# Factors contributing to sodium dodecyl sulphate (SDS) sedimentation in irrigation wheat

**By**

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# Declaration

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# Abbreviations

| | |
|---|---|
| µl | Microliter |
| $\mu l.ml^{-1}$ | Microliter per milliliter |
| AACC | American Association of Cereal Chemists |
| ACN | Acetonitrile |
| ANOVA | Analysis of variance |
| APS | Ammonium persulfate solution |
| ARC-SGI | Agricultural Research Council – Small Grain Institute |
| α | Alpha |
| BC | Before Christ |
| Bis | Bis-acrylamide |
| Bp | Base pair |
| β | Beta |
| °C | Degrees Celsius |
| cm | Centimetre |
| $cm^3$ | Cubic centimetre |
| d.f. | Degrees of freedom |
| $dH_2O$ | Distilled water |
| DNA | Deoxyribonucleotide acid |
| DO | Douglas |
| DTT | Dithiothreitol |
| EL | Elands cultivar |
| EM | Elite line means |
| Env | Environmental means |
| FAO | Food and Agricultural Organization |
| FN | Falling number |
| FPC | Flour protein content |
| g | Gram |
| $g\ m^{-2}$ | Gram per square metre |
| *Gli* | Gliadin locus |
| *Glu* | Glutenin locus |
| GPC | Grain protein content |
| GxE | Genotype by environment interaction |
| $H^2$ | Narrow sense heritability |
| *HA* | Hardness locus |

| | |
|---|---|
| HCl | Hydrochloric acid |
| HI | Hardness index |
| HLM | Hectolitre mass |
| HMW-GS | High molecular weight-glutenin subunits |
| HPLC | High performance liquid chromatography |
| hr | Hour |
| kDa | Kilo Dalton |
| $kg.h^{-1}$ | Kilogram per hectare |
| $kg.hl^{-1}$ | Kilogram per hectoliter |
| LMW-GS | Low molecular weight-glutenin subunits |
| LSD | Least significant differences |
| M | Protein marker |
| $m^2$ | Metre square |
| mA | Milli-absorbance |
| mM | millimolar |
| MA | Marydale |
| m.b | Moisture base |
| mg | Milligram |
| min | Minutes |
| ml | Millilitre |
| $ml.g^{-1}$ | Millilitre per gram |
| mm | Millimetre |
| MON | Total monomeric proteins |
| mRNA | Messenger ribonucleotide acid |
| MT | Million tonnes |
| N | Null subunit |
| NBC | Narrow bore column |
| nm | Nanometre |
| ns | Not significant |
| P | Probability |
| PC | Principal component |
| PCA | Principal component analysis |
| pH | Potential of hydrogen |
| RCBD | Randomised complete block design |
| RNA | Ribonucleotide acid |
| rpm | Revolutions per minute |
| SA | Sappo cultivar |

| | |
|---|---|
| SAGL | South African grain laboratory |
| SC | Scheepers cultivar |
| SDS | Sodium dodecyl sulphate |
| SDSVOL | SDS-sedimentation volume |
| SDS-PAGE | SDS-Polyacrylamide gel electrophoresis |
| SE-HPLC | Size exclusion-high performance liquid chromatography |
| Sec | Seconds |
| SKCS | Single kernel characterisation system |
| ST | Steenbras cultivar |
| TEMED | Tetramethylethylenediamine |
| Temp | Temperature |
| TFA | Trifluoroacetic acid |
| $Ton.ha^{-1}$ | Ton per hectare |
| Tris-HCl | Trisaminomethane hydrochloride |
| Tu | Tugela DN cultivar |
| V | Volts |
| VA | Vaalharts |
| v/v | Volume per volume |
| WGC | Wet gluten content |
| w/v | Weight per volume |

# Summary

The main objective of this study was to determine factors contributing to sodium dodecyl sulphate-sedimentation volume (SDSVOL) in elite wheat breeding lines grown at three different irrigation locations. This was possible through the use of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), mixograph analysis and size exclusion-high performance liquid chromatography (SE-HPLC) techniques. Genotype and environment effects were highly significant for SDSVOL, measured quality characteristics as well as the protein fractions, whereas genotype by environment (GxE) interaction was either significant or not significant for most of the measured variables and highly significant for almost all the protein fractions except the absolute value. Relationships between SDSVOL and other quality characteristics varied in combined and single location analysis of variance. The combined analysis shows a positive relationship between SDSVOL and flour protein content (FPC), high molecular weight-glutenin subunits (HMW-GS) and dough characteristics. Protein fractions also revealed positive relationships with SDSVOL and the measured characteristics such as FPC, envelope peak integral (EPI), midline right integral (MRI) and envelope peak time (EPT).

Principal component analysis (PCA) reduced 21 quality characteristics to five principle components, which explained 99.83% of total variance of envelope tail integral (ETI), midline left integral (MLI), midline peak integral (MPI), MRI, midline tail integral (MTI), SDSVOL and EPI. The SDSVOL and MRI showed the strongest discriminatory power compared to the other measured quality characteristics. Five of the elite lines (13, 12, 7, 2 and 1) can be recommended to breeders for further evaluation based on their good quality characteristics and strong relationship with some measured quality characteristics.



**Key words:** SDS-PAGE, mixograph parameters, bread making quality, SDSVOL, SE-HPLC, PCA

# Chapter 1

## General introduction

Wheat (*Triticum aestivum* sp.) is the second most important cereal crop in the world following maize and rice (Shewry, 2009). More than one-third of the world's population uses wheat as a staple food due to its diverse uses and nutritional value (Dachkevitch and Autran, 1989). It is ranked as one of the major sources of energy, protein and dietary fibre in human nutrition and animal feed. In South Africa, farmers produce about 1.4 million tonnes (MT) of wheat per year (SAGL, 2015). Of the wheat produced, most is used for human consumption and for livestock feed and the rest are used for some industrial productions (DAFF, 2010).

The success of wheat depends partly on its adaptability and high yield potential but also on the gluten protein, which confers the viscoelastic properties that allow dough to be processed into bread, pasta, noodles and other food products (Shewry, 2009). Several variables may determine wheat quality, including: physical grain properties, protein percentage and composition, as well as starch content and composition. Among the quality characteristics, seed storage protein content was found to be the most important baking quality feature for wheat (Dowell et al., 2008).

Gluten is produced by the interaction between wheat flour proteins in the presence of water. Bread making quality is influenced mainly by gluten proteins, which consist of two major fractions: gliadins and glutenins. These fractions have been found to have a greater effect on wheat quality than any other fractions found in wheat grain. Gliadins are monomeric proteins that form intra-molecular disulphide bonds (Esmaail et al., 2012) and glutenins are polymeric proteins made up of polypeptide chains that are cross-linked by disulphide bonds into higher level polymers (Ciaffi et al., 1996). When treated with a reducing agent such as 2-mercaptoethanol, glutenin dissociate into subunits of differing molecular weight: the HMW-GS and the low molecular weight-glutenin subunits (LMW-GS) (Tarekegne and Labuschagne, 2005). The HMW-GSs are encoded by *Glu-A1, Glu-B1* and *Glu-D1* loci which are located on the long arm of chromosomes 1A, 1B and 1D, respectively (Wang et al., 2006), whereas the main LMW-GSs are encoded by *Glu-A3, Glu-B3* and *Glu-D3* loci which are located on the short arm of chromosomes 3A, 3B and 3D (Gupta and Shepherd, 1990).

### 1.1 The origin of wheat

Wheat was and still is the staple food in the ancient and modern world for billions of people. It was first grown as a food crop about 10000-8000 BC (Hillman and Davies, 1990). Wheat originated from the Fertile Crescent encompassing present day Turkey and Iran, but today it is being produced in many different countries such as China, Lebanon, Syria, Northern Israel, Iraq, Eastern Turkey and South Africa (Matsuoka, 2011). Wheat breeding began in the early 1800s. Modern wheat cultivars belong primarily to two species: (1) hexaploid, bread wheat, *T. aestivum* spp. *aestivum* L. (2n = 42) and (2) tetraploid, hard or durum-type wheat, *T. turgidum* spp. *durum* Desf. em. Husn. (2n = 28) used for pasta and low rising bread (Nevo et al., 2002). Hexaploid wheat is made up of three genomes, A, B, and D, which were combined during hybridization events involving diploid and tetraploid descendants of a hypothesized diploid ancestors of all Triticeae, which includes wheat, rye and barley (Dvorak and Zhang, 1990; Nelson et al., 1995).

The origin of the bread wheat was first described and concluded as a hexaploid, resulting from hybridization between emmer type and wild grass species of grass (Orth and Shellenberger, 1988; Stagnari et al., 2008). Approximately 95% of wheat harvested worldwide today is hexaploid wheat with most of the remaining 5% being tetraploid durum wheat (Shewry, 2009).

### 1.2 Classification of wheat

Classification of wheat cultivars depend on a few factors such as growing season, gluten content and grain colour (Parker and Langridge, 2000; Drezner et al., 2007; Giacintucci et al., 2014). Bread wheat consists of winter and spring wheat. Winter wheat is planted in autumn and harvested in November. Winter wheat seeds need a cold treatment to initiate the next growth phase, thus the exposure to near freezing temperatures in the seedling stage is necessary before the flowering process can begin (Poehlman and Sleper, 1995). This wheat requires a longer period to reach full maturity. Winter wheat has good baking characteristics but is variable and has high water absorption. Spring wheat is planted in spring but some cultivars can be planted in winter and normally have a short growing season. Spring wheat contains good bread making characteristics and it has low water absorption compared to winter wheat (Bruckner et al., 2001). Hard wheat is known to have a higher level of protein content, known as a good quality trait and soft wheat has low protein content (Maghirang et al., 2006).

### 1.3 South African planting area and climate

Out of a total of 122 million hectares of land in South Africa, 100 million hectares is used for agricultural purpose, mostly for different crop plantings (Greyling et al., 2015). About 13% of this area receives sufficient rainfall for dry land crop production, of which only one-fifth is regarded as high potential land. This is due to the fact that South Africa is a dry country, which is subjected to periodic droughts and has been getting drier due to high temperatures and adverse rainfall patterns, with only 10% of the country receiving more than 750 mm of rain a year (Bernstein, 2013). Rainfall distribution also varies significantly and the available water resources is very irregular. Because of the above mentioned climate conditions, irrigated agriculture plays an important role in South Africa with over 60% of water used for farming in 1.3 million hectares (Aquastat, 2005, BusinessTech, 2015).

In the Western Cape region, wheat is planted from April until June, and then harvested October to December. The Free State region's planting start in mid-April until mid-August, depending on the cultivar and the specific production area. Harvesting takes place from November to January. In the Northern Cape region wheat is planted in July until August and harvesting occurs during November to January (SAGL, 2015).

### 1.4 Wheat production in South Africa

South Africa is ranked 37$^{th}$ on the list of the top wheat producing countries in the world, with China ranking first (FAO Stats, 2015). There are three major regions that produce wheat in South Africa namely: The Western Cape area where spring types are produced under rain fed conditions, Free State area is where winter and intermediate wheat are produced under rain fed conditions and the Northern area where spring types are cultivated under fully irrigated conditions (SAGL, 2015). Together these provinces are responsible for over 84% of the total wheat output. Because of the shortfall, most of the wheat that is used for local consumption is imported from other countries. Moreover, during the 2014/2015 market season, a total amount of 1 498 502 ton of wheat was imported, which was less than the previous season 2013/2014 by 169 000 ton (FAO Stats, 2015).

South Africa has three major rivers (the Vaal, Orange and Limpopo) and irrigation schemes were developed near the riverbanks of these rivers. Other irrigation schemes further away from these rivers are also supplied with water from these rivers (Dennis and Nell, 2006). Out of 36 crop production regions in South Africa, wheat is planted in about 28 of these. The total area planted in the 2009/2010 season was lower compared

to the total area production of the 2007/2008 season; hence the total production of wheat is over 1.4 MT (Agricultural Statistics, 2016). For the 2015/2016 season the commercial wheat crop was 17.7% lower than the previous season's crop. The total area used for wheat production was 482 150 ha with an average yield of 2.99 $t.ha^{-1}$ (SAGL, 2016).

The major aims of wheat breeding programmes are to improve quality and increase yield. To ensure the quality of cultivars, quality tests need to be performed on prospective cultivars in wheat breeding programmes. Flour yield, protein concentration and composition, kernel hardness, SDSVOL, dough mixing properties and loaf volume are some of the quality traits that are tested for bread wheat flour. However, what determines the end-use product quality depends on how the grain will be used. For instance, to obtain good bread, wheat flour with a high protein concentration, alkaline water retention capacity and dough strength is desired which is very different from the requirements for cakes and cookies (Nelson et al., 2006).

Understanding the relationship between proteins and mixing parameters can be beneficial to breeders when solving problems and encountering possible limitations. Mixograph analysis is one of the techniques used to predict the functional dough mixing properties of wheat genotypes in breeding programmes (Dong et al., 1992). During the mixing of flour, 44 parameters are measured from a single sample mixogram, using Mixsmart software. Most of these parameters have been strongly associated with bread making quality, measured as loaf volume and some protein fractions (Dachkevitch and Autran, 1989).

SE-HPLC is a powerful tool used to study protein aggregates and physicochemical properties of baking quality (Dachkevitch and Autran, 1989). Due to its speed, automation, quantitative capabilities and small sample size requirement, it is an ideal system for rapid screening of many wheat samples (Bietz, 1986). The results obtained with this technique have been highly correlated with bread making quality (Dachkevitch and Autran, 1989; Singh et al., 1990; Batey et al., 1991; Gupta et al., 1992, 1993).

### 1.5 Aim

The aim of the study was to evaluate selected characteristics, which play a role in the relationship between bread wheat quality, protein fractions and SDS-sedimentation volume (SDSVOL).

### 1.6 Objectives

Specific objectives of the study were to:

1. Determine the HMW-GS in each breeding line by SDS-polyacrylamide gel electrophoresis (PAGE)
2. Determine the relationship between protein fractions separated by SE-HPLC and baking quality in the breeding lines
3. Determine the correlation between the different breeding lines and quality characteristics using principle component analysis (PCA)

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# Chapter 2

## Quality testing parameters of bread wheat grain and flour with focus on sodium dodecyl sulphate-sedimentation volume

### 2.1 Introduction

Wheat is one of the staple cereal crops of humankind, which is usually consumed in the form of baked or cooked products (Nelson et al., 2006). It is a crop which was first cultivated for human consumption as bread 9000 years ago (Goutam et al., 2013). As the production of bread evolves and changed the expected quality of bread also increased. Which is why quality tests were introduced and their main function was to help improve the quality of wheat and the expected grain yield. These tests were also introduced to breeding programmes as a result of having small amount of grains to use during the early stages of a programme (Schuster et al., 1997).

One of the major targets when working on improving wheat cultivars in South Africa is baking quality (Kuchel et al., 2006) and therefore, loaf volume. The baking test has been found to be one of the most reliable methods for assessing bread making quality; however, this test is time consuming (Dhaka et al., 2012). Some other techniques have been implemented to help reach that target, such as SE-HPLC, SDS-sedimentation, mixograph, SDS-PAGE and rheological characteristics. Quality attributes of bread wheat that are usually measured using these tests are flour yield, protein concentration and composition, kernel hardness and dough mixing properties, and also properties of baked goods such as loaf volume and crumb structure (Nelson et al., 2006).

The quality of wheat for milling and baking use is determined by protein quantity and quality as well as the state of the carbohydrate-amylase complex, thus the degree of starch damage and amylase content. The determination of these features in both the cultivation process and manufacture is carried out by common standardised methods, the results of which are the main indicators of wheat quality.

These characteristics influence the quality of milling and baking products in a decisive way. In order for the millers and bakers to achieve their standard quality, they must contain a stable quality of flour in their established continuous manufacturing processes. Significant correlation between quality characteristics of wheat flour and the end-use products of wheat have been reported (Amjid et al., 2013).

Advanced lines are usually tested in multi-location trials to identify potential cultivars for production. The reason is that most quality traits, except for a few traits such as grain hardness, are controlled by several genes and are heavily affected by the growing environment (Langridge et al., 2001). In general, grain hardness, flour yield and gluten quality-associated traits, such as mixograph parameters, have relatively high heritability in comparison with protein content (Branlard et al., 2001). Factors such as genotype, environment and the interaction between them play an important role in determining wheat quality of different cultivars (Peterson et al., 1992, 1998; Graybosch et al., 1996; Mikhaylenko et al., 2000), although it is still not clear which one is more important. However, Denčić et al. (2011) pointed out that the importance of genetic and environmental effects depends on the genetic variability of the tested genotypes and variation of environmental conditions. Although end-use quality of common wheat is influenced by growing conditions and genotype, glutenin composition is still mostly responsible for the variability in wheat quality (Lagrain et al., 2013). The question still remains on which factor (genetic or environmental) will be dominant over the other in terms of determining the best bread making quality characteristics (Vázquez et al., 2012).

### 2.2 Hectolitre mass

Hectolitre mass (HLM) also known as test weight, is defined as a measure of the volume of grain per unit and expressed as kilograms per hectolitre ($kg.hl^{-1}$). It is a good indicator of grain soundness and is one of the oldest quality parameters used in wheat grading and serves as an indicator of other traits (Manley et al., 2009) such as wheat flour yield (Pushman and Bingham, 1975; Posner and Hibbs, 2005). The equipment for measuring HLM is cheap, the test is easy to perform, and it generates reliable results (Lockwood, 1960). South Africa and other grain producing and exporting countries' grading system uses and relies strongly on HLM as a guide to grain quality. The HLM values of sound wheat varies between 70 and 85 $kg.hl^{-1}$, but these values can increase or decrease as a result of environmental conditions and insect damage (Troccoli and Di Fonzo, 1999).

The HLM can be influenced by a number of factors, such as the presence of foreign materials and damaged wheat, which usually lowers the HLM values. Shrunken wheat kernels lower the HLM value and flour yield (Lockwood, 1960; Greenaway et al., 1971). Moisture content of the grain can also affect the HLM, the drier the grains the higher the HLM (Pushman, 1975).

### 2.3 Single kernel characterising system

Several factors are considered when grading or determining the quality of wheat grains. Those factors are kernel weight, width, moisture and hardness in wheat (Fang and Campbell, 2003). The single kernel characteristics system (SKCS) 4100was developed to help determine these factors, leading to a prediction of wheat texture, flour milling yield and starch damage (Sissons et al., 2000). The SKCS 4100 is a potentially useful tool to a plant breeder by maximizing the number of tests conducted simultaneously and providing useful mean and distribution information on bulk (300 kernels) samples.

Kernel hardness refers to the texture of the kernel (caryopsis), that is, the physical hardness or the softness of the endosperm. It is also known as a good indicator of milling and baking products. Increased kernel hardness requires higher milling forces, but results in higher flour yield and exhibits fracture planes that produce broken starch granules; hence the high levels of starch (Giroux and Morris, 1997) and better flowing and sifting properties during milling. Soft wheat kernels require less energy to mill, they yield smaller flour particles with less starch damage and absorbs less water (Hogg et al., 2004). Milling performance can be improved by constant kernel hardness; this was pointed out by Ohm et al. (1998) when he reported a negative correlation between milling score and SKCS hardness index (HI) standard deviation. Correlation between hardness and loaf volume was reported (Ohm et al., 1998) and a relationship between hardness and dough viscosity was also reported (Oda et al., 1980).

Based on the texture of the grain, wheat is classified into hard and soft wheat. Generally, bread is made from the hard wheat, whereas cookies, cakes and pastries are made from soft wheat. The difference between soft and hard classes of wheat is governed by the hardness locus (*Ha*). Kernel hardness is determined by two tightly linked puroindoline genes named *Pina-D1* and *Pinb-D1* (Giroux and Morris, 1998). These genes are located on the *Ha* locus of the short arm of chromosome 5D (Martin et al., 2001; Lillemo et al., 2006). According to Martin et al. (2001) wheat grain that had the *Pinb-d1b* allele group had traits such as softer grain, higher break flour yield, flour yield, milling score and loaf volume, lower flour ash and grain score as compared to the allele group *PinA-D1b*. To date, all known hard textured wheat have mutations on either *PinA* or *PinB* gene and wheat containing both the soft type *PinA-D1A* and *Pinb-d1a* sequences are soft (Hogg et al., 2004). Giroux and Morris (1998) suggested that the primary genetic element for grain hardness in wheat was the puroindolines, this was discovered after a strong relationship between the *Ha* locus and puroindolines was observed (Sourdille et al., 1996; Giroux and Morris, 1997). To investigate this hypothesis Hogg et al. (2004)

developed six unique transgenic lines in the Hi-Line background that have the addition of soft type *PinA*, *PinB* or *PinA* and *PinB* for the determination of puroindolines in controlling wheat kernel hardness. Hogg et al. (2004) reported the effect that the two puroindoline genes has on grain hardness when expressed independently or together. According to Giroux and Morris (1998) the absence of the *PinA* protein was linked consistently to grain hardness among 44 near- isogenic lines created between hard and soft varieties. Wheat kernel hardness is simply inherited.

### 2.4 Falling number

The falling number (FN) also known as the Hagberg FN and is characterised as one of the most important quality traits in wheat. This technique measures the alpha-amylase activity and also determines the sprout-damaged grain of wheat. Alpha-amylase activity can be due to the sprouting of wheat, which is caused by rain during harvesting time. The high levels of alpha amylase are the results of a grain sprouting when harvested during the rainy harvest season, which are detrimental to end-use quality (van Eeden and Labuschagne, 2012).

The level of alpha-amylase activity must be low in the wheat to have good bread making qualities. This activity is measured by the FN test. If the alpha-amylase activities of the wheat grain are high, the level of FN values is relatively low (Perten, 1964). This is often due to the cool and wet weather during grain filling (Smith and Gooding, 1996, 1999) and it make loaves to become sticky, discoloured and have poor texture (Gooding et al., 2003). The levels of alpha-amylase activity are strongly influenced by environmental factors during the developmental stage of the seed. FN has been proven to be a fast, reliable and an easy test to perform. The FN test has been and still is used worldwide as a general index in the grain grade for wheat and other cereals.

Hrušková et al. (2004) found that the FN of wheat and its flour significantly correlates with each other. Special attention was given to FN by van Eeden and Labuschagne (2012), as a result of its relationship with sprouting and the mutual effect both have on alpha-amylase activity. Moot and Every (1990) identified sprout damaged wheat using the FN method and alpha-amylase activities that resulted into flour with a less desirable end-product. Kruger and Tipples (1979) concluded that the level of alpha-amylase activity in sprouted wheat kernels can vary quite widely depending upon the severity of sprouting. Similarly, a bag of severely sprouted kernels may have higher levels of alpha-amylase than if the whole sample had been lightly sprouted (Barnard, 2001).

## 2.5 Vitreous kernels

For the determination of wheat quality vitreousity is one of the important factors because it reflects the texture of the endosperm and consequently the end-use product of wheat (Al-Saleh and Brennan, 2012). Kernel vitreousity mostly goes hand in hand with kernel hardness when it comes to predicting the quality of the cereal crop. Environmental factors such as climate change and soil characteristics during the development of grain play an important role in determining the vitreousity or starchy appearance of the kernel (El-Khayat et al., 2006). Brennan et al. (2012) demonstrated that the degree of vitreousity is indeed highly influenced by environmental factors, when they reported 37-100% of vitreousity in irrigated areas and 64-100% in rainfed areas. The protein content has also been associated with the degree of kernel vitreousity. The lower the kernel protein content the lower the kernel vitreousity (kernel starch increases) (Matsuo and Dexter, 1980). Positive correlations of ($r = 0.54^*$) were obtained between protein content and vitreousity in a study conducted by Al-Saleh and Brennan, (2012). Vitreous kernels have harder endosperm, higher protein content and greater density (Gaines, 1986).

## 2.6 Milling properties

Milling properties simply refer to the way the grain endosperm breaks down during the milling process. Different wheat types break down differently during milling. Milling times, milling energy requirements and the level of starch damage produced in the milled flour are all influenced by grain hardness. Hard wheat require longer milling times and more milling energy, and produce a larger amount of damaged starch (Peña, 2002).

Hard wheat produces better flour yield because the bran is easy to remove. This leads to a production of more gritty flour, whereas there is difficulty of bran removal in soft wheat, leading to finer sticky flour. The objective of milling is to produce the maximum quantity of flour of a given specification, from the wheat available. The milling process comprises of various grinding operations each followed by a sieving operation into different granulations with some flour being removed at each stage (Bass, 1988).

Milling wheat into flour consists of three essential processes: 1) the grinding process, 2) the sieving process and 3) the purification process. During the grinding process different rollers (corrugated and/or smooth) are used to shear open the kernels to release semolina, middling and flour from the bran and also to remove pieces of endosperm which still have bran and germ attached to it. The sieving process follows in order to remove flour and classify the different particle sizes into “bands” of different

granulation and qualities to make further grinding more specific and accurate. As for the last process it involves the purification of flour probably using different kinds of chemicals. The way the milling process is carried out and the type of instruments used can also help in determining the quality of the end-product.

For instance, in roller milling of wheat grains, each grain passes through the mill independently of the surrounding grains (Campbell et al., 2001; Fang and Campbell, 2003). Not only does the breakage pattern for each grain depend on the roller mill design and operation (such as roller diameter, fluting, roller gap, speed and differential) but also on the interaction between the grain's physical, chemical and structural characteristics (such as kernel size distribution, density, hardness, ash content and protein content) and not on the interaction with surrounding grains.

### 2.7 Sodium dodecyl sulphate-sedimentation

SDS-sedimentation tests have long been used to characterise wheat (*T. aestivum* and *T*. *turgidum* ssp. durum) flours with the aim of predicting processing and end-product qualities (Morris et al*.,* 2007; Oelofse et al., 2010; Si et al., 2013). The size of the samples under investigation plays a role in determining the bread wheat quality. In the early stages of a breeding programme, there is hardly ever enough grain for quality testing, and these bread making tests are labour intensive and require a lot of time to perform (Ayoub et al., 1993). Axford et al. (1979) implemented a simple sedimentation test using SDS to assess the wheat bread making quality on small samples and in a short period of time. The SDS sedimentation test is a low-cost and fast test that requires little manpower and no elaborate laboratory equipment when compared to other quality tests. It has proved to be a reliable, highly reproducible test that gives a good indication of the end-use quality of the flour (Oelofse et al., 2010).

There are two different small-scale sedimentation tests that require only an ear of the wheat crop that can be used to determine the quality of the wheat flour: the Zeleny and SDS tests. The SDS test is based on the potential behaviour of the sedimentation of whole meals and flours making use of a SDS lactic acid reagent (Axford et al., 1979). Thus, sedimentation values are a measure of the amount of swollen gluten protein and occluded starch in a flour-lactic acid suspension (Macri et al., 1986). In 1947 Dr Zeleny developed the Zeleny sedimentation test. After this method was implemented and studied it was modified for better separation of sediment and supernatant (Morris et al., 2007) and Pinckney et al. (1957) modified the test into the present form. Axford et al. (1979) modified the Zeleny test by introducing the SDS reagent into the protocol instead

of using iso-propanol, which was found by Zeleny to be effective in predicting end-use quality. After a comparison of the two different tests, good correlations were found with loaf volume. Axford et al. (1979) reported that, the SDS test was the best and the more convenient test to use.

Basically, the SDS sedimentation test involves the hydration of 5 g of flour with 50 ml of water in a 100 ml stopper measuring cylinders, with a rapid shaking for 10 sec to mix at 2 and 4 min, followed by the addition of 50 ml of 2% SDS, mixing by inverting five times, followed by the addition of 1 ml of diluted lactic acid (1:8 v/v) and again inverting five times to mix. The content in the cylinder is left to settle for 10 min before the reading of the sedimentation volume is taken (Axford et al., 1979).

One of the disadvantages of the standard sedimentation test is that it does not give a final indication of the bread wheat flour quality. Grinding effect can have a negative impact on the sedimentation values. Higher sedimentation values result from wheat flour produced by the slower grinding rate, possibly due to the differences in particle size distribution. This was confirmed by McDonald (1985) when he reported that the grinding rates have an effect on the sedimentation values.

Primarily, SDSVOL is genetically determined (van Lill et al. 1995), this was also confirmed by Kadar and Moldovan, (2003) when they reported genotype as the largest contributor of variance in SDSVOL by 89.98% followed by GxE with 9.73% and environment with 0.28%.

The SDSVOL is a good indicator of the protein content in wheat flour and bread volume, that is, the baking quality of wheat cultivars and the SDS-sedimentation test can differentiate between good bread-baking (high protein content and bread volumes) and poor bread making cultivars (low protein content and bread volumes). High SDSVOL is associated with good bread making quality whereas low SDSVOL is associated with poor baking quality (e Villiers and Laubscher, 1995).

Determination of wheat quality is very important to plant breeders, millers and consumers. The SDS sedimentation test was one of the tests introduced with the aim of testing for quality in wheat. It is a relatively simple test and has proved to be a good predictor of bread making potential and strength for wheat. This statement was proved by the study conducted by Fowler and de la Roche (1975) when they reported the quality of protein and rate of dough development based on the sedimentation values, as both basic quality measurements. However, Macri et al. (1986) showed that triticale flour had

lower sedimentation values than wheat flour, which were caused by the low percentage of the total protein content in triticale flour. Ayoub et al. (1993) proposed that the sedimentation test was not suitable for use in predicting wheat strength. This was after the sedimentation test was unable to differentiate between two seasons of Canadian bread wheat of different remix loaf volumes, especially when they have high grain protein content (GPC). Preston et al. (1982) reported a low correlation coefficient between SDSVOL and loaf volume using three seasons of Canadian bread wheat.

Good results have been see when various wheat quality tests are used together with the SDS-sedimentation test (Zeleny et al., 1960; Lebsock et al., 1964). Różylo and Laskowski (2011) have indicated that the best predictors of bread quality on the bread volume and crumb hardness are a combination of alveographic, physical and chemical parameters, Zeleny sedimentation index, FN and the strength of dough or FPC and the dough extensibility. In addition, Baker and Campbell (1971) also showed that of all the eight tests designated to screen early generations of wheat, SDS-sedimentation test, centrifuge absorption and nitrogen content were the most useful tests for the evaluation of the bread wheat quality.

### 2.7.1 Factors affecting SDS-sedimentation

#### 2.7.1.1 Environmental factors

Environmental factors do not greatly influence the SDS-sedimentation outcome. However, it is well known that protein concentration influences the SDS-sedimentation and the size of the effect differs according to the genotypes tested (Moonen et al., 1982; Lorenzo and Kronstad, 1987; de Villiers and Laubscher, 1995; Gooding, 2003; Morris et al., 2007).

Despite the longevity, relative economy and simplicity of these tests, relatively little is known about how the sediment forms or the reasons for differentiation between samples in terms of GxE interactions (Rittau et al., 2015). Even though Preston et al. (1982) reported that environmental factors played a less important role in the ability of the SDS-sedimentation test to predict dough strength, Drezner et al. (2007), Panozzo and Eagles (2000) as well as Tsenov et al. (2004) proved the importance of environmental effects on wheat quality characteristics while Atanasova et al. (2009) and Zhang et al. (2005) reported the higher effects genotype possess on the expression of sedimentation. Therefore, SDS-sedimentation value is highly heritable (Payne, 1986; van Lill et al., 1995; Kadar and Moldovan, 2003; Oelofse et al., 2010).

It is of importance to determine and quantify the extent to which environmental factors contribute to variation in SDS-sedimentation (Kaya and Akcura, 2014). SDS sedimentation test Pioneer Dr Zeleny, has made the following statement regarding factors which influence sedimentation results: “sedimentation values of wheat flour are mostly affected by the environmental factors during growing period of seed”. Sedimentation volume of affected wheat varieties may range between 20 ml to more than 60 ml depending on the conditions they grew under (Zeleny, 1947). Cultivation practices, soil fertility, heat, drought, disease and other factors can affect gluten quality, and thus influence sedimentation value (Foote, 2014). The effect of environmental factors cannot be ignored when breeding for end-use quality.

#### 2.7.1.2 Chemicals

These sedimentation techniques involve hydration of a small flour sample in a lactic acid solution containing either the detergent SDS or isopropyl alcohol (Zeleny sedimentation) (Zeleny, 1947). The presence of lactic acid in solution causes the hydrated flour particles to sink in the form of sediment, the level of which indicates the strength of the gluten (Oelofse, 2010).

#### 2.7.1.3 Sample size

Reduction of the sample size results in the reduction of SDSVOL. However, samples as small as 1 g is still sufficient to be able to perform the test with and still obtain a satisfactory outcome. Dexter et al. (1980) proved this statement true by using durum wheat to assess gluten strength through the sedimentation test. A 5 g of flour samples was reduced to a 1 g sample and still produced good results. The 1 g sedimentation test explained 43.1, 33.4 and 43.2% of variability in gluten breaking strength, cooking quality parameter and overcooking quality parameter respectively. When using 5 g of flour sample, the values were 57.4, 25.2 and 39.1%, respectively.

## 2.8 Rheology

The quality and the type of the gluten proteins are some of the properties that determine bread making quality. The balance between gliadins and glutenins is related to important rheological properties (Gomez et al., 2011). One of the most important rheological characteristics in the baking industries is elasticity, viscosity and extensibility. They are known to have good outcomes in predicting the processing parameters of dough and the quality of the final product (Hrušková and Šmejda, 2003).

For the measurement of all the rheological characteristics, instruments such as farinographs, mixographs, alveographs, extensigraphs and amylographs are used (Mirsaeedghazi et al., 2008; Codina et al., 2011; 2012). The farinograph and mixograph are rheological devices used to examine the reaction of dough during the process of mixing and kneading (Mironeasa and Codina, 2013; Popa et al., 2014). Instruments like the alveograph and extensigraph describe the resistance of dough to extension (Mirsaeedghazi et al., 2008; Chen et al., 2009; Codina et al., 2011).

### 2.8.1 Mixograph

The mixograph is an instrument that helps to predict the functional dough mixing properties of wheat genotypes in many wheat breeding programmes around the world (Dong et al., 1992). The instrument is very useful for estimating important physical dough properties in early-generation progenies and can also be used to predict bread loaf volume, mixing requirement, dough oxidation requirement and water absorption (Shogren and Finney, 1984).

By 1939 in Nebraska, the mixograph was first developed with the required mass of 35 g of flour, until it was improved to the 10 g mixograph (Finney and Shogren, 1972). Currently it can go as low as 5 g (Finney, 1989) and 2 g of flour (Rath et al., 1990) to evaluate the dough properties of early generation progeny of bread wheat cultivars (Martinant et al., 1998). The high resolution analysis of the mixograph mixing curve indicates that the mixing action in a pin mixer is the superposition of a large number of stretching and folding of the dough around the pins (Mann et al., 2008).

The mixograph gives many parameters on dough properties during mixing that can be analysed and selected as a function of their relationship and meaning (Martinant et al., 1998). Using Mixsmart® software, 44 parameters can be measured on a single curve (Pon et al., 1989). The software constructs a midline curve, which divides the mixogram into two envelope curves, the upper envelope as well as the midline curve (Walker and Walker, 1992; Dobraszczyk and Schofield, 2002). The 44 parameters result from measurements made at different heights, width and slopes as well as areas on the mixogram curve (Chung et al., 2001) (Figure 2.1).

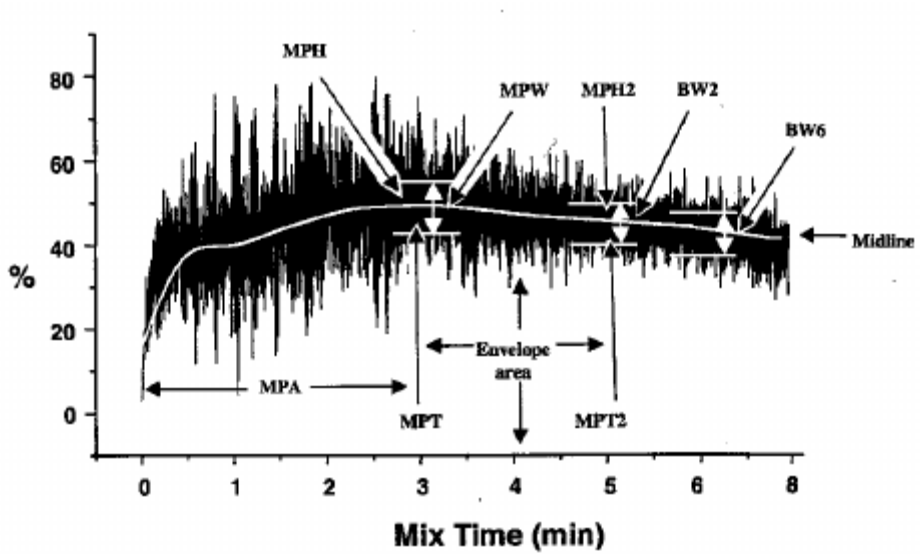


**Figure 2.1 An example of mixograph parameters**

Some selected parameters such as peak height, ascending angle from beginning until one min before peak time and total area under the curve showed a highly significant correlation with FPC and peak time was strongly associated with GPC and flour yield (Miles et al., 2013).

Most of the studies using the mixograph have reported results from only one or a few variables (often peak time and mixing tolerance). Peak time has been the most widely used parameter in terms of predicting the product end-used quality (Dobraszczyk and Schofield, 2002). Nevertheless, various studies have proved otherwise. Martinant et al. (1998) investigated relationships between various wheat grain quality indices and parameters obtained from an instrumented 10 g mixograph. They found that peak time was a poor parameter to explain bread making quality but found strong relationships between loaf volume and peak height and peak bandwidth. Also, Khatkar et al. (1996) found no correlation between loaf volume and peak time. Today, more detailed analyses can be carried out thanks to computer data acquisition and treatment.

### 2.9 Loaf volume

Loaf volume is the most important criteria of bread-baking quality. In South Africa and worldwide this is one of the most considered quality parameters, reflecting the gluten composition of the flour. Predicting the quality of bread wheat from kernel, flour or dough characteristics is not easy. To help predict the loaf volume of bread Millar (2003) developed an equation using a stepwise regression, which included glutenin quality, % gliadins, flour colour, protein content, glutenin elastic modulus, farinograph water

absorption, particle size index, moisture content and the ratio of HMW-GS to LMW-GS. The equation revealed the positive influence protein content and the ratio of HMW-GS to LMW-GS has on loaf volume and the negative influence flour colour and particle size index has on loaf volume (Dowell et al., 2008).

FPC has long been declared a primary factor influencing dough strength and loaf volume (Graybosch et al., 1993). Andersson et al. (1994) baked loaves from 750–950 g of flour and showed that loaf volume was consistently influenced by GPC and FPC, farinograph dough development, stability, and breakdown and extensigraph area, peak height and length. Not only is the loaf volume determined by the protein content in the flour but also by the SDS sedimentation. High SDSVOL has been associated with good loaf volume (Ayoub et al., 1993; Takata et al., 1999; Wang et al., 2002a, 2002b). This was also proved by several studies (Pinckney et al., 1957; Dexter et al., 1980; Moonen et al., 1982; de Villiers and Laubscher, 1995) where high correlations between SDS sedimentation and loaf volume were reported. However Preston et al. (1982) reported a low correlation coefficient between SDSVOL and loaf volume. Meanwhile, Hoshino et al. (1992; 1993) reported no correlation between SDS sedimentation and loaf volume.

### 2.10 Important storage proteins

The genetic make-up of wheat is the most complicated compare to the other domesticated species in the wheat family. Understanding the genetics and the genome organization of the wheat species is important and the development of markers has made an impact on genetics and plant breeding purposes. Bread wheat *T. aestivum* and durum wheat *T. durum* are composed of different genome complements. *T*. *durum* species are composed of four sets of chromosomes (tetraploid) while *T*. *aestivum* is an allohexaploid (2n = 6x = 42) with three diploid genomes AA, BB and DD, and has a very large genome of 16 x 10^9 bp/1C with more than 80% repetitive deoxyribonuclease (DNA) (Bennett and Smith, 1976; Bennett et al., 1982). It originated from the hybridization of three different diploid progenitors from the *Triticum* and *Aegilops* genera.

Firstly, the hybridization between two species *T*. *urartu* (source of the A genome) and *Ae. speltoides* or a closely related species (source of the B genome) took place. Resulting in a tetraploid wheat, *T. turgidum* (AABB), this hybridized with *T. tauschii* (source of the D genome) to produce the hexaploid bread wheat (Shewry, 2009). Each genome consists of seven pairs of chromosomes adding up to a total of 21 pairs in bread wheat. Each of these chromosome pairs is given a number followed by a letter referring to the genome from which the pair was inherited (Payne et al., 1984). The same number

is given to homoeologous chromosomes of different genomes, for examples 1A, 1B and 1D.

### 2.11 Genetics of wheat storage protein

Several studies have proved that there are more than 30 000 genes expressed in the development of wheat grain. The major seed storage proteins of wheat are called prolamins because they are rich in proline and glutamine (Malik, 2009; Anderson, 2014). Wheat gluten proteins account for about 80% of the total grain proteins in typical European wheats (Shewry, 2009) and are divided into two major groups: gliadins and glutenins. The gliadins are protein molecules that are classed into three structural types: α-, β-, and ω- gliadins. They are monomeric components that either lack inter-chain disulfide bonds or contain only intra-chain bonds (Esmaail et al., 2012) and they contribute mainly to the extensibility and viscosity of dough (Shewry and Halford, 2002). Whereas glutenins are polymeric proteins, made up of polypeptide chains that are cross-linked by disulphide bonds into higher level polymers, which aggregates into subunits of differing molecular weight: the HMW-GS and the LMW-GS. Glutenin molecules are responsible for the strength and elasticity of dough (Tarekegne and Labuschagne, 2005).

The HMW-GS are identified according to electrophoretic mobility within the group and according to chromosome coding for individual polypeptides (Lásztity and Abonyi, 2009). Cultivars of hexaploid bread wheat are made up of six HMW-GS genes, two each at the *Glu-1* loci on the long arms of homoeologous chromosomes 1A (*Glu-A1*), 1B (*Glu-B1*) and 1D (*Glu-D1*). Each of these loci encodes two tightly linked genes (Payne et al., 1981; Payne and Lawrence, 1983; Payne et al., 1987) that encode for two types of HMW-GS, the x-type with higher molecular weight, and the y-type with lower molecular weight (Gale, 2005). In the hexaploid wheat, the expression of the y-type gene on the A genome does not usually occur (Payne et al., 1981; Payne and Lawrence, 1983). The ratios of polymeric or monomeric and HMW-GS or LMW-GS are used to predict the quality of wheat for bread making purposes. A molecular-marker linkage map of hexaploid wheat provides a framework for integration with the classical genetic map and a record of the chromosomal rearrangements involved in the evolution of this crop species (Nelson et al., 1995).

## 2.12 Proteomics

Proteomics is the large-scale study of the entire complement of proteins and their function under specified growing conditions (Williams, 1999; Skylas et al., 2005). Proteins are the building blocks of living organisms and they account for more than 50% of dry mass of most cells and play an important part in the physical and chemical properties of an organism. Only a small part of potentially active genome is transcribed and translated into ribonucleic acid (RNA) which then initiate protein synthesis to take place in that particular cell (Gromov and Celis, 2000).

The synthesis of polypeptides in the living biological system is described by the central dogma of molecular biology where there is a flow of genetic information from DNA to RNA and then to proteins (Skylas et al., 2005). The newly developed technologies such as genomics (DNA), transcriptomics (mRNA), proteomics (proteins) and bioinformatics have been developed with the aim to support the interactions between biological molecular at all stages, during the flow of genetic information in a biological system (Maier et al., 2009; Horgan and Kenny, 2011).

Proteomics has positively impacted cereal science and also aided to understanding the quality of grain, by shedding light on the manner of gene expression during grain development under certain environmental conditions (Skylas et al., 2005). Studying wheat grains only on the genotype level does not exactly show the effects on the grain quality caused by environmental factors; however, this information can be obtained by looking at the biochemical level of analysing protein composition produced by the expression of genes under specific conditions (Skylas et al., 2000). Environmental factors can have a huge impact on the value of the wheat crop (both yield and quality). For the assessment of genetic variability, protein markers are good tools to use, not only for the above mentioned but also for establishment of genetic distance and similarities and dissimilarities between lines, species and genus's (Thiellement et al., 1999). In the same study Thiellement et al. (1999) stated that proteomics has become an essential field of research in plant biology, where the combined approaches of genetics, physiology and molecular biology will in the coming years, provide essential tools in understanding the mechanisms underlying plant growth and development.

## 2.13 Electrophoresis

Electrophoresis generally separates protein based on their size and charge; however, separation in SDS-PAGE is based on molecular size and isoelectric focusing, and is being used for the analysis of the glutenin-subunit composition (Uthayakumaran et al.,

2006). Disadvantages of this method are that it is a slow, labour intensive, non-quantitative technique and produces results that are sometimes difficult to interpret (especially the LMW-GS). Electrophoresis techniques can also be combined into two-dimensional methods of high resolving power (Bietz, 1985).

The functional activity of the genome can be monitored using the high resolution two-dimensional (2D) PAGE at protein level, which allows the study of thousands of proteins of an organism (Gromov and Celis, 2000). These proteins include gliadins (monomeric proteins coded by genes present at the *Gli-1* and *Gli-2* loci on the short arms of the group 1 and group 6 chromosomes, respectively and the glutenins, which are made up of the two important subunits: HMW-GS and LMW-GS. These subunits form large polymeric proteins and are encoded by genes at the *Glu-1* and *Glu-3* loci on the long arm of group 1 chromosomes, respectively (Lawrence and Shepherd, 1980; Vawser and Cornish, 2004). With two tightly linked genes located on *Glu-1* loci, there is an allelic variation in each of the *Glu-1* loci (*Glu-A1*, *Glu-B1*, and *Glu-D1*), encoding a higher relative molecular masses x-type HMW-GS and a lower relative molecular masses y-type HMW-GS (Ikeda et al., 2003; Vawser and Cornish, 2004).

The use of electrophoresis and the chromatography techniques in cereal protein research has opened up new possibilities for correlating the protein composition of gluten and bread making value of wheat and flours (Lásztity and Abonyi, 2009). For many years, HMW-GS have been important for quality screening, using the GLU-1 scoring system (Payne et al., 1987). Uthayakumaran et al. (2006) applied the GLU-1 system to score the HMW subunit (*Glu-1*) composition in Australian varieties and found that the identification of the LMW-GS was much more difficult than the HMW-GS; this was due to the large number of LMW-GS and the masking effects of non-glutenin proteins with similar mobility.

Payne et al. (1987) reported a positive and a negative influence that *Glu-D1d* (Dx5+Dy10) and *Glu-A1c* (null) alleles have on dough characteristics and bread making quality, respectively. Adding to the variation in allelic composition, there has been a few cases concerning variation in the expression levels of HMW-GS. The HMW-GS Bx7 protein is expressed at high levels in some wheat lines designated as containing the *Glu-B1a1* allele that confers and correlates directly with increased dough strength (Vawser and Cornish, 2004; Gale, 2005), and also this over-expression, has been associated with the positive relationship between the *Glu-B1a* allele and bread making quality (Gianibelli et al., 2002; Butow et al., 2004; Vawser and Cornish, 2004; Gale, 2005).

## 2.14 Size exclusion-high performance liquid chromatography

SE-HPLC is used to measure the main size classes of wheat flour proteins and to quantify the absolute and relative amounts of glutenin polymeric protein from total protein extraction obtained by sonication of flour samples (Singh et al., 1990; Batey et al., 1991). Research showed that the extraction of the total protein from wheat flour without the use of chemical reduction but by sonication, breaking down the large SDS-insoluble glutenin polymers into small SDS-soluble polymers, was successful (Singh et al., 1990). SE-HPLC can be applied to predict bread making quality (Ciaffi et al., 1996; Huebner et al., 1997; Labuschagne and Aucamp, 2004; Ohm et al., 2009). Wheat proteins are separated in a continuous range of decreasing molecular size. Sing et al. (1990) divided the SE-HPLC profile into three fractions. Of these three fractions the first, second and the third represent polymeric glutenin (albumin and globulin aggregates), monomeric gliadins and albumin/globulins, respectively (Singh et al., 1990). Peak 1 of the SE-HPLC profile is mainly made up of glutenins (Figure 2.2).

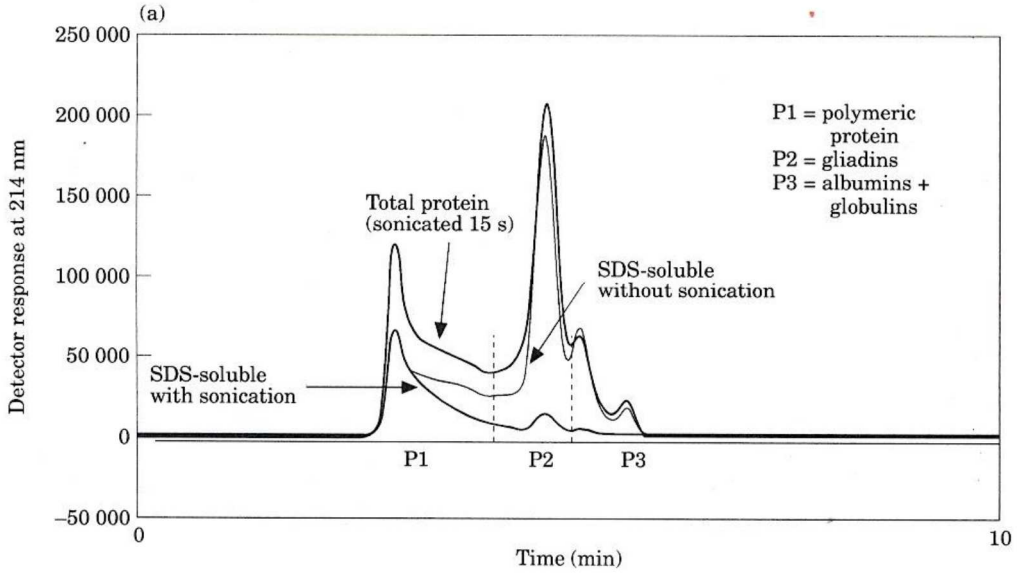


**Figure 2.2 An example of size exclusion-high performance liquid chromatography profile for soluble and insoluble fractions**

It has been accepted that the bread making quality of wheat flour is primarily determined by its protein composition (Oszvald et al., 2008; Morojele and Labuschagne, 2014). Based on their solubility in different solutions, proteins are classified into globulin, which is soluble in alkaline solution; albumin, which is soluble in water; gliadin, soluble in alcohol and glutenin, soluble in acidic solution. The molecular weight of every protein subunit is known from its sequence. Proteins can also be classified based on their molecular size: proteins of more than 100 kilo Daltons (kDa) were classified as glutenin,

those between 100 and 25 kDa as gliadin and those with molecular weight smaller than 25 kDa were classified as albumin or globulin (Bushuk and Wrigley, 1971).

High performance liquid chromatography is a very effective technique for analysing gluten protein. This is due to its speed, automation, quantitative capabilities and small sample size requirement. It also provides an ideal system for rapid screening of many wheat samples (Singh et al., 1990). The proteins in a solvent pass through a column of chromatographic medium with the molecules larger than the pores passing through in the void area to elute first (Bietz, 1985). Longer elution time is generated by the high permeation of smaller protein molecules in the pores. One of the disadvantages of using the SE-HPLC technique is that the materials such as column and chemicals are expensive.

Larroque et al. (2000) used a heating treatment of the extracts for two min at 80°C in a water bath immediately after filtration into the sample vials and before SE-HPLC analysis and found that it was sufficient to keep samples stable for at least 72 hrs before they deteriorate. Thus, a typical overlapping of the SE-HPLC profiles of unheated samples and a decrease in the area of peak 1 along with an increase in the area of peak 3. Huebner et al. (1997) reported a positive correlation between the amount of *Gli-3* (γ-gliadin) and loaf volume.

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# Chapter 3

## Determining the high molecular weight-glutenin subunits of cultivars using sodium dodecyl sulphate-polyacrylamide gel electrophoresis


### 3.1 Abstract

This study investigated the relationship between high molecular weight-glutenin subunits separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and the measured quality characteristics of 13 wheat elite lines across three locations Douglas, Marydale and Vaalharts. Significant correlations between quality characteristics and high molecular weight-glutenin subunits were observed. In single locations, subunits Dx2+Dy12 correlated significantly and negatively with midline left integral, midline left time, midline peak time and midline right time, while subunits Dx5+Dy10 correlated positively with them at all locations. At Marydale, SDSVOL, envelope tail value and midline tail value had a highly negative significant correlation with subunits Bx13+By16. For combined analysis, envelope peak time, midline left integral, midline left tail, midline peak time and midline right time correlated negatively and positively with subunits Dx2+Dy12 and Dx5+Dy10, respectively. Envelope peak integral and envelope peak value were positively correlated with subunits Bx13+By16.


### 3.2 Introduction

The most important factors in the production and marketing of wheat are grain yield and GPC. It has generally been accepted that the glutenin composition of wheat flour determines the outcome of the end-product, more especially during the bread making process. Glutenin constitutes about 45% of the total endosperm protein and is made up of single polypeptide subunits obtained after the reduction of its disulphide bonds (Žilić et al., 2011), some of which are linked by inter-polypeptide disulphide bonds. Glutenin can be further divided into two groups, the HMW-GS and the LMW-GS, with their molecular weight ranging from 100-140 kDa and 30-74 kDa, respectively, based on their mobility determined by SDS-PAGE (Žilić, 2013). The HMW-GS comprise about 5 to 10% of the total proteins, whereas LMW-GS account for about 20 to 30% of the total proteins (Payne, 1986). It is now possible to better isolate and characterize glutenin and relate its composition to quality and genotype, using an improved electrophoretic method (Bietz and Simpson, 1992). To support that, a significant relationship between specific

HMW-GS and bread making quality of bread wheat cultivars was reported by Payne et al. (1979).

For further examination of the relationship between glutenin subunits and bread making quality, a practical procedure is needed for estimating the molecular weights of the subunits. SDS-PAGE has become the most commonly used method for estimating HMW-GS of protein components in a complex mixture (Shapiro et al., 1967; Weber and Osborn, 1969; Bunce et al., 1985). Despite the limitations involving co-migration of some subunits, overestimation of molecular mass and difficulty in detecting differences in expression level, SDS-PAGE is still the simplest and cheapest technique suitable for large-scale and high-throughput HMW-GS screening for wheat genotypes in breeding programmes (Rasheed et al., 2014). Moreover, SDS-PAGE can be used for cultivar identification (du Cros et al., 1980; Gardiner and Forde, 1992).

van Lill and Purchase (1995) reported that in the 1960s the bread making quality of the South African wheat crop was of such a poor standard that good quality wheat had to be imported to supplement local wheat. After that, breeding programmes were implemented to develop good quality cultivars.

Major differences in the composition of HMW-GS are revealed when using SDS-PAGE. In all wheat, there are about 20 or more different HMW-GS but only four or five HMW-GS are found in each variety (Bietz and Simpson, 1992). These subunits comprise of alleles that are related to bread making quality (Burnouf and Bouriquet, 1980; Payne and Lawrence, 1983). The genetic variation of HMW-GS can lead to different combinations of subunits which leads to the quality of the end-product being influenced in a positive (good) or a negative way (Shewry et al., 1992), specifically Dx5+Dy10 subunits are generally known to be associated with good bread making quality and Dx2+Dy12 related to weaker bread making quality (Payne, 1987; Johansson et al., 1993). However, Johansson and Svensson (1999) pointed out that cultivars containing HMW-GS Dx5+Dy10 almost always had higher dough strength compared to those containing subunits Dx2+Dy12, but cultivars evaluated in 1994 and 1995 with Dx2+Dy12 subunits expressed stronger dough strength properties than cultivars with subunits Dx5+Dy10.

For an effective breeding programme, information regarding the extent and nature of genetic diversity within a crop species is essential. Protein electrophoresis is a useful method for describing the genetic structure of crop germplasm (Ciaffi et al., 1993). Xu et al. (2010) proved the usefulness of SDS-PAGE by identifying and characterizing the

HMW-GS of 43 *Ae. tauschii* hexaploid wheat genotypes. Using 8% and 12% SDS-PAGE gels, both 1Dx and a 1Dy subunits were identified (Sofalian and Validazeh, 2009).

High protein content is often a good predictor of the wheat end-use quality. However, studies proved that wheat varieties with the same FPC differed in their bread making quality (Cornish et al., 2001). This suggested that we cannot only rely on the protein quantity alone but also consider the variation of bread making quality (Weegels et al., 1996; Cornish et al., 2001). Various studies have proved the major influence that glutenins have on bread making quality, particularly the HMW-GS (Branlard and Dardevet, 1985; Payne et al., 1987; Gupta et al., 1994; Cornish et al., 2001).

The aim of this study was to analyse the HMW-GS patterns obtained from 13 wheat elite lines grown at three locations; Douglas, Marydale and Vaalharts, using SDS-PAGE, and correlate these patterns with baking quality characteristics in order to identify the lines that will, in future, be best to use for commercial exploitation.

## 3.3 Material and methods

### 3.3.1 Wheat samples

The experimental material consisted of 13 elite breeding lines from three different irrigated wheat production locations (Douglas, Marydale and Vaalharts), all located in the Northern Cape Province of South Africa. Weather data for the growing season over all locations are summarised in Appendix A, Table 1. The flour samples were kindly provided by the Agricultural Research Council-Small Grain Institute (ARC-SGI) in Bethlehem. Samples were taken from trials at the three locations in the 2014 growing season. The experiment was laid out in a randomized complete block design (RCBD), with three replications. Each plot measured 11.25 m² with inter-row and intra-row spacing of 45 cm and 5 cm, respectively. Normal agricultural practices were followed (Appendix A, Table 2).

### 3.3.2 Measured quality characteristics

#### 3.3.2.1 Protein content

The protein content of the flour was determined using the combustion method with a LECO FP-2000 following the AACC 46-30.01 protocol (AACC, 2010).

#### 3.3.2.2 Mixograph analysis

The AACC 54-40A method was applied on a 35 g mixograph (National Manufacturing Corporation, Lincoln, Nebraska) with Mixsmart® software in order to determine the mixing development time of the flour. Mixsmart® software parameters are listed in Appendix B, Table 1. Protein and moisture content of a flour sample are needed before a mixograph analyses can commence in order to determine the flour weight and water volume required.

The required weight of the flour and the volume of water were determined using the following formulas as described by Walker et al. (1997).

Firstly, protein content was converted to a 14% moisture basis (m.b.):

= [protein (as is) x 86] / (100 - moisture content)

Then, the required weight of flour was determined as follows:

= [86 / (100 - moisture content)] x 35

Lastly, the required volume of water was determined as follows:
= [(1.5 x protein content 14% m.b.) + 43.6] x 0.35

The mixograms were constructed as two envelope curves and one midline curve. The Mixsmart® software uses the midline as well as the top-envelope curve to analyse the mixograms. Different measurement points determined by Mixsmart® software included time values, heights, slopes, widths and areas. Time values were determined directly from the horizontal axis and were expressed in minutes. Heights expressed as value (%), were determined as the percentage of full scale. Slopes were calculated as the value (%) divided by the time in question. Width values are the differences in the values for the top of envelope and bottom of the envelope at a specific time. Midline widths obtain information from the envelopes. Areas (integrals values) were determined from starting to the specific time. Integral values are indicative of the work input for dough development and are determined as the vertical axis (% torque) multiplied by the horizontal axis (minutes), therefore it is expressed as torque*min (Walker and Walker, 1992). Only 19 of 44 Mixsmart® characteristics, which showed good repeatability and coefficient of variation, were used for this study.

#### 3.3.2.3 Sodium dodecyl sulphate-sedimentation test

For the measurements of the SDSVOL of wheat flour suspension after it has been acidified, AACC 56-70.01 method was followed (AACC, 2010), with certain modifications.

All chemicals were prepared a day before the experiment was conducted. With SDS sedimentation results recorded after 30 min and reported as sedimentation volume (ml 5 $g^{-1}$).

### 3.3.3 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

#### 3.3.3.1 Protein extraction

Glutenin proteins were extracted (Singh et al., 1991) by suspending 20 mg of flour sample in 1 ml of 50% n-propanol (extraction buffer), vortexed and then incubated at 60°C for 30 min with vortexing every 10 min. The samples were centrifuged for 2 min at 10 000 rpm and supernatant was discarded. Extraction buffer (80 Mm Tris-HCl, pH 8.0 made in 50% propanol) containing 1.25% freshly prepared dithiothreitol (DTT) (0.05 µl) was added to the pellet, then incubated at 60°C for 60 min followed by the addition of 84 µl of 16.8 µl $ml^{-1}$ 4-vinyl pyridine/extraction buffer and incubation for 60 min at 60°C. The samples were centrifuged again for 5 min at 10 000 rpm before 115 µl of supernatant and 105 µl of sample buffer (100 ml of 60 mM Tris-HCl, pH 8.0, 0.8 g $ml^{-1}$ SDS, 0.0002 g $ml^{-1}$ bromophenol blue) were transferred into new 1.5 ml Eppendorf tubes. Samples were briefly vortexed, placed in a water bath for 15 min at 60°C followed by loading (10 µl) on the gel.

#### 3.3.3.2 Electrophoresis

Protein subunits of the samples were detected by SDS-PAGE performed according to Singh et al. (1991) using a 10% separating gel and a 12% stacking gel in a vertical electrophoresis unit. The separating gel solution was made up of 1 M Tris-HCl pH 8.8; 1 M Tris-HCl pH 6.8; 10% SDS and a freshly prepared 1% APS, 40% Acrylamide, 2% bis-acrylamide, 17 ml distilled water ($dH_20$) and 0.030 ml of tetramethylethylenediamine (TEMED). Stacking gel solution comprised of stacking buffer (6.06 g Tris; 190 ml $dH_2O$; 0.4 g SDS; pH 6.8), stacking acrylamide (87.5 g acrylamide; 1.32 g Bis; 181 ml $dH_2O$), 30 µl TEMED and 1% APS.

Two gels were run simultaneously over night for 16 hrs in the same electrophoretic cell using a discontinuous vertical slab gel unit, Hoefer SE 600 System (Hoefer Scientific

Instruments, San Francisco, California, USA) with a running buffer (28.22 g Glycine; 6 g Tris; 5 g SDS; $dH_2O$ to a total volume of 2 l) using the following settings: migration at 18°C at 35 mA (per gel) and 90 V. After the run, gels were soaked in the fixing solution composed of acetic acid, methanol and distilled water at a 1:4:5 ratio by volume for three hours before they were submerged into a staining solution containing 0.58% (w/v) Coomassie brilliant blue G 250 mixed with 14% (w/v) trichloroacetic acid containing, 5% methanol and 900 ml distilled water for 16 hrs. The gel images were captured using the Hewlett Packard scanner.

### 3.3.4 Statistical analysis

All statistical analyses were done using Agrobase Generation II (2015) software. The relationships between quality characteristics and glutenin subunits were assessed using analysis of variance and correlations.

## 3.4 Results

### 3.4.1 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis high molecular weight-glutenin subunits identified from 13 selected wheat elite lines

A total of nine alleles of HMW-GS were detected at all three loci which resulted in a combination of eight different alleles shown in Figure 3.1 and Table 3.1. *Glu-A1* coded for three alleles (Ax1, Ax2* and null) and four subunit combinations (Bx7+By8, Bx7+By9, Bx13+By16 and Bx17+By18) were identified at *Glu-B1* and a combination of two subunits (Dx2+Dy12 and Dx5+Dy10) at the *Glu-D1* locus. From the *Glu-A1* locus only elite lines 1, 8, 9, 10, 11 and 12 expressed allele Ax1. Allele Ax2* was only observed in elite lines 2, 3, 4, 5, 7 and 13 leaving sample 6 the only elite line which expressed the null allele.

At the *Glu-B1* locus Bx7+By8 was the most dominant subunit combination (Table 3.2) expressed in nine genotypes. Elite lines 2, 4, 6 and 10 expressed subunits Bx7+By9, Bx17+By18, Bx17+By18 and Bx13+By16, respectively. The subunit combination Dx2+Dy12 of the *Glu-D1* locus was expressed in 10 elite lines, whereas three lines expressed subunits Dx5+Dy10.

Lines 1, 8, 9 and 12 expressed the same allele combinations of Ax1, Bx7+By8, and Dx2+Dy12 on all three loci. Subunits Dx5+Dy10 at the *Glu-D1* locus were observed in lines 7, 11 and 13 but they differed for *Glu-A1* subunits. Line 6 was the only line with a

null allele on the *Glu-A1* and Bx17+By18 at the *Glu-B1* locus while sharing the same expression of Dx2+Dy12 with 10 other lines. Lines 2, 3, 4 and 5 had the same expression at the *Glu-A1* and *Glu-D1* loci (Ax2* and Dx2+Dy12) but differed at the *Glu-B1* locus (Bx7+By9, Bx7+By8, Bx17+By18 and Bx7+By8), respectively. Large variability was observed at the *Glu-B1* locus with four different combinations (Table 3.1).

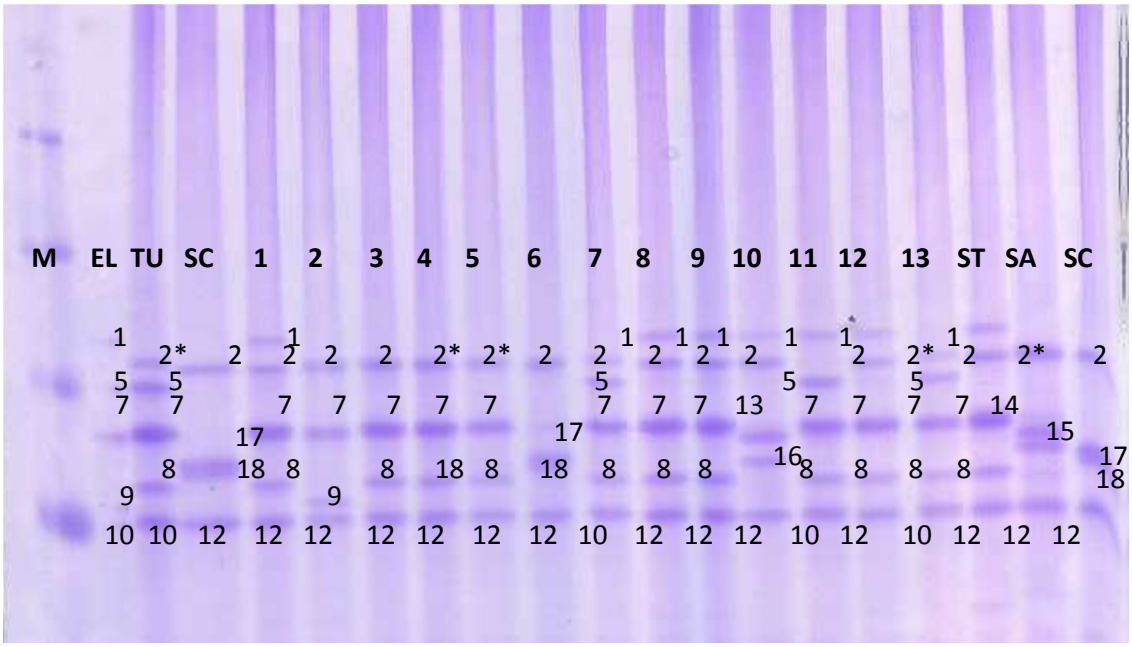


**Figure 3.1 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis high molecular weight-glutenin subunits band pattern.**

**M = protein marker; EL = Elands; TU = Tugela DN; SC = Scheepers; 1-13 = selected Elite lines; ST = Steenbras; SA = Sappo; SC = Scheepers. EL, TU, SC, ST and SA were used as reference cultivars with known banding patterns**

**Table 3.1 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis profile of high molecular weight-glutenin subunits composition obtained from 13 selected South African wheat elite lines**

| Elite lines | High molecular weight-glutenin subunits | | |
|---|---|---|---|
| | ***Glu-A1*** | ***Glu-B1*** | ***Glu-D1*** |
| **1** | Ax1 | Bx7+By8 | Dx2+Dy12 |
| **2** | Ax2* | Bx7+By9 | Dx2+Dy12 |
| **3** | Ax2* | Bx7+By8 | Dx2+Dy12 |
| **4** | Ax2* | Bx17+By18 | Dx2+Dy12 |
| **5** | Ax2* | Bx7+By8 | Dx2+Dy12 |
| **6** | Null | Bx17+By18 | Dx2+Dy12 |
| **7** | Ax2* | Bx7+By8 | Dx5+Dy10 |
| **8** | Ax1 | Bx7+By8 | Dx2+Dy12 |
| **9** | Ax1 | Bx7+By8 | Dx2+Dy12 |
| **10** | Ax1 | Bx13+By16 | Dx2+Dy12 |
| **11** | Ax1 | Bx7+By8 | Dx5+Dy10 |
| **12** | Ax1 | Bx7+By8 | Dx2+Dy12 |
| **13** | Ax2* | Bx7+By8 | Dx5+Dy10 |

**Table 3.2 Frequencies of the subunits found at the *Glu-1* loci**

| ***Glu-A1*** | | | ***Glu-B1*** | | | ***Glu-D1*** | | |
|---|---|---|---|---|---|---|---|---|
| **Subunits (A)** | **No. of lines** | **%** | **Subunits (B)** | **No. of lines** | **%** | **Subunits (D)** | **No. of lines** | **%** |
| | | 4 | | | 6 | | | 7 |
| Ax1 | 6 | 6 | Bx7+By8 | 9 | 9 | Dx2+Dy12 | 10 | 7 |
| | | 4 | | | | | | 2 |
| Ax2* | 6 | 6 | Bx7+By9 | 1 | 8 | Dx5+Dy10 | 3 | 3 |
| Null | 1 | 8 | Bx13+By16 | 1 | 8 | | | |
| | | | | | 1 | | | |
| | | | Bx17+By18 | 2 | 5 | | | |

### 3.4.2 Analysis of variance

#### 3.4.2.1 Combined analysis of variance for selected quality characteristics across three locations

Various mixograph parameters showed highly significant differences ($p \leq 0.001$) due to genotype (Table 3.3). A much larger variation was found between genotypes than between environments for all measured quality characteristics, indicating that environment had a smaller effect than genotype on variation in these characteristics (Table 3.4). GxE was significant for EPI, ETI, midline left time (MLT), midline peak time (MPT), midline peak width (MPW) and midline right time (MRT) and highly significant for midline left value (MLV), MTI and midline tail value (MTV) (Table 3.3).

#### 3.4.2.1.1 Flour protein content

The combined ANOVA (Table 3.3) showed highly significant (p≤0.001) differences for genotypes and also for the environments for the measured characteristics. GxE interaction was not significant. The largest variation was attributed to the environment (46.35%), genotype contributed 15.38% of total variation and GxE interaction contributed 7.69% (Table 3.4).

The FPC means for the lines varied between 10.30% (line 5) and 11.60% (line 11) (Table 3.5). Environmental means ranged between 9.90 (Vaalharts) and 11.30 (Marydale) (Table 3.6).

#### 3.4.2.1.2 SDS-sedimentation volume

Highly significant (p≤0.001) differences were revealed between genotypes and between environments. No significant GxE interaction effects were seen (Table 3.3). Genotypes were the largest contributor (66.41%) to the total variation while environment and GxE made the lowest contribution of 4.75% and 8.29% respectively (Table 3.4).

The SDSVOL means for the lines (Table 3.5) varied between 40.33 ml $g^{-1}$ (line 10) and 66.44 ml $g^{-1}$ (line 2) across the three locations. All elite lines contained desirable SDSVOL values except for line 10 that had a lower value than 50 ml $g^{-1}$. Environmental means (Table 3.6) varied between 55.15 ml $g^{-1}$ (Douglas) and 58.69 ml $g^{-1}$ (Marydale).

#### 3.4.2.1.3 Envelope peak integral

Genotype and environment effects were highly significant (p≤0.001) and GxE interaction significant (p≤0.05) for EPI (Table 3.3). Contribution to total variance was 45.93% for genotypes, 15.32% for environment and 13.29% for the GxE interaction (Table 3.4).

#### 3.4.2.1.4 Envelope peak time

Highly significant (p≤0.001) genotype and environment effects were evident for EPT. GxE interaction was not significant (Table 3.3). The largest variation was contributed by the genotype (40.04%), environment (21.01%) and GxE interaction 14.41% (Table 3.4).

EPT for the lines (Table 3.5) varied between 2.04 min (line 10) to 2.91 min (line 13) across the three locations. Environmental means ranged between 2.21 min (Douglas) and 2.61 min (Vaalharts) (Table 3.6).

#### 3.4.2.1.5 Envelope peak value

Genotype and environmental effects were highly significant (p≤0.001) for envelope peak value (EPV) (Table 3.3). There were no significant GxE interaction effects. Genotype contributed 45.38% to the total variation. Environment and GxE interaction contributed 21.49% and 11.6% to total variation, respectively (Table 3.4).

#### 3.4.2.1.6 Envelope peak width

Genotype effects were highly significant (p≤0.001) for envelope peak width (EPW). Environment and the GxE interaction effects were not significant (Table 3.3). Environment and GxE interaction contributed 2.1% and 20.77% of total variation, respectively, while genotypes were the largest contributor (26.82%) to total variation (Table 3.4).

#### 3.4.2.1.7 Envelope tail integral

Highly significant (p≤0.001) genotype and significant (p≤0.05) environment effects were seen for ETI (Table 3.3). Genotypes contributed the most (50.99%) to the total variation, GxE interaction and environment made similar contributions of 12.48% and 12.33% respectively (Table 3.4).

#### 3.4.2.1.8 Envelope tail value

Significant genotype effects (p≤0.01) were observed for envelope tail value (ETV) (Table 3.3) which contributed 55.00% to the total variation (Table 3.4). Highly significant (p≤0.001) environmental effects were evident (Table 3.3) which contributed 19.2% of total variation (Table 3.4). GxE interaction was not significant (Table 3.3) and contributed 10.6% to total variation (Table 3.4).

#### 3.4.2.1.9 Midline left integral

Genotype and environment effects were highly significant (p≤0.001) for MLI (Table 3.3), which contributed 52.32% and 19.11% to the total variation, respectively (Table 3.4). GxE interaction was significant (p≤0.01) for MLI (Table 3.3) and contributed 9.13% of total variation (Table 3.4).

#### 3.4.2.1.10 Midline left time

Significant genotype (p≤0.001) and environment (p≤0.01) effects and GxE interaction (p≤0.05) were seen for MLT (Table 3.3). Genotypes were the largest contributor (49.96%) to the total variation, while environment and GxE interaction contributed less at 22.94% and 9.13%, respectively.

Elite lines means ranged from 1.14 min (line 10) to 2.06 min (line 13). Environmental means ranged from 1.34 min (Douglas) to 1.72 min (Vaalharts) (Table 3.6).

#### 3.4.2.1.11 Midline left value

Genotype, environment and GxE interaction effects were all highly significant (p≤0.001) for MLV (Table 3.3). However, genotypes were the largest contributor (56.03%) to total variation. Environment and GxE interaction contributed 15.2% and 13.64% to variation, respectively (Table 3.4).

#### 3.4.2.1.12 Midline left width

Environment and GxE interaction effects were not significant for midline left width (MLW) but highly significant (p≤0.001) for genotypes (Table 3.3). About 27.33% of variation was contributed by the genotypes. Environment and GxE interaction contributed 3.19% and 19.14% (Table 3.4) to the total variation.

#### 3.4.2.1.13 Midline peak integral

Highly significant genotype and environment and GxE interaction effects (p≤0.001) were observed for MPI (Table 3.3). Genotype, environment and GxE contributed 18.74%, 4.02% and 27.36% to total variation, respectively (Table 3.4).

#### 3.4.2.1.14 Midline peak time

Genotype and environment effects were highly significant (p≤0.001) for MPT (Table 3.3). Genotype, GxE interaction and environment contributed 46.69%, 9.80% and 22.94%, respectively to total variation (Table 3.4). Elite line means ranged from 2.14 min (line 10) to 3.06 min (line 13) (Table 3.5). Environmental means varied between 2.34 min (Douglas) and 2.72 min (Vaalharts) (Table 3.6).

#### 3.4.2.1.15 Midline peak value

Genotype as well as environment effects were highly significant (p≤0.001) for midline peak value (MPV) (Table 3.3). Genotype contributed 44.39% to total variation. GxE interaction and environment contributed 8.1% and 29.65% respectively, to the total variation.

#### 3.4.2.1.16 Midline peak width

Genotype effects were highly significant (p≤0.001) for MPW (Table 3.3). Genotype, GxE interaction and environment contributed 27.51%, 21.67% and 6.44% respectively, to the total variation (Table 3.4).

#### 3.4.2.1.17 Midline right integral

Genotype and environment effects were highly significant (p≤0.001) for MRI (Table 3.3.). Contributions of the different components to the total variation were 58.24% for genotypes, 15.28% for environment and 11.22% for GxE interaction (Table 3.4).

#### 3.4.2.1.18 Midline right time

Genotype and environment effects were highly significant (p≤0.001) and GxE interaction significant (p≤0.05) for MRT (Table 3.3). Genotype, GxE interaction and environment contributed 49.96%, 9.13% and 22.94% to the total variation, respectively (Table 3.4).

MRT line means ranged between 3.14 min (line 10) and 4.06 min (line 13) across the three locations (Table 3.5). Environmental means varied between 3.34 (Douglas) and 3.72 min (Vaalharts) (Table 3.5).

#### 3.4.2.1.19 Midline right value

Only genotype and environment effects were highly significant (p≤0.001) for midline right value (MRV) (Table 3.3). Contributions of the different components to the total variation were 47.50% for genotypes, 10.51% for GxE interaction and 21.41% for environment (Table 3.4).

#### 3.4.2.1.20 Midline tail integral

Genotype, environment and GxE interaction effects were highly significant (p≤0.001) for MTI (Table 3.3). The largest contributor to variation was genotype (48.88%). GxE interaction and environment contributed 10.80% and 26.63% respectively (Table 3.4).

#### 3.4.2.1.21 Midline tail value

Genotype, environment and GxE interaction effects were highly significant (p≤0.001) for MTV (Table 3.3). Genotypes were the largest contributor (49.20%) to variation while environment and GxE interaction contributed 23.50% and 14.05% respectively (Table 3.4).

**Table 3.3 Mean square values for the combined analysis of variance for selected quality characteristics in three locations**

| Characteristics | Combined | | |
|---|---|---|---|
| | Genotype | Environment | GxE |
| **FPC** | 1.31*** | 23.77*** | 0.33$^{ns}$ |
| **SDSVOL** | 368.64*** | 158.19*** | 23.02$^{ns}$ |
| **EPI** | 254.27*** | 505.99*** | 36.69* |
| **EPT** | 0.56*** | 1.75*** | 0.10$^{ns}$ |
| **EPV** | 92.10*** | 261.62*** | 1.78$^{ns}$ |
| **EPW** | 31.84*** | 14.94$^{ns}$ | 12.33$^{ns}$ |
| **ETI** | 1098.05*** | 1586.71*** | 134.27* |
| **ETV** | 72.10** | 150.97*** | 6.96$^{ns}$ |
| **MLI** | 626.65*** | 1373.01*** | 64.68** |
| **MLT** | 0.56*** | 1.54** | 0.05* |
| **MLV** | 50.66*** | 82.46*** | 6.16*** |
| **MLW** | 27.64*** | 19.36$^{ns}$ | 9.68$^{ns}$ |
| **MPI** | 784.35*** | 1378.37*** | 78.44** |
| **MPT** | 0.56*** | 1.54*** | 0.05* |
| **MPV** | 57.93*** | 232.14*** | 5.28$^{ns}$ |
| **MPW** | 19.15*** | 26.90** | 7.54* |
| **MRI** | 1007.77*** | 1586.64*** | 97.10** |
| **MRT** | 0.56*** | 1.54*** | 0.05* |
| **MRV** | 44.14*** | 152.84*** | 4.88** |
| **MTI** | 1057.54*** | 3456.32*** | 116.85*** |
| **MTV** | 35.63*** | 102.13*** | 5.09*** |

*****p≤0.05, **p≤0.01, ***p≤0.001, ns=Not significant, FPC=Flour protein content, SDSVOL=SDS-sedimentation volume, EPI=Envelope peak integral, EPT=Envelope peak time, EPV=Envelope peak value, EPW=Envelope peak width, ETI=Envelope tail integral, ETV=Envelope tail value, MLI=Midline left integral, MLT=Midline left time, MLV=Midline left value, MLW=Midline left width, MPI=Midline peak integral, MPT=Midline peak time, MPV=Midline peak value, MPW=Midline peak width, MRI=Midline right integral, MRT=Midline right time, MRV=Midline right value, MTI=Midline tail integral, MTV=Midline tail value, GxE=Genotype by environment interaction

**Table 3.4 Percentages of contribution of variation of each characteristic to the total variation from combined analysis across the three locations**

| Source | Character | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **FPC** | **SDSVOL** | **EPI** | **EPT** | **EPV** | **EPW** | **ETI** | **ETV** | **MLI** | **MLT** |
| **Genotype** | 15.38 | 66.41 | 45.93 | 40.04 | 45.38 | 26.82 | 50.99 | 55.00 | 52.32 | 49.96 |
| **Environment** | 46.35 | 4.75 | 15.32 | 21.01 | 21.49 | 2.10 | 12.33 | 19.20 | 19.11 | 22.94 |
| **GxE** | 7.69 | 8.29 | 13.29 | 14.41 | 11.60 | 20.77 | 12.48 | 10.62 | 10.80 | 9.13 |
| | **MLV** | **MLW** | **MPI** | **MPT** | **MPV** | **MPW** | **MRI** | **MRT** | **MRV** | **MTI** | **MTV** |
| **Genotype** | 56.03 | 27.33 | 18.74 | 46.96 | 44.39 | 27.51 | 58.24 | 49.96 | 47.50 | 48.88 | 49.20 |
| **Environment** | 15.20 | 3.19 | 4.02 | 22.94 | 29.65 | 6.44 | 15.28 | 22.94 | 21.41 | 26.63 | 23.50 |
| **GxE** | 13.64 | 19.14 | 27.36 | 9.80 | 8.10 | 21.67 | 11.22 | 9.13 | 10.51 | 10.80 | 14.05 |

FPC=Flour protein content, SDSVOL=SDS-sedimentation volume, EPI=Envelope peak integral, EPT=Envelope peak time, EPV=Envelope peak value, EPW=Envelope peak width, ETI=Envelope tail integral, ETV=Envelope tail value, MLI=Midline left integral, MLT=Midline left time, GXE=Genotype by environment interaction, MLV=Midline left value, MLW=Midline left width, MPI=Midline peak integral, MPT=Midline peak time, MPV=Midline peak value, MPW=Midline peak width, MRI=Midline right integral, MRT=Midline right time, MRV=Midline right value, MTI=Midline tail integral, MTV=Midline tail value

**Table 3.5 Measured means of quality characteristics and combined analysis for Douglas, Marydale and Vaalharts**

| Elite lines | FPC | | | | SDSVOL | | | | EPI | | | | EPT | | | | EPV | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM |
| **1** | 11.90 | 11.30 | 10.70 | 11.30 | 53.67 | 61.00 | 57.33 | 57.33 | 36.77 | 53.54 | 39.88 | 43.39 | 1.94 | 2.55 | 2.25 | 2.25 | 62.21 | 65.98 | 58.11 | 62.10 |
| **2** | 11.50 | 11.90 | 9.80 | 11.10 | 65.00 | 69.00 | 65.33 | 66.44 | 37.43 | 44.18 | 40.67 | 40.76 | 2.12 | 2.47 | 2.29 | 2.29 | 60.90 | 62.44 | 56.10 | 59.82 |
| **3** | 11.50 | 11.10 | 9.50 | 10.70 | 53.33 | 56.33 | 58.33 | 56.00 | 36.77 | 44.13 | 48.00 | 42.96 | 2.15 | 2.50 | 2.79 | 2.48 | 60.68 | 59.76 | 56.52 | 58.99 |
| **4** | 10.90 | 11.20 | 9.90 | 10.70 | 59.00 | 64.67 | 64.67 | 62.78 | 30.50 | 39.27 | 38.32 | 36.03 | 1.95 | 2.28 | 2.39 | 2.21 | 55.82 | 62.48 | 54.15 | 57.48 |
| **5** | 10.50 | 11.20 | 9.20 | 10.30 | 51.67 | 59.00 | 57.00 | 55.89 | 32.49 | 36.46 | 37.70 | 35.55 | 2.12 | 2.24 | 2.37 | 2.25 | 51.28 | 58.69 | 51.23 | 53.73 |
| **6** | 11.10 | 10.60 | 9.40 | 10.40 | 64.33 | 63.67 | 58.33 | 62.11 | 41.12 | 42.79 | 36.61 | 40.17 | 2.40 | 2.52 | 2.39 | 2.44 | 56.94 | 57.77 | 50.27 | 54.99 |
| **7** | 11.30 | 11.20 | 9.90 | 10.80 | 51.00 | 51.00 | 53.33 | 51.78 | 38.74 | 48.48 | 45.80 | 44.34 | 2.35 | 2.80 | 2.69 | 2.61 | 56.53 | 57.74 | 53.12 | 55.80 |
| **8** | 11.40 | 11.50 | 9.80 | 10.90 | 59.00 | 62.00 | 63.33 | 61.44 | 42.65 | 44.10 | 43.69 | 43.48 | 2.41 | 2.61 | 2.73 | 2.58 | 56.26 | 56.89 | 52.99 | 55.38 |
| **9** | 11.00 | 11.30 | 10.20 | 10.90 | 54.67 | 58.00 | 61.67 | 58.11 | 39.25 | 42.08 | 45.83 | 42.39 | 2.37 | 2.43 | 2.81 | 2.54 | 55.64 | 57.86 | 55.57 | 56.35 |
| **10** | 11.00 | 10.80 | 10.30 | 10.70 | 35.67 | 39.33 | 46.00 | 40.33 | 24.73 | 30.09 | 30.92 | 28.58 | 1.80 | 2.05 | 2.27 | 2.04 | 50.78 | 50.20 | 47.37 | 49.45 |
| **11** | 11.50 | 12.50 | 10.90 | 11.60 | 54.00 | 61.67 | 62.00 | 59.22 | 42.10 | 43.87 | 54.74 | 46.90 | 2.35 | 2.62 | 3.47 | 2.81 | 56.13 | 55.02 | 48.70 | 53.28 |
| **12** | 11.10 | 11.00 | 9.10 | 10.40 | 56.33 | 58.67 | 51.67 | 55.56 | 36.23 | 43.42 | 45.86 | 41.83 | 2.19 | 2.58 | 2.35 | 2.38 | 59.18 | 54.67 | 55.65 | 56.50 |
| **13** | 11.40 | 11.20 | 10.00 | 10.90 | 59.33 | 58.67 | 62.67 | 60.22 | 42.79 | 53.38 | 51.14 | 49.11 | 2.54 | 3.02 | 3.18 | 2.91 | 57.15 | 55.33 | 50.61 | 54.36 |
| Env mean | 11.20 | 11.30 | 9.90 | 10.80 | 55.15 | 58.69 | 58.59 | 57.48 | 37.04 | 43.52 | 43.01 | 41.91 | 2.21 | 2.51 | 2.61 | 2.55 | 56.88 | 58.07 | 53.11 | 56.02 |
| LSD | 0.60 | | | | 3.90 | | | | 4.42 | | | | 0.02 | | | | 2.41 | | | |

FPC=Flour protein content, SDSVOL=SDS-sedimentation volume, EPI=Envelope peak integral, EPT=Envelope peak time, EPV=Envelope peak value, DO=Douglas, MA=Marydale, VA=Vaalharts, EM=Elite lines mean, Env=Environmental mean, LSD=Least significant difference

**Table 3.5 Continued**

| Elite lines | EPW | | | | ETI | | | | ETV | | | | MLI | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM |
| **1** | 23.51 | 27.66 | 22.25 | 24.47 | 66.14 | 88.37 | 67.79 | 74.10 | 38.33 | 43.16 | 36.25 | 39.25 | 27.94 | 49.59 | 33.40 | 36.98 |
| **2** | 23.06 | 24.06 | 21.81 | 22.98 | 79.31 | 92.25 | 93.06 | 88.21 | 40.39 | 45.40 | 40.62 | 42.14 | 34.76 | 43.04 | 42.68 | 40.16 |
| **3** | 23.00 | 21.65 | 21.23 | 21.96 | 66.16 | 75.86 | 81.61 | 74.54 | 37.90 | 38.86 | 39.13 | 38.63 | 30.84 | 37.98 | 49.40 | 39.40 |
| **4** | 18.87 | 24.38 | 20.03 | 21.10 | 59.11 | 71.56 | 68.02 | 66.23 | 35.04 | 40.11 | 33.91 | 36.35 | 24.31 | 33.65 | 32.59 | 30.18 |
| **5** | 16.96 | 21.59 | 16.66 | 18.40 | 57.42 | 69.58 | 67.85 | 64.95 | 31.76 | 38.33 | 34.25 | 34.78 | 23.96 | 33.68 | 31.78 | 29.81 |
| **6** | 21.64 | 21.48 | 18.36 | 20.49 | 78.39 | 85.65 | 70.04 | 78.03 | 38.64 | 41.35 | 33.94 | 37.97 | 33.66 | 41.28 | 32.63 | 35.85 |
| **7** | 20.70 | 21.93 | 22.56 | 21.73 | 79.10 | 92.10 | 96.18 | 89.13 | 39.15 | 41.97 | 40.28 | 40.47 | 37.25 | 51.31 | 54.75 | 47.77 |
| **8** | 20.36 | 20.91 | 21.11 | 20.79 | 80.31 | 86.33 | 82.96 | 83.20 | 38.72 | 42.63 | 38.45 | 39.94 | 39.35 | 48.07 | 47.47 | 44.96 |
| **9** | 21.40 | 21.95 | 21.71 | 21.69 | 76.18 | 82.17 | 80.22 | 79.52 | 37.37 | 40.18 | 37.94 | 38.49 | 40.10 | 39.28 | 47.11 | 42.16 |
| **10** | 16.78 | 17.25 | 17.45 | 17.16 | 49.81 | 56.27 | 57.45 | 54.51 | 31.28 | 32.40 | 31.30 | 31.66 | 17.85 | 25.94 | 26.72 | 23.50 |
| **11** | 21.98 | 20.10 | 18.20 | 20.09 | 83.13 | 88.14 | 90.16 | 87.14 | 38.47 | 41.91 | 40.07 | 40.15 | 37.86 | 43.28 | 57.50 | 46.21 |
| **12** | 20.23 | 18.92 | 26.77 | 21.97 | 69.01 | 91.19 | 111.45 | 90.55 | 37.75 | 41.33 | 41.77 | 40.28 | 28.22 | 45.51 | 44.28 | 39.34 |
| **13** | 21.44 | 20.06 | 18.48 | 19.99 | 77.63 | 93.54 | 89.37 | 86.85 | 39.02 | 42.63 | 40.56 | 40.74 | 45.94 | 58.60 | 59.33 | 54.63 |
| Env mean | 20.76 | 21.69 | 20.51 | 20.99 | 70.90 | 82.54 | 81.24 | 78.23 | 37.22 | 40.79 | 37.57 | 38.53 | 32.46 | 42.40 | 43.05 | 39.30 |
| LSD | 2.85 | | | | 8.37 | | | | 1.60 | | | | 2.53 | | | |

EPW=Envelope peak width, ETI=Envelope tail integral, ETV=Envelope tail value, MLI=Midline left integral, DO=Douglas, MA=Marydale, VA=Vaalharts, EM=Elite lines mean, Env=Environmental mean, LSD=Least significant difference

**Table 3.5 Continued**

| Elite lines | MLT | | | | MLV | | | | MLW | | | | MPI | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM |
| **1** | 1.15 | 1.72 | 1.41 | 1.43 | 36.95 | 44.28 | 36.43 | 39.22 | 20.89 | 26.35 | 21.78 | 23.00 | 73.57 | 99.33 | 76.36 | 83.09 |
| **2** | 1.36 | 1.62 | 1.61 | 1.53 | 40.10 | 42.37 | 40.44 | 40.97 | 22.11 | 22.36 | 21.49 | 21.98 | 81.03 | 90.95 | 86.47 | 86.15 |
| **3** | 1.31 | 1.55 | 1.84 | 1.57 | 37.45 | 39.23 | 39.73 | 38.80 | 19.52 | 21.86 | 20.30 | 20.56 | 75.54 | 83.36 | 92.62 | 83.84 |
| **4** | 1.12 | 1.44 | 1.38 | 1.31 | 34.70 | 38.76 | 34.94 | 36.13 | 18.93 | 21.35 | 17.63 | 19.30 | 66.41 | 79.71 | 72.73 | 72.95 |
| **5** | 1.14 | 1.37 | 1.41 | 1.31 | 32.24 | 37.64 | 34.55 | 34.81 | 18.26 | 19.02 | 19.19 | 18.82 | 62.14 | 77.79 | 71.14 | 70.35 |
| **6** | 1.36 | 1.62 | 1.45 | 1.48 | 37.09 | 39.50 | 34.05 | 36.88 | 20.43 | 20.91 | 17.40 | 19.58 | 75.95 | 85.51 | 70.71 | 77.39 |
| **7** | 1.48 | 1.86 | 2.04 | 1.80 | 38.12 | 40.65 | 38.41 | 39.06 | 20.27 | 19.91 | 21.70 | 20.63 | 80.00 | 95.46 | 95.26 | 90.24 |
| **8** | 1.54 | 1.79 | 1.90 | 1.75 | 37.76 | 39.42 | 37.03 | 38.07 | 20.75 | 23.06 | 20.35 | 21.39 | 81.92 | 91.76 | 88.10 | 87.26 |
| **9** | 1.59 | 1.54 | 1.89 | 1.67 | 38.00 | 38.16 | 37.68 | 37.95 | 20.06 | 21.26 | 20.06 | 20.46 | 82.63 | 82.73 | 88.82 | 84.73 |
| **10** | 0.93 | 1.21 | 1.28 | 1.14 | 30.01 | 32.54 | 31.90 | 31.48 | 15.21 | 17.87 | 15.45 | 16.18 | 55.55 | 64.19 | 62.65 | 60.79 |
| **11** | 1.54 | 1.70 | 2.24 | 1.83 | 37.44 | 38.08 | 36.20 | 37.24 | 20.64 | 18.53 | 17.75 | 18.97 | 79.78 | 85.43 | 95.47 | 86.89 |
| **12** | 1.19 | 1.72 | 1.68 | 1.53 | 37.06 | 39.22 | 39.33 | 38.54 | 19.05 | 19.92 | 26.84 | 21.94 | 72.04 | 88.31 | 85.42 | 81.92 |
| **13** | 1.76 | 2.14 | 2.26 | 2.06 | 39.07 | 39.60 | 36.85 | 38.51 | 20.13 | 20.67 | 18.42 | 19.74 | 89.58 | 101.36 | 98.50 | 96.48 |
| **Env mean** | 1.34 | 1.64 | 1.72 | 1.57 | 36.61 | 39.19 | 36.73 | 37.51 | 19.71 | 21.00 | 19.87 | 20.20 | 75.09 | 86.61 | 83.40 | 81.70 |
| **LSD** | 0.08 | | | | 1.35 | | | | 1.27 | | | | 5.52 | | | |

MLT=Midline left time, MLV=Midline left value, MLW=Midline left width, MPI=Midline peak integral, DO=Douglas, MA=Marydale, VA=Vaalharts, EM=Elite lines mean, Env=Environmental mean, LSD=Least significant difference

**Table 3.5 Continued**

| Elite lines | MPT | | | | MPV | | | | MPW | | | | MRI | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM |
| **1** | 2.15 | 2.72 | 2.41 | 2.43 | 51.32 | 52.75 | 47.25 | 50.44 | 18.64 | 25.43 | 19.99 | 21.35 | 121.32 | 149.83 | 120.69 | 130.61 |
| **2** | 2.36 | 2.62 | 2.61 | 2.53 | 49.67 | 50.61 | 45.40 | 48.56 | 20.09 | 22.77 | 19.29 | 20.72 | 128.56 | 139.88 | 130.29 | 132.91 |
| **3** | 2.31 | 2.55 | 2.84 | 2.57 | 49.42 | 49.16 | 45.48 | 48.02 | 20.32 | 20.58 | 20.95 | 20.62 | 121.66 | 129.59 | 135.95 | 129.07 |
| **4** | 2.12 | 2.44 | 2.38 | 2.31 | 46.78 | 50.56 | 43.32 | 46.89 | 16.52 | 21.82 | 20.02 | 19.46 | 110.29 | 127.36 | 113.57 | 117.07 |
| **5** | 2.14 | 2.37 | 2.41 | 2.31 | 42.19 | 48.19 | 42.42 | 44.26 | 16.75 | 19.24 | 16.46 | 17.48 | 101.40 | 123.36 | 111.06 | 111.94 |
| **6** | 2.36 | 2.62 | 2.45 | 2.48 | 45.67 | 46.82 | 40.57 | 44.35 | 20.04 | 20.17 | 17.95 | 19.39 | 119.60 | 130.19 | 109.15 | 119.65 |
| **7** | 2.48 | 2.86 | 3.04 | 2.80 | 45.71 | 46.19 | 41.94 | 44.62 | 19.39 | 21.48 | 19.51 | 20.13 | 123.60 | 139.80 | 135.71 | 133.04 |
| **8** | 2.54 | 2.79 | 2.90 | 2.75 | 45.76 | 46.22 | 42.90 | 44.96 | 18.99 | 19.20 | 17.72 | 18.64 | 125.48 | 136.16 | 129.31 | 130.32 |
| **9** | 2.59 | 2.54 | 2.89 | 2.67 | 45.21 | 46.70 | 44.40 | 45.43 | 18.45 | 20.94 | 20.88 | 20.09 | 125.79 | 127.26 | 131.16 | 128.07 |
| **10** | 1.93 | 2.21 | 2.28 | 2.14 | 42.44 | 41.84 | 38.18 | 40.82 | 15.51 | 15.46 | 17.04 | 16.00 | 95.07 | 103.47 | 99.09 | 99.21 |
| **11** | 2.54 | 2.70 | 3.24 | 2.83 | 44.69 | 44.61 | 39.13 | 42.81 | 19.19 | 19.33 | 17.18 | 18.57 | 122.61 | 128.56 | 133.76 | 128.31 |
| **12** | 2.19 | 2.72 | 2.68 | 2.53 | 48.02 | 45.05 | 42.37 | 45.15 | 20.16 | 17.68 | 22.22 | 20.02 | 117.26 | 131.66 | 126.49 | 125.13 |
| **13** | 2.76 | 3.14 | 3.26 | 3.06 | 46.57 | 44.73 | 40.82 | 44.04 | 18.40 | 19.05 | 18.22 | 18.56 | 134.06 | 144.51 | 138.10 | 138.89 |
| **Env mean** | 2.34 | 2.64 | 2.72 | 2.57 | 46.42 | 47.19 | 42.63 | 45.41 | 18.65 | 20.24 | 19.03 | 19.31 | 118.98 | 131.66 | 124.18 | 124.94 |
| **LSD** | 0.16 | | | | 1.77 | | | | 2.06 | | | | 5.89 | | | |

MPT=Midline peak time, MPV=Midline peak value, MPW=Midline peak width, MRI=Midline right integral, DO=Douglas, MA=Marydale, VA=Vaalharts, EM=Elite lines mean, Env=Environmental mean, LSD=Least significant difference

**Table 3.5 Continued**

| Elite lines | MRT | | | | MRV | | | | MTI | | | | MTV | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM |
| **1** | 3.15 | 3.72 | 3.41 | 3.43 | 43.51 | 47.61 | 40.79 | 43.97 | 230.71 | 247.85 | 214.69 | 231.09 | 36.09 | 40.45 | 34.03 | 36.86 |
| **2** | 3.36 | 3.62 | 3.61 | 3.53 | 44.83 | 46.53 | 41.47 | 44.28 | 234.22 | 241.49 | 219.66 | 231.79 | 36.44 | 39.82 | 35.15 | 37.14 |
| **3** | 3.31 | 3.55 | 3.84 | 3.57 | 42.09 | 42.81 | 40.65 | 41.85 | 223.06 | 224.32 | 217.18 | 221.52 | 35.57 | 36.34 | 35.74 | 35.88 |
| **4** | 3.12 | 3.44 | 3.38 | 3.31 | 40.62 | 44.24 | 38.03 | 40.96 | 212.92 | 229.93 | 201.76 | 214.87 | 32.81 | 37.57 | 31.48 | 33.96 |
| **5** | 3.14 | 3.37 | 3.41 | 3.31 | 35.86 | 42.36 | 37.07 | 38.43 | 191.58 | 223.47 | 197.89 | 204.31 | 29.58 | 35.93 | 31.51 | 32.34 |
| **6** | 3.36 | 3.62 | 3.45 | 3.48 | 41.15 | 41.94 | 35.94 | 39.68 | 217.92 | 221.76 | 192.12 | 210.60 | 35.15 | 36.40 | 30.54 | 34.03 |
| **7** | 3.48 | 3.86 | 4.04 | 3.80 | 40.98 | 42.06 | 38.59 | 40.54 | 217.45 | 223.38 | 206.41 | 215.75 | 35.12 | 37.27 | 34.43 | 35.61 |
| **8** | 3.54 | 3.79 | 3.90 | 3.75 | 40.96 | 42.08 | 39.13 | 40.72 | 216.65 | 221.37 | 205.57 | 214.53 | 34.83 | 36.84 | 34.60 | 35.43 |
| **9** | 3.59 | 3.54 | 3.89 | 3.67 | 40.52 | 41.94 | 39.81 | 40.76 | 214.49 | 220.90 | 208.43 | 214.61 | 34.46 | 35.95 | 34.49 | 34.97 |
| **10** | 2.93 | 3.21 | 3.28 | 3.14 | 36.09 | 36.18 | 34.31 | 35.53 | 191.92 | 193.28 | 183.61 | 189.60 | 29.36 | 30.19 | 29.25 | 29.60 |
| **11** | 3.54 | 3.70 | 4.24 | 3.83 | 40.52 | 41.30 | 37.18 | 39.67 | 213.92 | 216.00 | 196.46 | 208.80 | 35.04 | 35.85 | 34.42 | 35.11 |
| **12** | 3.19 | 3.72 | 3.68 | 3.53 | 41.87 | 41.17 | 39.31 | 40.78 | 222.30 | 217.85 | 211.64 | 217.26 | 34.98 | 35.92 | 34.83 | 35.24 |
| **13** | 3.76 | 4.14 | 4.26 | 4.06 | 41.85 | 41.14 | 37.95 | 40.31 | 219.83 | 216.15 | 200.11 | 212.03 | 36.11 | 36.85 | 34.29 | 35.75 |
| **Env mean** | 3.34 | 3.64 | 3.72 | 3.57 | 40.83 | 42.41 | 38.48 | 40.57 | 215.92 | 222.91 | 204.27 | 214.37 | 34.27 | 36.57 | 33.44 | 34.76 |
| **LSD** | 0.16 | | | | 1.34 | | | | 6.26 | | | | 1.12 | | | |

MRT=Midline right time, MRV=Midline right value, MTI=Midline tail integral, MTV=Midline tail value, DO=Douglas, MA=Marydale, VA=Vaalharts, EM=Elite lines mean, Env=Environmental mean, LSD=Least significant difference

**Table 3.6 Environmental means, minimum and maximum for measured quality characters in all three locations**

| Character | Environments | Mean | Min | Max |
|---|---|---|---|---|
| | Douglas | 11.20 | 10.50 | 11.90 |
| **FPC** | Marydale | 11.30 | 11.20 | 11.30 |
| | Vaalharts | 9.90 | 9.20 | 10.70 |
| | Douglas | 55.15 | 35.67 | 65.00 |
| **SDSVOL** | Marydale | 58.69 | 39.33 | 69.00 |
| | Vaalharts | 58.59 | 46.00 | 65.33 |
| | Douglas | 37.04 | 24.73 | 42.79 |
| **EPI** | Marydale | 43.52 | 30.09 | 53.54 |
| | Vaalharts | 43.01 | 30.92 | 54.74 |
| | Douglas | 2.21 | 1.80 | 2.54 |
| **EPT** | Marydale | 2.51 | 2.05 | 3.02 |
| | Vaalharts | 2.61 | 2.25 | 3.47 |
| | Douglas | 56.88 | 50.78 | 62.21 |
| **EPV** | Marydale | 58.07 | 50.20 | 65.98 |
| | Vaalharts | 53.11 | 47.37 | 58.11 |
| | Douglas | 20.76 | 16.78 | 23.51 |
| **EPW** | Marydale | 21.69 | 17.25 | 27.66 |
| | Vaalharts | 20.51 | 16.66 | 26.77 |
| | Douglas | 70.90 | 49.81 | 69.01 |
| **ETI** | Marydale | 82.54 | 56.27 | 91.19 |
| | Vaalharts | 81.24 | 57.45 | 111.45 |
| | Douglas | 37.22 | 31.28 | 40.39 |
| **ETV** | Marydale | 40.79 | 32.40 | 45.40 |
| | Vaalharts | 37.57 | 31.30 | 40.62 |
| | Douglas | 32.46 | 17.85 | 45.94 |
| **MLI** | Marydale | 42.40 | 25.94 | 58.60 |
| | Vaalharts | 43.05 | 26.72 | 59.33 |
| | Douglas | 1.34 | 0.93 | 1.76 |
| **MLT** | Marydale | 1.64 | 1.21 | 2.14 |
| | Vaalharts | 1.72 | 1.28 | 2.26 |

FPC=Flour protein content, SDSVOL=SDS-sedimentation volume, EPI=Envelope peak integral, EPT=Envelope peak time, EPV=Envelope peak value, EPW=Envelope peak width, ETI=Envelope tail integral, ETV=Envelope tail value, MLI=Midline left integral, MLT=Midline left time

**Table 3.6 Continued**

| Character | Environments | Mean | Min | Max |
|---|---|---|---|---|
| | Douglas | 36.61 | 30.01 | 40.10 |
| **MLV** | Marydale | 39.19 | 32.54 | 42.37 |
| | Vaalharts | 36.73 | 31.90 | 40.44 |
| | Douglas | 19.71 | 15.21 | 22.11 |
| **MLW** | Marydale | 21.00 | 17.87 | 22.36 |
| | Vaalharts | 19.87 | 15.45 | 21.49 |
| | Douglas | 75.09 | 55.55 | 89.58 |
| **MPI** | Marydale | 86.61 | 64.19 | 101.36 |
| | Vaalharts | 83.40 | 62.65 | 98.50 |
| | Douglas | 2.34 | 1.93 | 2.76 |
| **MPT** | Marydale | 2.64 | 2.21 | 3.14 |
| | Vaalharts | 2.72 | 2.28 | 3.26 |
| | Douglas | 46.42 | 42.19 | 51.32 |
| **MPV** | Marydale | 47.19 | 48.19 | 52.75 |
| | Vaalharts | 42.63 | 42.42 | 47.25 |
| | Douglas | 18.65 | 15.51 | 20.32 |
| **MPW** | Marydale | 20.24 | 15.46 | 20.58 |
| | Vaalharts | 19.03 | 17.04 | 20.95 |
| | Douglas | 118.98 | 95.07 | 134.06 |
| **MRI** | Marydale | 131.66 | 103.47 | 144.51 |
| | Vaalharts | 124.18 | 99.09 | 138.10 |
| | Douglas | 3.34 | 2.93 | 3.76 |
| **MRT** | Marydale | 3.64 | 3.21 | 4.14 |
| | Vaalharts | 3.72 | 3.28 | 4.26 |
| | Douglas | 40.83 | 35.86 | 44.83 |
| **MRV** | Marydale | 42.41 | 42.36 | 46.53 |
| | Vaalharts | 38.48 | 37.07 | 41.47 |
| | Douglas | 215.92 | 191.58 | 234.22 |
| **MTI** | Marydale | 222.91 | 223.47 | 241.49 |
| | Vaalharts | 204.27 | 197.89 | 219.66 |
| | Douglas | 34.27 | 29.36 | 36.44 |
| **MTV** | Marydale | 36.57 | 30.19 | 39.82 |
| | Vaalharts | 33.44 | 29.25 | 35.15 |

MLV=Midline left value, MLW=Midline left width, MPI=Midline peak integral, MPT=Midline peak time, MPV=Midline peak value, MPW=Midline peak width, MRI=Midline right integral, MRT=Midline right time, MRV=Midline right value, MTI=Midline tail integral, MTV=Midline tail value

**Table 3.7 Correlation for combined analysis between measured quality characteristics**

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **FPC** | **EPV** | 0.53*** | **EPI** | **EPT** | 0.84*** | **EPV** | **EPW** | 0.74*** | **ETI** | **ETV** | 0.83*** | **MLI** | **MLT** | 0.98*** | **MLW** | **MPV** | 0.44*** |
| | **ETV** | 0.30*** | | **EPW** | 0.30*** | | **ETI** | 0.24* | | **MLI** | 0.79*** | | **MLV** | 0.64*** | | **MPW** | 0.66*** |
| | **MLT** | -0.20* | | **ETI** | 0.78*** | | **ETV** | 0.57*** | | **MLT** | 0.72*** | | **MLW** | 0.27** | | **MRV** | 0.56*** |
| | **MLV** | 0.20* | | **ETV** | 0.71*** | | **MLV** | 0.68*** | | **MLV** | 0.74*** | | **MPI** | 0.30*** | | **MTI** | 0.57*** |
| | **MPT** | -0.20* | | **MLI** | 0.90*** | | **MLW** | 0.60*** | | **MLW** | 0.63*** | | **MPT** | 0.98*** | | **MTV** | 0.59*** |
| | **MPV** | 0.61*** | | **MLT** | 0.86*** | | **MPV** | 0.95*** | | **MPI** | 0.31*** | | **MPW** | 0.28** | **MPI** | **MPT** | 0.31*** |
| | **MRI** | -0.35*** | | **MLV** | 0.68*** | | **MPW** | 0.72*** | | **MPT** | 0.72*** | | **MRT** | 0.98*** | | **MRT** | 0.31*** |
| | **MRT** | -0.20* | | **MLW** | 0.40*** | | **MRI** | -0.29** | | **MPW** | 0.53*** | | **MTI** | 0.19* | | **MTV** | 0.22* |
| | **MRV** | 0.59*** | | **MPI** | 0.23* | | **MRV** | 0.94*** | | **MRT** | 0.72*** | | **MTV** | 0.52*** | **MPT** | **MPV** | -0.23* |
| | **MTI** | 0.54*** | | **MPT** | 0.86*** | | **MTI** | 0.93*** | | **MRV** | 0.35*** | **MLT** | **MLV** | 0.51*** | | **MPW** | 0.19* |
| | **MTV** | 0.47*** | | **MPW** | 0.46*** | | **MTV** | 0.80*** | | **MTI** | 0.37*** | | **MPI** | 0.31*** | | **MTV** | 0.40*** |
| **SDSVOL** | **EPI** | 0.44*** | | **MRT** | 0.86*** | **EPW** | **ETI** | 0.55*** | | **MTV** | 0.60*** | | **MPT** | 1.00*** | **MPV** | **MPW** | 0.49*** |
| | **EPT** | 0.35*** | | **MRV** | 0.30*** | | **ETV** | 0.57*** | **ETV** | **MLI** | 0.70*** | | **MPV** | -0.23* | | **MRI** | -0.32*** |
| | **EPV** | 0.33*** | | **MTI** | 0.31*** | | **MLI** | 0.22* | | **MLT** | 0.60*** | | **MPW** | 0.19* | | **MRT** | -0.23* |
| | **EPW** | 0.24** | | **MTV** | 0.59*** | | **MLV** | 0.62*** | | **MLV** | 0.89*** | | **MRT** | 1.00*** | | **MRV** | 0.93*** |
| | **ETI** | 0.40*** | **EPT** | **EPV** | -0.25** | | **MLW** | 0.81*** | | **MLW** | 0.58*** | | **MTV** | 0.40*** | | **MTI** | 0.92*** |
| | **ETV** | 0.54*** | | **ETI** | 0.52*** | | **MPV** | 0.52*** | | **MPI** | 0.27** | **MLV** | **MLW** | 0.62*** | | **MTV** | 0.74*** |
| | **MLI** | 0.41*** | | **ETV** | 0.43*** | | **MPW** | 0.84*** | | **MPT** | 0.60*** | | **MPI** | 0.27** | **MPW** | **MRI** | -0.22* |
| | **MLT** | 0.38*** | | **MLI** | 0.86*** | | **MRV** | 0.62*** | | **MPV** | 0.46*** | | **MPT** | 0.51*** | | **MRT** | 0.19* |
| | **MLV** | 0.53*** | | **MLT** | 0.90*** | | **MTI** | 0.62*** | | **MPW** | 0.57*** | | **MPV** | 0.59*** | | **MRV** | 0.60*** |
| | **MLW** | 0.22* | | **MLV** | 0.34*** | | **MTV** | 0.64*** | | **MRI** | -0.35*** | | **MPW** | 0.64*** | | **MTI** | 0.61*** |
| | **MPI** | 0.28** | | **MPI** | 0.26** | | | | | **MRT** | 0.60*** | | **MRI** | -0.31*** | | **MTV** | 0.64*** |
| | **MPT** | 0.38*** | | **MPT** | 0.90*** | | | | | **MRV** | 0.72*** | | **MRT** | 0.51*** | **MRI** | **MRV** | -0.38*** |
| | **MPV** | 0.30*** | | **MPV** | -0.31*** | | | | | **MTI** | 0.72*** | | **MRV** | 0.81*** | | **MTI** | -0.36*** |
| | **MPW** | 0.30*** | | **MRT** | 0.90*** | | | | | **MTV** | 0.90*** | | **MTI** | 0.83*** | | **MTV** | -0.39*** |
| | **MRT** | 0.38*** | | **MTV** | 0.26** | | | | | | | | **MTV** | 0.91*** | **MRT** | **MTV** | 0.40*** |
| | **MRV** | 0.46*** | | | | | | | | | | | | | **MRV** | **MTI** | 0.99*** |
| | **MTI** | 0.43*** | | | | | | | | | | | | | | **MTV** | 0.92*** |
| | **MTV** | 0.52*** | | | | | | | | | | | | | **MTI** | **MTV** | 0.91*** |

*****p≤0.05, **p≤0.01, ***p≤0.000, FPC=Flour protein content, SDSVOL=SDS-sedimentation volume, EPI=Envelope peak integral, EPT=Envelope peak time, EPV= Envelope peak value, EPW=Envelope peak width, ETI=Envelope tail integral, ETV=Envelope tail value, MLI=Midline left integral, MLT=Midline left time, MLV=Midline left value, MLW=Midline left width, MPI=Midline peak integral, MPT=Midline peak time, MPV=Midline peak value, MPW=Midline peak width, MRI=Midline right integral, MRT=Midline right time, MRV=Midline right value, MTI=Midline tail integral, MTV=Midline tail value

### 3.4.3 Correlations

#### 3.4.3.1 Correlation between observed high molecular weight-glutenin subunits and selected quality characteristics across three locations

In Vaalharts, subunits Bx13+By16 showed significant negative correlations ($p \leq 0.05$) with various quality characteristics (Table 3.8). Subunits Dx2+Dy12 were significantly and negatively correlated ($p \leq 0.01$) with EPT, MLI, MLT, MPT and MRT whereas subunits Dx5+Dy10 were significantly positively correlated ($p \leq 0.01$) with the same characteristics. Significant correlations ($p \leq 0.05$) between subunits Bx7+By8 and various quality characteristics were also observed (Table 3.8).

In Marydale, highly significant negative correlations ($p \leq 0.001$) were observed between SDSVOL, ETV as well as MTV respectively with subunits Bx13+By16. MLI, MLT, MPT and MRT showed significant negative and positive significant correlations ($p \leq 0.05$) respectively with subunits Dx2+Dy12 and Dx5+Dy10, while EPT with both subunit pairs were significantly correlated ($p \leq 0.01$) (Table 3.8).

Only MLW was highly negatively and significantly correlated ($p \leq 0.001$) with subunits Bx13+By16 at Douglas. Subunits Dx2+Dy12 and Dx5+Dy10 were significantly negatively and positively correlated ($p \leq 0.05$) with MLI, MLT, MPT and MRT, respectively (Table 3.8). A significant negative correlation ($p \leq 0.01$) between MLV and subunits Bx13+By16 was observed.

#### 3.4.3.2 Correlations across three locations of high molecular weight-glutenin subunits with measured quality characteristics

There was a negative correlation between subunits Bx13+By16 and SDSVOL, EPW, ETI, ETV, MLI, MLV, MLW, MPI, MPW, MRI, MRV, MTI and MTV, respectively (Table 3.9). EPI and EPV were positively correlated with subunits Bx13+By16. A negative correlation was found between subunits Dx2+Dy12 and EPT, MLI, MLT, MPT, and MRT, respectively. Subunits Bx7+By8 correlated positively with EPI, EPT, MLI, MLT, MPT and MRT. A positive correlation was observed between subunits Dx5+Dy10 and EPI, EPT, MLI, MLT, MPI, MPT and MRT, respectively.

**Table 3.8 Correlation between high molecular weight-glutenin subunits and the quality characteristics for three selected locations**

| Vaalharts | | | Marydale | | | Douglas | | |
|---|---|---|---|---|---|---|---|---|
| **Subunits** | **Character** | **Correlations** | **Subunits** | **Character** | **Correlations** | **Subunits** | **Character** | **Correlation** |
| **Bx13+By16** | **SDSVOL** | -0.67* | **Bx13+By16** | **SDSVOL** | -0.80*** | **Bx13+By16** | **EPI** | -0.70* |
| | **EPI** | -0.56* | | **EPI** | -0.64* | | **EPT** | -0.56* |
| | **ETV** | -0.57* | | **EPT** | -0.57* | | **EPW** | -0.56* |
| | **MLV** | -0.59* | | **EPV** | -0.58* | | **ETI** | -0.60* |
| | **MRT** | -0.61* | | **ETI** | -0.71** | | **ETV** | -0.64* |
| | **MRV** | -0.61* | | **ETV** | -0.80*** | | **MLI** | -0.56* |
| | **MTV** | -0.62* | | **MLI** | -0.57* | | **MLV** | -0.72** |
| **Dx2+Dy12** | **EPI** | -0.66* | | **MLV** | -0.74** | | **MLW** | -0.81*** |
| | **EPT** | -0.75** | | **MPI** | -0.68** | | **MPI** | -0.63* |
| | **MLI** | -0.75** | | **MPW** | -0.59* | | **MPW** | -0.62* |
| | **MLT** | -0.79** | | **MRI** | -0.74** | | **MRI** | -0.66* |
| | **MPI** | -0.65* | | **MRV** | -0.68** | | **MRV** | -0.57* |
| | **MPT** | -0.79** | | **MTI** | -0.69** | | **MTI** | -0.58* |
| | **MRT** | -0.79** | | **MTV** | -0.80*** | **Dx2+Dy12** | **MLI** | -0.57* |
| **Dx5+Dy10** | **EPI** | 0.66* | **Dx2+Dy12** | **EPT** | -0.70** | | **MLT** | -0.61* |
| | **EPT** | 0.75** | | **MLI** | -0.57* | | **MPT** | -0.61* |
| | **MLI** | 0.75** | | **MLT** | -0.64* | | **MRT** | -0.61* |
| | **MLT** | 0.79** | | **MPT** | -0.64* | **Dx5+Dy10** | **MLI** | 0.57* |
| | **MPI** | 0.65* | | **MRT** | -0.64* | | **MLT** | 0.61* |
| | **MPT** | 0.79** | **Dx5+Dy10** | **EPT** | 0.70** | | **MPT** | 0.61* |
| | **MRT** | 0.79** | | **MLI** | 0.57* | | **MRT** | 0.61* |
| **Bx7+By8** | **EPI** | 0.68* | | **MLT** | 0.64* | | | |
| | **MLI** | 0.61* | | **MPT** | 0.64* | | | |
| | **MLT** | 0.61* | | **MRT** | 0.64* | | | |
| | **MPI** | 0.62* | | | | | | |
| | **MPT** | 0.61* | | | | | | |
| | **MRI** | 0.63* | | | | | | |
| | **MRT** | 0.61* | | | | | | |
| | **MTV** | 0.63* | | | | | | |

*P≤0.05, **P≤0.01, ***P≤0.001, SDSVOL=SDS-sedimentation volume, EPI=Envelope peak integral, EPT=Envelope peak time, EPV=Envelope peak value, EPW=Envelope peak width, ETI=Envelope tail integral, ETV=Envelope tail value, MLI=Midline left integral, MLT=Midline left time, MLV=Midline left value, MLW=Midline left width, MPI=Midline peak integral, MPT=Midline peak time, MPW=Midline peak width, MRI=Midline right integral, MRT=Midline right time, MRV=Midline right value, MTI=Midline tail integral, MTV=Midline tail value, SDS-PAGE=Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

**Table 3.9 Significant correlations between high molecular weight-glutenin subunits and selected quality characteristics across locations**

| Combined | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Subunits** | **Character** | **Correlation** | **Subunits** | **Character** | **Correlation** | **Subunits** | **Character** | **Correlation** |
| **Bx13+By16** | **SDSVOL** | -0.81*** | **Dx2+Dy12** | **EPI** | -0.60* | **Dx5+Dy10** | **EPI** | 0.60* |
| | **EPI** | 0.71** | | **EPT** | -0.77** | | **EPT** | 0.77** |
| | **EPV** | 0.62* | | **MLI** | -0.70** | | **MLI** | 0.70** |
| | **EPW** | -0.61* | | **MLT** | -0.74** | | **MLT** | 0.74** |
| | **ETI** | -0.65* | | **MPI** | -0.58* | | **MPI** | 0.58* |
| | **ETV** | -0.73** | | **MPT** | -0.74** | | **MPT** | 0.74** |
| | **MLI** | -0.57* | | **MRT** | -0.74** | | **MRT** | 0.74** |
| | **MLV** | -0.76** | **Bx7+By8** | **EPI** | 0.63* | | | |
| | **MLW** | -0.68** | | **EPT** | 0.56* | | | |
| | **MPI** | -0.67* | | **MLI** | 0.57* | | | |
| | **MPW** | -0.68* | | **MLT** | 0.57* | | | |
| | **MRI** | -0.73** | | **MPT** | 0.57* | | | |
| | **MRV** | -0.68** | | **MRT** | 0.57* | | | |
| | **MTI** | -0.69** | | | | | | |
| | **MTV** | -0.78** | | | | | | |

*****p≤0.05, **p≤0.01, ***p≤0.001, SDSVOL=SDS-sedimentation volume, EPI=Envelope peak integral, EPV=Envelope peak value, EPW=Envelope peak width, ETI=Envelope tail integral, ETV=Envelope tail value, MLI=Midline left integral, MLV=Midline left value, MLW=Midline left width, MPI=Midline peak integral, MPW=Midline peak width, MRI=Midline right integral, MRV=Midline right value, MTI=Midline tail integral, MTV=Midline tail value, EPT=Envelope peak time, MPT=Midline peak time, MLT=Midline left time, MRT=Midline right time

### 3.5 Discussion

Protein quality is one of the most important quality factors in wheat grain (Dessalegn et al., 2011). Most genotypes analysed presented alleles that are mostly associated with good gluten strength and bread making quality. These results were in agreement with Khatkar (2006) who reported that HMW-GS Dx5+Dy10, Ax1, Ax2*, Bx17+By18 and Bx7+By8 were superior for bread making. In addition, Huang and Khan (1997) reported that subunits Dx5 and Ax2*, being large in molecular size, contribute most to the dough mixing properties.

Previous studies based on the segregation of subunits, state that variation is caused by the allelic genes occurring at five loci coding for high molecular weight-glutenin subunit. The long arm of chromosome 1D has two allelic genes, which control the 1Dx and 1Dy subunits; two on the long arm of chromosome 1B (Bietz et al., 1975) controlling the 1Bx and 1By subunits but only one allelic gene on the long arm of chromosome 1A controlling all three alleles (Payne et al., 1980). Payne et al. (1981) reported from their studies based on segregation, that the Bx and By genes of the Glu-B1 are tightly linked. The three alleles associated with the *Glu-A1* locus were all observed in this set of genotypes, even though the null allele was only found in one genotype (elite line 6).

Allelic combinations Dx2+Dy12 and Dx5+Dy10 have been associated with weak and strong gluten quality, respectively (Payne et al., 1987) and therefore have a crucial role in bread quality (Payne et al., 1987; Johansson et al., 1993) even though they vary in gluten strength. Subunits Dx2+Dy12 have been proven to cause lower gluten strength compared to subunits Dx5+Dy10 (Johansson and Svensson, 1999). Rasheed et al. (2012) reported that bread making quality is also influenced by the presence of some *Glu-B1* subunits such as Bx7+By8, Bx7+By9, Bx17+By18 and Bx13+By16 in a superior way. In this study, high levels of variation were reported at the *Glu-B1* locus compared to the other two loci.

Peak time is the most frequently used mixograph character in the industry, however, most of the studies using the mixograph reported the results from only one or few variables (mostly peak time and mixing tolerance) (Martinant et al., 1998). In this study 19 variables of the mixograph were reported. Most researchers reported the poor performance of MPT in explaining bread making quality as measured by loaf volume (Branlard et al., 1991; Dong et al., 1992; Khatkar et al., 1996). But Primard et al. (1991) reported a positive correlation between HMW-GS Dx5+Dy10, a subunit pair normally related to good bread making quality and MPT. These results are consistent with the

results of this study where MPT was positively correlated with subunits Dx5+Dy10 across locations.

Mixograph peak time, peak height and curve-width are determined by protein quality and protein quantity as well as the water-absorption of the specific flour (Martinant, 1998). Peak time has been reported to be largely genetically determined (van Lill, 1992) and the most frequently used mixograph parameter in literature. In this study across all environments there was a highly significant difference between genotypes and environments for EPT, with no significant GxE interaction. The same was observed for MPT. Nevertheless, MPT has been found to be a poor parameter to explain bread making quality (Branlard et al., 1991; Dong et al., 1992; Khatkar et al., 1996).

For determination of baking quality of breeding lines, SDSVOL is a good method, as it is rapid and not labour intensive. In this study, SDSVOL had no correlation with the FPC but significant correlations with several mixograph parameters were observed. Selecting genotypes that have higher SDSVOL may result in stronger dough characteristics in the next generations, which is not always ideal in all breeding programmes since the South African market prefers medium strength dough (Oelofse, 2008). van Lill et al. (1995) warned against breeding of excessively strong dough.

In this study, the wheat breeding lines exhibiting a positive correlation between measured quality characteristics and SDSVOL suggest that they are of good quality. The findings are consistent to those of Baker and Campbell, 1971 and Djojkovic et al. (2010).

### 3.6 Conclusions

Highly significant correlations between HMW-GS and some quality characteristics were observed. Subunits Bx13+By16 were the only subunits highly correlated ($p \leq 0.001$) with SDSVOL. Mixograph parameters which were included in this study showed their potential usefulness in predicting bread making quality. Both genotype and environment significantly influenced measured quality characteristics and the HMW-GS. Based on the results of this study, it is clear that some HMW-GS are more effective at conferring good bread making quality than others.

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# Chapter 4

## The relationship between SDS-sedimentation volume and protein fractions determined by size exclusion-high performance liquid chromatography

### 4.1 Abstract

The objective of this study was to determine the relationship between SDS-sedimentation volume and protein fractions of 13 wheat elite lines using size exclusion-high performance liquid chromatography (SE-HPLC). This technique fractionated proteins into four peaks. Peak 1 contained the glutenins, peak 2 the gliadins, peak 3 was signified as low molecular weight monomeric gliadins and peak 4 was albumins and globulins. The peak area percentage values were used to determine the correlation between different protein fractions and the selected quality characteristics. Relative and absolute amounts of total polymeric proteins, determined by SE-HPLC, after sonication, showed variable (significant to non-significant) relationships with selected mixograph characteristics. Genotype and environment had highly significant effects on protein fractions. In a combined analysis, flour protein content correlated highly and negatively with small polymeric protein of soluble protein fraction while it correlated highly and positively with small monomeric protein of soluble protein fraction. SDS-sedimentation volume correlated negatively with small polymeric protein and correlated positively with the large monomeric protein. Midline right integral showed a highly significant negative correlation with large monomeric protein. Negative correlations were observed between SDS-sedimentation volume and two fractions namely large monomeric protein and small monomeric protein, whereas small polymeric protein correlated positively with SDS-sedimentation volume. For relative value, large unextractable polymeric proteins correlated highly and significantly with envelope tail value, midline left value, midline right value, midline tail integral and midline tail value. Midline right integral was highly and negatively correlated with large unextractable polymeric proteins and unextractable polymeric proteins. Highly and significant correlation was observed between unextractable polymeric proteins and SDS-sedimentation volume. Large unextractable polymeric proteins, unextractable polymeric proteins and high molecular weight correlated positively with flour protein content. For absolute value, midline right integral correlated positively and negatively with total polymeric proteins and high molecular weight, respectively. A significant correlation between large unextractable polymeric

proteins and SDS-sedimentation volume was observed. Only high molecular weight proteins correlated highly and significantly with flour protein content. In single location analysis, relationship between SDS-sedimentation volume and some protein fractions (specifically small polymeric protein) was observed at all locations. Significant positive and negative correlations occurred for SDS-sedimentation volume with small polymeric protein and large monomeric protein for Douglas, respectively. The only negative correlation was observed between SDS-sedimentation volume and small monomeric protein for Vaalharts.

### 4.2 Introduction

Breeders often have to test many wheat lines before finding a high quality line suitable for bread making. Early generation lines are produced in limited quantities, which do not allow baking tests to be conducted. Therefore, the ability to estimate bread quality using limited sample sizes will be highly beneficial in wheat breeding programmes (Dowell et al., 2008). Protein composition has been used to determine bread making quality of wheat flour (Kim et al., 1988; Singh et al., 1990a; Gupta et al., 1992; Ciaffi et al., 1996; Gianibelli et al., 2001) and based on their solubility the proteins have been classified into four types: glutenins and gliadins, the latter being soluble in acid, and the former in alcohol and albumin which is soluble in water and globulin, soluble in alkaline solutions (Morojele and Labuschagne, 2014).

Wheat proteins differ in their state of solubility. There are the soluble and insoluble proteins. The insoluble proteins require sonication after extraction of the soluble proteins using a SDS buffer. After the stepwise extraction, both extractions can be fractionated by HPLC. Wheat protein is composed of polymeric and monomeric proteins, dominated by the HMW-GS. Different types of techniques have been used to study these proteins, and of these, SE-HPLC has been one of the most informative, particularly in characterising the effect of protein molecular weight distribution on baking potential (Millar, 2003). Therefore, it was essential to find a technique that is accurate and can be used as a tool in breeding programmes.

SE-HPLC is a valuable technique widely used for measuring the relative proportions of the main endosperm proteins (glutenins, gliadins, albumins, and globulins) of bread wheat and other cereals such as durum wheat and triticale (Larroque et al., 2000) and to study the functional effects of gluten components that differ in their degree of polymerization (Dachkevitch and Autran, 1989; Singh et al., 1990b; Gupta et al., 1993, 1995; Rao et al., 2001). This technique has been found to be most successful because

of its speed, automation, quantitative ability and the sample size it requires for analysis is small (Bietz, 1985). On top of that all, the technique can also be non-destructive if only a part of the endosperm is used in the extraction of the proteins.

Southan and MacRitchie (1999) did a review on the structure-functionality relationship of gluten. Each protein is represented by peaks produced during SE-HPLC separation. Peak 1 consist of large polymeric proteins (LPP) (glutenins) (Batey et al., 1991, Larroque et al., 1997), peak 2 is made up of smaller polymeric proteins (SPP), peak 3 is composed of large monomeric proteins (LMP), mainly gliadins, and small monomeric proteins (SMP) mainly albumins and globulins are represented by peak 4 (Labuschagne and Aucamp, 2004). The bread making quality of wheat flour has been associated with these fractions in several studies and they are considered the most important proteins in wheat grains (Singh et al., 1990b; Batey et al., 1991). These proteins are composed of polymers of HMW-GS joined by disulfide bonds. For over three decades HMW-GS have been widely studied (Payne et al., 1981; Shewry et al., 1992). According to Popineau et al. (1994) and Gupta et al. (1995) these subunits are the major proteins contributing to dough properties.

Most SE-HPLC fractions have been associated with some dough mixing properties and some of the bread making characteristics (Singh et al., 1990b; Batey et al., 1991; Larroque et al., 2000; Morel et al., 2000; Ohm et al., 2010; Tsilo et al., 2010). This was also supported by the study conducted by Ohm et al. (2009), in which they reported a significant correlation between the mixograph water absorption and mixing tolerance, and HMW polymeric protein fractions. The aim of this study was to determine correlations between protein fractions and selected measured quality characteristics using SE-HPLC in order to select breeding lines with good quality for further use in breeding programmes.

## 4.3 Material and methods

### 4.3.1 Plant material

Elite lines from the irrigation breeding programme were used as described in Chapter 3 (section 3.3.1).

### 4.3.2 Measured quality characteristics

Quality characteristics are the same as described in Chapter 3 (3.3.2).

### 4.3.3 Size exclusion-high performance liquid chromatography

For this study, in order to analyse the amount and size of distribution of polymeric proteins, the two-step extraction procedure for SE-HPLC developed by Gupta et al. (1993) was applied. For total protein analysis, samples were extracted using SDS-soluble (without sonication) and SDS-insoluble (with sonication) protein. Flour samples (17.0 mg) were extracted with 1.5 ml of 0.5% SDS-phosphate buffer (pH of 6.9) and vortexed for 10 sec. Samples were then shaken for 5 min using a mechanical shaker at 5000 rpm and centrifuged at 10 000 rpm for 30 min, to obtain a supernatant of SDS-soluble proteins. The supernatant was then filtered into glass vials using a 0.45 µm HT Tuffryn Acrodisc® syringe filter. Then to suppress protease activity, the glass vials were incubated at 80°C for 2 min (Larroque and Békés, 2000).

The remaining pellet was re-suspended in 1.5 ml of 0.5% SDS- phosphate buffer (pH of 6.9), vortexed for 10 sec then subjected to 30 sec of sonication in an ultrasonic disintegrator (Branson B12 Sonifier) fitted with a 3 mm exponential tip, at an amplitude of 5, and then centrifuged at 10 000 rpm for 30 min after it was shaken for 5 min at 5000 rpm. The supernatant was filtered through a 0.45 µm HT Tuffryn Acrodisc® syringe filter into a glass vial. Samples were immediately heated in a water bath for 2 min at 80°C (Larroque and Békés, 2000), and then placed in the HPLC auto-sampler for automatic injection.

A Biosep SEC-S 4000 column (Phenomenex, Torrance, CA, USA) in a LC-20AT Shimazu HPLC system was used. The system was equipped with Class VP chromatography data software for integration events and an automated sample injector and a binary pump which was used to fractionate 20 µl aliquots of each extracted protein sample. Separation was achieved after 15 min on an isocratic gradient of water with trifluoroacetic acid (TFA) *(*0.1%, v/v) and acetonitrile (LiChrosolv® Merck) + TFA (99.9/0.1%, v/v). Eluent was 50% acetonitrile and 50% water with a flow rate of 0.4 ml $min^{-1}$ ambient temperate. Protein fractions were detected at 210 nm.

Areas under the peaks were calculated according to Gupta et al. (1993). The following fractions were measured at approximate time intervals: F1, LPP (4.57 to 5.54 min), F2, SPP (5.54 to 6.98 min); F3, LMP (6.98 to 7.7 min) and F4, SMP (7.7 to 8.61 min up to where the trace cut the baseline) (Ohm et al., 2009).

The HPLC fractions were: (a) SDS-soluble (b) SDS-insoluble, where 1 = larger polymeric proteins (LPP), 2 = smaller polymeric proteins (SPP), 3 = larger monomeric proteins

(LMP) mainly gliadins, 4 = smaller monomeric proteins (SMP) mainly albumins and globulins (Figures 4.1 and 4.2).

**Protein fractions**

The protein fractions were calculated based on the percentage of the respective areas relative to the HPLC area. In all cases the protein fractions were determined as relative and absolute value corresponding to FPC.

**Relative value**

Relative value were calculated using the following formulas:

**Percentage of large unextractable polymeric proteins**

LUPP = [(F1 insoluble)/ (F1 soluble + F1 insoluble)] x 100

**Percentage unextractable polymeric proteins**

UPP = [(F1 insoluble + F2 insoluble)/ (F1 soluble + F2 soluble + F1 insoluble + F2 insoluble)] x 100

**Percentage total polymeric proteins**

POL = [(F1 soluble + F2 soluble + F1 insoluble + F2 insoluble)/ (F1 soluble + F2 soluble + F3 soluble + F4 soluble + F1 insoluble + F2 insoluble + F3 insoluble + F4 insoluble)] x 100

**Percentage high molecular weight**

HMW = [(F1 soluble + F1 insoluble)/ (F1 soluble + F2 soluble + F3 soluble + F4 soluble + F1 insoluble + F2 insoluble + F3 insoluble + F4 insoluble)] x 100

**Percentage low molecular weight**

LMW = [(F2 soluble + F2 insoluble)/ (F1 soluble + F2 soluble + F3 soluble + F4 soluble + F1 insoluble + F2 insoluble + F3 insoluble + F4 insoluble)] x 100

**Percentage total monomeric proteins**

MON = [(F3 soluble + F4 soluble + F3 insoluble + F4 insoluble)/ (F1 soluble + F2 soluble + F3 soluble + F4 soluble + F1 insoluble + F2 insoluble + F3 insoluble + F4 insoluble)] x 100

**Absolute value**

Absolute value were calculated as percentage of protein fractions of the grain multiplied by FPC divided by 100.

### 4.3.4 Statistical analysis

The analysis of variance was conducted to test the significance of genotype, environment and genotype by environment interactions across locations. Correlations between quality characteristics and protein fractions were determined. These statistical analyses were performed using Agrobase Generation II (2015).

## 4.4 Results

### 4.4.1 Size exclusion-high performance liquid chromatography graphs for SDS-soluble and SDS-insoluble fractions

Examples of chromatographs for SDS-soluble (Figure 4.1) and SDS-insoluble (Figure 4.2) separation by HPLC of the elite lines.

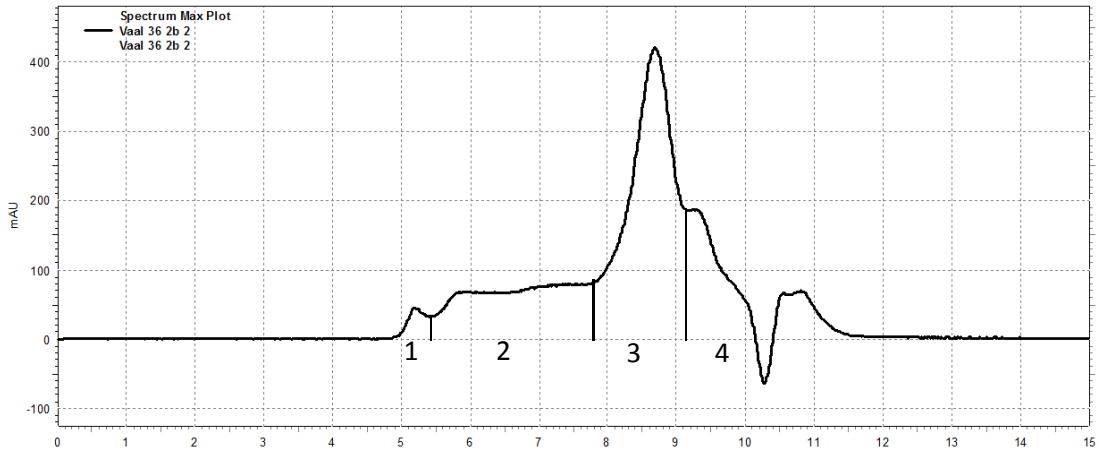


**Figure 4.1 Profile of size exclusion-high performance liquid chromatography soluble fractions.**

**1 = Large polymeric proteins, 2 = Small polymeric proteins, 3 = Large monomeric proteins, 4 = Small monomeric proteins**

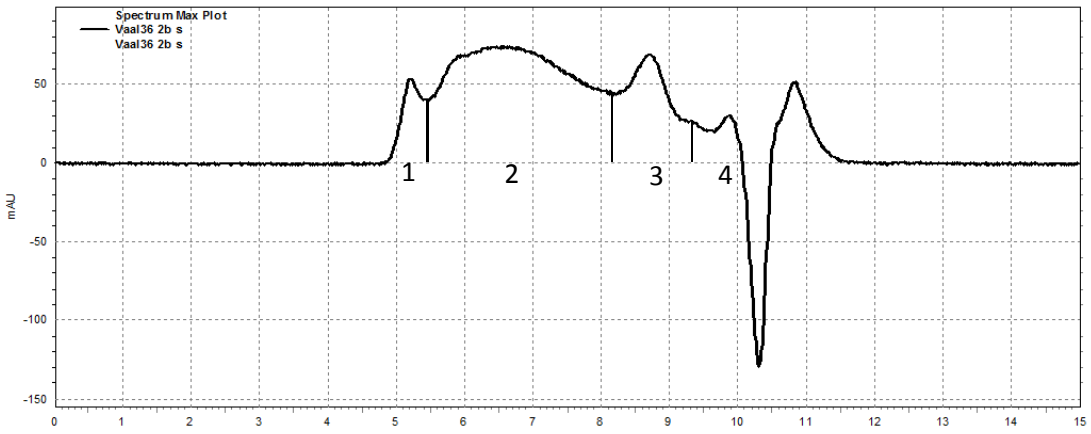


**Figure 4.2 Profile of size exclusion-high performance liquid chromatography insoluble fractions.**

**1 = Large polymeric proteins, 2 = Small polymeric proteins, 3 = Large monomeric proteins, 4 = Small monomeric proteins**

### 4.4.2 Analysis of variance

#### 4.4.2.1 Combined ANOVA of size exclusion-high performance liquid chromatography fractions across the three locations

**Soluble fractions**

Genotype, GxE interaction and environment effects were highly significant (p≤0.001) (Table 4.1) for LPP, SPP and SMP fractions. Environment effect was significant (p≤0.05) for the LMP fraction. The LPP, SPP, LMP and SMP means for the elite lines (Table 4.3) differed between 1.7-2.49, 24.34-28.05, 51.50-55.46 and 16.82-18.89, respectively. The largest variation was attributed to the environment (72.24%) for SMP and genotype (48.14%) for LMP (Table 4.2).

Environmental means ranged between 1.90 (Vaalharts) and 2.02 (Douglas) for LPP. For SPP it ranged between 24.05 (Marydale) and 27.78 (Douglas) and for LMP it varied between 53.19 (Douglas) and 53.80 (Marydale). Lastly environmental mean for SMP ranged between 16.57 (Vaalharts) and 20.20 (Marydale).

**Insoluble fractions**

Genotype and GxE interaction effects were highly significant (p≤0.01) for the LPP fraction. Genotype, GxE as well as environmental effects were highly significant (p≤0.001) for SPP and LMP fractions. Genotype and environment effects were highly

significant (p≤0.001) for the SMP fraction (Table 4.1). Environment was the largest contributor (68.78%) to the total variation for LPP and for LMP (50.55%) (Table 4.2).

**Relative value percentages**

Genotype, GxE interaction and environment effects were highly significant (p≤0.001) for LUPP, UPP, POL, HMW, LMW and MON (Table 4.1). Environment was the largest contributor (63.30%, 60.99% and 59.02%) to the total variation for HMW, LUPP and UPP, respectively, (Table 4.2).

**Absolute value percentages**

Genotype effect was significant (p≤0.05) for UPP. Environment effects were highly significant (p≤0.001) for POL, HMW and LMW fractions. Environment and GxE interaction effects were significant (p≤0.05) for the LUPP fraction (Table 4.1). The largest variation was contributed by the genotype (44.53%) for UPP and GXE (14.29%, 21.38%, 26.45% and 21.68%), respectively, Table 4.2.

**Table 4.1 Combined analysis of variance for protein fractions in the flour**

| | Protein fraction | Genotype | Environment | GxE |
|---|---|---|---|---|
| **SDS soluble** | LPP | 0.49*** | 0.99*** | 0.46*** |
| | SPP | 14.75*** | 174.92*** | 6.68*** |
| | LMP | 18.93*** | 5.76* | 5.60*** |
| | SMP | 3.66*** | 159.13*** | 1.64*** |
| **SDS insoluble** | LPP | 4.17** | 229.93*** | 2.76** |
| | SPP | 21.84*** | 61.70*** | 24.10*** |
| | LMP | 24.30*** | 593.52*** | 15.68*** |
| | SMP | 1.77*** | 7.43*** | 0.37* |
| **Relative value %** | LUPP | 48.87*** | 1789.27*** | 40.94*** |
| | UPP | 12.91*** | 301.29*** | 8.06*** |
| | POL | 15.85*** | 13.48*** | 7.72*** |
| | HMW | 1.42*** | 56.19*** | 0.97*** |
| | LMW | 9.93*** | 21.21*** | 7.66*** |
| | MON | 0.002*** | 0.003*** | 0.001*** |
| **Absolute value %** | LUPP | 0.73** | 0.84* | 0.47* |
| | UPP | 0.50* | $0.29^{ns}$ | $0.30^{ns}$ |
| | POL | $0.18^{ns}$ | 0.79*** | $0.14^{ns}$ |
| | HMW | 0.005** | 0.12*** | 0.003* |
| | LMW | $0.16^{ns}$ | 1.23*** | $0.14^{ns}$ |
| | MON | 0.00 | 0.00 | 0.00 |

*p≤0.05, **p≤0.01, ***p≤0.001, ns=Not significant, SE-HPLC=Size exclusion-high performance liquid chromatography, SDS=Sodium dodecyl sulphate, LPP=Large polymeric proteins, SPP=Small polymeric proteins, LMP=Large monomeric proteins, SMP=Small monomeric proteins, LUPP=Large unextractable polymeric proteins, UPP=Unextractable polymeric proteins, POL=Total polymeric proteins, HMW=High molecular weight, LMW=Low molecular weight, MON=Total monomeric proteins, GxE=Genotype by environment interaction

**Table 4.2 Contribution of sources of variation to total variation (%) for each measured protein fraction combined across the three locations**

| Source | Fractions | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | SDS soluble fraction | | | | SDS insoluble fraction | | | |
| | LPP | SPP | LMP | SMP | LPP | SPP | LMP | SMP |
| **Genotype** | 24.27 | 23.24 | 48.14 | 9.97 | 7.48 | 17.62 | 12.41 | 33.87 |
| **Environment** | 8.26 | 45.94 | 2.44 | 72.24 | 68.78 | 8.29 | 50.55 | 23.75 |
| **GxE** | 46.22 | 21.04 | 28.46 | 8.96 | 9.98 | 38.87 | 16.02 | 14.19 |

| | Relative value | | | | | | Absolute value | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HMW | LMW | LUPP | UPP | POL | MON | HMW | LMW | LUPP | UPP | POL | MON |
| **Genotype** | 9.59 | 23.53 | 9.99 | 14.40 | 30.77 | 33.33 | 12.24 | 11.87 | 20.59 | 44.53 | 13.47 | 0.00 |
| **Environment** | 63.30 | 8.38 | 60.99 | 56.02 | 10.19 | 9.68 | 48.98 | 15.70 | 3.95 | 1.81 | 8.90 | 0.00 |
| **GxE** | 13.12 | 36.30 | 16.75 | 17.99 | 29.99 | 33.33 | 14.29 | 21.38 | 26.45 | 23.00 | 21.68 | 0.00 |

SDS=Sodium dodecyl sulphate, GXE=Genotype by environment interaction, LPP=Large polymeric proteins, SPP=Small polymeric proteins, LMP=Large monomeric proteins, SMP=Small monomeric proteins, HMW=High molecular weight, LMW=Low molecular weight, LUPP=Large unextractable polymeric proteins, UPP=Unextractable polymeric proteins, POL=Total polymeric proteins, MON=Total monomeric proteins, GxE=Genotype by environment interaction

**Table 4.3 Means for soluble protein fractions at Douglas, Marydale and Vaalharts**

| Elite lines | LPP | | | | SPP | | | | LMP | | | | SMP | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM |
| **1** | 2.36 | 1.53 | 2.26 | 2.05 | 27.75 | 21.51 | 28.04 | 25.65 | 52.94 | 57.08 | 53.80 | 54.61 | 16.95 | 20.23 | 15.70 | 17.70 |
| **2** | 2.36 | 0.73 | 2.02 | 1.70 | 27.56 | 19.54 | 27.77 | 24.96 | 54.08 | 58.26 | 54.04 | 55.46 | 16.02 | 21.47 | 16.18 | 17.89 |
| **3** | 2.34 | 0.77 | 1.99 | 1.70 | 27.46 | 18.95 | 26.60 | 24.34 | 52.82 | 57.48 | 54.96 | 55.09 | 17.38 | 22.82 | 16.46 | 18.89 |
| **4** | 2.23 | 2.25 | 2.08 | 2.19 | 27.14 | 25.96 | 30.35 | 27.82 | 53.49 | 51.38 | 51.17 | 52.01 | 17.15 | 20.42 | 16.42 | 18.00 |
| **5** | 2.54 | 2.51 | 2.42 | 2.49 | 28.57 | 27.07 | 29.57 | 28.40 | 51.92 | 50.57 | 52.03 | 51.50 | 16.98 | 19.86 | 16.00 | 17.61 |
| **6** | 2.40 | 2.36 | 1.96 | 2.24 | 28.49 | 25.19 | 28.19 | 27.29 | 51.84 | 52.08 | 53.04 | 52.32 | 17.28 | 20.37 | 16.82 | 18.16 |
| **7** | 2.03 | 2.05 | 1.88 | 1.99 | 27.90 | 24.49 | 27.13 | 26.51 | 52.71 | 53.49 | 54.92 | 53.71 | 17.36 | 19.95 | 16.08 | 17.80 |
| **8** | 2.20 | 2.32 | 2.06 | 2.19 | 27.83 | 25.39 | 28.07 | 27.10 | 53.63 | 53.47 | 54.25 | 53.78 | 16.35 | 18.83 | 15.70 | 16.96 |
| **9** | 2.25 | 2.33 | 1.67 | 2.08 | 28.27 | 25.45 | 26.36 | 26.69 | 53.44 | 53.57 | 55.39 | 54.13 | 16.06 | 18.67 | 16.58 | 17.10 |
| **10** | 2.16 | 2.29 | 1.78 | 2.08 | 29.19 | 26.38 | 28.58 | 28.05 | 51.16 | 51.56 | 52.04 | 51.59 | 17.49 | 19.78 | 17.60 | 18.29 |
| **11** | 1.98 | 2.19 | 1.62 | 1.93 | 27.36 | 24.64 | 26.24 | 26.08 | 55.52 | 54.70 | 55.34 | 55.18 | 15.16 | 18.49 | 16.81 | 16.82 |
| **12** | 2.03 | 2.25 | 1.67 | 1.98 | 27.20 | 25.60 | 27.27 | 26.69 | 53.31 | 51.17 | 53.51 | 52.66 | 17.48 | 21.00 | 17.56 | 18.68 |
| **13** | 1.79 | 1.97 | 1.33 | 1.70 | 26.38 | 22.79 | 25.24 | 24.80 | 54.57 | 54.59 | 56.14 | 55.10 | 17.27 | 20.66 | 17.79 | 18.41 |
| Env mean | 2.02 | 1.96 | 1.90 | 2.02 | 27.78 | 24.05 | 27.65 | 26.49 | 53.19 | 53.80 | 53.90 | 53.63 | 16.84 | 20.20 | 16.57 | 17.87 |
| LSD | | | | 0.21 | | | | 0.78 | | | | 0.89 | | | | 0.56 |
| R-squared | | | | 0.79 | | | | 0.90 | | | | 0.80 | | | | 0.92 |

LPP=Large polymeric proteins, SPP=Small polymeric proteins, LMP=Large monomeric proteins, DO=Douglas, MA=Marydale, VA=Vaalharts, EM=Elite line means, Env=Environmental mean, LSD=Least significant difference

**Table 4.4 Means for insoluble protein fractions at Douglas, Marydale and Vaalharts**

| Elite lines | LPP | | | | SPP | | | | LMP | | | | SMP | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM |
| **1** | 4.44 | 6.94 | 5.14 | 5.51 | 58.36 | 58.62 | 54.33 | 57.10 | 29.10 | 25.62 | 32.56 | 29.09 | 8.11 | 8.83 | 7.99 | 8.31 |
| **2** | 5.05 | 7.77 | 6.29 | 6.37 | 55.77 | 63.12 | 54.91 | 57.93 | 31.26 | 20.67 | 30.24 | 27.49 | 7.93 | 8.46 | 8.27 | 8.22 |
| **3** | 5.51 | 10.08 | 5.78 | 7.12 | 55.53 | 59.19 | 57.23 | 57.32 | 30.75 | 21.83 | 29.46 | 27.35 | 8.23 | 8.91 | 7.55 | 8.23 |
| **4** | 6.15 | 11.58 | 4.65 | 7.46 | 56.05 | 57.66 | 62.23 | 58.65 | 29.78 | 22.32 | 25.08 | 25.73 | 8.03 | 8.45 | 8.05 | 8.18 |
| **5** | 7.38 | 10.44 | 4.27 | 7.36 | 55.86 | 58.45 | 56.94 | 57.08 | 28.85 | 22.69 | 30.36 | 27.3 | 7.91 | 8.44 | 8.45 | 8.27 |
| **6** | 5.36 | 10.50 | 4.52 | 6.80 | 61.33 | 56.77 | 60.83 | 59.64 | 25.68 | 23.71 | 26.83 | 25.41 | 7.65 | 9.03 | 7.82 | 8.07 |
| **7** | 5.97 | 9.89 | 4.53 | 6.80 | 54.98 | 56.36 | 57.18 | 56.17 | 30.84 | 24.66 | 30.54 | 28.68 | 8.22 | 9.09 | 7.76 | 8.36 |
| **8** | 5.27 | 9.27 | 4.65 | 6.39 | 60.08 | 59.46 | 59.99 | 59.84 | 27.76 | 23.21 | 28.42 | 26.46 | 6.91 | 8.07 | 6.94 | 7.31 |
| **9** | 3.98 | 9.35 | 3.86 | 5.73 | 60.90 | 56.86 | 53.17 | 56.98 | 28.07 | 25.22 | 34.77 | 29.35 | 7.06 | 8.57 | 8.20 | 7.94 |
| **10** | 4.36 | 9.48 | 4.31 | 6.05 | 57.36 | 54.55 | 52.53 | 54.81 | 29.69 | 26.04 | 34.01 | 29.91 | 8.61 | 9.94 | 9.16 | 9.24 |
| **11** | 5.01 | 8.98 | 4.72 | 6.24 | 54.2 | 58.54 | 51.37 | 54.70 | 32.89 | 24.32 | 35.23 | 30.81 | 7.91 | 8.17 | 8.68 | 8.25 |
| **12** | 4.88 | 7.88 | 4.00 | 5.59 | 56.84 | 61.35 | 52.37 | 56.86 | 30.00 | 21.64 | 34.63 | 28.76 | 8.30 | 9.14 | 9.00 | 8.81 |
| **13** | 5.51 | 7.04 | 4.31 | 5.62 | 60.16 | 56.94 | 52.74 | 56.61 | 26.68 | 27.05 | 34.45 | 29.24 | 8.13 | 8.97 | 8.51 | 8.54 |
| Env mean | 5.30 | 9.17 | 4.70 | 6.39 | 57.50 | 58.20 | 55.83 | 57.21 | 29.30 | 23.77 | 31.30 | 28.12 | 7.92 | 8.77 | 8.18 | 8.29 |
| LSD | | | | 0.84 | | | | 1.90 | | | | 1.95 | | | | 0.36 |
| R-squared | | | | 0.88 | | | | 0.72 | | | | 0.81 | | | | 0.75 |

LPP=Large polymeric proteins, SPP=Small polymeric proteins, LMP=Large monomeric proteins, DO=Douglas, MA=Marydale, VA=Vaalharts, EM=Elite line means, Env=Environmental mean, LSD=Least significant difference

**Table 4.5 Means for relative value (%) at Douglas, Marydale and Vaalharts**

| Elite lines | LUPP | | | | UPP | | | | POL | | | | HMW | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM |
| **1** | 65.86 | 82.06 | 69.22 | 72.45 | 67.56 | 74.09 | 66.25 | 69.30 | 46.45 | 44.12 | 44.88 | 45.15 | 3.40 | 4.24 | 3.70 | 3.78 |
| **2** | 68.22 | 91.38 | 75.49 | 78.36 | 67.03 | 77.76 | 67.26 | 70.68 | 45.36 | 45.58 | 45.49 | 45.48 | 3.70 | 4.25 | 4.15 | 4.03 |
| **3** | 69.97 | 92.20 | 73.66 | 78.61 | 67.18 | 77.84 | 68.79 | 71.27 | 45.42 | 44.49 | 45.79 | 45.23 | 3.92 | 5.42 | 3.88 | 4.41 |
| **4** | 73.20 | 83.63 | 69.12 | 75.32 | 67.93 | 71.10 | 67.34 | 68.79 | 45.78 | 48.72 | 49.65 | 48.05 | 4.19 | 6.92 | 3.36 | 4.82 |
| **5** | 74.42 | 80.57 | 63.84 | 72.94 | 67.02 | 69.95 | 65.67 | 67.55 | 47.18 | 49.23 | 46.59 | 47.66 | 4.96 | 6.47 | 3.34 | 4.92 |
| **6** | 69.13 | 81.64 | 69.77 | 73.51 | 68.33 | 70.94 | 68.42 | 69.23 | 48.78 | 47.41 | 47.75 | 47.98 | 3.88 | 6.43 | 3.34 | 4.52 |
| **7** | 74.03 | 82.84 | 70.74 | 75.87 | 67.06 | 71.39 | 68.01 | 68.82 | 45.44 | 46.40 | 45.36 | 45.73 | 4.00 | 5.97 | 3.20 | 4.39 |
| **8** | 70.50 | 79.93 | 69.34 | 73.26 | 68.51 | 71.27 | 68.22 | 69.33 | 47.69 | 48.21 | 47.36 | 47.75 | 3.73 | 5.79 | 3.35 | 4.29 |
| **9** | 63.89 | 80.14 | 69.73 | 71.25 | 68.01 | 70.46 | 67.04 | 68.50 | 47.69 | 46.99 | 42.53 | 45.74 | 3.11 | 5.84 | 2.77 | 3.91 |
| **10** | 66.64 | 80.55 | 70.75 | 72.65 | 66.31 | 69.07 | 65.17 | 66.85 | 46.53 | 46.58 | 43.60 | 45.49 | 3.26 | 5.88 | 3.05 | 4.06 |
| **11** | 71.38 | 79.96 | 74.17 | 75.17 | 66.86 | 71.56 | 66.80 | 68.41 | 44.27 | 47.17 | 41.98 | 44.47 | 3.50 | 5.59 | 3.17 | 4.09 |
| **12** | 70.61 | 77.36 | 70.49 | 72.82 | 67.85 | 71.30 | 66.05 | 68.40 | 45.47 | 48.53 | 42.65 | 45.55 | 3.50 | 5.06 | 2.84 | 3.78 |
| **13** | 75.38 | 78.07 | 76.37 | 76.61 | 69.98 | 72.07 | 68.23 | 70.09 | 46.53 | 44.37 | 41.81 | 44.36 | 3.65 | 4.51 | 2.82 | 3.66 |
| Env mean | 70.25 | 82.33 | 70.99 | 74.53 | 67.67 | 72.21 | 67.17 | 69.02 | 46.38 | 46.74 | 45.03 | 46.05 | 3.75 | 5.57 | 3.30 | 4.21 |
| LSD | | | | 2.47 | | | | 1.00 | | | | 1.18 | | | | 0.43 |
| R-squared | | | | 0.88 | | | | 0.89 | | | | 0.74 | | | | 0.88 |

LUPP=Large unextractable polymeric proteins, UPP=Unextractable polymeric proteins, POL=Total polymeric proteins, HMW=High molecular weight, DO=Douglas, MA=Marydale, VA=Vaalharts, EM=Elite line means, Env=Environmental mean, LSD=Least significant difference

**Table 4.6 Means for relative and absolute value (%) at Douglas, Marydale and Vaalharts**

| Elite lines | RELATIVE | | | | | | | | ABSOLUTE | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LMW | | | | MON | | | | LUPP | | | | UPP | | | |
| | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM |
| **1** | 43.05 | 39.89 | 41.18 | 41.37 | 0.54 | 0.56 | 0.55 | 0.55 | 4.22 | 6.03 | 4.52 | 4.92 | 4.30 | 5.22 | 4.25 | 4.59 |
| **2** | 41.66 | 41.33 | 41.33 | 41.44 | 0.55 | 0.54 | 0.55 | 0.55 | 4.35 | 5.39 | 4.80 | 4.85 | 4.23 | 4.53 | 4.26 | 4.34 |
| **3** | 41.49 | 39.06 | 41.91 | 40.82 | 0.55 | 0.56 | 0.54 | 0.55 | 4.38 | 5.10 | 4.82 | 4.77 | 4.27 | 4.35 | 4.67 | 4.43 |
| **4** | 41.59 | 41.80 | 46.28 | 43.23 | 0.54 | 0.51 | 0.50 | 0.52 | 4.77 | 4.70 | 4.35 | 4.60 | 4.34 | 4.03 | 4.30 | 4.22 |
| **5** | 42.22 | 42.75 | 43.25 | 42.74 | 0.53 | 0.51 | 0.53 | 0.52 | 4.32 | 4.21 | 4.26 | 4.26 | 4.02 | 3.66 | 4.30 | 3.99 |
| **6** | 44.90 | 40.98 | 44.51 | 43.46 | 0.51 | 0.53 | 0.52 | 0.52 | 4.56 | 4.57 | 4.46 | 4.53 | 4.27 | 3.96 | 4.32 | 4.18 |
| **7** | 41.43 | 40.43 | 42.15 | 41.34 | 0.55 | 0.54 | 0.55 | 0.54 | 4.79 | 4.69 | 4.67 | 4.71 | 4.59 | 4.09 | 4.54 | 4.41 |
| **8** | 43.95 | 42.42 | 44.01 | 43.46 | 0.52 | 0.52 | 0.53 | 0.52 | 4.68 | 4.67 | 4.44 | 4.60 | 4.66 | 4.16 | 4.37 | 4.40 |
| **9** | 44.58 | 41.15 | 39.76 | 41.83 | 0.52 | 0.53 | 0.57 | 0.54 | 4.32 | 4.59 | 4.06 | 4.32 | 4.55 | 3.99 | 3.81 | 4.11 |
| **10** | 43.27 | 40.46 | 40.55 | 41.43 | 0.53 | 0.54 | 0.56 | 0.55 | 4.30 | 4.67 | 4.28 | 4.42 | 4.27 | 4.03 | 3.97 | 4.09 |
| **11** | 40.77 | 41.58 | 38.80 | 40.39 | 0.56 | 0.53 | 0.58 | 0.56 | 4.75 | 4.99 | 4.32 | 4.68 | 4.35 | 4.49 | 3.91 | 4.25 |
| **12** | 42.01 | 43.47 | 39.82 | 41.77 | 0.55 | 0.51 | 0.57 | 0.54 | 4.75 | 3.93 | 4.16 | 4.28 | 4.56 | 3.66 | 3.85 | 4.02 |
| **13** | 43.26 | 39.86 | 38.99 | 40.70 | 0.53 | 0.56 | 0.58 | 0.56 | 4.99 | 4.88 | 5.90 | 5.26 | 4.61 | 4.64 | 5.23 | 4.83 |
| Env mean | 42.63 | 41.17 | 41.73 | 41.84 | 0.54 | 0.53 | 0.55 | 0.54 | 4.55 | 4.80 | 4.54 | 4.63 | 4.39 | 4.22 | 4.30 | 4.30 |
| LSD | | | | 1.07 | | | | 0.01 | | | | 0.39 | | | | 0.36 |
| R-squared | | | | 0.74 | | | | 0.74 | | | | 0.58 | | | | 0.52 |

LMW=Low molecular weight, MON=Total monomeric proteins, LUPP=Large unextractable polymeric protein, UPP=Unextractable polymeric proteins, DO=Douglas, MA=Marydale, VA=Vaalharts, EM=Elite line means, Env=Environmental mean, LSD=Least significant difference

**Table 4.7 Means for absolute value (%) at Douglas, Marydale and Vaalharts**

| Elite lines | POL | | | | HMW | | | | LMW | | | | MON | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM | DO | MA | VA | EM |
| **1** | 2.93 | 3.07 | 2.88 | 2.96 | 0.25 | 0.29 | 0.24 | 0.26 | 2.68 | 2.78 | 2.64 | 2.7 | 0.03 | 0.04 | 0.04 | 0.04 |
| **2** | 2.90 | 2.64 | 2.86 | 2.80 | 0.24 | 0.31 | 0.27 | 0.27 | 2.66 | 2.33 | 2.59 | 2.53 | 0.03 | 0.03 | 0.03 | 0.03 |
| **3** | 2.83 | 2.56 | 3.22 | 2.87 | 0.24 | 0.29 | 0.25 | 0.26 | 2.59 | 2.27 | 2.98 | 2.61 | 0.04 | 0.03 | 0.04 | 0.03 |
| **4** | 2.98 | 2.84 | 3.15 | 2.99 | 0.30 | 0.38 | 0.22 | 0.30 | 2.68 | 2.46 | 2.94 | 2.69 | 0.03 | 0.03 | 0.03 | 0.03 |
| **5** | 2.85 | 2.54 | 3.11 | 2.84 | 0.28 | 0.34 | 0.21 | 0.28 | 2.58 | 2.20 | 2.90 | 2.56 | 0.03 | 0.03 | 0.03 | 0.03 |
| **6** | 3.00 | 2.62 | 2.90 | 2.84 | 0.26 | 0.35 | 0.20 | 0.27 | 2.74 | 2.28 | 2.69 | 2.57 | 0.03 | 0.03 | 0.03 | 0.03 |
| **7** | 3.15 | 2.69 | 3.04 | 2.96 | 0.25 | 0.34 | 0.21 | 0.27 | 2.91 | 2.34 | 2.83 | 2.69 | 0.04 | 0.03 | 0.04 | 0.03 |
| **8** | 3.24 | 2.84 | 2.99 | 3.02 | 0.24 | 0.35 | 0.21 | 0.27 | 3.00 | 2.49 | 2.79 | 2.76 | 0.04 | 0.03 | 0.03 | 0.03 |
| **9** | 3.20 | 2.63 | 2.45 | 2.76 | 0.21 | 0.32 | 0.16 | 0.23 | 2.99 | 2.31 | 2.28 | 2.53 | 0.04 | 0.03 | 0.03 | 0.03 |
| **10** | 2.95 | 2.72 | 2.61 | 2.76 | 0.21 | 0.36 | 0.19 | 0.25 | 2.74 | 2.36 | 2.42 | 2.51 | 0.03 | 0.03 | 0.03 | 0.03 |
| **11** | 2.87 | 2.97 | 2.50 | 2.78 | 0.24 | 0.33 | 0.19 | 0.25 | 2.63 | 2.64 | 2.31 | 2.53 | 0.04 | 0.03 | 0.03 | 0.03 |
| **12** | 3.08 | 2.30 | 2.42 | 2.60 | 0.23 | 0.22 | 0.16 | 0.20 | 2.85 | 2.08 | 2.27 | 2.40 | 0.04 | 0.03 | 0.03 | 0.03 |
| **13** | 3.07 | 3.13 | 3.22 | 3.14 | 0.25 | 0.26 | 0.22 | 0.24 | 2.82 | 2.86 | 3.00 | 2.89 | 0.04 | 0.03 | 0.04 | 0.04 |
| Env mean | 3.00 | 2.73 | 2.87 | 2.87 | 0.24 | 0.32 | 0.21 | 0.26 | 2.76 | 2.42 | 2.66 | 2.61 | 0.04 | 0.03 | 0.04 | 0.03 |
| LSD | | | | 0.26 | | | | 0.03 | | | | 0.25 | | | | 0.003 |
| R-squared | | | | 0.50 | | | | 0.76 | | | | 0.55 | | | | 0.59 |

POL=Total polymeric proteins, HMW=High molecular weight, LMW=Low molecular weight, MON=Total proteins, DO=Douglas, MA=Marydale, VA=Vaalharts, EM=Elite line means, Env=Environmental mean, LSD=Least significant difference

### 4.4.3 Correlations

#### 4.4.3.1 Correlations between protein fractions and quality characteristics in Douglas

Soluble proteins: LPP correlated negatively with EPT, MLI, MLT, MPI and MRT. SDSVOL correlated negatively with SPP, whereas with LMP the correlation was positive. SPP correlated highly significantly (p≤0.001) with MLV, MRI, MRV and MTV. Only MTI showed a positive significant correlation (p≤0.01) with SPP. Significant negative correlations (p≤0.05) were observed between SMP fraction and quality characters ETI, MLI, MLT, MPI, MPT and MRT (Table 4.8).

Insoluble proteins: LPP significantly and negatively correlated (p≤0.5) with FPC. SDSVOL was significantly positively correlated with SPP and the reverse was true for SMP which correlated (P≤0.01) negatively with SDSVOL. EPI, EPT, MLI, MLT, MPT and MRT correlated positively and negatively with fractions SPP and SMP, respectively (Table 4.9).

Relative value: UPP highly and significantly correlated (p≤0.001) with SDSVOL as well as with EPI, EPT, MLI, MLT, MPI, MPT, MRI and MRT. HMW had a negative and significant correlation (p≤0.05) with FPC and MTV (Table 4.10).

Absolute value: LUPP, POL and LMW correlated (p≤0.05) significantly with EPT only. The correlations between FPC and UPP as well as between FPC and MON were significant. HMW showed a significant correlation (p≤0.05) only with SDSVOL (Table 4.11).

#### 4.4.3.2 Correlations between protein fractions and quality characteristics in Marydale

Soluble proteins: EPI, MLV, MPW and MTV significantly correlated (p≤0.01) negatively with SPP and positively with LMP. No correlation between soluble fractions and SDSVOL was evident at this location (Table 4.8).

Insoluble proteins: LPP exhibited a significant negative correlation (p≤0.01) with FPC, MLI and MTV. SDSVOL significantly and positively correlated (p≤0.05) with SPP. However, it showed a negative and significant correlation (p≤0.01) with SMP. MPI correlated positively with SPP and negatively with LMP (Table 4.9).

Relative value: MPI correlated positively with LUPP and POL (p≤0.05). Moreover it had a significant negative correlation with MON. A significant negative correlation (p≤0.01) was observed between HMW and FPC. Other quality characteristics such as EPI, ETV, MLI, MLV, MRV and MTV correlated (p≤0.01) negatively with HMW (Table 4.10).

Absolute value: Significant correlation (p≤0.01) between LUPP and EPV, EPW, MLV, MLW, MPW, MRV and MTV, respectively were observed. EPI and MLV showed a significant correlations (p≤0.01) with UPP. HMW correlated negatively (p≤0.05) with EPT. A highly significant correlation (p≤0.001) was only exhibited by EPI with MON. MLI and MLV also correlated (p≤0.01) significantly with MON (Table 4.7).

#### 4.4.3.3 Correlations between protein fractions and quality characteristics in Vaalharts

Soluble proteins: MLT, MPT and MRT were negatively and highly significantly (p≤0.001) correlated with LPP, while MPV showed a positive correlation (p≤0.05) with LPP. A highly significant and negative correlation (p≤0.001) was observed between SPP and EPI, EPT, ETV, MLI, MLT, MPI, MPT, MRT and MTV. EPI, EPT, ETV, MLI, MLT, MPI, MPT, MRT and MTV correlated positively and highly significantly (p≤0.001) with LMP. SMP was negatively correlated with SDSVOL, MRV and MTI (Table 4.8).

Insoluble proteins: FPC correlated (p≤0.05) negatively with SPP but positively with LMP. SDSVOL and ETV correlated significantly positively and negatively with SPP and the reverse was true for LMP, which correlated (p≤0.05) negatively and positively with SDSVOL and ETV. LPP only correlated (p≤0.05) significantly with MTI (Table 4.9).

Relative value: LUPP and UPP respectively correlated significantly (p≤0.01) and positively with MLI, MPT, MRI and MRT, while correlation between POL with the same characteristics were negative (p≤0.05). UPP also significantly correlated with SDSVOL. HMW only correlated significantly (p≤0.05) with MPV and MTI. A negative correlation between LMW and FPC was observed. EPI, ETI, ETV, MLI, MLT, MRT and MTV correlated significantly negatively and positively, respectively with LMW and MON (Table 4.10).

Absolute value: MLI correlated (p≤0.05) significantly with LUPP and MON. HMW showed a significant correlation with SDSVOL, MPV as well as with MTI (Table 4.11).

**Table 4.8 Significant correlations between soluble protein fractions and quality characters in each location**

| Douglas | | | Marydale | | | Vaalharts | | |
|---|---|---|---|---|---|---|---|---|
| Soluble fractions | | | | | | | | |
| **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** |
| **LPP** | **EPT** | -0.33* | **LPP** | **EPV** | -0.35* | **LPP** | **EPI** | -0.45** |
| | **MLI** | -0.42** | | **EPW** | -0.33* | | **EPT** | -0.47** |
| | **MLT** | -0.43** | | **MLV** | -0.34* | | **ETI** | -0.42** |
| | **MPI** | -0.39* | | **MPV** | -0.35* | | **ETV** | -0.41* |
| | **MRT** | -0.43** | | **MPW** | -0.35* | | **MLI** | -0.50** |
| **SPP** | **SDSVOL** | -0.44** | | **MRV** | -0.38* | | **MLT** | -0.53*** |
| | **EPI** | -0.42* | | **MTI** | -0.35* | | **MPI** | -0.43** |
| | **EPT** | -0.32* | | **MTV** | -0.35* | | **MPT** | -0.53*** |
| | **EPV** | -047** | **SPP** | **EPI** | -0.44** | | **MPV** | 0.40* |
| | **ETI** | -0.34* | | **EPV** | -0.40* | | **MRI** | -0.35* |
| | **ETV** | -0.48** | | **EPW** | -0.41* | | **MRT** | -0.53*** |
| | **MLI** | -0.41* | | **ETV** | -0.33* | **SPP** | **EPI** | -0.63*** |
| | **MLT** | -0.40* | | **MLV** | -0.48** | | **EPT** | -0.57*** |
| | **MLV** | -0.52*** | | **MLW** | -0.40* | | **ETI** | -0.43** |
| | **MLW** | -0.38* | | **MPV** | -0.36* | | **ETV** | -0.58*** |
| | **MPI** | -0.49** | | **MPW** | -0.46** | | **MLI** | -0.65*** |

*p≤0.05, **p≤0.01, ***p≤0.001, LPP=Large polymeric proteins, SPP=Small polymeric proteins, EPT=Envelope peak time, MLI=Midline left integral, MLT=Midline left tail, MPI=Midline peak integral, MRT=Midline right time, SDSVOL=SDS-sedimentation volume, EPI=Envelope peak integral, ETV=Envelope tail value, EPV=Envelope peak value, ETI=Envelope tail integral, MLV=Midline left value, MLW=Midline left width, MPV=Midline peak value, MPW=Midline peak width, MRV=Midline right value, MTI=Midline tail integral, MTV=Midline tail value, EPW=Envelope peak width, MPT=Midline peak time, MRI=Midline right integral

**Table 4.8 Continued**

| Douglas | | | Marydale | | | Vaalharts | | |
|---|---|---|---|---|---|---|---|---|
| Soluble fractions | | | | | | | | |
| **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** |
| **SPP** | **MPT** | -0.40* | **SPP** | **MRV** | -0.42* | **SPP** | **MLT** | -0.65*** |
| | **MPV** | -0.46** | | **MTI** | -0.41* | | **MLV** | -0.41* |
| | **MRI** | -0.54*** | | **MTV** | -0.43** | | **MPI** | -0.65*** |
| | **MRT** | -0.40* | **LMP** | **EPI** | 0.46** | | **MPT** | -0.65*** |
| | **MRV** | -0.52*** | | **EPV** | 0.38* | | **MRT** | -0.65*** |
| | **MTI** | 0.49** | | **EPW** | 0.41* | | **MTV** | -0.55*** |
| | **MTV** | -0.54*** | | **ETI** | 0.32* | **LMP** | **EPI** | 0.67*** |
| **LMP** | **SDSVOL** | 0.41* | | **ETV** | 0.39* | | **EPT** | 0.59*** |
| | **EPI** | 0.51*** | | **MLI** | 0.32* | | **ETI** | 0.41* |
| | **EPT** | 0.41* | | **MLV** | 0.46** | | **ETV** | 0.61*** |
| | **EPV** | 0.33* | | **MLW** | 0.41* | | **MLI** | 0.67*** |
| | **EPW** | 0.32* | | **MPW** | 0.49** | | **MLT** | 0.66*** |
| | **ETI** | 0.53*** | | **MRV** | 0.42** | | **MLV** | 0.49** |
| | **ETV** | 0.52*** | | **MTI** | 0.40* | | **MPI** | 0.70*** |
| | **MLI** | 0.55*** | | **MTV** | 0.43** | | **MPT** | 0.66*** |
| | **MLT** | 0.54*** | | | | | **MRT** | 0.66*** |

*p≤0.05, **p≤0.01, ***p≤0.001, SPP=Small polymeric proteins LMP=Large monomeric proteins, MPT=Midline peak time, MPV=Midline peak value, MRI=Midline right integral, MRT=Midline right time, MRV=Midline right value, MTI=Midline tail integral, MTV=Midline tail value, SDSVOL=SDS -sedimentation volume, EPI=Envelope peak integral, EPT=Envelope peak time, EPV=Envelope peak value, EPW=Envelope peak width, ETI=Envelope tail integral, ETV=Envelope tail value, MLI=Midline left integral, MLT=Midline left tail, MLV=Midline left value, MLW=Midline left width, MPW=Midline peak width, MPI=Midline peak integral

**Table 4.8 Continued**

| Douglas | | | Marydale | | | Vaalharts | | |
|---|---|---|---|---|---|---|---|---|
| **Soluble fractions** | | | | | | | | |
| **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** |
| **LMP** | **MLV** | 0.54*** | | | | **LMP** | **MRV** | 0.37* |
| | **MLW** | 0.39* | | | | | **MTI** | 0.34* |
| | **MPI** | 0.58*** | | | | | **MTV** | 0.65*** |
| | **MPT** | 0.54*** | | | | **SMP** | **SDSVOL** | -0.38* |
| | **MRI** | 0.58*** | | | | | **MRV** | -0.39* |
| | **MRT** | 0.54*** | | | | | **MTI** | -0.37* |
| | **MRV** | 0.45** | | | | | | |
| | **MTI** | 0.41* | | | | | | |
| | **MTV** | 0.51*** | | | | | | |
| **SMP** | **ETI** | -0.42* | | | | | | |
| | **MLI** | -0.34* | | | | | | |
| | **MLT** | -0.34* | | | | | | |
| | **MPI** | -0.32* | | | | | | |
| | **MPT** | -0.34* | | | | | | |
| | **MRT** | -0.34* | | | | | | |

*p≤0.05, **p≤0.01, ***p≤0.001, LPP=Large polymeric proteins, SPP=Small polymeric proteins, MLV=Midline left value, MLW=Midline left width, MPI=Midline peak integral, MPT=Midline peak time, MRI=Midline right integral, MRT=Midline right time, MRV=Midline right value, MTI=Midline tail integral, MTV=Midline tail value, ETI=Envelope tail integral, MLI=Midline left integral, MLT=Midline left time, SDSVOL=SDS-sedimentation volume

**Table 4.9 Significant correlations between insoluble protein fractions for three locations**

| Douglas | | | Marydale | | | Vaalharts | | |
|---|---|---|---|---|---|---|---|---|
| Insoluble fractions | | | | | | | | |
| **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** |
| **LPP** | **FPC** | -0.35* | **LPP** | **FPC** | -0.47** | **LPP** | **MTI** | 0.34* |
| **SPP** | **SDSVOL** | 0.32* | | **EPI** | -0.34* | **SPP** | **FPC** | -0.35* |
| | **EPI** | 0.34* | | **ETI** | -0.33* | | **SDSVOL** | 0.32* |
| | **EPT** | 0.32* | | **ETV** | -0.40* | | **ETV** | -0.33* |
| | **MLI** | 0.33* | | **MLI** | -0.44** | **LMP** | **FPC** | 0.35* |
| | **MLT** | 0.33* | | **MLT** | -0.40* | | **SDSVOL** | -0.33* |
| | **MPT** | 0.33* | | **MLV** | -0.37* | | **ETV** | 0.34* |
| | **MRT** | 0.33* | | **MPT** | -0.40* | **SMP** | **SDSVOL** | -0.35* |
| **SMP** | **SDSVOL** | -0.44** | | **MRT** | -0.40* | | **MPV** | -0.33* |
| | **EPI** | -0.48** | | **MRV** | -0.35* | | | |
| | **EPT** | -0.46** | | **MTV** | -0.39** | | | |
| | **ETI** | -0.41* | **SPP** | **SDSVOL** | 0.37* | | | |

*p≤0.05, **p≤0.01, LPP=Large polymeric proteins, SPP=Small polymeric proteins, SMP=Small monomeric proteins, FPC=Flour protein content, SDSVOL=SDS-sedimentation volume, EPI=Envelope peak integral, EPT=Envelope peak time, MLI=Midline left integral, MLT=Midline left time, MPT=Midline peak time, MRT=Midline right time, ETI=Envelope tail integral, ETV=Envelope tail value, MLV=Midline left value, MRV=Midline right value, MTV=Midline tail value, MTI=Midline tail integral, MPV=Midline peak value, LMP=Large monomeric proteins

**Table 4.9 Continued**

| Douglas | | | Marydale | | | Vaalharts | | |
|---|---|---|---|---|---|---|---|---|
| **Insoluble fractions** | | | | | | | | |
| **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** |
| **SMP** | **MLI** | -0.41* | **SPP** | **ETV** | 0.39* | | | |
| | **MLT** | -0.42** | | **MPI** | 0.46** | | | |
| | **MPI** | -0.37* | | **MRV** | 0.35* | | | |
| | **MPT** | -0.42** | | **MTI** | 0.34* | | | |
| | **MRT** | -0.42** | | **MTV** | 0.34* | | | |
| | | | **LMP** | **MPI** | -0.54*** | | | |
| | | | **SMP** | **SDSVOL** | -0.49** | | | |
| | | | | **ETV** | -0.39* | | | |
| | | | | **MRV** | -0.34* | | | |
| | | | | **MTI** | -0.33* | | | |
| | | | | **MTV** | -0.35* | | | |

*p≤0.05, **p≤0.01, ***p≤0.001, SMP=Small monomeric proteins, MLI=Midline left integral, MLT=Midline left time, MPI=Midline peak integral, MPT=Midline peak time, MRT=Midline right time, SPP=Small polymeric proteins, LMP=Large monomeric proteins, SMP=Small monomeric proteins, ETV=Envelope tail value, MRV=Midline right value, MTI=Midline tail integral, MTV=Midline tail value, SDSVOL=SDS-sedimentation volume

**Table 4.10 Significant correlations between relative value (%) and quality characteristics**

| Douglas | | | Marydale | | | Vaalharts | | |
|---|---|---|---|---|---|---|---|---|
| Relative fractions | | | | | | | | |
| **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** |
| **UPP** | **SDSVOL** | 0.53*** | **LUPP** | **MPI** | 0.33* | **LUPP** | **EPI** | 0.38* |
| | **EPI** | 0.53*** | **UPP** | **EPV** | 0.39* | | **EPT** | 0.36* |
| | **EPT** | 0.51*** | | **EPW** | 0.35* | | **ETI** | 0.32* |
| | **ETI** | 0.35* | | **ETV** | 0.33* | | **ETV** | 0.42* |
| | **ETV** | 0.37* | | **MLV** | 0.40* | | **MLI** | 0.48** |
| | **MLI** | 0.53*** | | **MPV** | 0.37* | | **MLT** | 0.45** |
| | **MLT** | 0.54*** | | **MPW** | 0.39* | | **MPI** | 0.47** |
| | **MLV** | 0.42** | | **MRV** | 0.42** | | **MPT** | 0.45** |
| | **MPI** | 0.53*** | | **MTI** | 0.41* | | **MRI** | 0.45** |
| | **MPT** | 0.54*** | | **MTV** | 0.41* | | **MRT** | 0.45** |
| | **MRI** | 0.51*** | **POL** | **EPI** | -0.38* | | **MTV** | 0.33** |
| | **MRT** | 0.54*** | | **MLW** | -0.34* | **UPP** | **SDSVOL** | 0.46** |
| | **MTV** | 0.40* | | **MPI** | 0.36* | | **EPI** | 0.38* |
| **HMW** | **FPC** | -0.38* | **HMW** | **FPC** | -0.46** | | **EPT** | 0.41* |
| | **MTV** | -0.33* | | **EPI** | -0.37** | | **MLI** | 0.49** |

*p≤0.05, **p≤0.01, ***p≤0.001, UPP=Unextractable polymeric proteins, HMW=High molecular weight, SDSVOL=SDS-sedimentation volume, EPI=Envelope peak integral, EPT=Envelope peak time, ETI=Envelope tail interval, ETV=Envelope tail value, MLI=Midline left integral, MLT=Midline left time, MLV=Midline left value, MPI=Midline peak integral, MPT=Midline peak time, MRI=Midline right integral, MRT=Midline right time, MTV=Midline tail value, LUPP=Large unextractable polymeric proteins, POL=Total polymeric proteins, FPC=Flour protein content, EPV=Envelope peak value, EPW=Envelope peak width, MPV=Midline peak value, MPW=Midline peak width, MRV=Midline right value, MTI=Midline tail integral

**Table 4.10 Continued**

| Douglas | | | Marydale | | | Vaalharts | | |
|---|---|---|---|---|---|---|---|---|
| Relative fractions | | | | | | | | |
| **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** |
| | | | **HMW** | **ETV** | -0.43** | **UPP** | **MPI** | 0.50** |
| | | | | **MLI** | -0.43** | | **MPT** | 0.46** |
| | | | | **MLT** | -0.39* | | **MRI** | 0.50** |
| | | | | **MLV** | -0.44** | | **MRT** | 0.46** |
| | | | | **MPT** | -0.39* | **POL** | **EPI** | -0.38* |
| | | | | **MPV** | -0.32* | | **ETI** | -0.37* |
| | | | | **MRT** | -0.39* | | **ETV** | -0.42* |
| | | | | **MRV** | -0.43** | | **MLI** | -0.34* |
| | | | | **MTI** | -0.39* | | **MLT** | -0.35* |
| | | | | **MTV** | -0.45** | | **MPT** | -0.35* |
| | | | **LMW** | **MPI** | 0.39* | | **MRT** | -0.35* |
| | | | **MON** | **EPI** | 0.38* | | **MTV** | -0.32* |
| | | | | **MLW** | 0.34* | **HMW** | **MPV** | 0.38* |
| | | | | **MPI** | -0.36* | | **MTI** | 0.34* |

*p≤0.05, **p≤0.01, HMW=High molecular weight, LMW=Low molecular weight, MON=Total monomeric proteins, ETV=Envelope tail value, MLI=Midline left integral, MLT=Midline left time, MLV=Midline left value, MPT=Midline peak time, MPV=Midline peak value, MRT=Midline right time, MRV=Midline right value, MTI=Midline time integral, MTV=Midline tail value, MPI=Midline peak integral, EPI=Envelope peak integral, MLW=Midline left width, UPP=Unextractable polymeric proteins, POL=Total polymeric proteins, MRI=Midline right integral, ETI=Envelope tail integral

**Table 4.10 Continued**

| Douglas | | | Marydale | | | Vaalharts | | |
|---|---|---|---|---|---|---|---|---|
| Relative fractions | | | | | | | | |
| **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** |
| | | | | | | **LMW** | **FPC** | -0.33* |
| | | | | | | | **EPI** | -0.38* |
| | | | | | | | **ETI** | -0.37* |
| | | | | | | | **ETV** | -0.44** |
| | | | | | | | **MLI** | -0.34* |
| | | | | | | | **MLT** | -0.34* |
| | | | | | | | **MPI** | -0.33* |
| | | | | | | | **MPT** | -0.34* |
| | | | | | | | **MRT** | -0.34* |
| | | | | | | | **MTV** | -0.37* |
| | | | | | | **MON** | **EPI** | 0.37* |
| | | | | | | | **ETI** | 0.37* |
| | | | | | | | **ETV** | 0.42* |
| | | | | | | | **MLI** | 0.34* |
| | | | | | | | **MLT** | 0.35* |
| | | | | | | | **MPT** | 0.35* |
| | | | | | | | **MRT** | 0.35* |
| | | | | | | | **MTV** | 0.32* |

*p≤0.05, LMW=Low molecular weight, MON=Total monomeric proteins, FPC=Flour protein content, EPI=Envelope peak integral, ETI=Envelope tail integral, ETV=Envelope tail value, MLI=Midline left integral, MLT=Midline left time, MPI=Midline peak integral, MPT=Midline peak time, MRT=Midline right time, MTV=Midline tail value

**Table 4.11 Significant correlations between absolute values (%) and quality characteristics for three locations**

| Douglas | | | Marydale | | | Vaalharts | | |
|---|---|---|---|---|---|---|---|---|
| Absolute fractions | | | | | | | | |
| **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** |
| **LUPP** | **EPT** | 0.38* | **LUPP** | **EPI** | 0.35* | **LUPP** | **MLI** | 0.35* |
| **UPP** | **FPC** | 0.37* | | **EPV** | 0.42** | | **MLT** | 0.33* |
| | **EPT** | 0.36* | | **EPW** | 0.49** | | **MPI** | 0.36* |
| | **MRI** | 0.32* | | **MLV** | 0.48** | | **MPT** | 0.33* |
| | **MTV** | 0.35* | | **MLW** | 0.45** | | **MRI** | 0.35* |
| **POL** | **EPT** | 0.33* | | **MPV** | 0.39* | | **MRT** | 0.33* |
| **HMW** | **SDSVOL** | 0.32* | | **MPW** | 0.49** | **HMW** | **SDSVOL** | 0.34* |
| **LMW** | **EPT** | 0.34* | | **MRV** | 0.46** | | **MPV** | 0.35* |
| **MON** | **FPC** | 0.47** | | **MTI** | 0.46** | | **MTI** | 0.33** |
| | **ETV** | 0.33* | | **MTV** | 0.41* | **MON** | **MLI** | 0.32* |
| | **MTV** | 0.39* | **UPP** | **EPI** | 0.45** | | | |
| | | | | **EPW** | 0.37* | | | |
| | | | | **MLI** | 0.35* | | | |
| | | | | **MLV** | 0.47** | | | |
| | | | | **MLW** | 0.41* | | | |
| | | | | **MPW** | 0.40* | | | |

*p≤0.05, **p≤0.01, LUPP=Large unextractable polymeric proteins, UPP=Unextractable polymeric proteins, POL=Total polymeric proteins, HMW=High molecular weight, LMW=Low molecular weight, MON=Total monomeric proteins, EPT=Envelope peak time, FPC=Flour protein content, EPT=Envelope peak time, MRI=Midline right integral, MTV=Midline tail value, SDSVOL=SDS-sedimentation volume, ETV=Envelope tail value, EPI=Envelope peak integral, EPV=Envelope peak value, EPW=Envelope peak value, MLV=Midline left value, MLW=Midline left width, MPV=Midline peak value, MPW=Midline peak width, MRV=Midline right value, MTI=Midline tail integral, MLI=Midline left integral, MLT=Midline left time, MPI=Midline peak integral, MPT=Midline peak time, MRI=Midline right integral, MRT=Midline right time

**Table 4.11 Continued**

| Douglas | | | Marydale | | | Vaalharts | | |
|---|---|---|---|---|---|---|---|---|
| Absolute fractions | | | | | | | | |
| **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** | **Fraction** | **Character** | **Correlation** |
| | | | **UPP** | **MRV** | 0.38* | | | |
| | | | | **MTI** | 0.36* | | | |
| | | | | **MTV** | 0.38* | | | |
| | | | **HMW** | **EPT** | -0.36* | | | |
| | | | **LMW** | **EPI** | 0.34* | | | |
| | | | | **MLI** | 0.32* | | | |
| | | | **MON** | **EPI** | 0.52*** | | | |
| | | | | **EPT** | 0.40* | | | |
| | | | | **ETI** | 0.34* | | | |
| | | | | **ETV** | 0.32* | | | |
| | | | | **MLI** | 0.44** | | | |
| | | | | **MLT** | 0.40* | | | |
| | | | | **MLV** | 0.43** | | | |
| | | | | **MLW** | 0.38* | | | |
| | | | | **MPT** | 0.40* | | | |
| | | | | **MPW** | 0.36* | | | |
| | | | | **MRT** | 0.40* | | | |

*p≤0.05, **p≤0.01, ***p≤0.001, UPP=Unextractable polymeric proteins, HMW=High molecular weight, LMW=Low molecular weight, MON=Total monomeric proteins, MRV=Midline right value, MTI=Midline tail integral, MTV=Midline tail value, EPT=Envelope peak time, EPI=Envelope peak integral, MLI=Midline left integral, ETI=Envelope tail integral, ETV=Envelope tail value, MLT=Midline left time, MLV=Midline left value, MLW=Midline left width, MPT=Midline peak time, MPW=Midline peak width, MRT=Midline right time

#### 4.4.3.4 Significant correlations between quality characteristics and soluble protein fraction across the three locations

Highly significant negative correlations (p≤0.001) were observed between LPP fractions and seven quality characteristics (Table 4.12). Significant correlation (p≤0.05) was seen between LPP and EPW only. SPP showed a highly significant negative correlation (p≤0.001) with all measured quality characteristics except for EPW and MLW, which were significantly negatively correlated (p≤0.01). SDSVOL correlated negatively and significantly (p≤0.05) with the SPP. LMP had a highly significant correlation with 13 of the selected quality characteristics and LMP also correlated significantly (p≤0.01) with SDSVOL, MLW and MPI. Significant correlations (p≤0.05) were observed between LMP and EPV and EPW. FPC, EPV, ETV, MLV, MPV, MRV, MTI and MTV showed a highly significant (p≤0.001) and positive correlation with SMP. MRI was highly negatively correlated with SMP. Significant positive correlation (p≤0.01) was seen between MPW and SMP.

#### 4.4.3.5 Significant correlations between insoluble protein fraction and quality characteristics across the three locations

A highly negative significant correlation (P≤0.001) was observed between LPP and MRI. FPC, EPV, ETV and MLV showed a significant positive correlation (p≤0.05) with LPP (Table 4.13). SPP exhibited a highly significant correlation (p≤0.001) with the SDSVOL but a significant correlation (p≤0.01) with EPV and MTV. MRI was negatively correlated (p≤0.05) with SPP. Quality characters MPV, MRV, MTI and MTV showed a highly negative and significant (p≤0.001) correlation with LMP but MRI showed high positive correlation. SDSVOL showed a significant negative (p≤0.05) correlation with LMP. SDSVOL revealed a significant (p≤0.01) negative correlation with SMP while MRI showed a highly significant (p≤0.001) negative correlation with the SMP.

#### 4.4.3.6 Correlation between relative value percentages and quality characteristics across the three locations

Highly significant (p≤0.001) and positive correlations were observed between LUPP respectively with ETV, MLV, MRV, MTI and MTV (Table 4.14). Only MRI showed a highly significant negative correlation with LUPP. Significant (p≤0.05) correlations were observed between LUPP and EPT, ETI, MLT and MRT. FPC and SDSVOL correlated positively and highly significantly with UPP, while MRI exhibited a negative and highly

significant correlation (p≤0.001) with UPP. Significant (p≤0.05) correlations were observed between UPP and EPT, EPW, MLT, MLW, MPT and MRT. POL significantly correlated negatively with ETI and MRI (p≤0.05) and with EPI, EPT, MLI, MLT, MPT and MRT (p≤0.01), respectively. HMW negatively and highly significantly (p≤0.001) correlated with MRI and correlated positively (p≤0.05) with FPC, EPV and MTV. Only EPI revealed a highly and negatively significant (p≤0.001) correlation with LMW. EPT, MLI, MLT, MPT and MRT showed a negative significant (p≤0.01) correlation with LMW. MON was significantly (p≤0.01) correlated with EPI, EPT, MLI, MLT and MRT.

#### 4.4.3.7 Significant correlation between the selected quality characteristics and the absolute value percentages of three locations

SDSVOL showed a significant (p≤0.05) correlation with LUPP. ETV, MLV, MRV and MTV were highly significantly (p≤0.001) correlated with LUPP (Table 4.15). Significant (p≤0.05) correlation was observed between UPP and six quality characteristics. POL showed a significant (p≤0.01) correlation only with MRI. HMW showed a significant (p≤0.001) negative correlation with MRI but correlated (p≤0.001) positively with FPC, EPV, MPV, MRV, MTI and MTV. MRI showed a highly significant (p≤0.001) correlation with LMW and MON, respectively. MLI, MLT, MPT and MRT showed a significant (p≤0.05) correlation with MON.

**Table 4.12 Significant correlations from combined analysis between soluble protein fractions and quality characteristics across three locations**

| Character 1 | Character 2 | Correlation | Character 1 | Character 2 | Correlation | Character 1 | Character 2 | Correlation | Character 1 | Character 2 | Correlation |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **LPP** | **EPI** | -0.36*** | **SPP** | **FPC** | -0.32*** | **LMP** | **SDSVOL** | 0.27** | **SMP** | **FPC** | 0.30*** |
| | **EPT** | -0.34*** | | **SDSVOL** | -0.22* | | **EPI** | 0.53*** | | **EPV** | 0.31*** |
| | **EPW** | -0.19* | | **EPI** | -0.46*** | | **EPT** | 0.42*** | | **ETV** | 0.33*** |
| | **ETI** | -0.30*** | | **EPT** | -0.33*** | | **EPV** | 0.22* | | **MLV** | 0.31*** |
| | **ETV** | -0.29** | | **EPV** | -0.39*** | | **EPW** | 0.23* | | **MPV** | 0.33*** |
| | **MLI** | -0.35*** | | **EPW** | -0.26** | | **ETI** | 0.40*** | | **MPW** | 0.25** |
| | **MLT** | -0.36*** | | **ETI** | -0.35*** | | **ETV** | 0.45*** | | **MRI** | -0.85*** |
| | **MLV** | -0.27** | | **ETV** | -0.56*** | | **MLI** | 0.48*** | | **MRV** | 0.37*** |
| | **MPI** | -0.29** | | **MLI** | -0.40*** | | **MLT** | 0.46*** | | **MTI** | 0.35*** |
| | **MPT** | -0.36*** | | **MLT** | -0.35*** | | **MLV** | 0.45*** | | **MTV** | 0.40*** |
| | **MPW** | -0.24** | | **MLV** | -0.55*** | | **MLW** | 0.25** | | | |
| | **MRT** | -0.36*** | | **MLW** | -0.29** | | **MPI** | 0.25** | | | |
| | **MTV** | -0.25** | | **MPT** | -0.35*** | | **MPT** | 0.46*** | | | |
| | | | | **MPV** | -0.38*** | | **MPW** | 0.31*** | | | |
| | | | | **MPW** | -0.39*** | | **MRT** | 0.46*** | | | |
| | | | | **MRI** | 0.63*** | | **MRV** | 0.32*** | | | |
| | | | | **MRT** | -0.35*** | | **MTI** | 0.30*** | | | |
| | | | | **MRV** | -0.51*** | | **MTV** | 0.44*** | | | |
| | | | | **MTI** | -0.48*** | | | | | | |
| | | | | **MTV** | -0.60*** | | | | | | |

*p≤0.05, **p≤0.01, ***p≤0.001, LPP=Large polymeric proteins, EPI=Envelope peak integral, EPT=Envelope peak time, EPW=Envelope peak width, ETI=Envelope tail integral, ETV=Envelope tail value, MLI=Midline left integral, MLT=Midline left time, MLV=Midline left value, MPI=Midline peak integral, MPT=Midline peak time, MPW=Midline peak width, MRT=Midline right time, MTV=Midline tail value, SPP=Small polymeric proteins, FPC=Flour protein content, SDSVOL=SDS-sedimentation volume, EPV=Envelope peak value, MLW=Midline left width, MPV=Midline peak value, MRI=Midline right integral, MTI=Midline tail integral, MRV=Midline right value, LMP=Large monomeric proteins, SMP=Small monomeric proteins

**Table 4.13 Significant correlations from combined analysis between insoluble protein fractions and quality characteristics across three locations**

| Character 1 | Character 2 | Correlation | Character 1 | Character 2 | Correlation | Character 1 | Character 2 | Correlation | Character 1 | Character 2 | Correlation |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **LPP** | **FPC** | 0.22* | **SPP** | **SDSVOL** | 0.30*** | **LMP** | **FPC** | -0.25** | **SMP** | **SDSVOL** | -0.28** |
| | **EPV** | 0.23* | | **EPV** | 0.22* | | **SDSVOL** | -0.23* | | **MRI** | -0.46*** |
| | **ETV** | 0.23* | | **MPV** | 0.27** | | **EPV** | -0.29** | | | |
| | **MLV** | 0.23* | | **MRI** | -0.23* | | **ETV** | -0.24** | | | |
| | **MPV** | 0.27** | | **MRV** | 0.27** | | **MLV** | -0.26** | | | |
| | **MRI** | -0.80*** | | **MTI** | 0.26** | | **MPV** | -0.35*** | | | |
| | **MRV** | 0.28** | | **MTV** | 0.21* | | **MRI** | 0.69*** | | | |
| | **MTI** | 0.28** | | | | | **MRV** | -0.36*** | | | |
| | **MTV** | 0.28** | | | | | **MTI** | -0.35*** | | | |
| | | | | | | | **MTV** | -0.32*** | | | |

*p≤0.05, **p≤0.01, ***p≤0.001, LPP=Large polymeric proteins, FPC=Flour protein content, EPV=Envelope peak value, EPW=Envelope peak width, ETV=Envelope tail value, MLV=Midline left value, MPV=Midline peak value, MRI=Midline right integral, MRV=Midline right value, MTI=Midline tail integral, MTV=Midline tail value, SPP=Small polymeric proteins, SDSVOL=SDS-sedimentation volume, LMP=Large monomer proteins, SMP=Small monomeric proteins

**Table 4.14 Significant correlation from combined analysis relative value percentages and selected quality characteristics in all three locations**

| Character 1 | Character 2 | Correlation | Character 1 | Character 2 | Correlation |
|---|---|---|---|---|---|
| **LUPP** | **FPC** | 0.25** | **POL** | **EPI** | -0.28** |
| | **EPI** | 0.27** | | **EPT** | -0.26** |
| | **EPT** | 0.21* | | **ETI** | -0.22* |
| | **EPV** | 0.28** | | **MLI** | -0.26** |
| | **ETI** | 0.23* | | **MLT** | -0.28** |
| | **ETV** | 0.42*** | | **MPT** | -0.28** |
| | **MLI** | 0.25** | | **MRI** | -0.24* |
| | **MLT** | 0.22* | | **MRT** | -0.28** |
| | **MLV** | 0.40*** | **HMW** | **FPC** | 0.22* |
| | **MPT** | 0.22** | | **EPV** | 0.21* |
| | **MPV** | 0.28** | | **MPV** | 0.26** |
| | **MPW** | 0.27** | | **MRI** | -0.77*** |
| | **MRI** | -0.74*** | | **MRV** | 0.25** |
| | **MRT** | 0.22* | | **MTI** | 0.25** |
| | **MRV** | 0.38*** | | **MTV** | 0.23* |
| | **MTI** | 0.35*** | **LMW** | **EPI** | -0.30*** |
| | **MTV** | 0.44*** | | **EPT** | -0.25** |
| **UPP** | **FPC** | 0.33*** | | **ETI** | -0.23* |
| | **SDSVOL** | 0.30*** | | **ETV** | -0.22* |
| | **EPI** | 0.34*** | | **MLI** | -0.25** |
| | **EPT** | 0.22* | | **MLT** | -0.25** |
| | **EPV** | 0.41*** | | **MLV** | -0.19* |
| | **EPW** | 0.20* | | **MLW** | -0.20* |
| | **ETI** | 0.25** | | **MPT** | -0.25** |
| | **ETV** | 0.50*** | | **MPW** | -0.20* |
| | **MLI** | 0.29** | | **MRI** | 0.19* |
| | **MLT** | 0.23* | | **MRT** | -0.25** |
| | **MLV** | 0.51*** | | **MTV** | -0.19* |
| | **MLW** | 0.20* | **MON** | **EPI** | 0.28** |
| | **MPT** | 0.23* | | **EPT** | 0.26** |
| | **MPV** | 0.43*** | | **ETI** | 0.22* |
| | **MPW** | 0.33*** | | **MLI** | 0.26** |
| | **MRI** | -0.71*** | | **MLT** | 0.28** |
| | **MRT** | 0.23* | | **MPT** | 0.28** |
| | **MRV** | 0.52*** | | **MRI** | 0.24* |
| | **MTI** | 0.50*** | | **MRT** | 0.28** |
| | **MTV** | 0.57*** | | | |

*p≤0.05, **p≤0.01, ***p≤0.001, LUPP=Large unextractable polymeric proteins, UPP=Unextractable polymeric proteins, FPC=Flour protein content, SDSVOL=SDS-sedimentation volume, EPI=Envelope peak integral, EPT=Envelope peak time, EPV=Envelope peak value, EPW=Envelope peak width, ETI=Envelope tail integral, ETV=Envelope tail value, MLI=Midline left integral, MLT=Midline left time, MLV=Midline left value, MLW=Midline left width, MPI=Midline peak integral, MPT=Midline peak time, MPV=Midline peak value, MPW=Midline peak width, MRI=Midline right integral, MRT=Midline right time, MRV=Midline right value, MTI=Midline tail integral, MTV=Midline tail value, POL=Total polymeric proteins, HMW=High molecular weight, LMW=Low molecular weight, MON=Total monomeric proteins

**Table 4.15 Significant correlation from combined analysis between absolute value percentages and selected quality characteristics across three locations**

| Character 1 | Character 2 | Correlation | Character 1 | Character 2 | Correlation |
|---|---|---|---|---|---|
| **LUPP** | **SDSVOL** | 0.18* | **HMW** | **FPC** | 0.41*** |
| | **EPI** | 0.27** | | **EPV** | 0.35*** |
| | **EPT** | 0.22* | | **ETV** | 0.27** |
| | **EPV** | 0.19* | | **MLV** | 0.25** |
| | **ETV** | 0.33*** | | **MPV** | 0.42*** |
| | **MLI** | 0.29** | | **MRI** | -0.65*** |
| | **MLT** | 0.25** | | **MRV** | 0.41*** |
| | **MLV** | 0.35*** | | **MTI** | 0.39*** |
| | **MPT** | 0.25** | | **MTV** | 0.35*** |
| | **MPV** | 0.21* | **LMW** | **MRI** | 0.38*** |
| | **MRT** | 0.25** | **MON** | **MLI** | 0.19* |
| | **MRV** | 0.30*** | | **MLT** | 0.20* |
| | **MTI** | 0.28** | | **MPT** | 0.20* |
| | **MTV** | 0.35*** | | **MRI** | 0.47*** |
| **UPP** | **MLI** | 0.21* | | **MRT** | 0.20* |
| | **MLT** | 0.185* | | | |
| | **MLV** | 0.21* | | | |
| | **MPT** | 0.19* | | | |
| | **MRT** | 0.19* | | | |
| | **MTV** | 0.19* | | | |
| **POL** | **MRI** | 0.26** | | | |

*p≤0.05, **p≤0.01, ***p≤0.001, LUPP=Large unextractable polymeric proteins, UPP=Unextractable polymeric proteins, POL=Total polymeric proteins, FPC=Flour protein content, SDSVOL=SDS-sedimentation volume, EPI=Envelope peak integral, EPT=Envelope peak time, EPV=Envelope peak value, EPW=Envelope peak width, ETI=Envelope tail integral, ETV=Envelope tail value, MLI=Midline left integral, MLT=Midline left time, MLV=Midline left value, MLW=Midline left width, MPI=Midline peak integral, MPT=Midline peak time, MPV=Midline peak value, MPW=Midline peak width, MRI=Midline right integral, MRT=Midline right time, MRV=Midline right value, MTI=Midline tail integral, MTV=Midline tail value, HMW=High molecular weight, LMW=Low molecular weight, MON=Total monomeric proteins

## 4.5 Discussion

Gluten proteins are the most important factor in determining bread making quality (MacRitchie et al., 1990). SE-HPLC was used to separate four main peaks of protein from the wheat flour (Larroque et al., 1997). In this study, the combined analysis of variance showed highly significant effects of genotype, environment and the interaction between them for most of the separated protein fractions, relative and absolute value. This indicates variable responses between the genotypes and environments for nearly all protein fractions, both relative and absolute value. Genotype and environment contributed almost an equal amount/percentage to the total variation of each protein fraction separated.

Across the three locations, SDSVOL correlated negatively with SPP of the SDS soluble fraction but it was highly significant and positively correlated with the SPP of the insoluble fractions. These results were in contrast with the results reported by Labuschagne and Aucamp (2004) where SDSVOL correlated negatively with small polymeric proteins across five locations tested.

In a single location analysis, large and SMP showed a positive and negative correlation with SDSVOL in Douglas and Vaalharts, of soluble fractions, respectively. In all three locations, SDSVOL showed a positive and a negative correlation between SPP and SMP of the insoluble fractions, respectively. This can indicates that an increase in the SPP might lead to a reduction in the SMP.

Sonication allows the evaluation of the insoluble protein fractions as possible predictors of bread making quality. It is also very important to produce cultivars with a stable polymeric-to-monomeric ratio across environments (Southan and MacRitchie, 1999). In this study, LMP and SMP correlated negatively with insoluble protein fractions. Labuschagne and Aucamp (2004) reported a successful extraction of insoluble proteins using sonication, leading to a very strong correlation between relative quantity of gluten and bread making quality when measured across locations.

Most of the fractions of both single locations and combined locations in this study revealed a correlation with FPC. Soluble and absolute value percentage fractions had no correlation with FPC in all three locations, except for UPP and MON at Douglas. HMW proteins correlated negatively and significantly with FPC at Douglas and Marydale for relative value fractions. The insoluble protein fractions at Douglas and Marydale both revealed a negative correlation with LPP while at Vaalharts LMP positively correlated with FPC.

In the combined analysis, FPC correlated positively with the HMW fractions (absolute value). These findings are in agreement with previous studies that have shown highly positive correlations between total protein content and absolute areas of individual peaks of both soluble and insoluble fractions (Ciaffi et al., 1996). They also found significant positive correlations between the percentage area of both monomeric and insoluble proteins and FPC. In this study a highly significant correlation was found between UPP. FPC and LUPP as well as HMW proteins (relative value) were significantly correlated. This indicated that as the FPC increased, the formation of large polymers may also have been positively affected. Consequently the relative amount of polymeric proteins easily extracted in SDS buffer decreased with increasing FPC. From this study, the fractions (especially absolute and soluble), were better predictors of bread making quality in the combined analysis compared to single location analysis.

Mixograph peak time, peak height and curve-width are determined by protein quality and protein quantity as well as the water-absorption of the specific flour (Martinant, 1998). Peak time has been reported to be largely genetically determined (van Lill, 1995) and is the most frequently used mixograph parameter reported in literature. In this study across all environments in both combined and single location analysis there was a negative correlation between MPT and LPP as well as SPP, but there was a highly significantly positive correlation with the LMP of the soluble proteins. Most researchers reported that the LPP are the main contributor to variation in dough properties (Weegels et al., 1996; MacRitchie and Lafiandra, 1997; Schober et al., 2006). Ohm et al. (2010) reported a correlation between peak time and FPC. The same correlations were observed for MPT of this study. Nevertheless, MPT has been found to be a poor parameter to explain bread making quality (Branlard et al., 1991; Dong et al., 1992; Khatkar et al., 1996).

Ohm et al. (2009) confirmed the applicability of SE-HPLC analysis of wheat protein for the evaluation of flour mixing characteristics by reporting a significant correlation between both mixograph water absorption and mixing tolerance and absorbance area and area percentage. For this study, EPV and EPI correlated positively and negatively with LMP and SPPs, respectively, at Douglas and Marydale for soluble fractions. There was no correlation between EPV and insoluble fractions. Whereas in a combined analysis, EPV highly and significantly correlated with most of the soluble fractions and LMP of the insoluble fraction.

Martinant et al. (1998) reported a relationship between the extractable protein content and MPV and another positive relationship between the unextractable proteins and the parameters describing the curve after peak time i.e. MRV. In this study, from the

combined analysis, MPV positively correlated highly and positively with SMP in soluble fractions and the MRV of insoluble fractions related positively with LPP and SPP. In reconstitution studies, monomeric protein fractions, in general, showed the same effects on dough functionality as the MON (Branlard and Dardevet, 1985; Dong et al., 1992). In this study the MON for the relative value revealed a highly significant difference for the genotypes, GxE interaction and environment, but no differences were observed for the absolute value. MON correlated positively with EPI and with relative value at Marydale and Vaalharts. Positive and significant correlations between absolute value and MON at each location was reported.

### 4.6 Conclusions

SE-HPLC has been used to relate the quantity of polymeric and monomeric protein fractions to bread making characteristics (Huebner and Beitz, 1985; Dachkevitch and Autran, 1989). This study was performed to investigate the relationships of quality characteristics of elite breeding lines with molecular weight distribution of proteins. This technique has proved to be a good indicator of the relation between protein fractions and mixing parameters as well as other measured quality characteristics such as FPC and SDSVOL. A much larger variation was found among genotypes, for all protein fractions: soluble, insoluble and relative value percentages except the absolute value percentages, than among environments. This indicates the higher heritability of these fractions. For soluble fractions, MTV was a good predictor of SPPs in both Douglas and Vaalharts and also in combined analysis. SDSVOL, EPI and MRI were very good predictors of UPP in relative value for Douglas and combined analysis.

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# Chapter 5

## Relationships of breeding lines with quality characteristics, determined by principle component analysis

### 5.1 Abstract

For the assessment of relationships between measured mixograph characteristics, flour protein content and SDS-sedimentation volume, a data matrix from 13 samples of wheat elite lines was subjected to principal component analysis. The applied method of principal component analysis allowed a complex assessment of the relations between the characteristics. The first five principal components contributed significantly to the variation. Principal component 1 explained 77.03% of the total variation, principal component 2 15.60% and principal components 3, 4 and 5 explained 3.73%, 3.15% and 0.32% of the total variation, respectively. All these principal components had an eigenvalue of more than 1. Principal component 1 variation was mainly due to envelope tail integral, midline left integral, midline peak integral, midline right integral and midline tail integral with a cumulative percentage of 77%. Both principal component 3 and principal component 4 were dominated by SDS-sedimentation volume and envelope peak integral. The highest negative loading was contributed by envelope peak integral in principal component 5 with a cumulative percentage of 99%. Principal component analysis biplot provided an overview of similarities and differences between measured characters and different genotypes. Midline peak value and envelope peak value were highly correlated. Envelope peak width, midline right value, midline peak width and midline tail integral were also highly correlated. Lines 9, 12 and 8 were grouped closely together and had higher value for envelope peak value, flour protein content and midline right integral. Line 2 had high value for midline left value and midline tail value. Over the locations, elite lines 13, 12, 2 and 1 have shown to be the best lines because of their closeness to the grouped characteristics.

### 5.2 Introduction

Analysis of multivariate data plays an important role in data analysis. These multivariate data consist of many different attributes or variables recorded for each observation. Summarizing multivariate attributes in two or three PCs that can be displayed graphically with minimal loss of information, is useful in knowledge discovery (Janmohammadi et

al., 2014). Because it is hard to visualize a multi-dimensional space, PCA, a multivariate technique that analyses a data table in which observations are described by several inter-correlated quantitative dependent variables (Nachimuthu et al., 2014) is very useful.

The objective of PCA is to identify a new set of orthogonal axes such that: 1) the coordinates of the observations with respect to each of the axes give the value for the new variables, PC's are the new axes or variables and PC scores are value of the new variable, 2) each new variable is a linear combination of the original variables, 3) the first new variable accounts for the maximum variance in the data, 4) the following new variable accounts for the maximum variance that has not been accounted for by the previous variables (Armanino and Festa, 1996; Sharma, 1996; Lewis and Lisle, 1998). PCA makes it possible to transform a given set of traits, which are correlated, into a new system of traits, known as PC's, which are not correlated. Each PC is a linear combination of the original variables, and so it is often possible to ascribe the meaning to what the components represent (Lewis and Lisle, 1998).

The proportion accounted for by each PC is expressed by the eigenvalue divided by the sum of the eigenvalues. The eigenvector defines the relation of the PC axes to the original data axes. The loadings indicate the relative contribution of the variables to each principal component: the higher the loading of a variable on a principal component, the more the variable has in common with this component (Armanino and Festa, 1996).

Correlation analysis helps in determining effective traits in order to indirectly select superior genotypes. On the other hand, PCA is a suitable multivariate technique in identifying and determining the independent PC that are effective for separate plant traits. Therefore, correlation and PCA helps breeders to distinguish significant relationships between traits such as mixograph parameters and SDSVOL, especially in early generations via indirect selection for traits (Tadesse and Bekele, 2001; Bennani et al., 2016).

PCA allows the relationship between variables and observations to be studied, as well as recognizing the data structure. However, there are few studies done on different wheat quality parameters using PCA. Veronesi and Falcinelli (1988) and Chozin (2007) showed a multivariate analysis to be a valid system to deal with germplasm collections and evaluation. The objective of this study was to determine the relationship between elite breeding lines and the measured quality characteristics through the use of PCA.

## 5.3 Material and methods

### 5.3.1 Plant material

Elite lines from the irrigation breeding programme were used as described in Chapter 3 (section 3.3.1).

### 5.3.2 Measured quality characteristics

Quality characteristics were described in Chapter 3 (section 3.3.2).

### 5.3.3 Statistical analysis

Correlations and PCA as well as biplot graphical display were performed using GenStats software (Payne et al., 2008) for all the traits of the tested bread wheat breeding lines across three locations.

#### 5.3.3.1 Principal component analysis

The PCA method explained by Harman (1976) was followed in the extraction of the components. Means of each variable were standardized prior to PCA as suggested by Ruiz et al. (1997) by subtracting from each observation the mean value of the character and subsequently dividing it by its respective standard deviation (Ruiz et al., 1997; Upadhyaya et al., 2002). PC plots were produced to determine the distribution of variation in the data, detect correlation between variables and ascertain which breeding lines had the desirable characteristics. The percentage variability explained by each component was determined (Harman, 1976; Sharma, 1996; Tadesse and Bekele, 2001).

## 5.4 Results

### 5.4.1 Correlations for combined analysis of measured quality characteristics

FPC correlated significantly ($p \leq 0.001$) and positively with EPV, ETV, MPV, MRV, MTI and MTV. SDSVOL correlated significantly ($p \leq 0.001$) with a number of characters and showed a significant correlation ($p \leq 0.01$ or $p \leq 0.05$) with EPW, MPI and MLW. A significant correlation was observed between EPI and MPI. MRI correlated negatively ($p \leq 0.001$) with FPC, ETV, MLV, MPV, MRV, MTI and MTV. EPV correlated positively with MPV. EPW positively correlated with MRV, MPW and MTI. MPV correlated negatively ($p \leq 0.05$) with MLT, MPT and MRT and correlated negatively ($p \leq 0.001$) with EPT (Table 5.1).

### 5.4.2 Relationships between quality characteristics and genotypes measured by principal component analysis

The first five PC's cumulatively explained 99.83% of the total variation. PC1 and PC2 explained 92.62% of the total variation, which shows a high degree of association among the bread making quality characteristics studied. PC3, 4 and 5 contributed 3.73%, 3.15% and 0.32% to the variation, respectively. These components had eigenvalues of more than 1 (Table 5.2).

The first PC alone explained 77.03% of the total variation, mainly due to variation in the ETI (0.46), MLI (0.34), MPI (0.41), MRI (0.48) and MTI (0.38). PC2 contributed 15.60% to the total variation and was dominated by characters such as MTI (0.71) and MLI (-0.38) which negatively contributed to the variation. The third PC was composed of ETI (-0.64) and SDSVOL (-0.58) with negative loadings and MRI (0.32) with positive loadings. ETI contributed the most of variation among the other characters in this PC with a negative loading. The fourth PC comprised of ETI and SDSVOL with negative and positive loadings, respectively. The eigenvectors of PC5 showed large negative loadings for the EPI (Table 5.2). The higher the eigenvector, regardless of the sign, the more effective they will be in discriminating between variables (Nachimuthu et al., 2014). For PC1, ETI, MLI, MPI, MRI and MTI contributed the most to the variation, while for PC2, MLI and MTI contributed most to the variation (Table 5.2).

In the PCA biplot, MPV and EPV were highly correlated. EPW, MRV, MPW and MTI were also highly correlated. EPT and MLI correlated with MRT, MPT and MLT (Figure 5.1).

Elite lines 10, 5 and 13 were furthest away from the centre of the biplot. Elite lines 6 and 9 were the closest to the PCA biplot, meaning that they recorded average value for most of the characters investigated. Elite lines 11, 7 and 13 recorded higher value for the MLI, EPI, MPI and ETI. Lines 9, 12 and 8 were grouped closely together and had higher percentages for ETV, FPC and MRI. Line 2 had high values recorded for MLV and MTV (Figure 5.1).

**Table 5.1 Correlations for quality characteristics measured on 13 elite lines combined across three locations**

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **FPC** | **EPV** | 0.53*** | **EPI** | **EPT** | 0.84*** | **EPV** | **EPW** | 0.74*** | **ETI** | **ETV** | 0.83*** | **MLI** | **MLT** | 0.98*** | **MLW** | **MPV** | 0.44*** |
| | **ETV** | 0.30*** | | **EPW** | 0.30*** | | **ETI** | 0.24* | | **MLI** | 0.79*** | | **MLV** | 0.64*** | | **MPW** | 0.66*** |
| | **MLT** | -0.20* | | **ETI** | 0.78*** | | **ETV** | 0.57*** | | **MLT** | 0.72*** | | **MLW** | 0.27** | | **MRV** | 0.56*** |
| | **MLV** | 0.20* | | **ETV** | 0.71*** | | **MLV** | 0.68*** | | **MLV** | 0.74*** | | **MPI** | 0.30*** | | **MTI** | 0.57*** |
| | **MPT** | -0.20* | | **MLI** | 0.90*** | | **MLW** | 0.60*** | | **MLW** | 0.63*** | | **MPT** | 0.98*** | | **MTV** | 0.59*** |
| | **MPV** | 0.61*** | | **MLT** | 0.86*** | | **MPV** | 0.95*** | | **MPI** | 0.31*** | | **MPW** | 0.28** | **MPI** | **MPT** | 0.31*** |
| | **MRI** | -0.35*** | | **MLV** | 0.68*** | | **MPW** | 0.72*** | | **MPT** | 0.72*** | | **MRT** | 0.98*** | | **MRT** | 0.31*** |
| | **MRT** | -0.20* | | **MLW** | 0.40*** | | **MRI** | -0.29** | | **MPW** | 0.53*** | | **MTI** | 0.19* | | **MTV** | 0.22* |
| | **MRV** | 0.59*** | | **MPI** | 0.23* | | **MRV** | 0.94*** | | **MRT** | 0.72*** | | **MTV** | 0.52*** | **MPT** | **MPV** | -0.23* |
| | **MTI** | 0.54*** | | **MPT** | 0.86*** | | **MTI** | 0.93*** | | **MRV** | 0.35*** | **MLT** | **MLV** | 0.51*** | | **MPW** | 0.19* |
| | **MTV** | 0.47*** | | **MPW** | 0.46*** | | **MTV** | 0.80*** | | **MTI** | 0.37*** | | **MPI** | 0.31*** | | **MTV** | 0.40*** |
| **SDSVOL** | **EPI** | 0.44*** | | **MRT** | 0.86*** | **EPW** | **ETI** | 0.55*** | | **MTV** | 0.60*** | | **MPT** | 1.00*** | **MPV** | **MPW** | 0.49*** |
| | **EPT** | 0.35*** | | **MRV** | 0.30*** | | **ETV** | 0.57*** | **ETV** | **MLI** | 0.70*** | | **MPV** | -0.23* | | **MRI** | -0.32*** |
| | **EPV** | 0.33*** | | **MTI** | 0.31*** | | **MLI** | 0.22* | | **MLT** | 0.60*** | | **MPW** | 0.19* | | **MRT** | -0.23* |
| | **EPW** | 0.24** | | **MTV** | 0.59*** | | **MLV** | 0.62*** | | **MLV** | 0.89*** | | **MRT** | 1.00*** | | **MRV** | 0.93*** |
| | **ETI** | 0.40*** | **EPT** | **EPV** | -0.25** | | **MLW** | 0.81*** | | **MLW** | 0.58*** | | **MTV** | 0.40*** | | **MTI** | 0.92*** |
| | **ETV** | 0.54*** | | **ETI** | 0.52*** | | **MPV** | 0.52*** | | **MPI** | 0.27** | **MLV** | **MLW** | 0.62*** | | **MTV** | 0.74*** |
| | **MLI** | 0.41*** | | **ETV** | 0.43*** | | **MPW** | 0.84*** | | **MPT** | 0.60*** | | **MPI** | 0.27** | **MPW** | **MRI** | -0.22* |
| | **MLT** | 0.38*** | | **MLI** | 0.86*** | | **MRV** | 0.62*** | | **MPV** | 0.46*** | | **MPT** | 0.51*** | | **MRT** | 0.19* |
| | **MLV** | 0.53*** | | **MLT** | 0.90*** | | **MTI** | 0.62*** | | **MPW** | 0.57*** | | **MPV** | 0.59*** | | **MRV** | 0.60*** |
| | **MLW** | 0.22* | | **MLV** | 0.34*** | | **MTV** | 0.64*** | | **MRI** | -0.35*** | | **MPW** | 0.64*** | | **MTI** | 0.61*** |
| | **MPI** | 0.28** | | **MPI** | 0.26** | | | | | **MRT** | 0.60*** | | **MRI** | -0.31*** | | **MTV** | 0.64*** |
| | **MPT** | 0.38*** | | **MPT** | 0.90*** | | | | | **MRV** | 0.72*** | | **MRT** | 0.51*** | **MRI** | **MRV** | -0.38*** |
| | **MPV** | 0.30*** | | **MPV** | -0.31*** | | | | | **MTI** | 0.72*** | | **MRV** | 0.81*** | | **MTI** | -0.36*** |
| | **MPW** | 0.30*** | | **MRT** | 0.90*** | | | | | **MTV** | 0.90*** | | **MTI** | 0.83*** | | **MTV** | -0.39*** |
| | **MRT** | 0.38*** | | **MTV** | 0.26** | | | | | | | | **MTV** | 0.91*** | **MRT** | **MTV** | 0.40*** |
| | **MRV** | 0.46*** | | | | | | | | | | | | | **MRV** | **MTI** | 0.99*** |
| | **MTI** | 0.43*** | | | | | | | | | | | | | | **MTV** | 0.92*** |
| | **MTV** | 0.52*** | | | | | | | | | | | | | **MTI** | **MTV** | 0.91*** |

*p≤0.05, ** p≤0.01, *** p≤0.001, FPC=Flour protein content, SDSVOL=SDS-sedimentation volume, EPV=Envelope peak value, ETV=Envelope tail value, MLT=Midline left tail, MLV=Midline left value, MPT=Midline peak time, MPV=Midline peak value, MRI=Midline right integral, MRT=Midline right time, MRV=Midline right value, MTI=Midline tail integral, MTV=Midline tail value, EPI=Envelope peak integral, EPT=Envelope peak time, EPW=Envelope peak width, ETI=Envelope tail integral, MLI=Midline left integral, MLW=Midline left width, MPI=Midline peak integral, MPW=Midline peak width

**Table 5.2 Principle component analysis of 21 quality characteristics showing eigenvalues, percentage variation and cumulative percentage of variation explained by the first five PC axes**

| | Eigenvectors | | | | |
|---|---|---|---|---|---|
| **Characters** | **PC1** | **PC2** | **PC3** | **PC4** | **PC5** |
| **EPI** | 0.22 | -0.13 | 0.14 | 0.14 | **-0.90** |
| **EPT** | 0.01 | -0.02 | 0.00 | 0.02 | -0.03 |
| **EPV** | 0.09 | 0.26 | 0.10 | -0.04 | -0.17 |
| **EPW** | 0.06 | 0.12 | 0.04 | -0.10 | -0.15 |
| **ETI** | **0.46** | -0.27 | **-0.64** | **-0.51** | -0.04 |
| **ETV** | 0.13 | 0.00 | -0.09 | -0.06 | 0.05 |
| **FPC** | 0.01 | 0.00 | 0.02 | 0.01 | -0.03 |
| **MLI** | **0.34** | **-0.38** | 0.21 | 0.16 | 0.15 |
| **MLT** | 0.01 | -0.01 | 0.01 | 0.01 | 0.00 |
| **MLV** | 0.10 | 0.07 | -0.01 | -0.06 | 0.09 |
| **MLW** | 0.06 | 0.09 | 0.00 | -0.09 | -0.09 |
| **MPI** | **0.41** | -0.21 | 0.26 | 0.14 | 0.19 |
| **MPT** | 0.01 | -0.01 | 0.01 | 0.01 | 0.00 |
| **MPV** | 0.06 | 0.22 | 0.10 | 0.00 | -0.06 |
| **MPW** | 0.05 | 0.09 | 0.02 | -0.07 | -0.14 |
| **MRI** | **0.48** | -0.03 | **0.32** | 0.13 | 0.19 |
| **MRT** | 0.01 | -0.01 | 0.01 | 0.01 | 0.00 |
| **MRV** | 0.08 | 0.14 | 0.02 | -0.01 | 0.04 |
| **MTI** | **0.38** | **0.71** | 0.09 | -0.22 | 0.07 |
| **MTV** | 0.09 | 0.06 | 0.02 | -0.01 | -0.07 |
| **SDSVOL** | 0.19 | 0.23 | **-0.58** | **0.76** | 0.02 |
| **Eigenvalues** | 482.06 | 97.61 | 23.35 | 19.73 | 2.02 |
| **% variation** | 77.03 | 15.60 | 3.73 | 3.15 | 0.32 |
| **cumulative %** | 77.00 | 92.63 | 96.36 | 99.51 | 99.83 |

PC=Principal component, EPI=Envelope peak integral, EPT=Envelope peak time, EPV=Envelope peak value, EPW=Envelope peak width, ETI=Envelope tail integral, ETV=Envelope tail value, FPC=Flour protein content, MLI=Midline left integral, MLT=Midline left time, MLV=Midline left value, MLW=Midline left width, MPI=Midline peak integral, MPT=Midline peak time, MPV=Midline peak value, MPW=Midline peak width, MRI=Midline right integral, MRT=Midline right time, MRV=Midline right value, MTI=Midline tail integral, MTV=Midline tail value, SDSVOL=SDS- sedimentation volume

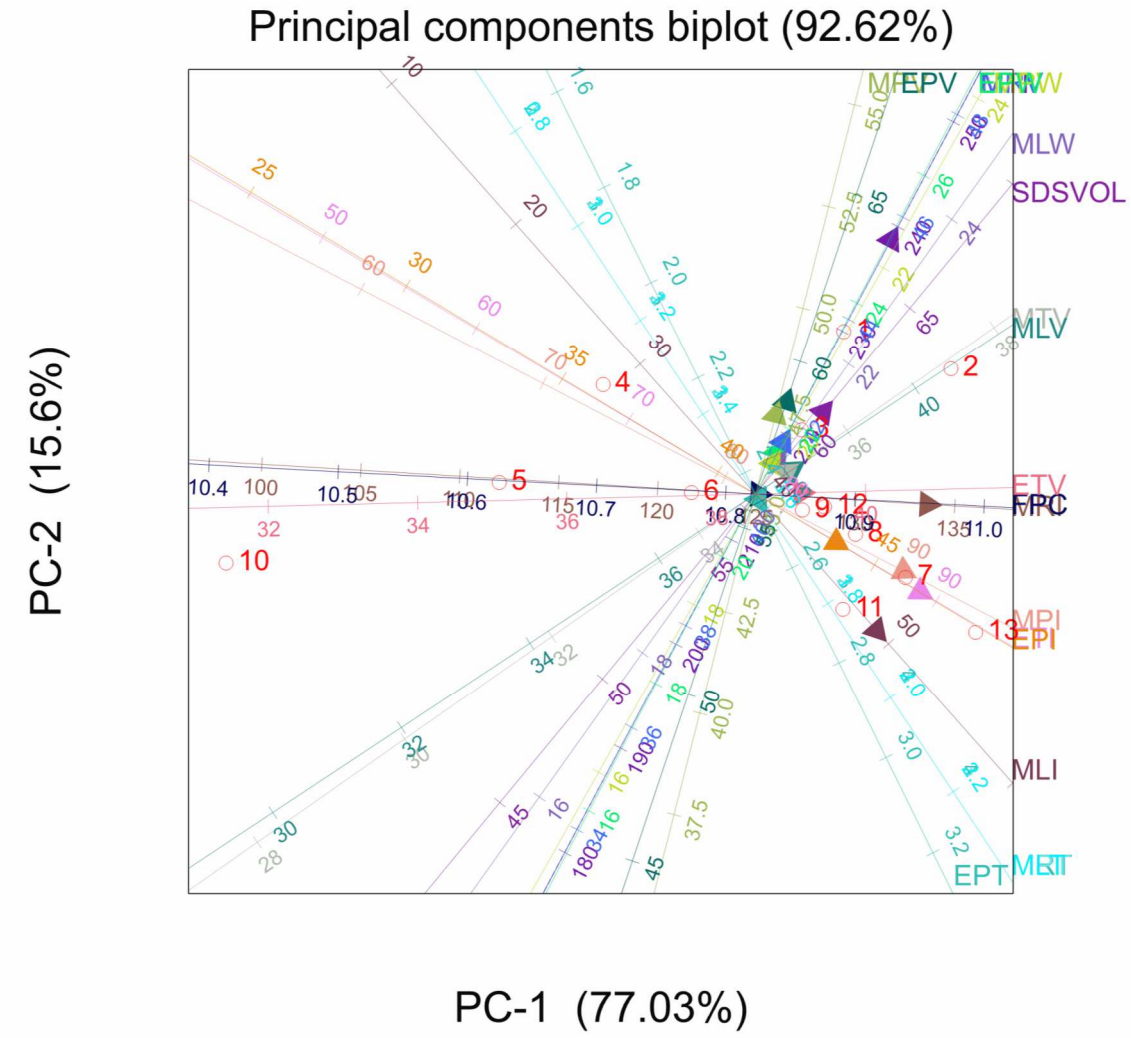


**Figure 5.1 Principle component analysis biplot of 21 quality characters and 13 wheat elite lines**

### 5.5 Discussion

PCA measures the importance and contribution of each component to total variance. It also helps a researcher to distinguish significant relationships between traits (Beheshtizadeh et al., 2013). The first PC accounts for maximum variability in the data with respect to succeeding components (Ajmal et al., 2013). In this study, the first PC accounted for 77.03% of total variation with positive contribution of ETI, MLI, MPI, MRI and MTI. Leilah and Al-Khateeb (2005) studied bread wheat genotypes and they showed that yield factor, biomass factor and harvest index factor accounted for 74.4% of total variation. In addition, Mohamed (1999) reported that two factors (grain yield and spike density) accounted for 80.8% of variation among traits in some bread wheat genotypes.

Total variation in each principal axis is determined by the number of variables (Caldo et al., 1996). The first PC associated positively with ETI, MLI, MPI, MRI and MTI, therefore it can be regarded as MRI component. Tadesse and Bekele (2001) regarded the first component as productivity per plant factory since it consisted of few variables that were associated with several traits which are components of yield. Barak et al. (2014) associate first and second components with solvent retention capacities damaged starch content and spread ratio. In this study, the second PC was associated with MTI because it was positively and negatively associated with MTI and MLI, respectively, which accounted for 15.60% of the total variation.

Many researchers use 1000-seed weight, which was accounted largely for in PC1, as a guide to selecting high yielding wheat cultivars and it was also reported as closely related to grain yield (Leilah and Al-Khateeb, 2005; Deyong, 2011; Rymuza et al., 2012; Beheshtizadeh et al., 2013). Caldo et al. (1996) found maturity, heading, plant height, culm length, leaf length, and tillering to be the highest contributing variables to variation of rice cultivars. Nachimuthu et al. (2014) and Sanni et al. (2012) have also reported similar results for phenological related variables. Tadesse et al. (2015) used PCA to indicate the importance quality parameters such as protein content, dough departure time, dough stability time and dough strength has in discriminating the genotypes.

Chatfield and Collins (1980) stated that components with eigenvalues of less than 1 should be eliminated to reduce the dataset, as eigenvalues greater than 1 are considered significant and component loadings greater than ±0.3 are regarded meaningful (Hair et al., 1998). Thus, the higher the loading of a variable on a PC, the more the variable has in common with this component (Armanino and Festa, 1996). In this study the first five PC's had an eigenvalue of more than 1 and only components loadings with ±0.3 were considered significant.

PCA can be used for measurement of independent impact of a particular trait to the total variance whereas each coefficient of proper vectors indicates the degree of contribution of every original variable with which each PC is associated. The higher the coefficients, regardless of the direction (positive or negative), the more effective they will be in discriminating between variables (Nachimuthu et al., 2014). Seiler and Stafford (1985) reported that the sign (+/-) of the coefficient indicate the direction of the relationship between a component and the variable. Tadesse and Bekele (2001) reported the same results.

PCA provides an overview of the similarities and differences between the quantitative characters of the different accessions and of the interrelationships between the measured characters (Tena et al., 2016). The closeness of variables to the centre of PCA indicates that the variables have an insignificant importance on other quality characteristics between the measured quality characteristics. In this study, elite lines 6 and 9 were the closest to the PCA biplot centre, meaning that they recorded average values for most of the characters investigated and also not significantly important to the measured quality characteristics. Furthest away from the centre of the PCA was elite lines 10, 5 and 13. Lines 9, 12 and 8 grouped closely together had highest percentages for ETV, FPC and MRI.

Over the locations, elite lines 13, 12, 2 and 1 have shown to be the best lines because they were closely situated to the grouped characteristics, indicating a strong correlation between these lines and quality characteristics; MPI, EPI, ETV, FPC, MLV, MTV, SDSVOL and MLW. Rheological characteristics of dough are used to predict the bread making quality of wheat (Bordes et al., 2008); therefore, the lines that are strongly correlated with the characteristics may be suggested for further evaluation or applied by breeders. MPI, ETI and EPI were closely related to Elite line 13 and 7. ETV, MRI and FPC were closely related to elite line 12. Elite line 2 was associated with MTV and MLV while line 1 was closely associated with SDSVOL and MLW. Using PCA, Shewry et al. (2013) reported a strong correlation between the total phenolic and bound phenolic acids compounds extracted from wheat. Also, Mironeasa and Codina (2013) found a close positive relationship between alveograph parameters for Romanian wheat flour.

PCA is a multivariate statistical analysis method, which can be used to reduce the large number of variables to manageable components while uncovering the similarities and the differences between variables. In this study, PCA reduced the number of 21 variables to five PC's. Westerlund et al. (1991) and Schung et al. (1993) used the PCA for the description of some wheat genotypes in regard to grain quality traits.

### 5.6 Conclusions

Characters assigned in the first and the second PCs are considered the most important when it comes to discrimination of characters in respect to succeeding components (Leilah and Al-Khateeb, 2005; Ajmal et al., 2013). In this study, PCA reduced 21 quality characters to five PCs, which explained 99.83% of total variance of the primary data. The first and the second PCs explained most of the variation among the measured bread making quality characteristics, suggesting a high degree of association among the

characters. MTI, SDSVOL and MRI had the strongest discriminatory power. Based on the strong relationship elite lines have with some of the measured quality characteristics, lines 13, 12, 7, 2 and 1 would be recommended to the breeders to place in the pre-release phases.

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# Chapter 6

# General conclusions

Large variation in SDSVOL among genotypes was observed, which varied from 40.33 - 66.44 ml and that for FPC ranged from 10.30 - 11.60%. Measured quality characteristics were more influenced by the genotype and the environment than by the GxE interaction effects. Highly significant correlations between HMW-GS and quality characteristics were observed. A significantly negative correlation between subunits Bx13+By16 and SDSVOL was observed in combined and also in single location (Vaalharts and Marydale) analysis. Douglas, Marydale and Vaalharts all had negative correlations between Bx13+By16 and Dx2+Dy12 subunits and quality characteristics. But HMW-GS Dx5+Dy10 showed a positive correlation with many quality characteristics. In a combined analysis, EPT correlated positively with subunits Bx7+By8 and Dx5+Dy10. There was no significant correlation between FPC and SDSVOL. In addition a large variation between SDSVOL and measured quality characteristics was observed in this study.

In terms of single locations, Douglas showed a negative and positive correlation between soluble protein fractions SPP and LMP and SDSVOL, respectively. Large variation was observed in the insoluble protein fraction and SDSVOL. In both combined and single location analysis of insoluble fraction, a positive and negative correlation between SDSVOL and protein fraction SPP and SMP was revealed, respectively. Highly significant correlations were observed in relative value with UPP and not in absolute value.

Furthermore, the highest correlations were observed between bread making characteristics and SE-HPLC fractions for relative value. Genotype, environment and GxE interaction had larger effects on the expression of soluble, insoluble and relative value fractions compared to the absolute value which had most of the effects not significant to fractions. SDSVOL was largely affected by genotype effect rather than the environment or GxE interactions. Significant relationships between SDSVOL and SE-HPLC protein fractions were observed, especially with UPP and SPP.

PCA reduced 21 quality characteristics to five PC's which explained 99.83% of total variance of the ETI, MLI, MPI, MRI, MTI, SDSVOL and EPI. SDSVOL, MTI and MRI had the strongest discriminatory power. Elite lines 13, 12, 7, 2 and 1 were recommended for the pre-release phase because of their strong relationship with some of the most important measured bread making quality characters.

# Appendices

## Appendix A

**Table 1 Weather data for 2014 and 2015 at three locations**

| Locality | Latitude | Longitude | Altitude | Month | Year | Tx | RHx | RHn | Rs | U2 | Rain | ETO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Douglas** | -29.0965 | 23.65636 | 882 | **1** | **2014** | 35.93 | 84.38 | 17.10 | 21.10 | 1.84 | 0.25 | 5.38 |
| | | | | **2** | | 33.98 | 92.97 | 26.43 | 16.04 | 1.28 | 0.29 | 3.83 |
| | | | | **3** | | 31.78 | 94.25 | 23.58 | 19.63 | 1.31 | 0.06 | 4.33 |
| | | | | **4** | | 28.02 | 94.63 | 20.85 | 13.89 | 1.03 | 0.10 | 3.00 |
| | | | | **5** | | 26.14 | 93.57 | 22.07 | 11.83 | 1.21 | 0.39 | 2.54 |
| | | | | **6** | | 20.80 | 92.71 | 22.47 | 8.78 | 1.71 | 0.07 | 3.06 |
| | | | | **7** | | 20.72 | 85.85 | 20.37 | 14.85 | 1.84 | 0.01 | 2.75 |
| | | | | **8** | | 22.50 | 88.35 | 24.38 | 20.44 | 2.20 | 0.66 | 3.60 |
| | | | | **9** | | 28.25 | 86.16 | 14.98 | 25.57 | 1.71 | 0.09 | 4.90 |
| | | | | **10** | | 31.06 | 85.08 | 13.12 | 31.04 | 1.86 | 0.72 | 6.30 |
| | | | | **11** | | 30.42 | 89.99 | 18.08 | 33.49 | 2.04 | 2.67 | 6.81 |
| | | | | **12** | | 36.58 | 82.13 | 12.68 | 36.49 | 2.44 | 0.45 | 8.69 |
| | | | | **1** | **2015** | 36.58 | 76.93 | 13.93 | 32.89 | 2.41 | 0.33 | 7.93 |
| | | | | **2** | | 35.10 | 80.90 | 11.88 | 35.67 | 2.50 | 0.81 | 8.13 |
| | | | | **3** | | 32.82 | 87.90 | 18.99 | 21.68 | 2.00 | 1.11 | 5.12 |
| | | | | **4** | | 27.00 | 91.91 | 24.55 | 22.50 | 1.31 | 1.07 | 4.29 |
| | | | | **5** | | 28.40 | 87.55 | 14.80 | 17.04 | 1.31 | 0.11 | 3.49 |
| | | | | **6** | | 20.10 | 92.00 | 28.55 | 12.57 | 1.67 | 0.39 | 3.64 |
| | | | | **7** | | 20.73 | 91.14 | 24.87 | 15.82 | 1.79 | 0.40 | 2.65 |
| | | | | **8** | | 24.96 | 85.23 | 18.89 | 17.30 | 1.85 | 0.11 | 3.38 |
| | | | | **9** | | 27.32 | 85.38 | 19.02 | 18.21 | 2.11 | 0.04 | 3.99 |
| | | | | **10** | | 33.62 | 81.37 | 11.36 | 22.97 | 1.99 | 0.23 | 5.43 |
| | | | | **11** | | 31.08 | 71.86 | 11.23 | 25.95 | 2.67 | 0.00 | 6.18 |
| | | | | **12** | | 36.08 | 74.56 | 11.82 | 24.38 | 2.31 | 0.50 | 6.49 |

Tx=Daily maximum temperature, Tn=Daily minimum temperature, Rain=Total rainfall, Rs=Total radiation, RHx=Daily maximum relative humidity, RHn=Daily minimum relative humidity, U2=Average wind speed, ETO=Total relative evapotranspiration

**Table 1 Continues**

| Locality | Latitude | Longitude | Altitude | Month | Year | Tx | Tn | RHx | RHn | Rs | U2 | Rain | ETO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Marydale** | -29.324 | 22.24615 | 928 | **1** | **2014** | 38.52 | 17.95 | 68.70 | 12.17 | 28.64 | 1.98 | 0.86 | 7.09 |
| | | | | **2** | | 37.78 | 17.93 | 81.15 | 15.26 | 24.98 | 1.80 | 1.35 | 5.92 |
| | | | | **3** | | 34.30 | 13.28 | 84.18 | 16.38 | 18.20 | 1.88 | 1.33 | 4.65 |
| | | | | **4** | | 30.25 | 8.37 | 82.27 | 17.54 | 14.58 | 1.48 | 0.39 | 3.44 |
| | | | | **5** | | 26.82 | 5.97 | 86.62 | 21.37 | 12.78 | 1.68 | 1.75 | 2.87 |
| | | | | **6** | | 21.67 | -0.42 | 90.87 | 20.83 | 12.54 | 1.53 | 0.06 | 15.95 |
| | | | | **7** | | 22.00 | -0.29 | 80.53 | 17.31 | 13.71 | 1.78 | 0.00 | 2.85 |
| | | | | **8** | | 23.86 | 3.55 | 82.90 | 18.71 | 15.26 | 2.29 | 0.34 | 3.25 |
| | | | | **9** | | 29.84 | 5.31 | 71.63 | 9.74 | 19.43 | 1.84 | 0.11 | 4.35 |
| | | | | **10** | | 33.28 | 11.36 | 64.22 | 9.42 | 25.45 | 2.49 | 0.03 | 6.04 |
| | | | | **11** | | 28.35 | 11.64 | 66.84 | 11.34 | 26.54 | 2.48 | 0.78 | 6.20 |
| | | | | **12** | | 31.22 | 13.05 | 54.72 | 6.87 | 30.03 | 2.48 | 0.19 | 7.58 |
| | | | | **1** | **2015** | 36.65 | 17.48 | 64.88 | 10.13 | 26.85 | 2.36 | 1.45 | 7.04 |
| | | | | **2** | | 34.07 | 14.21 | 62.47 | 7.91 | 28.10 | 2.36 | 0.06 | 6.89 |
| | | | | **3** | | 35.18 | 14.97 | 80.76 | 13.48 | 21.52 | 1.95 | 1.25 | 5.44 |
| | | | | **4** | | 28.65 | 9.13 | 79.49 | 17.71 | 16.40 | 1.52 | 0.61 | 3.57 |
| | | | | **5** | | 29.32 | 3.32 | 67.41 | 10.68 | 14.41 | 1.38 | 0.00 | 3.36 |
| | | | | **6** | | 20.75 | 2.04 | 86.45 | 24.00 | 11.13 | 1.72 | 0.66 | 4.21 |
| | | | | **7** | | 20.98 | 1.51 | 82.66 | 18.36 | 12.30 | 1.84 | 0.00 | 2.71 |
| | | | | **8** | | 26.17 | 3.96 | 74.95 | 12.27 | 14.65 | 1.91 | 0.12 | 3.44 |
| | | | | **9** | | 28.72 | 8.07 | 69.76 | 10.78 | 17.73 | 2.22 | 0.00 | 4.14 |
| | | | | **10** | | 35.98 | 11.67 | 62.00 | 5.31 | 23.71 | 2.09 | 0.03 | 5.36 |
| | | | | **11** | | 32.93 | 12.66 | 45.86 | 7.59 | 28.20 | 3.02 | 0.00 | 5.44 |
| | | | | **12** | | 38.52 | 16.44 | 44.66 | 7.94 | 27.07 | 2.33 | 0.21 | 5.20 |

Tx=Daily maximum temperature, Tn=Daily minimum temperature, Rain=Total rainfall, Rs=Total radiation, RHx=Daily maximum relative humidity, RHn=Daily minimum relative humidity, U2=Average wind speed, ETO=Total relative evapotranspiration

**Table 1 Continues**

| Locality | Latitude | Longitude | Altitude | Month | Year | Tn | RHx | RHn | Rs | U2 | Rain | ETO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Vaalharts** | -27.958 | 24.8399 | 1180 | **1** | **2014** | 17.64 | 72.14 | 15.41 | 25.8 | 1.59 | 0.43 | 6.13 |
| | | | | **2** | | | | | | | | |
| | | | | **3** | | | | | | | | |
| | | | | **4** | | | | | | | | |
| | | | | **5** | | | | | | | | |
| | | | | **6** | | | | | | | | |
| | | | | **7** | | -0.04 | 76.51 | 15.37 | 13.85 | 1.10 | 0.04 | 2.58 |
| | | | | **8** | | | | | | | | |
| | | | | **9** | | | | | | | | |
| | | | | **10** | | | | | | | | |
| | | | | **11** | | | | | | | | |
| | | | | **12** | | 16.63 | 85.04 | 22.71 | 27.52 | 1.35 | 5.21 | 6.03 |
| | | | | **1** | **2015** | 16.78 | 78.19 | 16.28 | 28.79 | 1.40 | 1.13 | 6.45 |
| | | | | **2** | | 14.04 | 75.92 | 14.46 | 27.77 | 1.47 | 0.68 | 6.23 |
| | | | | **3** | | 14.74 | 82.18 | 22.90 | 21.10 | 1.39 | 1.62 | 4.67 |
| | | | | **4** | | 8.73 | 83.10 | 21.60 | 17.58 | 0.94 | 0.49 | 3.53 |
| | | | | **5** | | 4.62 | 73.63 | 12.80 | 16.08 | 1.03 | 0.07 | 3.67 |
| | | | | **6** | | 1.37 | 87.93 | 23.04 | 13.38 | 1.07 | 0.96 | 2.32 |
| | | | | **7** | | 1.96 | 81.48 | 20.75 | 13.14 | 1.19 | 0.13 | 2.45 |
| | | | | **8** | | 4.71 | 61.48 | 12.24 | 15.86 | 1.27 | 0.03 | 3.43 |
| | | | | **9** | | 9.35 | 74.07 | 18.48 | 19.21 | 1.59 | 0.65 | 4.20 |
| | | | | **10** | | 13.32 | 59.87 | 9.54 | 23.91 | 1.55 | 0.29 | 5.67 |
| | | | | **11** | | 12.97 | 59.01 | 10.10 | 29.19 | 1.76 | 1.36 | 6.56 |
| | | | | **12** | | 16.81 | 63.23 | 10.77 | 27.58 | 1.38 | 1.06 | 6.66 |

Tn=Daily minimum temperature, Rain=Total rainfall, Rs=Total radiation, RHx=Daily maximum relative humidity, RHn=Daily minimum relative humidity, U2=Average wind speed, ETO=Total relative evapotranspiration

**Table 2 Fertilising programme for 2014 season**

| Locality | fertilizer source used | Total N (kg N ha) | Total P(kg N ha) | Total K (kg N ha) |
|---|---|---|---|---|
| Douglas | | 200 | 40 | 50 |
| Marydale | | 200 | 40 | 50 |
| Vaalharts | | 200 | 40 | 50 |

# Appendix B

## Table 1 Mixsmart parameters, description and units of measurement

| Mixsmart parameters | | Description | Unit |
|---|---|---|---|
| **EPI** | Envelope peak integral | Envelope area under curve from beginning until end of mixing process measured on envelope curve | %Torque*min |
| **EPT** | Envelope peak time | Time where envelope curve reaches a peak | min |
| **EPV** | Envelope peak value | Envelope curve height at envelope peak time | % |
| **EPW** | Envelope peak width | Envelope curve-width at envelope peak time | % |
| **ETI** | Envelope tail integral | Envelope area under curve from beginning until end of mixing process measured on envelope curve | |
| **ETV** | Envelope tail value | Envelope curve height at end of mixing process measured on envelope curve | % |
| **MLI** | Midline left integral | Midline area under curve from beginning until 1 min before midline peak time | %Toque*min |
| **MLT** | Midline left time | Time from starting point until 1 min before peak time measured on midline curve | min |
| **MLV** | Midline left value | Midline curve height at 1 min before midline peak time | % |
| **MLW** | Midline left width | Midline curve-width at 1 min before midline peak time | % |
| **MPI** | Midline peak integral | Midline area under curve from beginning until midline peak time | %Torque*mi |
| **MPT** | Midline peak time | Time where midline curve reaches a peak – Optimum dough development | min |
| **MPV** | Midline peak value | Midline curve height at midline peak time | % |
| **MPW** | Midline peak width | Midline curve-width at midline peak time | % |
| **MRI** | Midline right integral | Midline area under curve from beginning until 2 min after midline peak time | %Torque*min |
| **MRT** | Midline right time | Midline time from beginning until 2 min after midline peak time | min |
| **MRV** | Midline right value | Midline curve height at 2 min after midline peak time | % |
| **MTI** | Midline tail integral | Midline area under curve from beginning until end of mixing process | %Torque*min |
| **MTV** | Midline tail value | Midline curve height at end of mixing process (e.g. 6.5 min) | % |

%= Percentage, min=minute(s)

**Table 2 Size exclusion-high performance liquid chromatography protein fractions**

| Protein fractions | | Definitions |
|---|---|---|
| **SDS soluble** | **LPP** | Large polymeric protein |
| | **SPP** | Small polymeric protein |
| | **LMP** | Large monomeric protein |
| | **SMP** | Small monomeric protein |
| **SDS insoluble** | **LPP** | Large polymeric protein |
| | **SPP** | Small polymeric protein |
| | **LMP** | Large monomeric protein |
| | **SMP** | Small monomeric protein |
| **Relative value** | **LUPP** | Large unextractable polymeric protein |
| | **UPP** | Unextractable polymeric protein |
| | **POL** | Total polymeric protein |
| | **HMW** | High molecular weight |
| | **LMW** | Large molecular weight |
| | **MON** | Total monomeric proteins |
| **Absolute value** | **LUPP** | Large unextractable polymeric protein |
| | **UPP** | Unextractable polymeric protein |
| | **POL** | Total polymeric protein |
| | **HMW** | High molecular weight |
| | **LMW** | Large molecular weight |
| | **MON** | Total monomeric proteins |