The influence of heat and cold stress on gluten protein and starch in wheat

By

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Declaration

I Oskar Elago here by declare that the work on which this dissertation is based is my original work (except where acknowledgement indicated otherwise) and that neither the whole work or any part of it has been, is being, or has to be submitted for another degree in this or any other university.

Signature……………………………………………………

Date…………………………………………………………..

Dedication

This work is dedicated to my beloved mother Linda Amunime Elago and my late father as well as the entire Elago family.

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LIST OF ABBREVIATIONS

Amylo = Amylose content

A-PAGE = Acid-PAGE

ACN = Acetonitrile

CO_2 = Carbon dioxide

Corr = Correlation

Diam = Single kernel characteristic system seed diameter

DNA = Deoxyribonucleic acid

DP = Degree of polymerization

DPA = Days after anthesis

DTT = Dithiothratol

ELISA = Enzyme-linked immunosorbent assay

ER = Endosplasmic reticulum

FPC= Flour protein content

GBSS = Granule-bound starch synthase

GPC = Grain protein content

Ha = Hectare

HI = Single kernel characteristic system hardness index

HMW = High molecular weight

HMW-GS = High molecular weight glutenin subunits

HPLC = High performance liquid chromatography

IEF = Gel isoelectric focusing

KHz = Kilo Hertz

LMP = Large monomeric proteins

LMP1= SDS-soluble, larger monomeric proteins;

LMP2= SDS-insoluble larger monomeric proteins,

LMW = Low molecular weight

LMW-GS = Low molecular weight glutenin subunits

LPP = Larger polymeric proteins

LPP1= SDS-soluble larger polymeric proteins

LPP2= SDS-insoluble larger polymeric proteins

mAU = Milli absorption units

mg = Milligram

ml = Milliliter

MP = Monomeric proteins

MW = Molecular weight

N = Nitrogen

N:P:K = Nitrogen: Phosphate: Potassium

NIR = Near-infrared reflectance spectroscopy

Nm = Nanometer

P= Peak

PAGE = Polyacrylamide gel electrophoresis

PDI = Protein disulfide isomerase

PP = Polymeric proteins

PVDF = Polyvinylidene difluoride

QTL = Quantitative trait loci

Ratio = Amylose:amylopectin ratio

RP-HPLC = Reversed-phase high performance liquid chromatography

RPM = Revolutions per minute

RVA = Rapid Visco Analyser

SDS = Sodium dodecyl sulphate

SE-HPLC = Size-exclusion high performance liquid chromatography

SGP = Starch granule proteins

SKCS = Single kernel characteristic system

SMP = Smaller monomeric proteins

SMP1 = SDS soluble smaller monomeric proteins

SMP2 = SDS insoluble smaller monomeric proteins

SPP = Smaller polymeric proteins

SPP1 = SDS-soluble smaller polymeric proteins

SPP2 = SDS-insoluble smaller polymeric proteins

TFA = Trifluoroacetic acid

TKW = Thermal kinetic window

TUPP = Total unextractable polymeric protein

Var = Variable

Wght = Single kernel characteristic system seed weight

μm = Micrometer

CHAPTER 1

General introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops of the world, and its baking quality is increasingly important since it defines the uses and marketability of wheat derived products (Poehlman and Sleper, 1995). Millions of tonnes of wheat are grown in the world each year, making it one of the most important crops. Much of this is consumed by humans, almost exclusively after processing into bread, pasta and noodles and a range of other foods (Poehlman and Sleper, 1995). Bread, in particular, occurs in a vast range of forms in different cultures. The ability of wheat flour to be processed into these foods is largely determined by the gluten proteins, which confer unique visco-elastic properties to doughs. Grain protein content (GPC) is a critical quality factor for wheat. Protein content and quality are necessary for making good bread products and premiums are paid to growers for increased grain protein content (Shewry and Miflin, 1985; Wrigley and Bietz, 1988; Schofield, 1994).

The end use quality of wheat is influenced by genetic make up and the environment in which it is grown. Both genotype and environment influence gluten strength and bread-making quality of wheat (Peterson et al., 1992). The genetic background for variation in gluten strength and bread-making quality has been studied and correlations have been established between particular proteins and protein subunits and bread-making quality (Johansson et al., 1993). The environment does not influence the specific composition of wheat grain proteins. Instead it influences protein concentration and the amount of different protein groups and amount and size distribution of polymeric proteins (Graybosch et al., 1996). Environmental influences can be of different types and various effects on proteins have been reported, depending on the type of environmental influence, e.g. variation in fertilizer rate influences the amounts of different protein groups while weather variations influence the amount and size distribution of polymeric

proteins (Graybosch et al., 1995; Zhu and Khan, 2001). In South Africa heat and cold stress occurs frequently during wheat production, and this influences quality. Many traits used as indicators of milling and baking quality have shown reasonable to high heritability in wheat (Bretten et al., 1962; Loefler and Busch, 1982), however, the end use quality of any wheat genotype can vary significantly over production environments (Haunold et al., 1962). Wheat quality is a complex term and depends on its suitability for a specific purpose (Finney et al., 1987). The diversity of protein content exists because the amino acids are arranged in different sequences with different lengths. Wheat proteins are unique because they provide the unique gas retaining quality in the dough (Kent and Evers, 1994). The storage proteins i.e. gliadin and glutenins have a significant role in baking quality due to their quantitative and qualitative characteristics (Finney and Yamazaki, 1967; Van Lill et al., 1993). The increase in protein content can improve the baking quality but mostly differences in improvement are reflected as a function of the qualitative nature of gluten composition. The relative proportions of major protein fractions influence the quality of wheat (Anjum, 1976).

Equally important is starch which is the major component in the endosperm of cereal grains (60–70% of grain dry weight) and it determines the structure of the baked products. Starch is composed of two types of glucose polymers, amylose and amylopectin. Amylose consists of straight chains and behaves as a linear polymer. Amylopectin contains the same straight chains but includes branches, producing a larger molecule (Robin et al., 1974). Granule size distribution of wheat starch is an important characteristic that can influence its chemical composition, which in turn may affect its functionality (Manners and Matheson, 1981; French, 1984). The suitability of wheat flour for processing into a wide variety of forms is determined to a large extent by the gluten proteins which confer unique visco-elastic properties on the doughs (Ewart, 1979; Payne, 1987).

In order to be able to breed for and grow wheat of high and consistent quality, it is important to know what the effect of cultivar and environmental variation is on protein characteristics. The objective of this study was to gain a better understanding of the influences of different temperature treatments on the gluten

proteins, quality characteristics, starch and amylose/ amylopectin ratios in order to better understand the genetically and environmentally determined biochemical variation in bread-making quality. A better understanding of this will increase the possibilities for growing and breeding wheat cultivars with a good and consistent quality.

CHAPTER 2

Literature review

Environmental conditions during grain-fill can affect the duration of storage protein accumulation and starch deposition, and thus play an important role in determining grain yield and flour quality of wheat (Stone and Nicolas, 1995; Moffatt et al., 1990; Blumenthal et al., 1991a;b). To understand the genetic basis for the effects of environment on the complex programme of grain-fill, high throughput transcript and protein profiling techniques must be utilized to identify the many genes and gene products integral to this developmental programme. The modifications of gene expression due to different environmental conditions are a common response in the metabolism of plant cells. Gene activation due to environmental stimuli plays an extremely important role in the adaptation of plants to unfavourable conditions (Krishnan et al., 1989; Blumenthal et al., 1990). A common stress situation is exposure to low temperatures. It is already known that under low temperature conditions plant cells modify several physiological parameters leading to improved cold resistance and that cold-treatment leads to a modification in gene expression. There is, for example, evidence from several plant species that low, nonfreezing temperatures promote the appearance of specific proteins. However, it is not understood how the transcription of a new set of genes can lead to improved cold tolerance (Noctor et al., 1998).

Programmes of gene expression within the developing wheat grain control the timing of biochemical and physiological processes, including cell division, water uptake, accumulation of starch and protein, maturation and desiccation (Senioniti et al., 1986; Mahan et al., 1987). Environmental conditions during grain development influence these processes in unique ways, resulting in changes in grain yield and flour quality that are of considerable importance to growers and end-users. High temperature during maturation and ripening is a major stress in many wheat production areas (Burke, 1990). Elevated temperatures are a major cause of yield and quality loss in cereal crops throughout many of the world's

cereal growing areas, including North America, India and France as well as Australia (Wardlaw et al.,1989). Despite the fact that wheat is grown as a winter season crop in southern Australia and South Africa, the occurrence of heat stress (maximum daily temperatures above 35ºC) is a frequent phenomenon during the growing season particularly during the grain filling period (Blumenthal et al.,1991a). Since wheat is a crop that is adapted to cool, moist growing conditions, and has an optimum temperature for grain growth of approximately 15ºC (Paulsen, 1994), it is not surprising that high temperature has been found to be one of the major environmental factors which limit both the quantity and quality of wheat production in Australia and South Africa.

High temperature stress is often accompanied by a number of other environmental stress factors, including water stress, high solar irradiance and wind. While the interactions of heat stress and these other stresses have generally received a reasonable amount of attention in the literature, the interaction of heat stress and nutritional stress (with the exception of nitrogen) seems to have received little attention (Hawker and Jenner, 1993).

Wheat is vulnerable to high temperature during most reproductive stages (Nicolas et al., 1984; Wardlaw et al., 1989; Tashiro and Wardlaw, 1990a;b), and kernel number, kernel weight, or both can be diminished. Comparisons between favourable and high-temperature field environments found greater than fourfold differences in wheat yield (Midmore et al., 1984; Shipler and Blum, 1986; Zhong-hu and Rajaram, 1994). These differences were much greater than yield reductions by high temperature under controlled conditions (Chowdhury and Wardlaw, 1978; Wardlaw et al., 1989).

In some growth chamber studies, reduced yields were attributed mostly to lower kernel weight and only to some extent to lower kernel number (Sofield et al., 1977; Chowdhury and Wardlaw, 1978; Wardlaw et al., 1989; Tashiro and Wardlaw, 1990a;b). The responses of these yield components to temperature varied with timing and duration of treatments and among cultivars. Temperatures as high as 35ºC decreased kernel number up to 22% and kernel weight by as

much as 38% (Wardlaw et al., 1989), and a greater increase in temperature from seven days after anthesis until ripeness will decrease kernel weight up to 85% (Tashiro and Wardlaw, 1989). High temperatures during kernel filling (10 days after anthesis until ripeness) decreased wheat yield by reducing kernel weight (Tashiro and Wardlaw, 1990a; Stone and Nicolas, 1994).

SE-HPLC

SE-HPLC (size exclusion high performance liquid chromatography) separates the proteins in four major classes or fractions, the high molecular weight glutenins (HMW-GS), low molecular weight glutenins (LWW-GS), gliadins and albumins/globulins (Larroque et al., 1997). This separation can be achieved within 20-30 minutes and analysis of the resulting curve is simple (Autran, 1994).

Another advantage of SE-HPLC is that it has the potential of keeping large aggregates in a quasi-native state (no disruption of S-S bonds), which allows the examination of stability of protein complexes, interactive aspects, and structure of unreduced aggregates (Autran, 1994).

Due to complexity and insolubility of endosperm proteins, one of the major problems in the past has been accomplishing complete protein extraction, without altering their chemical state. This problem was resolved by the introduction of sonication. Peterson et al. (1992) found that using an ultrasonic probe solubilizes total protein from small flour samples. This method allows complete extraction of proteins with loss of only the very large glutenin polymers, because they require very little energy to degrade. He found that after sonication, a strong correlation existed between the proportion of main peaks and absolute areas and the percentage of protein recovered.

SE-HPLC was not used for quality prediction, until recent years, when the technology became more advanced. An increase in the concentration of high molecular weight proteins are correlated with improved quality in wheat. Some results showed that a correlation exists between dough mixing time and the

amount of HMW-GS present, or the ratio of polymeric to monomeric proteins, indicating possible use in breeding (Huebner and Bietz, 1985). SE-HPLC also proved to be a useful tool in studying the influences of changes in agro-climatic conditions on quality. Wieser et al. (1990) found that the amount of protein aggregates remained stable, even though nitrogen levels changed. The opposite was apparent for other cultivars, indicating that SE-HPLC has the potential to evaluate the stability of quality in response to environmental changes.

RP-HPLC

Reverse phase high performance liquid chromatography (RP-HPLC) has been widely applied to cereal proteins and has proven to be a highly efficient tool for the qualitative and quantitative investigation and isolation of gliadin and glutenin subunits (Wieser et al., 1994). Reverse phase high performance liquid chromatography (RP-HPLC) is becoming the method of choice to analyze wheat proteins accurately (Bietz, 1990). Its fast, optimal, high resolution separations generally take about one hour, and lower-resolution separations, which are sometimes adequate, can be much faster. Bietz and Cobb (1985) examined the possibility of more rapid RP-HPLC separations of wheat proteins. Shorter columns, faster flow rates, and steeper gradients significantly reduced analysis time. RP-HPLC is also sensitive: only a fraction of a kernel needs to be analyzed, making it potentially non destructive of valuable germplasm. It has excellent reproducibility, and many samples can be automatically analyzed (Bietz, 1990). Data are generated in vast amounts, and stored in a computer for subsequent evaluation. Data are, however, too complex for thorough visual analysis; it is therefore necessary to use computer assisted statistical programmes to correlate protein compositional information with quality (Huebner and Bietz, 1994). Since RP-HPLC fractionates proteins by surface hydrophobicity instead of size or charge, it complements other chromatographic and electrophoretic methods (Lasztity, 1996). Wheat quality can and does vary greatly among samples of a single variety grown in different environments. The qualitative and quantitative composition of its proteins can directly influence wheat functionality and quality or serve as markers of other quality-associated factors (Huebner and Bietz, 1994).

Various components make up a modern HPLC system. A thorough understanding of all the components is essential in order to optimize system performance and thus obtain the best protein separation and quantitation possible (Marchylo, 1994). The pumps represent the heart of the HPLC system. It forces eluting solvent through the system at a relatively constant flow rate and pressure. Factors that can impair proper functioning of the pumps include air bubbles in the head, check valve problems and pump seals (Dolan, 1991). Reproducibility of RP-HPLC separations can be influenced by column temperature fluctuations (Marchylo, 1994). Many early RP-HPLC studies of wheat proteins were done with columns maintained at ambient temperature. Eventually it became obvious that constant temperature was necessary to maximize reproducibility (Bietz and Cobb, 1985). The ability to control column temperature also led to tests of the effect of temperature on resolution. For many cereal proteins, elevating column temperature considerably enhanced resolution.

Proteins eluted from the RP-HPLC column are usually detected with UV detectors. 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 of about 100 times greater than at 280nm. The suggested wavelength for detecting protein is 210 nm since it is a good compromise between detection sensitivity and potential interference (Burke et al., 1991).

RP-HPLC did not become a suitable technique for the separation of proteins until columns prepared with wide-pore, “end-capped” spherical silica supports were developed in the early 1980s. End-capping of silanols has improved and minimized non specific adsorption. Before 1980, most RP-HPLC separations used irregular particles with relatively small (60-A) pores which did not efficiently separate high molecular weight proteins. Only after the development of large pore (300-A) supports (Lewis et al., 1980) could good separations be performed with high weight proteins such as those in cereals. The availability of columns packed with such a support first enabled Bietz (1983) to separate cereal proteins by RP-HPLC.

The application of reverse phase high performance liquid chromatography (RP-HPLC) to the identification of wheat cultivars is well documented (Marchylo et al., 1988). The analysis of gliadins and glutenins by RP-HPLC may also be used for predicting wheat quality (Huebner and Bietz, 1985; 1986). Using RP-HPLC as a separation method, prefractionation of gluten proteins into gliadin and glutenin is necessitated, since different group (e.g, α-gliadins and LMW subunit of glutenin or ω-gliadins and HMW subunits of glutenins) can overlap in the elution profile (Wieser et al., 1990). Quantitation of individual or groups of storage protein components is an important facet of the RP-HPLC analysis procedure in both of these applications. Consequently, variation in the relative contributions of individual or groups of peaks due to environmental influence could affect cultivar identification or quality prediction procedures (Marchylo et al., 1990). In a study (Kruger and Marchylo, 1985); using the cultivar Neepawa, it was found that environment did not qualitatively influence the RP-HPLC elution profiles of gliadins and glutenins. Differences in relative peak heights were observed, in elution profiles of Neepawa samples grown under different environmental conditions. This suggested that environmental conditions could affect quantitatively the relative proportions of storage protein components. More recently, it was shown that wheat grown under different sulphur fertilization levels exhibit major quantitative variations in gliadin components (Marchylo et al., 1990). A RP-HPLC study of gliadins from wheat grown under different conditions (Lookhart and Pomeranz, 1985) showed that lack of sulphur in the soil affects synthesis of some gliadins, and possibly of glutenins. Later, using a different column and higher column temperature (60°C), Huebner and Bietz (1986) showed significant and major differences in ratios of amounts of specific gliadins in wheat grown on sulphur-deficient soil. When there is a shortage of soil sulphur, there is increased synthesis of ω-gliadins, which elute early and contain no sulphur. Huebner and Bietz, (1985) found that the relative area of a group of late-eluting peaks, expressed as a percentage of the total area of the RP-HPLC chromatogram, increased with increased evapotranspiration.

Generally, quantitative variation in protein composition among samples of a variety grown in normal environments appears relatively small, and does not interfere with RP-HPLC varietal identification. Several reports have applied RP-HPLC to quality prediction because of relationships of some specific peaks or chromatographic regions to quality characteristics (Huebner and Bietz, 1985; 1986). Huebner and Bietz (1986) used RP-HPLC to characterize gliadins from hard red spring (HRS) wheat varieties grown in a uniform nursery.

RP-HPLC is a particularly powerful separation technique. The linkage of certain gliadins with LMW-glutenin subunits does not allow for a definite role of each constituent to be firmly established at this stage (Lafiandra et al., 1999). Each locus encoding gliadin component displays several alleles and it is important to establish the direct role of allelic gliadin variant in affecting dough properties and particularly extensibility (Lafiandra et al., 1999).

Huebner and Bietz (1986) and also Wieser et al. (1989) showed that high and low molecular weight glutenin subunits can be separated based on neutral 70% ethanol. RP-HPLC, optimized for each fraction, may yield better results than for the entire mixture of subunits. Sutton et al. (1989) tested the ability of quantitative RP-HPLC of high molecular weight glutenin subunits to predict bread making quality in a group of New Zealand grown wheats. Two HMW glutenin subunit peaks were resolved with subsequent RP-HPLC. Loaf volumes and bake scores correlated significantly with areas of these two peaks. Glutenin’s LMW subunits are also closely linked to bread making quality (Gupta et al., 1991), as shown by electrophoresis. RP-HPLC has had limited application to this somewhat difficult separation problem: LMW glutenin subunits are numerous and homologous and more difficult to resolve upon RP-HPLC (Huebner and Bietz, 1994). Yet, Wieser et al. (1989) achieved superior separations of LMW glutenin subunits by RP-HPLC, so it is certain that RP-HPLC of these polypeptides can and will provide another dimension in predicting wheat quality.

Not only specific compositions of high and low molecular weight glutenin subunits but also the quantitative ratio of these two polypeptide types can be a useful

predictor of quality. Huebner and Bietz (1985) found that this ratio, as determined by RP-HPLC, was related to general quality of hard red spring wheats.

RP-HPLC is also becoming a useful tool for detecting genes introduced during breeding that affect quality. One example is wheat/rye translocation lines. These genotypes have beneficial traits (e.g. disease resistance), but may have weaker gluten and cause dough stickiness (Huebner and Bietz, 1994). Lookhart and Pomeranz (1985) showed that ω-gliadins indicative of the 1RS translocation can be readily identified by RP-HPLC by their unique elution characteristics.

Structure of the kernel

The kernel has a somewhat vaulted shape with the germ or embryo (the future plantlet) at one end, and a bundle of hairs, which is referred to as the beard or brush at the other end. The endosperm is rich in starch and contains the proteins that will form the gluten during dough making. The endosperm is surrounded by the fused pericarp and seed coat. The endosperm mainly contains food reserves, which are needed for the growth of the seedling (Atwell, 2001).

Like any seed, the wheat kernel is a complex structure with many individual components. However with respect to processing (i.e., milling) the wheat kernel is divided into three general anatomical regions. The outer protective layers of the kernel are collectively called the bran. The bran comprises about 14% of the kernel weight, and is high in fiber and ash (mineral) content. The germ, the embryonic wheat plant, comprises only about 3% of the kernel. Most of the lipids and many of the essential nutrients in the kernel are concentrated in the germ. The remaining inner portion of the kernel is the starchy or storage endosperm, which provides the energy and protein for the developing wheat plant.

Wheat is characterized by its high starch and moderately high protein content. The endosperm constitutes the major portion of all kernels and is the primary constituent of flour. Finally a single, highly specialized layer of endosperm cells forms a border between the starchy endosperm and the bran. This layer, called

the aleurone, is usually considered part of the endosperm, but it is biologically much more active and, subsequently, contains high enzyme activity. Because of its composition, activity, and location, it can exert a variety of negative effects on the acceptability of flour. Consequently, it is generally removed as part of the bran during most flour milling operations; in fact, millers consider the aleurone to be part of the bran.

Grain filling

The grain filling stage is mainly dominated by starch and protein synthesis. Rate and duration are the two variable components of grain filling that display genetic and environmental influences. According to Jenner et al. (1990) grain filling starts at about 10 to 15 days after anthesis and occupies the last 20 to 30 days of the grain's development until it ripens. The precursors for starch and protein synthesis (i.e. sucrose for starch and amino acids for proteins) are supplied by the rest of the plant and are transported into the grain in the phloem during grain filling (Jenner et al., 1990). According to Jenner (1970) the pool of precursors in the grain for starch synthesis is less than required for one day's grain filling at any point in time, whereas enough amino acid is present to provide for one to two day's protein synthesis (Ugalde and Jenner, 1990). The supply of these precursors to the grain that regulate the rate of deposition of dry matter differs for starch and protein (Jenner et al., 1990).

Most of the carbohydrate deposited in the grain is derived from CO_2, fixed during the grain filling period (Evans et al., 1975). The rate of starch deposition is influenced mainly by sink-limited factors i.e. the capacity of the grain to utilize the substrate (Jenner et al., 1990). Aproximately 35 days after anthesis, starch synthesis ceases (Kumar and Singh, 1980).

According to Sofield et al. (1977) protein is deposited slightly faster than starch. Assimilated nitrogen is stored throughout the plant, either as vacuolar nitrate or as protein. It is remobilized later to provide nitrogen for deposition of protein in the grain (Austin and Nair, 1963). The deposition of grain protein is mainly a

source-limited process (Jenner et al., 1990) i.e. an increase in nitrogen supply causes a direct increase in deposition of grain protein. Most nitrogen is absorbed as nitrate from the soil, where the bulk is transported to the leaves. Here it's transformed to glutamate (utilized in the synthesis of protein) in the chloroplast (Dalling, 1985). As the older leaves senesce, their protein is mobilized and utilized for protein synthesis in younger leaves (Leopold, 1980).

During stress periods such as drought (Spiertz and Van de Haar, 1978) and leaf senescence (Blacklow et al., 1984), limitations are placed to photosynthetic supply. During such periods, soluble carbohydrates in the internodes of wheat can be mobilized to sustain growth (Jenner et al., 1990).

Increased temperature leads to an increase in the senescence rate which may reduce the accumulation of carbohydrates more than the accumulation of nitrogen. The number and size of starch granules in the endosperm is also reduced (Tester et al., 1995). During grain filling higher temperatures reduce the duration of grain growth and limit the maximum size of the grain. As nitrogen translocation is less affected, crude protein concentration would be increased (Evans et al., 1975).

Genetic variation in tolerance to environmental stress

Temperature is one environmental variable that cannot easily be manipulated in the field, and therefore crops are often selected on the basis of their response to the temperature conditions of a particular region (Chowdhury and Wardlaw, 1978). Some degree of heat tolerance may therefore already exist in many common wheats, since selection for performance in warm environments will have screened out any genotypes susceptible to high temperature. Rawson (1986) demonstrated some of the genetic variation in heat tolerance that exists between wheat genotypes in their study of 40 cultivars sown under high temperature conditions. A reduction in several yield components was observed in a number of genotypes, including number of days to heading, tillers per plant, plant height,

spikelets per spike, grains per spike and grain per yield plant. However no such response was observed in other, more thermo tolerant, genotypes.

When assessing genotypic differences in tolerance to high temperature stress, consideration must be given to the developmental stage at which the heat stress was imposed, the duration of the heat stress and the criteria used for evaluating tolerance (Paulsen, 1994). If the stress is applied prior to anthesis, then heat tolerance may be associated with a high kernel number per spike, as found by Shipler and Blum (1991) in an observation of 21 wheat cultivars grown under hot, irrigated conditions.

Wheat proteins

Proteins are the key quality components of wheat grains, governing end-use quality (Weegels et al., 1996). Variation in both protein content and composition significantly modify quality for bread-making (Weegels et al., 1996; Lafiandra et al., 1999; Branlard et al., 2001). Although grain protein composition depends primarily on genotype, it is significantly affected by environmental factors and their interactions (Graybosch et al., 1996; Huebner et al., 1997; Zhu and Khan, 2001). The wheat proteins are synthesized during the fruit period of the plant. The amount and content of the proteins in the grain is influenced by the availability of nitrogen in the fruiting period. If the nutrients are low, the proteins reduce storage proteins to maintain the metabolic proteins. The weather conditions, the variety and the environment influence the protein content in the wheat grain. The protein concentration is known to play the most essential role in bread-making (Morrison, 1988; Wall, 1979). Breadmaking quality correlates with the presence or absence of specific proteins and protein subunits (Gupta et al., 1989; Johansson, 1996; Johansson et al., 1993; Payne et al., 1987a). In addition, the quality depends on the ratio of monomeric to polymeric proteins and amount and size distribution of polymeric proteins (Gupta and Shepherd, 1993; Johansson et al., 2001). Environmental conditions during grain fill influence the accumulation of protein in the developing wheat kernel and can alter the functional properties of the resulting flour, but the precise effects of

environmental factors on the synthesis of the major gliadins and glutenins are not well understood. Quantitative studies of gene expression and protein accumulation under different environmental conditions are challenging because the complexity of the different groups of genes and proteins makes it difficult to distinguish and identify single components. Additionally, levels of gene expression and protein accumulation must be examined within the context of grain development since environmental factors such as temperature can alter the timing of grain development (Graybosch et al., 1996).

All gluten proteins are synthesised on the endosplasmic reticulum (ER) and they all contain a signal peptide, which, through analogy with animal systems (Kreil, 1981), were found to direct the nascent chain into the lumen of the ER (Grimwade et al., 1996). Some wheat storage proteins appear to follow the secretory pathway from the ER over the Golgi apparatus to protein bodies and lose their integrity as the grain matures, forming a protein matrix in the mature dry tissue (Parker, 1980). However, other proteins accumulate in the ER and are incorporated in vacuole-like compartments that surround the protein bodies (Levanony et al., 1992). Beside that, specifically for wheat, protein in the vacuole-like compartments is compressed between the starch granules (Levanony et al., 1992; Shy et al., 2001). The precise mechanism of intracellular transport of storage proteins from their site of synthesis to their site of deposition is still uncertain (Grimwade et al., 1996). Protein folding, together with both inter and intra-chain disulfide bond formation, are considered to occur within the lumen of the ER, and may be assisted by molecular chaperones and by the enzyme protein disulfide isomerase (PDI) respectively (Roden et al., 1982; Shimoni et al., 1995). Many attempts to reveal the structure of the gluten proteins have been carried out, although they have been troubled by the low solubility and lack of crystallinity of the proteins. The solubility properties of gluten proteins are determined by the primary structures of the individual proteins and their interactions by non-covalent forces (notably hydrogen bonds and hydrophobic interactions) (Belton et al., 1998) and by covalent disulphide bonds (Shewry and Halford, 2002). The whole protein structure is still far from being clear (Veraverbeke and Delcour, 2002).

Mature wheat grains contain 8-20% proteins. The gluten proteins, the gliadins and glutenins, constitute up to 80-85% of total flour protein, and confer properties of elasticity and extensibility that are essential for functionality of wheat flours (Shewry et al., 1995). The gliadins and glutenins each constitute around 50% of the gluten proteins. The importance of gluten proteins in bread making quality is largely related to the capability of forming a viscoelastic network, i.e. the gluten complex, which is responsible for expansion during fermentation. The distinction of gluten proteins is based on solubility (two groups, gliadins and glutenins) or on the basis of amino acid composition and structure.

Observations of crop statistics have indicated that there is a general increase in dough strength, associated with the increase in grain protein content (Randall and Moss, 1990; Blumenthal et al., 1991b; Stone et al., 1997). As dough strength increases so too does bread making quality. High dough strength is associated with a long development time, a slow rate of breakdown and a high resistance to extension, while doughs with very short development times and low resistance to extension generally perform poorly in bread making (MacRichtie et al., 1990). A decline in dough strength has been found to occur in response to just a few days of maximum temperatures above 32ºC, despite the fact that protein content may continue to increase (Finney and Fryer, 1958; Blumenthal et al., 1991a; Wrigley et al., 1994). This weakening of dough properties occurs as a result of changes in protein composition associated with high temperatures during grain filling, with these doughs having lower extensibility than doughs from grains of similar protein content produced at lower temperatures (Stone et al., 1997).

Seed storage proteins in wheat can be broadly classified into four groups: the albumins, which are soluble in water; globulins, which are insoluble in water but soluble in dilute salt solutions; gliadins, which are insoluble in water and dilute salt solutions but are soluble in 70% ethanol; and glutenins, which are insoluble in 70% ethanol but are soluble in dilute acid or alkali solutions (MacRichtie et al.,1990). The gliadins and glutenins together form the gluten, which posses the unique visco-elastic properties of doughs produced from wheat flour. Glutenins

are further divided into high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits, based on their separation by polyacrylamide gel electrophoresis.

The ratio of gliadin to glutenin proteins in wheat is generally reported to increase in response to high temperatures during grain filling (Blumenthal et al., 1991a; 1993; Stone et al.,1997), and increases in gliadin: gluten ratios have been found to be influenced by both genotype and environment, particularly the timing of heat stress. Stone and Nicolas (1995) in a survey of 75 wheat genotypes, found that some varieties showed an increase in gliadin:glutenin ratios when heat treated early in grain filling and a decrease when exposed to heat stress later, while the opposite was found to occur in other varieties.

Some authors have suggested that the increase in the gliadin:glutenin ratios observed as a result of heat stress occurs because the accumulation of glutenin decreases more than the accumulation of gliadins (which also decrease) (Graybosch et al.,1995; Stone et al.,1997). This conclusion was based on the observation that gliadins increased as a proportion of total flour protein, but their accumulation on a per kernel basis was found to decrease in response to increased temperature. In general, gliadin accumulation within the wheat endosperm has been found to be less sensitive to heat stress during grain filling period than glutenin accumulation (Stone and Nicolas, 1996).

Blumenthal et al. (1994) offer another explanation for the loss of dough strength as a result of high temperature. They observed a decrease in the size of glutenin polymers in the mature grain in response to a heat stress treatment, and suggested that this may be due to the heat sensitivity of the enzymes involved in the formation of the disulphide isomerase. High temperatures therefore restrict the formation of the complex protein aggregates responsible for superior dough mixing properties (Corbellini et al., 1997).

The number and size of subunits within the glutenin polymer is also affected by high temperature stress, resulting in a decrease in grain quality. Huebner and

Wall (1976) were among the first to show that the ratio of HMW to LMW subunits of glutenin could be used to indicate baking quality. An increased proportion of HMW glutenin subunits results in a higher proportion of elastic high molecular weight polymers within the total amount of glutenin, which in turn produce flours with increased dough strength (Gupta and Shepherd, 1993). Heat stress has been found to restrict the synthesis of these HMW subunits of glutenin, with the resulting flours having weaker dough properties (Blumenthal et al., 1994; Wrigley, 1994; Corbellini et al., 1997). Stone and Nicolas (1996) found that the synthesis of the intermediates of sodium dodecyl sulphate (SDS)-soluble polymers (containing mostly LMW glutenin subunits) continued unimpeded during heat stress, while the synthesis of the intermediates of the SDS-insoluble polymers (containing mostly HMW glutenin subunits) was reduced, resulting in a reduction in the percentage of SDS- insoluble polymers in the total polymers.

It is now known that the HMW subunits of glutenin have both quantitative and qualitative effects on bread making quality, with variation in baking quality correlated with allelic variation in HMW subunit composition (Payne, 1987). The loci encoding the HMW glutenins are designated Glu-A1, Glu-D1 and Glu-B1 for their positions on the long arms of chromosomes 1A, 1B and 1D, respectively. Correlation studies have indicated that strong or weak dough properties are associated with the combination of specific HMW subunits. For example, the alleles of the D genome (encoding either the 5+ 10 subunit combination or the 2+ 12 unit combination) have been found to provide much contrast in bread making quality. There is a general tendency among Australian wheat varieties with the Glu-Dla allele (coding for sub units 2+12) to have a lower gliadin:glutenin ratio and stronger doughs than those with the Glu-D1d allele which tend to be more sensitive to the dough weakening effects of heat stress, when compared to those with the Glu-D1a allele (Panozzo and Eagles, 2000). Gupta et al. (1996) observed that cultivars with the Glu-D1d allele accumulate large polymers more quickly than those with the Glu-D1a allele, and it may be hypothesized that this gives these cultivars an advantage when protein deposition coincides with a period of high temperature stress. Many important physiological and biochemical processes in plants are impaired by heat stress, resulting in a decrease in

growth, yield and grain quality of crop plants. Each plant species has its own temperature range for optimal function, with temperatures outside of this optimum being inhibitory to cellular metabolism and plant growth (Burke, 1990). This species-specific temperature range has been referred to as the thermal kinetic window (TKW), and is defined as "the range of plant temperatures at which the apparent Michaelis constant, Km, is at or below 200% of the minimum observed value" (Burke et al., 1988). Temperatures above those of the TKW induce changes in a number of physiological processes in plants, including photosynthesis, membrane integrity and enzyme stability (MacRitchie et al., 1990).

Bread making quality

Quality is a complex term depending on many factors and can not be expressed in terms of a single property, but depends on several milling, chemical, baking and rheological characteristics. The end-use quality is the summation effect of soil, climate and genotype on the wheat plant and kernel components. The simplest definition of wheat quality is in terms of its suitability for a specific use (Finney et al., 1987; Faridi and Finley, 1989).

Wheat quality is influenced both by genotype and growth environment (Kent and Evers, 1994; Quail, 1996). The protein content varies widely due to the effect of genotypes, environment and their interaction (Peterson et al., 1992; Huebner et al., 1995).

In the evaluation of new bread wheat varieties for their bread baking potential, it is extremely important to separate two closely related factors i.e. quantity and quality of the protein in the grain. Quality is that property of flour proteins, which gives rise to different baking performances with flours of the same protein content (Bushuk et al., 1969).

Factors affecting wheat quality

Protein quality under normal growing conditions is controlled genetically, whereas protein quantity depends on soil and climatic conditions prevailing during different growth stages (Bushuk et al., 1969). Paliwal and Singh (1985) and Finney et al. (1987) have demonstrated that various physiochemical and functional properties of wheat flour have been prejudiced either by genotypes and/or by other non-genetic factors. Kent (1983) reported that protein content ranges from 6-21% between different wheat varieties and is affected more by edaphic factors like soil, fertilizers and climatic conditions than heredity. The farinographic properties are also affected by growth conditions. The weather and soil conditions also affect the protein contents and quality, which indirectly affect the shape of the curve of farinograms (Ibrahim and D' Appolonia, 1979).

McGuire and McNeal (1974) and Baenziger et al. (1985) reported that the variation in years or environments have shown significant differences in quality and functional properties of individual wheat varieties. Baker and Kosmolak (1977) reported that both cultivars and environment had large effects on all the quality parameters. They further observed that cultivars x environment interaction effects were important for some quality characteristics i.e. mixograph development time, falling number and remix loaf volume but were relatively unimportant for other characteristics i.e. flour protein, flour yield, grinding time and sedimentation values. The season was found to be considerably more important than location in affecting cookie baking potential of flours within a variety, however, significant location effects in certain varieties were also found (Yamazaki and Lamb, 1962).

Peterson et al. (1992) reported that genotype, environment and interaction effects were found to significantly affect variation in all quality parameters. Variances of quality characteristic associated with environmental affects were generally larger that those of genetic factors. They concluded that environmental influences on end use quality attributes should be an important consideration in all cultivar improvement efforts towards enhancing marketing quality of hard red winter wheat.

Bajwa et al. (1983) recorded that, with the exception of protein content, significant difference due to locations for grain yield, protein harvest and thousand grains weight were evident. Ijaz et al. (1994) studied some new Pakistani wheat varieties and observed significant variation in protein content, ranging from 11.9 to 13.2%. Butt et al. (1997) conducted studies on 30 wheat varieties for their physiochemical characteristics grown during the 1993 - 1994 and 1994 - 1995 crop years. The results showed that thousand grain weight and particle size index did not vary significantly between the years. Highly signification variation was observed among the wheat varieties for all the tested physical characteristics of grain. The interaction of cultivars with years for thousand kernel weight and particle size index was also found significant. He further reported significant variation in moisture content and crude fiber between the years. The interaction of cultivars with years also showed significant variation. Ijaz et al. (2001) reported significant variation for different physical, chemical and mixograph characteristics and observed that these are dependent on genotype besides the influence of environmental conditions.

Slaughter et al. (1992) studied 12 quality parameters of hard red spring and hard red winter wheat grown over three crop years (1987, 1988 and 1989). They found wide variation in protein and moisture contents due to difference in crop years. They showed that the mean protein contents were 11.9, 12.6 and 13.7% while the mean moisture contents were 12.1, 10.4 and 13.7% during the crop years 1987, 1988 and 1989 respectively.

Bushuk et al. (1969) used two sets of multi-factorial wheat trials to illustrate the complex relations between genetic and environmental influence on baking quality. They observed that new high protein wheat varieties can help to improve protein content with increased nitrogen fertilizer application. Protein quality was found to be closely related to variety and can be a partial substitute for high protein content.

The wheat germination markedly affects the functional properties, particularly bread making (Selvaraj et al., 1986), which is also true in cases where wheat is damaged by unseasonal rain at the time of harvesting and threshing operations. High alpha-amylase activity is obtained from the germinated grain, which produces sticky dough and low quality bread with poor crumb and crust characteristics (Ibrahim and D' Appolonia, 1979).

Toma and Moraru (1993) studied high quality and low quality wheat varieties and gluten forming proteins during germination. They pointed out that glutenins were broken down by the proteolytic enzymes. Thus the poor baking quality of germinated grain resulted from the breakdown of protein. The high quality wheat showed a slower breakdown of glutenins into soluble form than did the low quality ones.

Subda (1991) reported that grain protein content in seven spring varieties and 12 varieties and two lines of winter habit depended to a great extent on variety, soil and climatic conditions. There was little variation in protein fractions soluble in acetic acid and SDS compared to those with poor quality. Results suggested that high molecular weight glutenins were responsible for good baking quality and proteins soluble in acetic acid and SDS, for poor baking quality.

Midmore et al. (1984) studied the effect of fertilizers on winter wheat varieties during three consecutive growing seasons (1987 - 1990) without NPK, with 308kg NPK/ha 89.8 - 225kg NPK/ha, using N:P:K ratio of 1:0.2:0.8. The average composition of the grain protein was 22.3% albumins and globulins, 34.3%, gliadins and 29.9% glutenins and 13% insoluble residue. However, NPK fertilizer showed significant effects for all protein fractions and for all growing seasons and remained significant when data from all seasons were pooled.

Tahir and Nakata (2005) reported that the decreased nitrogen application decreased the grain protein 1% nearly by and reduced loaf volume dough properties and Zeleny sedimentation values, but showed no effect on falling number, hectoliter weight or flour extraction.

Sato (1991) reported variation in the protein content of wheat grains due to location between 9 and 12%. Protein content also varied with cultivars. He further stated that the protein content was linearly related to the applied nitrogen and decreased with increased rate of applied phosphorous. Application of sulphur also increased the protein content.

Gliadins

Gliadins are heterogeneous mixtures of single-chained polypeptides which are, in their native state, soluble in 70% aqueous alcohol. In accordance with their mobility in A-PAGE (acid-PAGE), they are divided into four groups which are alpha-, beta-, gamma-, and omega-gliadins. The amino acid compositions of the alpha-, beta-, and gamma-gliadins are similar to each other and to that of the whole gliadin fraction (Tatham et al., 1990). The omega-gliadins contain little or no cysteine or methionine and only small amounts of basic amino acids. All gliadins are monomers with either no disulphide bonds (omega-gliadins) or intrachain disulphide bonds (alpha-, beta-, and gamma-gliadins). Although no complete sequences of omega-gliadins have been determined, Kasarda et al. (1983) purified a number of individual components from bread and pasta wheats and determined their relative molecular weights by sodium dodecyl sulphate polyacrylamide gel electrophoresis; and the molecular weights fell between 44000 and 74000, with most above 50000. The alpha-, beta-, and gamma-gliadins have lower molecular weights, ranging between about 30000 and 45000 by SDS-PAGE and by amino acid sequencing. The latter approach has shown that the alpha- and beta-gliadins are closely related, and both are now usually referred to as alpha-type (in contrast to the gamma-type) gliadins.

Most α- type gliadins contain six cysteine residues. Because of the monomeric character of α-type gliadins, and the absence of free sulphydryl groups, it has been assumed that the cysteine residues are linked by three intra-molecular disulphide bonds (Kasarda et al., 1987). The γ-type gliadins are single monomeric proteins with intra-chain disulphide bonds and are considered to be

the ancestral type of the S-rich prolamins (Shewry et al., 1986). Complete amino acid sequences of several γ-gliadins have been deduced from genomic and cDNA sequences (Okita et al., 1985). These sequences showed a clear domain structure, with a nonrepetitive sequence of 14 residues at the N-terminus, an N-terminal repetitive domain based on a heptapeptide repeat motif (consensus Pro Gln Gln Pro Phe Pro Gln) and a non-repetitive C-terminal domain which contained all the cysteine residues. Structural studies, using circular dichroism and structure prediction, indicated that the two domains adopt different conformations. While the repetitive domain adopts a β-reverse turn rich conformation, the non-repetitive domain is rich in α-helix (Tatham et al., 1990). In dough formation, the gliadins are thought not to become covalently-linked into large elastic networks as the glutenins but act as a 'plasticiser', promoting viscous flow and extensibility which are important rheological characteristics of dough. They may interact through hydrophobic interactions and hydrogen bonds (Belton, 1999).

Glutenins

Glutenins are heterogeneous mixtures of proteins comprising subunits linked by disulphide bonds. Although all wheat seed storage proteins are part of the gluten, the glutenin polymers are considered as the most important determinants of its viscoelastic property (Gupta et al., 1992). These proteins belong to a special class of oligomeric secretory proteins, like viral glycoproteins, which upon sequestration into the endoplasmic reticulum (ER) assemble by noncovalent interactions and intermolecular disulfide bonds (Levanony et al., 1992). In fact, the glutenins form the largest protein polymers known to occur in nature, reaching sizes as large as 11 million Daltons (Lew et al., 1992; MacRitchie et al., 1991; Khatkar et al., 2002). These polymers possess a highly elastic structure, similar to elastin and kitin. By convention, the glutenins are divided into two groups: high molecular weight subunits (HMW-GS) of about 80–100 kDa and low molecular weight subunits of about 30–40 kDa.

The HMW-GS are considered the most important determinants of the structure of these polymers. The HMW-GS consist of nonrepetitive domains of 88-104 and

42 residues at the N- and C-termini, respectively, separated by a longer repetitive domain (481-690 residues). Variation in the repetitive domain is responsible for most of the variation in the size of the whole protein, and it is based on random and interspersed repeats of hexapeptide and nonapeptide motifs, with tripeptides also present in x-type subunits only (Jiang et al., 2001; Shy et al., 2001; Chrispeels and Herman, 2000). Structure prediction indicated that the N- and C terminal domains are predominantly α-helical, while the repetitive domains are rich in β-turns (Shewry and Tatham, 1989). Many partial and full-length sequences of HMW-GS and LMW-GS have been determined (Shewry and Tatham, 1997). Despite the high degree of similarity in general structures and amino acid sequences of x- and y- type HMW-GS, some important differences are potentially critical for the structure and functionality of glutenin polymers (Shewry et al., 1992). There are three differences in: 1) molecular weight (x-type are bigger then y-type) due to a difference in length of the central repetitive domain; 2) the repeat structures in central domain; 3) the number and distribution of cysteine residues (Shewry et al., 1992).

Wheat starch

Starch is also a major determinant of yield, accounting for 65–75% of the grain dry weight and up to 80% of the endosperm dry weight (Rahman et al., 2000; Slattery et al., 2000). It is found in the endosperm in the form of discrete granules and consists of two carbohydrate polymers, amylose and amylopectin. The functional properties of starch, particularly the ability of starch or flour to take up water and form a paste in the presence of heat, are affected by variations in the proportions of amylose to amylopectin and in the size distribution of starch granules. Variations in starch composition and paste viscosity have been reported among Australian wheat cultivars (Moss, 1967; Moss and Miskelly, 1984; Lee et al., 1987). Starch quality is extremely important in producing marketable wheat for many end uses. Wheat varieties with high paste viscosity produce Japanese and Korean white salted noodles with good texture (Oda et al., 1980; Lee et al., 1987). Rapid gelatinization of starch affects the softness of Cantonese style noodles (Miskelly and Moss, 1985), is desirable for instant

noodle manufacture (Moss, 1983), and also for Japanese Udon noodles (Oda et al., 1980). Starch quality is important in Arabic bread production (Quail et al., 1990) and Chinese steamed bread (Huang et al., 1994). Wheat flour is also used for the commercial production of starch which has applications in food use as a thickener, gelling agent and fermentation substrate and as an industrial raw material for the production of paper, textiles and coatings (Simmonds, 1989).

A series of enzymes synthesize the amylose and amylopectin chains that comprise starch (Rahman et al., 2000; Ball et al., 1998). Within the amyloplast, ADP-glucose pyrophosphorylase converts glucose 1-phosphate to ADP-glucose, which is then converted into amylose and amylopectin polymers by starch synthases and branching enzymes. The starch polymers form layered granules within the amyloplasts. Large type A granules are initiated about 4–7 days after anthesis (DPA), and smaller type B granules appear around 10–12 DPA (Bechtel et al., 1990; Parker, 1985; Buleon et al., 1998; Peng et al., 1999; Langeveld et al., 2000). Many of the genes that encode enzymes required for starch biosynthesis have been sequenced (McCue et al., 2002; Murai et al., 1999; Nair et al., 1997; Vrinten and Nakamura, 2000; Li et al., 2000) and there are a few studies on transcriptional and post-transcriptional regulation of these genes in cereal grains (Morell et al., 1997). Some information is available on interactions between transcription factors and promoter binding sites of genes encoding starch biosynthetic enzymes in barley and maize endosperm (Zentella and Yamauchi, 2002; Kim and Guiltinan, 1999). Post-translational regulation of the starch biosynthetic enzymes has been shown to be important in chloroplasts and in potato tuber amyloplasts (Tiessen et al., 2002., Neuhaus and Emes, 2000). Much work remains to understand how starch biosynthesis and granule formation, size, and number are regulated in the wheat endosperm.

Wheat starch with a high amylose content and wheat flour containing such wheat starch are expected to provide new industrial and food applications. Therefore, attempts have been made to produce wheat starch with increased amylose content using crossbreeding and genetic engineering approaches. Amylose is an α(1,4)-linked glucose polymer which is essentially a linear chain without

branching. Amylopectin is a branched glucose polymer in which branch chains are linked to the main chain of α(1,4)-linked polymer by α(1,6)-linkages. The linear glucose polymers are synthesized by the action of starch synthases which produce α(1,4)-linkages. The α(1,6)-linkages of amylopectin are produced by the action of branching enzymes (Peng et al., 1999).

Studies in pea, maize, and wheat (Echt and Schwartz, 1981; Mu et al., 1994; Denyer et al., 1995) have shown that some enzymes for starch synthesis are tightly bound to starch granules from seed endosperms of maize and wheat and pea embryo.

The detailed mechanism for the binding of these enzymes to starch granules is unknown. However, it is believed that in wheat, at least four kinds of proteins, i.e., waxy protein and three starch granule proteins (SGP-1, SGP-2, SGP-3), are tightly bound to starch granules and are responsible for starch synthesis. Waxy protein, i.e., granule-bound starch synthase I (GBSS I) responsible for amylose synthesis, is the product of the waxy gene (Ainsworth et al., 1993). SGP-1, -2 and -3 correspond to starch granule-bound isozymes of about 100-105 kDa, about 90 kDa and about 77 kDa, respectively, reported by Denyer et al. (1995). Immunoblotting, amino acid sequencing and detection of starch synthase or branching activities (Denyer et al., 1995) suggest that SGP-2 is a homolog of maize branching enzyme IIb and that SGP-3 is a homolog of maize starch synthase I (Knight et al., 1998).

Immunoblotting studies on about 100-105 kDa protein (SGP-1) did not detect the protein in the soluble fraction. Thus, SGP-1 is exclusively bound to starch granules. This protein is presumed to be a starch synthase from the studies of antiserum recognition, enzymatic activity detected and homology in amino acid sequences (Denyer et al., 1995). However, information regarding the physiological function of SGP-1 in vivo has been limited. For maize, it has been reported that an apparent amylose content is increased in a mutant of dull 1 gene which is presumed to code for starch synthase II (Gao et al., 1998). However, there is no substantial amino acid sequence homology between the protein

coded by dull 1 (Gao et al., 1998) and the protein SGP-1 of wheat. Further, the protein coded by dull 1 is present in the soluble fraction. Thus, the starch synthase encoded by dull 1 is significantly different from SGP-1.

A hexaploid wheat has three isozymes of SGP-1, i.e., SGP-A1, SGP-B1 and SGP-D1. The gene coding for SGP-A1, SGP-A1, is located on chromosome arm 7A, SGP-B1 on 7B, and SGP-D1 on 7D (Denyer et al., 1995). Using SDS-polyacrylamide gel electrophoresis (SDS-PAGE), it has been found that a few wheat cultivars lacked either SGP-A1, -B1 or -D1, but no wheat cultivars lacked two or more SGP-1s.

Amylopectin

Amylopectin is a very large molecule with a molecular weight of 10^7 to 10^8, which consist of α-1,6 linked α-1,4 glucan chains. Four to six percent of the glycosidic linkages are of the α-1,6 type. The branched structure has been defined as consisting of three classes of chains termed A, B and C (Peat et al., 1952). A-chains are linked to inner B-chains and have no glycosidically linked chains. B-chains have glycosidically linked A-chains and B-chains and are themselves linked to other B-chains or a C-chain. There is only one C-chain in one amylopectin molecule, which thus has the sole reducing end of the amylopectin molecule.

Debranched amylopectins display a polymodal distribution of chain lengths consisting of overlapping distribution curves contrasting to the unimodal size distribution of chains observed in glycogen. Polymodal size distribution has been assumed to contain discrete normally distributed size fractions of chains. The interpreted classification, number of peaks and size distribution around peaks as well as chain length at peaks, differ to some extent depending on the source of starch and experimental conditions used. The similarities are more important than the differences though. The polymodal chain length distribution can be resolved into a short chain fraction of degree of polymerization (DP) 12 to 17 depending on species, a largely overlapping fraction with a peak at DP 18 to 24,

a third peak at DP 40 to 50 and a fourth at around DP 70 (Hizukuri, 1986; Blennow et al., 2000). Generally there is a periodicity to the peaks and an amylopectin with a peak at the lower end of the size range consistently are at the lower end of the range for the other peaks as well. This indicates that even though peaks are overlapping considerably there is a non-random nature to the molar distribution of chain lengths in amylopectin.

Amylose

The amylose content of most starches is 20 – 30%. However, certain mutant plants, commonly called waxy because of the waxy appearance of the seed endosperm, have a much lower amylose content or even lack the amylose component completely. Other types of mutant plants show increased amylose content (Bertoft, 2004). Amylose is mainly found as linear chains of about 2000 to 12000 units of α-glucopyranosyl residues linked by α-D-(1,4) glucosidic linkages (molecular weight around 0.3 to 2.0 x 10^6 Da). Its molecular size varies with plant species, stage of development and processing conditions employed in extracting the starch. Some of the amylose molecules are branched to a small extent at O-6 (α-1,6-D glucopyranose; one per 170 to 500 glucosyl units). About 0.3-0.5% of the total linkages are branch points (α1,6) (Blennow, 2004; Preiss, 2004)

At one end of the polymeric glucan chain, the anhydroglucose unit contains one primary and two secondary hydroxyls as well as an aldehydic reducing group in the form of a inner hemiacetal. This is called the reducing end of the molecule. The opposite end is called the nonreducing end. The abundance of hydroxyl groups imports hydrophilic properties to the polymer, giving it an affinity for moisture and dispersibility in water. However, because of their linearity, mobility and the presence of many hydroxyl groups, amylose polymers have a tendency to orient themselves in a parallel fashion and approach each other closely enough to permit hydrogen bonding between hydroxyl groups of adjacent polymers. As a result, the affinity of the polymer for water is reduced. In dilute solutions, the aggregate size of the associated polymers may increase to a point where precipitation may occur. At higher concentrations, steric hindrance may

interfere, so only partial orientation between segments of the polymers may occur, producing a gel consisting of a three-dimensional network held together by hydrogen at those sections where close alignment has occurred (Slaughter et al., 1992; Blennow, 2004).

CHAPTER 3

The influence of extreme temperature on proteins as measured by size exclusion high performance liquid chromatography

Introduction

Extreme temperatures during grain filling have been identified as a major source of variation in wheat quality characteristics. Reduced grain weight and modification of rheological properties are commonly reported as the consequences of heat stress on wheat. Another common stress situation is exposure to low temperatures.

It is already known that under low temperature conditions, plant cells modify several physiological parameters leading to improved cold resistance and that cold treatment leads to a modification in gene expression (Tipples,1980). There is, for example, evidence from several plant species that low, nonfreezing temperatures promote the appearance of specific proteins. Cold stress and immaturity are often closely related, since kernel immaturity may occur as a result of a freeze event. Immaturity is characterized by green or grass green kernels due to incomplete kernel maturation. The effect of cold stress on end use quality is dependent on the stage of crop maturity as well as the duration and severity of the freeze event (Dexter and Edwards, 1997; Preston et al., 1991). Frost during early filling affects physiological processes such as protein synthesis, resulting in immature kernels and a considerable reduction in end-use quality. At later stages near kernel maturity, frost has less of an effect on physiological development, but can cause wrinkling of the bran (Dexter et al.,1985).

Heat stress during grain filling has been reported to be one of the factors that can affect the dough properties and quality characteristics of wheat. Increased temperatures between stem elongation and anthesis in wheat tend to reduce grain number, while high temperatures during grain filling reduce grain size.

These yield reductions can be largely attributed to the twin effects of increased developmental rate and shortened developmental duration, which occurs as a result of high temperature stress. Furthermore high temperature events following anthesis also affect wheat grain quality, reducing starch content and altering protein composition. Under conditions of high temperatures, yield is depressed and grain protein concentration is elevated. Jenner (1970) found that a reduced number of B-type starch granules were formed in the grain at high temperature, and they concluded from this that the increase in grain protein as a result of high temperature is primarily a result of reduced starch content in the grain, rather than an increased amount of protein per grain.

Size exclusion high performance chromatography (SE-HPLC) has been one of the most useful techniques for analyzing cereal proteins. Separation occurs 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). 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 (Batey et al., 1991), as well as the overall molecular-size distribution within the polymeric fraction (Gupta and Shepherd,1993).

The aim of this study was to determine the effect of extreme high and low temperatures on protein fractions as measured by SE-HPLC.

Materials and methods

Two bread wheat cultivars, Kariega and SST86, one durum wheat cultivar, Oranje and a soft biscuit wheat cultivar, Snack, were used. The bread wheat cultivars both are very good quality, commercial bread wheat cultivars. Snack is the only biscuit wheat ever to be developed in South Africa. Oranje is a South

African durum wheat cultivar. The cultivars were grown two plants to a pot, with 12 pots for each replication, with three replications. Two treatments (cold and heat) were applied for each cultivar. The cold treatment was applied at the soft dough (grain filling) stage. The pots were placed in a cold room at 5°C. After 30 min the temperature was reduced to -3.5°C, and then it was reduced by 1°C every 30 min until it reached -5.5°C, where it was left for three hours. Then the temperature was increased to -2°C and after 30 min to 0°C, then to 2°C and after 30 min to 5°C. After another 30 min the pots were placed back in the greenhouse. The heat treatment consisted of a 32°C/15°C day/night treatment at the soft dough stage for three days. This trial was done in 2005 and was repeated in 2006. In 2005 the trial was planted on 26 April, and in 2006 on 24 February.

SE-HPLC

Proteins were extracted from the wheat flour with an extraction procedure developed by Gupta and Shepherd (1993). The first step extracts the proteins soluble in dilute SDS, while the second extracts 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 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 sec, 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 the HPLC system. Aliquots of 20 µl of each extract were injected into a BIOSEP SEC-4000 Phenomenex column on a Shimadzu Prominance System HPLC (Shimadzu Scientific instruments, Shimadzu Corporation,Tokyo, Japan) 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 filtered and

degassed prior to use. Proteins were detected by UV absorbance at 210nm. Areas of the different peaks were calculated. The measured HPLC fractions were: SDS-soluble (1) and SDS-insoluble (2), with each chromatogram subdivided into larger polymeric proteins (LPP), smaller polymeric proteins (SPP), larger monomeric proteins (LMP) mainly gliadins and smaller monomeric proteins (SMP) mainly albumins and globulins. The four major peaks eluted between 9 and 20 min.

Statistical analyses: Analysis of variance and correlation analyses were done with Agrobase (2000) and SAS.

Results

Year 1

Analysis of variance for measured characteristics

<u>SDS soluble fractions</u>:

There were significant differences between entries for all four the fractions (Table 3.1). The treatments were significant for the SDS soluble large and small monomeric proteins (LMP1 and SMP1). There was a significant interaction between the entry and treatment only for LMP1.

<u>SDS insoluble fractions</u>:

There were significant differences between all four the cultivars for all the measured protein fractions.

Table 3.1 Mean squares for entry, treatment and interaction for measured characteristics, year 1

	Entry	Treatment	Entry x treatment
LPP1	16.903*	24.056*	2.516
SPP1	490.520**	56.622	60.800
LMP1	532.776**	93.860*	69.157*
SMP1	17.878*	8.751	10.111
LPP2	214.579**	31.931	39.972
SPP2	297.469**	1.105	13.977
LMP2	648.322**	43.752	39.471
SMP2	37.991*	6.853	10.225

* $P \leq 0.05$, ** $P \leq 0.01$; LPP1= SDS-soluble larger polymeric proteins; SPP1= SDS-soluble smaller polymeric proteins; LMP1= SDS-soluble, larger monomeric proteins; SMP1= SDS-soluble smaller monomeric proteins, LPP2= SDS-insoluble larger polymeric proteins; SPP2= SDS-insoluble smaller polymeric proteins; LMP2= SDS-insoluble larger monomeric proteins, SMP2= SDS-insoluble smaller monomeric proteins

Mean values for different treatments for SE-HPLC fractions year 1

The protein fractions of neither Kariega nor Oranje were significantly influenced by the temperature treatments (Table 3.2). In Snack, the LPP1 and SPP1 were significantly reduced after cold treatment, while LMP1 and SMP1 were significantly increased. In SST86 LMP1 was significantly increased by cold treatment. LPP2 was increased significantly by heat treatment. SMP1 was significantly decreased for both heat and cold treatment.

Table 3.2 Values for different treatments for SE-HPLC fractions year 1

	Treat	LPP1	SPP1	LMP1	SMP1	LPP2	SPP2	LMP2	SMP2
Kariega	**Cold**	6.763a	28.893a	55.950a	8.230a	15.427a	33.017a	43.947a	7.380a
Kariega	**Heat**	6.130a	26.570a	59.867a	7.053a	18.840a	31.617a	41.077a	8.277a
Kariega	**Control**	9.140a	29.397a	52.560a	8.237a	22.753a	32.227a	37.087a	6.590a
Oranje	**Cold**	3.980a	16.253a	67.480a	12.180a	10.003a	20.620a	57.773a	11.597a
Oranje	**Heat**	4.103a	13.450a	70.747a	11.693a	6.610a	18.977a	63.503a	10.717a
Oranje	**Control**	5.477a	13.660a	71.157a	9.710a	7.927a	19.167a	58.880a	13.940a
Snack	**Cold**	5.013a	21.220a	61.847a	11.927a	9.130a	25.420a	54.237a	11.070a
Snack	**Heat**	6.077b	37.067b	48.467b	7.910b	12.083a	32.310a	48.307a	7.273a
Snack	**Control**	8.647b	32.350b	49.783b	9.230b	13.100a	29.947a	48.193a	7.883a
SST86	**Cold**	5.253a	25.673a	60.160a	7.803a	13.247a	31.940a	47.890a	6.943a
SST86	**Heat**	8.377a	31.490a	52.847b	7.100a	22.817b	30.457a	39.023a	6.930a
SST86	**Control**	8.863a	29.527a	49.737b	11.800b	13.417a	30.357a	45.463a	10.757a

LPP1= SDS-soluble larger polymeric proteins; SPP1= SDS-soluble smaller polymeric proteins; LMP1= SDS-soluble, larger monomeric proteins; SMP1= SDS-soluble smaller monomeric proteins, LPP2= SDS-insoluble larger polymeric proteins; SPP2= SDS-insoluble smaller polymeric proteins; LMP2= SDS-insoluble larger monomeric proteins, SMP2= SDS-insoluble smaller monomeric proteins; Values followed by different letters are significantly different at $P \leq 0.05$

Average values for cultivars and treatments year 1

Values across all treatments for each cultivar were calculated, and then average values of treatments across cultivars, to determine the relative effect of cultivar, and relative effect of treatment (Table 3.3). Oranje, which is a durum wheat cultivar, was significantly different from all the other cultivars for all the fractions except for SMP1, where it differed only from Kariega, and LPP2, where it differed only from SST86 and Kariega. This reflects the different genome composition (A and B) compared to bread and biscuit wheat (A, B and D).

When averaged for all the cultivars, there were no significant differences between the treatments for six of the eight fractions. LPP1 was reduced by cold and heat treatment and LMP1 was increased compared to the control under cold stress.

Table 3.3 Average values across cultivars and treatments, year 1

	LPP1	SPP1	LMP1	SMP1	LPP2	SPP2	LMP2	SMP2
Kariega	7.344a	28.287a	56.126a	7.840a	19.007a	32.287a	40.703a	7.416a
Oranje	4.520b	14.454b	69.794b	11.194b	8.180b	19.588b	60.052b	12.084b
Snack	6.579a	30.212a	53.366a	9.689a	11.438b	29.226a	50.246a	8.742a
SST86	7.498a	28.897a	54.248a	8.928a	16.493a	30.918a	44.126a	8.210a
Cold	5.253a	23.010a	61.359a	10.035a	11.952a	27.749a	50.962a	9.248a
Heat	6.172a	27.144a	57.982b	8.439a	15.088a	28.340a	47.978a	8.299a
Control	8.032b	26.233a	55.809b	9.764a	14.299a	27.924a	47.406a	9.793a

LPP1= SDS-soluble larger polymeric proteins; SPP1= SDS-soluble smaller polymeric proteins; LMP1= SDS-soluble, larger monomeric proteins; SMP1= SDS-soluble smaller monomeric proteins, LPP2= SDS-insoluble larger polymeric proteins; SPP2= SDS-insoluble smaller polymeric proteins; LMP2= SDS-insoluble larger monomeric proteins, SMP2= SDS-insoluble smaller monomeric proteins; Values followed by different letters are significantly different at $P \leq 0.05$

Year 2

Analysis of variance for measured characteristics

SDS soluble fractions:

There were highly significant differences between the entries and between the treatments for all but LMP1 (Table 3.4). There was a significant entry with treatment interaction for SPP1.

SDS insoluble fractions:

There were highly significant differences between all the fractions for entry, and for all the fractions except for SPP2 for treatment. There was a significant interaction between entry and treatment for LMP2 and SMP2. The treatment effect was larger in year 2 than year 1 probably because the trial was planted earlier in the second year.

Table 3.4 Mean squares for entry, treatment and interaction for measured characteristics, year 2

	Entry	Treatment	Entry x treatment
LPP1	27.92**	4.19**	0.614
SPP1	111.97**	21.34**	4.50*
LMP1	3.08	0.63	5.83
SMP1	240.92**	48.73**	7.64
LPP2	378.61**	182.64**	22.88
SPP2	199.29**	7.29	20.48
LMP2	432.85**	197.63**	29.51**
SMP2	15.65**	5.56**	3.27**

* P ≤ 0.05, ** P ≤ 0.01; LPP1= SDS-soluble larger polymeric proteins; SPP1= SDS-soluble smaller polymeric proteins; LMP1= SDS-soluble, larger monomeric proteins; SMP1= SDS-soluble smaller monomeric proteins, LPP2= SDS-insoluble larger polymeric proteins; SPP2= SDS-insoluble smaller polymeric proteins; LMP2= SDS-insoluble larger monomeric proteins, SMP2= SDS-insoluble smaller monomeric proteins

Mean values for different treatments for SE-HPLC fractions year 2

In Kariega, the LMP1 for the heat treatment was significantly lower than that of the cold treatment (Table 3.5). Heat and cold treatment caused a significant increase in LMP2, and cold significantly decreased SMP2. The treatment effects in Kariega were more pronounced than in the first year. In Oranje, SPP1 was significantly decreased and SMP1 increased due to both treatments. LMP2 was increased due to heat, and SMP2 increased due to cold treatment.

In Snack, SPP1 and SMP1 were decreased and increased respectively, by cold treatment. LPP2 was decreased, and LMP2 and SMP2 increased due to cold treatment. Snack was therefore very sensitive to cold treatment. In SST86, all the effects were also due to cold treatment. LPP1 and SPP1 were decreased, SMP1, LMP2 and SMP2 were increased and LPP2 was decreased. SPP2 was also significantly lower under cold, than heat stress.

Average values for cultivars and treatments year 2

As in year 1, Oranje was very different from the other cultivars for all the fractions except for LMP1. The LPP1, SPP1 and LPP2 were significantly lower, and the SMP1 and LMP2 significantly higher than for the other cultivars (Table 3.6). The LPP1, SPP1 and SPP2 of Kariega were significantly higher, and SMP1 and SMP2 significantly lower than that of the other cultivars.

LPP1, SPP1 and LPP2 were significantly reduced, and SMP1 and LMP2 increased by both heat and cold stress, but SPP1, LPP2 and LMP2 were much more affected by cold than heat stress. SMP2 was increased only under cold stress.

Table 3.5 Values for different treatments for SE-HPLC fractions year 2

	Treat	LPP1	SPP1	LMP1	SMP1	LPP2	SPP2	LMP2	SMP2
Kariega	**Cold**	6.90a	23.16a	55.07a	14.39a	21.29a	44.47a	29.31a	4.65a
Kariega	**Heat**	7.58a	23.98a	51.40b	16.43a	22.56a	41.69a	29.52a	5.98b
Kariega	**Control**	7.74a	23.79a	53.85b	13.80a	25.22a	44.84a	23.55b	6.14b
Oranje	**Cold**	2.88a	13.18a	53.04a	29.41a	15.17a	34.50a	42.08a	7.62a
Oranje	**Heat**	3.18a	14.74a	52.44a	28.10a	15.40a	33.83a	44.39b	6.04b
Oranje	**Control**	3.99a	18.89b	52.11a	22.88b	18.03a	37.67a	38.26a	5.70b
Snack	**Cold**	6.32a	20.49a	53.13a	19.39a	19.38a	36.20a	34.59a	9.48a
Snack	**Heat**	5.53a	22.53b	54.53a	17.04b	27.66b	36.74a	27.73b	7.43b
Snack	**Control**	6.73a	22.63b	54.13a	16.15b	33.49b	34.23a	24.70b	7.25b
SST86	**Cold**	5.84a	20.91a	51.60a	20.95a	26.03a	28.85a	34.94a	9.84a
SST86	**Heat**	6.10b	21.51b	53.89a	17.84b	32.28b	36.28b	24.03b	7.14b
SST86	**Control**	7.78b	23.09b	53.96a	15.59b	36.33b	33.25b	21.96b	8.12b

LPP1= SDS-soluble larger polymeric proteins; SPP1= SDS-soluble smaller polymeric proteins; LMP1= SDS-soluble, larger monomeric proteins; SMP1= SDS-soluble smaller monomeric proteins, LPP2= SDS-insoluble larger polymeric proteins; SPP2= SDS-insoluble smaller polymeric proteins; LMP2= SDS-insoluble larger monomeric proteins, SMP2= SDS-insoluble smaller monomeric proteins, Values followed by different letters are significantly different at $P \leq 0.05$

Table 3.6 Average values across cultivars and treatments, year 2

	LPP1	SPP1	LMP1	SMP1	LPP2	SPP2	LMP2	SMP2
Kariega	7.41a	23.64a	53.44a	14.87a	23.02a	43.66a	27.46a	5.59a
Oranje	3.35b	15.60b	52.53a	26.80b	16.20b	35.33b	41.58b	6.45b
Snack	6.19c	21.88c	53.93a	17.53c	26.84c	35.73b	29.00c	8.05c
SST86	6.57c	21.84c	53.15a	18.13c	31.55d	32.79b	26.98a	8.37c
Cold	5.48a	19.43a	53.21a	21.03a	20.47a	36.00a	35.23a	7.90a
Heat	5.60a	20.69b	53.06a	19.85a	24.48b	37.13a	31.42b	6.65b
Control	6.56b	22.10c	53.51a	17.11b	28.27c	37.50a	27.12c	6.80b

LPP1= SDS-soluble larger polymeric proteins; SPP1= SDS-soluble smaller polymeric proteins; LMP1= SDS-soluble, larger monomeric proteins; SMP1= SDS-soluble smaller monomeric proteins, LPP2= SDS-insoluble larger polymeric proteins; SPP2= SDS-insoluble smaller polymeric proteins; LMP2= SDS-insoluble larger monomeric proteins, SMP2= SDS-insoluble smaller monomeric proteins; Values followed by different letters are significantly different at P≤0.05

Year 1 and year 2 combined

Analysis of variance for measured characteristics

SDS soluble fractions:

The mean squares for entry were highly significant for all measured fractions (Table 3.7). For treatment, mean squares were highly significant for LPP1 and significant for SMP1. The year effect was highly significant for SPP1, LMP1 and SMP1. The entry x year interaction was highly significant for LMP1 and SMP1 but significant for SPP1. There was a treatment x year interaction only for SMP1.

SDS insoluble fractions:

Mean squares for entry were highly significant for all measured protein fractions except for SMP2 which was significant. For treatment, mean squares were highly significant for LPP2 and LMP2. The year effect was highly significant for all fractions. Entry x year was highly significant for LPP2 and SPP2 and significant for SMP2.

Table 3.7 Mean squares for entry, treatment and interaction for measured characteristics, year 1 and 2

	Entry	Treatment	Entry x treatment	Year	Entry x year	Treatment x year
LPP1	43.45**	23.88**	1.18	6.61	1.38	4.36
SPP1	523.07**	64.02	31.66	401.155**	79.42*	13.95
LMP1	232.93**	42.86	21.18	472.27**	302.93**	51.62
SMP1	190.59**	27.36*	10.70	1770.82**	68.21**	30.12*
LPP2	453.78**	163.07**	37.87	2031.61**	139.40**	51.50
SPP2	334.64**	5.75	14.33	1417.69**	162.12**	2.64
LMP2	1031.35**	206.05**	58.68	5529.14**	49.82	35.34
SMP2	24.25*	7.85	7.46	71.82**	29.40*	4.57

* P ≤ 0.05, ** P ≤ 0.01; LPP1= SDS-soluble larger polymeric proteins; SPP1= SDS-soluble smaller polymeric proteins; LMP1= SDS-soluble, larger monomeric proteins; SMP1= SDS-soluble smaller monomeric proteins, LPP2= SDS-insoluble larger polymeric proteins; SPP2= SDS-insoluble smaller polymeric proteins; LMP2= SDS-insoluble larger monomeric proteins, SMP2= SDS-insoluble smaller monomeric proteins

Values for different treatments for SE-HPLC fractions year 1 and 2

Across the two years the protein fractions for Kariega and Oranje were not influenced by any of the treatments (Table 3.8). In Snack there were significant decreases in LPP1 and LPP2 for both cold and heat treatments. There was also a decrease in SPP1 for cold treatment. LPP1 was significantly reduced by cold stress in SST86.

Table 3.8 Values for different treatments for SE-HPLC fractions year 1 and 2

	Treat	LPP1	SPP1	LMP1	SMP1	LPP2	SPP2	LMP2	SMP2
Kariega	**Cold**	6.83a	26.03a	55.51a	11.31a	18.36a	38.74a	36.63a	6.01a
Kariega	**Heat**	6.86a	25.28a	55.64a	11.74a	20.70a	36.65a	35.30a	7.13a
Kariega	**Control**	8.44a	26.60a	53.21a	11.02a	23.99a	38.53a	30.32a	6.36a
Oranje	**Cold**	3.43a	14.72a	60.26a	20.80a	12.59a	27.56a	49.93a	9.61a
Oranje	**Heat**	3.64a	14.10a	61.59a	19.90a	11.01a	26.40a	53.95a	8.38a
Oranje	**Control**	4.73a	16.27a	61.63a	16.30a	12.98a	28.42a	48.57a	9.82a
Snack	**Cold**	5.67a	20.86a	57.49a	15.66a	14.26a	30.81a	44.41a	10.27a
Snack	**Heat**	5.80a	29.80b	51.50a	12.48a	19.87b	34.53a	38.02a	7.35a
Snack	**Control**	7.69b	27.49b	51.96a	12.69a	23.30b	32.09a	36.45a	7.57a
SST86	**Cold**	5.55a	23.29a	55.88a	14.38a	19.64a	30.39a	41.42a	8.39a
SST86	**Heat**	7.24b	26.50a	53.37a	12.47a	27.55a	33.37a	31.53a	7.03a
SST86	**Control**	8.32b	26.31a	51.85a	13.74a	24.87a	31.81a	33.71a	9.44a

LPP1= SDS-soluble larger polymeric proteins; SPP1= SDS-soluble smaller polymeric proteins; LMP1= SDS-soluble, larger monomeric proteins; SMP1= SDS-soluble smaller monomeric proteins, LPP2= SDS-insoluble larger polymeric proteins; SPP2= SDS-insoluble smaller polymeric proteins; LMP2= SDS-insoluble larger monomeric proteins, SMP2= SDS-insoluble smaller monomeric proteins;Values followed by different letters are significantly different at $P \leq 0.05$

Values for cultivars and treatments year 1 and 2

When summed over treatments (Table 3.9), there were very large differences between cultivars, with Kariega and SST86 having significantly higher LPP1 than the other two cultivars, with Oranje having a very low value. Oranje had significantly lower SPP1, LPP2 and SPP2 and higher SMP1 than the other cultivars, and a higher SMP2 than Kariega. Across cultivars, heat and cold stress caused a reduction in LPP1, and cold stress caused a significant reduction in SPP1 and LPP2, and an increase in LMP1 and SMP1. There were significant differences between years for all fractions except LPP1.

Table 3.9 Values for cultivars and treatments year 1 and 2

	LPP1	SPP1	LMP1	SMP1	LPP2	SPP2	LMP2	SMP2
Kariega	7.38a	25.97a	54.78a	11.36a	21.02c	37.98a	34.08a	6.50a
Oranje	3.94b	15.03b	61.16b	19.00b	12.19b	27.46b	50.81b	9.26b
Snack	6.38c	26.05a	53.65a	13.61c	19.14c	32.48c	39.63c	8.40b
SST86	7.03a	25.37a	53.70a	13.53c	24.02d	31.86c	35.55a	8.29b
Cold	5.37a	21.22a	57.28a	15.53a	16.21a	31.88a	43.10a	8.57a
Heat	5.88a	23.92b	55.52b	14.15b	19.78b	32.74a	39.70b	7.47a
Control	7.30b	24.17b	54.66b	13.44b	21.28b	32.71a	37.26b	8.30a
Year1	6.49a	25.46a	58.38a	9.41a	13.78a	28.00a	48.78a	9.11a
Year2	5.88a	20.74b	53.26b	19.33b	24.40b	36.88b	31.26b	7.12b

LPP1= SDS-soluble larger polymeric proteins; SPP1= SDS-soluble smaller polymeric proteins; LMP1= SDS-soluble, larger monomeric proteins; SMP1= SDS-soluble smaller monomeric proteins, LPP2= SDS-insoluble larger polymeric proteins; SPP2= SDS-insoluble smaller polymeric proteins; LMP2= SDS-insoluble larger monomeric proteins, SMP2= SDS-insoluble smaller monomeric proteins; Values followed by different letters are significantly different at $P \leq 0.05$

Discussion

It is a known fact that frost damage has an influence on the quality of wheat. These quality defects are dependant on the temperature of the frost, severity, duration and to the growth stage of the plant, all of which will influence the amount of damage to the seeds in the emerged ear (Single, 1985). Previous researchers have suggested that the effects of temperature on protein composition and dough properties become most important above a certain threshold (usually 32-35℃) (Finney and Fryer,1958; Blumenthal et al., 1991; Graybosch et al., 1995) and that larger monomer (gliadin) is the protein fraction most responsive to temperature (Blumenthal et al.,1990; 1991a). In this study the effects of the temperature stress was the most pronounced in the second year. This may be due to the fact that the material was planted earlier in the second year, when day light length was longer, and the growing season was a bit shorter than in the first year. In both years the softest cultivar, Snack showed the largest effect on low temperature stress specifically, where the monomeric proteins were significantly increased, and the polymeric proteins were significantly decreased. In the second year SST86 showed the same trend as Snack under cold stress conditions. Oranje, the durum wheat cultivar, reacted to both heat and cold stress in the second year, where the small polymeric proteins (SDS soluble) were significantly reduced, and the small monomeric proteins were increased with stress, and the SDS insoluble large monomeric proteins were increased only under heat stress. When the two years were combined, the only significant effects were for Snack under cold stress conditions, where three of the four polymeric protein fractions were reduced significantly due to cold stress. All the monomeric fractions were increased, but none of the increases were significant. In SST86 the large polymeric proteins (SDS soluble) were also significantly reduced under cold stress conditions.

When summed across all the treatments, the effect of the type of wheat involved became very clear. The protein fractions of the tetraploid Oranje was very different from the other three hexaploid cultivars, as can be expected. The polymeric proteins were much lower, and the monomeric proteins much higher in Oranje. This is probably due to the lack of the D genome. The soft wheat Snack

had significantly lower large polymeric protein, and higher large monomeric protein fractions (SDS insoluble) than the bread wheat cultivars across both years.

Across the cultivars, per treatment across the two years, all the fractions, except for the small polymeric and small monomeric proteins (SDS insoluble) were affected by the cold treatment. The monomeric proteins were consistently increased, and the polymeric proteins decreased. Only the SDS soluble large polymeric proteins were significantly affected by the heat treatment, where it was significantly decreased.

Bettge et al. (1989) and Souza et al. (1994) also concluded that the flours of weak dough soft wheat generally have lesser amounts of high molecular weight glutenins, protein content and weaker gluten strength.

When frost damage occurs when the grain is physiologically immature, it can have pronounced effects on end-use quality as a result of altered protein composition, presumably as a result of termination of glutenin synthesis (Dexter et al., 1985; Preston et al., 1991; Tipples, 1980). Altered protein composition may help to partially explain the poor bread making quality of frost-damaged wheat.

CHAPTER 4

The influence of temperature extremes on starch and starch components and quality characteristics

Introduction

Starch, which accounts for 65 - 75% of wheat grain weight and can exceed 80% of the endosperm weight, is a major determinant of wheat yield. The two major components of starch are amylose and amylopectin. Amylose consists of long linear chains of α-1,4 linked glucose residues with relatively few α-1,6 linked branches whereas amylopectin is a highly branched molecule of shorter α-1,4 linked glucose molecules and more frequent α-1,6 branches. These two molecules are assembled together to form a semi-crystalline starch granule. Amylose content is used as one of the parameters characterizing starch, and plays an important role in wheat quality because it affects starch properties. Oda et al. (1980), and Shibanuma et al. (1994) have shown that the size and branching of amylase and average chain length of amylopectin may also determine quality. The functional properties of starch, particularly the ability of starch or flour to take up water and form a paste in the presence of heat, are affected by variations in the proportions of amylose to amylopectin and in the size distribution of starch granules (Dengate, 1984).

Environmental temperatures affect the amylose content, structural features and gelatinization properties of starch in several cereal species. In rice, higher ambient temperatures reduce amylose content and increase amylopectin chain length (Asaoka et al., 1989). Starch accumulation and starch granule size were reduced at high temperatures in barley but amylose contents were little affected, and no changes in amylopectin fine structures were detected (MacLeod and Duffus, 1988; Tester et al., 1991). Maize grown at high temperature during endosperm cell division had reduced kernel mass due to reduced number of

endosperm cells, starch granules or both (Jones et al., 1985). In wheat, environmental temperature affects grain yield, protein content, size and number of starch granules (Sofield et al., 1977; Bhullar and Jenner, 1985; Tester et al., 1995). Shi et al. (1994) reported that with increasing temperature during grain filling, amylose was slightly increased. Two of the key enzymes of starch biosynthesis, the starch synthase and branching enzyme, have been proposed to play a significant role in limiting starch deposition when temperature exceed 25℃ (Keeling et al., 1994). High and low temperatures during grain-fill decrease starch production, reducing the final weight of the wheat grain, and diminishing yield. High temperatures decreased levels of fructose, hexose phosphate, and sugar nucleotides (Jenner et al., 1991) and reduced the activity of some enzymes in the starch biosynthetic pathway, especially soluble starch synthase (Keeling et al., 1993; Rijven, 1986). Diminished rates of starch production in wheat endosperm at high temperatures were hypothesized to be due mainly to heat inactivation of starch synthase, a key enzyme in the starch biosynthetic pathway.

The aim of this study was to determine the effect of extreme high and low temperatures on starch and its components as well as quality characteristics.

Materials and methods

Total starch

Starch content was determined using a Total Starch Amyloglucosidase/ α-Amylase Assay Kit (Megazyme International Ireland Ltd, Bray, Ireland). One hundred milligram of wheat flour sample was weighed into a glass tube (16 x 120mm). The samples were wetted with 0.2 ml of 80% (v\v) aqueous ethanol to aid dispersion, and stirred on a vortex mixer. Three milliliters of thermostable α-amylase in 3-Morpholinopropanesulfonic acid (MOPS) buffer was added to the samples and vigorously stirred on a vortex mixer. The samples were incubated in a boiling water bath for 6 min with stirring after 2 min and 4 min. The samples were placed in a water bath at 50°C; and sodium acetate buffer (4 mL, 200 mM, pH 4.5) was added followed by amyloglucosidase (0.1 ml). The samples were

stirred on a vortex mixer and incubated at 50°C for 30 minutes. The entire contents of the test tubes were transferred to 50 ml tubes and the volume was adjusted with 0.7 ml distilled water. The sample was mix thoroughly and centrifuged at 3000 rpm for 10 minutes. One millilitre aliquots of the diluted solution was transferred in duplicate to the bottom of glass test tubes (16 x 100 mm) and 3.0 ml of glucose oxidase peroxidase 4-aminoantipyrine (GOPOD) reagent was added to each tube (including the glucose controls and reagent blanks), and incubated at 50°C for 20 min. Glucose controls consisted of 0.1 ml of glucose standard solution and 3.0 ml of GOPOD Reagent. Reagent Blank Solutions consisted of 0.1 ml of distilled water and 3.0 ml of GOPOD reagent. The absorbance was read against the reagent blank at 510nm for each sample including the glucose control.

Amylose and amylopectin

Amylose content was determined using an Amylose / Amylopectin Assay Kit (Megazyme International Ireland Ltd, Bray, Ireland). Wheat flour (20-25 mg) was weighed into a 50 ml screw capped sample tube and the sample weight was recorded. One ml of Dimethyl sulphoxide (DMSO) was added to the samples while gently stirring it at low speed on a vortex mixer. The tube was capped and heated in a boiling water bath until the sample was completely dispersed (approx. 1 min), ensuring that no gelatinous lumps of starch were remaining. Samples were vigorously mixed at high speed on a vortex mixer and placed in a boiling water bath and heated for 15 min, with intermittent high-speed stirring on a vortex mixer. The samples were stored at room temperature for approximately 5 min and 2 ml of 95 % (v\v) ethanol was added with continuous stirring on a vortex mixer. A further 4 ml of ethanol was added, and the tube was capped and inverted to mix. A starch precipitate was formed. The samples were allowed to stand for 30 min, then centrifuged at 2000 rpm for 5 min and the supernatant was discarded and dried for 10 min. The pellet was used in the subsequent amylose and starch determinations. Two millilitres of DMSO was added to the starch pellet with gentle vortex mixing. The samples were placed in a boiling water bath for 15 min and mixed occasionally to ensure that there are no gelatinous lumps in the

tubes. After removing the samples from the boiling water bath, 4 ml of concanavalin A (Con A) solvent was added, mixed thoroughly and then the tube contents was transferred quantitatively (by repeated washing with Con A solvent) to a 25 ml volumetric flask and diluted to volume with Con A solvent (this is Solution A).

Con A precipitation of amylopectin and determination of amylose was performed when 1.0 ml of Solution A was transferred to a 2.0 ml Eppendorf microfuge tubes and 0.50 ml of Con A solution was added to the samples and then mixed gently by repeated inversion without frothing of the sample. The tubes were allowed to stand for 1 hour at room temperature and then centrifuged at 14000 rpm for 10 min in a microfuge at room temperature. One mililitre of the supernatant was transferred to a 50 ml centrifuge tube and 3 ml of a 100 mM sodium acetate buffer, pH 4.5 was added to reduce the pH to ~ 5. The samples were then mixed and heated in a boiling water bath for 5 min to denature the Con A. The samples were placed in a water bath at 40℃ and allowed to equilibrate for 5 min before 0.1 ml of amyloglucosidase/ -amylase enzyme mixture was added and incubated at 40℃ for 30 min. Tubes were centrifuged at 2000 rpm for 5 min. To 1.0 ml aliquots of the supernatant 4 ml of GOPOD Reagent was added incubated at 40℃ for 20 min concurrently with the Reagent Blank and the D-Glucose controls.

Total starch was determined by mixing 0.5 ml of Solution A with 4 ml of 100 mM sodium acetate buffer, pH 4.5 and add 0.1 ml of amyloglucosidase/ -amylase solution and incubated at 40℃ for 10 min. One millilitre aliquots of this solution were then transfered in duplicate to glass test tubes and 4 ml of GOPOD Reagent was added and mixed well before being incubated at 40℃ for 20 minutes. This incubation was performed concurrently with the samples and standards. The absorbance was read against the reagent blank at 510 nm for each sample, including the glucose control.

Quality analysis

The following was measured in both years: SKCS (single kernel characteristic system)-seed weight (AACC method 53-31), SKCS-seed diameter (AACC 53-

31), SKCS-hardness index (AACC 53-31), flour protein content (AACC 39-11) and sodium dodecyl sulphate sedimentation (SDSS).

Results

Year 1

Mean squares for entry, treatment and interaction for measured characteristics, year 1

The mean squares were highly significant for SDS sedimentation, seed weight, diameter and hardness index; and significant for starch (Table 4.1). The treatment was highly significant for seed weight and seed diameter; and significant for SDS sedimentation, amylose and amylose:amylopectin ratio. There was a significant interaction between entry and treatments for seed weight, seed diameter and starch.

Table 4.1 Mean squares for entry, treatment and interaction for measured characteristics, year 1

	Entry	Treatment	Entry x treatment
SDS	2963.66**	389.528*	103.935
FPC	6.359	4.737	6.369
Wght	190.457**	17.00**	3.19*
Diam	0.481**	0.626**	0.140*
HI	2125.145**	82.587	60.371
Starch	93.005*	65.489	77.248*
Amylose	7.207	370.907*	59.128
Ratio	7.212	370.955*	59.131

* $P \leq 0.05$, ** $P \leq 0.01$; SDS= Sodium Dodecyl Sulphate Sedimentation, FPC= flour protein content, Wght= single kernel characteristic system seed weight, Diam= single kernel characteristic system seed diameter, HI= single kernel characteristic system hardness index, Starch= starch content, Amylose= amylose content, Ratio= amylose:amylopectin ratio

Average values for three treatments, year 1

Average values of the three treatments were compared for each cultivar. SDS sedimentation was significantly reduced under cold treatment for Kariega compared to the control. The SDS sedimentation of the cold treated Snack was significantly lower than that of the control and the heat treatment (Table 4.2). FPC was significantly increased in the bread wheat cultivar Kariega and SST86 for both stress treatments, but for the soft wheat Snack, the FPC was significantly reduced under both treatments. Kernel weight and diameter was significantly reduced under both stress treatments for both Kariega and SST86, while for Oranje they were only significantly reduced under heat stress. Hardness index was significantly reduced in Oranje for both stress treatments. Starch content was significantly reduced for both stress treatments in Kariega, while amylose content and consequently the amylose:amylopectin ratio was significantly increased by cold treatment in Snack.

Table 4.2 Average values for three treatments, year 1

	Treat	SDS	FPC	Wght	Diam	HI	Starch	Amylose	Ratio
Kariega	**Cold**	68.33a	18.43a	32.08a	2.13a	29.32a	43.76a	30.31a	0.453a
Kariega	**Heat**	78.67b	18.40a	32.30a	2.15a	34.33a	49.02a	40.83a	0.710a
Kariega	**Control**	83.00b	15.00b	41.52b	2.65b	33.63a	61.50b	28.72a	0.440a
Oranje	**Cold**	38.00a	18.57a	32.51a	2.17a	53.34a	51.98a	37.60a	0.603a
Oranje	**Heat**	38.67a	19.30a	24.61b	1.74b	56.48a	44.50a	40.37a	0.687a
Oranje	**Control**	32.67a	18.50a	38.25a	2.31a	69.00b	49.72a	27.84a	0.423a
Snack	**Cold**	37.67a	17.73a	24.73a	1.82a	25.73a	44.89a	40.63a	0.723a
Snack	**Heat**	59.67b	18.77a	24.46a	1.81a	26.02a	46.91a	37.15b	0.603b
Snack	**Control**	52.33b	20.43b	24.01a	1.76a	23.55a	45.03a	22.66b	0.293b
SST86	**Cold**	61.33a	18.50a	27.39a	2.03a	47.63a	52.64a	33.02a	0.503a
SST86	**Heat**	70.33a	18.40a	28.89a	1.99a	43.54a	52.90a	36.60a	0.590a
SST86	**Control**	73.67a	16.00b	39.55b	2.73b	49.80a	53.23a	32.31a	0.533a

SDS= Sodium Dodecyl Sulphate Sedimentation, FPC= flour protein content, Wght= single kernel characteristic system seed weight, Diam= single kernel characteristic system seed diameter, HI= single kernel characteristic system hardness index, Starch= starch content, Amylose= amylose content, Ratio= amylose:amylopectin ratio; Values followed by different letters are significantly different at $P \leq 0.05$

Average values for cultivars and treatments, year 1

Average values summed over the treatments showed that SDS sedimentation for the two bread wheats were similar, and significantly higher than that of the soft and durum wheats (Table 4.3). Summed over cultivars, SDS sedimentation values were significantly reduced by cold treatment. FPC was significantly higher for the durum and soft wheat, compared to the bread wheats. Across cultivars, heat and cold treatment significantly increased protein content. Kernel weight and diameter was significantly lower in Snack than the other cultivars. Heat and cold treatment significantly reduced kernel weight, diameter and hardness index. Hardness index was significantly different for all cultivars, ranging from Oranje (the hardest) to Snack (the softest). Starch content was significantly lower for Snack than SST86. Amylose content was significantly increased by both treatments, and amylose:amylopectin ratio was increased by heat treatment.

Table 4.3 Average values for cultivars and treatments, year 1

	SDS	FPC	Wght	Diam	HI	Starch	Amylose	Ratio
Kariega	76.67a	17.28a	35.30a	2.31a	32.43a	51.43b	33.28a	0.534a
Oranje	36.44b	18.79b	31.79a	2.07b	59.61b	48.73b	35.27a	0.571a
Snack	49.89c	18.98b	24.40b	1.79c	25.10c	45.61a	33.48a	0.540a
SST86	68.44a	17.63a	31.94a	2.25a	46.99d	52.92b	33.98a	0.542a
Cold	51.33a	18.31a	29.18a	2.036a	39.00a	48.32a	35.39a	0.571b
Heat	61.83b	18.72a	27.56a	1.922a	40.10a	48.33a	38.74a	0.648a
Control	60.42b	17.48b	35.83b	2.362b	43.99b	52.37a	27.88b	0.423b

SDS= Sodium Dodecyl Sulphate Sedimentation, FPC= flour protein content, Wght= single kernel characteristic system seed weight, Diam= single kernel characteristic system seed diameter, HI= single kernel characteristic system hardness index, Starch= starch content, Amylose= amylose content, Ratio= amylose:amylopectin ratio; Values followed by different letters are significantly different at $P \leq 0.05$

Year 2

Mean squares for entry, treatment and interaction for measured characteristics, year 2

The mean squares were highly significant for SDS sedimentation, flour protein content, seed weight, seed diameter and hardness index; and significant for starch, amylose and ratio between amylose and amylopectin. The treatment was highly significant for flour protein content and significant for seed weight, seed diameter, starch, amylase and ratio (Table 4.4). There was a significant interaction between entry and treatments for SDS sedimentation and starch.

Table 4.4 Mean squares for entry, treatment and interaction for measured characteristics, year 2

	Entry	Treatment	Entry x treatment
SDS	2904.99**	61.08	101.27*
FPC	8.57**	9.11**	0.502
Wght	185.13**	145.80*	21.99
Diam	0.496**	0.415*	7.08
HI	2732.44**	25.02	72.40
Starch	30.48*	47.72*	26.11*
Amylose	57.30*	109.44*	11.14
Ratio	0.00245*	0.00459*	0.00037

* P ≤ 0.05, ** P ≤ 0.01; SDS= Sodium Dodecyl Sulphate Sedimentation, FPC= flour protein content, Wght= single kernel characteristic system seed weight, Diam= single kernel characteristic system seed diameter, HI= single kernel characteristic system hardness index, Starch= starch content, Amylose= amylose content, Ratio= amylose:amylopectin ratio

Comparison of temperature treatments within cultivars, year 2

SDS sedimentation was significantly reduced by cold treatment for Kariega, and SST86, and for Oranje the SDS sedimentation value was significantly increased by cold conditions. FPC was significantly increased by cold and heat for Kariega, and for Snack it was increased only with heat treatment. Kernel weight was

reduced under heat stress for Oranje. For kernel diameter there was a significant decrease under cold stress for Kariega and Snack. Starch content was significantly reduced under both stress conditions for Kariega and Oranje. Amylose content was significantly increased under cold stress for Kariega, Oranje and Snack. The same was true for the amylose:amylopectin ratio.

Average values for cultivars and treatments, year 2

Kariega had significantly higher SDS sedimentation values than the other cultivars, while Oranje had a significantly lower value than the others (Table 4.6). Flour protein content was the highest for Oranje, followed by SST86, Kariega and Snack. Across cultivars, heat treatment caused an increase in protein content. Kernel weight and diameter was the lowest for Snack and SST86, and both stress treatments caused a significant decrease in kernel weight and diameter. Hardness index was far higher for Oranje than the other cultivars and the soft wheat Snack was significantly lower than the other cultivars. Starch content of Oranje and Snack was lower than for the other two cultivars, and starch was significantly reduced by both stress treatments. Amylose content of Oranje was significantly lower than for other cultivars, and it was significantly increased by both stress treatments. Amylose:amylopectin ratio was the lowest for Oranje and it was significantly increased by heat and cold stress.

Table 4.5 Comparison of cold and heat treatments within cultivars, year 2

	Treat	SDS	FPC	Wght	Diam	HI	Starch	Amylose	Ratio
Kariega	**Cold**	75.00a	14.33a	34.97a	2.33a	46.60a	52.08a	36.52a	0.575a
Kariega	**Heat**	82.67b	15.10a	37.03a	2.47b	35.03a	51.17a	34.16a	0.519a
Kariega	**Control**	84.00b	12.77b	43.69a	2.93b	35.76a	56.30b	30.49b	0.439b
Oranje	**Cold**	44.00a	15.77a	39.47	2.47a	63.37a	49.68a	33.30a	0.500a
Oranje	**Heat**	33.33b	16.97a	33.77a	2.27a	64.2a	44.97b	29.91b	0.427b
Oranje	**Control**	37.67b	15.53a	43.43b	2.55a	71.65a	55.52c	26.03b	0.353b
Snack	**Cold**	64.67a	13.47a	25.60a	1.83a	25.80a	52.58a	41.06a	0.644a
Snack	**Heat**	67.33a	15.30b	30.13a	2.07	26.50a	54.09a	34.91b	0.547b
Snack	**Control**	65.00a	13.33a	34.23a	2.40b	21.60a	50.91a	31.52b	0.465b
SST86	**Cold**	60.33a	14.90a	33.07a	2.13a	45.63a	47.53a	32.97a	0.495a
SST86	**Heat**	75.00b	15.70a	28.90a	2.03a	45.07a	49.15a	34.37a	0.527a
SST86	**Control**	74.00b	14.60a	34.10a	2.20a	51.07a	51.55a	31.72a	0.466a

SDS= Sodium Dodecyl Sulphate Sedimentation, FPC= flour protein content, Wght= single kernel characteristic system seed weight, Diam= single kernel characteristic system seed diameter, HI= single kernel characteristic system hardness index, Starch= starch content, Amylose= amylose content, Ratio= amylose:amylopectin ratio; Values followed by different letters are significantly different at $P \leq 0.05$

Table 4.6 Average values for cultivars and treatments, year 2

	SDS	FPC	Wght	Diam	HI	Starch	Amylose	Ratio
Kariega	80.56a	14.07a	38.56a	2.58a	39.13a	53.18a	33.72a	0.52a
Oranje	38.33b	16.09b	38.89a	2.43a	66.40b	50.05b	29.75b	0.43b
Snack	65.67c	14.03a	29.99b	2.10b	24.63c	52.52a	35.83a	0.55a
SST86	69.78c	15.07c	32.02b	2.12b	47.26a	49.41b	33.02a	0.50a
Cold	61.00a	14.62a	33.28a	2.19a	45.35a	50.47a	35.96a	0.55a
Heat	64.58a	15.77b	32.46a	2.21a	42.70a	49.85a	33.34a	0.50a
Control	65.17a	14.06a	38.86b	2.52b	45.02a	53.57b	29.94b	0.43b

SDS= Sodium Dodecyl Sulphate Sedimentation, FPC= flour protein content, Wght= single kernel characteristic system seed weight, Diam= single kernel characteristic system seed diameter, HI= single kernel characteristic system hardness index, Starch= starch content, Amylose= amylose content, Ratio= amylose:amylopectin ratio; Values followed by different letters are significantly different at $P \leq 0.05$

Combined data for year 1 and 2

Mean squares for entry, treatment and interaction for measured characteristics, year 1 and 2

The mean squares for entry were highly significant for all characteristics but insignificant for starch, amylose and amylose:amylopectin ratio (Table 4.7). For treatment, mean squares were highly significant for flour protein content, seed weight, seed diameter, amylose and amylase:amylopectin ratio; and significant for SDS sedimentation and starch. The entry x treatment interaction was significant only for flour protein content and starch content. The year effect was highly significant for flour protein content, seed weight and seed diameter; and significant for SDS sedimentation and hardness index. There was a year x treatment interaction for flour protein content, seed diameter and starch content. There was a treatment x year interaction only for amylose.

Table 4.7 Mean squares for entry, treatment and interaction for measured characteristics, year 1 and 2

	Entry	Treatment	Entry x treatment	Year	Entry x year	Treatment x year
SDS	5661.0**	374.60*	152.10	589.39*	207.65	76.01
FPC	9.53**	13.0**	4.23**	202.68**	5.40**	0.85
Wght	333.55**	370.93**	37.63	289.12**	42.04	5.24
Diam	0.76**	1.01**	0.10	0.73**	0.22*	0.03
HI	4786.71**	62.81	95.62	199.03*	70.87	44.81
Starch	41.82	111.79*	70.54*	47.22	81.67*	1.42
Amylo	13.87	386.88**	52.71	15.39	50.63	93.47*
Ratio	0.01	0.16**	0.03	0.05	0.02	0.04

P ≤ 0.05, ** P ≤ 0.01; SDS= Sodium Dodecyl Sulphate Sedimentation, FPC= flour protein content, Wght= single kernel characteristic system seed weight, Diam= single kernel characteristic system seed diameter, HI= single kernel characteristic system hardness index, Starch= starch content, Amylose= amylose content, Ratio= amylose:amylopectin ratio

Average values for three treatments, year 1 and 2

In Kariega, SDS sedimentation was significantly reduced after cold treatment (Table 4.8). Flour protein content was significantly increased for both cold and heat treatments while seed weight, seed diameter and starch were significantly decreased for both cold and heat treatments. In Oranje, amylose was significantly increased for both cold and heat treatments, while hardness index was significantly reduced for both cold and heat treatments. Seed weight, seed diameter and starch were significant reduced by heat treatment. Like Kariega the SDS sedimentation was reduced in Snack and SST86 for cold treatment. Amylose and amylase:amylopectin ratio were significantly increased for both cold and heat treatments for Snack. There were significant reductions in seed weight and seed diameter for both cold and heat treatments for SST86.

Table 4.8 Average values for three treatments, year 1 and 2

	Treat	SDS	FPC	Wght	Diam	HI	Starch	Amylose	Ratio
Kariega	**Cold**	71.67a	16.38a	33.53a	2.23a	37.96a	47.92a	33.41a	0.51a
Kariega	**Heat**	80.67b	16.75a	34.67a	2.31a	34.68a	50.10a	37.50a	0.61a
Kariega	**Control**	83.50b	13.88b	42.60b	2.79b	34.70a	58.90b	29.60a	0.44a
Oranje	**Cold**	41.00a	17.17a	35.99a	2.32a	58.35a	50.83a	35.45a	0.55a
Oranje	**Heat**	36.00a	18.13a	29.19b	2.01b	60.34a	44.74b	35.14a	0.56a
Oranje	**Control**	35.17a	17.02a	40.84a	2.43a	70.32b	52.62a	26.94b	0.39a
Snack	**Cold**	51.17a	15.60a	25.17a	1.83a	25.76a	48.74a	40.85a	0.68a
Snack	**Heat**	63.50b	17.03a	27.30a	1.94a	26.26a	50.50a	36.03a	0.58a
Snack	**Control**	58.67a	16.88a	29.12a	2.08a	22.58a	47.97a	27.09b	0.38b
SST86	**Cold**	60.83a	16.70a	30.23a	2.08a	46.63a	50.09a	33.00a	0.50a
SST86	**Heat**	72.67b	17.05a	28.90a	2.01a	44.31a	51.03a	35.49a	0.56a
SST86	**Control**	73.83b	15.30a	36.82b	2.46b	50.43a	52.39a	32.01a	0.50a

SDS= Sodium Dodecyl Sulphate Sedimentation, FPC= flour protein content, Wght= single kernel characteristic system seed weight, Diam= single kernel characteristic system seed diameter, HI= single kernel characteristic system hardness index, Starch= starch content, Amylose= amylose content, Ratio= amylose:amylopectin ratio; Values followed by different letters are significantly different at $P \leq 0.05$

Average values for cultivars and treatments, year 1 and 2

Across treatments Kariega had the highest SDS sedimentation values and Oranje had significantly lower values than all the other cultivars (Table 4.9). SDS sedimentation was significant decreased by cold and the values between the years were significantly different. Oranje had significantly higher flour protein content than the other cultivars and Kariega significantly lower values. Flour protein content was significantly increased by both cold and heat treatments and the values between the years were significantly different. Kariega and Oranje had significantly higher seed weight values and Snack significantly lower values than the other cultivars. Kernel weight was significantly reduced by both heat and cold treatments and the values between the years were significantly different. Kariega had the highest seed diameter values and Snack the lowest values compared to other cultivars. Seed diameter was significantly reduced by both cold and heat treatment and the values between the years were significantly different. Oranje had the highest hardness index value and Snack the lowest. Starch was significantly reduced but amylose and amylose:amylopectin ratio were significantly increased by both heat and cold treatments.

Table 4.9 Average values for cultivars and treatments, year 1 and 2

	SDS	FPC	Wght	Diam	HI	Starch	Amylose	Ratio
Kariega	78.61a	15.67a	36.93a	2.44a	35.78a	52.30a	33.50a	0.52a
Oranje	37.39b	17.44b	35.34a	2.25b	63.01b	49.39a	32.51a	0.50a
Snack	57.78c	16.51c	27.19b	1.95c	24.87c	49.07a	34.66a	0.55a
SST86	69.11d	16.35c	31.98c	2.19b	47.12d	51.17a	33.50a	0.52a
Cold	56.17a	16.46a	31.23a	2.11a	42.18a	49.39a	35.68a	0.56a
Heat	63.21b	17.24b	30.01a	2.07a	41.40a	49.09a	36.04a	0.58a
Control	62.79b	15.77c	37.35b	2.44b	44.51a	52.97b	28.91b	0.43b
Year1	57.86a	18.17a	30.86a	2.11a	41.03a	49.67a	34.00a	0.55a
Year2	63.58b	14.81b	34.87b	2.31b	44.36a	51.29a	33.08a	0.50a

SDS= Sodium Dodecyl Sulphate Sedimentation, FPC= flour protein content, Wght= single kernel characteristic system seed weight, Diam= single kernel characteristic system seed diameter, HI= single kernel characteristic system hardness index, Starch= starch content, Amylose= amylose content, Ratio= amylose:amylopectin ratio; Values followed by different letters are significantly different at $P \leq 0.05$

Discussion

The effect of frost on quality test results is usually much more evident during the early maturity stages. Very high starch damage was reported above 37% moisture, but little change in starch below 37%. According to Tottman (1987) the soft dough stage contains approximately 50% moisture and the hard dough stage approximately 30% moisture. The critical stages will therefore be during the late milk, early dough, and soft dough stages, with the early hard dough stages also being subject to frost damage. A temperature below approximately –3℃ is required to bring out this response and maximum response is attained over a narrow temperature range (Preston et al*.,* 1991).

This study showed that between treatments within cultivars, cold treatment caused a significant reduction in SDS sedimentation in Kariega and SST86, while in Snack SDS sedimentation was significantly higher under heat than cold conditions. This suggests that the bread wheat cultivars experienced a decrease

in baking quality due to low temperatures. A significant increase in SDS sedimentation volumes found under the high temperature conditions in the biscuit wheat contrasts with other studies (Corbellini et al., 1997; Stone et al., 1997). Peterson et al. (1998) observed a curvilinear association of SDS volume with hours of high temperature stress (>32°C) during grain filling. The increase in protein content in most of the studies, where decreases in SDS volume were reported, was mainly due to an increase in the soluble (gliadin) protein content.

Flour protein content was increased by heat stress in all the cultivars over both seasons, but only for Kariega the increase was significant. The increase in flour protein content due to the effect of high temperatures is in agreement with other studies (Wrigley et al., 1994; Ciaffi et al., 1996; Corbellini et al., 1997; Stone et al., 1997; Daniel and Triboi, 2000).

Both kernel weight and diameter were significantly decreased at both stress treatments for the two bread wheat cultivars, indicating that their kernel characteristics are very sensitive to extreme temperatures. Kernel characteristics of the biscuit wheat were thermo stable across the two seasons. Marcellos and Single (1984) and Single (1985) showed that ice nucleation occurring at below -4°C caused disruption of immature seed cell membranes and tracheary elements of the rachis and rachilla where translocation of nutrients from vegetative tissue to the growing seed would occur. In Oranje, seed weight and diameter were only reduced under heat, but it was not sensitive to low temperatures. The negative effect of high temperature during grain filling on grain yield has been found to be closely associated with the reduction in grain weight as found in this study (Gibson and Paulsen, 1999; Tahir and Nakata, 2005).

Wheat kernel hardness is known to be sensitive to heat and frost (Hoseney et al., 1966; Tipples, 1980; Dexter et al., 1985). In this study this was only the case for the durum wheat cultivar, and only for the heat treatment. For the soft wheat, the hardness index was actually increased by extreme temperatures, although not significantly.

Across the two seasons, the starch content in Kariega was significantly reduced by both heat and cold temperatures, as is also reflected in the reduction of weight and diameter of these kernels. In the durum wheat, only heat caused a significant reduction in starch content which is again reflected in the reduction of kernel weight and diameter. This result is consistent with the findings of Rijven (1986) who suggested that starch synthase, which is located in the amyloplast, might be the enzyme responsible for the unusual sensitivity of starch synthesis to temperature. Amylose content and the amylose:amylopectin ratio increased under heat and cold treatment. Shi et al. (1994) also reported that with increased temperature during grain filling amylose was slightly increased and starch gelatinization temperature increased.

Kariega had a significantly higher diameter than the other cultivars, with Snack having the lowest. Hardness index was much higher for Oranje than for the other cultivars. This is due to the fact that Oranje is a durum wheat. Heat stress reduces grain weight by decreasing grain growth duration (Stone and Nicolas, 1995; Ishag and Mohamed, 1996) and grain growth rate (Sofield et al., 1977; Tashiro and Wardlaw, 1989). Increased temperatures during grain filling results in a steady decrease in the duration of dry matter accumulation. At temperatures up to 20℃ there is a compensating increase in the rate of dry-matter accumulation, but above 25℃ the rate begins to decrease. Thus, reduced grain size at high temperature results from the failure of the rate of dry-matter accumulation to compensate for a shorter duration of grain filling. Wheat cultivars differ in their response of grain filling to increased temperature (Rawson, 1986; Wardlaw et al.,1989a;b), but it is not clear whether these differences are due to changes in grain-filling rate or grain-filling duration.

CHAPTER 5

The relationship between gluten proteins, starch and starch components, and quality characteristics

Introduction

The properties of wheat flour are strongly dependent on wheat genotype and growing conditions, and are mainly determined by both structure and quantity of gluten proteins. Numerous amino acid sequences of gluten proteins, mostly deduced from nucleotide sequences, have been determined and, except for the ω-gliadins, all gluten protein types are now known in complete primary structures. Accordingly, gluten proteins can be classified into three main groups, each consisting of two or three protein types (Shewry et al., 1986): a high molecular weight (HMW) group including the x- and γ -type of HMW subunits of glutenin; a medium molecular weight (MMW) group (S-poor group) containing the ω5– and ω1,2-type gliadins; and a low molecular weight (LMW) group (S-rich group), consisting of LMW glutenin subunits, and α and γ -type gliadins. Certainly, wheat cultivars differ in the primary structures of single gluten protein components, but differences are considered to be small and restricted to only a few substitutions, deletions, or insertions of single amino acid residues or oligopeptides in the peptide chain.

Protein content is traditionally recognized as the most influential factor affecting wheat bread making quality (Bushuk et al., 1969; Shewry et al., 1986), protein quality is also an important consideration. The two protein fractions that constitute gluten protein, namely gliadin and glutenin, each have unique effects on end-use quality (Gupta et al., 1992; Schofield, 1994). Gliadin, which is a very heterogeneous protein fraction, is responsible for the viscous properties of dough during mixing. Conversely, glutenin is a polymeric protein that exhibits a high degree of inter-molecular bonding that reduces dough extensibility and is the protein fraction responsible for dough strength (Wall, 1979). Interestingly, the

accumulation of various protein fractions in the developing grain is both highly ordered and asynchronous (Daniel and Triboi, 2000; Stone and Nicolas, 1996). The gliadin fraction is detectable within the first seven to 10 days after anthesis. It is the first storage protein fraction to accumulate in quantity, and is synthesized most rapidly during mid development of the wheat kernel (Gupta et al., 1996; Panozzo and Eagles, 2000; Stone and Nicolas, 1996). The glutenin fraction is not present in the kernel in large quantities until the latter half of the filling period, and is readily detectable around 20 days after anthesis (Gupta et al. 1996; Panozzo et al., 2000; Stone and Nicolas 1996). Given these results, the ratio of gliadin to glutenin has been shown to increase during the first half of filling and then decrease considerably, particularly at the end of filling when a steep decline in kernel moisture content occurs. Clearly, conditions during the filling period are likely to have pronounced effects on protein quality. For example, a reduction in the duration of the filling period due to high temperature stress has been demonstrated to shorten the duration of glutenin synthesis, in turn reducing dough strength (Ciaffi et al., 1996; Corbellini et al., 1997; Stone et al., 1997; Stone and Nicolas, 1996). Altered protein composition may help to partially explain the poor bread making quality of frost-damaged wheat observed by many researchers (Dexter et al., 1985; Preston et al., 1991; Tipples, 1980). When frost damage occurs when the grain is physiologically immature, it can have pronounced effects on end-use quality as a result of altered protein composition, presumably as a result of termination of glutenin synthesis. The aim of this chapter was to investigate the relationship between gluten protein, starch and starch and starch components, and quality.

Material and methods

Proteins were extracted, using a modified method of Marchylo et al. (1989). Flour samples (100 mg) were extracted with 1 ml 70% (v/v) ethanol for 30 min at room temperature with continuous shaking. Following centrifugation at 17000 g for 4 min, the supernatant, consisting of monomeric proteins were transferred to a new vial and stored at 4℃. The remaining pellet was washed twice with 1 ml 50% (v/v) propan-1-ol with continuous shaking for 30 min at room temperature.

Following centrifugation at 17000 g for 4 min, the supernatant was discarded and the pellet re-suspended in 1ml buffer (pH 6.6) containing 50% (v/v) propan-1-ol, 2 M urea, 0.2 M Tris and 1% (w/v) dithiothratol (DTT). Samples were vortexed and placed in a waterbath at 60℃ for 1 h. The samples were alkylated with the addition of 10 µl of 4-vinylpyridine at 60℃ for 15 min. The supernatant recovered after centrifugation at 17 000 g for 4 min, was filtered through polyvinylidene difluoride (PVDF) syringe filters (0.45 µm) into glass vials.

RP-HPLC

Reversed phase HPLC analysis was performed on a Shimadzu Prominence LC System using a Supelcosil LC-308 column (C8, 300 Å pore size, 5 µm particle size, 250 x 4.6 mm). Samples (80 µl) were injected and run at a solvent flow rate of 0.800 ml/min or 0.500ml/min, alternatively, using a column temperature of 50℃. The two eluants used were: (A) 95% acetonitrile (ACN) containing 0.1% (v/v) trifluoroacetic acid (TFA); (B) water containing 5% (v/v) ACN and 0.11% (v/v) TFA.

The following linear solvent gradient was used for the glutenin analysis: 20-40% (A), 0-40 min; 40-56% (A), 40-42 min; 56-90% (A), 42-60 min; 90—20% (A), 70-80 min. Effluent was detected at 210 nm, glutenin components were quantified by integration of the chromatogram areas using Shimadzu Class-VP. Only the most distinct peaks were taken into account, and they were numbered according to their elution times.

Results

Correlations year 1

Under cold conditions there were very few significant correlations between subunits or protein fractions and quality characteristics (Table 5.1). Hardness index correlated negatively with P36, amylose content correlated negatively and amylose:amylospectin ratio positively with P18. SDS soluble large polymeric proteins correlated negatively with starch content, and P42 correlated positively with amylase:amylopectin ratio. The other correlations were between RP-HPLC and SE-HPLC fractions, and give an indication of how the RP-HPLC and SE-HPLC proteins relate to each other.

Under heat treatment RP-HPLC peak P18b was significantly negatively correlated with single kernel weight and kernel diameter (Table 5.1). Hardness index was significantly correlated with P34 and P20. SDS sedimentation was significantly positively correlated with SDS insoluble large polymeric proteins, and negatively with SDS insoluble large monomeric proteins. P14 correlated significantly with starch content.

Under control conditions, flour protein content was negatively correlated with kernel weight and kernel diameter. P19 and P22 were both negatively correlated with hardness index. Starch content and SDS sedimentation were positively correlated. Hardness index was negatively correlated with both SDS soluble and insoluble small polymeric protein fractions, and positively correlated with both SDS soluble and insoluble large monomeric protein fractions.

Table 5.1 Significant correlations between characteristics for year 1

Cold			Heat			Control		
Var 1	**Var 2**	**Corr**	**Var 1**	**Var 2**	**Corr**	**Var1**	**Var2**	**Corr**
HI	P36	-0.689*	Wght	P18b	-0.646*	Wght	FPC	-0.772**
Amylo	P18	0.640*	Diam	P18b	-0.649*	Diam	FPC	-0.834**
Ratio	P18	-0.652*	SDS	P36	0.748*	Diam	Wght	0.942**
Ratio	P42	0.677*	HI	P34	0.689*	P19	HI	-0.743*
LPP1	Starch	-0.731*	HI	P20	-0.728*	P22	HI	-0.667*
			SDS	LPP2	0.749*	Starch	SDS	0.662*
			HI	LMP1	0.77*	LPP2	SDS	0.711*
			HI	SPP2	-0.64*	LMP2	SDS	-0.827**
			SDS	LMP2	-0.788**	HI	SPP1	-0.775**
			P14	Starch	0.638*		LMP1	0.737*
							SPP2	-0.677*
							LMP2	0.655*

Var= variable; corr= correlation; P ≤ 0.05, ** P ≤ 0.01; P= RP-HPLC bands (Peak); SDS= Sodium Dodecyl Sulphate Sedimentation, FPC= flour protein content, Wght= single kernel characteristic system seed weight, Diam= single kernel characteristic system seed diameter, HI= single kernel characteristic system hardness index, Starch= starch content, Amylo= amylose content, Ratio= amylose:amylopectin ratio, LPP1= SDS-soluble larger polymeric proteins; SPP1= SDS-soluble smaller polymeric proteins; LMP1= SDS-soluble, larger monomeric proteins; LPP2= SDS-insoluble larger polymeric proteins; SPP2= SDS-insoluble smaller polymeric proteins; LMP2= SDS-insoluble larger monomeric proteins, SMP2= SDS-insoluble smaller monomeric proteins

Stepwise regression year 1

Under cold stress, 57.53% of flour protein content was explained by the small monomeric proteins, followed by SDS insoluble large polymeric proteins and hardness index (Table 5.2). Under heat stress, kernel diameter explained most variation, followed by amylose:amylopectin ratio, P22, P14 and SDS insoluble large monomeric proteins. Under control conditions, kernel diameter was again the most important factor, followed by P22, P36, P20 and SDS soluble large monomeric proteins. SDS sedimentation was explained, under cold stress by the SDS insoluble small monomeric proteins followed by P42, and then the SDS insoluble small and large monomeric proteins, and amylose content. Under heat stress the SDS insoluble large monomeric fractions explained 62.14% of variation, followed by protein content and amylose:amylopectin ratio. Under control conditions, SDS insoluble large monomeric and large polymeric proteins explained most variation followed by kernel diameter.

Table 5. 2 Stepwise regression of flour protein content and SDS sedimentation year 1

FPC

Cold		**Heat**		**Control**	
SMP2	27.14	Diam	44.21	Diam	68.57
SMP1	57.53	Ratio	55.82	P22	81.91
LPP2	84.90	P22	65.18	P36	93.19
HI	96.18	P14	77.57	P20	98.97
		LMP2	97.57	LMP1	99.67

SDS

Cold		**Heat**		**Control**	
SMP1	60.46	LMP2	62.14	LMP2	68.14
P42	80.16	FPC	83.99	LPP1	86.91
SPP2	92.99	Ratio	91.84	Diam	94.24
LMP2	97.96				
Amylo	99.18				

P= RP-HPLC bands (Peak); SDS= Sodium Dodecyl Sulphate Sedimentation, FPC= flour protein content, Wght= single kernel characteristic system seed weight, Diam= single kernel characteristic system seed diameter, HI= single kernel characteristic system hardness index, Starch= starch content, Amylo= amylose content, Ratio= amylose:amylopectin ratio, LPP1= SDS-soluble larger polymeric proteins; SPP1= SDS-soluble smaller polymeric proteins; LMP1= SDS-soluble, larger monomeric proteins; SMP1= SDS-soluble smaller monomeric proteins, LPP2= SDS-insoluble larger polymeric proteins; SPP2= SDS-insoluble smaller polymeric proteins; LMP2= SDS-insoluble larger monomeric proteins, SMP2= SDS-insoluble smaller monomeric proteins

Correlations year 2

SDS sedimentation was strongly negatively correlated with flour protein content for both cold and heat stress (Table 5.3). For all three treatments SDS sedimentation was significantly correlated with P35, P38, P43 and P46 and negatively correlated with SDS soluble small monomeric proteins, SDS insoluble large monomeric proteins and P38. Under heat stress SDS was also correlated with P37. Under heat and the control SDS sedimentation was significantly correlated with SDS soluble large polymeric proteins, SDS soluble small polymeric proteins, P23 and P29 and negatively with hardness index. Under heat stress SDS was also correlated with SDS insoluble large polymeric proteins. Flour protein content was significantly negatively correlated with SDS soluble small monomeric proteins for all three treatments. Under heat stress and the control flour protein content was also correlated with hardness index. Under heat and cold stress flour protein content was significantly correlated with P40 and negatively with P35.

Under heat stress flour protein content was further correlated with starch content, SDS soluble large polymeric proteins, SDS soluble small polymeric proteins, P23, P43 and P46 (negatively) and positively with SDS insoluble large monomeric proteins, P37, P38 and P39. Single kernel weight, hardness and diameter were significantly interrelated under cold stress. Under heat and control conditions diameter and weight were related. Under control single kernel weight was further correlated with starch content, SDS insoluble small polymeric proteins, and P41 and negatively with SDS insoluble small monomeric proteins, P29.5, P32, P42 and P43.5. Under heat conditions, hardness was negatively correlated with starch and P23 and positively correlated with SDS insoluble small monomeric proteins. Under heat stress starch content was positively related with SDS soluble small polymeric proteins, P23 and P35 and negatively to SDS insoluble large monomeric proteins, and P38. Under heat and cold stress hardness was significantly correlated with SDS soluble small monomeric proteins and P38 and negatively correlated with P35. Under cold stress hardness was further correlated with SDS insoluble large monomeric proteins, P39 and P40

and negatively correlated with SDS insoluble small polymeric proteins, P29, P43 and P46.

Under control conditions starch content was positively related to SDS insoluble small polymeric proteins and negatively to P29.5 and P43.5. Diameter and starch content were significantly positively correlated; diameter was further positively correlated with SDS insoluble small polymeric proteins and negatively with SDS insoluble small monomeric proteins, P29.5 and P32. Amylose content was positively correlated with SDS soluble small polymeric proteins. For all three treatments amylose:amylopectin ratio content was positively correlated with P42.5.

Table 5.3 Correlations between characteristics in year 2

Cold			Heat			Control		
Var 1	**Var 2**	**Corr**	**Var 1**	**Var 2**	**Corr**	**Var1**	**Var2**	**Corr**
SDS	FPC	-0.648*	SDS	FPC	-0.855**	SDS	HI	-0.640*
	Smp1	-0.911**		HI	-0.699*		LPP1	0.872**
	Lmp2	-0.863**		LPP1	0.881**		SPP1	0.946**
	P35	0.852**		SPP1	0.928**		SMP1	-0.954**
	P37	-0.871**		SMP1	-0.922**		LMP2	-0.838**
	P38	-0.831**		LPP2	0.669*		P23	0.771**
	P43	0.767**		LMP2	-0.843**		P29	0.958**
	P46	0.867**		P23	0.860**		P35	0.750*
FPC	SMP1	0.647*		P29	0.723*		P38	-0.901**
	P35	-0.793**		P35	0.856**		P43	0.912**
	P40	0.743*		P38	-0.932**		P46	0.953**
Wght	Diam	0.976		P43	0.857**	FPC	HI	0.661*
	HI	0.699*		P46	0.920**		SMP1	0.647*
Diam	HI	0.681*	FPC	HI	0.689*	Wght	Diam	0.864**
Ratio	P42.5	0.718*		Starch	-0.653		Starch	0.765**
				LPP1	-0.850**		SPP2	0.734*
				SPP1	-0.925**		SMP2	-0.877**
				SMP1	0.837**		P29.5	-0.808**
				LMP2	0.775**		P32	-0.768**
				P23	-0.868**		P41	0.782**
				P35	-0.912**		P42	-0.780**
				P37	0.794**		P43.5	-0.753*
				P38	0.904**	Diam	Starch	0.695*
				P39	0.719*		SPP2	0.885**
				P40	0.740*		SMP2	-0.753*
				P43	-0.810**		P29.5	-0.722*
				P46	-0.782**		P32	-0.843**

Table 5.3 Correlations between characteristiscs in year 2 (Cont.)

Wght	Diam	0.927**	HI	SPP1	-0.737*
HI	Starch	-0.884**		SMP1	0.756*
	SMP1	0.841**		LMP2	0.705*
	SMP2	0.675*		P29	-0.721*
	P23	-0.747*		P35	-0.819**
	P35	-0.886**		P38	0.819**
	P38	0.788**		P39	0.730*
Starch	SPP1	0.769**		P40	0.840**
	LMP2	-0.704*		P43	-0.748*
	P23	0.719*		P46	-0.780**
	P35	0.788**	Starch	SPP2	0.680*
	P38	-0.738*		P29.5	-0.788**
				P43.5	-0.734*
			Amylose	SPP1	0.633*

Var= variable; corr= correlation; * P ≤ 0.05, ** P ≤ 0.01; P= RP-HPLC bands (Peak); SDS= Sodium Dodecyl Sulphate Sedimentation, FPC= flour protein content, Wght= single kernel characteristic system seed weight, Diam= single kernel characteristic system seed diameter, HI= single kernel characteristic system hardness index, Starch= starch content, Amylo= amylose content, Ratio= amylose:amylopectin ratio, LPP1= SDS-soluble larger polymeric proteins; SPP1= SDS-soluble smaller polymeric proteins; LMP1= SDS-soluble, larger monomeric proteins; SMP1= SDS-soluble smaller monomeric proteins, LPP2= SDS-insoluble larger polymeric proteins; SPP2= SDS-insoluble smaller polymeric proteins; LMP2= SDS-insoluble larger monomeric proteins, SMP2= SDS-insoluble smaller monomeric proteins

Stepwise regression year 2

Under heat stress, the small polymeric proteins was by far (85.59%) the largest contributing factor to flour protein content, followed by kernel weight, and both the small monomeric fractions, followed by small polymeric and P29 (Table 5.4). Under control conditions, amylose content was the most important contributor to variation in flour protein content, followed by P41. The SDS soluble small monomeric proteins were the important in SDS sedimentation under cold and control conditions. Under cold stress SDS soluble small monomeric proteins was followed by SDS insoluble large monomeric proteins while under control treatment was followed by P38. Under heat stress SDS soluble small polymeric proteins was the most important determining factor in SDS sedimentation, followed by P37, kernel weight, SDS insoluble small polymeric proteins and SDS soluble large polymeric protein.

Table 5.4 Stepwise regression of flour protein content and SDS sedimentation in year 2

FPC

Cold		Heat		Control	
P35	62.90	SPP1	85.59	Amylo	87.73
		Wght	92.43	P41	99.98
		SMP2	96.84		
		SMP1	98.59		
		SPP2	99.46		
		P29	99.79		

SDS

Cold		Heat		Control	
SMP1	83.02	SPP1	86.19	SMP1	99.99
LMP2	90.74	P37	91.90	P38	99.99
		Wght	94.42		
		SPP2	97.53		
		LPP1	99.06		

P= RP-HPLC bands (Peak); SDS= Sodium Dodecyl Sulphate Sedimentation, FPC= flour protein content, Wght= single kernel characteristic system seed weight, Diam= single kernel characteristic system seed diameter, HI= single kernel characteristic system hardness index, Starch= starch content, Amylose= amylo content, Ratio= amylose:amylopectin ratio, LPP1= SDS-soluble larger polymeric proteins; SPP1= SDS-soluble smaller polymeric proteins; LMP1= SDS-soluble, larger monomeric proteins; SMP1= SDS-soluble smaller monomeric proteins, LPP2= SDS-insoluble larger polymeric proteins; SPP2= SDS-insoluble smaller polymeric proteins; LMP2= SDS-insoluble larger monomeric proteins, SMP2= SDS-insoluble smaller monomeric proteins

Correlations year 1 and year 2 combined

The peak elution times for the reversed phase HPLC were different for the two years as flow rates were adapted in the second year to improve separation profiles. This made comparison of peaks for the two years difficult. For this reason the data were discussed separately for the two years, and were not combined for analysis.

SDS sedimentation was significantly positively correlated with SDS soluble and insoluble large polymeric proteins and negatively correlated with SDS insoluble large monomeric proteins for all three treatments (Table 5.5). Under cold stress SDS sedimentation was also positively correlated with SDS insoluble small polymeric proteins and negatively correlated with SDS insoluble small monomeric proteins. For all three treatments, flour protein content was significantly negatively correlated with SDS insoluble large and small polymeric proteins and positively correlated with SDS insoluble large monomeric proteins. Under both heat and cold stress treatments flour protein content was also negatively correlated with SDS soluble small monomeric proteins and positively correlated with SDS soluble large monomeric proteins under cold stress. Kernel weight, hardness index and kernel diameter were significantly interrelated under cold stress. Under heat stress and control, SDS sedimentation was significantly negatively correlated with flour protein content and hardness index and positively correlated with starch. Under heat stress SDS sedimentation was also positively correlated with SDS soluble and insoluble small polymeric proteins. Flour protein content was negatively correlated with kernel weight and diameter and positively correlated with amylose and amylose:amylopectin ratio under heat stress. Kernel weight and kernel diameter were directly related under heat and control conditions. Hardness index and starch content were negatively correlated under heat stress, and hardness index was negatively correlated with SDS soluble large and small polymeric and positively correlated with SDS soluble small monomeric proteins under heat stress. Starch content was positively correlated with SDS soluble and insoluble large polymeric proteins and negatively

correlated with SDS insoluble large monomeric proteins under heat stress. Under control conditions, SDS sedimentation was positively correlated with SDS soluble small polymeric proteins and negatively correlated with SDS soluble large monomeric proteins. Flour protein content was negatively correlated with kernel weight, diameter, starch content, amylose and amylose:amylopectin ratio. Kernel weight was related to kernel diameter. Kernel weight and diameter were both correlated with starch content. Diameter was related to SDS insoluble small polymeric proteins and hardness index to SDS soluble large monomeric proteins.

Table 5.5 Significant correlations between characteristics for year 1 and 2 combined (RP-HPLC data excluded)

Cold			Heat			Control		
Var 1	**Var 2**	**Corr**	**Var 1**	**Var 2**	**Corr**	**Var1**	**Var2**	**Corr**
SDS	LPP1	0.641**	SDS	FPC	-0.462*	SDS	FPC	-0.512*
	LPP2	0.607**		HI	-0.648**		HI	-0.545**
	SPP2	0.565**		Starch	0.520*		Starch	0.443*
	LMP2	-0.658**		LPP1	0.680**		LPP1	0.598**
	SMP2	-0.585**		SPP1	0.500*		SPP1	0.458*
FPC	LMP1	0.558**		LPP2	0.632**		LMP1	-0.503*
	SMP1	-0.570**		SPP2	0.442*		LPP2	0.520*
	LPP2	-0.613**		LMP2	-0.649**		LMP2	-0.605**
	SPP2	-0.455*	FPC	Wght	-0.573**	FPC	Wght	-0.550**
	LMP2	0.711**		Diam	-0.625**		Diam	-0.676**
Wght	Diam	0.974**		Amylose	0.476*		Starch	-0.438*
	HI	0.563**		Ratio	0.495*		Amyl	-0.527*
Diam	HI	0.562**		SMP1	-0.539**		Ratio	-0.454*
				LPP2	-0.620**		LPP2	-0.591**
				SPP2	-0.605**		SPP2	-0.580**
				LMP2	0.716**		LMP2	0.696**
			Wght	Diam	0.954**	Wght	Diam	0.916**
				SPP2	0.526*		Starch	0.590**
			Diam	SPP2	0.618**	Diam	Starch	0.587**
			HI	Starch	-0.519*		SPP2	0.432*
				LPP1	-0.435*	HI	SPP1	-0.614**
				SPP1	-0.676**		LMP1	0.443*
				SMP1	0.495*			
			Starch	LPP1	0.486*			
				LPP2	0.480*			
				LMP2	-0.509*			

Var= variable; corr= correlation; * $P \leq 0.05$, ** $P \leq 0.01$; SDS= Sodium Dodecyl Sulphate Sedimentation, FPC= flour protein content, Wght= single kernel characteristic system seed weight, Diam= single kernel characteristic system seed diameter, HI= single kernel characteristic system hardness index, Starch= starch content, Amylose= amylose content, Ratio= amylose:amylopectin ratio, LPP1= SDS-soluble larger polymeric proteins; SPP1= SDS-soluble smaller polymeric proteins; LMP1= SDS-soluble, larger monomeric proteins; SMP1= SDS-soluble smaller monomeric proteins, LPP2= SDS-insoluble larger polymeric proteins; SPP2= SDS-insoluble smaller polymeric proteins; LMP2= SDS-insoluble larger monomeric proteins, SMP2= SDS-insoluble smaller monomeric proteins, SMP2= SDS-insoluble smaller monomeric proteins

Stepwise regression year 1 and 2

Under cold stress flour protein content was explained by SDS insoluble large monomeric proteins and SDS insoluble small monomeric proteins, followed by kernel diameter, amylose content, SDS soluble small polymeric protein, kernel weight, SDS soluble large monomeric and hardness index (Table 5.6). Under heat stress SDS insoluble large monomeric proteins, SDS soluble small monomeric proteins and SDS sedimentation explained 83.34% of variation. Under control conditions, SDS insoluble large monomeric proteins were again the most important explaining factor, followed by kernel diameter and amylose content. When everything was combined, SDS insoluble large monomeric proteins were the most important factor, followed by diameter, amylose, SDS soluble small monomeric proteins and hardness index. SDS sedimentation was also explained largely by SDS insoluble large and small monomeric proteins, kernel diameter and hardness index. Under heat conditions SDS soluble and insoluble large polymeric proteins explained the most variation followed by hardness index. Under control conditions, SDS insoluble large monomeric proteins and SDS soluble large polymeric proteins explained most variation followed by SDS soluble small monomeric proteins and kernel diameter. When everything was combined, SDS insoluble monomeric proteins and SDS soluble large polymeric proteins explained most variation, followed by SDS soluble small monomeric proteins and flour protein content. When a stepwise regression was done across the treatments and the two years, the main explaining factor for both the flour protein content and SDS sedimentation was the SDS insoluble large monomeric proteins fraction. For flour protein content kernel diameter was also important, followed by amylose content, and the SDS soluble small monomeric proteins fraction and hardness index. SDS sedimentation was further explained by SDS insoluble large polymeric and small monomeric proteins followed by flour protein content.

Table 5.6 Stepwise regression of year 1 and 2 combined for flour protein content and SDS sedimentation (RP-HPLC data excluded)

FPC

Cold		Heat		Control		Combined	
LMP2	50.58	LMP2	51.20	LMP2	48.47	LMP2	45.61
SMP2	65.87	SMP1	67.11	Diam	72.36	Diam	59.00
Diam	71.01	SDS	83.34	Amylose	81.07	Amylose	65.44
Amylose	77.21					SMP1	70.03
SPP1	81.14					HI	72.69
Wght	86.69						
LMP1	89.74						
HI	92.64						

SDS

Cold		Heat		Control		Combined	
LMP2	43.23	LPP1	46.17	LMP2	36.54	LMP2	41.27
SMP1	64.60	LPP2	66.25	LPP1	72.28	LPP1	62.84
Diam	72.14	HI	74.98	SMP1	80.47	SMP1	69.68
HI	76.08			Diam	86.05	FPC	71.10

SDS= Sodium Dodecyl Sulphate Sedimentation, FPC= flour protein content, Wght= single kernel characteristic system seed weight, Diam= single kernel characteristic system seed diameter, HI= single kernel characteristic system hardness index, Starch= starch content, Amylose= amylose content, Ratio= amylose:amylopectin ratio, LPP1= SDS-soluble larger polymeric proteins; SPP1= SDS-soluble smaller polymeric proteins; LMP1= SDS-soluble, larger monomeric proteins; SMP1= SDS-soluble smaller monomeric proteins, LPP2= SDS-insoluble larger polymeric proteins; SPP2= SDS-insoluble smaller polymeric proteins; LMP2= SDS-insoluble larger monomeric proteins, SMP2= SDS-insoluble smaller monomeric proteins

Discussion

The significant influences of genotype and environment on bread-making quality have been reported previously (Peterson et al., 1992). However, only a few investigators (Graybosch et al., 1995; 1996; Zhu and Khan 2001) have tried to understand the biochemical background on a protein level for the variation in bread-making quality determined by genotype and different types of environments. Weaker dough properties were reported to occur in years with a cooler grain-filling period, while a warmer grain-filling period led to stronger gluten (Johansson and Svensson, 1999). There is evidence that gluten strength increases with increasing temperature during grain filling up to about 30℃ and then decreases as temperatures rise above 30℃. In earlier investigations it has

been shown that the quantity of specific glutenin protein fractions measured by RP-HPLC correlates with baking performance in wheat cultivars (Sutton et al., 1990); the quantity of total HMW and LMW glutenin subunits measured by RP-HPLC correlate with dough properties (Andrews et al., 1994); the quantity of HMW protein amounts determined by enzyme-linked immunosorbent assay (ELISA) correlate with rheological parameters, such as strength and extensibility (Skerritt, 1991); and the proteins that are most difficult to extract are of high importance for determining the gluten strength (Huebner and Wall, 1976).

An increase in the concentration of high molecular weight proteins correlated with improved quality in wheat has been indicated by size exclusion high performance liquid chromatography. Some results showed that a correlation exists between dough mixing time and the amount of HMW-GS present, or the ratio of polymeric to monomeric proteins, indicating possible use in breeding (Huebner and Bietz, 1985). A significant positive correlation was found between the amount of most protein groups as well as in the amounts of monomeric and polymeric proteins and wheat cultivars ranged in order of increasing protein concentration. This is in accordance with findings by Wieser and Seilmeier (1998), showing increases of amounts of gliadins and glutenins when the protein concentration is increased. Variation in protein concentration between cultivars is thereby explained by differences in all protein parameters containing glutenins and gliadins.

Single-kernel hardness was positively correlated with kernel diameter and kernel weight. Pomeranz et al. (1985) and Ohm et al. (1998) reported similar results of significant correlation between wheat protein and other measurements of endosperm texture which concurred their finds. They also found very strong negative correlations for SKCS hardness and flour protein. On the contrary, a very strong positive correlation was found between SKCS hardness and flour protein content. The relationship between kernel weight and protein content observed here is in agreement with the findings of others (Ohm et al., 1998). The analysis of protein by reverse phase high performance liquid chromatography

has been used for predicting wheat quality and varietal identification (Huebner and Bietz, 1985, 1986). Reversed phase HPLC fractionates based on the protein hydrophobicities has proven to be a highly efficient tool for qualitative and quantitative studies (Wieser et al., 1994). The sensitivity of the RP-HPLC technique makes it suitable for use on single kernels, giving it the potential of non-destructive analysis. Fractionation occurs due to differences in protein surface hydrophobicity. Many peaks identified by RP-HPLC have been correlated to baking quality. Studies by Sutton et al. (1989) found two HMW-GS peaks correlating to loaf volume. One of the major contributions to protein studies by RP-HPLC was the fractionation of subunits with similar SDS-PAGE mobilities (Sutton et al., 1990). P14 correlated significantly with starch content and it also correlated significantly positively with the large polymeric proteins, and negatively with the monomeric proteins. This indicates that it is probably in the high molecular range. P19 correlated positively with both small polymeric fractions, and highly negatively with the large monomeric proteins. It is therefore probably a low molecular weight (LMW) gluten protein. Some low molecular weight subunit peaks have also been identified linked to bread-making quality (Gupta et al., 1991). LMW-GS have higher surface hydrophobicity than HMW-GS, but similar hydrophobicity to that of gliadins. Unfortunately the application of RP-HPLC in studying LMW-GS was inhibited by the homologous nature and the number of these subunits. Improving separation of both HMW and LMW gluten subunits will allow the use of this technique in predicting and breeding for quality. Not only in determining specific composition of proteins, but also the quantitative ratio of LMW and HMW gluten subunits can be a useful predictor. The prediction of wheat functionality is an essential step of breeding programmes to produce improved wheat varieties (Pawlinsky and Williams, 1998).Grain protein is of primary importance in determining the bread making quality of wheat flour. Although total protein is the primary factor in determining the end use of wheat, there is often a need to measure properties that are indicative of the protein quality (Delwiche, 2004). The positive correlations of protein fractions with the quality characteristics observed in this study are advantageous to the breeder.

This will make it easier for the breeder to determine and evaluate the basic quality of wheat in breeding programmes and promote efficient selection for needed-quality bases, which would result in the possibility of shortening the breeding programme. There is a need as plant breeders to expand our knowledge and understanding of our raw material to be able to focus on specific end uses and seek out new opportunities in the food industry. Specifically we need to look more closely at the functionality of starch and the variability of storage proteins (Dewey, 1984). Significant advances in technology and methodology now mean we are able to look at an increasing range of quality parameters whilst still only utilizing small volumes of seed. One of the greatest advances in plant breeding now is the use of molecular markers to assist in breeding for wheat quality. Molecular markers are small sequences of wheat DNA that can be located to specific regions of the wheat genome -the genetic information determining all aspects of the wheat plant (Branlard et al., 2001). These markers can be linked to specific characteristics, such as HMW protein sub-units, and used to track these desired traits through breeding programmes (Delwiche, 2004). By using molecular markers to track specific quality traits, plant breeders can follow traits through the breeding programme, ensure that crucial genes are not lost in the breeding process and be much more precise in targeting specific quality characters. Markers can now be used to stack combinations of genes that control quality traits of interest that, due to complex genetic inheritance, would be virtually unachievable through standard breeding methodology. Markers also enable these programs of quality breeding to progress at a significantly faster rate (Ainsworth et al., 1993). The challenge now is for plant breeders to work in conjunction with end-users to more closely relate specific end-use qualities with effective molecular markers. This will enable plant breeders to more precisely determine the output of their breeding programmes to produce varieties more suited to specific end-use requirements, and potentially unlocking new opportunities for the baking industry.

CHAPTER 6

General conclusions

In this study it was indicated that the high and low temperature treatments significantly affected the measured characteristics. The year effect was significant for all characteristics except for starch, amylose and amylose:amylopectin ratio, this could be due to the fact that the trial was planted earlier in the second year, which caused more distinct differences between the cultivars. There was a year x treatment interaction for flour protein content, diameter, starch and both monomeric and polymeric proteins and there was also a treatment x year interaction for amylose. Cold treatment caused a reduction in SDS sedimentation in Kariega and SST86, while in Snack SDS sedimentation was higher under the heat treatment. Flour protein content was increased under heat treatment in Kariega. Kernel weight and diameter were decreased at both stress treatments in Kariega and SST86, but for Oranje it was only reduced under heat. Hardness index was reduced in Oranje by both cold and heat stress. Starch content was significantly reduced in Kariega for both stress treatments, and for Oranje under heat. Amylose content was significantly increased under stress in Oranje and Snack, and amylose:amylopectin ratio was increased significantly under both treatments in Snack. It was concluded that Kariega had higher SDS sedimentation values than the other cultivars, with Oranje having the lowest value. Kariega is also known to have very good bread making quality, while Oranje is a durum wheat with poor bread making quality. Cold treatment caused a significant reduction in SDS sedimentation. There were very large differences between cultivars, with Kariega and SST86 having more polymeric proteins than the other cultivars, with Oranje having the lowest value. Both heat and cold stress caused a reduction in polymeric proteins and an increase in monomeric proteins, although the cold stress had a much larger effect than heat stress. The biscuit wheat, Snack was the most sensitive to especially low

temperature stress. The protein fractions of the tetraploid wheat Oranje were very different from that of the other three hexaploid cultivars. SDS sedimentation was positively correlated with large polymeric proteins and negatively correlated with monomeric proteins. Flour protein content was positively correlated with polymeric proteins and large monomeric proteins, and negatively correlated with small monomeric proteins. Kernel weight, hardness and diameter were interrelated. The reverse phase HPLC yielded peaks that were highly correlated to quality characteristics, but peaks could only be defined by their elution times, which differed for the two years. More work is needed in this area, to standardize HPLC conditions so that peaks can be compared. Fraction collection could be an option to define peaks and determine their molecular weight. This will allow a selection strategy for peaks that are strongly related to quality characteristics. Starch content was significantly reduced by the stress treatments, which was related to the reduction in kernel size and diameter.

CHAPTER 7

SUMMARY

The ability of wheat flour to be processed into bread and other products is largely determined by the gluten proteins, which confer unique visco-elastic properties to dough. These proteins are influenced by genetic make up and the environment in which the plants are grown. The objective of this study was to gain a better understanding of the influence of extreme temperatures on the gluten proteins, quality characteristics, starch and starch components. Two bread wheat cultivars, a soft biscuit wheat and a durum wheat were grown two plants to a pot, with 12 pots for each replication, with three replications in a greenhouse for two consecutive years. Cold and heat treatments were applied during grain filling. Size exclusion and reverse phase HPLC were used to measure the different protein fractions. Starch, amylose, amylopectin and various quality characteristics were measured on all entries after harvest in both years.

The results from this study showed that in both years the softest cultivar, Snack showed the largest reaction to low temperature stress specifically, where the monomeric proteins were significantly increased, and the polymeric proteins were significantly decreased. In the second year SST86 showed the same trend as Snack under cold stress conditions. Oranje, the durum wheat cultivar, reacted to both heat and cold stress in the second year, where the small polymeric proteins (SDS soluble) were significantly reduced, and the small monomeric proteins were increased with stress, and the SDS insoluble large monomeric proteins were increased only under heat stress. The protein fractions of the tetraploid Oranje were very different from that of the other three hexaploid cultivars. The soft wheat Snack had significantly lower large polymeric protein, and higher large monomeric protein fractions (SDS insoluble) than the bread wheat cultivars for both years. Summed over cultivars, per treatment for the two years, all the fractions, except for the small polymeric and small monomeric proteins (SDS insoluble) were affected by the cold treatment. The monomeric proteins were consistently increased, and the polymeric proteins decreased. Only

the SDS soluble large polymeric proteins were significantly affected by the heat treatment, where it was significantly decreased.

Both kernel weight and diameter were significantly decreased at both stress treatments for the two bread wheat cultivars, indicating that their kernel characteristics were sensitive to extreme temperatures. Across the two seasons, the starch content in Kariega was significantly reduced by both heat and cold temperatures, as is also reflected in the reduction of kernel weight and diameter. Amylose content and the amylose:amylopectin ratio increased under heat and cold treatment. There were highly significant positive and negative correlations between certain RP-HPLC peaks and quality characteristics for all three the temperature treatments in both years. The number of significant correlations was reduced under the two stress conditions, but there is a possibility to use certain peaks for quality selection. The profiles must, however, be standardised so that peaks can be scored and compared on any gel according to their elution times. Stepwise regression also emphasized the importance of some of the RP-HPLC peaks in explaining variation in flour protein content and SDS sedimentation for all three temperature treatments.

OPSOMMING

Die vermoë van koring meel om verwerk te word na brood en ander produkte, word grootliks bepaal deur die gluten proteïene, wat visko-elastiese eienskappe aan deeg gee. Hierdie proteïene word geneties bepaal, maar word ook deur die omgewing beïnvloed waarin die plante groei. Die doel van hierdie studie was om ‘n beter begrip te kry van die invloed van uiterste temperature op die gluten proteïene, kwaliteitseienskappe, stysel en stysel komponente. Twee broodkoring kultivars, ‘n sagte koekiekoring en ‘n durum koring is geplant, twee plante per pot, met 12 potte per herhaling, en drie herhalings, in die glashuis, in twee opeenvolgende jare. Koue en hitte behandeling is toegedien tydens die graanvul periode. SE- en RP-HPLC is gebruik om die verskillende proteïenfraksies te skei. Stysel, amilose en amilopektien, en verskeie kwaliteits eienskappe is gemeet na oes in beide jare.

Die resultate van hierdie studie het getoon dat die sagte koring kultivar, Snack, die grootste reaksie getoon het op lae temperatuur stremming spesifiek, waar die monomeriese proteïene betekenisvol toegeneem en die polimeriese proteïene betekenisvol afgeneem het. In die tweede jaar het SST86 dieselfde tendens getoon as Snack onder koue temperatuur stremming. Oranje, die durum koring kultivar, het gereageer op beide hitte en koue stremming in die tweede jaar, waar SDS oplosbare klein polimeriese proteïene afgeneem het en klein monomeriese proteïene toegneem het. Die SDS onoplosbare groot monomeriese proteïene het slegs onder hitte stremming toegeneem. Die proteïen fraksies van die tetraploïede Oranje was baie verskillend van die van die drie heksaploïede koring kultivars. Die sagte koring Snack het betekenisvol minder groot polimeriese proteïene en meer monomeriese proteïene (SDS onoplosbaar) gehad as die broodkorings in beide jare. Waar data saamgevoeg is oor cultivars oor jare vir die verskillende behandelings, is al die proteïen fraksies behalwe die klein polimeriese en klein monomeriese (SDS onoplosbaar) proteïene geaffekteer deur die koue behandeling. Die monomeriese proteïene het konstant toegeneem,

en die polimeriese proteïene het konstant afgeneem. Net die SDS oplosbare groot polimeriese proteïene is deur die hitte behandeling beïnvloed, waar dit betekenisvol afgeneem het.

Beide korrel massa en deursnee is betekenisvol verminder deur beide stremmings behandelings in die twee broodkoring cultivars, wat aandui dat die twee culivars sensitief is vir uiterste temperature. Oor beide die jare, het die styselinhoud van Kariega betekenisvol afgeneem onder beide die stremmmings behandelings, wat ook gereflekteer is in die afname van die korrelmassa en deursnee. Amilose inhoud en amilose:amilopektien verhouding het toegneem onder beide stremming behandelings. Daar was hoogs betekenisvolle positiewe en negatiewe korrelasies tussen sekere RP-HPLC pieke en kwaliteits eienskappe vir al drie die temperatuur behandelings in beide jare. Die aantal betekenisvolle korrelasies was minder onder stremmings toestande, maar daar bestaan moontlikhede om sekere pieke te gebruik om kwaliteits seleksie te doen. Die proteïenprofiele sal egter gestandardiseer moet word sodat sodat pieke van verskillende profiele met mekaar vergelyk kan word op grond van tye waar pieke vorm. Stapsgewyse regressie het ook die belangrikheid van sommige RP-HPLC pieke uitgewys in die verduideliking van variasie in meel proteïen inhoud en SDS sedimentasie vir al drie temperaturuur behandelings.

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