Interrelationship between storage protein, vitamin E and quality characteristics of selected South African bread wheat cultivars

By

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

Bloemfontein
Republic of South Africa
June 2014

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DECLARATION

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

N.N. Mkhatywa ..... **Date: .....**

Acknowledgements

I wish to extend my sincere appreciation to the following people and organisations for their contribution towards the success of this study

- Our heavenly Father for giving me the strength, perseverance and insight to complete this study.
- Dr Angeline van Biljon, my study leader for all her time, continued encouragement and dedication, I could not have completed this thesis without her support and help.
- My co-promoters, Professor Maryke Labuschagne and Mr Barend Wentzel, for their encouragement and advice.
- Mrs Sadie Geldenhuys for all the administration and motivation.
- My family and the Ngobeni family, for their constant support, love, prayers and encouragement throughout this study.
- Nhlanhla, my best friend, for his constant support, love, encouragement and prayers.
- My friends, for their support, listening and always putting a smile on my face.
- The Winter Cereal trust for financial assistance.
- ARC-Small Grain Institute and The Swedish University of Agricultural Sciences (SLU) for the providing facilities.
- Prof Eva Johansson and Me Maria Prieto-Linde for their contribution in this study and lab work at SLU.
- This work is based on the research supported in part by the National Research Foundation of South Africa (UID) (83909).

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Abbreviations

µl	Microlitre
µm	Micrometre
µg	Micrograms
Alb	Albumins
AACC	American Association of Cereal Chemists
ACN	Acetonitrile
AlvDIST	Dough distensibility
AlveoW	Dough strength
AlvP/L	AlvSTAB to AlvDIST ratio
AlvSTAB	Dough stability
ANOVA	Analysis of variance
ARC-SGI	Agricultural Research Council-Small Grain Institute
B	Bethlehem
BFY	Break flour yield
C	Clarens
℃	Degrees Celsius
cm	centimetre
cm^3	cubic centimetre
DIA	Kernel diameter
DTT	Dithiothreitol
FABS	Farinograph water absorption
FC	Flour colour
FN	Falling number
FP	Flour protein
FY	Flour yield
g	Gram
g/L	Gram per litre

Glo	Globulins
G X E	Genotype by environmental interaction
HI	Hardness index
HLM	Hectolitre mass
HMW-GS	High molecular weight - glutenin subunits
HPLC	High performance liquid chromatography
HPP	High molecular weight polymeric proteins
Kg/hl	Kilogram per hectolitre
L	Ladybrand
LMW-GS	Low molecular weight - glutenin subunits
LPP	Low molecular weight polymeric proteins
LV	Loaf volume
LV12	Loaf volume expressed on a 12% protein basis
m	Metre
mg	Milligram
mg/kg	Milligram per kilogram
min	Minutes
mm	Millimetre
ml	Millilitre
ml/L	Millilitre per litre
nm	nanometre
PC	Protein content
RP-HPLC	Reversed phase-high performance liquid chromatography
rpm	Revolutions per minute
sec	Seconds
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate - polyacrylamide gel electrophoresis
SDSVOL	Sodium dodecyl sulphate sedimentation volume
SE-HPLC	Size exclusion-high performance liquid chromatography

SKCS	Single Kernel Characterisation System
TKM	Thousand kernel mass
VK	Vitreous kernels
v/v	volume per volume
v/v/v	volume per volume per volume
w/v	weight per volume
WGC	Wet gluten content

Chapter 1

Introduction

Wheat (*Triticum aestivum* L.) is counted among the important cereal crops dominating agriculture (Shewry, 2009) and is widely consumed by humans; it is a staple food for 40% of the world's population due to its diverse uses (Peng et al., 2011). The earliest cultivated forms of wheat were essentially landraces selected by farmers from wild populations but domestication of wheat was associated with genetic traits (Shewry, 2009). Hexaploid bread wheat accounts for about 95% of the world wheat production and producers have an ongoing need to improve flour quality for bread-making and protein quality for better nutrition (Huebner and Bietz, 1985; Peng et al., 2011).

The qualitative and quantitative aspects of wheat proteins both have to be addressed in order to define wheat quality (Suchy et al., 2003). Baking quality is the final result of an interaction between the genetic potential of cultivars and the environment where the cultivar was grown (Tlanu et al., 1996). Of all the flour components, protein and protein related characteristics determine bread-making quality to the greatest extent (Weegels et al., 1996). Wheat breeders select for protein content (PC) in breeding programmes.

Storage proteins are important because they make up a part of the PC of seed as well as quality for various end use products. The gluten fraction of the storage protein contributes about 85% to the total flour protein (FP) and confers elasticity and extensibility, essential characteristics for bread-making (Shewry et al., 1995). Gliadins and glutenins are two prolamine groups of gluten contributing towards visco-elastic properties of gluten (Rasheed et al., 2012). These two groups have been found to have a greater effect on wheat quality than albumins and globulins (Ahmad et al., 2000). The unique protein properties of wheat flour allow it to be processed into bread, cakes, biscuits, pasta and noodles (Rakszegi et al., 2005). These properties are not shared by the storage proteins of other cereals (Shewry et al., 1995). A higher quality variety produces good bread over a fairly wide range of protein percentages, whereas a low quality variety produces relatively poor quality bread even when PC is high (Pomeranz, 1988). The grain PC of wheat is genetically controlled but varies for a given cultivar according to environment, soil fertility, rainfall and temperature (Johansson et al., 2001).

Knowledge of the relationship between proteins and baking quality parameters can be utilised to solve problems and limitations that still exist, as well as to form a basis for manipulating important properties of dough in breeding programmes. Selection and breeding

processes in food crops have caused losses of many favourable alleles in released varieties (Rasheed et al., 2012). The major aims of wheat breeding programmes are to increase yield and improve quality. Breeders seek to develop varieties that will be suitable for multiple uses within the domestic market (Zećević et al., 2007).

Wheat quality has been judged on the basis of functionality and to a lesser extent on nutritional value (Adom et al., 2003). Wheat breeding programmes have traditionally targeted white flour bread quality as prime selection criteria (Bruckner et al., 2001). A study has associated the consumption of whole grains with reduced incidence of chronic diseases (Jacobs et al., 1998). These health benefits have been attributed to the unique phytochemical content of grains (Adom et al., 2003). Cereal grains contribute significant biological substances, such as vitamin E, which are important for actions such as inhibiting lipid peroxidation in biological membranes (Liu, 2007).

Vitamin E is a term used to describe a family of eight lipid-soluble tocochromanols; four tocopherols (α-, β-, γ-, and δ-tocopherol) and four tocotrienols (α-, β-, γ-, and δ-tocotrienol) (Hussain et al., 2012). The most important chemical property of tocochromanols is antioxidant activity. An antioxidant is a substance that has the capacity and ability to act as free radical scavenger; it delays and prevents oxidation (Delgado-Zamarreño et al., 2009). Tocopherols show vitamin E activity to various degrees while tocotrienols do not exhibit vitamin E activity. The content of tocochromanol compounds in wheat has been shown to be influenced by genotype and environment (Lampi et al., 2008; Hejtmánková et al., 2010). The content of tocochromanol compounds in wheat has been investigated (Panfili et al., 2003; Lampi et al., 2008; Hussain et al., 2012) and whole grain and milling fractions have been reported to contain a higher amount of these compounds than white flour (Liyana-Pathirana and Shahidi, 2006a; 2006b).

Size exclusion - high performance liquid chromatography (SE-HPLC) is a powerful tool used to study protein aggregates and physicochemical properties on baking quality. It is a method which gives information of the structure, size-distribution and interactions of protein components (Dachkevitch and Autran, 1989). Reversed phase - high performance liquid chromatography (RP-HPLC) differentiates proteins by surface hydrophobicity and has been applied successfully to separate wheat proteins (Bietz et al., 1984). It is also valuable for separating gliadins and glutenin subunits (Bietz et al., 1984; Burnouf and Bietz, 1984; Bietz and Burnouf, 1985).

The objectives of this study were to:

- Evaluate baking quality characteristics and content of proteins and vitamin E in 10 different wheat cultivars grown at 3 different locations.
- Separate proteins fractions using RP-HPLC and SE-HPLC analyses on white flour and whole wheat flour.
- Determine the amount of genetic and environmental impact on the quality characteristics.
- Correlate baking quality characteristics to protein content and vitamin E levels in white flour and whole wheat.

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Chapter 2

Bread wheat quality and vitamin E

2.1 Introduction

Wheat is the second most important cereal in South Africa. It is among the oldest and most extensively grown crops. Wheat is a species adapted to diverse environments and is used to make different food products. Hexaploid wheat is used in the production of bread while durum wheat is used for pasta production. Wheat bread in developing countries has become preferred over rice, sorghum and millet based foods (Wrigley, 2009). Bread is a useful source of nutrients such as carbohydrates, protein and antioxidants in a diet.

Flour quality is the ability of flour to produce a uniformly good end-product (Mailhot and Patton, 1988). Quality of wheat cannot be expressed using a single property; it depends on a number of characteristics such as milling, rheological and processing characteristics (Pomeranz, 1988). Qualitative and quantitative aspects of wheat proteins also have to be dealt with in order to define wheat quality (Suchy et al., 2003). Improving quality in wheat cannot be achieved successfully if grain hardness, PC and several glutenin and gliadin alleles are not taken into consideration (Branlard et al., 2001). Improving quality is also dependent on understanding the complexities of the storage proteins.

There are important parameters that need to be considered for good bread quality, they include high FP, high water absorption, good dough extensibility, tolerance to mixing and high loaf volume (LV). There has been little research devoted to understanding the relationship between flour and dough quality as measured on white flour versus that measured using whole grain flour (Bruckner et al., 2001). Some components found in whole wheat flour may interfere with baking performance. Bran added to white flour bound a large amount of water and the gluten was not properly hydrated. Poor hydrated gluten results in lower LV and changes in dough properties (Lai et al., 1989).

Protein quality and quantity are considered primary factors in measuring the potential of flour in relation to its end use and protein quality is more involved with the physical rather than nutritional characteristics of bread (Zeleny, 1964). Wheat quality and grain development are influenced by genotype, environment as well as genotype x environment interaction (G X E) (Mailhot and Patton, 1988; Barnard et al., 2002; Finlay et al., 2007). Wheat quality is also influenced by the polygenic nature of the characteristics involved (Barnard et al., 2002). Environment is the main factor that causes large variation in quality.

2.2 Baking quality

Wheat grain quality is influenced by factors that are classified into different groups namely: grain characteristics, milling characteristics, rheological characteristics as well as baking characteristics. Grain characteristics include hectolitre mass (HLM), kernel characteristics (hardness, kernel texture, kernel weight, thousand kernel mass (TKM), kernel diameter (DIA) and size), falling number (FN) and vitreous kernels (VK). Milling characteristics are break flour yield (BFY), flour yield (FY) and flour colour (FC). Rheological characteristics include values from a farinograph and an alveograph. Baking characteristics also include characteristics which are related to baking quality such as SDS sedimentation volume (SDSVOL), wet gluten content (WGC), LV and PC.

2.2.1 Hectolitre mass

HLM or test weight is a measure of soundness of wheat (Marshall et al., 1986; Matsuo and Dick, 1988; Bordes et al., 2008) and estimates flour extractability (Nel et al., 1998). The grain weight is measured per unit volume and is influenced by two non-endosperm components; the packing efficiency of grain and the density of the individual kernels (Zeleny, 1964; Farrer et al., 2006; Koen, 2006). Packing efficiency depends on genotype while kernel density is influenced by the environment (Gaines et al., 1996a; Farrer et al., 2006; Koen, 2006; Bordes et al., 2008; Mut et al., 2010) that influences the biological structure of grain as well as the chemical composition thereof (Zeleny, 1964). Test weight is influenced mostly by packing efficiency which is affected by kernel size and shape. Fully mature, plump kernels, undamaged by biotic or abiotic stress factors have a high test weight. Factors such as water stress, heat stress, frost damage and disease which cause changes in kernel size and shape also effect test weight (Farrer et al., 2006). Low kernel weight tends to result in low test weight (Matsuo and Dick, 1988) where plump kernels fill more uniformly giving rise to high test weight (Czarnecki and Evans, 1986; Matsuo and Dick, 1988). Test weight is significant in the wheat industry because of its influence on the transportation of grain costs. The higher the test weight the greater the weight of grain that can be loaded into a fixed volume container (Fowler and De la Roche, 1975; Bordes et al., 2008).

Differences in test weight have been observed among genotypes cultivated under the same growth conditions (Marshall et al., 1986) and among wheat of different classes and varieties within a class (Carson and Edwards, 2009). Higher HLM is indicative of grain plumpness (Nel et al., 1998). Test weight may also be influenced by time of harvest, especially harvesting after the grain is ripe (Farrer et al., 2006). A value of 74 kg hl^{-1} is required in order for a cultivar to be suitable for bread-making (Nel et al., 1998).

Test weight provides an indication of FY, soundness and density of wheat. Grain and flour PC provides an indication of final quality and functionality (Maghirang et al., 2006). It is used by millers as a crude predictor of potential FY especially for wheat varieties from the same location (Carson and Edwards, 2009). Test weight is a good measurement with which to estimate the weight of a specific volume of grain (Posner, 2009).

2.2.2 Kernel characteristics

Hardness is the ability of grain to resist deformation and is determined by endosperm properties, associated with PC. It influences the bond between starch granules and the protein matrix (Symes, 1965; Surma et al., 2012). Anatomy, structure and mechanical properties of the grain are factors which make up grain hardness (Surma et al., 2012). According to Kulp (1988), hardness is genetically controlled and is not directly correlated with PC of the kernel. Although grain hardness is a genetic trait, its expression is greatly influenced by environmental conditions during grain filling and the conditions before flour milling (Pomeranz et al., 1985; Bechtel et al., 1996; Delwiche, 2000).

Hard wheat kernels require greater force to cause it to break up (Kulp, 1988). Hardness is said to influence milling behaviour, performance and is used as a grading factor to classify the type of wheat. It is a major factor determining end use quality (Morris, 2002; Greffeuille et al., 2006; Bordes et al., 2008; Bhave and Morris, 2008; Pasha et al., 2010). Hardness is known to indicate the rate and quantity of water uptake during the tempering process (Delwiche, 2000). Unfavourable weather conditions may reduce grain hardness and the grain is subject to more fracturing during harvest (Czarnecki and Evans, 1986). Gaines et al. (1996b) reported that high moisture content may increase the hardness index (HI) value. The HI is calculated from measurements of the force that is required to crush a kernel (Maghirang and Dowell, 2003). Hardness and vitreousness are often mixed up because they both refer to texture (Greffeuille et al., 2006).

Kernel texture is simply inherited and controlled by a single locus *Hardness (Ha)* which comprises of three genes on chromosome 5D with two major texture classes. The location of the major gene was determined using chromosome substitution lines (Doekes and Belderok, 1976; Chantret et al., 2005; Bhave and Morris, 2008). Two major genes and other minor genes were reported by Yamazaki and Donelson (1983) to control kernel texture. Symes (1965) reported that a single gene was responsible for the hardness differences, although some modifying genes could play a role. However, Martin et al. (2001) found that the *Ha* locus does not explain all the differences seen in wheat populations when grain hardness is tested. Hard wheat carry the recessive form (*ha*) while soft wheat have the dominant form (Gazza et al., 2005; Bhave and Morris, 2008).

Density and vitreousness of grain are factors that influence hardness while texture of grain influences rheological properties of dough (Martinant et al., 1998; Branlard et al., 2001). In a study by Surma et al. (2012), they reported that grain hardness is influenced by genotype rather than location. Genotype was observed to have a larger influence on the variability of wheat. Wheat that is very hard is likely to produce non-extensible dough because of starch damage (Bordes et al., 2008). Drier climates tend to produce large, better filled and hard kernels that have superior milling characteristics (Gaines et al., 1996a).

Kernel weight is a function of kernel size and kernel density (Halverson and Zeleny, 1964; Zeleny, 1964; Koppel and Ingver, 2008). Large kernels usually have a higher ratio of endosperm to non-endosperm component (Koppel and Ingver, 2008). Environmental effect has been said to affect kernel size and composition (DuPont and Altenbach, 2003). Kernel weight and size are significant because of their correlation with milling quality (Tsilo et al., 2010). Yoon et al. (2002) reported that kernel length and width are influenced by independent quantitative trait loci (QTL). Kernel size uniformity or its distribution allows for a more efficient milling process and quality control. QTL clusters that influence kernel weight, DIA and kernel size are located on chromosomes 2A, 5B and 7A (Tsilo et al., 2010). DIA measures a physical property of wheat. Large DIA is expected to correlate positively with milling yield (Yoon et al., 2002).

TKM measures the grain size and density; it is an indicator of yield (Bordes et al., 2008) and is a measure of average kernel size. It can be useful as a reliable guide to predict FY (Koppel and Ingver, 2008). TKM is affected by growing conditions especially during the grain filling period (Pomeranz et al., 1985) cultivation practices and fertilisation (Mut et al., 2010) and was found to be lower when harvesting was delayed (Czarnecki and Evans, 1986). Heavier kernels have a higher percentage of endosperm than lighter ones (Posner, 2009).

2.2.3 Falling number

The FN has proved to be more practical for measuring α-amylase activity than conventional chemical methods. FN is a measure of α-amylase enzyme activity and a lower FN indicates higher enzyme activity and a possibility of the kernels to sprout (Farrer et al., 2006). FN can be defined as the time in seconds required to stir and allowing a viscometer-stirrer to fall at a fixed distance through a hot aqueous flour suspension being liquidified by the enzyme in a standardised apparatus. It is useful in evaluating the quality of wheat especially that which may have been exposed to wet conditions at harvest (Halverson and Zeleny, 1964). FN values of 400 indicates the deficiency of α-amylase in flour and in order to achieve the level of desired enzyme activity, flour must be supplemented (Maghirang et al., 2006; Valentina et al., 2007). High levels of α-amylase activity may be caused by high levels of enzyme that

occurs naturally or may be caused by premature germination which causes α-amylase to be synthesized (Koppel and Ingver, 2008).

2.2.4 Vitreous kernels

Vitreousness of wheat kernels can be used to predict the end use product and reflects the texture of the endosperm (Al-Saleh and Brennam, 2012). Kernel vitreousness is related to the endosperm microstructure (Greffeuille et al., 2006). VK have a translucent endosperm that appears almost waxy which is a sign of hardness (Bass, 1988). When the vitreousness of kernels decline, the granules are finer, the colour will be less and PC will be lower. Grains that are less vitreous produce more flour; they appear glossy and translucent. A harder endosperm has been observed in VK, as well as higher PC (Zeleny, 1964; Gaines, 1986) and greater density (Gaines, 1986). Vitreousness is affected more by environmental conditions than hardness of the kernel. Some authors reported that vitreousness does not correlate directly with other known quality characteristics (Halverson and Zeleny, 1964). Environmental conditions, mainly water availability, have a great influence on vitreousness of grain (Rharrabti et al., 2003). Weather conditions, soil fertility and heredity are some of the factors that may cause wheat kernels to be non-vitreous. It is influenced by growing conditions rather than genetic factors (Greffeuille et al., 2006).

2.2.5 Break flour yield

BFY is a function of the number of particles that pass through the sieve during the first three or four break rollers. It is a function of wheat kernel texture, if wheat kernels are soft more flour particles pass through and more break flour is produced (Gaines, 1986; Gaines et al., 1996b). Break flour is produced when wheat is broken open in the first break system. During the breaking, the rollers separate the endosperm and germ from bran (Bass, 1988). Break flour of soft wheat was reported to be higher than break flour of hard wheat (Labuschagne et al., 1997). According to Pasha et al. (2010) BFY is positively correlated with grain hardness.

2.2.6 Flour yield

FY is the percentage of flour recovered during the milling process from a known quantity of grain (Bass, 1988; Dewettinck et al., 2008) and flours differ in their extraction rate (Dewettinck et al., 2008). FY is thought to be reduced by variation in DIA or kernel hardness (Yoon et al., 2002). Softer wheat produces lower FYs (Labuschagne et al., 1997) as well as less plump kernels (Pumphrey and Rubenthaler, 1983). Both environment and genotype were reported to have an influence on FY (van Lill and Smith, 1997).

Three QTL influencing FY were identified on chromosome 3A, 5A and 7D (Parker et al., 1998). Studies have showed that increasing kernel weight (Wiersma et al., 2001) and kernel

size results in an increase in FY (Marshall et al., 1984; Berman et al., 1996). Pasha et al. (2010) observed that BFY and FY were positively correlated with grain hardness.

2.2.7 Flour colour

FC is an important parameter when assessing quality of flour and end use product. For bread-making, white flour with little pigmentation is a requirement. Genetic variability for FC within wheat genotypes is present and is expressed as a quantitative trait (Parker et al., 1998). Several wheat crosses showed that there were two major genes controlling FC and the mode of action on yellow pigment was changed by the presence of minor genes (Bhatt and McMaster, 1976). Other authors also identified QTL influencing FC (Mares and Campbell, 2001; Kuchel et al., 2006). When improving FC, other kernel characteristics such as test weight, kernel weight, and PC, DIA and kernel size distribution are also improved (Tsilo et al., 2011).

Moisture content of flour affects colour, the lower the moisture the brighter the flour appears (Hugh and Meade, 1964). Increasing PC can result in duller looking flour (Carson and Edwards, 2009). FC is thus a parameter that affects end-user results (Posner, 2009).

2.2.8 Farinograph

Water absorption, dough development time, dough stability and dough mixing tolerance index characterise farinograph curves. Farinograph water absorption (FABS) is the amount of water that can be added to flour to produce dough of a fixed consistency (Holas and Tipples, 1978). Dough development time is the time taken to reach maximum consistency. Longer development time, longer stability and lower mixing tolerance index indicate that the dough has stronger properties (Cenkowski et al., 2000). Dough stability shows the tolerance of flour to mixing and the stronger the flour; the more stable the dough (Miralbés, 2004). When PC in grain increases, water absorption also increases (Van Lill and Smith, 1997). The farinograph has been previously used to study the effect of temperature and mixing speed on some rheological properties (Bayfield and Stone, 1960; Hlynka, 1962).

Water absorption is an important quality factor to the baker because it is related to the amount of bread that can be produced from a given weight of flour. High absorption values are desirable in bread baking as added moisture delays staling (Koppel and Ingver, 2010). Water absorption is an important parameter when purchasing flour for bread-making and is influenced by wheat cultivar and by the amount and type of grinding performed during milling (Maghirang et al., 2006).

2.2.9 Alveograph

The different alveograph curve measurements give information about the strength and extensibility of the dough (Bordes et al., 2008). The alveograph is sensitive to flour maturation and the decline in quality during flour storage. Dough becomes less extensible when temperature increases, this is due to oxidation (Cenkowski et al., 2000). Resistance is strongly affected by the water absorption of the flour. Oxidation of flour increases the dough resistance to deformation and decreases extensibility (Bloksma and Bushuk, 1988). The alveograph test is effective in identifying flours of weak to medium strength (Maghirang et al., 2006).

The dough resistance to deformation (P) indicates the dough's ability to retain gas. A low P value indicates that the dough is less elastic and a high P value indicates that the dough can retain gas. Dough extensibility (L) provides information about processing characteristics, the ability to expand without breaking down. Dough that is easily stretched and extensible has high L values. Alveograph P/L shows the balance of elasticity and extensibility. The W-value gives information on the behavior of the dough during the baking process, it summarises all parameters of the alveograph (Banu et al., 2012). Wheat flour with high PC produces dough that has high elasticity and low extensibility. The effect of genotype and environment appeared to have a similar influence on protein quality measured the alveograph W parameter (Surma et al., 2012). PC content and composition have an influence on the alveograph. As starch damage increases, L of dough decreases and P increase (Addo et al., 1990).

2.2.10 SDS sedimentation

Sodium dodecyl sulphate (SDS) sedimentation was developed to estimate bread baking quality. The SDS sedimentation test was developed for differentiating protein quality but may not be controlled by protein quality only. Differences observed in SDSVOL of different wheats also show that these wheats differ in SDS-insoluble glutenins (Moonen et al., 1981). SDSVOL is known to be influenced by PC but the effect depends on the wheat variety as well as protein quality (Morris et al., 2007). The composition of wheat flour affects sedimentation values and the value correlates with PC and wheat hardness (Pasha et al., 2010). Wheat with higher SDS sedimentation value tends to have a higher baking quality and correlates better with LV (Zhao et al., 2012). SDS sedimentation that is higher than 70 ml is indicative of superior baking strength (De Villiers and Laubscher, 1995), although it would be advisable to screen entries against an approved quality standard.

2.2.11 Wet gluten content

Grausgruber et al. (2000) and Surma et al. (2012) reported that WGC is correlated to PC and PC is strongly influenced by growing conditions. With higher gluten content, higher peak heights and work input resulted in order to obtain full dough development in the mixograph (Lang et al., 1992).

2.2.12 Loaf volume

LV has been considered as the most important criterion for bread-making quality. Quality of bread is altered by variation in composition and the content of protein in the flour (Pomeranz, 1988; Branlard et al., 2001). This variation then causes differences in performance of dough during bread-making. Baking quality of wheat is also determined by the baking process applied (Švec and Hrušková, 2010). Good baking flour exhibits stronger resistance to extension, greater strain hardening and greater extensibility (Kokelaar et al., 1996). High strength dough can inhibit the extensibility of dough films between gas cells causing LV to decrease (Sliwinski et al., 2004). The glutenin fraction is responsible for dough mixing time and dough development (Hrušková and Faměra, 2003). Flour from sprouted wheat grains results in low LV and poor texture even if the cultivar is of good quality (Koen, 2006).

LV is measured by rapeseed displacement and indicates the capacity of the dough to retain gas during fermentation (Shogren and Finney, 1984) and it increases with an increase in PC (Khatkar et al., 1996) but the relationship is not completely linear (Huebner et al., 1997). It can be predicted precisely if all the flour samples have a similar origin and same extraction rate (Bloksma and Bushuk, 1988). FP percentage is a good predictor of LV which is influenced by environmental conditions. LV seemed to be strongly influenced by environment only (Salmanowicz et al., 2012). For the milling and baking industry, it is desirable that quality traits be maintained under all environments (Koppel and Ingver, 2010).

Gluten proteins determine the bread-making quality differences observed between wheat cultivars (Khatkar et al., 1996). Quantitative effects of gluten proteins cause most variation in LV (Chung et al., 2003). LV potential of bread flour depends on the quantities of two types of glutenin. The glutenin proteins control LV potential of wheat flour and LV is a function of glutenin quality (Pomeranz, 1988; Hrušková and Faměra, 2003). Specific glutenins account for up to 80% of the differences observed in LV (Van Lonkhuijsen et al., 1992).

2.2.13 Protein content

The PC of wheat ranges from about 6%-20%, depending on variety, class and environmental conditions during the growth period (Halverson and Zeleny, 1964). Environment plays a greater role in PC than genotype (Hrušková and Faměra, 2003; Mut et al., 2010; Surma et al., 2012) however, other reports state that PC is more influenced by genotype (Yong et al.,

2004). Total rainfall and seasonal distribution of rainfall have a great effect on the amount of protein (Pomeranz, 1988). When conditions during the growing season are dry, PC is high (Zeleny, 1964). Environmental conditions such as soil type, crop rotations, use of nitrogen fertilisation also influence PC but protein quality is determined by wheat genotypes (Al-Saleh and Brennam, 2012). Mut et al. (2010) reported that in low rainfall environments, the PC in wheat is high. Moisture stress increases PC in grain (Rharrabti et al., 2003).

According to Gupta et al. (1992), an increase in PC of flour can lead to better protein composition and also improve all flour quality parameters. Wheat containing high content of protein is hard with strong gluten and produces good quality bread (Pasha et al., 2010). Wheat of the same PC produces flours which behave differently during the baking process (Zeleny, 1964). PC positively correlates with WGC and LV (Fredriksson et al., 1997; 1998). PC is also positively correlated with bread-making quality (Kolster et al., 1991).

Weather conditions during the grain filling period have a large effect on PC. The weather also affects gluten quality. PC and quality are important for baking quality (Johansson and Svensson, 1998).

2.3 Storage proteins

Bread-making quality of wheat arises from interrelated characteristics, such as grain hardness and PC. Quality is determined by composition and molecular structure of the storage proteins of wheat (Wieser et al., 1998; Gianibelli et al., 2001) and these storage proteins control interactions of proteins during the bread-making process (Gianibelli et al., 2001). The variation in amount and composition of proteins contributes to variation in bread-making quality between genotypes (Kolster et al., 1991). Wheat varieties differ in their bread-making ability and the endosperm proteins have a major influence on bread-making quality (Békés et al., 2006). Quantity and quality of proteins are the main factors that determine flour quality (DuPont and Altenbach, 2003). In the past, relating wheat proteins to dough properties were difficult because it was a challenge to solubilise all proteins that need to be characterised. The use of sonication with SE-HPLC made it possible to accurately determine all the protein classes in flour samples (Singh et al., 1990a).

Bread-making quality correlates with the presence or absence of specific proteins and protein subunits (Gupta et al., 1989). Improving bread-making quality can be done by either altering the protein composition or increasing the protein concentration of cultivars (Meintjes, 2004). Environmental factors affect concentration of protein but protein subunit composition is genetically controlled (Huebner et al., 1997). According to Labuschagne et al. (2006), it may be better to breed for improved protein composition rather than breeding for high protein concentration when improving bread-making quality. Statistical analyses of protein

components have linked certain gliadins and glutenin subunits to bread-making quality parameters. Research on the biochemical basis of bread-making quality of wheat flour has increased the need for separating polymeric glutenin from the monomeric wheat FPs accurately (Fu and Sapirstein, 1996).

Protein quantity is strongly influenced by the environment and quality is determined by the genotype and environment. Protein quality as well as the amount is important for good bread-making quality (Hrušková and Faměra, 2003; Békés et al., 2006). Higher protein percentage is often associated with better quality. Wheat gluten proteins are very heterogeneous and their synthesis does not differ under a wide range of conditions (Bietz, 1985). In cereals, the synthesis and expression of storage proteins is directly associated with genotype (Rodriguez-Nogales et al., 2006). The effects of growing conditions on the expression levels on the individual genes are a direct cause of the large variation of protein composition (Békés et al., 2006). According to Bietz and Kruger (1994), plant storage proteins have been found difficult to work with for many reasons besides their heterogeneity. Wheat contains one of the most complex groups of proteins ever characterised. Qian et al. (2008) stated several difficulties that are associated with protein analysis of wheat storage proteins, problems such as the limited databases available, limited number of basic residues (especially in the Low Molecular Weight [LMW] glutenin subunits [GS]), the complexity resulting from the presence of sets of homologous proteins and the presence of repeating motifs. Extraction conditions can change the correlation between protein fractions and quality parameters (Preston et al., 1992).

Storage proteins constitute 80-85% of the total wheat protein (Shewry and Tatham 1997; Meintjes, 2004). The protein is highly heterogeneous in composition and in molecular weight (Wrigley et al., 2006). Proteins usually constitute 7-15% of common flour on a 14% moisture basis in wheat. Wheat proteins is classified into albumins (15%), which are water-soluble proteins, globulins (3%) proteins that are soluble in salt solutions, prolamin proteins that are generally soluble in 70% aqueous ethanol and gliadin (33%) proteins, which is one of the major components of the wheat gluten complex. The other major component of gluten is glutenin (16%), which is classified as glutelins which are soluble in dilute acid or bases (Atwell, 2001). The ability of flour to produce dough with good gas-holding properties is attributed to gluten. Gluten proteins (gliadins and glutenins) are the reason wheat is the most important source of protein in the human diet.

Ohm et al. (2010) reported that specific protein fractions had distinct effects on quality characteristics, especially the high molecular weight (HMW) proteins that are not extractable with SDS; they had greater positive correlations with dough characteristics. Gluten is

recognised as the wheat protein fraction most closely linked with bread-making quality (Huebner and Bietz, 1994). The major groups of these proteins include gliadins and glutenins consisting of LMW and HMW subunits, associated through inter-chain disulfide bonds (Zhu and Khan, 1999; Qian et al., 2008; Mamone et al*.,* 2009). LMW-GS are present in gluten at about three times the amount of the HMW-GS and have also been difficult to separate because of their complexity, heterogeneity and overlap with other polypeptides in sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) analysis (Juhasz and Gianibelli, 2006).

Gluten protein composition determines the rheological characteristics (strength and extensibility) of flour dough and is the main component responsible for differences in end-use suitability (Bietz, 1985; Liu et al., 2009). Gluten proteins are major determinants of the unique viscoelastic dough characteristics and influence mixing and baking performance (Gianibelli et al., 2001). Gluten is a very large component composed of polymeric glutenins and monomeric gliadins and comprise about 78-85% of total wheat proteins (Horvat et al., 2009). Gluten proteins are the reason wheat is the most important source of protein in the human diet. Gluten is elastic; wheat dough expands and retains gas generated during fermentation (Bietz and Kruger, 1994; Atwell, 2001). Major gluten protein groups, the monomeric gliadins and polymeric glutenins are associated with quality differences among wheat cultivars (Zhu and Khan, 1999; Liu et al., 2009; Mamone et al., 2009). This variation that exists in protein components which contain glutenins and gliadins leads to a variation in protein concentration and bread volume. The gluten strength of a cultivar influences the bread volume of that cultivar (Johansson et al., 2001). Gluten quality depends primarily on genotype (Luo et al., 2000; Meintjes, 2004) and environmental factors are known to affect the content, composition and polymerisation of gluten proteins (Graybosch et al., 1995; Luo et al., 2000). According to Bietz (1985), a large variation in flour quality may be caused by a variation in gluten PC and composition.

2.3.1 Gliadin

Gliadins are one of the major components of the gluten complex and the most abundant storage protein in wheat seed, amounting to about 40%, by weight of wheat-flour protein (Metakovsky and Graybosch, 2006). Gliadins are categorised in α/β, γ and ω subunits. The main blocks of gliadin genes are located on the short arms of group-1 and group-6 chromosomes and they are referred to as the *Gli-1* and *Gli-2* loci respectively (Wrigley et al., 2006; Mamone et al., 2009). With increasing PC, gliadin proteins tend to increase more than other protein fractions (Gupta et al., 1992). Although some authors have associated some particular alleles with bread-making quality, in terms of dough strength these proteins may not have a direct effect on wheat quality. They have a minor effect on baking performance

when compared to that of glutenin. The effect of gliadin on dough is attributed to the LMW-GS associated with them (Nieto-Taladriz et al., 1994) and due to their tight genetic linkage to the gliadins; this causes the effect of gliadins on quality to be difficult to interpret. LMW-GS have the ability to form large aggregates that are related to dough strength (Gianibelli et al., 2001; Gil-Humanes et al., 2012). Huebner and Bietz (1987) demonstrated that the presence of specific gliadins can be correlated with measured quality characteristics statistically. Gliadins may have an important contribution to the quality variation of many parameters and providing viscosity and gluten extensibility which is a constituent of dough strength (Branlard et al., 2001; Gianibelli et al., 2001; Gao et al., 2010).

2.3.2 Glutenin

Glutenin is recognised as a protein fraction which is closely related to bread-making quality (Meintjes, 2004). Analysis of glutenin in the past has been said to be more complex than that of gliadin (Huebner and Bietz, 1987). According to Gianibelli et al. (2001) research has been conclusive about the importance of glutenin, with emphasis on those subunits of HMW especially those controlled by the D genome. The HMW-GS are encoded by *Glu-1* loci on the long arms of group 1 chromosomes, while the LMW-GS are encoded by the *Glu-3* loci. These loci are named *Glu-A1*, *Glu-B1* and *Glu-D1* on account of their chromosome location. They are located on long arms of chromosomes 1A, 1B, and 1D respectively. Two genes are linked together on each locus and they encode two different types of HMW-GS, x- type that encodes a larger subunit and y-type that encodes smaller subunits (Cunsolo et al., 2002; Wrigley et al., 2006; Gao et al., 2010). Genetic analysis showed allelic variation in HMW-GS composition exist and it is well known that different alleles at the *Glu-1* locus confer different end-use quality, due to variation in the structures and properties of subunits encoded by the various genes (Cunsolo et al., 2002; Gao et al., 2010). Composition and amount of glutenin solubilised is influenced by extraction method as well as denaturation conditions (Huebner and Bietz, 1987).

Despite the massive amount of proteomic research done in grain proteins of wheat, analysis of glutenin proteins is still hindered by several difficulties such as the limited database information available and the complexity resulting from the presence of sequence repeating motifs (Mamone et al., 2009). It is still a challenge to separate protein fractions due to its dependency on extraction conditions (Preston et al., 1992).

2.3.2.1 High molecular weight - glutenin subunits

HMW-GS play a crucial role in bread-making quality although they represent only about 10% of the total storage protein in the grain (Gao et al., 2010; Ji et al., 2012). HMW-GS are the major determinants of gluten elasticity and they comprise about 20-30% of gluten (Shan et

al., 2007). The HMW-GS are encoded by *Glu-1* loci on the long arms of homologous group 1 chromosomes and the LMW-GS are encoded by *Glu-3* loci on the short arms of homologous group 1 chromosomes in hexaploid wheat.

It is difficult to separate HMW-GS that contain the same molecular weight and similar hydrophobicities by SDS-PAGE and RP-HPLC; as a result errors were obtained when determining HMW-GS using approximate molecular weights (Zhang et al., 2008). Bread-making quality has been found to be associated with certain types of HMW-GS, while other HMW-GS mostly correlate with low quality (Veraverbeke and Delcour, 2002). It is accepted that the differences in number, amount and properties of HMW subunits are linked with variation in the bread-making quality of different wheat cultivars. These differences then lead to variation in size distribution of the glutenin polymers. Size distribution of gluten polymers and properties of polymeric to monomeric protein ratios affect dough strength and bread-making quality (Singh et al., 1990a; Gupta et al., 1993; Gupta and MacRitchie, 1994; Johansson et al., 2001; Cunsolo et al., 2002). Quantitative variations have an important effect in determining bread-making quality differences among bread wheat cultivars (Khan et al., 1989; Ji et al., 2012).

2.3.2.2 Low molecular weight - glutenin subunits

Although LMW-GS have received less attention than HMW-GS, they are of great importance in determining the quality and end-use properties of grain (Juhasz and Gianibelli, 2006). LMW-GS account for about 70-80% of glutenins and affect dough extensibility. The role of HWM-GS is well characterised when compared to that of LMW-GS. This is because large numbers of LMW-GS display similar mobilities in SDS-PAGE analysis (Ikeda et al., 2003). LMW-GS are derived from more genes than HMW-GS; these cause difficulty in characterisation and have a higher surface hydrophobicity than HMW-GS (Gianibelli et al., 2001; Juhasz and Gianibelli, 2006). They have RP-HPLC elution characteristics that are similar to those of γ-gliadins (Huebner and Bietz, 1993).

Glutenin polymers are mainly composed of LMW-GS (Masci et al., 1998). According to a system proposed by Lew et al. (1992), LMW-GS can be divided based on the N-terminal sequences. The first group indicates the first amino acid in the sequence corresponding to LMW-m and LMW-s (m for methionine and s for serine); the second group has sequences similar to α- and γ-gliadins. The LMW-s is the most abundant type of LMW-GS. Genes that code for LMW glutenins at the Glu-3 loci are closely linked to those which control the majority of gliadins at short arms of chromosomes of homologous group 1 (Jackson et al., 1983).

LMW-GS form disulphide linked aggregates and form a large portion of total endosperm proteins and LMW-GS alleles have been found to be significantly correlated with dough properties in bread (Gupta et al., 1989).

2.4 Tocochromanols

Whole grain wheat contains compounds such as proteins, phytate, polysaccharides, phenolics, lignans and tocopherols to control the effect of oxidation reactions (Baublis et al., 2000). Wheat grain contains a significant amount of energy, protein and selected minerals, all contributing to the human diet. Wheat also contains a variety of biological active substances such as vitamin E (Hill, 1998). Tocochromanols or tocols are a group of eight tocopherols and tocotrienols that collectively constitute vitamin E (DellaPenna and Pogson, 2006; Dörmann, 2007). Tocochromanols occur in all plant species and they are also produced in green algae and cyanobacteria (Dörmann, 2007).

Tocopherols and tocotrienols can be distinguished based on the number and positions of methyl groups on the chromanol ring. Alpha-tocopherol is the most abundant form of tocopherol in green leaves and *γ*-tocopherol is found mostly in seeds. Seeds contain 10-20 times more tocochromanols when compared to photosynthetic tissue content of tocochromanols (DellaPenna and Pogson, 2006). Tocopherols are widely distributed in higher plants and tocotrienols are mainly found in non-photosynthetic tissues such as seeds (Lampi et al., 2010). The antioxidant activity increases in the following order, α-tocopherol, β-tocopherol, *Y*-tocopherol (Trebst et al., 2002). Antioxidants are compounds that inhibit and delay the oxidation of other molecules by inhibiting the initiation of oxidising chain reaction (Velioglu et al., 1998). Among the tocochromanols, α-tocopherol has been determined as the most efficient for breaking free radical driven chain reactions (Packer, 1995).

2.4.1 Importance of tocochromanols

Tocotrienols are abundant in cereals and are the primary form of vitamin E. A lot of work has been done on tocotrienols due to the fact that they contribute with several health promoting properties that differ from those of tocopherols (Schaffer et al., 2005). Tocochromanols differ in their vitamin E activity, α-tocopherol shows highest activity as compared to other tocopherols and tocotrienols. Tocopherol protects photosystem ii against singlet oxygen (Trebst et al., 2002). Tocopherols show vitamin E activity to various degrees while tocotrienols do not exhibit vitamin E activity or they have a substantially lower vitamin E activity when compared with tocopherols (Valentin and Qi, 2005; Delgado-Zamarreno et al., 2009; Tiwari and Cummins, 2009). This could be one of the reasons why there is a high quantity of some of the tocotrienols in samples. Some authors found that tocotrienols have different properties from the properties of tocopherols and the differences might be due to the

structural differences between tocotrienols and tocopherols (Delgado-Zamarreno et al., 2009). Tocopherols play a role in preventing diseases such as cancer and heart related diseases as well as lowering cholesterol level (Tiwari and Cummins, 2009). Tocochromanols also contribute to regulation of cellular signaling and gene expression (Lampi et al., 2008). Antioxidants in general play a role in preventing undesirable changes in flavour and nutritional quality of foods (Zielinski and Kozlowska, 2000).

Tocochromanols are antioxidants that have been studied intensively for their effect on human health but there is less work that has focused on the contribution of wheat to food regarding tocochromanol intake. An increase in the quantity of antioxidants in wheat is important not only for human health but for the plant as well. In a number of studies it was proved that wheat antioxidants react directly and quench free radicals (Onyeneho and Hettiarachchy, 1992; Zielinski and Kozlowska, 2000; Yu et al., 2002a; 2002b; Yu et al., 2003). Antioxidants protect polyunsaturated fatty acids in membranes against lipid peroxidation in both animals and plants (Di Mascio et al., 1991, Dörmann, 2007). Understanding the effect of tocochromanols on bread wheat quality will help breeders to select for better cultivars, which not only produce more but also have the benefits of providing nutrients that are very important for health.

Chemical reactions form free radicals, peroxides and secondary oxidation breakdown products that react and cause damage to cellular membranes, proteins and nucleic acids (Baublis et al., 2000). Peroxidising agents damage lipids and produce secondary intermediates, lipid hydroperoxides which can decompose into alkoxyl and organic peroxyl radicals and thus lead to a chain reaction of lipid peroxidation. Tocopherols protect lipids by destroying peroxyl radicals (Di Mascio et al., 1991). Alpha-tocopherol content is important because it is the most active form of vitamin E. It is often the minor component of tocochromanols in plant seeds (Van Eenennaam et al., 2003; DellaPenna and Pogson, 2006).

Tocochromanols decompose easily in the presence of light, oxygen, alkaline pH or traces of transition metal ions. Other treatments such as post harvesting treatment or processing may have an effect on the antioxidant properties of wheat based products (Zhou et al., 2004). Tocopherols and tocotrienols act as antioxidants because they stabilise and stop the propagation phase of the oxidation chain reaction of radicals by donating hydrogen from their phenolics group (Bramley et al., 2000).

2.4.2 Quantity of tocochromanols in wheat grain

According to Hejtmankova et al. (2010) and Lampi et al. (2008), the content of tocochromanols depends on the cultivated genotype, location as well as the growing

conditions. Lampi et al. (2008) stated that tocochromanols are influenced by geographic location and some genotypes contain more tocochromanols than others, therefore selection can be applied to cultivars than have a higher content of tocochromanols. The amount of tocopherol in plants increases strongly during stress such as high light intensity, drought, salt or heat treatment. Plants that are exposed to stressful conditions will have a higher quantity of tocopherols than tocotrienols. The increase in tocopherol is associated with the induction of expression of tocopherol biosynthetic genes (Collakova and DellaPenna, 2003; Kanwischer et al., 2005). Both genetic and environmental factors have a strong and important impact on tocochromanols in wheat genotypes (Lampi et al., 2010).

Environmental factors and G X E can alter the antioxidant activity of wheat (Yu et al., 2003; Yu and Zhou, 2004; Zhou and Yu, 2004). Antioxidant activity of wheat products depends on the nature of antioxidant species, wheat variety, extraction method, type of antioxidant activity assay as well as the extraction medium used (Fardet et al., 2008). The antioxidant activity of different fractions of wheat usually depends on the variety examined (Yu et al., 2002a; 2002b) and the area where it is grown (Yu et al., 2003; Yu and Zhou, 2004). In a study by Liyana-Pathirana and Shahidi (2006), activity of antioxidant and properties varied among the different milling fractions that were examined.

The fraction with the highest antioxidant quantity is the aleurone layer followed by the bran fraction and whole grain (Zhou et al., 2004; Esposito et al., 2005). Generally vitamin E is located within the germ of whole grain cereal from which vitamin E rich oils are extracted. Refined cereals or grain contains less quantities of vitamin E because a significant amount of vitamin E is lost during the refining process. The main contributors of antioxidant potential are the bran and germ, but both are removed during milling (Fardet et al., 2008). Wheat bran is consequently an excellent source of natural antioxidants (Zhou et al., 2004; Fardet et al., 2008) so bran and whole grain may contribute significantly to daily dietary intake of vitamin E to prevent several chronic diseases (Zhou et al., 2004).

2.5 Fractionation of wheat proteins

Various methods have been used to determine the size distribution of the gluten proteins. Gel filtration chromatography was initially used to separate wheat FP based on size and for comparison in their native states. However, this method was time consuming, lacked reproducibility and had poor peak resolutions. HPLC has replaced it and it provides a better understanding of protein functionality. It separates the three main classes of wheat endosperm proteins (glutenin, gliadin, and albumins-globulins) accurately (Gianibelli et al., 2001). The technique has also proved useful for the study of LMW-GS and it showed that

LMW-GS have higher hydrophobic surfaces than HMW-GS and hydrophobic surface of gliadins (Juhasz and Gianibelli, 2006).

HPLC provides a suitable method for characterisation and quantification of vitamin E (Panfili et al., 2003). Several methods have been employed to separate proteins in cereals. The ability to easily and accurately quantify and identify cereal proteins is another reason why automated HPLC and other related methods have gained favour in cereal research. These methods allow the determination of cereal proteins in the presence of co-existing compounds and without interference (Rodriguez-Nogales et al., 2006).

2.5.1 Size exclusion-high performance liquid chromatography

SE-HPLC has been one of the most useful techniques for analysing cereal proteins. Separations occur on the basis of molecular size: Proteins, larger than the pores, elute from the column. Smaller proteins are slowed down by the support in opposite relation to their molecular size (Bietz, 1985; Rodriguez-Nogales et al., 2006). SE-HPLC separations are fast, permitting larger numbers of samples to be analysed and can also characterise and compare isolated subunit fractions. It is also valuable for determining cereal protein molecular weight, for estimating purity, for comparing proteins and for demonstrating disulphide or non-covalent bonds (Bietz, 1985).

SE-HPLC separates the proteins in four major classes or fractions, the HMW-GS, LMW-GS (polymeric proteins), gliadins and albumins/globulins (monomeric protein). It is also used for the analysis of molecular size distribution of polymeric proteins. Parameters such as percentage of polymeric protein (PPP), percentage of gliadins (PG), gluten-to-gliadin ratio (GLU/GLI), polymeric protein in the flour (FPP) and percentage of unextractable polymeric protein (%UPP) are obtained for those analyses and are useful markers for assessing bread-making quality.

SE-HPLC has not been used for prediction of quality on regular basis, but promising results have been reported using a small number of samples (Dachkevitch and Autran, 1989; Larroque and Bekes, 2000). The application has been difficult due to its complex and time consuming procedure (Ohm et al., 2009). The insoluble fraction can be extracted with SDS-solution and sonication. This insoluble fraction usually correlates positively with bread-making quality parameters (Dachkevitch and Autran, 1989). The greatest challenge in studying wheat protein is caused by the difficulty of dissolving them completely without chemically altering them. The use of sonication and SE-HPLC has enabled accurate determination of the three major protein classes in flour samples (Singh et al., 1990b).

2.5.2 Reversed phase – high performance liquid chromatography

RP-HPLC fractionates proteins based on hydrophobicity. It is efficient for qualitative and quantitative studies. RP-HPLC can identify specific proteins that are associated with quality. RP-HPLC may be utilised for determining evolutionary relationships or hybrid percentage; it may also point out misidentified proteins (Bietz, 1985). RP-HPLC enables us to explore more effectively the wealth of information encoded in the proteins of a kernel. It can help assure introduction of superior varieties through breeding and can help optimize their marketing and use (Bietz and Kruger, 1994).

RP-HPLC has many advantages. It is fast and high resolution separations generally take about one hour, and lower resolutions separations, which are sometimes adequate, can be much faster and it also has an excellent reproducibility (Bietz and Kruger, 1994; Rodriguez-Nogales et al., 2006). It can be automated and it can differentiate some varieties that other methods cannot differentiate. RP-HPLC can accurately quantitate data, which is more difficult in electrophoresis (Bietz, 1985; Bietz and Kruger, 1994).

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Chapter 3

The use of reversed phase – high performance liquid chromatography to determine quality from whole wheat and white flour of ten South African bread wheat cultivars

3.1 Abstract

Protein quality and quantity are considered primary factors in measuring the potential of flour in relation to its end use.Gluten proteins are essential for bread production because elasticity and extensibility are considered most important for bread-making. Reversed phase – high performance liquid chromatography was used to investigate the influence of gliadin and glutenin subunits in bread-making quality. Genotype and environment had highly significant effects on protein fractions obtained from both whole wheat and white flour. The correlation between protein content, wet gluten content and ωb-gliadins was negative in both white flour and whole wheat. A highly significant correlation between gliadin peaks with dough strength and dough stability was observed. High molecular weight - glutenin subunits correlated negatively with dough strength to dough stability ratio while the association with low molecular weight - glutenin subunits was positive. Dough distensibility correlated positively with high molecular weight - glutenin subunits and sodium dodecyl sulphate sedimentation correlated negatively with low molecular weight - glutenin subunits. The association between protein fractions and quality characteristics differed between both types of flours and the analysis for quality performance shows that variation of correlation depends on the traits as well.

3.2 Introduction

Quality potential in wheat is determined by both genotype and environment, and baking quality of cultivars is a fulfillment of the potential of the cultivar and is also influenced by both genotype and environment (Johansson and Svensson, 1998). Quality variation is not only attributed to the protein constituents but is highly influenced by the amount and interaction of the different fractions of proteins (Singh et al., 1990; Hruškova and Faměra, 2003), protein concentration and size distribution (Southan and MacRitchie, 1999). It is important to point out that a variation in all classes of gluten proteins may influence the end-use product's quality of wheat lines in some way (Primard et al., 1991).

PC can vary greatly in wheat; it can be as low as 6% to as high as 20%. The content depends on wheat class, soil fertility and environment during a growing season. High protein wheat has higher water absorbing capacity and greater LV potential (Carson and Edwards,

2009). Protein quality is influenced by both environment and genotype while quantity is largely influenced by environment (DuPont and Altenbach, 2003). Conditions that occur during grain filling period have pronounced effects on protein quality. Kernels that do not fill properly will give low yields however, the PC in these grains are usually high. Very high temperatures during grain filling also cause great variation in wheat quality characteristics (Labuschagne et al., 2009; Edwards et al., 2010).

Gluten proteins are essential for bread production because elasticity and extensibility are considered most important for bread-making. Glutenins appear to contribute to mixing time, strength and elasticity where gliadins contribute to extensibility and stickiness. The concept of wheat protein quality is involved with physical rather than nutritional characteristics of bread (Mailhot and Patton, 1988). The amount of unextractable protein mainly glutenin polymers, the amount of polymers extracted by sonication and the amount of HMW-GS contained within the glutenin polymers have all been reported to correlate with bread-making quality (Gupta et al, 1991).

Quality of bread-making has been said to be correlated with specific allelic variants of HMW-GS which can either be present or absent (Wieser and Zimmermann, 2000) and a few specific LMW-GS (Gupta et al., 1989). RP-HPLC can be used to separate these prolamins according to hydrophobic properties (Labuschagne and Aucamp, 2004). It has been successful in separating HMW subunits and has the advantage that individual and groups of components can be quantitated and manipulated with a computer (Burnouf and Bietz, 1984). Protein fractions for RP-HPLC have been found to correlate significantly with wheat quality parameters (Wieser and Kieffer, 2001).

Standard methods for measuring quality characteristics have been done based on white flour. However, the consumption of whole wheat has been increasing in recent years due to health benefits found in whole wheat grain. There has been little research devoted to understanding the relationship between FP and quality characteristics measured on white flour and those measured on whole wheat. The aim of this study was to analyse proteins obtained from white and whole wheat flour using RP-HPLC and correlate these fractions from three different locations with baking quality characteristics.

3.3 Materials and methods

3.3.1 Plant materials

Ten South African bread wheat cultivars: Betta-DN, Caledon, Elands, Gariep, Komati, Limpopo, Matlabas, PAN3118, PAN3349 and PAN3377 replicated four times each were used. The trials were conducted at three different locations in 2007: Bultfontein

(28°16'53.14"S 26°27'02.77"E, north western Free St ate with low rainfall, high temperatures, high evaporation requirements and deep, yellow sandy loam soils with a water table present), Ladybrand (29°14'30.75"S 27°20'18.55"E, central Free State, moderate rainfall, moderate temperatures, a lower evaporation requirement and relatively shallow duplex soils) and Clarens (28°24'26.63"S 27°20'18.55"E, eastern F ree State, higher rainfall, lower temperatures, lower evaporation requirement with predominantly yellow soils of average effective depth). Weather data was recorded at all three locations (Appendix, Table 1). The trials were planted under dryland conditions, according to a randomised complete block design. Trial plots consisted of five rows of 5 m length and inter-row spacing of 5 cm.

3.3.2 Measured quality characteristics

The following quality characteristics were measured using AACC (2000) methods at the laboratories of the Agricultural Research Council - Small Grain Institute (ARC-SGI), Bethlehem.

3.3.2.1 Hectolitre mass

HLM (kg/hl) was determined according to AACC 55-10 and was performed using a two-level funnel (AACC, 2000).

HLM = Obtained mass / 5 = kg/hl

3.3.2.2 Break flour yield and flour yield

BFY and FY were determined according to approved method AACC 26-21A (AACC, 2000). Wheat samples were milled on a laboratory, pneumatic mill, Bühler model MLU-202. The percentage BFY was determined for each sample by dividing the value of total break flour obtained by total flour and bran (Bass, 1988).

3.3.2.3 Loaf volume

LV was determined by applying the rapeseed displacement procedure according to AACC 10-05 (AACC, 2000).

3.3.2.4 Flour colour

A Martin series III colour grader was used to determine FC at the wavelength of 540nm according to the AACC 14-30 (AACC, 2000) procedure.

3.3.2.5 Flour protein content

FP content was determined using the protocol according to AACC 46-30 (AACC, 2000). FP content for both white flour and whole wheat was determined using LECO FP-2000 (Combustion method).

3.3.2.6 Farinograph water absorption

A Brabender® $_{OHG}$ Duisburg was used to measure constant flour weight according to AACC 54-21 (AACC, 2000).

3.3.2.7 Hardness index, thousand kernel mass and kernel diameter

By using the Single Kernel Characterisation System (SKCS) 4100, HI, TKM and DIA were determined according to the AACC 55-31 (AACC, 2000) protocol.

3.3.2.8 Falling number

FN was determined according to AACC 56-81B (AACC, 2000) and is the measure of α-amylase enzyme activity.

3.3.2.9 Vitreous kernels

A farinator is a cutter used to slice kernels longitudinally and vitreousness is determined visually.

3.3.2.10 SDS sedimentation

The method AACC 56-70 was followed in order to determine SDS sedimentation (AACC, 2000).

3.3.2.11 Alveograph

Dough resistance was measured using a Chopin Alveograph NG and followed according to AACC 54-30A method (AACC, 2000).

3.3.3 Reversed-phase high-performance liquid chromatography

Gliadin extraction

Proteins were extracted using a modified method of Marchylo et al. (1989). White flour and whole wheat were weighed (0.1 mg) into a 2 ml reaction tube and 1 ml of 70% ethanol (v/v) was added into tubes. The tubes were vortexed for 2 min at room temperature until the pellet was well suspended. Samples were shaken for 30 min and centrifuged for 5 min at 14000 rpm. The supernatant was filtered through a 0.45 µm HT Tuffryn Acrodisc® syringe filter into a glass vial. The extractions were done in duplicate; the average was then used in the statistical analysis.

Glutenin extraction

Glutenins were extracted from the pellet remaining after gliadin extraction. Samples were vortexed after the addition of 1 ml 50% propan-1-ol (v/v) then shaken for 30 min, centrifuged for 1 min at 14000 rpm and the supernatant was discarded. The pellet was washed for the second time using 1 ml 50% propan-1-ol (v/v) and shaken for 20 min. Tubes were

centrifuged again for 1 min after which the supernatant was discarded. RP-extraction buffer (1 ml) (containing 50% v/v propan-1-ol, 2M urea, 0.2M Tris pH 6.6) and freshly prepared 1% dithiothreitol was added. Samples were incubated at 60℃ for 60 min, with intermitted vortexing every 20 min. After first incubation, 10 µl 4-vinylpyridine was added to each sample, mixed and incubated at 60℃ for another 15 min. Samples were centrifuged for 5 min at 14000 rpm. Supernatants were filtered through a 0.45 µm HT Tuffryn Acrodisc® syringe filter into a glass vial for auto-sampler injection.

Analyses were performed on a Shimadzu 20A HPLC system with a photodiode array (PDA) detector; Phenomenex® Jupiter 5u C_{18} column 300A with 250 x 4.60 mm inner diameter; with column temperature of 50℃; injection volume was 25 µl. Protein fractions were detected at a wavelength of 210 nm.

Elution system: A) 95% acetonitrile containing 5% water and 0.1% (v/v) trifluoroacetic acid (TFA); B) 95% water containing 5% (v/v) acetonitrile and 0.11% (v/v) TFA. Linear gradient for gliadin analysis: 0 – 2 min 0% A; 2 – 15 min 20 - 26% A; 15 – 30 min 26 - 46% A; 30 – 35 min 46 - 60% A; 35 – 40 min 60 - 90% A; 40 – 45 min 90% A; 45 – 55 min 90 - 20% A; 55 – 60 min 20% A. Linear gradient for glutenin analysis: 0 – 40 min 20 - 40% A; 40 – 42 min 40 - 56% A; 42 – 60 min 56 - 90% A; 70 – 80 min 90 - 20% A. The flow rate was 0.8 ml/min or 0.5 ml/min.

Gliadins were measured from 15.60 - 38.73 min. Gliadin peaks that appeared consistently in all the samples were selected and used for statistical analysis. Seven gliadin peaks were selected at 15.38 min for peak 1, 19.72 min for peak 2, 25.74 min for peak 3, 28.59 min for peak 4, 33.95 min for peak 5, 37.02 min for peak 6 and 38.38 min for peak 7. Quantities for glutenin fractions included ωb-gliadins, HMW-GS and LMW-GS, ωb-gliadins were measured from 14.82 - 18.17 min; HMW-GS, 18.17 - 24.93 min and LMW-GS, 24.93 - 41.54 min. The gliadin and glutenin components were quantified by integration of the chromatogram area percentage.

3.3.4 Statistical analysis

All statistical analyses were done with Agrobase (2005) software. The relationships between protein fractions and quality characteristics were investigated using analysis of variance (ANOVA) and correlations.

3.4 Results

3.4.1 Reversed phase-high performance liquid chromatography

Figure 3.1 and 3.2 are examples of gliadin and glutenin RP-HPLC profiles.

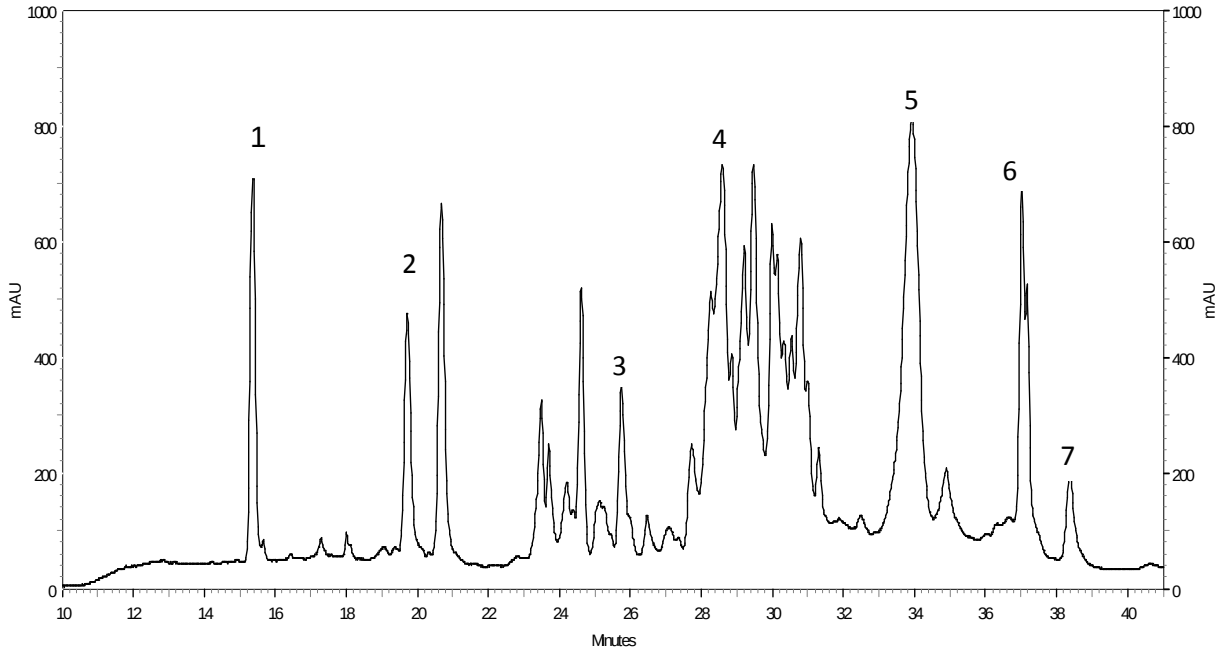

Figure 3.1: An example of RP-HPLC profile of gliadin proteins. 1 = peak 1 (15.38 min), 2 = peak 2 (19.72 min), 3 = peak 3 (25.74 min), 4 = peak 4 (28.59 min), 5 = peak 5 (33.95 min), 6 = peak 6 (37.02 min), 7 = peak 7 (38.38 min).

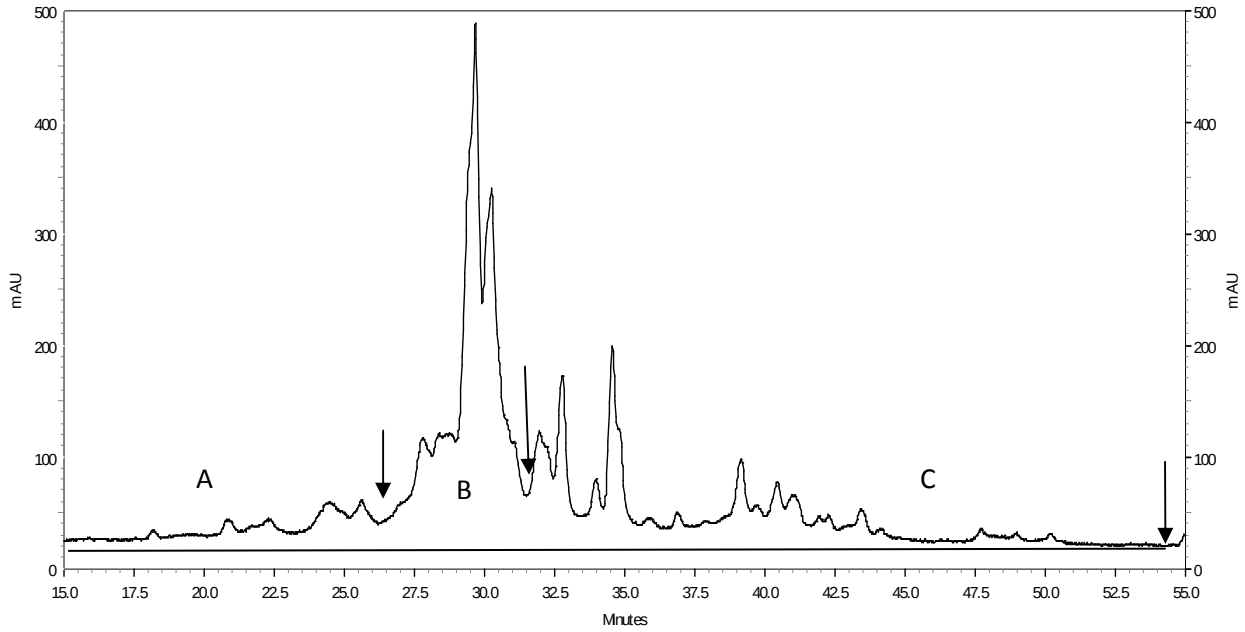

Figure 3.2: An example of RP-HPLC profile of glutenin proteins. A = ωb-gliadins, B = high molecular weight glutenin subunits (HMW-GS), C = low molecular weight glutenin subunits (LMW-GS). The arrows indicate the cutting point for the different subunits.

3.4.2 Analysis of variance for quality characteristics and protein fractions at three locations

ANOVA for alveograph parameters showed significant ($p \leq 0.01$) differences for genotype (entry) and environment (location). The G X E had a highly significant effect on alveograph parameters except for dough distensibility (AlvDIST). Genotype effect and environmental effect were highly significant for LV12 (LV at 12% PC) while G X E had no significant effect.

LV, BFY, FY, FN, FABS, HLM, FP, DIA, HI, TKM and VK all showed significant ($p \leq 0.01$) differences for genotype, environment as well as G X E. ANOVA for SDSVOL and WGC showed highly significant differences for genotype and environment while no significant differences were observed for G X E (Table 3.1).

Table 3.1: Mean squares for quality characteristics across three locations

Characteristic	Genotype	Environment	G X E
AlvDIST	3810.76**	58559.31**	369.22
AlveoW	8998.14**	88616.223**	2713.07**
AlvP/L	1.39**	23.48**	0.39**
AlvSTAB	1367.72**	14205.26**	215.48**
LV12	10062.78**	68152.10**	1735.6
LV	19462.52**	429660.20**	2568.77**
BFY	7.74**	24.67**	7.93**
FY	6.06**	13.67**	0.89**
FN	24829.36**	38750.00**	3606.78**
FABS	15.50**	10.29**	6.62**
HLM	6.27**	77.72**	4.38**
SDSVOL	219.34**	106.34**	17.13
WGC	66.78**	895.18**	9.71
FP	2.09**	105.23**	0.45**
DIA	0.04**	0.08**	0.006**
HI	156.17**	851.93**	28.10**
TKM	26.10**	135.49**	3.42**
VK	189.44**	1362.02**	62.47**

* p≤0.05; ** p≤0.01, AlvDIST=dough distensibility, AlveoW=dough strength, AlvP/L=AlvSTR to AlvSTAB ratio, AlvSTAB=dough stability, LV12=LV at 12% PC, LV=loaf volume, BFY=break flour yield, FY=flour yield, FN=falling number, FABS=farinograph water absorption, HLM=hectolitre mass, SDSVOL=SDS sedimentation volume, WGC=wet gluten content, FP=protein content in flour, DIA=kernel diameter, HI=hardness index, TKM=thousand kernel mass and VK=vitreous kernels.

ANOVA for HMW-GS extracted in whole wheat showed highly significant differences for genotype and environment, while no significant differences were observed for G X E. LMW-GS in whole wheat showed highly significant differences for genotype, environment and G X

E. Peak 1, 2, 5 in whole wheat had highly significant differences for genotype, environment and G X E. Peak 3 showed highly significant differences for genotype only and there were no significant differences for environment and G X E. ANOVA values for peak 6 showed highly significant differences for genotype and G X E while peak 7 had highly significant differences for genotype and environment (Table 3.2).

Table 3.2: Analysis of variance for RP-HPLC fractions from white flour and whole wheat

Type of flour	Characteristic	Genotype	Environment	G X E
Whole wheat	HMW-GS	78.126**	50.702**	14.606
	LMW-GS	20.213**	51.003**	8.37**
	Peak1	4.452**	83.679**	1.852**
	Peak2	12.137**	1.149**	0.847**
	Peak3	46.364**	0.277	0.346
	Peak5	4.797**	5.408**	2.050**
	Peak6	14.293**	2.181	3.687**
	Peak7	10.785**	7.080**	0.611
White flour	HWM-GS	85.398**	34.398*	12.124
	LMW-GS	38.591**	113.832**	17.441
	Peak1	0.727**	2.278**	0.129*
	Peak2	12.983**	1.667*	0.809*
	Peak3	27.7**	21.302**	2.898
	Peak6	10.933**	4.491**	2.395**
	Peak7	14.419**	4.797**	9.738**

* p≤0.05; ** p≤0.01, HMW-GS=high molecular weight - glutenin subunits, LMW-GS=low molecular weight - glutenin subunits. Peaks are gliadin fractions while HMW-GS and LMW-GS are glutenin fractions.

There was a highly significant genotype effect, significant environmental effect and no G X E effect on HMW-GS extracted in white flour. The environment and genotype effect were both highly significant for LMW-GS in white flour, but there were no significant differences in G X E. ANOVA for peak 1 in white flour showed highly significant differences for genotype and environment, the G X E was significant ($p \leq 0.05$). There was a highly significant genotype, environment and G X E effect for peak 2. Peak 3 had highly significant differences for genotype, environment and no significant differences for G X E. Genotype, environment and G X E showed highly significant differences for peak 6 and peak 7 (Table 3.2).

3.4.3 Quality characteristics for the ten cultivars at Bultfontein, Clarens and Ladybrand

LV value of BettaDN (1020.00 cm^3) at Clarens, PAN3377 (1020.00 cm^3) at Clarens and Bultfontein ranked high while PAN3349 had the lowest value for LV in all three localities (Table 3.3).

BFY of BettaDN (22.27%) was the highest at Clarens and Bultfontein while the lowest value was obtained from Caledon (16.52%) at Ladybrand.

Caledon (65.50%) at Ladybrand had the highest value for FABS and Gariep (59.38%) had the lowest value at Bultfontein.

Highest value for WGC was obtained from Caledon (42.51%) at Bultfontein. PAN3118 (26.23%) at Ladybrand had the lowest value.

HLM for Elands (80.35 kg/hl) was the highest at Ladybrand and lowest at Clarens for PAN3349 (74.11 kg/hl).

Matlabas (96 ml) at Clarens had the highest value for SDSVOL and the lowest value was at Bultfontein from Caledon (77.75 ml).

Matlabas (471.00) at Clarens had the highest value for FN. PAN3377 at both Clarens (243.50) and Bultfontein (258.00) had the lowest values for this characteristic.

DIA was highest at Bultfontein and Ladybrand for cultivar Matlabas (2.96 mm). Limpopo (2.68 mm) had the lowest value for DIA at Bultfontein as well as the cultivar Komati (2.68 mm) at Bultfontein.

The lowest value for TKM was for Limpopo (31.08 mg) at Bultfontein and the highest value was for Matlabas (40.78 mg) at Ladybrand.

HI for Caledon (79.75) was highest at Bultfontein and PAN3377 (59.19) at Clarens gave the lowest HI value.

Highest value for VK was obtained from Elands (30%) at Clarens while the lowest was from Caledon (3%) at Ladybrand.

Pan3377 in Bultfontein had 176.00 as the highest value for AlvDIST while Pan3349 (44.50) from Ladybrand had the lowest value.

The lowest value for AlveoW was for Pan3349 at Ladybrand and the highest value was for Pan3377 (428.75) at Clarens.

Table 3.3: Measured means for quality characteristics at Bultfontein, Clarens and Ladybrand for white flour

Cultivar	LV	BFY	FABS	WGC	HLM	SDSVOL	FN	DIA	HI	TKM	VK	AlvDIST	AlveoW	AlvP/L	AlvSTAB	AlvSTR
	cm^3	%	%	%	Kg/hl	ml	Sec	mm		mg	%					
Betta(B)	1010.00	22.27	60.25	38.50	76.26	87.25	345.75	2.71	74.49	32.75	13.75	164.5	373.25	0.43	70.25	57.07
Betta(C)	1020.00	22.27	62.53	38.58	77.92	86.25	447.00	2.83	64.86	36.62	23.75	127.25	335.75	0.65	81.25	54.40
Betta(L)	787.50	19.22	61.30	27.85	78.93	86.75	383.00	2.80	72.28	37.04	12.25	58.75	245.25	2.14	109.25	37.50
Caledon(B)	993.75	20.02	62.61	42.51	76.72	77.75	390.25	2.74	79.75	33.24	12.25	170.00	296.75	0.40	66.75	45.37
Caledon(C)	1010.00	19.83	62.79	39.92	75.94	86.00	380.00	2.84	70.79	35.96	24.5	142.25	332.75	0.56	78.75	50.88
Caledon(L)	851.25	16.52	65.50	34.17	80.17	86.50	443.25	2.91	78.61	39.00	3.00	83.00	283.75	1.24	101.75	43.39
Elands(B)	946.25	20.03	62.53	35.15	77.45	82.25	316.25	2.76	74.08	34.31	16.25	107.75	370.00	0.93	98.75	56.57
Elands(C)	908.75	20.43	63.75	38.02	76.82	86.25	292.50	2.86	61.26	36.78	30.00	91.75	402.50	1.25	113.75	61.54
Elands(L)	746.25	17.28	62.53	27.62	80.35	85.00	384.00	2.80	77.17	36.53	9.50	47.25	243.00	2.90	128.50	37.16
Gariep(B)	1016.00	21.18	59.38	37.76	77.30	82.00	333.25	2.74	74.72	33.46	15.50	137.75	321.25	0.52	70.00	49.12
Gariep(C)	953.75	21.41	62.44	36.28	77.16	83.50	342.50	2.84	64.88	36.91	23.25	102.75	324.00	0.86	87.50	49.54
Gariep(L)	842.50	20.01	59.99	28.66	79.62	80.75	413.50	2.83	73.47	37.70	15.50	68.50	251.50	1.44	97.50	38.46
Komati(B)	1010.00	21.94	61.21	39.66	75.25	80.75	335.25	2.68	75.99	32.63	10.25	136.00	350.25	0.61	80.50	53.56
Komati(C)	963.75	21.06	60.95	37.18	76.86	85.75	344.25	2.80	66.60	35.28	18.75	111.00	326.75	0.76	83.00	49.96
Komati(L)	838.75	17.96	63.84	33.92	79.77	86.50	390.50	2.82	75.10	37.04	12.25	67.50	288.75	1.72	113.00	44.15
Limpopo(B)	1002.50	20.86	60.51	36.75	76.79	79.25	316.50	2.68	79.64	31.08	8.25	133.25	329.25	0.58	76.50	50.34
Limpopo(C)	890.00	20.99	60.95	36.75	78.06	85.00	369.75	2.82	69.17	35.18	22.25	105.75	299.25	0.77	80.50	45.76
Limpopo(L)	755.00	18.45	60.43	27.42	79.76	84.00	369.00	2.77	76.60	35.26	10.00	64.75	255.00	1.84	105.50	38.99
Matlabas(B)	1020.00	22.06	61.74	37.39	77.96	93.25	364.75	2.96	68.30	38.71	5.25	132.50	408.25	0.64	82.50	62.42
Matlabas(C)	940.00	19.94	61.91	34.60	75.99	96.00	471.00	2.92	67.93	38.47	14.00	108.50	368.00	0.88	95.00	56.27
Matlabas(L)	781.25	19.24	63.40	26.74	77.28	95.00	447.50	2.96	68.06	40.78	16.25	56.50	285.00	2.21	122.00	43.58

B=Bultfontein, C=Clarens, L=Ladybrand, LV=Loaf volume, BFY=Break flour yield, FABS=Farinograph water absorption, WGC=Wet gluten content, HLM=Hectolitre mass, SDSVOL=SDS sedimentation volume, FN=Falling number, DIA=Kernel diameter, HI=Hardness index, TKM=Thousand kernel mass, VK=vitreous kernels, AlvDIST=dough distensibility, AlveoW=dough strength, , AlvP/L= AlveoW to AlvSTAB ratio, AlvSTAB=dough stability, AlvSTR=dough strength.

Table 3.3: Continued

Cultivar	LV	BFY	FABS	WGC	HLM	SDSVOL	FN	DIA	HI	TKM	VK	AlvDIST	AlveoW	AlvP/L	AlvSTAB	AlvSTR
	cm^3	%	%	%	Kg/hl	ml	Sec	mm		mg	%					
PAN3118(B)	953.75	19.28	63.31	32.65	78.07	92.25	301.75	2.81	76.84	34.24	20.25	130.00	349.75	0.65	82.25	53.48
PAN3118(C)	931.25	20.34	61.65	34.10	75.07	90.00	300.25	2.85	65.34	36.09	25.50	114.25	369.75	0.85	94.50	56.54
PAN3118(L)	757.50	21.94	62.18	26.23	77.80	87.75	344.25	2.86	68.83	37.24	19.75	56.00	289.50	2.40	131.00	44.27
PAN3349(B)	915.00	18.80	63.58	33.04	76.55	86.75	359.00	2.88	72.60	36.97	14.25	94.75	355.75	1.12	106.25	54.40
PAN3349(C)	883.75	19.42	61.21	34.18	74.11	90.25	426.00	2.87	66.47	36.55	25.25	104.75	305.00	0.82	85.75	46.64
PAN3349(L)	732.50	19.12	65.41	27.28	78.04	91.00	426.75	2.89	70.10	39.00	9.75	44.50	239.25	3.22	136.25	36.58
PAN3377(B)	1020.00	18.81	62.47	35.49	76.21	89.67	258.00	2.83	69.18	34.36	22.00	176.00	381.00	0.40	70.33	58.26
PAN3377(C)	1020.00	20.38	65.06	38.42	74.99	93.25	243.50	2.94	59.19	37.26	28.00	139.00	428.75	0.65	90.50	65.56
PAN3377(L)	845.00	22.33	63.14	26.64	77.66	92.00	341.25	2.93	61.72	38.50	22.50	87.50	328.00	1.24	120.25	50.15
Mean	**911.53**	**20.11**	**62.29**	**34.12**	**77.36**	**86.96**	**362.68**	**2.83**	**70.80**	**36.16**	**16.80**	**105.47**	**324.57**	**1.16**	**95.32**	**49.73**

B=Bultfontein, C=Clarens, L=Ladybrand, LV=Loaf volume, BFY=Break flour yield, FABS=Farinograph water absorption, WGC=Wet gluten content, HLM=Hectolitre mass, SDSVOL=SDS sedimentation volume, FN=Falling number, DIA=Kernel diameter, HI=Hardness index, TKM=Thousand kernel mass, VK=vitreous kernels, AlvDIST=dough distensibility, AlveoW=dough strength, AlvP/L= AlveoW to AlvSTAB ratio, AlvSTAB=dough stability, AlvSTR=dough strength.

Pan3349 at Ladybrand had the highest value for AlvP/L (3.22) while Pan3377 and Caledon (0.40) had lowest values for AlvP/L.

Dough distensibility had the highest value at Ladybrand for Pan3349 (136.25) and the lowest value from Bultfontein for Caledon (66.75).

Pan3377 (65.56) obtained from Caledon had the highest value for AlvSTR while the lowest value was from Ladybrand for Pan3349

3.4.4 Significant correlations between gliadins and quality characteristics

White flour

Bultfontein: Peak 1 and peak 2 correlated negatively with BFY, FC and PC. The correlation between peak 3 and FC as well as BFY was positive. Peak 1, peak 4 and peak 5 correlated negatively with WGC and the reverse was true for peak 3 which correlated positively with WGC and peak 4 with PC. Peak 1 correlated positively with VK and FY and negatively with FP. Peak 2 correlated positively with FABS, AlvSTAB, AlvP/L and peak 2 as well as peak 4 correlated negatively with LV. Peak 3 correlated negatively with FY, TKM and DIA. Peak 2 correlated negatively with LV12 while correlation was positive with peak 6. Correlation between peak 4 and FN was negative. For peak 1 all significant correlations were negatively. Peak 5 correlated negatively with AlveoW and SDSVOL (Table 3.4).

Clarens: Peak 2 correlated negatively with HLM while correlation with peak 3 was positive. FABS correlated positively with peak 6 and peak 7 but correlation with peak 1 was negative. SDSVOL correlated significantly with only peak 3 although this correlation was negative. Peak 6 correlated with AlveoW positively. Peaks 7 correlated negatively with FN and positively with LV, BFY, FP, AlveoW and WGC. Peak 5 also correlated negatively with BFY and peak 4 correlated positively with WGC.

Ladybrand: Peak 1 correlated positively with AlvSTAB and DIA. HI correlated negatively with peak 1, 2 and positively with peak 3. Peak 2 correlated positively with SDSVOL. Peak 3 correlated positively with HLM and BFY and negatively with VK. FN positively correlated with peak 6 and 7. Peak 5 correlated positively with TKM and DIA. Peak 4 and 5 had no significant correlations with quality characteristics.

Table 3.4: Significant correlations between gliadins and quality characteristics in white flour for three localities

Bultfontein			Clarens			Ladybrand		
Character 1	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**
Peak1	VK	0.4916**	Peak1	WGC	-0.4459**	Peak1	AlvSTAB	0.4505**
	BFY	-0.4423**		FP	-0.3819*		DIA	0.4602**
	FY	0.4288**		PC	-0.4067*		HI	-0.3921*
	FC	-0.4751**		FABS	-0.3725*	Peak2	HI	-0.3760*
	FP	-0.4387**	Peak2	HLM	-0.5480***		SDSVOL	0.3845*
	PC	-0.5438***		FY	0.3673*		HLM	0.4810**
	WGC	-0.5491**		HLM	0.5194***	Peak3	VK	-0.4046*
Peak2	BFY	-0.4651**	Peak3	SDSVOL	-0.4701**		BFY	0.3999*
	FC	-0.3782*		WGC	0.3334*		HI	0.5740***
	FABS	0.6061***	Peak4	BFY	-0.3219*		TKM	0.3344*
	AlvSTAB	0.5089**	Peak5	FABS	0.4482**		DIA	0.3478*
	AlvP/L	0.4617**	Peak6	AlveoW	0.6041***	Peak6	FN	0.4263**
	LV	-0.3935*						
	PC	-0.4500**						
	LV12	-0.4045*						

* p≤0.05; ** p≤0.01; *** p≤0.001, VK=vitreous kernels, BFY=break flour yield, FY=flour yield, FC=flour colour, FP=protein content in flour, PC=protein content in whole wheat, WGC=wet gluten content, FABS=farinograph water absorption, AlvSTAB=dough stability, AlvP/L=AlveoW to AlvSTAB ratio, LV=loaf volume, TKM=thousand kernel mass, DIA=kernel diameter, FN=falling number, AlveoW=dough strength, SDSVOL=SDS sedimentation volume, HI=hardness index and HLM=hectolitre mass.

Table 3.4: Continued

Bultfontein			Clarens			Ladybrand		
Character 1	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**
Peak3	FC	0.6437***	Peak7	FN	-0.5411***	Peak 7	FN	0.4263**
	BFY	0.3737*		LV	0.5509***			
	FY	-0.4101*		BFY	0.4115*			
	WGC	0.4609**		FP	0.3809*			
	TKM	-0.4631**		FABS	0.3819*			
	DIA	-0.5419***		AlveoW	0.4101*			
Peak4	PC	0.4172**		WGC	0.3950*			
	FN	-0.3607*						
	LV	-0.3330*						
Peak5	WGC	-0.4157**						
	AlveoW	-0.4263**						
Peak6	SDSVOL	-0.3432*						
	LV12	0.4284**						

* p≤0.05; ** p≤0.01; *** p≤0.001, VK=vitreous kernels, BFY=break flour yield, FY=flour yield, FC=flour colour, FP=protein content in flour, PC=protein content in whole wheat, WGC=wet gluten content, FABS=farinograph water absorption, AlvSTAB=dough stability, AlvP/L=AlveoW to AlvSTAB ratio, LV=loaf volume, TKM=thousand kernel mass, DIA=kernel diameter, FN=falling number, AlveoW=dough strength, SDSVOL=SDS sedimentation volume, HI=hardness index and HLM=hectolitre mass.

Whole wheat

Bultfontein: Peak 1 and peak 2 correlated negatively with BFY, PC and LV. Peak 1 correlated negatively with FC while correlation with peak 3 was positive. Peak 2 (positive), 3 and 7 (negative) correlated with FABS. Peak 1 correlated positively with VK and negatively with FP. Peak 2 correlated negatively with AlvDIST, LV12 and WGC. Peak 3 negatively correlated with FY, TKM, DIA, and SDSVOL, and correlated positively with HI. Peak 7 correlated negatively with AlvSTAB and AlvP/L (Table 3.5).

Clarens: Peak 1 and peak 2 correlated positively with FY and peak 1 correlated positively and peak 3 negatively with DIA. Peak 1, 2 and 5 correlated negatively with HLM while correlation between peak 3 and HLM was positive. FP correlated positively with peak 3 and negatively with peak 1 and peak 5. Peak 2 negatively correlated with BFY and correlation with peak 3 and 5 was positive. Peak 1 negatively correlated with FABS, PC and WGC. SDSVOL correlated negatively with peak 3. Peak 4 correlated negatively with WGC. Peak 5 correlated negatively with LV, WGC and PC. Peak 6 was correlated positively to AlveoW, LV and FABS but correlation with FN was negative and FN correlated negatively with peak 7.

Ladybrand: Dough stability had negative correlation with peak 3 and 7 but correlated positively with peak 2. BFY correlated positively with peak 1, 2 and 6. Peak 1 and 6 correlated positively with VK. HI correlated negatively with peak 1, 2, 6 and positively with peak 3. Peak 2 correlated negatively with HLM and positively with FABS and SDSVOL while peak 3 correlated positively with HLM and negatively with TKM, DIA, SDSVOL and FABS. Peak 6 negatively correlated with FN and positively correlated with AlveoW, AlvDIST, LV and LV12. Peak 7 correlated negatively with AlvP/L, FABS and positively correlated with AlvDIST. Peak 4 and 5 had no significant correlations with quality characteristics.

Table 3.5: Significant correlations between gliadins and quality characteristics in whole wheat for three localities

Bultfontein			Clarens			Ladybrand		
Character 1	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**
Peak1	BFY	-0.4648**	Peak1	FP	-0.5019**	Peak1	VK	0.4294**
	VK	0.5161***		PC	-0.4418**		BFY	0.4885**
	FC	-0.4329**		FABS	-0.4211**		HI	-0.5741***
	FP	-0.5416***		WGC	-0.4135*	Peak2	HLM	-0.5033**
	LV	-0.5019**		HLM	-0.3687*		BFY	0.3714*
	PC	-0.4748**		FY	0.3943*		FABS	0.4720**
Peak2	BFY	-0.4724**		DIA	0.4536**		AlvSTAB	0.5362***
	FABS	0.6430***	Peak2	HLM	-0.4331**		HI	-0.4950**
	AlvSTAB	0.6015***		BFY	-0.3377*		SDSVOL	0.3480*
	AlvDIST	-0.4165**		FY	0.3444*	Peak3	HLM	0.6911***
	AlvP/L	0.5493***	Peak3	BFY	0.6009***		AlvSTAB	-0.4336**
	LV	-0.4501**		HLM	0.6873***		TKM	-0.4407**
	LV12	-0.4271**		DIA	-0.5663***		DIA	-0.5374***
	WGC	-0.3740*		SDSVOL	-0.7371***		HI	0.6757***
	PC	-0.4108*		FP	0.3858*		SDSVOL	-0.5659***
				WGC	-0.3276*		FABS	-0.4505**
			Peak4	WGC	-0.3276*			

* p≤0.05; ** p≤0.01; *** p≤0.001, BFY=break flour yield, VK=vitreous kernels, FC=flour colour, FP=protein content in flour, LV=loaf volume, PC=protein content in whole wheat, FABS=farinograph water absorption, AlvSTAB=dough stability, AlvDIST=dough distensibility, AlvP/L= AlveoW to AlvSTAB ratio, WGC=wet gluten content, FY=flour yield, TKM=thousand kernel mass, DIA=kernel diameter, HI=hardness index, SDSVOL=SDS sedimentation volume, HLM=hectolitre mass, FN=falling number.

Table 3.5: Continued

Bultfontein			Clarens			Ladybrand		
Character 1	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**
Peak3	FY	-0.4628**	Peak5	HLM	-0.4558**	Peak6	BFY	0.5169***
	FC	0.4111*		FP	-0.5439***		VK	0.4784**
	FABS	-0.4881**		PC	-0.5120***		FN	-0.3940*
	TKM	-0.7170***		LV	-0.4298**		AlveoW	0.4996**
	DIA	-0.7702***		WGC	-0.4389**		AlvDIST	0.5391***
	HI	0.5894***		BFY	-0.4745**		LV	0.3959*
	SDSVOL	-0.5491***	Peak6	AlveoW	0.4683**		LV12	0.5613***
Peak7	FABS	-0.4389**		FN	-0.4001*		HI	-0.6128***
	AlvSTAB	-0.4571**		LV	0.3536*	Peak7	AlvSTAB	-0.4563**
	AlvP/L	-0.4378**		FABS	0.3982*		AlvDIST	0.4031*
			Peak7	FN	-0.3795*		AlvP/L	-0.4850**
							FABS	-0.4015*

* p≤0.05; ** p≤0.01; *** p≤0.001, BFY=break flour yield, VK=vitreous kernels, FC=flour colour, FP=protein content in flour, LV=loaf volume, PC=protein content in whole wheat, FABS=farinograph water absorption, AlvSTAB=dough stability, AlvDIST=dough distensibility, AlvP/L= AlveoW to AlvSTAB ratio, WGC=wet gluten content, FY=flour yield, TKM=thousand kernel mass, DIA=kernel diameter, HI=hardness index, SDSVOL=SDS sedimentation volume, HLM=hectolitre mass, FN=falling number.

3.4.5 Significant correlations between glutenins and quality characteristics

White flour

Bultfontein: HMW-GS correlated positively with AlvDIST, LV, WGC, and PC. HMW-GS correlated negatively with AlvP/L and AlvSTAB. LMW-GS extracted in white flour correlated negatively with AlvDIST and LV while correlation with AlvP/L was positive. Correlation between ωb-gliadins and BFY, FC, PC, FP was negative and a positive correlation was observed with FABS (Table 3.6).

Clarens: LMW-GS correlated negatively with FN and SDSVOL while correlation with peak 3 and peak 7 was positive. Correlation between ωb-gliadin and HLM, BFY, FP as well as WGC was negative and with SDSVOL the correlation was positive.

Ladybrand: HMW-GS correlated positively with peak 6 and peak 7.

Whole wheat

Bultfontein: The correlation between HMW-GS and WGC was negative. LMW-GS correlated negatively with FN and positively with WGC. The correlation between ωb-gliadins and FY and FABS was positive. LV, FC and PC correlated negatively with these gliadins (Table 3.7).

Clarens: HMW-GS correlated positively with HLM, WGC, BFY and FP while ωb-gliadins correlated negatively with HLM, BFY, FP, WGC and LV.

Ladybrand: HMW-GS correlated negatively with BFY and FY. LMW-GS correlated positively with BFY. The ωb-gliadin fractions correlated negatively with HLM and positively with FY.

Table 3.6: Significant correlations between glutenin, gliadin fractions and quality characteristics in white flour at three localities

Bultfontein			Clarens			Ladybrand		
Character 1	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**
HMW	AlvDIST	0.4454**	LMW	FN	-0.4337**	HMW	Peak 6	0.4619**
	AlvP/L	-0.4644**		SDSVOL	-0.3878*		Peak 7	0.4457**
	WGC	0.4013*		Peak 3	0.3564*			
	AlvSTAB	-0.4104*		Peak 7	0.3358*			
	LV	0.5188**	ωb-gliadin	HLM	-0.6445***			
	PC	0.3760*		BFY	-0.4374**			
LMW	LV	-0.3920*		FP	-0.4124**			
	AlvDIST	-0.4089*		WGC	-0.3580*			
	AlvP/L	0.3650*		SDSVOL	0.4129**			
ωb-gliadin	BFY	-0.4595**						
	FC	-0.3345*						
	PC	-0.4425**						
	FP	-0.3436*						
	FABS	0.3954*						

* p≤0.05; ** p≤0.01; *** p≤0.001, HMW=high molecular weight - glutenin subunits, AlvDIST=dough distensibility, AlvP/L=AlveoW to AlvSTAB ratio, WGC=wet gluten content, AlvSTAB=dough stability, LV=loaf volume, PC=protein content in whole wheat, BFY=bread flour yield, FC=flour colour, FP=protein content in flour, FABS=farinograph water absorption, FN=falling number, SDSVOL=SDS sedimentation volume and HLM=hectolitre mass.

Table 3.7: Significant correlations between glutenin fractions and quality characteristics in whole wheat at three localities

Bultfontein			Clarens			Ladybrand		
Character 1	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**
HMW	WGC	-0.3506*	HMW	HLM	0.6531***	HMW	BFY	-0.4295**
LMW	FN	-0.4917**		BFY	0.3995*		FY	-0.4524**
	WGC	0.3680*		FP	0.3407*	LMW	BFY	0.3433*
ωb-gliadin	PC	-0.3589*		WGC	0.3204*	ωb-gliadin	HLM	-0.3359*
	FY	0.6405***	ωb-gliadin	HLM	-0.6392***		FY	0.3360*
	FC	-0.3345*		BFY	-0.4057**			
	FABS	0.3744*		FP	-0.4067**			
	LV	-0.3395*		WGC	-0.3307*			
				LV	-0.3395*			

* p≤0.05; ** p≤0.01; *** p≤0.001, LMW=low molecular weight - glutenin subunits, HMW=high molecular weight - glutenin subunits, FN=falling number, WGC=wet gluten content, PC=protein content in whole wheat, FY=flour yield, FC=flour colour, FABS=farinograph water absorption, LV=loaf volume, BFY=break flour yield, FP= protein content in flour and HLM=hectolitre mass.

3.4.6 Significant correlations from combined analysis between gliadin fractions and quality characteristics over three locations

White flour:

Results showed a positive correlation between peak 1 with HLM, AlvSTAB, AlvP/L, TKM, DIA and SDSVOL; a negative correlation was observed for peak 1 with BFY, FC, PC, FP, AlveoW, AlvDIST, LV and WGC. Peak 2 correlated negatively with only HLM and correlated positively with FY, FABS and SDSVOL. The correlation between peak 3 and VK, FABS, TKM, DIA, SDSVOL was negative while a positive correlation was found with FC, WGC and HI. Peak 5 was negatively correlated with FY, FC, PC, FP, AlveoW, AlvDIST and correlated positively with only AlvSTAB and AlvP/L. HI correlated negatively with peak 6 and AlveoW, TKM, DIA and SDSVOL correlated positively with peak 6. Peak 4 and 5 had no significant correlations with quality characteristics (Table 3.8).

Whole wheat:

Positive correlation was observed for peak 1 with HLM, FN, PC, AlvSTAB, AlvP/L, TKM, DIA and a negative correlation was observed with BFY, FY, FC, FP, AlveoW, AlvDIST, LV, LV12 and WGC. Peak 2 had a positive correlation with HLM, FABS, FC, AlvSTAB, AlvP/L, DIA, SDSVOL and had negative correlation with LV and WGC. Peak 3 correlated positively with WGC and HI but correlation with TKM, DIA and SDSVOL was negative. FN, TKM, DIA all correlated positively with peak 5 while BFY, FC, PC, FP, AlveoW, LV and LV12 correlated negatively. Peak 6 correlated negatively with FN and HI but was positively correlated with VK, BFY, AlveoW and LV12. A negative correlation was observed for peak 7 with FN, FABS, AlvSTAB, AlvP/L, TKM and DIA; a positive correlation was observed between peak 7 and PC, FP, AlveoW, AlvDIST, LV, LV12 and WGC.

Table 3.8: Significant correlations from combined analysis between quality characteristics and gliadin fractions for three locations

White flour			Whole wheat		
Character 1	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**
Peak 1	HLM	0.2037*	Peak 1	HLM	0.3689***
	BFY	-0.2969**		FN	0.2452**
	FC	-0.3367***		BFY	-0.3478***
	PC	-0.6125***		FY	-0.2213*
	FP	-0.5772***		FC	-0.4843***
	AlveoW	-0.3923***		PC	0.7961***
	AlvSTAB	0.4256***		FP	-0.7988***
	AlvDIST	-0.4118***		AlveoW	-0.6029***
	AlvP/L	0.4570***		AlvSTAB	0.5314***
	LV	-0.5414***		AlvDIST	-0.6155***
	WGC	-0.6077***		AlvP/L	0.5608***
	TKM	0.2993**		LV	-0.7238***
	DIA	0.2500**		LV12	-0.2976**
	SDSVOL	0.2373**		WGC	-0.7228***
Peak 2	HLM	-0.2573**		TKM	0.3369***
	FY	0.2099*		DIA	0.1924*
	FABS	0.2669**	Peak 2	HLM	0.3103***
	SDSVOL	0.2298*		FABS	0.3820***
				FC	0.2005*

* p≤0.05; ** p≤0.01; *** p≤0.001, HLM=hectolitre mass, BFY=break flour yield, FC=flour colour, PC=protein content in whole wheat, FP=flour protein, AlveoW=dough strength, AlvDIST=dough distensibility, AlvP/L=AlveoW to AlvSTAB ratio, LV=loaf volume, LV12=loaf volume at 12% protein basis, WGC=wet gluten content, TKM=thousand kernel mass, DIA=kernel diameter, SDSVOL=SDS sedimentation volume, VK=vitreous kernels, FABS=farinograph water absorption, HI=hardness index and FY=flour yield.

Table 3.8: continued

White flour			Whole wheat		
Character 1	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**
Peak 3	VK	-0.2917**	Peak 2	AlvSTAB	0.3429***
	FC	0.3926***		AlvP/L	0.2518**
	FABS	-0.2288*		LV	-0.1994*
	WGC	0.2154*		WGC	-0.2137*
	TKM	-0.3564***		DIA	0.2063*
	DIA	-0.4814***		SDSVOL	0.2549**
	HI	0.4528***	Peak 3	WGC	0.2567**
	SDSVOL	-0.3950***		TKM	-0.4286***
Peak 5	FY	-0.2081*		DIA	-0.5826***
	FC	-0.2113*		HI	0.3731***
	PC	-0.3163***		SDSVOL	-0.5903***
	FP	-0.3244***	Peak 5	FN	0.2811**
	AlveoW	-0.2918**		BFY	-0.2098*
	AlvSTAB	0.1912*		FC	-0.2266*
	AlvDIST	-0.2497**		PC	-0.2743**
	AlvP/L	0.2047*		FP	-0.2635**
				AlveoW	-0.2719**
				LV	-0.2604**
				LV12	-0.2283*
				TKM	0.2541**
				DIA	0.1972*

* p≤0.05; ** p≤0.01; *** p≤0.001, HLM=hectolitre mass, BFY=break flour yield, FC=flour colour, PC=protein content in whole wheat, FP=flour protein, AlveoW=dough strength, AlvDIST=dough distensibility, AlvP/L=AlveoW to AlvSTAB ratio, LV=loaf volume, LV12=loaf volume at 12% protein basis, WGC=wet gluten content, TKM=thousand kernel mass, DIA=kernel diameter, SDSVOL=SDS sedimentation volume, VK=vitreous kernels, FABS=farinograph water absorption, HI=hardness index and FY=flour yield.

Table 3.8: Continued

White flour			Whole wheat		
Character 1	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**
Peak 6	AlveoW	0.2422**	Peak 6	VK	0.2315*
	TKM	0.2575**		FN	-0.2441**
	DIA	0.3125***		BFY	0.3182***
	HI	-0.2200*		AlveoW	0.2753**
	SDSVOL	0.3149***		LV12	0.2104*
				HI	-0.3336***
			Peak 7	FN	-0.3547***
				PC	0.3423***
				FP	0.3173***
				FABS	-0.2767**
				AlveoW	0.2117*
				AlvSTAB	-0.4244***
				AlvDIST	0.3435***
				AlvP/L	-0.4377***
				LV	0.4115***
				LV12	0.3943***
				WGC	0.3067***
				TKM	-0.3006***
				DIA	-0.2547**

* p≤0.05; ** p≤0.01; *** p≤0.001, HLM=hectolitre mass, BFY=break flour yield, FC=flour colour, PC=protein content in whole wheat, FP=flour protein, AlveoW=dough strength, AlvDIST=dough distensibility, AlvP/L=AlveoW to AlvSTAB ratio, LV=loaf volume, LV12=loaf volume at 12% protein basis, WGC=wet gluten content, TKM=thousand kernel mass, DIA=kernel diameter, SDSVOL=SDS sedimentation volume, VK=vitreous kernels, FABS=farinograph water absorption, HI=hardness index and FY=flour yield.

3.4.7 Significant correlations from combined analysis between glutenin fractions and quality characteristics over three locations

White flour:

HMW-GS correlated positively with HLM and correlation with FY was negative. The correlation between PC, FP, AlvDIST, LV, WGC and LMW-GS was positive and it was negative with AlvSTAB, AlvP/L and TKM. PC, FP, LV and WGC all correlated negatively with ωb-gliadins (Table 3.9).

Whole wheat:

HLM and HI correlated positively with HMW-GS; correlation with VK and FY was negative. The correlation between LMW-GS with HLM and HI was negative but correlation was positive with VK and AlveoW. HLM, WGC and HI all negatively correlated with ωb-gliadins but correlation with VK, FY, DIA and SDSVOL was positive.

Table 3.9: Significant correlations from combined analysis between quality characteristics and glutenin fractions for three locations

White flour			Whole wheat		
Character 1	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**
HMW	HLM	0.1891*	HMW	HLM	0.3292***
	FY	-0.2512**		VK	-0.3441***
LMW	PC	0.2941**		FY	-0.2676**
	FP	0.3239***		HI	0.2518**
	AlvSTAB	-0.2064*	LMW	HLM	-0.2634**
	AlvDIST	0.1950*		VK	0.3315***
	AlvP/L	-0.2329*		AlveoW	0.2074*
	LV	0.2608**		HI	-0.3118***
	WGC	0.2745**	ωb-gliadins	HLM	-0.2640**
	TKM	-0.2041*		VK	0.2021*
ωb-gliadins	PC	-0.2457**		FY	0.3113***
	FP	-0.2710**		WGC	-0.2026*
	LV	-0.2219*		DIA	0.1916*
	WGC	-0.2450**		HI	-0.1888*
				SDSVOL	0.2421**

* p≤0.05; ** p≤0.01; *** p≤0.001, HMW=high molecular weight - glutenin subunits, LMW=low molecular weight – glutenin subunits, HLM=hectolitre mass, FY=flour yield, PC=protein content in whole wheat, FP=flour protein, AlvSTAB=dough stability, AlvDIST=dough distensibility, AlvP/L=AlveoW to AlvSTAB ratio, AlveoW=dough strength, LV=loaf volume, WGC=wet gluten content, TKM=thousand kernel mass, VK=vitreous kernels, HI=hardness index, DIA=kernel diameter and SDSVOL=SDS sedimentation volume.

3.5 Discussion

Gliadins and glutenin proteins of ten bread wheat cultivars were analysed and determined using RP-HPLC.

Genotype had a highly significant effect on all protein fractions obtained from white flour and whole wheat flour. Genotype and environment had a highly significant influence on quality characteristics measured. The environmental effect becomes noticeable when the same protein fractions across environments influence different traits. Some of the peaks consistently influenced the same quality parameters across environments. These results were confirmed by several authors who found that environmental conditions affect the amount, composition and polymerization of the gluten proteins (Graybosch et al., 1995; Huebner et al., 1997; Wieser and Seilmeier, 1998; Johansson et al., 2001; DuPont and Altenbach 2003).

Genotypes varied significantly for HMW-GS, LMW-GS and gliadin peaks and variation was also observed due to environment in both whole wheat and white flour. Genotype and environment interaction showed significant influences for LMW-GS in whole wheat and gliadin peaks in both white flour and whole wheat. There were significant differences between RP-HPLC extracted protein fractions across different environments, indicating that the environment largely influenced these fractions. The distribution of total gliadin among different types of wheat flour is strongly dependent on wheat genotype and growing conditions. It can be said that α-, β- and γ-gliadins are major components while ω-gliadin (ω-gli) occurs in much lower proportions (Wieser and Kieffer, 2001).

Xue et al. (2011) said that FY and PC were all affected by the main effects of environment, seeding rate, genotype and their interactions. Barnard et al. (2002) found a significant genotype effect for grain PC, FY, BFY, HLM, LV and TKM. According to Khatkar et al. (1995), farinograph parameters are largely influenced by environment and less by genotype. Tlanu et al. (1996) reported that SDSVOL was least influenced by environment and WGC had the largest environmental influence, in this study, both characteristics were influenced significantly by environment. Grain PC is a primary component in determining milling and baking quality (Xue et al., 2011).

PC showed a significant positive correlation with LV and WGC (Khan et al., 1989; Fredriksson et al., 1997, 1998; Wieser and Kieffer, 2001; Hanell et al., 2004; Koppel and Ingver, 2008). Similar results were obtained in this study. SDSVOL is also significantly influenced by PC and the degree of effect varies according to genotype (De Villiers and Laubscher, 1995; Morris et al., 2007). No significant association between SDSVOL and PC was found in this study.

Large variation in PC was observed and ranged from 8.7%-14.4%. Most gliadin peaks correlated negatively with FP, PC and WGC with the exceptions of peak 3 and 7, this association varied across environments. The correlation between FP, PC, WGC and ωb-gliadins was negative in both white flour and whole wheat. Similar observations were found by Wieser and Kieffer (2001) who concluded that gliadins correlated better with crude PC than glutenins.

Although some authors have associated specific gliadin alleles with bread-making quality, it is now accepted that these proteins may not have a direct effect on wheat (Gianibelli et al., 2001). The high number of correlations between gliadin peaks and quality parameters might be due to LMW-GS contamination in the extracts (Gupta et al., 1989).

Highly significant correlations were found between individual gliadin peaks and quality characteristics. Gliadin peaks correlated significantly with rheological properties. Ahmad et al. (2000) did not find correlations between gliadins and certain rheological properties. In contrast, other authors in previous RP-HPLC studies showed correlations between gliadin fractions and rheological properties (Huebner et al., 1997; Wieser and Kieffer, 2001; Peña et al., 2005). In this study, gliadin peaks varied in concentration as well as presence between cultivars. A highly significant correlation was observed between dough strength and gliadin peaks. In a study by Peña et al. (2005), it was found that dough strength correlated with glutenins especially with HMW-GS. They suggested that the correlation existed because of the presence of active HMW type x glutenin alleles coded by the gene *Glu-A1* and over-expression of some alleles of the gene *Glu-B1*x.

A decrease in dough strength occurs whenever gliadins are added (Wieser and Kieffer, 2001; Khatkar et al., 2002). Effect of gliadins on dough quality could be attributed to the LMW glutenin subunits associated with them (Gupta et al., 1989; Shewry and Tatham, 1990; Nieto-Taladriz et al., 1994). According to Peña et al. (2005), the gliadins and LMW-GS seem to act as solvents for the HMW-GS, interfering with the formation of intermolecular linkages between them. Uthayakumaran et al., (1999; 2003) found a relationship between extensibility and gliadin quantity. The findings were that dough extensibility is affected when changes in LMW-GS and gliadin composition occurs (Uthayakumaran et al., 2002).

Wieser and Kieffer (2001) stated that the correlation of LMW-GS and HMW-GS with rheological properties is similar. These results differ from those observed in this study. The correlation between HMW-GS and dough distensibility in white flour was positive while dough distensibility and LMW-GS correlation was negative. Dough stability and HMW-GS showed a negative association. Contrary to the results obtained in this study, Sadouki et al. (2005) found no significant correlation between dough properties and HMW-GS and LMW-GS also

correlated with some gliadin peaks. This association shows that there is a distinct interaction between glutenin and gliadin protein present in flour.

According to Peña et al. (2005), there is positive correlation between P/L and different types of glutenins. They discovered the ratio P/L correlated only with the total amount of HMW-GS but in this study both HMW and LMW-GS correlated with the ratio. HMW-GS correlated negatively with AlvP/L and the correlation with LMW-GS was positive. A negative correlation was observed between LMW-GS and SDSVOL. Over expression of certain LMW-GS lead to a reduction in SDSVOL and Masci et al. (2003) stipulate that low SDS sedimentation values are an indication of poor gluten strength.

The association between gliadin fractions and dough stability and AlvP/L was positive for fractions obtained from both white flour and whole wheat in the combined analysis. LV, PC, FP had a negative correlation with most of the gliadin peaks except for peak 7 (whole wheat) which also correlated positively with PC, FP, WGC and LV at 12% protein basis. Positive association was observed between LMW-GS from white flour with PC, FP, LV and WGC while no significant correlation from whole wheat was observed. The ωb-gliadins correlated negatively with all these characteristics and no significant association with HMW-GS was obtained in this study.

3.6 Conclusions

There was a distinct difference between gliadin and glutenin fractions that could be fractionated from white flour and whole wheat. The association between protein fractions and quality characteristics differed between both flours. Analysis for white flour and whole wheat quality performance shows that variation of correlation depends on the traits as well. The cause of difference between whole wheat and white flour is not clear but bran might be causing variability. Breeders may have to use white flour data in order to estimate the expected performance of whole wheat.

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Chapter 4

Variation of size exclusion-high performance liquid chromatography protein fractions of South African bread wheat cultivars and the effect on quality characteristics of whole wheat flour

4.1 Abstract

Ten South African bread wheat cultivars were used and the trials were conducted at three different locations, under dryland conditions. Analysis of proteins extracted from whole wheat flour was performed using SE-HPLC. There was variation between the effect caused by protein fractions based on total protein (% p) and those based on flour protein (% f). Loaf volume correlated negatively with low molecular weight polymeric protein (LPP) while no significant correlation was observed with high molecular weight polymeric proteins (HPP). The extractable LPP and, albumins and globulins could have a detrimental effect on increased loaf volume while ω-gliadins based on flour had a positive effect. The HPP correlated negatively with protein content, flour protein and wet gluten content in Ladybrand while at Clarens the HPP correlated positively with protein content. Protein content showed a significant positive correlation with loaf volume and wet gluten content. Flour protein correlated positively with loaf volume in all three locations. Protein content, flour protein and wet gluten content all correlated negatively with ω-gliadins. Only negative correlations were observed between vitreous kernels and other quality characteristics while grain hardness was significantly correlated with loaf volume. Extractable and unextractable polymeric proteins correlated positively with SDS sedimentation volume. Dough strength and dough stability correlated positively with extractable and unextractable LPP. Genotype and environmental effect had significant influences on SE-HPLC protein fractions in whole wheat. Protein fractions had varying effects on the same baking quality traits.

4.2 Introduction

Wheat is one of the most important cereal crops in the world when it comes to production and utilisation. Wheat storage proteins determine the different food products that can be processed using wheat flour (Žilic et al., 2011). Protein concentration and composition of proteins influences bread-making quality. This is also due to the variability caused by genotype, growing conditions and processing. The relationship between composition of each class of proteins and its effect on quality of bread-making has been investigated extensively. Researchers concluded that gliadins and glutenins have a greater effect on quality than albumins and globulins (Ahmad et al., 2000).

Baking quality is an interaction between the different protein components and it cannot be expressed in terms of a single property (Pomeranz, 1988). Identification of proteins with significant effects will help with the improvement of wheat lines and to produce higher quality wheat (Primard et al., 1991). Flours with higher total PC would also have higher contents of all protein subclasses than flours with lower PC (Park et al., 2006). According to Gupta et al. (1992) an increase in PC resulted into an increase in gliadin proteins, this increase is at a greater rate than that of the other protein fractions. Environmental conditions are likely to influence the amount and size distribution of polymeric proteins (Labuschagne et al., 2006). During grain filling period, temperatures that are higher than 30℃ (Labuschagne et al., 2009) and moisture variation have been revealed as a major source of variation in wheat quality characteristics (Tlanu et al., 1996).

Studies using SE-HPLC revealed that the amount and size distribution of polymeric proteins were important in bread-making quality (Gupta et al., 1993). SE-HPLC can reveal variations in amounts of wheat protein molecular weight fractions (Batey et al., 1991, Graybosch et al., 1995). It is a powerful tool to study native protein aggregates as well as the physical and chemical basis of baking strength and has potential for rapid assessment of baking quality of bread wheat genotypes in breeding programmes. Results obtained with this technique have been highly correlated with bread-making quality (Dachkevitch and Autran, 1989).

The use of 100% whole wheat is perceived desirable by health conscious consumers. Whole wheat products depend on wheat varieties developed on the basis of traditional quality screening conducted on white flour (Bruckner et al., 2001). The aim of this study was to determine the correlation between wheat proteins and wheat quality characteristic of ten South African bread wheat cultivars using SE-HPLC analysis on whole wheat flour.

4.3 Material and methods

4.3.1 Plant material

As described in Chapter 3 (Section 3.3.1).

4.3.2 Measured quality characteristics

As described in Chapter 3 (Section 3.3.2).

4.3.3 Size exclusion - high performance liquid chromatography

Proteins were extracted twice using a procedure by Gupta et al. (1993) with modifications. Solvents were of HPLC grade and eluants were prepared using deionised water prepared with a Millipore water purification system. The first step in the procedure extracts the proteins which are soluble in dilute SDS, while the second extract contains proteins that are soluble

after sonication. For the extraction of SDS soluble proteins, 17 mg whole wheat flour was suspended in 1.5 ml of 0.5% (w/v) SDS-phosphate buffer (pH 6.9) and vortexed for 10 sec. Samples were then shaken for 5 min and centrifuged for 30 min at 10000 rpm. A 0.45 µm HT Tuffryn Acrodisc® syringe filter was used to filter the supernatant into a glass vial. The glass vials were then placed for 2 min in hot water (80℃) to suppress protease activity (Larroque et al., 2000).

The pellet was resuspended in 1.5 ml SDS-phosphate buffer, vortexed for 10 sec and sonicated in an ultrasonic disintegrator (Branson B12 Sonifier) for 30 sec, amplitude 5 and fitted with a 3 mm exponential tip. The samples were centrifuged for 30 min at 10000 rpm to obtain a supernatant of unextractable proteins. A 0.45 µm HT Tuffryn Acrodisc® syringe filter was used to filter the supernatant into a glass vial. The glass vials were then placed for 2 min in hot water (80℃).

SE-HPLC analyses were performed using a Thermo Finnigan™ Surveyor Plus (Thermo Electron, San Jose, CA), with ChromQuest™ 4.2 chromatography data system for integration events; 300 × 4.60 mm BioSep-SEC-S 4000 Phenomenex® column was used. Separation was achieved in 15 min by loading a 20 µl sample. Elution system A) TFA (0.1%, v/v), B) ACN (ROMIL-SpS™ acetonitrile 200 far UV) and TFA (99.9/0.1%, v/v). Eluant was 50% B and the flow rate was 0.4 ml/min. Proteins were detected at 210 nm with a PDA detector.

The SE-HPLC profiles were divided into five fractions: F1 (3.6-4.3 min), F2 (4.3-6.0 min), F3 (6.0-6.5 min), F4 (6.5-6.9 min) and F5 (6.9-7.7 min). Primary components of each fraction were HPP for F1, LPP for F2; ω-gli for F3; γ-, β- and α-gliadins for F4 and, albumins and globulins for F5 (Morel et al., 2000; Samson et al., 2005). The values of these five protein fractions were converted into percentage values based on wheat weight [% flour (% f)] and total protein [% protein (% p)] (Ohm et al., 2010).

Protein fraction in flour:

Soluble fractions:

HPP (% f) = [(F1 soluble)/(F1 soluble + F2 soluble + F3 soluble + F4 soluble + F5 soluble)]*100

Insoluble fractions:

HPP (% f) = [(F1 insoluble)/(F1 insoluble + F2 insoluble + F3 insoluble + F4 insoluble + F5 insoluble)]*100

Protein fraction in protein

Soluble fractions:

HPP (% p) = [(F1 soluble)/(F1 soluble + F2 soluble + F3 soluble + F4 soluble + F5 soluble + F1 insoluble + F2 insoluble + F3 insoluble + F4 insoluble + F5 insoluble)]*100

Insoluble fractions:

HPP (% p) = [(F1 insoluble)/(F1 soluble + F2 soluble + F3 soluble + F4 soluble + F5 soluble + F1 insoluble + F2 insoluble + F3 insoluble + F4 insoluble + F5 insoluble)]*100

Percentage of large unextractable polymeric proteins (LUPP):

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

4.3.4 Statistical analysis

All statistical analyses were done with Agrobase (2005) as described in section 3.2.4.

4.4 Results

Figure 4.1 and 4.2 are examples of SDS-soluble and SDS-insoluble SE-HPLC profiles

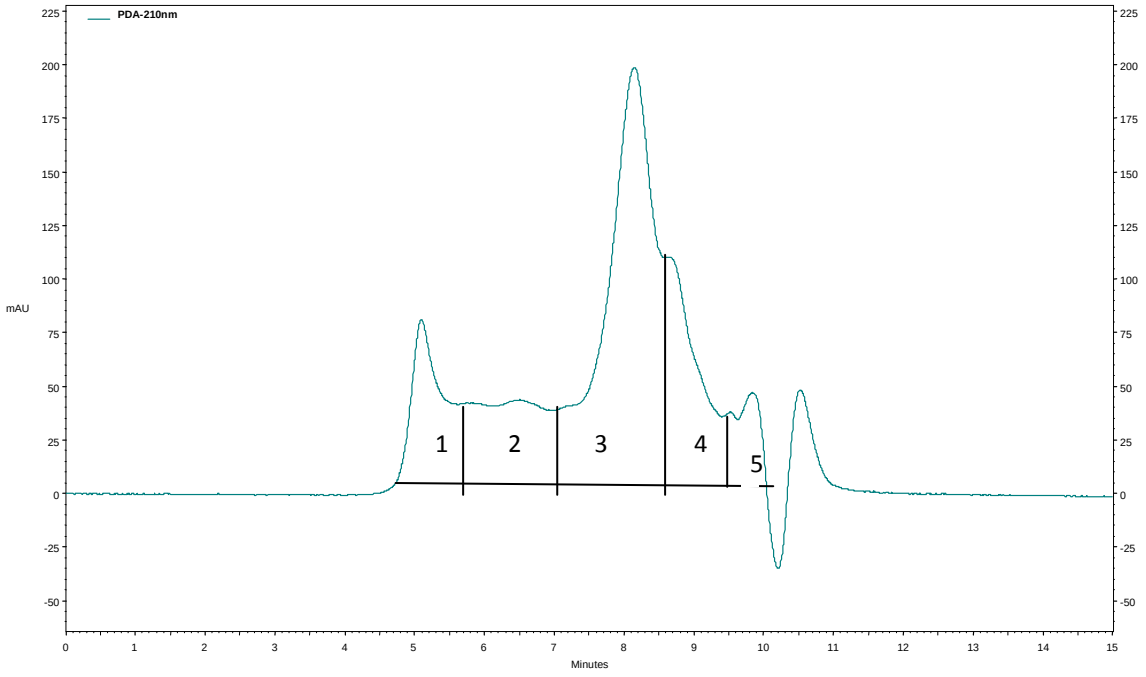

Figure 4.1: SE-HPLC profile for SDS-soluble fractions of whole wheat flour. Fraction 1 = HPP fractions, Fraction 2 = LPP fractions, Fraction 3 = ω-gli, Fractions 4 = γ-, β-, α-gliadins and Fractions 5 = albumins and globulins.

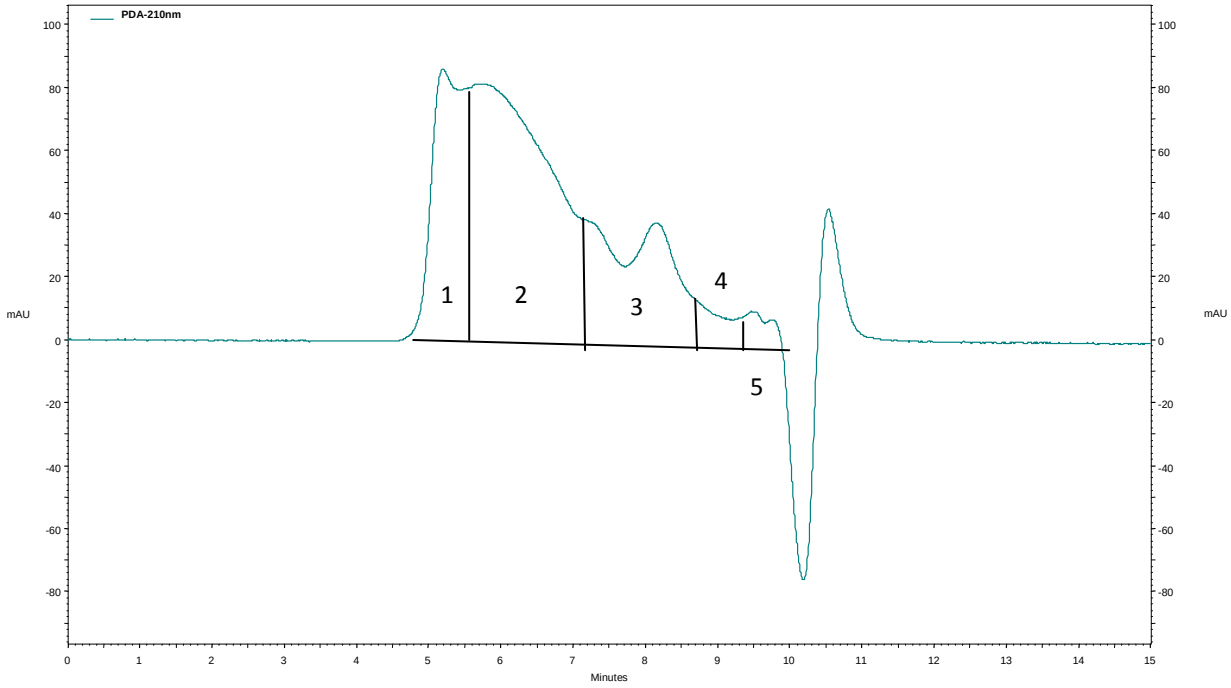

Figure 4.2: SE-HPLC profile for SDS-insoluble fractions (unextractable proteins) of whole wheat flour. Fraction 1 = HPP fractions, Fraction 2 = LPP fractions, Fraction 3 = ω-gli, Fractions 4 = γ-, β-, α-gliadins and Fractions 5 = albumins and globulins.

4.4.1 Analysis of variance for size exclusion - high performance liquid chromatography fractions

SDS-extractable: Analysis of variance (ANOVA) for HPP protein fractions based on total protein (% p) differed highly significantly ($p \leq 0.01$) for genotype, environment as well as G X E. Only environmental effect was significantly ($p \leq 0.05$) different for LPP based on total protein. Genotype, environment and G X E caused highly significant differences for protein fraction based on flour (% f) (Table 4.1).

SDS-unextractable: ANOVA for protein fractions based on total protein (% p) revealed significant ($p \leq 0.01$) differences for genotype and G X E; environment was non-significant for LPP but highly significant for albumins and globulins. All protein fractions based on flour (% f) differed significantly for genotype, environment and G X E.

Table 4.1: Analysis of variance for SE-HPLC fractions of whole wheat flour

	Protein fraction	Genotype	Environment	G X E
SDS extractable	HPP (% p)	1.80**	12.44**	2.19**
	LPP (% p)	10.11**	3.13*	3.79**
	ω-gli (% p)	77.47**	83.36**	67.02**
	γ-,β-,α-gli (% p)	68.67**	11.49**	55.03**
SDS extractable	LPP (% f)	21.23**	2.23**	7.58**
	γ-,β-,α-gli (% f)	146.69**	93.18**	133.79**
SDS unextractable	LPP (% p)	15.38**	0.51	8.35**
	Alb & Glo (% p)	1.54**	2.70**	0.48*
SDS unextractable	HPP (% f)	18.59**	192.90**	26.02**
	LPP (% f)	120.05**	57.99**	45.86**
	γ-,β-,α-gli (% f)	68.82**	186.03**	34.96**

* p≤ 0.05; ** p≤0.01, SDS=sodium dodecyl sulphate, HPP=high molecular weight polymeric proteins, LPP= low molecular weight polymeric proteins, ω-gli=ω-gliadins, γ-, β-, α-gli= γ-, β-, α-gliadins, Alb & Glo=albumins and globulins, % p=protein fraction based on total protein, % f=protein fraction based on flour.

4.4.2 Significant correlations between quality characteristics and protein fractions at Bultfontein, Clarens and Ladybrand

Results are given in Table 4.2.

Bultfontein: WGC correlated negatively with unextractable LPP (unLPP) in flour (% f) and extractable LPP (exLPP) in total protein (% p). A positive correlation between HLM and unLPP (% f) was observed. VK and exLPP (% p) and exHPP (% p) showed a positive correlation and a negative correlation with unextractable albumins and globulins (% p & % f), unLPP (% p) and unextractable γ-, β-, α-gli (% p). FY showed a negative correlation with extractable γ-, β-, α-gliadins (% p). The correlation between TKM and unHPP (% f) was positive while the correlation between HI and unHPP (% f) was negative. The significant correlations between LV and extractable protein fractions were negative. Similar results were observed with PC and significant protein fractions. FABS correlated positively with albumins and globulins and LPP based on flour.

Clarens: HLM correlated negatively with LPP (% f) and unLPP based on flour and proteins based on wheat while the correlation with γ-, β-, α-gli (extractable, % f) and unHPP (% f) was positive. A negative correlation was showed between VK and unextractable albumins and globulins (% p) as well as extractable albumins and globulins (% f) while correlation with γ-, β-, α-gli (extractable, % p) was positive. BFY correlated negatively with LPP (% f), LPP (% p), unLPP (% f), unLPP (% p) and the correlation with HPP (% p) and unHPP (% f) was positive.

A significant positive correlation was revealed between AlveoW with LPP (% f) and ω-gli (% p). UnLPP (% f) and ω-gli (% p) showed a positive correlation with AlveoW and AlvSTAB correlated positively with unLPP (% f). LPP (% f), unLPP (% f), ω-gli (% p) showed a positive correlation with DIA while other protein fraction i.e. extractable γ-,β-,α-gli (% f) and unextractable γ-,β-,α-gli (% f) showed a negative correlation with DIA. Similar results were obtained with SDSVOL, where SDSVOL revealed a positive correlation with LPP (% f), unLPP (% f), ω-gli (% p) and a negative correlation with extractable γ-,β-,α-gli (% f) and unextractable γ-,β-,α-gli (% f). TKM correlated positively with unLPP (% f) and ω-gli (% p) while correlation with unextractable γ-, β-, α-gli (% f) was negative.

PC was significantly correlated with unHPP (% f) (positive) and negatively correlated with unextractable and extractable albumins and globulins based on flour and total protein. FP correlated positively with γ-, β-, α-gli (% f) and negatively with unextractable and extractable albumins and globulins (% f) and unHPP (% p). LV correlated positively with unextractable and extractable ω-gli (% f) and correlated negatively with albumins and globulins (% p). FABS had significant and positive correlation with LPP and unLPP based on flour and total protein, extractable and unextractable ω-gli (% f) and correlated negatively with albumins and globulins (% f and % p).

Ladybrand: HLM correlated negatively with unLPP (% p) and albumins and globulins (% p). LPP (% f), unHPP (% f), unLPP (% f), HPP (% p), LPP (% p), ω-gli (% p) and unLPP (% p) all correlated negatively with FP. Similar results were obtained for WGC, were LPP (% f), unHPP (% f), unLPP (% f),LPP (% p), ω-gli (% p) and unLPP (% p) correlated negatively with WGC.

PC correlated negatively with all protein fractions that were revealed to be significant, similar results were obtained for FP. HI correlated with unLPP (% p) negatively. LV had negative correlation with LPP (% p and % f) and, albumins and globulins (% p). A positive correlation was observed between FN and unextractable albumins & globulins (% f) while correlation with unextractable ω-gli (% p) was negative. FABS correlated negatively with extractable ω-gli (% f), γ-, and β-, α-gli (% p and % f).

Table 4.2: Significant correlations between quality characteristics and protein fractions of whole wheat flour for Bultfontein, Clarens and Ladybrand

Bultfontein			Clarens			Ladybrand		
Character 1	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**
WGC	unLPP (% f)	-0.4970**	HLM	LPP (% f)	-0.6593***	HLM	unLPP (% p)	-0.4713**
	LPP (% p)	-0.4194**		γ-,β-,α-gli (% f)	0.4845*		Alb & Glo (% p)	-0.4567**
HLM	unLPP (% f)	0.5023***		unHPP (% f)	0.5129***		ω-gli (% f)	-0.3900*
VK	LPP (% p)	0.4544**		unLPP (% f)	-0.5778***		Alb & Glo (% f)	-0.5735***
	Alb & Glo (un,% p)	-0.4483**		unLPP (% p)	-0.5171***		Alb & Glo (un,% f)	-0.4840**
	Alb & Glo (un,% f)	-0.3231*	VK	Alb & Glo (% f)	-0.4334**		Alb & Glo (% p)	-0.4381**
	exHPP (% p)	0.4039*		γ-,β-,α-gli (% p)	0.4056*		unHPP (% p)	-0.4958**
	unLPP (% p)	-0.3493*		Alb & Glo (un,% p)	-0.4010*	BFY	ω-gli (% f)	0.4365**
	γ-,β-,α-gli (un,% p)	-0.3851*	BFY	LPP (% f)	-0.7014***		Alb & Glo (% f)	0.3761*
FY	γ-,β-,α-gli (% p)	-0.4518**		unHPP (% f)	0.5913***		ω-gli (un, % f)	-0.3949*
TKM	unHPP (% f)	0.4862**		unLPP (% f)	-0.5352***		unHPP (% p)	0.4398**
HI	unHPP (% f)	-0.4643**		HPP (% p)	0.4887**		ω-gli (un, % p)	0.4768**
LV	LPP (% f)	-0.3819*		LPP (% p)	-0.4533**	WGC	LPP (% f)	-0.4716**
	LPP (% p)	-0.3513*		unLPP (% p)	-0.4186**		unHPP (% f)	-0.5084***
	Alb & Glo (% f)	-0.4236**	AlveoW	LPP (% f)	0.4922**		unLPP (% f)	-0.5074***
	Alb & Glo (% p)	-0.3325*		ω-gli (% p)	0.4414**		LPP (% p)	-0.4802**
PC	unLPP (% f)	-0.3335*		unLPP (% f)	0.4922**		ω-gli (% p)	-0.5273***
	LPP (% f)	-0.3294*					unLPP (% p)	-0.4283**

* p≤0.05; ** p≤0.01; *** p≤0.001; un=unextractable, % p=protein fraction based on total protein, % f=protein fraction based on flour, WGC=wet gluten content, HLM=hectolitre mass, VK=vitreous kernels, FY=flour yield, TKM=thousand kernel mass, HI=hardness index, LV=loaf volume, PC=protein content in whole wheat, FABS=farinograph water absorption, BFY=break flour yield, AlveoW=dough strength, AlvSTAB=dough stability, DIA=kernel diameter, SDSVOL=SDS sedimentation volume, FP=protein content in flour, FN=falling number, unLPP=unextractable low molecular weight polymeric proteins, LPP=extractable low molecular weight polymeric proteins, Alb & Glo=albumins and globulins, unHPP=unextractable high molecular weight polymeric proteins, γ-,β-,α-gli = γ-,β-,α-gliadins, ω-gli= ω-gliadins.

Table 4.2: Continued

Bultfontein			Clarens			Ladybrand		
Character 1	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**
FABS	Alb & Glo (% f)	0.3342*	AlvSTAB	unLPP (% f)	0.4628**	FP	LPP (% f)	-0.5240***
	LPP (% f)	0.3853*	DIA	LPP (% f)	0.4858**		HPP (% f)	-0.4553**
				γ-,β-,α-gli (% f)	-0.5304***		ω-gli (% f)	-0.5777***
				unLPP (% f)	0.5198***		Alb & Glo (% f)	-0.4682**
				γ-,β-,α-gli (un,% f)	-0.4435**		ω-gli (un, % f)	-0.4937**
				ω-gli (% p)	0.4927**		γ-,β-,α-gli (% p)	-0.3778*
			SDSVOL	LPP (% f)	0.4835**	PC	HPP (% f)	-0.3995*
				γ-,β-,α-gli (% f)	-0.6480***		LPP (% f)	-0.4899**
				unLPP (% f)	0.5372***		ω-gli (% f)	-0.4636**
				γ-,β-,α-gli (un,% f)	-0.4724**		Alb & Glo (% f)	-0.4664**
				ω-gli (% p)	0.4855**		unHPP (% f)	-0.4585**
			TKM	unLPP (% f)	0.4141**		unLPP (% f)	-0.4358**
				γ-,β-,α-gli (un,% f)	-0.4473**		ω-gli (un, % f)	-0.3490*
				ω-gli (% p)	0.4213**		HPP (% p)	-0.4418**
							LPP (% p)	-0.5146***
							ω-gli (% p)	-0.4893**
							Alb & Glo (% p)	-0.5095***
							unHPP (% p)	-0.3947*

* p≤0.05; ** p≤0.01; *** p≤0.001, un=unextractable ,% p=protein fraction based on total protein, % f=protein fraction based on flour, WGC=wet gluten content, HLM=hectolitre mass, VK=vitreous kernels, FY=flour yield, TKM=thousand kernel mass, HI=hardness index, LV=loaf volume, PC=protein content in whole wheat, FABS=farinograph water absorption, BFY=break flour yield, AlveoW=dough strength, AlvSTAB=dough stability, DIA=kernel diameter, SDSVOL=SDS sedimentation volume, FP=protein content in flour, FN=falling number, unLPP=unextractable low molecular weight polymeric proteins, LPP=extractable low molecular weight polymeric proteins, Alb & Glo=albumins and globulins, unHPP=unextractable high molecular weight polymeric proteins, γ-,β-,α-gli = γ-,β-,α-gliadins, ω-gli= ω-gliadins.

Table 4.2: Continued

Bultfontein			Clarens			Ladybrand		
Character 1	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**
			PC	Alb & Glo (% f)	-0.3817*	FP	Alb & Glo (% p)	-0.5038***
				unHPP (% f)	0.3412*		unHPP (% p)	-0.4756**
				Alb & Glo (un,% f)	-0.4478**		γ-,β-,α-gli(un,% p)	-0.4039*
				Alb & Glo (% p)	-0.5111***		unHPP (% f)	-0.5290**
			FP	γ-,β-,α-gli (% f)	0.3918*		unLPP (% f)	-0.5372***
				Alb & Glo (% f)	-0.4353**		HPP (% p)	-0.4974**
				Alb & Glo (un,% f)	-0.4086*		LPP (% p)	-0.5521***
				unHPP (% p)	-0.5417***		ω-gli (% p)	-0.5869***
			LV	ω-gli (% f)	0.4560**		unLPP (% p)	-0.4156**
				ω-gli (un, % f)	0.5227***	HI	unLPP (% p)	-0.4379**
				Alb & Glo (% p)	-0.3515*	LV	LPP (% p)	-0.3520*
			FABS	LPP (% f)	0.3363*		LPP (% f)	-0.3234*
				ω-gli (% f)	0.4645**		Alb & Glo (% p)	-0.3367*
				Alb & Glo (% f)	-0.3251*	FN	Alb & Glo (un,% f)	0.3421*
				unLPP (% f)	0.3685*		ω-gli (un,% p)	-0.3536*
				ω-gli (un,% f)	0.4467**	FABS	γ-,β-,α-gli (% f)	-0.3373*
				Alb & Glo (% p)	-0.3769*		ω-gli (un, % f)	-0.3541*
				unLPP (% p)	0.3507*		γ-,β-,α-gli (% p)	-0.4066*

* p≤0.05; ** p≤0.01; *** p≤0.001, un=unextractable,% p=protein fraction based on total protein, % f=protein fraction based on flour, WGC=wet gluten content, HLM=hectolitre mass, VK=vitreous kernels, FY=flour yield, TKM=thousand kernel mass, HI=hardness index, LV=loaf volume, PC=protein content in whole wheat, FABS=farinograph water absorption, BFY=break flour yield, AlveoW=dough strength, AlvSTAB=dough stability, DIA=kernel diameter, SDSVOL=SDS sedimentation volume, FP=protein content in flour, FN=falling number, unLPP=unextractable low molecular weight polymeric proteins, LPP=extractable low molecular weight polymeric proteins, Alb & Glo=albumins and globulins, unHPP=unextractable high molecular weight polymeric proteins, γ-,β-,α-gli = γ-,β-,α-gliadins, ω-gli= ω-gliadins.

4.4.3 Significant correlations between quality characteristics and protein fractions of whole wheat flour at three locations combined

Results are given in Table 4.3.

HLM correlated negatively with LPP (% f) and unLPP (% p), and a positive correlation was observed between FN and unextractable albumins and globulins (% p). Unextractable HPP (% f), HPP (% p) and ω-gli (% p) all correlated positively with BFY. FY also correlated positively with HPP (% f), HPP (% p) but γ-, β-, α-gli (% p) was negatively correlated to FY. A positive correlation was observed between unHPP (% f), HPP (% p) and unextractable albumins and globulins (% p) correlated negatively FP. FABS correlated negatively with both HPP (% p) and ω-gli (% p). Unextractable HPP (% f) and HPP (% p) correlated positively with AlveoW, AlvDIST, LV, LV12 and unextractable albumins and globulins (% p) yielded a negative correlation with these characteristics. AlvSTAB and AlvP/L correlated negatively with unHPP (% f), HPP (% p) and correlated positively with unextractable albumins and globulins (% p). AlvSTAB correlated negatively with ω-gli (% p) and correlation with ω-gli (% p) was positive.

Unextractable HPP (% f) had a positive correlation with WGC while unLPP (% f) and unLPP (% p) yielded a negative association with WGC. TKM correlated with unHPP (% f), HPP (% p), extractable ω-gli (% p) negatively and correlated positively with unextractable LPP (% p) and unextractable albumins and globulins (% p). Kernel DIA correlated positively with extractable LPP (% f) and unextractable albumins and globulins (% p); the correlation with ω-gli (% p) was negative. HI correlated with only unextractable LPP (% p) negatively. A positive correlation was observed between SDSVOL and extractable LPP (% f), unextractable LPP (% p) and unextractable LPP (% f). Correlation with SDS and γ-, β-, α-gli (% p) as well as unextractable γ-, β-, α-gli (% f) was negative.

Table 4.3: Significant correlations from combined analysis between quality characteristics and protein fractions of whole wheat flour at the three locations

Character 1	Character 2	Correlation
HLM	LPP (% f)	-0.3079***
	unLPP (% p)	-0.2887**
FN	Alb & Glo (s, % p)	0.3860***
BFY	unHPP (% f)	0.2976**
	HPP (% p)	0.2165*
	ω-gli (% p)	0.2712**
FY	unHPP (% f)	0.3127***
	HPP (% p)	0.2087*
	γ-,β-,α-gli (% p)	-0.2434**
FP	unHPP (% f)	0.3718***
	HPP (% p)	0.2165*
	Alb & Glo (un, % p)	-0.2616**
FABS	HPP (% p)	-0.2311*
	ω-gli (% p)	-0.2147*
AlveoW	sHPP (% f)	0.2413**
	HPP (% p)	0.1939*
	Alb & Glo (un, % p)	-0.2261*
	Alb & Glo (un, % p)	-0.2261*
AlvSTAB	unHPP (% f)	-0.3214***
	HPP (% p)	-0.3103***
	ω-gli (% p)	-0.2249*
	Alb & Glo (un, % p)	0.2198*
AlvDIST	unHPP (% f)	0.3497***
	HPP (% p)	0.2978**
	ω-gli (% p)	0.3120***
	Alb & Glo (un, % p)	-0.2448**
AlvP/L	unHPP (% f)	-0.2929**
	HPP (% p)	-0.2798**
	Alb & Glo (un, % p)	0.2380**

* p≤0.05; ** p≤0.01; *** p≤0.001, % p=protein fraction based on total protein, % f=protein fraction based on flour, HLM=hectolitre mass, FN=falling number, BFY=break flour yield, FY=flour yield, FP=flour protein, FABS=farinograph water absorption, AlveoW=dough strength, AlvSTAB=dough stability, AlvDIST=dough distensibility, AlvP/L=AlveoW to AlvSTAB ratio, LV=loaf volume, LV12=loaf volume at 12% protein basis, WGC=wet gluten content, TKM=thousand kernel mass, DIA=kernel diameter, HI=hardness index, SDSVOL=SDS sedimentation volume.

Table 4.3: Continued

Character 1	Character 2	Correlation
LV	sHPP (% f)	0.3692***
	HPP (% p)	0.2945**
	Alb & Glo (s, % p)	-0.2533**
LV12	s HPP (% f)	0.2982***
	HPP (% p)	0.3292***
	Alb & Glo (s, % p)	-0.3022***
WGC	sHPP (% f)	0.2341*
	sLPP (% f)	-0.2553**
	sLPP (% p)	-0.1956*
TKM	sHPP (% f)	-0.1977*
	HPP (% p)	-0.2154*
	ω-gli (% p)	-0.2556**
	sLPP (% p)	0.1911*
	Alb & Glo (s, % p)	0.3577***
DIA	LPP (% f)	0.2338*
	ω-gli (% p)	-0.2544**
	Alb & Glo (s, % p)	0.3199***
HI	sLPP (% p)	-0.2418**
SDSVOL	LPP (% f)	0.2500**
	sLPP (% f)	0.2225*
	γ-,β-,α-gli (s, % f)	-0.1945*
	γ-,β-,α-gli (% p)	-0.2003*
	sLPP (% p)	0.2810**

* ≤p=0.05; ** p≤=0.01; *** p≤=0.001, % p=protein fraction based on total protein, % f=protein fraction based on flour, HLM=hectolitre mass, FN=falling number, BFY=break flour yield, FY=flour yield, FP=flour protein, FABS=farinograph water absorption, AlveoW=dough strength, AlvSTAB=dough stability, AlvDIST=dough distensibility, AlvP/L=AlveoW to AlvSTAB ratio, LV=loaf volume, LV12=loaf volume at 12% protein basis, WGC=wet gluten content, TKM=thousand kernel mass, DIA=kernel diameter, HI=hardness index, SDSVOL=SDS sedimentation volume.

4.5 Discussion

Quality characteristics had diverse responses to protein fractions at Clarens and Bultfontein but the same response was observed in Ladybrand. There was a variation between the effect caused by protein fractions based on total protein and those based on flour.

LV correlated negatively with exLPP while the effect of HPP was not significant in this study, this indicates that a high proportion of polymeric proteins that are extractable affect LV negatively (Ohm et al., 2010). The exLPP and, albumins and globulins could have a detrimental effect on increased LV while ω-gli (% f) had a positive effect. Huebner et al. (1997) showed positive correlation between LV and γ-gliadin, while ω-, α, β-gliadins correlated negatively with LV. Greater quantities and proportions of extractable protein eluted in F3 (ω-gli) seemed to contribute to LV (Ohm et al., 2010) and variation of ω-gli has been said to have positive association with LV (Khatkar et al., 2002; Uthayakumaran et al., 2002). Other studies discovered that unHPP have positive correlation with LV (Gupta et al., 1993; Park et al., 2006). According to Khatkar et al. (2002), the addition of total gliadins and the subgroups (γ-, β-,α- & ω-gliadins) improved bread-making quality while Labuschagne and Aucamp (2004) observed a negative effect caused by gliadin on important quality traits.

The HPP correlated negatively with PC, FP and WGC at Ladybrand while at Clarens the HPP correlated positively with PC. Highly significant correlations were observed for HPP from the combined analysis. The HPP in unextractable proteins should contribute to increasing bread-making quality independently of the variation of total wheat proteins, while exHPP did not have significant associations with bread-making characteristics (Ohm et al., 2010). Quantitative variations of unextractable HPP have positive effects on LV than extractable polymeric protein fractions (Tsilo et al., 2010). The HPP had significant but negative correlation with PC that had positive correlation with bread-making characteristics. The most likely explanation for this is that when PC increases, it may also positively affect the formation of large polymers (Ciaffi et al., 1996). Extractable and unextractable polymeric proteins correlated positively with SDSVOL.

Wheat PC affects variations in protein fractions and quality characteristics (Ohm et al., 2010). In wheat genotypes with large differences in protein quality, the correlation between PC and LV was not significant (Johansson and Svensson, 1998). According to Morel et al. (2000), the total amount of gliadins (F4) would be a good indicator of FP content but in this study the findings were contradictory. PC, FP and WGC all correlated negatively with ω-gli. They also discovered that polymeric proteins (F1 and F2) from total protein extracts were highly correlated to FP content.

FP correlated positively with LV in all three locations, similar finding were observed by Ohm et al. (2010). Protein fractions that correlated with FP were also significantly correlated with WGC, this suggest that the association between these quality traits shown were directly affected by the variation of these protein fractions. Bread-making parameters such as WGC and FP had a negative correlation with proteins eluted at F5; this reveals that albumins and globulins have a detrimental effect on bread-making quality. FP correlated negatively with albumins and globulins and polymeric proteins. Similar results were reported by Morel et al. (2000). Contrary to this, the levels of albumins and globulins, and soluble polymeric proteins based on flour were correlated positively to FP but to a much lesser degree in a study by Park et al. (2006). Where protein increased in flour, albumins and globulins decreased due to the building of storage proteins at the expense of albumins and globulins. According to Labuschagne and Aucamp (2004), an increase in polymeric proteins will lead to a reduction in monomeric proteins.

At Clarens, VK had negative association with albumins and globulins, and positive association with γ-, β-, α-gliadins but at Bultfontein the correlation between VK and these protein fractions was negative and positive with exHPP, LPP and unLPP. VK were associated with variations in extractable F4 (γ-, β-, α-gliadins), F5 (Alb & Glo) and unHPP. Protein fractions that were associated with kernel characteristics also had significant association with bread-making parameters (Ohm et al., 2010).

At Bultfontein, FABS correlated positively with albumins and globulins (% f) and exLPP (% f) while the correlation at Ladybrand was negative with extractable γ-, β-, α-gliadins (% f & p) and unextractable ω-gli (% f). At Clarens, FABS correlated positively with ω-gli and LPP but correlation with albumins and globulins was negative. Ohm et al. (2010) observed that protein fractions that eluted around F3 and F4 were associated with optimum water absorption. The variation in gliadins might be responsible for the variation in water absorption. Lai et al. (1989) found that white flour with added bran binds a large amount of water. Bran could also be the cause of variation in water absorption. Environment also had an effect because protein fractions had a varying effect on the same characteristic from one location to the other. The varying effect might also be caused by contamination from other protein fractions. It is desirable that quality traits be maintained as stable as possible through all environments (Koppel and Ingver, 2010). Variation of wheat proteins caused by environmental effect is related to changes in monomeric gliadins (Saint Pierre et al., 2008). Since variation in wheat protein molecular weight distribution is related to bread-making quality characteristics, variation in kernel wheat PC is also most likely to have significant association with bread-making quality characteristics (Ohm et al., 2010).

Dough strength and dough stability all positively correlated with extractable and unLPP (% f). This positive association might be due to the increase of dough strength when the size distribution of polymeric proteins increases (Uthayakumaran et al., 2002). Gupta et al. (1993) found that SDS-insoluble polymeric proteins were more strongly correlated with dough strength. Dachkevitch and Autran (1989) reported a high correlation between the amount of SDS unextractable proteins and alveograph values. According to Labuschagne and Aucamp (2004), LPPs which are mainly glutenins, correlated positively with FN, DIA and negatively with LV. In this study the gliadins correlated negatively with FN and positively with albumins and globulins.

4.6 Conclusions

A very large variation was observed in the protein fractions extracted from whole wheat flours and the effect on quality characteristics. Environment and genotype had significant effects on these protein fractions. The LPP correlated positively with LV while no significant association was observed for HPP. There was a negative association between HPP and PC and other protein related quality characteristics.

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Chapter 5

Determination of vitamin E content of white flour and whole wheat flour of South African wheat cultivars

5.1 Abstract

Wheat is an important cereal crop that is a good source of tocochromanol compounds. Tocochromanols are only synthesized in plants, thus emphasizing its importance in a diet. They are important because of their antioxidant activity. Milled grain samples were freeze dried prior to extraction. Saponification and normal phase-high perfomance liquid chromatography was used for extraction of tocochromanols. In this study, whole wheat and white flour were investigated for tocochromanol content in ten South African wheat cultivars. Whole wheat had higher tocochromanol content than white flour. The major tocochromanol compounds found in the samples were α- and β-tocotrienols and α- and β-tocopherol. The samples also contained more tocotrienols than tocopherols and only traces of δ-tocochromanol compounds were found in samples. Tocochromanols extracted from white flour correlated positively with loaf volume, protein content and wet gluten content. No significant correlation was observed for tocochromanols extracted from whole wheat with protein related baking quality characteristics. Hardness index and hectolitre mass were consistently correlated with tocochromanol compounds. Grain size is presumed to be a cause of variation for tocochromanols in wheat. Environment and genotype and G X E interaction influenced tocochromanols significantly. Tocochromanols extracted from whole wheat showed less significant association with baking quality characteristics. Cultivars that were analysed in this study contained small amounts of tocochromanols when compared to results obtained by other researchers.

5.2 Introduction

Wheat is a major crop and considered as a staple food for a majority of the world's population. Wheat provides a significant amount of energy, proteins and selected micronutrients (Zielinski and Kozlowska, 2000). It serves as a source of health improving components such as vitamin E, fiber, phenolics and carotenoids. Vitamin E prevents oxidation of double bonds by reacting with peroxyl radicals and protects lipids and membrane proteins against oxidation stress (Wolf, 2005; Dörmann, 2007). Vitamin E consists of eight lipid-soluble antioxidants: α-, β-, γ-, δ-tocotrienol and α-, β-, γ-, δ-tocopherol. The tocopherols and tocotrienols are also called tocochromanols or tocols. Tocochromanols cannot be produced by humans; this makes vitamin E an important component in any diet. Cereals are one of the main sources of vitamin E. According to Hussain et al. (2012), about 20% of

vitamin E daily requirement is contributed by wheat. Cereals are considered to be a moderate source of vitamin E, providing 6-23 µg of α-tocopherol. Dörmann (2007) found tocotrienols to be abundant in cereals.

Tocopherols are widely distributed in higher plants whereas tocotrienols occur mainly in some non-photosynthetic tissues such as seeds and endosperm of monocot seeds (Lampi et al., 2010). Many foods including wheat grain contain tocochromanols. Tocochromanols in wheat germ consist mainly of α- and β-tocopherols. Tocotrienols are concentrated in the pericarp, testa, aleurone and a significant concentration is found in the endosperm. Tocotrienols might be similar to or even have more potential than tocopherols as antioxidants. Cereals contain tocotrienols with low vitamin E activity. They are more valuable as a source of tocotrienols and tocopherols than as a source of vitamin E (Piironen et al., 2009).

In the near future, a need for more processed foods with greater emphasis on food quality will arise. The fractionation of the crushed grain during milling has a critical implication for the distribution of many nutrients (Wrigley, 2009). The acceptance of whole grain flour simplifies processing and the consumption of whole grain is a healthy alternative (Bramley et al., 2000). There will be an interest among plant breeders in developing cultivars having a significant level of tocols when more research results on the benefits of tocols become available (Peterson and Qureshi, 1993). There is limited information available about the effect of G X E and environment (e.g. soil type, temperature and precipitation) on the content and composition of tocochromanols in wheat. Little is also known on the variation among different wheat genotypes. In the development of new cultivars, environmental effect is significant therefore controlled studies with wheat genotypes are essential (Lampi et al., 2010).

The aim of the study was to determine the quantity of vitamin E in white and whole wheat flour of ten selected South African cultivars grown at three different locations and to correlate vitamin E with quality characteristics of wheat.

5.3 Material and methods

5.3.1 Plant material

As described in Chapter 3 (Section 3.3.1).

5.3.2 Measured quality characteristics

As described in Chapter 3 (Section 3.3.2).

5.3.3 Extraction of tocochromanols

Milled grain samples (1 g) were freeze dried for three days before the extraction of tocochromanols commenced. The extraction of tocochromanol was performed using the method of Fratianni et al. (2002) with modifications. Wheat flour (white and whole wheat) (1 g) was accurately weighed into a screw cap teflon tube and 2.5 ml ethanol pyrogallol (60 g/L), 1 ml sodium chloride (10 g/L), 1 ml ethanol (95 ml/L) and potassium hydroxide (600 g/L) were added. This was mixed with a vortex-mixer for a few seconds and then flushed with nitrogen for 2 min. The tubes were transferred to a water bath (70℃) for 30 min. The tubes were vortexed every 10 min.

After saponification the tubes were placed in an ice-water bath. After cooling, 7.5 ml sodium chloride and 7.5 ml n-hexane/ethyle acetate (9:1 v/v) were added. The tubes were centrifuged at 1500 rpm for 5 min and the organic layer was collected. After the first extraction, two additional extractions of the suspension with 5 ml n-hexane/ethyle acetate (9:1 v/v) were done. The collected organic layer was evaporated to dryness in the presence of nitrogen and the dry residue was dissolved in 2 ml n-hexane. Four replicates were prepared separately for each sample.

Analytical HPLC

A Normal Phase-HPLC method described by Panfili et al. (2003) with modification was used to separate the tocochromanol compounds. A Phenomenex LUNA Silica column (250 mm x 4.6 mm inner diameter (i.d.), 5µm particle size) was used. The mobile phase was *n*-hexane/ethyle acetate/acetic acid (97.3:1.8:0.9 v/v/v) at a flow rate of 1.6 ml/min. All peaks were detected by fluorescence and the wavelength of detection was set to excitation 290 nm and emission wavelength of 330 nm. HPLC injection volume was 10 µl per injection.

A standard solution was used to carry out the linearity test over the different concentration ranges (ng per µl) close to the amounts of tocochromanols found in the samples: α-tocopherol 0.47-9.57; β-tocopherol 0.23-4.7; γ-tocopherol 0.65-13.1; δ-tocopherol 0.62-12.4; β-tocotrienol 0.54-10.82. The percentage recovery was determined to ensure the precision of the method. The percentage recovery of tocochromanols was >95%.

5.3.4 Statistical analysis

All statistical analyses were done with Agrobase (2005) as described in section 3.3.4.

5.4 Results

Figure 5.1 is an example of tocochromanol compounds detected by NP-HPLC in a wheat sample.

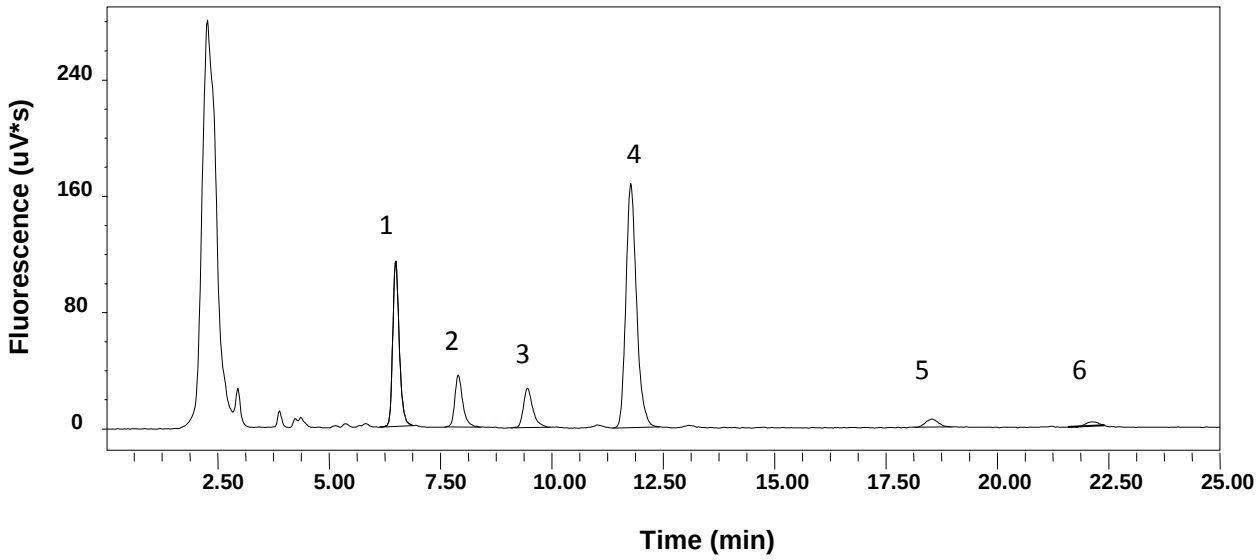

Figure 5.1: Chromatogram of tocochromanols in wheat sample. Peak 1 = α-tocopherol, Peak 2 = α-tocotrienol, Peak 3 = β-tocopherol, Peak 4 = β-tocotrienol, Peak 5 = δ-tocopherol, Peak 6 = δ-tocotrienol.

5.4.1 Mean values and mean squares of tocochromanol compounds from white flour and whole wheat

Total tocochromanols were the sum of α-tocopherol, β-tocopherol, α-tocotrienol, β-tocotrienol and δ-tocotrienol. Larger quantities of tocochromanols were extracted from whole wheat flour than from white flour (Table 5.1 and 5.2) and major tocochromanols found were α- and β-tocopherol and, α- and β-tocotrienol. Delta-tocochromanols and γ-tocochromanols were only found in small traces in the samples (Table 5.3 and 5.4). Tocochromanol content from both white flour and whole wheat varied significantly among the bread wheat cultivars that were tested. Whole wheat had significantly higher concentrations of tocochromanols than white flour in all three locations. Total tocochromanol concentration found in genotypes ranged from an average of 16.49-25.49 mg/kg for white flour (Table 5.1) and 41.92-54.87 mg/kg for whole wheat (Table 5.2).

Table 5.1: Total tocochromanol compounds (mg/kg) mean values of ten cultivars in three locations from white flour

Cultivar	Bultfontein	Clarens	Ladybrand	Average
Betta-DN	21.25	17.55	19.05	19.28
Caledon	26.43	24.13	25.91	25.49
Elands	16.45	20.57	23.84	20.29
Gariep	20.10	20.01	24.69	21.60
Komati	20.65	21.42	23.65	21.91
Limpopo	17.87	20.70	17.37	18.65
Matlabas	18.83	18.74	18.29	18.62
PAN3118	17.11	17.02	15.33	16.49
PAN3349	17.99	16.60	15.91	16.83
PAN3377	18.22	17.78	17.15	17.72
Average	19.49	19.45	20.12	

Table 5.2: Total tocochromanol compounds (mg/kg) mean values of ten cultivars in three locations from whole wheat

Cultivar	Bultfontein	Clarens	Ladybrand	Average
Betta-DN	48.00	51.19	52.85	50.68
Caledon	52.03	54.99	57.58	54.87
Elands	49.94	51.38	59.76	53.69
Gariep	40.90	48.57	49.62	46.36
Komati	43.80	51.81	52.29	49.30
Limpopo	55.75	49.47	55.74	53.65
Matlabas	39.41	52.81	52.01	48.08
PAN3118	43.50	42.86	45.38	43.91
PAN3349	40.44	41.75	43.57	41.92
PAN3377	50.21	47.68	54.47	50.79
Average	46.40	49.25	52.33	

Caledon had the highest concentration for total tocochromanols with an average of 25.49 mg/kg for white flour (Table 5.1) and 54.87 mg/kg for whole wheat (Table 5.2). PAN3118 had the lowest concentration in white flour and PAN3349 had the lowest concentration in whole

wheat with an average of 16.49 mg/kg and 41.92 mg/kg respectively (Table 5.1 and 5.2). Caledon ranked the highest for most of the tocochromanol compounds. It had the highest concentration of α-, β tocopherol, α-tocotrienol for white flour and whole wheat and the highest concentration of β-tocotrienol in white flour. PAN3377 had the highest concentration for β-tocotrienol extracted from whole wheat only. Highest average concentration for δ-tocotrienol was obtained from Limpopo in both white flour and whole wheat. Genotypes with low concentration of tocols were PAN3118, PAN3349, PAN3377, Gariep and Matlabas. When comparing the concentration of total tocochromanols found in three locations, Ladybrand had the highest values with an average of 20.12 mg/kg for white flour (Table 5.1) and 52.33 mg/kg for whole wheat (Table 5.2).

Results showed significant variation when comparing locations for the quantity of each tocochromanol compound found in samples. Clarens had the highest concentration of α-tocopherol and β-tocopherol from white flour and whole wheat. Highest average concentrations of β-tocotrienol were found in Ladybrand for white flour and whole wheat, while high concentrations α-tocotrienol were found in Bultfontein for white flour samples and at Ladybrand for whole wheat samples. Good average values for δ-tocopherol were obtained at Bultfontein from both white flour and whole wheat samples (Table 5.3).

Table 5.3: Average content of each tocochromanol compound (mg/kg) in three locations from white and whole wheat

Type of flour	Characteristic	Bultfontein	Clarens	Ladybrand
Whole wheat	α-Tocotrienol	4.83	4.45	5.52
	α-Tocopherol	11.85	14.10	14.00
	β-Tocotrienol	22.66	23.38	26.04
	β-Tocopherol	6.33	6.62	6.33
	δ-Tocotrienol	0.73	0.71	0.44
White flour	α-Tocotrienol	1.32	1.12	1.28
	α-Tocopherol	3.02	3.59	3.36
	β-Tocotrienol	12.69	12.39	12.95
	β-Tocopherol	1.88	2.08	2.02
	δ-Tocotrienol	0.58	0.27	0.51

In Bultfontein, variation among genotypes was highly significant ($p \leq 0.01$, $p \leq 0.05$) for most of the tocols found in white flour and whole wheat. Differences for content of δ-tocotrienol in whole wheat were not significant. Similar results were obtained from cultivars in Clarens but

the quantity of δ-tocotrienol extracted from whole wheat and white flour was not significantly different among genotypes. Significant differences (p≤ 0.01) for all tocols were observed for genotypes in Ladybrand (Table 5.4).

Table 5.4: Mean squares of entries in three locations for tocochromanol compounds (mg/kg)

Type of flour	Characteristic	Bultfontein	Clarens	Ladybrand
Whole wheat	α-Tocotrienol	3.03**	1.72**	1.96**
	α-Tocopherol	14.51**	10.34**	11.16**
	β-Tocotrienol	24.90**	17.51**	25.90**
	β-Tocopherol	3.16**	2.70**	2.25**
	δ-Tocotrienol	0.012	0.007	0.15**
White flour	α-Tocotrienol	0.39**	0.15**	0.63**
	α-Tocopherol	1.61**	1.73*	5.31**
	β-Tocotrienol	14.47**	7.67**	18.05**
	β-Tocopherol	0.62**	0.39**	0.87**
	δ-Tocotrienol	0.02*	0.002	0.05**

* p≤0.05; ** p≤0.01.

The ANOVA showed significant (p≤ 0.05 and p≤0.01) differences between tocol fractions for genotype, environment and G X E in both whole wheat and white flour. The environmental effect was not significant for β-tocopherol in whole wheat and β-tocotrienol in white flour in a combined analysis of three locations (Table 5.5).

Table 5.5: Mean squares of tocochromanol compounds (mg/kg) for combined analysis for three locations

Type of flour	Characteristic	Genotype	Environment	G X E
Whole wheat	α-Tocotrienol	5.552**	11.784**	0.588**
	α-Tocopherol	23.06**	64.63**	6.47**
	β-Tocopherol	5.770**	1.074	1.176**
	β-Tocotrienol	55.165**	126.802**	6.578**
	δ-Tocotrienol	0.074**	1.051**	0.052**
White flour	α-Tocopherol	4.805**	3.339**	1.930**
	α-Tocotrienol	0.950**	0.450**	0.118**
	β-Tocopherol	1.117**	0.437*	0.385**
	β-Tocotrienol	33.705**	3.1	3.248*
	δ-Tocotrienol	0.027**	1.052**	0.026**

* p≤0.05; ** p≤0.01.

The total content of tocotrienols (T3) and tocopherols (T) varied across locations and whole wheat had the highest total content of tocotrienols and tocopherols. Content of tocotrienols was more than that of tocopherols in both white flour and whole wheat in all three locations (Figure 5.2).

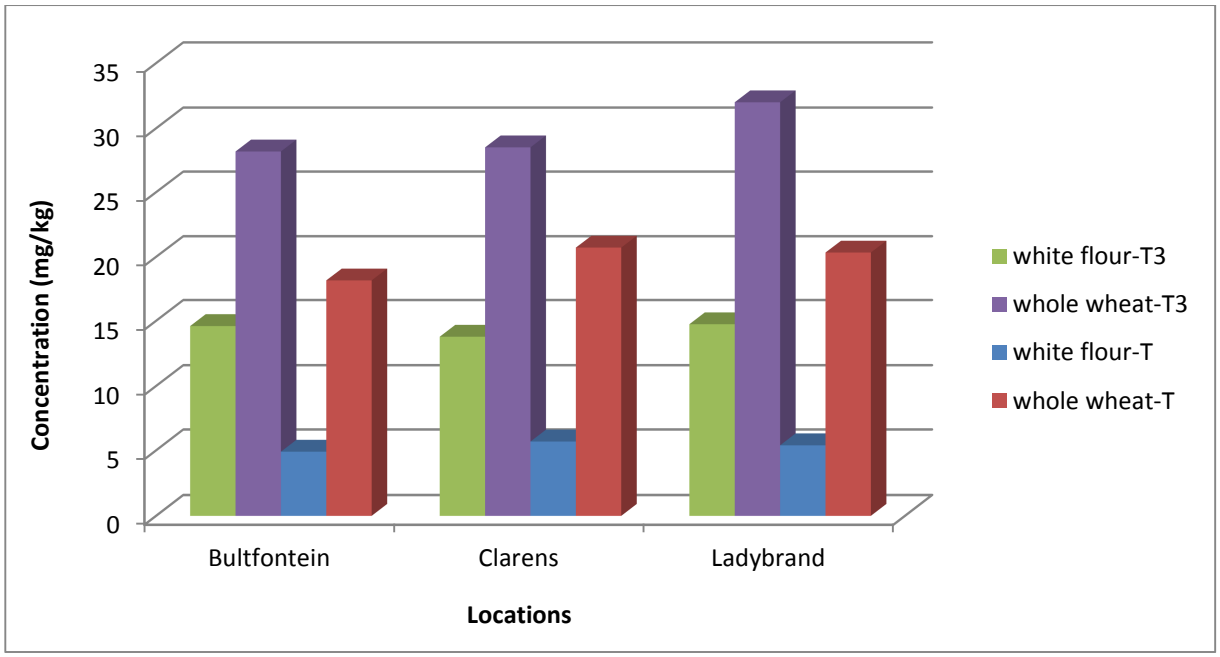

Figure 5.2: Total tocopherol and tocotrienol content from three different locations extracted from both whole wheat and white flour.

In both white flour and whole wheat, highest average content of tocotrienols (T3) was obtained in Ladybrand followed by Bultfontein. However, Clarens had the highest content of tocopherols (T) followed by Ladybrand. Caledon was the cultivar with the highest average content of total tocotrienols in all three locations ranging from 16.99 to 19.95 mg/kg in white flour (Table 5.6) and had the highest average only in Clarens for total tocopherols. Betta-DN in Bultfontein had the highest average for total tocopherols and Gariep in Ladybrand. In whole wheat (Table 5.7), Limpopo had the highest average content for both tocotrienols and tocopherols in Bultfontein, while Caledon had the highest average in Clarens and Elands in Ladybrand.

Table 5.6: Mean values of tocochromanol compounds (mg/kg) in white flour for three locations

White flour						
	Tocotrienols			Tocopherols		
Cultivars	**Bultfontein**	**Clarens**	**Ladybrand**	**Bultfontein**	**Clarens**	**Ladybrand**
Betta-DN	14.71	12.9	13.32	6.54	4.65	5.73
Caledon	19.95	16.99	18.89	6.48	7.14	7.02
Elands	12.7	14.62	17.34	3.75	5.95	6.5
Gariep	15.55	14.81	17.36	4.55	5.2	7.33
Komati	15.64	14.66	16.7	5.01	6.76	6.95
Limpopo	13.56	14.02	13.28	4.31	6.68	4.09
Matlabas	13.15	13.01	12.81	5.68	5.73	5.48
PAN3118	13.02	11.72	12.03	4.09	5.3	3.3
PAN3349	14.07	12.13	12.66	3.92	4.47	3.25
PAN3377	13.59	12.92	12.96	4.63	4.86	4.19
Average	14.59	13.78	14.74	4.90	5.67	5.38

Table 5.7: Mean values of tocochromanol compounds (mg/kg) in whole wheat for three locations

Whole wheat						
	Tocotrienols			Tocopherols		
Cultivars	**Bultfontein**	**Clarens**	**Ladybrand**	**Bultfontein**	**Clarens**	**Ladybrand**
Betta-DN	29.08	29.4	31.52	18.92	21.79	21.33
Caledon	30.97	30.65	34.86	21.06	24.34	22.72
Elands	29.09	29.49	35.46	20.85	21.89	24.3
Gariep	26.58	30.32	31.23	14.32	18.25	18.39
Komati	27.1	30.53	32.36	16.7	21.28	19.93
Limpopo	33.74	29.58	33.67	22.01	19.89	22.07
Matlabas	24.97	28.6	32.32	14.44	24.21	19.69
PAN3118	25.48	25.17	28.35	18.02	17.69	17.03
PAN3349	24.23	23.43	26.43	16.21	18.32	17.14
PAN3377	30.95	28.19	33.75	19.26	19.49	20.72
Average	28.22	28.54	32.00	18.18	20.72	20.33

5.4.2 Significant correlations between tocochromanol compounds and baking quality characteristics

White flour

Results are given in Table 5.8

Bultfontein: Alpha-tocopherol correlated positively with BFY, FY, AlvDIST and LV. A negative correlation was observed between α-tocopherol with AlvP/L and AlvSTAB. Alpha-tocotrienol was negatively correlated with DIA, SDSVOL and alveograph characteristics with the exceptions of AlvDIST but positively correlated with FN, WGC and HI. Beta-tocopherol correlated significantly positively with BFY, FP, WGC, LV and AlvDist. Dough stability correlated negatively with this tocochromanol compound so did AlvP/L. A negative correlation was observed between β-tocotrienol and SDSVOL, AlveoW, AlvP/L as well as AlvSTAB, while correlation with WGC and AlvDIST was positive.

Clarens: The only significant correlation at this location was for the following: Alpha-tocopherol correlated negatively with VK. Similar results were observed between α-tocotrienol and DIA while correlation with HLM was positive. The correlation between β-tocotrienol and WGC was also positive.

Ladybrand: Alpha-tocopherol correlated significantly positively with HLM, FN, WGC, HI, FP and LV but correlation was negative with BFY and AlvSTAB. Alpha-tocotrienol correlated positively with HLM, HI, FN and WGC but correlated negatively with VK, BFY, SDSVOL and AlveoW. Correlation between β-tocopherol with AlvSTAB and BFY was negative while a positive correlation was observed with HLM, LV, LV12 and HI. Beta-tocotrienol was positively correlated HLM, WGC, HI, FN and LV but correlation with BFY and VK was negative. Delta-tocotrienol was positively correlated with HLM and HI, while negatively correlated with BFY and DIA.

Table 5.8: Significant correlations between tocochromanol compounds (mg/kg) and quality characteristics in white flour

Bultfontein			Clarens			Ladybrand		
Character 1	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**
α-Tocopherol	BFY	0.3885*	α-Tocopherol	VK	-0.3394*	α-Tocopherol	HLM	0.5064***
	FP	0.3678*	α-Tocotrienol	DIA	-0.4339**		FN	0.3888*
	AlvP/L	-0.4540**		HLM	0.4017*		BFY	-0.4613**
	AlvSTAB	-0.4860**	β-Tocotrienol	WGC	0.4512**		WGC	0.4205**
	AlvDIST	0.4907**					HI	0.5012***
	LV	0.3726*					FP	0.3677*
α-Tocotrienol	AlvDIST	0.4907**					AlvSTAB	-0.3878*
	FN	0.3828*					LV	0.4045*
	AlvP/L	-0.4540**				α-Tocotrienol	HLM	0.6681***
	AlveoW	-0.5048***					VK	-0.4079*
	WGC	0.4720**					FN	0.3394*
	DIA	-0.4247**					BFY	-0.5402***
	HI	0.5983***					WGC	0.4099*
	SDSVOL	-0.4678**					HI	0.7416***
β-Tocopherol	BFY	0.4666**					SDSVOL	-0.4179**
	FP	0.3908*					AlveoW	-0.3665*
	WGC	0.6075***				β-Tocopherol	HLM	0.3726*
	AlvSTAB	-0.5762***					BFY	-0.3484*
	AlvDIST	0.6288***					AlvSTAB	-0.4667**
	AlvP/L	-0.5866***					LV	0.4092*
	LV	0.3980*					LV12	0.4036*
							HI	0.3358*

* p≤0.05; ** p≤0.01; *** p≤0.001, BFY=break flour yield, WGC=wet gluten content, AlvSTAB=dough stability, AlvDIST=dough distensibility, AlvP/L=AlveoW to AlvSTAB ratio, DIA=kernel diameter, HI=hardness index, SDSVOL=SDS sedimentation volume, FY=flour yield, AlveoW=dough strength, HLM=hectolitre mass, FP=flour protein, LV=loaf volume and LV12=loaf volume at 12% protein basis.

Table 5.8: Continued

Bultfontein			Clarens			Ladybrand		
Character 1	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**
β-Tocotrienol	AlveoW	-0.4472**				β-Tocotrienol	HLM	0.5724***
	AlvSTAB	-0.4047**					VK	-0.3583*
	AlvDIST	0.3753*					FN	0.3495*
	AlvP/L	-0.3388*					BFY	-0.4982**
	WGC	0.5563***					LV	0.3998*
	SDSVOL	-0.4162**					WGC	0.4808**
δ-Tocotrienol	FY	0.3741*					HI	0.5677***
						δ-Tocotrienol	HLM	0.4299**
							BFY	-0.4287**
							DIA	-0.4190**
							HI	0.5439***

* p≤0.05; ** p≤0.01; *** p≤0.001, BFY=break flour yield, WGC=wet gluten content, AlvSTAB=dough stability, AlvDIST=dough distensibility, AlvP/L=AlveoW to AlvSTAB ratio, DIA=kernel diameter, HI=hardness index, SDSVOL=SDS sedimentation volume, FY=flour yield, AlveoW=dough strength, HLM=hectolitre mass, FP=flour protein, LV=loaf volume and LV12=loaf volume at 12% protein basis.

Whole wheat

Results are given in Table 5.9.

Bultfontein: Alpha-tocotrienol correlated negatively with AlvSTAB and β-tocopherol correlated negatively but highly significant (p≤0.01) with FY. Beta-tocotrienol was negatively correlated with FY and TKM. Delta-tocotrienol was negatively correlated with HLM, AlveoW, AlvSTAB, AlvP/L, TKM and DIA while a positive correlation was observed with FC, AlvDIST and LV.

Clarens: Alpha-tocotrienol correlated positively with BFY and HLM. It correlated negatively with AlveoW, TKM, DIA and SDSVOL. Beta-tocopherol positively correlated with FN. Beta-tocotrienol was positively correlated with BFY and HLM.

Ladybrand: Alpha-tocotrienol was positively correlated with HLM and HI but was negatively correlated with VK, BFY, AlveoW, TKM, DIA and SDSVOL. Beta-tocopherol was positively correlated with HLM and HI but negatively correlated with BFY and FY. Correlation with β-tocotrienol and FY was negative and positive with AlvDIST. Delta-tocotrienol was positively correlated HLM, HI and FC while negatively correlated with BFY, VK, DIA and SDSVOL.

Table 5.9: Significant correlations between tocochromanol compounds (mg/kg) and quality characteristics in whole wheat

Bultfontein			Clarens			Ladybrand		
Character 1	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**
α-Tocotrienol	AlvSTAB	-0.3364*	α-Tocotrienol	HLM	0.5644***	α-Tocotrienol	HLM	0.7474***
β-Tocopherol	FY	-0.5154***		AlveoW	-0.4482**		VK	-0.4558**
β-Tocotrienol	FY	-0.4138**		TKM	-0.4429**		BFY	-0.6100***
	TKM	-0.4892**		DIA	-0.6900***		AlveoW	-0.4297**
δ-Tocotrienol	FC	0.3690*		BFY	0.3943*		TKM	-0.3866*
	HLM	-0.4860**		SDSVOL	-0.5867***		DIA	-0.5519***
	AlveoW	-0.5541***	β-Tocopherol	FN	0.4347**		HI	0.7771***
	AlvSTAB	-0.4014*	β-Tocotrienol	HLM	0.5080***		SDSVOL	-0.4788**
	AlvDIST	0.3515*		BFY	0.3402*	β-Tocopherol	HLM	0.5687***
	AlvP/L	-0.3965*					BFY	-0.5398***
	LV	0.3638*					FY	-0.5849***
	TKM	-0.7054***					HI	0.4832**
	DIA	-0.6913***				β-Tocotrienol	FY	-0.4269**
							AlvDIST	0.4153**
						δ-Tocotrienol	HLM	0.6047***
							VK	-0.3849*
							BFY	-0.5500***
							DIA	-0.4138**
							HI	0.6258***
							FC	0.4229**
							SDSVOL	-0.3640*

* p≤0.05; ** p≤0.01; *** p≤0.001, FY=flour yield, TKM=thousand kernel mass, FC=Flour colour, HLM=hectolitre mass, AlveoW=dough strength, DIA=kernel diameter, SDSVOL=SDS sedimentation volume, FN=falling number, VK=vitreous kernels, BFY=break flour yield, HI-hardness index, AlvDIST= dough distensibility and FC=flour colour.

5.4.3 Significant correlations from combined analysis between tocochromanol compounds and quality characteristics

Results are given in Table 5.10.

White flour:

HLM, VK and WGC all correlated positively with α-tocopherol. A positive correlation was observed between HLM, FN, WGC, HI and α-tocotrienol; while VK, BFY, AlveoW, TKM, DIA and SDSVOL all correlated negatively with this tocochromanol compound. Beta-tocopherol correlated negatively with AlvSTAB. HLM, WGC and HI had positive correlation with β-tocotrienol and BFY, AlveoW, SDSVOL were correlated with β-tocotrienol negatively. A positive association between δ-tocotrienol and HLM, FC, HI was observed and VK, TKM, DIA all correlated negatively with δ-tocotrienol.

Whole wheat:

Alpha-tocotrienol showed a positive correlation with HLM, FN, AlvP/L, HI and the correlation with VK, BFY, FY, FP, AlveoW, AlvDIST, LV, WGC, TKM and DIA was negative. Results showed a negative correlation between β-tocopherol with BFY, FY but correlation with HI was positive. Beta-tocotrienol correlated positively with HLM, AlvSTAB and AlvP/L and a negative correlation was observed between BFY, FY, FC, FP, AlveoW, AlvDIST, LV and WGC with β-tocotrienol. HLM, FN, FABS, AlvSTAB, AlvP/L, TKM and SDSVOL all showed a negative correlation with δ-tocotrienol and FY, FC, FP, AlveoW, AlvDIST, LV, LV12, WGC correlated positively.

Table 5.10: Significant correlations from combined analysis between tocochromanol compounds (mg/kg) and quality characteristics at three locations

White flour			Whole wheat		
Character 1	**Character 2**	**Correlation**	**Character 1**	**Character 2**	**Correlation**
α-Tocopherol	HLM	0.1980*	α-Tocotrienol	HLM	0.5748***
	VK	0.1971*		VK	-0.3348***
	WGC	0.2305**		FN	0.2309*
α-Tocotrienol	HLM	0.3205***		BFY	-0.3354***
	VK	-0.3069***		FY	-0.2916**
	FN	0.1992*		FP	-0.3837***
	BFY	-0.2285*		AlveoW	-0.6128***
	AlveoW	-0.3271***		AlvDIST	-0.2893**
	WGC	0.2002*		AlvP/L	0.3076***
	TKM	-0.2619**		LV	-0.3701***
	DIA	-0.3988***		WGC	-0.2289*
	HI	0.6000***		TKM	-0.4558***
	SDSVOL	-0.4420***		DIA	-0.4558***
β-Tocopherol	AlvSTAB	-0.2492**		HI	0.6103***
β-Tocotrienol	HLM	0.2381**	β-Tocopherol	BFY	-0.2494**
	BFY	-0.2264*		FY	-0.2873**
	AlveoW	-0.2415**		HI	0.2343*
	WGC	0.2577**	β-Tocotrienol	HLM	0.4777***
	HI	0.3628***		BFY	-0.2256*
	SDSVOL	-0.3225***		FY	-0.4912***
				FC	-0.3479***
				FP	-0.3467***

* p≤0.05; ** p≤0.01; *** p≤0.001, HLM=hectolitre mass, VK=vitreous kernels , FN=falling number, BFY=break flour yield, FC=flour colour, AlveoW=dough strength, AlvSTAB=dough stability, WGC=wet gluten content, TKM=thousand kernel mass, DIA=kernel diameter, HI=hardness index, SDSVOL=SDS sedimentation volume, FY=flour yield, FP=flour protein, FABS=farinograph water absorption, LV=loaf volume and LV12=loaf volume at 12% protein basis.

Table 5.10: Continued

White flour			Whole wheat		
Character 1	**Character 2**	**Character 1**	**Character 2**	**Character 1**	**Character 2**
δ-Tocotrienol	HLM	0.3693***	β-Tocotrienol	AlveoW	-0.2774**
	VK	-0.5308***		AlvSTAB	0.2444**
	FC	0.3990***		AlvDIST	-0.2528**
	TKM	-0.2148*		AlvP/L	0.2335*
	DIA	-0.2978**		LV	-0.2832**
	HI	0.6227***		WGC	-0.2486**
			δ-Tocotrienol	HLM	-0.3123***
				FN	-0.1964*
				FY	0.2617**
				FC	0.4593***
				FP	0.6115***
				FABS	-0.2857**
				AlveoW	0.3876***
				AlvSTAB	-0.6220***
				AlvDIST	0.5542***
				AlvP/L	-0.5656***
				LV	0.6291***
				LV12	0.3471***
				WGC	0.6175***
				TKM	-0.3404***
				SDSVOL	-0.2467**

* p≤0.05; ** p≤0.01; *** p≤0.001, HLM=hectolitre mass, VK=vitreous kernels , FN=falling number, BFY=break flour yield, FC=flour colour, AlveoW=dough strength, AlvSTAB=dough stability, WGC=wet gluten content, TKM=thousand kernel mass, DIA=kernel diameter, HI=hardness index, SDSVOL=SDS sedimentation volume, FY=flour yield, FP=flour protein, FABS=farinograph water absorption, LV=loaf volume and LV12=loaf volume at 12% protein basis.

5.5 Discussion

In this study, samples from whole wheat showed a higher tocochromanol compound content than samples from white flour. Similar results were shown in a study by Zielinski et al. (2001), where it was found that whole grain had 10-30% higher tocochromanols content than white flour fractions. Other authors found there was significantly more antioxidant activity in products manufactured with whole grains than products of refined wheat (Baublis et al., 2000; Perez-Jimenez and Saura-Calixto, 2005). Tocochromanols are found in large concentrations in those fractions that contain germ or the outer layers of kernels and the content in the endosperm fraction is lower than in whole grain (Piironen et al., 2009). According to Saadi et al. (1998), grain antioxidants are concentrated in the bran fraction. The bran and germ fraction of cereal kernels may contribute to the total antioxidant activities of wheat (Onyeneho and Hettiarachchy, 1992; Fardet et al., 2008). This may be the reason for a higher concentration of tocochromanol compounds from whole wheat than samples from white flour. In this study, whole wheat samples contained the highest concentration of tocotrienols. Tocopherols are said to be more concentrated in the germ fraction, while tocotrienols are found more in bran and also observed in the endosperm (Bramley et al., 2000; Hidalgo and Brandolini, 2008). Miller and co-workers (2000) found that antioxidant activity of whole grain bread was double that of white bread.

Major tocochromanol compounds in samples were reported to be α- and β-tocotrienols and α- and β-tocopherol (Zielinski et al., 2001; Panfili et al., 2003; Hidalgo et al., 2006; Lampi et al., 2008; Okarter et al., 2010; Hussain et al., 2012). Similar results were found in this study. Authors also found that the amount of α and β-tocotrienol is generally higher than α and β-tocopherol. The β-tocotrienol in this study was found to be the main tocochromanol compound in the samples analysed. These results were confirmed by Lampi et al. (2008; 2010) and Hussain et al. (2012). Results were similar to those obtained in this study but α-tocopherol was generally higher in quantity when compared to α-tocotrienol. Vitamin E content of South African cultivars varied from 0.76-2.16 mg for whole wheat to bran and 0.15 mg for white bread flour (Danster et al., 2008). The content is less than when compared with cultivars from other countries and the cultivars in this study.

The cultivars in this study varied in tocotrienol content, suggesting that some genotypes might contain more tocotrienols than other genotypes and more tocotrienols than tocopherols. Okarter et al. (2010) found that all varieties of wheat contained more tocotrienols than tocopherols. A high level of β-tocotrienol has been reported in genotypes which are rich in tocochromanols and that of α-tocopherol was generally lower as was found by a study done by Lampi et al. (2008). About 60% of total tocochromanol compounds are represented by β-tocotrienol (Hidalgo and Brandolini, 2010) and a study showed that α-

tocopherol is most prone to degradation (Wennermark et al., 1994). This may be the cause of a reduced concentration of tocopherols found in samples. Cultivation practices and conditions of extraction and analysis may also cause variation (Hussain et al., 2012). In milled wheat products, the concentration of vitamin E depends on the extraction rate, milling method, bread-making and storage (Nielsen and Hansen, 2008). The type, degree of fractionation and the extraction medium used also determines the antioxidant potential of cereals (Fardet et al., 2008). Milling of grain causes significant losses of tocochromanol compounds. The content of vitamin E tends to concentrate in certain fractions during milling (Bramley et al., 2000).

Traces of δ-tocochromanol compounds were found in wheat samples in this study, while no γ-tocols were detected. A study conducted by Zielinski et al. (2001) also showed a small quantity of δ-tocochromanol compounds can be extracted from wheat samples and γ-tocochromanol compounds were not present in the analyzed samples, while Okarter et al. (2010) and Hussain et al., (2012) found traces of γ-tocochromanol. The efficiency of extraction and analytical methods used may play a crucial role in the capacity to detect γ-tocols. Among the tocochromanols, δ-tocopherol has the strongest antioxidant activity, followed by γ, β and α-isomers (Wennermark et al., 1994; Zhou et al., 2004). More vitamin E content is lost when wheat is stored as flour than if it is stored as intact grains (Bramley et al., 2000) as damaged grains are more susceptible to oxidation, causing more losses in vitamin E content (Shin et al., 1997). During the processing of wheat flour, lipid oxidation destroys vitamin E (Wennermark et al., 1994).

Alpha- and β-tocopherol correlated positively with LV, FP and WGC in white flour. Beta-tocotrienol also correlated positively with LV and α- and β-tocotrienol had positive correlations with WGC and HI. In whole wheat no significant correlations were observed between PC, LV and α- and β-tocotrienol but δ-tocotrienol was positively correlated with LV. Wheat bran has been identified as being detrimental to LV (Lai et al., 1989). HI and HLM were consistently correlated positively with tocochromanol compounds. Tocochromanol compounds also correlated negatively with DIA. Grain size is presumed to be the cause of variation in tocochromanol content. Smaller grain sizes yield a larger proportion of tocochromanol compounds than larger grains (Lampi et al., 2010).

Tocopherols show vitamin E activity to various degrees while tocotrienols does not exhibit vitamin E activity or they have a substantially lower vitamin E activity when compared with tocopherols (Valentin and Qi, 2005; Delgado-Zamarreño et al., 2009; Tiwari and Cummins, 2009). This could be another reason why there is a high quantity of some of the tocotrienols in the samples. Alpha-tocopherol has been said to be the most active form of vitamin E but α-

tocotrienol had the highest biological activity of all eight tocols (Šramková et al., 2009). It has one third of the activity of α-tocopherol (Miyazawa et al., 2011).

There is a lot of data available about tocochromanols in genotypes, wheat fractions and commercial products but there are few studies that have compared the content of tocochromanols of diverse genotypes and environmental factors on tocochromanols. Tocochromanol compounds in this study were all influenced significantly by genotype effect and G X E effect. The environment also influenced tocochromanol compound content except for β-tocopherol and β-tocotrienol. Yu et al. (2003) stated that content of certain antioxidants in wheat varies according to growth location. They showed that environmental conditions had a significant effect on the antioxidant activity in wheat.

Lampi et al. (2010) found significant differences in tocochromanol amounts among genotypes. Hidalgo et al. (2009) also reported a significant genotype and year effect on total tocochromanols and tocotrienols, but only genotype had a significant effect on tocopherols. He observed that when there was a high rainfall season the level of tocochromanols was low. In diverse genotypes, the concentration of tocochromanols in wheat has been reported to be different (Panfili et al., 2003; Lampi et al., 2008; Hejtmánková et al., 2010). This implies the possibility of selecting genotypes with higher tocol content for human consumption. Results in a study by Peterson and Qureshi (1993) showed that location had a significant effect on total tocochromanols in oats but not in barley. There were significant differences between wheat samples obtained from different growing locations and samples from the same variety grown at different locations differ significantly in tocochromanol content. Similar results were reported by a number of authors (Yu et al., 2003; Yu et al., 2004; Piironen et al., 2009).

Cereals have low levels of α-tocopherol and this makes them moderate sources of vitamin E but they contain other tocochromanol compounds (Tiwari and Cummins, 2009). This is because cereals have a low content of lipids and they tend to have a low content of fat-soluble vitamins (Dewettinck et al., 2008). Elimination of bran and germ for the production of white flour lowers the concentration of tocols (Hidalgo and Brandolini, 2010). A significant proportion of minerals and vitamins are located in the endosperm. Nutrients are generally found in highest concentrations in the germ or embryo and in the aleurone cells surrounding the starch endosperm. Wheat bran does affect the bioavailability of vitamin E (Betschart, 1988).

Stressful conditions have been shown to promote α-tocopherol and total tocopherol synthesis in a number of crops (Munné-Bosch and Alegre, 2002; Konopka et al., 2012). This could be the case for other tocochromanol compounds as well and the reason for a large variation in tocochromanol content in wheat grain among genotypes. Tocochromanols are also easily

oxidised by oxidising agents in the presence of heat, light and alkali. During bread-making the dough is extensively mixed and aerated and subjected to lipoxygenases, which can cause oxidation losses of tocochromanols (Piironen et al., 2009).

5.6 Conclusions

This study showed that whole wheat contains more tocochromanols than white flour but tocochromanols extracted from white flour correlated more positively with important quality characteristics than those from whole wheat. This is probably due to the fact that baking quality was evaluated on white flour. Cultivars that were analysed in this study contained small amounts of tocochromanols when compared to results obtained by other researchers. Limited research had been done on tocochromanols in South African wheat and various reasons may have led to these low, such as the genetic composition of the germplasm, the environment in which the wheat was grown, and laboratory protocols used. Different extraction solvents and methods were used from previous studies; this may have influenced results. In general, the variation in tocochromanol content between genotype groups suggests that some genotypes might be better source of tocochromanols in the human diet than others.

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Chapter 6

General conclusions

Large variation in PC among genotypes was observed and ranged from 8.7% - 14.4%. Genotype had a highly significant effect on all protein fractions obtained from both white flour and whole wheat flour. Most gliadin fractions correlated negatively with baking quality traits. Gliadins had significant association with dough strength; it is suggested that it might be influenced by the presence of LMW-GS. Glutenin subunits also correlated with gliadin peaks, showing that there is a relationship between these gluten proteins. Quality characteristics that correlated positively with HMW-GS tended to correlate negatively with LMW-GS.

There was a variation between the effect caused by SE-HPLC protein fractions based on total protein and those based on flour. A large variation was observed in the proteins fractions extracted from whole wheat flours and the effect on quality characteristics. There was a difference between protein fractions that could be fractionated from white flour and whole wheat flour. The difference might be caused by the bran in whole wheat flour. Bran binds a large amount of water and thus results in changed dough properties such as LV. Albumins and globulins correlated negatively with WGC and FP; it shows that these protein fractions have a detrimental effect on bread-making quality. Combined analysis of protein fractions revealed similar results. There was a strong genotype effect for protein fractions. The ω-gli correlated negatively with important quality traits. Bultfontein and Ladybrand had a higher proportion of negative correlations between protein fractions and quality traits.

Major tocochromanol compounds (α- and β-tocopherol and, α- and β-tocotrienol) correlated positively with baking quality characteristics. Highly significant correlations were observed in white flour but not in whole wheat. Large variation was found among genotypes for tocochromanol content therefore selection can be applied to cultivars that contain higher content of tocochromanols. Environment also made a contribution to variation among tocochromanol compounds.

Tocochromanol compounds had no negative effect on important quality baking characteristics. Therefore it is possible to select cultivars that have a high proportion of tocochromanol compounds as well as good baking quality. Results in this study reveal that bran in whole wheat had significant but negative effects on quality baking characteristics including tocochromanol compounds. Breeders and the wheat industry might have to use white flour data to estimate expected perfomance of whole wheat and take advantage of cultivars with high tocochromal content and good baking quality to produce cultivars with high

nutritional value and good baking quality. However it is not clear if selecting for high quality based on white flour will result in high quality in whole wheat flour.

Summary

The main objective of this study was to determine the relationship between protein fractions, vitamin E and quality characteristics in white flour and whole wheat flour grown at different geographical locations. Genotype, environment and G X E effect was highly significant for protein fractions as well as tocochromanols. Analysis of white flour and whole wheat in relation to baking quality performance showed that correlation may vary depending on characteristics. Most gliadins correlated negatively with important baking quality characteristics but some correlated positively with loaf volume, protein content and wet gluten content. A combined analysis shows positive correlations between LMW-GS and protein content, loaf volume and dough distensibility. Combined analysis of protein fractions also revealed that a high amount of high molecular weight polymeric proteins correlated with baking quality characteristics. Variability in content of tocochromanols was found among wheat cultivars from different environments. Caledon had the highest content of tocochromanols for both white and whole wheat flour. In this study it was shown that vitamin E does not compromise baking quality.

Key words: Baking quality, bread wheat, whole wheat, white flour, tocochromanols, RP-HPLC, SE-HPLC

Opsomming

Die hoofdoel van hierdie studie was om die verhouding tussen proteïenfraksies en kwaliteitseienskappe met behulp van RP-HPLC en SE- HPLC te bepaal. Vitamien E (o.a. tocochromanole) inhoud van tien Suid-Afrikaanse brood koring kultivars is ondersoek. Genotipe, omgewing en G X E effek was hoogs betekenisvol vir proteïenfraksies sowel as tocochromanole. Ontleding vir wit meel en volgraan meel koring in verhouding tot die bak gehalte prestasie toon dat die korrelasies kan wissel, afhangende van eienskappe. Die meeste gliadien was negatief gekorreleer met belangrike bakkwaliteiteienskappe, maar sommige was positief gekorreleer met brood volume, proteïen-inhoud en nat gluten inhoud. 'n Gesamentlike ontleding toon positiewe korrelasie tussen lae molekulêre gewig - gluten subeenhede en proteïen-inhoud, brood volume en deeg vervormbaarheid. Gekombineer ontleding van proteïenfraksies het ook getoon dat 'n groot hoeveelheid van die hoë molekulêre gewig polimeriese proteïene korreleer is met van die bakkwaliteitseienskappe. Variasie in inhoud van tocochromanole is gevind onder die koring kultivars van verskillende omgewings. Caledon het die hoogste tocochromanol inhoud vir beide wit meel en volgraan meel gehad. In hierdie studie is getoon dat vitamien E nie bakkwaliteit benadeel nie.

Sleutel woorde : Bak gehalte, brood koring, hele koring, wit meel, tocochromanols, RP-HPLC, SE- HPLC

Appendix

Table 1: Weather data for 2007 at three locations

Location	Year	Latitude	Longitude	Altitude	Month	Tx °C	Tn °C	Rain mm
Bultfontein	2007	-28.15268	26.06357	1302	1	31.82	15.76	1.59
					2	32.56	14.45	0.65
					3	29.90	11.79	0.53
					4	25.68	10.42	1.23
					5	22.10	1.64	0.00
					6	17.48	1.34	0.68
					7	18.41	-1.58	0.00
					8	21.90	0.89	0.00
					9	28.38	7.45	2.05
					10	24.67	11.75	3.09
					11	28.36	12.29	2.62
					12	27.51	15.25	1.89
Clarens	2007	-28.5038	28.58383	1851	1	24.93	Error	1.48
					2	28.13	Error	1.79
					3	29.12	Error	3.20
					4	19.91	Error	1.56
					5	19.23	1.78	0.21
					6	15.05	0.75	1.09
					7	16.23	-0.73	0.10
					8	18.72	1.68	0.00
					9	24.00	8.67	1.55
					10	19.23	8.92	6.71
					11	22.31	10.40	4.39
					12	21.92	11.78	3.21
Ladybrand	2007	-29.48301	27.1346	1500	1	30.44	14.38	1.29
					2	31.03	14.04	0.75
					3	27.75	11.17	1.45
					4	23.87	9.03	0.67
					5	20.41	0.94	0.14
					6	16.03	-0.20	0.81
					7	17.04	-2.58	0.08
					8	20.22	0.58	0.11
					9	26.20	7.10	1.53
					10	22.37	9.88	2.59
					11	25.85	10.50	3.91
					12	25.61	13.20	2.62

Tx=average maximum temperature, Tn=average minimum temperature