield and grain protein content (refer to Section 8.3.1.4) as both flour and grain protein content have strong and positive correlations with insoluble larger polymeric glutenin in irrigation (refer to Chapter 4).

Insoluble smaller polymeric glutenin in total insoluble protein (0.83*) and soluble smaller polymeric glutenin in total protein (0.60***) correlated positively with low yield of rainfed WRR and irrigation respectively. The insoluble smaller polymeric glutenin in total smaller polymeric protein, however, correlated negatively with low yield of irrigation trials (-0.51***). Water stress and soil properties influence the concentrations and particle size of glutenin. Park et al. (2014)

concluded that variation in soil and climate in South Dakota impacted yields, protein composition, and dough quality whereas Jiang et al. (2009) also reported water stress to increase the concentration of glutenin. Clay soil in relation to loam and sandy soil furthermore was also found to promote higher accumulation of glutenin (Liang et al. 2008).

In table 8.5 concentration of soluble ω gliadin in total soluble protein correlated positively with the high yield ranking group (0.61**) but negatively with the low yield ranking group (-0.89*) in wheat of rainfed WRR. Soluble ω gliadin in total protein also correlated positively with the high yield ranking group (0.71***) but negatively with the low yield ranking group (-0.89*) of rainfed WRR. Insoluble larger monomeric ω gliadin (LMP) in total ω gliadin exhibited a reverse trend and correlated negatively with the high yield ranking group (-0.45*) but positively with the low yield ranking group (0.86*) of rainfed WRR. These cross-over interactions between high and low yield ranking groups indicate a sensitivity of ω gliadin towards environmental influences (G x E interaction). The concentration of insoluble ω gliadin in total insoluble protein furthermore correlated strongly and positively with high yield (0.64***) of rainfed WRR. The large monomeric and soluble α/β, γ gliadin in total soluble protein correlated positively with the low yield ranking group (0.82*) of rainfed WRR. Insoluble α/β, γ gliadin in total protein also correlated positively with the low yield ranking group (0.87*) but negatively with the high yield ranking group (-0.48*) for rainfed WRR (-0.48***) and low yield ranking group of irrigation (-0.48***). Both ω and α/β, γ gliadin seem to be sensitive to G x E interaction as cross-over interaction within a production region only occurred for this protein fraction (Table 8.5). A similar trend was reported for winter wheat grown in South Dakota as gliadin correlated negatively with yield loss under water stress conditions (Park 2011).

Table 8.5 Correlations of protein fractions with grain yield

Solubility classes	Protein fractions	Irrigation		Rainfed SRR		Rainfed WRR	
		High yield	Low yield	High yield	Low yield	High yield	Low yield
Soluble fraction in total soluble protein	Glutenin (LPP)	NS	NS	NS	NS	NS	NS
	Glutenin (SPP)	NS	NS	NS	NS	NS	NS
	ω Gliadin (LMP)	NS	NS	NS	NS	0.61**	-0.89*
	α/β, γ Gliadin (LMP)	NS	NS	NS	NS	NS	0.82*
	Albumin/globulin (SMP)	NS	0.54***	0.70*	NS	-0.55**	NS
Insoluble fraction in total insoluble protein	Glutenin (LPP)	NS	NS	NS	NS	NS	NS
	Glutenin (SPP)	NS	NS	NS	NS	NS	0.83*
	ω Gliadin (LMP)	NS	NS	NS	NS	0.64***	NS
	α/β, γ Gliadin (LMP)	NS	NS	NS	NS	NS	NS
	Albumin/globulin (SMP)	NS	NS	NS	NS	NS	-0.86*
Soluble fraction in total protein	Glutenin (LPP)	NS	NS	NS	NS	NS	NS
	Glutenin (SPP)	NS	0.60***	NS	NS	NS	NS
	ω Gliadin (LMP)	NS	NS	NS	NS	0.71***	-0.89*
	α/β, γ Gliadin (LMP)	NS	NS	NS	NS	NS	NS
	Albumin/globulin (SMP)	NS	0.70***	NS	NS	-0.46*	NS
Insoluble fraction in total protein	Glutenin (LPP)	NS	-0.62***	NS	NS	NS	NS
	Glutenin (SPP)	NS	NS	NS	NS	NS	NS
	ω Gliadin (LMP)	NS	NS	NS	NS	NS	NS
	α/β, γ Gliadin (LMP)	NS	-0.48***	NS	NS	-0.48*	0.87*
	Albumin/globulin (SMP)	NS	NS	NS	NS	NS	NS
Insoluble fraction in total same fraction	Glutenin (LPP)	NS	-0.61***	NS	NS	NS	NS
	Glutenin (SPP)	NS	-0.51***	NS	NS	NS	NS
	ω Gliadin (LMP)	NS	NS	NS	NS	-0.45*	0.86*
	α/β, γ Gliadin (LMP)	NS	NS	NS	NS	NS	NS
	Albumin/globulin (SMP)	NS	-0.50***	NS	NS	NS	NS
Insoluble glutenin in total glutenin		NS	-0.56***	NS	NS	NS	NS

LPP: Large polymeric proteins, SPP: small polymeric proteins, LMP: large monomeric proteins, SMP: small monomeric proteins

*** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05, NS - not significant

The concentration of soluble albumin/globulin (SMP) in total soluble protein correlated positively with low yield (0.54***) of irrigation, positively with high yield (0.70*) for rainfed SRR

but negatively with high yield (-0.55**) for rainfed WRR. Insoluble albumin/globulin in total insoluble protein also correlated negatively with low yield (-0.86*) of rainfed WRR. Soluble albumin/globulin in total protein correlated positively with low yield (0.70***) of irrigation but negatively with high yield (-0.46*) of rainfed WRR. The concentration of insoluble albumin/globulin (SMP) in total albumin/globulin correlated negatively with low yield (-0.50***) of irrigation (Table 8.5). The quantity of albumins/globulins is rarely influenced by nitrogen nutrition (Wieser and Seilmeier 1998; Johansson et al. 2001) but rather by total quantity of nitrogen accumulated in the grain (Triboï et al. 2003) as albumins/globulins start accumulating during cell division in the grain, shortly after anthesis.

8.3.3 Concentrations of proteins in high, medium and low yield groups

8.3.3.1 Highest and lowest concentrations of protein fractions

Soluble α/β, γ gliadin was the protein fraction with the highest concentration in total protein and ranged between 25.18% and 30.42%. In contrast, the protein fraction with the lowest concentration was insoluble albumin/globulin with a range between 1.67% and 2.03% (Figure 8.2).

8.3.3.2 Soluble and insoluble glutenin

Soluble glutenin concentration in genotypes of the high, medium and low yield groups differed significantly only for irrigation conditions and the concentration in low yield (6.47%) was significantly less than in medium yield (6.75%) and high yield (6.79%). An inverse trend occurred for insoluble glutenin, as the concentration in low yield (5.58%) was significantly higher than in medium yield (4.97%) and high yield (5.17%) groups. Interestingly in rainfed SRR, concentrations of insoluble glutenin in medium yield (5.68%) was significantly higher compared to high yield (4.87%) and low yield (5.08%) groups. No significant differences for glutenin occurred between the three yield-groups of rainfed WRR (Figure 8.2).

8.3.3.3 Soluble and insoluble gliadin

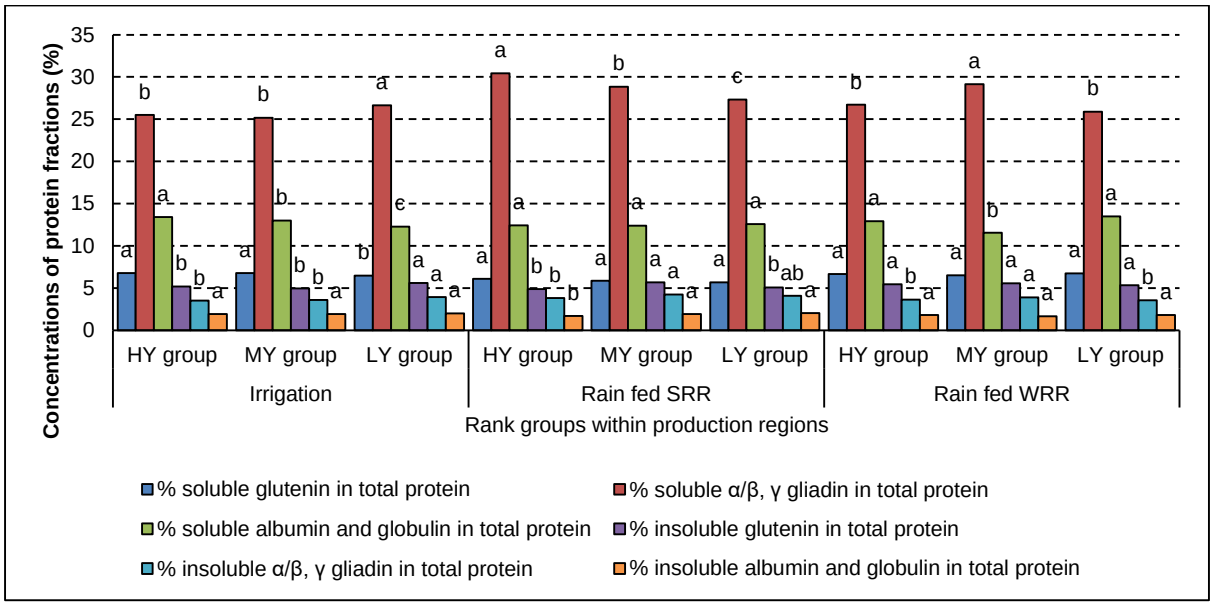
Soluble gliadin (α/β, γ) concentrations in the three production regions followed different trends. Concentration of soluble gliadin (α/β, γ) in low yield (26.64%) for irrigation was significantly more than in medium yield (25.18%) and high yield groups (25.52%). In contrast, for rainfed SRR production, the soluble gliadin in high yield (30.42%), medium yield (28.86%) and low yield (27.32%) groups were significantly different from each other and declined in step with yield. Genotypes of the medium yield group for rainfed WRR had significantly higher concentrations of soluble gliadin (29.16%) than high yield (26.70%) or low yield (25.90%) groups of which the latter two did not differ significantly. Insoluble gliadin concentrations in genotypes for irrigation production followed a similar trend than soluble gliadin, as insoluble gliadin in low yield (3.91%) was significantly higher than in medium yield (3.60%) and high yield (3.49%) groups. Concentration of insoluble gliadin in medium yield of rainfed SRR (4.25%) were significantly higher than in the high yield group (3.81%) whereas the concentration of gliadin in low yield were not significantly different from the high or medium yield groups. Insoluble gliadin concentrations in rainfed WRR were similar to soluble gliadin as medium yield (5.54%) had significantly higher concentrations than high yield (3.63%) or low yield (3.53%) groups which did not differ significantly from each other (Figure 8.2).

8.3.3.4 Soluble and insoluble albumin/globulin

Soluble albumin/globulin concentrations were the second highest protein concentration in total protein. Concentration of albumin/globulin in the irrigation region differed significantly between high yield (13.40%), medium yield (13.00%) and low yield (12.28%) groups. No significant differences occurred for rainfed SRR whereas concentrations of high yield (12.91%) and low yield (13.49%) groups in rainfed WRR were not significantly different from each other, but significantly higher than concentration of the medium yield (11.53%) group. Concentrations of insoluble albumin/globulin for irrigation were not significantly different from each other whereas concentrations in medium yield (1.91%) and low yield (2.03%) groups of rainfed SRR (not significantly different from each other) were significantly higher than in high yield (1.69%)

groups. As in irrigation, concentrations of insoluble albumin/globulin in rainfed WRR did not vary significantly (Figure 8.2).

According to results by Triboï et al. (2006) the effect of variations in total nitrogen per grain affects protein concentrations differently. Although the percentage glutenin at harvest was not affected by variations in the total quantity of nitrogen per grain, concentration of gliadin was positively correlated with the total quantity of nitrogen per grain (for grain nitrogen ranges of 560 to 1000 mg N grain^{-1}). An opposite trend occurred for albumins/globulins as percentages decreased when the total quantity of nitrogen per grain increased (range of 560 to 1000 mg N^{-1}).



HY = high yield, MY = medium yield, LY = low yield

Different letters within a production region indicate significant statistical difference only within that production region

Figure 8.2 Percentage concentrations of protein fractions

8.4 Conclusions

For the irrigated, rainfed SRR and rainfed WRR production regions, environment contributed the most to variability of grain yield, grain protein yield, flour protein content, grain protein

content and loaf volume. Grain protein yield per ha for the irrigated production region was approximately double than for the rainfed WRR and rainfed SRR production regions, although at a substantially higher rate of nitrogen ha^{-1} per season. Few strong correlations ($r > 0.45$***) occurred between the high and low grain yield groups and flour protein content, grain protein content and loaf volume of wheat from the three production regions. Environment (E) of all three production regions were mostly significantly different for both soluble and insoluble glutenin, α/β, γ gliadin and albumin/globulin in total protein and contributed most to variability of all the protein fractions under irrigation. A similar trend occurred for rainfed SRR and rainfed WRR, although variation in concentrations of protein fractions was determined by G, E and G x E. Correlations between grain yield and protein fractions were more frequent for irrigation and rainfed WRR production regions and cross-over interactions of ω gliadin (high yield and low yield groups) for rainfed WRR indicate a sensitivity towards environmental influences. Soluble α/β, γ gliadin was the protein fraction with the highest concentration in total protein (25.18% and 30.42%) compared to insoluble albumin/globulin (1.67% and 2.03%) with the lowest concentration. Bergman et al. (1998) concluded that it is due to large environmental influences on quality traits that screening methods should be developed that rather employ genotype than phenotype. Application of grain protein yield or protein per grain (Brunori et al. 1980) and analyses of grain protein deviation (Monaghan et al. 2001; Oury and Godin 2007; Marinciu and Săulescu 2008) focussing on genotypes with higher grain protein concentration than predicted from grain yield, has been proposed. All these selection criteria seem to have a partly genetic basis, but effective separation and comprehension of the role of protein fractions will be essential.

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Chapter 9

Conclusions and recommendations

9.1 Introduction

An unresolved debate continues regarding grain protein content and its application as true gauge of bread making quality, which indirectly challenges its role in determining the price a farmer receives for wheat. For grain protein content to be a valid measurement, two assumptions are made, namely, that wheat grain consists mainly of elements containing nitrogen which are associated with high bread making quality and secondly, all nitrogen is incinerated during the measurement process. Grain protein levels appear not to be the primary factor determining the loaf volume of local and imported wheat (SAGL 2014) which is confirmed by results of different genotypes, particularly for irrigated wheat in 2012 and 2013 (Chapter 7). Genotypes with protein levels only meeting B3 grade specifications were able to produce loaf volumes within the 10% variation range determined by the quality standard. These observations lead to the conclusions that (i) protein content, as applied in wheat grading, cannot accurately predict bread making quality (as defined through loaf volume) and (ii) wheat farmers in this case would have received a lower price for wheat, producing loaf volumes equal to the top grade (B1).

9.2 The primary climatic and production environments of the three production regions

An attempt to understand the major factors affecting the composition and concentrations of proteins important in bread quality of the three production regions requires comprehension of their rainfall patterns. Composition and concentration of proteins depend on the availability of

nitrogen and water during critical growth stages and shortages will eventually mirror in results of most of the primary baking parameters and rheological properties.

9.2.1 Irrigated wheat of the cooler region (along the Orange River of the Northern Cape)

Despite highly regulated and frequent application of irrigation (a total of between 600 to 900 mm throughout the growing season) and fertiliser (Figure 9.1a), the grain yields and bread making quality of irrigated wheat were still affected by seasons with lower rainfall and high temperatures (as in 2013). Two irrigation systems occur namely, demand-driven centre pivot and supply driven flood irrigation. The former is used in commercial farming practises whereas the latter is more suited for small scale and low input farming. The water delivering capacity of centre pivot systems is limited to approximately 12 mm day^{-1} in contrast to supply driven flood irrigations generally exceeding 50 mm. Common irrigation intervals practised under production conditions are either bi-daily, weekly, or fortnightly with rates of 12, 80 or 100 mm per application, respectively. For successful seeding, the soil moisture level should be as close as possible to field capacity (10 kPa tension) which would necessitate an initial irrigation of 50 mm to fill the 1.8 m rooting zone (Annandale et al. 2011). A smaller deviation from the nine year mean for rainfall between post-anthesis and grain filling in 2012 (Chapter 3) resulted in higher wheat yields and quality.

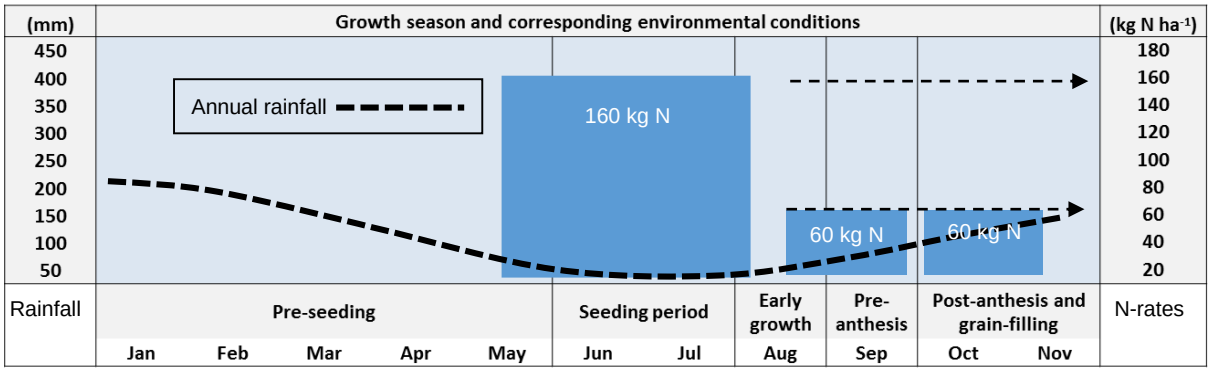

Figure 9.1a Primary fertilisation stage(s) in relation to annual rainfall for irrigated wheat

9.2.2 Rainfed wheat from the summer rainfall region

Rainfed wheat from the SRR has the strongest rheological quality of the three production regions of SA (SAGL 2014) which can probably be attributed to high summer temperatures coupled with high rainfall benefitting longer growth periods of winter and intermediate type wheat. Seeding densities and fertiliser rates are relatively low compared to the other two production regions and winter and/or facultative wheat types are cultivated. A commercial fertiliser mixture of 4 (N):2 (P):1 (K) (28) was applied during seeding at an amount of 60 kg N ha^{-1} (ARC-SGI 2014a). The most important and significant element affecting grain yield and grain quality of rainfed wheat in 2012 and 2013 in the SRR was rainfall (Chapter 3). Although rainfall in the pre-seeding period of 2012 for Bethlehem and Clarens was 132.28 mm and 68.99 mm, respectively, less than the nine year mean of the EFS, the critical period determining grain yield was during pre-anthesis. In 2012 Bethlehem and Clarens respectively received 30.42 mm and 61.97 mm more rainfall in the pre-anthesis period compared to the nine year mean which, in contrast, was lower than the corresponding period in 2013. Showers occurring in September are generally considered an important factor for high yield of rainfed wheat in the SRR. Another important rainfall period in the growth season of 2012 at Bethlehem and Clarens was the pre-seeding period from June to July. These two months are in the middle of the SA winter and coincides with seeding time of wheat and rainfall in these two months is

uncommon but contributes significantly to higher yields in seasons when it occurs (Figure 9.1b).

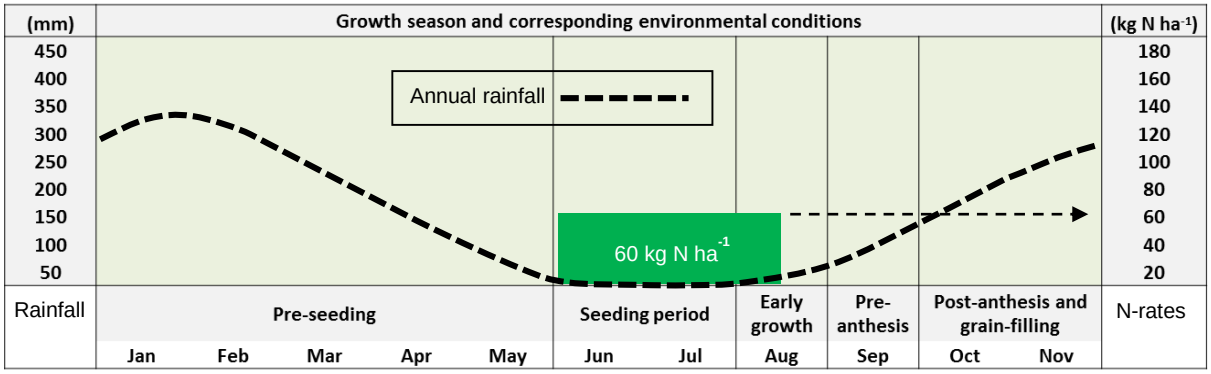

Figure 9.1b Primary fertilisation stage(s) in relation to annual rainfall for rainfed wheat in the summer rainfall region

9.2.3 Rainfed wheat from the winter rainfall region

In 2013 when the highest grain yields occurred in the Swartland, an increase of more than 85 mm rain was recorded between the early growth stages and pre-anthesis (Figure 9.1c). This higher rainfall followed after the pre-seeding and seeding periods during which relatively small deviations from the nine-year mean occurred. Spring type genotypes are seeded at high densities and fertiliser rates. Record yields were achieved over the last few seasons as implementation of conservation agriculture reduced the traditional risks associated with varying rainfall and high maximum temperatures. A total nitrogen amount of 130 kg N ha^{-1} was supplied through a commercial mixture of 4 (N):1 (P):1 (K) (31), (ARC-SGI 2014b). Fertiliser application was split between 100 kg N^{-1} during seeding and the remaining 30 kg N ha^{-1} applied as K.A.N (28) between tillering (GS20) and stem elongation (GS39) as described by Zadoks (Anderson and Garlinge 2000).

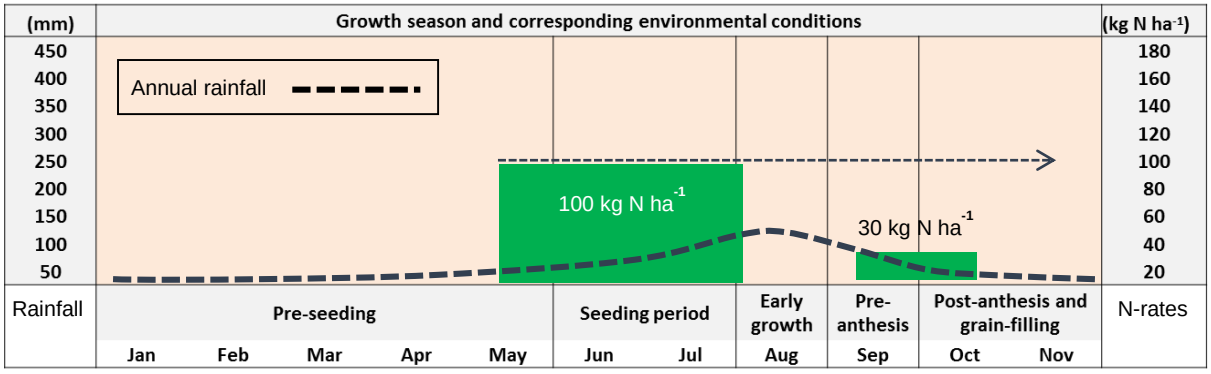

Figure 9.1c Primary fertilisation stage(s) in relation to annual rainfall for rainfed wheat of the winter rainfall region (Swartland)

Interestingly, differences occurred between rainfall patterns of the Rûens and Swartland. Rainfall in the Rûens follows a summer rainfall pattern with the peak occurring during the pre-seeding period tapering down to a low in pre-anthesis. The Swartland region experiences a typical winter rainfall peak that coincides with seeding in May and June. These differences will have a significant effect on the accumulation of proteins in the grain.

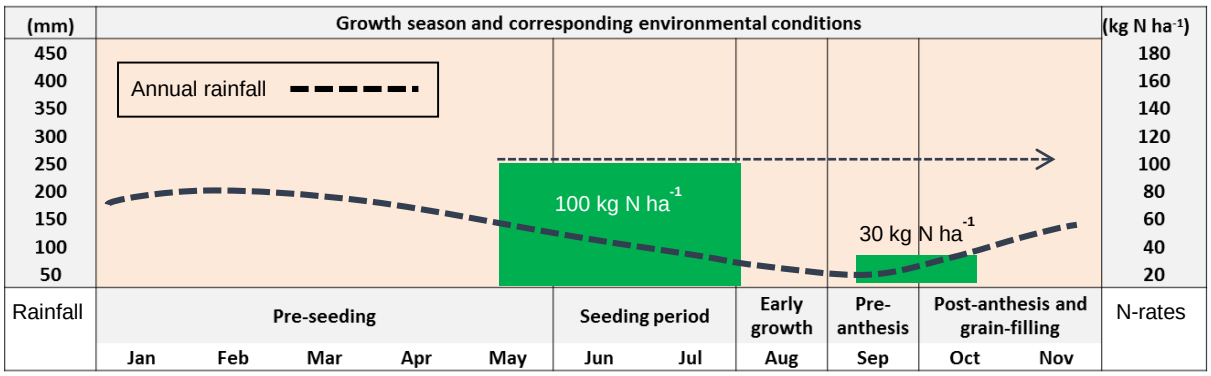

Figure 9.1d Primary fertilisation stage(s) in relation to annual rainfall for rainfed wheat in the winter rainfall region (Rûens)

9.3 Study objectives and their outcomes

Several objectives were set at the onset of the study. Although the predominant commercial genotypes were used for each production region, the study focus was placed on high, medium and low ranking groups (each group consisting of several genotypes) for flour protein, grain

protein and loaf volume and not individual genotypes. Outcomes in each of the objectives are discussed below:

9.3.1 Determine the variation in the composition of gluten over the three different wheat production regions of South Africa

Glutenin concentrations were highest in wheat from the rainfed WRR compared to α/β, γ gliadin concentration in total protein that was highest for the rainfed SRR although α/β, γ gliadin concentrations were higher in low grain protein than high grain protein content groups. Albumin/globulin concentrations were higher in wheat of the irrigated regions (14.3-15.2%) and also followed an inverse trend to grain protein content.

In contrast to results reported in this study Peña (1996) found that genotype determines composition and concentration of glutenin and gliadin but that genotypic variation becomes irrelevant when grain protein content decreases below 9%. It would therefore be expected that at lower grain protein content environment plays a more prominent role in determining glutenin and gliadin concentrations. Nitrogen availability during advanced growth stages furthermore influences the extent to which the genetic potential for gluten composition and concentration of a genotype is achieved.

9.3.2 Establish associations (correlations) between flour protein, grain protein, loaf volume and (i) bread quality (primary quality parameters) and (ii) gluten composition in the three production regions

9.3.2.1 Correlations of flour protein, grain protein and loaf volume with bread quality

Irrigated wheat

In irrigated wheat, E had the largest impact on flour protein (72.08%) and grain protein (76.18%) with relatively small effects from genotype (10.78% and 8.22% respectively). Loaf volume was primarily determined by E (50.73%) although genotype (18.25%) and G x E interaction (16.04%) also had a prominent role.

The highest ranking group average of flour protein (mean of 12.57%) correlated strongly and positively with grain protein, wet gluten, alveograph strength, dough strength and loaf volume whereas the lowest ranking group average (10.45%) correlated with the same parameters with the addition of dough distensibility. Although the highest ranking group of grain protein (mean of 13.30%) correlated positively with flour protein, wet gluten, alveograph strength and dough strength, low grain protein (mean of 11.42%) only correlated positively with flour protein, wet gluten, alveograph strength, dough strength and loaf volume. The highest ranking group average of loaf volume (average of 933 cm^3) correlated strongly and positively with flour protein, wet gluten, grain protein, alveograph strength and dough strength whereas low loaf volume (839 cm^3) correlated strongly and positively with flour protein, wet gluten, grain protein, alveograph strength, dough strength and dough distensibility.

Rainfed wheat of the summer rainfall region

E (test sites) had the largest impact on flour protein content (55.01%) and grain protein content (74.93%). The effect of G was larger on variation of flour protein (19.89%) than grain protein (8.15%). Variances in loaf volume were primarily determined by E (46.51%) although contributions of G (20.42%) and G x E interaction (19.86%) were also important. The effect of G was larger on variation of flour protein (19.89%) than grain protein (8.15%). Variances in loaf volume were primarily determined by E (46.51%) although contributions of G (20.42%) and G x E interaction (19.86%) were also substantial.

Both high (mean of 15.21%) and low (mean of 13.64%) flour protein groups correlated more strongly with loaf volume than the high and low average groups of grain protein content. High

and low flour protein, grain protein and loaf volume furthermore had strong positive correlations with alveograph (Wf), dough strength and wet gluten. Significant negative correlations occurred between falling number and high (mean of 15.21%) and low (mean of 13.64%) flour protein, high (mean of 15.54%) and low (mean of 14.04%) grain protein and high (mean of 1012.8 cm^3) and low (mean of 959.8 cm^3) loaf volume group averages. Negative correlations occurred between the highest ranking group average of flour protein content and highest ranking group of grain protein content with hectolitre mass, which can probably be attributed to the negative association between grain yield (density) and protein content.

The highest ranking group average for loaf volume (1012.8 cm^3) correlated positively with wet gluten, flour protein, grain protein, dough strength and alveograph strength whereas low loaf volume (959.8 cm^3) correlated with flour protein, grain protein, alveograph strength, dough strength, wet gluten, flour extraction and dough stability but negatively with falling number.

Rainfed wheat of the winter rainfall region

E (test sites) had the largest impact on flour protein (43.81%) followed by the G x E interaction (16.89%) and G (15.03%). E also had the largest effect on variation of grain protein (49.70%) followed by G (23.23%) and G x E interaction (8.46%). Variations in loaf volume were approximately equally determined by E (29.68%), G x E interaction (24.14%) and G (20.71%).

The highest ranking group average for flour protein content (mean of 13.04%) correlated positively with wet gluten, grain protein content, dough strength, alveograph strength and loaf volume, whereas the lowest ranking group average (11.84%) correlated with wet gluten, alveograph strength and grain protein content but negatively with flour extraction. The highest ranking group average of grain protein content (14.03%) correlated strongly with wet gluten, flour protein content, alveograph strength, loaf volume and dough strength. The lowest ranking group average of grain protein (1.99%) correlated positively with wet gluten, falling number and flour protein but negatively with flour extraction and dough P/L. The highest ranking group

average of loaf volume (980.8 cm^3) correlated strongly with dough strength, flour protein content, wet gluten, hectolitre mass, grain protein content, peak time minutes and alveograph strength. The lowest ranking group for loaf volume (mean of 908.7 cm^3) had no correlations with any of the quality parameters.

9.3.2.2 Correlations of flour protein, grain protein and loaf volume with gluten composition

Large glutenin proteins

In irrigated wheat the primary positive correlations occurred between high flour protein group (mean of 12.57%) with large insoluble glutenin in total insoluble protein, in total protein and in total glutenin. No correlations occurred between the low flour protein group and large glutenin proteins. No significant correlations occurred between large soluble glutenin and low flour protein (mean average of 10.45%), low grain protein (mean average of 11.42%) and low loaf volume (mean average of 839 cm^3) groups.

For rainfed wheat of the SRR strong and positive correlations occurred between large insoluble glutenin in total glutenin and high ranking groups of flour protein content (15.21%), grain protein content (15.54%) and loaf volume (1012.8 cm^3). In the low ranking groups, large insoluble glutenin in total protein and in total glutenin correlated strongly and positively with the low ranking groups of grain protein (14.04%) and loaf volume (959.8 cm^3).

Significant correlations between glutenin of rainfed wheat of the WRR and high flour protein (mean of 13.01%), high grain protein (mean of 14.03%) and high loaf volume (980.8 cm^3) group averages were common. Large insoluble glutenin in total insoluble protein and in total protein correlated positively and strongly with the high ranking groups of flour protein (mean average of 13.01%), grain protein (mean average of 14.03%) and loaf volume (mean average of 980.8 cm^3). Large soluble glutenin in total protein correlated positively with high ranking groups of grain protein and loaf volume groups. Large insoluble glutenin in total insoluble

protein and in total protein and in total glutenin correlated positively with low ranking groups of flour protein (mean average of 11.84%) and grain protein (mean average of 11.99%).

Small glutenin proteins

In irrigated wheat no significant correlations occurred between the small glutenin proteins and high ranking groups of flour protein (mean average of 12.57%), grain protein (mean average of 13.30%) and loaf volume (mean average of 933 cm^3). Insoluble small glutenin in total protein and in total glutenin correlated strongly and positively with only the low ranking group for loaf volume (839 cm^3).

In contradiction to irrigated wheat, small soluble glutenin in total soluble protein and in total protein and small insoluble glutenin in total insoluble protein of rainfed SRR correlated negatively with the high ranking group of flour protein (average of 15.21%). Small insoluble glutenin in total insoluble protein correlated strongly but negatively with the low ranking groups of flour protein (mean average of 13.64%) and loaf volume (average of 959.8 cm^3) groups.

In rainfed wheat from the WRR, small soluble glutenin in total soluble protein correlated strongly but negatively with the high ranking group of grain protein (average of 14.03%) whereas small insoluble glutenin in total insoluble protein correlated negatively with high ranking groups of flour protein (average of 13.01%) and grain protein. Small insoluble glutenin in total protein correlated significantly but negatively with the high ranking group for grain protein.

ω Gliadin (large monomeric proteins)

In irrigated wheat none of the ω gliadin proteins correlated strongly with either the high- or low ranking groups for flour protein, grain protein and loaf volume whereas several correlations occurred in wheat of the rainfed SRR. Soluble ω gliadin in total soluble protein and insoluble

ω gliadin in total protein correlated positively and strongly with the high ranking groups of flour protein and grain protein.

Soluble ω gliadin in total insoluble protein and soluble ω gliadin in total protein correlated positively and strongly with the high ranking group of flour protein from the rainfed SRR. Soluble ω gliadin in total protein, insoluble ω gliadin in total protein and insoluble ω gliadin in total protein correlated positively and strongly with the low ranking group of grain protein.

In wheat from the rainfed WRR, soluble ω gliadin in total soluble protein and in total protein correlated positively and strongly with the high ranking group for grain protein. In contrast, insoluble ω gliadin in total insoluble protein correlated strongly but negatively with high ranking group for loaf volume and insoluble ω gliadin in total ω gliadin correlated strongly but negatively with the high ranking group for grain protein. Soluble ω gliadin in total soluble protein, insoluble ω gliadin in total insoluble protein and soluble ω gliadin in total protein correlated negatively with the low ranking group for loaf volume. Insoluble ω gliadin in total ω gliadin correlated strongly but positively with the low ranking group for loaf volume.

α/β, γ Gliadin (large monomeric proteins)

Several of the α/β, γ gliadin fractions (large monomeric proteins) correlated with high ranking groups of flour- and grain protein and loaf volume from the three production regions. For the irrigation region, large soluble α/β, γ gliadin in total soluble protein and in total protein correlated strongly and positively with the high ranking groups for flour protein, grain protein and loaf volume whereas large insoluble α/β, γ gliadin in total protein correlated positively and strongly with the high ranking group of loaf volume. Large soluble α/β, γ gliadin in total soluble protein correlated with the low ranking groups of flour protein and grain protein, whereas large soluble α/β, γ gliadin in total protein correlated positively and strongly with the low ranking groups of flour protein and loaf volume. Large insoluble α/β, γ gliadin in total insoluble protein correlated strongly and positively with low ranking groups of flour protein and loaf volume,

whereas large insoluble α/β, γ gliadin in total protein correlated strongly and positively with the low ranking groups for flour protein, grain protein and loaf volume.

In wheat of the rainfed SRR, insoluble α/β, γ gliadin in total insoluble protein correlated strongly and positively with the high ranking group of flour protein whereas insoluble α/β, γ gliadin in total protein and in total α/β, γ gliadin correlated with both the high ranking groups of flour protein and grain protein. Only insoluble α/β, γ gliadin in total α/β, γ gliadin correlated strongly and positively with the low ranking group of grain protein.

For rainfed WRR, soluble α/β, γ gliadin in total soluble protein correlated positively and strongly with the high ranking groups of grain protein and loaf volume and soluble α/β, γ gliadin in total protein correlated positively and strongly with high ranking group for grain protein. None of the soluble or insoluble α/β, γ gliadin proteins correlated strongly with the low ranking groups of flour protein, grain protein and loaf volume.

Albumin/globulin (small monomeric proteins)

In irrigated wheat, the soluble albumin/globulin proteins in total soluble protein and in total protein correlated strongly but negatively with high ranking groups of flour protein, grain protein and loaf volume. Insoluble albumin/globulin in total insoluble protein correlated strongly but negatively with the high ranking groups of flour protein and grain protein, whereas insoluble albumin/globulin in total protein correlated strongly but negatively with the high ranking group of grain protein. Soluble albumin/globulin in total soluble protein and in total protein correlated significantly but negatively with low ranking groups of flour protein, grain protein and loaf volume. Insoluble albumin/globulin in total insoluble protein correlated strongly but negatively with high ranking groups of grain protein and loaf volume.

In rainfed SRR wheat, no correlations occurred between insoluble and soluble albumin/globulin and high ranking groups of flour protein, grain protein and loaf volume.

Soluble albumin/globulin in total soluble protein correlated strongly but negatively with low ranking groups of flour protein, grain protein and loaf volume. Insoluble albumin/globulin in total insoluble protein and in total protein and soluble albumin/globulin in total protein correlated strongly but negatively with low ranking groups of grain protein and loaf volume. Insoluble albumin/globulin in total albumin/globulin correlated strongly but negatively with the low ranking group of grain protein.

In rainfed wheat of the WRR, soluble albumin/globulin in total soluble protein and in total protein correlated strongly but negatively with high ranking groups of flour protein and grain protein. Insoluble albumin/globulin in total insoluble protein and in total protein correlated negatively with the high ranking group of grain protein. Soluble albumin/globulin in total soluble protein and in total protein correlated negatively with low ranking groups of flour protein and grain protein.

9.3.3 Establish correlations between (i) flour protein, grain protein, loaf volume and (ii) gluten with grain yield

9.3.3.1 Correlations of flour protein, grain protein, loaf volume with grain yield

Irrigated wheat

In irrigation wheat the low ranking group (average yield of 9.37 ton ha^{-1}) for grain yield correlated strongly but negatively with flour protein and grain protein.

Rainfed wheat of the summer rainfall region

A single strong correlation ($r > 0.45$***) occurred and the high yield ranking group (mean of 3.79 ton ha^{-1}) correlated positively with only loaf volume.

Rainfed wheat of the winter rainfall region

In rainfed wheat from the WRR, neither the high ranking group of yield (mean average of 5.15 ton ha^{-1}) nor low ranking group of yield (mean average of 4.38 ton ha^{-1}) correlated with flour protein and grain protein.

9.3.3.2 Correlations of proteins with grain yield

E contributed most to the variation of soluble glutenin in total protein for irrigation (31.36%) whereas G x E interaction resulted in the highest variation for rainfed SRR (12.26%) and rainfed WRR (7.20%). The factors contributing most to variation of soluble α/β, γ gliadin in total protein were E (36.78%) for irrigated production and G for both rainfed SRR (13.72%) and rainfed WRR (22.77%). E contributed most to the variation in soluble albumin/globulin in total protein for irrigation (59.63%), rainfed SRR (27.32%) and rainfed WRR (37.97%). For the insoluble fractions, E largely determined the variations of insoluble glutenin in total protein for irrigation (54.80%), rainfed SRR (32.08%) and rainfed WRR (72.24%) as illustrated in Table 8.5. The amounts of insoluble α/β, γ gliadin in total protein were also determined primarily by E of the irrigation (8.26%) and rainfed SRR (23.77%) regions whereas G determined variation in the rainfed WRR (7.71%). The amounts of insoluble albumin/globulin in total protein were primarily determined by the E of the irrigation region (13.03%) and rainfed WRR (15.36%) whereas G x E interaction determined most of the variation in the rainfed SRR region (15.19%).

Large glutenin proteins

No correlations occurred for the large soluble glutenin in total soluble protein, large insoluble glutenin in total insoluble protein or soluble glutenin in total protein in any of the three production regions. The large fraction of insoluble glutenin in total protein, in total glutenin and in total glutenin correlated negatively with the lowest yielding rank group average of irrigation.

Small glutenin proteins

Insoluble small polymeric glutenin in total insoluble protein and soluble small polymeric glutenin in total protein correlated positively with the low yield ranking group of irrigation and rainfed WRR. The insoluble small polymeric glutenin in total small polymeric protein, however, correlated negatively with the low yield group of irrigation.

ω Gliadin (large monomeric proteins)

Insoluble small polymeric glutenin in total insoluble protein and soluble small polymeric glutenin in total protein correlated positively with the low yield rank group of rainfed WRR and irrigation respectively. The insoluble small polymeric glutenin in total small polymeric protein, however, correlated negatively with the low yield ranking group of irrigation.

α/β, γ Gliadin (large monomeric proteins)

The large monomeric and soluble α/β, γ gliadin in total soluble protein correlated positively with the low yield ranking group of rainfed WRR. Insoluble α/β, γ gliadin in total protein also correlated positively with the low yield ranking group but negatively with the high yield ranking group for rainfed WRR and low yield ranking group of irrigation. Both ω and α/β, γ gliadin seem to be sensitive to G x E interaction as cross-over interaction within a production region only occurred for this protein fraction.

Albumin/globulin (small monomeric proteins)

Concentrations of soluble albumin/globulin in total soluble protein correlated positively with the low yield ranking group of irrigation, positively with the high yield ranking group of rainfed SRR but negatively with the high yield ranking group of rainfed WRR. Insoluble albumin/globulin in total insoluble protein also correlated negatively with the low yield ranking group of rainfed WRR. Soluble albumin/globulin in total protein correlated positively with the low yield ranking group of irrigation but negatively with the high yield ranking group of rainfed

WRR. The concentration of insoluble albumin/globulin in total albumin/globulin correlated negatively with the low yield ranking group of irrigation.

9.3.4 Can genotypes with low protein content achieve good baking quality?

Comparing wheat grades with loaf volumes

An important fact is firstly that grain protein content often does not correlate with good bread making quality, which is also reported in international literature (Morgounov et al. 2013; Gómez et al. 2009; Souza et al. 1993). Secondly, baking results in this study are based purely on analyses of wheat flours without addition of any industrial additives for promoting bread making quality.

The first point was clearly illustrated by genotypes of the irrigation region in the medium and low ranking group averages for grain protein. Although these genotypes obtained Grade 2 (< 12% grain protein content) and Grade 3 (< 11% grain protein content) they still produced loaf volumes well within the allowed 10% variation from the loaf volume of the quality standard.

9.3.5 Does the composition and concentration of gluten differ for each production region and what are the effects of genotype and environment on protein quality?

9.3.5.1 Gluten composition and concentration

Variations in the ratio of grain protein to flour protein content

Hypothetically, the grain protein content to flour protein content ratio indicates the levels of protein content in the endosperm (flour protein content) in relation to total protein content from the endosperm and outer layers of the grain (grain protein content). This ratio is important for interpreting trends in ratios of different protein fractions. Variation between flour protein content and grain protein content was substantial for the three wheat production regions

(Figure 7.1). Grain protein content is higher than flour protein content as the germ (embryo) and bran (pericarp, testa, nucellar layer, and aleurone layer) are removed during milling for production of white flour (Shewry and Halford 2002). White flour relates to the starchy endosperm cells in wheat grain and contains high amounts of starch and gluten (Tosi et al. 2011). The concentration of protein is low near the endosperm cavity and progressively increases outwards (Farrand and Hinton 1974; Shewry and Halford 2002) potentially allowing extraction of high protein flour from the outer layers and low protein flour from the central part of the grain endosperm (Tosi et al. 2011).

Differences between the flour protein/ grain protein content ratio of irrigated wheat were large and ranged between 5.5% and 8.5%. Of particular interest are the smaller differences between flour protein to grain protein content of the high and medium ranking groups for grain protein compared to the larger differences of the low ranking groups of grain protein in irrigated and rainfed wheat from the SRR. In rainfed wheat of the SRR differences in the flour protein/ grain protein ratio were small (between 1.8% and 2.9%) and noteworthy are the smaller differences between flour protein/ grain protein content of the high and medium ranking groups for grain protein compared to the low ranking group of grain protein. Differences in the flour protein/ grain protein ratio of rainfed wheat in the WRR were large (between 1.2% and 7.3%) and decreased sharply between the high- to low ranking groups meaning an inverse trend occurred compared to irrigation and rainfed SRR. The largest differences occurred in the high ranking group of grain protein (7.3%) reducing sharply to 1.2% of the low ranking group for grain protein.

Grain protein yield

Due to higher yields under irrigation, the grain protein yield (GPY) per ha for this production region (ranging from 1, 168 to 1, 223 kg ha^{-1}) was approximately double than for the rainfed WRR (551.3 to 675.8 kg ha^{-1}) and rainfed SRR (439.5 to 534.8 kg ha^{-1}) production regions (Table 8.3). The differences between GPY of the high-, medium- and low ranking groups for

yield were significant for all three production regions (Table 8.3) and possibly reflected the fact that the groups were selected on the basis of significant differences in grain yield. Even considering the effect of current input cost ha^{-1} (data not shown) for irrigated wheat (GrainSA 2015) the production of protein under irrigation remains the most feasible and cost effective production system for wheat calories in SA. Important though is that GPY for irrigation comes from a total of approximately 280 kg N ha^{-1} per season, rainfed WRR from approximately 130 kg N ha^{-1} per season and rainfed SRR from only approximately 60 kg N ha^{-1} per season.

Variations in the concentrations of soluble and insoluble glutenin, α/β, γ gliadin and albumin/globulin in total protein of genotypes with varying grain protein content and loaf volume

Concentrations of soluble glutenin followed an inverse trend to insoluble glutenin in the high, medium and low ranking groups for grain protein of irrigation and rainfed SRR wheat. Rank groups with medium grain protein content for irrigated and rainfed SRR production had significantly lower concentrations of insoluble glutenin in total protein than rank groups with high protein content. This trend also occurred in low ranking groups for high, medium and low loaf volume in rainfed production in the SRR. The insoluble to soluble ratio for glutenin were approximately one for grain protein content and ranged from 0.88 to 0.72 for irrigated production, 0.99 to 0.87 for rainfed SRR and 0.80 to 0.82 for rainfed WRR. The insoluble to soluble ratio of glutenin for loaf volume ranged from 0.79 to 0.75 for irrigated production, 0.99 to 0.83 for rainfed SRR and 0.82 and 0.83 for rainfed WRR.

The concentrations of insoluble and soluble α/β, γ gliadin mirrored results of insoluble glutenin. Medium and/or low grain protein content and loaf volume had equal or lower concentrations of insoluble and soluble α/β, γ gliadin compared to high grain protein content and loaf volume. The ratio of insoluble to soluble α/β, γ gliadin were much larger compared to glutenin, indicating larger variation. Values of high, medium and low grain protein ranged from 0.15-0.14 for irrigated, 0.15-0.14 for rainfed SRR and 0.14 for rainfed WRR. Ratios of insoluble

to soluble α/β, γ gliadin in high-, medium- and low ranking groups of loaf volume ranged from 0.15-0.13 for irrigated, 0.15-0.13 for rainfed SRR and 0.13-0.14 for rainfed WRR indicating slightly larger differences.

The concentrations of insoluble and soluble albumin/globulin tend to incline towards a trend in comparison to percentage grain protein content and loaf volume. Soluble concentrations of albumin/globulin in the low ranking group of grain protein content were significantly higher in the irrigated and rainfed WRR regions and insoluble concentrations in the rainfed WRR region. In the low ranking group of loaf volume, soluble concentrations of albumin/globulin were significantly higher for rainfed SRR and rainfed WRR and insoluble concentrations if albumin/globulin in rainfed WRR. The ratios of insoluble to soluble albumin/globulin were large and as with α/β, γ gliadin, indicated large variation between concentrations of soluble and insoluble fractions. For high-, medium- and low ranking groups of grain protein content the ratios ranged from 0.14-0.17 for irrigation, 0.14-0.16 for rainfed SRR and 0.13-0.14 for rainfed WRR. Genotypes ranked for loaf volume ranged from 0.15-0.16 for irrigation, 0.15-0.16 for rainfed SRR and 0.14-0.13 for rainfed WRR.

9.4 Summary of results and trends

As grain protein of irrigated wheat decreased, the ratio between grain protein content and flour protein content increased, indicating that in genotypes with low grain protein content flour protein content of the endosperm decreased even more dramatically (Table 9.1). The same trend, although not as substantial as in irrigation, occurred in rainfed SRR where the ratio between grain protein content and flour protein content increased in the lower ranked average for grain protein (mean average of 14.04%). This trend may indicate a varietal response of genotypes to the production environment as both environments are subjected to fairly high rainfall (Figure 9.1a and 9.1b) between flowering to physiological maturity. In irrigated wheat, rainfall varies between low (47 mm to 63 mm at Upington) to medium (102 mm to 120 mm at

Vaalharts) during this late period of the growth season but are supplemented by intensive irrigation (an average of 600 to 900 mm throughout the whole season) coinciding with the last application of nitrogen to the wheat crop. In rainfed SRR, rainfall was much higher and between 190 mm to 352 mm at Bethlehem in 2012 and 2013 respectively and between 290 mm to 302 mm at Clarens in 2012 and 2013 respectively. These amounts of water available during grain filling can result in leaching of nutrients and particularly nitrogen from primary root zones when it is critical for determining the composition and concentrations of protein fractions accumulating in the grain. In contrast, however, the highest ranking average group for grain protein of rainfed wheat from the WRR had the largest grain protein to flour protein ratio, whereas the smallest ratio occurred in low grain protein content. In rainfed WRR (Rûens) primary rainfall occurs early in the season during seeding with adequate nitrogen supplemented by a split application usually during anthesis. Moisture stress in rainfed WRR generally occurs from flag leaf stage to physiological ripening and has a major effect on the composition and concentrations of protein fractions in grain seed.

Another very important factor is the genetic ability of genotypes to accumulate the required assimilates necessary for high grain protein and concentrations of protein fractions. A possible trend from the grain protein content and grain protein content: flour protein content ratio results is that most variation in irrigation and rainfed SRR occurs from 11% to 14% (Table 9.1).

Table 9.1 Important parameters and their trends in wheat from the three production regions in South Africa

Parameter	Irrigation		
	Higher ranking group	**Medium ranking group**	**Low ranking group**
Ratio of flour protein/ grain protein	5.5	5.6	8.5
Grain protein yield (kg ha^{-1})	1223	1172	1168
Grain protein content (%)	13.3	12.01	11.42
Grain yield (ton ha^{-1})	10.63	10.17	9.37
Loaf volume (cm^3)	933	893	839
Correlation of wheat grades with loaf volumes	Good	Medium	Poor
	Rainfed SRR		
	Higher ranking group	**Medium ranking group**	**Low ranking group**
Ratio of flour protein/ grain protein	2.1	1.8	2.9
Grain protein yield (kg ha^{-1})	535	510	440
Grain protein content (%)	15.54	14.77	14.04
Grain yield (ton ha^{-1})	3.79	3.54	3.14
Loaf volume (cm^3)	1012.8	989.0	959.8
Correlation of wheat grades with loaf volumes	Good	Good	Good
	Rainfed WRR (Rûens)		
	Higher ranking group	**Medium ranking group**	**Low ranking group**
Ratio of flour protein/ grain protein	7.3	3.4	1.2
Grain protein yield (kg ha^{-1})	651	676	551
Grain protein content (%)	14.03	12.69	11.99
Grain yield (ton ha^{-1})	5.15	4.91	4.38
Loaf volume (cm^3)	980.8	939.7	908.7
Correlation of wheat grades with loaf volumes	Good	Good	Medium

Correlations between two traits are not an indication that one trait results in changes in the other trait but merely imply that the two traits are associated. It may, however, mean that both traits respond to a range of factors and conditions mutually affecting both traits. With a decline in the grain protein of irrigated and rainfed SRR wheat, the grain protein to flour protein ratio increased accordingly, indicating that in genotypes with low grain protein content flour protein content of the endosperm was even lower. This could indicate that accumulation of proteins commences from the outer part of the grain seed towards the internal endosperm and if the source is limited, as in the case of the low ranking average group for grain protein, flour protein (originating solely from the endosperm) will be substantially less than the grain protein. In the case of irrigation wheat, a large portion of assimilates are also required for materialising the

high yield potential. This may partially be the reason why the low ranking average groups of flour protein, grain protein and loaf volume had no strong correlations with the large glutenin proteins and only a few with the small glutenin proteins (Figure 9.2b). Based on the grain protein content, it is interesting to note that the profiles of the low rank group of rainfed SRR (14.04%) and high rank group of rainfed WRR (14.03%) match well. Both profiles (Tables 9.2a and 9.2b) have positive correlations of insoluble large glutenin, negative correlations of insoluble small glutenin, positive correlations of small glutenin and negative correlations of soluble and insoluble albumin/globulin.

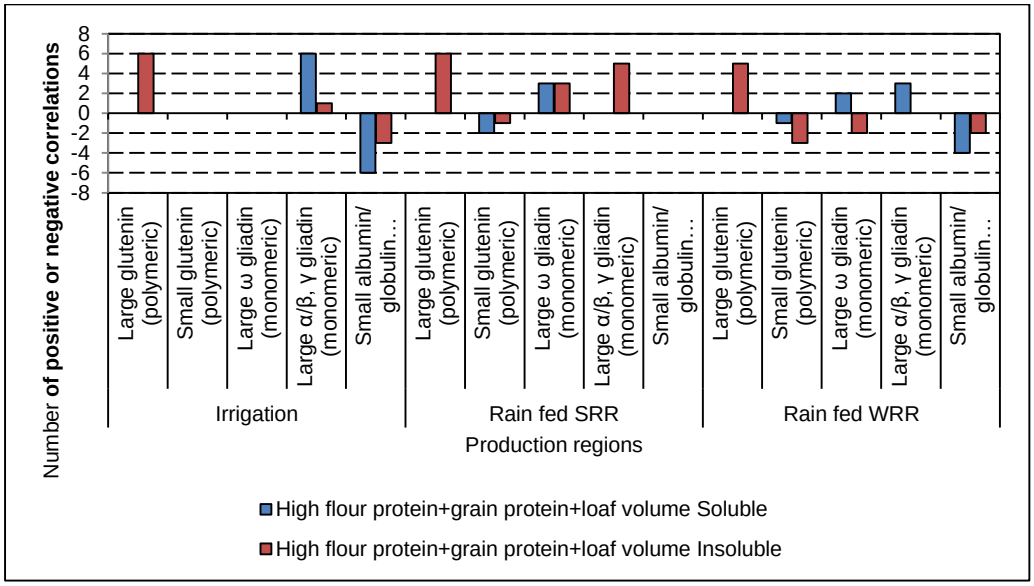

Figure 9.2a The number of correlations occurring in high ranking groups of flour protein, grain protein and loaf volume groups of wheat from the three production regions in South Africa

Comparison of the profiles with the highest grain protein content (rainfed SRR 15.54%) and lowest grain protein content (irrigated 11.42%) reveal interesting trends in regard to the number of correlations (Figures 9.2a and 9.2b). The former had positive correlations of insoluble large glutenin, insoluble and soluble ω gliadin, no positive correlation of soluble α/β, γ gliadin and no negative correlations of albumin/globulin compared to the low rank groups of flour protein, grain protein and loaf volume of irrigated wheat.

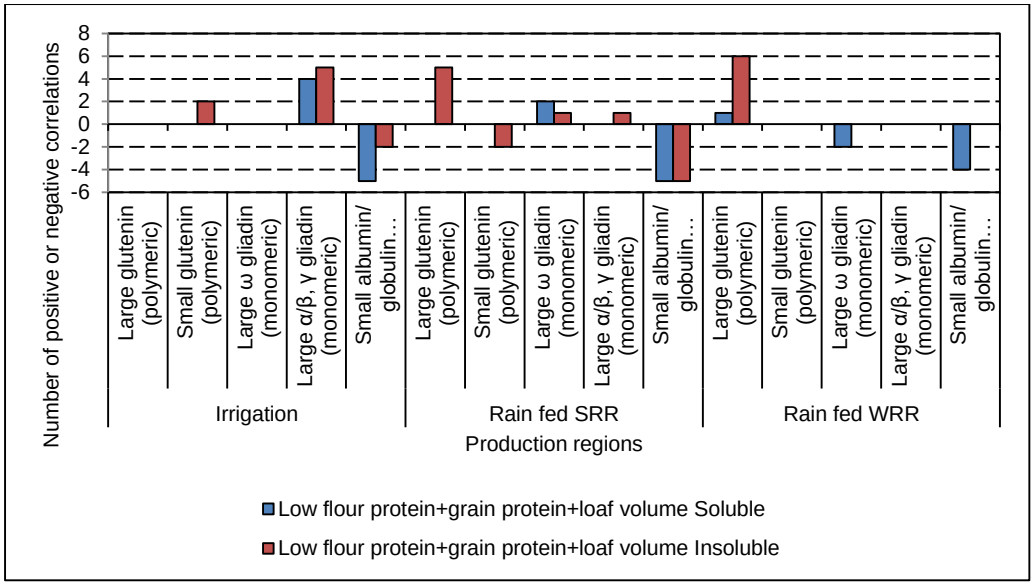

Figure 9.2b The number of correlations occurring in low ranking groups of flour protein, grain protein and loaf volume groups of wheat from the three production regions in South Africa

9.5 General conclusions and remarks

Several of the trends observed in this study can be manipulated and exploited through well directed breeding, although the large effect of E on composition and concentration of gluten needs to be taken into account. Glutenin is determined by HMW-GS and LMW-GS encoded by loci *Glu-1* and *Glu-2.* The quality of gluten is also linked to nitrogen levels of the soil, soil moisture and stress in plants with leaching, inadequate fertiliser rates and poor nitrogen use efficiency (NUE) in genotypes. Chope et al. (2014) reported that grain protein had a small effect on the proportions of HMW-GS or large glutenin polymers and that the proportion of total protein present in polymers in grain decreased with increasing nitrogen levels. Adequate water from flag leaf stage to grain filling coupled with high nitrogen levels available to wheat plants throughout the growth season will contribute substantially towards higher yields and better bread making quality.

Biological phenotyping is a modern and all-inclusive approach for assessing the intricacies in living organisms, in both plants and animals. Recently, metabolomics was added to the

existing “omics” (proteomics and genomics) and are the study of the complete set of metabolites/low molecular weight intermediates varying according to the physiology and developmental state of the cell (Oliver et al. 1998). Metabolomics focus on differences in test samples resulting from varying responses to biochemical and/or environmental stresses and food processing. Baker et al. (2006) applied metabolomics to determine if the insertion of transgenes have unpredicted effects on the expression of other endogenous genes, by evaluating non-GM and GM sister lines. They reported that the environment affects the metabolome and differences between the non-GM and GM lines are generally within the same range of each test site and year with no significant effects by the transgene on gene expression.

Another novel approach is by deleting transcription sites for HMW-GS and Jondiko et al. (2012) reported that allelic variations in *Glu-1* loci of wheat can be manipulated to produce flour protein profiles and dough making attributes for specific end product attributes. Tortillas from wheat lines encoding subunits 2 + 12 at *Glu-D1* had large diameter and acceptable flexibility that are good for development of wheats optimised for tortilla production. Similarly, to this research, Zhen et al. (2014) reported that deletion of *Glu-A3a* had no apparent effects on morphological and yield traits but significantly reduced gluten strength and bread making quality, meaning that *Glu-A3* genes code for LMW-GS and not HMW-GS.

Results from this study may only form a small component in a more detailed study in future research on bread making quality of SA wheat. It is clear that numerous variables affecting the quality of wheat products will have to be taken into account in future research in order to develop wheat meeting the demands of consumers in SA.

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Executive summary

In field crops the measurement of any yield or quality parameter by a single factor that is inherently sensitive to the environment, may skew the result and have no application. Two classical examples exist in the wheat industry where firstly, grain yield alone does not fully reflect and determine the on-farm profitability of a wheat variety. The parameters that are part of the grading scale (hectolitre mass, grain protein content and falling number) together with grain yield and price per ton form the components responsible for the ultimate farm gate price. Secondly, grain protein content is mostly inadequate for explaining flour quality of wheat which is critical for determining sustainability of the milling and baking industry. Protein quality, referring to the concentrations and ratios of glutenin, gliadin and albumin protein fractions are proving to be more important than protein quantity. Wheat varieties from the irrigation region in the medium and low ranking group were a clear example thereof as these genotypes obtained Grade 2 (< 12% grain protein content) and Grade 3 (< 11% grain protein content) but produced loaf volumes well within the allowed 10% variation from the loaf volume of the quality standard.

In irrigated wheat and rainfed wheat of the summer rainfall region and winter rainfall region, E had the largest impact on flour protein and grain protein content. E contributed most to the variation of soluble glutenin in total protein for irrigation whereas G x E interaction resulted in the highest variation for rainfed SRR and WRR. Soluble α/β, γ gliadin in total protein were primarily affected by E for irrigation and G for both rainfed in the SRR and in the WRR. E contributed most to the variation in soluble albumin/globulin in total protein for irrigation, rainfed SRR and rainfed WRR wheat. For the insoluble fractions, E largely determined the variations of insoluble glutenin in total protein for irrigation, rainfed SRR and rainfed WRR. Insoluble α/β, γ gliadin in total protein were primarily determined by E in irrigation and rainfed SRR whereas G determined variation in the rainfed WRR. Insoluble albumin/globulin in total

protein were primarily determined by the E of irrigation and rainfed WRR whereas G x E determined variation in the rainfed SRR region.

Glutenin concentrations were highest in wheat from the rainfed WRR, α/β, γ gliadin in rainfed SRR wheat and albumin/globulin in irrigated wheat. No clear and repeating trends regarding the concentrations and ratios of protein fractions could be established with correlations in any of the three production regions, which underlines the enormous role of the environment on protein quality. For example, large insoluble glutenin in total protein of irrigated and rainfed SRR wheat only correlated strongly and positively with high flour protein, whereas in rainfed wheat of the WRR large insoluble glutenin in total protein correlated positively and strongly with high flour protein, high grain protein and high loaf volume. The examples become more extreme with insoluble small glutenin proteins of irrigated wheat correlating strongly and positively with low loaf volume, whereas in rainfed SRR small soluble and insoluble glutenin correlated negatively with low loaf volume. In rainfed wheat from the WRR, small insoluble glutenin in total protein correlated negatively with high grain protein, but positively with low loaf volume.

The climatic environment of the three production regions differs significantly in regard to annual rainfall and seasonal temperatures which wheat producers cannot control. Management of the environment by wheat farmers should focus on the production environment through selection of adapted wheat varieties, soil moisture conservation and optimal fertilising practises. These factors determine grain yield but also determine the protein composition that eventually affects wheat flour quality.

Algemene opsomming

Die akkurate meting van opbrengs of kwaliteit in gewasproduksie deur ‘n enkel parameter wat gevoelig is vir omgewingstoestande, kan ‘n verwronge beeld skep en eindelik die resultate ontoepaslik maak. Twee baie duidelike voorbeelde hiervan bestaan in die koringbedryf waar eerstens, die plaashek winsgewendheid van ‘n koringkultivar nie alleenlik deur graanopbrengs bepaal word nie. Graderingskriteria soos hektolitermassa, proteïeninhoud en valgetal bepaal saam met graanopbrengs en die heersende koringprys die finale prys. Tweedens, blyk proteïeninhoud onakkuraat te wees om die meelkwaliteit van koring, noodsaaklik vir die voortbestaan van die bak en maalbedryf, te omskryf. Proteïenkwaliteit, verwys na die konsentrasie en verhoudings van glutenien, gliadien en albumien/globulien, lyk belangriker te wees as die hoeveelheid proteïen. ‘n Sprekende voorbeeld hiervan is die koringkultivars uit die besproeiingsgebied in die medium en lae proteïeninhoud groepe wat ten spyte van ‘n Graad 2 (< 12% graanproteïen) en Graad 3 (< 11% graanproteïen) broodvolumes geproduseer het wat gemaklik binne die 10% variasie van die kwaliteitstandaard se broodvolume val.

Die omgewing (E) het die grootste invloed gehad op die variasie in graanproteïen en meelproteïen van besproeiingskoring en droëlandkoring uit die somerreënval en winterreënvalgebiede. Omgewing het ook die meeste bygedra tot die variasie in oplosbare glutenien in totale proteïen vir besproeiingskoring terwyl die omgewing en genotipe interaksie (G x E) die mees prominente faktor was wat variasie van droëlandkoring in die somerreënval en winterreënvalgebiede bepaal het. Variasie in die oplosbare α/β, γ gliadien in totale proteïen in besproeiingskoring is primêr deur E bepaal terwyl genotipe (G) die primêre faktor was wat variasie in droëlandkoring van die somerreënval en winterreënvalgebiede bepaal het. E was ook primêr verantwoordelik vir die variasie in oplosbare albumien/globulien in totale proteïen van koring afkomstig uit die besproeiingsgebiede en droëlandkoring van die somerreënval en winterreënvalgebiede. Vir die onoplosbare fraksies was E weereens verantwoordelik vir die

variasie van onoplosbare glutenien in totale proteïen van koring uit al drie die produksiegebiede. Variasie in die onoplosbare α/β, γ gliadien in totale proteïen van besproeiingskoring en droëlandkoring uit die somerreënvalgebied is hoofsaaklik deur E bepaal terwyl G die variasie in droëlandkoring van die winterreënvalgebied bepaal. Variasie in die onoplosbare albumien/globulien in totale proteïen van besproeiingskoring en droëlandkoring uit die winterreënvalgebied is primêr deur E bepaal terwyl G x E hoofsaaklik verantwoordelik was variasie in droëlandkoring van die somerreënvalgebied.

Die glutenien konsentrasies was die hoogste in droëlandkoring uit die winterreënvalgebied, α/β, γ gliadien die hoogste in droëlandkoring van die somerreënvalgebied en albumien/globulien die hoogste in besproeiingskoring. Geen duidelike of herhalende tendense in die konsentrasies en verhoudings van die proteïenfraksies in koring uit enige van die drie produksiegebiede is deur korrelasies uitgewys nie, wat die groot invloed van omgewing op proteïenkwaliteit beklemtoon. ‘n Voorbeeld hiervan is dat die groot onoplosbare glutenien in totale proteïen van besproeiingskoring en droëlandkoring uit die somerreënvalgebied slegs positief met hoë meelproteïen gekorreleer het terwyl groot onoplosbare glutenien in totale proteïen van droëlandkoring uit die winterreënvalgebied positief met hoë meelproteïen, hoë graanproteïen en hoë bakvolume gekorreleer het. ‘n Ander voorbeeld van uiterstes is die sterk en positiewe korrelasie van klein onoplosbare glutenien van besproeiingskoring met lae broodvolume terwyl droëlandkoring uit die somerreënvalgebied negatief met lae broodvolume korreleer. Klein onoplosbare glutenien in totale proteïen van droëlandkoring uit die winterreënvalgebied het weer negatief met hoë graanproteïen gekorreleer maar positief met lae broodvolume.

Die klimaatsomgewing van die drie produksiestreke verskil drasties van mekaar in terme van jaarlikse reënval en temperature waaraan ‘n koringprodusent niks kan doen nie. Bestuur van die omgewing berus dus grootliks op die produksie-omgewing wat ‘n koringboer kan manipuleer deur keuse van kultivars wat goed aangepas is, vogbewaring en optimale

bemestingtoediening. Hierdie faktore bepaal nie alleen net graanopbrengs nie maar ook die samestelling van proteïen wat noodsaaklik is vir goeie meelkwaliteit.