# THE EFFECT OF SOFTNESS GENES ON THE BISCUIT-MAKING QUALITY OF SOFT WHEAT

by

ANSA CLAASSEN

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Study Leader: Prof. M.T. Labuschagne
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I declare that this dissertation is my own work. It is being submitted for the degree Master of Agricultural Science (Plantbreeding) at the University of the Orange Free State, Bloemfontein. It has not been submitted for any degree or examination at any other faculty or University. Furthermore, I renounce copyright on the dissertation in favour of the University of the Orange Free State.

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# CHAPTER 1

## INTRODUCTION

Wheat is probably the most important of all cultivated plants with respect to human nutrition. Estimated 1992 world wheat production was 553 million tons. Most of the production of wheat is consumed directly as flour. Bran by-products are fed to animals, but relatively little whole grain goes into animal feeds. Wheat is also one of the most nutritious cereals and its contribution to the human diet clearly puts it in the first rank of plants that feed the world.

In the past, research has focused primarily on one of the two major wheat classes, namely hard wheat, which is used to produce bread. Soft wheat is used to produce more tender and less dense products, which include cake, biscuits and pastries (Gaines, Kassuba and Finney, 1994). Economic growth in developed countries has led to a higher demand for processed, high quality food. In South Africa, the production of soft wheat is limited to the Northern Cape irrigation areas with a projected domestic consumption of approximately 10 percent of the national wheat crop or ± 250 000 tons per annum (Labuschagne and Van Deventer, 1993).

Research of soft wheat products had to be focused on quality, in order to satisfy the consumer's growing needs. Nearly all modern wheat breeding programs consider quality as well as yield to be a high priority. Wheat quality characteristics are numerous and complex and breeding goals for quality are usually aimed toward achieving acceptable standards for the trade. Breeding for quality is complicated by the many uses of wheat (Allan, 1987). Kernel texture is an important quality characteristic of wheat, because it influences milling and baking quality parameters (Rogers, Hoseney, Lookhart, Curran, Lin and Sears, 1993). A soft textured wheat usually has a low protein content with weak gluten that produces products that are more tender, less dense and larger than products made from hard textured wheats (D'Appolonia, 1993).

Although general assumptions can be made about the effect of kernel texture on the quality of soft wheat, the relationship of this quality characteristic to other quality parameters, such as protein and kernel appearance, complicates the prediction of end-use quality (Finney, Yamazaki, Youngs and Rubenthaler, 1987). The exact effect of kernel texture on the biscuit-making quality of soft wheat could be clarified by the development of near-isogenic lines that differed with regard only to kernel texture. Heritability of kernel texture in wheat has been studied extensively (Symes, 1961, 1965 and 1969; MacRitchie, 1980; Yamazaki and Donelson 1983; Greenwell and Schofield, 1986 a,b). Although it has been generally concluded that kernel softness is inherited simply and is directly controlled by one or two major genes and perhaps one or more minor genes, different studies suggest that other genes may also be involved.

The purpose of this study was:

1. To determine the segregation ratios of softness genes in wheat.
2. To determine the effect of softness genes on the biscuit-making quality of soft wheat.

# CHAPTER 2

## LITERATURE REVIEW

### 2.1 The inheritance and expression of kernel texture in wheat

Heritability in wheat and in particular kernel texture has been studied extensively. (Symes 1961, 1965 and 1969, MacRitchie 1980, Yamazaki and Donelson 1983; Greenwell and Schofield 1986 a,b). These authors have all concluded that kernel softness is inherited simply and that it is probably directly controlled by one or two major genes and perhaps one or more minor genes.

Different studies suggest that other genes might also be involved, but the identification of a major gene has stimulated the search for a chemical explanation of grain texture - a quality characteristic that is used to distinquish between wheat classes in world trade and that is also an important indication of end-use quality (Du Cros, MacRitchie and Wrigley, 1990).

In an effort to determine the inheritance of quality in a soft and hard wheat, Worzella (1934) used the wheat-meal fermentation test to determine gluten strength (baking strength). He found that soft wheats had a weak gluten, while hard wheats had a strong gluten. When a soft, female parent was crossed with a hard male parent, the $F_1$ had a predominantly weak gluten, but when the parents were reversed, the opposite was found. In the $F_2$ generation, plants exhibited a wide range of variation from the weak to the strong gluten parent. The quality of the $F_1$ seed would therefore depend on which way the cross was made, since the female contributes factors from two nuclei during the formation of the endosperm, while the male contributes factors from only one nucleus (Worzella, 1934). Aamodt, Torrie and Wilson (1935) were of the opinion that the kernel texture of wheat was determined primarily by the climatic conditions under which it was grown and they have proved that inherent differences for kernel texture exist among different wheat varieties. The material that was used to study the inheritance of kernel texture, was classified by assigning values from one to ten, one being completely starchy and ten completely vitreous. The inheritance of kernel texture appeared to be due to the presence of polymeric factors and starchy texture was dominant over vitreous texture (Aamodt *et al.*, 1935)

Davis, Middleton and Hebert (1961) studied the inheritance of protein, texture and yield in wheat and used the pearling test to determine kernel texture. The estimates of heritability for texture varied greatly from population to population. The estimated heritability values obtained for protein were larger in all the populations than for yield and texture. Only one of the four populations that were evaluated, showed a positive correlation between protein and texture (Davis *et al.*, 1961).

Symes (1961) did preliminary work on the inheritance of kernel softness as measured by particle size index. In two crosses between soft and hard wheats, excellent agreement to a 1:2:1 ratio was obtained, while in a backcross programme, further evidence of the action of a single gene was found. An indication of one or more genes acting independently within major groupings and thereby influencing the particle size index was also reported (Symes, 1961).

Extensive research was done to show that the difference in particle size index between a hard wheat and a soft wheat was definitely due to a single major gene. The existence of minor genes which modify the action of the major gene in determining the hardness or softness of wheat grain, was demonstrated (Symes, 1965). In the light of this research it was found that the conversion of a hard wheat to a soft wheat could be achieved by backcrossing. The grain hardness of the new wheat would be influenced by both the hardness of the donor parent and by the degree to which modifying genes are carried over (Symes, 1965).

Heritability of three soft wheat quality characters, namely alkaline water retention capacity, pearling index (an indication of kernel softness) and flour yield was studied by Briggle, Yamazaki and Hanson (1968). It was found that the expected genetic gain for pearling index was very high and it was concluded that selection for any one of the three quality characteristics could be introduced into a soft wheat breeding programme at the $F_2$ level, where genotypes could be effectively screened before expending considerable effort on testing for agronomic characters (Briggle *et al.*, 1968).

Wrigley (1972) suggested that grain hardness is largely determined by the water-soluble material surrounding the starch granule. This material acts as a cementing substance between storage protein and starch. When adhesion is weak, starch is released more cleanly, with less protein adhering than when adhesion is strong, as in the case of a hard wheat. Simmonds, Barlow and Wrigley (1973) investigated the biochemical basis of grain hardness in wheat and presented evidence to suggest that adhesion between starch and storage protein is more important in determining grain hardness than the composition of the protein matrix. They found that the starch granules of hard wheats had a larger amount of water-soluble material of uniform composition associated with them and suggested that this may provide an explanation for greater adhesion in hard than soft wheats. Although it seemed unlikely that any single factor would provide a complete explanation of grain hardness, Simmonds *et al.* (1973) offered adhesion between starch and protein as one important aspect of this phenomenon.

Doekes and Belderok (1976) attempted to identify the chromosomal location of genetic control of a few components of wheat quality, using chromosome substitution lines. In this investigation, the damaged starch content of flour was used as a measure of kernel hardness. Major factors for kernel hardness and increased baking absorption (requiring more water) were identified on chromosome 5D of each of the hard wheats as well as on chromosomes 3B and 7D of another cultivar. The presence of only one of these chromosomes was sufficient to make the wheat hard and to increase baking absorption (Doekes and Belderok, 1976).

Grinding time was used to measure kernel softness in an inheritance study by Baker (1977). This study showed that the difference between a hard wheat and a soft wheat was due to the presence of two major genes and one or more minor genes. However, a single major gene and one or more minor genes accounted for the difference between a hard wheat and a very hard wheat.

Stenvert and Kingswood (1977) investigated the influence of a range of factors on wheat hardness with particular reference to the physical structure of the endosperm protein matrix. Differences in hardness were found to involve the continuity of the protein matrix and the strength with which it physically entrapped starch granules. The primary determinant of wheat hardness was found to be genetically controlled and appeared to relate to factors influencing the degree of compactness of endosperm cell components. Environment and protein content were also of significance in determining the extent to which an ordered structure formed.

Pearson, Rosielle and Boyd (1981) studied the heritabilities of five wheat quality traits for early generation selection and found that pearling resistance exhibited high standard-unit heritabilities (80 percent). This data suggested that selection for pearling resistance would be highly effective at the single plant stage.

Sampson, Flynn and Jui (1983) performed genetic studies on kernel texture in wheat using grinding time and near infrared reflectance spectroscopy to measure kernel softness. Kernel softness was measured in 600 random lines from five crosses and in seven control cultivars of spring wheat grown over two years. The parents of the five crosses represented a range in softness and were themselves from a hard x soft cross. A hard and hard cross gave only hard lines, a medium and soft cross gave mostly soft lines and three soft or medium crosses gave a wide range of softness types that in two crosses suggested a single gene difference (Sampson *et al.*, 1983).

Williams (1986) studied the influence of chromosome number and species on wheat softness. Cereals of different species, varieties and genotypes of diploid, tetraploid or hexaploid genetic constitution were tested for softness using the particle size index method. Diploid types were all very soft, tetraploid wheats all very hard and the combination of AABB with the DD genome in hexaploid wheats resulted in a complete spectrum of hardness, from very hard to very soft.

Sampson and Flynn (1987) measured kernel softness in terms of grinding time and found that a cultivar which was thought to have had medium-hard kernels, was in fact a mixture of soft and hard plants plus a few intermediates. Apparently this was due to the fact that when the plants were originally selected, a heterozygous plant was selected in the $F_4$ generation (one chance in eight). If this was true, segregation in later generations would result in the cultivar yielding a 1:1 mixture of soft and hard lines, however Sampson and Flynn (1987) found a 4:1 mixture of soft and hard lines. They indicated that this was either due to a shift in the proportions of soft and hard lines that occurred during sampling and selection or that the single gene hypothesis was wrong. They concluded that this phenomenon was probably due to a major shift in the proportions of soft and hard components of the cultivar.

O'Brien and Ronalds (1987) studied the heritabilities of small-scale and standard measures of wheat quality for early generation selection. Grain hardness was measured by grinding time and particle size index. It was found that despite the effects of genotype-environment interactions in reducing heritability, the estimates reported indicated that where seed quantity was limited, good average response to early generation selection for quality could be expected using tests to estimate grain hardness, flour protein content and a measure of protein quality.

Lukow, McKenzie and De Pauw (1989) investigated the genetic implications of kernel hardness variation in Canada prairie spring wheats with a view to developing wheats with medium kernel-hardness which could fulfil milling requirements in new overseas markets. Grinding time was used as a measurement of kernel softness in this study. The results of this study suggested that a medium kernel-hardness wheat could be developed only by the accumulation of minor (modifier) genes which either soften the effect of the major gene for hardness or conversely harden the effects of the major gene for soft kernels. Developing a true breeding medium kernel-hardness genotype may involve the accumulation of these minor (modifier) genes in one plant. Selecting for minor genes in plant breeding is difficult because of the low frequency of the desired genotype among the segregating population. It was concluded that a major gene conferring medium hardness properties would be more desirable since a high frequency of segregants would be homozygous for the desired genotype.

Possibly the most significant hypothesis regarding kernel softness inheritance and expression, was initiated by Greenwell and Schofield (1986 b). They demonstrated the presence of a protein with a molecular weight of about 15 000 dalton on the surface of starch granules washed from soft wheats, but not on those from hard-grained varieties. The protein was extracted in the presence of sodium dodecyl sulphate and was identified following sodium dodecyl sulphate gel electrophoresis. Analysis of the starch granule proteins (SGP) showed that all the soft wheats possessed the prominent 15-k dalton band, the hard bread wheats had a faint or very faint 15-k dalton band and the very hard durum wheats lacked the band completely. Glenn and Saunders (1990) supported this theory when they found a 15-k dalton polypeptide from sodium dodecyl sulphate-extracted starch only evident in soft wheat samples. They concluded that the intensity of the 15-k dalton polypeptide band did not necessarily reflect the textural hardness of wheat endosperm.

Robson and Skerritt (1980) did preliminary experiments with an antibody specific for the softness protein and found that it is also present in the endosperm of hard wheats, but often at lower levels than for soft varieties. Probing the grain softness protein with antibodies for various classes of gluten protein, indicated that this starch granule protein is immunologically distinct from the gluten proteins. Du Cros *et al.* (1990) reported that since all these experiments involved water-washed starch granules and not whole flour or wheat meal, it would be important to establish whether the correlation was due to the absence of the softness protein from the endosperm of hard wheats or merely to its distribution between starch granules and wash water during their preparation.

Further research done on this protein showed a heterogenous character which consisted of one or more $\alpha$-amylase inhibitor subunits and a fraction largely composed of a previously uncharacterised polypeptide(s) referred to as the "grain softness protein" (GSP). Jolly, Rahman, Kortt and Higgins (1993) used an antiserum specific for GSP to show that GSP accumulated in both hard and soft wheat grains, but the GSP in soft grains associated more strongly with starch granules, than the GSP in hard grains. A positive correlation between grain softness and the accumulation of GSP in the seed was demonstrated, which differed from the qualitative relationship, based on the isolated starch fraction, between GSP and grain softness that had already been reported. Analysis also showed that the accumulation of GSP in the seed was dependent on the short arm of chromosome 5D, which also encodes the *Ha* locus. Examinations of near-isogenic lines differing in hardness indicated that the gene(s) controlling GSP, was (were) linked to the *Ha* locus. All of these findings indicated that GSP may be the product of the *Ha* locus and therefore may be the major factor that determines the milling and ultimately also the baking characteristics of wheat.

## 2.2 Quantifying kernel texture in wheat

Tests for determining kernel texture in wheat can either utilize single kernels or bulk samples. Single kernel tests can either be done on the whole grain or on a section of the grain. These tests may include the penetration, abrasion, crushing or cutting of wheat kernels. Tests on bulk samples measure the power or time required to grind the kernels, resistance to grinding, percentage of abraded material formed and the particle size of the abraded material.

Obuchowski and Bushuk (1980) questioned the practical application and low reproducibility of these tests due to variability among kernels, as well as variability among various parts of the endosperm. Significant differences can be expected between methods that rely on pearling the kernel, which depend strongly on bran properties and methods based on milling properties, which depend mainly on endosperm characteristics.

### 2.2.1 Bulk sample testing

The testing of bulk samples of wheat for kernel texture involves the use of mechanical procedures during which failure is caused under four different kinds of stress: tension, compression, shearing and bending. A hard wheat kernel requires more force to be fractured, maintains a larger particle size, passes through sieves more easily and has more damaged starch in the resultant flour than a soft wheat kernel (Anjum and Walker, 1991).

Instruments defining texture by measuring some physical property of the wheat as it is ground, include those measuring abrasion, energy needed to grind and time to grind. Methods defining texture by measuring a property after it is ground, are usually measuring some aspect of the resulting particle size distribution, since harder wheats have a larger mean particle size after grinding than softer wheats (Norris, Hruschka, Bean and Slaughter, 1989). Near-infrared reflectance (NIR) spectroscopy provides a rapid measurement of certain compositional factors of a ground sample of grain. The reflectance signal is affected by particle size (near-infrared absorption increases with particle size) and particle size of wheat increases with hardness. The NIR method can be used to give an indication of kernel texture as well as other factors relating to flour composition.

Williams and Sobering (1986) reported on a collaborative study that was undertaken to test wheat for hardness, using near-infrared spectroscopy, particle size index (PSI) and a grinding/sieving method as the test procedures. Nine collaborators assisted in the project and their results intercorrelated with an average coefficient of 0.995. The study indicated that the PSI test could clearly distinguish between wheat varieties of different textures. Grinders affected the results and the actual results obtained for individual samples in some laboratories differed widely from others, despite their excellent overall correlation. Although very precise, the PSI test is not very fast and takes about 20 minutes, which makes it unsuitable for use at receival points. The technique is very sensitive to variations in mean particle size, shape and particle size distribution. Norris *et al.* (1989) concluded that the near-infrared reflectance procedure provided a score that separated durum from all other classes. It also separated soft red winter, soft white winter and club varieties from all other classes. It did not, however, distinguish hard red winter from hard red spring and it did not distinguish within the soft wheat classes, neither could it detect mixtures.

Sampson *et al.* (1983) measured kernel texture in 600 random lines from five crosses and in seven control cultivars of spring wheat grown over two years, using grinding time and near-infrared reflectance spectroscopy. Grinding time was determined by the method of De La Roche and Fowler (1975) and is the time in seconds required to pass 20g of seed through a Wiley laboratory mill fitted with a 28-mesh screen. Time was manually determined, using a stop watch. The results represented the mean of two grindings per plot. It was concluded that both methods clearly differentiated between soft and hard cultivars. Grinding time was found to be the most accurate method, since it gave lower coefficients of variation and higher correlations between years, but it also required five times more grain than near-infrared reflectance spectroscopy.

Pomeranz, Afework and Lai (1985) evaluated four methods used to determine wheat texture: time to grind, resistance to grinding, particle size index and near-infrared reflectance. Twelve soft red winter varieties and 12 hard red winter wheat varieties that differed widely in texture, were used to evaluate the methods. There was little, if any overlap in analytical hardness parameters, but none of the methods could be used to determine precisely the admixture of small amounts of soft to hard wheats or hard to soft wheats. The estimation of the amount of admixed wheat depends on the hard : soft wheat ratio, hardness characteristics and number of wheat varieties in the blend as well as the method used to determine kernel texture.

Obuchowski and Bushuk (1980) compared several methods of wheat texture evaluation and concluded that the wheat hardness index and the flour yield obtained on the two-step Brabender Hardness Tester and the wheat hardness index from the one-step Brabender Hardness Tester, provided a rapid and sensitive measure of the physico-mechanical properties of wheat related to texture. On the basis of these results, the cultivars were properly grouped in wheat classes of known hardness. The two-step Brabender Hardness Tester is an apparatus where the first burr mill is used to produce a cracked grain product of fairly uniform particle size for the measuring (second) grinder, which is connected to a farinograph torque measuring and recording device. In the one-step Brabender Hardness Tester, the grinder was connected to a farinograph dynometer.

Alternative methods, for instance the particle size index, average particle size and the energy input on the two-step Brabender Hardness Tester, ranked the wheat classes in proper order, but were either less sensitive or more time-consuming. The pearling resistance index did not rank the wheat classes in the same order as the other methods that were evaluated. This discrepancy is presumed to be the result of differences in bran properties. Results of some of the methods evaluated were significantly influenced by the moisture content; the best discrimination was achieved at an "optimum" moisture content. This is an important factor which will be discussed in more depth later.

Williams (1979) screened two series of wheats, which varied widely in protein content and hardness, with near-infrared reflectance spectroscopy and concluded that the analysis of hard wheats was more accurate than that of soft wheats. The high starch content of soft wheats and their floury nature may interfere with protein measurement more than variations in mean particle size do. It was found that both the particle size index and the protein predictability was satisfactory for screening early generations of wheat for protein and texture in breeding programmes.

Anjum and Walker (1991) reported on a more recent technique, where starch, protein and water solubles are reconstructed and compressed into small tablets, which can then be crushed. This permits the study of the effects of individual constituents by selecting the source prior to reconstruction. The tensile strength of reconstructed flour tablets gave fair correlations with other grain hardness procedures. Davis and Eustace (1984) used the scanning electron microscope to provide visual evidence of the great variability in the milling properties of different classes of wheat under commercial milling conditions. The visual evidence produced by this study supports laboratory-scale studies that previously indicated that hard wheat endosperm and soft wheat endosperm have quite different patterns of disintegration. It was suggested that soft wheat endosperm is more readily removed from its bran than is the endosperm from the hard wheats. Disintegration of the soft wheat endosperm is also more quickly accomplished, a fact confirmed by the requirement of soft wheat mills for increased sifting surface areas early in the mill flow.

### 2.2.2 Single kernel testing

The evaluation of single wheat kernels for texture requires more advanced equipment and sophisticated techniques than for bulk samples, mainly because of the limited availability of experimental material. Pomeranz, Martin, Rousser, Brabec and Lai (1988) determined the hardness in 33 samples representing varieties from six wheat classes. Individual kernels of various sizes and moisture contents were evaluated by a specially designed compression instrument equipped with a semi-automated kernel feeder. Software was developed to automatically compute, print and analyze the data. Estimation of the amounts of soft and hard wheats in a blend was affected, among other things, by the wide heterogeneity in hardness among individual kernels in a variety or class. The range in texture among kernels within a variety or class was found to be larger than the difference between individual hard kernels of a soft wheat and soft kernels of a hard wheat.

An inexpensive, fast and simple method was developed by Mattern (1988) for texture evaluation of single wheat kernels. Wheat grains were crushed and viewed with a dissecting microscope after which a hardness index was established with ratings from one to ten (very soft to very hard). Crushed soft wheat endosperm exhibited no apparent cell structure, as opposed to hard wheat which broke sharply along cell walls and across endosperm cells, to produce angular pieces. Although rating with a microscope can be a subjective test, no difficulty was experienced differentiating between true hard and soft types and a single soft kernel could readily be identified in a true hard wheat sample.

Williams and Sobering (1986) found that the coefficient of correlation for the microscopic hardness test versus the particle size index, near-infrared reflectance and damaged starch were -0.94, -0.95 and 0.93 respectively. Spillman (1989) used resistance to shearing as a technique for arriving at an objective evaluation of the texture of an individual kernel. The tester consisted of a feeding device which delivers kernels to a rotating plate, where they fall into holes which orientate them for slicing by a rotary cutting edge. The force on the cutting edge is then recorded at intervals, providing an almost continuous record during the slicing event. Parameters measured during the cutting event are used to determine the hardness of individual kernels in a maximum of a 300-kernel sample.

Eckhoff, Supak and Davis (1988) designed an instrument which achieved texture evaluation by shearing individual kernels and recording the associated force breakage curves, allowing continuous data acquisition via computer. The results were affected by variations in kernel moisture content, size and orientation during cutting. Slaughter (1989) investigated an alternative method where an acoustical technique was used to analyze the sounds emitted during the rupture of wheat kernels as a measure of individual kernel texture, and found the method to be successful in over 80 percent of the cases when mixtures of hard and soft wheat had to be detected. Digital image analysis has also been used to distinguish between the starch granules of hard and soft red winter wheats and proved to be useful in assisting plant breeders in selections (Zayas, Bechtel, Wilson and

Dempster, 1994). When considering the wide spectrum of tests available to measure kernel texture, it is useful to remember that each method or test is influenced by variables peculiar to the equipment used (Miller, Afework, Pomeranz, Bruinsma and Booth, 1982). In order to diminish the amount of variance present in these tests, researchers have tried to optimize existing techniques through modification and combinations with other methods, but the particle size index (PSI) and variations thereof still appear to be the most widely used method for distinguishing between hard and soft wheat varieties.

## 2.3 Non-genetic factors that may influence the expression of kernel texture in wheat

Symes (1961) drew attention for the first time to the confusion of hardness, strength and protein content in the literature. Another factor which can be misleading when dealing with kernel texture, is the morphological appearance of wheat kernels. Finney *et al.* (1987) found that vitreosity in soft wheat has been a cause of misapprehension on the part of a number of workers who have been led to believe that all vitreous grain has hard wheat milling and baking properties. This belief apparently arose because in the past almost all vitreous grains were hard wheat cultivars and soft wheat cultivars were usually grown in areas in which low protein, and hence mealy kernels, was the rule.

### 2.3.1 Wheat protein

The word *protein* was proposed, with the meaning of primary substance, around 1838 - long after the acceptance of the terms *gluten, gliadin* and *albumin*. Gluten was actually one of the first proteins to be studied because it can be readily prepared (by washing of dough) as a reasonably pure protein. Because of the importance of gluten, much of the variation in quality among wheat samples can be explained in terms of its quantity and quality. Total protein content is generally taken as an indication of gluten quantity, although about 20% of grain protein is non-gluten, including the range of enzymes and metabolic proteins (Wrigley, 1994).

Gluten consists of two components, namely glutenin and gliadin. When protein composition of wheat is electrophoretically analyzed, glutenin will appear at the top of the gel as high molecular weight (HMW) subunits. The HMW subunits represent only about 25% of glutenin, the remainder being the low-molecular-weight (LMW) ones that appear further down the SDS gel pattern. Gliadin is the other major constituent of wheat gluten. Unlike the glutenin subunits, the gliadins form most of their disulfide bonds intramolecularly, leaving them essentially monomeric (non-aggregated with respect to covalent bonding). The ratio of monomeric to polymeric gluten proteins (thus gliadin to glutenin) is an important determinant of dough properties; the large aggregates apparently determining resistance to extension and the smaller gliadins contributing plasticity. The dough properties provided by the gluten proteins in the mature grain are determined by the genotype (built in by the breeder) and by growing conditions. The environment may alter protein composition at all stages after gene expression, with respect to quantities of polypeptides synthesized and the ways in which they associate to produce the combination of aggregated and less aggregated gluten proteins (Wrigley, 1994).

Wheat starch comprises large lenticular (A-type) and small spherical (B-type) granules, with some intermediate granules (underdeveloped A-type), composed of two structurally different polysaccharides, amylose (20 - 30%) and amylopectin (70 - 80%). In addition plus small amounts of lipids, nitrogen and phosphorus are present (Anjum and Walker, 1991). Simmonds *et al.* (1973) presented evidence that suggested that the adhesion between starch and the storage protein in the wheat endosperm is more important in determining grain hardness than the composition of the protein matrix. It was also shown that the starch granules of hard wheats have a larger amount of water-soluble material of uniform composition associated with them, which may explain the greater adhesion in hard wheats than in soft wheats. Anjum and Walker (1991) suggested three basic mechanisms of grain hardness:

a) chemically induced adhesion between the protein matrix and starch granule;
b) continuity of the protein matrix
c) and the net charge on the protein.

a) Greenwell and Schofield (1986 a) demonstrated an unbroken positive association between the presence of a $M_r$ 15K starch protein and endosperm softness, the dominantly inherited type of endosperm texture, for some 150 wheats of widely different genetic backgrounds. It was also suggested that since this protein associates with the surface of the starch granules, it may have some sort of "non-stick" property that reduces the adhesion between the starch granule and the protein matrix of the endosperm.

b) Stenvert and Kingswood (1977) found that the extent to which the endosperm structure is ordered could determine hardness. They felt that this would be dependent primarily on the state of the protein matrix which functions as the connecting matter within mature endosperm cells. A continuous protein matrix physically entrapping the starch granules would result in difficulty in separating the starch granules from the protein as is characteristic in hard wheats. A discontinuous matrix structure would allow the ready release of starch granules as found with soft wheats. Seckinger and Wolf (1970) studied endosperm from hard and soft wheats with an electron microscope and found that differences between protein particles of hard and soft wheats existed. Particles from hard wheat were found to be compact structures difficult to disrupt, whereas the protein particles from soft wheat were expanded and easy to disrupt.

c) Anjum and Walker (1991) proposed another mechanism in which hardness is caused by the wheat protein fractions that have a charge. If the net charge of these proteins is high, the proteins will repel each other and the grain will be soft. If the net charge is low, there is no repulsion and the grain is hard.

According to Williams (1979), the amount of protein incorporated in the wheat kernel is controlled to a great extent by environmental factors. Weather conditions during maturation, soil nitrogen status, cultivation practice in general and the use of fertilizers account for about ninety five % of the reasons underlying variance in the protein content of wheat. Miller, Pomeranz and Afework (1984) investigated whether wheats retained their inherent hardness characteristics when they are grown in areas where they may not be ideally adapted. They concluded that correlations between hardness and protein content were either very low or totally insignificant. Pomeranz, Peterson and Mattern (1985) also found that grain hardness and protein content was not correlated when it was calculated for 15 varieties over 11 localities. Hong, Rubenthaler and Allan (1989) supported these findings when they reported that harder grains may be attributable to the effects of environment on the responsive changes of water-soluble pentosans and endosperm protein levels. Pomeranz, Czuchajowska, Shogren, Rubenthaler, Bolte, Jeffers and Mattern (1988) concluded that the wheat milling hardness score was correlated with protein content, a reflection of the fact that hard wheats possessed high protein levels, rather than that protein and hardness were related. It is worthy to note that in all of the cases the methods that were used to determine kernel texture, were either particle size index or near-infrared reflectance spectroscopy, the correlation of which was highly significant.

#### 2.3.1.1 Kernel morphology vs. kernel texture vs. protein content

Moss (1978) declared that hardness in wheat was associated with vitreous appearance, although Parish and Halse (1968) proved that samples of wheat grain of the same genotype at the same protein level differed markedly in translucency according to environmental conditions during grain filling and grain desiccation. Vitreosity is the degree of translucency shown by wheat kernels and its measurement is essentially subjective, although efforts toward objectivity has been made (Yamazaki and Donelson, 1983). Anjum and Walker (1991) concluded that a vitreous (translucent or hornlike) appearance was generally associated with hardness and high protein content and opaqueness (mealiness or flouriness) with softness and low protein content. Hard wheats generally have high protein contents and tend to be vitreous, but the *causes* for hardness and vitreousness are different (Anjum and Walker, 1991). Vitreous character is the result of a lack of air spaces within the kernel. Air spaces make the opaque grain less dense and are formed during grain drying. The protein shrinks, ruptures and leaves air spaces upon drying, whereas in vitreous kernels, the protein shrinks, but remains intact.

Miller *et al.* (1982) pointed out that in marketing channels, wheat hardness is judged by appearance rather than an objective test.

### 2.3.1.2 Factors affecting protein content

#### 2.3.1.2.1 Season, Location and Climate

Trupp (1976) found that the effect of the environment, in general, was much higher on protein percentage, than on kernel texture. He used the example of a cultivar, which had consistently higher than average protein levels, but also a softer than average kernel texture. The milling and baking industries accepted this cultivar, which proved that a higher level of protein could be tolerated in new cultivars of pastry quality wheats, if that protein was in a form which did not interfere with quality parameters. Miller *et al.* (1982) also suggested that unknown factor(s) in the environment may affect the hardness of wheat, but most importantly found that samples from irrigated plots had a consistently higher protein content than samples from non-irrigated plots.

Miller *et al.* (1984) wanted to determine whether wheats retain their inherent hardness characteristics when grown in areas where they were not ideally adapted. The found that protein content was not consistently different, but wheats from the soft and hard classes grown in the "hard wheat area" were higher in protein than wheats grown at other locations. Baenziger, Clements, McIntosh, Yamazaki, Starling, Sammons and Johnson (1985) detected highly significant differences among environments and cultivars for whole grain protein percent as well as other quality parameters. Pomeranz *et al.* (1985) also proved that the effects of location were larger than those for variety on protein content. Pomeranz and Mattern (1987) indicated great stability among varieties for hardness characteristics and large variability for protein content when they determined the environmental effects on hardness of hard red winter wheat. Rao, Smith, Jandhyala, Papendick and Parr (1993) concluded that a general rise in temperature resulted in higher protein contents, when they investigated fluctuating protein levels of wheat grown in the Pacific Northwest.

#### 2.3.1.2.2 Fertilization

Boquet and Johnson (1987) studied the effects of fertilizer on yield, grain composition and foliar diseases of doublecrop soft red winter wheat. It was found that nitrogen, at the rates applied in their study (0, 34, 56, 78 and 101 kg $ha^{-1}$), did not affect grain protein content but did increase total protein per hectare by increasing yield. Phosphorus and potassium had no effect on grain protein or mineral composition.

Bruckner and Morey (1988) also studied the effects of nitrogen on soft red winter wheat yield, agronomic characteristics and quality. Nitrogen was applied at rates of 0, 33.6, 67.2, 100.8 and 134.4 kg N $ha^{-1}$. Nitrogen and cultivar interaction was important only for grain protein content. Nitrogen rates in excess of 67 kg N $ha^{-1}$ contributed to undesirable grain protein increases, which led to poor milling and baking quality.

#### 2.3.1.2.3 Plant physiology

Huebner and Gaines (1992) commented on the increase of variation in kernel hardness, which made classification difficult. To assess the effects of growing conditions on protein composition and hardness, wheat grown in a greenhouse and commercial field-grown wheats were examined. Mature kernels from greenhouse plants were harvested and segregated according to origin from wheat heads. Differences in hardness among single kernels of a cultivar could have resulted from variation in protein synthesis in kernels from different head locations, from variation between heads of the same plant that developed at different dates and from multiple biotypes within cultivars.

### 2.3.2 Moisture

Anjum and Walker (1991) reported that grain moisture content and kernel hardness measurements were well correlated. Although opposing results were represented, it would appear as though the moisture effect was more pronounced in soft than in hard wheats. Softness increased in soft wheats at higher moisture levels, but in hard wheats showed little response to high moisture (Orth, 1977). Yamazaki and

Donelson, (1983) found high positive correlation coefficients between particle size index and the moisture content of wheat samples within a soft wheat cultivar.

### 2.3.3 Lipids

Anjum and Walker (1991) studied lipids in wheat starches and found that the surface lipids were mostly free fatty acids in amounts correlated with starch granule surface area. The true (internal) lipids are lysophospholipids and appear to be correlated with amylose content. Starch lipids form complexes with amylose and modify some starch granule properties. More recent studies have also indicated that free polar lipids are associated with increased endosperm softness.

### 2.3.4 Delayed harvest

Pool, Patterson and Bode (1958) investigated the effect of delayed harvest on quality of soft red winter wheat. In the eastern United States, harvesting is frequently delayed by rains. Changes in chemical and physical properties of the kernels and changes in milling and baking properties during the post-maturity period were studied to determine the nature and extent of the changes and to examine the differences in varieties and the rate of change in different varieties. It was found that soft red winter wheats increased significantly in kernel softness over a delayed harvest period of about 45 days.

## 2.4 The effect of kernel texture on the milling quality of wheat

The products manufactured from wheat determine its quality requirements, and in the case of soft wheat, they are mainly pastries, cakes, wafers, biscuits, biscuits and variations thereof. Finney *et al.* (1987) defined soft wheat of good milling quality as a wheat that should fracture into particles of significantly smaller median diameter than hard wheats, but not be so soft as to cause poor flowability through ducts or inhibit proper bolting (sieving). It should be low to medium-low in protein content and should have a high weight per bushel (depending on class). High flour yield, normal flour ash content and minimum power requirement are important prerequisites for good milling quality. Bingham (1961) noted that grain quality in the case of *Triticum aestivum*, should be considered as a "complex of characters".

He also stated that milling quality was determined principally by the cellular structure of the endosperm.

Minor (1966) observed that nearly all soft wheat millers produced flours designed especially for the production of pies, pastries and biscuits, all of which products could be made from the relatively wide range of flours that is offered to bakers under various brand and type designations. The trend towards more complete automation, especially in the larger bakery plants, has brought about a corresponding reduction in the bakery's flexibility of production and has created the need for a larger number of flours with more clearly defined functional characteristics and greater uniformity between shipments. The wide variations in performance of soft wheat biscuit flours are attributable to four primary factors, namely: the blend of wheat varieties used in milling; the degree of extraction; flour granulation and chemical treatment, or its absence, of the flour. In general, the miller is required to meet certain biscuit flour specifications that the bakers consider essential to their production efficiency and product quality. These specifications are based on a series of tests which the miller can apply to control the properties and uniformity of the biscuit flour he produces.

### 2.4.1 Testing for milling quality

Yamazaki (1959 a,b,c) reported that flour testing was done mainly on an empirical basis. In spite of several physico-chemical tests currently in use, none were as satisfactory as a test bake of the actual product for which the flour is to be used. The major tests used by millers to control the properties of the biscuit flour produced, are as follows (Minor, 1966):

#### 2.4.1.1 Ash

The level of mineral substances, or ash, present in the flour is considered primarily an index of the flour grade or degree of refinement (Minor, 1966). Kaldy and Rubenthaler (1987) found the ash content of a spring wheat flour to be lower than that of a winter wheat flour, which indicated a higher soft wheat quality. Significant correlations were also found between ash content and cake crumb grain (pooled correlation coefficient = 0.48), as well as falling number (pooled correlation coefficient = 0.55) ($p \leq 0.05$).

### 2.4.1.2 Viscosity

This property is another indicator of the flour's strength, with the viscosity of flour batters prepared under standardized conditions increasing as the flour's protein content increases. The viscosity value, a measure of the extent of protein swelling in a lactic acid medium, is influenced by the quantity of protein as well as soluble ash (Finney *et al.*, 1987). Viscosity gives no insight into the flour's baking characteristics (Minor, 1966). Kaldy and Rubenthaler (1987) reported that lower viscosity readings, usually meant less resistance, which indicated better soft wheat quality. Finney *et al.* (1987) concluded that for most soft wheat applications, it would appear that a low adjusted viscosity value is desired in cultivars, but in some cases, such as saltines, optimum values may be higher.

### 2.4.1.3 Protein content

Minor (1966) reported that a flour's protein content is generally taken as a measure of its strength. Finney *et al.* (1987) supported this by remarking that in the United States, the soft wheat industry requires low protein content in order to maximize product tenderness. Kaldy and Rubenthaler (1987) reported that higher protein content resulted in greater viscosity and smaller biscuit spread with firmer, tougher cake textures. It was also demonstrated that biscuits with the same protein content could differ in size, when one was baked from spring and the other from wheat cultivars. The spring wheats yielded somewhat smaller biscuits, which indicates that protein quantity is not necessarily the only cause of smaller biscuit size, increased viscosity, lesser cake volume or heavier cake crumb structure. These results contradicts earlier findings by Minor (1966), who found very little correlation between protein content of soft wheat and quality.

A recent approach to breeding is to seek experimental lines that break the traditional protein-product performance relationship by having higher protein but performing as those wheats with lower protein content. This approach may have merit for regions exporting soft wheat to nations where protein is needed in the diet and where wheat represents a large portion of food intake (Finney *et al.*, 1987). Testing grain for protein content is a basic procedure, generally carried out using the Kjeldahl methods or near-infrared reflectance spectroscopy.

#### 2.4.1.4 Alkaline water retention capacity

The alkaline water retention capacity test is a standardized method of measuring the water retention ability of a flour against centrifugal force. It is recognized that the lower the percent of water retained, the better the pastry quality (Kaldy and Rubenthaler, 1987). Results of these tests have been found to be inversely correlated ($p < 0.05$) with biscuit quality as determined by the biscuit baking test. A microversion of this physicochemical test has thus been applied to an early generation screening evaluation programme (Finney *et al.*, 1987).

#### 2.4.1.5 Falling number

Falling number indicates sprout damage. The enzyme $\alpha$-amylase, synthesized in the grain has the ability to liquefy the starch. The falling number apparatus measures the time in seconds required for a plunger to fall through the flour slurry after stirring for 60 seconds in a boiling water bath. The higher the value, the lower the enzyme activity and the better the flour quality (the falling number should not exceed 400 units). At higher enzyme activity a greater portion of the starch is liquefied and the plunger falls faster (Kaldy and Rubenthaler, 1987).

#### 2.4.1.6 $\alpha$-Amylase test

The Cibacron method measures $\alpha$-amylase activity in cereals and a higher value means a higher enzyme activity and poorer flour quality (Kaldy and Rubenthaler, 1987).

#### 2.4.1.7 Mixograph

Mixograph absorption reflects the optimum amount of water required to produce a dough of optimum consistency for handling and baking performance. Lower absorption indicates better quality for pastry flour according to Kaldy and

Rubenthaler (1987). It was found that within a variety, flours with higher protein content produce curves of larger areas and the usual method for comparing flours is to apply an area correction for protein content. This is done because the effect of protein content on mixogram area is greater than its effect on biscuit spread (Yamazaki, 1959 c). A significant correlation between mixograph and sponge cake crumb grain and cake score was found by Kaldy and Rubenthaler (1987).

#### 2.4.1.7 Farinograph

The farinograph, an instrument used to determine the absorption and mixing requirements of flour, principally for bread purposes, is also useful in determining flour absorption as related to biscuit potential. It has also been applied to evaluate flour for specific soft wheat products in certain private laboratories, but has not been extensively used in breeding programmes, possibly because of the need for larger size samples than for most other tests for early generation evaluation and because of the relative high cost of the apparatus (Finney *et al.*, 1987).

Finney and Andrews (1986) reported that in an effort to save time and money, microtests had been developed to predict important milling and baking qualities so that undesirable lines could be eliminated before reaching an advanced generation. Andrews, Blundell and Skerrit (1993) developed an antibody-based method for discrimination of wheat flours or whole meals on the basis of differences in dough strength and modified it for use in large-scale screening to predict dough quality. Highly significant correlations were reported between color developed in the assay and rheological measurements of dough strength, such as farinograph development time and extensograph maximum resistance.

#### 2.4.1.9 Particle size

Minor (1966) reported that granulation or particle size was, in the past, equated with different flour grades. The methods employed on soft wheat milling, the type of wheat being milled and the particular mill stream selection, all tend to produce flour that has smaller average particle size than is the case with flour made from hard wheats. As a rule, the softer the wheat, the higher the percentage of fine particles produced during milling and the greater are the differences in biscuit spreads obtained with the fine and coarse fractions of the parent flour. Harder wheats produce smaller amounts of fine particles and the difference in biscuit spread between fine and coarse fractions is found to be smaller (Minor, 1966). Yamazaki (1959 a) reported that a mixture of coarse and fine fractions baked biscuits larger than would be expected from a calculation of the weighted mean diameters of the fractions. This augmentation of spread, called the interaction effect, had its maximum value when approximately equal quantities of coarse and fine fractions were present. Yamazaki (1959 b) also reported that flour granularity as measured by yield of fine fractions appeared to be a varietal character unaffected by protein content within a variety. He concluded that factors other than flour granularity are also important in determining biscuit quality.

### 2.4.1.10 Break flour yield

Kaldy and Rubenthaler (1987) reported that the milling industry claim to recover a higher quality flour fraction from soft wheats than hard wheats. Significant correlations were also determined between break flour and biscuit diameter, cake volume and external cake factors. Yamazaki and Donelson (1972) found flour granularity as determined by the particle size index (PSI), to be highly correlated with break flour yield during milling and with cake quality potential. Rogers *et al.* (1993) concluded that soft wheats produced higher percentages of break flour and bran than hard wheats.

### 2.4.1.11 Starch damage

Stenvert (1972) first reported that the starch of soft wheats remains relatively unaffected by the milling process. Rogers *et al.* (1993) investigated the milling and biscuit baking quality of near-isogenic lines of wheat that differed in kernel hardness. They found that the wheats that had been classified as being hard, had greater amounts of starch damage after milling than did the soft wheats. Moss, Edwards and Goodchild (1973) investigated tests for softness in flour and found that starch damage was significantly related to each aspect of processing quality at most of the sites in the experiment, with protein quantity less frequently involved.

### 2.4.1.12 Separation of the bran from the endosperm

Everson and Seeborg (1958) reported on a technique where the separation of the bran from the endosperm meal is used as a measure of milling quality. Poor milling samples have high bran weights because of adhering endosperm, whereas low bran weights are indicative of good separation of bran from endosperm and thus, high flour yield. Yamazaki and Andrews (1982) called this method the ESI (endosperm separation index) test and found that it could be calculated before milling was completed, making it at least partially independent of flour yield and quality. It was found to be unrelated to kernel texture or temper level (within reasonable limits) but appeared to be associated with inherent wheat properties, making it a varietal trait.

### 2.4.2 Factors influencing milling quality and the testing thereof

Several authors have reported that milling quality of soft wheat and the tests designed to measure it, is highly dependant on the kernel texture of wheat. It would therefore appear, that the factors that may influence the expression of kernel texture in wheat, would also effect the milling quality.

### 2.4.2.1 Nitrogen fertilizer

Baenziger *et al.* (1985) studied the role of time and rate of nitrogen fertilization in influencing milling and baking quality of a soft red winter wheat. It was found that the location-years interaction played a major role in affecting the milling and baking quality. Nitrogen applications at the recommended rate in the spring did not adversely effect the overall milling and baking quality, except at one location, during one year. The trend towards higher protein content with increased nitrogen rate may result in deterioration in milling and baking quality. Cultivar differences in such responses may be of increasing importance to breeders as management levels intensify in small grains.

### 2.4.2.2 Effect of pre-ripe harvest and artificial drying

Kirleis, Housley, Emam, Patterson and Okos (1982) evaluated several quality factors, including test weight, protein and ash content, kernel texture, milling quality, alkaline water retention capacity and biscuit baking. It was concluded that any tendency to combine soft red winter wheat at moisture levels above 25 to 30% would result in a large percentage of broken kernels and lower milling and baking quality as compared to fully combined ripe wheat. Finney, Gaines and Andrews (1987) supported this data by stating that if wheat was harvested at too high a moisture content, insufficient seed germination could result; which could be highly detrimental to the quality of some soft wheat products, such as soups, batters, gravies and fermented crackers.

### 2.4.2.3 Effect of preharvest sprouting

Damage due to preharvest sprouting can cause major economic losses in regions where precipitation occurs frequently at harvest time (Sorrels, Paterson and Finney, 1989). Research was conducted to evaluate the effects of preharvest sprouting on milling and baking characteristics of resistant and susceptible soft white genotypes subjected to conditions inducing preharvest sprouting. Flour protein, sugar-snap biscuit diameter, ash content, kernel hardness and top grain were not affected by any of the treatments. The effects of preharvest sprouting on the tested soft wheat milling and baking characteristics were relatively minor, even with high levels of sprouting damage.

### 2.4.2.4 Genetic effects

Everson and Seeborg (1958) used the separation of the bran from the endosperm to measure milling quality and found that this specific quality characteristic was controlled by more than four major factors. May, Sanford and Finney (1989) investigated a single cross between a soft red winter and a hard red winter population.

The results of the study indicated that hard wheat cultivars could be used in a soft wheat breeding programme as sources of new germplasm in an effort to achieve acceptable milling and baking quality.

## 2.5 The effect of kernel texture on the baking quality of wheat

Finney *et al.* (1987) defined the prerequisites for good soft wheat quality as the conformity of such flours to the baking performance of widely grown commercial cultivars. It is generally agreed in the industry that such cultivars are satisfactory. Thus, a flour of good quality should have a low to medium-low protein content and a low water absorption, and should bake large-diameter test biscuits and large-volume cakes, both with good external appearance and satisfactory internal grain structure. It should also bake good quality saltines and flat breads, as well as make good non-baked products, such as noodles.

### 2.5.1 Evaluating baking quality

#### 2.5.1.1 Biscuit test

Biscuit doughs are cohesive, but lack the extensibility and elasticity of bread doughs. Relatively high quantities of fat and sugar allow dough plasticity and cohesiveness without the formation of a gluten network. Wade (1972) determined that the addition of sodium metabisulphate (SMS) reduce the elasticity of the doughs. The purpose of adding such an agent would be to facilitate the production of a uniform sheet of dough from which biscuits may be cut. Depending on the formulation, biscuit dough tends to become larger and wider as it bakes, rather than to shrink as does cracker dough. This increase in size, or "spread", is the greatest single problem in process control (Faridi, 1990). Finney, Morris and Yamazaki (1950) described a biscuit baking test, where only 40 g of untreated flour was required. The principal criterion of quality was the increase in diameter of the product. A flour from which the biscuits were of a larger diameter than those of another was said to be superior in its response to the test. Yamazaki (1955) has shown that the increase in biscuit diameter is inversely correlated with the water absorption requirement of the flour and to a lesser extent with protein content. Both biscuit quality and water absorption capacity are heritable traits. Yamazaki

(1955) investigated a factor in soft wheat flours which affected biscuit quality. It was found that any component of flour which could absorb relatively large quantities of water, would have a detrimental effect on biscuit spread and further that the biscuit baking potential of a flour was determined by the sum of the hydrophillic components, regardless of their chemical composition.

Thompson and Whitehouse (1962) proposed that biscuit wheat should have a low resistance and high extensibility. Flours may be subjectively classified depending on the shape of the graphical relationship between resistance and extensibility. Another way of integrating resistance and extensibility is to take the maximum value of the product resistance x extension (Bingham, unpublished according to Thompson and Whitehouse, 1962). This value should be high for bread wheats and low for biscuit wheats. Finney and Yamazaki (1953) reported on an alkaline viscosity test for soft wheat flours. The acid viscosity test, which had been used previously, was of value, but was found to be inconsistent as an index of soft wheat quality. The alkaline viscosity test gave a more accurate evaluation of varieties. This is probably because the doughs or batters prepared from soft wheat flours have a pH value greater than seven as a result of the chemical leavenings employed. The differentiation or spread between varieties measured by the alkaline viscosity test was about twice that of the acid test. Yamazaki (1953) reported a correlation coefficient of -0.847 for the alkaline water retention capacity vs. biscuit diameter for 506 samples.

Wainwright, Cowley and Wade (1985) showed that flours from soft milling wheats required less water to give biscuit doughs of standard consistency than did flours from hard milling wheats. The effect of flour particle size on biscuits was studied by regrinding a number of flours. With flours from both soft milling wheats and hard milling wheats, reduction in particle size resulted in hard sweet biscuits of higher density and soft sweet biscuits of lower density.

Gaines and Donelson (1985) evaluated biscuit spread potential of whole wheat flours from soft wheat cultivars and found that test baking and biscuit diameter were the best evaluation methods. Cultivars with a soft kernel texture produced larger whole wheat biscuits. Within a cultivar, whole wheat flour biscuit size was significantly affected by flour particle size and moisture content. Gaines (1985) studied associations among several quality parameters across cultivars and found biscuit diameter and cake volume to be positively correlated with soft textured wheats with lower protein content, which produced more break flour and flour having smaller particle size.

Alveography was used in the quality assessment of soft white winter wheat cultivars by Rasper, Pico and Fulcher (1986). This rheological technique was based on subjecting a piece of dough to biaxial extension until rupture. Concern has been expressed about performing a stretchability test on doughs of constant water content without allowing for differences in the hydration capacity of the tested flours. However, the alveograph still proved to be a useful tool in testing and quality ranking of the soft white winter wheats that were tested.

Gaines, Donelson and Finney (1988) found that decreased flour moisture, increased starch damage, longer holding time, warmer dough temperature, increased dough handling and flour chlorination caused doughs to handle as if they were more plastic; these doughs were also stiffer and more cohesive, had a greater consistency, had less flow and adhesion and made smaller biscuits.

In some cases the nature of the end-use product may warrant the development of a new technique for evaluating quality, as in the case of rotary moulded biscuits. Control of biscuit thickness and density are major problems associated with the commercial packaging of rotary moulded biscuits. It is for this reason that a standardized method to evaluate the quality of these biscuits on the basis of biscuit thickness and density was developed by Gaines and Tsen (1980).

Gaines (1990) found that longer mixing time increased the sensory ranking of biscuit hardness, although hardness increased without a significant change in dough consistency. Any gluten developed during mixing was relatively small compared with the increase observed in biscuit hardness. It was concluded that soft wheat proteins functioned by affecting sugar-snap biscuit size, weight and texture without forming an extensive gluten network. Abboud, Rubenthaler and Hoseney (1985) reported that a good correlation was found between protein content and biscuit diameter, although it appeared as though protein content had a minor effect on biscuit spread, compared with the presence of genetic factors.

Kaldy, Kereliuk and Kozub (1993) studied the influence of gluten components and flour lipids on soft wheat quality. Statistical analysis indicated that among the gluten variables, yield of gluten and pentosan in gluten were the variables most associated with biscuit diameter corrected for protein content. However, when the correction for protein was not taken into account, total protein was shown to be negatively correlated with biscuit diameter. Rogers *et al.* (1993) concluded that when protein quantity varied in different wheat samples, the factors associated with kernel texture had a major influence on biscuit diameter, milling characteristics, and starch damage. Among the components of flour lipid, polar lipids had the highest correlation with cake volume. Kissell, Pomeranz and Yamazaki (1971) also found that flours which had been defatted, produced smaller biscuits with reduced top-grain definition. When the unfractionated free lipids were returned, original spread and top-grain quality were restored. Yamazaki, Donelson and Clements (1979) found that the lipids from bran probably increased diameter by the same mechanism as that reported for flour lipids, soy lecithin, and similar emulsifiers in test biscuits. An attractive feature of using bran lipids as emulsifiers to improve biscuit quality, was the relatively low price of the source material. Moreover, the yield of lipids from bran was higher than that from flour. Abboud *et al.* (1985) came to the conclusion that it was not so much the type of fat that was used, but the amount, that affected biscuit spread. Sugar was found to have no influence on biscuit spread, except in non-creamed systems.

#### 2.5.1.2 Sponge cake test

A second test that is used to measure end-use quality is the sponge cake test. Kaldy and Rubenthaler (1987) reported that cakes with greater volume, finer crumb grain structure and softer, more tender texture are considered superior. Sponge cakes are made with a batter system, instead of a dough system as in biscuit production. Flour protein with a strong gluten matrix formation disrupts the foam structure in the batter system (Kaldy and Rubenthaler, 1987). Sponge cake score is negatively correlated with flour protein as protein influences cake volume and crumb properties. Unfortunately, the sponge cake test has not yet been developed for micro-testing (Finney *et al.*, 1987).

### 2.5.1.3 Layer cake test

The white layer cake test is also a baking test that is used in several laboratories, termed high ratio because of the relatively large quantity of sugar in the formulation. The flour used in this test is usually of short extraction, pin milled to reduce average particle size and treated with chlorine gas to a given pH. The evaluation is based on cake volume. Flour fineness closely parallels kernel hardness, which can be measured by the PSI test when applied to only a few grams of grain early in breeding line development (Finney *et al.*, 1987).

Yamazaki and Donelson (1972) found varietal differences in eastern soft wheat cake potential, as measured by the white layer cake test, to be associated largely with inherent differences in endosperm friability. It was also suggested that since such a high correlation was found between particle size index and cake volume, this test, together with protein and alkaline water retention capacity tests, could be used to screen lines early in a breeding program for quality. Chaudhary, Yamazaki and Gould (1981) studied the relation of cultivar and flour particle size distribution to cake volume. It was found that in addition to particle size itself, heritable endosperm-fracturing properties were important in influencing layer-cake quality.

### 2.5.2 Factors affecting baking quality

The factors that may influence soft wheat baking quality, as defined by different tests, are in some cases the same factors that influence milling quality and kernel texture in wheat, since high correlations between kernel texture and quality have been found.

### 2.5.2.1 Nitrogen fertilization

Mixogram areas of the doughs made from various wheats indicate that greater gluten strengths are associated with higher rates, as well as late applications of nitrogen (Long and Sherbakoff, 1951).

### 2.5.2.2 Season and location

Yamazaki and Lamb (1961) determined that although biscuit baking potential of wheat appeared to be a varietal trait, it could be modified in the grain by location or season of growth. In the evaluation of a new wheat for biscuit quality, its baking performance is usually compared with that of one or more standard varieties grown in the same year at the same location. Climatic, soil and cultural variations as well as inherent varietal tendencies may bring about a range in protein content that may also affect the biscuit potential of a flour. It would therefore be rather difficult to compare baking performances of different varieties, unless these variables are eliminated or minimized.

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 Materials

### Parental material

Two spring wheat genotypes with opposing kernel textures were selected to develop near-isogenic lines (NIL's). Edwall is a soft white wheat variety with good biscuit-making quality, but is not very well adapted to the Northern Cape irrigation areas of South Africa. M29519 is a hard red wheat variety with poor biscuit-making quality, but is agronomically well adapted to the Northern Cape irrigation area. The countries of origin and kernel textures and colours of the two parental lines used in this study are listed in Table 3.1.

**Table 3.1** **Countries of origin and kernel textures and colours of the parental lines used in this study.**

| **Parents** | **Country of origin** | **Kernel texture and colour** |
|---|---|---|
| Edwall | U.S.A. | Very soft,white |
| M29519 | Mexico | Very hard, red |

### Development of near isogenic lines

A backcross procedure was used to incorporate the dominant endosperm softness genes from the donor parent, Edwall, to the recurrent parent, M29519.

A schematic representation of the crossing procedure used, is given in Figure 3.1. The parental lines were planted in the greenhouse at the University of the Orange Free State, Department of Plant Breeding. Three seeds were planted to a two litre plastic pot. Environmental conditions were optimized. Three plantings were made at two-weekly intervals to synchronize pollen availability for fertilization. During flowering, several crosses were made. At the end of the growing season the $F_1$ seeds were harvested and stored until the following season.

The $F_1$ seed was germinated in petri dishes at room temperature and planted in two litre plastic pots in the greenhouse during March. It was planted together with the recurrent parental line M29519. Three plantings were made at two-weekly intervals to synchronize pollen availability for fertilization. As soon as flowering started, intended female parents were emasculated and covered with plastic bags. Three days after emasculation the female plants were fertilized using pollen from the male plants. The $F_1$ plants were backcrossed to M29519, the recurrent parental line.

Seeds from both the parents, Edwall and M29519, their $F_1$ progeny and the first backcross (BC1) were harvested separately and evaluated for kernel texture using the method described in **3.2.1.** The results obtained were used to determine the segregation patterns of kernel texture in this particular genetic background. Only soft textured kernels were selected from the seeds of the first backcross and germinated in petri dishes at room temperature before being planted with the recurrent parental line M29519 in the greenhouse. M29519 was backcrossed to the soft textured progeny during the flowering period. This second backcross (BC2) took place during the same year as the first backcross.

The seeds resulting from the second backcross (BC2) were evaluated for kernel texture (**3.2.1.**) and soft textured seeds were selected for the third and final backcross (BC3). These seeds were germinated at room temperature and planted in the greenhouse, with the recurrent parental line M29519, during the first half of the second year. During the flowering period, the plants from the second backcross were backcrossed for the third time to the recurrent parent, M29519.

Edwall x M29519
Soft Hard
(AA) (aa)

↓

BC1 Hybrid (Aa) x M29519 (aa)
(50% M29519)
↓
Soft kernels selected
↓
BC2 Selected plants x M29519 (aa)
(75% M29519)
↓
Soft kernels selected
↓
BC3 Selected plants x M29519 (aa)
(87.5% M29519)
↓
Soft and hard kernels selected
Plants allowed to self pollinate
↓
AA Aa aa

The plants obtained from the selected soft kernels were used as females, while the recurrent hard cultivar, M29519 was used as the male in every backcross.

Figure 3.1 A schematic representation of the backcross procedure followed (Poehlman, 1987).

The seeds from the third and final backcross (BC3) were randomly evaluated and the half kernels that remained after texture evaluation, were surface sterilized and placed on commercial water agar in small tissue culture vessels in a laminar flow cabinet. The vessels were left at room temperature until germination. All abnormal kernels (i.e. air bubbles, uneven endosperm surfaces) that were found, were discarded. This was done to increase the frequency of viable seedlings. Kernels representative of soft and hard texture classes were selected and the seedlings were transplanted to the greenhouse where they were left to self-pollinate. The seed was collected after harvest from the lines consisting of soft and hard kernel texture classes, that were developed, and was then stored in a germplasm bank until the commercial planting season in June.

### Planting procedure

During June, planting commenced at the Northern Cape Agricultural School, which is situated in the Northern Cape irrigation area. The seed was mechanically planted with a precision plot planter (Wintersteiger Plotmaster) in a randomized block design with three replications. The size of the plots was 8.75 m $^2$, with four rows per plot and the planting density was 51 kg ha$^{-1}$, equalling about 200 plants per plot. Moisture levels were kept at optimum levels with flood irrigation. Nitrogen fertilization was applied in the form of 3:2:0 N.P.K., according to the guidelines for the area and totalled 120 kg N ha$^{-1}$· Additional fertilization requirements as well as weed and insecticide control was applied to achieve optimum yield.

At the end of the growing season, when the wheat had physiologically ripened and dried off, the plots were individually harvested with a mechanical plot harvester (Wintersteiger Plotmaster). The grain was then cleaned and weighed. Representative samples of each plot were randomly numbered and sent to the South African Wheat Board in Pretoria for quality analysis.

## 3.2. The quality characteristics that were measured

### 1. Kernel texture

The method used to determine kernel texture was adapted from Glenn and

Saunders (1990). It involved the transverse halving of a single wheat kernel with a razor blade, after which the germ end was numbered and stored in an eppendorf tube, to be used as a possible source for later backcrosses. The other end of the kernel was glued to an aluminium pin stub G329 (Wirsam Scientific and Precision Equipment, Pty., Ltd.) 12.5 mm in diameter. The kernel was positioned so that the crease faced upwards. The glue was a commercial type, Superglue Pen (General Chemical Corporation Ltd.) and was applied to the pin stub, the surface of which had been roughened with fine sandpaper. This was done to improve the adhesion of the half kernel to the pin stub. After the glue had been allowed to harden, the pin stub was marked and levelled with sandpaper to a flat surface.

The pin stub was then inserted in a microtome and sectioned with a glass knife, until the entire cross-sectional surface of the grain was in the cutting plane. Glass knives were obtained from cutting glass strips first into squares and then halving the squares into triangles. The triangles had a blunt and sharp edge. The left hand side of the sharp edge was used to cut each kernel. For every kernel that was cut, a new glass knife was used. Figure 3.3 shows the knife-maker that was used to cut the glass knives. Section thickness, as determined by the microtome setting, was progressively reduced and recorded when the thinnest possible intact section for each kernel was achieved. Microtome settings between seven and in some cases higher than ten micron, were used to classify floury soft to crumbling soft kernels. Microtome settings between one and six micron were used to classify very hard, brittle and translucent, glassy kernels. The thinnest possible cross section that remained intact, when agitated with a camel hair brush, was taken as a measurement of endosperm cohesiveness. This method was used to determine the presence of soft and hard texture classes in the kernels that were evaluated. This method of determining kernel texture can not be recommended for use in a large commercial breeding programme, since it is expensive and time consuming. It has application value in research projects, where only small amounts of genetic material is available to be tested for kernel texture. Figure 3.2 shows the microtome that was used to classify the wheat kernels.

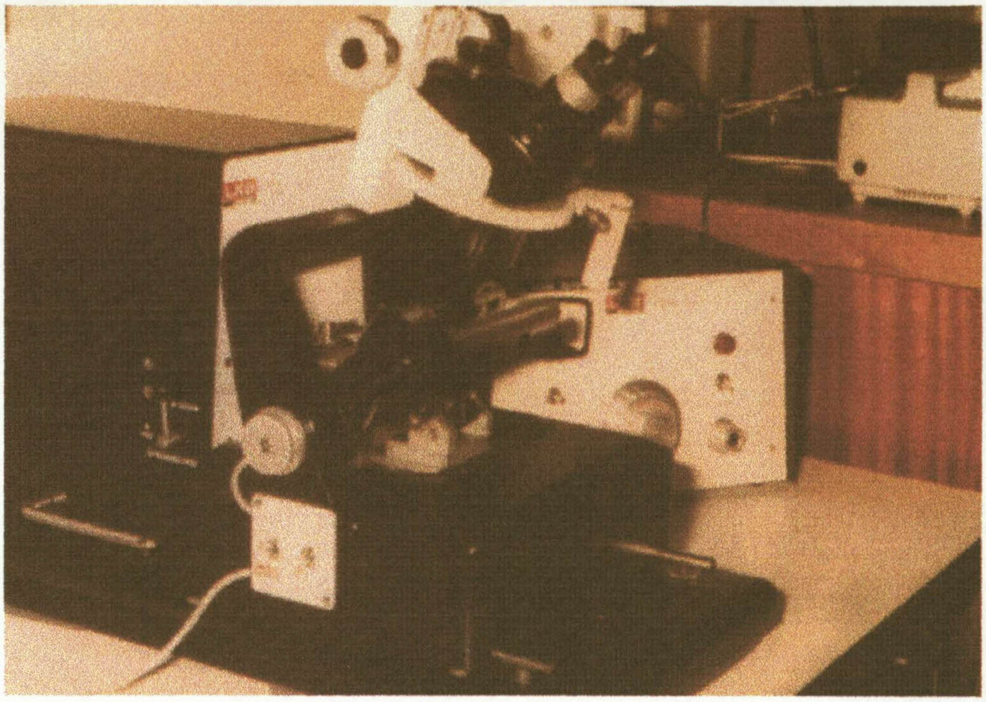


Figure 3.2 The microtome used to classify wheat kernels according to texture.

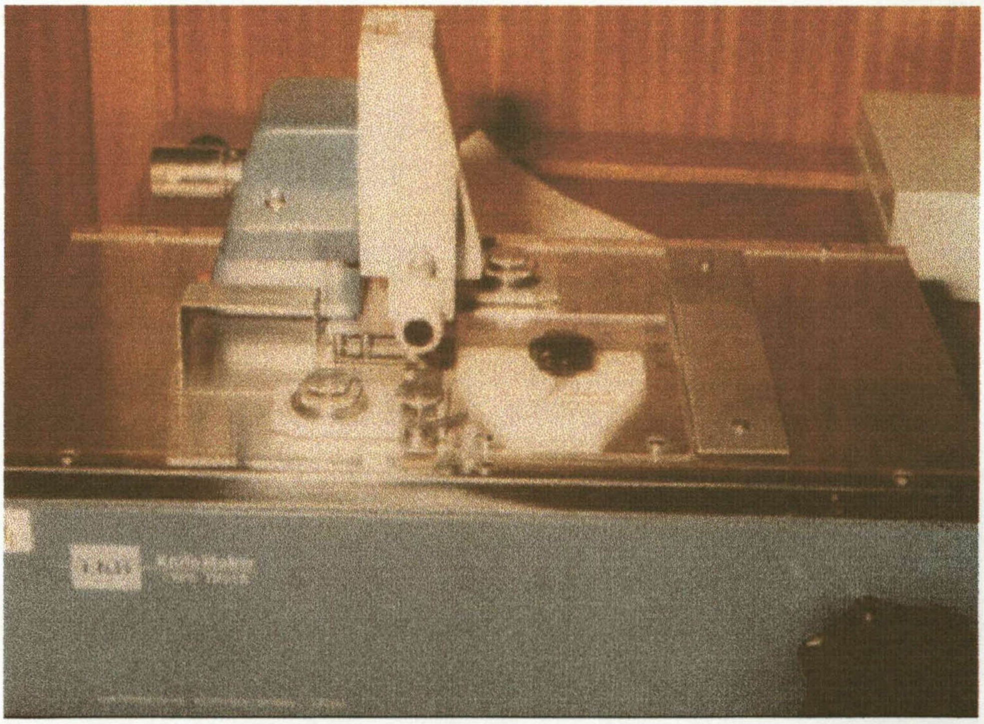


Figure 3.3 The knife-maker used to cut glass knives for the microtome.

2. Hectolitre mass / test weight (HLM)

This characteristic is a relative measure of kernel density. A chondrometer is used to isolate a set volume of wheat after which test weight is determined by weighing.

3. Falling number value (VN)

Utilizes the principle of rapid gelatinization of a flour suspension with a subsequent measurement of the liquefaction of the starch by $\alpha$-amylase. This process corresponds to chemical reactions that occur during baking.

4. Vitreous kernels (VK)

The percentage of kernels not showing signs of floury spots.

5. Wheat protein (WP)

The Kjeldal method was used to determine the protein content in whole wheat samples (AACC method 48-12).

6. Break flour yield (BFY)

The flour obtained from the break rolls of a Brabender mill, as a percentage of total flour regained.

7. Milling flour yield (EX)

The amount of flour extracted as a percentage of total flour regained.

8. Kent Jones value (KJ)

Measured with the Kent-Jones and Martin colour grader. The apparatus ignores whiteness of flour and concentrates on the influence of the bran-like material present in the flour by measuring the reflectance in the green band of the light spectrum.

9. Flour protein (FLP)

The Kjeldal method was used to determine the quantity of protein present in the flour.

10. Mixograms (MM = mixogram mixing time, MA = mixogram absorption)

The effect of quality and quantity of flour proteins on mixing properties is objectively determined by 10g mixograms obtained from a mixograph. The mixograph is composed of two planetary pins that revolve in a bowl containing flour and optimum water. As mixing proceeds, water is absorbed and the dough is progressively developed so that resistance to the planetary pins passing through the dough is recorded on paper. Water absorption is adjusted to protein content for each sample.

11. Alveograph (AST = Alveograph strength, ASB = Alveograph stability, ADI = Alveograph extensibility, APL = ASB/ADI)

Measures the resistance of dough to extension and the extent to which it can be stretched (AACC Method 54-30).

12. Alkaline water retention capacity (AWRC)

This is a standardized method of measuring the quantity of sodium bicarbonate solution, absorbed by flour and held against a centrifugal force.

13. Biscuit score (SC = Grain score, ASC = Grain score with 0.2 $NH_4HCO_3$ added)

Provides a basic baking method for evaluation of the quality of biscuit flour by measuring the spread of sugar-snap biscuits. South African Wheat Board - Technical Service adapted method.

14. Biscuit spread (DIAM = sugar snap biscuit spread, ADIAM = sugar snap biscuit spread with 0.2 $NH_4HCO_3$ added)

Average point score is determined in each baking test. 9 = extremely poor, 0 = excellent.

## 3.3 Statistical analysis

### 3.3.1 Chi - squared test

The chi-squared test was used to study the segregation ratios of the kernel texture classes observed in the various backcross generations.

### 3.3.2 Analyses of variance (ANOVA)

Analysis of variance, mean squares, coefficients of variance (CV), standard errors and missing values were calculated using Genstat 5, Release 3.1 (Genstat 5 Committee, 1997) for the progeny of the third backcross in a randomized block design. The least significant differences for the means were calculated by the method of Bonferroni at a 95% confidence level.

### 3.3.3 Stepwise regression

A forward selection, stepwise regression, with biscuit diameter as dependant variable was calculated using Genstat 5, Release 3.1 (Genstat 5 Committee, 1997).

# CHAPTER 4

## RESULTS AND DISCUSSION

### 4.1 Segregation ratios of softness genes

Two different classes of kernel textures were identified when the progeny of the first, second and third backcrosses were studied using the method as described in **3.2.1**. Microtome settings of seven and higher were required to obtain intact endosperm cuts with minimum break-away endosperm on the glass knife from kernels with textures ranging from soft and floury to crumbling soft (S). A setting between one and six micron obtained intact cuts from kernels with hard to glassy, brittle and mirror-like textures (H). Examples of the observed kernel textures of the parents and their classified progeny are given in Figures 4.3 to 4.6.

Abnormal kernels with air bubbles and uneven endosperm surfaces were found at a frequency of approximately 2% in all of the wheat kernels that were evaluated. Examples of abnormal kernels are shown in Figure 4.2.

Figure 4.1 shows the selected, soft wheat kernels germinating on commercial water agar. The two parent spring wheat genotypes, Edwall and M29519, as well as their F1 progeny, were evaluated for kernel texture classification with the microtome. Ten kernels of each genotype were evaluated. The results of this evaluation are shown in Table 4.1.1.

Figure 4.1 Selected, halved wheat kernels germinated *in vitro* on commercial water agar.

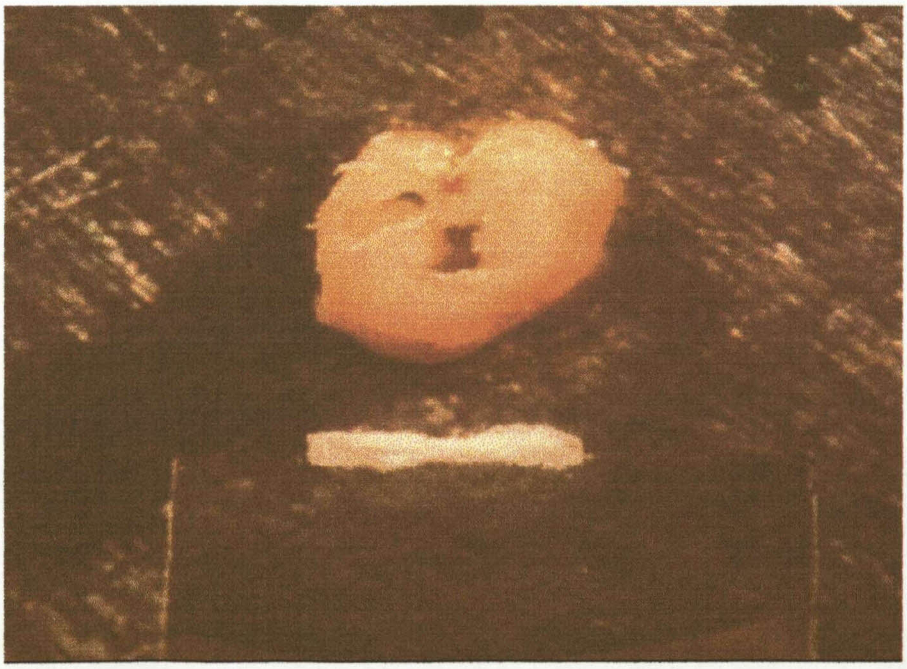


Figure 4.2 Discarded, abnormal wheat kernel with air bubbles.

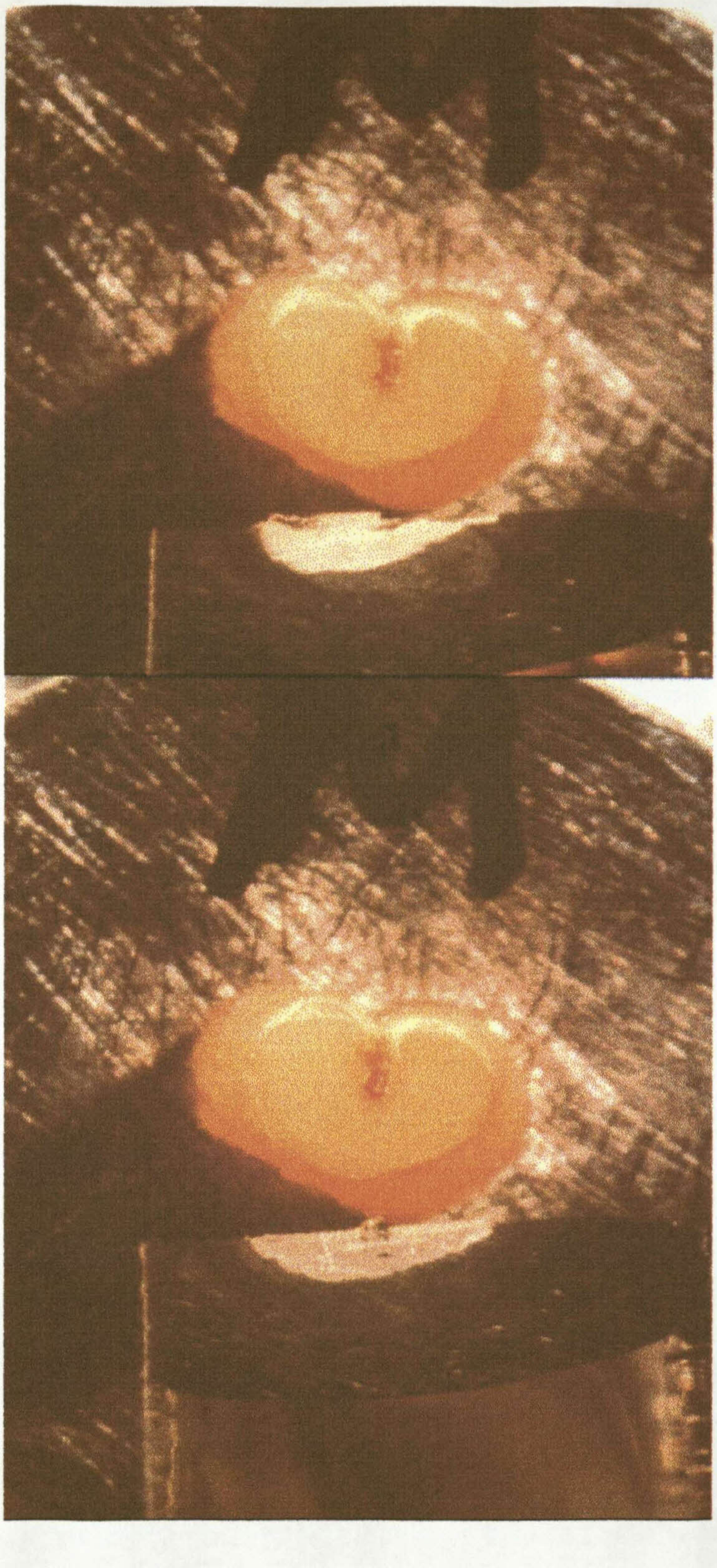


Figure 4.3 M29519, the recurrent parent at one and three micron respectively.

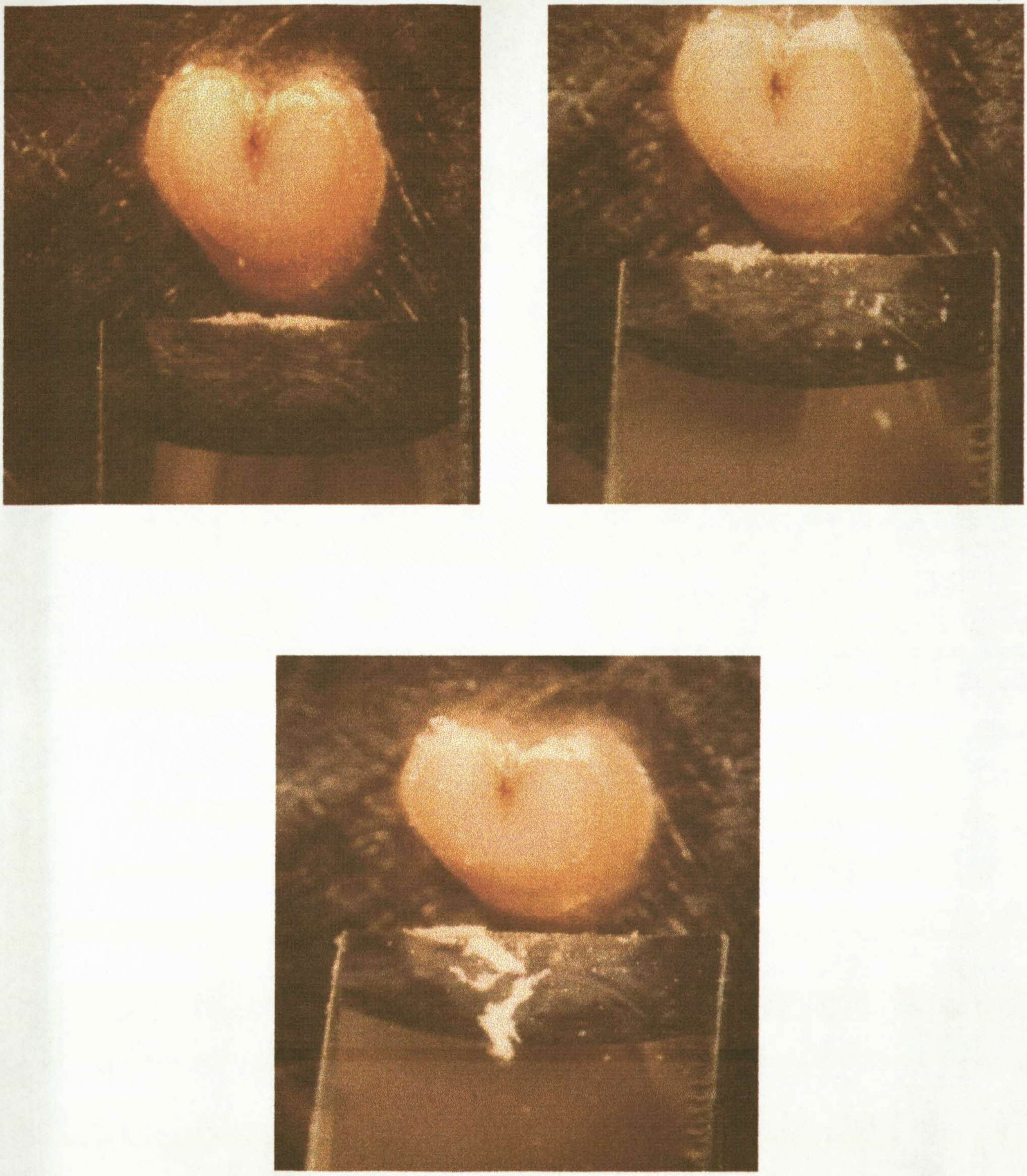
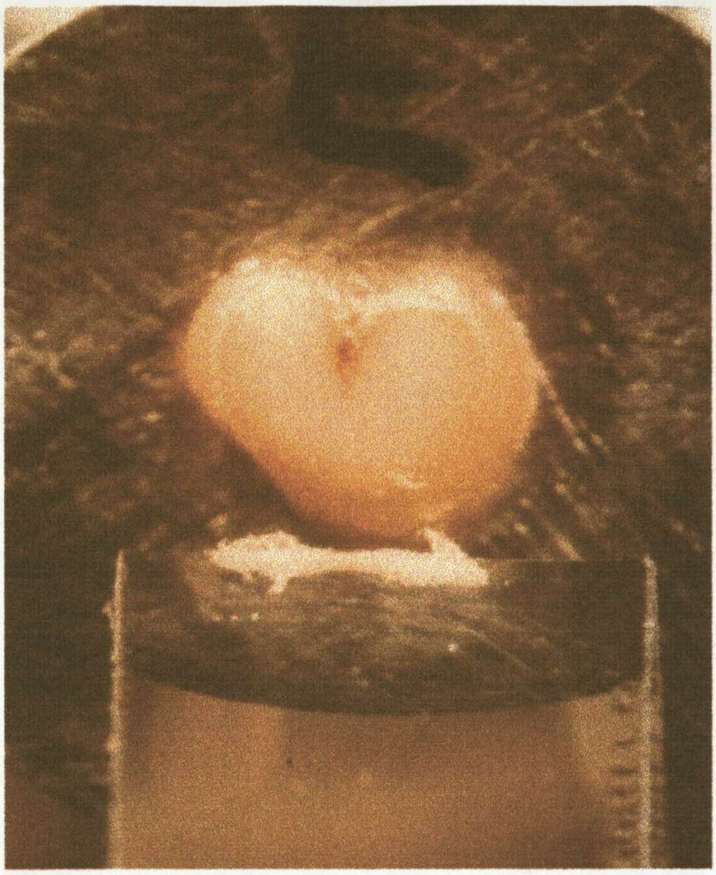
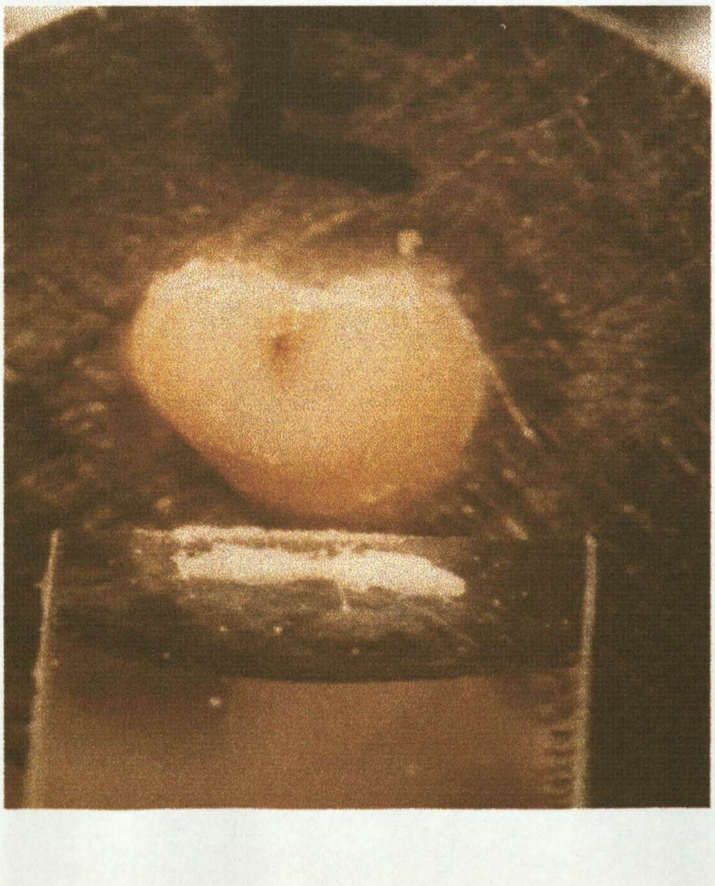


Figure 4.4 Edwall, the donor parent, at one, three, five, eight and ten micron respectively (left to right, clockwise).





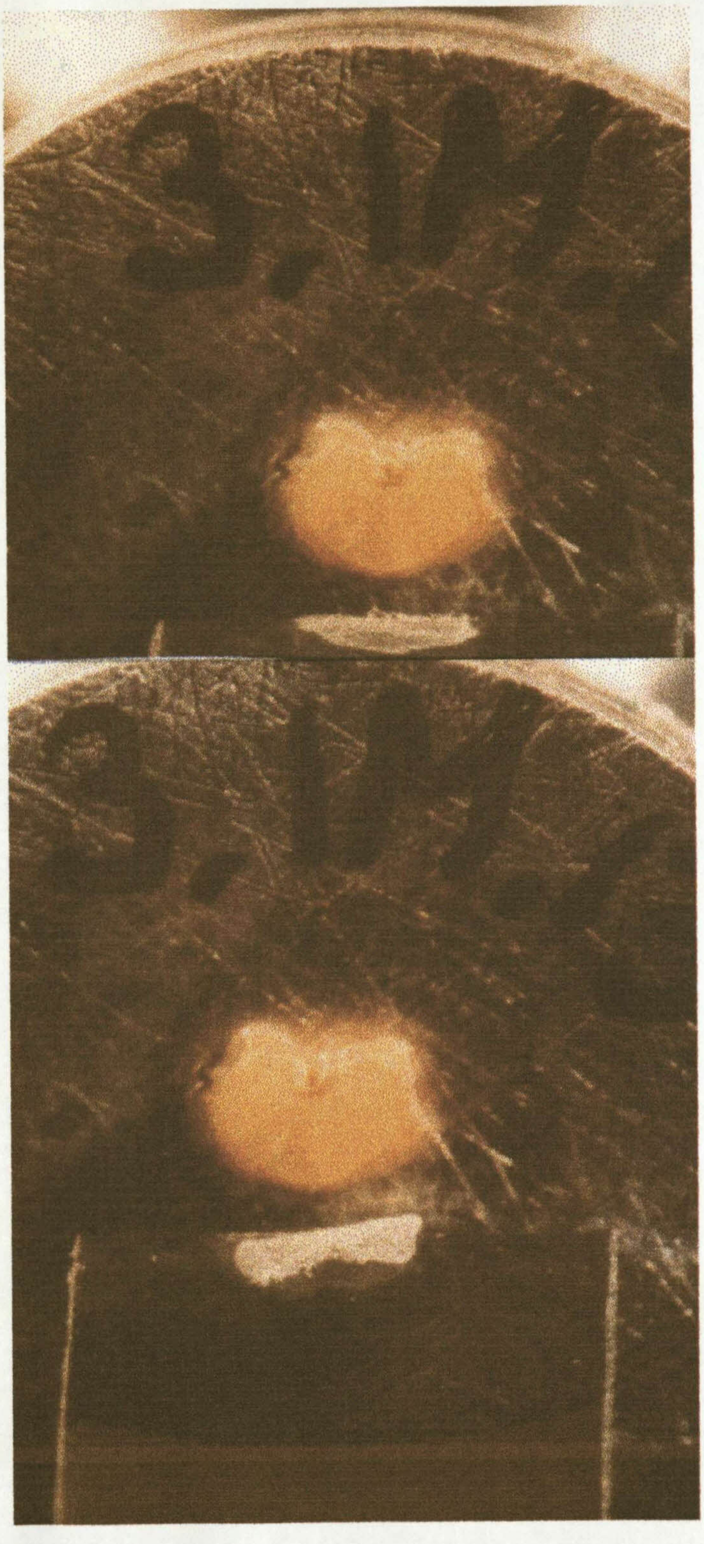


Figure 4.5 A classified hard wheat kernel from the third backcross at one and three micron (top to bottom).

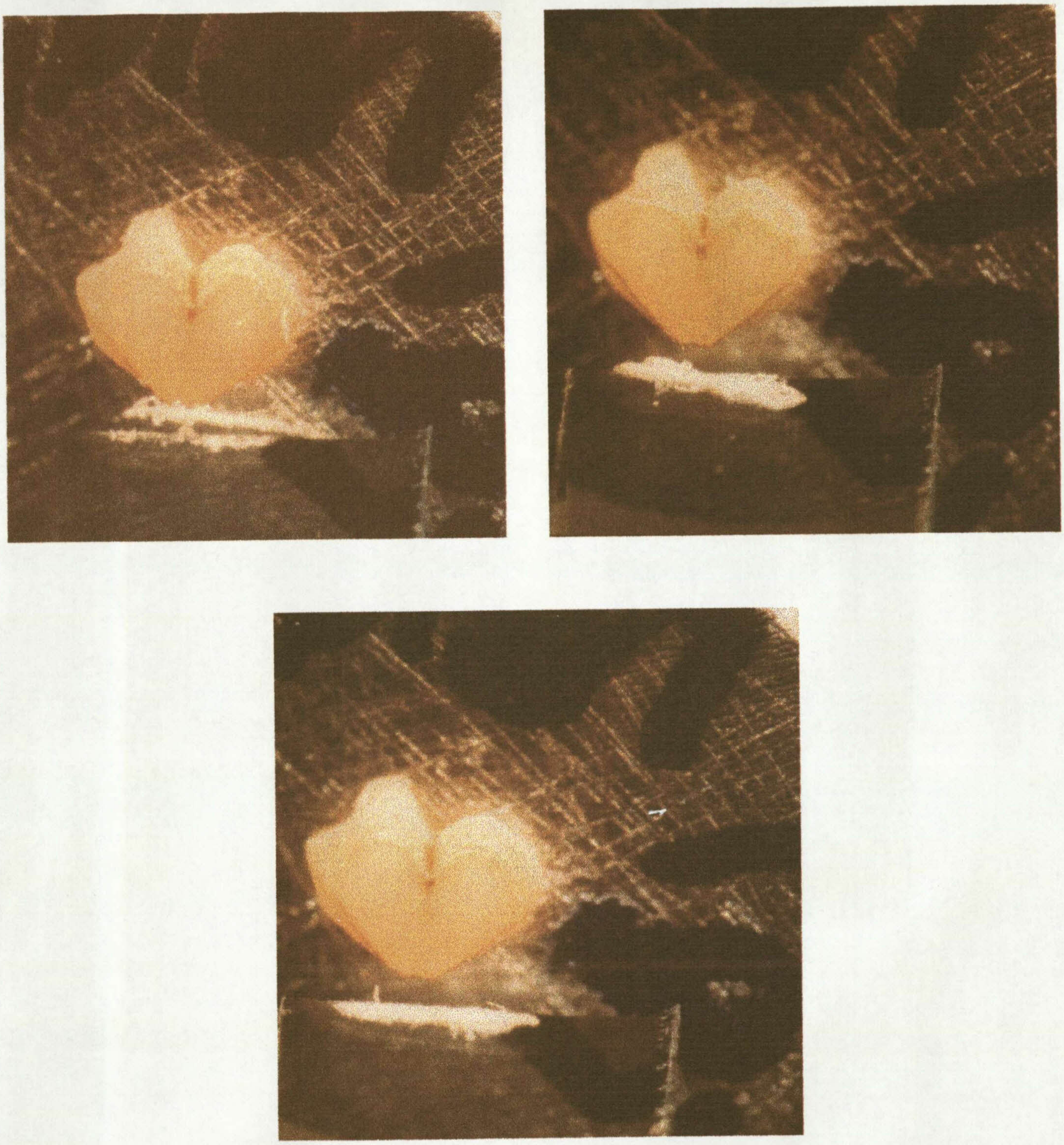


Figure 4.6 A classified soft wheat kernel from the second backcross at three, six and ten micron (left to right, clockwise).

**Table 4.1.1** **Kernel texture evaluation of the two parent spring wheat genotypes and their F1 progeny.**

| **Entry** | **Soft (seven micron and higher)** | **Hard (between one and six micron)** |
|---|---|---|
| Edwall | ten kernels | |
| M29519 | | ten kernels |
| Edwall\M29519 | ten kernels | |

In Table 4.1.1 it is shown that the parents Edwall and M29519 were homozygous for their individual kernel textures. The kernel texture of the resulting $F_1$ progeny was evaluated as being soft, although the texture was of a slightly firmer nature than that of the soft parent, Edwall. In Table 4.1.2 the results of a chi-squared analysis are given. The chi-squared analysis was performed on the progeny of the crosses between the F1 (EDWALL/M29519), used as the female parent and the recurrent line, M29519, used as the male parent. The chi-squared analysis was performed on the progeny of three backcrosses.

Table 4.1.2 **Test for equally distributed frequencies of kernel textures among progeny of the first backcross (BC1), second backcross (BC2) and third backcross (BC3) (p = 0.05).**

| **Entry** | **Backcross** | **Soft** | **Hard** | **Chi-squared values** | **Results** |
|---|---|---|---|---|---|
| $BC_1$/M29519 | 1 | 54 | 45 | 0.776 | N.S.D. |
| $BC_2$/M29519 | 2 | 58 | 41 | 2.772 | N.S.D. |
| $BC_3$/M29519 | 3 | 47 | 52 | 0.281 | N.S.D. |

N.S.D. = values not significantly different

### Discussion

In Table 4.1.2 no significant differences between the two pooled classes for any of the backcrosses could be shown. The segregation ratio of soft and hard kernels remained 1:1 after each backcross. It would appear that, in this particular genetic background, wheat kernel softness is determined by the presence of one major gene pair (AA) present in the soft wheat, Edwall. The hard M29519 (aa), used as the recurrent parent, when crossed with Edwall (AA), resulted in soft (Aa) $F_1$ progeny. The selected, soft progeny (Aa) was repeatedly backcrossed to M29519 (aa) in an effort to reconstruct the agronomical qualities of M29519 to the soft textured wheat (Symes, 1965, 1969; Yamasaki and Donelson, 1983; Greenwell and Schofield, 1986 a,b).

## 4.2 The effect of softness genes on the biscuit-making quality of wheat

In Table 4.2.1 the average performance of the parents and progeny of the third backcross (BC3), grouped in two different texture classes, as well as the significant differences, standard errors and coefficients of variances are given for the soft wheat quality characters measured.

Table 4.2.1 Averages, LSD's and mean squares of quality characteristics measured on the parents and the progeny of the third backcross (BC3). p = 0.05

| | Edwall soft | M29519 hard | $BC_3$/M29519 soft | $BC_3$/M29519 hard | $LSD_B$ | Mean | SE (mean) | CV(%) |
|---|---|---|---|---|---|---|---|---|
| Break flour yield | 25.10 | 17.37 | 22.90 | 17.67 | 2.13 | 20.45 | 0.42 | 3.5 |
| Vitreous kernels | 14.67 | 30.00 | 15.33 | 33.00 | 12.77 | 22.30 | 2.51 | 19.5 |
| Hectolitre mass | 69.17 | 75.90 | 74.27 | 75.90 | 4.58 | 74.58 | 0.90 | 2.1 |
| Flour extraction | 69.70 | 77.00 | 73.83 | 76.53 | 2.98 | 74.32 | 0.59 | 1.4 |
| Flour protein | 11.63 | 12.60 | 12.33 | 12.80 | 0.54 | 12.35 | 0.11 | 1.5 |
| Mixing time | 1.53 | 2.40 | 2.30 | 2.40 | 0.56 | 2.11 | 0.11 | 9.1 |
| Alveograph distensibility | 155.30 | 163.70 | 182.50 | 160.00 | 78.38 | 166.20 | 15.38 | 16.0 |
| stability | 31.90 | 52.83 | 37.70 | 52.53 | 8.36 | 43.57 | 1.64 | 6.5 |
| strength | 11.67 | 27.67 | 20.33 | 26.67 | 6.27 | 21.37 | 1.23 | 10.0 |
| P/L ratio | 0.20 | 0.33 | 0.23 | 0.33 | 0.16 | 0.27 | 0.03 | 20.2 |
| AWRC | 59.84 | 71.09 | 60.90 | 70.90 | 8.72 | 64.18 | 1.71 | 4.6 |
| Biscuit diameter | 8.79 | 8.39 | 8.75 | 8.46 | 0.26 | 8.59 | 0.05 | 1.0 |

In Table 4.2.1, the average values of characteristics are given. The break flour yield of the soft backcross derivative was significantly higher than that of the hard backcross derivative. The amount of vitreous kernels present in the soft backcross derivative sample was significantly less than the amount of vitreous kernels present in the hard backcross derivative sample. The soft backcross derivative had an alveograph stability, alveograph strength and alkaline water retention capacity (AWRC) significantly lower than that of the hard backcross derivative. The soft backcross derivative showed a significant increased biscuit diameter over the hard backcross derivative.

Although the protein content, mixing time, extraction and hectolitre mass of the soft backcross derivative were lower than that of the hard backcross derivative, the difference was not significant.

There were no significant differences between the measured characteristics of the recurrent parent, M29519 and the backcrossed hard derivative, which indicates that the genotype of the recurrent parent was satisfactorily reconstituted after the three backcrosses and one selfing. There were significant differences in hectolitre mass, extraction, protein, mixing time, break flour yield and alveograph strength between the soft backcross derivative and the donor parent, Edwall. This was unexpected, since the dominant gene for softness would have to be present in all the $BC_3$ kernels, which should have caused the same expression of characteristics as in the donor parent, Edwall. The genetic background into which the softness gene had been inserted, probably modified the degree to which it was expressed as measured by the quality characteristics. This could also explain the fact that although the backcrossed progeny yielded kernels that had a softer texture than the hard recurrent parent, M29519, the kernels were in most cases of a slightly firmer nature than that of the donor parent, Edwall.

Table 4.2.2 **Significant correlations (r) between biscuit diameter and other quality characteristics for the soft and the hard group (pooled data). p =0.05**

| **Quality characteristic (Soft group)** | **Correlation value** |
|---|---|
| Break flour yield | 0.835 |
| Flour protein | -0.797 |
| Mixing time | -0.695 |
| **Quality characteristic (Hard group)** | **Correlation value** |
| Alveograph strength | -0.795 |
| AWRC | -0.713 |

When the data from the soft lines (pooled) was considered, significant correlations with biscuit diameter were found with break flour yield (positive), flour protein (negative) and mixing time (negative). The pooled data from the hard lines showed significant correlations with biscuit diameter and AWRC (negative) and alveograph strength (negative) (Table 4.2.2).

Table 4.2.3 **Results of the forward selection stepwise regression on biscuit diameter within the soft group (pooled data).**

| **Quality characteristic** | **Percentage variance** | **Standard error** | **Probability** |
|---|---|---|---|
| Break flour yield | 65.4 | 0.0874 | 0.005 |
| Alveograph strength | 83.3 | 0.0607 | 0.002 |
| Flour extraction | 94.2 | 0.0359 | <0.001 |

**Table 4.2.4** **Results of the forward selection stepwise regression on biscuit diameter within the hard group (pooled data).**

| Quality characteristic | Percentage variance | Standard error | Probability |
|---|---|---|---|
| Alveograph strength | 58.0 | 0.332 | 0.010 |
| Flour extraction | 73.8 | 0.0262 | 0.008 |
| Flour protein | 81.1 | 0.0223 | 0.009 |
| Break flour yield | 89.1 | 0.0170 | 0.009 |
| Mixing time | 92.9 | 0.0137 | 0.014 |

Results from the forward stepwise regression on biscuit diameter with the data from the soft derivatives (pooled), showed that break flour yield, alveograph strength and flour extraction contributed 94.2% of the total variation observed.

Results from the forward stepwise regression on biscuit diameter with the data from the hard derivatives (pooled), showed that alveograph strength, flour extraction, flour protein, break flour yield and mixing time contributed 92.9% of the total variation observed.

## Discussion

The significant increase in the break flour yield of the soft backcross derivative is consistent with earlier reports by Rogers *et al.* (1993) that soft wheats produce higher percentages of break flour and bran than hard wheats. Kaldy and Rubenthaler (1987) confirmed that the factors controlling break flour yield similarly contribute to greater biscuit spread which explains the significantly positive correlation between biscuit diameter and break flour yield found with the soft derivatives.

It is recognized that the lower the percentage of water retained by a flour against centrifugal force, the better the pastry quality (Kaldy and Rubenthaler, 1987). The significant decrease in AWRC of the soft derivatives and the significantly negative correlation with biscuit diameter confirmed that the soft derivatives retained a lower percentage of water. Wainwright *et al.* (1985) also found that flours from soft milling wheats required less water to give biscuit doughs of standard consistency than did flours from hard milling wheats.

Although it was found that flour protein content was significantly negatively correlated with biscuit diameter, the presence of the softness genes did not significantly lower the protein content of the soft derivatives. The fact that soft kernels were selected on the basis of their endosperm performance when cut by the microtome may explain the dramatic decrease in the amount of vitreous kernels present. In this study, translucent, brittle and vitreous kernels were classified as hard in most cases and floury, white kernels were classified as soft. It would therefore be incorrect to assume that soft wheats with opaque kernels will simultanously be low in protein. In other words, a soft wheat cultivar mills like a soft wheat whether it is low or high in protein content Finney *et al.* (1987). Rogers *et al.* (1993) concluded that when protein quantity varied in different wheat samples, the factors associated with kernel texture (e.g. break flour yield and AWRC) had the biggest impact on biscuit diameter.

Minor (1966) remarked that biscuit spread, the ability of a flour to impart the desired spread to a biscuit as it bakes, is probably the best test the miller has, to judge the merits of a biscuit flour. Biscuits will vary from large diameters with open and well developed cracks, as are produced by very soft wheats, to biscuits with reduced spreads, smooth top grain, and hairline surface cracks, that are obtained from flours of increasing hardness or strength. Figure 4.7 and Figure 4.8 show the biscuits that were baked from the soft backcross derivatives, compared to a biscuit from a hard backcross selection. Gaines and Donelson (1985) also found that test baking and biscuit diameter were the best methods for end-use quality evaluation. Cultivars with a soft kernel texture tend to produce larger biscuits. To this end, Gaines (1990) developed a best-fit multiple regression equation to predict biscuit diameter.

$$BD = 21.42 - 0.067(P) + 0.023(BFY) - 0.074(AWRC),$$

where BD = biscuit diameter, P = flour protein, BFY = break flour yield and AWRC = alkaline water retention capacity. This equation could explain up to 59% ($r = 0.77$) of the variation in biscuit diameter, with a standard error of estimate of 0.27cm.

The importance of break flour yield and AWRC as parameters of end-use quality for soft wheat is reconfirmed in the results of this study. It is also significant that although the transfer of the softness genes into a different genetic background, did not simultanously transfer all factors generally associated with good biscuit quality, it did produce a biscuit that did not differ significantly in diameter from the donor parent's. Alveograph strength was significantly negatively correlated with biscuit diameter and alveograph strength and stability was significantly lower in the soft derivatives than the hard derivatives. This is in accordance with Thompson and Whitehouse (1962) who proposed that biscuit wheat should produce a low resistance (weak) dough. Although mixing time was significantly negatively correlated with biscuit diameter, it did show a significant decrease in the soft derivative. Mixing time, tolerance, dough elasticity and other factors considered to be important in hard wheat flour testing, are not given as prominent a place in biscuit testing, because the relatively large quantities of shortening in pastry formulations inhibit gluten development and the use of sugar brings about changes in water distribution among ingredients and flour components (Yamazaki, 1959).

The fact that hectolitremass was not influenced by the softness genes is probably due to the fact that hectolitremass can be seen as a yardstick of environmental influences (Gaines, 1991). The environment was constant for all entries in this study.

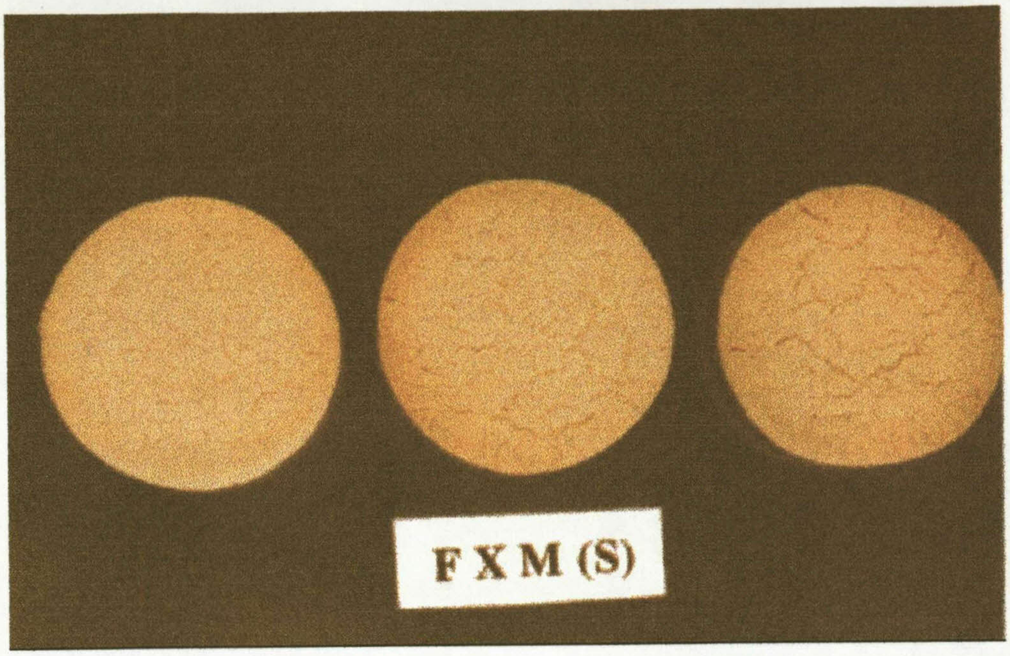


Figure 4.7 Biscuits baked from the soft textured near-isogenic line that was produced after the progeny of the third backcross self-pollinated (three replications).

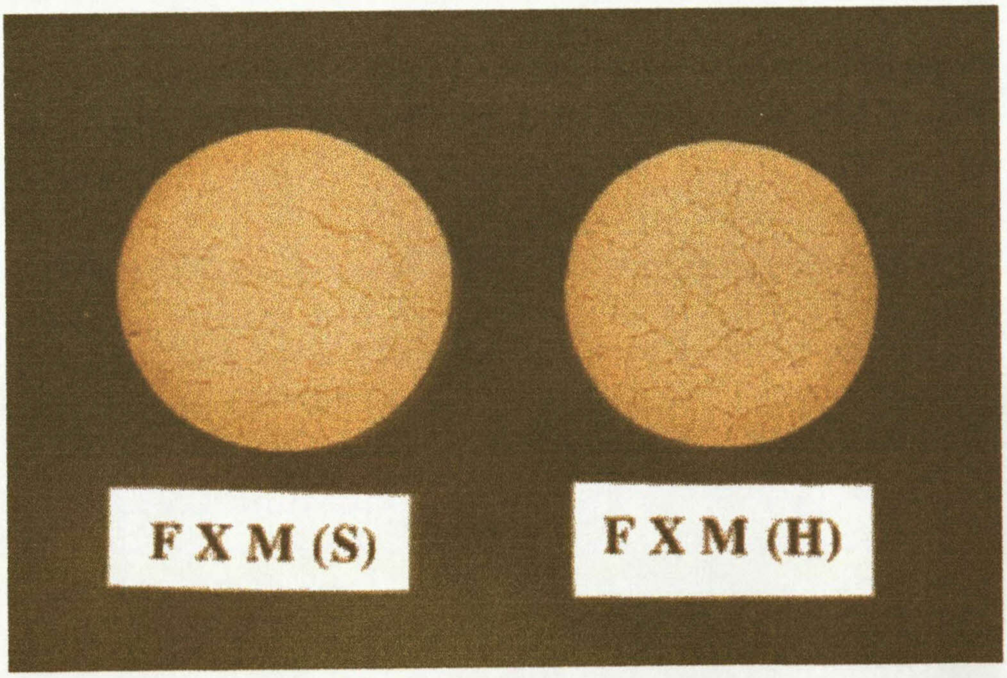


Figure 4.8 Biscuits baked from the soft (left) and hard (right) textured lines that were produced after the third backcross progeny self-pollinated.

# CHAPTER 5

## CONCLUSION

The purpose of this study was firstly to determine the segregation ratios of the gene(s) responsible for kernel softness in this particular genetic background and secondly, to determine the effect of the softness gene(s) on the biscuit-making quality of wheat.

Symes (1961, 1965 and 1969), MacRitchie (1980), Yamazaki and Donelson (1983) and Greenwell and Schofield (1986 a,b) are a few of the authors that studied the heritability of kernel texture in wheat. Kernel texture was measured in these studies with a wide spectrum of techniques that included chemical and mechanical procedures. It was concluded that kernel softness is inherited simply and that it is probably controlled by one or two major genes and perhaps one or more minor genes.

Bushuk and Obuchowski (1980) reported that significant differences can be expected between methods that utilize different parts of the wheat kernel.The technique used to classify kernel texture in this study was adapted from a similar method used by Glenn and Saunders (1990). Although this technique is time consuming, it is descriptive, accurate and can be recommended for classifying a small number of single kernels. In this study kernel softness was determined by a single, dominant gene. The progeny of a cross between a hard wheat variety and soft wheat variety was classified as soft, using the technique described earlier. The progeny was not as floury and soft as the donor parent, although such individuals were occasionally identified. It was concluded that the genetic background into which the softness gene was inserted could influence the expression of the gene.

Gaines and Donelson (1985) found that biscuit diameter was the best evaluation method to determine the end-use quality of soft wheat. In this study, biscuit diameter was positively correlated with breakflour yield and negatively with flour protein, which agree with the findings of Gaines (1985) and Kaldy and Rubenthaler (1987). Finney *et al.* (1987) found that the lower the percentage water retained by a flour, the better the quality of the end product. Alkaline water retention capacity was found to be negatively correlated with biscuit diameter, as was alveograph strength. Differences in dough strength between soft and hard wheat could not be detected when measured by the mixogram, but the alveogram values did indicate significant differences in dough strength and stability between soft and hard wheat. Biscuits baked from the near-isogenic soft textured line produced significantly larger biscuits than those baked from near-isogenic hard textured line.

The quality differences between the near-isogenic soft line and the donor parent may be due to the genetic background into which the gene was inserted. In this study the presence of the softness gene did improve the biscuit-making qualities of wheat as determined by the most important quality characteristics.

# CHAPTER 6

## SUMMARY

* The purpose of this study was firstly to determine the segregation ratios of softness genes in this particular genetic background.

* Secondly, the effect of these softness genes on bisquit-making quality had to be determined.

* A soft, white wheat cultivar was crossed with a hard red wheat cultivar.

* The soft white wheat cultivar had the ability to produce biscuits with an acceptable diameter while the hard red wheat cultivar had agronomic qualities that rendered it suitable for production in the Northern Cape irrigation area of Southern Africa.

* Soft wheat kernels were selected from the progeny using a microtome and micron settings as an indication of kernel softness.

* Micron settings were used as an indication of kernel softness. Soft textured kernels had a setting of seven micron or higher. Hard textured kernels had a setting of between one and six micron.

* The selected soft kernels were agronomically reconstructed to the hard red wheat cultivar for three generations, using the backcross method.

* Results showed that kernel softness is determined by a single locus and two alleles, with the dominant gene determining kernel softness.

* The two near-isogenic lines that differed with regard to kernel texture, were evaluated for milling and baking qualities.

* The genes for kernel softness had no effect on dough strength as measured by the mixogram.

* The stability and dough strength as measured by the alveogram were significantly influenced by the genes for kernel softness. The AWRC was significantly lower in soft wheats.

* The genes for kernel softness significantly increased biscuit diameter.

* Break flour yield was highly correlated with biscuit diameter (r = 0.835), while the percentage flour protein (r = -0.797) and mixing time (r = -0.695) were significantly negatively correlated with biscuit diameter in the soft group.

* In the group with hard kernels, alveograph strength (r = -0.795) and AWRC (r = -0.713) showed a significantly negative correlation with biscuit diameter.

* The stepwise regression on biscuit diameter within the soft group showed high variances for flour extraction, break flour and alveograph strength. Within the hard group, high variances were found for flour protein, flour extraction, break flour and mixing time.

# HOOFSTUK 6

## OPSOMMING

* Die doel van hierdie studie was eerstens om die segregasie patrone van koring sagtheidsgene in hierdie spesifieke genetiese agtergrond te bepaal.

* Tweedens, moes die effek van hierdie sagtheidsgene op die kwaliteit van beskuitjies bepaal word.

* 'n Sagte wit koring kultivar is met 'n harde rooi kultivar gekruis.

* Die sagte wit koring kultivar het beskuitjies met 'n aanvaarbare deursnee geproduseer, terwyl die harde rooi koring kultivar agronomies aangepas was vir die besproeiingsgebied in die Noordelike Kaapprovinsie van Suidelike Afrika.

* Sagte koringpitte is uit die nageslag geselekteer deur van 'n mikrotoom gebruik te maak.

* Mikrotoomlesings is gebruik as 'n aanduiding van pitsagtheid. Koringpitte wat as sag geklassifiseer is, het mikrotoomlesings van sewe mikron en hoër gehad. Koringpitte wat as hard geklassifiseer is, het mikrotoomlesings van tussen een en ses mikron gehad.

* Die geselekteerde sagte pitte is agronomies gerekonstrueer na die harde rooi kultivar vir drie generasies, deur van die terugkruisingstegniek gebruik te maak.

- Die resultate toon dat pitsagtheid deur 'n enkel lokus met twee allele bepaal word, waarvan die dominante geen uitdrukking gee aan pitsagtheid.

- Die twee naby-isogeniese lyne wat ontwikkel is, het slegs ten opsigte van pitsagtheid van mekaar verskil. Hierdie lyne is geëvalueer vir maal- en bakeienskappe.

- Die gene vir pitsagtheid het geen effek gehad op die deegsterkte soos gemeet deur die miksogram nie.

- Die stabiliteit en deegsterkte soos gemeet deur die alveogram is betekenisvol beïnvloed deur die gene vir pitsagtheid. Die AWRC was ook betekenisvol laer in die sagte koring.

- Die gene vir sagtheid het die beskuitjie deursnee betekenisvol verhoog.

- Breekmeel opbrengs was hoogs gekorreleer met beskuitjie deursnee ($r = 0.835$), terwyl die persentasie meel proteïen ($r = -0.797$) en mengtyd ($r = -0.695$) betekenisvol negatief gekorreleer is met beskuitjie deursnee in die sagte groep.

- In die harde groep toon alveogram sterkte ($r = -0.795$) en AWRC ($r = -0.713$) betekenisvolle negatiewe verwantskap met beskuitjie deursnee.

- Die stapsgewyse regressie op beskuitjie deursnee binne die sagte groep toon hoë variansie vir meel ekstraksie, breekmeel en alveogram sterkte. Binne die harde groep is hoë variansies verkry vir meel proteïen, meel ekstraksie, breekmeel en mengtyd.

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