The use of HPLC for quality prediction of South African wheat cultivars

By

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CONTENTS

Page

1. **Introduction** 1

References 3

2. Literature review

2.1 The structure of the wheat kernel 5

2.2 Milling 7

2.2.1 Dry milling 7

2.2.2 Milling products 10

2.2.3 Milling of soft and hard wheat 11

2.3 The composition of wheat proteins 12

2.3.1 Classification of wheat proteins 12

2.3.2 Protein structure 16

- Wheat storage proteins 18

2.3.3 Low molecular weight storage proteins (Gliadins) 19

2.3.4 High molecular weight storage proteins (Glutenins) 23

a. The high molecular weight glutenin subunits (HMW-GS) 25

b. The low molecular weight glutenin subunits (LMW-GS) 31

2.4 Wheat Quality 36

2.4.1 Protein composition (functionality of the gluten components) 36

a. High molecular weight glutenin subunits (HMW-GS) 38

b. Low molecular weight glutenin subunits (LMW-GS) 44

c. Gliadins 47

2.4.2 Protein content 48

2.4.3 Hard and soft wheat quality 54

2.4.4 Environmental effect on quality 59

2.5 Quality testing parameters 61

- Basic analysis 62

2.5.1 Test weight 62

2.5.2 Kernel hardness 62

2.5.3 Flour yield 63

2.5.4 Moisture 63

2.5.5 Protein content 64

- Flour Performance tests 66

2.5.6 Farinograph 66

2.5.7 Mixograph 70

2.5.8 Alveograph 72

2.5.9 Gluten washing tests 73

2.5.10 Alkaline water retention capacity 73

- Enzyme analyses 74

2.5.11 Falling number (Endosperm starch content determination) 74

- Viscosity methods 75

2.5.12 Sodium dodecyl sulphate (SDS) – sedimentation test 76

- Baking tests 76

2.5.13 Loaf volume 76

2.6 High performance liquid chromatography of wheat proteins 77

2.6.1 SE-HPLC and wheat quality 78

2.7 References **84**

3. The effect of nitrogen fertilization on the quality of hard and soft wheat cultivars as determined by SE-HPLC

3.1 Introduction **111**

3.2 Materials and Methods **113**

3.2.1 Materials **113**

3.2.2 Methods **115**

a. Quality analysis **115**

b. Size-exclusion HPLC **115**

c. Statistical analysis **116**

3.3 Results and discussion for 2001 **117**

3.3.1 Results **117**

3.3.2 Discussion **143**

3.4 Results and discussion for 2002 **161**

3.4.1 Results **161**

3.4.2 Discussion **184**

3.5 Results and discussion for the combined data of 2001 and 2002 **199**

3.5.1 Results **199**

3.5.2 Discussion **226**

3.6 Conclusion **239**

3.7 References **244**

4. Summary 249

Opsomming 251

LIST OF ABBREVIATIONS

AACC	=	American Association of Cereal Chemists
A-PAGE	=	acidic polyacrylamide gel electrophoresis
ANOVA	=	analysis of variance
AWRC	=	alkaline water retention capacity
BFLY	=	breakflour yield
BU	=	brabender units
DNA	=	deoxyribonucleic acid
FLN	=	falling number
FLY	=	flour yield
FPC	=	flour protein content
IE-HPLC	=	ion-exchange high performance liquid chromatography
IEF	=	gel isoelectric focusing
pI	=	isoelectric point
ha	=	hectare
HLM	=	hectoliter mass
HMW	=	high molecular weight
HMW-GS	=	high molecular weight glutenin subunits
HPLC	=	high performance liquid chromatography
HRS	=	hard red spring wheat
kg/hl	=	kilograms per hectoliter
KHz	=	kiloHertz
L	=	alveograph extensibility
LMW	=	low molecular weight
LMW-GS	=	low molecular weight glutenin subunits
LPP	=	larger polymeric proteins
LSD	=	least significant difference
LUPP	=	larger unextractable polymeric protein
mAU	=	milli absorption units
mb	=	moisture balance
MP	=	monomeric proteins

MTI = mixing tolerance index

MW = molecular weight

NIR = near-infrared reflectance spectroscopy

N = nitrogen

nm = nanometer

N:P:K = Nitrogen: Phosphate: Potassium

p = probability of significance

PP = polymeric proteins

PAGE = polyacrylamide gel electrophoresis

P/L = alveograph ratio

QTL = quantitative trait loci

rpm = revolutions per minute

RVA = Rapid Visco Analyser

RP-HPLC = reversed-phase high performance liquid chromatography

SDS = sodium dodecyl sulphate

SDS-PAGE = sodium dodecyl sulphate-polyacrylamide gel electrophoresis

SDS-sedimentation = sodium dodecyl sulphate – sedimentation test

SE-HPLC = size-exclusion high performance liquid chromatography

SK-diam = single kernel diameter

SK-hard = single kernel hardness index

SK-wght = single kernel weight

SKCS = single kernel characteristic system

SMP = smaller monomeric proteins

SPP = smaller polymeric proteins

W = strength (area under the alveograph curve)

TFA = Trifluoroacetic acid

TUPP = total unextractable polymeric protein

μm = micrometre

VK = vitreous kerne

CHAPTER 1

Introduction

Wheat (*Triticum aestivum* L. and *T. turgidum* L.) is the world's leading cereal grain and most important food crop. Its importance derives from the properties of wheat gluten, a cohesive network of tough endosperm proteins that stretch with the expansion of fermenting dough, yet coagulate and hold together when heated to produce a 'risen' loaf of bread. Only wheat, and to a lesser extent rye and triticale, has this property. Wheat is utilized for making bread, flour confectionary products (e.g., cakes, cookies, crackers, pretzels), unleavened bread, semolina, bulgar, and breakfast cereals. Its diversity of uses, nutritive content, and storage qualities has made wheat a staple food for more than one-third of the world's population (Poehlman & Sleper, 1995).

In wheat, like other cereal grains, carbohydrate compounds in the form of starch are the major storage compounds which have a big influence on the yield. Having proteins as the second largest storage compound are what makes wheat unique (in terms of physical and biochemical properties) (Mamuya, 2000). The storage proteins constitute about 85% of the endosperm proteins in wheat kernels, and are traditionally classified into gliadins and glutenins, according to their solubility properties (Osborne, 1907). Currently, it is customary to think in terms of monomeric and polymeric proteins, although gliadins and glutenins are known to be the main components of these two groups, repectively. The monomeric proteins consist of single chain polypeptides. In contrast, the polymeric proteins are multiple chain polymers in which the individual polypeptides of subunits are linked by disulfide bonds (Southan & MacRitchie, 1999).

The two protein groups impart different properties to the dough: glutenin bestows elasticity, whereas gliadin is viscous and confers extensibility (Payne et al., 1984). It is the unique combination of dough viscosity and dough elasticity that comprises the functional properties of dough. Glutenin is

subdivided according to molecular weight into high molecular weight (HMW) and low molecular weight (LMW) subunits (Payne et al., 1981). About 50% of the storage proteins consist of gliadin, while 10% and 40% are HMW and LMW subunits of glutenin, respectively (Payne et al., 1984).

Gliadins arise from multigene families located on the short arms of homologous group 1 and group 6 chromosomes (Bietz, 1988). Glutenins are produced by genes located only on group 1 chromosomes; the HMW glutenin subunits arise from genes on the long arms, while LMW subunits are produced by genes tightly linked to gliadin genes on the short arms. Genetic variation, based on mobilities in polyacrylamide gel or chromatographic separations, for both gliadins and glutenin subunits, is extensive. Exhaustive experimentation has been conducted in attempts to explain wheat quality variation as a function of genetic variation in gluten protein subunit composition (Bietz, 1988; MacRitchie et al., 1990).

Size-exclusion high performance liquid chromatography (SE-HPLC) is the method of choice to give information of the structure, size-distribution and interactions of protein components (Singh & MacRitchie, 1989) without the disruption of large polymers. In addition, SE-HPLC exhibits high resolution and reproducibility, its other most attractive features being automation and quantification due to its computer capabilities (Bietz, 1986).

Subunit composition is genetically fixed; still there is a portion of wheat quality variation that is dependent upon environmental factors (Graybosch et al., 1996). High yield and good bread-making quality are important features in today's wheat market. Both can be improved through nitrogen (N) fertilization strategies, such as the rates and timing of N-fertilization (Martin et al., 1992). Many studies have shown that the increase of flour protein content resulting from N application can lead to changes in protein composition (Gupta et al., 1992). The effects of various timings of nitrogen fertilization show that the application at an early stage increases yield, but the

N supply at a later stage (boot or head-emergence stage) increases significantly the amounts of all the protein fractions, and thus improves the baking–quality properties. However, the responses to increasing N fertilization of diverse types of glutenin polymers are very different according to the environmental conditions. The growing location and maturation conditions are the origin of the differences in distribution among glutenin polymers and in the mode of glutenin polymerization. This results in different contributions to the potential bread-making quality properties (Jia et al., 1996).

In this study the influence of N-applications on the different protein fractions and various quality characteristics of hard and soft wheats were studied with the use of SE-HPLC. Most of the quality characteristics are polygenically inherited, and were therefore influenced by the environment to a large extent. The quality potential of the cultivars was predicted from these results.

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CHAPTER 2

Literature Review

2.1 The structure of the wheat kernel

The wheat kernel or grain, known botanically as a caryopsis, is the fruit of the plant and is normally about 4 – 8 mm long, depending on the variety and condition of growth. The kernel contains only one seed, which is not shed at maturity, in common with other grasses (Cornell & Hoveling, 1998). Simplistically, the grain can be divided into three constituents, the bran, germ, and endosperm (Worland & Snape, 2001).

The colour of the kernel is governed primarily by materials present in the seed coat or pericarp. This consists of an epidermis (outer layer) and a hypodermis, next to a layer of thin-walled cells and several other types of cells (Fig. 2.1). Altogether, this pericarp is about 50 µm thick. Then we find another thin seed coat (testa), covering a nucellular epidermis, and then an aleurone layer, before coming to the starch-rich endosperm. Bran is chiefly the outer material down to and including the aleurone layer (Cornell & Hoveling, 1998). The bran constitutes about 14% of the kernel by weight and is high in fiber and ash (mineral) content (Atwell, 2001).

The wheat germ, the embryonic wheat plant, constitutes only about 3% of the kernel. Most of the lipid and many of the essential nutrients in the kernel are concentrated in the germ (Atwell, 2001). The germ consists of several parts. The plumule, which forms the shoot when the seed germinates, has a stem attached to it and to the coleoptile, which functions as a protective sheath. There is also the scutellum, the storage, digestive, and absorbing organ, which is attached to the plumule. It contains food for the plant, which is supplied at germination, and also transfers food from the endosperm. The germ is readily separated from the endosperm and bran by milling. It is an important dietary supplement, providing a rich source of vitamin E (Cornell & Hoveling, 1998).

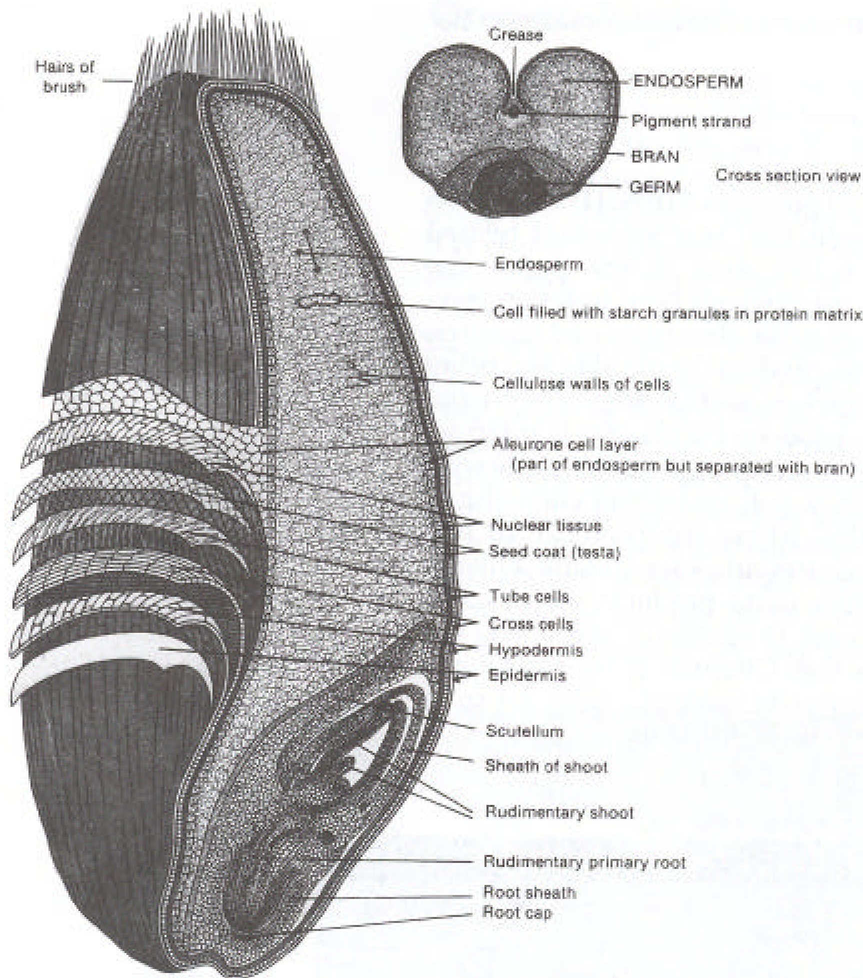

Figure 2.1. Schematic diagram of the wheat kernel, illustrating the major anatomical parts (Courtesy Millers National Federation, Atwell, 2001).

The endosperm composition has received most attention with respect to the genetic analysis of quality traits since, proportionally; this is, by far, the greatest component. Eighty percent of the endosperm is made up of starch, and most of the remainder is protein. To date, the greatest contribution of genetics to quality breeding has been to improve the understanding of the control of endosperm constituents (Worland & Snape, 2001).

The starchy endosperm is the material from which white flour is made. It comprises starch granules embedded in a matrix of proteins. The proteins consist of albumins, globulins, gliadins, and glutenins. The combination of the gliadins and glutenins is referred to as the "gluten complex" and is regarded as storage protein. It is formed in discrete particles by proteoplasts, which are able to be seen in developing kernels, and can be separated from the interstitial protein. The starch is present as lenticular granules varying in size from 10 to 50 μm diameter (major axis) and smaller, more spherical granules of 2 – 5 μm diameters. The starch granules also contain protein and lipids as minor constituents and the amounts are related to the size of the granules (Cornell & Hoveling, 1998).

2.2 Milling

Milling is simply the reduction of wheat kernels to smaller particles that can be made into more palatable products. In modern times, it involves, more specifically, the separation of the germ and bran from the endosperm and the reduction of the endosperm to flour. The concept of gradual reduction, which persists today, originated in the 19th century in Hungary. The sophisticated means of grinding, separating, and conveying used in modern mills are based on the same basic processes employed in these early mills. Wheat is subjected to some standard wheat quality tests (e.g., protein, moisture, test weight, and flour yield), and milled on small mills in laboratories. The resultant flour is evaluated using a number of standard testing procedures (e.g., protein, moisture, ash, wet gluten, falling number, farinogram characteristics, baking quality, and sedimentation) (Atwell, 2001). These testing procedures are described in section 2.5.

2.2.1 Dry milling

A modern milling operation involves much more than grinding wheat to a powder. Three general operations are usually involved: cleaning, tempering, and milling. Cleaning removes unwanted material; tempering softens the grain, making it easier to separate and grind; and milling involves grinding the wheat and isolating wheat components of a specific size (Atwell, 2001).

Wheat is received and stored in that part of the mill known as the elevator. In general, mills are equipped to receive wheat by road, rail, water, or combinations of all three. The flow of wheat through the elevator is as follows: Incoming wheat is weighed, sampled, and immediately analyzed. Analyses are for foreign material such as other seeds, sand, straw, stones; insects; damaged kernels such as burnt, shrivelled, immature, or sprouted kernels; moisture and protein content; and usually also a-amylase activity by some mechanical means, such as falling number. The wheat is weighed as received, dumped through a grate to remove coarse foreign material, passed over a magnet, then usually passed through a preliminary cleaner known as a "receiving separator" on its way to a storage bin, where it is stored according to class, grade, and protein content (Bass, 1988).

A conveying system is used to transfer the wheat to intermediate storage bins, from which different grades may be blended to give a desired milling grist. The conveying system may be arranged to feed backward, to permit the "turning" of wheat from one bin to another during long storage periods. This turning over is sometimes necessary to keep the wheat in sound condition. Provision is often also made for drying of wheat. This requires a separate conveying system to move the wheat through an automatic dryer and back to the dried-wheat bins and main storage block. Usually, transfer systems that turn grain include facilities to fumigate the grain. The fumigant can be in liquid form but more commonly now is the form of pellets that are dispensed as the grain moves along a conveyor (Bass, 1988).

Tempering is the addition of predetermined amounts of water to wheat during specific holding periods. It toughens the bran making it easier to separate from the endosperm and germ. It also softens the endosperm, allowing it to break apart with less force. Tempering involves adjusting the moisture level of the wheat. For soft wheat, optimal tempering brings the grain to 13.5 – 15.0% moisture and takes 6 – 10 hours. For hard wheat, the final moisture is 15.5 – 16.5%, and tempering times are 12 – 18 hours. Incoming wheat is generally lower in moisture content than this; hence, water is usually added and the grain is allowed to equilibrate for a period of time. This time varies considerably based on the hardness of the wheat. Conditioning of wheat refers to the application of heat in the tempering process to increase the rate of penetration of moisture into the kernels. Temperatures lower than 50°C are employed during conditioning to ensure that the functionality of the flour components, especially the gluten, is maintained (Atwell, 2001).

At this point, the wheat is ready for milling and starts through the various systems in the mill. The first machine in almost every mill is the roller mill. Wheat kernels fall into the grinding zone formed by a pair of rolls rotating towards each other at different speeds and is subjected to the grinding action. Flour milling involves several pairs of rolls used in sequence. From the first to the last pair of rolls the roll gap is set successively narrower as the particle size of the feed stock becomes smaller. Generally there are about five roller mills or five "breaks" in the system. The germ is removed in the first two breaks, as is much of the bran. The germ is pliable and tends to flatten when it goes through the rollers. Bran particles are usually in the form of low-density small flakes. These properties allow millers to separate the germ and bran fractions from the endosperm fraction (Atwell, 2001).

After each break, a set of sieves and/or purifiers (aspirators) separates the ground material by size and density. Small particles are channelled into the flour, and large particles are either removed (as in the case with the germ and

bran) or sent to the next break (as occurs for large endosperm pieces) (Atwell, 2001).

Once the endosperm is isolated, the large particles that result (called middlings) are reduced in the reduction system to a particle size distribution consistent with flour. This means they must be able to pass through a 136 μm opening. The rollers in the reduction system are smooth and are operated at low differentials, providing a crushing action that yields the fine particles of a flour (although a small amount of shear is still important). A large percentage of the particles composing the final flour come off the reduction rolls. Flour from the break and reduction rolls may be combined in many ways to create different types of flour, but it is usually sifted again in the flour dressing system. Material that passes through the 10XX sieves (apertures of 136 μm) in this process meets the particle size standard for flour. Larger particles are recirculated back to the appropriate point in the grinding process. The flour may be further treated with chlorine or supplemented with nutrients, malt (germinated barley to bolster enzymatic activity and improve flavour), and/or a bleaching agent (whiten flour) depending on the requirements of the customer (Atwell, 2001).

In the millfeed system, the germ and bran are separated from each other, and adhering endosperm is removed. The coarse bran from the early breaks is termed "bran" and composes about 11% of the total products from the mill. The finer branny material from the later steps is called "shorts"; it represents about 15% of the total. Germ is generally recovered at the rate of about 0.5 – 2.0% of the total wheat depending on the type of equipment used. These products are usually sold separately as animal feed, specialty products, or ingredients for human consumption (Atwell, 2001).

2.2.2 Milling products

If the entire wheat kernel is ground, separated, and recombined, the resultant product is called whole wheat flour. The extraction rate for whole wheat flour

is essentially 100%, because all of the wheat has been recovered as flour. A flour with most of the bran and germ removed, representing about 72% of the kernel (i.e., an extraction rate of 72%) is termed straight-grade flour. Patent flours are those from which many of the flour streams containing high bran content have been removed. These flours contain the lowest amount of bran. Their extraction rates are always less than 72%, generally ranging from 65% extraction for long-patent flour to 45% extraction for short-patent flour. Flour produced solely from the fractions between 45 and 65% extraction is termed "cutoff flour". Clear flour (or low-grade flour) is composed of the flour streams between 65 and 72% extraction. Clear flour is usually dark because these fractions are quite high in bran. Thus, the extraction rate is an estimate of the "purity" of the endosperm, or more accurately, its freedom from non-endosperm components. Therefore, the extraction rate is a rough first reflection of certain quality aspects because the non-endosperm components can have adverse effects on processing or product quality (Atwell, 2001).

The less bran and germ in a flour, the lower the mineral content because minerals are concentrated in these fractions. Hence, the ash test is often used to quantify the purity of a sample. High-extraction flour has a higher ash content than lower-extraction flour. Similarly, the endosperm is white, whereas the bran and germ are not, so visual tests can also be used to determine the general composition of flour (Atwell, 2001).

2.2.3 Milling of soft and hard wheat

Wheat of the different classes varies significantly in kernel hardness and consequently in how much power is required to grind the grain. Hardness of wheat is a result of the strength of the adhesion between starch and protein in the endosperm. In durum and hard wheat, the interactions between protein and starch are strong, whereas the interactions are weaker in soft wheat. Hence, when the kernel fractures during milling, it breaks apart in different ways. For example, starch occurs in the cells of the endosperm in partially

crystalline aggregates called starch granules. The space between the granules is filled primarily with the amorphous gluten proteins. When soft wheat is milled, the endosperm cells are converted to flour consisting largely of free starch granules and small particles containing both starch granules and protein. In hard wheat, however, the force holding the starch to the protein may be so strong that, in some cases, the starch granules fracture before the protein-starch interactions are severed. Granules broken in this manner compose what is termed "damaged starch". In hard wheat, damaged starch may constitute 8% or more of the total starch in the flour. If durum wheat is milled to the particle size of flour, the amount of damaged starch is considerably higher because the interactions between gluten and starch are even stronger (Atwell, 2001).

The description of the milling process given above applies to all types of wheat. Clearly, there are differences in how the process is operated depending on process flow details, the type of wheat, and the particular mill. In general, compared to a hard wheat mill, a soft wheat mill will temper the wheat for a shorter time since the endosperm is already softer. The kernel breaks apart easily so there are usually fewer roller mills in the reduction system. Because the endosperm breaks up more easily, the particle size distribution of soft wheat is smaller and narrower. Thus, there are differences in the sizes of the apertures in the sieves used in soft versus hard wheat mills. The smaller particle sizes of soft wheat often make sifting more difficult because of the tendency of these small particles to attract each other, aggregate, and subsequently not pass through a sieve. This can become a major problem in the operation of a soft wheat mill (Atwell, 2001).

Wheat is treated as a commodity that is classified by bran colour (red or white), growth habit (spring and winter) and kernel hardness (hard or soft). Although the concept of wheat milling and baking will vary with the type of wheat and end product, high quality baked goods begin with good quality wheat (Atwell, 2001).

2.3 The composition of wheat proteins

2.3.1 Classification of wheat proteins

The endosperm is by far the largest component of the grain - for this reason it has received the most attention with respect to the genetic analysis of quality traits. Eighty percent of the endosperm is made up of starch, and most of the remainder is protein. To date, the greatest contribution of genetics to quality breeding has been to improve understanding of the control of endosperm constituents (Worland & Snape, 2001). Protein usually constitutes 7 – 15% of common flour on a 14% moisture basis (Table 2.1) (Atwell, 2001).

The classical fractionation procedure of Thomas Osborne (1907) has been used for years to divide cereal proteins into four major groups on the basis of their solubilities (Wrigley & Bietz, 1988). The albumins are soluble in water; the globulins are soluble in dilute salt solutions but not in water; the prolamins are soluble in aqueous alcohols but not in water or salt solutions; and some glutelins are soluble in acid or alkali but not in alcohol, water, or salt solutions. Although much criticism has been levelled against this procedure, and even though many improved methods are available, it has provided the basis for, and been most useful in, both structural and functional investigations of cereal proteins (Bushuk, 1981). It must be understood that each of these fractions is a complex mixture of different polypeptides and also that these polypeptide overlap in their solubilities. This is particularly true for the gliadins and glutenin proteins (Gianibelli, 2001).

The water-soluble proteins or albumins, such as many of the enzymes of wheat, make up about 15% of the flour proteins (Table 2.1). The globulins are relatively minor, making up only about 3% of the total protein (Atwell, 2001). It seems to be a common opinion that the composition of albumins and globulins does not vary between wheat varieties, and there is no correlation

between the amount of albumins and globulins and baking performance (MacRitchie, 1984). It is thus not possible to use the albumins and globulins to identify cultivars or to discriminate between cultivars differing in baking performance.

Table 2.1. Analytical composition of flour and its primary components (Compiled from data in the text, Atwell, 2001)

Property	Percent
Moisture	14 (of flour)
Protein	7 – 15 (of flour)
Osborne classification	
Albumins	15 (of protein)
Globulins	3 (of protein)
Prolamin (gliadin)	33 (of protein)
Glutenlin (glutenin)	16 (of protein)
Residue	33 (of protein)
Gluten	6 – 13 (of flour)
Gliadin	30 – 45 (of gluten)
Glutenin	55 – 70 (of gluten)
Starch	63 – 72 (of flour)
Nonstarchy polysaccharides	4.5 – 5.0 (of flour)
Lipids	1 (of flour)

Prolamins are cereal proteins generally soluble in 70% aqueous ethanol. Gliadin, one of the two major components of the wheat gluten complex, is a prolamin; it constitutes about 33% of all the proteins in flour. The other major component of gluten, glutenin, is classified as a glutelin. Glutelins are proteins that are generally soluble in dilute acids or bases. Glutenin accounts for about 16% of the flour protein. The Osborne classification of proteins is helpful in that proteins with significantly different properties may be isolated based on their solubility in the solvents above. In practice, however, the

separations are not absolute. For example, wheat protein, if treated successively with these solvents, does not totally dissolve. Some wheat protein defies dissolution in any of these solvents; hence, an unclassified residue, which can account for 33% of the total protein, is always left (Atwell, 2001).

It is thus understandable that better classification schemes are needed. Biologists and biochemists suggest classification according to biological function. According to this proposition, proteins of cereal grains can be divided into two classes: metabolically active (or cytoplasmic) proteins and storage proteins. The former corresponds roughly to the group consisting of albumins and globulins (according to Osborne's classification), and the latter is comprised of the prolamins and glutelins. However, some overlapping of properties and function is possible (Lásztity, 1996). Concerning the distribution of metabolically active and storage proteins it can be stated that the proteins of the aleurone layer and germ belong mainly to the group of metabolically active proteins, and the storage proteins are presumably located in the endosperm. Cytoplasmic and storage proteins differ considerably in physical properties and amino acid composition. Generally the cytoplasmic proteins are easily soluble in water or salt buffer solutions, their molecular weight is relatively small, and the molecules have a globular form. The cytoplasmic protein group includes the most important metabolically active proteins, the membrane proteins, nonenzymic regulatory proteins, proteins of organelles, etc. The storage proteins of the endosperm are generally insoluble in water and salt solutions. It is characteristic for the endosperm storage proteins to include two types of proteins: a low molecular weight protein consisting of single polypeptide chains and having only intramolecular disulfide bonds, and high molecular weight proteins consisting of many polypeptide chains cross-linked by intermolecular disulfide bonds (Lásztity, 1996).

On the basis of the morphology of cereal grains the proteins may be divided into three groups: endosperm proteins, proteins of the aleurone layer, and proteins of the embryo (or germ). The protein concentration in the different morphological parts (endosperm, aleurone layer, and embryo) of the cereal grains shows big variations. The protein content of the germ is the highest (about 30%); a relatively high concentration (about 20%) may be observed in the aleurone layer; and the lowest protein concentration is evident in the endosperm. The ultrastructure of the endosperm of different cereal grains is similar. The storage proteins form a matrix and/or protein granules surrounding the starch granules. It has been observed by many research workers that the protein concentration varies from the inner to the outer endosperm parts. As a consequence of the differences in protein concentration and amino acid composition, the nutritive value of the whole grain is generally higher than that of the endosperm. Storage proteins are typically endosperm proteins. Nevertheless, smaller amounts of storage proteins may also be found in the aleurone layer and in the embryo (Lásztity, 1996). The different classification possibilities are summarized in Table 2.2.

Table 2.2. Possibilities for the classification of Cereal Proteins (Lásztity, 1996).

On the basis of morphology	On the basis of biological function	According to solubility (Osborne)	On the basis of chemical composition
Endosperm proteins Proteins of the aleurone layer Proteins of the embryo	Metabolically active cytoplasmic proteins Enzymes • Membrane proteins • Proteins of ribosomes • Regulatory proteins • Other proteins Storage proteins	Albumins Globulins Prolamins Glutelins Residue proteins	Simple proteins Complex proteins • Lipoproteins • Glycoproteins • Nucleoproteins • Metalloproteins • Chromoproteins

	• Low molecular weight proteins • High molecular weight proteins		• Phosphoprotein s

From the chemical point of view the existence of complex proteins is also interesting. In Osborne's classification the complex proteins were not taken into account. The newer investigations show that the protein-lipid and protein-carbohydrate interactions and the lipo- and glycoproteins play a very important role in the properties and technological value of different cereals (Lásztity, 1996).

2.3.2 Protein structure

Amino acids are the building blocks of proteins. Twenty of them occur naturally in most proteins, and each contains an amino group, a carboxylic acid group, and a side group (referred to as the R group). The amino groups and carboxylic acid groups are bound together in proteins to form peptide bonds and consequently string amino acids together to form long protein chains. The sequence of amino acids in the protein chain is called the primary structure of the protein. The R group is not involved in the peptide bond. However, the character of the R group influences how the protein interacts with other protein chains or other constituents in the system. The amino acid composition of the gluten proteins, gliadin and glutenin, is remarkable in that relatively few amino acids predominate. Glutamine, an amino acid that contains an amide side group that binds water well, constitutes over 40% of all the amino acids composing these proteins on a molar basis. Another amino acid composing about 15% of gliadin and 10 – 12% of glutenin is proline, which has a cyclic R group structure that puts a bend in a chain of amino acids (Atwell, 2001).

Often, protein chains coil and form helices that are considered a secondary structure of the protein. Another secondary structure of proteins is the “pleated sheet”, which occurs when chains fold back upon themselves. Proline inhibits the formation of these types of secondary structures in gluten. Although it constitutes only 1 – 3% of gluten proteins, another amino acid of significance is cysteine. Cysteine is unique in its ability to form bonds connecting protein chains with its sulphur-containing R group, which constitutes another type of secondary structure of proteins. The connections are called disulfide bonds, and the formation or destruction of them has a major effect on the size of gluten molecules. These three amino acids (glutamine, proline, and cysteine) play a major role in explaining the characteristics of gluten proteins, but gluten does contain other amino acids as well. They can be characterized into four types depending on the structure of their R group: acidic, basic, neutral hydrophilic and neutral hydrophobic (Table 2.3). The acidic and basic amino acids enter into interactions involving electrostatic attraction or repulsion, and the neutral amino acids exert and influence on how well the protein binds water (Atwell, 2001).

Table 2.3. The amino acids, grouped by charge and hydrophobicity (Morrison, 1988).

Acidic	**Basic**	**Neutral Hydrophilic**	**Neutral Hydrophobic**
Glutamic acid	Lysine	Glutamine	Valine, Cysteine, Glycine
Aspartic acid	Histidine	Asparagine	Leucine, Cystine
	Arginine	Serine	Isoleucine, Proline
	Tryptophan	Threonine	Alanine, Methionine
			Tyrosine, Phenylalanine

The tertiary structure of a protein involves the three-dimensional structure of the protein as a whole. How the R groups are oriented in space in such a structure dictates how the protein interacts with other molecules in its environment. If the tertiary structure is destroyed (e.g., by heat of shear) the protein is said to be denatured. Denatured proteins do not have the same characteristics as native or unaffected proteins, even though the primary and secondary structures are the same (Atwell, 2001).

The albumins and globulins of wheat have a significantly different amino acid composition than the gluten proteins have. There is less glutamine and proline and more of the basic amino acids and cysteine. Albumins and globulins usually have more secondary structure as a result. Many albumins and globulins are enzymes, which have a much defined tertiary structure. The spatial orientation of amino acids at the catalytic site of an enzyme is critical to the activity of the enzyme with respect to the reaction it catalyzes. In general, the molecules of albumins and globulins are smaller than those of the gluten proteins (Atwell, 2001).

Wheat storage proteins

Wall (1979) suggested that the unique, cohesive properties of wheat dough are due to its water-insoluble proteins. This conclusion is evidenced by the fact that one can extract lipids from a flour and wash away its starch, water-soluble carbohydrates and proteins, and other components and still retain a hydrated rubbery mass, the gluten, which is 80% protein (Lásztity, 1996).

The two storage protein fractions or groups, prolamins and glutelins, make up the bulk of the proteins in all cereals. In general, storage proteins constitute 80 – 85% of the total wheat and barley proteins (in nearly equal proportions) and 85 – 90% of the rice proteins (mainly glutelins). Rice and

oats have the lowest amounts of prolamin of the five major cereals. Analysis of the relative amounts of these protein classes may indicate general differences in quality type (e.g., prolamin-glutenlin ratio and dough properties in wheat), but information useful for identifying grain varieties has been provided mainly by qualitative aspects of composition within classes, particularly the prolamins (Lookhart, 1991).

Bietz and Wall (1973) showed that gluten can be divided into low-molecular-weight (alcohol-soluble; gliadins) and high-molecular-weight (alcohol-insoluble; glutenin) gluten-subunits. Gliadins are present chiefly as single polypeptide chains with intramolecular disulfide bonds and glutenins as high-molecular-weight aggregates stabilized by intermolecular disulphide bonds and noncovalent interactions (Wieser et al., 1994). The two fractions have quite different physical properties when hydrated, the gliadin fraction behaving as a viscous liquid and the glutenin fraction as a cohesive elastic solid (Schofield, 1986).

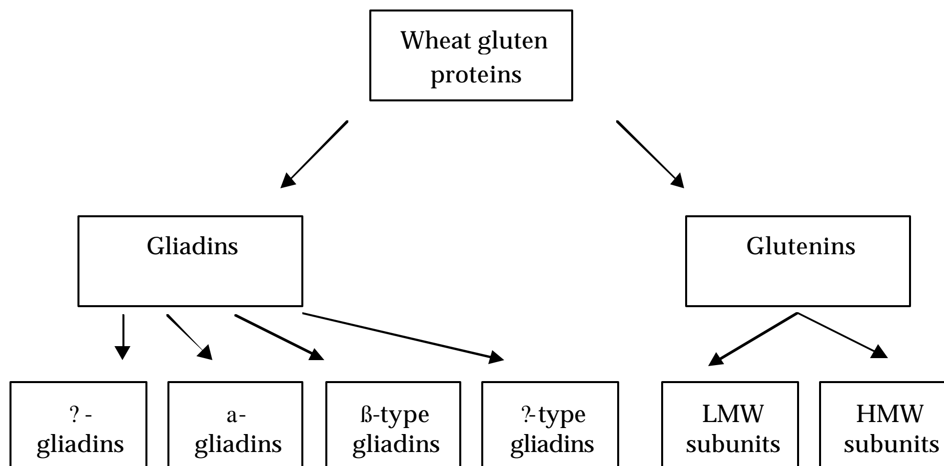

Figure 2.2. The classification of wheat gluten proteins (Compiled from data in the text).

It has long been established that rheological and bread-making quality differences among wheat varieties reside in the gluten proteins. The quantity can be analyzed easily and is strongly influenced by the environment and fertilizer application. Gluten quality depends primarily on the genotype and, hence, on the protein composition, and is difficult to define. The degree of glutenin aggregation, ratio of gliadin/glutenin, disulfphide and thiol content or presence of quality-associated proteins, in particular specific HMW subunits of glutenin, have been suggested as important factors for gluten quality (Wieser et al., 1994).

2.3.3 Low Molecular Weight Storage Proteins (Gliadins)

Kaczkowski and Tkachuk (1980) proposed the following definition for gliadins: "proteins of wheat endosperm soluble in alcohol such as 70 percent ethanol at room temperature, and which migrate in polyacrylamide and starch gels without reduction as reasonable discrete bands, and which are not excluded during gel filtration on Sephadex-G-100". Gliadins are monomeric proteins characterized by their high glutamine and proline content and by their solubility in neutral solutions containing high concentrations of alcohols. When fractionated by gel electrophoresis at low pH, they separate into four groups designated a-, ß-, ?- and ?-gliadins, in order of decreasing mobility (Lafiandra et al., 1994). An approach developed by Bushuk and Sapirstein (1991) defines three arbitrary gliadin bands (40.4, 53.2, 68.6) of a reference cultivar (Neepawa) as limits for the determination of the four groups: ? (<40.4), ? (40.4 – 53.2), ß (53.2 – 68.6), and a (>68.6).

All the gliadin components have an extremely high glutamic acid content. The ?-gliadins contain the highest proportion - in some ?-gliadins the glutamic acid content is higher than 50%. Almost all of the glutamic acid content of the gliadins is present as glutamine. Since glutamine provides a concentrated source of nitrogen that can be used readily by the germinating seed, it seems that the cereal grains, such as wheat, have a highly concentrated glutamine

reserve in the form of gliadins and other storage proteins. It may also be mentioned that the nitrogen reserve in the form of glutamine is the most economical from the perspective of biochemical pathways (Atwell, 2001).

The gliadins are also characterised by a high proline content (Atwell, 2001). The high proline and glutamine contents are the predominant basis for the name prolamins, the name being derived from the combined names of these amino acids (Gianibelli, 2001). Proline may be present in such large proportions for the same reason that glutamine is present in large quantities – it has a close connected pathway of biosynthesis to glutamic acid. The high proline content has an effect on the secondary structure of gliadin polypeptides because the formation of a-helices is hindered by the presence of proline side chains (Lásztity, 1996). Gliadin exists as single chains. Disulfide linkages exist, but they link cysteine R groups in the same chain. Because of the high level of proline, only about 20% of gliadin chains exist in a helical structure, and there is little evidence of pleated sheets. The tertiary structure is thought to be compact, with many binding interactions occurring between R groups within gliadin molecules (Atwell, 2001).

Gliadins are poor in basic amino acids, especially lysine. From the nutritional point of view, lysine is the limiting essential amino acid in the strorage proteins (and also in the whole grain) of wheat. The low levels of lysine, arginine, and histidine, along with the low levels of free carboxyl groups, place the gliadins among the least charged proteins known (Lásztity, 1996). The ?-gliadins have relatively high levels of phenylalanine in addition to their high content of glutamine and proline. Phenylalanine residues make up about 10% of the total residues in ?-gliadins. Low amounts of S-containing amino acids are also typical for ?-gliadins (Lásztity, 1996).

Due to the differences in cysteine content the gliadin components may be divided into S-rich (a-, ß-, and ?-gliadins) and S-poor (?-gliadins) components (Fig. 2.2) (Lásztity, 1996). The ?-gliadins are totally lacking in cysteine and are

not able to produce SS-type bonding (Gianibelli et al., 2001). Their surface hydrophobicity is lower than that of the a- and ?-type gliadins and they are the first peptides to elute from the RP-HPLC column (Popineau & Pineau, 1987). The aß- and ?-gliadins are rich in sulfur with six and eight cysteine residues, respectively. As a result, three and four disulfide bonds are formed. There are no free cysteines, and all S-S linkages are intramolecular, preventing gliadins from participating in the polymeric structure of glutenin (Müller & Wieser, 1995; 1997; Gianibelli, 2001).

On the basis of molecular weight the gliadin components may be divided into two groups. The a-, ß-, and ?-gliadins have molecular weights of about 30 kDa. The molecular weights of ?-gliadins, determined as well by ultracentrifugation as by SDS-PAGE or gel filtration, are approximately twice as high as those of a-, ß-, and ?-gliadins. The ?-gliadins are found in the smallest amount, followed by the a-, ß-, and ?-gliadins, respectively, in increasing amounts (Lásztity, 1996).

The gliadin composition is characteristic of the wheat variety. The gliadin pattern is not affected by growth conditions, by total protein content, or by sprouting (Lásztity, 1996). Using one-dimensional electrophoresis, gliadins of a single wheat grain can be separated into 20-25 components (Bushuk & Zillman, 1978; Metakovsky et al., 1984a). Two-dimensional electrophoresis allows better separation with a resolution of up to 50 components (Payne et al., 1982). Due to extensive polymorphism, these proteins have been widely used for cultivar identification.

The gliadin proteins of wheat are encoded by six complex loci (Law & Payne, 1983). *Gli-A1*, *Gli-B1*, and *Gli-D1*, coding for the ?- and ?-gliadins, are located distally on the short arms of chromosomes 1A, 1B, and 1D, respectively. The remaining three loci, predominantly coding for the a- and ß-gliadins and some of the ?-gliadins, are on the short arms of 6A, 6B and 6D and are designated *Gli-A2*, *Gli-B2*, and *Gli-D2*. Variation at each of these loci gives an

enormous range of proteins. Each of the *Gli-1* loci is closely linked to a locus coding for LMW glutenin subunits (*Glu-3*) (Rogers et al., 1989).

The number of gliadin encoding genes at the *Gli-1* and *Gli-2* loci has been estimated to be between 9 and 15 and between 9 and 12, respectively. This is a higher number than the number of major polypeptides expressed by these loci (Payne, 1987). One explanation for this discrepancy might be the presence of one or more pseudogenes at each *Gli* locus. This was similar to what was found in corresponding studies for the *Glu-1* loci (Payne et al., 1985). It might also be possible that some of the frequent polypeptides, in particular the ?-gliadins, are products of two or more different genes (Payne, 1987). For the *Gli-1* loci, 18 (A-genome), 16 (B-genome) and 12 (D-genome) alleles were described by Metakovsky (1991). Metakovsky (1991) also described 24 (*Gli-A2*), 22 (*Gli-B2*) and 19 (*Gli-D2*) alleles for the a- and ß-gliadins (*Glu-2* loci).

Genetic analyses of electrophoretic gliadin patterns have proved that gliadins are inherited in the form of definite groups or blocks (Sozinov & Poperelya, 1980; Metakovsky et al., 1984b; Payne et al., 1984a). A nomenclature has been developed for these blocks (Sozinov & Poperelya, 1980; Metakovsky et al., 1984b; Metakovsky, 1991). Several investigations on correlations between specific gliadins (Campbell et al. 1987) or gliadin blocks (Sozinov & Poperelya, 1980; Rogers et al., 1989; Mosleth & Uhlen, 1990) and bread-making quality have been carried out. Nevertheless, it has been suggested that most of the effects of gliadins, encoded on the *Gli-1* loci, on dough quality should be attributed to the LMW glutenin subunits associated with them (Gupta, 1994).

The proportions of both gliadin and glutenin in flour protein are influenced by both genotype and environment, with gliadin more sensitive to the environment (Panozzo & Eagles, 2000).

2.3.4 High Molecular Weight Storage proteins (Glutenins)

Glutenin is generally recognized as the wheat protein fraction most closely associated with bread-making quality. It is a highly aggregated, polymeric protein in its native (unreduced) state, with molecular weight estimations from 100 000 up to 20 million (large polymers) (Huebner & Wall, 1976; Bietz & Huebner, 1980; Tatham et al., 1985). Glutenin makes up approximately 55 - 70% of the gluten complex (Atwell, 2001). The glutenin subunits have two main characteristics: they are not soluble in dilute salt solutions and 70% ethanol, and the macromolecule is composed of polypeptide chains bound by disulfide bonds (Lásztity, 1996). The disulfide bonding occurs towards the end of the chains, so, in effect, the glutenin molecule is linear. The tertiary structure is thought to be one containing repetitive ß-turns, which form a ß-spiral structure. This type of structure is stabilized by hydrogen bonding and may explain the elastic nature of glutenin. When stress is applied, this stable conformation is disrupted, but it returns when the stress is absent (Tatham et al., 1985).

Native glutenin is, because of their high molecular weight, difficult to solubilize completely and to characterize. To do so, native glutenin must be treated with a reducing agent (to break disulfide bonds) (Gianibelli, 2001). The high molecular weight storage proteins may be separated by different techniques into fractions. One possibility for separation is based on solubility. Acetic acid is frequently used to solubilize glutenin. This method yields a soluble glutenin and residue protein. The ratio of these fractions may vary widely (MacRitchie et al., 1990). A major advance was made in characterizing glutenin subunits by using sodium dodecyl sulphate-polyacrylamide gel electrophoreses (SDS-PAGE) (Woychik et al., 1964). The component subunits, when reduced, can be separated by SDS-PAGE into two distinct groups,

termed high- (HMW) and low-molecular weight (LMW) glutenin subunits (Lafiandra et al., 1994).

Jackson et al. (1983) classified glutenin subunits according to their mobility in SDS-PAGE (after reduction of S-S bonds) and their relative acidity. The “A” group includes the entire group of HMW-GS, which have the slowest mobility (Fig. 2.3). Low molecular weight glutenin subunits collectively form the “B”, “C”, and “D” groups (Lásztity, 1996).

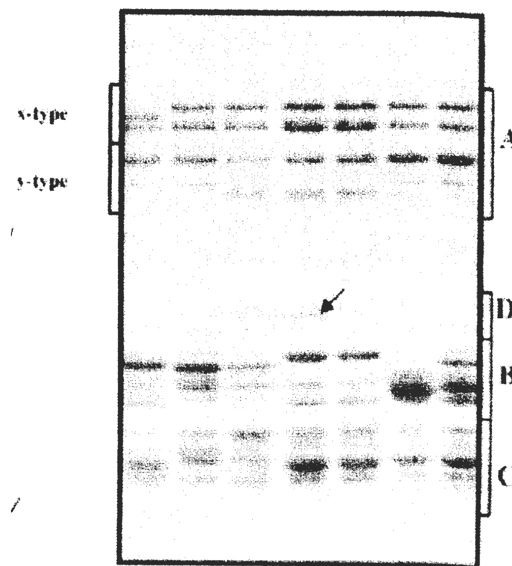

Figure 2.3. SDS-PAGE of polymeric protein (after reduction to subunits), performed according to the one-step one-dimensional procedure of Gupta and MacRitchie (1991) (Gianibelli unpublished results).

Group A: HMW glutenin subunits showing x- and y-type glutenin subunits. Group B, C & D: LMW glutenin subunits. Arrow indicates subunit D.

The “B” group is comprised of the greatest number of subunits, which are the most basic of the endosperm storage proteins and which are much slower in mobility than a-, ß-, and ?-gliadins. The “C” group present a wide range of

isoelectric points and overlaps with a-, ß-, and ?-gliadins in SDS-PAGE. The "D" subunits are the most acidic and are the slowest among LMW subunits (Lásztity, 1996).

The amino acid composition of high molecular weight storage proteins of wheat endosperm is in general similar to those of low molecular weight (gliadin) storage proteins. A slightly higher content of basic amino acids and lower amount of glutamic acid and proline may be observed. The average content of the amino acids having hydrophobic side chains is also smaller. Generally, glutenin preparations have a more hydrophilic character. The high level of glutamine residues has a very high capacity to form both intra- and intermolecular hydrogen bonds. Belton (1999) has postulated that this feature may be involved in elasticity through formation of intermolecular hydrogen bonds. In the dough, some of these bonds break on stretching, giving rise to unbonded mobile regions (loops) and bonded regions (trains). The loops can be stretched and then reform when the stress is removed, which accounts for the elastic restoring force, as in rubber elasticity.

HMW-GS and LMW-GS are cross-linked to form the so-called glutenin polymers, which are amongst the largest molecules in nature. HMW-GS comprise only a few components and have been widely studied, whereas LMW-GS include a large number of polypeptides and their structure, organisation and relationship to grain processing quality have not yet been investigated to the same degree as for the HMW-GS (D'Ovidio & Masci, 2004). Each cultivar contains 3 – 5 HMW and 7 – 16 LMW subunits of glutenin (Gupta & Shepherd, 1990).

a. The high molecular weight glutenin subunits (HMW-GS)

The HMW glutenin subunits are encoded by three loci, *Glu-A1*, *Glu-B1* and *Glu-D1*, located on the long arms of chromosomes 1A, 1B and 1D, respectively (Rogers et al., 1989). A catalogue of genes coding for HMW subunits of wheat is given by Payne and Lawrence (1983). At this time more than 40 different

HMW-GS have been found; they may be dassified into two subgroups: *x*-type and *y*-type subunits (Lásztity, 1996). Each locus includes two genes linked together encoding two different types of HMW-GS, *x*-type and *y*-type subunits (Payne et al., 1981; Shewry et al., 1992). The *x*-type subunits generally have a slower electrophoretic mobility in SDS-PAGE and higher molecular weight than the *y*-type subunits (see Figure 2.3) (Lafiandra et al., 1994). The *Glu-D1* locus encodes both *x*- and *y*-types of subunits, the *Glu-B1* locus can code for both types or only the *x*-type, and the *Glu-A1* locus can code only for the *x*-type subunit or neither (Payne et al., 1981). Molecular studies have demonstrated the presence of two genes at each of the three bci (Harberd et al., 1986). SDS-PAGE separation of HMW glutenin subunits from several bread wheat cultivars demonstrates a number of alleles at each locus (Payne & Lawrence, 1983). For the three *Glu-1* loci, 3 (*Glu-A1*), 11 (*Glu-B1*) and 6 (*Glu-D1*) alleles have been described by Payne and Lawrence (1983). Later, some additional HMW subunits of glutenins have been identified.

The HMW glutenin subunits can be easily distinguished by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Payne et al., 1979) and each cultivar contains between three and five subunits. Of these three to five subunits, two are encoded at *Glu-D1*, one or two at *Glu-B1* and none or one at *Glu-A1*. Despite the fact that bread wheats (hexaploid wheats) possess six HMW-GS genes, the number of expressed subunits ranges from three to five because of gene silencing (gene inactivation) processes which have occurred during wheat evolutionary history (Forde et al., 1985; Lafiandra et al., 2000b). The y-type gene present at the *Glu-A1* locus is always silent in tetraploid and hexaploid cultivated wheats, whereas the x-type gene at the same locus and the y-type gene at the *Glu-B1* locus are expressed only in some cultivars; this leads to variation in the number of subunits from three to five in bread wheat and from two to three in durum wheat (Lafiandra et al., 2000b).

The numbering system developed by Payne and Lawrence (1983) to identify HMW-GS according to electrophoretic mobility also provides a chromosomal location of the genes and is the system in current use (Fig. 2.4). The HMW-GS designated 1A1 is a subunit coded by chromosome 1A having the lowest electrophoretic mobility (Lásztity, 1996). Originally, the assignment of ascending numbers was related to the mobility in SDS-PAGE, lower numbers equating to lower mobility. As new subunits have been identified, there has been difficulty in following this logical order. Thus, there are some subunits, such as 21, with lower mobility and higher number than the original subunits. When identifying subunits numerically, it is customary to include both the genome from which the subunit is derived and the indication of whether it is an *x*-type or *y*-type subunit (e.g., D*x*5) (Gianibelli, 2001).

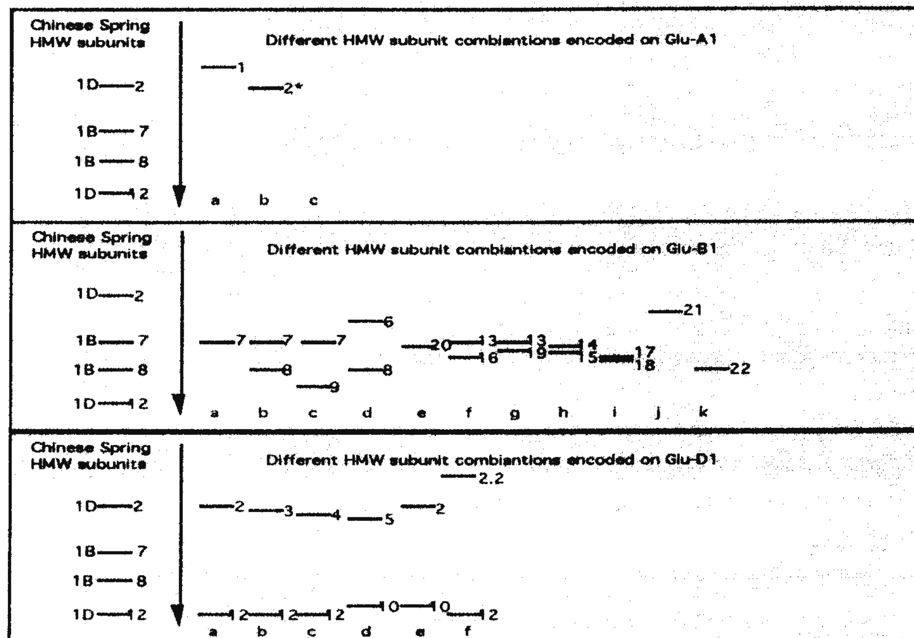

Figure 2.4. Allelic variation of the HMW subunits of glutenin. The subunits are split into three groups according to the localization of the encoding genes. The arrows indicate the direction of electrophoresis. On the left hand side the

subunits present in Chinese Spring is given as a standard pattern. The HMW subunits of glutenin are denoted by numbers and the alleles by letters (Payne & Lawrence, 1983).

Important work by Payne and coworkers (1987) established that dough strength and baking performance of wheat cultivars were related to allelic variation in HMS-GS. As a result of correlation of different alleles with dough properties, a system of quality scores was assigned to HMW-GS. Each HMW-GS has been assigned a number, and the presence of certain combinations of these subunits is related to different quality aspects (Payne et al., 1981). The HMW-GS 5+10 are said to be present in varieties of good baking performance, whereas 2+12 are present in varieties of poor performance (Payne et al., 1981). On the basis of these results, quality scores are assigned to each of the HMW subunits, and by adding these scores for the different HMW subunits present, a quality score, called the *Glu-1* score, is obtained for each wheat variety. The highest score is obtained for the subunits 5+10. The *Glu-1* score is positively correlated with baking performance in the case of bread, and 47 to 60 percent of the variation in bread-making qualities could be accounted for by the variation in HMW subunits. The quality score is negatively correlated with the baking performance in the case of biscuits, which is in accordance with what is expected (Payne et al., 1987).

The quality scores assigned to the HMW-GS range from 0 (null allele) to 4 (Table 2.4). The HMW-GS pair 5+10 coded by *Glu-D1* has been assigned a score of 4 as this pair has been associated with the greatest dough strength. Its *Glu-D1* counterpart, the pair 2+12, on the other hand has been assigned a score of 2, reflecting its association with dough weakness. Based on similar correlations at the *Glu-B1* locus, the pair 17+18 was given a score of 3, whereas the subunit 20x+20y, also coded at *Glu-B1*, was given a score of 1 (Southan & MacRitchie, 1999).

Table 2.4. Quality scores assigned to HMW glutenin subunits encoded on the different chromosomes according to Payne et al. (1987).

Score	Chromosome		
	1A	1B	1C
4	-	-	5+10
3	1	17+18	-
3	2*	7+8	-
2	-	7+9	2+12
2	-	-	3+12
1	null	7	4+12
1	-	6+8	-

One aspect that is sometimes overlooked when using this scoring system is that subunits with the same electrophoretic mobility in SDS-PAGE differ in some other features like small differences in protein sequences and surface hydrophobicity (Gianibelli, 2001). For example, after the *Glu-1* score was established, Sutton (1991) found differences in retention time for subunit 8 in some cultivars when subjected to RP-HPLC. He concluded that two different subunits 8 were involved (8 and 8*). Also, different electrophoretic mobilities were recorded for subunit 7 (7 and 7*). Thus, four different alleles, instead of just one, are expected for this pair (7+8; 7*+8; 7+8*; 7*+8*) (Marchylo et al., 1992).

It is significant that subunits 5+10 and subunits 2+12 are coded by genes on the D-genome. This is the genome that distinguishes bread wheats from durums. This probably explains why HMW glutenin subunits have not been found to be associated in any way with dough properties in durum wheats.

The differential effects of the HMW subunits of glutenin appear to be strongest for those coded by chromosome 1D, followed closely by chromosome 1A with the 1B chromosomes the least effective (Payne et al., 1981). Although durum wheat is mostly used for pasta production, its use for the preparation of bread is also widespread, especially in many Mediterranean countries, in spite of the fact that durum wheat bread-making quality is inferior to that of bread wheat. The poor performance of durum wheat in bread-making has been attributed to the absence of the D-genome related proteins (Lafiandra et al., 2000a).

Among allelic HMW subunits controlled by the *Glu-A1* locus on chromosome 1A, bands 1 and 2* have an equal positive effect over the null allele, suggesting a quantitative effect. Similarly, among several alleles at the *Glu-B1* locus on chromosome 1B, those producing double bands or intensely staining bands (for example subunits 7+8, 13+16, and 17+18) are associated with superior bread-making quality compared with those with single or faint bands (for example subunits 7, 20, and 6+8) (Singh et al., 1990a). From the above it is clear that chromosome 1A consists of band null, band 1, and band 2*. Bands 6+8, 7+8, 7+9, 13+16, and bands 17+18 are found on chromosome 1B, while bands 5+10 and 2+12 are found on chromosome 1D.

The mature HMW glutenin subunit has three distinct domains (Shewry & Tatham, 1989); a central domain, composed of repetitive sequences (Harberd et al., 1986), flanked by non-repetitive domains at the N- and C-terminal ends (Halford et al., 1987). One of the features of HMW subunits is that they contain large amounts of glutamine (35 mol%), glycine (20 mol%) and proline (10 mol%) (Tatham et al., 1990).

The role of the HMW subunits in gluten elasticity relates to their presence in high molecular weight glutenin polymers. These polymers are stabilised by the formation of inter-chain disulphide bonds between cysteine residues. The number and distribution of these bonds will have an influence on their size

and biophysical properties (Shewry et al., 1992). The amino acid sequences of the HMW subunits show the presence of four to seven cysteine residues, which are predominantly located in the N-terminal and C-terminal domains (Buonocore et al., 1996; Shewry et al., 1992). One cysteine residue is present in the C-terminal domains of all the HMW subunits. In the N-terminal domain three cysteine residues (in the *x*-type subunits) or five (in the *y*-type subunits) are present. The 1B*y* and 1D*y* subunits contain single cysteine residues towards the C-terminal ends of their repetitive domains. Subunit 1D*x*5 has an additional cysteine residue at the N-terminal end of this domain. The latter subunit is part of the quality-related subunit pair 1D*x*5 and 1D*y*10. The additional cysteine residue is not present in the poor quality related subunit 1Dx2 (Shewry et al., 1992). The disulphide bonds are very important. Cleavage of disulphide bonds causes a decrease in viscosity, because the polymers break into smaller pieces (Bietz & Lookhart, 1996).

The existence of ß-turns has been suggested as the other reason for the elasticity of glutenins. The conformation of the HMW glutenin is similar to that of the ?-gliadins (Tatham et al., 1985). HMW glutenins are linear proteins and the conformation is characterised by a large proportion of ß-turns in the central domain. The terminal domains contain some a-helix structure (Tatham et al., 1985). HMW subunits are characterised by high levels of mobility in the presence of water and they consist of ß-turns and ß-sheets in proportions that vary with water content (Belton, 1994). In the absence of water the chains will tend to hydrogen bond to each other to form a dense mass. As water is added, there will be an increase in the number of water-protein hydrogen bonds formed, but the number of interchain hydrogen bonds will ensure that it is very unlikely that all the interchain hydrogen bonds will break simultaneously. There will always be a balance between the residues involved in interchain hydrogen bonds and those that are hydrated (Belton, 1999).

b. The low molecular weight glutenin subunits (LMW-GS)

The low molecular weight glutenin subunits have been shown to be very heterogeneous in size and charge and have been subdivided into B, C and D groups according to their biochemical characteristics (Lafiandra et al., 2000b). The LMW-GS represent about one-third of the total seed protein and about 60% of the total glutenins (Bietz & Wall, 1973). Despite their abundance, they have received much less research attention than the HMW-GS. It has been mainly due to the difficulty in identifying them in one-dimensional SDS-PAGE gels (Gianibelli, 2001), or by other one-dimensional systems such as isoelectric focusing (IEF) or electrophoresis in aluminium lactate buffer at pH 3.1. On one-dimensional SDS-PAGE gels many LMW glutenin subunits overlap with gliadins (Zhen & Mares, 1991) and until recently, solubility fractionation methods for wheat flour proteins did not yield these subunits free of appreciable contamination with other gluten proteins (Skerritt & Robson, 1990). The attempts to purify LMW glutenin subunits presented considerable difficulties, because of the heterogeneous and insoluble nature of LMW glutenin subunits and their strong tendency to aggregate (Melas et al., 1994).

Studies to date suggest that the LMW glutenin subunits share similarities with both gliadins and HMW glutenin subunits (Bietz & Wall, 1973), since a-, ß-, ?-gliadins and LMW glutenin subunits have similar electrophoretic mobilities (similar molecular size) and are both soluble in aqueous ethanol (Bietz & Rothfus, 1970). Jackson et al. (1983) showed that all the 14 major LMW glutenin subunits have different positions to the a-, ß-, ?- and ?-gliadins on the composite two-dimensional map and their conclusion was that they are different and distinctive proteins. At this time no generally accepted nomenclature for LMW-GS is available. This fact is connected with the relatively fewer number of studies about this type of subunit and to the problems caused by overlapping of LMW-GS and gliadin electrophoretic spectra. It seems that a system similar to that used for HMW-GS must be developed (Lásztity, 1996).

The introduction of a two-step, one-dimensional SDS-PAGE procedure by Singh and Shepherd (1988) provided a rapid method for analysing a large number of samples in a single gel in a gliadin-free background. Using this method, they were able to map the genes controlling some of these subunits at the *Glu-3* loci, and further investigations revealed extensive variation for these subunits in durum and bread wheats (Gupta & Shepherd, 1988). This two-step method also separated some globulins and albumins with similar mobility to the B and C subunits of glutenin. The procedure has now been modified to allow the latter subunits to be analysed in a background free from globulins and albumins (Gupta & Shepherd, 1990). RP-HPLC has also proved useful for the study of LMW-GS, showing that these proteins have higher hydrophobic surfaces than those from HMW-GS and comparable with the hydrophobic surfaces of gliadins (Gianibelli, 2001).

Acidic polyacrylamide gel electrophoresis (A-PAGE) or high performance liquid chromatography (HPLC) analyses of gliadins can uncover more variation than one-dimensional SDS-PAGE, largely because they can detect biochemical changes (amino acid substitutions) that occur in the absence of molecular weight alterations. However, SDS-PAGE may provide molecular weight estimations. Knowledge of the molecular weight of various endosperm storage proteins will facilitate the identification of both the proteins and the gene sequences responsible for their production. Molecular weight estimations also provide information regarding relationships between the proteins of different classes (Graybosch & Morris, 1990).

The genes controlling the synthesis of the major, basic structures of LMW glutenin are located on the short arms of chromosome 1A, 1B, and 1D (*Glu-3* loci), near the gliadin-coding loci (?-gliadins and ?-gliadins) (Jackson et al., 1983). Chromosome 1A encodes relatively few LMW-GS. Some cultivars do not exhibit any LMW-GS encoded by *Glu-A3*. On the other hand, there is extensive polymorphism for LMW-GS encoded by chromosome 1B (Lew et al., 1992; Gupta & Shepherd, 1993). On the basis of screening a collection of

222 hexaploid wheats from different countries, Gupta and Shepherd (1990) detected 20 different band patterns (LMW-GS blocks), six for the *Glu-A3* locus, nine for the *Glu-B3* locus, and five for the *Glu-D3* locus. The only genes of LMW glutenin subunits not located on group 1 chromosomes are the minor components which migrate to similar positions on IEF and SDS-PAGE gels as the group 6 a- and ß-gliadins (Jackson et al., 1983). These LMW-GS are controlled by genes on group 6 chromosomes (*Glu-2* loci) (Lew et al., 1992; Gupta & Shepherd, 1993; Rogers et al., 1989).

Because the actual number of electrophoretic band combinations in LMW-GS is much lower than the total of randomly possible combinations, it is assumed that the genes controlling LMW-GS are closely linked. They form clusters that are inherited together, similar to those controlling gliadin blocks (Gupta & Shepherd, 1990). Genes for LMW glutenin subunits on the short arms of homoeologous group 1 chromosomes (*Glu-3* loci) have been found to be closely linked to the group 1 gliadin genes (*Gli-1* loci) (Singh & Shepherd, 1988). The *Gli-1* multigene loci encode ?- and ?-gliadins and some ß-gliadins at the distal ends of the short arms of chromosomes 1A, 1B, and 1D. This close linkage (estimated as 2cM between *Glu-B3* and *Gli-B1* on the short arm of chromosome 1B in both bread and durum wheat) is useful for identifying the *Glu-B3* alleles and some of the *Glu-D3* alleles in breeding programs. Because the gliadin composition can be screened more readily than specific LMW-GS, the gliadins are potentially useful as indicators of LMW-GS alleles (Singh et al., 1991b, Jackson et al., 1996).

Shepherd (1988) stressed the importance of achieving genetic separations between products of genes encoding LMW glutenin subunits, and those controlling the production of linked gliadin subunits, as a means of ascertaining the quality effects of LMW subunits. Since a number of LMW glutenin subunits and gliadin bands are controlled by a cluster of very tightly linked genes, for practical screening purposes any protein band of a specific gene cluster (or “block”) should give an indication of the LMW subunit allele

present. The inherent danger with this approach is that a low level of recombination can occur within some gliadin/LMS subunit blocks. Consequently, the gliadin bands can only be used as an indicator rather than confirmatory evidence for the presence of specific LMW subunit alleles, especially when dealing with cultivars or lines of unknown origin, where rare recombinants may have been fixed. This approach is very convenient for analysing segregating progenies from crosses between cultivars of known LMW subunits/gliadin patterns, because the level of recombination is extremely low (Singh et al., 1991a).

LMW glutenin subunits form B, C and D groups (Payne & Corfield, 1979). Most of the typical LMW subunits are present in the B group (Masci et al., 2002; Tao & Kasarda, 1989). On the basis of N-terminal amino acid sequences, three subgroups of typical LMW-GS can be recognized, called LMW-s, LMW-m, and LMW-i types, according to the first amino acid residue of the mature protein: serine, methionine, or isoleucine, respectively. LMW-s type subunits are the most abundant in all genotypes analysed and their average molecular mass (35 000 – 45 000) is higher than that of LMW-m type subunits (30 000 – 40 000). The N-terminal amino acid sequences of LMW-s type subunits is SHIPGL-, whereas the N-termainal sequences of LMW-m type subunits are more variable and include METSHIGPL-, METSRIPGL-, and METSCIPGL- (Tao & Kasarda, 1989; Lew et al., 1992; Masci et al., 1995). However, both LMW-s and LMW-m type subunits contain eight cysteine residues, two of which are involved in intermolecular disulphide bonds. Among typical LMW-GS, the LMW-i type can be considered as a variant form. These LMW-GS lack the N-terminal region, starting directly with the repetitive domain after the signal sequence, with ISQQQQ- being the deduced N-terminal sequence of all LMW-I type genes isolated so far. Although the N-terminal region is missing, the typical eight cysteine residues are all present in the C-terminal domain (Cloutier et al., 2001). The B group LMW-GS are the most

basic of the major endosperm storage proteins and have lower mobilities than a-, ß- and ?-gliadins in SDS-PAGE (Payne et al., 1985).

The C group consists of a minor amount of typical LMW-GS and mainly of a- and ?-gliadin type LMW-GS (Kasarda et al., 1988). This group has a wider range of isoelectric points and SDS-PAGE mobility similar to that of the a-, ß- and ?-gliadins. Subunits in the D group are the most acidic and have the lowest mobilities among the LMW glutenin subunits (Payne et al., 1985). Genes encoding D-subunits are completely linked to the *Gli-D1* locus on chromosome 1D (Payne et al., 1986; Pogna et al., 1995). On chromosome 1B, they are located at a separate locus originally designated as *Glu-B2*. Masci et al. (1993) has shown that D glutenin subunits are in fact mutated ?-gliadins that have acquired at least one cysteine residue. The LMW glutenins have relatively large amounts of cysteine and their disulphide bonds are highly reactive. The identification of cystein residues in ?-gliadins, which typically lack this amino acid residue, was the first evidence that gliadin-like subunits were present in the glutenin polymers. It is probable that the D-subunits form part of the glutenin fraction because the numbers of cysteine residues is different from that in the a- and ?-gliadins (Masci et al., 1999). Among the three LMW-GS groups, the C-subunits are the least characterised, because of their low level of expression compared to B-subunits.

On the basis of the structural characteristics of the B, C and D groups, Kasarda (1989) suggested the existence of two functional groups of LMW-GS. One group, which includes the majority of the B-type subunits, acts as chain extenders of the growing polymers because of their ability to form two intermolecular disulphide bonds. The second group, which includes most of the C and D-type LMW subunits, act as chain terminators of the growing polymer, having only one cysteine available to form an intermolecular disulphide bond.

The average molecular weight of the LMW glutenins (40 kDa) is about half the average of the HMW glutenin subunits (average 85kDa). There are thus about six molecules of LMW glutenin subunits for every HMW glutenin molecule. The molar ratio is 6:1. With characters such as formation of large polymers and dough strength, the HMW glutenins have a greater influence than the LMW glutenin subunits and it can be calculated that the effect of one HMW subunit molecule is four times that of one LMW subunit molecule (Gupta et al., 1995).

2.4 Wheat Quality

Wheat flour is an organic complex in which starch interacts with both gluten and non-gluten proteins (largely albumins and globulins), lipids, and non-starch carbohydrates. A large portion of variation observed in flour quality may be attributed to variation in gluten protein content and composition (Bietz, 1988). Thus, breeding for improved bread-making quality can be done either by increasing the protein concentration or altering the protein composition of cultivars. Increased grain protein concentration will improve the volume and the texture of baked loaves considerably. Unfortunately, increasing of the protein concentration is hampered by a negative correlation between the yield and the protein concentration of the grains (Halloram, 1981; Johnson et al., 1985). Breeding for increased protein concentration is therefore a difficult task, although not impossible.

Flour protein composition, or the distribution of flour protein in classes based on molecular size and solubility, has been reported to be a major variable influencing wheat processing quality (Graybosch et al., 1996). A large part of the variation in bread-making quality between cultivars has been ascribed to the HMW glutenin subunit genotype (Payne et al., 1987). The contribution of the HMW glutenin subunit alleles to the quality has been determined by relating the presence or absence of an allele in cultivars or in segregating progenies to the bread-making quality. So far, only the contribution of the most frequent alleles has been determined. The identified HMW glutenin subunit alleles, influencing the dough gluten strength in a positive or negative way, can be used by plant breeders for improving bread-making quality.

2.4.1 Protein composition (functionality of the gluten components)

The protein fraction is the most important in determining the bread-making quality in wheat (Wall, 1979). When wheat flours are mixed with water, the

water-insoluble storage poteins hydrate and form a gluten matrix, a complex coherent network in which components as starch and gas cells are embedded. During kneading, the protein strands are further extended into protein films. Thus, gluten constitutes the skeleton of the dough. Its protein components, giving rise to the viscoelastic properties, are responsible for the gas retention during processing and determine the flour's suitability for bread-making (Eliasson & Lundh, 1989; Amend & Belitz, 1990). Good elasticity and aggregation behaviour appear to be a basic requirement for bread dough formation.

The importance of gliadins and glutenins in influencing gluten rheological properties is well established (MacRitchie et al., 1990). Gliadins are responsible for dough extensibility and viscosity while glutenins are important for dough strength (resistance to extension) and elasticity (Bietz et al., 1973; Bietz & Huebner, 1980). The commercially desirable viscoelastic poperties required for good performance in processing wheat flour doughs result from the combined contributions of these two main types of proteins (Southan & MacRitchie, 1999).

The molecular weight distributions of glutenin polymers play an important role in determining flour viscoelastic properties (Wrigley & Bietz, 1988). According to Huebner and Wall (1976), cultivars possessing good bread-making quality have high ratios of higher molecular weight to lower molecular weight glutenins. Previous studies have indicated a direct correlation between the size and the amount of the glutenin polymers and quality characteristics (Dachkevitch & Autran, 1989). It has been found that higher molecular weight glutenin polymers contain a higher proportion of HMW-GS (Huebner & Wall, 1976). Flours of weak-dough wheats generally have lesser amounts of HMW glutenins and unextracted protein. HMW glutenin molecules result in long mixing times and high dough stability (Bietz et al., 1973; Bietz & Huebner, 1980). A variety with a very short mixing requirement almost invariably has a low loaf volume potential. Similarly, a

variety with a medium to medium-long mixing requirement almost invariably has a good to very good loaf volume potential (Finney & Yamazaki, 1967).

A major problem in working with gluten proteins has been the difficulty of solubilising the total protein. Gluten has strong aggregation tendencies generally assumed to result from the hydrogen bonding potential of the usually large numbers of glutamine side chains. Also of importance is the large potential for apolar bonding of the many nonpolar side chains and gluten's low ionic character. The gluten from poor-quality flour has higher solubility than the gluten from good-quality flour. The differences in protein solubilisation between different quality flours suggest that the proteins in poor-quality flour might have smaller molecular weights or possess less of a tendency to interact with themselves (He & Hoseney, 1990).

Because loaf volume is a function of gliadin quality and the mixing requirement is a function of glutenin quality, good- and poor-quality glutenins are related to or associated with good- and poor-quality gliadins, respectively. These relationships are diagrammed in Figure 2.5.

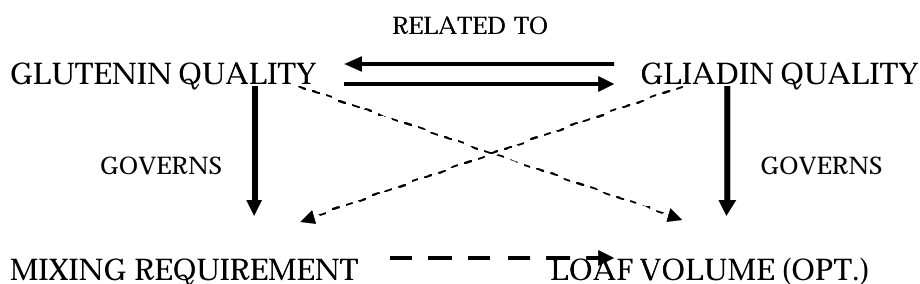

Figure 2.5. Diagram of likely direct and indirect relationships between glutenin quality, gliadin quality, and the functional properties, mixing requirement and loaf volume (optimum). Broken lines indicate indirect relationships. The wider the line, the stronger the relationships. Also (not shown), glutenin quality governs mixing tolerance and dough absorption

(attributable to flour proteins). Oxidation requirement and dough stability are strongly related indirectly to mixing requirement (Finney et al., 1982).

a. High molecular weight glutenin subunits (HMW-GS)

The 21 chromosomes (gametic number) of hexaploid wheat have been divided into seven homoeologous groups. Each homoeologous group contains three partially homologous chromosomes, one chromosome from each of the A, B, and D genomes. Chromosomes are identified by the homoeologous group number (1 to 7), and the genome (A, B, or D) from which the chromosome originated (Poehlman & Sleper, 1995). As mentioned previously, the genes coding for HMW subunits are located on the long arms of chromosomes 1A, 1B, and 1D.

Within the context of improving protein quality (e.g., high extensibility, appropriate dough strength) by wheat breeding, research has been conclusive about the importance of glutenin, with emphasis on those subunits of high molecular weight (HMW), particularly those controlled by the D genome (Payne et al., 1981; 1987; Branlard & Dardevet, 1985). The emphasis on the HMW glutenin subunits initially arose from accessibility for analysis, appearing as they do at the top of an electroporesis gel pattern (using the routine method with SDS); well separated from all the other polypeptide bands (Fig. 2.3) (Gianibelli et al., 2001).

The first subunit reported to be associated with good bread-making quality was subunit 1 encoded on chromosome 1A. It was compared with the null-allele from chromosome 1A, in cultivars grown in Great Britian (Payne et al., 1979). The positive effects of subunit 1 were suggested to be due either to its unique structure, enabling larger and more stable protein aggregates to be formed, or to an increased total amount of HMW subunits of glutenin. Later, strong correlations were also detected between the presence of subunits 5+10

encoded on chromosome 1D, and good bread-making quality (Payne et al., 1981). Several other studies, analyzing other sets of wheat cultivars and breeding lines, have confirmed the correlation between HMW glutenin subunits 5+10 and high gluten strength (Branlard & Dardevet, 1985; Campbell et al., 1987; Cressey et al., 1987). The allelic counterparts, subunits 2+12 were found to be negatively correlated with good bread-making quality (Payne et al., 1981). Slight differences in the amino acid sequences of subunits 2 and 5, causing differences in their three-dimensional structures, were suggested as an explanation. Evidence that glutenin subunits 5 and 10 produce "stronger" wheats than glutenin subunits 2 and 12 have been confirmed in laboratories elsewhere. For example, a study by Lawrence et al. (1987) demonstrated that subunits 5+10 at the *Glu-D1* locus confer significantly greater dough resistance than subunits 2+12.

The relationships between subunits encoded on chromosome 1B and quality were less clear in the study by Payne et al. (1981). Subunits 13+16 and 7+9 were found to provide better quality than subunits 6+8 based on analyses of progenies from one cross segregating for these alleles. Other allelic variant pairs had similar results: Subunits 14+15 showed a trend towards better quality compared with subunit 7 in progenies from another cross (Payne et al., 1981). *Glu-B1* subunits 17+18 showed greater dough strength than 20x+20y. These differences in dough strength were due to differences in molecular size of glutenin polymers deduced from solubility measurements (Gupta & MacRitchie, 1994). Branlard and Dardevet (1985) also reported that the alveograph parameters W (gluten strength) and P (tenacity), and the Zeleny sedimentation value are correlated positively with subunits 7+9 and 5+10, and negatively with bands 2+12, whereas subunit 1 is correlated to W and subunits 2* and 17+18 with G (extensibility). Sabine et al. (1997) reported that the *Glu-B1* allele 7+9 and the *Glu-D1* allele 5+10 were more frequent in the cultivars with better bread-making quality, and no cultivar with good quality contained the subunits 6+8 and 2+12. Lukow et al. (1989) having

studied the HMW subunit composition of a great number of Canadian varieties, obtained data for the high positive effect of the allele 5+10 on the wheat quality. Their conclusion is that the best quality of a wheat variety is composed when 1A subunits 1 or 2*; or 1B subunits 7+8, 7+9, 13+16; and 1 D subunits 5+10 were invariably present.

A study by Peter et al. (1998b), that showed that the allele 2* had a better effect in comparison to allele 1 controlled by locus 1A, disagree with the data of Schepers et al. (1993), showing an advantage of allele 1 against allele 2* concerning their effect on sedimentation value. According to Gyula et al. (1998) the old Hungarian variety Bánkúti 1201 possesses excellent technological quality parameters despite the fact that it bears the HMW-subunits 2+12 on chromosome 1D. This signifies the importance of breeders in different geographic areas to evaluate their genetic material so as to know which subunits has more influence on quality. The presence of high molecular weight subunit 1 or 2* in a hard wheat is usually an indication of a strong wheat for good bread making quality (Payne et al., 1987).

A new high Mr subunit, 21*, has been found in some Swedish wheats. The new subunit was designated 21* since it migrated similarly to high Mr glutenin subunit 21 on SDS-PAGE. However, band 21 and band 21* had consistently different mobilities on SDS-PAGE (Johansson et al., 1993). The presence of subunit 21* gave a higher sedimentation volume, shorter mixogram development times, more optimal alveogram values, and higher loaf volumes than the presence of the other 1A encoded subunits (Johansson & Svensson, 1995). Presence of the subunit 21* did not only give longer mixogram development times than the presence of subunit 1 or 2* but has also shown good bread-making quality (MacRitchie et al., 1990). The Swedish baking industy has problems with not only too weak gluten, but also with too strong gluten. Cultivars containing subunits 5+10, which are strongly positively correlated with gluten strength often give too strong flour for the Swedish baking requirements (Johansson & Svensson, 1995). An optimal

HMW subunit combination for the Swedish baking conditions might be: 21*=21*y subunits, encoded on chromosome 1A, conferring positive properties to the dough; 14+15, encoded on chromosome 1B, and positively correlated to gluten strength (although not to the same extent as for 5+10); 2+12 encoded on chromosome 1D, negatively correlated with gluten strength and thus not giving the overstrong properties of 5+10. However, the best subunit combination is dependent on the protein concentration of the cultivar, the rest of the genetical background, the yearly weather fluctuations and other factors.

There are two types of high molecular weight subunits, termed x- and y-type subunits, in order of decreasing molecular weight. Whether bread-making quality is mainly influenced by x- or y-subunits or if both play an important role has not been ascertained (Flavell et al., 1989). x-Type subunits, which include 2*, 7, and 5, have a lower mobility than their counterpart y-type subunits (9 and 10) on the SDS-PAGE gel (both x and y subunits are controlled by one gene locus), because of their higher molecular weight (Huang & Khan, 1997). Subunits 5 and 2* have the largest molecular sizes (827 and 794 amino acid residues, respectively) among all gluten protein subunits identified so far (Shewry et al., 1992) and show negative correlations with dough mixing tolerance and mixing time (Huang & Khan, 1997). Subunit 10 (with a smaller molecular size) shows positive correlations with flour dough strength (0.83* with stability and 0.86* with mixing time). Based on these correlations, subunits with a relatively higher molecular weight, such as subunits 5 and 2*, seems to contribute more to the strength of the gluten polymer network than those with a lower molecular weight, such as subunit 10. Comparing the two subunits with the highest molecular weight, 5 and 2*, we can also see the effects of differences in molecular size of these two subunits on flour strength. Subunit 5 has a higher molecular weight than subunit 2* and a stronger correlation ($r > 0.9$, significant at 0.01 level) with flour strength than subunit 2* ($r = \sim 0.8$, significant at 0.05 level). Therefore,

the functionality (mixing properties) of individual HMW glutenin subunits seems to be related to their molecular chain length (Huang & Khan, 1997).

Ever since Payne et al. (1979) published their findings that the presence or absence of HMW glutenin subunits was related to bread-making quality, numerous investigations have been done to find out why some HMW subunits have a “better quality” than others. The chemical characterization of HMW glutenin subunits reported so far shows that subunits do not differ substantially from each other in their overall molecular structures (MacRitchie et al., 1990; Shewrey et al., 1992). Little molecular basis could be found to differentiate their functionality by comparing their molecular structures. Green et al. (1988) reported that subunits 5 and 2 were 98.7% identical and subunits 10 and 12 were 97.8% identical in DNA-derived sequence homology. The observation that subunit 1Dx5 has an extra-cysteine compared to 1Dx2, explains its capability to form higher molecular weight polymers (Lafiandra et al., 1993; Gupta & MacRitchie, 1994). Recently evidence has been reported that supports the hypothesis that both x- and y-subunits might be necessary for an efficient polymerisation process (Shimoni et al., 1997).

Based on the work of Payne et al. (1987) that related individual HMW glutenin subunits to bread-making quality by genetic analysis, quality scores were assigned to each of the commonly occurring subunits (Table 2.4). The quality scores of each of the subunits were summed to create a *Glu-1* quality score for each variety. Between 47 and 60% of the variation in bread-making qualities could be accounted for by variation in HMW subunits of glutenin (Refer back to 2.3.4a). However, limited variation in HMW glutenin subunit composition among hard red spring wheats (Khan et al., 1989) may restrict the application of the *Glu-1* score to certain classes of wheat or breeding programs. Thus, there are significant limitations in using either *Glu-1* scores or protein solubility characteristics in hard red winter wheat breeding. When working with wheats with average to above average quality, these predictors have little useful value.

Usually, the combined use of gliadin and glutenin markers give the best results in predicting baking quality. For example, gliadin marker 1B3 and glutenin markers 1B6+8 and 1D2+12 were associated with low baking quality during a study of winter wheat varieties by Sasek et al. (1986). Campbell et al. (1987) also selected wheat lines for a study of statistical correlations between quality attributes and the protein composition of grain. The protein components most consistently implicated were the HMW glutenin subunits 5 and 10 and gliadin 59 (correlated with high resistance to dough extension) and glutenin subunits 2 and 12 and gliadin 58 (with dough weakness).

HMW glutenin subunit combinations could not always explain many differences in baking quality and other factors must be responsible. Studies showed that one of these factors could be the aggregative tendency of glutenin with nonprotein constituents such as carbohydrate and lipids (Bekes et al., 1983; Zawistowska, 1985). It is generally accepted that nonprotein components play an important role in the functionality of wheat proteins during the dough-making process. The water and salt soluble fractions (albumins and globulins) are not significantly related to loaf volume, but together with endogenous lipids are considered to enhance loaf volume (Panozzo et al., 1990). Indeed, probably 50% of the variability between varieties in bread-making quality can be accounted for by variation in grain storage proteins. The challenge now is to understand the remaining 50% which is probably due to other grain constituents (Worland & Snape, 2001).

Another important parameter which has been suggested to influence gluten viscoelastic properties is the glutenin to gliadin ratio, as it has been suggested that a change in this ratio toward higher values would result in stronger doughs (Lafiandra et al., 2000b).

b. Low molecular weight glutenin subunits (LMW-GS)

The presence or absence of certain high molecular weight (HMW) glutenin subunits in relation to baking quality have been well documented (Payne et al., 1987; Shewry et al., 1992), while only limited investigations have been made on the functionality of low molecular weight (LMW) glutenin subunits (Gupta et al., 1995) because of the difficulties of identifying LMW subunits from gliadin proteins on SDS-PAGE gels of total protein extracts. Yet the LMW-GS play a significant role in gluten structure (Gianibelli et al., 2001). LMW-GS have the ability to form large aggregates that are related to dough strength (Gupta et al., 1994). The cysteine residues in the primary structure of the LMW-GS allow us to identify two different polymer-building subunits: chain extenders (subunits with two or more cysteine residues that form intermolecular disulfide bonds) and chain terminators (with only one residue of cysteine available for intermolecular disulfide bonding). While chain extenders allow the formation of stronger doughs, chain terminators have the opposite effect (Masci et al., 1998).

Differences in the total amount of LMW-GS, associated with specific allelic forms, have been reported to be an important cause of quality differences in bread wheat (Gupta & MacRitchie, 1994). In support of this hypothesis, Wieser and Kieffer (2001) found that twice the amount of LMW-GS was necessary to obtain the same dough resistance as they achieved with HMW-GS, as determined by rheological measurements and baking tests on a micro-scale.

A number of studies have revealed that the allelic variation at the LMW-GS loci is associated with significant differences in dough quality in bread (Gupta et al., 1989; Gupta et al., 1994). In general, the LMW-GS are associated with dough resistance to extensibility (Andrews et al., 1994; Cornish et al., 2001), and some allelic forms of LMW-GS show even greater effects on these properties than HMS-GS (Gupta et al., 1989; 1994). Different allelic forms of

LMW-GS seem to play different roles in determining different quality parameters (Luo et al., 2001). A very close linkage has been found between the LMW glutenin genes at the *Glu-3* loci and the ?- and ?-gliadin genes at the *Gli-1* loci (Payne et al, 1984a; Singh & Shepherd, 1988). Despite this, it has been concluded that, in comparison with the gliadins, it is primarily the LMW subunits that are responsible for the differences in bread-making quality (Gupta, 1994).

Most qualitative evaluations of LMW-GS have been perfomed on B-type subunits, as they are the most abundant and easiest to detect. Very little is known about the role of C-type subunits, probably because a procedure allowing their detailed study has only recently been developed (Masci et al., 2002). A few studies have dealt with the role of D-type subunits on quality characteristics with contrasting results: their reported presence in smaller polymers or in the propanol-soluble fraction suggests that they should be negatively correlated with quality. Similarly, the presence of a single cysteine residue that terminates growth of the glutenin polymer may indicate a role as a chain terminator (Masci et al., 1993; Tao & Kasarda, 1989; Gianibelli et al., 2002a, b). SDS-sedimentation tests on two biotypes of the bread wheat cultivar Newton differing at the *Gli-D1/Glu-D3* loci showed lower values for the biotype that possessed D-type subunits, providing further support for their negative contribution to visco-elastic properties (Masci et al., 1991). However, Nieto-Taladriz et al. (1998) suggested that the presence of a 1B-coded D-type subunit might be responsible for the higher SDS-sedimentation test value of the bread wheat cultivar Prinqual. The apparently contrasting effects reported by these authors may be due to the different genetic backgrounds analysed. Reports of correlations between particular allelic forms of LMW-GS and quality parameters in bread wheat are often contradictory, a possible explanation being that gene interactions and environmental effects may play a fundamental role (Gupta et al., 1994; Killermann & Zimmermann, 2000; Nieto-Taladriz et al., 1994).

The limited variation in LMW-GS in cultivated wheats, which may result from the intense selection over recent decades, forces breeders to look for new allelic variants that can be found in landraces or wild wheat relatives (D'Ovidio & Masci, 2004). Genetic transformation allows new allelic variants or additional copies of LMW-GS genes to be introduced into wheat, in order to increase the amount of the encoded proteins. The effect of the introduction of multiple copies of a LMW-GS gene into bread wheat by means of particle bombardment, on glutenin polymer organisation and quality predictive tests has been evaluated (Masci et al., 2003). Although the rationale of this work was to improve gluten strength by increasing the amount of LMW-GS, the very high expression of the transgenic polypeptide (up to 16 fold with respect to an average LMW-GS) resulted in a lower SDS-sedimentation volume of the transgenic wheat with respect to untransformed lines. Such behaviour has been interpreted in terms of an optimum size for glutenin polymers that, when exceeded, negatively affects gluten strength. In support of this hypothesis, expression of a lower amount of a transgenic LMW-GS in pasta wheat increased dough strength, although a second line expressing the same gene showed reduced dough strength due to partial suppression of endogenous LMW-GS genes (Tosi, 2002; Tosi et al., 2004). It has been suggested that the effect of the LMS-GS alleles on quality will become more accurately assessed if they are considered in conjunction with the HMW-GS (Gupta et al., 1994).

It is generally accepted that LMW glutenin subunits are primarily responsible for the determination of pasta-making properties (Payne et al., 1984b). *Triticum durum* cv. Lira is particularly suitable for studying the mechanism involved in quality differences, because its two biotypes differ only in the electrophoretic patterns of 1B-coded ?-gliadins, ?-gliadins and LMW glutenin subunits. The group of LMW glutenin subunits designated LMW-1 in one biotype correlates with poor quality, and the group designated LMW-2 in the other correlates with good quality (Masci et al., 1995). The LMW-2 allele on

chromosome 1B also seems to be important for determining bread-making properties (Pena et al., 1994). LMW-2 comprises a group of polypeptides, encoded by the *Glu-B3* locus, which is genetically linked to the *Gli-B1* locus, which contains genes encoding ?- and ?-gliadins, designated 45 and 35, respectively. Most commonly grown durum wheat cultivars have either the LMW-2/?-45 (plus ?-gliadin 35) or the LMW-1/?-42 (plus ?-gliadins 33, 35 and 38) allelic forms, the latter being associated with poor quality pasta-making properties. Because of the close association between ?-42 and ?-45 with LMW-1 and LMW-2, respectively, it was initially believed that quality characteristics were dependent on the presence of the specific ?-gliadins rather than the associated LMW-GS. However, many more studies have demonstrated the importance of LMW-GS, and it is now commonly accepted that ?-42 and ?-45 are only genetic markers for quality (Boggini & Pogna, 1989; Pogna et al., 1988).

There are indications that the better quality associated with the presence of LMW-2 in durum wheat is mainly due to the fact that the subunits are more abundant than the LMW-1 subunits (Autran et al., 1987; D'Ovidio et al., 1992; Masci et al., 1995) and that structural differences may play a minor role (D'Ovidio et al., 1999; Masci et al., 1998). In support of this, D'Ovidio et al. (1999) showed that allelic genes encoding major components of the LMW-1 and LMW-2 groups differed only by 15 amino acid substitutions within the repetitive domain. The main difference between the LMW-1 and LMW-2 protein groups is the presence of a slow moving *Glu-B3* coded LMW-GS in the latter (D'Ovidio et al., 1999; Masci et al., 1995). This slow moving LMW-GS corresponds to the 42K LMW-GS (Masci et al., 1998) in most genotypes and it is consistently the most abundant LMW-GS polypeptide (Masci et al., 1995).

c. Gliadins

Gliadins are generally considered to contribute to the viscosity and extensibility of gluten (Gianibelli et al., 2001) in the dough system. Purified,

hydrated gliadins have little elasticity and are less cohesive than glutenins (Wall, 1979). According to Bietz et al. (1973) and Bietz and Huebner (1980), in the dough, the glutenin interacts with gliadin and other proteins to produce optimum flour performance.

The relationships between specific gliadin and glutenin components and quality attributes have mostly been studied using electrophoretic techniques (Payne et al, 1981). Similar studies have been carried out using RP-HPLC. Huebner and Bietz (1986) found that the amount of a late eluting gliadin fraction was negatively correlated to bread wheat quality. Dal Belin Peruffo et al. (1985) purified and partially characterized the ?-gliadins 40 and 43.5, the presence of which correlates with bread-making quality. These proteins had the same molecular weight (42 000), as determined by SDS-PAGE, and similar amino acid compositions. Peptide mapping, after enzymic hydrolysis, suggested that these proteins resemble each other closely with respect to their primary structures but are not identical. An analysis of the electrophoretic components of gliadins and their significance in defining flour quality was conducted by Bebyakin et al. (1984). In soft wheats, the gliadins were inherited as a group, and this affected flour quality. A block of component gliadins identified in one cultivar affected the protein and gluten positively, and blocks identified in gliadin of another cultivar resulted in a high flour sedimentation value. Sozinov and Poperelya (1980) were able to find consistent associations between several gliadin blocks and Zeleny sedimentation volume, used as a bread-making quality parameter. Metakovsky et al. (1990) showed that the correlation between the gliadins encoded on the chromosome group 1 and quality parameters might be attributed to the LMW subunits of glutenin.

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 quality in terms of dough strength. This role may instead be

due to the LMW-GS because of their tight genetic linkage to the gliadins (Gianibelli et al., 2001).

2.4.2 Protein content

The protein content of wheat grains is important for two reasons. First, cereal protein is an important nutrient. Second, the amount and type of protein are important in the functional uses of the flour (Wall, 1979; Lásztity, 1996). A strong positive relationship has been demonstrated between grain protein concentration and the volume and texture of the baked loaves (Finney, 1985; Pomeranz, 1988). An increase in the protein concentration of flours from 10 to 14% results in an increase in loaf volume of almost 50% (Finney & Barmore, 1948; Tipples & Kilborn, 1974).

The wheat plant requires a basic amount of nitrogen (N) from the soil to accumulate dry mass and nitrogen content in the vegetative tissue to reach acceptable yield and protein content (Dechard et al., 1984). The limiting factor in protein production appears to be the amount of available nitrogen in the soil at different stages of crop development in relation to soil moisture, mineral nutrients in the soil, and environmental factors that determine yield (Pomeranz, 1988). In areas where cereals are grown, the level of available soil N is often low during the grain-filling period (Simpson et al., 1983). Fertilization is then of importance (Sosulski et al., 1963). Both high yield and good bread-making quality can be improved through nitrogen (N) fertilization strategies, such as the rates and timings of N fertilization (Martin et al., 1992). High protein concentration is very important, but a low fertilizer input is also desired, because of fertilizer cost and risk of N leakage which causes environmental pollution.

By using different breeding lines and different N regimes, factors determining the grain protein accumulation were investigated. The grain protein concentration is basically the combined result of grain growth and nitrogen accumulation. Grain growth is mainly related to starch deposition in

endosperm cells and is genetically determined by cell number, cell size, grain cavity size and the capacity of the cultivar for sustained metabolism (Jenner et al., 1991). Since the access of assimilates in most cases is not limiting (Evans & Rawson, 1970; Rawson & Evans, 1971), the number of endosperm cells in the grain and the space in the cavity mainly determine the storage capacity of the grain. However, grain size can be influenced by environmental factors as for example the N regime. This is probably due to subsequent changes in the metabolism and senescense caused by the availability of N (Thomas et al., 1978). Thus, during field conditions with a relatively low supply of N during post-anthesis the grain size will be limited mainly by the metabolism and senescense which are partly genetically determined and partly environmentally influenced.

Also the N accumulation is influenced by the N regime employed. The effects of various timings of nitrogen fertilization show that the application at the early stage (1-cm head stage) increases yield, but the N supply at the later stage (boot and head-emergence stage) significantly increases the amounts of all the protein fractions, resulting not only in a significant increase in yield and in grain protein content, but also in a significant improvement in the bread-making properties (Jia et al., 1996). A high availability of nitrogen after anthesis also generally leads to a high protein concentration in the kernel (Mitra & Bhatia, 1973). The late N regimes gave higher N contents per grain than early N regimes. The explanation of this is obviously a higher rate of N transported to the developing grains with increased amounts of N applied during post-anthesis.

Available N for transport can originate either from root uptake of N or from translocation of N from the green parts of the plant. No differences in uptake capacity between cultivars have been detected so far (Mattsson et al., 1992; Oscarson et al., 1995), but differences in uptake can be caused by differences in N supply. The reason for differences in N supply can either be due to differences in N application or to differences in root size and volume. The

latter might be genetically determined. Concerning N transport from the green tissues, there are genetially determined differences between cultivars in capacity for sustained metabolism and early or late senescense, but the metabolism is also influenced by the N supply. The N accumulation is to a large extent based on sink activity in the grain and there is an optimum that can be reached. This means that there is an optimal N content per grain, and if there is more N available it will be used for other purposes. The maximal N content per grain is genetically determined. When maximal N content per grain is reached, the protein concentration in the grains can be increased by decreasing the grain size – which is very difficult. Also, smaller grain size leads to a low flour yield and might also be related to lower yield, which is not desirable. However, the likelihood of reaching the maximal N content per grain is frequently not feasible under conventional field cultivation conditions due to the low supply of N during post-anthesis. To increase protein concentration in cultivars and at the same time maintain the large grain size and high yield, with decreased fertilizer input, is a difficult task for the breeder. Controlled conditions during both vegetative and generative growth are required in order to have a reliable comparison of grain protein concentration of wheat cultivars grown under different N regimes. This can be achieved by experiments in climatic chambers using solution culture techniques.

Protein is synthesized throughout the fruiting period or the plant. Starch synthesis, on the other hand, starts later during fruiting and accelerates as maturity approaches. If growing conditions in the late fruiting period are good, starch yield will be good and grain yields high, but protein content will be relatively low. The availability of nitrogen throughout the growing period is of course of major importance, as mentioned above (Lásztity, 1996).

Nitrogenous compounds accumulate in the grain during most of the grain filling period, and there are changes in the composition of amino acids which are consistent with an increase in the proportion of storage proteins as

development progresses (Martin del Molino et al., 1988). The synthesis of proteins involved in gluten formation, as far as loaf volume potentialities are concerned, begins generally three weeks before hard red winter wheat is ripe (Finney, 1965; Scott et al., 1957). The wheat reaches optimum potentialities of volume, crumb gain, mixing requirements, and mixing tolerance as early as two weeks before it ripens. Although the protein content of wheat changes relatively little during the last two weeks of ripening, the gluten-forming capacity of the proteins improves dramatically. The transformation is assumed to result from increases in the size and complexity of the proteins (Hoseney et al., 1966). The amount of protein in wheat harvested at various stages of development was determined by the biuret method and it did not change during maturation. However, mixograms and pictures of loaves baked from wheat harvested at various stages of maturity showed that changes in the bread-making properties of the flours occurred and that the ideal loaf volume was only achieved 10-14 days before the wheat was ripe. This was due to changes in the formation of gluten proteins during maturation (Pomeranz, 1988).

Many studies have shown that the increase of flour protein content resulting from N application can lead to changes in protein composition (Gupta et al., 1992). When the protein content of a cereal grain changes, the relative proportions of the various protein classes also change. Generally at a low protein content the amount of metabolically active proteins (albumins and globulins), expressed as a percentage of total protein, is much higher than at a higher protein content. In contrast, a relative decrease in the ratio of storage proteins might be observed. This appears logical if we remember the biological roles of the two groups of proteins. As the plant produces more protein, less is required for physiological functions and more is available as storage protein (Lásztity, 1996). The baking quality will thereby be improved to a certain level by the increased protein concentration in the grains (Long & Sherbakoff, 1951). Since only the amount of the gluten proteins is increased

with an increase in protein concentration (MacRitchie, 1984), the improvement of the loaf volume will be due to a higher amount of gluten. Thus, in breeding for improved bread-making quality, it might be better to breed for improved protein composition than to breed for higher protein concentration.

Major effects on loaf quality have been demonstrated due to overall protein content (MacRitchie, 1992) and the glutenin-to-gliadin ratio (MacRitchie, 1987; Gupta et al., 1992; Blumenthal et al., 1994; Pechanek et al., 1997). This is supported by Uthayakumaran et al. (1999) who concluded that the protein content and glutenin-to-gliadin ratio (a measure of molecular weight distribution or protein size) have different roles in determining the various dough and bread quality parameters.

Understanding the genetics of grain protein content in wheat has proven to be quite difficult, since it has a low heritability and is very sensitive to environmental conditions. This has made the discovery of genes affecting protein content, using classical genetical approaches, quite difficult (Worland & Snape, 2001). To date, six genes or more correctly QTL have been identified, *Pro-A1* and *Pro-D1* associated with the *Vrn1* loci on the long arms of chromosomes 5A and 5D, respectively (Snape et al., 1993), and an unnamed gene on chromosome 2D. *Pro-A2* and *Pro-D2* are on the short arms of chromosomes 5A and 5D. A QTL, *QGpc.ndsu-6B* is on chromosome 6B. No major genes have been discovered which give discontinuous variation. However, alleles at *Pro-1* may increase protein by up to 1%, and it seems that the greater the protein content, the bigger the allelic difference that is found at this locus (Worland & Snape, 2001). How these genes work is not understood, although there is evidence that *Pro-1* appears to increase post-anthesis nitrogen uptake (Monaghan, 1997). Probably less than 30% of the protein content variation between UK winter wheats can presently be accounted for by known genes, and this is probably characteristic of the world's germplasm. Thus, it is a great challenge to identify much more of the genetical variation in

grain protein content, so that genes independent of yield can be pyramided by marker-assisted selection into new varieties with precision, rather than by chance, as at present. The development of molecular genetic maps is now making this easier, as with other such low heritability traits. Sources of genetic variation for protein content from other species, particularly *Triticum dicoccoides*, may be useful in increasing the protein content dramatically in bread wheat (Worland & Snape, 2001).

The protein concentration is determined by the genetic background, but also, to a large extent, by environmental factors such as nitrogen, water access, and temperature conditions (Sosulski et al., 1963; Benzian et al., 1983). Higher soil temperatures have been shown to favour the mineralization and uptake of nitrogen (Smika & Greb, 1973). Water can increase nitrogen availability to the crop as it increases root growth; the mass flow of water, and therefore nitrogen, towards the plant; mineralization of N from soil organic matter; and movement of N fertilisers into the root zone (Sander et al., 1987). According to Pawlson et al. (1992), rainfall prior to grain filling may accelerate nitrogen leaching and other forms of nitrogen loss. As a result they found a negative relationship between rainfall in the three weeks following nitrogen application and nitrogen availability to the crop.

Researchers have observed the negative correlation between yield and protein content of cereals, and generally the following explanation is accepted. The energy needed to produce proteins is approximately twice that required for starch synthesis. Therefore, an increase in protein concentration at a constant yield level will require either a higher photosynthetic rate and duration or more efficient partitioning of assimilates into grain (higher harvest index). Application of nitrogen fertilizer leads to an increase in yield and protein content. Low-protein cultivars did not necessarily give a greater protein response to nitrogen fertilizer than high-protein cultivars (Lásztity, 1996). Johnson et al. (1985) reported that although the amount of grain proteins tends to be negatively correlated with yield, the correlation coefficients

seldom exceed (r = 0.60) indicating that much of the variation in protein is independent of yield and that simultaneous breeding advances in yield and protein are possible. This is supported by a study on selection strategy for combining high grain yield and high protein content in South African wheat cultivars (Koekemoer et al, 1999). They concluded that selection for grain protein yield would give the best solution towards a simultaneous improvement of both grain yield and protein content.

2.4.3 Hard and soft wheat quality

Cereal chemists use the term "quality" to describe the suitability of a wheat flour for producing specific end products, such as bread, pastry, cakes, macaroni, or crackers. Quality of wheat cannot be expressed in terms of a single property (Pomeranz, 1988); it depends on many properties of the wheat kernel that affect its utilization for specific products. The properties are inherent in the cultivar, yet may be strongly influenced by the environment in which the wheat is grown (Poehlman & Sleper, 1995). From the standpoint of utilization, three basic market groups of wheat cultivars are grown, each with specific properties:

- hard (common or bread) wheat of the hexaploid species, *Triticum aestivum*,
- soft wheat of the hexaploid species, *Triticum aestivum*, and
- durum wheat of the tetraploid species, *Triticum turgidum* (Poehlman & Sleper, 1995).

A major classification of bread wheats is based on milling texture, and they are generally divided into two groups, hard and soft, which relates to the fractionation of the endosperm cells and starch granules, and the size of fragments obtained (Worland & Snape, 2001). A hard wheat kernel requires greater force to cause it to disintegrate than does a soft wheat kernel. Therefore, the flour obtained from a hard wheat kernel has a coarser particle size than does flour from a soft wheat kernel and more of the hard wheat starch is damaged (Hoseney et al., 1988). For commercial purposes, wheat is also classified as red or white, and spring or winter so that, for example, we refer to a wheat as being a "hard red winter wheat" (Cornell & Hoveling, 1998).

The hard (common or bread) wheats have "strong" gluten strands and hard kernel texture. When made into a dough using yeast as the leavening agent, large amounts of water are absorbed by the gluten and CO_2 is released by

chemical action of the leavening agent. As the gluten strands become solidified by baking, the CO_2 becomes trapped in the cellular structure of the gluten, resulting in a large well-piled loaf of bread. Because hard wheats in the United States are generally grown in drier climates than the softer wheats, they normally have a higher grain protein content which complements the greater strength of the gluten strands so that loaf volume becomes an important criterion for measuring quality in hard wheat flours (Poehlman & Sleper, 1995). For bread-making, the cultivar should yield flour with high capacity for water absorption, medium-long mixing time, and high loaf volume (Poehlman & Sleper, 1995).

Flours from different hard wheat cultivars differ inherently in capacity for flour yield, water absorption, particle size index, dough mixing time, and other quality components. Laboratory tests have been devised to measure the individual components of hard wheat quality, but the final test is in the loaf of bread that can be baked from the different cultivars and breeding lines (refer to 2.4 Quality testing parameters). Large loaf volume indicates superior water absorption and gas retention of the wheat gluten, a property desired in flour used for baking bread (Poehlman & Sleper, 1995).

On the basis of their suitability for the manufacture of yeast-leavened bread, common or vulgare wheats and the flours milled therefrom are classified broadly into two groups: strong and weak. Strong wheat flours contain a relatively high percentage of proteins, which forms a tenacious, elastic gluten of good gas-retaining properties and are capable of being baked into well-risen, shapely loaves possessing good crumb grain and texture. They require considerable water to make a dough of proper consistency to give a high yield of bread. The doughs have excellent handling qualities and do not have critical mixing and fermentation requirements; for this reason, they yield good bread over a wide range of baking conditions and have good fermentation tolerance (Pomeranz, 1988).

In contrast, weak flours have a relatively low protein content and form a soft, weak, relatively nonelastic gluten of poor gas-retaining properties. They have relatively low water-absorbing capacity and yield doughs of inferior handling quality (which give trouble in machine baking), and the fulfilment of exact mixing and fermentation requirements is very critical, so that they are more likely to fail in baking. Weak flours require less mixing and fermentation than strong flours to give optimum baking results (Pomeranz, 1988). Hard red spring wheats (wheats with a higher protein content) do normally sell for a higher price, and are often blended with wheats of lower protein content or bread-making quality (Huebner & Bietz, 1994).

Strong wheats surpass weak wheats for bread-making, but the reverse is true for chemically leavened products. In the latter, there is no fermentation to "mellow" or "ripen" the gluten, and the low protein content and the soft, mellow characteristics of the gluten formed by weak flours produce a lighter, more tender product than do strong flours. Various degrees of strength or weakness are required by the bakery trade. Thus, the general strength of bread flours sold for commercial pan bread is greater than that of bread flour sold for the family trade, and flour for commercial hearth bread is even stronger. Home baking involves rather mild treatment (hand mixing or very slow-speed mixing and gentle fermentation), so that good results are obtained with a flour of lower protein content and more easily conditioned gluten than would be satisfactory for commercial bakeries. For the manufacture of pan bread, a medium-strong flour is required, to withstand high-speed mixing and produce a dough possessing the physical characteristics that permit machine manipulation. Bread baked on the hearth of the oven without pans requires a flour of still higher protein content to yield a strong dough that does not flatten unduly under its own weight. Other types of bread flours must be supplied by the flour miller to suit different markets (Pomeranz, 1988).

The soft wheats mill into a fine, silky flour, with "weak" gluten strength, suited for making confectionery products, such as cakes, cookies, crackers, wafers, pretzels, waffles, pancakes, doughnuts, or flatbread (Hoseney et al., 1988; Poehlman & Sleper). Soft wheat products generally require flour with low protein content and weak gluten strength (Bettge et al., 1989; Souza et al., 1994). The soft wheats are generally adapted in higher rainfall areas than the hard wheats, resulting in lower protein, complementary to the weaker gluten (Poehlman & Sleper, 1995). Starch damage is often mentioned as an important quality factor for soft wheat flour, and thus its measurement is considered important. A high level of starch damage is undoubtedly detrimental in cookies and is generally viewed as a negative characteristic for soft wheat flours (Hoseney et al., 1988). Damaged starch is well known to absorb much higher levels of water than does undamaged starch. The amount of water absorbed by the flour is an important quality of cookie flours: one that absorbs small amounts of water is desired (Hoseney et al., 1988).

With soft wheat flours, the protein content and the desirable gluten quality also vary widely. For example, in cake making, very weak flours, with protein contents ranging from about 7 to 9% and yielding batters that have a pH value of 5.1 – 5.3, give the best results (Pomeranz, 1988). Protein quality does not appear to be strongly correlated with cookie-baking quality. Gaines (1985) proved that both cookie diameter and cake volume were positively correlated with flour softness and lower protein content. One of the most important properties of a cake flour is its ability to support a high sugar content. Cake flour must be able to develop a strong protein structure without making the product tough and the protein and starch components of the flour must also hydrate rapidly (Hoseney et al., 1988).

Flour for cracker products is higher in protein content (9 – 10%) and generally stronger than cookie flour. The quantity and quality of protein are important, because cracker dough contains relatively low levels of soluble ingredients such as sugar and salts, and thus the protein becomes hydrated and forms

gluten during sheeting. Proper development of the gluten during sheeting is essential for gas retention, volume, and final product texture. If too much protein is present or if it is too strong, excessive product shrinkage and cracker toughness result. The ideal cracker flour generally has poor spread in the cookie test (Hoseney et al., 1988). The rheology of a cracker dough is important to the processor; therefore, a battery of rheological tests (farinograph, alveograph, mixograph) have been applied. Uniformity is the most critical attribute of flour in the cracker process (Hoseney et al., 1988).

The hardness of a given cultivar of wheat is genetically controlled and is not directly correlated with the protein content of the kernel (Miller et al., 1984). Many of the new varieties of wheat in the United Kingdom have hard-texture kernels and, although they have relatively low protein content, yield flours unsuitable for the manufacture of soft wheat products (Hoseney et al., 1988). Low-protein flour (7 – 9% protein) milled from soft cultivars of *T. aestivum* is most suitable for making cakes and biscuits. Flours from hard wheats of the species *T. aestivum*, if of suitable protein content (11 – 13%, N x 5.7), is primarily used for bread-making (Hoseney et al., 1988).

With most of the products made from soft wheat flours, the doughs are not mixed to development. The exceptions to this rule are certain cracker doughs, which are developed by sheeting, and sheeted cookie doughs. Therefore, it would appear unusual to use techniques designed for hard wheat flours to evaluate soft wheat flours. The use of physical dough-testing machines such as the mixograph, farinograph, and alveograph to evaluate soft wheat flours appears to be based on the assumption that the rheological properties of soft wheat flour are the opposite of those of hard wheat flours. Stated in other terms, if a good hard wheat gives a strong curve, then a good soft wheat flour should give a weak curve. There appears to be little evidence to support such an assumption. The physicochemical properties of a good-quality soft wheat flour are a unique blend of properties. They are not just the properties of a poor-quality hard wheat flour. It would appear on theoretical grounds that

physical dough instruments would be of little value in characterizing soft wheat flours. This has been found to be generally true (Hoseney et al., 1988).

Testing criteria of soft wheats are significantly different from those of hard wheats. In hard wheats the experimentally milled flour is tested for dough mixing and bread-baking properties. In soft wheats, in which the gluten strands are weaker than in the hard wheats, a cookie test that measures spread of the dough rather than volume is utilized to evaluate baking quality (Poehlman & Sleper, 1995). Typical quality tests for soft wheat flours include tests for moisture, protein, ash, alkaline-water-retention capacity (AWRC) tests, and baking tests for sugar cookies and cakes. Probably the most useful test to evaluate soft wheat flours is the AWRC test. This relatively simple test does a reasonable job of evaluating soft wheat flours and roughly predicts flour performance in cookie baking. It essentially measures how much water is bound or held by the flour. Cake-baking tests involve milling the flour to produce a 50% patent flour, which is then impact-milled, chlorinated, and baked into a cake. Scores are based on crumb quality and cake volume. Baking tests remain the ultimate quality test. Cookie dough expands and flows as it is heated. The expansion is the result of leavening. The flowing is due to the force of gravity. Doughs made with good-quality flour flow much faster than those made with poor-qualify flour. Because all cookie doughs are cut to the same size, the difference between cookies produced from good-quality flours and those form poor-quality flours does not express itself until the dough is heated (Hoseney et al., 1988).

There is little information available regarding the contributions of the quantities of HMW-GS and LMW-GS to soft wheat end-use qualities (Hou et al., 1996).

2.4.4 Environmental effect on quality

The composition of proteins and protein subunits is genetically determined (Payne et al., 1987; Johansson et al., 1993; MacRitchie, 1999). However, the

relative quantity of specific proteins, protein subunits, and protein groups, as well as amount and size-distribution of polymeric proteins varies due to environmental conditions. Robert et al. (1996) noted that flour protein concentration and the percentage of protein present as gliadin and non-gluten proteins were most sensitive to environmental fluctuations. The glutenin part was found to be almost totally genotype dependent (Graybosch et al., 1996). The same results were found by Zhu and Khan (2001): the total flour protein content and SDS-soluble glutenin content were influenced more by environmental than genetic factors, while SDS-insoluble glutenin content was controlled more by genetic than environmental factors.

The variations in total flour protein content, the content of different protein fractions and size distributions of glutenin polymers, in turn, influenced properties of dough mixing (Peterson et al., 1992; Zhu & Khan, 2001). In theory, by knowing the relation between protein composition and different functional properties, it should be possible to design a genotype with the optimum allelic composition for a given end-use. However, even if this could be done, environmental conditions during growth of the plant can alter the protein composition in ways that may not have been anticipated. The general effects of certain environmental variables are understood to some extent. These include nitrogen and sulphur fertilizer levels and the effect of temperature during plant growth (Southan & MacRitchie, 1999).

Nitrogen fertilization is a significant factor influencing protein content (Hunter & Stanford, 1973; Memon & Jamro, 1988). High nitrogen availability translates into high protein contents in the grain and flour (Altenbach et al., 2002; Luo et al., 2000). Increased protein content usually results in higher dough extensibility and bread-making potential. However, changes in protein composition also occur. With increasing protein content, gliadin proteins tend to increase at a greater rate than other proteins (Gupta et al., 1992). This can lead to decreases in the polymeric-to-monomeric protein ratio and may mean

that dough strength as measured by a parmeter such as R_{max} could decrease as the flour protein content is raised (Southan & MacRitchie, 1999).

When sulphur fertilizer level is limiting, this can lead to a rearrangement of the relative quantities of different groups of proteins with dramatic effect on the molecular weight distribution and, as a result, on functional properties (Wrigley et al., 1984). Under suphur deficiency conditions, the relatively sulphur-poor proteins increase in amounts, while the synthesis of the sulphur-rich proteins is negatively affected (Shewry et al., 2001; Wrigley et al., 1984; Zhao et al., 1999). The ?-gliadins are the most suphur-poor of the proteins, while the a- and ß-gliadins, albumins, and globulins are relatively sulphur-rich. Most importantly, the HMW-GS are relatively sulphur-poor compared with the LMW-GS. The result is that, when sulphur availability is limiting, the HMW/LMW-GS ratio increases dramatically (Southan & MacRitchie, 1999). This effect is even more marked if high levels of nitrogen are supplied (Wooding et al., 1994).

Temperatures during grain development have significant influences on end-use quality (Randall & Moss, 1990). Johnson et al. (1972) found that a slight positive correlation between increased temperatures during the early stages of grain filling and protein content existed, while higher average temperatures in grain filling showed no effect on protein content. However, the study by Rao et al. (1993) indicated that protein content of soft white winter wheats was positively associated with maximum temperatures during grain filling but the relationships varied among locations. They concluded that the primary environmental factors conditioning protein content differed from location to location. Blumenthal et al. (1994) found that heat stress during grain filling caused the decrease of glutenin-to-gliadin ratio because gliadin synthesis continued during heat stress while there was a greatly decreased synthesis of glutenin protein. Heat stress reduced the size of glutenin polymers as measured by reversed-phase HLPC (Ciaffi et al., 1995) and, as a result, weakened the dough. Van Lill and Purchase (1995) reported that for winter

wheat increased values for mixograph dough development time was associated with favourable growth conditions during grain filling. Temperature (Blumenthal et al., 1990) and fertilisation conditions (Shewry et al., 2001) are the most important environmental factors that influence gluten protein synthesis.

Thus, in order to compare baking quality of different cultivars and experimental lines, it is necessary that the cultivars be grown under similar environmental conditions (Poehlman & Sleper, 1995).

2.5 Quality testing parameters

This discussion is limited to the procedures most commonly used in the testing of wheat to specify flour for end-user needs. Not all the tests discussed should be run on every flour. Some of the tests mentioned are appropriate only for certain types of flours and/or certain types of flour uses.

Hard wheat flour is usually used in the production of yeast-leavened, dough-based products. Consequently, it is usually important to include at least one testing procedure that describes the water relationships and viscoelastic properties of a flour-water dough (i.e., the farinograph or alveograph test). In yeasted products, the inclusion of malt may be specified. Unless it is heat treated, malt has a high amount of a-amylase, and any testing procedure to measure it is affected. A falling number, Rapid Visco Analyser (RVA), or amylograph test may be appropriate in such cases to ensure that the malt was added at the appropriate levels. Another test that may relate well to the performance of a flour in a yeasted system is starch damage. In addition to affecting water relationships in a dough, damaged starch can provide a substrate for yeast gas production and therefore may be important to measure (Atwell, 2001).

Market quality refers to grain that is plump, heavy, sound, and disease-free. Selection for these characteristics must always be foremost in the breeding programme, the breeder ruthlessly discarding strains with light, shrivelled, or diseased seeds (Poehlman & Sleper, 1995). Wheat grain characteristics that account for the milling performance (e.g., high flour extraction yielding flour of the right colour) of bread wheat cultivars are kernel morphology, test weight (an international grading standard), kernel weight and size, bran content, wheat kernel hardness and moisture content. Flour quality is mainly considered as flour yield, flour colour and flour protein content (Atwell, 2001).

Basic analysis

2.5.1 Test weight

High test weight indicates sound wheat. As test weights (hectoliter mass) drop, the percentage of small, malformed, and broken kernels usually increases. Hence, this test is used in the grading of wheat in many countries (Atwell, 2001). During grain filling, growth conditions, which affect test weight, are moisture stress, high temperature, nitrogen supply and diseases (Evans et al., 1975). Test weight of wheat is determined by weighing clean wheat occupying a given volume. Test weights may range from about 57.9 kg/hl (kilograms per hectoliter) for a poor wheat to about 82.4 kg/hl for a sound wheat (Atwell, 2001). Some researchers like Charles et al. (1996) have indicated that higher test weight is an indication of higher protein content, which is one of the quality parameters. Kernel plumpness (an indication of higher test weight), which is favoured by high photosynthetic rates and/or long grain filling periods, may influence flour extraction yield (Planchon, 1969).

2.5.2 Kernel hardness

Hardness is highly hereditary and wheat cultivars are specified either to be hard or soft (Mamuya, 2000). Endosperm hardness can affect the amount of starch damaged during the milling process and subsequently the water requirements of the resulting flour. It varies significantly between wheat classes and even between varieties within a class. Growing conditions and moisture content of the kernel can also affect hardness. Measurements are based on near-infrared absorption, particle size of milled fractions, or on the force required to crush individual kernels (Atwell, 2001). Van Lill and Smith (1997) reported that grains containing higher protein content were inclined to be harder, which in turn increased flour yield.

2.5.3 Flour yield

This test is of importance to the miller because it is a general indication of how much flour can be made from a given wheat. It is conducted on a laboratory mill (e.g., Buhler or Miag Multomat) under a standard operating procedure. The value obtained (i.e., the percentage of flour based on the initial weight of wheat) relates to the extraction rate on a commercial mill. It should be understood, however, that a small laboratory mill is not as flexible as a commercial mill. Often, a miller can make adjustments in a commercial mill to make better separations and obtain yield higher than those obtainable on a laboratory mill (Atwell, 2001).

2.5.4 Moisture

The complexity involved in the analysis of moisture is often underestimated. Moisture is easily driven out of a sample by heat when there is excess water in the system, but as a sample becomes drier, the remaining water can be tenaciously bound and, consequently, more difficult to remove. For this reason, it is very important to follow any moisture method exactly, especially with respect to temperature and time for oven-based methods. The most commonly applied moisture-testing method for flour and wheat involves the use of an air oven. Flour is analyzed directly, but wheat is ground in a

laboratory mill before the analysis. If the sample is below 13% moisture, 2 – 3 g is weighed into a tared (pre-weighed) vessel. It is then heated for exactly 60 min at 130 ± 1 °C, allowed to cool under desiccation, and weighed again. Percent moisture is calculated as the moisture loss divided by the original sample weight multiplied by 100. For samples over 13% moisture, an air-drying stage precedes this procedure. Replicate determinations should agree within 0.2% moisture (Atwell, 2001).

Moisture content over 14% affects the storage quality of flour and wheat. At higher moisture contents, mould growth, increases in microbial content, and infestation by insects are favoured. High moisture can also lead to production problems because flour agglomerates more readily as it becomes wetter. This often causes hoppers and other devices with bottlenecks to "bridge" when flour clumps, resulting in blocked passageways (Atwell, 2001).

Moisture should be included in every analysis of wheat or flour. It should also be the first test run, because it provides a basis for comparison for all other tests. Usually a 14% moisture basis is used for comparisons, but tests may also be compared on a dry basis or any other moisture basis. To assure accurate communication, it is important to always include the moisture basis when reporting wheat or flour analyses. Additionally, for other testing procedures where the dry matter in the sample governs the results (e.g., the farinograph test), it is important to know the moisture content of a sample to ensure that the appropriate amount of sample is analyzed. The following equation may be used to convert any analysis to any moisture basis:

$$A = B \; \frac{(100-C)}{(100-D)}$$

in which A = the analysis percentage at the desired moisture basis, B = the analysis percentage as originally analyzed, C = the desired moisture basis, and D = the moisture percentage as originally analyzed. For example: A sample is analyzed and shown to contain 11.2% moisture and 12.5% protein.

It is necessary to report the protein content on a 14% moisture basis (Atwell, 2001).

$$A = 12.5 \ \frac{(100-14)}{(100-11.2)} = 12.1\%$$

2.5.5 Protein content

Protein quantity and quality are both considered primary factors in measuring the potential of a flour in relation to its end use. The quantitative expression of crude protein is related to total organic nitrogen in the flour, whereas quality evaluations relate specifically to physicochemical characteristics of the gluten-forming component (Mailhot & Patton, 1988). The primary procedure for determining protein content in wheat and flour has traditionally been the Kjeldahl method. There are several modifications of this basic procedure, but all are based on the same principle of converting protein nitrogen to ammonia, complexing it, and titrating it against a standardized sulphuric acid solution. Because the method measures nitrogen and not protein directly, the data must be converted. For wheat and wheat flour, the percent nitrogen obtained by the analysis is converted to percent protein by multiplying by a factor of 5.7. This factor varies for other proteins depending on amino acid composition (Atwell, 2001).

Another method more commonly employed today is the Leco combustion method. This method employs high-temperature pure-oxygen atmospheres to liberate nitrogen form the protein. Combustion methods are fully automated and are capable of measuring protein amounts in a range from 0.2 – 20.0%. Conversion from nitrogen to cereal protein by multiplying by 5.7 is also required for combustion procedures (Atwell, 2001).

The most common spectroscopic means of determining nitrogen is near-infrared (NIR) reflectance spectroscopy. This type of analysis has broad application and is widely used in mills to measure protein as well as many other paramers. Protein content is an important criterion for marketing and

purchasing wheat and, as such, is included in almost every flour specification. In general, for hard wheats, the higher the protein content, the better the bread-making characteristics of the flour. High-protein flour is also likely to require more water and mixing time to reach an optimum consistency for processing or product purposes. Bread doughs with high protein levels are generally more resistant to overworking during mixing. It is important to note, however, that for any processing or product parameter, protein quality plays at least as important role as protein level. For example, high-protein flours with poor bread-making qualities do exist, as do wheats with all combinations of protein level and processing characteristics (Atwell, 2001).

Flour performance tests

2.5.6 Farinograph

The farinograph is a type of recording mixer. It measures and records the resistance offered over time by a dough against mixing blades operating at a constant speed (rpm) and a constant temperature. Parameters obtained from the resultant curve {i.e., resistance in Brabender units (BU) versus time in minutes} relate to the amount of water required to reach a desired peak consistency, the amount of time required to mix a dough to a desired consistency, and the amount of resistance a dough formulated with the flour will have to overmixing. Although the faringraph is but one of many recording dough mixers, it is the one that is most commonly used in the flour industry (Atwell, 2001).

For the test most widely used, the equivalent of 300 g of flour (14% mb) is placed in the faringraph bowl. The instrument is turned on, and water is added from a burette. As the flour hydrates and the dough forms, the resistance on the mixing blades increases, and the pen on the chart recorder and/or the curve on the computer screen rises. The mixing curve obtained

generally rises to a maximum and then slowly falls from that point. To ensure that farinograms from different samples can be compared, the midpoint of the farinograph bandwidth at the maximum resistance is always centered on the 500-BU line. This is accomplished by adjusting the amount of flour and water used. An experienced operator can generally achieve this in the second or third run for any given flour sample (Atwell, 2001).

A number of parameters can be derived from a farinograph curve (Fig. 2.6); the ones mentioned here are the most widely used to assess flour properties. The amount of water added to balance the curve on the 500-BU line, expressed as a percentage of the flour (14% mb), is the **farinograph absorption** (Atwell, 2001). Water absorption gives an indication of the potential of the protein molecules to absorb moisture. Higher protein content flour generally results in higher water absorption (Finney & Shogren, 1972). Van Lill and Smith (1997), who noted that grains containing higher protein were inclined to be harder, support this. Ash content is liable to increase when hard wheat is milled, consequently improving the water absorption. In South Africa the ideal absorption value should reach approximately 60% as the optimum, but it can go as high as 63% (Mamuya, 2000).

Dough development time, which is also called mixing time or peak time, is the time between the origin of the curve (water addition) and its maximum. The maximum of the farinogram curve or any mixing curve is commonly believed to be the point at which the dough is optimally developed and best able to retain gas. Another parameter, called the **arrival time**, is the time between the origin and the point where the curve first reaches the 500-BU line. The **tolerance index** (also called mixing tolerance index, MTI) is measured as the difference in Brabender units between the top of the curve at the optimum and the point on the curve 5 min later. **Stability** is defined as the difference in minutes between the arrival time and the time the top of the curve falls below the 500 BU line (i.e., the **departure time**) (Atwell, 2001). Dough stability estimates the ability of dough to resist mechnical mixing

(Brunori et al., 1989). A peak time of 4 – 4.5 min is desirable and it is better if the graph will remain for 4 min after peak at a higher level. Therefore the stability should be around 9 min and above (Mamuya, 2000).

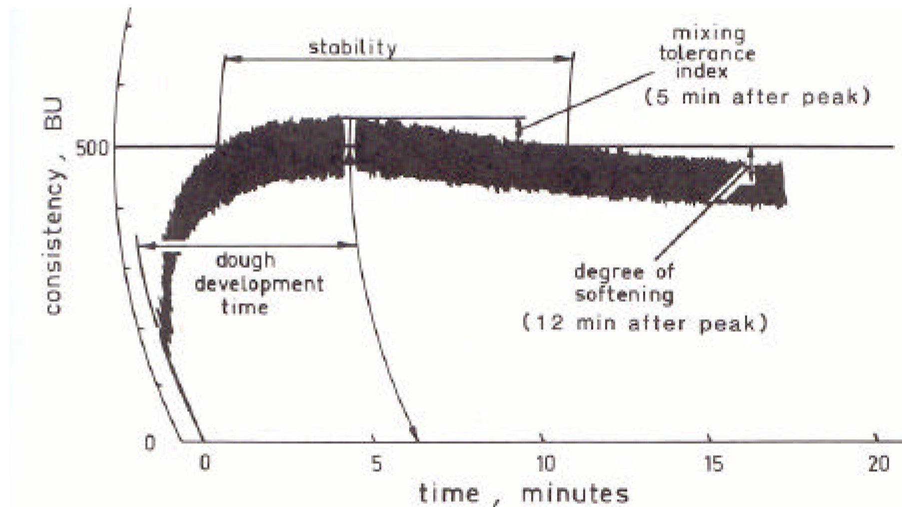

Figure 2.6. A typical hard wheat farinogram, with some commonly measured indices indicated (Bloksma & Bushuk, 1988).

C.W. Brabender Instruments, the manufacturer of the farinograph, has developed software to aid in the interpretation of farinograms. Generally, flour with good bread-making characteristics has higher absorption, takes longer to mix, and is more tolerant to overmixing than poor-quality flour (Fig. 2.7). The parameters obtained from a farinogram are useful in determining the direction for adjustments required when flour changes, but they should not be taken as absolutes. For example, if the standard flour used in a commercial process has a farinograph mixing time of 4 min and a new shipment of this flour has a farinograph mixing time of 5 min, there is an indication that the mixing time in the commercial mixer where the new flour is used should be increased. However, it does not indicate that an increase of 1 min is required, because the mixers and ingredients in the mixers are different. Similarly, higher absorption indicates that more water is required to reach a desired

consistency in a commercial process, but it doesn't indicate exactly how much. The mixing tolerance index and stability parameters indicate how well a flour resists additional work after it has been mixed to optimum. Long stability values and low tolerance index values are representative of flours that can be overmixed with little change in the consistency of the dough, but again, comparison of these parameters between flour shipments implies only directional changes. Sometimes useable relationships between farinograph parameters and commercial parameters can be developed, but it is important to ensure that the contributions of other ingredients in a formula and processing parameters such as temperature are also included (Atwell, 2001).

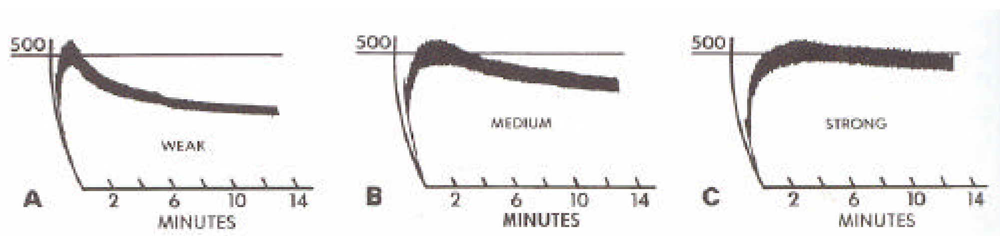

Figure 2.7. Farinograms of three flours exhibiting weak (A), medium (B), and strong (C) mixing characteristics. The weak flour has a development time of 1.25 min and a tolerance index of 180. The strong flour has a development time of 5.0 min and a tolerance index of 30 (Preston & Kilborn, 1984).

As commonly used, the farinograph assesses flour properties through simple flour-water dough mixing. In addition to evaluating flour, the farinograph can be used to adjust flour-water ratios of fully formulated doughs in a commercial process. In this test, 480g of dough is taken directly out of the commercial mixer at the end of the mixing cycle and put in the farinograph bowl, and a "remix" curve is run. The maximum of this curve can be used to specify the target consistency of a dough. Once the optimum consistency is identified, successive doughs can be evaluated and flour-water adjustments can be made to maintain this target consistency (Atwell, 2001).

Randall et al. (1993) reported that significant relationships were identified between rheological (farinograph inclusive) parameters and the high molecular weight glutenin subunit patterns. Band combinations 5+10, 13+16 and 7+9 were suggested to be predominant in conferring good rheological quality characteristics. The presence of subunit 9, coded by locus *Glu-B1*, shortened the dough development time and increased loaf volume, farinograph water absorption and gluten content (Khan et al., 1989). Peter et al. (1998a) also noted that the HMW glutenin subunits very clearly control the main qualitative features of the gluten, namely sedimentation value, farinographic data and loaf volume. It is therefore clear that the genotype has more influence on farinograph parameters, but still the environment and genotype by environment interaction may contribute to the final expression of the parameters.

It has been shown for both a group of hard bread wheats and a group of durum wheats that there is a strong positive relationship between the glutenin:gliadin ratio and dough tolerance to mixing. Since the glutenin:gliadin ratio can easily be analysed for large numbers of small samples, it may, in combination with selection for desirable high molecular weight glutenin subunits, prove a useful tool in early generation selection within wheat breeding programs. The selection of cultivars with a greater tolerance to overmixing would overcome some of the processing problems encountered for both bread wheat and durum wheats as a consequence of environmental effects. For example, the increased synthesis of gliadin protein at higher temperatures during grain filling may be less significant in cultivars with a high glutenin:gliadin ratio. Similarly, analysis of the glutenin:gliadin ratio may assist in the selection of any 1B/1R translocation lines with better dough mixing tolerance and less dough stickiness (Blumenthal et al., 1990).

2.5.7 Mixograph

The mixograph is also a recording dough mixer, albeit a smaller, simpler piece of equipment than the farinograph. Samples as small as 2g and as large as 35g of flour can be evaluated. Although the curve obtained from a mixograph also results from the developing dough exerting force on mixing pins, the curve and the interpretation of it are not the same as for a farinogram. A "centre curve" through the centre of the mixogram band is first drawn to facilitate interpretation. All parameters are measured relative to this line (Atwell, 2001).

The **height of the centre curve** at the highest point relates to the absorption as well as the protein content of the flour analyzed. The determination of optimum absorption (water addition) requires some experience and is best accomplished by comparing the test curve to the shape of curves with optimal absorption that have been produced previously (Atwell, 2001). The height of the curve increases with increasing protein content (Hendriks, 1992).

The time in minutes from the origin to the highest point on the centre curve is the **dough development time** (also called time to maximum height, time to peak, and time to the point of minimum mobility). As on a farinogram, this is the point at which gas retention and ultimately bread wheat quality will be the best (Atwell, 2001). It is also considered as the point where dough is optimally mixed (Finney et al., 1987). The suggested mixing time (e.g., in South Africa) is 2 min to 3 min, with 2.5 min as the optimum. A shorter mixing time will result in sub-optimal dough development, whereas longer mixing time is not desirable due to time, energy, and financial loss. The optimum mixing time of 2.5 min is, however, strongly influenced by both the protein content and oxidation ability of the flour (Finney and Yamazaki, 1967). Mixing time decreases as flour protein content increases to about 12%, thereafter remaining approximately constant with increases in flour protein. Generally, as mixing time increases, dough extensibility decreases and dough stability, elasticity and mixing tolerance increase (Hendriks, 1992).

The range of stability is the time at which the mixogram band encompasses a line drawn parallel to the baseline at the highest point on the center curve (Atwell, 2001). Mixograph mixing time, peak height and bandwidth are dependent on both protein quality and quantity (Khathar et al., 1994). This in turn, is strongly influenced by the amount of nitrogen fertiliser (Kilian et al., 1990) as well as water stress (Neales et al., 1963) and high temperatures during kernel filling (Campbell and Read, 1968).

The shape of mixograph curves varies dramatically as the strength of the flour changes (Fig. 2.8). As with farinograms, software programs are available to analyze mixograms. Analogue and digital data collection, recording, and analysis are becoming increasingly common for both the farinograph and the mixograph. Plant breeders commonly use the mixograph because small samples can be run (Atwell, 2001).

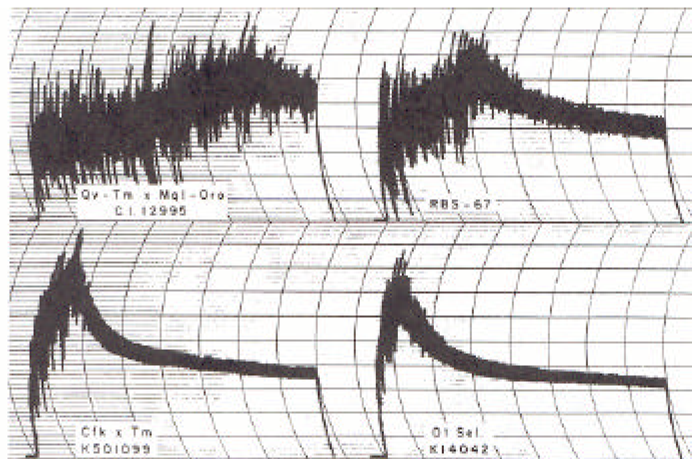

Figure 2.8. Mixograms of hard winter wheat, showing strong (top left), good (top right), weak (bottom left), and extremely weak (bottom right) characteristics (Hoseney & Finney, 1974).

The quality laboratory at the Small Grain Institute established a scale (1 - 5) for scoring the mixograms. The scale takes into consideration the mixing time, break-down process, easiness of determining the mixing time and the thickness of the breakdown band (Mamuya, 2000).

2.5.8 Alveograph

The alveograph test involves mixing flour with a standardized salt solution, extruding the dough as a thin sheet, and expanding the dough sheet as a bubble. The pressure inside the bubble is recorded with time until the bubble bursts. Usually, the test is repeated several times, and the parameters for the curves are averaged. The height of the peak relates to the resistance of the dough to deformation, while the length of the curve relates to its extensibility. The area under the curve represents the energy required to expand the dough and is related to the baking strength of the flour. This area is generally much larger for hard wheat flours than for soft wheat flours. Thus, the parameters obtained from an alveogram also describe the viscoelastic properties of a dough, but the measurement is distinctly different from those of the recording dough mixers or the extensigraph (Atwell, 2001).

Hou et al. (1996) studied the relationships of glutenin subunit quality of selected U.S. soft wheat flours to rheological and baking properties. They noted the high molecular weight glutenin subunit (HMW-GS) 1 to be correlated positively with alveograph extensibility (L), subunit 2* with pressure inside the bubble (P) and P/L values, and subunit pair 5+10 with P and strength (W) values. These results are consistent with those of Branlard and Dardevet (1985) who found that subunits 2* and 1 were positively correlated with P and L values respectively. Payne et al. (1987) assigned the same quality score to subunits 1 and 2*, and the presence of these subunits in a hard wheat usually indicates a strong wheat for good bread-making.

2.5.9 Gluten washing tests

Gluten washing tests produce information concerning the quantity and quality of gluten in a flour or ground-wheat sample. These tests involve forming a dough and washing the starch and water-soluble components out of it. Wet gluten is left following the washing and centrifuge procedure, and the amount (mass) is an indication of both gluten quantity and quality. This is based on the observation that good-quality gluten binds more water than does poor-quality gluten (Atwell, 2001). To obtain a dry gluten amount, the gluten is dried between two Teflon-coated hotplates (Mamuya, 2000). Wet gluten and dry gluten criteria are included in flour specification in many countries as a primary test of flour quality. This is likely due to the simplicity of the test and the quantitative information obtained relating to both gluten content and quality (Atwell, 2001).

A large portion of variation observed in flour quality may be attributed to variation in gluten protein content and composition (Bietz, 1988). The gluten consists mainly of aggregating glutenins and monomeric gliadins. The glutenins are responsible for the dough elasticity, while the extensibility and viscosity of the dough is determined by the gliadins (Shewry et al, 1995). Dough testing with blends of constant glutenin-to-gliadin ratios showed increases in the mixing time, mixograph peak resistance, maximum resistance to extension, and loaf volume as the protein content increased. Also, at a constant protein content, increases in glutenin-to-gliadin ratios were associated with increases in mixing time, mixograph peak resistance, maximum resistance to extension, and loaf volume. Thus the total protein content and the glutenin-to-gliadin ratio independently affected dough and baking properties (Uthayakumaran et al., 1999).

2.5.10 Alkaline water retention capacity

The alkaline water retention capacity (AWRC) is the amount of alkaline water retained by flour (14% mb) after controlled centrifugation. Flour is slurried with sodium bicarbonate, it is then shaken, allowed to hydrate, and centrifuged under specified and constant conditions of time and centrifugal force. The supernatant is decanted, the weight of the wet flour is determined and AWRC is calculated. This parameter is important when the water relationships in a product are critical to product quality. One specific application of this test is as a flour specification to predict cookie spread. As AWRC increases, cookie spread decreases (Atwell, 2001).

Enzyme analyses

Flour contains many active enzymes. Most of them do not cause problems in processing flour-based products or in determining final product quality. The one notable exception is α-amylase, and so the methods discussed below are commonly required in flour specifications. Other enzyme tests are usually not specified. However, if a specific enzyme such as polyphenol oxidase or lipoxygenase is important, tests are available (Atwell, 2001).

2.5.11 Falling number (Endosperm starch content determination)

The falling number procedure provides an index of the amount of α-amylase in a flour or ground-wheat sample. The procedure relies on the reduction in viscosity caused by the action of α-amylase on a starch paste and the fact that native wheat α-amylase is active above the gelatinization temperature of wheat starch. The procedure involves the use of the falling number apparatus, which contains a boiling water bath, stirring apparatus, a viscometer tube, and a timer. A flour slurry containing 7g of flour and 25g of water is added to the viscometer tube. The tube is immersed in the water bath and stirred. The starch gelatinizes, and the α-amylase liquefies the resultant paste. The time it

takes (in seconds) for the viscometer stirring rod to fall through the starch paste is the falling number (Atwell, 2001).

Under rainy conditions prior to harvesting wheat grain may begin to germinate, a phenomenon known as preharvest sprouting (Derera et al., 1977). The alpha-amylase in sprouted wheat results in degradation of starch into simple sugars. Flour made from sprout-damaged wheat can have a falling number of 100 seconds or lower. A bread wheat with average a-amylase activity has a falling number of about 250 seconds. The upper limit for the falling number test is about 400 seconds, which occurs for a flour devoid of a-amylase activity. The addition of malt or a-amylase from another source also affects falling numbers. Consequently, this test can be used as a means to monitor and control these additions (Atwell, 2001).

When flour, water and all the other ingredients required for bread-making are being mixed, the storage proteins hydrate and yield a continuous film-like matrix in which the starch granules are embedded (Hoseney, 1985). The alpha-amylase concentration gives an indication of the starch to sugar conversion in the wheat grain (Lukow & Bushuk, 1984). A higher falling number implies no or less conversion of starch into sugar. Unsprouted grains will have more starch which will absorb water and thus higher falling number values, whereas sprouted grains will have less starch and more sugar, resulting in low falling number values (Mamuya, 2000).

Because a-amylase hydrolyzes starch linkages, more free sugars are liberated and lower starch paste viscosity results when enzyme activity is high. The implications of this for a baked product can be very significant, considering the functional roles that starch plays in most products. High a-amylase activity can lead to excessive browning since the reducing sugars liberated enter into Maillard browning reactions. Reduced viscosity caused by a-amylase can have devastating effects on batter products by reducing volume and producing an undesirable crumb structure. In bread products, the

hydrolysis of the starch can lead to sticky crumbs and reduced volumes (Atwell, 2001). Flour with a higher falling number, results in higher loaf volume and good (fine) texture, whereas lower falling number flours result in lower loaf volumes and poor (coarse) texture (Mamuya, 2000). The effects of a-amylase on a flour-water system can also be evaluated with an amylograph or a Rapid Visco Analyser (RVA) (Atwell, 2001).

Viscosity methods

Specifications for flours used in batter-based products often contain a viscosity test. Changes in the viscosity of a batter have dramatic effects on the volume and texture of a final product (Atwell, 2001).

2.5.12 Sodium dodecyl sulphate (SDS) – sedimentation test

The gluten proteins of wheat are chiefly responsible for the visco-elastic structure of the dough. The differences in the functional properties of gluten are due to the differences in the properties of gluten protein groups, their interactions with each other and with other constituents of wheat flour. The SDS-sedimentation test is used for measuring relative gluten strength as it indicates differences in the quantities of the polymeric glutenins (gel protein). SDS-sedimentation values can range from 20 or less for low protein wheat of inferior bread-baking strength to as high as 70 or more for high protein wheat of superior bread-baking strength. The high protein (and gluten content) helps to retain gas by forming a continuous film together with starch granules during fermentation and this result in higher loaf volumes (Mamuya, 2000).

Baking tests

2.5.13 Loaf volume

The best test for the quality of a flour as it relates to a baked product is to produce the baked product with the flour and measure the important quality criteria. In conducting a test of this type, it is very important to strictly control all the other ingredients in the formula and to ensure that none of the processing steps vary. This ensures that the observed effects are due to the flour. Of course, the test must be of a scale that can be performed before the flour is run on the commercial process, and it must also approximate the product and process as closely as possible. For these reasons, baking tests are often performed at the mill (Atwell, 2001). The loaf volume method provides a basic baking test by a straight dough process that employs long fermentation and in which all ingredients are incorporated in the initial mixing step (Mamuya, 2000).

Bread-making is primarily based on protein concentration and quality (Finney et al., 1987). A linear correlation between protein content and loaf volume generally exists, indicating protein content to be a measure of wheat quality (Finney, 1945). High protein content (especially gluten) results in high loaf volume and good (fine) texture. High loaf volume also shows that there was no sprouting damage, as flour from sprouted wheat grains result in low loaf volumes and poor texture regardless of a cultivar being of good quality (Mamuya, 2000).

Loaf volume and water absorption are among the indicators of baking quality (Finney et al., 1987). Loaf volume gives an indication of the gas retention capacity of the dough during the fermentation process and indicates the volume of bread determined by rapeseed displacement (Shogren & Finney, 1984). Water absorption gives an indication of the potential of the proteins to absorb moisture. Generally, higher protein content flour results in higher water absorption (Finney & Shogren, 1972). The more water absorbed, the bigger the volume of dough per unit flour that can be produced.

The SDS-sedimentation volumes (a measure of flour aggregative ability) have been found to be positively correlated with both protein content and bread volume (De Villiers and Laubsher, 1995). It therefore appears that SDS-sedimentation values are good parameters for estimating (and predicting) the baking quality of wheat cultivars.

2.6 High performance liquid chromatography of wheat proteins

Conventional chromatography has serious drawbacks: it is slow, the column beds frequently are unstable and results may be difficult to reproduce and quantitate (Bietz, 1985b). In recent years, chromatographic methods providing superior separations have been developed. These methods, termed high performance liquid chromatography (HPLC), basically represent improved instrumentation and columns. Chromatographic systems that are highly reliable, and small silica-based columns that can withstand relatively high pressures (as indicated by the original definition of HPLC as "high-pressure" liquid chromatography) and flow rates, due to their small, uniform, and stable packings, have become available. These columns provide improved speed, sensitivity, resolution, reproducibility, and ease of use. However, the relatively small (generally 80 – 100 Å) pore size of normal silica in the columns used to separate low molecular weight (LMW) organic compounds does not permit penetration and optimal separation of proteins having molecular weights in excess of approximately 15 000. More recently, silica-based HPLC columns suitable for HMW poteins have become available (Regnier & Gooding, 1980). These columns, generally designated "large-pore" or "wide-pore" columns, contain silica packings with pore sizes of 300 Å or larger, permitting the total silica surface to be accessible to and penetrated by polypeptides of molecular weights up to several hundred thousand. Various bonded phases may be covalently attached to silica silanol groups, resulting

in reversed-phase (RP-), ion-exchange (IE-), and size-exclusion (SE-) HPLC columns.

HPLC techniques are successfully used for separation (Bietz, 1985a), detection of quality differences (Huebner et al., 1990, Huebner & Bietz, 1985), and variety identification (Bietz, 1985a) because the fractionation of plant endosperm proteins yield a complex “fingerprint”, which is highly specific of a genotype (Bietz & Kruger, 1994).

2.6.1 SE-HPLC and wheat quality

Size-exclusion chromatography was the first mode of liquid chromatography to be adapted to high performance methods for protein analysis. New silica-based packings, prepared from 3 - 5 micron-sized ultra-pure silica, bonded with a patented hydrophilic coating, were largely developed that reduced interactions with the support and approximated ideal conditions of size-exclusion. In addition, various improvements such as selection of proper mobile phase, appropriate adjustment of pH and ionic strength, and especially the use of detergents counteracting hydrophobic interactions, made resolution and analysis time in high performance systems superior and generally allowed any protein that could be separated on carbohydrate columns to be analyzed by SE-HPLC (Autran, 1994). Unlike the types of columns that are used in reversed-phase HPLC in which silica is modified to incorporate hydrophobic groups, SE-HPLC requires, in ideal conditions, that the stationary phase does not interact with proteins. Consequently, in SE-HPLC columns, residual silanols are usually locked by “end-capping” to minimize adsorption and allow sample recoveries greater than 95% (Autran, 1994).

SE-HPLC has several other major advantages. It is very sensitive – less than a single kernel can easily be analyzed. It is reproducible and easily automated. Data can be accurately quantified (Bietz & Kruger, 1994). SE-HPLC equipment is much simpler than, for example, the equipment of RP-HPLC - a single

pump and no gradient controller are needed since an isocratic elution is used (Autran, 1994). The most important advantage of SE-HPLC is, however, speed: a 20 – 30 minute analysis could give far better information than that achieved in a day or more on a conventional column (Bietz & Kruger, 1994) - allowing larger numbers of samples to be analyzed. For these reasons, SE-HPLC has been used extensively to analyze cereal proteins (Bietz, 1985b). Also, in view of routine use for predicting baking quality in breeding programs, the interpretation of the elution curve is simpler, since it consists of four major fractions very easy to identify (instead of 20 – 30 in RP-HPLC), two of them being related to quality (Autran, 1994).

SE-HPLC allows examination of the size distribution of protein polypeptides and protein aggregates (e.g., estimation of aggregating vs. monomeric proteins), and assessment of various characteristics related to size distribution. Because quality is often associated with the occurrence of large protein aggregates, it is essential to assess the size range of gluten proteins and to determine the proportions of aggregating and monomeric proteins in flour or grain (Autran, 1994). Whereas many other biochemical techniques (e.g., SDS-PAGE and RP-HPLC) are based on reduced or dissociated proteins (via the reduction of S-S bonds), resulting in loss of much information concerning structure, interactive aspects and stability of the protein complexes, SE-HPLC allows studies of protein composition of native unreduced aggregates, and also quantitation that has often been neglected in SDS-PAGE. A major advantage of SE-HPLC, therefore, is its potential to keep relatively large aggregates in a quasi-native state, to retain information on the aggregate level, and to be more likely to approach the physico-chemical and structural basis of wheat quality (Autran, 1994).

SE-HPLC accurately separates the three main classes of wheat endosperm proteins: glutenin, gliadin, and albumins-globulins (Larroque et al., 1997). The results obtained with this technique have been highly correlated with bread-making quality, particularly focusing on the first peak of the chromatogram

(polymeric protein) (Batey et al., 1991), as well as the overall molecular-size distribution within the polymeric fraction (Gupta et al., 1993). Studies using SE-HPLC indicate that the amount of polymeric proteins and their size distribution correlate positively with technological properties (Gupta et al., 1993; Dachkevitch & Autran, 1989). The proteins can be detected at wavelengths of 280, 254 or < 220 nm. At wavelengths below 220 nm, proteins are detected on the basis of peptide bonds at a sensitivity about 100 times greater than at 280 nm. The suggested wavelength for detecting protein is 210 nm since it is a good compromise between detection sensitivity and potential detection interference (Burke et al., 1991).

Size-exclusion chromatography has been one of the most useful techniques for analyzing cereal proteins. Separations occur on the basis of molecular size: if proteins are larger than the support pores, they rapidly elute from the column. Smaller proteins are retarded by the support in inverse relation to their molecular size. Proteins are thus sorted by size, and accurate molecular weight (MW) estimations can be obtained from elution times (Bietz, 1985b). To determine the molecular size of proteins separated by SE-HPLC, it is necessary to calibrate the column using known protein standards. When the logarithm of molecular weight for standard proteins in either native or reduced states is plotted against elution time (or volume) for each protein, a straight line is obtained under ideal conditions, the equation for which can be used to estimate MW of unknown proteins (Bietz, 1985b). Computer programs can be used to indicate start, maximum and end time, relative area and relative percentage, and apparent molecular weight of each peak (Autran, 1994).

SE-HPLC methods are still evolving, and improvements may be expected in the resolution and pore size of the columns, permitting analysis of larger and larger protein aggregates. We are also learning better ways to use these methods. For instance, it is no longer acceptable to work on only partially solubilized material, as in studies reported between 1985 and 1990. It is

essential that measurements are based on a complete protein extract. Because methods for complete solubilization are now available (Singh et al, 1990a), size distribution for the range of molecular species thus extracted can be determined quantitatively by SE-HPLC and more reliable correlations with potential quality of genotypes or end-use quality parameters can now be determined (Autran, 1994).

The difficulty of completely dissolving the storage proteins from flour without using conditions that chemically alter them remained unresolved. Until 1990, no chromatographic solvent had been developed that could extract more than 90% of total proteins without scission of disulfide bonds and, in addition, protein extractability was quite variable, proteins from strong wheat flours being much less extractable than those from weak flours (Danno et al., 1974). Considering that dough mixing could allow more efficient solubilization of unreduced proteins without affecting their size-based fractionation into polymeric glutenin, monomeric gliadin and albumin/globulin, Singh et al. (1990a) tried to achieve similar shear degradation of large gluten polymers using ultrasonic probes in order to solubilize total proteins from small flour samples and allow a more reliable pattern of protein aggregates to be obtained through SE-HPLC fractionations. Using a sonifier generating ultrasonic vibrations with a frequency of 20 KHz in 1.5 ml Eppendorf tubes, Singh et al. (1990b) clearly demonstrated that complete dissolution of unreduced proteins from strong and weak flours was possible in a 2% SDS solution (pH 6.9). It showed the following major advantages: 1) a very short time (30 sec) is needed to completely extract proteins, 2) a very small quantity of flour (11 mg) is required, 3) only very large glutenin polymers – that require much less energy for their shear degradation – are degraded and the resulting products elute from the column without affecting the size-based fractionation into polymeric and monomeric groups. The use of sonication combined with size-exclusion HPLC (SE-HPLC) has enabled fairly accurate determinations of the proportions of the three major protein classes in flour samples (Singh et al., 1990a).

In the earlier SE-HPLC fractions, a dramatic instability of the protein extracts was noticed, resulting in a continuous decrease of the percentage of the excluded peak during the first hours after extraction. Reproducible and comparable results could be obtained only upon storage of the extracts for one day (Autran, 1994). Dachkevitch and Autran (1989) speculated that such instability could be related to the dissociating effect of SDS resulting in a relatively slow disruption of large noncovalently bound aggregates until the extract contains only the more stable S-S-bonded complexes. Alternatively, as proposed by Huebner and Bietz (1985), it could not be ruled out that proteases remain active in phosphate-SDS buffer and are involved in the decrease of the excluded peak. Whereas no clear improving effect was found by adding various protease inhibitors in the extracting solution, it was reported by Dachkevitch (1989) that a higher extraction temperature (e.g., 60°C for 2 hours) could totally overcome the problem of instability and make the extracts ready for SE-HPLC analysis without any equilibration or other treatment. This minor change yielded an extremely stable elution curve, even with supernatants that had been stored for 48 hours after extraction, making possible the comparison of samples extracted at different times and full use of an automatic sampler for injection (Dachkevitch & Autran, 1989).

In order to protect the column, protein extracts must be centrifuged and elution solvents must be filtered to remove particles (Autran, 1994). The major problem with SE-HPLC is the loss of resolution and increased pressure that occur after a few hundred injections. According to Huebner and Bietz (1986), once problems occur, lost resolution or increased pressure can seldom be reversed. These factors make SE-HPLC more expensive than other HPLC modes, and limit the use of SE-HPLC as a routine procedure (Autran, 1994). It is recommended to completely remove SDS from the elution solvent and to use 59% (v/v) aqueous acetonitrile containing 0.1% TFA, which is likely to extend the column life (Autran, 1994). On the other hand, introduction of 50% acetonitrile as a new elution solvent (Batey et al., 1991) considerably increased

column life and resulted in much improved resolution of different protein classes. This is likely to make SE-HPLC a routine procedure.

Many elution solvents can theoretically be used provided they are compatible with the column packing as far as pH range and viscosity are concerned. They must also have a low absorbance in the 210 nm region and they must have a moderate but non-zero ionic strength to reduce affinity of proteins to the column and allow elution (Autran, 1994). In recent years, several solvents have been reported for elution in SE-HPLC, including detergents (SDS) or hydrogen bond dissociating agents (urea, DMF). In studies on cereal proteins, 0.1M sodium phosphate, pH 6.9, containing 0.05 or 0.1% SDS, has been the most frequently used since it allows good compromise between low absorbance, low viscosity and protein extractability. It also keeps in solution a relatively high proportion of medium-size native aggregates, and gives a good relationship between molecular size and elution volume (Huebner & Bietz, 1985). Because hydrophobic interactions often play an important role in the formation of aggregates, detergents such as SDS are acceptable. They help counteract hydrophobic interactions, although certain manufacturers state that they tend to shorten column life. Batey et al. (1991) finally recommended the use of 50% (v/v) aqueous acetonitrile containing 0.1% TFA, which, without calling into question again the presence of SDS in the sample extract, resulted in a reduction by a factor of 200 in the amount of SDS to which the column was exposed and extended the column life, while further improving resolution.

Because there is considerable variation in grain quality and protein composition depending on environmental and physicological factors, HPLC fractionations have been performed by Huebner et al. (1990) to follow changes induced by stages of maturity, kernel size and spike location. From glutenin samples extracted at various stages of maturity and redissolved in phosphate buffer containing 5% acetonitrile, 2% SDS and 0.01% dithiothreitol, two major peaks were observed in SE-HPLC, corresponding to high (peak B) and low

(peak C) molecular weight subunits, with some albumins and globulins present (peak D) and unreduced material also present at the void volume (peak A). Only fraction C increased as wheat matured, indicating that the accumulation of LMW subunits may parallel synthesis of gliadins and formation of protein bodies. In contrast, the amount of HMW subunits and other constituents remained nearly constant and may be largely non-storage proteins. Thus, although both types of subunits of glutenin are present during kernel development, they differ in rates of accumulation.

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CHAPTER 3

The effect of nitrogen fertilization on the quality of hard and soft wheat cultivars as determined by SE-HPLC

3.1 Introduction

Of all the cereal grains, wheat is unique because wheat flour alone has the ability to form dough that exhibits the rheological properties required for the production of leavened bread and for the wider diversity of foods that have been developed to take advantage of these attributes. The unique properties of the wheat grain reside primarily in the gluten-forming storage proteins of its endosperm (Gianibelli et al., 2001). When water is added to wheat flour and mixed, the water-insoluble proteins hydrate and form gluten, a complex coherent mass, in which starch, added yeast, and other dough components are embedded (Pomeranz, 1988). Because gluten is elastic, wheat flour dough expand and retain gas generated during fermentation. Gluten then sets upon heating, giving bread its unique texture (Bietz & Kruger, 1994).

The gluten can be fractionated with aqueous alcohols into the soluble, predominantly monomeric gliadins and the insoluble, aggregated glutenins (Bietz & Wall, 1973). Both fractions are cohesive, but their contribution to other functional properties of dough is different. Gliadins determine viscosity and dough extensibility, while glutenins regulate strength and elasticity (Khathar & Schofield, 1997; Sabine et al., 1997). The glutenin fraction consists of two main protein subgroups: high molecular weight (HMW) and low molecular weight (LMW) subunits (Southan & MacRitchie, 1999). According to Pomeranz (1988), although the HMW-glutenins make up only 10% of the total gluten and only 1% of the whole endosperm, they are nevertheless of

fundamental significance in determining the rheological properties of the dough.

The direct impact of these protein fractions on flour technological properties has stimulated intensive genetical and biochemical studies, aimed at understanding and manipulating the molecular basis underlying qualitative aspects. Most of these studies have been carried out using electrophoretic techniques (Lafiandra et al., 1994). However, when sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and reversed-phase high performance liquid chromatography (RP-HPLC) are used to separate gluten subunits, it causes a reduction of S-S bonds and a loss of information on how individual polypeptides interact to form large glutenin polymers (Autran, 1994). Size-exclusion high performance liquid chromatography (SE-HPLC) is the technique most commonly used to retain information at the level of protein aggregates and to give insight into structure and interactions between components. For example, the largest glutenin molecules can be broken down and solubilized by sonication (Singh & MacRitchie, 1989). SDS is used for the first protein extraction step, and the rest of the proteins are extracted by sonication. It is important to optimise solubilisation of wheat polymeric proteins with the minimum possible changes from their native state.

With the application of size-exclusion HPLC to wheat proteins pioneered by Bietz and developed by many workers (e.g., Dachkevitch & Autran, 1989), it has become possible to measure the relative quantities of monomeric and polymeric proteins with accuracy. SE-HPLC separates the three main classes of wheat endosperm proteins, glutenins, gliadins and albumins-globulins (Larroque et al., 1997).

The HMW subunits of glutenin are considered to be the most important components with respect to baking quality (Payne et al., 1979; 1981b; 1987). Correlations have been established between particular HMW glutenin subunits and bread-making quality (Payne et al., 1981b; 1987; Branlard &

Dardevet, 1985; Johansson et al., 1993). Besides the HMW-GS composition there are other factors, such as the amounts of the individual subunits, the proportions of HMW- to LMW-glutenins and the ratio of insoluble to soluble polymer fractions, which determine quality differences among wheat varieties.

Dough properties and baking performance of wheat are strongly dependent on both the genotype and the environment (Peterson et al., 1992). The composition of proteins and protein subunits is genetically determined (Payne et al., 1987; Johansson et al., 1993). However, the total flour protein content, the content of different protein fractions, and also the amount and size-distribution of polymeric proteins can be modified by environmental factors (Zhu & Khan, 2001) such as nitrogen applications, water access, and temperature conditions (Sosulski et al., 1963; Benzian et al., 1983). The identification of biochemical components that are highly influenced by the environment would assist in the development of wheats with enhanced quality and stability over diverse environmental conditions.

The aim of this study was to investigate the influence of cultivar and nitrogen application on the protein concentration, protein composition, and the amount and size-distribution of different protein components using SE-HPLC. The influence of these different components and N-applications on the quality characterstics was also investigated.

3.2 Materials and Methods

3.2.1 Materials

The material comprised of two hard wheat cultivar and two soft wheat cultivar flours. The type and origin for the genotypes used in this study are indicated in Table 3.1.

Table 3.1 . Entries of irrigation wheat cultivars included in the study.

Cultivar	Type	End use
Snack	Soft white spring wheat	Biscuits
Cracker	Hard white spring wheat	Crackers
E94/8	Soft white spring wheat	Biscuits
SST876	Hard red spring wheat	Bread

The wheat, analysed in this study, were obtained from trials conducted in an irrigation area in the Rietriver district, during 2001 and 2002. A total of 600mm irrigation was given during the growing season. The trials were planted at an experimental station where the soil was representative of the red Hutton soil profile. Each of the plots was 5.2m^2, with 6 rows per plot and 17cm between the rows. The seeding rate was 120 kg per hectare.

The cultivars were planted according to a randomised block design with three replicates in 2001 and 2002. However, because of high costs, only two replicates were used for quality analysis.

During the first year (2001) six different nitrogen applications (N-treatments) were used:

- P1 - 80 kg N/ha with planting (total = 230 kg)
  120 kg N/ha six weeks after planting
  30 kg N/ha during flag leave stage
- P2 - 80 kg N/ha with planting (total = 200 kg)
  120 kg N/ha six weeks after planting
- P3 - 80 kg N/ha with planting (total = 200 kg)
  90 kg N/ha six weeks after planting
  30 kg N/ha during flag leave stage

- P4 - 80 kg N/ha with planting (total = 170 kg)
  90 kg N/ha six weeks after planting
- P5 - 80 kg N/ha with planting (total = 170 kg)
  60 kg N/ha six weeks after planting
  30 kg N/ha during flag leave stage
- P6 - 80 kg N/ha with planting (total = 140 kg)
  60 kg N/ha six weeks after planting

The fertilizers used were N:P:K 3:2:1 (32) and urea (46).

During the second year (2002) the same six treatments as in 2001 were used, and one treatment (P0) was added, where P0 = 80 kg N/ha with planting.

3.2.2 Methods

a. Quality analysis

The following quality parameters were measured in duplicate at the laboratories of the Small Grains Institute, Bethlehem, South Africa. Quality analyses were done using mostly AACC procedures (AACC, 1995).

i SKCS (single kernel characterization system)-seed weight/SK-wght was measured using AACC method 53-31
ii SKCS-seed diameter/SK-diam (AACC 53-31)
iii SKCS-hardness index/SK-hard (AACC 53-31)
iv. Hectoliter mass/HLM was measured with the DICKEY-JOHN Grain analysis Computer II
v. Flour protein content/FPC (AACC 39-11)
vi. Falling number/FLN (AACC 56–81B)
vii. Vitreous kernels/VN (kernels were sliced and counted)
viii. Breakflour yield/BFLY (AACC 26-21A)
ix. Flour yield /FLY (AACC 26-21A)

x. Alveograph P/L ratio and Strength (AACC 54-30A)

The moisture content of all samples varied between 11.4 and 12.2%.

b. Size exclusion HPLC

Proteins were extracted from the wheat flour with a two-step extraction procedure developed by Gupta et al. (1993) – the same method described by Singh et al. (1990) and Batey et al. (1991), with a few modifications. The first step extracts the proteins soluble in dilute SDS, while the second extract contains proteins soluble only after sonication.

For the first extraction 0.017g of flour was suspended in 1.5 ml of 0.5% (w/v) SDS-phosphate buffer (pH 6.9) and vortexed for 10 seconds. Samples were then stirred for 5 min and centrifuged for 30 min at 10 000 rpm to obtain the supernatant protein. The supernatant, containing extractable proteins (mostly monomeric), was passed through a 0.45 µm filter and submitted to SE-HPLC fractionation.

The pellet was subsequently resuspended in SDS buffer as above and sonicated in an ultrasonic desintegrator (Branson B12 sonifier) for 30 seconds, amplitude 5, while moving the tube slowly up and down. The samples were centrifuged as above, to obtain a supernatant containing "unextractable" proteins The extracts were filtered through 0.45 µm filters before running on HPLC.

Aliquots of 20 µl of each extract were injected into a BIOSEP SEC-4000 Phenomenex column on a System Gold HPLC (Beckman Instruments Inc., Fullerton, CA, USA) and run for 30 min with a flow rate of 0.2 ml/min. The elution solvent used was 50% acetonitrile in water (v/v) with 0.1% of trifluoroacetic acid (v/v). The solvent was previously filtered and degassed.

Proteins were detected by UV absorbance at 210nm. Areas of the different peaks were calculated. The percentage of total unextractable polymeric protein in the total polymeric protein [(SDS-insoluble large and smaller

protein polymers)/SDS-soluble and insoluble large and smaller protein polymers)] and the percentage of large unextractable polymeric protein in the total large polymeric protein [(SDS-insoluble large protein polymers)/(SDS-soluble and SDS-insoluble large protein polymers)] was calculated according to the method of Gupta et al. (1993).

The measured HPLC fractions were: SDS-soluble (Fig. 3.1) and SDS-insoluble (Fig. 3.2), with each chromatogram subdivided into a = larger polymeric proteins (LPP), b = smaller polymeric proteins (SPP), c = larger monomeric proteins (LMP) mainly gliadins, d = smaller monomeric proteins (SMP) mainly albumins and globulins. The four major peaks, referred to as 'a' to 'd', were eluted between 9 and 20 min (Fig. 3.1 & 3.2). Peak 'b' did not make up a real peak and it is likely to consist of smaller aggregates with a continuous range of molecular size.

c. Statistical Analysis

All statistical analysis was done with Agrobase 2000 software. Relations between SE-HPLC protein fractions and quality characteristics were investigated by carrying out analysis of variance (ANOVA) and linear correlation coefficients.

SE-HPLC analysis was performed in triplicate for each of the two replicates (2 x 3 = 6). The mean of these six replicates was calculated and used in the statistical analysis. This was done to obtain more accurate representations of profiles for each replication.

3.3 Results and discussion for 2001

3.3.1 Results

Graphic representations of the SDS-soluble and SDS-insoluble protein elution profiles are given in Figures 3.1 and 3.2.

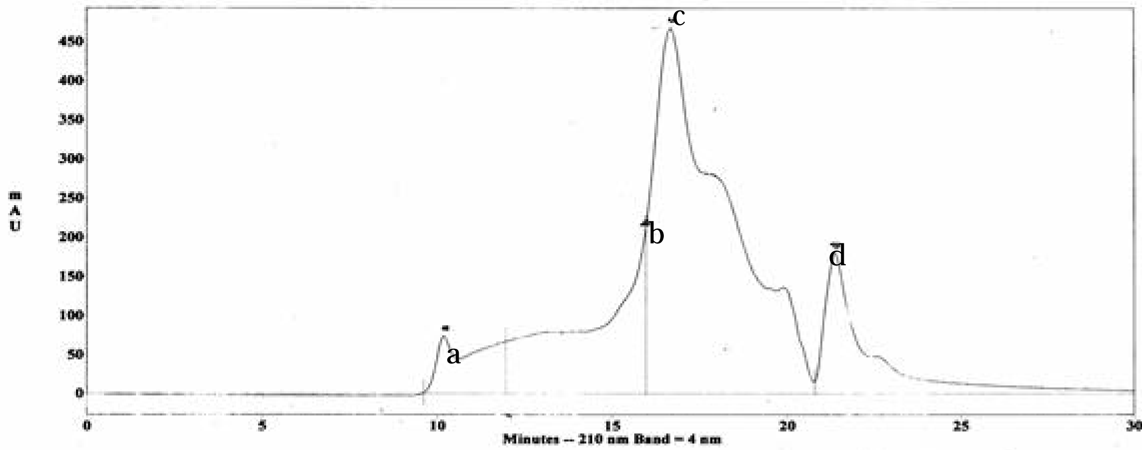

Figure 3.1. SDS-soluble proteins as separated with SE-HPLC, where a = larger polymeric proteins (LPP), b = smaller polymeric proteins (SPP), c = larger monomeric proteins (LMP) mainly gliadins, d = smaller monomeric proteins (SMP) mainly albumins and globulins (mAU = milli adsorption units).

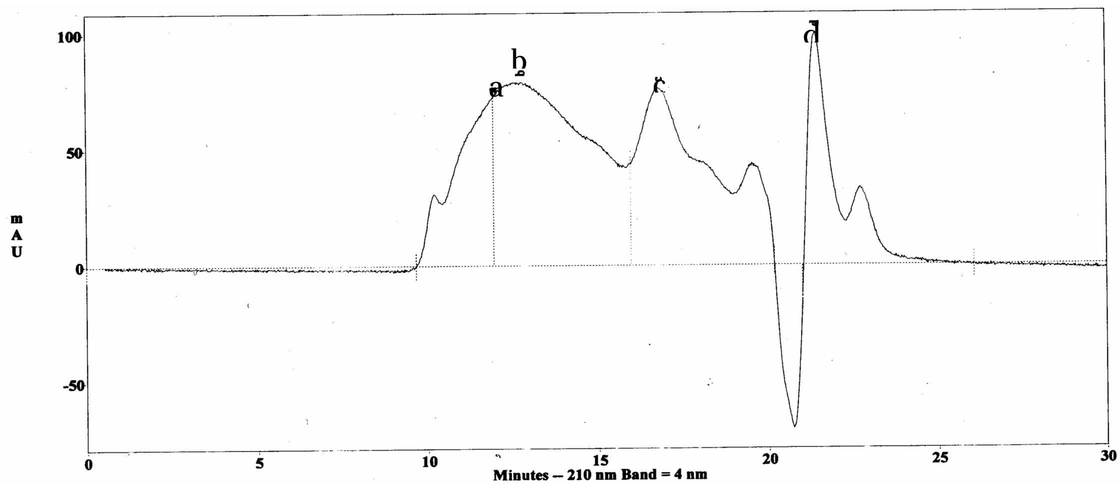

Figure 3.2. SDS-insoluble proteins as separated with SE-HPLC (after sonication), where a = larger polymeric proteins (LPP), b = smaller polymeric proteins (SPP), c = larger monomeric proteins (LMP) mainly gliadins, d = smaller monomeric proteins (SMP) mainly albumins and globulins (mAU = milli adsorption units).

Table 3.2. Means of protein fractions for four cultivars at six different nitrogen treatments (2001).

	LPP1	SPP1	LMP1	SMP1	LPP2	SPP2	LMP2	SMP2	TUPP	LUPP	LPP	LMP	SPP	SMP	PP	MP
Snack P1	4.06	13.83	63.26	17.81	14.15	33.73	32.86	15.31	77.77	72.91	18.21	96.11	47.55	33.11	65.76	129.22
Snack P2	3.62	12.29	63.63	18.91	12.47	31.56	32.48	15.75	77.51	73.49	16.08	96.11	43.85	34.66	59.93	130.76
Snack P3	3.87	12.50	64.65	17.03	12.48	29.62	32.58	18.27	76.41	72.06	16.35	97.23	42.12	35.30	58.47	132.53
Snack P4	3.96	12.19	62.17	19.64	11.27	29.68	33.13	17.72	74.09	71.76	15.22	95.30	41.87	37.36	57.09	132.65
Snack P5	4.27	12.57	62.32	19.20	12.33	30.31	32.79	17.87	74.32	71.73	16.60	95.11	42.88	37.06	59.47	132.17
Snack P6	3.72	11.69	62.17	21.29	9.89	29.44	34.45	22.99	72.68	71.85	13.61	96.61	41.13	44.28	54.74	140.89
CrackerP1	5.43	14.23	59.55	19.79	14.45	34.70	31.42	15.07	72.59	71.40	19.88	90.97	48.93	34.86	68.80	125.83
CrackerP2	5.39	12.93	59.81	19.91	15.34	34.51	31.09	16.31	73.92	73.13	20.73	90.90	47.43	36.21	68.16	127.11
CrackerP3	5.82	14.03	60.25	18.75	15.40	35.67	30.97	15.86	72.55	71.99	21.22	91.21	49.70	34.60	70.92	125.81
CrackerP4	5.79	12.63	59.49	19.30	14.43	34.22	31.76	17.02	71.24	72.50	20.21	91.25	46.85	36.32	67.06	127.57
CrackerP5	5.76	14.06	58.09	21.23	12.32	30.97	36.52	16.89	68.38	68.48	18.07	94.61	45.02	38.12	63.09	132.73
CrackerP6	4.94	11.79	56.52	24.31	12.01	30.07	35.42	19.02	71.32	71.61	16.94	91.94	41.86	43.32	58.80	135.26
E94/8 P1	4.34	13.31	64.39	16.41	13.23	31.20	36.13	17.15	75.79	71.78	17.57	100.52	44.51	33.56	62.07	134.07
E94/8 P2	4.21	12.81	62.68	18.71	11.26	28.61	39.11	18.43	73.18	70.16	15.47	101.79	41.42	37.14	56.89	138.92
E94/8 P3	3.98	13.48	64.57	16.21	11.92	30.55	36.62	17.00	74.99	70.90	15.90	101.19	44.03	33.21	59.93	134.40
E94/8 P4	3.78	13.01	62.70	19.14	12.83	33.15	33.27	17.95	77.28	73.29	16.60	95.97	46.16	37.09	62.76	133.06
E94/8 P5	5.09	15.15	60.13	18.21	11.07	31.55	37.21	16.54	68.26	67.72	16.15	97.33	46.70	34.75	62.85	132.08
E94/8 P6	4.70	13.50	60.25	19.86	12.28	31.28	33.69	19.32	72.36	70.51	16.98	93.93	44.78	39.18	61.76	133.11
SST876P1	4.43	13.60	62.71	17.84	11.89	35.02	33.51	17.08	72.93	72.40	16.31	96.22	48.62	34.92	64.93	131.13
SST876P2	5.17	14.10	59.51	19.52	12.41	32.75	35.16	16.20	70.54	70.12	17.58	94.67	46.85	35.71	64.42	130.38
SST876P3	4.83	12.82	60.04	21.01	11.90	30.52	39.10	14.90	71.24	70.53	16.73	99.14	43.34	35.91	60.07	135.05
SST876P4	5.65	13.21	55.95	23.51	11.84	30.60	36.51	18.00	67.77	69.11	17.49	92.46	43.80	41.50	61.29	133.96
SST876P5	4.85	14.14	59.98	19.41	12.09	32.13	38.33	14.77	71.64	70.12	16.94	98.30	46.27	34.18	63.20	132.48
SST876P6	4.63	12.90	57.29	23.43	11.46	30.93	36.54	17.09	71.09	70.62	16.09	93.83	43.83	40.52	59.92	134.35
Average	4.676	13.20	60.92	19.60	12.53	31.78	34.61	17.19	72.91	71.26	17.20	95.53	44.98	36.78	62.18	132.31
LSD (0.05)	1.646	2.855	2.919	3.302	2.749	4.919	3.958	4.928	6.233	4.496	3.865	5.064	6.674	7.563	10.19	10.98

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble) mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric

proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins, LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins

Protein fractions for four cultivars at six different nitrogen treatments (2001)

The results are given in Table 3.2.

SDS-soluble LPP and SPP: There were no significant differences within any of the tested cultivars for LPP and SPP.

SDS-soluble LMP: Significant differences were visible among the cultivars. Cracker P6 had a significantly lower LMP than Cracker P3 and E94/8 P1 and P3 had a significantly higher LMP than P5 and P6. SST876 P1 had a significantly higher LMP than SST876 P2, P4 and P6.

SDS-soluble SMP: There were significant differences among the cultivars. The SMP of Snack P6 was significantly higher than for Snack P3 and P1. Cracker P6 had a significantly higher SMP than P1, P2, P3 and P4 and the SMP of SST876 P4 and P6 were also significantly higher than the SMP of P1, P2 and P5. The SMP of E94/8 P6 was also higher than the rest of the E94/8 treatments, not significantly though. From the above it is clear that the P6 treatment gave the highest proportion of SMP.

SDS-insoluble LPP: Snack P1 had a significantly higher LPP than Snack P6 and P4. The LPP of Cracker P2 and P3 were significantly higher than for Cracker P5 and P6.

SDS-insoluble SPP: Cracker P3 had a significantly higher SPP than Cracker P6.

SDS-insoluble LMP: The LMP of Cracker P1, P2 and P3 were significantly lower than for Cracker P5 and P6. E94/8 P2 had a significantly higher LMP than P4 and P6. SST876 P1 had a significantly lower LMP than P3 and P5.

SDS-insoluble SMP: Snack P6 had a significantly higher value of SDS-insoluble SMP than all the other Snack treatments, except for Snack P3.

TUPP: E94/8 P5 had a significantly lower amount of total unextractable polymeric proteins (TUPP) than P1, P3 and P4.

LUPP: Cracker P5 had a significantly lower LUPP than P2. E94/8 P5 had a significantly lower LUPP than P4. It seems as if treatment P5 has given the lowest amount of LUPP for all the cultivars.

LPP: The total LPP of Snack P6 and Cracker P6 were significantly lower than the LPP of Snack P1 and Cracker P3.

LMP: There were significant differences among the cultivars for LMP. The LMP of E94/8 P4 and P6 were significantly lower than for P2 and P3. The LMP of SST876 P4 and P6 were also significantly lower than for P3.

SPP: The total SPP of Cracker P3 was significantly higher than for P6.

SMP: The SMP of Snack P1, P2 and P3 were significantly lower than for P6. The SMP of Cracker P3 was also significantly lower than for Cracker P6.

PP: Cracker had the highest and Snacker the lowest amount of PP. The PP of both Cracker P6 and Snack P6 were significantly lower than for Cracker P3 and Snack P1.

MP: The MP of Snack P6 was significantly higher than for Snack P1.

Table 33. Means of measured quality characteristics for four cultivars at six different nitrogen treatments (2001).

	SK-wght	SK-diam	SK-hard	HLM	FPC	FLN	VK	BFLY	FLY	P/L	Strength
Snack P1	36.48	2.45	13.47	76.35	11.25	208.50	49	31.35	75.30	0.31	17.59
Snack P2	35.95	2.40	14.35	77.35	11.55	196.50	49	32.00	75.20	0.32	15.22
Snack P3	40.81	2.62	10.66	77.20	11.60	202.50	54	31.60	75.75	0.31	15.98
Snack P4	39.79	2.60	7.60	76.00	10.40	201.50	40	33.30	76.00	0.38	15.37
Snack P5	38.76	2.54	13.96	75.95	10.90	191.00	38	31.90	75.55	0.37	16.74
Snack P6	39.50	2.64	9.63	76.30	10.65	202.00	40	33.20	74.50	0.32	15.14
CrackerP1	33.42	2.39	66.30	78.70	11.85	352.00	69	24.85	79.55	0.52	34.25
CrackerP2	33.99	2.43	62.79	79.20	10.95	347.50	68	25.85	79.55	0.55	27.22
CrackerP3	33.28	2.40	64.03	78.85	11.70	373.00	73	24.60	78.75	0.55	36.62
CrackerP4	36.16	2.53	62.84	78.90	10.90	344.00	68	25.15	79.25	0.56	30.66
CrackerP5	36.82	2.58	60.31	79.45	10.65	379.50	67	25.20	79.30	0.58	30.36
CrackerP6	35.80	2.55	59.93	79.15	10.15	303.50	55	25.85	78.90	0.65	23.32
E94/8 P1	37.67	2.52	16.25	75.70	11.50	198.00	47	29.90	74.85	0.36	17.51
E94/8 P2	38.31	2.62	14.34	75.45	11.85	184.50	44	30.85	74.20	0.27	16.06
E94/8 P3	38.42	2.56	14.90	75.80	12.35	182.00	54	30.45	74.55	0.27	14.76
E94/8 P4	38.69	2.61	14.86	76.00	10.80	186.50	40	31.25	74.95	0.40	15.75
E94/8 P5	38.19	2.60	16.49	76.00	11.65	186.50	39	30.80	76.05	0.33	13.99
E94/8 P6	39.76	2.63	14.41	75.50	11.35	172.50	47	31.15	74.85	0.35	13.53
SST876P1	38.34	2.51	52.32	80.45	12.00	387.50	58	24.55	80.10	0.38	26.61
SST876P2	37.79	2.57	53.56	80.00	11.45	381.00	51	25.25	80.00	0.39	24.54
SST876P3	39.66	2.66	47.68	80.00	11.95	354.50	56	24.35	79.95	0.39	26.22
SST876P4	38.42	2.61	47.33	79.10	12.15	336.00	45	25.85	79.65	0.30	22.56
SST876P5	37.05	2.51	52.57	79.05	12.10	318.50	53	24.40	79.20	0.41	24.16
SST876P6	36.94	2.50	42.73	78.40	10.65	368.00	35	28.80	79.95	0.32	20.87
Average	37.50	2.54	34.72	77.70	11.35	273.21	52	28.44	77.33	0.40	21.46
LSD (0.05)	3.412	0.191	5.050	1.497	0.859	53.030	14.16	0.999	1.123	0.08	4.462

SK-wght = single kernel chracterization system weight, SK-diam = single kernel characterization system diameter, SK-hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength

Quality characteristics for four cultivars at six different nitrogen treatments (2001)

Results are given in Table 3.3.

There were significant differences among the cultivars for SK weight, SK hardness, HLM, FPC, FLN, VK, BFLY, FLY, alveograph P/L ratio and alveograph strength.

SK weight: The SK weight of Snack P2 was significantly lower than for P3, P4 and P6. The SK weight of Cracker P3 was significantly lower than for P5. The SK weight of Cracker was low in comparison with the SK weight of Snack, E94/8 and SST876.

SK diameter: The SK diameters of Snack P6, P4 and P3 were significantly higher than for P2.

SK hardness: The SK hardness of Snack P1, P2 and P5 was significantly higher than for P4. Cracker P1 was significantly harder than P6. The SK hardness of SST876 P6 was significantly lower than for SST876 P1, P2 and P5.

HLM: The HLM of SST876 P6 was significantly lower than for P1, P2 and P3.

FPC: The FPC values of Snack P2 and P3 were significantly higher than for P4 and P6. The FPC values of Cracker P1 and P3 were higher than for P5 and P6 and the FPC of E94/8 P2 and P3 were significantly higher than for P4. SST876 P6 had a significantly lower FPC than all of the other SST876 treatments, but P2.

FLN: There were significant differences among the cultivars for FLN. The FLN values of Cracker P3 and P5 were significantly higher than for P6. The FLN of SST876 P1 and P2 were higher than for P5.

VK: The amount of vitreous kernels (VK) of Snack P3 was significantly higher than for P5. The VK values of Cracker P3 and E94/8 P3 were significantly higher than for Cracker P6 and E94/8 P5, respectively.

The VK of SST876 P6 was significantly lower than for P1, P2, P3 and P5.

BFLY: The BFLY values of Snack P4 and P6 were significantly higher than for P1, P2, P3 and P5. The BFLY of Cracker P1 and P3 were significantly lower than for P2 and P6. The BFLY of E94/8 P1 was significantly lower than for P4 and P6. The BFLY of SST876 P6 was significantly higher than for all the other SST876 treatments. Also, SST876 P4 had a higher BFLY than P1, P3 and P5.

FLY: Snack P6 had a significantly lower FLY than Snack P3 and P4. E94/8 P5 had a significantly higher FLY than all the others in the E94/8 group.

Alveograph P/L ratio: There were significant differences among the cultivars for the alveograph P/L ratio. The P/L ratio of Cracker P6 was higher than for P1, P2, P3 and P4. The P/L ratios of E94/8 P4 and SST876 P5 were higher than for E94/8 P2 and P3 and SST876 P6 and P4.

Alveograph strength: The strength of Cracker P3 was higher than for P2, P4, P5 and P6. The alveograph strength of SST876 P6 was significantly lower than for P1 and P3.

Table 3.4. Means of protein fractions for six different nitrogen treatments (2001).

	LPP1	**SPP 1**	**LMP1**	**SMP 1**	**LPP2**	**SPP 2**	**LMP2**	**SMP 2**	**TUPP**	**LUPP**	**LPP**	**LMP**	**SPP**	**SMP**	**PP**	**MP**
P1	4.56	13.74	62.47	17.96	13.43	33.66	33.48	16.15	74.77	72.12	17.99	95.95	47.40	34.11	65.39	130.06
P2	4.59	13.03	61.40	19.26	12.87	31.85	34.46	16.67	73.78	71.72	17.46	95.86	44.88	35.93	62.35	131.79
P3	4.63	13.21	62.38	18.25	12.92	31.59	34.82	16.51	73.80	71.37	17.55	97.19	44.80	34.75	62.35	131.95
P4	4.79	12.76	60.08	20.40	12.59	31.91	33.67	17.67	72.59	71.66	17.38	93.74	44.67	38.07	62.05	131.81
P5	4.99	13.98	60.13	19.51	11.95	31.24	36.21	16.52	70.65	69.51	16.94	96.34	45.22	36.03	62.15	132.36
P6	4.50	12.47	59.06	22.22	11.41	30.43	35.02	19.60	71.87	71.15	15.90	94.08	42.90	41.82	58.80	135.90
Average	4.68	13.20	60.92	19.60	12.53	31.78	34.61	17.19	72.91	71.26	17.20	95.53	44.98	36.78	62.18	132.31
LSD(0.05)	0.864	1.202	2.176	1.792	1.455	2.368	2.533	2.146	3.153	1.978	2.059	3.211	3.093	3.096	4.882	4.909

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins, LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins

Protein fractions for six different nitrogen treatments (2001)

Results are given in Table 3.4.

SDS-soluble LPP: There were no significant differences between the different N-treatments for LPP. P5 gave the highest proportion of LPP and P6 the lowest.

SDS-soluble SPP: Both P1 and P5 gave significantly higher amounts of SPP than P6.

SDS-soluble LMP: The LMP of P1, P2 and P3 were significantly higher than for P6.

SDS-soluble SMP: P6 gave a significantly higher SMP than all the N-treatments. P1 gave a significantly lower SMP than P4 and P6.

SDS-insoluble LPP: P6 gave a significantly lower LPP than P1, P2 and P3. The LPP of P5 was also significantly lower than for P1.

SDS-insoluble SPP: P6 gave a significantly lower SPP than P1.

SDS-insoluble LMP: P1 gave a significantly lower LMP than P5.

SDS-insoluble SMP: P6 gave a significantly higher SMP than P1.

TUPP & LUPP: The TUPP and LUPP values of P1 were significantly higher than for P5.

LPP: P1 gave a significantly higher total LPP than P6.

LMP: The LMP of P4 was significantly lower than for P3.

SPP: The SPP of P1 was significantly higher than for P5.

SMP: There were significant differences between the different N-treatments for SMP. The SMP values of P6 and P4 were significantly higher than for P1.

PP: The PP of P6 was significantly lower than for P1.

MP: The MP of P6 was significantly higher than for P1.

From the above results it is clear that the treatment P6 usually gave the lowest proportion of SDS-soluble LPP, SPP and LMP, SDS-insoluble LPP and SPP, and TUPP, LUPP, LPP, SPP and PP when P1 gave the highest proportion of

SDS-soluble LMP and SDS-insoluble LPP and SPP, and TUPP, LUPP, LPP, SPP and PP. However, treatment P6 gave the highest and P1 gave the lowest proportion of SDS-soluble SMP and SDS-insoluble LMP and SMP, and SMP and MP.

Table 3.5. Means of measured quality characteristics for six different nitrogen treatments (2001).

	SKwght	SKdiam	SKhard	HLM	FPC	FLN	VK	BFLY	FLY	P/L	Strength
P1	36.47	2.47	37.08	77.80	11.65	286.50	55.75	27.66	77.45	0.39	23.99
P2	36.51	2.50	36.26	78.00	11.45	277.38	53.00	28.49	77.24	0.38	20.76
P3	38.04	2.56	34.32	77.96	11.90	278.00	59.25	27.75	77.25	0.38	23.39
P4	38.26	2.58	33.16	77.50	11.06	267.00	48.25	28.89	77.46	0.41	21.08
P5	37.70	2.56	35.83	77.61	11.33	268.88	49.26	28.08	77.53	0.42	21.31
P6	38.00	2.58	31.67	77.34	10.70	261.50	44.25	29.75	77.05	0.41	18.21
Average	37.50	2.54	34.72	77.70	11.35	273.21	51.63	28.44	77.33	0.40	21.46
LSD (0.05)	2.080	0.093	20.055	1.603	0.518	77.435	10.167	2.886	2.076	0.100	6.103

SK-wght = single kernel chracterization system weight, SK-diam = single kernel characterization system diameter, SK-hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength

The effect of the different N-treatments on wheat quality characteristics (2001)

Results are given in Table 3.5.

There were no significant differences between the N-treatments for SK weight, SK hardness, HLM, FLN, BFLY, FLY, alveograph P/L and alveograph strength. P6 gave the lowest SK hardness, HLM, FPC, FLN, FLY and alveograph strength, although not significantly. There were significant differences between the N-treatments for FPC. The FPC of P6 was significantly lower than for P1, P2, P3, and P5. The VK of P6 was significantly lower than for P1 and P3. The VK of P4 was also significantly lower than for P3. P4 and P6 gave significantly higher SK diameters than P1.

Table 3.6. Means of protein fractions for four cultivars during 2001.

Cultivar	LPP1	SPP1	LMP1	SMP1	LPP2	SPP2	LMP2	SMP2	TUPP	LUPP	LPP	LMP	SPP	SMP	PP	MP
Snack	3.92	12.51	63.03	18.98	12.10	30.72	33.05	17.98	75.46	72.30	16.01	96.08	43.23	36.96	59.24	133.04
Cracker	5.52	13.28	58.95	20.55	13.99	33.35	32.86	16.69	71.67	71.52	19.51	91.81	46.63	37.24	66.14	129.05
E94/8	4.35	13.54	62.45	18.09	12.09	31.06	36.00	17.73	73.64	70.73	16.44	98.45	44.60	35.82	61.04	134.27
SST876	4.92	13.46	59.25	20.78	11.93	31.99	36.52	16.34	70.87	70.48	16.85	95.77	45.45	37.12	62.30	132.89
LSD (0.05)	0.545	0.987	1.440	1.564	1.096	1.873	1.745	1.845	2.370	1.607	1.393	2.084	2.484	3.063	3.697	3.859

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins, LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins

Differences between the cultivars for the different protein fractions (2001)

Results are given in Table 3.6.

SDS-soluble LPP: Cracker had the higest proportion of LPP and Snack the lowest.

SDS-soluble SPP: E94/8 had a significantly higher amount of SPP than Snack.

SDS-soluble LMP: Snack had a significantly higher amount of LMP than Cracker.

SDS-soluble SMP: SST876 and Cracker had significantly higher amounts of SMP than Snack and E94/8.

SDS-insoluble LPP and SPP: Cracker possessed significantly higher amounts of LPP and SPP than Snack, E94/8 and SST876.

SDS-insoluble LMP: SST876 and E94/8 had significantly higher amounts of LMP than Snack and Cracker.

SDS-insoluble SMP: There were no significant differences between the cultivars for SMP. Snack possessed the highest and SST876 the lowest amount of SMP.

TUPP: The TUPP of Snack was significantly higher than for Cracker and SST876.

LUPP: The LUPP of Snack was significantly higher than for E94/8 and SST876.

LPP: The LPP of Cracker was significantly higher than for SST876, E94/8 and Snack.

LMP: E94/8 and Snack had significantly higher proportions of LMP than Cracker.

SPP: The SPP of Cracker was significantly higher than for Snack.

SMP: There were no significant differences between the cultivars for SMP. Cracker had the highest proportion of SMP and E94/8 the lowest.

PP: The PP fraction of Cracker was significantly higher than for SST876, E93/8 and Snack.

MP: The MP fraction of E94/8 was significantly higher than for Cracker.

From the above results it is clear that cultivars Cracker and SST876 had the highest amounts of SDS-soluble LLP and SMP, SDS-insoluble SPP, LPP, SPP and SMP and PP, while Snack and E94/8 had the lowest amounts of these fractions. Cracker and SST876 had the lowest amounts of SDS-soluble LMP, SDS-insoluble SMP, TUPP, LMP and MP and Snack and E94/8 the highest.

Table 3.7. Means of measured quality characteristics for four cultivars during 2001.

Cultivar	SKwght	SKdiam	SKhard	HLM	FPC	FLN	VK	BFLY	FLY	P/L	Strength
Snack	38.55	2.54	11.61	76.53	11.06	200.33	45.00	32.23	75.38	0.33	16.00
Cracker	34.91	2.48	62.70	79.04	11.03	349.92	66.67	25.25	79.22	0.56	30.40
E94/8	38.51	2.59	15.21	75.74	11.58	185.00	45.17	30.73	74.91	0.33	15.26
SST876	38.03	2.56	49.36	79.50	11.72	357.58	49.67	25.53	79.81	0.37	24.16
LSD (0.05)	1.353	0.075	2.373	0.558	0.448	20.234	6.089	0.731	0.453	0.038	2.250

SK-wght = single kernel chracterization system weight, SK-diam = single kernel characterization system diameter, SK-hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strengh

Differences between the cultivars for the different quality characteristics (2001)

Results are given in Table 3.7.

SK weight: Cracker had a significantly lower SK weight than all three other cultivars.

SK diameter: E94/8 and SST876 had significantly higher SK diameters than Cracker.

SK hardness: Cracker was significantly the hardest, followed by SST876, E94/8 and Snack.

HLM, FLN and FLY: For all three quality parameters the cultivars were significantly different ($p>0.01$). SST876 and Cracker had significantly higher values for HLM, FLN and FLY than Snack and E94/8. Snack had a significantly higher HLM and FLY than E94/8 and SST876 had a significantly higher FLY than Cracker.

BFLY: SST876 and Cracker had significantly lower breakflour yields than Snack and E94/8. The BFLY of Snack was significantly higher than for E94/8.

FPC: SST876 and E94/8 had significantly higher flour protein contents than Snack and Cracker.

VK, alveograph P/L ratio and alveograph strength: There were significant differences between the cultivars for these three parameters. Cracker had a significantly higher VK, P/L ratio and alveograph strength than SST876, E94/8 and Snack. However, there were also significant differences between SST876 and Snack for P/L ratio and alveograph strength – with SST876 having higher values.

Table 3.8. Means of protein fractions for hard and soft wheats during 2001.

	LPP1	SPP 1	LMP1	SMP 1	LPP2	SPP 2	LMP2	SMP 2	TUPP	LUPP	LPP	LMP	SPP	SMP	PP	MP
Hard	5.22	13.37	59.10	20.66	12.96	32.67	34.69	16.51	71.27	71.00	18.18	93.79	46.04	37.18	64.22	130.97
Soft	4.13	13.03	62.74	18.53	12.10	30.89	34.53	17.86	74.55	71.51	16.23	97.26	43.91	36.39	60.14	133.65
LSD(0.05)	0.398	0.706	1.001	1.092	0.840	1.317	1.465	1.276	1.674	1.158	1.073	1.655	1.745	2.125	2.660	2.759

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins, LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins

Table 3.9. Means of measured quality characteristics for hard and soft wheats during 2001.

	SKwght	SKdiam	SKhard	HLM	FPC	FLN	VK	BFLY	FLY	P/L	Strength
Hard	36.47	2.52	56.03	79.27	11.38	353.75	58.17	25.39	79.51	0.46	27.28
Soft	38.53	2.57	13.41	76.13	11.32	192.67	45.08	31.48	75.15	0.33	15.63
LSD (0.05)	1.086	0.054	2.943	0.418	0.345	14.298	5.171	0.572	0.340	0.044	1.912

SK-wght = single kernel chracterization system weight, SK-diam = single kernel characterization system diameter, SK-hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength

Differences between the hard and soft wheat cultivars for the different protein fractions (2001)

Results are given in Table 3.8.

The hard wheat cultivars had significantly higher amounts of SDS-soluble LPP and SMP, SDS-insoluble LPP and SPP, LPP, SPP and PP than the soft wheat cultivars. The hard wheat cultivars also had higher amounts of SDS-soluble SPP, SDS-insoluble LMP and SMP, although not significantly. However, the soft wheat cultivars had significantly higher amounts of SDS-soluble LMP, SDS-insoluble SMP, TUPP and LMP than the hard wheat cultivars.

Differences between the hard and soft wheat cultivars for the different quality characteristics (2001)

Results are given in Table 3.9.

The hard wheat cultivars had significantly higher SK hardness, HLM, FLN, VK, FLY, alveograph P/L ratio and alveograph strength values than the soft wheat cultivars. The hard wheat cultivars also had higher FPC values than the soft wheats, however not significantly. The soft wheat cultivars had significantly higher SK weight and BFLY values than the hard wheat cultivars.

Mean square values for all measured characteristics are given in Table 3.10.

Table 3.10. Mean square values for protein fractions and quality characteristics of four cultivars (2001).

Trait	Mean Squares					
	Across cultivars and treatments		Only across N-treatments		Only across cultivars	
	Entry	**Blocks**	**Treatment**	**Blocks**	**Number**	**Blocks**
LLP1	1.015	3.271	0.265	3.271	5.823**	3.271*
SPP1	1.439	8.141	2.650	8.141	2.663	8.141
LMP1	12.357**	186.532**	15.349	186.532**	53.903**	186.532**
SMP1	8.592*	182.586**	19.409**	182.586**	19.885*	182.586**
LPP2	3.686	0.048	4.282	0.048	11.466**	0.048
SPP2	7.865	6.593	9.135	6.593	16.646	6.593
LMP2	12.547*	99.677**	7.937	99.677**	44.423**	99.677**
SMP2	6.225	107.700**	13.325	107.700**	7.588	107.700**
TUPP	15.733	36.331	18.065	36.331	51.064**	36.331
LUPP	4.424	8.560	6.720	8.560	8.142	8.560
LPP	6.493	4.114	4.153	4.114	29.711**	4.114
LMP	20.368*	558.920**	14.421	558.920**	90.916**	558.920**
SPP	12.530	0.082	16.620	0.082	24.579	0.082
SMP	18.430	570.747**	63.632**	570.747**	5.127	570.747**
PP	32.205	3.038	34.871	3.038	102.384*	3.038
MP	26.103	0.062	29.726	0.062	61.371	0.062
SK-wght	8.048*	5.894	5.124	5.894	36.402**	5.894
SK-diam	0.013	0.029	0.017	0.029	0.026	0.029
SK-hard	1011.3**	89.735**	33.711	89.735	7648.32**	89.735**
HLM	5.825**	0.827	0.557	0.827	41.023**	0.827
FPC	0.741**	0.152	1.453**	0.152	1.497*	0.152
FLN	14282.4**	4961.333*	658.233	4961.333	140379.**	4961.333*
VK	234.620**	24.083	237.550	24.083	1262.75**	24.083
BFLY	21.716**	0.317	5.012	0.317	152.841**	0.317
FLY	10.482**	0.853	0.261	0.853	77.423**	0.853
P/L	0.023**	0.000	0.002	0.000	0.152**	0.000
Strength	94.419**	3.950	34.109	3.950	621.753**	3.950

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric protein, LUPP = larger unextractable polymeric protein, LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins, SK-wght = single kernel chracterization system weight, SK-diam = single kernel characterization system diameter, SK-hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength

$^{*}p < 0.05$, $^{**}p < 0.01$

Table 3.11. Significant correlations between protein fractions and measured characteristics (2001).

SDS-soluble proteins			SDS-insoluble proteins			TUPP and combined protein fractions					
LPP	Sk-wght	-0.311*	LPP	Sk-wght	-0.505**	TUPP	Sk-hard	-0.404**	SPP	Sk-wght	-0.350*
	Sk-hard	0.602**		Sk-diam	-0.396**		HLM	-0.386**		Sk-hard	0.359*
	HLM	0.475**		Skcs-hard	0.371*		FLN	-0.356*		HLM	0.302*
	FLN	0.422**		VK	0.492**		BFLY	0.360*		VK	0.336*
	VK	0.389**		BFLY	-0.348*		FLY	-0.436**		BFLY	-0.386**
	BFLY	-0.549**		P/L	0.422**		Strength	-0.303*		FLY	0.311*
	FLY	0.548**		Strength	0.483**	LPP	Sk-wght	-0.499**		Strength	0.468**
	P/L	0.450**	SPP	Sk-wght	-0.379**		Sk-diam	-0.307*	PP	Sk-wght	-0.432 **
	Strength	0.580**		Sk-hard	0.393**		Sk-hard	0.524**		Sk-hard	0.448**
LMP	Sk-hard	-0.538**		HLM	0.303*		HLM	0.343*		HLM	0.336*
	HLM	-0.441**		VK	0.388**		FLN	0.312*		FLN	0.306*
	FLN	-0.389**		BFLY	-0.392**		VK	0.523**		VK	0.433**
	BFLY	0.415**		FLY	0.328*		BFLY	-0.485**		BFLY	-0.449**
	FLY	-0.572**		P/L	0.327*		FLY	0.411**		FLY	0.370*
	P/L	-0.360*		Strength	0.473**		P/L	0.497**		P/L	0.376*
	Strength	-0.338*	SMP	BFLY	0.315*		Strength	0.597**		Strength	0.548*
SMP	Sk-hard	0.307*		Strength	-0.308*	LMP	Sk-hard	-0.417**	MP	Sk-wght	0.353*
	FLY	0.356*					HLM	-0.306*		Sk-hard	-0.311*
							FPC	0.328*		VK	-0.343*
							FLY	-0.405**		FLY	-0.294 *
							P/L	-0.421 **		P/L	-0.310*
							Strength	-0.327*		Strength	-0.413**

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins, LPP = larger polymeric proteins, LMP = larger monomeric proteins, SPP = smaller polymeric proteins, SMP = smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins, SK-wght = single kernel chracterization system weight, SK -diam = single kernel characterization system diameter, SK -

hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength
*$p < 0.05$, ** $p < 0.01$

Correlations between protein fractions and quality characteristics (2001)

Results are given in Table 3.11.

The large polymeric proteins of the SDS-soluble fraction (LPP) were significantly positively correlated ($p<0.01$) with SK hardness, HLM, FLN, VK, FLY, P/L ratio and alveograph strength, and significantly negatively correlated with SK weight ($p<0.05$) and BFLY ($p<0.05$). The large monomeric proteins of the SDS-soluble fraction (LMP) were significantly positively correlated ($p<0.01$) only with BFLY and significantly negatively correlated ($p<0.01$) with SK hardness, HLM, FLN, FLY, P/L ($p<0.05$) and alveograph strength ($p<0.05$). The small monomeric proteins of the SDS-soluble fraction (SMP1) was significantly positively correlated ($p<0.05$) with SK hardness and FLY.

The SDS-insoluble large polymeric proteins (LPP) were significantly positively correlated ($p<0.05$) with SK hardness, VK ($p<0.01$), P/L ratio ($p<0.01$) and alveograph strength ($p<0.01$) and significantly negatively correlated ($p<0.01$) with SK weight, SK diameter and BFLY ($p<0.05$). The small polymeric proteins of the SDS-insoluble fraction (SPP2) were significantly positively correlated ($p<0.01$) with SK hardness, HLM ($p<0.05$), VK, FLY ($p<0.05$), P/L ($p<0.05$) and alveograph strength and significantly negatively correlated ($p<0.01$) with SK weight and BFLY. The small monomeric proteins of the SDS-insoluble fraction (SMP2) was significantly positively correlated ($p<0.05$) with BFLY and significantly negatively correlated with alveograph strength.

The total unextractable polymeric proteins (TUPP) were significantly positively correlated ($p<0.05$) with BFLY and significantly negatively correlated ($p<0.01$) with SK hardness, HLM, FLN ($p<0.05$), FLY and alveograph strength ($p<0.05$). The total large polymeric protein fraction (LPP) was significantly positively correlated ($p<0.01$) with SK hardness, HLM

($p<0.05$), FLN ($p<0.05$), VK, FLY, P/L and alveograph strength. The total LPP was also significantly negatively correlated ($p<0.01$) with SK weight, SK diameter ($p<0.05$) and BFLY. The total large monomeric proteins (LMP) were significantly positively correlated ($p<0.05$) with FPC, and significantly negatively correlated ($p<0.01$) with SK hardness, HLM ($p<0.05$), FLY, P/L and alveograph strength ($p<0.05$). The total small polymeric proteins (SPP) were significantly positively correlated ($p<0.05$) with SK hardness, HLM, VK, FLY and alveograph strength ($p<0.01$), but significantly negatively correlated with SK weight ($p<0.05$) and BFLY ($p<0.01$).

The total polymeric proteins (PP) were significantly positively correlated with SK hardness ($p<0.01$), HLM ($p<0.05$), FLN ($p<0.05$), VK ($p<0.01$), FLY ($p<0.05$), P/L ($p<0.05$) and alveograph strength ($p<0.05$) and significantly negatively correlated ($p<0.01$) with SK weight and BFLY. The total monomeric proteins (MP) were significantly positively correlated with SK weight ($p<0.05$) and significantly negatively correlated ($p<0.05$) with SK hardness, VK, FLY, P/L and alveograph strength ($p<0.01$).

Table 3.12. Correlations between ratios and quality characteristics for 2001 (p<0.01).

LPP:LMP	Sk-wght	-0.469	LPP1:LMP1	Sk-hard	0.616	LMP1:SMP1	Sk-hard	-0.399
	Sk-hard	0.559		HLM	0.490		FLY	-0.438
	HLM	0.376		FLN	0.427	LMP1:SPP2	Sk-hard	-0.564
	VK	0.497		BFLY	-0.550		HLM	-0.443
	BFLY	-0.476		FLY	0.578		FLN	-0.384
	FLY	0.462		P/L ratio	0.448		FLY	-0.521
	P/L ratio	0.534		Strength	0.550		P/L ratio	-0.443
	Strength	0.588	LPP1:SMP1	Strength	0.438		Strength	-0.522
LPP:SPP	Sk-wght	-0.449	LPP1:LPP2	Sk-hard	0.390	SMP1:SMP2	Sk-hard	0.545
	Sk-hard	0.473		FLY	0.417		HLM	0.567
	VK	0.490	LPP1:SPP2	Sk-hard	0.478		FLN	0.480
	BFLY	-0.391		HLM	0.379		BFLY	-0.534
	P/L ratio	0.516		BFLY	-0.419		FLY	0.654
	Strength	0.482		FLY	0.454		Strength	0.422
LPP:SMP	Sk-wght	-0.412		Strength	0.415	LPP2:SPP2	Sk-wght	-0.398
	Strength	0.423	LPP1:LMP2	Sk-hard	0.571	LPP2:LMP2	Sk-wght	-0.482
LMP:SPP	Sk-hard	-0.433		HLM	0.421		Sk-diam	-0.387
	FLY	-0.387		VK	0.436		VK	0.485
	P/L ratio	-0.388		BFLY	-0.472		P/L ratio	0.449
	Strength	-0.464		FLY	0.495		Strength	0.452
PP:MP	Sk-wght	-0.427		P/L ratio	0.512	LPP2:SMP2	Sk-wght	-0.423
	Sk-hard	0.429		Strength	0.577		Sk-diam	-0.345
	VK	0.417	LPP1:SMP2	Sk-hard	0.526		Strength	0.451
	BFLY	-0.420		HLM	0.498	SPP2:LMP2	VK	0.400
	Strength	0.536		FLN	0.419		P/L ratio	0.388
LPP1:SPP1	Sk-hard	0.630		BFLY	-0.546		Strength	0.417
	HLM	0.468		FLY	0.528	SPP2:SMP2	BFLY	-0.386
	FLN	0.419		Strength	0.575		Strength	0.426
	VK	0.405	SPP1:LMP1	FLY	0.377			
	BFLY	-0.522	LMP1: LPP2	Sk-wght	0.456			
	FLY	0.563		Sk-hard	-0.475			
	P/L ratio	0.515		VK	-0.443			
	Strength	0.529		BFLY	0.412			
				FLY	-0.383			
				P/L ratio	-0.485			
				Strength	-0.501			

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins, LPP = larger polymeric proteins, LMP = larger monomeric proteins, SPP = smaller polymeric proteins, SMP = smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins, SK-wght = single kernel chracterization system weight, SK-diam = single kernel characterization system diameter, SK-hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength

Correlations between ratios of protein fractions and quality characteristics (2001)

The correlations in Table 3.12 show how the different protein fraction ratios are related to the quality characteristics.

LPP:LMP was significantly positively correlated with SK hardness, HLM, VK, FLY, P/L ratio and alveograph strength and negatively with SK weight and BFLY.

LPP:SPP was significantly positively correlated with SK hardness, VK, P/L ratio and alveograph strength and negatively with SK weight and BFLY.

LPP:SMP was significantly positively correlated with alveograph strength and negatively with SK weight.

LMP:SPP was significantly negatively correlated with SK hardness, FLY, P/L ratio and alveograph strength.

PP:MP was significantly positively correlated with SK hardness, VK and alveograph strength and negatively with SK weight and BFLY.

LPP1:SPP1 was significantly positively correlated with SK hardness, HLM, FLN, VK, FLY, P/L ratio and alveograph strength and negatively with BFLY.

LPP1:LMP1 was significantly positively correlated with SK hardness, HLM, FLN, FLY, P/L ratio and alveograph strength and negatively with BFLY.

LPP1:SMP1 was significantly positively correlated with alveograph strength.

LPP1:LPP2 was significantly positively correlated with SK hardness and FLY.

LPP1:SPP2 was significantly positively correlated with SK hardness, HLM, FLY and alveograph strength and negatively with BFLY.

LPP1:LMP2 was significantly positively correlated with SK hardness, HLM, VK, FLY, P/L ratio and alveograph strength and negatively with BFLY.

LPP1:SMP2 was significantly positively correlated with SK hardness, HLM, FLN, FLY and alveograph strength and negatively correlated with BFLY.

SPP1:LMP1 was significantly positively correlated with FLY.

LMP1:LPP2 was significantly positively correlated with SK weight and BFLY and negatively with SK hardness, VK, FLY, P/L ratio and alveograph strength.

LMP1:SMP1 was significantly negatively correlated with SK hardness and FLY.

LMP1:SPP2 was significantly negatively correlated with SK hardness, HLM, FLN, FLY, P/L ratio and alveograph strength.

SMP1:SMP2 was significantly positively correlated with SK hardness, HLM, FLN, FLY and alveograph strength and negatively with BFLY.

LPP2:SPP2 was significantly negatively correlated with SK weight.

LPP2:LMP2 was significantly positively correlated with VK, P/L ratio and alveograph strength and negatively with SK weight and SK diameter.

LPP2:SMP2 was significantly positively correlated with alveograph strength and negatively with SK weight and SK diameter.

SPP2:LMP2 was significantly positively correlated with VK, P/L ratio and alveograph strength.

SPP2:SMP2 was significantly positively correlated with alveograph strength and negatively with BFLY.

3.3.2 Discussion

There were significant differences among the blocks in this trial for SDS-soluble LMP and SMP, SDS-insoluble LMP, total LMP and SMP (Table 3.10). When looking at the separate effects of the N-treatments and cultivars on the different protein fractions, differences were again found for SDS-soluble LMP and SMP, SDS-insoluble LMP and SMP, and total LMP and SMP. These results indicate significant differences between replicates, only for the large and small monomeric proteins, not the polymeric proteins. This could be due to the fact that monomeric proteins (gliadins) are most sensitive to the environment (e.g., temperature and fertilisation conditions) (Robert et al., 1996), while the polymeric proteins (glutenins) are almost totally genotype dependent (Graybosch et al., 1996).

Wheat cultivar and nitrogen (N) applications influenced the amount and size-distribution of several of the monomeric and polymeric proteins differentiable with SE-HPLC.

The hard wheat cultivars had significantly higher amounts of SDS-soluble and SDS-insoluble large polymeric proteins than the soft wheat cultivars, with Cracker having the higest proportion of SDS-soluble LPP and Snack the lowest. The SDS-insoluble LPP and total LPP of Cracker was significantly higher than for SST876, E94/8 and Snack. From the above it is obvious that the harder the wheat, the higher the SDS-soluble and SDS-insoluble LPP fractions. This is reflected in the significant positive correlations between all LPP fractions with SK hardness.

There were no significant differences among the cultivars for SDS-soluble LPP, possibly because there were no significant effects between the different N-treatments. The SDS-soluble LPP fraction appeared thus relatively independent of environmental changes. However, differences were visible among the cultivars for SDS-insoluble LPP and total LPP. N-treatment P6

gave a significantly lower SDS-insoluble LPP than P1, P2 and P3; the SDS-insoluble LPP of P5 was significantly lower than for P1; and P6 gave a significantly lower LPP than P1. This can explain why Snack P1 had a significantly higher proportion of SDS-insoluble LPP than Snack P4 and P6, why the SDS-insoluble LPP of Cracker P2 and P3 were significantly higher than for Cracker P5 and P6, and why the LPP of Snack P6 and Cracker P6 were significantly lower than for Snack P1 and Cracker P3. N-treatments P1, P2 and P3 could be responsible for the higher LPP values because of the higher total amounts of N applied (200 – 230 kg N/ha) during the growth season. Conversely, P5, P6 and possibly P4 could be responsible for the lower LPP values because of the lower total amounts of N applied (140 – 190 kg N/ha).

The SDS-insoluble LPP fraction was significantly positively correlated with SK hardness, VK, alveograph P/L ratio and alveograph strength, while the SDS-soluble LPP and the total LPP fractions showed significant positive correlations with SK hardness, HLM, FLN, VK, FLY, aleograph P/L ratio and alveograph strength. Thus, both the SDS-soluble and SDS-insoluble LPP fractions were associated with good bread-making quality characteristics. The SDS-soluble LPP, SDS-insoluble LPP and total LPP fractions were significantly negatively correlated with SK weight and BFLY. The SDS-insoluble LPP and the total LPP were also significantly negatively correlated with SK diameter.

The hard wheat cultivars had significantly higher amounts of smaller polymeric proteins (SDS-insoluble SPP and total SPP) than the soft wheat cultivars. The hard wheat cultivars also had higher amounts of SDS-soluble SPP, although not significantly. E94/8 had a significantly higher proportion of SDS-soluble SPP than Snack. Cracker possessed significantly higher amounts of SDS-insoluble SPP than Snack, E94/8 and SST876. The SPP of Cracker was significantly higher than for Snack. It is obvious that the harder the wheat, the higher the SPP fractions (especially the SDS-insoluble SPP fraction) because of

the significant positive correlations between the SDS-insoluble SPP and total SPP fractions with SK hardness.

There were no significant differences among the cultivars for SDS-soluble SPP, although there were significant differences between the N-treatments. Both P1 and P5 gave significantly higher SDS-soluble SPP values than P6; P1 gave significantly higher SDS-insoluble SPP values than P6; and the total SPP of P1 was significantly higher than for P5. The total SPP and SDS-insoluble SPP of Cracker P3 were significantly higher than for P6. It seems as if the N-treatment P6 was responsible for the lowest amounts of the SPP fractions, possibly because of the lowest total amount of N applied during the growth season (140 kg N/ha). Except for the higher total amounts of N applied with treatments P1, P3 and P5 (170 – 230 kg N/ha), the higher SPP fractions could also be the result of later N-applications (30 kg N/ha during the flag leave stage) characteristic of P1, P3 and P5. It is known from studies described in the literature that late N applications increase significantly the amounts of all the protein fractions, the yield, the grain protein content, and thus the bread-baking quality properties (Jia et al., 1996).

The SDS-insoluble SPP and total SPP fractions were significantly positively correlated with SK hardness, HLM, VK, FLY, alveograph P/L ratio (only SDS-insoluble SPP) and alveograph strength and significantly negatively correlated with SK weight and BFLY. A high SDS-insoluble SPP fraction is thus also characteristic of good bread-making quality.

Both the SDS-soluble LMP and total LMP fractions (large monomeric proteins) were significantly negatively correlated with SK hardness. This explains why the soft wheat cultivars (e.g., Snack) had significantly higher amounts of SDS-soluble LMP and total LMP than the hard wheat cultivars (e.g., Cracker). The cultivars SST876 and E94/8 gave significantly higher proportions of SDS-insoluble LMP than Snack and Cracker, thus there were

no clear differences between the hard and soft wheat cultivars for SDS-insoluble LMP.

Significant differences were visible among the cultivars for both LMP fractions and total LMP. Cracker P3 had a significantly higher SDS-soluble LMP than Cracker P6; E94/8 P1 and P3 had significantly higher SDS-soluble LMP values than P5 and P6; and SST876 P1 had a significantly higher SDS-soluble LMP than SST876 P2, P4 and P6. These resuls are confirmed by the significant differences between the N-treatments. The SDS-soluble LMP of P1, P2 and P3 were significantly higher than for P6, which indicates that the higher the total amount of N applied (e.g., P1, P2), the higher the SDS-soluble LMP value. E94/8 P2 had a significantly higher SDS-insoluble LMP than P4 and P6, which can also be explained by the above assumption. However, totally opposite results were obtained for the rest of the SDS-insoluble LMP values. Cracker P1, P2 and P3 had significantly lower SDS-insoluble LMP values than Cracker P5 and P6 and SST876 P1 had a significantly lower SDS-insoluble LMP than P3 and P5. In this case the N-treatment P1 gave a significantly lower SDS-insoluble LMP than P5. A higher total amount of N applied most probably had a lower SDS-insoluble LMP value as a result. The results of the total LMP values were very similar to results obtained from SDS-soluble LMP. The LMP of N-treatment P4 was significantly lower than for P3, the LMP values of E94/8 P4 and P6 were significantly lower than for P2 and P3 and the LMP of SST 876 P4 and P6 were also significantly lower than for P3.

The SDS-soluble LMP fraction was significantly positively correlated with BFLY and the total LMP was significantly positively correlated with FPC. Both these fractions were significantly negatively correlated with SK hardness, HLM, FLN (only total LMP fraction), FLY, alveograph P/L ratio and alveograph strength. There were no significant correlations between SDS-insoluble LMP and the quality characteristics, which indicates that the different results obtained from SDS-insoluble LMP did not have significant

effects on the quality characteristics and that the results obtained from SDS-soluble LMP and total LMP are more reliable.

The hard wheat cultivars (SST876 and Cracker) had significantly higher amounts of SDS-soluble small monomeric proteins (SMP) and total SMP (not significantly) than the soft wheat cultivars (Snack and E94/8), while the soft wheat cultivars had significantly higher amounts of SDS-insoluble SMP than the hard wheat cultivars. Only the SDS-soluble SMP fraction was significantly positively correlated with SK hardness.

The SDS-soluble SMP of Snack P6 was significantly higher than for Snack P1 and P3; Cracker P6 had a significantly higher SDS-soluble SMP than P1, P2, P3 and P4; the SDS-soluble SMP values of SST876 P4 and P6 were significantly higher than for P1, P2 and P5; and the SDS-soluble SMP of E94/8 P6 was also higher than for the rest of the E94/8 treatments, not significantly though. From the above it is clear that the P6 N-treatment gave a significantly higher proportion of SDS-soluble SMP than the other treatments. P1 gave a significantly lower SDS-soluble SMP than P4 and P6. These results are similar to the results of SDS-insoluble SMP and SMP (total). The total SMP of Snack P6 was significantly higher than for P1, P2 and P3; the total SMP of Cracker P6 was significantly higher than for Cracker P3; and Snack P6 had a significantly higher proportion of SDS-insoluble SMP than all the other Snack treatments, except for Snack P3. The SMP of N-treatments P6 and P4 were significantly higher than for P1, and P6 gave a significantly higher SDS-insoluble SMP than P1. The higher total amounts of N applied by treatments P1, P2 and P3 (200 – 230 kg N/ha) had a negative effect on the amounts of the SMP fractions, while lower total N amounts (P6 = 140 kg N/ha) gave higher SMP fractions.

There were no significant correlations between the total SMP fraction and the quality characteristics. The SDS-soluble SMP fraction was significantly positively correlated with SK hardness and FLY. The SDS-insoluble SMP

fraction was significantly positively correlated with BFLY and significantly negatively correlated with alveograph strength. These relatively few correlations of the SMP fractions with quality characteristics agrees with MacRitchie (1984) who concluded that there is no (or few) correlations between the amount of albumins and globulins and baking performance.

The soft wheat cultivars had significantly higher amounts of total unextractable polymeric proteins (TUPP) than the hard wheat cultivars as can be seen from the significant negative correlation between TUPP and SK hardness. For example, the TUPP of Snack was significantly higher than for Cracker and SST876. For the soft wheat cultivars to have higher TUPP values they had to posess higher amounts of SDS-insoluble LPP and SPP and/or lower amounts of SDS-soluble LPP and SPP than the hard wheat cultivars (refer to 3.2.2b for the formula of Gupta et al., 1993). It was already shown that the hard wheat cultivars posessed significantly higher amounts of SDS-soluble and SDS-insoluble LPP and SPP than the soft wheat cultivars. The only explanation for the higher TUPP values could be that the soft wheats possessed lower amounts of all these fractions but with a high SDS-insoluble:SDS-soluble ratio. This was definitely the case as can be seen in Tables 3.2, 3.6 and 3.8. The hard wheats also showed high SDS-insoluble:SDS-soluble ratios, but still their TUPP values remained lower than for the soft wheats. According to the literature (He & Hoseney, 1990), the gluten from poor-quality flour has higher solubility than the gluten from good-quality flour because of the occurrence of smaller molecular weight proteins. The hard wheat cultivars were thus supposed to give higher TUPP values than the soft wheat cultivars.

The N-treatment P1 gave a significantly higher TUPP than P5, which can explain why E94/8 P5 had a significantly lower TUPP than P1, P3 and P4. Thus, the higher the total amount of N applied (e.g., P1), the higher the TUPP value.

TUPP was significantly positively correlated with BFLY and significantly negatively correlated with SK hardness, HLM, FLN, FLY and alveograph strength (the quality characteristics associated with hard wheats).

There were no significant differences in the large unextractable polymeric proteins (LUPP) between the hard and soft wheat cultivars and also no significant correlations between LUPP and the quality characteristics. The LUPP of Snack was significantly higher than for E94/8 and SST876. Cracker P5 and E94/8 P5 had significantly lower LUPP values than Cracker P2 and E94/8 P4. It seems as if treatment P5 has given the lowest amount of LUPP for all the cultivars, not significantly though in all cases. The LUPP of N-treatment P1 was significantly higher than for P5 – possibly because of the higher amount of N applied during treatment P1.

The hard wheat cultivars had significantly higher amounts of polymeric proteins (PP) than the soft wheat cultivars as already proven by the higher LPP and SPP fractions of the hard wheats. Cracker had the highest and Snack the lowest amount of PP. The PP fraction of Cracker was significantly higher than for SST876, E93/8 and Snack. The PP fraction was also significantly positively correlated with SK hardness, just like the LPP and SPP fractions.

The N-treatment P6 gave a significantly lower PP than P1. This can explain why the PP of both Cracker P6 and Snack P6 were significantly lower than for Cracker P3 and Snack P1 . A rough estimation can be made that the P1, P2 and P3 treatments, with their higher total amounts of N applied, could be responsible for the higher LPP, SPP and PP fractions. Conversely, the P4, P5 and P6 treatments, with their lower total amounts of N applied, could be responsible for the lower LPP, SPP and PP fractions.

The soft wheat cultivars had higher amounts of monomeric proteins (MP) than the hard wheat cultivars, not significantly though. Still, the MP fraction showed a negative correlation with SK hardness which proves why the MP fraction of E94/8 was significantly higher than for Cracker.

N-treatment P6 gave a significantly higher MP than P1. This explains why the MP of Snack P6 was significantly higher than for Snack P1. A lower amount of total N applied (e.g., P6) could be responsible for a higher MP, and vice versa.

The MP fraction was significantly positively correlated with SK weight and significantly negatively correlated with SK hardness, VK, FLY, alveograph P/L ratio and alveograph strength.

From the above results it is clear that cultivars Cracker and SST876 (the hard wheat cultivars) had the highest amounts of all LLP fractions, SDS-insoluble SPP, total SPP and PP, while Snack and E94/8 (the soft wheat cultivars) had the lowest amounts of these fractions. Cracker and SST876 had the lowest amounts of SDS-soluble LMP, SDS-insoluble SMP, TUPP, LMP and MP, and Snack and E94/8 the highest. It is obvious that higher amounts of polymeric proteins and lower amounts of monomeric proteins are characteristic of the hard wheats, while lower amounts of polymeric proteins and higher amounts of monomeric proteins are characteristic of the softer wheats. These results suggest significant negative correlations between LPP and LMP (an increase in polymeric proteins will lead to a reduction in monomeric proteins). Bettge et al. (1989) and Souza et al. (1994) also concluded that flours of weak-dough soft wheats generally have lesser amounts of HMW glutenins, lower protein contents and weaker gluten strengths.

According to Table 3.10, the cultivar effect was mostly responsible for the significant differences between the protein fractions, while the N-treatments had a very little effect.

From the above results and Table 3.4 it is clear that the N-treatment P6 usually gave the lowest amounts of SDS-soluble LPP, SPP and LMP, SDS-insoluble LPP and SPP, total LPP, SPP and PP and the highest amounts of SDS-soluble SMP, SDS-insoluble SMP, total SMP and MP. P1 was responsible for the highest amounts of SDS-soluble LMP, SDS-insoluble LPP and SPP, TUPP, LUPP, LPP, SPP and PP and the lowest amounts of SDS-soluble SMP, SDS-

insoluble LMP and SMP, total SMP and MP. This could indicate that the higher the total amount of N applied (e.g., P1), the higher the polymeric fractions (and also SDS-soluble LMP) and the lower the monomeric protein fractions (especially the smaller monomeric proteins). Opposite results were obtained from lower total amounts of N applied, namely, lower polymeric fractions and higher SMP fractions. Different levels of nitrogen (N) fertilisation are known to cause changes in flour protein quantities and proportions (Wieser & Seilmeier, 1998). However, the N-treatments did have an effect on the SMP fractions, which is in contrast with the foundings of Wieser and Seilmeier (1998). They concluded that different N-treatments have strong influences on the quantity of storage proteins (gliadins, glutenin subunits) in wheat flour, but not on that of other endosperm proteins (albumins, globulins).

Wieser and Seilmeier (1998) also believed that the effects on total gliadins are more pronounced than on total glutenin subunits, and that major protein types (a-gliadins, ?-gliadins, LMW subunits of glutenin) are more affected than minor types (?-gliadins, HMW subunits). However, the percentage increase of gliadin (LMP) was lower than that of glutenin (LPP & SPP) (Table 3.4; calculations not shown).

The timings of the N-applications did not have much of an effect on the protein fractions. Maybe if the amount of N applied during the flag leave stage was increased, the timings of N-applications would have had a greater impact than the total amount of N applied during the growth season.

The polymeric protein fractions showed similar positive correlations with SK hardness, HLM (not SDS-insoluble LPP), FLN (not SDS-insoluble LPP and SPP), VK, FLY, alveograph P/L ratio (not SPP) and alveograph strength. This indicates the big influence of the polymeric proteins on the quality characteristics. Studies by Dachkevitch and Autran (1989) have shown a direct correlation between the size and the amount of the glutenin polymers and

quality characteristics. Higher amounts of polymeric proteins will thus have better quality as a result. The SDS-soluble and SDS-insoluble polymeric proteins were found to be equally important in quality prediction.

The large monomeric proteins (SDS-soluble LMP and total LMP), which are mainly gliadins, had a consistent negative effect on quality. These proteins were significantly negatively correlated with SK hardness, HLM, FLN (only total LMP fraction), FLY, alveograph P/L ratio and alveograph strength. The MP fraction was also significantly negatively correlated with SK hardness, VK, FLY and alveograph P/L ratio and both the MP and SDS-insoluble SMP fractions were significantly negatively correlated with alveograph strength.

The SDS-soluble LMP and SDS-insoluble SMP fractions were significantly positively correlated with BFLY, MP was significantly positively correlated with SK weight, and the total LMP was significantly positively correlated with FPC.

All of the significant differences among the quality characteristics were due to the cultivar effect. Table 3.10 indicates that only the differences in FPC were partly due to the N-treatments.

There were significant differences between the replicates for SK-hardness and FLN (Table 3.10).

Many statistically significant differences were found among flours for the different quality parameters. Because the hard and soft wheat cultivars had statistically different SK hardnesses (hard wheats significantly harder), they exhibited different rheological and functional characteristics. Cracker was significantly the hardest, followed by SST876, E94/8 and Snack. The harder the cultivars, the higher the LPP fractions, SDS-insoluble SPP, total SPP, SDS-soluble SMP and PP fractions which could be explained by the significant positive correlations of these fractions with SK hardness. The SDS-soluble LMP, total LMP, TUPP and MP fractions were significantly negatively

correlated with SK hardness. Thus, higher amounts of these fractions were characteristic of the soft wheat cultivars. SK hardness was significantly positively correlated with HLM, FLN, VK, FLY, alveograph P/L ratio and alveograph strength, which gives harder wheats higher quality characteristics for bread-baking. SK hardness was also significantly negatively correlated with BFLY (Table 3.36).

The hardness of a given cultivar of wheat is genetically controlled and is not directly correlated with the protein content of the kernel (Miller et al., 1984). Thus it is understandable that the different N-treatments had no significant effect on the SK hardness of the cultivars. In contrast with the above findings, Van Lill and Smith (1997) reported that grains containing higher protein contents were inclined to be harder. Although the N-treatments had no significant effect on the SK hardness of the cultivars, there were significant differences among the cultivars for SK hardness. For example, the SK hardnesses of Snack P1, P2 and P5 were significantly higher than for P4. Cracker P1 was significantly harder than Cracker P6 and SST876 P1, P2 and P5 were significantly harder than SST876 P6. This was the result of higher polymeric protein fractions due to higher total amounts of N applied. It is obvious that the N-treatments did have an indirect effect on the SK hardness of the cultivars.

Cultivars with a low to medium SK diameter could indicate increased kernel hardness, because of the compactness of the endosperm (Mamuya, 2000). This fact was confirmed when Cracker, the hardest cultivar, had a significantly lower SK diameter than E94/8 and SST876, with relatively soft kernel hardnesses. The SDS-insoluble LPP and total LPP fractions were significantly negatively correlated with SK diameter. This shows that the harder the cultivar (the higher the SDS-insoluble LPP and LPP fractions), the lower the SK diameter.

The different N-treatments had significant effects on the SK diameters of the cultivars. For example, P4 and P6 gave significantly higher SK diameters than P1 and the SK diameters of Snack P6, P4 and P3 were significantly higher than for P2. It was already shown that N-treatments that applied a higher total amount of N (e.g., P1 = 230 kg N/ha) gave significantly higher amounts of polymeric protein fractions, which are negatively correlated with SK diameter. The higher SK diameters of N-treatments P3, P4 and P6 could thus be the result of lower amounts of LPP fractions. The kernel diameter is an indication of how plump the wheat kernels are, and therefore is associated with kernel mass (SK weight), HLM and FLY (Mamuya, 2000).

The soft wheat cultivars had significantly higher SK weight values than the hard wheat cultivars. The SK weight of Cracker (the hardest cultivar) was significantly the lowest. The SDS-soluble LPP, SDS-insoluble LPP, total LPP, SDS-insoluble SPP, total SPP and PP fractions (positively correlated with SK hardness) were significantly negatively correlated with SK weight. In most cases a higher amount of these fractions would lead to harder grain, smaller SK diameters and lower SK weights. The MP fraction was significantly positively correlated with SK weight, which explains why the soft wheat cultivars had higher SK weights (the higher the MP fraction, the softer the wheat, and the higher the SK weight). Usually the endosperm of soft wheats tend to be less compact, leading to a larger SK diameter and reduced mass, but in this case the soft wheats still had higher SK weights than the hard wheats. SK weight was significantly positively correlated with SK diameter and BFLY and significantly negatively correlated with SK hardness, FLN, VK and alveograph strength.

There were no significant differences between the N-treatments for SK weight, which indicates that the SK weight is mostly genotype dependent. Still there were a few significant differences among the cultivars. The SK weight of Snack P2 was significantly lower than for P3, P4 and P6, and the SK weight of Cracker P3 was significantly lower than for P5. A possible

explanation could be that the higher the total amount of N applied (e.g., P1, P2), the higher the LPP fractions, the harder the grain, and the lower the SK diameters and SK weights.

Hectoliter mass (HLM) is one of the parameters used to predict expected flour yield during milling. A high HLM value indicates sound wheat. As hectoliter mass drop, the percentage of small, malformed, and broken kernels usually increases (Atwell, 2001). In South Africa, a minimum hectoliter mass of 76 kg/hl is required for wheat to be graded as suitable for bread-making (Mamuya, 2000). The hard wheat cultivars (SST876 and Cracker) had significantly higher HLM values than the soft wheat cultivars (Snack and E94/8). Both the mean HLM for the hard wheats (79.27 kg/hl) and the mean HLM for the soft wheats (76.13 kg/hl) were above the recommended value for bread-baking. Snack had a significantly higher HLM than E94/8. The SDS-soluble LPP, total LPP, SDS-insoluble SPP, total SPP and PP fractions, which are significantly positively correlated with SK hardness, were also significantly positively correlated with HLM. Harder wheats are thus supposed to give higher HLM values. The SDS-soluble LMP, total LMP and TUPP fractions were significantly negatively correlated with HLM. This supports the above conclusion. HLM was significantly positively correlated with FLN, VK, FLY, alveograph P/L ratio and alveograph strength.

Although the N-treatments gave no significant differences for HLM, there were significant differences among the cultivars. The HLM of SST876 P6 was significantly lower than for P1, P2 and P3. The low total amount of N applied by P6 gave indirectly a lower HLM because of its direct effect on the polymeric protein fractions.

Low-protein flour (7 – 9% protein) milled from soft cultivars of *T. aestivum* is most suitable for making cakes and biscuits (Hoseney et al., 1988). The flour protein content (FPC) of Snack varied from 10.4 to 11.3%, and the FPC of E94/8 from 10.8 to 12.4%. N-treatments P2 and P3 were responsible for the

highest FPC values in Snack and E94/8, with P4 and P6 responsible for the lowest FPC values. Flour for cracker products is higher in protein content (9 – 10%) and generally stronger than cookie flour (Hoseney et al., 1988). The FPC of Cracker varied from 10.1 to 11.9%, which is stronger than for Snack and E94/8. Flours from hard wheats with a protein content of 11 – 13% are primarily used for bread-making (Hoseney et al., 1988). The FPC of SST876 ranged from 10.6 to 12.2% which could have been higher. Under ideal circumstances the hard wheat cultivars would have had significantly higher FPC values than the soft wheat cultivars, but in this study it was not the case. According to Table 3.11, the total LMP was significantly positively correlated with FPC and significantly negatively correlated with SK hardness, HLM, FLY, alveograph P/L ratio and alveograph strength. FPC was also significantly negatively correlated with the alveograph P/L ratio (Table 3.36).

Significant differences between the different N-treatments were noted. P6 gave a significantly lower FPC than treatments P1, P2, P3 and P5. A possible explanation can be that regimes with a high proportion of total N resulted in the highest FPC values. N-treatment P6 consisted of the least total amount of N (140 kg N/ha) in contrast with P1 (230 kg/ha), P2 (200 kg/ha), P3 (200 kg/ha), P4 (170 kg/ha) and P5 (170 kg/ha). This could also explain why Snack P2 and P3 gave significantly higher FPC values than P4 and P6, why the FPC of Cracker P1 and P3 were higher than for P5 and P6, why the FPC of E94/8 P2 and P3 were significantly higher than for P4, and why SST876 P6 had a significantly lower FPC than of all the other SST876 treatments, but P2. The higher N-applications also led to higher amounts of the LMP fraction, which is positively correlated with FPC. N-treatments P1, P3 and P5 gave late N-applications (30 kg N/ha during flag leave stage) in contrast with P2, P4 and P6 (only with plant and six weeks after planting). These late N-applications did not have a significant effect on the FPC values.

Even though the different N-treatments showed significant effects for FPC, it is obvious that the N-treatments had a greater effect on the different protein fractions than on the total FPC.

The hard wheat cultivars (SST876 and Cracker) had significantly higher falling number (FLN) values than the soft wheat cultivars (Snack and E94/8). Flour with a higher FLN results in higher loaf volume with a good (fine) texture, whereas lower FLN flours result in lower loaf volumes and poor (coarse) texture (Mamuya, 2000), which is suitable for the soft wheats. A bread wheat with average α-amylase activity has a falling number of about 250 seconds. The upper limit for the falling number test is about 400 seconds, which occurs for a flour devoid of α-amylase activity (Atwell, 2001). The falling number of the bread cultivar SST876 was 357.58 s and of Cracker was 349.92 s. The average falling number of the two soft wheat cultivars was 192.67 s, which is well below the standard for bread wheats, but still well above the falling number of sprout-damaged wheat (100 s). The FLN values obtained during this study are suitable for the hard and soft wheat cultivars. FLN was significantly positively correlated with FLY, alveograph P/L ratio and alveograph strength and negatively correlated with BFLY.

There were no significant differences between the N-treatments for FLN; still there were significant differences among the cultivars. The FLN of Cracker P3 and P5 were significantly higher than for P6 and the FLN of SST876 P1 and P2 were higher than for P5. This could be due to the fact that the SDS-soluble LPP, total LPP and PP fractions were significantly positively correlated with FLN. Thus, the lower the total amounts of N-applied (e.g., P6), the lower the LPP fractions, the softer the wheat, and the lower the FLN values. The SDS-soluble LMP and TUPP fractions were significantly negatively correlated with FLN. This proves the previous conclusion to be right, because the higher the SDS-soluble LMP and TUPP values, the lower the total amount of N-applied, the lower the FLN and the softer the wheat.

Vitreous kernels (VK) indicate the extent (percentage) to which a certain genotype appears to be hard (Mamuya, 2000). The hard wheat cultivars had significantly higher amounts of VK than the soft wheat cultivars which are in accordance with what can be expected. Cracker, the hardest cultivar, had significantly higher VK values than SST876, E94/8 and Snack. These results indicate a definite positive correlation between VK and SK hardness. All the LPP fractions, SDS-insoluble SPP, total SPP and PP fractions (which are positively correlated with SK hardness) were significantly positively correlated with VK. The MP fraction was significantly negatively correlated with VK.

There were also significant differences between the N-treatments for VK. P6 gave a significantly lower VK than P1 and P3. The amount of VK caused by P4 was also significantly lower than for P3. A possible explanation is again the lower total N applied by P4, P5 and P6 (140 - 170 kg/ha) in contrast with P1, P2 and P3 (200 - 230 kg/ha) and the resultant lower polymeric protein fractions The later N-applications of treatments P1, P3 and P5 (30 kg N/ha during flag leave stage) could also have had an influence on the above results. The VK amounts of Snack P3, Cracker P3 and E94/8 P3 were significantly higher than for Snack P5, Cracker P6 and E94/8 P5, respectively. The VK value of SST876 P6 was significantly lower than for P1, P2, P3 and P5, all of which could be explained by the total amounts of N applied in the different N-treatments.

Breakflour yield (BFLY) is related to wheat hardness, with higher BFLY values representative of soft wheats. The soft wheat cultivars (Snack and E94/8) had significantly higher BFLY values than the hard wheat cultivars (SST876 and Cracker) as could be expected. The BFLY of Snack (the softest cultivar) was significantly higher than for E94/8. The SDS-soluble LPP, SDS-insoluble LPP, total LPP, SDS-insoluble SPP, total SPP and PP fractions were significantly negatively correlated with BFLY, while the SDS-soluble LMP and TUPP fractions were significantly positively correlated with BFLY. This

agrees with data which indicates that SK hardness was significantly negatively correlated with BFLY (Table 3.36). There was also a significant negative correlation between BFLY and FLY.

There were no significant differences between the N-treatments for BFLY, but still the following significant differences among the cultivars were recorded: the BFLY values of Snack P1, P2, P3 and P5 were significantly lower than for P4 and P6; the BFLY values of Cracker P1 and P3 were significantly lower than for P2 and P6; the BFLY of E94/8 P1 was significantly lower than for P4 and P6; and the BFLY of SST876 P6 was significantly higher than for all the other SST876 treatments. Also, SST876 P4 had a higher BFLY than P1, P3 and P5. The late N-applications (30 kg N/ha during the flag leave stage) given by P1, P3 and P5 had a definite effect on these results. The later N-applications were most likely responsible for the lower BFLY values.

Higher flour yield (FLY) is the primary objective for millers, and in South Africa, an extraction of more or less 76% for white flour is used as a selection norm for new cultivars (Mamuya, 2000). The hard wheat cultivars had significantly higher FLY values than the soft wheat cultivars, with SST876 having a significantly higher FLY than Cracker, and Snack having a significantly higher FLY than E94/8. The hard wheat cultivars had a mean flour yield (79.51) above the recommended value, with the mean flour yield of the soft wheats slightly under (75.51). The SDS-soluble LPP, total LPP, SDS-insoluble SPP, total SPP, SDS-soluble SMP and PP fractions were significantly positively correlated with FLY. The SDS-soluble LMP, total LMP, TUPP and MP fractions were significantly negatively correlated with FLY. There was also a significant negative correlation between BFLY and FLY. All the above correlations indicate that harder wheats posess higher amounts of polymeric protein fractions and have higher flour yields, while soft wheats posess higher amounts of monomeric proteins and have lower flour yields. FLY was also significantly positively correlated with alveograph P/L ratio and alveograph strength, quality characteristics associated with bread-baking quality.

Although there were significant differences among the cultivars for FLY, there were no significant differences between the N-treatments for FLY. Snack P6 had a significantly lower FLY than Snack P3 and P4. E94/8 P5 had a significantly higher FLY than all the others in the E94/8 group. The late N-applications (30 kg N/ha during the flag leave stage) given by P1, P3 and P5 had a definite negative effect on the BFLY results and thus a positive effect on the FLY values was expected. The higher FLY values of Snack P3, Snack P4 and E94/8 P5 can possibly be explained by the later N-applications but also by the higher total amounts of N applied by these treatments in contrast with P6.

Significant differences in alveograph P/L ratios were observed between the hard and soft wheat cultivars. "P" relates to the dough's tenacity and elastic resistance and it predicts the dough's ability to retain gas. Weak and strong doughs show low and high "P" values, respectively. On the other hand "L" gives information about dough extensibility with longer "L" values indicating weak doughs. A high P and a short L value would denote very elastic dough. In South Africa P/L values between 0.5 and 0.8 produce good quality bread, with 0.8 being the optimum (Mamuya, 2000). The hard wheat cultivars had significantly higher P/L ratios (0.46) than the soft wheat cultivars (0.33) with Cracker having the highest and SST876 having a higher P/L ratio than Snack. These results are in accordance to what could be expected, because soft wheats for cake and biscuit production should have lower P/L ratios than hard wheats. However, the P/L ratio of SST876 (0.37) is far below the recommended value for bread-baking. A positive correlation with the SDS-soluble LPP, SDS-insoluble LPP, total LPP, SDS-insoluble SPP and PP fractions and a negative correlation with the SDS-soluble LMP, total LMP and MP fractions agrees with the fact that harder wheats posess higher P/L ratios. The P/L ratio was also significantly positively correlated with alveograph strength.

There were significant differences among the cultivars – even though there were no significant differences between the N-treatments. The alveograph P/L ratio of Cracker P6 was higher than for P1, P2, P3 and P4. The alveograph P/L ratio of E94/8 P4 and SST876 P5 were also higher than for E94/8 P2 and P3 and SST876 P6 and P4. Most of these results can be explained by the indication that lower total amounts of N (P4, P5, P6) gave higher P/L ratios, while higher total amounts of N applied (P1, P2 and P3) gave lower P/L ratios.

The alveograph strength is an indication of the amount of work required for the deformation of the dough and is related to the baking strength of the dough. Alveograph strength is always derived from the alveographic parameter "W" under the curve. Lower and higher "W" values indicate weak and strong flours, respectively. A "W"-value close to 250 is usually recommended so that it, when divided by a constant 6.54, will result in a strength value of about 35 joules which is popular with bakers (Mamuya, 2000). The hard wheat cultivars had significantly higher alveograph strength values than the soft wheat cultivars. Cracker had significantly the highest alveograph strength and the alveograph strength of SST876 was significantly higher than for Snack. The low alveograph strength values of the soft wheats (15.26 and 16.00) is recommended for cake and biscuit production, but the strength value of SST876 of 24.16 seems low for bread-baking. All LPP values, SDS-insoluble SPP, total SPP and PP fractions were significantly positively correlated with alveograph strength and the SDS-soluble LMP, total LMP, TUPP and MP fractions were significantly negatively correlated with alveograph strength. This agrees with the fact that harder wheats (with higher polymeric protein fractions) should have higher alveograph strengths.

The different N-treatments did not have a significant effect on the alveograph strength of the cultivars; however, there were significant differences among the cultivars. The alveograph strength of Cracker P3 was higher than for P2, P4, P5 and P6 and the alveograph strengths of SST876 P1 and P3 were

significantly higher than for P6. The higher total amounts of N applied by P1 and P3 could be the cause of these results.

The relative amounts of the protein fractions showed enormous effects on the quality characteristics of the cultivars, but according to the literature, various protein fraction ratios can also give information about a cultivar's quality. The values in Table 3.8 were used to calculate the ratios (calculations not shown).

According to Huebner and Wall (1976), cultivars possessing good bread-making quality have high ratios of higher molecular weight (HMW) to lower molecular weight (LMW) glutenins. The LPP1:SPP1, LPP1:SPP2, LPP2:SPP2 and LPP:SPP ratios were calculated separately for the hard and soft wheat cultivars. The hard wheat cultivars had a higher LPP1:SPP1 ratio (0.39) than the soft wheat cultivars (0.32), a higher LPP1:SPP2 ratio (0.16) than the soft wheats (0.13), a higher LPP2:SPP2 ratio (0.40) than the soft wheats (0.39), and a higher LPP:SPP ratio (0.39) than the soft wheat cultivars (0.37), indicating better bread-making quality. Table 3.12 showed significant positive correlations for LPP1:SPP1 with SK hardness, HLM, FLN, VK, FLY, P/L ratio and alveograph strength; for LPP1:SPP2 with SK hardness, HLM, FLY and alveograph strength; and for LPP:SPP with SK hardness, VK, P/L ratio and alveograph strength, proving that higher ratios are associated with better bread-making quality.

Another important parameter which has been suggested to influence gluten viscoelastic properties is the glutenin/gliadin ratio. It has been suggested that a change in this ratio towards higher values would result in stronger doughs (Lafiandra et al., 2000). The following ratios were calculated to illustrate the glutenin/gliadin ratio: LPP1:LMP1, SPP1:LMP1, LPP1:LMP2, LPP2:LMP2, SPP2:LMP2, SPP:LMP and LPP:LMP. The hard wheat cultivars had a higher LPP1:LMP1 ratio (0.09) than the soft wheats (0.07), a higher SPP1:LMP1 ratio (0.23) than the soft wheats (0.21), a higher LPP1:LMP2 ratio (0.15) than the soft

wheats (0.12), a higher LPP2:LMP2 ratio (0.37) than the soft wheats (0.35), a higher SPP2:LMP2 ratio (0.94) than the soft wheats (0.89), a higher SPP:LMP ratio (0.49) than the soft wheats (0.45) and a higher LPP:LMP ratio (0.19) than the soft wheats (0.17). From the above it is obvious that the harder wheats had stronger doughs. In Table 3.12 all of the above ratios showed significant positive correlations with bread-baking quality characteristics such as SK hardness, HLM, VK, FLN, FLY, P/L ratio and alveograph strength.

According to the literature (He & Hoseney, 1990), the gluten from poor-quality flour has a higher solubility than the gluten from good-quality flour because of the occurrence of smaller molecular weight proteins. The LPP1:LPP2 ratio was calculated to illustrate the SDS-soluble:SDS-insoluble ratio for the hard wheat cultivars (0.40) and for the soft wheat cultivars (0.34). LPP1:LPP2 was significantly positively correlated with SK hardness and FLY.

3.4 Results and discussion for 2002 data

3.4.1 Results

Table 3.13. Means of protein fractions for four cultivars at seven different nitrogen treatments (2002).

	LPP1	SPP1	LMP1	SMP1	LPP2	SPP2	LMP2	SMP2	TUPP	LUPP	LPP	LMP	SPP	SMP	PP	MP
Snack P0	3.82	16.01	51.70	24.29	33.82	16.77	8.57	11.92	71.92	66.84	37.63	60.27	32.77	36.20	70.40	96.47
Snack P1	3.50	16.57	52.34	21.64	36.62	18.41	10.18	11.99	73.60	66.38	40.12	62.52	34.97	33.62	75.09	96.14
Snack P2	2.95	15.73	53.98	21.04	29.48	22.53	16.29	9.92	73.78	56.83	32.43	70.28	38.26	30.96	70.68	101.24
Snack P3	4.21	16.88	51.53	20.87	24.54	25.43	22.81	10.39	70.35	49.25	28.75	74.34	42.30	31.25	71.05	105.59
Snack P4	3.72	17.40	53.56	20.29	32.51	19.35	12.34	10.25	71.00	62.98	36.22	65.90	36.75	30.53	72.96	96.43
Snack P5	3.86	17.14	52.45	20.65	27.30	26.83	16.49	5.36	71.85	49.91	31.17	68.94	43.98	26.01	75.14	94.94
Snack P6	3.96	15.33	50.24	26.23	21.69	27.36	23.17	8.23	72.36	44.39	25.66	73.41	42.68	34.46	68.33	107.87
CrackerP0	3.68	15.34	53.18	22.42	34.53	18.98	14.15	8.15	74.14	64.58	38.21	67.33	34.33	30.56	72.53	97.89
CrackerP1	3.44	15.93	52.51	22.59	36.14	15.70	10.66	11.02	72.82	69.35	39.57	63.17	31.62	33.56	71.19	96.77
CrackerP2	4.02	15.84	51.28	21.22	31.32	21.80	14.40	10.60	72.94	58.97	35.35	65.69	37.63	31.82	72.98	97.50
CrackerP3	4.32	16.77	50.80	21.09	24.89	24.82	19.92	10.15	70.27	50.20	29.21	70.72	41.59	31.24	70.79	101.96
CrackerP4	4.60	17.04	52.92	21.40	26.15	22.89	16.63	9.34	69.62	52.55	30.75	69.55	39.93	30.74	70.67	100.28
CrackerP5	3.88	16.44	53.91	20.86	35.76	18.90	8.07	8.19	72.94	64.97	39.64	61.98	35.34	29.05	74.97	91.02
CrackerP6	4.85	17.86	51.01	23.26	32.07	26.16	14.63	6.02	71.63	55.50	36.91	65.64	44.01	29.27	80.92	94.91
E94/8 P0	2.92	15.97	53.07	21.08	36.08	15.31	8.84	14.29	73.26	69.60	38.99	61.90	31.27	35.36	70.26	97.27
E94/8 P1	4.19	17.00	53.95	18.84	31.80	20.01	12.85	8.06	71.25	61.35	35.99	66.79	37.01	26.89	72.99	93.68
E94/8 P2	3.95	16.54	52.04	19.49	23.93	20.08	18.88	12.86	68.02	53.87	27.88	70.92	36.62	32.35	64.50	103.27
E94/8 P3	3.58	18.47	52.43	20.36	31.97	18.39	13.72	12.29	69.55	62.22	35.55	66.14	36.85	32.65	72.39	98.79
E94/8 P4	3.57	16.28	53.19	23.19	20.21	21.75	19.76	14.37	67.72	48.07	23.48	72.95	38.03	37.55	61.50	110.50
E94/8 P5	3.49	17.70	53.52	20.11	31.19	21.26	15.16	6.57	71.18	59.27	34.68	68.67	38.96	18.73	73.64	95.35
E94/8 P6	4.03	18.32	49.77	21.78	35.27	21.97	11.37	9.26	71.97	61.55	39.30	61.14	40.29	31.03	79.58	92.18
SST876P0	3.22	16.71	48.57	23.50	33.35	20.42	11.49	11.83	72.97	61.64	36.56	60.05	37.13	35.32	73.68	95.37
SST876P1	3.43	16.27	52.84	21.97	39.28	20.03	9.80	9.48	74.96	65.62	42.71	62.63	36.30	34.08	79.01	96.70
SST876P2	3.71	16.43	52.80	21.13	29.35	20.48	15.92	11.54	71.40	58.51	33.06	68.72	36.90	32.67	69.96	101.38
SST876P3	4.74	17.42	52.61	18.95	34.50	20.06	12.79	7.24	71.12	61.54	39.24	65.41	37.49	26.19	76.72	91.59
SST876P4	4.08	18.07	52.31	20.86	35.74	20.20	13.80	10.62	71.59	62.89	39.81	66.11	38.26	31.48	78.07	97.58
SST876P5	4.43	17.23	52.80	20.13	32.49	21.92	9.42	8.04	71.79	59.96	36.64	62.21	39.15	28.16	75.79	90.37
SST876P6	5.05	18.57	50.30	20.95	38.03	21.52	12.42	8.83	71.56	65.05	43.07	62.78	40.09	29.77	83.16	92.49
Average	3.90	16.83	52.20	21.43	31.43	21.05	14.08	9.88	71.70	59.42	35.30	66.29	37.87	31.12	73.18	97.70
LSD (0.05)	1.430	2.708	5.010	4.666	9.533	6.897	7.215	5.794	4.409	13.15	9.344	9.223	7.823	9.486	8.647	10.331

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric

proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins, LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins

Protein fractions for four cultivars at seven different nitrogen treatments (2002)

The results are given in Table 3.13.

There were no significant differences among the cultivars for SDS-soluble SPP and SDS-soluble LMP.

SDS-soluble LPP: SST876 P6 had a significantly higher LPP than P1 and P0.

SDS-soluble SMP: Snack P6 had a significantly higher SMP than P2, P3, P4 and P5.

SDS-insoluble LPP: Snack P1 had a significantly higher LPP than P3 and P6. Cracker P1 and P5 had significantly higher amounts of SDS-insoluble LPP than P3 and P4. The LPP of Cracker P0 was also higher than for P3. E94/8 P4 had a significantly lower LPP than the rest of the E94/8 treatments, except for P2. E94/8 P0 and P6 also had significantly higher LPP values than P2. The LPP of SST876 P1 was significantly higher than for P2.

SDS-insoluble SPP: Snack P3, P5 and P6 had significantly higher amounts of SPP than P1 and P0. The SPP of Snack P5 and P6 were also significantly higher than for P4. Cracker P3, P4 and P6 had significantly higher amounts of SPP than P1. Cracker P6 also had a significantly higher SPP than P0 and P5.

SDS-insoluble LMP: Snack P2, P3, P5 and P6 had significantly higher amounts of LMP than Snack P0. The LMP of Snack P3 and P6 were also significantly higher than for P1 and P4. Cracker P3 and P4 had significantly higher amounts of LMP than P5. The LMP2 of Cracker P3 was also significantly higher than for P1. E94/8 P2 and P4 had significantly higher amounts of LMP than P0 and P6.

SDS-insoluble SMP: Snack P0 and P1 had significantly higher amounts of SMP than P5. The SMP values of E94/8 P0 and P4 were significantly higher than for P1 and P5, with P2 also higher than P5.

TUPP: Cracker P0 had a significantly higher TUPP than P4, and E94/8 P0 had a higher TUPP than P2 and P4.

LUPP: Snack P0 and P1 had significantly higher LUPP values than P3, P5 and P6. Snack P4 also had a higher LUPP than P3 and P6. Cracker P1 had a significantly higher LUPP than Cracker P2, P3 and P4. The LUPP values of Cracker P0 and P5 were also significantly higher than for P3. E94/8 P0 had a significantly higher LUPP than P2 and P4. The LUPP values of E94/8 P1, P3 and P6 were also significantly higher than for P4.

LPP: The total LPP of Snack P0, P1 and P4 were significantly higher than for P6. Snack P1 had a significantly higher LPP than P3. Cracker P1 and P5 had significantly higher LPP values than Cracker P3. E94/8 P4 had significantly the lowest LPP of all the E94/8 N-treatments, except for P2. SST876 P1 and P6 had significantly higher LPP values than P2.

LMP: The total LMP of Snack P2, P3 and P6 were significantly higher than for Snack P0. Snack P3 and P6 also had significantly higher LMP values than P1. E94/8 P2 and P4 had significantly higher LMP values than P6 , and E94/8 P4 also had a higher LMP value than P0.

SPP: The total SPP of Snack P3, P5 and P6 were significantly higher than for Snack P0. Snack P5 and P6 also had significantly higher SPP values than P1. Cracker P3, P4 and P6 had significantly higher SPP values than P1 , and the SPP of Cracker P6 was also higher than for P0 and P5. E94/8 P0 had a significantly lower SPP value than P6.

SMP: Snack P0 had a significantly higher SMP than P5 ; E94/8 P4 had a higher SMP than P1 and P5; and E94/8 P0 had a higher SMP than P5.

PP: The PP of Cracker P6 was significantly higher than for P1, P3 and P4 ; E94/8 P6 was significantly higher than P0, P2 and P4; and E94/8 P4 was significantly the lowest, except for P2. SST876 P6 had a significantly higher PP value than SST876 P0 and P2 ; and SST876 P1 also had a higher PP value than SST876 P2.

MP: Snack P6 had a significantly higher MP than P0, P1, P4 and P5; and Snack P3 had a significantly higher MP than P5. The MP values of Cracker P3 and SST876 P2 were significantly higher than for Cracker P5 and SST876 P5, respectively. The MP of E94/8 P4 was significantly higher than for P0, P1, P3, P5 and P6; and the MP of E94/8 P2 was significantly higher than for P6.

Table 3.14. Means of measured quality characteristics for four cultivars at seven different nitrogen treatments (2002).

	SK-wght	SK-diam	SK-hard	HLM	FPC	FLN	VK	BFLY	FLY	P/L	Strength
Snack P0	40.30	2.59	44.00	78.15	9.55	247.50	15.50	30.35	76.35	0.58	12.92
Snack P1	39.85	2.48	44.50	79.15	10.45	287.50	14.00	28.95	75.35	0.65	15.06
Snack P2	36.83	2.42	45.00	77.20	11.10	263.50	16.50	29.90	74.30	0.46	18.51
Snack P3	38.00	2.42	44.50	77.90	10.75	331.50	13.00	27.95	74.40	0.64	16.52
Snack P4	38.23	2.44	44.50	77.80	10.65	280.00	13.00	28.15	74.25	0.58	18.35
Snack P5	42.55	2.68	43.50	78.65	10.25	294.00	18.50	27.55	74.95	0.74	13.61
Snack P6	39.37	2.50	43.50	77.35	10.35	303.00	15.00	29.10	74.45	0.52	15.68
Cracker P0	36.24	2.52	58.50	82.50	9.15	379.00	60.00	22.70	77.25	1.25	27.98
CrackerP1	35.22	2.44	62.00	81.75	11.05	378.50	77.50	23.10	77.00	0.98	30.74
CrackerP2	35.27	2.48	60.50	82.50	10.35	380.50	70.00	22.85	77.20	0.89	30.97
CrackerP3	34.20	2.41	60.50	81.95	11.25	360.00	73.00	23.40	76.65	0.83	37.85
CrackerP4	33.78	2.37	60.00	82.10	10.30	358.00	73.50	21.85	76.65	1.12	32.96
CrackerP5	38.83	2.71	60.00	82.45	10.60	395.00	75.50	22.90	77.05	1.08	31.96
CrackerP6	35.17	2.47	60.50	81.75	10.25	268.50	83.50	22.30	76.90	1.24	38.92
E94/8 P0	40.37	2.58	44.50	77.45	9.50	248.00	16.00	29.65	75.45	0.63	11.62
E94/8 P1	40.06	2.55	45.50	77.25	11.00	252.00	11.00	28.75	74.80	0.45	18.27
E94/8 P2	40.77	2.61	47.00	78.10	10.60	248.50	13.00	27.05	74.95	0.56	15.83
E94/8 P3	39.78	2.62	46.00	78.15	11.05	270.50	14.50	27.75	74.30	0.51	18.96
E94/8 P4	40.78	2.61	46.50	77.10	10.70	329.00	15.00	27.35	75.00	0.53	17.51
E94/8 P5	37.70	2.38	44.00	75.85	11.20	312.00	13.50	29.25	73.90	0.44	22.25
E94/8 P6	40.61	2.51	44.00	77.10	10.05	252.00	13.50	28.80	74.60	0.54	16.82
SST876 P0	40.84	2.64	57.50	81.05	9.00	341.50	41.50	25.25	77.50	0.71	20.04
SST876P1	38.78	2.58	60.50	82.45	10.25	410.00	70.00	21.30	78.20	0.69	27.37
SST876P2	39.00	2.53	61.50	82.15	10.35	410.00	67.00	21.75	78.65	0.80	24.55
SST876P3	38.66	2.46	60.50	82.40	10.60	410.00	55.00	21.50	78.25	0.66	31.58
SST876P4	35.83	2.32	59.50	81.35	10.50	410.00	39.00	21.60	77.80	0.66	29.74
SST876P5	36.84	2.39	59.50	82.05	10.45	410.00	48.50	22.00	78.10	0.69	29.44
SST876P6	35.43	2.34	58.00	80.75	10.05	396.00	43.50	23.15	77.50	0.67	27.37
Average	38.19	2.50	52.36	79.80	10.41	329.50	38.55	25.58	76.13	0.72	23.33
LSD (0.05)	3.759	0.247	2.070	1.470	0.813	70.768	16.56	1.083	1.187	0.186	6.056

SK-wght = single kernel chracterization system weight, SK-diam = single kernel characterization system diameter, SK-hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength

The effect of the different cultivars and N-treatments on wheat quality characteristics (2002)

There were significant differences among the cultivars for SK weight, SK hardness, HLM, FPC, FLN, VK, BFLY, FLY, P/L ratio and alveograph strength (Table 3.14).

SK weight: Snack P5 had a significantly higher SK weight than Snack P2, P3 and P4. Cracker P5 had a significantly higher SK weight than P3 and P4, and the SK-weight of SST876 P0 was higher than for P4, P5 & P6.

SK diameter: Snack P5 had a significantly higher SK diameter than P2 and P3. The SK diameter of Cracker P5 was significantly higher than for P1, P3 and P4 ; and of SST876 P0 was higher than for P4, P5 and P6.

SK hardness: Cracker P1 was significantly harder than P0; E94/8 P2 was harder than P0, P5 and P6; E94/8 P4 was harder than P5 and P6 ; and SST876 P1 , P2 and P3 were significantly harder than P0 and P6.

HLM: Snack P1 had a significantly higher HLM than P2 and P6. The HLM of E94/8 P0, P2 and P3 were significantly higher than for P5; and SST876 P1 and P3 had higher HLM values than P6.

FPC: The FPC of Snack P1, P2, P3 and P4 were significantly higher than for P0, and Snack P2 gave a significantly higher FPC than P5. The FPC of Cracker P3 was significantly higher than for P0, P2, P4 and P6. The FPC of Cracker P0 was significantly the lowest. The FPC of E94/8 P1, P3 and P5 were higher than for P0 and P6. The FPC of E94/8 P0 was significantly lower than for all the others, but P6. SST876 P0 gave significantly the lowest FPC of all the SST876 N-treatments.

FLN: The FLN of Snack P3 was significantly higher than for P0, and the FLN of E94/8 P4 was significantly higher than for P0, P1, P2 and P6. The FLN of Cracker P6 was significantly lower than for all the other Cracker treatments.

VK: The VK of Cracker P1 and P6 were significantly higher than for P0. The VK of SST876 P1 and P2 were significantly higher than for P0, P4, P5 and P6.

BFLY: Snack P0 had a significantly higher BFLY than P1, P3, P4, P5 and P6. Cracker P3 had a significantly higher BFLY than P4 and P6; E94/8 P0 had a significantly higher BFLY than P2, P3 and P4; and SST876 P0 and P6 had significantly higher BFLY values than all the other SST876 treatments.

FLY: The FLY of Snack P0 was significantly higher than for all the others, except P1. The FLY of E94/8 P0 was higher than for P5.

Alveograph P/L ratio: Snack P5 had a significantly higher P/L ratio than P2 and P6; Cracker P0 and P6 had significantly higher P/L ratios than P1, P2 and P3; and E94/8 P0 had a higher P/L ratio than P5.

Alveograph strength: Cracker P6 had a significantly higher alveograph strength than P0, P1, P2 and P5. The alveograph strength of Cracker P3 was also significantly higher than for P0, P1 and P2. The alveograph strength of E94/8 P0 was significantly lower than for P1, P3 and P5. SST876 P0 had a significantly lower alveograph strength than all the others, but P2. The alveograph strength of SST876 P3 was also significantly higher than for P0 and P2.

Table 3.15. Means of protein fractions for seven different nitrogen treatments (2002).

	LPP1	SPP 1	LMP1	SMP 1	LPP2	SPP 2	LMP2	SMP 2	TUPP	LUPP	LPP	LMP	SPP	SMP	PP	MP
P0	3.41	16.00	51.63	22.82	34.44	17.87	10.76	11.55	73.07	65.66	37.84	62.39	33.87	34.36	71.72	96.75
P1	3.64	16.44	52.91	21.26	35.96	18.54	10.87	10.14	73.15	65.67	39.60	63.78	34.97	32.04	74.57	95.82
P2	3.66	16.13	52.52	20.72	28.52	21.22	16.37	11.23	71.53	57.04	32.18	68.90	37.35	31.95	69.53	100.85
P3	4.21	17.38	51.84	20.32	28.97	22.17	17.31	10.02	70.32	55.80	33.18	69.15	39.56	30.33	72.74	99.48
P4	3.99	17.19	52.99	21.43	28.65	21.04	15.63	11.14	69.98	56.62	32.56	68.63	38.24	32.57	70.80	101.20
P5	3.91	17.13	53.17	20.44	31.68	22.23	12.28	7.04	71.94	58.53	35.53	65.45	39.35	23.60	74.88	92.92
P6	4.47	17.52	50.33	23.05	31.76	24.25	15.40	8.08	71.88	56.62	36.23	65.74	41.77	31.13	78.00	96.86
Average	3.90	16.83	52.20	21.43	31.43	21.05	14.09	9.88	71.70	59.42	35.30	66.29	37.87	30.85	73.18	97.70
LSD (0.05)	0.637	1.175	2.035	2.036	5.121	3.232	3.902	2.589	1.964	6.783	5.128	4.460	3.421	5.053	4.623	5.247

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins, LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins

Differences between the N-treatments for the different protein fractions (2002)

There were highly significant differences between the N-treatments for SDS-insoluble SPP, LMP and SMP and LUPP, SPP and SMP (Table 3.15).

SDS-soluble LPP: P6 gave a significantly higher LPP fraction than P0, P1 and P2. The LPP of P3 was also significantly higher than for P0.

SDS-soluble SPP: P6 gave a significantly higher SPP1 fraction than P0 and P2. The SPP1 of P0 was significantly lower than for P3 and P4.

SDS-insoluble LMP: The LMP of P6 was significantly lower than for P1, P2, P4 and P5.

SDS-insoluble SMP: The SMP of P0 and P6 was significantly higher than for P2, P3 and P5.

SDS-insoluble LPP: P0 and P1 gave significantly higher LPP values than P2, P3 and P4.

SDS-insoluble SPP: P3, P5 and P6 gave significantly higher SPP values than P0 and P1. The SPP of P2 was also significantly higher than for P0.

SDS-insoluble LMP: P2 and P3 gave significantly higher LMP values than P0, P1 and P5. The LMP values of P4 and P6 were also significantly higher than for P0 and P1.

SDS-insoluble SMP: P5 gave a significantly lower SMP than all the N-treatments, but P6. The SMP of P6 was also significantly lower than for P0, P2 and P4.

TUPP: P0 and P1 gave significantly higher TUPP values than P3 and P4.

LUPP: P0 and P1 gave significantly the highest LUPP values of all the N-treatments.

LPP: P1 gave a significantly higher total LPP than P2, P3 and P4. The LPP of P0 was also significantly higher than for P2 and P4.

LMP: P0 and P1 gave significantly lower total LMP values than P2, P3 and P4.

SPP: P0 gave significantly the lowest total SPP of all the N-treatments, except for P1. The SPP of P1 was significantly lower than for P3, P5 and P6. The SPP of P6 was significantly higher than for P0, P1, P2 and P4.

SMP: P5 gave significantly the lowest total SMP of all the N-treatments.

PP: P2 gave a significantly lower PP than P1, P5 and P6. The PP of P6 was also significantly higher than for P0, P3 and P4.

MP: P2, P3 and P4 gave significantly higher MP values than P5. The MP of P4 was also significantly higher than for P1.

Table 3.16. Means of measured quality characteristics for seven different nitrogen treatments (2002).

	SKwght	SKdiam	SKhard	HLM	FPC	FLN	VK	BFLY	FLY	P/L	Strength
P0	39.44	2.58	51.13	79.79	9.30	304.00	33.25	26.99	76.64	0.79	18.14
P1	38.47	2.51	53.13	80.15	10.69	332.00	43.13	25.53	76.34	0.69	22.86
P2	37.97	2.51	53.50	79.99	10.60	325.63	41.63	25.39	76.28	0.68	22.46
P3	37.66	2.47	52.88	80.10	10.91	343.00	38.88	25.15	75.90	0.66	26.22
P4	37.15	2.44	52.63	79.59	10.54	344.25	35.13	24.74	75.93	0.72	24.64
P5	38.98	2.54	51.75	79.75	10.63	352.75	39.00	25.43	76.00	0.73	24.31
P6	37.64	2.45	51.50	79.24	10.18	304.88	38.88	25.84	75.86	0.74	24.70
Average	38.19	2.50	52.35	79.80	10.41	329.50	38.55	25.58	76.13	0.72	23.33
LSD (0.05)	2.415	0.123	6.935	2.087	0.385	58.017	24.297	2.854	1.386	0.212	7.045

SK-wght = single kernel chracterization system weight, SK -diam = single kernel characterization system diameter, SK -hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength

Differences between the N-treatments for the different quality characteristics (2002)

The results are given in Table 3.16.

SK weight and SK hardness: There were no significant differences between the different N-treatments for SK weight and SK hardness.

SK diameter: P0 gave a significantly higher SK diameter than P4 and P6.

FLN: P0 and P6 gave the lowest and P5 gave the highest FLN, not significantly though.

HLM, BFLY, VK, FLY and alveograph P/L ratio: There were no significant differences between any of the N-treatments for HLM, BFLY, VK, FLY and P/L ratio (Table 3.21).

FPC: There were highly significant differences between the treatments for FPC. P0 gave significantly the lowest FPC. The FPC of P6 was also significantly lower than for P1, P2, P3 and P5.

Alveograph strength: P3 gave a significantly higher alveograph strength than P0.

Table 3.17. Means of protein fractions for four cultivars during 2002.

Cultivar	LPP1	SPP1	LMP1	SMP1	LPP2	SPP2	LMP2	SMP2	TUPP	LUPP	LPP	LMP	SPP	SMP	PP	MP
Snack	3.71	16.43	52.25	22.14	29.42	22.38	15.69	9.72	72.12	56.65	33.14	67.95	38.81	31.86	71.95	99.81
Cracker	4.11	16.46	52.23	21.83	31.55	21.32	14.06	9.07	72.05	59.44	35.66	66.29	37.78	30.89	73.43	97.19
E94/8	3.68	17.18	52.56	20.69	30.06	19.82	14.37	11.10	70.42	59.42	33.69	66.93	37.00	29.57	70.69	98.72
SST876	4.09	17.24	51.74	21.07	34.68	20.66	12.23	9.65	72.20	62.17	38.73	63.98	37.90	31.09	76.63	95.07
LSD (0.05)	0.500	0.907	1.603	1.593	3.941	2.658	3.207	2.111	1.547	5.489	3.873	3.527	2.988	4.226	3.523	4.090

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins, LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins

Differences between the cultivars for the different protein fractions (2002)

The results are given in Table 3.17.

There were no significant differences between the cultivars for SDS-soluble LPP, SPP, LMP and SMP, SDS-insoluble SPP and SMP, and total SPP and SMP.

SDS-insoluble LPP: Snack had a significantly lower LPP fraction than SST876.

SDS-insoluble LMP: Snack had a significantly higher LMP fraction than SST876.

LPP: The total LPP of SST876 was significantly higher than for E94/8 and Snack.

LMP: Snack had a significantly higher LMP than SST876.

TUPP: The TUPP of E94/8 was significantly lower than for the rest of the cultivars.

LUPP: The LUPP of SST876 was significantly higher than for Snack.

PP: SST876 had a significantly higher PP fraction than Snack and E94/8.

MP: Snack had a significantly higher MP fraction than SST876.

Table 3.18. Means of measured quality characteristics for four cultivars during 2002.

Cultivar	SKwght	SKdiam	SKhard	HLM	FPC	FLN	VK	BFLY	FLY	P/L	Strength
Snack	39.30	2.50	44.21	78.03	10.44	286.71	15.07	28.85	74.86	0.59	15.81
Cracker	35.53	2.48	60.29	82.14	10.42	359.93	73.29	22.73	76.96	1.05	33.05
E94/8	40.01	2.55	45.36	77.29	10.59	273.14	13.79	28.37	74.71	0.52	17.32
SST876	37.91	2.46	59.57	81.74	10.17	398.21	52.07	22.36	78.00	0.70	27.15
LSD (0.05)	1.445	0.093	0.881	0.561	0.422	27.802	6.333	0.701	0.453	0.080	2.644

SK-wght = single kernel chracterization system weight, SK -diam = single kernel characterization system diameter, SK -hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BF LY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength

Differences between the cultivars for the different quality characteristics (2002)

The results are given in Table 3.18.

SK weight: E94/8, Snack and SST876 had significantly higher SK weights than Cracker.

SK diameter: There were no significant differences between the cultivars for SK diameter.

SK hardness: Cracker and SST876 were significantly harder than E94/8 and E94/8 was significantly harder than Snack.

HLM: The HLM values of Cracker and SST876 were significantly higher than for Snack and E94/8. The HLM of Snack was significantly higher than for E94/8.

FPC: There were no significant differences between the cultivars for FPC.

FLN: SST876 had significantly the highest FLN. The FLN of Cracker was also significantly higher than for Snack and E94/8.

VK: Cracker had significantly the highest amount of VK. The VK of SST876 was also significantly higher than for Snack and E94/8.

BFLY: The BFLY of Snack and E94/8 were significantly higher than for Cracker and SST876.

FLY: SST876 had significantly the highest FLY, followed by Cracker with a significantly higher FLY than Snack and E94/8.

Alveograph P/L ratio: Cracker had significantly the highest P/L ratio, followed by SST876 with a significantly higher P/L ratio than Snack and E94/8.

Alveograph stength: Cracker had a significantly higher alveograph strength than all the other cultivars, followed by SST876 with a higher alveograph strength than E94/8 and Snack.

Table 3.19. Means of protein fractions for the hard and soft wheats during 2002.

	LPP1	SPP1	LMP1	SMP1	LPP2	SPP2	LMP2	SMP2	TUPP	LUPP	LPP	LMP	SPP	SMP	PP	MP
Hard	4.10	16.85	51.99	21.45	33.11	20.99	13.15	9.36	72.12	60.81	37.19	65.14	37.84	30.72	75.03	96.13
Soft	3.69	16.81	52.41	21.42	29.74	21.10	15.03	10.41	71.27	58.03	33.41	67.44	37.91	30.99	71.32	99.26
LSD(0.05)	0.347	0.653	1.115	1.136	2.780	1.892	2.253	1.484	1.108	3.857	2.732	2.479	2.092	2.953	2.505	2.861

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins, LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins

Table 3.20. Means of measured quality characteristics for the hard and soft wheats during 2002.

	SKwght	SKdiam	SKhard	HLM	FPC	FLN	VK	BFLY	FLY	P/L	Strength
Hard	36.72	2.47	60.29	81.94	10.30	379.07	62.68	22.55	77.48	0.87	30.10
Soft	39.66	2.53	19.82	77.66	10.51	279.93	14.43	28.61	74.79	0.56	16.56
LSD (0.05)	1.080	0.065	3.242	0.412	0.296	20.372	5.587	0.496	0.358	0.082	2.083

SK-wght = single kernel chracterization system weight, SK-diam = single kernel characterization system diameter, SK-hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength

Differences between the hard and soft wheat cultivars for the different protein fractions (2002)

Results are given in Table 3.19.

The hard wheat cultivars had significantly higher amounts of SDS-soluble LPP, SDS-insoluble LPP, total LPP and PP than the soft wheat cultivars. The hard wheat cultivars also had higher amounts of SDS-soluble SPP and SMP, total SMP, TUPP and LUPP, although not significantly. However, the soft wheat cultivars had significantly higher amounts of MP than the hard wheat cultivars.

Differences between the hard and soft wheat cultivars for the different quality characteristics (2002)

Results are given in Table 3.20.

The hard wheat cultivars had significantly higher SK hardness, HLM, FLN, VK, alveograph P/L ratio and alveograph strength values than the soft wheat cultivars. The hard wheat cultivars had also higher FLY values than the soft wheats, however not significantly. The soft wheat cultivars had significantly higher SK weight and BFLY values than the hard wheat cultivars.

Means squares for all measured characteristics are given in Table 3.21.

Table 3.21. Mean square values for protein fractions and quality characteristics of four cultivars (2002).

Trait	Mean Squares					
	Across cultivars and treatments		Only across N-treatments		Only across cultivars	
	Entry	**Replicates**	**Treatment**	**Replicates**	**Number**	**Replicates**
LLP1	0.559	0.686	1.071	0.686	0.773	0.686
SPP1	1.651	70.179**	3.090	70.179**	2.738	70.179**
LMP1	3.628	612.218**	8.165	612.218**	1.603	612.218**
SMP1	5.146	105.875**	9.763	105.875**	6.281	105.875**
LPP2	50.371	3.347	69.345	3.347	76.802	3.347
SPP2	18.700	7.755	39.140*	7.755	16.335	7.755
LMP2	33.675	7.674	59.137*	7.674	28.486	7.674
SMP2	10.582	0.799	23.446*	0.799	10.370	0.799
TUPP	5.708	53.998**	11.947	53.998**	10.202	53.998**
LUPP	90.252	5.179	151.014*	5.179	71.064	5.179
LPP	50.488	6.291	63.447	6.291	89.289	6.291
LMP	33.694	756.168**	57.334	756.168**	39.532	756.168**
SPP	21.817	124.564*	60.007**	124.564**	7.715	124.564*
SMP	46.294	20.570	94.443*	20.570	12.679	20.570
PP	42.87	187.026*	65.835	187.026*	91.652*	187.026*
MP	48.580	354.263**	71.036	354.263**	59.139	354.263**
SK-wght	11.048*	10.833	5.284	10.833	54.671**	10.833
SK-diam	0.022	0.039	0.020	0.039	0.019	0.039
SK-hard	121.55**	7.143*	6.476	7.143	1074.3**	7.143
HLM	10.446**	1.031	0.816	1.031	87.375**	1.031
FPC	0.656**	2.200**	2.287**	2.200**	0.415	2.200*
FLN	7439.8**	1874.6	2957.71	1874.57	49720.**	1874.571
VK	1418.7**	70.875	94.161	70.875	11918.**	70.875
BFLY	21.076**	1.446	4.008	1.446	172.46**	1.446
FLY	4.519**	3.159*	0.673	3.159	36.341**	3.159*
P/L	0.106**	0.049	0.016	0.049	0.786**	0.049
Strength	124.94**	13.416	54.461	13.416	941.86**	13.416

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric protein, LUPP = larger unextractable polymeric protein, LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins, SK-wght = single kernel chracterization system weight, SK-diam = single kernel characterization system diameter, SK-hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength

$^{*}p < 0.05$, $^{**}p < 0.01$

Table 322. Significant correlations between protein fractions and measured characteristics (2002).

SDS-soluble proteins			SDS-insoluble proteins			TUPP, LUPP and combined protein fractions		
LPP	Sk-wght	-0.494**	LPP	HLM	0.270*	TUPP	FLY	0.318*
	Sk-diam	-0.556**		FLY	0.327*		Sk-diam	0.392**
	FLN	0.296*	LMP	FLY	-0.271*	LUPP	FLY	0.291*
	BFLY	-0.306*	SMP	Sk-wght	0.345*	LPP	HLM	0.288*
	Strength	0.437**		Sk-diam	0.313*		FLY	0.333*
LMP	FPC	0.345*		Strength	-0.377**	SPP	FLY	-0.272*
SPP	Sk-wght	-0.344*					Sk-wght	-0.311*
	Sk-diam	-0.466**					Sk-diam	0.371**
SMP	FPC	-0.498**				PP	Sk-wght	-0.306*
							Sk-diam	-0.316*
							Strength	0.349**

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins, LPP = larger polymeric proteins, LMP = larger monomeric proteins, SPP = smaller polymeric proteins, SMP = smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins, SK-wght = single kernel chracterization system weight, SK-diam = single kernel characterization system diameter, SK-hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength
*$p < 0.05$, ** $p < 0.01$

Correlations between protein fractions and measured characteristics (2002)

Results are given in Table 3.22.

Only highly significant correlations ($p<0.01$) were discussed. There were relatively few correlations in contrast with year 1.

The large polymeric proteins of the SDS-soluble fraction (LPP1) were significantly positively correlated with FLN ($p<0.05$) and alveograph strength ($p<0.01$), and significantly negatively correlated with SK weight ($p<0.01$), SK diameter ($p<0.01$) and BFLY ($p<0.05$). The large monomeric proteins of the SDS-soluble fraction (LMP1) were significantly positively correlated ($p<0.05$) only with FPC and the small polymeric proteins (SPP1) were significantly negatively correlated with SK weight ($p<0.05$) and SK diameter ($p<0.01$). The small monomeric proteins of the SDS-soluble fraction (SMP1) were significantly negatively correlated ($p<0.01$) with FPC.

The SDS-insoluble large polymeric proteins (LPP2) were significantly positively correlated ($p<0.05$) with HLM and FLY. The large monomeric proteins of the insoluble fraction (LMP2) were significantly negatively correlated with FLY ($p<0.05$). The small monomeric proteins of the SDS-insoluble fraction (SMP2) were significantly positively correlated ($p<0.05$) with SK weight and SK diameter and significantly negatively correlated with alveograph strength ($p<0.01$).

The total unextractable polymeric proteins (TUPP) were significantly positively correlated with FLY ($p<0.05$) and SK diameter ($p<0.01$). The total unextractable large polymeric proteins (LUPP) were significantly positively correlated with FLY ($p<0.05$) and the total large polymeric protein fraction (LPP) was significantly positively correlated ($p<0.05$) with HLM and FLY. The total SPP was significantly positively correlated with SK diameter ($p<0.01$) and negatively correlated ($p<0.05$) with SK weight and FLY.

The total polymeric proteins (PP) were significantly positively correlated with alveograph strength ($p<0.01$) and negatively correlated ($p<0.01$) with SK weight and SK diameter.

There were no significant correlations between the SPP (SDS-insoluble), total LMP, total SMP and MP and the quality characteristics.

Table 3.23. Correlations between ratios and quality characteristics for 2002 ($p<0.01$).

LPP:SPP	FLY	0.361
LPP1:SPP1	Strength	0.415
	Sk-wght	-0.365
	Sk-diam	-0.376
LPP1:LMP1	Sk-wght	-0.492
	Sk-diam	-0.542
	Strength	0.375
LPP1:SMP1	Sk-wght	-0.416
	Sk-diam	-0.532
	Strength	0.402
LPP1:LPP2	Sk-diam	-0.388
LPP1:SMP2	Sk-wght	-0.376
	Sk-diam	-0.440
SPP1:LMP1	Sk-diam	-0.360
SPP1:SMP1	FPC	0.437
	Sk-diam	-0.468
SPP1:LPP2	FLY	-0.396
SPP1:SMP2	Sk-diam	-0.356
LMP1:SMP1	FPC	0.486
SMP1:SPP2	FPC	-0.413

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble),

SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins, LPP = larger polymeric proteins, LMP = larger monomeric proteins, SPP = smaller polymeric proteins, SMP = smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins, SK-wght = single kernel chracterization system weight, SK-diam = single kernel characterization system diameter, SK-hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength

Correlations between protein fraction ratios and quality characteristics (2002)

Table 3.23 show how the protein fraction ratios are related to the quality characteristics. There were relatively few correlations in contrast with year 1. Only correlations significant at $p<0.01$ were discussed.

LPP:SPP was significantly positively correlated with FLY.

LPP1:SPP1 was significantly positively correlated with alveograph strength and negatively correlated with SK weight and SK diameter.

LPP1:LMP1 was significantly positively correlated with alveograph strength and negatively with SK weight and SK diameter.

LPP1:SMP1 was significantly positively correlated with alveograph strength and negatively with SK weight and SK diameter.

LPP1:LPP2 was significantly negatively correlated with SK diameter.

LPP1:SMP2 was significantly negatively correlated with SK weight and SK diameter.

SPP1:LMP1 was significantly negatively correlated with SK diameter.

SPP1:SMP1 was significantly positively correlated with FPC and negatively with SK diameter.

SPP1:LPP2 was significantly negatively correlated with FLY.

SPP1:SMP2 was significantly negatively correlated with SK diameter.

LMP1:SMP1 was significantly positively correlated with FPC.

SMP1:SPP2 was significantly negatively correlated with FPC.

3.4.2 Discussion

Wheat cultivar and nitrogen (N) applications influenced the amount and size-distribution of several of the monomeric and polymeric proteins differentiable with SE-HPLC.

The hard wheat cultivars had significantly higher amounts of SDS-soluble LPP, SDS-insoluble LPP and total LPP than the soft wheat cultivars. For example, SST876 had a significantly higher SDS-insoluble LPP than Snack and the total LPP of SST876 was significantly higher than for E94/8 and Snack. It is obvious that there is a definite cultivar effect on the LPP fraction because the harder the cultivar, the higher the amounts of large polymeric proteins, both SDS-soluble and SDS-insoluble.

The environment, and specifically the N-treatments, also had a great impact on the LPP fractions. N-treatment P6 gave a significantly higher SDS-soluble LPP value than P0, P1 and P2, which is possibly the explanation why SST876 P6 had a significantly higher SDS-soluble LPP than SST876 P1 and P0. The SDS-soluble LPP value obtained from N-treatment P3 was also significantly higher than for P0. It seems as if the highest and lowest amounts of N applied by P1 and P0 (230 and 80 kg N/ha), were responsible for the lowest SDS-soluble LPP fractions.

The N-treatments P0 and P1 gave significantly higher SDS-insoluble LPP values than P2, P3 and P4. This can explain most of the following results: Snack P1 had a significantly higher SDS-insoluble LPP than Snack P3 and P6; Cracker P1 and P5 had significantly higher amounts of SDS-insoluble LPP than Cracker P3 and P4; Cracker P0 had a higher SDS-insoluble LPP than P3; E94/8 P0 and P6 had significantly higher amounts of SDS-insoluble LPP than P2; SST876 P1 had a significantly higher SDS-insoluble LPP than P2; and E94/8 P4 had a significantly lower SDS-insoluble LPP than the rest of the E94/8 treatments, except for P2. The highest and lowest N-applications (P1

and P0) were responsible for the highest SDS-insoluble LPP values. These results are totally the opposite than for SDS-soluble LPP.

P1 gave a significantly higher total LPP than P2, P3 and P4; P0 gave a significantly higher LPP than P2 and P4; Snack P0, P1 and P4 gave higher LPP values than P6; Snack P1 gave a significantly higher LPP than P3; Cracker P1 and P5 gave significantly higher LPP values than Cracker P3; E94/8 P4 gave significantly the lowest LPP of all the E94/8 N-treatments, except for P2; and SST876 P1 and P6 gave significantly higher LPP values than P2.

The SDS-soluble LPP was significantly positively correlated with FLN and alveograph strength, and significantly negatively correlated with SK weight, SK diameter and BFLY. Both the SDS-insoluble LPP and total LPP fractions were significantly positively correlated with HLM and FLY. Thus, even though the SDS-soluble and SDS-insoluble LPP fractions showed different reactions to the N-treatments, all LPP fractions were associated with good bread-baking quality characteristics.

There were no significant differences between the cultivars for SDS-soluble SPP, SDS-insoluble SPP or total SPP (small polymeric proteins). Thus, the hard and the soft wheat cultivars had almost the same amounts of these fractions. During 2001, however, the hard wheat cultivars possessed significantly higher amounts of SDS-insoluble SPP and total SPP than the soft wheat cultivars.

There were highly significant differences between the N-treatments, especially for SDS-insoluble SPP and total SPP. These differences are very difficult to explain, because no constant patterns were found between the results.

N-treatment P6 gave a significantly higher SDS-soluble SPP value than P0 and P2 and the SDS-soluble SPP of P0 was also significantly lower than for P3 and P4. N-treatments P3, P5 and P6 gave significantly higher SDS-insoluble SPP

values than P0 and P1. The SDS-insoluble SPP of P2 was also significantly higher than for P0. Snack P3, P5 and P6 had significantly higher amounts of SDS-insoluble SPP than P0 and P1; the SDS-insoluble SPP of Snack P5 and P6 were significantly higher than for P4; Cracker P3, P4 and P6 had significantly higher amounts of SDS-insoluble SPP than P1; and Cracker P6 had a significantly higher SDS-insoluble SPP than P0 and P5. N-treatment P0 gave significantly the lowest total SPP of all the N-treatments, except for P1. The SPP of P1 was significantly lower than for P3, P5 and P6; the SPP of P6 was significantly higher than for P0, P1, P2 and P4; the SPP of Snack P3, P5 and P6 were significantly higher than for Snack P0; Snack P5 and P6 had significantly higher SPP values than P1; Cracker P3, P4 and P6 had significantly higher SPP values than P1; the SPP of Cracker P6 was higher than for P0 and P5; and E94/8 P0 had a significantly lower SPP value than P6.

It seems as if N-treatments P0 and P1 were usually responsible for the lowest SPP fractions, while P3, P4, P5 and P6 were responsible for the highest SPP fractions (not in all the cases, and not significantly). N-treatments P0 and P1 applied the lowest (80 kg N/ha) and highest (230 kg N/ha) total amounts of N during the growth season, while N-treatments P3, P4, P5 and P6 were responsible for intermediate levels of N (140 – 200 kg N/ha).

SDS-soluble SPP was significantly negatively correlated with SK weight and SK diameter; SDS-insoluble SPP showed no significant correlations with the quality characteristics; and the total SPP fraction was significantly positively correlated with SK diameter and negatively correlated with SK weight and FLY. The SPP fractions showed almost contrasting correlations and cannot be associated with either the hard or the soft wheat cultivars.

There were no significant differences between the cultivars for SDS-soluble LMP, but Snack had significantly higher SDS-insoluble LMP and total LMP values than SST876, indicating that the higher the LMP (large monomeric protein) fractions, the softer the wheat. This assumption agrees with the

results of 2001, when both the SDS-soluble LMP and total LMP fractions were significantly negatively correlated with SK hardness. The SDS-soluble LMP fraction was significantly positively correlated with FPC and the SDS-insoluble LMP fraction was significantly negatively correlated with FLY (associated with good bread-making quality).

The SDS-insoluble LMP of N-treatment P6 was significantly lower than for P1, P2, P4 and P5. Thus, a higher total amount of N applied resulted in higher SDS-soluble LMP values. This agrees with the results obtained during 2001.

There were highly significant differences between the N-treatments for SDS-insoluble LMP. P2 and P3 gave significantly higher total LMP values than P0, P1 and P5. The SDS-insoluble LMP values of P4 and P6 were also significantly higher than for P0 and P1. The total LMP values of P2, P3 and P4 were significantly higher than for P0 and P1. A possible explanation could be that the highest (e.g., P1 = 230 kg N/ha) and lowest (e.g., P0 = 80 kg N/ha) N-treatments were responsible for the lowest SDS-insoluble LMP and LMP values, while intermediate N-applications were responsible for higher SDS-insoluble LMP and LMP values. This explains only a few of the following results: Snack P2, P3, P5 and P6 had significantly higher amounts of SDS-insoluble LMP than Snack P0; the SDS-insoluble LMP of Snack P3 and P6 were significantly higher than for P1 and P4; Cracker P3 and P4 had significantly higher amounts of SDS-insoluble LMP than P5; the SDS-insoluble LMP of Cracker P3 was significantly higher than for P1; E94/8 P2 and P4 had significantly higher amounts of SDS-insoluble LMP than P0 and P6; the total LMP of Snack P2, P3 and P6 were significantly higher than for Snack P0; Snack P3 and P6 had significantly higher LMP values than P1; E94/8 P2 and P4 had significantly higher LMP values than P6; and E94/8 P4 had a higher LMP value than P0.

There were no significant differences between the cultivars for any of the small monomeric protein fractions (SDS-soluble and insoluble SMP, or total

SMP). During 2001 however, the hard wheat cultivars (STT876 and Cracker) had significantly higher amounts of SDS-soluble SMP than the soft wheat cultivars (Snack and E94/8), while the soft wheat cultivars had significantly higher amounts of SDS-insoluble SMP than the hard wheat cultivars. The quality characteristics correlated with SDS-insoluble SMP (2002) are representative of the soft wheat cultivars. SDS-insoluble SMP was significantly positively correlated with SK weight and SK diameter and significantly negatively correlated with alveograph strength. The total SMP showed no significant correlations with the quality characteristics.

There were highly significant differences between the N-treatments, especially for the SDS-insoluble SMP and total SMP fractions. The SDS-soluble SMP values of N-treatments P0 and P6 were significantly higher than for P2, P3 and P5, explaining why Snack P6 had a significantly higher SDS-soluble SMP than P2, P3, P4 and P5. N-treatments P0 and P6 applied the lowest total amounts of N (80 – 140 kg N/ha) during the growth season and were responsible for the highest SDS-soluble SMP values. The lowest N-applications were also responsible for the highest SDS-soluble SMP values during 2001.

N-treatment P5 gave significantly the lowest total SMP. P5 was also responsible for the lowest SDS-insoluble SMP of all the N-treatments, but P6. The SDS-insoluble SMP of P6 was significantly lower than for P0, P2 and P4. These results indicate that lower total amounts of N-applied (e.g., P5) gave lower SDS-insoluble SMP and total SMP values. The following results, however, did not give a constant pattern. Snack P0 and P1 had significantly higher amounts of SDS-insoluble SMP than P5; the SDS-insoluble SMP values of E94/8 P0 and P4 were significantly higher than for P1 and P5, with P2 also higher than P5; Snack P0 had a significantly higher SMP than P5; E94/8 P4 had a higher SMP than P1 and P5; and E94/8 P0 had a higher SMP than P5.

The hard wheat cultivars had higher amounts of total unextractable polymeric proteins (TUPP), although not significantly. The TUPP of E94/8 was significantly lower than for the rest of the cultivars. According to He and Hoseney (1990) the gluten from poor-quality flour has a higher solubility than the gluten from good-quality flour because of the occurrence of smaller molecular weight proteins. Thus, the reason why the hard wheat cultivars showed higher TUPP values was the higher amount of large molecular weight proteins (e.g., SDS-insoluble LPP). The formula of Gupta et al. (1993) supports the above assumption because a higher TUPP value is the result of higher amounts of SDS-insoluble LPP and/or SPP. TUPP was significantly positively correlated with FLY (associated with good bread-making quality) and SK diameter (associated with soft wheat quality according to 2001).

N-treatments P0 and P1 gave significantly higher TUPP values than P3 and P4. For example, Cracker P0 had a significantly higher TUPP than P4, and E94/8 P0 had a significantly higher TUPP than P2 and P4. From these limited results it seems as if the lowest total amount of N applied by P0 (80 kg N/ha) and the highest total amount of N applied by P1 (230 kg N/ha) gave higher TUPP values than intermediate N-applications by P2 (200 kg N/ha) and P4 (170 kg N/ha).

The hard wheat cultivars posessed higher amounts of large unextractable polymeric proteins (LUPP), although not significantly. The LUPP of SST876 was significantly higher than for Snack. The hard wheat, SST876, is probably a better quality wheat with higher molecular weight proteins than Snack. Snack, in turn, posessed higher amounts of smaller molecular weight proteins (e.g., LMP) which are more soluble in SDS. LUPP was significantly positively correlated with FLY. This correlation also indicates that harder wheats are associated with better bread-making quality.

There were highly significant differences between the N-treatments for LUPP. P0 and P1 gave significantly the highest LUPP values of all the N-treatments.

This proves most of the following results: Snack P0 and P1 had significantly higher LUPP values than P3, P5 and P6; Snack P4 had a higher LUPP than P3 and P6; Cracker P1 had a significantly higher LUPP than Cracker P2, P3 and P4; the LUPP values of Cracker P0 and P5 were significantly higher than for P3; E94/8 P0 had a significantly higher LUPP than P2 and P4; and the LUPP values of E94/8 P1, P3 and P6 were also significantly higher than for P4. Thus, the lowest total amount of N applied by P0 (80 kg N/ha) and the highest total amount of N applied by P1 (230 kg N/ha) were responsible for the highest LUPP values.

The hard wheat cultivars (e.g., SST876) had significantly higher amounts of PP than the soft wheat cultivars (e.g., Snack and E94/8). The higher PP of the hard wheat cultivars was probably due to higher LPP fractions. The total polymeric proteins (PP) were significantly positively correlated with alveograph strength and negatively correlated with SK weight and SK diameter, as could be expected.

The following results were obtained from the different N-treatments: P2 gave a significantly lower PP than P1, P5 and P6. The PP of P6 was also significantly higher than for P0, P3 and P4. The PP of Cracker P6 was significantly higher than for P1, P3 and P4; E94/8 P6 was significantly higher than P0, P2 and P4; and E94/8 P4 was significantly the lowest, except for P2. SST876 P6 had a significantly higher PP value than SST876 P0 and P2; and SST876 P1 had a higher PP value than SST876 P2. N-treatment P6 seems to be responsible for the highest PP values.

The monomeric protein (MP) fraction of the soft wheat cultivars was significantly higher than the MP fraction of the hard wheat cultivars. For example, Snack had a significantly higher MP fraction than SST876. The higher MP fraction of the soft wheat cultivars is most probably the result of higher SDS-insoluble LMP and total LMP values obtained by them. There

were no significant correlations between MP and the quality characteristics (Table 3.22).

N-treatments P2, P3 and P4 gave significantly higher MP values than P5 and the MP of P4 was also significantly higher than for P1. Snack P6 had a significantly higher MP than P0, P1, P4 and P5; Snack P3 had a significantly higher MP than P5 ; the MP values of Cracker P3 and SST876 P2 were significantly higher than for Cracker P5 and SST876 P5, respectively; the MP of E94/8 P4 was significantly higher than for P0, P1, P3, P5 and P6; and the MP of E94/8 P2 was significantly higher than for P6 . No constant pattern was found between these results.

From the above results it is clear that cultivars Cracker and SST876 (the hard wheat cultivars) had the highest amounts of all the LLP fractions and PP while Snack and E94/8 (the soft wheat cultivars) had the lowest amounts of these fractions. Cultivars Cracker and SST876 had the lowest amounts of SDS-insoluble LMP, total LMP and MP, and Snack and E94/8 the highest. These results suggest significant negative correlations between the LPP and LMP fractions, with LPP characteristic of the hard wheat cultivars, and LMP characteristic of the soft wheat cultivars. According to Table 3.21, the N-treatments were largely responsible for the significant differences between SDS-insoluble SPP, LMP and SMP, LUPP, total SPP and SMP. The cultivar effect was responsible for the significant differences between the PP fractions.

Wieser and Seilmeier (1998) stated that different N-treatments have strong influences on the quantity of storage proteins (gliadins, glutenin subunits) in wheat flour, but not on that of other endosperm proteins (albumins, globulins). However, the N-treatments did have significant effects on the SMP fractions Wieser and Seilmeier (1998) also believed that major protein types (a-gliadins, ?-gliadins, LMW subunits of glutenin) are more affected than minor types (? -gliadins, HMW subunits). This appears to be true, because the LPP (HMW-glutenin) fractions showed results independent of the N-

treatments, while the SPP (LMW-glutenin) and SDS-insoluble LMP (gliadins) fractions were significantly influenced by the N-treatments.

N-treatments P0 and P6 applied the lowest total amounts of N (80 – 140 kg N/ha) during the growth season and were responsible for the highest SDS-soluble SMP and lowest SDS-soluble LMP values. The higher total N-applications of P1 to P5 (170 – 230 kg N/ha) resulted in higher SDS-soluble LMP and lower SMP values.

It seems as if the lowest total amount of N applied by P0 (80 kg N/ha) and the highest total amount of N applied by P1 (230 kg N/ha), were usually responsible for the lowest SDS-soluble LPP and SPP, SDS-insoluble LMP and SPP, and total SPP and LMP fractions. Average N-applications applied by P3, P4, P5 and P6 (140 – 200 kg N/ha) were responsible for the highest SDS-soluble LPP and SPP, SDS-insoluble LMP and SPP, total SPP and LMP fractions (not in all the cases, and not significantly). The highest and lowest N-applications (P1 and P0) were responsible for the highest SDS-insoluble LPP, TUPP and LUPP values. The N-treatments did not always give the same pattern of results.

The timings of the N-applications did not have a noticeable effect on the protein fractions. N-treatments P1, P3 and P5 applied N at a later stage (30 kg N/ha during flag leave stage), while the rest of the treatments applied N only during plant and 6 weeks later.

The SDS-soluble LPP was significantly positively correlated with FLN and alveograph strength, SDS-insoluble LPP and total LPP were significantly positively correlated with HLM and FLY, TUPP and LUPP were significantly positively correlated with FLY, and PP was significantly positively correlated with alveograph strength. The positive correlations of the large polymeric protein fractions indicate the big influence of the large polymeric proteins on the quality characteristics associated with bread-baking quality. Studies by Dachkevitch and Autran (1989) showed a direct correlation between the size

and the amount of the glutenin polymers and quality characteristics. Higher amounts of polymeric proteins (LPP fractions) will thus have better quality as a result. The SDS-soluble and SDS-insoluble polymeric proteins were found to be equally important in quality prediction.

SDS-soluble LPP, total SPP and PP were significantly negatively correlated with SK weight, SK diameter and BFLY (only SDS-soluble LPP), the quality characteristics associated with soft wheats. The SDS-soluble LMP was significantly positively correlated with FPC, SDS-insoluble LMP was significantly negatively correlated with FLY (associated with good bread-making quality), and SDS-insoluble SMP was significantly positively correlated with SK weight and SK diameter (associated with soft wheats) and significantly negatively correlated with alveograph strength (associated with good bread-making quality).

All of the significant differences among the quality characteristics were due to cultivar effects. Table 3.21 indicates that only the differences in FPC were partly due to the N-treatments.

The hard wheat cultivars had significantly higher SK hardness values than the soft wheat cultivars. The following significant differences were visible between the cultivars: Cracker and SST876 were significantly harder than E94/8, and E94/8 was significantly harder than Snack. SK hardness was significantly positively correlated with HLM, FLN, VK, FLY, alveograph P/L ratio and alveograph strength and negatively correlated with BFLY. Harder wheats are thus associated with better quality.

There were significant differences among the cultivars for SK hardness even though there were no significant differences between the N-treatments. Cracker P1 was significantly harder than P0; E94/8 P2 was harder than P0, P5 and P6; E94/8 P4 was harder than P5 and P6; and SST876 P1, P2 and P3 were significantly harder than P0 and P6. A simple explanation would be that N-treatments P1, P2, P3 and P4 applied a higher total amount of N (170 – 230 kg

N/ha) during the growth period, which in turn, led to harder grains. N-treatments P0, P5 and P6 applied lower total amounts of N (80 – 170 kg N/ha) and were responsible for softer wheats.

The soft wheat cultivars had significantly higher SK weight values than the hard wheat cultivars. E94/8, Snack and SST876 had significantly higher SK weights than Cracker. The higher SK weights of the softer wheats can be explained by the significant negative correlation of SK weight with SK hardness. The SDS-soluble LPP and SPP, total SPP and PP fractions were significantly negatively correlated with SK weight, proving that higher amounts of these fractions lead to harder wheats with lower SK weights. SDS-insoluble SMP was significantly positively correlated with SK weight, indicating an association with softer wheats. SK weight was also significantly positively correlated with SK diameter and BFLY and negatively correlated with FLN, VK, HLM, alveograph P/L ratio and alveograph strength.

Although there were no significant differences between the N-treatments for SK weight, there were significant differences among the cultivars. Snack P5 had a significantly higher SK weight than Snack P2, P3 and P4; Cracker P5 had a significantly higher SK weight than P3 and P4; and the SK weight of SST876 P0 was significantly higher than for P4, P5 and P6. A lower total amount of N-applied (e.g., P0 and P5) is therefore responsible for a higher SK weight.

There were no significant differences between the cultivars for SK diameter. Because of the significant positive correlation of SK weight with SK diameter, the softer wheats would have been expected to have higher SK diameters than the hard wheats. The polymeric protein fractions (e.g., SDS-soluble LPP, SPP and PP), characteristic of the harder wheats, were significantly negatively correlated with SK diameter, which confirms the above assumption. SDS-insoluble SMP, TUPP and the total SPP was significantly positively correlated with SK diameter. SK diameter was significantly negatively correlated with

FLN and alveograph strength, quality characteristics associated with harder wheats.

Snack P5 had a significantly higher SK diameter than P2 and P3; the SK diameter of Cracker P5 was significantly higher than for P1, P3 and P4; and the SK diameter of SST876 P0 was higher than for P4, P5 and P6. N-treatment P0 gave a significantly higher SK diameter than P4 and P6. A higher total amount of N applied had a positive effect on the polymeric protein fractions (only clearly visible during 2001) and thus a negative effect on SK weight and SK diameter. A lower total amount of N applied (e.g., P0), had a negative effect on the polymeric protein fractions, resulting in higher SK weight and SK diameter values.

The hard wheat cultivars (Cracker and SST876) had significantly higher hectoliter mass (HLM) values than the soft wheat cultivars (Snack and E94/8). The HLM of Snack was also significantly higher than for E94/8. This can be explained by the significant positive correlations of SDS-insoluble LPP and total LPP with HLM. Thus, higher proportions of large polymeric proteins lead to harder cultivars with higher HLM values. HLM shared the same correlations as the LPP fractions; it was significantly positively correlated with FLN, VK, FLY, P/L ratio and alveograph strength and negatively correlated with BFLY. The HLM values of all the cultivars were above 76 kg/hl, the recommended value for wheat to be graded as suitable for bread-making (Mamuya, 2000). The high HLM values indicated the presence of sound wheat (Atwell, 2001).

There were significant differences among the cultivars for HLM, but no significant differences between the N-treatments. Snack P1 had a significantly higher HLM than P2 and P6, the HLM of E94/8 P0, P2 and P3 were significantly higher than for P5, and the HLM of SST876 P1 and P3 were higher than for P6. Most of these results can be explained by the positive effect of higher total N-applications on the HLM values of the cultivars.

There were no significant differences between the cultivars for flour protein content (FPC). The average FPC values of the hard and soft wheat cultivars were almost the same, ranging between 10.17% and 10.59%, according to Table 3.18. The average FPC of the two soft wheat cultivars was 10.52%, which is very high for the production of cakes and biscuits (Hoseney et al., 1988). Hoseney et al. (1988) suggested a FPC of 7 – 9% for biscuit production, a FPC of 9 – 10% for cracker production and a FPC of 11 – 13% for bread-baking. The average FPC of Cracker (10.42) could still be suitable for the production of crackers, although very high, but the FPC of SST876 (10.17%) was definitely very low for bread-baking. The SDS-soluble LMP fraction was significantly positively correlated with FPC, while the SDS-soluble SMP fraction was significantly negatively correlated with FPC.

There were highly significant differences between the N-treatments for FPC. For example, P0 gave significantly the lowest FPC and the FPC of P6 was also significantly lower than for P1, P2, P3 and P5. Thus, a lower total amount of N applied by P0 and P6 (80 – 140 kg N/ha) gave lower FPC values. This explanation is proved right by the significant differences among the cultivars: the FPC of Snack P1, P2, P3 and P4 were significantly higher than for P0; Snack P2 gave a significantly higher FPC than P5; the FPC of Cracker P3 was significantly higher than for P0, P2, P4 and P6; the FPC of Cracker P0 was significantly the lowest; the FPC of E94/8 P1, P3 and P5 were higher than for P0 and P6; the FPC of E94/8 P0 was significantly lower than for all the others, but P6; and SST876 P0 gave significantly the lowest FPC of all the SST876 N-treatments.

The hard wheat cultivars had significantly higher falling numbers (FLN) than the soft wheat cultivars. SST876 had significantly the highest FLN, followed by Cracker with a significantly higher FLN than Snack and E94/8. SDS-soluble LPP, which was significantly positively correlated with SK hardness, was also significantly positively correlated with FLN. This proves that a harder cultivar posess a higher SDS-soluble LPP fraction and also a higher

FLN value. All four cultivars had FLN values above 250 seconds, the average for bread wheat (Atwell, 2001). The upper limit for the falling number test is about 400 seconds, which occurs for a flour devoid of a-amylase activity (Atwell, 2001), such as the flour of the bread cultivar SST876 (398.21 s). The average falling number of the two soft wheat cultivars was 279.93 s. FLN was significantly positively correlated with VK, FLY and alveograph strength and negatively with BFLY. A higher FLN value is thus responsible for better bread-baking quality characteristics.

There were significant differences among the cultivars for FLN. The FLN of Snack P3 was significantly higher than for P0; the FLN of E94/8 P4 was significantly higher than for P0, P1, P2 and P6 ; and the FLN of Cracker P6 was significantly lower than for all the other Cracker treatments. It is possible that lower amounts of total N applied (e.g., P0 and P6) were responsible for the lower FLN values.

The hard wheat cultivars had significantly higher amounts of vitreous kernels (VK) than the soft wheat cultivars. Cracker had significantly the highest amount of VK, with the VK of SST876 significantly higher than for Snack and E94/8. These results indicate a definite positive correlation between VK and SK hardness. VK was significantly positively correlated with FLY, alveograph P/L ratio and alveograph strength and negatively with BFLY. A higher VK is thus associated with higher bread-baking quality characteristics.

The VK of Cracker P1 and P6 were significantly higher than for P0 and the VK of SST876 P1 and P2 were significantly higher than for P0, P4, P5 and P6. It seems as if a higher total amount of N applied could be responsible for higher VK values, and vice versa.

The soft wheat cultivars (Snack and E94/8) had significantly higher breakflour yields (BFLY) than the hard wheat cultivars (Cracker and SST876). Lower BFLY values are characteristic of the harder wheats, as can be seen from the significant positive correlation of SDS-soluble LPP with SK hardness,

and its negative correlation with BFLY. BFLY was significantly negatively correlated with FLY, P/L ratio and alveograph strength, the quality characteristics required for bread-baking.

There were significant differences among the cultivars for BFLY, even though there were no significant differences between the N-treatments. Snack P0 had a significantly higher BFLY than P1, P3, P4, P5 and P6; Cracker P3 had a significantly higher BFLY than P4 and P6; E94/8 P0 had a higher BFLY than P2, P3 and P4; and SST876 P0 and P6 had significantly higher BFLY values than all the other SST876 treatments. Snack, E98/8 and SST876 indicated that lower total N-applications by P6 (140 kg N/ha) and especially P0 (80 kg N/ha) resulted in higher BFLY values, while higher N-applications gave lower BFLY values.

There were significant differences between the flour yield (FLY) values of the different cultivars. SST876 had significantly the highest FLY, followed by Cracker with a significantly higher FLY than Snack and E94/8. According to the negative correlation between BFLY and FLY (Table 3.36), the hard wheat cultivars were supposed to have higher FLY values than the soft wheat cultivars. The SDS-insoluble LPP, total LPP, TUPP and LUPP protein fractions, which are positively correlated with SK hardness, were also significantly positively correlated with FLY. This emphasise the fact that harder wheats give higher FLY values. FLY was significantly positively correlated with alveograph P/L ratio and alveograph strength. The SDS-insoluble LMP and total SPP fractions were significantly negatively correlated with FLY.

E94/8 P0 had a significantly higher FLY than E94/8 P5 and Snack P0 had a significantly higher FLY than P2, P3, P4, P5 and P6. It seems as if lower total amounts of N-applied by P0 were responsible for higher FLY values.

The hard wheat cultivars were characterised by significantly higher alveograph P/L ratios than the soft wheat cultivars. Cracker had a

significantly higher P/L ratio than all other entries, followed by SST876 with a significantly higher P/L ratio than Snack and E94/8. South African wheat cultivars with P/L ratios between 0.5 and 0.8 produce good quality bread, with 0.8 being the optimum (Mamuya, 2000). Cracker and SST876, the hard wheat cultivars, had P/L ratios of 1.05 and 0.70, respectively. The P/L ratio of SST876 is relatively good for the production of high quality bread. The P/L ratio was significantly positively correlated with alveograph strength.

Although there were no significant differences between the N-treatments, the following significant differences were visible among the cultivars: Snack P5 had a significantly higher P/L ratio than P2 and P6; Cracker P0 and P6 had significantly higher P/L ratios than P1, P2 and P3; and E94/8 P0 had a higher P/L ratio than P5. Most of these results can be explained by the indication that lower total amounts of N (P0, P5, P6) gave higher P/L ratios, while higher total amounts of N applied (P1, P2 and P3) gave lower P/L ratios. The same conclusion was made for the results of 2001.

The hard wheat cultivars had significantly higher alveograph strength values than the soft wheat cultivars. Cracker had a significantly higher alveograph strength than all other entries, followed by SST876 with a significantly higher strength value than E94/8 and Snack. The SDS-insoluble LPP and PP fractions (associated with harder wheats) were significantly positively correlated with alveograph strength. SDS-insoluble SMP was significantly negatively correlated with alveograph strength.

The following significant differences were found among the cultivars for alveograph strength: Cracker P6 had a significantly higher alveograph strength than P0, P1, P2 and P5; the alveograph strength of Cracker P3 was significantly higher than for P0, P1 and P2; E94/8 P0 had a significantly lower alveograph strength than P1, P3 and P5; SST876 P3 had a significantly higher alveograph strength than P0 and P2; and the strength value of SST876 P0 was significantly lower than for all the others, but P2. N-treatment P3 was

responsible for significantly higher strength values than P0. P0, P1 and P2 (lowest and highest N-applications) might be responsible for the lowest alveograph strength values.

Various protein fraction ratios can give information about a cultivar's quality. The values in Table 3.19 were used to calculate these ratios.

A high ratio of HMW- to LMW-glutenins is an indication of good bread-making quality (Huebner & Wall, 1976). The LPP1:SPP1, LPP2:SPP1 and LPP:SPP ratios were calculated separately for the hard and soft wheat cultivars. The hard wheat cultivars had a higher LPP1:SPP1 ratio (0.24) than the soft wheat cultivars (0.22), a higher LPP2:SPP1 ratio (1.96) than the soft wheat cultivars (1.77), and a higher LPP:SPP ratio (0.98) than the soft wheat cultivars (0.88), indicating better bread-making quality. The correlations in Table 3.23 also prove that the harder wheat cultivars (higher ratios) are associated with better bread-making quality. LPP1:SPP1 was significantly positively correlated with alveograph strength and negatively correlated with SK weight and SK diameter. LPP2:SPP1 and LPP:SPP were significantly positively correlated with FLY.

A high glutenin to gliadin ratio is, according to the literature, one of the features related to good bread-making quality (Lafiandra et al., 2000). The LPP1:LMP1 and SPP1:LMP1 ratios were calculated to illustrate the glutenin/gliadin ratio. The hard wheat cultivars had a higher LPP1:LMP1 ratio (0.08) than the soft wheat cultivars (0.07) and a higher SPP1:LMP1 ratio (0.324) than the soft wheat cultivars (0.320), indicating stronger doughs. Table 3.23 show significant positive correlations for LPP1:LMP1 with alveograph strength and significant negative correlations for LPP1:LMP1 with SK weight and SK diameter. The SPP1:LMP1 ratio was also significantly negatively correlated with SK diameter.

According to He and Hoseney (1990), the gluten from poor-quality flour (soft wheats) has a higher solubility than the gluten from good-quality flour (hard

wheats). The LPP1:LPP2 ratio was calculated to illustrate the SDS-soluble:SDS-insoluble ratio. The hard and the soft wheat cultivars both had a ratio of 0.12, but still the positive correlation of LPP1:LPP2 with SK hardness and FLY proved the harder cultivars to posess lower soluble gluten.

3.5 Results and discussion for the combined data of 2001 and 2002.

3.5.1 Results

Table 3.24. Protein fractions for all entries (2001 and 2002 combined).

	LPP1	**SPP1**	**LMP1**	**SMP1**	**LPP2**	**SPP2**	**LMP2**	**SMP2**	**TUPP**	**LUPP**	**LPP**	**LMP**	**SPP**	**SMP**	**PP**	**MP**
Snack P1	3.78	15.20	57.80	19.72	25.39	26.07	21.52	13.65	75.68	69.64	29.16	79.31	41.26	33.37	70.42	112.68
Snack P2	3.28	14.01	58.80	19.97	20.97	27.04	24.39	12.84	75.64	65.16	24.25	83.19	41.05	32.81	65.30	116.00
Snack P3	4.04	14.69	58.09	18.95	18.51	27.52	27.70	14.33	73.38	60.65	22.55	85.78	42.21	33.28	64.76	119.06
Snack P4	3.84	14.80	57.86	19.96	21.89	24.51	22.74	13.98	72.55	67.37	25.72	80.60	39.31	33.94	65.03	114.54
Snack P5	4.07	14.86	57.38	19.92	19.81	28.57	24.64	11.61	73.08	60.82	23.88	82.02	43.43	31.53	67.30	113.55
Snack P6	3.84	13.51	56.20	23.76	15.79	28.40	28.81	15.61	72.52	58.12	19.63	85.01	41.90	39.37	61.53	124.38
CrackerP1	4.43	15.08	56.03	21.19	25.29	25.20	21.04	13.04	72.70	70.37	29.27	77.07	40.27	34.21	69.99	111.30
CrackerP2	4.70	14.38	55.54	20.56	23.33	28.15	22.75	13.45	73.43	66.05	28.04	78.29	42.53	34.02	70.57	112.30
CrackerP3	5.07	15.40	55.52	19.92	20.15	30.24	25.44	13.00	71.41	61.10	25.21	80.96	45.64	32.92	70.85	113.88
CrackerP4	5.19	14.83	56.20	20.35	20.29	28.55	24.19	13.18	70.43	62.52	25.48	80.40	43.39	33.53	68.87	113.92
CrackerP5	4.82	15.25	56.00	21.05	24.04	24.93	22.29	12.54	70.66	66.72	28.85	78.29	40.18	33.59	69.03	111.87
CrackerP6	4.89	14.82	53.77	23.78	22.04	28.11	25.02	12.52	71.50	63.55	26.93	78.79	42.93	36.29	69.86	115.08
E94/8 P1	4.26	15.15	59.17	17.62	22.51	25.60	24.49	12.60	73.52	66.56	26.78	83.65	40.76	30.22	67.53	113.88
E94/8 P2	4.08	14.67	57.36	19.10	17.60	24.35	29.00	15.64	70.60	62.02	21.67	86.35	39.02	34.74	60.69	121.09
E94/8 P3	3.78	15.97	58.50	18.29	21.94	24.47	25.17	14.65	72.27	66.56	25.72	83.67	40.44	32.93	66.16	116.60
E94/8 P4	3.67	14.64	57.95	21.16	16.52	27.45	26.52	16.16	72.50	60.68	20.04	84.46	42.09	37.32	62.13	121.78
E94/8 P5	4.29	16.43	56.82	19.16	21.13	26.40	26.18	11.55	69.72	62.50	25.42	83.00	42.83	22.96	68.24	113.71
E94/8 P6	4.37	15.91	55.01	20.82	23.77	26.63	22.53	14.29	72.17	66.03	28.14	77.54	42.54	35.10	70.67	112.64
SST876P1	3.93	14.94	57.77	19.90	25.58	27.52	21.65	13.28	73.94	69.01	29.51	79.42	42.46	34.50	71.97	113.92
SST876P2	4.44	15.26	56.16	20.32	20.88	26.61	25.54	13.87	70.97	64.31	25.32	81.69	41.87	34.19	67.19	115.88
SST876P3	4.78	15.12	56.33	19.98	23.20	25.29	25.95	11.07	71.18	66.04	27.98	82.27	40.41	31.05	68.40	113.32
SST876P4	4.86	15.64	54.13	22.18	23.79	25.40	25.15	14.31	69.68	66.00	28.65	79.28	41.03	36.49	69.68	115.77
SST876P5	4.64	15.68	56.39	19.77	22.29	27.03	23.87	11.40	71.72	65.04	26.79	80.26	42.71	31.17	69.49	111.42
SST876P6	4.84	15.73	53.80	22.19	24.75	26.23	24.48	12.96	71.32	67.83	29.58	78.30	41.96	35.14	71.54	113.42
Average	4.33	15.08	56.61	20.40	21.73	26.68	24.63	13.40	72.19	64.82	26.04	81.23	41.76	33.53	67.80	115.08
LSD (0.05)	1.009	1.772	2.811	2.562	5.423	4.191	4.889	3.662	3.858	7.257	5.441	5.115	5.115	6.432	6.874	7.355

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble) mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins,

LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins

Protein fractions for all entries (2001 and 2002 combined)

Results are given in Table 3.24.

SDS-soluble LPP: There were no significant differences.

SDS-soluble SPP: The SPP of E94/8 P5 was significantly higher than the SPP of P4.

SDS-soluble LMP: E94/8 P1, P3 and P4 had significantly higher LMP values than P6. SST876 P1 had a significantly higher LMP than P4 and P6.

SDS-soluble SMP: Snack P6 and Cracker P6 had significantly the highest SMP values of all the Snack and Cracker treatments. E94/8 P4 and P6 had significantly higher SMP values than P1. E94/8 P4 had a higher SMP than P3.

SDS-insoluble LPP: The LPP of Snack P1 was significantly higher than for Snack P3, P5 and P6. Snack P4 also had a higher LPP than P6. E94/8 P6 had a significantly higher LPP than P2 and P4. E94/8 P1 had a significantly higher LPP than P4.

SDS-insoluble SPP: Cracker P3 had a significantly higher SPP than P1 and P5.

SDS-insoluble LMP: The LMP of Snack P3 and P6 were significantly higher than for P1 and P4. E94/8 P2 had a significantly higher LMP than P6.

SDS-insoluble SMP: The SMP of Snack P6 was significantly higher than for P5. E94/8 P2 and P4 had significantly higher SMP values than P5.

TUPP: SST876 P1 had a significantly higher TUPP than P4.

LUPP: Snack P1 had a significantly higher LUPP than P3, P5 and P6. The LUPP of Snack P4 was also higher than for Snack P6. Cracker P1 had a significantly higher LUPP than P3 and P4.

LPP: The total LPP of Snack P1 was significantly higher than for P3 and P6. Snack P4 had a significantly higher LPP than P6. The LPP of E94/8 P6 was significantly higher than for P2 and P4. The LPP of E94/8 P1 and P3 were also significantly higher than for E94/8 P4.

SPP: Cracker P3 had a significantly higher total SPP than P1 and P5.

LMP: Snack P3 had a significantly higher total LMP than P1 and P4 . Snack P6 also had a significantly higher LMP than P1. E94/8 P6 had significantly the lowest LMP of all the E94/8 treatments.

SMP: The total SMP of Snack P6 was significantly higher than for P2 and P5. The SMP of E94/8 P5 was significantly the lowest of all the Snack treatments. The SMP of E94/8 P1 was also significantly lower than for P4.

PP: Snack P1 had a significantly higher PP than Snack P6. The PP of E94/8 P6 was significantly higher than for P2 and P4, and the PP of E94/8 P5 was also significantly higher than for P2.

MP: Snack P6 had a significantly higher MP than all the other Snack treatments, but P3. E94/8 P2 and P4 had significantly higher MP values than P5 and P6. E94/8 P4 also had a higher MP than E94/8 P1.

Table 3.25. Quality characteristics for 2001 and 2002.

	SK-wght	SK-diam	SK-hard	HLM	FPC	FLN	VK	BFLY	FLY	P/L	Strength
Snack P1	38.16	2.46	17.61	77.75	10.85	248.00	31.50	30.15	75.33	0.48	16.32
Snack P2	36.39	2.41	16.53	77.28	11.33	230.00	32.75	30.95	74.75	0.39	16.86
Snack P3	39.40	2.52	15.64	77.55	11.18	267.00	33.50	29.78	75.08	0.47	16.25
Snack P4	39.01	2.52	14.69	76.90	10.53	240.75	26.50	30.73	75.13	0.48	16.86
Snack P5	40.65	2.61	16.68	77.30	10.58	242.50	28.25	29.73	75.25	0.55	15.18
Snack P6	39.43	2.57	12.75	76.83	10.50	252.50	27.50	31.15	74.48	0.42	15.41
CrackerP1	34.32	2.42	66.05	80.23	11.45	365.25	73.25	23.98	78.28	0.75	32.49
CrackerP2	34.63	2.45	64.94	80.85	10.65	264.00	69.00	24.35	78.38	0.72	29.09
CrackerP3	33.74	2.40	65.33	80.40	11.48	366.50	73.00	24.00	77.70	0.69	37.23
CrackerP4	34.97	2.45	64.96	80.50	10.60	351.00	70.75	23.50	77.95	0.84	31.81
CrackerP5	37.83	2.64	61.02	80.95	10.63	387.25	71.25	24.05	78.18	0.83	31.16
CrackerP6	35.48	2.51	62.80	80.45	10.20	286.00	69.25	24.08	77.90	0.94	31.12
E94/8 P1	38.86	2.53	16.93	76.48	11.25	225.00	29.00	29.33	74.83	0.40	17.89
E94/8 P2	39.54	2.61	17.03	76.78	11.23	216.50	28.50	28.95	74.58	0.42	15.94
E94/8 P3	39.10	2.59	16.63	76.98	11.70	226.25	34.25	29.10	74.43	0.39	16.86
E94/8 P4	39.74	2.61	17.64	76.55	10.75	257.75	27.50	29.30	74.98	0.47	16.63
E94/8 P5	37.94	2.49	16.82	75.93	11.43	249.25	26.25	30.03	74.98	0.38	18.12
E94/8 P6	40.19	2.57	15.43	76.30	10.70	212.25	30.25	29.98	74.73	0.44	15.18
SST876P1	38.56	2.54	54.72	81.45	11.13	398.75	64.00	22.93	79.15	0.54	26.99
SST876P2	38.39	2.55	54.29	81.08	10.90	395.50	59.00	23.50	79.33	0.59	24.54
SST876P3	39.16	2.56	51.58	81.20	11.28	382.25	55.50	22.93	79.10	0.53	28.90
SST876P4	37.12	2.46	51.34	80.23	11.33	373.00	42.00	23.73	78.73	0.48	26.15
SST876P5	36.95	2.45	55.48	80.55	11.28	364.25	50.75	23.20	78.65	0.55	26.80
SST876P6	36.19	2.42	48.40	79.58	10.35	382.00	39.25	25.98	78.73	0.50	24.12
Average	37.74	2.51	37.30	78.75	10.97	303.48	45.53	26.89	76.69	0.55	22.83
LSD (0.05)	2.368	0.149	4.018	1.098	0.601	45.084	16.66	0.991	0.956	0.121	4.122

SK-wght = single kernel chracterization system weight, SK-diam = single kernel characterization system diameter, SK-hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength

Quality characteristics combined for 2001 and 2002

Results are given in Table 3.25.

There were significant differences among the cultivars for SK weight, SK hardness, HLM, FPC, FLN, VK, BFLY, FLY, P/L ratio and alveograph strength (see Table 3.34).

SK weight: Snack P2 gave a significantly lower SK weight than Snack P3, P4, P5 and P6. The SK weight of Snack P5 was also higher than for Snack P1. Cracker P5 gave a significantly higher SK weight than P1, P2, P3 and P4; and the SK weight of SST876 P3 was higher than for P6.

SK diameter: Snack P5 gave a significantly higher SK diameter than P1 and P2. The SK diameter of Snack P6 was also higher than for Snack P2. The SK diameter of Cracker P5 was significantly higher than for P1, P2, P3 and P4.

SK hardness: Snack P1 was significantly harder than Snack P6; Cracker P1 was significantly harder than P5; SST876 P5 was significantly harder than P4 and P6; and SST876 P1 was significantly harder than P6.

HLM: SST876 P1 gave a significantly higher HLM than P4 and P6. The HLM of SST876 P3 was also significantly higher than for P6.

FPC: The FPC of Snack P2 was significantly higher than for P4, P5 and P6; and Snack P3 gave a significantly higher FPC than P4 and P6. The FPC values of Cracker P1 and P3 were significantly higher than for P2, P4, P5 and P6. The FPC values of E94/8 P3 and P5 were significantly higher than for P4 and P6.

FLN: The FLN of Cracker P6 was significantly lower than for all the other Cracker treatments. The FLN of E94/8 P4 was significantly higher than for P6.

VK: The VK values of SST876 P1 and P2 were significantly higher than for P4 and P6. SST876 P3 had a higher VK than P6.

BFLY: Snack P6 had a significantly higher BFLY than P1, P3 and P5. Snack P2 also had a significantly higher BFLY than P3 and P5, and Snack P4 had a significantly higher BFLY than P5. E94/8 P5 had a higher BFLY than P2, and SST876 P6 had a significantly higher BFLY than all the SST876 treatments.

FLY: There were no significant differences among the cultivars for FLY.

Alveograph P/L ratio: Snack P5 had a significantly higher P/L ratio than P2 and P6; Cracker P6 had a significantly higher P/L ratio than P1, P2 and P3; and Cracker P4 and P5 had significantly higher P/L ratios than P3.

Alveograph strength: Cracker P3 ranked significantly the highest of all the Cracker treatments for alveograph strength. The alveograph strength of SST876 P3 was significantly higher than for P2 and P6.

Table 3.26. Protein fractions for six different nitrogen treatments (2001 and 2002 combined).

	LPP1	SPP 1	LMP1	SMP 1	LPP2	SPP 2	LMP2	SMP 2	TUPP	LUPP	LPP	LMP	SPP	SMP	PP	MP
P1	4.10	15.09	57.69	19.61	24.69	26.10	22.18	13.14	73.96	68.90	28.97	79.86	41.19	33.07	69.98	112.94
P2	4.13	14.58	56.96	19.99	20.69	26.54	25.42	13.95	72.66	64.38	24.82	82.38	41.12	33.94	65.94	116.32
P3	4.42	15.29	57.11	19.28	20.95	26.88	26.06	13.26	72.06	64.14	25.37	83.17	42.18	32.54	67.54	115.71
P4	4.39	14.98	56.53	20.91	20.62	26.48	24.65	14.41	71.29	64.02	24.97	81.18	41.45	35.32	66.42	116.50
P5	4.45	15.55	56.65	19.97	21.82	26.73	24.25	11.78	71.29	63.88	26.23	80.89	42.28	29.81	68.52	112.64
P6	4.48	14.99	54.69	22.64	21.58	27.34	25.21	13.84	71.87	63.59	26.07	79.91	42.33	36.48	68.40	116.38
Average	4.33	15.08	56.61	20.40	21.73	26.68	24.63	13.40	72.19	64.82	26.04	81.23	41.76	33.53	67.80	115.08
LSD(0.05)	0.534	0.858	1.437	1.273	2.783	2.069	2.408	1.741	1.881	3.568	2.873	2.696	2.419	3.134	3.504	3.712

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins, LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric protein

The differences between the N-treatments for the different protein fractions (2001 and 2002 combined)

Results are given in Table 3.26.

There were no significant differences between the N-treatments for SDS-soluble LPP, SDS-insoluble SPP and total SPP.

SDS-soluble SPP: P5 gave a significantly higher SPP fraction than P2.

SDS-soluble LMP: There were significant differences between the N-treatments for LMP (Table 3.34). P6 gave significantly the lowest LMP.

SDS-soluble SMP: There were significant differences between the N-treatments for SMP (Table 3.34). P6 gave significantly the highest SMP, and the SMP of P4 was significantly higher than for P1 and P3.

SDS-insoluble LPP: P1 gave a significantly higher LPP than all other N-treatments.

SDS-insoluble LMP: The LMP of P1 was significantly lower than for P2, P3, P4 and P6.

SDS-insoluble SMP: The SMP of P2, P4 and P6 was significantly higher than for P5.

TUPP: P1 gave a significantly higher TUPP than P3, P4, P5 and P6.

LUPP: P1 gave significantly the highest LUPP of all the treatments.

LPP: P1 gave a significantly higher total LPP than P2, P3 and P4.

LMP: The LMP of P3 was significantly higher than for P1 and P6.

SMP: There were significant differences between the N-treatments for SMP (Table 3.34). P6 gave a significantly higher SMP than P1, P3 and P5. P5 gave a significantly lower SMP than the rest of the treatments, but P3.

PP: P1 gave a significantly higher PP than P2 and P4.

MP: P4 and P6 gave significantly higher MP values than P5.

Table 3.27. Quality characteristics for six different nitrogen treatments (2001 and 2002 combined).

	SKwght	SKdiam	SKhard	HLM	FPC	FLN	VK	BFLY	FLY	P/L	Strength
P1	37.47	2.49	38.83	78.98	11.17	309.25	49.44	26.59	76.89	0.54	23.42
P2	37.24	2.51	38.20	78.99	11.03	301.50	47.31	26.94	76.76	0.53	21.61
P3	37.85	2.52	37.29	79.03	11.41	310.50	49.06	26.45	76.58	0.52	24.81
P4	37.71	2.51	37.16	78.54	10.80	305.63	41.69	26.81	76.69	0.56	22.86
P5	38.34	2.55	37.50	78.68	10.98	310.81	44.13	26.75	76.76	0.58	22.81
P6	37.82	2.51	34.84	78.29	10.44	283.19	41.56	27.79	76.46	0.57	21.45
Average	37.74	2.51	37.30	78.75	10.97	303.48	45.53	26.89	76.69	0.55	22.83
LSD (0.05)	1.582	0.079	13.42	1.279	0.314	46.408	13.18	1.962	1.221	0.109	4.582

SK-wght = single kernel chracterization system weight, SK-diam = single kernel characterization system diameter, SK-hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength

Differences between the N-treatments for the different quality characteristics (2001 and 2002 combined)

Results are given in Table 3.27.

SK weight, SK diameter and SK hardness: There were no significant differences between the different N-treatments for SK weight, SK diameter and SK hardness (Table 3.34).

FPC: There were highly significant differences between the N-treatments for FPC. P6 had a significantly lower FPC than all the other treatments. P3 gave a significantly higher FPC than all the other N-treatments, but P1.

HLM, FLN, VK, BFLY, FLY, alveograph P/L ratio and alveograph strength: There were no significant differences between any of the N-treatments for HLM, FLN, VK, BFLY, FLY, alveograph P/L ratio and alveograph strength (Table 334).

Table 3.28. Protein fractions for four cultivars (2001 and 2002 combined).

Cultivar	LPP1	SPP 1	LMP1	SMP 1	LPP2	SPP 2	LMP2	SMP 2	TUPP	LUPP	LPP	LMP	SPP	SMP	PP	MP
Snack	3.81	14.51	57.69	20.38	20.39	27.02	24.96	13.67	73.81	63.63	24.20	82.65	41.53	34.05	65.72	116.70
Cracker	4.85	14.96	55.51	21.14	22.52	27.53	23.45	12.96	71.69	65.05	27.37	78.97	42.49	34.09	69.86	113.06
E94/8	4.07	15.46	57.47	19.36	20.58	25.82	25.65	14.15	71.79	64.22	24.63	83.11	41.28	32.21	65.90	116.62
SST876	4.58	15.39	55.76	20.72	23.41	26.34	24.44	12.81	71.47	66.37	27.97	80.20	41.74	33.75	69.71	113.95
LSD (0.05)	0.387	0.682	1.151	1.122	2.263	1.649	2.000	1.441	1.515	2.978	2.268	2.090	1.956	2.715	2.734	3.001

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins, LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins

Differences between the cultivars for the different protein fractions (2001 and 2002 combined)

Results are given in Table 3.28.

SDS-soluble LPP: Cracker and SST876 had significantly higher LPP values than E94/8 and Snack.

SDS-soluble SPP: E94/8 and SST876 had significantly higher SPP values than Snack.

SDS-soluble LMP: Snack and E94/8 had significantly higher SDS-soluble LMP values than SST876 and Cracker.

SDS-soluble SMP: Cracker and SST876 had significantly higher SMP values than E94/8.

SDS-insoluble LPP: SST876 had a significantly higher LPP than E94/8 and Snack.

SDS-insoluble SPP: Cracker had a significantly higher SPP than E94/8.

SDS-insoluble LMP: The LMP of E94/8 was significantly higher than for Cracker.

TUPP: The TUPP value of Snack was significantly the highest.

LPP: The total LPP values of SST876 and Cracker were significantly higher than for E94/8 and Snack.

LMP: E94/8 and Snack had significantly higher LMP values than SST876 and Cracker.

PP: Cracker and SST876 had significantly higher PP values than E94/8 and Snack.

MP: Snack and E94/8 had significantly higher MP values than Cracker.

There were no significant differences between the cultivars for SDS-insoluble SMP, LUPP, total SPP and SMP.

Table 3.29. Quality characteristics for four cultivars (2001 and 2002 combined).

Cultivar	SKwght	SKdiam	SKhard	HLM	FPC	FLN	VK	BFLY	FLY	P/L	Strength
Snack	38.84	2.51	15.65	77.27	10.83	246.79	30.0	30.41	75.00	0.46	16.14
Cracker	35.16	2.48	64.18	80.56	10.83	353.33	71.08	23.99	78.06	0.79	32.15
E94/8	39.23	2.57	16.75	76.50	11.18	231.17	29.29	29.45	74.75	0.42	16.77
SST876	37.73	2.49	52.63	80.68	11.04	382.63	51.75	23.71	78.95	0.53	26.25
LSD (0.05)	1.017	0.062	1.677	0.445	0.286	18.935	6.442	0.479	0.365	0.051	1.679

SK-wght = single kernel chracterization system weight, SK -diam = single kernel characterization system diameter, SK -hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BF LY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strengh

Differences between the cultivars for the different quality characteristics (2001 and 2002 combined)

Results are given in Table 3.29.

There were highly significant differences between the cultivars for SK weight, SK hardness, HLM, FLN, VK, BFLY, FLY, alveograph P/L ratio and alveograph strength.

SK weight: E94/8 and Snack had significantly higher SK weights than SST876, and the SK weight of SST876 was significantly higher than for Cracker.

SK diameter: The SK diameter of E94/8 was significantly higher than for SST876 and Cracker.

SK hardness: Cracker was significantly harder than SST876, and SST876 was significantly harder than E94/8 and Snack.

HLM: The HLM of SST876 and Cracker were significantly higher than for Snack and E94/8. The HLM of Snack was significantly higher than for E94/8.

FPC: The FPC of E94/8 and SST876 were significantly higher than for Cracker and Snack.

FLN: SST876 had significantly the highest FLN. The FLN of Cracker was also significantly higher than for Snack and E94/8.

VK: Cracker had significantly the highest amount of VK. The VK of SST876 was also significantly higher than for Snack and E94/8.

BFLY: The BFLY of Snack was significantly higher than for E94/8, and the BFLY of E94/8 was significantly higher than for Cracker and SST876.

FLY: SST876 had significantly the highest FLY, followed by Cracker with a significantly higher FLY than Snack and E94/8.

Alveograph P/L ratio: Cracker had significantly the highest P/L ratio, followed by SST876 with a significantly higher P/L ratio than Snack and E94/8.

Alveograph strength: The cultivars were significantly different at a 99.99% level of significance. Cracker had significantly the highest alveograph strength value, followed by SST876 with a higher alveograph strength than E94/8 and Snack.

Table 3.30. Protein fractions for the hard and soft wheats (2001 and 2002 combined).

	LPP1	SPP1	LMP1	SMP1	LPP2	SPP2	LMP2	SMP2	TUPP	LUPP	LPP	LMP	SPP	SMP	PP	MP
Hard	4.72	15.18	55.64	20.93	22.97	26.94	23.95	12.88	71.58	65.71	27.67	79.59	42.11	33.92	69.79	113.51
Soft	3.94	14.99	57.58	19.87	20.48	26.42	25.30	13.91	72.80	63.92	24.41	82.88	41.40	33.13	65.81	116.66
LSD(0.05)	0.275	0.494	0.806	0.796	1.586	1.171	1.406	1.009	1.088	2.090	1.588	1.470	1.371	1.912	1.911	2.101

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins, LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins

Table 3.31. Quality characteristics for the hard and soft wheats (2001 and 2002 combined).

	SKwght	SKdiam	SKhard	HLM	FPC	FLN	VK	BFLY	FLY	P/L	Strength
Hard	36.44	2.49	58.41	80.62	10.94	367.98	61.42	23.85	78.50	0.66	29.20
Soft	39.03	2.54	16.20	76.88	11.00	238.98	29.65	29.93	74.88	0.44	16.46
LSD (0.05)	0.780	0.044	1.848	0.325	0.206	13.855	5.096	0.357	0.279	0.049	1.383

SK-wght = single kernel chracterization system weight, SK-diam = single kernel characterization system diameter, SK-hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength

Differences between the hard and soft wheat cultivars for the different protein fractions (2001 and 2002 combined)

Results are given in Table 3.30.

The hard wheat cultivars had significantly higher amounts of SDS-soluble LPP and SMP, SDS-insoluble LPP, total LPP and PP than the soft wheat cultivars. The hard wheat cultivars also had higher amounts of SDS-soluble SPP, SDS-insoluble SPP, total SPP, SMP and LUPP, although not significantly. However, the soft wheat cultivars had significantly higher amounts of SDS-soluble LMP, SDS-insoluble SMP, TUPP, LMP and MP than the hard wheat cultivars.

Differences between the hard and soft wheat cultivars for the different quality characteristics (2001 and 2002 combined)

Results are given in Table 3.31.

The hard wheat cultivars had significantly higher SK hardness, HLM, FLN, VK, FLY, alveograph P/L ratio and alveograph strength values than the soft wheat cultivars. The soft wheat cultivars had significantly higher SK weight, SK diameter and BFLY values than the hard wheat cultivars.

Mean square values for all measured characteristics are given in Table 3.32.

Table 3.32. Mean square values for protein fractions and quality characteristics of four cultivars (2001 and 2002 combined).

Trait	Mean Squares					
	Across cultivars and treatments		Only across N-treatments		Only across cultivars	
	Entry	**Replicates**	**Treatment**	**Replicates**	**Number**	**Replicates**
LLP1	1.018	5.121**	0.464	5.121**	5.379**	5.121**
SPP1	1.648	133.535**	1.714	133.535**	4.693	133.535**
LMP1	9.188	855.068**	16.727*	855.068**	30.608**	855.068**
SMP1	8.791	131.752**	23.970**	131.752**	13.925	131.752**
LPP2	29.466	2711.22**	37.532	2711.22**	52.645	2711.22**
SPP2	9.548	835.410**	2.813	835.410**	13.576	835.410**
LMP2	18.285	3227.92**	29.369	3227.92**	20.495	3227.92**
SMP2	7.172	495.919**	13.535	495.919**	9.389	495.919**
TUPP	10.138	42.082*	16.292	42.082**	28.419*	42.082**
LUPP	39.806	1331.22**	64.991	1331.22**	33.917	1331.22**
LPP	33.900	2505.90**	34.256	2505.90**	87.068*	2505.90**
LMP	29.216	6995.14**	28.228	6995.14**	93.882**	6995.14**
SPP	8.709	347.933**	5.152	347.933**	6.570	347.933**
SMP	37.082	552.161**	86.558*	552.161**	18.963	552.161**
PP	40.102	1051.01**	35.359	1051.01**	126.398*	1051.01**
MP	45.353	9582.21**	51.722	9582.21**	82.657	9582.21**
SK-wght	15.802**	5.047	2.257	5.047	80.700**	5.047
SK-diam	0.021	0.038	0.006	0.038	0.036	0.038
SK-hard	1941.77**	245.084**	29.537	245.084	14791.5**	245.084**
HLM	15.616**	35.814**	1.441	35.814**	114.157**	35.814**
FPC	0.682**	5.702**	1.745**	5.702**	0.695	5.702**
FLN	19578.4**	31584.6**	1781.267	31584.6**	137536.**	31584.6**
VK	1347.25**	1244.15**	202.894	1244.15**	9572.21**	1244.15**
BFLY	40.822**	76.815**	3.603	76.815**	299.709**	76.815**
FLY	14.443**	14.069**	0.381	14.069*	108.738**	14.069**
P/L	0.102**	0.768**	0.010	0.768**	0.681**	0.768**
Strength	198.535**	71.430**	24.485	71.430	1439.89**	71.430**

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric protein, LUPP = larger unextractable polymeric protein, LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins, SK-wght = single kernel chracterization system weight, SK-diam = single kernel characterization system diameter, SK-hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels,

BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength
*$p < 0.05$, ** $p < 0.01$

Table 3.33. Significant correlations between protein fractions and measured characteristics for 2001 and 2002 at p<0.01.

SDS-soluble proteins			**SDS-insoluble proteins**			**TUPP, LUPP and combined fractions**		
LPP	Sk-wght	-0.413	LPP	HLM	0.496	TUPP	FLN	-0.277
	Sk-hard	0.332		FPC	-0.559	LUPP	FPC	0.286
	FPC	0.263		FLN	0.373		FLY	0.300
	VK	0.290		BFLY	-0.437		P/L	-0.411
	FLY	0.424		P/L	0.605	LPP	HLM	0.515
	Strength	0.403	SPP	HLM	-0.358		FPC	-0.550
LMP	Sk-hard	-0.269		FPC	0.516		FLN	0.400
	HLM	-0.430		VK	0.299		BFLY	-0.468
	FPC	0.591		FLY	0.261		P/L	0.613
	FLN	-0.400		P/L	-0.488	LMP	HLM	-0.481
	BFLY	0.415	LMP	HLM	-0.460		FPC	0.631
	P/L	-0.559		FPC	0.589		FLN	-0.382
SPP	Sk-diam	-0.324		FLN	-0.335		BFLY	0.422
	HLM	0.312		BFLY	0.384		P/L	-0.641
	FPC	-0.353		P/L	-0.620	SPP	Sk-wght	-0.330
	FLN	0.338	SMP	Sk-diam	0.292		HLM	-0.276
	BFLY	-0.372		HLM	-0.404		FPC	0.441
	P/L	0.432		FPC	0.315		P/L	-0.374
SMP	FPC	-0.478		FLN	-0.364	PP	Sk-diam	-0.323
	P/L	0.318		BFLY	0.441		Sk-hard	0.319
				P/L	-0.525		HLM	0.466
				Strength	-0.319		FPC	-0.395
							FLN	0.419
							BFLY	-0.512
							P/L	0.522
							Strength	0.395
						MP	HLM	-0.475
							FPC	0.541
							FLN	-0.388
							BFLY	0.445
							P/L	-0.633

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total unextractable polymeric proteins, LUPP = larger unextractable polymeric proteins, LPP = larger polymeric proteins, LMP = larger monomeric proteins, SPP = smaller polymeric proteins, SMP = smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins, SK-wght = single kernel chracterization system weight, SK-diam = single kernel characterization system diameter, SK-hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength

Correlations between protein fractions and quality characteristics (2001 and 2002 combined)

Only correlations significant at p<0.01 were discussed (Table 3.33).

The large polymeric proteins of the SDS-soluble fraction (LPP1) were significantly positively correlated with SK hardness, FPC, VK, FLY and alveograph strength, and significantly negatively correlated with SK weight. The large monomeric proteins of the SDS-soluble fraction (LMP1) were significantly positively correlated with FPC and BFLY, and significantly negatively correlated with SK hardness, HLM, FLN and alveograph P/L ratio. The small polymeric proteins of the SDS-soluble fraction (SPP1) were significantly positively correlated with HLM, FLN and P/L ratio and negatively correlated with SK diameter, FPC and BFLY. The small monomeric proteins of the SDS-soluble fraction (SMP1) were significantly positively correlated with alveograph P/L ratio and negatively correlated with FPC.

The SDS-insoluble large polymeric proteins (LPP2) were significantly positively correlated with HLM, FLN and alveograph P/L ratio, and negatively correlated with FPC and BFLY. The large monomeric proteins of the SDS-insoluble fraction (LMP2) were significantly positively correlated with FPC and BFLY and negatively correlated with HLM, FLN and alveograph P/L ratio. The small polymeric proteins of the SDS-insoluble fraction (SPP2) was significantly positively correlated with FPC, VK and FLY, and significantly negatively correlated with HLM and alveograph P/L ratio. The small monomeric proteins of the SDS-insoluble fraction (SMP2) were significantly positively correlated with SK diameter, FPC and BFLY, and negatively correlated with HLM, FLN, P/L ratio and alveograph strength.

The total unextractable polymeric proteins (TUPP) were significantly negatively correlated with FLN. The total unextractable large polymeric proteins (LUPP) were significantly positively correlated with FPC and FLY

and negatively correlated with the alveograph P/L ratio. The total large polymeric protein fraction (LPP) was significantly positively correlated with HLM, FLN and alveograph P/L ratio, and negatively correlated with FPC and BFLY. The total large monomeric protein fraction (LMP) was significantly positively correlated with FPC and BFLY, and negatively correlated with HLM, FLN and alveograph P/L ratio. The total SPP was significantly positively correlated with FPC and negatively correlated with SK weight, HLM and alveograph P/L ratio.

The total polymeric proteins (PP) were significantly positively correlated with SK hardness, HLM, FLN, alveograph P/L ratio and alveograph strength, and negatively correlated with SK diameter, FPC and BFLY. The total monomeric proteins (MP) were significantly positively correlated with FPC and BFLY, and negatively correlated with HLM, FLN and alveograph P/L ratio.

There were no significant correlations between total SMP and the quality characteristics.

Table 3.34. Correlations between quality characteristics year 1, 2 and combined for the two years (p<0.01).

Year 1 (2001)			Year 2 (2002)			Combined		
Sk-wght	Sk-diam	0.876	Sk-wght	Sk-diam	0.815	Sk-wght	Sk-diam	0.799
	Sk-hard	-0.542		Sk-hard	-0.584		Sk-hard	-0.539
	FLN	-0.406		FLN	-0.485		FLN	-0.363
	VK	-0.476		VK	-0.514		VK	-0.498
	BFLY	0.431		BFLY	0.468		BFLY	0.359
	Strength	-0.559		HLM	-0.352		FLY	-0.258
Sk-hard	HLM	0.836		P/L	-0.367		Strength	-0.636
	FLN	0.896		Strength	-0.727	Sk-diam	FLN	-0.331
	VK	0.666	Sk-diam	FLN	-0.362		Strength	-0.396
	BFLY	-0.943		Strength	-0.407	Sk-hard	Strength	0.848
	FLY	0.898	Sk-hard	HLM	0.907	HLM	FPC	-0.330
	P/L	0.726		FLN	0.685		FLN	0.763
	Strength	0.893		VK	0.893		VK	0.534
HLM	FLN	0.835		BFLY	-0.887		BFLY	-0.898
	VK	0.523		FLY	796		FLY	0.579
	BFLY	-0.841		P/L	0.722		P/L	0.739
	FLY	0.874		Strength	0.816		Strength	0.689
	P/L	0.512	HLM	FLN	0.677	FPC	P/L	-0.580
	Strength	0.738		VK	0.874	FLN	VK	0.355
FPC	P/L	-0.437		BFLY	-0.913		BFLY	-0.834
FLN	VK	0.511		FLY	0.874		FLY	0.606
	BFLY	-0.869		P/L	0.724		P/L	0.489
	FLY	0.898		Strength	0.703		Strength	0.690
	P/L	0.554	FLN	VK	0.599	VK	BFLY	-0.543
	Strength	0.838		BFLY	-0.755		FLY	0.572
BFLY	FLY	-0.872		FLY	0.615		P/L	0.370
	P/L	-0.594		Strength	0.590		Strength	0.692
	Strength	-0.850	VK	BFLY	-0.859	BFLY	FLY	-0.609
FLY	P/L	0.532		FLY	0.732		P/L	-0.680
	Strength	0.745		P/L	0.788		Strength	-0.789
P/L	Strength	0.709		Strength	0.590	FLY	Strength	0.569
			BFLY	FLY	-0.810		P/L	0.516
				P/L	-0.657			
				Strength	-0.793			
			FLY	P/L	0.481			
				Strength	0.570			
			P/L	Strength	0.556			

SK-wght = single kernel chracterization system weight, SK-diam = single kernel characterization system diameter, SK-hard = single kernel hardness, HLM = hectoliter mass, FPC = flour protein content, FLN = falling number, VK = vitreous kernels, BFLY = breakflour yield, FLY = flour yield, P/L = alveograph (P/L) ratio, Strength = alveograph strength

Correlations between quality characteristics for year 1, year 2 and combined

Data is given in Table 3.34.

2001: SK weight was significantly positively correlated with SK diameter and BFLY and significantly negatively correlated with SK hardness, FLN, VK and alveograph strength.

SK hardness was significantly positively correlated with HLM, FLN, VK, FLY, alveograph P/L ratio and alveograph strength. SK hardness was also significantly negatively correlated with BFLY.

HLM was significantly positively correlated with FLN, VK, FLY, alveograph P/L ratio and alveograph strength and negatively correlated with BFLY.

FPC was significantly negatively correlated with alveograph P/L ratio.

FLN was significantly positively correlated with VK, FLY, alveograph P/L ratio and alveograph strength and negatively correlated with BFLY.

BFLY was significantly negatively correlated with FLY, alveograph P/L ratio and alveograph strength.

FLY was significantly positively correlated with alveograph P/L ratio and alveograph strength.

The alveograph P/L ratio was significantly positively correlated with alveograph strength.

2002: SK weight was significantly positively correlated with SK diameter and BFLY and negatively correlated with SK hardness, FLN, VK, HLM, alveograph P/L ratio and alveograph strength.

SK diameter was significantly negatively correlated with FLN and alveograph strength.

SK hardness was significantly positively correlated with HLM, FLN, VK, FLY, alveograph P/L ratio and alveograph strength and negatively with BFLY.

HLM was significantly positively correlated with FLN, VK, FLY, alveograph P/L ratio and alveograph strength and negatively with BFLY.

FLN was significantly positively correlated with VK, FLY and alveograph strength and negatively with BFLY.

VK was significantly positively correlated with FLY, alveograph P/L ratio and alveograph strength and negatively with BFLY.

BFLY was significantly negatively correlated with FLY, alveograph P/L ratio and alveograph strength.

FLY was significantly positively correlated with alveograph P/L ratio and alveograph strength.

Alveograph P/L ratio was significantly positively correlated with alveograph strength.

Years 2001 and 2002 combined: SK weight was significantly positively correlated with SK diameter and BFLY and negatively with SK hardness, FLN, VK, FLY and alveograph strength.

SK diameter was significantly negatively correlated with FLN and alveograph strength.

SK hardness was significantly positively correlated with alveograph strength.

HLM was significantly positively correlated with FLN, VK, FLY, P/L ratio and alveograph strength and negatively with FPC and BFLY.

FPC was significantly negatively correlated with alveograph P/L ratio.

FLN was significantly positively correlated with VK, FLY, P/L ratio and alveograph strength and negatively with BFLY.

VK was significantly positively correlated with FLY, P/L ratio and alveograph strength and negatively with BFLY.

BFLY was significantly negatively correlated with FLY, P/L ratio and alveograph strength.

FLY was significantly positively correlated with P/L ratio and alveograph strength.

Table 3.35. Correlations between ratios and quality characteristics for 2001 and 2002 ($p<0.01$) (abbreviations as in Table 3.33).

LPP:LMP	HLM	0.514	LPP1:LPP2	HLM	-0.348	SPP1:LMP2	HLM	0.465	SMP1:LMP2	HLM	0.486
	FPC	-0.519		FPC	0.467		FPC	-0.459		FPC	-0.489
	FLN	0.426		BFLY	0.305		FLN	0.394		FLN	0.385
	BFLY	-0.497		FLY	0.381		BFLY	-0.447		BFLY	-0.440
	P/L	0.627		P/L	-0.559		P/L	0.550		P/L	0.567
LPP:SPP	HLM	0.512	LPP1:SPP2	Sk-hard	0.348	SPP1:SMP2	Sk-diam	-0.321	SMP1:SMP2	FPC	-0.342
	FPC	-0.450		HLM	0.429		P/L	0.388		BFLY	-0.310
	FLN	0.403		FLN	0.450	LMP1:SMP1	HLM	-0.354		P/L	0.465
	BFLY	-0.468		BFLY	-0.479		FPC	0.527	LPP2:SPP2	HLM	0.478
	P/L	0.586		P/L	0.410		FLN	-0.341		FPC	-0.436
LMP:SPP	Sk-diam	0.309		Strength	0.380		BFLY	0.349		FLN	0.383
	HLM	-0.365	LPP1:LMP2	Sk-hard	0.328		P/L	-0.477		BFLY	-0.436
	FPC	0.466		HLM	0.527	LMP1:LPP2	HLM	-0.503		P/L	0.552
	FLN	-0.362		FPC	-0.420		FPC	0.534	LPP2:LMP2	HLM	0.476
	BFLY	0.427		FLN	0.470		FLN	-0.427		FPC	-0.411
	P/L	-0.520		BFLY	-0.535		BFLY	0.516		FLN	0.377
	Strength	-0.335		P/L	0.568		P/L	-0.675		BFLY	-0.435
PP:MP	Skcs-diam	-0.323		Strength	0.383	LMP1:SPP2	P/L	0.300		P/L	0.495
	HLM	0.486	LPP1:SMP2	Sk-diam	-0.374	LMP1:LMP2	HLM	0.455	LPP2:SMP2	HLM	0.358
	FPC	-0.481		BFLY	-0.311		FPC	-0.359		FPC	-0.366
	FLN	0.434		P/L	0.318		FLN	0.349		FLN	0.326
	BFLY	-0.509		Strength	0.352		BFLY	-0.404		BFLY	-0.382
	P/L	0.620	SPP1:LMP1	HLM	0.378		P/L	0.483		P/L	0.508
	Strength	0.347		FPC	-0.490	LMP1:SMP2	P/L	0.269	SPP2:LMP2	HLM	0.467
LPP1:SPP1	FPC	0.329		FLN	0.387	SMP1:LPP2	Sk-diam	0.300		FPC	-0.419
	VK	0.346		BFLY	-0.427		HLM	-0.397		FLN	0.377
	FLY	0.516		P/L	0.549		FPC	0.333		BFLY	-0.447
	P/L	-0.336	SPP1:SMP1	Sk-diam	-0.305		FLN	-0.300		P/R	0.533
LPP1:LMP1	Sk-wght	-0.377	SPP1:LPP2	HLM	-0.478		BFLY	0.412			
	Sk-hard	0.446		FPC	0.506		P/L	-0.542			
	FLN	0.342		FLN	-0.362	SMP1:SPP2	HLM	0.408			
	BFLY	-0.374		BFLY	0.457		FPC	-0.507			

	FLY	0.373		P/L	-0.641		FLN	0.348			
	Strength	0.429	SPP1:SPP2	HLM	0.402		BFLY	-0.350			
LPP1:SMP1	Sk-wght	-0.340		FPC	-0.426		P/L	0.534			
	FPC	0.344		FLN	0.365						
	FLY	0.265		P/L	0.523						

Correlations between ratios of protein fractions and quality characteristics (2001 and 2002 combined)

The correlations between ratios and quality characteristics are given in Table 3.35.

The ratios LPP:LMP and LPP:SPP were significantly positively correlated with HLM, FLN and P/L ratio and negatively with FPC and BFLY.

LMP:SPP was significantly positively correlated with SK diameter, FPC and BFLY and negatively with HLM, FLN, P/L ratio and alveograph strength.

PP:MP was significantly positively correlated with HLM, FLN and alveograph P/L ratio and negatively with SK diameter, FPC and BFLY.

LPP1:SPP1 was significantly positively correlated with FPC, VK and FLY and negatively with alveograph P/L ratio.

LPP1:LMP1 was significantly positively correlated with SK hardness, FLN, FLY and alveograph strength and negatively with SK weight and BFLY.

LPP1:SMP1 was significantly positively correlated with FPC and FLY and negatively with SK weight.

LPP1:LPP2 was significantly positively correlated with FPC and BFLY and negatively with HLM and alveograph P/L ratio.

LPP1:SPP2 was significantly positively correlated with SK hardness, HLM, FLN, P/L ratio and alveograph strength and negatively with BFLY.

LPP1:LMP2 was significantly positively correlated with SK hardess, HLM, FLN, P/L ratio and alveograph strength and negatively with FPC and BFLY.

LPP1:SMP2 was significantly positively correlated with SK diameter, P/L ratio and alveograph strength and negatively with BFLY.

SPP1:LMP1 was significantly positively correlated with HLM, FLN and alveograph P/L ratio and negatively with FPC and BFLY.

SPP1:SMP1 was significantly negatively correlated with SK diameter.

SPP1:LPP2 was significantly positively correlated with FPC and BFLY and negatively with HLM, FLN and alveograph P/L ratio.

SPP1:SPP2 was significantly positively correlated with HLM, FLN and alveograph P/L ratio and negatively with FPC.

SPP1:LMP2 was significantly positively correlated with HLM, FLN and alveograph P/L ratio and negatively with FPC and BFLY.

SPP1:SMP2 was significantly positively correlated with alveograph P/L ratio and negatively with SK diameter.

LMP1:SMP1 and LMP1:LPP2 were significantly positively correlated with FPC and BFLY and negatively with HLM, FLN and alveograph P/L ratio.

LMP1:SPP2 was significantly positively correlated with alveograph P/L ratio.

LMP1:LMP2 was significantly positively correlated with HLM, FLN and alveograph P/L ratio and negatively with FPC and BFLY.

LMP1:SMP2 was significantly positively correlated with alveograph P/L ratio.

SMP1:LPP2 was significantly positively correlated with SK diameter, FPC and BFLY and negatively with HLM, FLN and alveograph P/L ratio.

SMP1:SPP2 and SMP1:LMP2 were significantly positively correlated with HLM, FLN and alveograph P/L ratio and negatively with FPC and BFLY.

SMP1:SMP2 was significantly positively correlated with alveograph P/L ratio and negatively with FPC and BFLY.

LPP2:SPP2, LPP2:LMP2 and LPP2:SMP2 were significantly positively correlated with HLM, FLN and alveograph P/L ratio and negatively with FPC and BFLY.

SPP2:LMP2 was significantly positively correlated with HLM, FLN and alveograph P/L ratio and negatively with FPC and BFLY.

3.5.2 Discussion

Wheat cultivar and nitrogen (N) applications influenced the amount and size-distribution of the monomeric and polymeric proteins differentiable with SE-HPLC. The hard wheat cultivars had significantly higher amounts of large polymeric proteins (SDS-soluble LPP, SDS-insoluble LPP and total LPP) than the soft wheat cultivars. For example, SST876 and Cracker had significantly higher SDS-soluble LPP and total LPP values than E94/8 and Snack. The SDS-insoluble LPP value of SST876 was also significantly higher than for E94/8 and Snack. From the above it is evident that the harder the grain, the higher the LPP fractions. However, only the SDS-soluble LPP fraction showed significant positive correlations with SK hardness.

There were no significant differences among the cultivars for SDS-soluble LPP – possibly because there were no significant differences between the N-treatments. P1 gave significantly the highest SDS-insoluble LPP of all the treatments and also a significantly higher total LPP than P2, P3 and P4. A higher total amount of N applied (e.g., P1 = 230 kg N/ha) is thus responsible for higher SDS-insoluble LPP and LPP values. This can explain why Snack P1 had a significantly higher SDS-insoluble LPP than Snack P3, P5 and P6 ; why E94/8 P1 had a higher SDS-insoluble LPP than P4 ; why Snack P4 had a higher SDS-insoluble LPP than P6 ; why the total LPP of Snack P1 was significantly higher than for P3 and P6 ; why Snack P4 had a significantly higher LPP than P6 ; and why the LPP of E94/8 P1 and P3 were significantly higher than for E94/8 P4.

SDS-soluble LPP was significantly positively correlated with SK hardness, FPC, VK, FLY and alveograph strength, and significantly negatively correlated with SK weight. Both SDS-insoluble LPP and total LPP were significantly positively correlated with HLM, FLN and alveograph P/L ratio and significantly negatively correlated with FPC and BFLY. The quality characteristics, HLM, FLN, VK, FLY, alveograph P/L ratio and alveograph strength are thus associated with hard wheat quality.

The hard wheat cultivars had higher amounts of small polymeric proteins (SDS-soluble SPP, SDS-insoluble SPP, and total SPP) than the soft wheat cultivars, although not significantly. SST876 and E94/8 had significantly higher SDS-soluble SPP values than Snack and the SDS-insoluble SPP values of Cracker were significantly higher than for E94/8. The fractions showed significant positive correlations with FPC (SDS-insoluble SPP and SPP), VK (SDS-insoluble SPP), HLM (SDS-soluble SPP), FLN (SDS-soluble SPP), FLY (SDS-insoluble SPP) and alveograph P/L ratio (SDS-soluble SPP), suggesting that the SPP fractions are associated with good bread-baking quality characteristics. Significant negative correlations were found between SDS-soluble SPP and SK diameter, FPC and BFLY; SDS-insoluble SPP and HLM and P/L ratio; and the total SPP and SK weight, HLM and P/L ratio. The negative correlations with SK diameter, SK weight and BFLY agrees with the above conclusion that these fractions are associated with good bread-making quality and weak soft wheat quality, but the negative correlations with HLM and P/L ratio indicate that the SPP fractions are also associated with weak bread-baking quality characteristics

There were no significant differences between the N-treatments for SDS-insoluble SPP and total SPP. Still, the following differences were visible among the cultivars: P5 gave a significantly higher SDS-soluble SPP than P2; E94/8 P5 had a significantly higher SDS-soluble SPP than P4; and Cracker P3 had a significantly higher SDS-insoluble SPP and SPP than P1 and P5.

The soft wheat cultivars had significantly higher amounts of large monomeric proteins (SDS-soluble LMP and total LMP) than the hard wheat cultivars. Both the SDS-soluble LMP and total LMP values of Snack and E94/8 were significantly higher than for SST876 and Cracker. The SDS-insoluble LMP value of E94/8 was also significantly higher than for Cracker. From the above it is clear that the harder the grain, the lower the LMP fractions – even though only the total LMP showed a significant negative correlation with SK hardness (Table 3.33). SDS-soluble LMP, SDS-insoluble LMP and total LMP were significantly positively correlated with FPC and BFLY, characteristics of soft wheat cultivars, and significantly negatively correlated with HLM, FLN and P/L ratio, characteristics associated with harder wheats.

There were significant differences between the N-treatments for the LMP fractions P6 gave significantly the lowest SDS-soluble LMP, indicating that higher total N-applications were responsible for higher SDS-soluble LMP values. E94/8 P1, P3 and P4 had significantly higher SDS-soluble LMP values than P6 and the SDS-soluble LMP of SST876 P1 was significantly higher than for P4 and P6. These results prove the above explanation to be true.

No constant pattern was found between the results of SDS-insoluble LMP and total LMP. The SDS-insoluble LMP of P1 was significantly lower than for P2, P3, P4 and P6; Snack P3 and P6 had significantly higher SDS-insoluble LMP values than P1 and P4; E94/8 P2 had a significantly higher SDS-insoluble LMP than P6; P3 had a significantly higher total LMP than P1 and P6; Snack P3 had a significantly higher total LMP than P1 and P4; Snack P6 had a significantly higher LMP than P1; and E94/8 P6 had significantly the lowest LMP of all the E94/8 treatments.

The hard wheat cultivars (Cracker and SST876) had significantly higher SDS-soluble SMP and total SMP values than the soft wheat cultivars (e.g., E94/8). The SDS-insoluble SMP fraction gave just the opposite results, namely, the soft wheat cultivars had significantly higher amounts of SDS-insoluble SMP

than the hard wheat cultivars. This is probably due to the results of 2001, because there were no differences between the SMP fractions during 2002.

SDS-soluble SMP showed significant positive correlations with characteristics associated with hard wheat quality (bread-baking), such as the alveograph P/L ratio. SDS-insoluble SMP was significantly positively correlated with SK diameter, FPC and BFLY, and negatively correlated with HLM, FLN, P/L ratio and alveograph strength. The SDS-insoluble SMP fraction was thus associated with soft wheat quality.

There were significant differences between the N-treatments for the SMP fractions P6 gave significantly the highest SDS-soluble SMP, and the SDS-soluble SMP of P4 was significantly higher than for P1 and P3. Thus, the lower the total amount of N applied (e.g., P6), the higher the SDS-soluble SMP fraction. This explains why Snack P6 and Cracker P6 had significantly the highest SDS-soluble SMP values of all the Snack and Cracker treatments, why E94/8 P4 and P6 had significantly higher SDS-soluble SMP values than P1, and why E94/8 P4 had a higher SDS-soluble SMP than P3.

As expected, the SDS-insoluble SMP fraction gave totally opposite results. Higher total N-applications were responsible for higher SDS-insoluble SMP values. The SDS-insoluble SMP of P2, P4 and P6 was significantly higher than for P5, and E94/8 P2 and P4 had significantly higher SDS-insoluble SMP values than P5.

Because of the opposite effects of the N-treatments on SDS-soluble SMP and SDS-insoluble SMP, the results of total SMP cannot be explained. P6 gave a significantly higher total SMP than P1, P3 and P5; P5 gave a significantly lower SMP than the rest of the treatments, but P3; the total SMP of Snack P6 was significantly higher than for P2 and P5; the SMP of E94/8 P5 was significantly the lowest of all the Snack treatments; and the SMP of E94/8 P1 was significantly lower than for P4.

The soft wheat cultivars had significantly higher amounts of total unextractable polymeric proteins (TUPP) than the hard wheat cultivars. The TUPP value of Snack was significantly the highest. TUPP was significantly negatively correlated with FLN, one of the quality characteristics associated with hard wheats. P1 gave a significantly higher TUPP than P3, P4, P5 and P6, which explains why SST876 P1 had a significantly higher TUPP than P4.

There were no significant differences between the cultivars for LUPP (large unextractable polymeric proteins). P1 gave significantly the highest LUPP of all the treatments, indicating that a higher total N-application by P1 (230 kg N/ha) would result in higher LUPP values. Snack P1 had a significantly higher LUPP than P3, P5 and P6; the LUPP of Snack P4 was higher than for Snack P6; and Cracker P1 had a significantly higher LUPP than P3 and P4. LUPP was significantly positively correlated with FPC and FLY and negatively correlated with the P/L ratio. This fraction showed characteristics of both hard and soft wheat cultivars.

The hard wheat cultivars (Cracker and SST876) posessed significantly higher amounts of polymeric proteins (PP) than the soft wheat cultivars (E94/8 and Snack). This can be explained by the significant positive correlation of PP with SK hardness. PP was also significantly positively correlated with HLM, FLN, P/L ratio and alveograph strength, the quality characteristics associated with hard wheat cultivars. PP was significantly negatively correlated with SK diameter, FPC and BFLY, illustrating a negative association with soft wheat cultivars. P1 gave a significantly higher PP than P2 and P4. Thus, the higher the total amount of N applied (e.g., P1), the higher the PP fraction. Snack P1 had a significantly higher PP than Snack P6, which agrees with the above conclusion.

The soft wheat cultivars had significantly higher amounts of monomeric proteins (MP) than the hard wheat cultivars. For example, Snack and E94/8 had significantly higher MP values than Cracker. The MP fraction was

significantly positively correlated with FPC and BFLY (associated with soft wheat cultivars), and negatively correlated with HLM, FLN and alveograph P/L ratio (associated with hard wheat cultivars).

N-treatments P4 and P6 were responsible for significantly higher MP values than P5. Snack P6 had a significantly higher MP than all the other Snack treatments, but P3; E94/8 P2 and P4 had significantly higher MP values than P5 and P6; and E94/8 P4 had a higher MP than E94/8 P1.

From the above results it is clear that cultivars Cracker and SST876 (the hard wheat cultivars) had the highest amounts of SDS-soluble LLP and SMP, SDS-insoluble LPP, total LPP and PP, while Snack and E94/8 (the soft wheat cultivars) had the lowest amounts of these fractions. Cultivars Cracker and SST876 had the lowest amounts of SDS-soluble LMP, SDS-insoluble SMP, TUPP, LMP and MP, and Snack and E94/8 the highest. It is obvious that higher amounts of large polymeric proteins and lower amounts of large monomeric proteins are characteristic of the hard wheats, while lower amounts of large polymeric proteins and higher amounts of large monomeric proteins are characteristic of the softer wheats. These results suggest a significant negative correlation between LPP and LMP.

According to Table 3.34, the cultivar effect was mostly responsible for the significant differences between the protein fractions (SDS-soluble LPP and LMP, TUPP, LPP, LMP and PP), while the N-treatments affected only the SDS-soluble LMP, SDS-soluble SMP and total SMP fractions. According to Wieser and Seilmeier (1998), the N-treatments were not expected to cause changes in the SMP fractions (albumins and globulins).

A higher total amount of N applied (e.g., P1 = 230 kg N/ha) was responsible for higher SDS-soluble LMP, SDS-insoluble LPP, SDS-insoluble SMP, TUPP, LUPP and LPP values. Lower total N-applications (e.g., P6) resulted in higher SDS-soluble SMP fractions. No constant pattern was found between the different N-treatments on SDS-insoluble LMP, total LMP, SPP, PP and MP.

Different levels of nitrogen (N) fertilisation are known to cause changes in flour protein quantities and proportions (Wieser & Seilmeier, 1998).

The timings of the N-applications (e.g., P1, P3 and P5 applied N during flag leave stage) did not have a visible effect on the protein fractions. The total amount of N applied during the growth season gave more significant results.

The polymeric protein fractions showed similar positive correlations with SK hardness (SDS-soluble LPP and PP), HLM (SDS-soluble SPP, SDS-insoluble LPP, total LPP and PP), FPC (SDS-soluble LPP, SDS-insoluble SPP, LUPP and SPP), FLN (SDS-soluble SPP, SDS-insoluble LPP, LPP and PP), VK (SDS-soluble LPP and SDS-insoluble SPP), FLY (SDS-soluble LPP, SDS-insoluble SPP and LUPP), P/L ratio (SDS-soluble SPP, SDS-insoluble LPP, LPP and PP) and alveograph strength (SDS-soluble LPP and PP). This shows the big influence of the polymeric proteins on the quality characteristics. Studies by Dachkevitch and Autran (1989) have indicated a direct correlation between the size and the amount of the glutenin polymers and quality characteristics. Higher amounts of polymeric proteins will thus have better quality as a result. The SDS-soluble and SDS-insoluble polymeric proteins were found to be equally important in quality prediction.

The monomeric proteins (LMP fractions and SDS-insoluble SMP) had a consistent negative effect on hard wheat quality. SDS-soluble LMP, SDS-insoluble LMP, SDS-insoluble SMP, total LMP and MP were significantly negatively correlated with SK hardness (only SDS-soluble LMP), HLM, FLN, P/L ratio and alveograph strength (only SDS-insoluble SMP).

The SDS-soluble LMP, SDS-insoluble LMP, SDS-insoluble SMP, total LMP and MP fractions were significantly positively correlated with SK diameter (only SDS-insoluble SMP), FPC and BFLY. These protein fractions and quality characteristics are associated with soft wheat quality. SDS-soluble SPP, SDS-insoluble LPP, total LPP and PP were significantly negatively correlated with SK diameter (only SDS-soluble SPP and PP), FPC and BFLY. SDS-soluble LPP

was significantly negatively correlated with SK weight. The polymeric protein fractions are thus associated with poor soft wheat quality.

All of the significant differences among the quality characteristics were due to the cultivar effect. Table 3.32 indicates that only the differences in FPC were partly due to the N-treatments.

The hard wheat cultivars had significantly higher SK hardness values than the soft wheat cultivars. Cracker was significantly harder than SST876, and SST876 was significantly harder than E94/8 and Snack. SK hardness was significantly positively correlated with alveograph strength, a quality characteristic associated with hard wheat cultivars. The SDS-soluble LPP and PP fractions were significantly positively correlated with SK hardness. There were a negative correlation between SDS-soluble LMP and SK hardness, proving that softer wheat cultivars have lower SK hardness values.

There were no significant differences between the N-treatments for SK hardness. The significant differences among the cultivars can be explained by higher total N-applications which resulted in harder wheat grains. Snack P1 was significantly harder than Snack P6; Cracker P1 was significantly harder than P5; SST876 P5 was significantly harder than P4 and P6; and SST876 P1 was significantly harder than P6.

The SK diameters of the soft wheat cultivars were significantly higher than for the hard wheat cultivars. E94/8 had a significantly higher SK diameter than SST876 and Cracker. The SDS-insoluble SMP fraction, positively associated with soft wheat cultivars, was significantly positively correlated with SK diameter. SK diameter was significantly negatively correlated with FLN and alveograph strength and also with the SDS-soluble SPP and PP fractions

There were no significant differences between the N-treatments for SK diameter. Snack P5 had a significantly higher SK diameter than P1 and P2; Snack P6 had a higher SK diameter than Snack P2, and the SK diameter of

Cracker P5 was significantly higher than for P1, P2, P3 and P4. A lower total amount of N applied by P5 and P6 (140 – 170 kg N/ha) resulted in higher amounts of the SDS-insoluble SMP fraction and thus also in higher SK diameters.

The soft wheat cultivars had significantly higher SK weight values than the hard wheat cultivars. E94/8 and Snack had significantly higher SK weights than SST876, and the SK weight of SST876 was significantly higher than for Cracker. SK weight was positively correlated with quality characteristics associated with soft wheat cultivars, for example, SK diameter and BFLY. It was negatively correlated with hard wheat quality characteristics, such as SK hardness, FLN, VK, FLY and alveograph strength. The significant negative correlations of SDS-soluble LPP and total SPP with SK weight emphasise the fact that harder wheats have lower SK weight values. The SDS-insoluble SMP fraction and SK diameter were positively correlated with SK weight.

Lower total amounts of N applied resulted in higher SDS-insoluble SMP fractions and higher SK diameters and should also be responsible for higher SK weight values. Most of the following differences among the cultivars prove this conclusion to be correct: Snack P2 had a significantly lower SK weight than Snack P3, P4, P5 and P6; the SK weight of Snack P5 was higher than for Snack P1; Cracker P5 had a significantly higher SK weight than P1, P2, P3 and P4; and SST876 P3 had a higher SK weight than P6.

The hard wheat cultivars had significantly higher hectoliter mass (HLM) values than the soft wheat cultivars. Thus, the harder the wheat cultivar, the higher the HLM value. For example, the HLM of SST876 and Cracker were significantly higher than for Snack and E94/8 and the HLM of Snack was significantly higher than for E94/8. The SDS-soluble LMP, SDS-insoluble LMP, SPP and SMP, total LMP, total SPP and MP fractions were significantly negatively correlated with HLM. Most of these fractions are associated with soft wheat cultivars. The SDS-soluble SPP, SDS-insoluble LPP, total LPP and

PP fractions were significantly positively correlated with HLM, proving that harder wheats posess higher HLM values.

HLM shared almost the same correlations as the SDS-soluble SPP, SDS-insoluble LPP, total LPP and PP fractions. It was significantly positively correlated with FLN, VK, FLY, P/L ratio and alveograph strength and negatively with FPC and BFLY. Test weight (HLM) may range from about 57.9 kg/hl (kilograms per hectoliter) for a poor wheat to about 82.4 kg/hl for a sound wheat (Atwell, 2001). All the cultivars showed HLM values between 76.5 and 80.7 kg/hl, proving that the wheats were sound.

Although there were no significant differences between the N-treatments for HLM, there were significant differences among the cultivars. SST876 P1 had a significantly higher HLM than P4 and P6 and the HLM of SST876 P3 was significantly higher than for P6. A higher total amount of N applied by P1 and P3 (200 – 230 kg N/ha) could be responsible for the higher HLM values.

The flour protein contents (FPC) of E94/8 and SST876 were significantly higher than for Cracker and Snack. Thus, there were no distinct differences between the hard and soft wheat cultivars for FPC. SDS-soluble LPP and LMP, SDS-insoluble SPP, LMP and SMP, LUPP, total SPP, LMP and MP were significantly positively correlated with FPC. SDS-soluble SPP and SMP, SDS-insoluble LPP, total LPP and PP were significantly negatively correlated with FPC. FPC was significantly negatively correlated with alveograph P/L ratio.

There were highly significant differences between the N-treatments for FPC. P6 gave significantly the lowest FPC and P3 gave a significantly higher FPC than all the other N-treatments, but P1. The FPC of Snack P2 was significantly higher than for P4, P5 and P6; Snack P3 gave a significantly higher FPC than P4 and P6 ; the FPC values of Cracker P1 and P3 were significantly higher than for P2, P4, P5 and P6; and the FPC values of E94/8 P3 and P5 were significantly higher than for P4 and P6. It seems as if higher total N-applications (e.g., P1) resulted in higher FPC values.

The hard wheat cultivars possessed significantly higher falling numbers (FLN) than the soft wheat cultivars. SST876 had significantly the highest FLN. The FLN of Cracker was also significantly higher than for Snack and E94/8. Protein fractions associated with harder wheats (SDS-soluble SPP, SDS-insoluble LPP, total LPP and PP) were significantly positively correlated with FLN. SDS-soluble LMP, SDS-insoluble LMP, SDS-insoluble SMP, TUPP, total LMP and MP fractions were significantly negatively correlated with FLN, indicating that these monomeric proteins are associated with soft wheat quality. FLN was significantly positively correlated with VK, FLY and alveograph P/L ratio and negatively with BFLY.

There were no significant differences between any of the N-treatments for FLN. The significant differences among the cultivars indicate that lower N-applications were responsible for lower FLN values. For example, the FLN of Cracker P6 was significantly lower than for all the other Cracker treatments and the FLN of E94/8 P4 was significantly higher than for P6.

The hard wheat cultivars had significantly higher VK (vitreous kernel) values than the soft wheat cultivars. Cracker had significantly the highest amount of VK, followed by SST876 with a significantly higher VK than Snack and E94/8. The higher VK values of the hard wheat cultivars can be explained by the significant positive correlations of SDS-soluble LPP and SDS-insoluble SPP fractions (fractions associated with hard wheat cultivars) with VK. VK was significantly positively correlated with FLY, P/L ratio and alveograph strength and negatively with BFLY.

The following significant differences among the cultivars were due to higher N-applications than resulted in higher VK values: the VK values of SST876 P1 and P2 were significantly higher than for P4 and P6; and the VK of SST876 P3 was higher than for P6.

The soft wheat cultivars had significantly higher breakflour yield (BFLY) values than the hard wheat cultivars. The BFLY of Snack was significantly

higher than for E94/8, and the BFLY of E94/8 was significantly higher than for Cracker and SST876. The SDS-soluble LMP, SDS-insoluble LMP, SDS-insoluble SMP, total LMP and MP fractions were significantly positively correlated with BFLY. The SDS-soluble SPP, SDS-insoluble LPP, total LPP and PP fractions were significantly negatively correlated with BFLY. BFLY was significantly negatively correlated with FLY, P/L ratio and alveograph strength, quality characteristics associated with hard wheat quality (bread-baking).

It seems as if a higher total N-application (e.g. P6 = 140 kg N/ha) was responsible for lower BFLY values (not always). For example, Snack P6 had a significantly higher BFLY than P1, P3 and P5; Snack P2 had a significantly higher BFLY than P3 and P5; Snack P4 had a significantly higher BFLY than P5; E94/8 P5 had a higher BFLY than P2; and SST876 P6 had a significantly higher BFLY than all the SST876 treatments.

The hard wheat cultivars had significantly higher flour yields (FLY) than the soft wheat cultivars, as could be expected from the significant negative correlation between FLY and BFLY. SST876 had significantly the highest FLY, followed by Cracker with a significantly higher FLY than Snack and E94/8. FLY was significantly positively correlated with the polymeric protein fractions (e.g., SDS-soluble LPP, SDS-insoluble SPP and LUPP), alveograph P/L ratio and alveograph strength, which are associated with hard wheat quality.

There were no significant differences among the cultivars for FLY – possibly because there were no significant differences between any of the N-treatments.

The combined data of 2001 and 2002 showed significantly higher P/L ratios for the hard wheat cultivars. This is in agreement with the separate results obtained for 2001 and 2002. Cracker had a significantly higher P/L ratio than the other entries, followed by SST876 with a significantly higher P/L ratio

than Snack and E94/8. In South Africa P/L ratios between 0.5 and 0.8 produce good quality bread, with 0.8 being the optimum (Mamuya, 2000). Cracker's average P/L ratio was 0.79, the P/L ratio of SST876 was 0.53 and the average P/L ratio of Snack and E94/8 was 0.44. The SDS-soluble SPP, SDS-soluble SMP, SDS-insoluble LPP, total LPP and PP fractions were significantly positively correlated with the P/L ratio. The SDS-soluble LMP, SDS-insoluble SPP, LMP and SMP, LUPP, total SPP, total LMP and MP were significantly negatively correlated with the P/L ratio.

There were no significant differences between any of the N-treatments for alveograph P/L ratio. Snack P5 had a significantly higher P/L ratio than P2 and P6; Cracker P6 had a significantly higher P/L ratio than P1, P2 and P3; and Cracker P4 and P5 had significantly higher P/L ratios than P3. The above differences among the cultivars indicate that lower total amounts of N applied by P4, P5 and P6 (140 – 170 kg N/ha) are responsible for higher P/L ratios.

The hard wheat cultivars (Cracker and SST876) had significantly higher alveograph strength values than the soft wheat cultivars (E94/8 and Snack). The SDS-soluble LPP and PP fractions were significantly positively correlated with alveograph strength, while the SDS-insoluble SMP fraction was significantly negatively correlated with alveograph strength.

Table 3.27 showed that N-treatments P1 and P3 gave the highest alveograph strengths, although not significantly. This proves why Cracker P3 ranked the highest of all the Cracker treatments for alveograph strength and why the alveograph strength of SST876 P3 was significantly higher than for P2 and P6. The higher alveograph strength values could be the result of higher total N-applications or due to later N-applications by P1, P3 and P5 (applied N during flag leave stage).

The relative amounts of the protein fractions showed enormous effects on the quality characteristics of the cultivars, but according to the literature, various

protein fraction ratios can also give information about a cultivar's quality. The values in Table 3.30 were used to calculate the ratios.

According to Huebner and Wall (1976), cultivars possessing good bread-making quality have high ratios of higher molecular weight (HMW) to lower molecular weight (LMW) glutenins. The LPP1:SPP1, LPP1:SPP2, LPP2:SPP1, LPP2:SPP2 and LPP:SPP ratios were calculated separately for the hard and soft wheat cultivars. The hard wheat cultivars had a higher LPP1:SPP1 ratio (0.31) than the soft wheat cultivars (0.26), a higher LPP1:SPP2 ratio (0.18) than the soft wheats (0.15), a higher LPP2:SPP1 ratio (1.51) than the soft wheats (1.37), a higher LPP2:SPP2 ratio (0.85) than the soft wheats (0.78), and a higher LPP:SPP ratio (0.66) than the soft wheat cultivars (0.59), indicating better bread-making quality. Table 3.35 showed significant positive correlations for LPP1:SPP1 with FPC, VK and FLY; for LPP1:SPP2 with SK hardness, HLM, FLN, P/L ratio and alveograph strength; and for LPP2:SPP1, LPP2:SPP2 and LPP:SPP with HLM, FLN and alveograph P/L ratio, proving that higher ratios are associated with better bread-making quality. Lower ratios would indicate lower quality for soft wheat products, as shown by the negative correlations of LPP1:SPP2 with BFLY; and of LPP2:SPP1, LPP2:SPP2 and LPP:SPP with FPC and BFLY.

Lafiandra et al. (2000) suggested that a higher glutenin/gliadin ratio would result in stronger doughs. Ten ratios were calculated to illustrate the glutenin/gliadin ratio. The hard wheat cultivars had a higher LPP1:LMP1 ratio (0.08) than the soft wheats (0.07), a higher SPP1:LMP1 ratio (0.27) than the soft wheats (0.26), a higher LPP1:LMP2 ratio (0.20) than the soft wheats (0.16), a higher SPP1:LMP2 ratio (0.63) than the soft wheats (0.59), a higher LPP2:LMP1 ratio (0.41) than the soft wheats (0.36), a higher SPP2:LMP1 ratio (0.48) ratio than the soft wheats (0.46), a higher LPP2:LMP2 ratio (0.96) than the soft wheats (0.81), a higher SPP2:LMP2 ratio (1.12) than the soft wheats (1.04), a higher SPP:LMP ratio (0.53) than the soft wheats (0.50) and a higher LPP:LMP ratio (0.35) than the soft wheats (0.29). From the above it is obvious

that the harder wheats had stronger doughs. In Table 3.37 all of the above ratios showed significant positive correlations with bread-baking quality characteristics. The ratios SPP1:LMP1, SPP1:LMP2, LPP2:LMP1, LPP2:LMP2, SPP2:LMP2 and LPP:LMP were all significantly positively correlated with HLM, FLN and alveograph P/L ratio. SPP:LMP was significantly positively correlated with HLM, FLN, P/L ratio and alveograph strength; LPP1:LMP1 was significantly positively correlated with SK hardness, FLN, FLY and alveograph strength; LPP1:LMP2 was significantly positively correlated with SK hardness, HLM, FLN, P/L ratio and alveograph strength; and SPP2:LMP1 was significantly positively correlated with the alveograph P/L ratio.

All of these ratios were negatively correlated with FPC, BFLY, SK weight (only LPP1:LMP1) and SK diameter (only SPP:LMP), indicating that lower ratios are associated with softer wheats.

According to the literature (He & Hoseney, 1990), the gluten from poor-quality flour has a higher solubility than the gluten from good-quality flour. The LPP1:LPP2, LMP1:LMP2 and SMP1:SMP2 ratios were calculated to illustrate the SDS-soluble:SDS-insoluble ratios for the hard and soft wheat cultivars. The hard wheat cultivars had a higher LPP1:LPP2 ratio (0.21) than the soft wheats (0.19), a higher LMP1:LMP2 ratio (2.32) than the soft wheats (2.28) and a higher SMP1:SMP2 ratio (1.63) than the soft wheat cultivars (1.43). The LMP1:LMP2 and SMP1:SMP2 ratios were significantly positively correlated with HLM (only LMP1:LMP2), FLN (only LMP1:LMP2) and alveograph P/L ratio and were negatively correlated with FPC and BFLY. The hard wheat cultivars were thus characterised by protein fractions with lower solubilities.

3.6 Conclusions

During the first year of this study (2001) some definite differences were observed between the hard and soft wheat cultivars. The hard wheat cultivars were characterised by higher amounts of SDS-soluble and SDS-insoluble polymeric protein fractions and lower amounts of monomeric proteins. The higher amounts of polymeric proteins (LPP and SPP) also awarded the harder cultivars with quality characteristics more suitable for the production of bread and crackers. For example, they showed higher hectoliter mass (HLM), flour yield (FLY), alveograph P/L ratio and alveograph strength values. Smaller SK diameters and lower SK weights were characteristic of harder wheats. SST876 showed high hectoliter mass (HLM), falling number (FLN) and flour yield (FLY) values, but the flour protein content (FPC), alveograph P/L ratio and alveograph strength values could have been higher. The flour protein content (FPC) of Cracker was relatively high and could lead to excessive product shrinkage and cracker toughness (Hoseney et al., 1988).

The soft wheat cultivars were characterised by lower amounts of polymeric proteins and higher amounts of monomeric proteins, especially SDS-soluble large monomeric and SDS-insoluble small monomeric proteins. Most of the quality parameters were negatively correlated with soft wheats and gave relatively low values. For example, the hectoliter mass (HLM), flour yield (FLY), alveograph P/L ratio and alveograph strength values were relatively low for the soft wheats. However, these low values are what make soft wheats suitable for the production of biscuits. Flours with low falling numbers (FLN), low amounts of vitreous kernels (VK) and high breakflour yields (BFLY) are essensial for the production of soft wheat products and were obtained by the soft wheat cultivars in this study.

There were six different nitrogen (N) treatments during 2001. The N-treatments P1, P2, and P3 applied a higher total amount of N during the growth season (200 – 230 kg N/ha), while N-treatments P4, P5 and P6 applied a relatively lower total amount of N (140 – 170 kg N/ha). These significant differences between the N-treatments were responsible for most of the

variations in protein fractions and quality parameters. Higher total amounts of N applied (e.g., P1) usually gave higher amounts of polymeric protein fractions (e.g., LPP and SPP) than lower total amounts of N. This in turn led to harder cultivars with higher hectoliter mass (HLM), falling number (FLN), vitreous kernels (VK), flour yield (FLY), alveograph P/L ratio and alveograph strength values. Opposite results were obtained from lower total amounts of N applied, namely, lower polymeric fractions, higher amounts of monomeric protein fractions (especially the smaller monomeric proteins) and higher SK diameter and SK weight values (positively associated with the soft wheat cultivars).

During N-treatments P1, P3 and P5, N was applied at a later stage (30kg N/ha during the flag leave stage). These later N applications did not have much of an effect on the different protein fractions or the quality characteristics. The only quality characteristics that were visibly influenced by the later N applications, were breakflour yield (BFLY) and its negative correlation with flour yield (FLY). For the later N-applications to have more significantly visible effects, it should probably be increased.

Year 2 (2002) showed almost the same results as year 1 (2001), however, not as clearly. The hard wheat cultivars were characterised by higher amounts of large polymeric proteins (SDS-soluble LPP, SDS-insoluble LPP, total LPP and PP) and lower amounts of monomeric proteins than the soft wheat cultivars. The higher amounts of large polymeric proteins resulted in harder grains with quality characteristics suitable for the production of breads and crackers. The hard wheat cultivars had significantly higher SK hardness, hectoliter mass (HLM), falling number (FLN), vitreous kernel (VK), alveograph P/L ratio and alveograph strength values than the soft wheat cultivars. They were also characterised by lower SK weight and BFLY values. A higher flour protein content (FPC) of SST876, could however result in higher bread-baking quality.

The soft wheat cultivars were characterised by lower amounts of polymeric proteins and higher amounts of monomeric proteins The hectoliter mass (HLM), falling number (FLN), vitreous kernel (VK), flour yield (FLY), alveograph P/L ratio and alveograph strength values were again negatively correlated with soft wheat cultivars and relatively low values were obtained for these quality parameters. The soft wheat cultivars had significantly higher SK weight and BFLY values than the hard wheat cultivars.

Seven different N-treatments were given during 2002. The N-treatments P1, P2, and P3 applied a higher total amount of N during the growth season (200 – 230 kg N/ha), while N-treatments P0, P4, P5 and P6 applied a relatively lower total amount of N (80 – 170 kg N/ha). The lower total amounts of N applied by P0 and P6 were responsible for higher SDS-soluble SMP, lower SDS-soluble LMP, and lower SDS-insoluble SMP and total SMP values.

It seems as if the lowest total amount of N applied by P0 (80 kg N/ha) and the highest total amount of N applied by P1 (230 kg N/ha), were usually responsible for the lowest SDS-soluble LPP and SPP, SDS-insoluble LMP and SPP, and total SPP and LMP fractions. Average N-applications applied by P3, P4, P5 and P6 (140 – 200 kg N/ha) were responsible for the highest SDS-soluble LPP and SPP, SDS-insoluble LMP and SPP, total SPP and LMP fractions (not always). The highest and lowest N-applications (P1 and P0) were responsible for the highest SDS-insoluble LPP, TUPP and LUPP values.

Higher total N-applications (e.g., P1) led to harder grains with higher flour protein content (FPC), falling number (FLN) and vitreous kernel (VK) values than low total N-applications. However, lower total N-applications were responsible for softer grains with higher SK weight, SK diameter and breakflour yield (BFLY) values. Lower N-applications also resulted in higher alveograph P/L ratios and flour yields (FLY).

The total amounts of N applied during the treatments had a more significant effect on protein fractions and quality characteristics than later N-applications (N applied during the flag leave stage) during treatments P1, P3 and P5.

In conclusion, the combined data of 2001 and 2002 showed the following results:

Cracker and SST876 (the hard wheat cultivars) had the highest amounts of SDS-soluble LLP and SMP, SDS-insoluble LPP, total LPP and PP, while Snack and E94/8 (the soft wheat cultivars) had the lowest amounts of these fractions. Cultivars Cracker and SST876 had the lowest amounts of SDS-soluble LMP, SDS-insoluble SMP, TUPP, LMP and MP, and Snack and E94/8 the highest. It is obvious that higher amounts of large polymeric proteins and lower amounts of large monomeric proteins are characteristic of the hard wheats, while lower amounts of large polymeric proteins and higher amounts of large monomeric proteins are characteristic of the softer wheats. The cultivar effect was mostly responsible for the significant differences between the protein fractions (SDS-soluble LPP and LMP, TUPP, LPP, LMP and PP), while the N-treatments affected only the SDS-soluble LMP, SDS-soluble SMP and total SMP fractions.

A higher total amount of N applied (e.g., P1 = 230 kg N/ha) was responsible for higher SDS-soluble LMP, SDS-insoluble LPP, SDS-insoluble SMP, TUPP, LUPP and LPP values. Lower total N-applications (e.g., P6) resulted in higher SDS-soluble SMP fractions.

The timings of the N-applications (e.g., P1, P3 and P5 applied N during flag leave stage) did not have a visible effect on the protein fractions. The total amount of N applied during the growth season gave more significant results.

The polymeric protein fractions showed positive correlations with SK hardness, hectoliter mass (HLM), flour protein content (FPC), falling number (FLN), vitreous kernels (VK), flour yield (FLY), alveograph P/L ratio and

alveograph strength. Higher amounts of polymeric proteins will thus have better quality as a result. The monomeric protein fractions (e.g., SDS-soluble LMP, SDS-insoluble LMP, SDS-insoluble SMP, total LMP and MP) had a consistently negative effect on these quality parameters. The monomeric proteins were significantly positively correlated with SK diameter, flour protein content (FPC) and breakflour yields (BFLY).

Higher total N-applications resulted in harder wheat grains with higher hectoliter mass (HLM), flour protein content (FPC), falling number (FLN), vitreous kernels (VK) and alveograph strength values. Lower total amounts of N applied resulted in higher SK diameters, SK weight, BFLY and alveograph P/L values.

Even though the different N-treatments affected the quality characteristics, the effect was relatively small compared to the effect of the cultivars.

The flour protein content (FPC) did not have a great impact on the protein fractions and quality characteristics. There were almost no correlations between FPC and the protein fractions and FPC and the other quality characteristics. Although the various N-treatments did have significants effects on the FPC value, they had a more visible and definite influence on the different protein fractions.

Cultivars possessing good bread-making quality (such as the hard wheat cultivars) had higher ratios of higher molecular weight (HMW) to lower molecular weight (LMW) glutenins, higher glutenin:gliadin ratios and less soluble gluten. The soft wheat cultivars, however, had lower HMW:LMW glutenin ratios, lower glutenin: gliadin ratios and more soluble gluten.

SE-HPLC could effectively distinguish between the hard and soft wheat cultivars, and was very usefull in determining effects of nitrogen treatments on polymeric and monomeric proteins, and their ratios.

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CHAPTER 4

Summary

Keywords: baking quality, monomeric proteins, polymeric proteins, SE-HPLC, wheat

- The primary aim of this study was to determine whether size-exclusion high performance liquid chromatography (SE-HPLC) could be used for quality prediction of South African wheat cultivars.
- A bread cultivar, a cracker cultivar and two soft wheat cultivars were used in this two-year experiment.
- Protein extracts of wheat flour were analysed by SE-HPLC.
- SDS was used for the first protein extraction step, and the rest of the proteins were extracted by sonication.
- The SE-HPLC method fractionated the storage proteins (both SDS-soluble and SDS-insoluble) into four distinct peaks of decreasing molecular size range, representing mainly larger polymeric proteins (mainly HMW-glutenins), smaller polymeric proteins (mainly LMW-glutenins), larger monomeric proteins (mainly gliadins), and smaller monomeric proteins (mainly albumins and globulins).
- The wheat samples were also analysed for a total of 11 important quality characteristics.
- Relationships between the amount and size-distribution of polymeric and monomeric proteins and flour quality properties were established.
- The influence of different nitrogen (N) treatments on protein fractions and quality characteristics was also determined by SE-HPLC.

- The results showed that both the genotype and N-treatments had a significant influence on quality characteristics.
- However, the genotype (cultivar effect) had a more significant influence on the protein fractions and quality characteristics than the N-treatments.
- The total amounts of N applied during the treatments had a more significant effect on protein fractions and quality characteristics than later N-applications (N applied during the flag leave stage).
- The SDS-soluble and SDS-insoluble polymeric proteins were found to be equally important in quality prediction.
- The hard wheat cultivars were characterised by an increase of polymeric protein as opposed to monomeric protein.
- The polymeric proteins (glutenins) were strongly positively correlated with dough strength properties, such as alveograph P/L ratio and alveograph strength.
- The large monomeric proteins, which are mainly gliadins, were positively associated with the soft wheat cultivars, and had a consistently significant negative effect on quality characteristics, such as falling number (FLN), vitreous kernels (VK), alveograph P/L ratio and alveograph strength.
- The large monomeric proteins showed positive correlations with single kernel weight (SK weight), single kernel diameter (SK diameter) and breakflour yield (BFLY).
- SE-HPLC provided a simple and an objective test for measuring the relative size-distributions of wheat storage proteins.
- SE-HPLC also proved to be very usefull in predicting quality.

Opsomming

Sleutelwoorde: bakkwaliteit, koring, monomeriese proteïene, polimeriese proteïene, SE-HPLC

- Die hoofdoel van die studie was om te bepaal of SE-HPLC aangewend kan word vir kwaliteitsbepaling van Suid-Afrikaanse koring kultivars.
- ‘n Broodkoring kultivar, ‘n kultivar vir cracker produksie en twee sagte koring kultivars is ontleed gedurende hierdie twee jaar eksperiment.
- SE-HPLC is gebruik om proteïen ekstrakte van die meel ontleed.
- SDS is gebruik tydens die eerste ekstraksie prosedure. Die SDS-onoplosbare proteïene was daarna met behulp van sonifisering geëkstraheer.
- The SE-HPLC metode het die bergingsproteïene (beide SDS-oplosbare en SDS-onoplosbare) in vier duidelike fraksies verdeel volgens hul molekulêre gewig. Die vier fraksies (of pieke) het bestaan uit groter polimeriese proteïene (hoofsaaklik hoë molekulêre gewig gluteniene), kleiner polimeriese proteïene (hoofsaaklik lae molekulêre gewig gluteniene), groter monomeriese proteïene (hoofsaaklik gliadiene) en kleiner monomeriese proteïene (hoofsaaklik albumiene en globuliene).
- Die koringmonsters is getoets vir 11 belangrike kwaliteitseienskappe.
- Die verwantskappe tussen die hoeveelhede en grootte-verspreiding van polimeriese en monomeriese proteïene en kwaliteitseienskappe is bepaal.
- Die invloed van verskillende stikstofbehandelings op die proteïenfraksies en kwaliteitseienskappe is vasgestel met behulp van SE-HPLC.

- Die resultate het getoon dat beide die genotipe en die verskillende stikstofbehandelings gelei het tot betekenisvolle verskille in die kwaliteitseienskappe.

- Die effek van die genotipe of kultivar op die proteïenfraksies en kwaliteitseienskappe was egter baie belangriker en duideliker as die effek van die verskillende stikstofbehandelings.

- Die totale stikstof-hoeveelhede wat toegedien is, het meer betekenisvolle resultate getoon as latere stikstoftoedienings.

- Die SDS-oplosbare en SDS-onoplosbare polimeriese proteïene was ewe belangrik in die voorspelling van koringkwaliteit.

- Die harde korings was gekenmerk deur 'n verhoogde polimeriese:monomeriese proteïen-verhouding.

- Die polimeriese proteïene (gluteniene) was sterk gekorreleer met deegsterkte en kwaliteitseienskappe soos alveogram P/L verhouding en alveogram sterkte.

- Die groter monomeriese proteïene (gliadiene) was positief geassosieer met die sagte kultivars en het betekenisvol negatief gekorreleer met kwaliteitseienskappe soos valgetal, glasagtige korrels, alveogram P/L verhouding en alveogram sterkte.

- Die groter monomeriese proteïene het positiewe korrelasies getoon met korrel-massa, korrel-deursnee en breekmeel.

- SE-HPLC was 'n relatief eenvoudige en objektiewe metode om die grootte-verspreiding van bergingsproteïene te bepaal.

- SE-HPLC was ook baie effektief vir die voorspelling van koringkwaliteit.