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17/5/2006

Sedimentation Testing of Wheat:

Methodology, Efficacy and Biochemistry

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

Department of Agriculture Food and Natural Resources
University of Sydney
March, 2006

Statement of Original Authorship

The work contained in this thesis has not previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Signature ____________________

Date 7/8/2006

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Abstract

Sedimentation testing of wheat has long been accepted as a simple, rapid, inexpensive small-scale test of wheat protein quality, but there are ongoing questions about its predictive value and about the mechanistic basis of the test. The test works by hydrating small wheatmeal or flour samples in water, followed by the addition of a solution containing either sodium dodecyl sulfate (SDS) and lactic acid or isopropyl alcohol and lactic acid.

The wheat sample, swells in the acidified solution causing a 'sediment' to form. The volume of this sediment, after settling for a specified time, gives a measure of the quality of the protein present, which has been shown to correlate with the dough and/or baking quality of the meal or flour sample tested.

The environmental (E) conditions under which grain is grown and stored influence the expression of genotype (G) and the G x E interactions determine the quality characteristics of the grain and as such are taken in to consideration both during breeding and after harvest. There is thus a need to develop tests capable of assessing quality changes due to these interactions.

This study aimed to investigate current sedimentation testing methodology to improve the robustness of the test, and assess the factors that can influence the test results. Furthermore, this work aimed to investigate the nature of the 'sediment' with regard to its structure and composition, and to extend the application of sedimentation testing beyond the evaluation of wheat grain and flour.

Improvements were made to the procedure and equipment for sedimentation testing, making it a robust and reproducible quality test that was unaffected by small changes in testing parameters, such as sample size, reagent concentration, mixing speed and temperature. The factors found to affect the outcome of sedimentation testing included the pH of the reaction solution and the particle size of the sample. The procedure was optimised by consistent preparation of samples and reaction solution, and by regular analysis of a reference sample of known sedimentation volume.

Sedimentation testing proved useful as a means of assessing grain quality regardless of the effects of G x E interactions, particularly with regard to heat stress. The decrease in sedimentation volume that accompanies environmental stresses reflects the poor grain quality associated with the environmental stress. Severe sprout damage caused a rise in sediment volume, but this was not a significant effect for milling-grade grain. Storage conditions of 40°C following harvest caused decreases in the volume of sediment, especially when samples were stored as flour rather than either grain or wheatmeal.

Examination of the form and composition of the sediment indicated that the sediment results from complex interactions between a diversity of flour components, not only gluten as is suggested in the literature. The sediment is stabilised by disulphide linkages and ionic bonding and agents that interrupt these bonds destroy the form of the sediment.

Finally, sedimentation testing was compared with small-scale quality tests developed since sedimentation testing was developed and currently in routine use. Sedimentation testing reflected dough quality results as effectively as some methods that are more complex and more expensive to perform. Results for sedimentation testing were similar to those achieved using the swelling-index-of-glutenin test, except that when maximum and minimum cut-off limits were applied sedimentation resulted in 50% more false negative results than the swelling-index-of-glutenin test.

Sedimentation testing was demonstrated to be a robust, reproducible test that correlates well with dough qualities and is capable of predicting changes in grain quality as a result of genotypic and environmental factors. The comparative simplicity, economy and speed of sedimentation testing, compared to alternative small-scale tests, indicate that sedimentation testing is still a viable routine test for grain and flour quality, with possibilities for wider application in cereal chemistry.

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Abbreviations

A	Aleurone
A-PAGE	Acid polyacrylamide gel electrophoresis
APH	Australian prime hard wheat grade
APW	Australian premium white wheat grade
AS-PCR	Allele specific polymerase chain reaction markers
ASW	Australian standard white wheat grade
AUH	Australian hard wheat grade
BU	Brabender units
C	Cuticle
CL	Cross layer
CTAB	Cetyl trimethyl ammonium bromide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribose nucleic acid
E	Endosperm
Em	Embryo
EOF	Electroendosmotic flow
Ext.	Dough extensibility
Ext.45	Dough extensibility following 45 minutes resting
FN	Falling number
GSP-1	Grain softness protein
GBSS	Granule bound starch synthase
GS	Glutenin subunits
G x E	Genotype by environment interactions
Ha	Hardness locus
HPLC	High performance liquid chromatography
HMW	High molecular weight
HMW-GS	High molecular weight glutenin subunit
IEF	Isoelectric focussing
IE	Ion exchange
IPA	Isopropyl alcohol
LA	Lactic acid
LMW	Low molecular weight
LMW-GS	Low molecular weight glutenin subunit

M	Mesocarp
MS-SDS-PAGE	Multi stacking sodium dodecyl sulphate polyacrylamide gel electrophoresis
N	Nitrogen
NIR	Near infrared reflectance spectroscopy
NIT	Near infrared transmittance spectroscopy
PIN-a	Puroindoline-a protein peptide
PIN-b	Puroindoline-b protein peptide
PLN	Perten liquefaction number
PQV	Protein quality value
PSI	Particle size index
QTL	Quantitative trait locus
RFLP	Restriction fragment length polymorphisms
Rmax	Maximum resistance to extension
RP-HPLC	Reversed phase high performance liquid chromatography
RPM	Revolutions per minute
RVA	Rapid ViscoAnalyser
S	Scutellum
SDM	Standard deviation from the mean
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SE	Scutellar epithelium
SE-HPLC	Size exclusion high performance liquid chromatography
SIG	Swelling index of glutenin
SKCS	Single kernel characterisation system
SRC	Solvent retention capacity
SV	Sedimentation volume
T	Testa
TC	Tube cell
TFA	Trifluoroacetic acid
UPP	Unextractable polymeric protein
WSN	White salted noodle
Wx	Waxy
YAN	Yellow alkaline noodle

Chapter 1

Introduction and Literature Review

1. Introduction

The measurement of wheat grain quality is of enormous importance to both local and global grain markets as it is the quality of the grain that determines both its end use application and its price. Thus it is a priority to develop and implement consistent, reproducible and accurate tests of wheat quality across the industry. This review discusses grain quality determination with particular emphasis on protein quality. The composition and quality of flour protein is discussed along with the current tests for protein quality. Finally, sedimentation testing as a means of determining protein quality will be discussed with regard to past and current methodology as well as the advantages and disadvantages of this type of testing versus other protein quality tests.

1.1 Introduction to wheat

Wheat has been used as a primary food grain around the world for centuries and is a staple food source in many countries. Wheat is the most heavily farmed grain in the world today covering more of the world's surface that any other grain crop (Anonymous, 2002).

Each year Australia produces in excess of 20 million tonnes of wheat. While this represents only 3-4% of worldwide production, more than two thirds of this wheat is exported making Australia one of the leading wheat exporters in the World.

Wheat export contributes approximately $4 billion each year to the Australian economy.

It is the unique structure and composition of wheat grains, as illustrated in Figure 1.1, which has played a critical role in making wheat one of the worlds most heavily consumed foods.

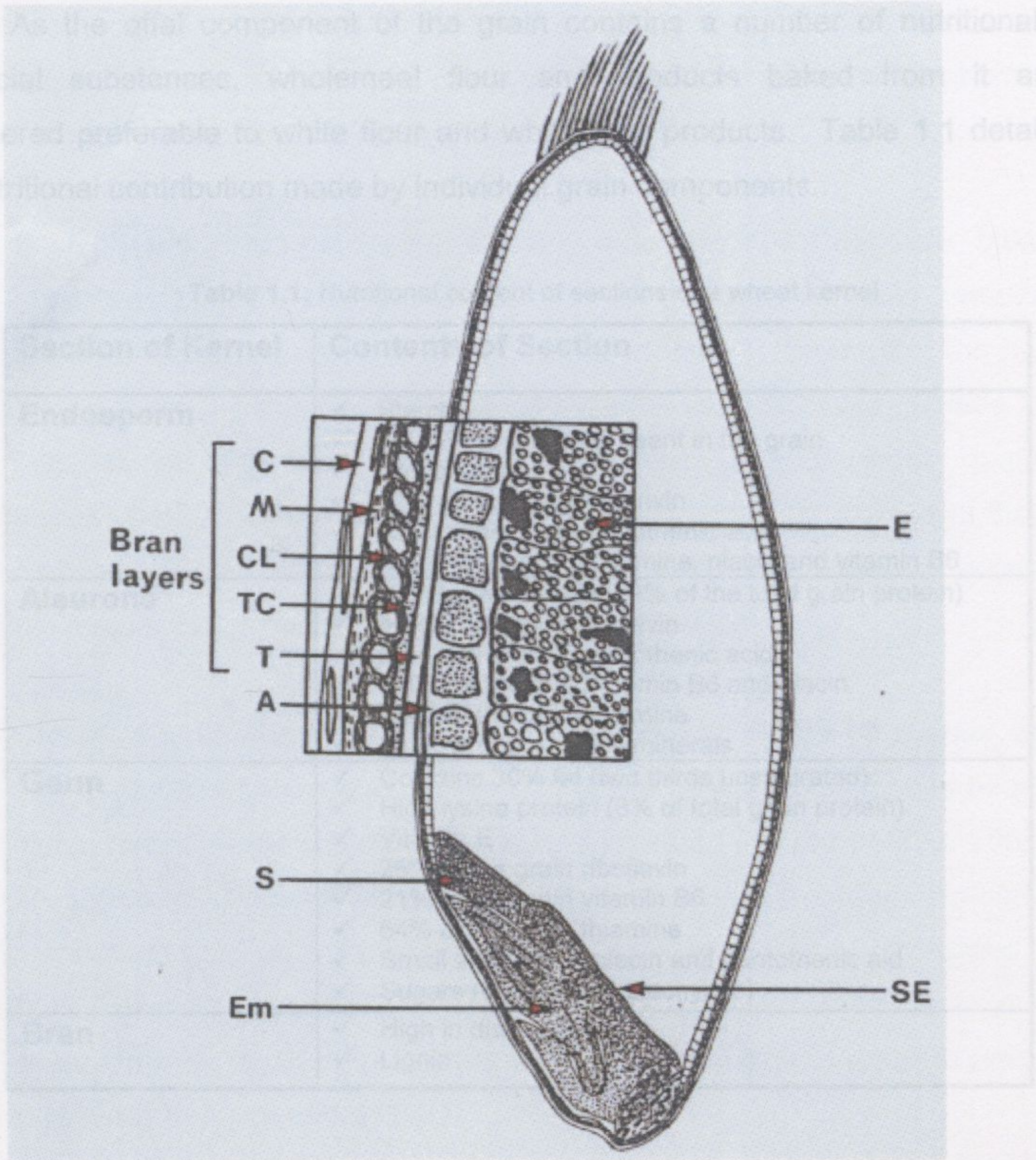

Figure 1.1: The structure of a wheat grain (Saxelby and Venn-Brown, 1980)
Abbreviations: C, cuticle; M, mesocarp; CL, cross layer; TC, tube cells; T, testa; A, aleurone; E, endosperm; S, scutellum; SE, scuttelar epithelium; Em, embryo.

The grain endosperm occupies the centre of the kernel and acts as a storage facility for the proteins and energy sources (mainly in the form of starch) required for grain growth and development. It is this endosperm that, when isolated and

ground during the milling process, results in the production of white flour. During the milling process the bran and germ (known as offal), are removed and the endosperm is crushed to flour. Wholemeal flour, however, is made either by grinding the whole grain kernel complete with offal or milling to white flour and adding bran and pollard fractions back to the flour. Enzymes present in the germ fraction can detrimentally affect shelf life and so are not usually added back to the flour. As the offal component of the grain contains a number of nutritionally beneficial substances, wholemeal flour and products baked from it are considered preferable to white flour and white-flour products. Table 1.1 details the nutritional contribution made by individual grain components.

Table 1.1: Nutritional content of sections of a wheat kernel

Section of Kernel	Contents of Section
Endosperm	✓ Starch ✓ 70-75% of protein present in the grain ✓ Glucofructan ✓ 32% of the grain riboflavin ✓ 43% of the grain pantothenic acid ✓ Small amounts of thiamine, niacin and vitamin B6
Aleurone	✓ High lysine protein (19% of the total grain protein) ✓ 42%of the grain riboflavin ✓ 50% of the grain pantothenic acid ✓ >70% of the grain vitamin B6 and niacin ✓ 33% of the grain thiamine ✓ 50-80% of the grains minerals
Germ	✓ Contains 30% fat (two thirds unsaturated) ✓ High lysine protein (8% of total grain protein) ✓ Vitamin E ✓ 26% of the grain riboflavin ✓ 21% of the grain vitamin B6 ✓ 64% of the grain thiamine ✓ Small amounts of niacin and pantothenic aid ✓ Sugars (eg sucrose, stachyose)
Bran	✓ High in dietary fibre ✓ Lignin

The range of products that can be made from wheat is diverse with different varieties of bread being the most commonly consumed wheat-based food product worldwide. The most popular of these bread varieties, in the Australian market, is loaf or pan-style bread (AACC, 2002d; Gianibelli et al., 2001). This type of bread is also widely consumed throughout North America and Great Britain.

Other popular bread products include flat or pocket bread which are widely consumed in the Middle East. This type of bread is baked under extreme heat for a very short period of time. The extreme heat causes the bread to blow up like a balloon, at which point it is removed from the heat and subsequently deflates, forming a flat, circular-shaped bread with a pocket.

Different again, Asian-style steamed bread is widely used in both sweet and savoury applications. To produce this type of bread dough, it is steamed in bamboo steamers to produce a spongy, crustless style of bread

Wheat is also used in the production of many other foods, for example, baked products such as biscuits, cakes and pastries. In the case of these products, wheat with a softer endosperm and lower protein content is preferred to the hard wheats required for the making of bread. Wheat-based Asian style noodles are made from hexaploid wheats, preferably those with special starch quality, whereas pasta products are made from tetraploid durum wheats. . Each of these different wheat products requires different flour and dough characteristics in order to achieve the desired product quality (Anonymous, 2003b).

The ability of ground wheat to produce such a diverse range of popular and predominantly nutritious leavened breads and wheat-based products, has been a major driving force behind continued worldwide interest in wheat (AACC, 2002d; Anonymous, 2003b; Gianibelli et al., 2001). In turn, this interest has stimulated ongoing research into optimisation of the quality and cost-effectiveness of production of wheat-based food products. To this end, a number of techniques and instruments have been developed to assess grain quality in order to predict the most suitable end-use of the flour.

The remainder of this chapter addresses current quality specifications and testing techniques as well as protein composition effects on grain quality. Further to this, current testing techniques will be investigated, particularly those tests looking at the sedimentation of wheatmeal or flour samples, with a view to further development and optimisation of testing techniques for future protein quality testing.

1.2 Wheat Quality

Wheat quality within the food industry is defined as the suitability of grain for efficient production of flour to give the intended end product (Wrigley et al., 1998). A number of testing parameters are currently used to gauge the quality of grain from harvest to milling. It is important that grain quality is tested at a number of stages along the production line in order to attain the highest possible grain quality throughout the process.

Parameters currently used for assessment of grain quality in Australia are detailed in this section.

1.2.1 Milling Quality

The milling of flour from grain originally involved the grinding of the grains between rocks or in a primitive mortar and pestle. Following on from this large, manually-operated grinding stones were used for larger-scale grinding before windmills became common in the twelfth century. This was followed by steam power in the eighteenth century. The concept of using large rollers to grind wheat was first considered in the eighteenth century; however, they did not become common until the 1870's. The development of these rollers not only improved the colour of the resulting flour but also allowed millers to get rid of the large and cumbersome millstones they had been using until then.

The modern milling process uses numerous rollers structured into a 'break reduction' system involving the flow of grain from a hopper down onto corrugated rollers known as break rolls which are set close together. These rollers then crack open the individual grains removing the large chunks of endosperm while removing the external bran layer. This process is repeated several times with the corrugations on the roles becoming finer with each successive break roll. Flour particles are sifted out and purifiers remove the bran and pollard from the endosperm fragments. The endosperm particles are ground to flour by a series of smooth-surfaced rolls. This process is repeated a number of times with flour being progressively sifted out. In most modern mills, it is common to use four or

five break rolls and the same number of reduction rolls, though this can range from three to nine of each roll.

In order to remove as much of the bran etc as possible, the grain is 'conditioned' to a standard moisture (generally around 14.5-16%) prior to milling. This softens the seed coat, making it easier to remove, resulting in larger sections of bran being removed.

Milling quality is an important quality consideration in that protein content and quality of the flour is tailored to achieve suitable characteristics. A major factor in achieving the desired flour characteristics is the level of damage done to the starch granules during milling. Heavily damaged starch (generally seen during the milling of hard grain) requires more water during dough formation. Alternatively, starch with relatively little damage (from soft grain) has lower water absorption. These differences in water absorption can result in significant differences to processing and end product quality.

1.2.2 Flour Yield

Flour yield refers to the percentage by weight of white flour extracted from the whole grain during the milling process detailed in section 1.2.1. This yield is calculated using the calculation displayed below.

$$\% \text{ Extraction} = \frac{\text{Weight of Flour (g)}}{\text{Flour + Bran + Pollard (g)}} \times 100$$

Most commercial mills maintain extraction rates at approximately 75-80%.

A number of factors affect flour yield, including the size, shape and density of the grain. These factors are determined by a combination of genotype and environmental factors. Mechanical factors such as roll speed differential can also affect the extraction ratio (Hareland, 1998).

Plants exposed to drought or heat-stress conditions during grain-filling can result in grains that are smaller that usual and shrivelled in appearance. This increases the surface-to-volume ratio of the grain, leading to diminished flour extraction

(Williams, 1998). On the other hand, adequate water during grain filling often results in rounder plumper grains and thus increased flour yield.

Image analysis has been used to measure potential milling quality and it has been suggested following image analysis assessment of grain, that two thirds of the variation in flour yield is caused by factors relating to grain size, shape and test weight (Berman et al., 1996; Williams, 1998). Image analysis conveys information on factors including grain density, crease depth and bran thickness of wheat kernels. This information can be used as a means of predicting flour yield (Berman et al., 1996).

1.2.3 Flour Colour

Interest In flour colour as a quality parameter has increased recently in response to the increasing popularity and manufacture of products, such as noodles and steamed breads, for the Asian market. These types of products have very strict flour colour specifications and as such it has become increasingly important to define the colour characteristics of flours to be considered for production of these products (Parker and Langridge, 2000).

There are two features of flour that contribute to differences in flour colour; bran speckiness from residual bran/aleurone layers and actual endosperm colour (Mares and Campbell, 2001). Three factors have commonly been used to measure overall flour colour: brightness of the flour or L* value, the yellowness of the flour (b* value) and the redness of the flour (a* value) (Mares and Campbell, 2001).

There are two major types of wheat-based Asian noodles, white salted noodles (WSN) and yellow alkaline noodles (YAN). These noodle types have very different flour colour specifications. Flour that is to be used for noodles must be bright (i.e. have an L* value 89-94) and, in the case of WSN, be creamy in colour, (i.e. have a b* value $\leq$ 10.5). Flour required for YAN production on the other hand, should have yellow colouring just below that detectable by eye but with good yellow colour development on addition of alkaline salts (Mares, 2003).

Naturally occurring yellow pigments are known as carotenoids while those responsible for yellow colour development under basic conditions are known as flavonoids (Parker and Langridge, 2000).

The most common method for measuring the colour characteristics of flour is with a Minolta Colorimeter (Williams, 1998). These instruments use one of two methods to measure colour. The tristimulus method mimics the way the human eye perceives colour by measuring the amount of red, blue and yellow light reflected from an object/substance, while the spectrophotometric method uses spectral sensors to give highly accurate readings. The tristimulus method is the most effective method for measuring colour differences between objects/substances.

1.2.4 Test Weight

The test or hectolitre weight, also known as the bulk density of wheat, is defined as the weight of 100 litres of grain (Anonymous, 2003a; Talbert et al., 2001). This measurement is generally accepted as a strong quality indicator of wheat particularly those wheats of the durum varieties (Troccoli and di Fonzo, 1999). Test weight is an indicator of the density and the soundness of the wheat being tested (Williams, 1998). That is, wheat cultivars with a greater test weight will generally achieve significantly higher flour yields when milled. Thus, increased test weight is a highly desirable characteristic.

Australian guidelines indicate that for wheat to be accepted into most grades, the test weight on receival must exceed 74 kg/hL (71kg/hL in the case of Australian Hard (AUH)). To be considered high quality however, the test weight wheat should exceed 80 kg/hL (Wurst, 1999).

Wheats with high test weight tend to be visually appealing, while those samples with low test weight tend to be small and pinched in appearance (Czarnecki and Evans, 1986). Grain size and shape, however, are controlled to some degree by cultivar and thus the grain cultivar can have a significant influence on the test weight achieved by the grain (Wurst, 1999). Another factor contributing to test

weight is the level of rain damage the grain has undergone. Rain damage causes grain to swell, reducing the density of the grains and so the test-weight (Williams, 1998). Low test weight can be attributed to a number of different factors, including lack of tolerance to weathering, damage caused by insects, heat stress, defoliation of plants, frost and late harvest (Troccoli and di Fonzo, 1999). A number of environmental factors are also believed to increase the test weight of grain, including earlier heading of plants and optimal growing temperatures (Talbert et al., 2001). In Australia, wheats with an unacceptably low test weight are generally used in animal feeds (Wurst, 1999).

1.2.5 Grain Moisture

Grain moisture is an important consideration in wheat quality as it can affect the storage capability of grain by promoting infection by fungal pathogens and insect infestation (Williams, 1998). Thus, it is advantageous for the moisture level of the grain to be no greater than 12.5% to maintain grain quality during storage and in Australia this is the upper limit for acceptance of wheat (Wurst, 1999).

1.2.6 Grain Hardness

Grain hardness is of critical importance when selecting grain for an intended end product (Williams, 1998), (Galleschi et al., 2003; Morris, 2002). Importantly, the physical 'hardness' of the grain kernel is not necessarily related to wheat being classified as'hard' wheat. The hard wheat classification is generally based on the variety of the wheat whereas grain 'hardness' or grain texture refers to the physical hardness of the kernel when crushed.

Grain hardness is a genetically inherited characteristic. In 1986, Greenwell and Schofield (1986) reported a 15kDa protein in conjunction with the surface of starch granules from soft wheats. This protein was absent from the surface of starch granules water-washed from hard wheats (Greenwell and Schofield, 1986). Later it was discovered that the 15kDa protein was composed of three separate polypeptide chains and that the two most significant of these chains were puroindoline-a (PIN-a) and puroindoline-b (PIN-b) (Morris, 2002). The third polypeptide is known as the grain softness protein (GSP-1) (Turnbull et al., 2000). The presence of PIN-a and PIN-b in its native form are indicative of kernel

softness, while the absence of PIN-a or mutations of PIN-b indicate kernel hardness (Igrejas et al., 2001). Hardness is a strongly heritable characteristic with the locus responsible (termed the *Ha* locus) located on the short arm of chromosome 5D (Corona et al., 2001; Morris, 2002) of wheat (Wurst, 1999). As such, grain hardness is, in part, dependent upon the cultivar of the wheat (Turnbull et al., 2000). It is speculated that the *Ha* locus may be tightly linked to the puroindolines and GSP-1 while not actually producing them (Turnbull et al., 2000).

Generally, high soil fertility and low rainfall conditions favour the development of hard grained wheat and the resulting grain kernel will be vitreous and translucent in appearance. This is due to the high fertility soil conditions of the harder wheat favouring the synthesis of proteins as opposed to starch. Light when passed through a wheat kernel will pass unrefracted through protein leading to the vitreous appearance of high protein grain. Soft-grained wheats, however tend to grow in conditions of lower soil fertility and high rainfall and often appear opaque as lower fertility conditions favour starch production and the starch-protein interface causes light to be refracted giving the kernel a starchy opaque appearance (Simmonds, 1989).

The hardness of grain is important with regard to how the grain responds to the milling process. (Wurst, 1999). Hard grain results in a higher level of starch damage and subsequently the flour will require the addition of more water during dough mixing. Soft-grained wheats, on the other hand, yield flours with less starch damage following milling and therefore less water is absorbed during dough formation.

The hardness of the harvested grain can be measured in a number of ways, the most common of which is measurement of the particle size index (PSI) of the grain (Morris, 2002). This test requires the grain to be either ground or milled and then sieved at a particular aperture size. The percentage of material which passes through the sieve is then used to allocate the PSI of the material (Morris, 2002). This test works on the basis that softer grains will be more friable. As such, when pressure is applied to the kernel, less energy will be required to crush

it to smaller flour particles. Hard grains on the other hand will not crush as easily as soft wheats and the resulting fragments will be larger. Thus more material will pass through the sieve when testing soft wheats and consequently the PSI will be larger for these wheats (Morris, 2002).

NIR spectroscopy (described in Section 1.4.2.5) has also been used to measure grain hardness (Morris, 2002), while a third technique involves the crushing and subsequent characterisation of a single wheat kernel, using the single-kernel characterisation system (SKCS). The PSI and NIR techniques are used more frequently than the single kernel method (Morris, 2002).

1.2.7 Starch Properties

Starch is the second most abundant carbohydrate found in the biosphere with only the cell wall carbohydrate cellulose being more abundant. Starch is used by plants as an energy storage reservoir particularly by legumes and cereals such as wheat. Figure 1.2 shows the composition of the wheat kernel.

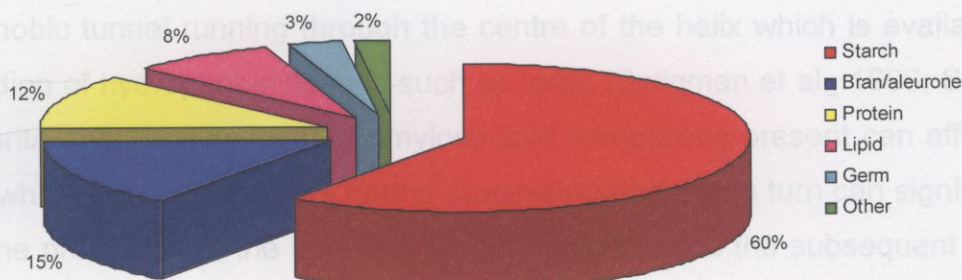

Figure 1.2. Composition of wheat grain (dry basis). Starch represents the majority of the grain (Orth and Shellenberger, 1988)

Starch represents 60-70% of the grain kernel and is stored in the endosperm of the grain as either A or B-type granules. A-type granules are the larger of the granules with a diameter greater than 10μm and a lenticular shape (Batey et al., 2001). These granules are synthesised four days after anthesis with granule growth continuing over 20 days. The smaller (<10μm) B-type granules are spherical in shape. The synthesis of these granules is initiated more than 10 days after anthesis and major growth doesn't occur for a further 20 days after

synthesis. B-type granules constitute 15-35% of the total grain starch (Shinde et al., 2003)

Starch granules are composed of two major glucose polymers, amylose and amylopectin (McCormick et al., 1995). Amylose is a predominantly linear molecule and is helical in structure whereas amylopectin is extensively branched and does not form a helix (Demeke et al., 1999). Each of these two polymers plays an important role in defining the quality characteristics of the flour, dough and end-product (McCormick et al., 1995). Aside from differences in size, shape and biosynthetic pathway, starch granules also differ in composition with A-type granules generally containing higher amylose levels than the B-type granules. B-type granules, however, are more likely to contain amylose complexed with lipid molecules (Shinde et al., 2003).

This complexing of lipids with amylose molecules arises as a result of the structure of the amylose molecule itself. The helical structure of amylose polymers involves six glucose units per turn of the helix. This leaves a long hydrophobic tunnel running through the centre of the helix which is available for the binding of hydrophobic ligands such as lipids (Seligman et al., 1998; Siswoyo and Morita, 2003). The level of amylose-lipid complexing present can affect the way in which the flour behaves during processing and this in turn can significantly affect the properties of the resulting dough or batter, and the subsequent quality of the end-product.

One of the most important characteristics of wheat starches is the ability of the starch to absorb water and gelatinise to form a paste when exposed to heat. During this process, the hydroxyl groups of the starch bind water, leaving less free water in suspension and subsequently increasing the suspension viscosity. A and B type granules behave differently with respect to this important quality parameter. The degree to which gelatinisation occurs, and the viscosity of the resulting paste is controlled primarily by the ratio of amylose to amylopectin. Amylose is generally considered to be responsible for the gelling properties of the starch while amylopectin contributes to the gel viscosity (Demeke et al., 1999). Further pasting and gelatinisation differences can be attributed to the large

surface-area-to-volume ratio of the smaller B-type granules as compared with the larger A-type granules. This results in more water being required by the B granules. Tests of starch pasting and gelatinisation include differential scanning calorimetry, Amylograph and the Rapid ViscoAnalyser (RVA).

Pasting behaviour is not necessarily predictable and there are a number of conflicting reports on starch gelatinisation and pasting characteristics with regard to A- and B-type granules. It has been speculated that the reason for the variation between results may be attributable to the cultivar and/or isolation procedures used within each study.

A focus of much research in recent years has been the concept of 'waxy' wheat. This refers to wheat with a reduced ratio of amylose to amylopectin. As mentioned above, amylose represents approximately 20-30% of the total grain starch in a standard wheat flour with amylopectin making up the remainder (Baik et al., 2003). Recent studies have investigated the development of wheat with reduced amylose levels. These wheat varieties are known as waxy or partial waxy wheats. The proteins responsible for amylose synthesis during growth are known as waxy or granule bound starch synthase (GBSS) and three loci are responsible for their expression; the *wx-A1*, *wx-B1* and *wx-D1* loci. To be considered 'waxy' all three alleles must be null, while to be 'partially waxy' requires only one or two null alleles. If any or all of these alleles are made null, amylose content will be altered causing changes in functional physical and compositional properties of the wheat (Baik et al., 2003).

1.2.8 Grain Protein

The proteins present in the wheat endosperm are termed storage proteins due to their propensity to store nitrogen for nutrition during growth and it is the quantity and quality of these proteins that is responsible for the functionality of the grain for processing (Bean and Tilley, 2003). Grain protein content is the strongest indicator of grain quality with regard to intended end-product (Wesley et al., 2001). Figure 1.3 indicates the protein content required for successful production of a number of different wheat products (Williams, 1998).

Flours that are high in protein are often blended with flours with a lower protein content in order to stabilise the dough and reduce mixing time and required work input.

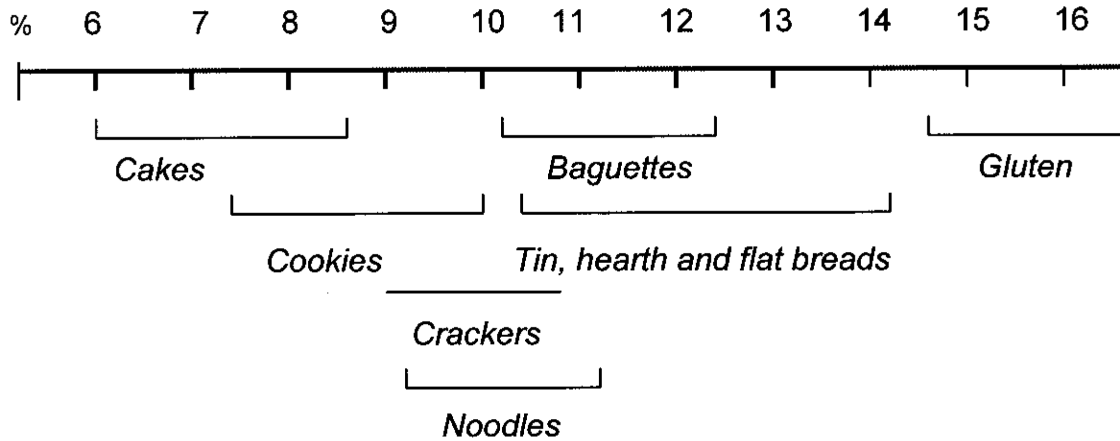

Figure 1.3: Protein Content Requirements for Major End-Products (Williams, 1998)

Along with protein content, protein quality is an important indicator of overall wheat quality (Wesley et al., 2001). Protein quality refers to the manner in which environmental conditions and genotypic protein composition interact to affect the quality of the gluten. A number of factors both genetic and environmental can contribute to protein content including cultivar and rainfall post anthesis among others (Wurst, 1999). Protein composition and quality will be considered in more detail in Section 1.3.

1.3 Wheat Protein Composition

There are four major groups of proteins found in wheat: albumins, globulins, prolamins and glutelins. Albumins and globulins are relatively small proteins that are soluble in water and saline, respectively (Gianibelli et al., 2001). These easily extractable proteins are generally grouped together. The other two groups comprise the 'gluten' proteins, with the third group being easily extractable with 60/70% alcohol and characterised by high levels of proline and glutamine residues in the primary protein structure. For this reason this group is termed the 'prolamins' known by the wheat-specific name of gliadin proteins (Figure 1.4).

The fourth group, soluble in dilute acid or alkali, is classed as glutelin, known by the wheat-specific name of 'glutenin'.

Approximately half of the gluten proteins fall into the gliadin category while the other half represents the glutenins. These gluten proteins are significantly larger and less soluble in water than the albumins and globulins. A schematic of these wheat proteins is shown in Figure 1.4.

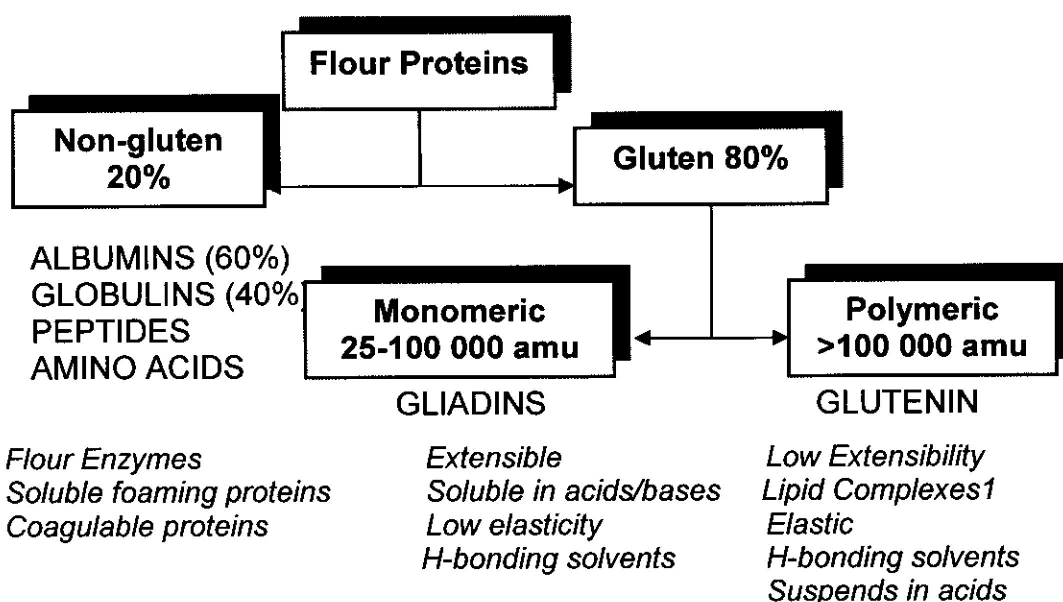

Figure 1.4: Flour protein classification

1.3.1 Gliadins

Gliadins are a group of monomeric wheat endosperm proteins (Daniel and Triboi, 2001; Gianibelli et al., 2001). These proteins make up approximately 40% of the total endosperm protein and are soluble in alcohol (Daniel and Triboi, 2001; Gianibelli et al., 2001). Gliadins are heterogeneous comprising four groups: α, β, γ and ϖ gliadins. Each gliadin type expresses a different pattern of constituent amino acids. As there are strong structural similarities between the α and β type gliadins these two groups are often considered together.

Though the various gliadin types differ in their constituent amino acids, all gliadins are characteristically low in the ionic amino acids, lysine, arginine,

histidine, aspartic acid and glutamic acid (Gianibelli et al., 2001). This reduced level of the essential amino acid lysine has been identified as an important nutritional disadvantage of wheat products (Gianibelli et al., 2001). The ϖ-gliadins are distinct in having virtually no sulfur-containing amino acids.

The basic structure of the ϖ-gliadins incorporates a 19-amino-acid long signal sequence, 10-11 amino acid long residue sequences at both the C- and N-terminals of the molecule as well as greater than 90% repetition throughout the molecule. This differs from the α/β and γ gliadins which consist of repetitive and non-repetitive domains with amino acid sequences of variable length (5-13 amino acids) at the N-terminal. Another important structural feature of the α/β gliadins is the absence of free cysteine molecules for intermolecular bonding. This means that gliadins are unable to form part of the gluten macropolymer but remain monomeric as opposed to the polymeric glutenin proteins.

The ϖ group of gliadins can be separated into a further three groups (Gianibelli et al., 2001). These groups are named using the first three N-terminal amino acid residues for each: ARQ, KEL and SRL (see Table 1.2 for full names of amino acids). ARQ type ϖ-gliadins are believed to be the ancestral sequence type, while the KEL ϖ-gliadins, while similar to these ARQ ϖ-gliadins, have a different and highly conserved 10 base amino acid sequence at the N-terminal (Gianibelli et al., 2001). The SRL ϖ-gliadins are different again and are coded for by genes on the 1B chromosome (Gianibelli et al., 2001)

Table 1.2: Full names and abbreviations of the twenty most common amino acid residues (Stryer, 1995)

1 Letter Abbrev.	Name of Amino Acid	3 Letter Abbrev.	1 Letter Abbrev.	Name of Amino Acid	3 Letter Abbrev.
A	Alanine	Ala	M	Methionine	Met
C	Cysteine	Cys	N	Asparagine	Asn
D	Aspartic Acid	Asp	P	Proline	Pro
E	Glutamic Acid	Glu	Q	Glutamine	Gln
F	Phenylalanine	Phe	R	Arginine	Arg
G	Glycine	Gly	S	Serine	Ser
H	Histidine	His	T	Threonine	Thr
I	Isoleucine	Iso	V	Valine	Val
K	Lysine	Lys	W	Tryptophan	Trp
L	Leucine	Leu	Y	Tyrosine	Tyr

The four groups of gliadins can be distinguished by differences in mobility on acidic cathodic electrophoresis, with α and β-gliadins having the highest mobilities followed by the γ- gliadins, and the ϖ-gliadins displaying the lowest mobility. Gliadin types are separated and identified using acidic polyacrylamide gel electrophoresis (A-PAGE), reversed phase- or size exclusion-high performance liquid chromatography (RP-HPLC, SE-HPLC) (Daniel and Triboi, 2001), (Siriamornpun et al., 2001) and more recently with capillary electrophoresis (CE) (Siriamornpun et al., 2001). In turn gliadins, once identified, can be used as markers to identify the variety of the wheat being tested (Cornish et al., 2001a; Cornish et al., 2001b; Siriamornpun et al., 2001). The use of proteins as markers will be discussed further in Section 1.4.1.4.

Gliadin proteins can affect the rheology of doughs significantly thereby contributing to the quality of the resulting baked product (Panozzo and Eagles, 2000; Panozzo et al., 2001). This important role has generated interest in those gliadins capable of imparting desirable quality characteristics and the conditions under which wheat quality is optimised (Panozzo and Eagles, 2000; Panozzo et al., 2001).

The major effects of gliadin proteins on dough properties include increased viscosity and extensibility, so that an excess of gliadins (compared to glutenin) can be accompanied by a decrease in dough strength (Daniel and Triboi, 2001; Gianibelli et al., 2001). Results of Huebner et al. (1999) have shown that in hard red winter wheat, γ-gliadins correlate well with loaf volume while gliadins have generally been known to have a favourable affect on Alveograph values (Alveograph being a measure of the tenacity, strength and extensibility of dough when blown up like a bubble) (Khelifi and Branlard, 1992).

An important consideration when investigating gliadin effects however, is the speculation that the allele coding for ϖ- and γ-gliadins, which are believed to contribute to some of these quality traits, may in fact have been attributable to LMW-GS (Khelifi and Branlard, 1992). This is due to the relative proximity of the loci coding for the gliadins and LMW-GS (Khelifi and Branlard, 1992).

Gliadins are coded for on chromosomes 1 and 6. The loci *Gli-A1*, *Gli-B1* and *Gli-D1* are found on the short arm of chromosome 1 and code for the ϖ- and most of the γ- gliadins, whereas *Gli-A2, Gli-B2* and *Gli-D2* are found on the short arm of chromosome 6, and code for the remaining γ- gliadins and all of the α- and β-gliadins (Gianibelli et al., 2001). Multiple allelism has been observed at both the *Gli-1* and *Gli-2* loci (Gianibelli et al., 2001).

A number of factors can be responsible for fluctuations in gliadin levels within the wheat endosperm including environmental aspects such as temperature, soil nitrogen levels at and after anthesis (Daniel and Triboi, 2001), and atmospheric carbon dioxide levels (Rogers et al., 1998). Wheat genotype (Daniel and Triboi, 2001; Panozzo and Eagles, 2000; Panozzo et al., 2001) and stage of kernel development (Huebner et al., 1999) can also have a major impact. It has been demonstrated however (Panozzo and Eagles, 2000; Panozzo et al., 2001) that environmental considerations generally have a greater quantitative effect on gliadin levels than genotype.

1.3.2 Glutenins

Glutenins are extremely large molecules, some aggregates reaching molecular weights of greater than 20 000 000Da (Gianibelli et al., 2001). This phenomenal size makes glutenin proteins some of the largest molecules in nature (Gianibelli et al., 2001).

The glutenin fraction of wheat proteins consists of many subunits joined via disulfide-linkages to form massive polymer structures. The subunits that make up the polymeric glutenin molecules consist of two major categories: the low-molecular-weight glutenin subunits (LMW-GS) and high-molecular-weight glutenin subunits (HMW-GS). These categories are described in detail below.

1.3.2.1 Low Molecular Weight Glutenin Subunits

LMW-GS are more abundant than the HMW-GS (Cornish et al., 2001a; Gianibelli et al., 2001), though the exact quantity is dependent on cultivar and growth conditions (Antes and Wieser, 2001; Wieser et al., 1994). Generally LMW-GS

make up ~60-80% of the gluten protein fraction (Bietz and Wall, 1973; Gianibelli et al., 2001),

LMW-GS are coded for by a closely linked family of genes known as the *Glu-3* alleles (Cornish et al., 2001a). These alleles are found on group-1 chromosomes and, as with the alleles for gliadins, involve the presence of three separate loci, *Glu-A3*, *Glu-B3* and *Glu-D3* (Gianibelli et al., 2001). The LMW-GS coded by the chromosome 1B locus exhibit particularly wide-ranging polymorphism. In contrast, relatively few LMW-GS are coded by the chromosome 1A locus (Gianibelli et al., 2001).

The LMW subunits are integral components of the glutenin polymer structure (Gianibelli et al., 2001; Gupta et al., 1989; Payne et al., 1984), with two LMW-GS types contributing. LMW subunits with more than one free cysteine residue are capable of forming intermolecular sulfhydryl bonds with more than one other subunit (LMW or HMW), thereby extending the polymer structure. These LMW-GS are thus known as chain extenders (Gianibelli et al., 2001; Masci et al., 1996; Sissons et al., 1998). Alternatively, those LMW-GS with only one cysteine residue available for cross linking may form only one intermolecular bond, after which there is no remaining binding site for ongoing polymerisation. These LMW subunits are thus known as chain terminators (Gianibelli et al., 2001; Gupta et al., 1989).

A branch of research into this area has involved the sequencing of the primary structure of the LMW-GS. Studies have shown that wheat varieties containing LMW-GS with the N-terminal amino acid sequence METSH correlate to increased dough strength (Gianibelli et al., 2001). This increase in dough strength is however, minimal when compared with the strength imparted by the HMW-GS (section 1.3.2.2).

Originally LMW-GS containing the METSH sequence were thought to be chain terminators as they contained just one cysteine residue in the N-terminal region (Sissons et al., 1998) making them incapable of extending the polymerisation process. If this were the case, dough strength would not be increased in the

presence of these subunits (Gianibelli et al., 2001; Masci et al., 1996; Sissons et al., 1998). Further study resulted in the discovery of a second cysteine residue in a repeat sequence close to the N terminal sequence. This complexity of the structure-function relationships of gluten proteins highlights the need for further research and deeper understanding of the quality implications.

Despite the relative abundance of LMW-GS, research has often been complicated by difficulties with successful isolation of the proteins using standard separation procedures. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is the standard separation procedure and has been found to be useful when separating the larger HMW-GS. However, the identification of large numbers of LMW-GS with similar sizes and gel mobilities has resulted in many subunits appearing to be similar to one another and to other protein types, particularly gliadins. This has made identification of individual LMW proteins problematic. The SDS-PAGE technique is described in more detail in Section 2.3.1.

Several techniques are being developed to increase the resolution of these LMW-GS, for example the extension of SDS-PAGE into two dimensions. The use of RP-HPLC as an alternative to SDS-PAGE has also been addressed as preliminary studies have indicated that LMW-GS resolution may be increased using RP-HPLC (El Haddad et al., 1995). Another technique currently in use involves the removal of the gliadins from the sample via extraction with various solvents such as dimethyl sulfoxide (DMSO). Removing the gliadins before electrophoresis vastly decreases the number of proteins amassed in a small area of the gel, allowing the LMW-GS to be seen more clearly (Cornish et al., 2001a; Gianibelli et al., 2001; Gupta and Shepherd, 1990).

These advances have helped promote further research into LMW-GS, leading to the discovery that LMW subunits may play a more important role in grain quality than was previously supposed.

This has been seen particularly for the tetraploid durum wheat varieties with durum wheat quality and subsequent pasta quality being more heavily reliant on

LMW-GS composition than the baking quality of hexaploid wheats (Ciaffi, 1995). This theory came about with the discovery of two LMW-GS alleleic groups initially named LMW-1 and LMW-2. These two allelic groups are coded for at the *Glu-B3* locus and have been shown to correlate with poor and good gluten viscoelasticity respectively (Carillo et al., 1990).

Further, LMW-GS variability has been indicated as a potential factor responsible for dough extensibility (Cornish et al., 2001a) which is an important factor in determining end-product viability of flours. The result of research so far, has been the discovery that LMW-GS expressed at the *Glu*-A3b, *Glu*-B3b and *Glu*-D3b alleles are capable of increasing extensibility, allowing breeders to produce varieties with characteristics suitable for their target market (Cornish et al., 2001a).

1.3.2.2 High Molecular Weight Glutenin Subunits

As mentioned above, ease of identification of the HMW subunits has resulted in them being considered as more attractive research foci than gliadins or LMW-GS. The HMW-GS, being the group of glutenin subunits of highest molecular weight, separate clearly at the top of an SDS-PAGE gel, making them comparatively simple to identify. This has promoted further research into these subunits both individually and collectively.

HMW-GS represent 20-40% of the glutenin fraction of wheat endosperm, the exact amount being dependent on cultivar and growth conditions (Antes and Wieser, 2001; Wieser et al., 1994). Although this represents significantly less HMW-GS than LMW-GS by weight, the HMW-GS fraction contributes more to the quality of the flour (Gianibelli et al., 2001), dough (Radovanovic, 2002) and baked end product (Khelifi and Branlard, 1992) than any other gluten polypeptides.

Much of this contribution is due to the capability of several HMW-GS to significantly increase gluten elasticity (defined as the resistance of dough to being stretched) (Gianibelli et al., 2001; Radovanovic, 2002). Increased elasticity results in a substantial increase in the strength of dough, and dough strength is important for the production of high quality bread as it correlates strongly with the

volume of the subsequent baked loaves. Loaf volume is also a primary quality indicator (Radovanovic, 2002) and so the importance of HMW-GS to overall end-product quality can be seen down the production line. On the other hand, excessive dough strength is a potential difficulty in bread production, especially as it is likely to increase work input and require longer mixing time.

Research has indicated that certain HMW-GS are more effective than others at enhancing the elasticity of gluten proteins (Gianibelli et al., 2001). Dough mixing times (time required to reach maximum resistance) have also been seen to improve in the presence of certain HMW-GS. This has led to further research into how manipulation of HMW-GS composition could potentially lead to improved flour and end-product quality characteristics (Bekes et al., 2001).

HMW-GS are coded for at the *Glu-1* loci on the long arm of the group one chromosomes (Gianibelli et al., 2001; Radovanovic, 2002). Hence *Glu-A1, Glu-B1* and *Glu-D1* respectively, indicate loci on chromosomes 1A, 1B and 1D (Radovanovic, 2002). Each of these loci incorporates genes for an ***x*** and a ***y*** subunit. Therefore, bread wheats are capable of expressing six HMW-GS types, 1Ax, 1Ay, 1Bx, 1By, 1Dx and 1Dy (Barro et al., 2003; Gianibelli et al., 2001).

Commonly, wheats express three to five of these subunits with almost all wheats containing the 1Bx, 1Dx and 1Dy subunits. Certain cultivars also contain the 1By and 1Ax subunits, though both of these subunits are capable of remaining null, and so not being expressed. The allele translating the 1Ay subunit is usually silent and thus this subunit is very rarely expressed, although this subunit has been observed in certain species including the wild diploid wheat varieties *T. monococcum* (einkorn) and *T. urartu.* (Gianibelli et al., 2001; Johansson et al., 1993).

HMW-GS also display significant polymorphism, as observed with SDS-PAGE, resulting in multi-allelism of the HMW-GS. This gives rise to the variations of HMW-GS composition observed and thus variations in the dough quality of wheat genotypes are partially dependent on the HMW-GS encoded on group-1 chromosomes.

The resulting HMW-GS variation can lead to quantitative and qualitative differences between wheat varieties (Barro et al., 2003). This can include the improved viscoelastic properties and loaf volumes mentioned above. Among the HMW-GS capable of enhancing quality parameters such as viscosity and elasticity are the 5x+10y (Gianibelli et al., 2001), 17x+18y (Gianibelli et al., 2001) and 7x+8y subunits (Radovanovic, 2002). Other subunits, for example, the 2+12 subunit, are capable of weakening the dough strength. It is generally accepted that the presence of either the 5x+10y or the 2x+12y subunit is a major determinate of dough strength or weakness (Czuchajowska et al., 1996; Radovanovic, 2002). Bekes et al, (1995) demonstrated that addition of the 5+10 subunit pair into a null *Glu-D1* flour resulted in significantly stronger dough than did the incorporation of similar amounts of 2+12 subunits. The hypothesised reason for this was the higher number of cysteine residues present in the structure of the *Glu* 1-Dx5 and *Glu* 1-Dy10 alleles as opposed to the *Glu*-1-Dx2 and *Glu* 1-Dy12 alleles.

The presence of different HMW-GS in different wheat varieties is believed to result in significant variation between flours in a number of quality testing parameters. For example it has been observed that lines expressing subunits 7+8 also produce flour with strong gluten while lines expressing the 5+10 subunits have higher mixing times than flours without these subunits. Further, lines capable of over-expressing subunit 7, display very strong dough characteristics, sedimentation levels and increased energy to peak dough resistance (Radovanovic, 2002).

As with all of the prolamines, HMW-GS have high levels of proline and glutamic acid (Gianibelli et al., 2001). Along with this, HMW-GS also have high glycine levels and are very low in lysine, which is a nutritional disadvantage. The structure of the HMW-GS includes a hydrophilic, repetitive central domain. Greater than ninety percent of this repetitive domain is made up of the two amino acid sequences PGQGQQ and GYYPTSPQQ (Shewry, 1997). This repetitive central domain is flanked by two non-repetitive, hydrophobic terminals both of which are rich in cysteine (Gianibelli et al., 2001). There is relatively good conservation, between HMW-GS in the first 15 amino acid residues of the N-

terminal (Wrigley, 1996). Differences between subunits, however, can often come down to the alteration of just several amino acids in the repetitive domain of the protein (Radovanovic, 2002).

Understanding these variations in HMW-GS structure, function and composition and their effects on flour, dough and end product quality is essential to the breeding of wheat varieties which are reliable and suitable for their intended purpose.

1.3.3 Protein Ratios

1.3.3.1 Gliadin:Glutenin

Glutenins and gliadins both play important and distinct roles in determination of end-product quality. The relationship between glutenins and gliadins has been described in a number of ways. Wieser and Keiffer (Wieser and Kieffer, 2001) describe gliadins as behaving as solvents for the glutenins. On the other hand Uthayakumaran et al, (2000a), describe gliadins as being responsible for the flow of the bread dough, while glutenins are responsible for the strength and elasticity of the dough. Either way, gliadins and glutenins work together to define the quality characteristics of a flour.

Of equal importance however is the ratio of gliadin to glutenin. This ratio is believed to be an important parameter in quality testing of wheat varieties. Uthayakumaran et al (2000a) studied the manner in which the results of quality tests such as elongational testing and viscosity testing varied with different ratios of glutenin to gliadin. It was observed that increased glutenin-to-gliadin ratio resulted in an increase in rupture viscosity and a decrease in rupture strain. Similarly, a decrease in glutenin-to-gliadin ratio would result in the opposite effect.

Several studies (Wieser and Kieffer, 2001; Wieser et al., 1994) have indicated that an increased ratio of gliadin to glutenin is a major contributing factor in both increased extensibility and decreased maximum resistance displayed of dough. These studies also found that when considering baking characteristics such as

loaf volume, the major contributing factor was total gluten protein content rather than ratios of protein types.

1.3.3.2 HMW-GS:LMW-GS

The relationship between larger and smaller glutenin subunits has also been studied in recent years (Uthayakumaran et al., 2000a; Uthayakumaran et al., 2000b; Wieser and Kieffer, 2001). These studies have aimed to gauge the manner in which glutenin-subunit ratios can reflect the possible end uses of wheat cultivars and to better understand the manner by which these protein ratios affect rheological properties of doughs.

Wieser and Kieffer (2001) observed that LMW-GS and HMW-GS correlate with rheological properties similarly. For example, an increase in either HMW-GS or LMW-GS will result in increased dough strength and decreased dough extensibility. Therefore, if one glutenin type results in a dough property being enhanced, so will the other; however double the amount of LMW-GS were required in order for the dough properties to be affected similarly.

Uthayakumaran et al (2000b), however, observed that changes in HMW-GS-to-LMW-GS ratio resulted in a large number of changes in the properties of the dough. It was observed that increasing HMW-GS-to-LMW-GS ratio brought about changes such as increased mixing time, peak resistance, maximum resistance to extension, loaf height and dough extensibility while resistance to breakdown was observed to decrease.

1.3.3.3 x-Type HMW-GS : y-Type HMW-GS

Studies have also been conducted into the contribution to rheological properties of the different HMW-GS types x- and y- (Wieser and Kieffer, 2001; Wieser et al., 1994). Thus far, it has been observed that the x-type subunits, for example individual 5 and 2 subunits, have a greater effect on dough rheological properties than do their y-type counterparts such as 10 or 12 subunits. This is seen particularly in relation to dough strength with x-type subunits having a far greater positive affect on dough strength than the y-type subunits. This may be because x-subunits are generally larger than y-subunits.

1.4 Current tests for protein quality

A number of different test methods and instruments are used in wheat chemistry in order to identify and quantify the protein content, composition and quality of individual flours. Those techniques that are most commonly employed are described in detail in this section.

1.4.1 Tests for Genotype

Tests for genotype are particularly useful for wheat breeders.

1.4.1.1 Electrophoresis

Electrophoresis refers to the separation and molecular structure analysis of molecules such as proteins, based on the movement of the molecules through a colloidal suspension while under the influence of an electric field (Anonymous, 2000).

The versatility of the technique has made it one of the most important protein analyses today as it can be applied to a diverse array of proteins from many plants and animals. Proteins to be analysed can be of almost any type or size, can be native or in reduced form and can have been extracted by any number of techniques. Electrophoresis can also be carried out under acidic, basic and neutral conditions further increasing this versatility (Shewry, 2003).

There are a number of electrophoretic techniques commonly used in wheat chemistry including sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), multi-stacking SDS-PAGE (MS-SDS-PAGE), lactic acid-polyacrylamide gel electrophoresis (A-PAGE) and isoelectric focussing (IEF) as well as two dimensional techniques and western blotting.

SDS-PAGE is the most common electrophoretic technique used to fractionate proteins and accomplishes separation largely based on molecular size (Figure 1.5). As shown, a small aliquot of sample mixture is mixed with the anionic detergent SDS and sometimes a reducing agent such as mercaptoethanol. The SDS complexes with proteins disrupting the hydrogen and disulfide linkages present in the protein structure. This causes individual proteins to unfold and

polymers to 'depolymerise' releasing constituent subunits (Stryer, 1995). A dye such as Coomassie Blue is also commonly added in order to mark the advancing front and to indicate when the run has reached completion. A small amount of this mixture is then pipetted into each small sample well at the top of the polyacrylamide gel. Polyacrylamide is the suspension of choice due to its porous nature. The size of these pores is inversely proportional to the concentration of acrylamide and this factor along with the wide range of buffers that can be used allows enormous flexibility of protein separations. Following addition of the sample, an electrical voltage is applied to the gel. This results in the migration of the sample proteins into the gel. The smaller proteins migrate faster through the gel matrix, and so appear further down the gel than larger molecules. Once the gel is stained, the protein bands become visible. In the case of wheat proteins, it is usual to conduct SDS-PAGE on fully reduced extracts of flour, so that the disulfide bonds linking the glutenin subunits are cleaved. Apart from the HMW subunits of glutenin (separated at the top of the gel pattern), the remaining polypeptides (e.g. LMW-GS and gliadins) assemble as a complex pattern in the lower part of the gel. It is this complexity that has made isolation of these proteins more difficult than that of the HMW-GS.

SDS-PAGE is employed in grain science as a means of identifying individual protein subunits, which in turn can be used as varietal markers. It can also be used to measure seed sample purity prior to sowing, at harvest and before processing, if necessary.

Multi-stacking SDS-PAGE (MS-SDS-PAGE) works on a similar basis to standard SDS-PAGE with the differences being that only non-reducing solutions are used and several stacking layers are incorporated to fractionate very large proteins. Successive layers include sharp changes in pore size resulting in polymers becoming trapped at the layer interfaces (Khan and Huckle, 1994).

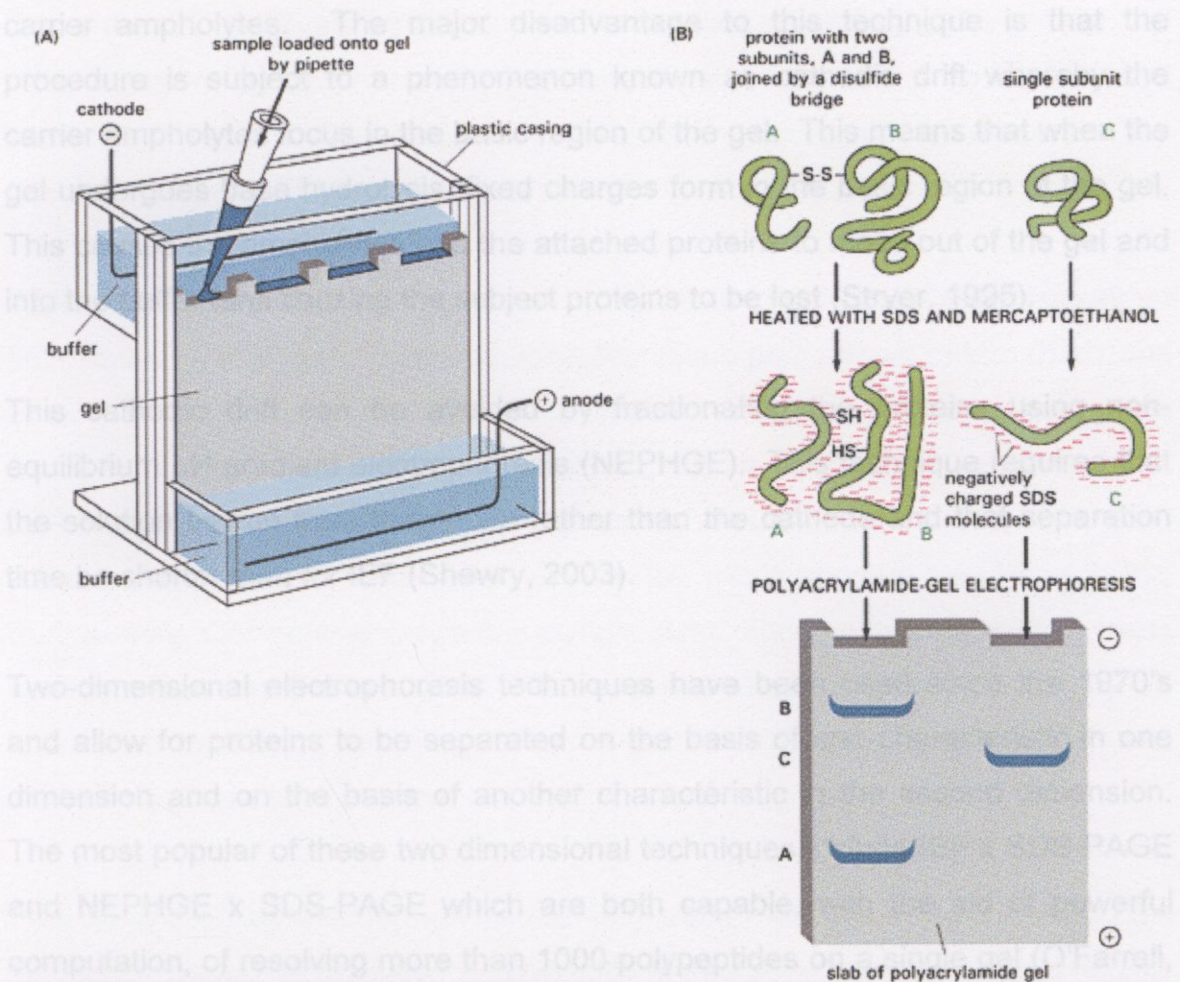

Figure 1.5: Schematic of polyacrylamide gel electrophoresis as a means of separating proteins

A-PAGE (acidic-polyacrylamide gel electrophoresis) again follows the same electrophoretic principals; however the current is applied in the opposite direction (i.e. migration towards the cathode) and tracking dyes are required to ascertain when the run has reached completion. The technique is used predominantly as a means of varietal identification, particularly with respect to separation of gliadins for this purpose. A-PAGE is a sensitive and stable technique and provides complementary information to that obtained from SDS-PAGE.

Isoelectric focussing refers to the separation of proteins on the basis of their isoelectric points. The isoelectric point of a protein refers to the pH at which the net charge of the protein molecule is zero. This point is significant to isoelectric focussing as it is also the point at which the mobility of the protein is zero and thus the protein will accumulate (focus) at that pH. This is carried out by establishing a pH gradient between the electrodes and stabilising proteins with

carrier ampholytes. The major disadvantage to this technique is that the procedure is subject to a phenomenon known as cathodic drift whereby the carrier ampholytes focus in the basic region of the gel. This means that when the gel undergoes base hydrolysis, fixed charges form in the basic region of the gel. This causes the ampholytes and the attached proteins to move out of the gel and into the buffer tank causing the subject proteins to be lost (Stryer, 1995).

This cathodic drift can be avoided by fractionating the proteins using non-equilibrium pH gradient electrophoresis (NEPHGE). This technique requires that the solution be run from the anode rather than the cathode and that separation time be shorter than for IEF (Shewry, 2003).

Two-dimensional electrophoresis techniques have been used since the 1970's and allow for proteins to be separated on the basis of one characteristic in one dimension and on the basis of another characteristic in the second dimension. The most popular of these two dimensional techniques include IEF x SDS-PAGE and NEPHGE x SDS-PAGE which are both capable, with the aid of powerful computation, of resolving more than 1000 polypeptides on a single gel (O'Farrell, 1975).

Finally, Western blotting is a technique involving electroelution as a means of blotting proteins from a polyacrylamide gel to a membrane in order to stabilise the proteins. This is generally achieved by either simple diffusion, capillary action, vacuum blotting or electroblotting.

1.4.1.2 Capillary Electrophoresis

Capillary electrophoresis (CE) is a relatively new procedure having been developed over the last 10-15 years (Siriamornpun et al., 2001). CE can be used to separate proteins, peptides, amino acids, nucleic acids, inorganic ions, organic acids and bases and even whole cells (Perez, 2000). Methods of capillary electrophoresis have recently been developed for possible use in the wheat industry to distinguish between different wheat cultivars. This has involved fractionation of gliadin proteins using buffers such as phosphate buffer (Bietz and Lookhart, 1997). Capillary electrophoresis employs capillaries with small inner

diameters (50 to 100μm) as anti-convective media as opposed to the gel slabs of other types of electrophoresis. The small diameters of the capillaries used facilitates the use of high voltages which results in high resolution separation in a comparatively short time (Shewry, 2003).

There are a number of advantages of using CE to identify cultivars versus other techniques. CE is a significantly quicker technique than certain others (Bietz and Lookhart, 1997) being capable of completing analyses in approximately ten minutes. The technique does not require extensive skilled manpower (Siriamornpun et al., 2001) and also gives high resolution separation and good reproducibility (Bietz and Lookhart, 1997; Siriamornpun et al., 2001). Furthermore, CE requires only small sample sizes, and can be coupled to a mass spectrophotometer (Perez, 2000). Two modes of wheat protein separation by CE have been developed thus far; free zone capillary electrophoresis (FZ-CE) and SDS capillary electrophoresis.

Free zone capillary electrophoresis (FZ-CE) is the most commonly used and the simpler of the two techniques. A schematic of free zone capillary electrophoresis is shown in Figure 1.6 below. The FZ-CE technique is analogous to A-PAGE (Shewry, 2003)

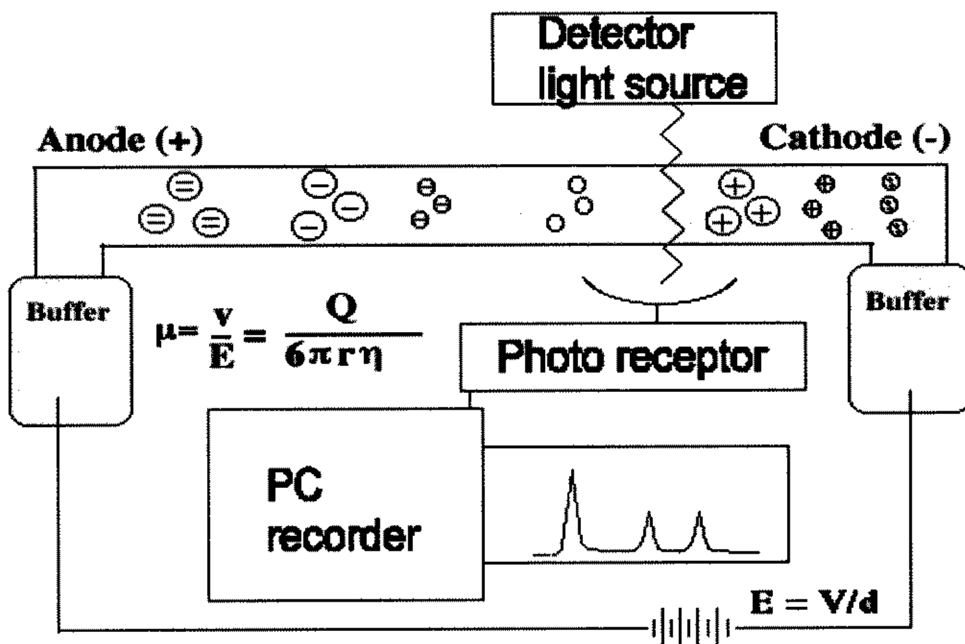

Figure 1.6: Schematic of Free Zone Capillary Electrophoresis Instrumentation (Perez, 2000)

The procedure begins by placing the extracted test sample in a buffer solution. This is then injected either electromigrationally, hydrostatically or pneumatically into a capillary. The capillary is made of fused silica, the hydroxyl group of which is dissociated leaving the tube with a net negative charge. A voltage is then applied across the length (30-100cm) of the capillary between the anode and the cathode. With the application of the voltage along the negatively charged capillary, anion electrophoretic migration will be toward the anode, while cation electrophoretic migration will be in the direction of the cathode. The latter is generally applicable to wheat protein separations.

FZ-CE separates on the basis of charge and then size. Molecules with high charge will migrate faster than molecules of low charge while larger molecules will migrate slower than smaller molecules (Perez, 2000). Thus when voltage is applied the smaller more highly charged molecules will migrate the most rapidly followed by the highly charged larger molecules. The last molecules to migrate will be the large negatively charged molecules. The migration pattern within the capillary is illustrated in Figure 1.7.

As the molecules migrate along the length of the capillary, a light source, for example UV/Vis or fluorescent is directed across the capillary tube toward a photo-receptor. The manner in which this light is deflected on to the photo-receptor is then transmitted through to a computer which interprets the data graphically.

Several problems exist when using FZ-CE to separate proteins particularly in relation to attaining reproducibility. A serious concern is the condition of the capillary as the amphoteric nature of proteins can cause them to adhere to the capillary wall. Over time this will cause deterioration of the capillary resulting in a decrease in separation efficiency thus the capillary must be constantly monitored and preparation must be carried out carefully in order to detect this deterioration. Increased salt concentrations can help to overcome this however heat will be generated as a result of increased current. It is essential to maintain correct pH when operating the apparatus. This pH will need to be above the isotonic pH

(IpH) of all the proteins in solution. Other factors that can affect reproducibility and resolution when separating proteins versus other types of molecules, include sample vial humidification, capillary end-cut, capillary equilibration, sample injection amount, voltage ramp-up time and sample preparation (Perez, 2000; Shewry, 2003).

In the same way that FZ-CE is analogous to A-PAGE, so SDS-CE carries out separations in the same manner as SDS-PAGE (Shewry, 2003). A schematic of the SDS-CE technique is seen in Figure 1.7.

The technique separates protein mixtures based on size using an entangled polymer solution inside the capillary. This solution effectively sieves the proteins as the current moves the proteins along the negatively charged capillary. SDS-CE has been shown to work with a number of polymers, for example un-crosslinked polyacrylamide, dextran and polyethylene oxide and is generally carried out at basic or neutral pH.

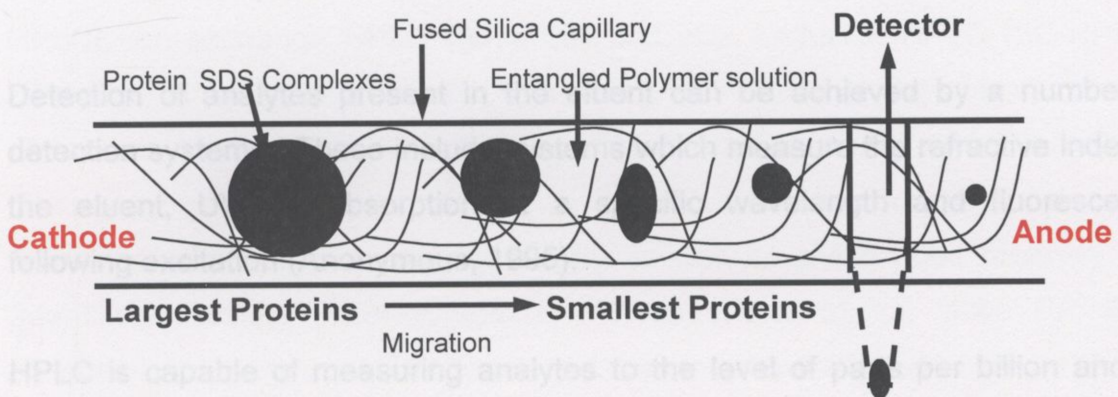

Figure 1.7: Diagram of an SDS-CE separation of gluten proteins (Shewry, 2003)

The major problem encountered with this technique is the occurrence of electroendosmotic flow (EOF). This term refers to the bulk flow within the capillary and is generally considered a disadvantage as the magnitude of the EOF is difficult to reproduce. Several solutions to this problem have been

discovered including using permanently coated capillaries as well as polymers which bind to the capillary walls such as un-crosslinked polyacrylamide (Shewry, 2003).

1.4.1.3 High Performance Liquid Chromatography (HPLC)

Another methodology that is regularly used to separate wheat proteins is high performance liquid chromatography (HPLC). This technique involves the injection of an aliquot of sample solution onto a separation column containing a specifically bonded phase. Solvents are then applied to the column by means of a liquid pump system, effectively forcing the sample down the column and separating protein fractions based on partitioning behaviour.

Figure 1.8 shows a schematic of HPLC instrumentation. Two solvents are pumped from solvent reservoirs into a pressurised liquid line and thus into a pre-column and finally down an analytical column. The sample or mobile phase is injected into the instrument at the sample injection port at which point solvent will begin to move the sample down the column. Solvent should be running down the column prior to injection of sample and the solvents require degassing on entry to the instrument to prevent the formation of air bubbles which can affect results.

Detection of analytes present in the eluent can be achieved by a number of detection systems. These include systems which measure the refractive index of the eluent, UV-VIS absorption at a specific wavelength and fluorescence following excitation (Anonymous, 1995).

HPLC is capable of measuring analytes to the level of parts per billion and as such is considered extremely sensitive. HPLC is, however, comparatively time consuming and lacks the ability to distinguish between certain wheat cultivars (Siriamornpun et al., 2001).

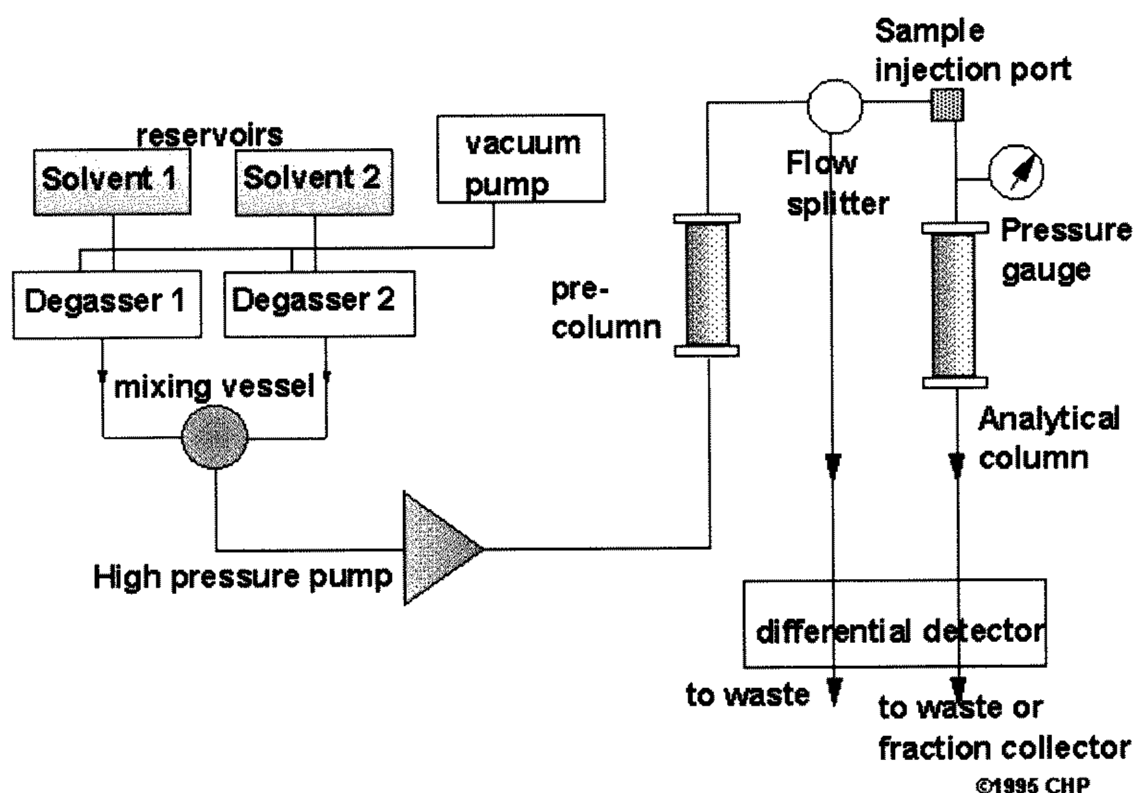

Figure 1.8: Schematic of an HPLC instrument.(Anonymous, 1995)

A number of different HPLC protocols exist including reversed phase HPLC (RP-HPLC), ion exchange HPLC (IE-HPLC) and size exclusion HPLC (SE-HPLC). RP-HPLC is thus named due to the non-polar nature of the column bonded phase as opposed to the polar bonded phases normally used in column chromatography. The technique has many advantages including very high resolution separations, speed, sensitivity as well as giving good recovery and quantifiable results (Shewry, 2003). These characteristics make RP-HPLC suitable not only for analytical separations but for preparative separations as well i.e., the separated proteins can be collected as well as quantified. RP-HPLC separates proteins based on hydrophobicity, making column selection the most important consideration when using RP-HPLC. The columns must have the correct hydrophobic ligand, support type, dimensions, particle size and pore size for the target proteins if successful separations are to be achieved.

RP-HPLC has been used to study protein accumulation, aggregation, structure and functional properties such as viscoelasticity. The technique can also be used two-dimensionally with electrophoresis (Bean and Lookhart, 1997). RP-HPLC has also been used successfully in characterising polypeptide components and glutenin subunits as well as being invaluable in identification of HMW-GS, particularly those subunits displaying identical SDS-PAGE mobilities. The high resolution of the technique is also a valuable asset for identification of LMW-GS (Shewry, 2003).

The IE-HPLC technique involves the running of a liquid or mobile phase, including the proteins to be isolated, down a stationary matrix column that is either charged anionically or cationically. The proteins will bind to the column with different affinities dependent on the protein charge thus separating out the individual proteins (Lee, 2001).

Prior to RP-HPLC, ion exchange chromatography was the leading technique for separation of wheat proteins prior to the advent of RP-HPLC. The reason for this is the lower reproducibility and resolution of the IE-HPLC separation technique (Shewry, 2003).

Size exclusion HPLC is another useful application of HPLC. This technique is regularly used in cereal chemistry to characterise the size of wheat proteins, particularly those that are polymeric in structure. The technique works using a column packed with a highly porous material. In this way, the sample is fractionated on the column on the basis of the size of the molecule or complex and the manner in which the molecule or complex can penetrate the pores of the column material (Shewry, 2003). Figure 1.9 shows the graphical output of the SE-HPLC technique for three wheat varieties.

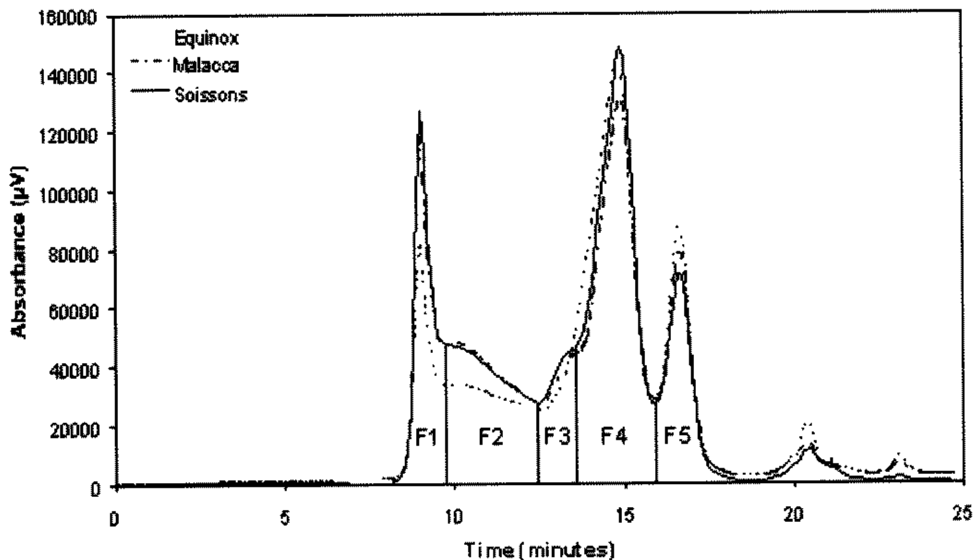

Figure 1.9: SE-HPLC chromatogram showing the different fractions present for three varieties. F1 represents the larger glutenin fraction, F2 the smaller glutenin fraction, F3 and F4 the gliadin fractions and F5 the combined albumin and globulin fraction (HGCA, 2002).

The proteins to be investigated are generally extracted using buffers containing phosphate and SDS (2%) at pH 6.9. Studies have shown that an extraction, involving sonication, results in extraction of greater than 95% of the proteins present in the wheat samples. The procedure used in this work thus involves an initial extraction using the above buffer solution in order to remove the 'extractable' proteins followed by a second extraction in the same buffer solution including sonication of the sample aliquot in order to extract the usually 'unextractable' proteins. Each of these samples is then run by SE-HPLC and the results used to quantify the relative amounts of the different protein types.

The columns used for SE-HPLC are generally short rigid silica columns capable of withstanding pressure of 3000 psi. The particle size of the HPLC columns is also important, with the smaller particle size resulting in greater resolution of proteins on the column. The aqueous nature of the protein separations requires the silica column to be of the hydrolysed glycidoxypropylsilane column type, whereas organic separations require tri-methyl silane silica columns.

The requirements for the mobile phase of this technique for protein separation include the ability to reduce inter-protein interactions in solution, thus maintaining

the proteins in their native form. The mobile phase must also be active in reducing reactions between the components to be measured, that is, the proteins and the stationary phase. When using SE-HPLC to separate proteins, the most common mobile phase comprises 0.05-0.1% SDS in a 50:50 acetonitrile/water solution with 0.05-0.1% TFA (Autran, 1994).

A number of other factors are important in order for proteins to be separated by SE-HPLC at appropriate resolution. These include column temperature, flow rate, sample filtration and sample volume; each must be optimised for effective protein separation.

1.4.1.4 Protein Markers

Genes code for characteristics capable of enhancing wheat quality. Therefore, it is important to understand the mechanism of genetic inheritance and how it can be manipulated via breeding for the benefit of the industry.

One way of achieving this is identification of certain genetic traits which are indicative of the presence of a second trait. For example, Sax observed that bean seed colour was linked to seed weight (Sax, 1923). Observations such as these lead progressively to the discovery of 'linked' genes and the eventual implication that it was possible to use easily characterised genes as a means of 'marking' the presence of other linked genes (Eagles et al., 2001). This phenomenon allows breeders to select for the easily characterised gene and in so doing select for the desired trait as well.

In order for a marker to be used for selection purposes, it must display several characteristics. Firstly, the marker must be closely linked with a gene coding for a trait with potential economic importance. Secondly, the marker must be polymorphic in order for the desirable allele to be distinguished from the undesirable allele. Lastly, the marker must be cost effective in order to be useful to breeders (Eagles et al., 2001).

Currently, there are four groups of markers being used for marker assisted selection in the Australian wheat industry. These categories include morphological, disease resistance, biochemical and DNA-based markers.

Morphological markers are markers producing easily identified phenotypes that are linked to economically important traits such as rust resistance. An example of this type of marker is pseudo-black chaff. Selection for this phenotypically identifiable trait has been shown to result in conferring of resistance to stem rust (Brown, 1993).

Linked disease resistance genes are directly selected resulting in the indirect selection of other genes capable of conferring resistance to a number of common diseases. This works on the basis that the presence of the selected gene indicates that the other gene(s) will be present also. McIntosh et al. (1998) list a number of examples of this type of genetic selection.

Biochemical and DNA-based markers are both grouped in the category of molecular markers, with biochemical markers producing enzymes or storage proteins which can then be analysed by bioassay. DNA-based markers identify molecular changes in DNA segments or genes coding for important traits.

Biochemical markers can include 'perfect' or 'diagnostic' markers whereby the marker is itself the determinant. An example of this is the 5+10 HMW-GS, the presence of which indicates that dough made from this wheat will have increased dough strength. In this case the 5+10 subunit combination is both the marker for increased dough strength and the physical reason for the increase (Eagles et al., 2001). As well as storage proteins, enzymes can be used as biochemical markers; however, this technique is currently not used in Australian wheat breeding programs due to the difficulty involved in development of suitable assays.

DNA-based markers are considered to be the area of greatest potential in marker assisted selection (Eagles et al., 2001). There are three major areas of DNA-based markers. These include restriction fragment length polymorphisms

(RFLP's), allele-specific polymerase chain reaction markers (AS-PCR's) and microsatellites. DNA-based markers are used extensively in the area of cereal cyst nematode resistance. This resistance is conferred by the genes *Cre1* and *Cre3* and so a marker was developed which is strongly linked to the *Cre1* gene. This type of marker assisted selection justifies its significant development costs as the enhancement of wheat production has such importance to the industry.

Other DNA-based markers of note are microsatellites or simple sequence repeats (SSRs). These are short, repetitive DNA segments (approximately 1-6 base pairs long) that are highly polymorphic, co-dominant and abundant. These characteristics have made microsatellites very useful as molecular markers and they are now used for marker assisted selection, genetic mapping and varietal identification among other uses (Harker, 2001b). This diversity has now led to the development of a number of new microsatellites as well as a microsatellite database (Harker, 2001b). In addition, the relative ease of scoring and interpretation of these microsatellites makes them promising for future development (Harker et al., 2001a).

Microsatellites are currently being used for the development of waxy wheat cultivars. These microsatellites are closely linked to the waxy genes *Wx-A1*, *Wx-B1* and *Wx-D1* and can be linked individually or in multiplex assays. This linkage allows triple null genotypes to be identified for backcross breeding regimes (Eagles et al., 2001). Microsatellites are being investigated currently as markers for characteristics such as grain protein content (Prasad et al., 2003), sensitivity to vernalisation (Salina et al., 2003) and powdery mildew (Bougot et al., 2003), among others. Microsatellite markers have the ability to permit fast, high throughput fingerprinting of large numbers of accessions from a germplasm collection and so to assess genetic diversity.

1.4.2 Tests for Genotype x Environment Interactions

These quality tests provide relevant information on grain receival as well as providing appropriate information to millers and bakers.

1.4.2.1 Falling Number

An important consideration when ascertaining grain quality is the presence of the enzyme α-amylase in the mature grain. This α-amylase results from either late season rainfall or the presence of one or two genes coding for late maturity α-amylase. The active enzyme interferes with starch gelatinisation by effectively liquefying the starch. This prevents the formation of the characteristic viscous gel on heating of a wheatmeal-water mixture. The test for the presence of α-amylase in a sample is known as its falling number. This test involves heating a mixture of wheatmeal and flour in a water bath using a mechanical stirring device. The stirrer is then left to fall a measured distance through the, now gelatinised, mixture. The time in seconds for the stirrer to complete falling is known as the falling number. In the presence of α-amylase, these will have thinner consistencies and therefore the stirrer will fall rapidly resulting in a low falling number. Australian wheat is required to have a falling number greater than 300 for all grades besides Australian Prime Hard which requires a falling number greater than 350.

1.4.2.2 Test Baking and Loaf Volume

It is desirable, when testing for quality that the end-product quality should be tested as well as the flour and dough quality. It is generally impractical, however for baking quality tests to be carried out on the large scale found in commercial bakeries (Zounis and Quail, 1997). Test baking provides an alternative if definitive means of evaluating end-product suitability is not available. Test baking involves mixing, proofing and baking a small number (3-6) of loaves (or other baked product), which can then be evaluated for staling and crumb properties, loaf volume and bread texture or softness.

Variations in flour variety/blend, mixing times, water absorption and dough development after mixing can all have significant effects upon the baking characteristics (Zounis and Quail, 1997). This testing technique allows for a number of variables to be checked in order to discover the optimal mixing and baking conditions for production of the highest quality baked product.

1.4.2.3 Dough Testing

1.4.2.3.1 Farinograph

One of the most common quality tests carried out in grain science utilizes the Brabender® Farinograph. This test involves the mixing of either 300, 50 or 10 g flour samples (10 g instrument is known as a Microfarinograph) of flour with enough water to make a dough. In so doing, information on the mixing properties of the flour can be ascertained.

Figure 1.10 shows a standard trace from a bread flour. This trace begins at the point at which water is added to the flour (C). This is when the flour begins to offer resistance to the mixers. The amount of water required for the dough to reach a resistance of 500 BU or Brabender Units (B) on the y-axis is termed the water absorption, due to a combination of factors, including the protein content and the level of starch damage, related in turn to grain hardness. From this point, the dough is left to develop to peak resistance (E). After peak resistance is reached, the dough begins to break down. This breakdown process is considered complete when the top of the curve moves below the 500 BU line (departure time (F)). The length of time from when the curve first reaches the 500 BU line (arrival time (A)) to the departure time is termed stability. High stability is a desirable characteristic as it limits changes to product quality if over-mixing occurs.

1.4.2.3.2 Extensograph

Another test of dough properties is the Brabender® Extensograph (Bangur et al., 1997). This test measures the extensibility of the dough when stretched at constant force (in centimetres) as well as the resistance to extension of the dough (in Brabender units)(Stampfli et al., 1996). Dough extensibility refers to the degree to which the dough can be stretched without rupturing (Wurst, 1999).

Extensograph testing involves the mixing of a salted dough in a Farinograph bowl using the water absorption calculated from the Farinograph. Following dough development, the doughs are cut into smaller portions and moulded. The moulded dough pieces are left to develop at constant temperature for a period of 45 minutes (and sometimes at 90 and 135 minutes) before being stretched at

constant elongation until the dough breaks. A graphical representation (Extensogram) is recorded based on resistance and extensibility (Figure 1.11). Extension testing can be carried out in large-scale using the Brabender Extensograph or for small-scale analysis a Keiffer Rig attachment to a texture analyser can be used (following microfarinograph mixing of dough).

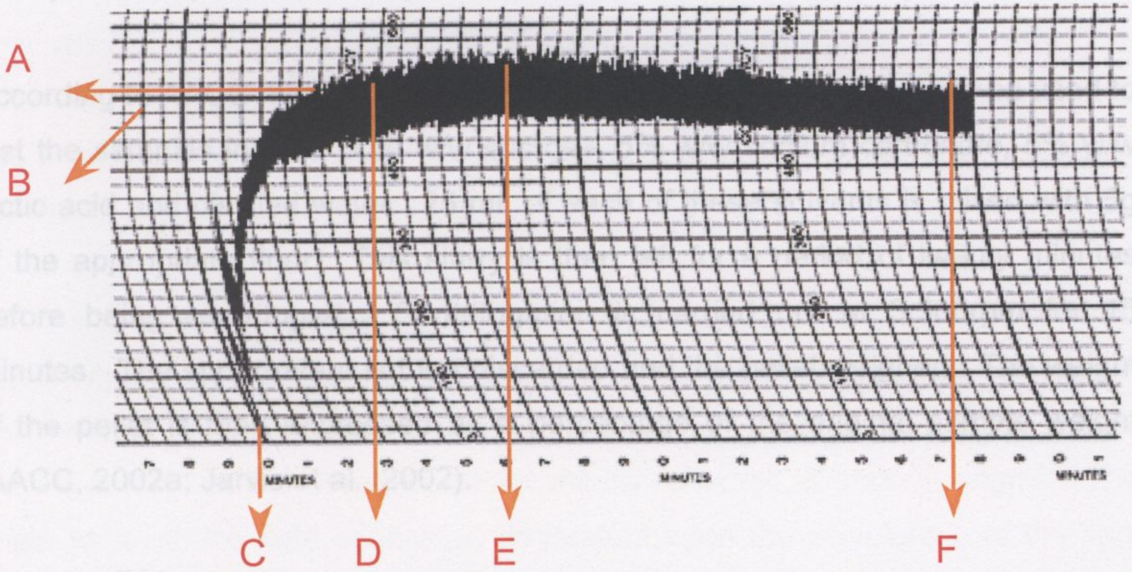

Figure 1.10: A Farinograph trace illustrating (A) arrival time, (B) 500 BU line, (C) point of water addition, (D) dough development time, (E) point of maximum resistance, (F) departure time

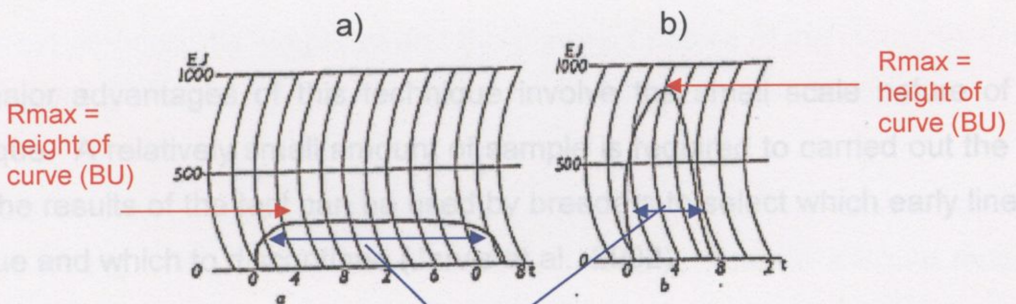

Figure 1.11: Extensograph traces indicating a) a dough with low resistance to extension (Rmax) as measured in Brabender units(BU)) and high extensibility (cm) and b) a dough with high Rmax and low extensibility

1.4.2.4 Solvent Retention Capacity

Jarvis et al. (2002) defines this test as a measure of the ability of a flour sample to hold a specific solvent following centrifugation. Currently four samples of the flour to be tested are mixed individually with four different aqueous solvents. The manner in which the flour behaves with each of these solvents allows a profile of the flour to be created (Jarvis et al., 2002). This profile is indicative of the flour quality and the potential baking quality of the sample.

According to AACC method 56-11 (AACC, 2002a), the four solvents to be used to test the samples include 50% w/w sucrose, 5% w/w sodium carbonate, 5% w/w lactic acid and distilled water. 25 mL of each of these solvents is mixed with 5g of the appropriate flour. This slurry is then left for a period of twenty minutes before being centrifuged. Centrifugation is carried out at 3,500rpm for 15 minutes. The supernatant is then discarded and the pellet weighed. The weight of the pellet is then expressed as a percentage of the original sample weight (AACC, 2002a; Jarvis et al., 2002).

As described by the AACC (AACC, 2002a), results indicate that in most cases, the lactic acid SRC will correlate with glutenin quality, while the sodium carbonate result correlates with the starch damage of the sample, and the sucrose SRC correlates with pentosan content. Water on the other hand, affects all of these constituents.

The major advantages of this technique involve the small scale nature of the technique. A relatively small amount of sample is required to carried out the test while the results of the test can be used by breeders to select which early lines to continue and which to discontinue (Jarvis et al., 2002).

1.4.2.5 Near Infrared Reflectance Spectroscopy (NIR)

NIR has become one of the most common techniques used in the wheat industry. This has been predominantly due to the ease of operation of the instrument and also to the manner in which computation is applied resulting in calculations being accomplished rapidly and accurately. NIR also involves minimal risk to the operator unlike its predecessor, known as the Kjeldahl method, which measures

the nitrogen content of the flour or ground grain sample. A determination of total nitrogen content is still needed for calibrating the NIR procedure. The Kjeldahl method is relatively time consuming, requiring the use of a number of hazardous and toxic chemicals, and it is not widely applicable to testing a large number of samples (Fox et al., 1999). The Kjeldahl method, was largely replaced by the Leco or gas combustion procedure. This procedure involves incineration of a small sample of wheatmeal or flour at ~1000°C in an oxygen rich environment. This results in the production of nitrogen oxide compounds from which an estimate of nitrogen content and thus protein content can be obtained. Though this procedure is very accurate it is also quite time consuming and requires a significant level of operator training.

NIR instrumentation is very simple involving filling a cell with a glass face with whole wheat kernels, ground whole wheat or the milled flour. This cell is then inserted into the instrument for analysis by the NIR beam. Depending on the protein content of the flour, the beam will be refracted at various angles. The angle at which the light scatters is dependent upon the wavelength of the light and upon the composition of the sample. In this manner, a characteristic spectrum is obtained from which physical and chemical properties of grain can be ascertained (Wang et al., 2001).

NIR is widely used to measure the protein, moisture and lipid content of grain and cereal products. Its simplicity and the compact nature of the instrument have seen it become a standard method in silos and laboratories worldwide.

NIR has also been used to distinguish wheat class, colour class and insect damage (Wang et al., 2001) as well as being used to analyse a broad range of non-grain products throughout the grain industry.

Near infrared transmittance (NIT) spectroscopy works via similar principles as NIR however it is most often used to analyse whole grain samples as opposed to the flour/wheatmeal samples analysed using NIR.

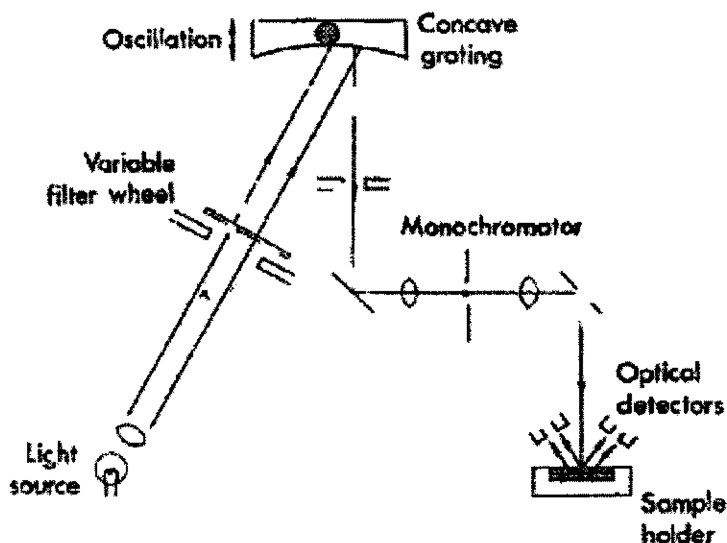

Figure 1.12: Schematic of NIR instrumentation for analysis.

1.4.2.6 Sedimentation Tests

1.4.2.6.1 History of Sedimentation

Research to determine the processing quality of the gluten proteins of wheat has been carried out since the late nineteenth century. One of the major areas of interest regarding these proteins is the degree to which the gluten within a flour sample imbibes water causing swelling of the protein in a colloidal fashion. This swelling facilitates dough formation hence allowing for the production of the myriad products made from wheat flour (Upson and Calvin, 1916).

Early studies had demonstrated the importance of protein content to grain quality and in 1947 the most common test for protein content was the Kjeldahl method. This technique involved the use of a number of dangerous reagents (including boiling sulfuric acid and mercury) and was unable to differentiate adequately between flours of poor gluten quality (Zeleny, 1947). Protein quality, with regard to breadmaking potential, was commonly tested using the Pelshenke method (Pelshenke, 1930). This test involved observing the length of time required for a heavily yeasted dough ball to disintegrate when placed underwater. Balls taking longer to disintegrate were considered to have greater breadmaking potential

than those which disintegrated quickly. While this test was considered a useful indicator of potential bread flours the technique is relatively long and inconvenient (Axford et al., 1978).

For these reasons, Lawrence Zeleny (Zeleny, 1947) formulated a new quality test capable of exploiting the natural propensity of gluten proteins in flour to swell and in so doing, invented sedimentation as a means of measuring the protein quality of flour.

This original sedimentation test involved suspending 4 g flour samples in 50 mL aliquots of water in a 100 mL graduated measuring cylinder. The measuring cylinder was then shaken for 30 seconds before being left to stand for a five minute period. Following this, 25 mL of a dilute (~21%) solution of lactic acid was added to the measuring cylinder which was then inverted ten times and left to stand for a further five minutes. Following this period of time, the level to which the flour sediment reaches in the measuring cylinder can be read from the cylinder graduations (see Table 1.3 (a)). Those samples with strong baking characteristics resulted in sediment that reached a higher point in the cylinder while the flours with poor baking characteristics resulted in much lower sedimentation volumes (SV). Zeleny analysed the results in terms of these sedimentation volumes as well as by a value he termed the "specific sedimentation" value. The specific sedimentation was calculated by dividing the sedimentation volume by the protein content of the flour. The aim of this was to ensure that the results were independent of the quantity of protein present in the sample but rather, reflected only the *quality* of the gluten proteins.

Results from the testing of 135 flour samples in this manner indicated that the test was highly repeatable with duplicate determinations often agreeing within 0.1 mL and rarely at greater than 1 mL.

Despite the promise of Zeleny's report, there were a number of problems with this procedure. For example, the test was ineffective in testing wholemeal samples because differences in grinding technology resulted in variable particle size distributions, which in turn were capable of significantly influencing the resulting

sedimentation volumes. Further to this, the test had been known to fail particularly in regard to soft flours. However, arguably the most important limitation of the test was the poor reproducibility between laboratories (Pinckney et al., 1957).

Taking this into consideration, Pinckney et al., investigated the effects of various modifications to the original technique. The most effective of these changes included reduction of the lactic acid and the addition of isopropyl alcohol. Further modifications (Table 1.3 (b)) involved the time schedule of the method (lengthened to 15 minutes) and the sample size (decreased to 3.2 g). The new sedimentation technique involved the mixing of the 3.2 g flour or wholemeal samples in 50 mL of water containing 4mg/L of bromphenol blue indicator. Each cylinder was then shaken to disperse the flour before being left on a mechanical mixer for 5 minutes. Following this mixing, 25 mL of reagent, containing 4.5% lactic acid and 20% isopropyl alcohol, was added to each cylinder and the cylinders were then placed back on the mechanical mixer for a further 5 minutes. Following this mixing, the cylinders were allowed to rest for 5 minutes more before the sediment level was read.

This new technique was tested on over 6000 wheat samples from the crop years 1947-1955. The range of the new test (13 – 83 mL) proved to be far broader than that of the original test (20 – 55 mL) and, importantly, was much more effective at grading those samples with inferior gluten quality. Also of major consequence was the discovery that, when the procedure was followed carefully, interlaboratory reproducibility increased considerably from the original test, with nine individual laboratories each testing 6 different wheat samples and resulting in agreement generally within 1 mL.

A major outcome of this study was the determination of a wheat classification system based on sedimentation results. The authors graded grain into the following four categories:

- **SV ≥ 60** Almost entirely hard wheat with greater than 14% protein content. Superior baking strength, often able to be blended with weaker wheat for bread making.
- **SV 40 – 59** Also almost entirely hard wheat, generally 12-14% protein, good quality gluten, providing good bread making quality.
- **SV 20 – 39** Low protein hard wheat, high protein soft wheat, weather damaged wheat. Suited to production of 'all purpose' flour.
- **SV ≤ 20** Almost entirely soft wheat, useful for production of biscuits, cakes and pastry.

In 1961 this modified technique was accepted by the AACC as an official method which is still widely used in both the United States and Great Britain (AACC, 2002b, c).

Over time however it became apparent that this method does not adequately distinguish between samples of unusually high protein content. Thus, further modifications of the test were developed (AACC, 2002c), involving the concentrations of reagents. The lactic acid concentration was reduced to 3% and the isopropyl alcohol concentration was reduced to 13.3% while the amount of solution added to each cylinder was doubled to 50 mL.

This modification proved useful in distinguishing between flours of high protein. However, if the technique is used for a range of flours it is necessary to consider that those flours with a standard SV between 30 and 50 are likely to register results approximately 1-3 mL lower than when using the Pinckney test. Similarly those samples with standard SV between 50 and 65 were likely to register results 1-3 mL higher than when the Pinckney test was employed (AACC, 2002c).

In 1978, however, Axford et al, published a paper in Milling, Feed and Fertiliser describing a sedimentation test using sodium dodecyl sulfate (SDS) solution as well as a lactic acid solution (Axford et al., 1978). This new sedimentation test involved larger sample sizes than the previous test (5 g of flour or 6 g of wholemeal). After these samples were added to the water in the cylinders, the

cylinders were shaken and left to stand for 4 minutes with additional shaking at 2 minutes and following the 4 minutes standing. At this point, 50 mL 2% SDS solution was added and the cylinders inverted four times. The cylinders were similarly inverted at 2, 4 and 6 minutes following addition of the SDS solution. Following the final inversion, 1 mL 10.5% lactic acid was added to the cylinders, which were then inverted at 0, 2, 4 and six minutes as was the case following addition of SDS solution. After the final inversion at 6 minutes, the cylinders were left to equilibrate for 40 minutes in the case of flour samples or 20 minutes for wholemeal samples. It was noted by the authors that it may be possible to add the SDS and lactic acid simultaneously.

While this test took much longer to carry out than previous sedimentation tests it did possess the added benefit of performing well on wholemeal samples. This had proven a problem for the Zeleny style tests.

The authors subsequently published a further paper modifying their original test by addition of the lactic acid and SDS together as previously suggested. The study compared the modified new sedimentation test with both the Zeleny test and the widely used Pelshenke test (Axford et al., 1979). The modified test was identical to the original SDS sedimentation test with the exception that both reagents were added together in a 2% SDS / 0.2% lactic acid following the shaking of the cylinders at 4 minutes. This allowed the length of the test to be reduced by six minutes.

Results indicated that both the new SDS sedimentation test and the Zeleny sedimentation test were superior to the Pelshenke test (or the sole use of protein content) in predicting loaf volume for a long fermentation baking technique. In the case of a mechanical-development baking process, the SDS sedimentation and the Pelshenke test proved to be equally strong predictors of baking quality while the Zeleny test was a significantly poorer indicator. Overall the SDS sedimentation technique proved to be a strong all round indicator of baking quality regardless of the baking technique used. The test also proved useful as a means of gauging protein quality independent of high alpha-amylase activity, which had been a problem for some years. Furthermore, SDS sedimentation

testing proved superior when characterising wholemeal samples for potential baking quality of corresponding flours.

Up until this time sedimentation tests had been used only as a test of the breadmaking potential of standard wheat (*Triticum aestivum*). However, following the success of the SDS sedimentation test, interest was stimulated in its application to durum grain (*Triticum durum*), for which small scale quality tests were being sought to predict pasta-making quality. Several research groups reported the possibility of using the SDS sedimentation test for durum gluten quality (Dexter et al., 1980; McDonald, 1985; Quick and Donnelly, 1980).

Dexter et al. began by trialling the test using the original SDS sedimentation test described by Axford et al. (1978), with a reduced sample size (5 g wholemeal). The test correlated strongly ($r=0.8$, $P<0.05$) with mixograph development time (a good indicator of durum gluten strength) while being a simpler, cheaper test with a reduced chance of interference from personal bias. It was also shown in this study that increasing the concentration of SDS in solution could increase the range of results. Even more important, though was the discovery that it is possible to decrease the size of the sample to 1 g while still ranking the samples in the same order.

Following this study, it was shown that a stable test with an increased range of volumes could be achieved by modifying a number of factors. These included increasing lactic acid concentration to 3%, maintaining constant laboratory temperatures (or correcting for temperature changes), and grinding grain on similar mills at similar grinding speed (2-3 g/minute) (McDonald, 1985; Quick and Donnelly, 1980).

The SDS Sedimentation Test for Durum Wheat was accepted as an AACC approved method in 1984 (AACC, 2002d). This test involves the addition of 6.3 g of ground durum wheat to 50 mL of water in a graduated measuring cylinder which is then shaken for fifteen seconds to disperse the grain, inverted 12 times, then placed on a mixer ten seconds before being left to stand. Mixing is repeated at 2, 4 and 6 minutes. After the four minutes a solution of 3% SDS and 2% lactic

acid is added to the cylinder and the cylinder is then shaken for fifteen seconds. Shaking is repeated for six seconds at 2 and 4 minutes after the solution is added. The cylinders are then left to stand for twenty minutes before the sediment level is recorded.

It is from this SDS sedimentation test for durum that the test currently in use at Allied Mills Australia, Summer Hill, was developed. However, the Allied sedimentation test has been significantly simplified.

To overcome operator-related factors, the test (Table 1.3) is carried out on an automated mixing rack so that the only manual handling required is the shaking of cylinders to disperse grain prior to starting the test. The test uses 6.3g wholemeal sample which is shaken in 50 mL of water to disperse before being placed on a mixing rack and mixed for 10 seconds. Following this, cylinders are left to sediment for a period of 4 minutes 45 seconds, before addition of a 3%SDS 2% lactic acid solution. The cylinders are then left again to settle for five minutes at which point the sediment level is measured. This test is the simplest of the SDS sedimentation techniques, yet it also results in the largest range of sedimentation volumes (20-100 mL). A summary of the techniques described here is given in Table 1.3.

1.4.6.2.1 Mechanism of sedimentation

Sedimentation tests rely on the swelling power of the gluten complexes of different flours in the presence of lactic acid (Williams, 1998). This type of testing was first described by L. Zeleny in 1947 (Zeleny, 1947) and has since become one of the most commonly used techniques for the testing of the gluten strength of different wheat varieties (Carter et al., 1999).

The Zeleny test involves the mixture of a sample of flour sieved from ground wheat or white flour with a solution containing lactic acid in isopropyl alcohol. The lactic acid solution ruptures the endosperm cells within the flour and subsequently hydrates the particles within the flour (Adayemi and Muller, 1983).

Hydration of the particles stimulates the formation of fibrils which bind the flour particles together.

Table 1.3: Summary of sedimentation testing techniques used since the development of the Zeleny test in 1947

Method	Sample Preparation	Sample Size	Reagent	Total Time	Shaking	Inverting	Range (mL)
a) Zeleny, 1947	Flour only	4 g	25 mL of 21% LA	~ 11 min.	30 sec. to disperse flour	10 times after LA added	20 – 55
b) Pinckney et al, 1957 ↓ **AACC Methods 56-60 & 56-61a**	Flour and wheat (run 5x through 0.023 inch rolls, 100 mesh sieve)	3.2 g	25 mL of 4.5% LA 21% IPA	~ 15 min.	By hand then on mixer x 2 for 5 min.	None	13 – 83
c) AACC Method 56-62	**As above**	3.2 g	50 mL of 3% LA 13.3% IPA	~15 min.	By hand then on mixer x 2 for 5 min.	None	As above ±1-3 mL*
d) Axford et al, 1978	Flour – Bühler milled Wholemeal – KT mill with falling no. screen	5 g flour 6 g meal	50 mL of 2% SDS followed by 1 mL of 10.5% LA	1hr for flour, 40 min. for meal	15 sec at 0, 2 and 4 min. after sample added	4 times at 0, 2 ,4 and 6 min. after **each** solution added	20-70 mL
e) Axford et al, 1979	Flour – Bühler milled Wholemeal – Udy cyclone mill with falling no. screen	5 g flour 6 g meal	50 mL of 2% SDS, 0.2% LA	50 min. for flour, 30 min. for meal	15 sec at 0, 2 and 4 min. after sample added	4 times at 0, 2, 4 and 6 min. after solution added	25-75 mL
f) AACC Method 56-70	Durum – ground at 2-3g/min, sieved through 1mm screen	6.3 g	50 mL of 3% SDS, 2% LA	30 min..	15 sec to disperse, then 10 sec. at 0, 2, 4 and 6 min. then 15 sec. after sol'n added and 6 sec. at 2 and 4 min.	12 times after sample dispersed.	20-65 mL
g) Current test used at Allied Mills Australia	Wholemeal – Falling number mill Flour – Bühler milled	6.3 g of meal 5 g flour	50 mL of 3% SDS, 2% LA **or** 1.5% SDS 2% LA**	10 min.	15 sec. by hand then 10 sec. by mixer	15 times after sol'n added	25-100 mL

The fibrillar structure is stabilised by the disulfide bonds of the flour proteins resulting in flocculation of the flour particles such that on settling they can not become resuspended in the supernatant (Adayemi and Muller, 1983). Due to the stability provided by the disulfide bonds, addition of any substances capable of breaking disulfide bonds will prevent floc formation thus rendering the Zeleny sedimentation test useless (Orth et al., 1973).

The presence of the lactic acid in solution is essential in order for the sedimentation test to be useful. If the lactic acid is absent from the isopropyl alcohol solution the flour particles will not sediment effectively making the results unreadable. This is due to the important role the lactic acid plays in both cell wall rupture and the subsequent swelling and flocculation of the flour proteins. Without the lactic acid, the solution will be unable to flocculate these proteins.

In 1978 this Zeleny test was modified (Axford et al., 1978) giving rise to the SDS-sedimentation test. As the name suggests, this test differs from the Zeleny test by the addition of the detergent sodium dodecyl sulfate to the solution in place of isopropyl alcohol (Carter et al., 1999). SDS enjoys widespread use in biochemistry due to its ability to disperse and denature proteins as well as its involvement in protein fractionation (Vereijken et al., 2000). Since then, several other slight modifications to the technique have also occurred. The result of this has been the optimisation of a second sedimentation technique capable of greater separation of sedimentation values. However, the modified test is still simple to perform and highly reproducible (Carter et al., 1999).

The SDS-sedimentation test works in a similar manner to the Zeleny sedimentation test. That is, glutenin strands swell extensively (Carter et al., 1999) causing flocculation of the saturated flour. Formation of this floc also follows a similar formation mechanism to that of the Zeleny sedimentation flocculation and again addition of disulfide bond breaking substances renders the test useless. The advantage of the SDS sedimentation over the Zeleny sedimentation test however is that the floc formed when the SDS sedimentation technique is employed exhibits greater variance between cultivars allowing more accurate identification of particular cultivars. For this reason, SDS sedimentation

is used much more commonly today than Zeleny sedimentation as a small-scale quality test.

The AACC Approved Methods (AACC, 2002d) indicate that the SDS sedimentation test is predominantly used for the protein analysis of durum wheat varieties while the Zeleny sedimentation test is indicated for the hexaploid (standard) varieties. This is not always the case with a number of studies on hexaploid wheat employing the SDS technique (Carter et al., 1999; Faergestad et al., 1999; Huebner et al., 1999; Stone et al., 1997; Vereijken et al., 2000).

Basically the SDS sedimentation test involves the addition of a set aliquot of either flour or ground wheat (or occasionally the flour sifted from ground wheat) to water in a measuring cylinder. The sample is mixed and allowed to rest at which time sediment will start to form. Following this rest period, the solution containing SDS (commonly at 3%) and 2% lactic acid is added to the measuring cylinder. A dye such as toluidine blue is also added regularly to the solution in order to allow the sedimentation level to be read from the cylinder more clearly. The cylinder is then inverted a number of times and allowed to rest once more. Once the specified time has elapsed, the level of sedimentation can be read from the measuring cylinder. The higher the level of settled sediment in the cylinder, the stronger the flour will generally be. The test is usually done either in duplicate or quadruplicate. The Zeleny sedimentation test uses a very similar method, the only differences being that the test requires less flour or ground wheat (approximately half that needed for the SDS sedimentation) and the solution added to the cylinders following the first rest period, contains isopropyl alcohol rather than SDS, although lactic acid levels remain the same.

SDS sedimentation testing is used in order to predict the gluten strength of different wheat cultivars and as gluten strength correlates very strongly with loaf volume, the subsequent quality of the end-product following baking (Carter et al., 1999). SDS sedimentation values are highly heritable and therefore can be used for selection among early generation progeny (Carter et al., 1999) and thus SDS testing has become an extremely handy tool for wheat breeders .

One of the difficulties commonly associated with the SDS sedimentation tests has been to obtain reproducibility of the several steps, namely, the shaking, inverting and resting of the measuring cylinders (Sapirstein and Suchy, 1999). Timetables pertaining to this have been known to be quite arbitrary (Sapirstein and Suchy, 1999). Such techniques can vary widely between different organisations making standardisation of results and the suppositions subsequently taken from them difficult to compare.

An example of this variance between studies is seen in the case of Carter et al. who employed a solution of 3% w/v SDS (Carter et al., 1999) whereas Alvarez et al. used a solution containing 2% w/v (Alvarez et al., 1994). Studies have also shown vast differences in lactic acid concentrations, and the presence or absence of dyes used for clarifying sediment lines. The most variable area of the technique however is the time scheme. Length of mixing and resting times particularly may affect results quite dramatically between studies.

Sedimentation testing shows promise as a means of evaluating wheat protein quality; however, currently there is insufficient information on test methods, reproducibility and potential applications to exploit the test to the full extent.

1.5 Conclusions and Research Objectives

Current tests of protein quality, while effective, are subject to numerous limitations including high set-up and maintenance costs, limited availability, and the requirement of skilled instrument operators. Thus there is significant interest in the development of techniques for testing of wheat quality which minimise these limitations.

Sedimentation is a simple, inexpensive, small-scale, protein quality test currently used in both the United States and the United Kingdom. Sedimentation testing is reported to correlate well with both dough quality and loaf volume and current methods include tests for both wheatmeal and flour samples. Current sedimentation methodology however, has a number of limitations. Firstly for the

test to be useful tool for breeding programs it is necessary to better understand both the composition and form of the sediment and how these factors relate to grain quality characteristics. Meanwhile to maximise the effectiveness of sedimentation testing for research purposes it is important to develop consistent methodologies for comparison between laboratories. It would also be beneficial to extend the application of sedimentation testing (i.e. SDS test methods currently exist only for wheat) to other sample types.

Taking the limitations of current sedimentation methods into account, this study aims to:

- Examine current sedimentation test methods with regard to the robustness and reproducibility of standard operating procedures and to use this information to improve current methods

- Develop SDS sedimentation test methods for samples other than wheatmeal

- Investigate the composition, protein profile and bonding structure present within the sediment

- Compare sedimentation testing as a predictor of dough quality with recently developed small-scale test methods

Chapter 2

Methods and Materials

2.1 Sample acquisition

Unless otherwise stated all samples were obtained from Allied Mills Australia Toowoomba.

2.2 Sample Preparation

2.2.1 Grain cleaning

Grain was cleaned using a wheat-cleaner consisting of an elevated grain hopper with a controlled rate exit passage at its base through which grain flows down on to a series of two screens. The first screen consists of ovoid slits of aperture size 3.6 mm x 20 mm while the second screen consists of round holes 2 mm in diameter. The screens vibrate thereby sieving out unwanted material while letting grain pass through.

2.2.2 Grinding

Wholemeal samples were produced by grinding whole grain using a Perten Falling number Mill. Hard grain was ground on the Number 1 setting, while soft grain was ground on the Number 2 setting to avoid fine material obstructing the grinder.

2.2.3 Oven moisture

In order to ascertain the moisture of either wholemeal or flour samples AACC method 44-19 was used. This procedure involved weighing 2.000 g ± 0.001 g of wholemeal or flour samples into 55 mm aluminium moisture dishes. These dishes were then placed, uncovered, in an oven at exactly 135°C for exactly two hours. The dishes were then removed, covered and placed in a desiccator for 30 minutes before reweighing. The difference in sample weight is then measured and calculated as a percentage of total sample moisture (Equation 1).

$$\% \text{ Moisture} = \left[\frac{(\text{original sample weight} - \text{sample weight after heating})}{\text{original sample weight}}\right] \times 100$$

Equation (1)

2.2.4 Grain Conditioning

Prior to milling, clean grain samples were conditioned in order to assist the milling process. The samples were weighed out to the nearest kilogram (minimum 1 kg) for large-scale milling or to the nearest 10 g for small scale milling. The sample size and grain moisture (as ascertained by oven moisture procedure) were used to calculate the amount of water required to condition the grain (Equation 2).

$$\text{Weight of water required} = \left[\frac{100 - \text{original moisture (\%)}}{100 - \text{desired moisture (\%)}}\right] -1 \times \text{sample weight}$$

Equation (2)

This water was then added to the grain in a thick plastic bag and mixed thoroughly before being left overnight. Hard wheat was conditioned to 14.5% moisture while soft wheat was conditioned to 13.5% moisture.

2.2.5 Test milling

2.2.5.1 Small Scale

Small scale test milling was carried out using a Brabender Quadrumat Junior laboratory mill using AACC Method 26-50. Samples weighed no less than 50g and were conditioned to 14.5% moisture prior to milling.

2.2.5.2 *Large Scale*

Test milling was carried out on grain samples of no less than one kilogram in weight using a Bühler laboratory pneumatic mill (model MLU-202) using AACC method 26-21A (Cleanout Method (A)).

2.3 Sample Analysis

2.3.1 Protein Content

2.3.1.1 *Near infrared reflectance (NIR) spectrophotometry*

Protein content of flour and wholemeal was generally ascertained using NIR spectroscopy. The analyses were carried out on a Foss NIR Systems 6500 instrument using AACC method 39-11.

2.3.1.2 *Leco*

Protein content was also analysed using a Leco FP 428 Nitrogen Determination System 601-700-300. This study employed RACI Method 02-03 (2003) with instrument calibration as per the manufacturer's instructions.

2.3.2 Dough Rheology

2.3.2.1 *Farinograph*

Farinograph analyses were carried out using Brabender Farinograph instrumentation with a 300 g mixing bowl attachment using RACI Method 06-02 (2003). Graphical interpretations of results were obtained using a manual data recorder.

2.3.2.2 *MicroFarinograph*

Smaller flour samples were analysed using a Brabender Microfarinograph with a 10g mixing bowl attachment. Graphical interpretations of results and data information were obtained using the program Rf Mixer High Speed Dough Test © 1999 by Feather Engineering, Australia.

2.3.2.3 *Extensograph*

Extensograph analyses were carried out using a Brabender Farinograph instrument to mix salted doughs and a Brabender Extensograph instrument for moulding, resting and extension of dough pieces. RACI Method 06-01 (2003) was employed for these analyses.

2.3.2.4 *Keiffer Rig*

9.73 g of flour was mixed with 0.2 g of salt and mixed dry in a 10 g Microfarinograph bowl. An appropriate amount of water (~5-6 mL depending on water absorption) was then added and the samples mixed to a dough of maximum resistance 500 BU. Following mixing the dough was removed from the bowl and left to relax for 20 minutes at constant humidity and briefly kneaded to reincorporate any separated water. The dough was then rolled into a ball and shaped into a sausage shape and placed on the grooved plastic strip shaper provided. The top of the shaper was then placed on top of the dough and pressed down until the top and bottom met. Any dough extruded from the side of the shaper was removed with a spatula and the shaper was left to relax for 40 minutes at constant temperature and humidity.

Following this resting time strips were removed from the shaper using the tools provided and placed into the clamp on the Keiffer Rig attachment of the *TA.XT2 Texture Analyser* instrument. The instrument was activated causing the hook attachment to ascend, stretching the dough strip. Results (extensibility (cm)) were obtained electronically using Texture Expert Exceed software.

2.3.2.5 *Dough Stickiness*

Dough Stickiness was measured using a Chen-Hoseney Dough Stickiness Cell and 25 mm Perspex cylinder probe attached to a TA-XT2 Texture Analyser. Doughs were prepared as for Keiffer Rig and a dough piece no larger than the chamber placed into the cell. The internal screw of the cell was then rotated until dough extruded to 1mm through holes at the top of the cell. This dough was scraped off using a spatula and a further 1 mm extruded. The screw was then wound backwards slightly to allow the dough to relax after the extrusion process

and rested for 30 seconds. The cell was then placed under the probe and the test was commenced.

2.3.2.6 Grain Hardness

Grain hardness was ascertained using NIR spectroscopy. The analyses were carried out on a Foss NIR Systems 6500 instrument using AACC method 39-11.

2.4 Falling Number

Falling number analyses were carried out using AACC Method 56-81B however results were not corrected for 14% moisture but reported without change.

2.5 SDS-PAGE

SDS-PAGE analyses of HMW-GS identification were carried out at the SARDI Laboratories, Adelaide and employed the method used by Singh et al., (1991). Firstly, three solutions are made up for this technique (A) 50% (v/v) propan-1-ol, (B) 50% (v/v) propan-1-ol, 0.08 M Tris-HCl, pH 8.0 and (C) sample buffer, 2% (w/v) SDS, 40% (w/v) glycerol, 0.02% (w/v) bromophenol blue, 0.08 M Tris HCl, pH 0.8. All extractions, alkylations and treatment of propanol extracts with SDS sample buffer were carried out in Eppendorf tubes at 65°C.

Flour (20 mg) was tested for each sample. Gliadins were extracted by adding 1 mL of A to the 20 mg of flour in an Eppendorf tube. This solution was left for 30 minutes and vortexed several times during this period. The tubes were then centrifuged (1 minute at 10,000 x g) and the supernatant discarded. This process was then repeated and the resulting pellet washed in a further 0.5 mL of A and centrifuged at 10,000 x g for 5 minutes. The supernatant was then removed via aspiration. Glutenin proteins were extracted into 0.1 mL of solution B plus 1% (w/v) dithiothreitol (30 minutes) and then centrifuged for 5 minutes. Following centrifugation, 0.1 mL of solution B plus 1.4% vinylpyridine was then added and the tube/s incubated for 15 minutes for protein alkylation. Samples were then centrifuged for two minutes, then 0.1 mL of supernatant was removed and mixed with 0.1 mL solution C in a new tube. Tubes were incubated for 15 minutes in

order for SDS to complex with reduced, alkylated polypeptides. The tubes were then centrifuged for 2 minutes and 10-20 µL of the supernatant loaded into a sample well of an SDS-PAGE gel.

2.6 Size exclusion high performance liquid chromatography (SE-HPLC)

SE-HPLC analyses were carried out using the method of Batey et al. (1991). This procedure involves extraction of the low molecular weight proteins from a 10 µg flour or wholemeal sample using 1 mL of 0.5% SDS buffer in an Eppendorf tube. The samples were then vortexed to disperse the sample before being centrifuged at 10,000 x g for 10 minutes. The supernatant was then removed using a syringe and filtered (pore size 0.45 µm) into a small glass vial. The pellet was resuspended in 0.9 mL of 0.5% SDS buffer and revortexed to resuspend the pellet. Tubes were then sonicated for approximately 10 seconds. Samples were centrifuged again (10,000 x g for 10 minutes) and the supernatant filtered into fresh glass vials. Samples were analysed on a Phenomenex BIOSEP-SEC 4000 column with a running time of 10 minutes (flow rate 2 mL/ minute) instead of the standard 35 minutes run (0.5 mL/ minutes) as described by Batey *et al.* (1991). The eluent used was aqueous acetonitrile buffer (0.05% trifluoroacetic acid in water and 0.05% in acetonitrile). The proteins were detected at a wavelength of 214 nm. The areas of the glutenin peak (> 70,000) and gliadin peak (< 70,000) were measured by Gold Nouveau software (Beckman Instruments, Inc., Fullerton, CA, USA). The 'unextractable' polymeric protein (UPP) was determined as the ratio of the glutenin peak of the 'insoluble glutenin' extract to the sum of the glutenin peaks of both insoluble and soluble extracts (Gupta et al., 1993)

2.7 Fractionation of Flour

2.7.1 Lipid Extraction

Non starch lipids were removed from flour samples via chloroform extraction (MacRitchie and Gras, 1973). Flour samples (100 g) were mixed with analytical

grade chloroform (200 mL) for ten minutes using a magnetic stirrer before being filtered by suction. This process was repeated three times and the resulting flour was air-dried.

2.7.2 Starch and Gluten Separation

Flours (defatted and whole) were separated into starch and gluten by mixing either three hundred or ten grams of flour to a dough (approximately 500 BU) in the appropriate farinograph bowl. The bowl was then filled with water and mixed for ~1 minute. Following this, the water, now containing starch, was suctioned off using a large plastic pipette and filtered through a 180 μm screen to remove any floating gluten and other insoluble material. The contents of the screen were discarded following filtering leaving only the single gluten ball. This process was repeated a minimum of six times or until the water remained clear following mixing. The gluten ball was removed from the bowl, washed to remove any remaining starch and frozen at -18 °C in small segments. These segments were then freeze dried. The filtered starchy water was centrifuged at 1000 x g for 2 minutes and the supernatant discarded while the starch pellet was frozen and then freeze dried. Dried starch and gluten samples were ground to powder using a Waring blender. Samples were then passed though a 180 μm screen to obtain a uniform particle size.

2.8 Sedimentation Tests

2.8.1 SDS Sedimentation

Wheatmeal samples (6.300 ± 0.005 g, ground on a falling number mill) were weighed out in duplicate and each sample added to a 100 mL graduated measuring cylinder containing 50.0 ± 0.5 mL of distilled water (Figure 3.6). The cylinders were capped and shaken briefly before being placed on a measuring cylinder (two samples in duplicate per test). The mixing rack was activated at which time stirrers descended into the cylinders stirring the slurry at ~900 rpm for 15 seconds. The cylinders were left for 4 minutes 45 seconds at which time 50±

0.5 mL of 3% SDS / 2% lactic acid solution (plus 0.012% toluidine blue dye) was added and the cylinders inverted 15 times. The sediment was allowed to settle for 5 minutes before the sedimentation volume was read from the cylinder graduations. Sedimentation methods are described in more detail in Chapter 3.

2.8.2 Isopropyl Alcohol Sedimentation

This sedimentation test was carried out as for SDS sedimentation; however, the lactic acid solution contained 20% isopropyl alcohol / 1.5% lactic acid (plus 0.012% toluidine blue dye).

2.9 Statistical Analysis

Unless otherwise stated all statistical analyses were carried out using Microsoft Excel software (including Analysis Toolpack add-in option).

Chapter 3

Validation, Development and Modification of Current Sedimentation Methodology

3.1 Introduction.

Although sedimentation testing has been widely used as a means of gauging protein quality since the 1950s, various problems have been noted for a number of different sedimentation testing methodologies. These include problems with inter-laboratory repeatability (Pinckney et al., 1957), use of different reagent solutions and concentrations of reagents in solutions (McDonald, 1985), the sample preparation i.e. the type of grinder used (Ward et al., 1979), sample size (Silvela et al., 1993) and type of instrumentation. A number of studies have involved carrying out sedimentation testing manually (Preston et al., 1982), while others have employed automated instruments (McDonald, 1985).

To elucidate the robustness of sedimentation tests in general and the test currently used at Allied Mills Australia in particular, it is important to understand how variations in the testing procedure affect the results of the test. Therefore this chapter aims to investigate the impact on sedimentation results following variations in testing parameters with a view to improving the robustness of the sedimentation test.

Further, this chapter investigates the potential for manipulation of these procedural variations to broaden the scope of current sedimentation testing for use with the testing of flour samples as well as triticale and gluten samples.

3.2 Methods and Materials

3.2.1 Method Validation

3.2.1.1 Variations due to the operator

Six operators were selected on the basis of a wide range of experience with sedimentation testing. The least experienced operator had never used the sedimentation instrument nor seen it demonstrated while the most experienced operator had used the instrument to analyse in excess of 10,000 samples. Testing was carried out as described in Section 2.8.1 with all required solution having been made in a single batch. In order to achieve ranges in sedimentation volume representative of the range of values commonly observed in Australian grain (~50-90 mL, based on 2000-2005 harvest results at Allied Mills Australia), ten samples were chosen covering this range. Each sample was ground on a falling number mill as described in Section 2.2.2 and all ten samples were analysed in duplicate.

3.2.1.2 Sample preparation - grinding

Grain samples were selected representing five Australian grades (Australian Standard White (ASW1), Australian Premium White (APW2), Australian Hard (AH2), Australian Utility Hard (AUH2) and Australian Prime Hard (APH2)) grown at two sites (ten samples in total). Samples were divided into nine sub-samples using a sample splitter and ground in one of nine different ways as detailed below.

1) Perten 3100 Falling Number mill (0.8 mm screen) –setting 1
2) Perten 3100 Falling Number mill (0.8 mm screen) – mill feeder setting 2
3) Perten 3100 Falling Number mill (0.8 mm screen) – mill feeder setting 3
4) Perten 3100 Falling Number mill (0.5 mm screen) – mill feeder setting 1

5) Perten 3100 Falling Number mill (0.5 mm screen) – mill feeder setting 2
6) Perten 3100 Falling Number mill (0.5 mm screen) – mill feeder setting 3
7) Perten 3100 Falling Number mill (0.8 mm screen) – only 1 mill feeder setting available (hereafter referred to as QC mill).
8) Udy cyclone mill
9) Perten 3303 laboratory scale mill (no screen) – mill feeder setting 1 (finest setting available)

Each sample was then tested for particle size distribution using a Malvern Mastersizer (Malvern Instruments, Worcester, UK) instrument with software of the same name. Sedimentation testing was carried out in duplicate as described in Section 2.8.1.

3.2.1.3 Sample size

In order to investigate the effect of sample size on sedimentation results, six samples of matching wheat and flour were selected and each was tested in quadruplicate using the SDS sedimentation techniques detailed in Section 2.8.1 and 3.3.2.1 respectively. The standard sample sizes of 6.300 g ± 0.005 g and 5.000 g± 0.005 g were varied in 0.1 g increments/decrements from 5.8 g to 6.8 g for wheatmeal and from 4.5 g to 5.5 g for flour samples.

3.2.1.4 Automation versus manual testing

Single variety samples were selected, representing five Australian grades grown at two sites (as for Section 3.2.1.2). Each sample was tested in quadruplicate using the automated mixing rack apparatus. Manual testing was carried out by addition of 6.300 ± 0.005 g samples to 50.0 ± 0.5 mL distilled water. Cylinders were shaken to disperse the material and agitated by hand, by rapid shaking for 15 seconds before being left for a further 4 minutes and 45 seconds. A solution containing 3% SDS / 2% lactic acid (50.0 ± 0.5 mL) was added to each cylinder and the cylinders inverted 15 times in 30 seconds. Samples were left again for a further 4.5 minutes after which the sediment level was read from the cylinder graduations. Manual testing was carried out in quadruplicate.

3.2.1.5 Mechanical stirring

Samples representing a range of sedimentation values (55-85 mL) were selected and tested using the sedimentation test described in Section 2.8.1 adjusting the rotation speed of the stirrers to 0, 200, 400, 600, 800, 1000 revolutions per minute (rpm) with each test. Each sample was tested in quadruplicate at each stirrer speed.

3.2.1.6 Reagents

3.2.1.6.1 SDS

In order to ascertain the importance of SDS on sedimentation results, SDS was omitted from solution (i.e. the solution contained only 2% lactic acid), and 10 samples of known sedimentation volume retested. To investigate the impact of SDS versus another detergent, solutions were made up containing cetyl trimethyl ammonium bromide (CTAB) in place of SDS and the same samples tested again.

3.2.1.6.2 Lactic acid

The samples indicated in Section 3.2.1.6.1 were retested in the absence of lactic acid i.e. in solution containing 3% SDS. Lactic acid was then replaced in the reaction solution with formic acid. Formic acid was selected on the basis of it having a pKa value similar to that of lactic acid. The pKa of lactic acid is 3.86 whereas the pKa of formic acid is 3.75. Following this, the effect of the pH of the solution was tested by altering lactic acid concentration to achieve pH values for the solution of 1.7, 2.4, 2.7 and 3.7 and the sedimentation volumes determined as per Section 2.8.1.

3.2.1.7 Temperature

In order to gauge the effect of temperature on sedimentation results, solutions were allowed to equilibrate to 12°C and 37°C and 10 samples, representing a range of sedimentation values at ambient temperature, were tested in quadruplicate. Final temperatures in cylinders (following sedimentation) were approximately 15°C and 35°C respectively.

3.2.2 Method Development and Modification

3.2.2.1 Modification of SDS sedimentation test for wheat flour

A sub-set of 45 grain/flour samples representative of a broad protein range (8-16%) was selected and the grain ground to wholemeal (Section 2.2.2). Both the wholemeal and flour samples were tested for NIR protein and moisture (Sections 2.3.1.1) and an SDS sedimentation test was performed (Section 2.8.1).

To develop an SDS sedimentation test for flour, a range of solutions containing different reagent concentrations was trialled. Sedimentation tests were then carried out as described in Section 2.8.1 using 6.300g ± 0.005 g sub-samples of each flour. The resulting sedimentation values were too high to be read accurately from the cylinders and so the sub-sample weight was reduced to 5.000g ± 0.005 g.

3.2.2.2 Sedimentation test for triticale

Triticale samples were tested as per Section 2.8.1 (wholemeal) and Section 3.3.2.2 (flour). Samples were then retested using reagent solutions of varying lactic acid and SDS concentrations in order to optimise the sedimentation test for a range of triticale samples. Sedimentation results were correlated against protein content (%) as this was the only data on grain quality available for this sample set.

3.2.2.3 Sedimentation test for wheat gluten

Gluten samples were obtained from Food Science Australia, North Ryde and were tested, as for triticale samples, under a range of different sedimentation conditions in order to gauge which conditions result in the most consistent floc and also the widest range of values. These variations included changes in reagent concentrations, settling times and mixing regimes.

3.3 Results and Discussion

All data discussed in Chapter 3 are presented in full in Appendix A

3.3.1 Method Validation

3.3.1.1 Operator Effects

As illustrated in Figure 3.1, all operators returned similar results with operator O2 the only exception. Data were sorted in ascending order for operator O1 who was the most experienced operator. Excepting operator O2, all other operators returned results within ± 2.5 mL (2.5 - 5 %) of operator O1 for all but one sample, the exception being the sample labelled WW which returned results ranging from 79-93mL over the fourteen recorded results indicating substantial intrasample variability.

Figure 3.1: Comparison of sedimentation volume (SDS-SV) results for six operators with varying degrees of experience in the use of the testing apparatus

Operator O2 consistently returned higher sedimentation values than all other operators with only two of ten results concurring with the other operators. It is worth noting here that operator O2 was not an inexperienced operator having used the apparatus prior to this study.

A two-way analysis of variance for these data (Minitab, 2003) indicated that despite the results of the five remaining operators (O1, O3-O6) apparently being

very similar, the results were, in fact, statistically different (F=16.71, P<0.001). However, taking into account that the average results for all operators were within 2.5 – 7.5%, despite that significant variability was apparent within the test, it can be considered that the results were not substantially different for these five operators. The results of operator O2 however, were significantly different from the other operators (F=95.4. P<0.001), differing by between 10 and 20%. Even taking into account variability both within samples and due to handling differences, these results are outside the acceptable range to be considered experimental error. The consistently high nature of the results of operator O2 indicates that a systematic error is responsible for these differences in results.

Observation of the operator led to the finding that the source of error originated as a result of misinterpretation of the standard operating procedure, as distributed to each operator prior to commencement of testing. This resulted in sample tubes being shaken manually for a far greater time prior to being placed in the mixing rack (tubes were shaken for approximately 40 seconds rather than the recommended three quick shakes). This resulted in the first pair of tubes to be shaken being left to hydrate in the water for significantly longer than the second pair (i.e. in excess of sixty seconds). Further, the time between addition of the samples to the cylinders and commencement of the test increased to over two minutes (an approximately four fold increase in the time taken by other operators). The excess hydration time experienced by the first cylinders to be shaken (which is experienced by the second pair) could potentially explain the fact that some of the samples originally tested resulted in similar sedimentation volumes to that of the other operators while others gave significantly different results. Following this finding, the operator repeated analyses on all of the samples under double-blind conditions and using the correct shaking regime. The result of these repeat analyses is illustrated in Figure 3.2 which indicates that when the procedure was adjusted following detection of the testing discrepancy, results for O2 came into line with those of the other operators. This result was further supported by a second two-way analysis of variance (F=12.96, P<0.001).

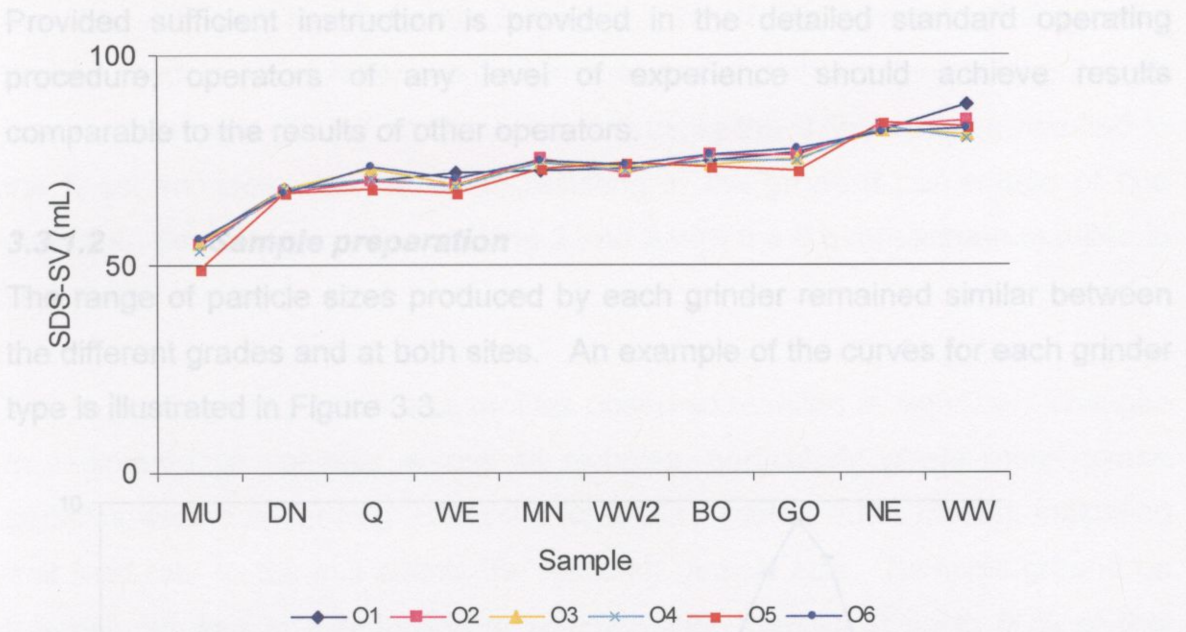

Figure 3.2: Comparison of sedimentation results (SDS-SV) for six operators of varying degrees of experience in using testing apparatus following adjustment of the procedure for operator O2.

The results of this study indicate that clarification of the exact test method is essential in order to achieve consistent, reproducible results. In particular, it was important to emphasise the need for the cylinder to be kept on the mixing rack at all times as less experienced operators tended to remove the cylinders from the rack to facilitate addition of the reagent solution. The subsequent disruption of the sediment led to variations in results far exceeding the acceptable experimental error (< 5%) particularly for the soft-grained samples. Further, variations in hand shaking of each cylinder prior to placement of cylinders in the mixing rack can result in substantial differences in sedimentation results. A number of operators took several minutes to carry out this step as material tended to adhere beneath the cap of the cylinders and was difficult to dislodge. The extended hydration time resulted in slightly higher than expected volumes in those cylinders that had already been shaken but had been standing the longest. In order to overcome this problem a uniform manual shaking protocol should be clearly stipulated in the procedure. The suggested protocol involves two rapid shakes i.e. two single movements, of the cylinders. Should any sample material remain, the stopper should be removed with a twisting motion to dislodge the material, before being replaced and further shakes administered. Completion of this procedure for all four cylinders should take no longer than 30 seconds.

Provided sufficient instruction is provided in the detailed standard operating procedure, operators of any level of experience should achieve results comparable to the results of other operators.

3.3.1.2 Sample preparation

The range of particle sizes produced by each grinder remained similar between the different grades and at both sites. An example of the curves for each grinder type is illustrated in Figure 3.3.

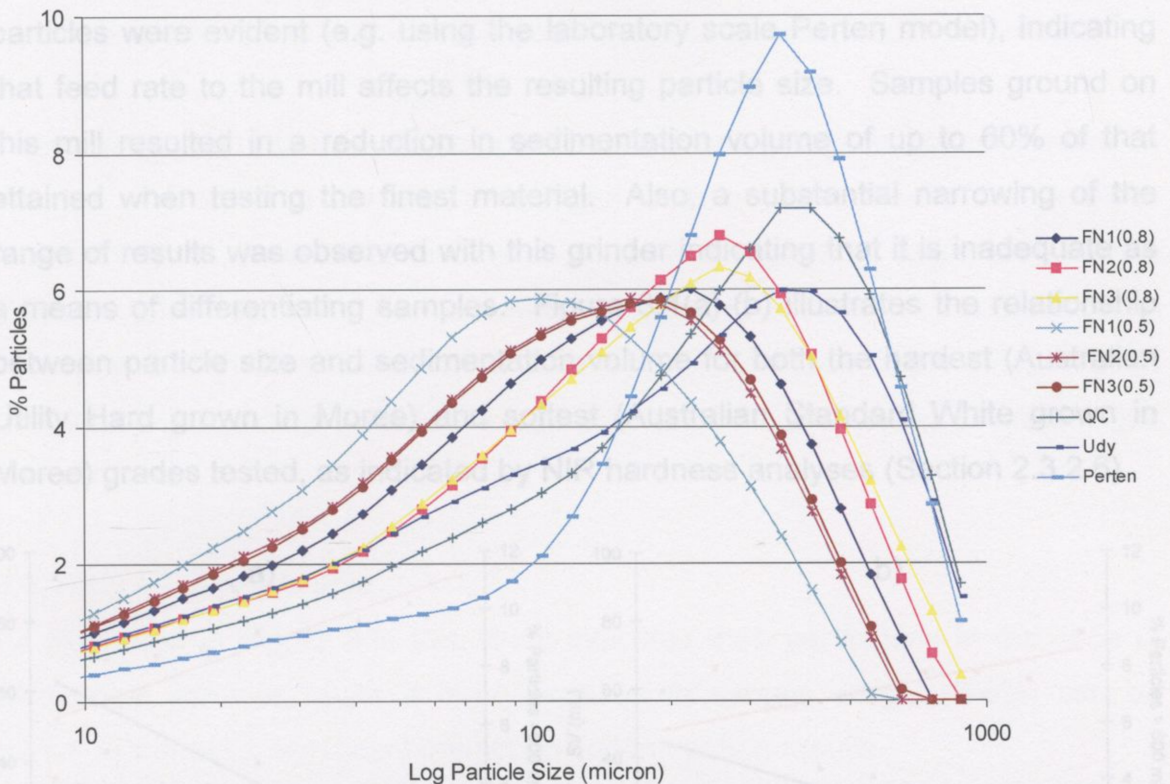

Figure 3.3: Particle size distribution profiles of hard grain (Australian Hard grade) grown in Gunnedah for nine different grinding regimes (FN1 = falling number grinder, setting 1, FN2 = falling number grinder, setting 2, FN3 = falling number grinder, setting 3, (0.8) = 0.8 mm screen fitted to grinder, (0.5) = 0.5 mm screen fitted to the grinder, QC = falling number grinder with one setting only, Udy = Udy cyclone grinder, Perten = Perten 3303 laboratory scale grinder.)

The curves in Figure 3.3 can be divided into several groups, with the curves for the Udy, QC and Perten mills all representing the highest percentage of coarse particles present in the tested sample. Samples ground on the falling number grinder using the 0.8 mm screen at either setting 2 or 3 (FN2 (0.8) and FN3 (0.8) respectively) resulted in slightly finer material than that indicated by the curves of

the Udy, QC and Perten mills. Setting 1 (FN1 (0.8)), however (the grinding regime used at Allied Mills) resulted in finer particles than either setting 2 or 3; however samples ground on the same mill using the 0.5mm screen resulted in the finest samples with FN1 (0.5) resulting in the greatest percentage of fine particles. Samples ground on setting 2 and 3 with the 0.5 mm screen resulted in very similar curves.

The large range of particle size profiles observed resulted in significant changes in sedimentation volumes across all samples, particularly where more coarse particles were evident (e.g. using the laboratory scale Perten model), indicating that feed rate to the mill affects the resulting particle size. Samples ground on this mill resulted in a reduction in sedimentation volume of up to 60% of that attained when testing the finest material. Also, a substantial narrowing of the range of results was observed with this grinder indicating that it is inadequate as a means of differentiating samples. Figure 3.4(a)-(b) illustrates the relationship between particle size and sedimentation volume for both the hardest (Australian Utility Hard grown in Moree) and softest (Australian Standard White grown in Moree) grades tested, as indicated by NIR hardness analyses (Section 2.3.2.6).

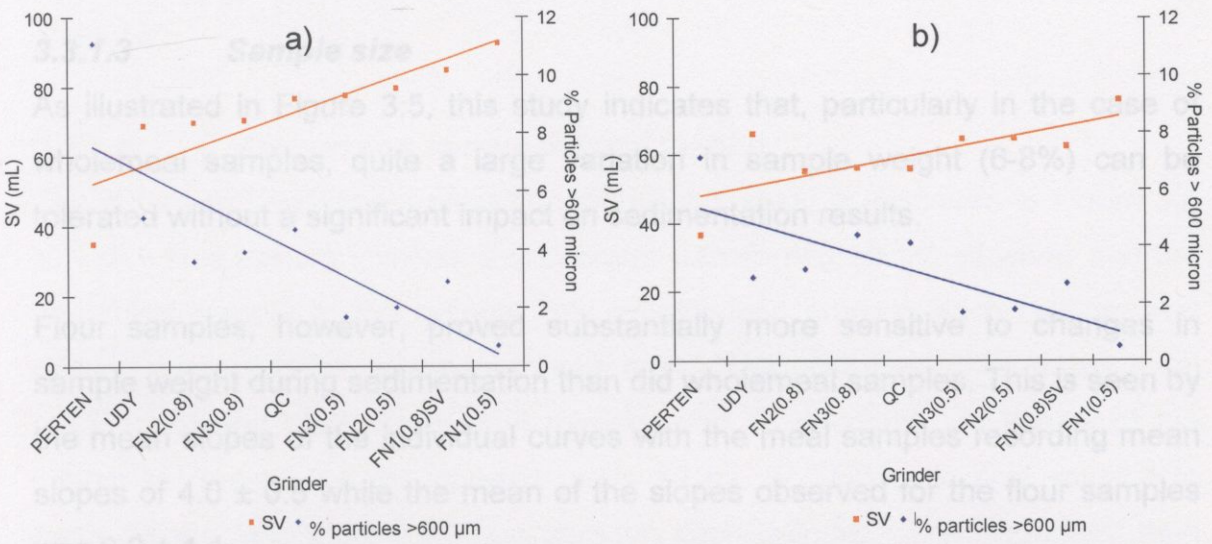

Figure 3.4: The inverse relationship between sedimentation volume (SV) and the percentage of particles exceeding 600µm of (a) a hard-grained sample and (b) a soft grained sample for a range of grinders

The results in Figure 3.4 indicate that there is a highly significant negative correlation between particle size and SV regardless of whether or not the material is hard or soft ($r = -0.951$, $p<0.001$, $r = -0.957$, $p<0.001$ respectively).

It was also observed that, in the case of the Udy, QC and Perten grinders, variability within the wholemeal samples resulted in less repeatable sedimentation volume. That is, that between the four quadruplicates, greater variability was observed indicating that not only are there a higher proportion of very large particles present in the ground sample but also a larger range of particle sizes resulting in less stable sedimentation results (results not shown).

These results emphasise the importance of grinding regime on sedimentation volume. In order to achieve meaningful sedimentation results and achieve repeatability, it is essential to have a consistent regime in conjunction with a reliable control system. Further, when comparing results from sedimentation experiments with those cited in the literature, consideration of the differences in grinding and resulting particle size is essential to the drawing of meaningful comparisons.

3.3.1.3 Sample size

As illustrated in Figure 3.5, this study indicates that, particularly in the case of wholemeal samples, quite a large variation in sample weight (6-8%) can be tolerated without a significant impact on sedimentation results.

Flour samples, however, proved substantially more sensitive to changes in sample weight during sedimentation than did wholemeal samples. This is seen by the mean slopes of the individual curves with the meal samples recording mean slopes of 4.0 ± 0.5 while the mean of the slopes observed for the flour samples was 6.2 ± 1.1.

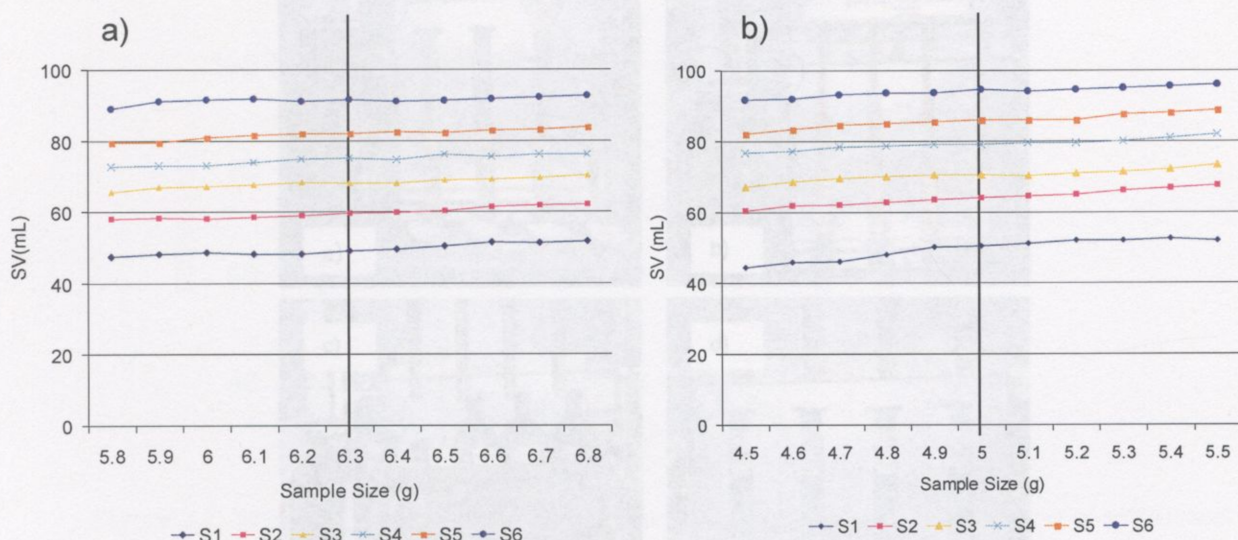

Figure 3.5: Sedimentation volume as a function of sample weight for (a) wholemeal and (b) flour samples weights used currently are marked in the centre, S1-S6 = samples covering a range of sedimentation volumes.

The weight specifications currently employed in the AACC Standard Methods, as well as the method employed at Allied Mills Australia, require samples to be within ± 0.005 g of the required weight. These results indicate that this threshold is more than adequate with regard to the sensitivity of the test to sample weight changes.

3.3.1.4 Automation versus manual testing

The automated instrument used throughout this study is illustrated in Figure 3.6 a) - d). This system involves the operator transferring 50 mL of water and the flour/meal sample to the cylinder (a), capping and shaking each cylinder and placing each onto the automated instrument. The instrument then mixes each cylinder (b) and rests them for 4 minutes and 45 seconds. At this point the instrument beeps and the operator adds 50 mL of SDS/lactic acid solution to each cylinder and places the cap back on each cylinder. The instrument then inverts each cylinder 15 times by tipping the entire back board backwards (c). Following a further 4 minute and 45 second resting period, sedimentation volume can be read directly from each cylinder (d). This process allows for consistent treatment of each sample throughout testing.

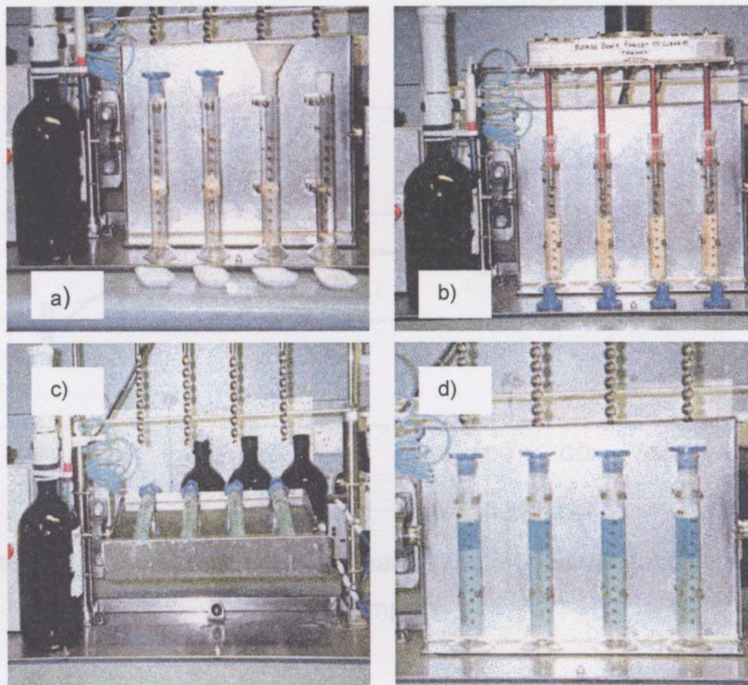

Figure 3.6: Automated instrument currently used at Allied Mills Australia (Summer Hill) showing addition of sample (a) stirring of samples by mechanical stirrers (b) inversion of cylinders (c) and the sediment volume ready to be read from the cylinders following testing (d)

The effect of using an automated mixing rack to carry out sedimentation tests versus using a hand mixing system is illustrated in Figure 3.7. These results indicate that while the correlation between the two methods is significant ($r = 0.67$, $p<0.05$) the order in which the samples are ranked is different. Further, a greater level of variability was observed between the manually tested quadruplicate samples with an average standard deviation from the mean (SDM) of 2.479 versus those tested using the automated mixing rack (SDM = 1.034). This is likely to be a result of the more consistent treatment each cylinder receives using automation, particularly during the mixing and stirring elements of the test. It is also likely that reproducibility between operators will be detrimentally affected by using the manual method, as factors such as the speed of inversions and the vigour employed during shaking are likely to change between operators. As detailed in section 3.3.1.1, this can result in significant differences in sedimentation volume.

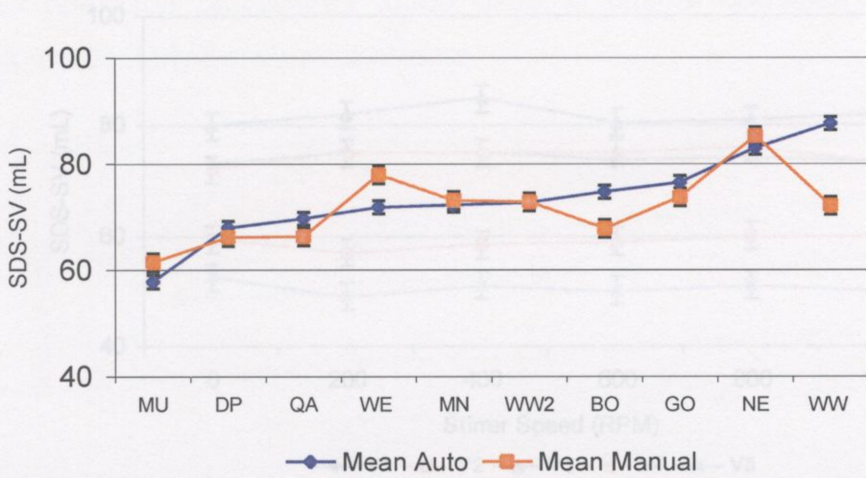

Figure 3.7: Effects of automation versus manual testing on sedimentation volume of wheatmeal samples

Ideally automatic testing would be used as opposed to manual testing, as this type of testing maintains the treatment of each cylinder under strict control, thus reduces the variation due to inconsistent sample treatment. Manual testing, on the other hand, introduces a number of areas of variability making results less reliable. It is likely that operator training would need to be extended for a manual method and that testing parameters would need to be more rigid to reduce the variability within samples and increase the reproducibility of results.

The use of an automated instrument, as opposed to manual testing, is an important consideration when analysing sedimentation results as the means of testing is likely to impact both variation between samples as well as the comparison between replicates of the same sample. This variation in results is likely to become particularly problematic when comparing results from different studies.

3.3.1.5 *Mechanical stirring*

The effect of stirrer speed on sedimentation volume is illustrated in Figure 3.8. These results indicate that stirrer speed does not have a significant effect on the formation and settling of the sediment with this trend remaining constant throughout the range of hard and soft varieties tested.

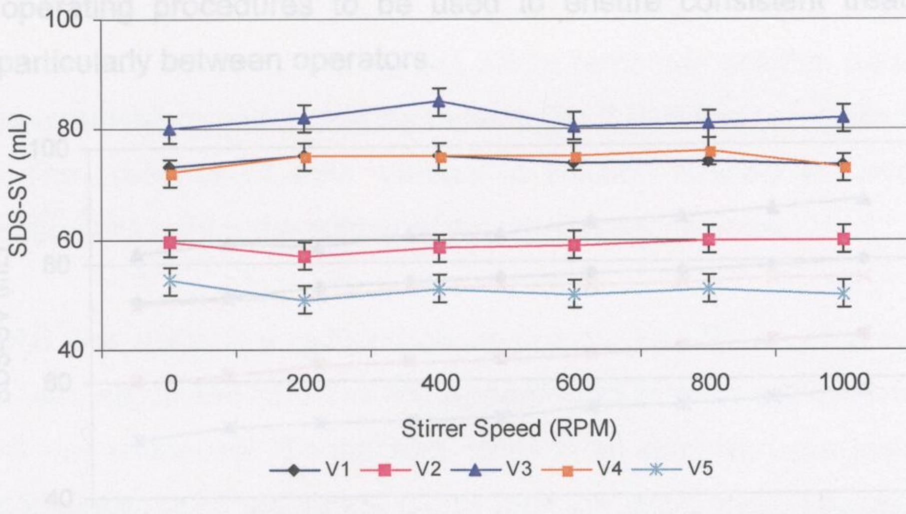

Figure 3.8: Effect of stirrer speed on sedimentation volume of wholemeal samples for five single variety samples (V1-V5)

This experiment maintained a constant length of time from addition of sample to the cylinders until the start of the mechanical stirring (~30 seconds) while varying only the vigour of the stirring. This resulted in no significant changes to sedimentation volumes. This indicates that vigorous stirring of cylinders is not required though the entire sample needs to be hydrated prior to testing.

Taking into account the significant differences seen in Section 3.3.1.1 following modification of the shaking regime, it can be hypothesised that the important factor in achieving consistent sedimentation volume is not, in fact, the vigour of stirring but rather the time taken to disperse the dry matter into the water. However, inconsistency in the shaking carried out by the operator (prior to placement of cylinders on the mixing rack, as observed in Section 3.3.1.1) also affects the length of time samples are left to hydrate prior to the start of mixing. Figure 3.9 illustrates the effect of increasing this hydration period.

These results indicate that sedimentation volume is affected by increasing the length of time the sample is hydrated in water prior to the beginning of the test with all five varieties experiencing increased sedimentation volumes with increasing hydration time. The increase in sedimentation volume as a result of increased hydration time further emphasises the need for clear and specific

standard operating procedures to be used to ensure consistent treatment of samples, particularly between operators.

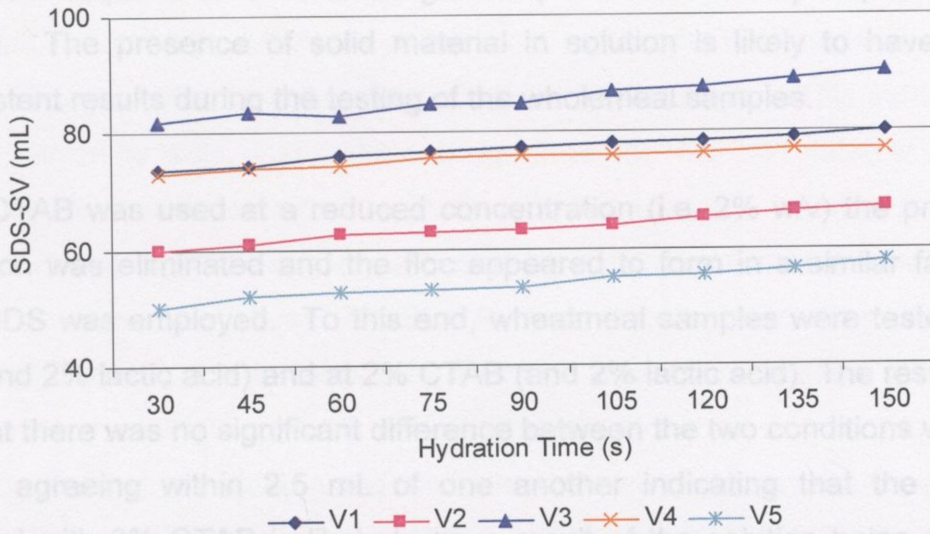

Figure 3.9: Effect of hydration time on sedimentation volume of wholemeal samples for five single variety samples (V1-V5)

3.3.1.6 Reagents

3.3.1.6.1 SDS

Samples were first tested using a solution containing only 2% lactic acid without SDS present. The result of this was that the sample did not flocculate at all but rather the sample partially dispersed through the solution while the remainder of the sample sunk to the base of the cylinder. Subsequently SDS was replaced with CTAB in order to observe the effect of replacing an anionic detergent with a cationic detergent. As indicated by Figure 3.10, sedimentation behaviour was largely unaffected for the flour samples while wholemeal samples were affected appreciably.

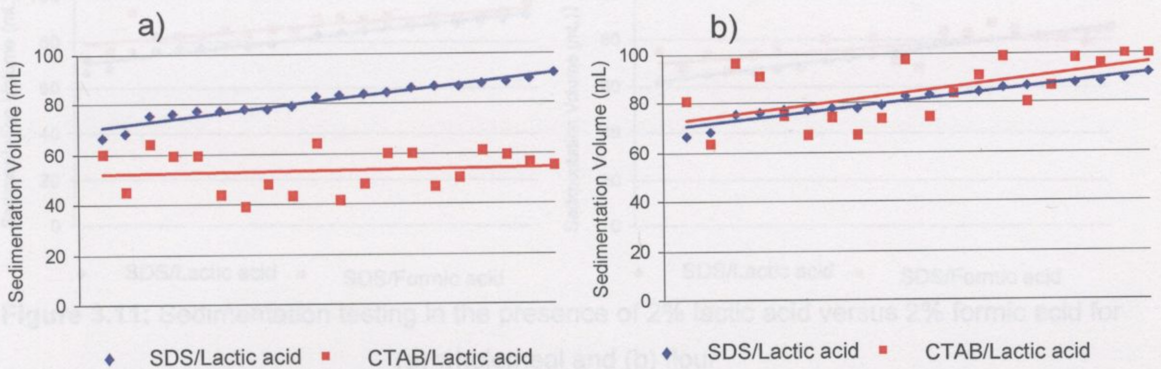

Figure 3.10: Sedimentation results in the presence of SDS versus CTAB for (a) wheatmeal (reagents added at 3%) and (b) flour samples (reagents added at 1.5%)

The result observed for wholemeal samples is likely to be a result of the concentration of CTAB being too high at 3% to remain in solution meaning that the solution required constant shaking to keep the CTAB from precipitating out of solution. The presence of solid material in solution is likely to have lead to inconsistent results during the testing of the wholemeal samples.

When CTAB was used at a reduced concentration (i.e. 2% w/v) the problem of saturation was eliminated and the floc appeared to form in a similar fashion to when SDS was employed. To this end, wheatmeal samples were tested at 2% SDS (and 2% lactic acid) and at 2% CTAB (and 2% lactic acid). The result of this was that there was no significant difference between the two conditions with each sample agreeing within 2.5 mL of one another indicating that the variation observed with 3% CTAB is likely to be a result of the solution being saturated leading to inconsistent concentrations being used throughout testing.

3.3.1.6.2 Lactic Acid

As for SDS, samples were originally tested using a solution that did not contain any lactic acid, while keeping SDS concentrations at 3% for meal and 1.5% for flour. The result of this was that the sediment failed to settle in the column at all. The contents of the cylinders remained a continuous cloudy suspension throughout the solution and thus readings could not be taken. Lactic acid is therefore, an essential factor in floc formation and the sedimentation test in general. Whether this result is due to the specific acid or the pH of the solution was unclear.

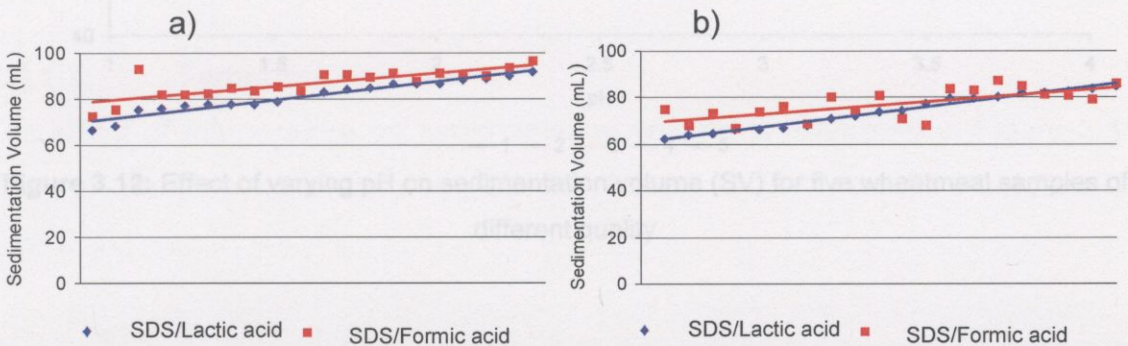

Figure 3.11: Sedimentation testing in the presence of 2% lactic acid versus 2% formic acid for (a) wholemeal and (b) flour

In order to ascertain whether the lactic acid is itself critical to sedimentation or whether it is a result of the pH of solution, lactic acid was replaced in solution by formic acid (which has a similar pKa). The results of this substitution (Figure 3.11) indicate that little difference arises from replacement of the lactic acid with an acid of similar pKa for either flour or wholemeal samples. This indicates that the role played by lactic acid in the settling of the floc may be related to the pH of the solution versus an effect of the lactic acid in particular. In order to test this, solutions containing SDS at the level used for regular sedimentation testing (3% SDS for wholemeal samples, 1.5% SDS for flour samples) were prepared and the pH adjusted to 1.7, 2.4, 2.7 and 3.7. Samples were then retested. The results are illustrated in Figures 3.12 and 3.13 (wheatmeal and flour respectively) indicating that pH is critical to sedimentation volume particularly in the pH range between 2.4 and 2.7 where an inflection point is evident. At 2% lactic acid in solution (the concentration used for wheatmeal and flour sedimentation tests), the pH is 2.4. At this pH the range of sedimentation values is broad giving strong differentiation between samples.

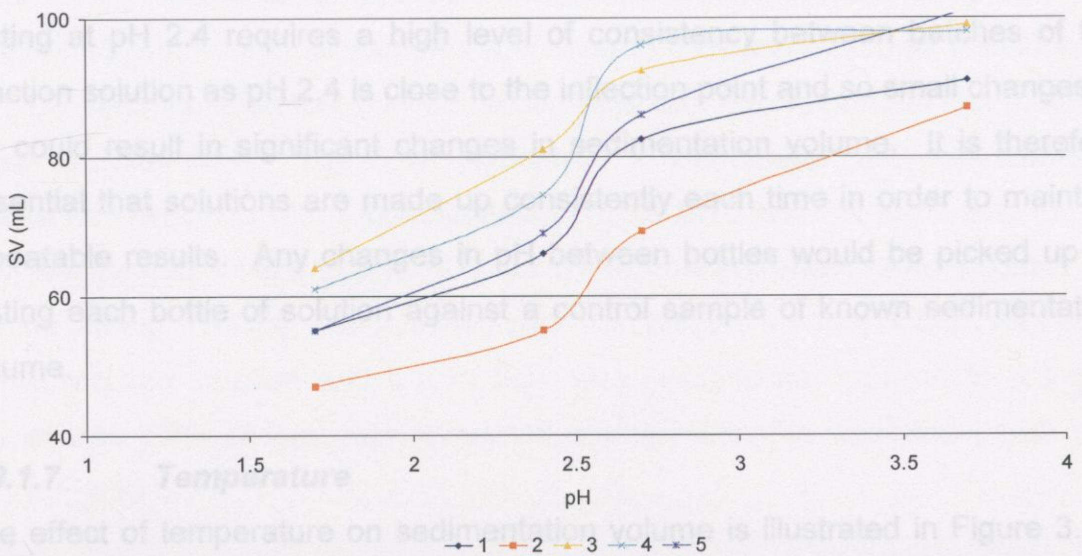

Figure 3.12: Effect of varying pH on sedimentation volume (SV) for five wheatmeal samples of different quality

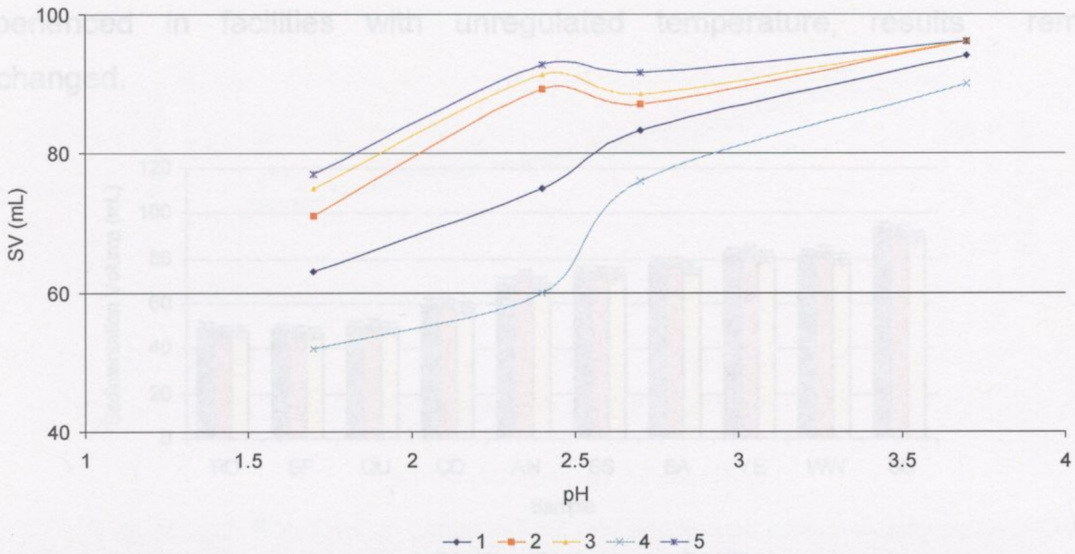

Figure 3.13: Effect of varying pH on sedimentation for five flour samples of different quality

At the high and low pH values tested the range of sedimentation volumes decreased substantially indicating that carrying out sedimentation testing under these conditions would result in inadequate differentiation between samples for the purposes of quality identification. However, carrying out sedimentation testing at pH 2.4 requires a high level of consistency between batches of the reaction solution as pH 2.4 is close to the inflection point and so small changes in pH could result in significant changes in sedimentation volume. It is therefore essential that solutions are made up consistently each time in order to maintain repeatable results. Any changes in pH between bottles would be picked up by testing each bottle of solution against a control sample of known sedimentation volume.

3.3.1.7 Temperature

The effect of temperature on sedimentation volume is illustrated in Figure 3.14. Each of the ten wheatmeal samples was tested at 12ºC, ambient temperature and 37ºC.

Results indicate that fluctuations of temperature within the range tested have no significant effect on the results of sedimentation testing. It is likely that sedimentation testing would be carried out, for the most part, in temperature controlled environments (~19 - 21ºC) however this study indicates that in the

range of temperatures tested, i.e. the range of temperature likely to be experienced in facilities with unregulated temperature, results remain unchanged.

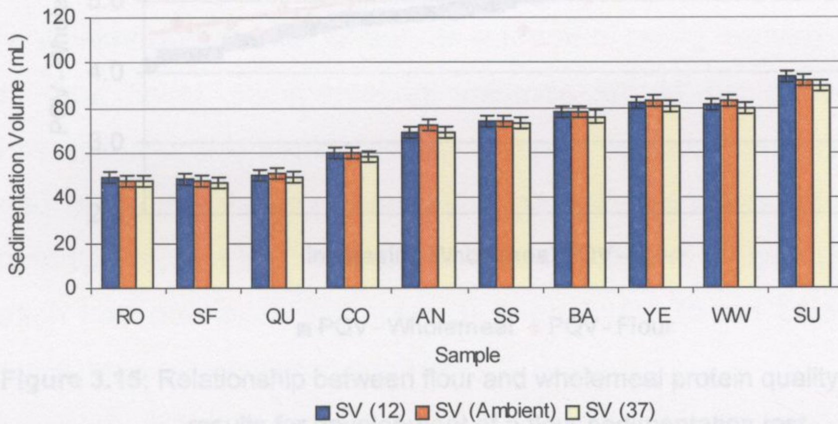

Figure 3.14: Effect of temperature variation on sedimentation results for ten wheatmeal samples, SV(12), sedimentation volume at 12°C; SV(Ambient), sedimentation volume at ambient temperature; SV(37), sedimentation volume at 37°C.

3.3.2 Method Development and Modification

3.3.2.1 Modification of SDS sedimentation test for flour samples

A set of 45 grain-and-flour samples was used to determine the best solutions for an SDS sedimentation test that would correlate with the wheatmeal test routinely used by Allied Mills. The best relationship to wheatmeal SDS sedimentation values was obtained with a combination of 1.5% SDS and 2% lactic acid for the flour sedimentation test (Table 3.1), so these concentrations were adopted for routine testing. The test was modified such that half the amount of SDS was used in the reaction solution and 5 g of flour was required as opposed to 6.3 g of wholemeal. The chosen test correlated strongly with the wholemeal test (r^2 = 0.87) (Figure 3.15).

It proved possible to achieve results for flour sedimentation testing comparable with those observed for the routine wheatmeal sedimentation method, without the need for new equipment or alterations to existing equipment. These results can be achieved for flour by reducing the sample weight used for testing and halving the concentration of SDS used for wheatmeal testing.

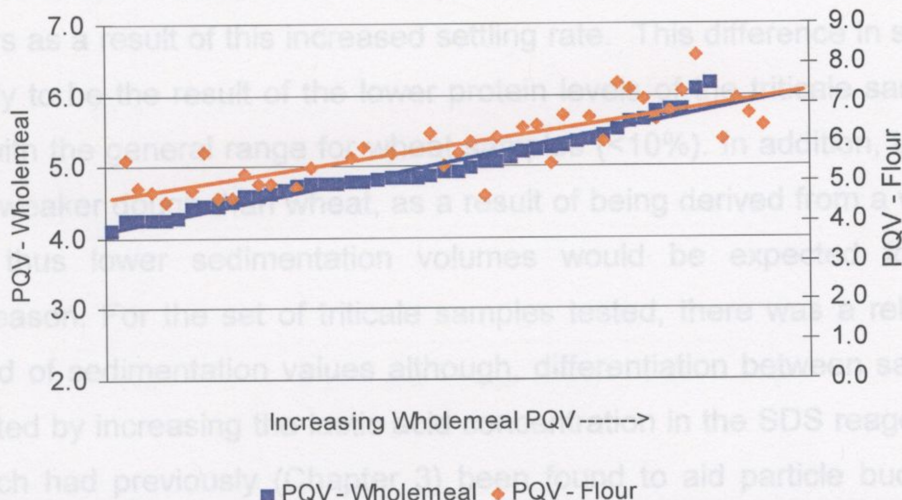

Figure 3.15: Relationship between flour and wholemeal protein quality value (PQV) results for development of a flour sedimentation test

Table 3.1: Correlation coefficients (r^2) relating to SDS-sedimentation results for corresponding wheatmeal and flour samples, using the solution combinations indicated for the flour samples.

Lactic Acid Concentration (%)	Concentration SDS (%) 1	1.5	2	3
1.2	-	-	0.82***	-
2	0.67***	0.87***	0.81***	N/A #
3	-	-	0.86***	-

Sedimentation values were too high to read accurately, *** $P<0.001$

3.3.2.2 *Sedimentation test for triticale*

Triticale samples, both flour and wheatmeal, show promise for sedimentation testing, producing a sediment similar to that seen for soft wheat. The range of values, however, is significantly lower than for wheat samples, with all samples tested giving results within 15 mL of one another. Despite the particle size of the samples being approximately the same as that of wholemeal (due to having been ground on the same grinder at the same setting), the floc settled to the bottom of the cylinder much more quickly in the case of triticale samples, both flour and meal, than did wheat flour and meal.

Triticale meal samples resulted in much lower sedimentation volumes than the triticale flours as a result of this increased settling rate. This difference in settling rates is likely to be the result of the lower protein levels of the triticale samples, compared with the general range for wheat samples (<10%). In addition, triticale produces a weaker dough than wheat, as a result of being derived from a wheat-rye cross; thus lower sedimentation volumes would be expected for this additional reason. For the set of triticale samples tested, there was a relatively small spread of sedimentation values although, differentiation between samples was attempted by increasing the lactic acid concentration in the SDS reagent, as this approach had previously (Chapter 3) been found to aid particle buoyancy thereby increasing sedimentation volumes.

A number of different SDS and lactic acid concentrations were tested as described in section 3.2.2.2. These included: 1% SDS/1% lactic acid, 1.5% SDS/2% lactic acid, 3% SDS/2% lactic acid, 3% SDS/5% lactic acid, 3% SDS/10% lactic acid, 5% SDS/2% lactic acid, 5% SDS/5% lactic acid, 5% SDS/10% lactic acid and 10% SDS/2% lactic acid.

Of these, the solution containing 5% SDS and 5% LA resulted in the floc that most resembled that of wheat flour in terms of consistency while also resulting in the largest spread of values (27.5 – 38 mL). These results provided a strong correlation with protein content (R^2 = 0.95; Figure 3.16). Given this relationship with protein content, it can be assumed that sedimentation volume is likely to be a better indicator of protein quantity than protein quality for triticale.

Samples were also tested using the Zeleny reaction solution containing lactic acid and isopropyl alcohol. A larger range (40-70 mL) of values was achieved using the solution containing isopropyl alcohol than that resulting from the SDS solution, however, the upper level of the sediment was sometimes difficult to detect, resulting in less accurate readings. If this problem could be overcome, e.g. by using a different dye to aid accurate reading of the sediment, this type of sedimentation testing may prove useful for triticale samples.

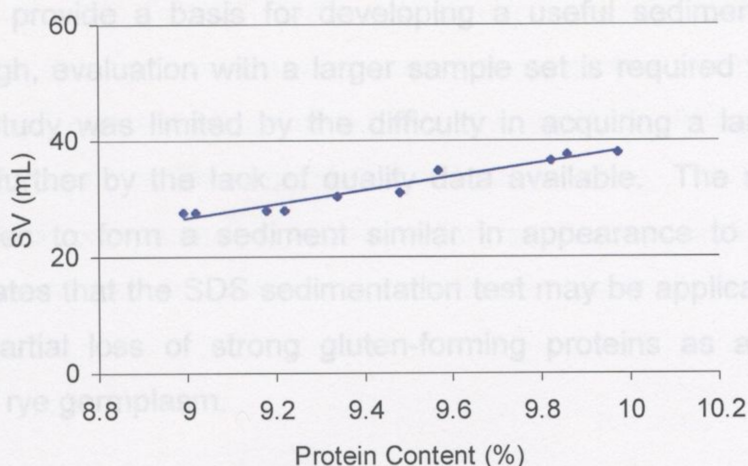

Figure 3.16: Relationship between protein content and SV for triticale meal samples

Testing of triticale flour samples (Figure 3.17) resulted in a smooth homogeneous sediment, not dissimilar in appearance to the sediment of wheat flours. Increasing the concentration of SDS in solution resulted in an increase in sedimentation volume for all samples; however, the range of values was significantly narrower making differentiation between samples difficult. For this reason, solutions containing decreased amounts of SDS (e.g., 2%, 1%) were investigated. The best results for testing triticale flours was achieved using a solution containing 3% SDS and 5% lactic acid.

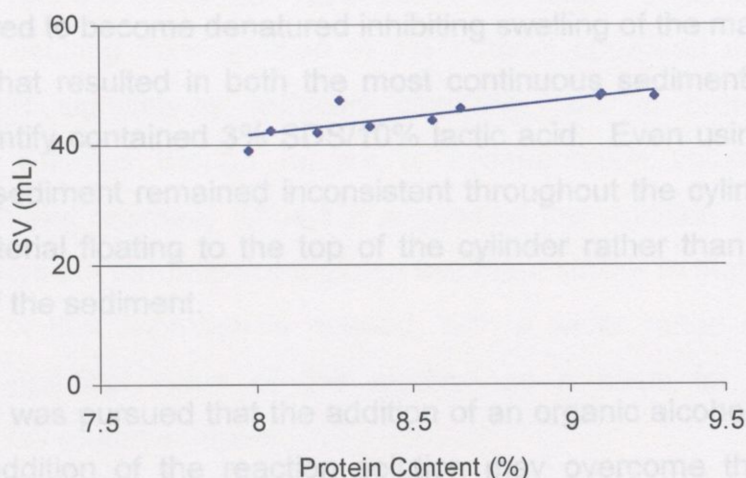

Figure 3.17: Relationship between protein content and SV for triticale flour samples

These results provide a basis for developing a useful sedimentation test for triticale although, evaluation with a larger sample set is required to establish its value. This study was limited by the difficulty in acquiring a large number of samples and further by the lack of quality data available. The ability of these triticale samples to form a sediment similar in appearance to that of wheat samples indicates that the SDS sedimentation test may be applicable to triticale, despite the partial loss of strong gluten-forming proteins as a result of the introduction of rye germplasm.

3.3.2.3 Sedimentation test for wheat gluten

Vital dry-gluten samples were tested using the reagents developed for wheatmeal and flour (3% SDS / 2% lactic acid and 1.5% SDS / 2% lactic acid, respectively); however the resulting floc differed markedly from that of wheatmeal and flour samples. The most obvious difference was that the gluten sediment was not homogenous in nature, unlike the sediment from flour. The floc formed large, dense, dough–like structures interspersed throughout the solution as opposed to the cloudy continuous matrix of the flour/meal sediment. This trend remained much the same regardless of the concentrations of reagents used in solution. It was noted that decreasing concentrations of lactic acid or SDS led to more marked clumping of the protein material in the cylinder. When both reagent concentrations were increased significantly (i.e., 5% SDS/10% lactic acid) the protein appeared to become denatured inhibiting swelling of the material.

The solution that resulted in both the most continuous sediment and the least difficult to quantify contained 3% SDS/10% lactic acid. Even using this solution however, the sediment remained inconsistent throughout the cylinder with some of the dry material floating to the top of the cylinder rather than hydrating and forming part of the sediment.

The possibility was pursued that the addition of an organic alcohol to the sample prior to the addition of the reaction solution may overcome this problem by minimising the surface tension between the gluten and the solution, thereby allowing the gluten sample to disperse more evenly throughout the solution. To this end, 1-butanol was incorporated into the distilled water at the beginning of the test at levels of 1, 2, 5 and 10%. Sedimentation testing continued thereafter

as normal with the 3% SDS/2% lactic acid solution used for testing wheatmeal samples. While the appearance and consistency of the sediment improved significantly at all concentrations of butanol, the most significant improvement was seen in the case of 3% butanol (v/v of total solution). This is illustrated in Figure 3.18 which compares the sedimentation results from addition of 3% and 5% butanol as related to gluten extensibility (as determined by Keiffer rig analysis).

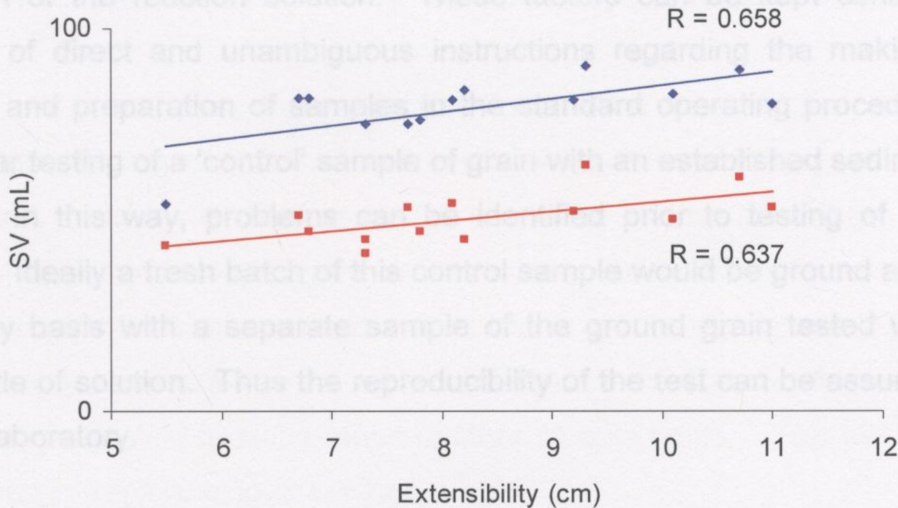

Figure 3.18: Relationship between sedimentation volume (SV) and Keiffer rig extensibility (Ext) in the presence of 3% (blue) and 5% (red) 1-butanol.

While both 3% and 5% levels resulted in similar correlation coefficients ($p < 0.01$), the sediment resulting from addition of 3% butanol appeared more consistent throughout with significantly reduced clumping, and a broader range of sedimentation volumes though ranges of sedimentation volume were quite narrow for both test regimes. Thus it is suggested that addition of 3% 1-butanol solution facilitates the formation of a gluten sediment similar to that of wheat flour. An extension of this approach is needed, with a wider range of samples, to establish the practical value of this method as a basis for predicting the extensibility of dry-gluten samples.

3.4 Conclusions

This chapter demonstrates that, in a single laboratory situation, SDS sedimentation testing is a robust, small-scale quality test. When carried out

under controlled, consistent conditions the test remains insensitive to small changes in sample size, stirrer speed and the temperature under which testing is conducted. The test can also be expected, when operating procedures are adhered to, to return highly reproducible results regardless of the experience level of the operator.

The procedure is however, sensitive to the particle size range of the sample and to the pH of the reaction solution. These factors can be kept consistent by inclusion of direct and unambiguous instructions regarding the making up of solutions and preparation of samples in the standard operating procedures and the regular testing of a 'control' sample of grain with an established sedimentation volume. In this way, problems can be identified prior to testing of unknown samples. Ideally a fresh batch of this control sample would be ground and tested on a daily basis with a separate sample of the ground grain tested with each fresh bottle of solution. Thus the reproducibility of the test can be assured within a single laboratory.

Reproducibility becomes a more significant problem, however, with regard to the use of the test across different laboratory locations. Currently, literature suggests that each laboratory uses different grinding protocols which will result in a large range of particle size profiles and so a range of sedimentation results. Inconsistency also exists between laboratories with regard to the concentration of reagents used in solution and also the volume of solution used for each sample. If the results of the sedimentation testing are to be meaningful as a basis for comparison of wheat protein quality, standard procedures need to be followed closely.

This chapter also indicates the potential for sedimentation testing to be expanded for use as a test of quality for other sample types including triticale and gluten samples. Minor manipulation of test parameters resulted in a quantifiable sediment not dissimilar in appearance to that of wheat samples. The speed, simplicity and relative economy of sedimentation testing along with the small sample size required are all major advantages in favour of this type of test and thus extension of the application of sedimentation testing is likely to be

advantageous. While significant further testing of large sample sets of trítical and gluten is required sedimentation testing could prove useful in situations requiring large numbers of samples to be tested, e.g., triticale breeding programs or starch and gluten plants.

As a result of these studies a standard protocol for sedimentation of wheat flour and meal samples has been determined for use with the current instrumentation at Allied Mills Australia (Summer Hill). This protocol is presented in Appendix B.

In order to take advantage of this information and better understand sedimentation as a useful, robust testing technique, it is necessary to understand how the test works on a molecular and structural level. This information is also of significant importance with regard to maximising the potential for expansion of sedimentation across a range of applications within the grain industry, as understanding the mechanism of action of the test is the first step to manipulating the testing parameters to suit a range of other sample types. This will be further investigated in the following chapters.

Chapter 4

Factors Affecting Sediment Formation and Composition

4.1 Introduction

As described in Chapter 3, current sedimentation testing techniques are robust methods with the potential for use in various applications, mainly to predict the processing potential of wheat grain. Further applications include that of Cressey and McStay (Cressey and McStay, 1987) who investigated a sedimentation test capable of gauging the affect on quality as a result of grain damage caused by bug damage.

Despite the many reports on the use of sedimentation testing, relatively little information is currently available as to the nature and composition of the sediment material or the process by which the sediment forms. It has been reported that while the monomeric gliadin fraction is almost completely soluble in SDS, the polymeric protein material present in the sample remains insoluble. Thus it has been implied that the polymeric glutenin fraction is solely responsible for the sediment volume observed during sedimentation testing (Wang and Kovacs, 2002; Cressey and McStay, 1987). Eckert et al. (1993) concur, reporting that in microscopic studies of the swelling capability of flour particles and their constituent protein fractions, only the polymeric glutenins were capable of swelling in the SDS/lactic acid solution. It was also observed that the swelling of flour particles was greater in the solution containing SDS, as opposed to the

Zeleny solution containing isopropyl alcohol (Eagles et al., 2001; Eckert et al., 1993).

Numerous studies have challenged the theory that the sediment is a result only of the propensity of flour particles to swell in acidified solutions (Zeleny, 1963). An alternative theory proposed by Frazier et al. (1969) suggests that rather than the flour particles simply swelling, flocculation of particles takes place causing aggregation and precipitation of the sample components, resulting in the formation of the floc. In agreement with the theory of flocculation as opposed to protein swelling, Adeyemi and Muller (1983) proposed that the protein molecules present in the sedimentation solution formed long fibrous structures ("fibrils") that were responsible for connecting the suspended flour particles in a matrix structure. It has also been hypothesised that these fibrils may be the same proteinaceous material responsible for dough viscoelasticity and that the number of fibrils produced may correlate positively with sedimentation volume (Adeyemi and Muller, 1975).

It is widely reported that each fraction of wheat flour plays a specific role in the overall functionality of the flour with regard to dough formation. For example, the gliadin proteins promote the viscous flow and extensibility of dough (Shewry, 2003), while lipids are known to complex with the structural protein network thereby contributing to the matrix structure of the dough (Van Der Borght et al., 2005). There is less information, however, on the effects of and interactions between, these fractions with regard to sedimentation behaviour.

To this end, the primary aims of this chapter were to investigate the contribution of different protein bonds and of the individual flour fractions, in order to probe the structure and composition of the sediment during and following sedimentation.

4.2 Materials and Methods

4.2.1 Flour fractionation and reconstitution

4.2.1.1 Isolation of the water soluble fraction

The water-soluble fraction of the flours was obtained as for a typical starch-and-gluten separation (Section 2.7.2) except that only 100 mL of water was used to wash the starch from each 10 g of flour tested. Following gentle centrifugation of the starch/water mixture at 1000 x g for 5 minutes the supernatant, containing the water soluble flour components, was collected and made up to 100 mL exactly. This fraction was used in place of the 50 mL of distilled water generally used for SDS sedimentation of flour samples.

4.2.1.2 Reconstitution and sedimentation

For starch and gluten tests, gluten was added at the appropriate percentage (based on protein content, as determined by NIR), and made up to 5 g with starch. Where appropriate, the water-soluble fraction was added in place of the 50 mL of distilled water. Sedimentation was carried out, in all cases, using an automated mixing rack as described in Section 2.8.1.

4.2.1 Contribution of bond types to sedimentation behaviour

4.2.2.1 Disulphide bonds

Reaction solutions were made up as normally (3% SDS/2% lactic acid for meal, 1.5% SDS/2% lactic acid for flour – see Appendix B). L-Cysteine was added to the distilled water at both 0.01 M and 0.1 M concentrations in order to disrupt the disulphide linkages present in the flour/wholemeal samples. Sedimentation was carried out on a set of six samples representing a wide range of sedimentation volumes (45-92 mL), as described in Sections 2.8.1 (meal) and 3.3.1 (flour).

4.2.2.2 Ionic bonds

This procedure was carried out as for Section 4.2.2.1, except that ammonium chloride was added to the distilled water at concentrations of 0.1 M and 1.0 M in order to disrupt the ionic bonds present in the flour/wholemeal samples.

4.2.2.3 Hydrogen bonds

This procedure was carried out as for Section 4.2.2.1, except that urea was added to the distilled water at a concentration of 0.1 M and 1 M in order to disrupt the ionic bonds present in the flour/wholemeal samples.

4.2.2.4 van der Waals forces

In order to observe the effect of van der Waals forces on sedimentation outcomes, solutions and cylinders were heated to 60°C prior to testing. Solutions were heated using water baths while the equipment was heated in 60°C ovens. Sedimentation testing was carried out using the heated solutions and glassware, at ambient laboratory temperature, according to Sections 2.8.1 (meal) and 3.3.1 (flour). The temperature in each cylinder following the sedimentation test was measured at 43-46°C.

4.2.2.5 Lipid-protein interactions

This procedure was carried out as for Section 4.2.2.2, except that CTAB was added to the distilled water at a concentration of 0.0001 M in order to disrupt the bonds present between the lipids and proteins present in the wheat samples.

4.2.3 Protein profile throughout sediment

In order to investigate the protein composition of the sediment throughout the cylinder during sedimentation testing, three flours were selected representing high, medium and low sedimentation volumes. Sedimentation testing was carried out as described in Section 3.3.2.1. Following sedimentation testing, 10 mL samples of the sediment were taken from the top, middle and bottom of the sediment (from three separate cylinders) using a syringe fitted with an 18 cm, 6 gauge needle. The aliquots were centrifuged at 1,000 x g for 2 minutes and the pellet was resuspended in 10 mL of water and re-centrifuged. This process was repeated three times in order to wash away as much of the residual SDS as possible. The final pellet was drained and frozen before being freeze-dried. Freeze-dried pellets were ground in a mortar and pestle, and size exclusion HPLC used as described in Section 2.6 to ascertain protein composition.

4.3 Results and Discussion

All data discussed in Chapter 4 are presented in full in Appendix C.

4.3.1 Flour fractionation and reconstitution

As illustrated in Figure 4.1, sedimentation behaviour of individual flour fractions is considerably different to the sedimentation behaviour of the whole flour (Figure 4.2). The gluten samples resulted in the highest sedimentation volume with negligible volume provided by the starch fraction. The water-soluble fraction alone did not produce any sediment at all. While the high reading of the gluten indicates that this fraction plays an important role in the formation of sediment, it is important to consider that this result was achieved by sedimentation of a 5 g sample of isolated gluten, representing an approximate ten-fold increase in the expected protein content of unfractionated flour.

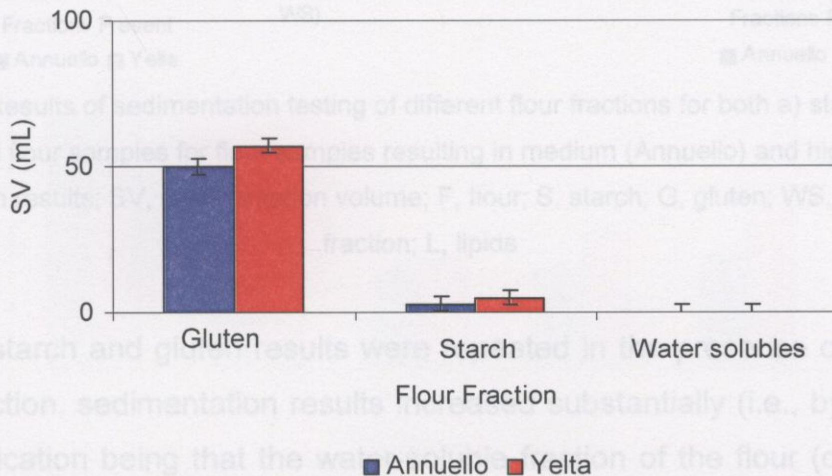

Figure 4.1: SDS sedimentation volume of individual flour fractions for flour samples resulting in medium (Annuello) and high (Yelta) sedimentation results

In order to probe the effects of fractionation and the interaction between wheat flour fractions, the fractions were sampled in combination with each other. These results are illustrated in Figure 4.2 which represents interactions between flour fractions for whole flour and defatted flour samples. No significant difference was observed between the whole and defatted flours indicating that the lipid component of the flour plays a relatively minor role in formation and composition of the sediment.

When starch and gluten were reconstituted at the levels found in the original flour, it was observed that the level of sediment in the cylinder following testing measured only 3-5mL. Similar results were not indicated by literature reports, as previous studies of quality tests have resulted in similar values for reconstituted flours as those observed for original flours (DuPont et al., 2005; Van Der Borght et al., 2005).

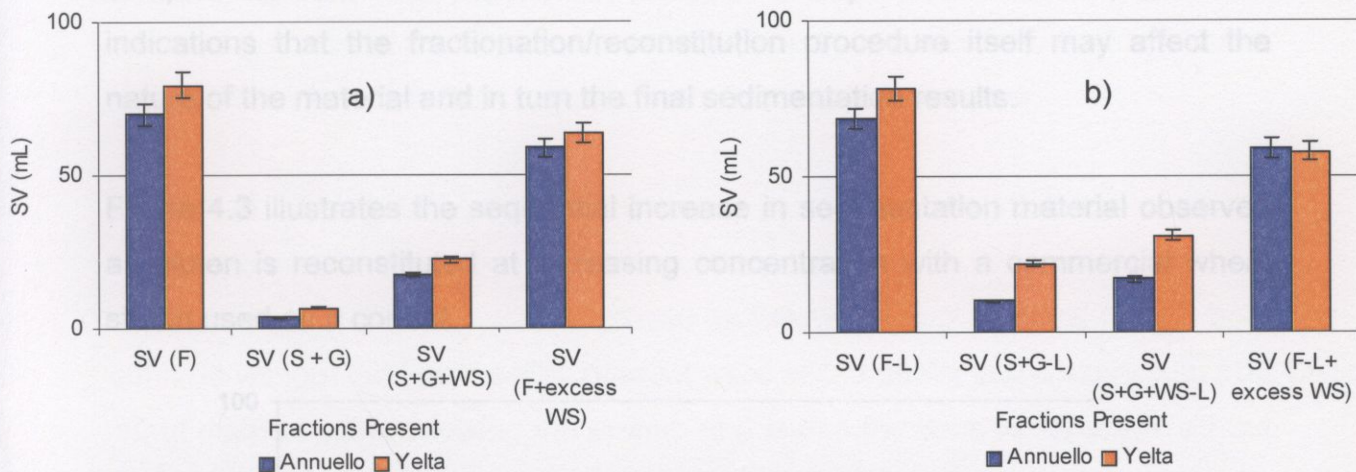

Figure 4.2: Results of sedimentation testing of different flour fractions for both a) standard and b) de-fatted flour samples for flour samples resulting in medium (Annuello) and high (Yelta) sedimentation results; SV, sedimentation volume; F, flour; S, starch; G, gluten; WS, water-soluble fraction; L, lipids

When the starch and gluten results were repeated in the presence of the water-soluble fraction, sedimentation results increased substantially (i.e., by a factor of 5) the implication being that the water-soluble fraction of the flour (consisting of albumins, globulins and soluble starch) contains components that play a significant role in the formation of the sediment complex. However, Frazier et al. (1969) and Adayemi and Muller (1983), have reported that the insoluble protein fraction (i.e., glutenin) is solely responsible for the sediment.

It is worth noting however, that when the water-soluble fraction is used in place of the water to test the whole and defatted flour samples (i.e. the water soluble components are in excess), sediment levels are actually substantially lower than those observed when testing was carried out using water only. The implication here is that while water-soluble components play an important role in the composition of the protein matrix, and thus in sedimentation volume, if they are

present in excess of the level present in the grain/flour sample, they are capable of detrimentally affecting protein quality as determined by sedimentation volume.

These results, particularly the unexpectedly low starch-gluten sedimentation volume, indicate that the formation of the sediment and the overall sediment volume are determined not only by simple swelling of HMW proteins but by a complex series of interactions between flour components. Further, there are indications that the fractionation/reconstitution procedure itself may affect the nature of the material and in turn the final sedimentation results.

Figure 4.3 illustrates the sequential increase in sedimentation material observed as gluten is reconstituted at increasing concentration with a commercial wheat starch used as a control.

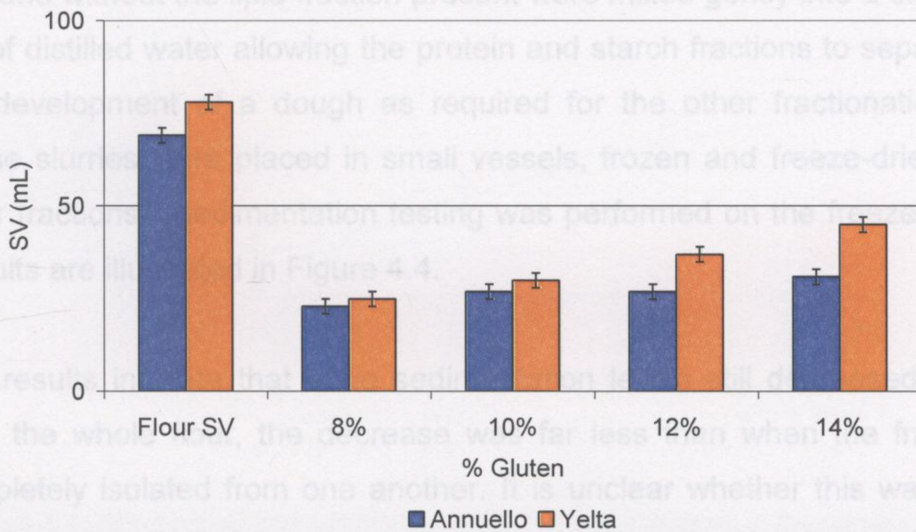

Figure 4.3: Effect on sedimentation volume (SV) of differing gluten concentrations in a standard starch for both a medium (Annuello) and a high (Yelta) performing flour

While the volume of sediment would be expected to increase with increasing protein content in the test sample, even at 14% gluten, a protein content higher than is present in either of the test samples, the sedimentation volumes are still very low when compared with those of the whole flour samples.

The unexpectedly low sedimentation volumes observed indicate that although the protein content clearly plays a role in the volume of sediment present at the

conclusion of the test, it is certainly not the only interaction involved. It is worth noting that the sediment levels reported here, using a commercial starch as a background, are significantly higher than those reported in Figure 4.2 where the isolated starches of each sample were used. It is likely that the nature of the starch material and its ability to interact as it does in whole flour are affected by the extraction, isolation and/or drying procedures. During sedimentation testing of matching starch and gluten pairs, the liquid phase in the sedimentation columns (i.e., above the sediment) was significantly cloudier for the reconstituted samples than for either flour samples or the commercial starches.

In order to investigate how the fractionation procedure may affect the nature of the fractions and thus the sediment volume, a procedure involving minimal interference with the flour was employed as follows. Flour samples (20 g), both with and without the lipid fraction present were mixed gently into a slurry with 100 mL of distilled water allowing the protein and starch fractions to separate without the development of a dough as required for the other fractionation methods. These slurries were placed in small vessels, frozen and freeze-dried as for the other fractions. Sedimentation testing was performed on the freeze-dried flours. Results are illustrated in Figure 4.4.

The results indicate that while sedimentation levels still decreased significantly from the whole flour, the decrease was far less than when the fractions were completely isolated from one another. It is unclear whether this was due to the polymeric protein fraction not undergoing development (an irreversible process) prior to being isolated from the starch or as a result of the isolation itself.

Figure 4.5, indicates how the presence of the bran fraction (collected following Quadrumat Junior milling and reconstituted at 18% as per the presence in whole ground grain) affects sedimentation levels at varying gluten addition levels.

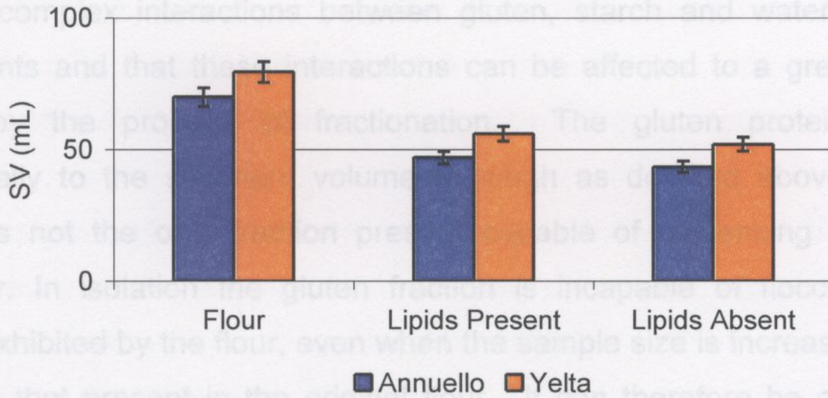

Figure 4.4: Effects on sedimentation volume (SV) of mixing and freeze drying of a flour/water slurry involving medium (Annuello) and high (Yelta) performing flours.

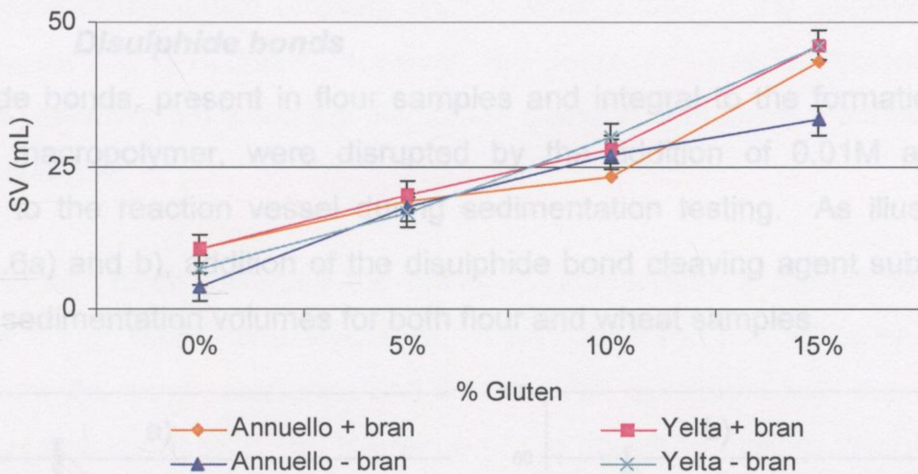

Figure 4.5: Effect on sedimentation volume (SV) of gluten content in the presence of bran for a medium (Annuello) and a high (Yelta) performing flour

These results indicate that the presence of bran makes very little difference to the overall sedimentation volume with the exception of the Annuello sample, which recorded a significant difference between the sample tested with bran versus without bran at 15% gluten addition. It is also worth noting that there is no significant difference between the sedimentation volumes for the Annuello and Yelta samples despite their having been selected on the basis of significant differences originally.

It can be concluded that the sediment formed during SDS sedimentation tests involves complex interactions between gluten, starch and water soluble flour components and that these interactions can be affected to a greater or lesser degree by the process of fractionation. The gluten proteins contribute substantially to the sediment volume although as detailed above, the protein fraction is not the only fraction present capable of influencing sedimentation behaviour. In isolation the gluten fraction is incapable of flocculating to the volume exhibited by the flour, even when the sample size is increased by a factor of ten on that present in the original flour. It can therefore be concluded that protein-starch interactions also play an important role in sediment flocculation as does the water soluble fraction.

4.3.2 Contribution of bond types to sedimentation behaviour

4.3.2.1 Disulphide bonds

Disulphide bonds, present in flour samples and integral to the formation of the glutenin macropolymer, were disrupted by the addition of 0.01M and 0.1M cysteine to the reaction vessel during sedimentation testing. As illustrated in Figure 4.6a) and b), addition of the disulphide bond cleaving agent substantially lowered sedimentation volumes for both flour and wheat samples.

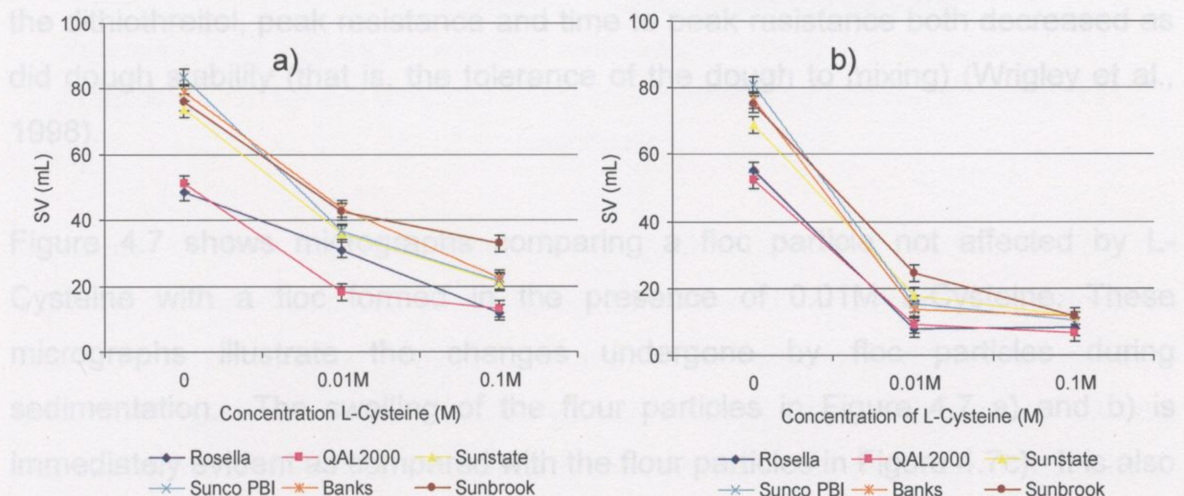

Figure 4.6: Effect on sedimentation volume (SV) of addition of L-Cysteine for a) wheatmeal and b) flour for six varieties varying in quality characteristics

Decreased sedimentation volume was observed at both concentrations of L-Cysteine although, the majority of the decrease observed occurred with the addition of 0.01 M L-Cysteine indicating that samples are sensitive to the presence of the disulphide cleaving agent even at low concentration.

Flour samples exhibited particular sensitivity to the effects of disulphide bond cleavage even at low concentrations perhaps due to the large bran particles in the wholemeal sample providing a level of protection to the flour particles against penetration by the L-Cysteine.

Studies reported by Adayemi and Muller (1983) and Wrigley et al. (1998) also found substantial deterioration to Zeleny sedimentation volume and dough quality respectively. Adayemi and Muller observed decreases in sedimentation volume with addition of disulphide cleaving agents when added to the distilled water in which the sample is hydrated; however, the decrease in sedimentation volume was much less marked when the disulphide cleaving agents were added with the reaction solution.

Wrigley et al. (1998) observed the effect of dithiothreitol, a disulphide cleaving agent, on dough mixing characteristics. It was observed that in the presence of the dithiothreitol, peak resistance and time to peak resistance both decreased as did dough stability (that is, the tolerance of the dough to mixing) (Wrigley et al., 1998).

Figure 4.7 shows micrographs comparing a floc particle not affected by L-Cysteine with a floc formed in the presence of 0.01M L-Cysteine. These micrographs illustrate the changes undergone by floc particles during sedimentation. The swelling of the flour particles in Figure 4.7 a) and b) is immediately evident as compared with the flour particles in Figure 4.7c). It is also evident that the floc particle not exposed to the disulphide cleaving agent (Figure 4.7a)) is significantly smaller and more compact than the particle exposed to the agent. It is likely that the floc particle exposed to the disulphide cleaving agent undergoes significant protein denaturation leading to a less organised protein matrix. The looser structure resulting from the observed protein denaturation

facilitates exposure of a greater surface area to the reaction solution creating larger and heavier particles that settle more rapidly than the smaller tighter particles formed in the solution that does not contain L-Cysteine. The studies of Adayemi and Muller (1983) concur, observing that the fibrous structure of the Zeleny sediment, as viewed by light microscopy, was reduced to an amorphous structure following addition of a disulphide cleaving agents.

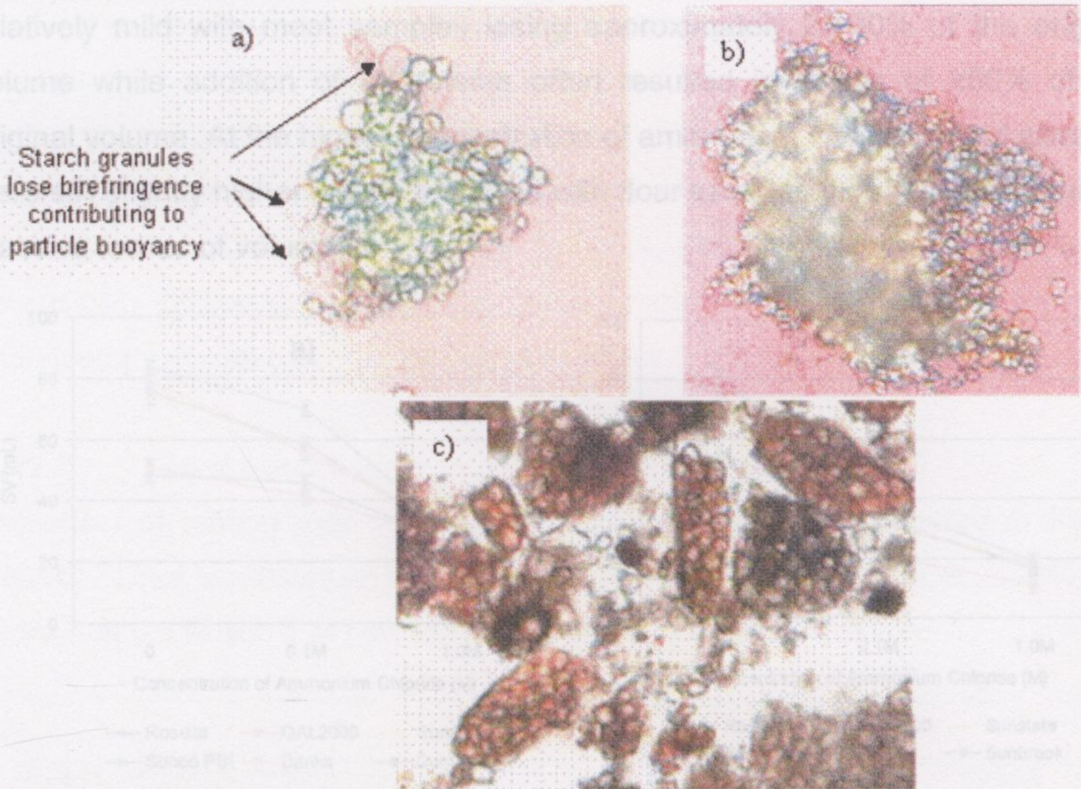

Figure 4.7: Floc particles following SDS sedimentation of flours in a) the absence and b) presence of L-Cysteine as compared with c) unsedimented flour particles, all viewed at x10 magnification

A number of clear starch granules are evident surrounding the floc particle not exposed to the disulphide cleaving agent representing starch granules which have lost birefringence. These granules, which are not present to the same extent in Figure 4.7b) provide the particle with a level of buoyancy thereby increasing the sedimentation volume in the cylinder. The particles affected by L-Cysteine, without the aid of this buoyancy, settle much more rapidly in the cylinders thus contributing to the decreased sedimentation volume. (Lukaszewski and Gustafson, 1987)

4.3.2.2 Ionic bonds

As illustrated in Figure 4.8a)-b), ionic bonding in flour and meal particles is also essential to the sedimentation behaviour of the sample. As in the case of the disulphide bonds, flour samples experience a greater decrease in sedimentation volume at low concentrations of ammonium chloride than do meal samples. The effect of low concentrations of ammonium chloride on wheatmeal samples was relatively mild with most samples losing approximately 20-30% of the original volume while addition of L-Cysteine often resulted in losses of ≥50% of the original volume. At the higher concentration of ammonium chloride, meal samples were still slightly higher when compared with flour samples though both sustained massive losses of volume.

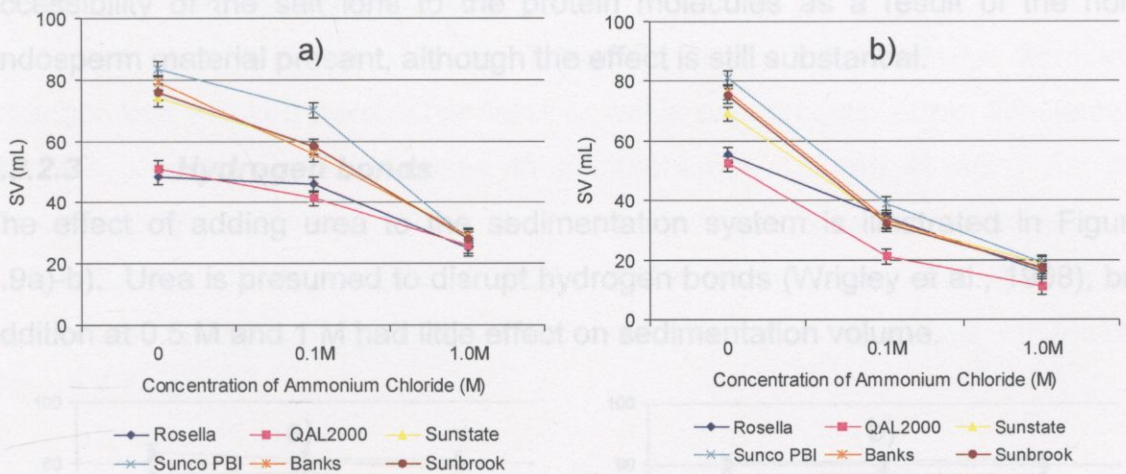

Figure 4.8: Effect on sedimentation volume (SV) of addition of ammonium chloride for a) wheatmeal and b) flour for six varieties varying in quality characteristics

Belitz et al., (1990) also observed that dough quality characteristics were affected at high concentrations of an ionic bond cleaving agent (NaCl) with the resulting dough samples being weak and sticky while at low concentration (< 0.05M) mild increases in peak resistance and stability were observed.

Ionic bonding plays a role in both the tertiary and quaternary structure of native proteins thus it can be concluded that both of these structural characteristics are of importance to the formation and structure of the sediment.

Quaternary structure refers to the clustering of several individual peptide or protein chains into a specific functional shape (Stryer, 1995). Quaternary structure results in the binding of protein subunits as present in the gluten macropolymer. Cleaving the ionic bonds present in the native protein results in the partial breakdown of this structure. Accompanying this structural change would also be an unfolding of the tertiary structure of the native proteins resulting in critical denaturation of the proteins. Thus addition of any ionic salt (i.e. such as ammonium chloride) is likely to result in massive changes to the functional nature of the gluten proteins, preventing swelling and thus reducing sedimentation volume significantly. The reduction in sedimentation volume is less pronounced for wholemeal samples than for flour samples, perhaps as a result of reduced accessibility of the salt ions to the protein molecules as a result of the non-endosperm material present, although the effect is still substantial.

4.3.2.3 Hydrogen bonds

The effect of adding urea to the sedimentation system is illustrated in Figure 4.9a)-b). Urea is presumed to disrupt hydrogen bonds (Wrigley et al., 1998), but addition at 0.5 M and 1 M had little effect on sedimentation volume.

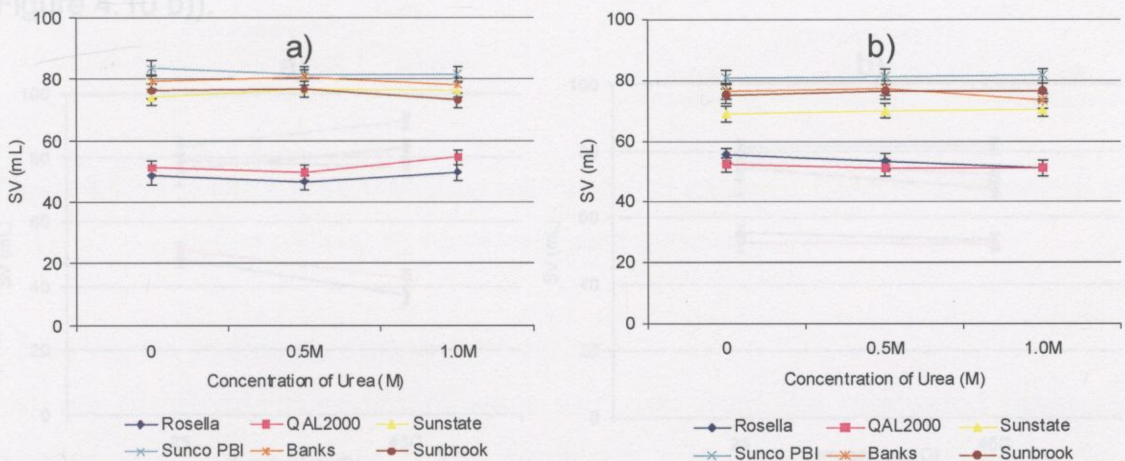

Figure 4.9: Effect on sedimentation volume (SV) of addition of urea for a) wheatmeal and b) flour samples for six varieties varying in quality characteristics

No significant changes in sedimentation volume were observed for both flour and wheatmeal samples and across all samples tested with the possible exception of the wheatmeal sample of the soft variety QAL2000 which experienced an increase in sedimentation volume in the presence of 1M urea. Wrigley et al.

(1998) also observed minimal changes in dough characteristics at 0.5M and 1M concentration of urea with small decreases observed for peak resistance and time to peak resistance, as measured by Mixograph.

Hydrogen bonding plays an important role in protein structure particularly with regard to the secondary structure of the molecules. Hydrogen bonding occurs between two hydrophilic amino acid residues thus contributing to protein folding and thus functionality. Yet disruption of hydrogen bonds had negligible effects on the swelling of the flour particles indicating that hydrogen bonding does not play a major role in the structure of the sediment.

4.3.2.4 *van der Waals forces*

Heat was used to break van der Waals forces in the flour/meal samples. Changes that resulted were either small or within experimental error. Wholemeal samples showed small increases in sedimentation volume at 45ºC for the stronger-dough varieties, while the soft varieties showed decreases in sedimentation volume (Figure 4.10a)). Flour samples on the other hand experienced either no significant differences or minor decreases in volume (Figure 4.10 b)).

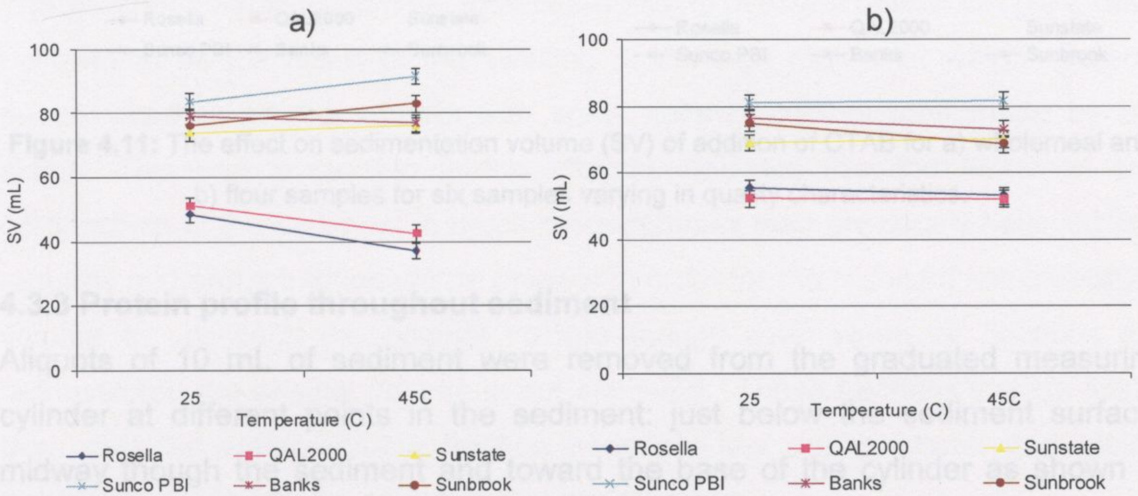

Figure 4.10: Effect on sedimentation volume (SV) of temperature for a) wheatmeal and b) flour samples for six samples varying in quality characteristics

Van der Waals forces are much weaker than the other bond types described above; however, they are more numerous. Nevertheless, van der Waals bonds do not appear to contribute to the swelling associated with the sedimentation test.

4.3.2.5 Lipid-Protein Interactions

As illustrated in Figure 4.10 the lipid-protein interactions present within the meal and flour samples resulted in little change in sedimentation results, the exception being the wheatmeal sample of Banks for which a significant decrease in sedimentation volume was evident. The other hard-grained wheatmeal samples all resulted in minor increases in SV while no significant change was observed for the soft-grained samples.

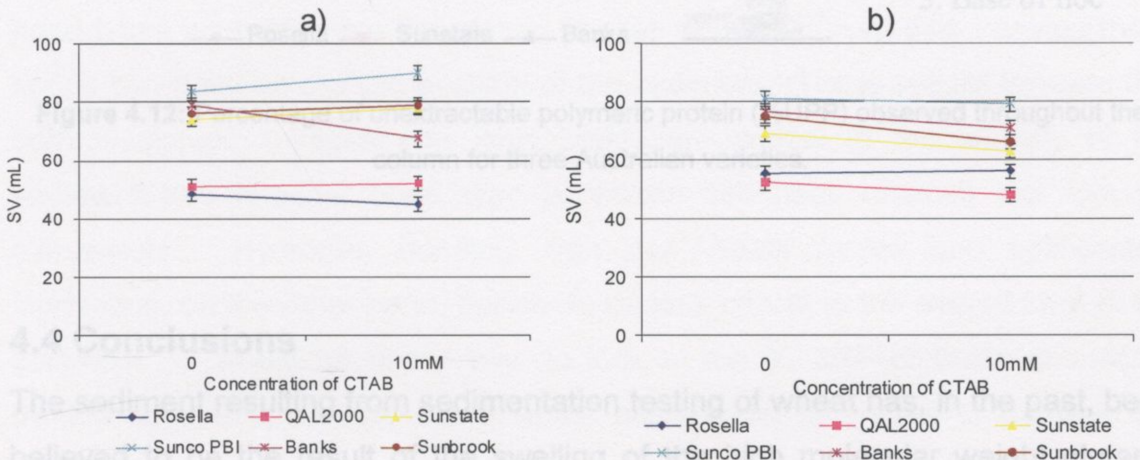

Figure 4.11: The effect on sedimentation volume (SV) of addition of CTAB for a) wholemeal and b) flour samples for six samples varying in quality characteristics.

4.3.3 Protein profile throughout sediment

Aliquots of 10 mL of sediment were removed from the graduated measuring cylinder at different points in the sediment: just below the sediment surface, midway though the sediment and toward the base of the cylinder as shown in Figure 4.12.

The unextractable polymeric protein (UPP) analyses of the dried solid material from these samplings indicate that protein composition is not homogenous throughout the column; however, the pattern of composition change is unclear,

since changes in UPP content differed for the three samples tested (Figure 4.12). Differences were relatively large with the trends indicating that the harder-grained varieties Sunstate and Banks may result in a different protein profile throughout the column than that of the soft-grained Rosella samples. Testing of a greater number of varieties may help to clarify this.

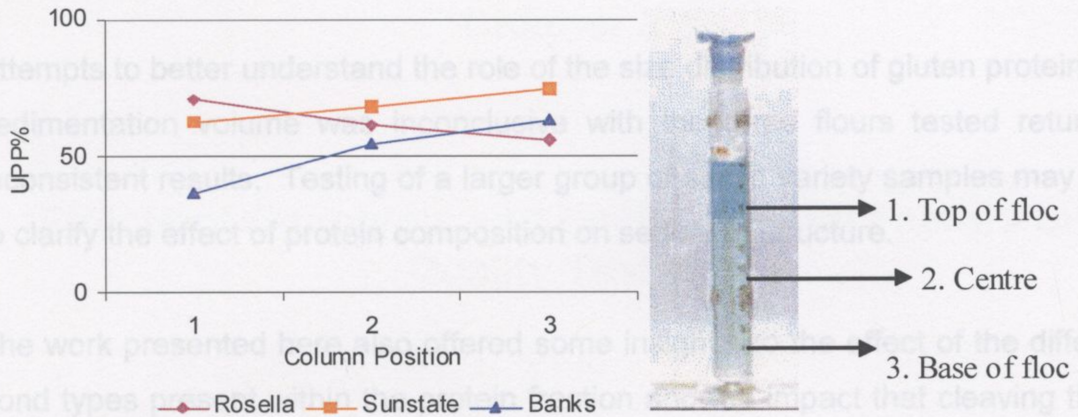

Figure 4.12: Percentage of unextractable polymeric protein (%UPP) observed throughout the column for three Australian varieties.

4.4 Conclusions

The sediment resulting from sedimentation testing of wheat has, in the past, been believed to be the result of the swelling of the high molecular weight glutenin subunit fraction of the sample with small volume contributions made by the starch. The work presented in this chapter indicates that the formation and composition of the sediment is much more complex than previously believed and requires numerous fractions to be present in order for the resultant sedimentation volume to approach the original volume though even when all fractions are reconstituted the original volume is not achieved. These fractions include lipids, bran and the water soluble fraction generally lost during starch and gluten separation as well as the starch and gluten fractions.

All of these fractions contribute to the sediment and decreases in sediment volume are observed, to a greater or lesser degree, in the absence of any one fraction. The gluten fraction is, predictably, the major contributor to swelling volume. However sedimentation volume is significantly higher in the presence of

the water soluble fraction. The lipid and starch fractions make minor but significant contributions to volume while the bran fraction appears to provide bulk and stabilise the sediment structure. The separation procedure involved in the fractionation also appears to affect the sedimentation volume as compared with the whole flour.

Attempts to better understand the role of the size distribution of gluten proteins on sedimentation volume was inconclusive with the three flours tested returning inconsistent results. Testing of a larger group of single variety samples may help to clarify the effect of protein composition on sediment structure.

The work presented here also offered some insight into the effect of the different bond types present within the protein fraction and the impact that cleaving these bonds can have on the composition of the sediment. These results indicate that both disulphide linkages and ionic bonds are critical to the stability of the sediment and if either bond type is broken sediment volumes are reduced extensively. Hydrogen bonding, van der Waals forces and lipid-protein interaction, on the other hand, appear to be less critical to the overall form of the sediment. It would be useful also to look at the % UPP of these chemically treated sediments however care would need to be taken not to damage the column.

Having now looked at the method itself with regard to variability and reproducibility (Chapter 3), as well as sediment composition and formation (this chapter), it is next pertinent to examine the technique with regard to the factors responsible for sample variation. The major causes of sample variability are genetic, environmental or a result of interactions between the two (G x E interactions). Therefore Chapter 5 aims to investigate the effects these factors have on sedimentation test results.

Chapter 5

Effects of Genotypic and Environmental Factors on Quality Testing

5.1 Introduction

As indicated in Chapter 4, the formation of the sediment and thus the sedimentation results are dependent on numerous components of the grain and the complex interactions between these components. Therefore the factors capable of affecting the development of the grain, and therefore the nature of the material, are also of critical importance to the sedimentation behaviour of the sample. For this reason it is important to investigate the interactions between genotypic and environmental factors and their potential effect on sedimentation as these interactions are essential to the understanding of grain quality.

In Australia alone, tens of thousands of new wheat lines are propagated in breeding programs every year. It would be impossible to grow each of these lines on a scale appropriate to large-scale quality testing and therefore the responsibility for selection of lines appropriate to the desired market falls on wheat breeders. Preferably this selection occurs in the early generations of breeding lines.

In order to carry out these selections successfully, breeders must obtain information pertaining to each new line. The first basis for selection is the genotype of the line. The genotype provides breeders with information relating to the presence or absence of a number of genes known to affect the quality of the

resulting grain. For example, HMW-GS composition of the variety is considered as recent studies indicate that the presence of the 5+10 glutenin subunit (present in varieties such as Diamondbird, Hartog and Sunbrook) in the genotype often results in significantly stronger dough properties than the 2+12 subunit (present in varieties such as Rosella, Batavia and Sunsoft '98). The selection of grain on the basis of genotype subsequently leads to better breadmaking performance (Gianibelli et al., 2001). Other genotypic characteristics exploited by wheat breeders include yield, protein content, abiotic stress resistance and protein quality kernel hardness and disease resistance (Gedye et al., 2005).

Genotypic variation, as observed for most organisms including wheat, is a result of the segregation of multiple quantitative trait loci (QTL). Each of these QTL explains a percentage of the variation experienced for the particular phenotypic characteristic (e.g., variation in kernel hardness and protein composition). QTL have proven a useful tool for identification of genes or genomic regions responsible for aspects of many attributes.

The second critical consideration for wheat breeders is the response of the grain to the environmental conditions likely to be experienced both throughout cultivation and following harvest. Environment is of critical importance to grain quality in general with certain quality attributes being more heavily dependent upon environmental conditions than others (Shah and Paulsen, 2003). Figure 5.1 indicates the relative dependence of some quality attributes on both genotype and environment.

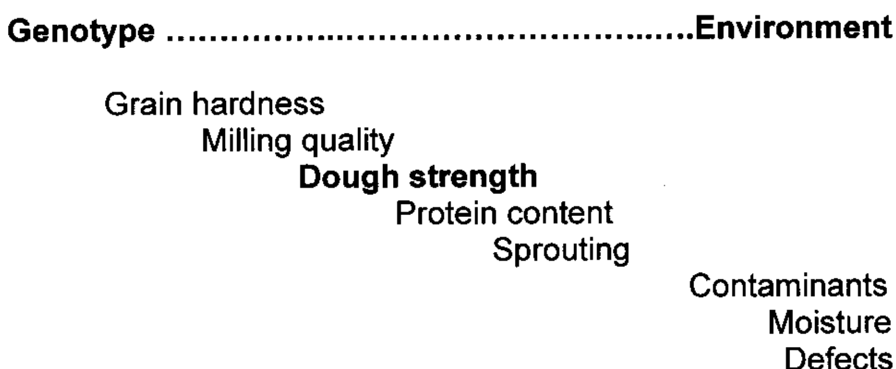

Figure 5.1: Grain-quality attributes, listed in position from left to right, according to the relative influences of genotype and growth environment on each attribute (Wrigley and Batey, 2003).

From the time grain is sown (and potentially before), environmental factors shape the growth pattern and final characteristics of the grain. These environmental conditions include the nitrogen and sulphur content of the soil, growth temperatures, rainfall (throughout the growth of the plant and particularly around harvest), and even atmospheric carbon dioxide levels.

Consideration of the manner by which these genotypic (G) and environmental (E) factors interact with one another is an important part of the modern wheat industry. These interactions are referred to as G x E interactions (Panozzo and Eagles, 2000).

The significance of G x E interactions is apparent when considering global warming and the subsequent increase in atmospheric carbon dioxide levels. Elevated CO_2 levels result in an increase in temperature as well as a decline in soil (and thus plant) nitrogen levels (Kimball et al., 2001). Reduced soil nitrogen levels in turn lead to a reduction in the protein content of the grain (Groos et al, 2003) and can also impact on the glutenin-to-gliadin ratio present in the grain endosperm (Tea et al., 2004). The decreased protein content and altered protein composition impacts substantially on the dough quality and the end-use potential of the resulting flour.

The occurrence of greater than three days of temperatures exceeding 35°C following anthesis (defined as 'heat stress') can reduce dough strength (Blumenthal et al., 1995a) and subsequently affect the baking quality of the resulting flour (Shah and Paulsen, 2003). Heat stress can cause a number of other detrimental effects on grain quality including decreased kernel size, (due to a reduction in the rate and duration of grain filling), kernel abortion and interruption of starch synthesis, causing the grain to appear mottled and opaque (Shah and Paulsen, 2003; Zahedi and Jenner, 2003). Certain cultivars exhibit a level of tolerance to heat shock while other varieties appear more susceptible, resulting in a more severe impact on grain quality (Blumenthal et al., 1995a; Hunt et al., 1991; Rawson, 1986).

Similarly, drought conditions have a significant effect on the quality of wheat grain by reducing leaf expansion and conductance by the stomata. This occurs due to breakdown of the photosynthetic pathway as a result of water deprivation (Passioura, 1994). Too much rainfall, particularly late in the growing season can also influence grain quality causing a reduction in total protein content and kernel bleaching as well as stimulating germination of the seed causing sprout damage capable of severely affecting baking quality.

Environmental conditions are not just an important consideration during plant growth but also following the harvest of the grain. Long-term storage of grain can cause significant changes in grain quality as determined by dough and baking tests, depending on the specific storage conditions (Gras and O'Riordan, 1994).

This chapter examines how both genetic and environmental conditions as well as the G x E interactions impact on the resulting quality of the grain, particularly the quality of the wheat proteins as predicted by SDS and Zeleny sedimentation testing.

5.2 Methods and Materials

5.2.1 Effects of HMW-GS alleles in a biscuit wheat background

A multi-null sample set (Table 5.1) based on the soft-wheat variety Tincurrin was provided by the Plant Breeding Institute, University of Sydney. These lines were obtained by crossing Tincurrin with a Gabo-Olympic set of multi-null lines from Lawrence et al. (1987). The HMW-GS identities of the samples were verified by SDS-PAGE, carried out at the SARDI Grain Quality Research Laboratory, Adelaide, and a sub-set of lines were chosen, based on these results. Genotypic data for this subset is summarised in Table 5.1. Small amounts of each sample were ground to wholemeal using a Perten 3100 Falling Number mill and the remaining grain samples (1-2kg) were milled to flour using a laboratory-scale Buhler test mill (Section 2.2.5.2).

NIR protein and SDS sedimentation testing were performed on the wholemeal samples as detailed in Sections 2.3.1.1 and 2.8.1 respectively. The flour samples underwent the following tests: Leco protein content (Section 2.3.1.2), large and small scale Farinograph testing (Sections 2.3.2.1 and 2.3.2.2 respectively), Extensograph (Section 2.3.2.3), Keiffer rig (Section 2.3.2.4) and dough stickiness (Section 2.3.2.5) using a TAXT2 texture analyser, Zeleny sedimentation (Section 2.8.2) and SE-HPLC (Section 2.6).

Table 5.1: Genotypic data and protein content for multi-null samples

HMW-GS present	HMW-GS Abbreviation	No of samples used	Mean flour protein content (%)
All 3 genomes	+ + +	2	6.7
B & D genomes	- + +	2	7.5
A & B genomes	+ + -	4	8.4
B genome	- + -	3	7.7

5.2.2 Identification of quantitative trait loci (QTL) for sedimentation and protein quality value

QTL analyses were carried out by Adele Schmidt of CSIRO Plant Industry, St Lucia, Queensland. Seed from 180 Janz-Kukri doubled-haploid lines was obtained and phenotypic data was generated over two growing seasons (2001, 2002) at two replicate sites (Biloela and Lundavra) by the Queensland Department of Primary Industries, Toowoomba (Schmidt et al., 2005).

DNA samples were extracted from samples using the method of Schmidt et al. (2004) and diluted to ~25ng/μl. Line genotyping was carried out using recently developed microsatellite markers (Gupta et al., 2002; Leigh et al., 2003; Petsova et al., 2000; Roder et al., 1998; Song et al., 2002; Varshney et al., 2000); AFLP markers (Life Technologies/Gibco BRL); PCR-markers specific for HMW-GS and LMW-GS alleles (Ahmad, 2000; Butow et al., 2003); and PCR markers specific for the Rht1 and Rht2 genes (Ellis et al., 2002).

These marker groups were individually amplified and electrophoretically visualised as per Schmidt et al. (2005). The data was then amassed into a genetic map using *MapManager QTXb20* software (Manly and Cudmore, 1996). The phenotypic data from the 30 lines tested for PQV (PQV = sedimentation volume/protein content (%)) and the genetic map were then imported into *QTL Cartographer for Windows Version 2.0* and QTL were detected using multiple interval mapping

5.2.3 Effects of heat-shock versus genotype

Flour samples of 36 wheat genotypes were obtained from a previous research project (Blumenthal et al., 1995a). The samples represented commercially grown Australian genotypes, as well as international varieties reputed to display either tolerance or resistance to heat stress conditions during grain filling. The samples were grown in the phytotron at the CSIRO, Division of Plant Industry, Canberra. Each cultivar was grown in duplicate under control conditions (18°C/13°C, day/night) until 29 days after anthesis. At this point half of the plants of each variety were removed and subjected to heat-stress conditions of 40°C during each of three consecutive days, with 25°C as the night temperature (Blumenthal et al., 1995a) while the other half of the plants remained under the original regime. Pots were regularly rotated to eliminate differences due to position and grain was harvested 60 days after anthesis. Each grain sample was milled to flour using a Quadrumat Junior mill as described in Section 2.2.5.1. The flour samples were stored at -20°C prior to use.

Protein and moisture content were determined for each flour sample using NIR spectroscopy (Section 2.3.1.1) and flour sedimentation tests were carried out using 5.000 ± 0.005g sub-samples and 1.5% SDS - 2% lactic acid as described in Section 3.3.2.1.

5.2.4 Effects of climate conditions in the six weeks prior to harvest

Following the 2002-2003 harvest, 72 samples from northern New South Wales and southern Queensland were obtained. Samples were obtained as both flour

and grain. Grain samples were ground on a Perten 3100 falling number mill and SDS sedimentation testing carried out as detailed in Section 2.8.1 (wholemeal). Flour samples were test milled at Allied Mills Australia, Toowoomba using a Bühler Laboratory mill (Section 2.2.5.2) and were tested using Zeleny sedimentation (Section 2.8.2). Climate data for the growing area was retrieved from the Bureau of Meteorology for the six-week period preceding harvest.

5.2.5 Effects of long term storage conditions on a number of varieties

Eight single-variety samples were selected; Rosella, QAL2000, Sunco, SuncoPBI (see below) Sunstate, Lang, Banks and Sunbrook. Two different Sunco samples were used in this work with one sample being commercially grown and characteristic of Sunco, while the other (grown at the Plant Breeding Institute, Narrabri) displayed uncharacteristically high protein content. This sample is hereafter referred to as SuncoPBI.

Samples were divided equally into three sub-samples, one of which was milled to flour using a Buhler Laboratory Mill, while a second was ground to wheatmeal using a Perten falling number mill (setting 1, 0.8mm screen) and the third sub-sample remained as whole grain. Each sub-sample of each variety was then sub-divided again into 3 smaller samples to represent the three storage conditions (4°C, ambient temperature and 40°C). Lastly each of the three samples for each variety was divided between 14 individual plastic screwtop containers. These represented each sample in replicate to be tested after being stored for 0, 2, 4, 6, 8, 10 and 12 months (Figure 5.2). Flour samples were not available for the QAL2000 sample.

In order to minimise the effect of the increased carbon dioxide levels due to CO_2 release by the grain, containers were selected to have a large headspace (approximately 6:1 ratio of air to sample). Screw top lids were used to limit effects due to humidity.

Samples were stored in darkness for a period of twelve months as grain, meal and flour and at three treatment conditions: under refrigeration at 4 ± 1.5°C, in a storage cupboard at ambient temperature (22 ± 6.2°C) and in an incubation oven

at 40 ± 4.4°C. Every two months two replicate samples of grain, meal and flour for each variety and temperature were removed, NIR protein and moisture content assessed and sedimentation testing carried out.

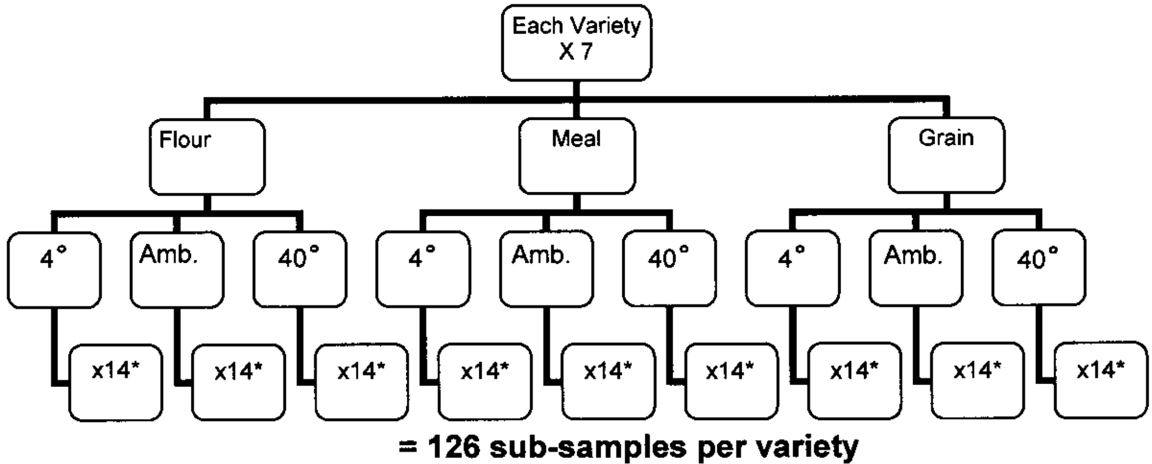

Figure 5.2: Division of single variety samples for long term storage project

* ~50g duplicate samples of each variety to be tested at 0, 2, 4, 6, 8, 10 and 12 months

5.2.6 Effect of sprouting on varieties differing in end-product use

Three varieties were selected on the basis of poor, medium and strong SDS sedimentation results. These varieties were Rosella, Sunstate and Sunco respectively. Each of the single-variety grain samples was halved using a sample splitter and one of each of these sub-samples was ground on a falling number mill and falling number were determined as outlined in Section 2.4. The second set of sub-samples were artificially sprouted by soaking in water for one hour before being drained and patted dry. These samples were then spread out on damp paper towels in a shallow tray, covered with plastic wrap and left at 25°C for 24 hours. Following this, both the plastic wrap and paper towels were removed and the samples dried for 24 hours at 35°C. Falling number evaluations were then carried out on these sprouted samples.

The falling number values of both the sound and sprouted grain were then used in conjunction with the scale of Perten Liquefaction Number (PLN) where PLN = 6000 / (falling number – 50) to calculate the proportion of sprouted grain required for blending with sound grain to achieve falling number values of 100 and 200

seconds. The meals were then blended in the appropriate ratios and again tested for falling number and sedimentation volume.

5.3 Results and Discussion

All data discussed in Chapter 5 is presented in full in Appendix D.

5.3.1 Effects of HMW-GS alleles in a biscuit wheat background

Dough quality for the multi-null flours (lacking specific HMW-GS) was examined using the Extensograph (Figures 5.3 and 5.4) and Farinograph (10g). The most significant results were observed for the Extensograph although, due to the extreme softness of the doughs, individual dough pieces required hand rolling as opposed to the mechanical moulding and shaping generally used for this and thus the reproducibility was affected. As certain flour samples were relatively small (<l kg) and thus large-scale extensibility testing could not be repeated, further extensibility results were obtained using a 10g Farinograph and a TAXT-2 texture analyser with a Keiffer Rig attachment. This procedure proved significantly simpler than the Extensograph due to the smaller dough pieces and the tools provided to avoid contact with the very sticky doughs. A strong correlation was observed between the two extensibility techniques ($r = 0.76$, $P < 0.01$) and correlations with sedimentation data showed similar trends, though at reduced magnitude.

It was observed that the dough strength (resistance to extension) decreased significantly for all null lines when compared with the lines expressing all three HMW-GS alleles. However, lines exhibiting two null alleles (double nulls), on average, did not result in the weakest doughs observed (Figure 5.3) as those with ++- configuration were in fact weaker as described by Farinograph results. This trend may be a result of an increase in total protein content observed when two of the alleles were made null (Table 5.2).

Dough extensibility decreased with deletion of HMW-GS alleles. Deletion of the allele in the A genome resulted in the smallest loss of extensibility, deletion of the

allele in the D genome resulted in higher decreases in sedimentation. The most substantial decrease in extensibility was observed in the double-null line. These results are consistent with those observed by Lawrence et al. (1987) who also found that the most substantial decreases in grain quality occurred whn the allele in the D genome was made null while making the allele in the A genome null resulted in less substantial decreases in grain quality.

Table 5.2: Correlation coefficients demonstrating the relationship between sedimentation results (sedimentation volume, (SV)), % of unextractable polymeric protein (%UPP) and dough properties (resistance to extension (Rmax), extensibility (Ext.), water absorption (WA), dough development time (DDT)).

Attribute	Extensograph			Keiffer Rig		Farinograph		
	Rmax (BU)	Ext. (cm)	Ext./ Prot.	Rmax (BU)	Ext. (cm)	WA	DDT	Stability
SDS SV (meal)	0.33	0.63*	0.30	0.32	0.38	0.25	0.36	0.19
Zeleny SV (flour)	0.79***	0.98***	0.83***	0.52*	0.54**	-0.13	0.09	-0.33
%UPP	0.49	0.89***	0.75**	0.29	0.35	0.05	0.43	0.21

*** P < 0.05, ** P < 0.01, *** P < 0.001**

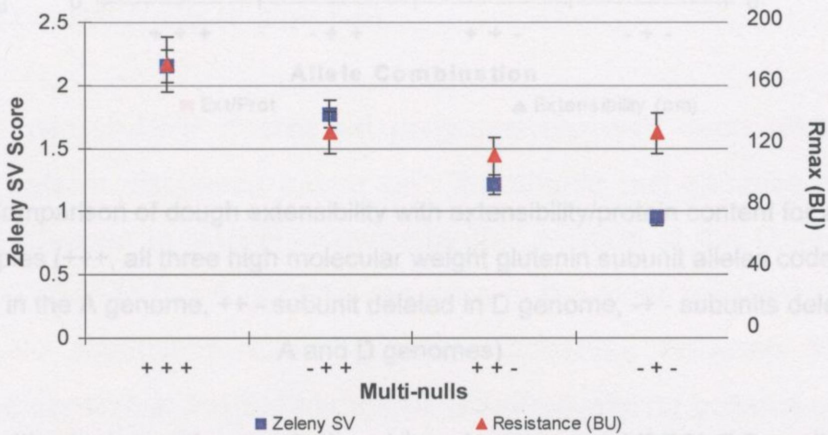

Figure 5.3: Comparison of Zeleny sedimentation results and dough resistance to extension for a range of multi-null samples (+++, all three high molecular weight glutenin subunit alleles coded for, -++, subunit deleted in the A genome, ++ - subunit deleted in D genome, -+- subunits deleted at both A and D genomes).

Figure 5.4 illustrates the relationship between extensibility and protein content by comparison of the extensibility values with extensibility divided by protein content (Ext/P). A positive correlation exists between these two values.

The Zeleny sedimentation test also ranked the multi-null deletions in the order mentioned above (Figure 5.3) with deletion of alleles at both the A and D genomes resulting in the greatest decrease in sedimentation volume followed by deletion of the allele in the D genome only. Deletion of the allele in the A genome resulted in the smallest decrease in sedimentation volume. This result may be influenced by the fact that the A genome is responsible for expressing only one HMW-GS while the D genome is responsible for the expression of 2 HMW-GS. Further, although only a small number of samples were available, the Zeleny sedimentation results provided strong correlations with both extensibility (r = 0.98 and 0.83, p<0.001) and dough strength (r = 0.79, p<0.001) (Table 5.2).

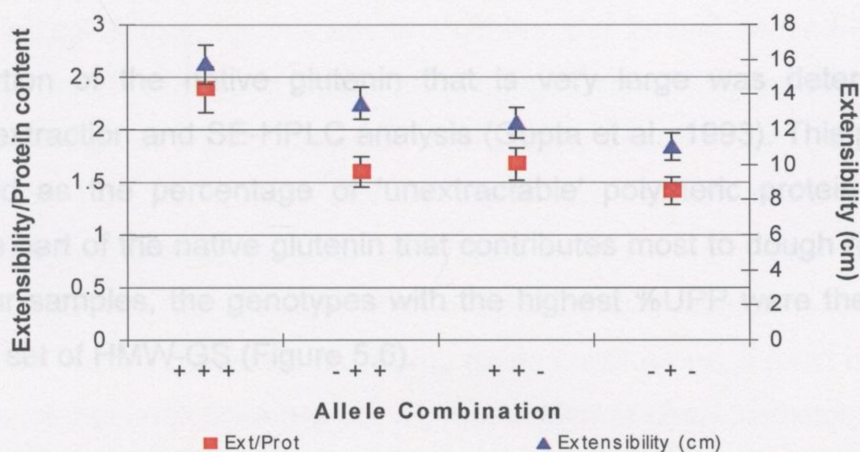

Figure 5.4: Comparison of dough extensibility with extensibility/protein content for a range of multi-null samples (+++, all three high molecular weight glutenin subunit alleles coded for, -++, subunit deleted in the A genome, ++ - subunit deleted in D genome, -+ - subunits deleted at both A and D genomes)

Figure 5.5 illustrates the relationship between HMW-GS alleles and sedimentation results (as both Zeleny and SDS protein quality values (PQV)). Again, those samples with allele deletions at both A and D genomes experienced the most significant decreases in PQV for both sedimentation test types. Figure 5.5 also indicates that SDS sedimentation ranks samples slightly differently to

Zeleny sedimentation with deletion of the allele in the A genome resulting in a higher PQV than samples with all HMW-GS alleles coded for. This is likely to be a result of the different composition of the Zeleny solution.

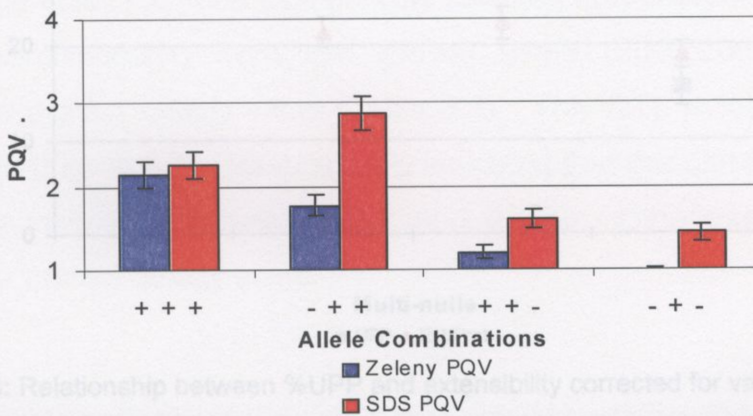

Figure 5.5: Effects of allele combinations on SDS and Zeleny sedimentation test results for a range of multi-null samples (+++, all three high molecular weight glutenin subunit alleles coded for, -++, subunit deleted in the A genome, ++ - subunit deleted in D genome, -+- subunits deleted at both A and D genomes)

The proportion of the native glutenin that is very large was determined by sequential extraction and SE-HPLC analysis (Gupta et al., 1993). This proportion is measured as the percentage of 'unextractable' polymeric protein (%UPP), which is the part of the native glutenin that contributes most to dough resistance. For the flour samples, the genotypes with the highest %UPP were the samples with the full set of HMW-GS (Figure 5.6).

For the null lines, %UPP decreased progressively with each allele deletion following the pattern discussed above with the double null samples resulting in the highest decreases in %UPP followed by the deletion at the D genome with deletion of the allele in the A genome resulting in the least substantial decrease in %UPP. No significant relationship was observed between %UPP and resistance to extension in the Extensograph although, strong positive correlations (r = 0.89 and r = 0.75, P<0.01) were obtained for %UPP with dough extensibility and extensibility/protein content, respectively (Figure 5.6, Table 5.2).

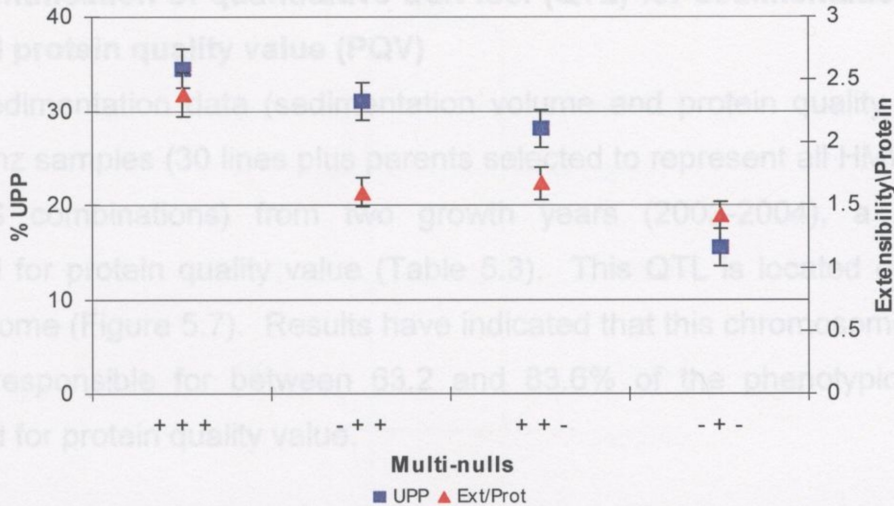

Figure 5.6: Relationship between %UPP and extensibility corrected for variations in protein content for a range of multi-null samples (+++, all three high molecular weight glutenin subunit alleles coded for, -++, subunit deleted in the A genome, ++ - subunit deleted in D genome, -+ - subunits deleted at both A and D genomes).

This study indicates that, for this set of samples, the Zeleny sedimentation and %UPP tests proved to be good indicators of dough extensibility for the genotypes tested, while dough strength correlated with Zeleny sedimentation only. These results indicate that the Zeleny sedimentation test may be more capable of predicting dough characteristics for soft flours or those samples experiencing some level of genetic manipulation, than the SDS sedimentation test commonly used for bread flours. However, in drawing these conclusions, it must be realised that the sets of multi-null lines are not representative of characteristics for typical commercial wheat samples.

The loss of sedimentation volume due to loss of HMW-GS indicates the importance of this aspect of glutenin composition in contributing to the sediment volume. Furthermore, specific HMW subunits (e.g., of the D genome) are more important than others (e.g., of the A genome) in contributing to the sedimentation volume.

5.3.2 Identification of quantitative trait loci (QTL) for sedimentation volume (SV) and protein quality value (PQV)

Using sedimentation data (sedimentation volume and protein quality value) on Kukri-Janz samples (30 lines plus parents selected to represent all HMW-GS and LMW-GS combinations) from two growth years (2003-2004), a QTL was identified for protein quality value (Table 5.3). This QTL is located on the 3BL chromosome (Figure 5.7). Results have indicated that this chromosome contains a QTL responsible for between 63.2 and 83.6% of the phenotypic variation observed for protein quality value.

Table 5.3: QTL for Sedimentation and Protein Quality Value Detected via Multiple Interval Mapping

Dataset	Chromosome	QTL Position (cM)	Marker	LRS[a]	%[b]
PQV 2003	1B	11.9229	Glu-B1	12.5693	10.6
	1D.1	0.1001	Glu-D1	13.3509	2.2
	3BL	24.6398	gwm247	37.4026	63.2
	gwm533 (chr)	24.0001	gwm533.1	14.435	12.1
		1B x 3BL	AA	6.6206	0.9
		1D.1 x gwm533	AA	5.8296	4
PQV2004	3BL	23.6398	gwm247	30.8943	83.6
	6A	25.5873	ACT/CTC.2	2.0035	5.1

SDS: $\sigma^2 = 0.94$, PQV: $\sigma^2 = 0.97$
[a] likelihood ration score, [b] phenotypic variation due to QTL

This QTL is associated with the gwm247 marker observed at 26.2cM on the long arm of chromosome 3B at the telomere region, and may potentially provide breeders with a useful tool for selection of grain lines on the basis of sedimentation results.

Chromosomes 3A and 3B have been shown to have the largest impact on overall loaf volume as well as significant positive correlations with both gluten viscosity and gluten elasticity moduli, farinograph stability and Spiral crumb score (Law et al., 2005).

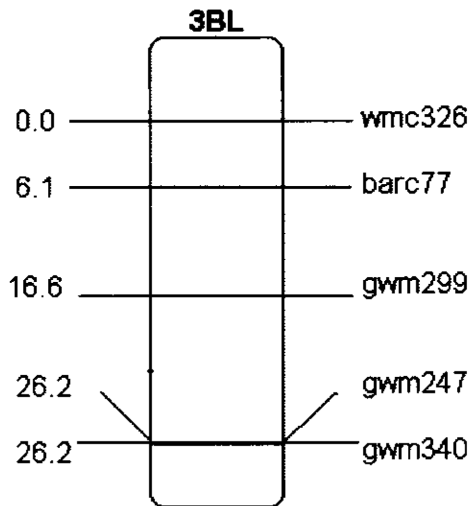

Figure 5.7: Positions of molecular markers on chromosome 3BL

This finding concurs with the finding in Section 4.3.1 that non-gluten components of wheat samples play a significant part in the volume of the sediment, as the 3B chromosome is not responsible for gluten protein expression. This indicates that the QTL for PQV discovered here is likely to be a result of other phenotypic factors, for example, the water soluble proteins discussed in Chapter 4.

5.3.3 Effects of heat-shock versus genotype

The results of this study indicated that both protein content and sedimentation volumes of heat-shocked samples were higher than those of the corresponding control samples. These results are not entirely consistent with those of Stone et al (1997), who reported a decrease in sedimentation values following heat shock. That study however, utilised a micro-sedimentation test (AACC 2002; Method 56-63), which does not include SDS. Presumably the AACC method is less sensitive to the contribution of protein content, which over-rides the loss of protein quality. When the protein-quantity effect was removed by calculating protein quality values, it was evident that many of the genotypes suffered loss of sedimentation volume potential due to heat stress.

Although heat shock had a detrimental effect on the protein quality values of all genotypes tested, the severity of this effect differed markedly between the genotypes. This can be seen with the relatively small difference between control

and heat shock exhibited with the more tolerant varieties (e.g. Halberd) while the more susceptible varieties (e.g. Meering) have a significantly larger difference between control and heat shock protein quality value (results not shown). These findings are in accordance with the findings of Hunt et al. (1991) however the ranking of the genotypes with respect to susceptibility to heat stress differed from the original results of Blumenthal et al. (1995a), which were based on time to peak resistance in the Mixograph.

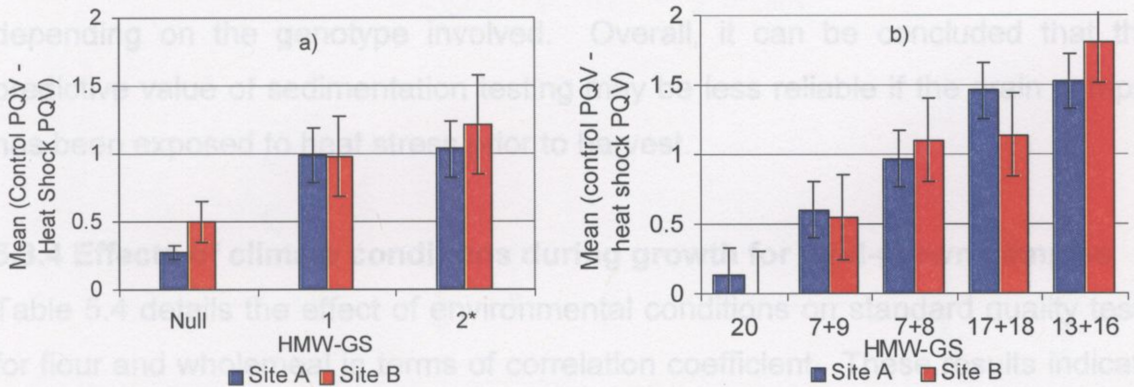

Figure 5.8: Mean results for control PQV – heat shock PQV for HMW-GS at the a) *Glu-A1* and b) *Glu-B1* loci

The high-molecular-weight glutenins (HMW-GS) make an important contribution to dough strength, so it is relevant to see if subunit composition relates to the apparent susceptibility to heat stress, as indicated by PQV. This study indicates that these subunits, in particular, may play an important role in the resulting protein quality of the wheat.

The HMW-GS coded for at the *Glu-B1* locus had the most significant effect on the PQV result of the sedimentation test (Figure 5.8b)) with subunits 20 and 13+16 conferring the greatest levels of tolerance and susceptibility, respectively. Subunits coded for at the *Glu-A1* locus also appeared to contribute to the intervarietal variations observed (Figure 5.8a), while subunits coded for at the *Glu-D1* allele accounted for virtually none of the variation in PQV results (results not shown). These results contrast with previous studies (applied to these genotypes, but using a different means of assessing quality, namely time to peak in the Mixograph). These previous studies indicated that the most significant

impact on dough characteristics, as assessed by Mixograph results, was due to the subunits coded for at the *Glu-D1* locus (Blumenthal et al., 1995b). The difference in results between these studies is likely to be a result of the PQV test measuring baking quality directly while Mixograph results indicate dough quality.

The extension of these studies to include sedimentation testing indicates that heat stress during grain filling may alter the usual relationship between grain quality and sedimentation results, and that this relationship may be different depending on the genotype involved. Overall, it can be concluded that the predictive value of sedimentation testing may be less reliable if the grain sample has been exposed to heat stress prior to harvest.

5.3.4 Effects of climate conditions during growth for field-grown samples

Table 5.4 details the effect of environmental conditions on standard quality tests for flour and wholemeal in terms of correlation coefficient. These results indicate that, while a larger samples set is required for conclusive trends to be observed, a positive correlation between quality test results and rainfall (mm) exists while an increase in the number of days exceeding 35°C was accompanied by a decrease in quality.

Rainfall correlated positively with protein and moisture content as determined by NIR, SDS sedimentation volume, Zeleny sedimentation volume, dough stability and resistance to extension. Conversely, dough stability was negatively impacted by an increase in the number of days exceeding 35° C, as were Zeleny sedimentation volume and PQV. The strongest of these correlations were observed for Zeleny sedimentation of flour. These results indicate that Zeleny sedimentation (both as SV and protein quality value (PQV)) is strongly affected by the environmental conditions to which the grain is exposed prior to harvest.

Table 5.4: Correlations of quality test results including sedimentation volume (SV) and protein quality value (PQV) for both meal and flour, with number of days over 35°C and rainfall in the six weeks prior to harvest.

	% Meal Protein	Meal SV	% Flour Protein	Flour SV	Flour PQV	Dough Stability (min.)	Rmax 45min.
Days >35°C	0.08	-0.05	-0.04	-0.45***	-0.30**	-0.31**	-0.24*
Rainfall (mm)	0.24*	0.26*	0.26*	0.32*	0.17	0.31**	0.26*

P < 0.05, ** P < 0.01, *** P < 0.001

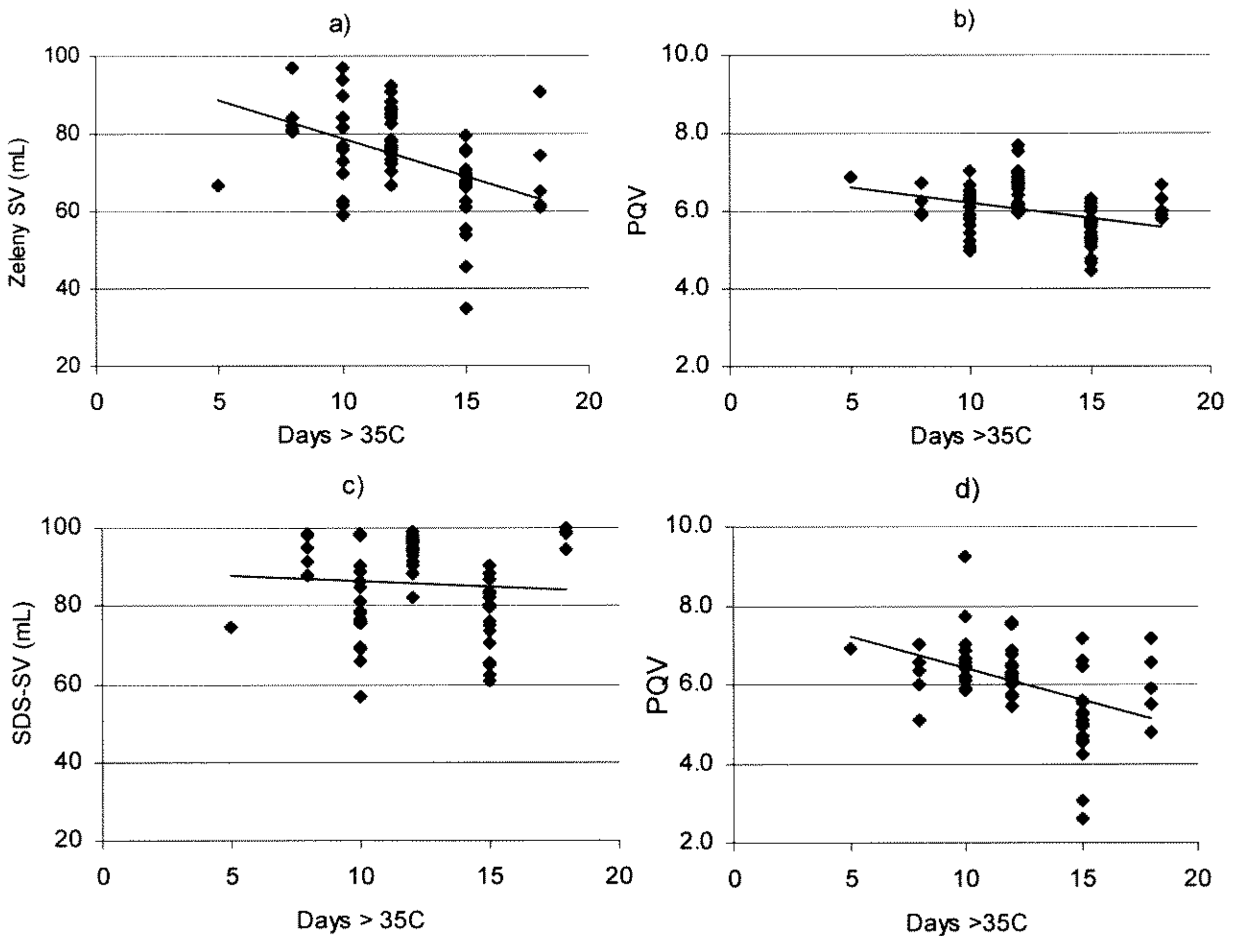

Figure 5.9: Effect of heat stress on a) Zeleny sedimentation volume (SV), b) Zeleny protein quality value (PQV), c) sodium dodecyl sulfate (SDS) SV and d) SDS PQV

Figure 5.9 illustrates the strong negative correlations between heat stress and protein quality value for both Zeleny and SDS sedimentation. There is however, no significant relationship between the number of days exceeding 35°C and SDS

sedimentation volume indicating that the increase in protein content of wholemeal samples observed during heat stress conditions may influence sedimentation results unexpectedly. Graybosch et al. (1995) described a curvilinear response for a version of the Zeleny sedimentation test which employed a smaller sample size. The influential effect of the increased protein content can be minimised by utilising a normalising methodology such as PQV.

The finding that protein quality is susceptible to heat stress is as expected and concurs with the findings of numerous reports (Blumenthal et al., 1993) (Blumenthal et al., 1995a). However results did differ somewhat from those of Graybosch et al. (1995), as mentioned above, whose results indicated that sedimentation volumes increased slightly under mild heat stress conditions (i.e. exposure to <90 hr of temperatures in excess of 32°C) before decreasing rapidly at high stress conditions (i.e. exposure to >90 hr of temperatures in excess of 32°C). Studies by Graybosch et al. however, differed from the current study in a number of ways. Firstly, fewer samples were selected and these samples were grown in small scale in as opposed to the commercial samples used here. Further, sedimentation was carried out only on the flour from these samples and using 2 g of sample. Samples were also not tested in duplicate. These conditions limit the level to which small differences in quality can be measured by significantly reducing the range of sedimentation results achievable (range = ~17 mL (Graybosch et al., 1995) versus ~ 62 mL in this study). Further, it is unclear what concentration of SDS was employed for the sedimentation testing thus making comparison of the results difficult.

The result that protein quality, as determined by SDS sedimentation testing, decreases following heat stress concurs with the results of Blumenthal et al (1993) who found similar trends when investigating quality as determined by dough properties.

5.3.5 Effects of long term storage conditions on a number of varieties

The effect on sedimentation behaviour of long term storage of grain, wheatmeal and flour samples is illustrated in Figures 5.10, 5.11 and 5.12 respectively. Storage of samples for a 12 month period had a significant detrimental impact on

almost all varieties tested and under all storage conditions. In the case of samples stored as grain (Figure 5.10), storage at 40ºC resulted in the greatest decreases in sedimentation volume. When stored at 4ºC however, five of the seven varieties tested experienced a higher or unchanged level of deterioration than when stored at ambient temperature. This trend was not as pronounced for the samples stored as wheatmeal (Figure 5.11) with wheatmeal samples, excepting QAL2000 and Sunstate, experiencing greater deterioration when stored under ambient conditions than when stored at 4ºC.

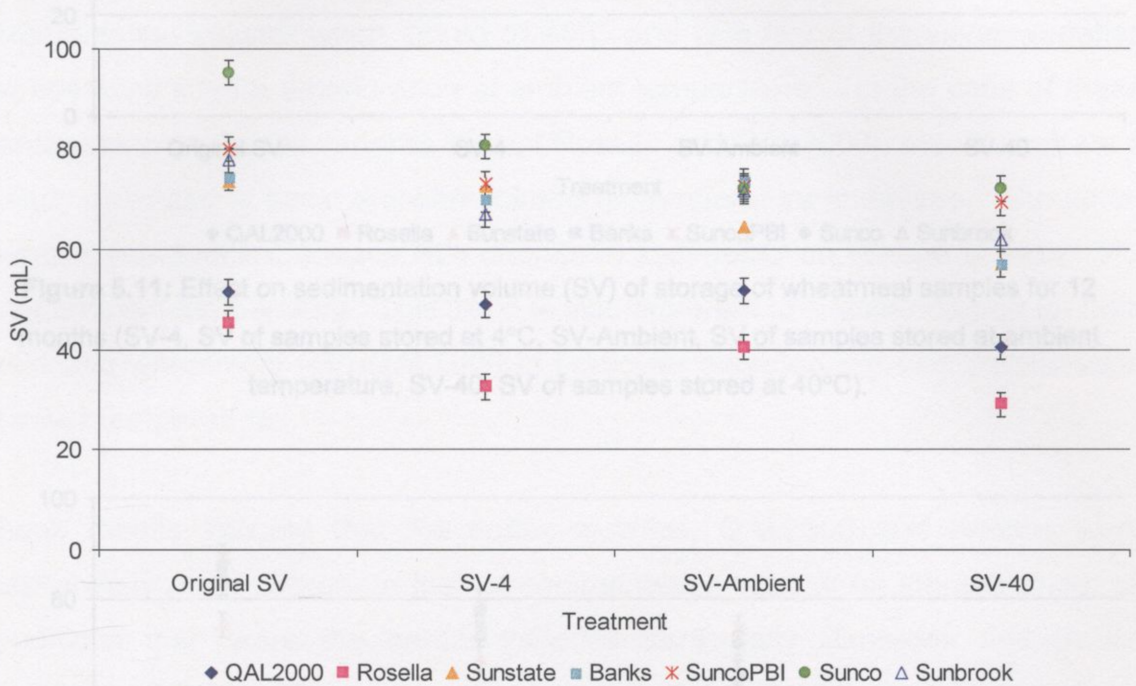

Figure 5.10: Effects on sedimentation volume (SV) of storage of grain samples for 12 months (SV-4, SV of samples stored at 4°C, SV-Ambient, SV of samples stored at ambient temperature, SV-40, SV of samples stored at 40°C).

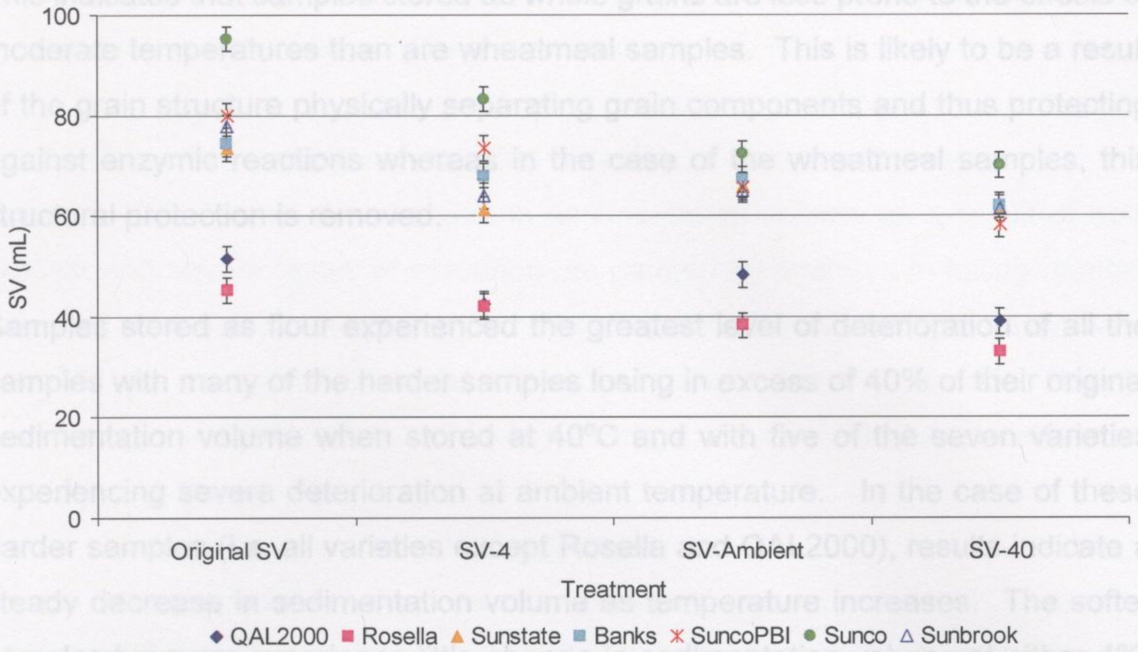

Figure 5.11: Effect on sedimentation volume (SV) of storage of wheatmeal samples for 12 months (SV-4, SV of samples stored at 4°C, SV-Ambient, SV of samples stored at ambient temperature, SV-40, SV of samples stored at 40°C).

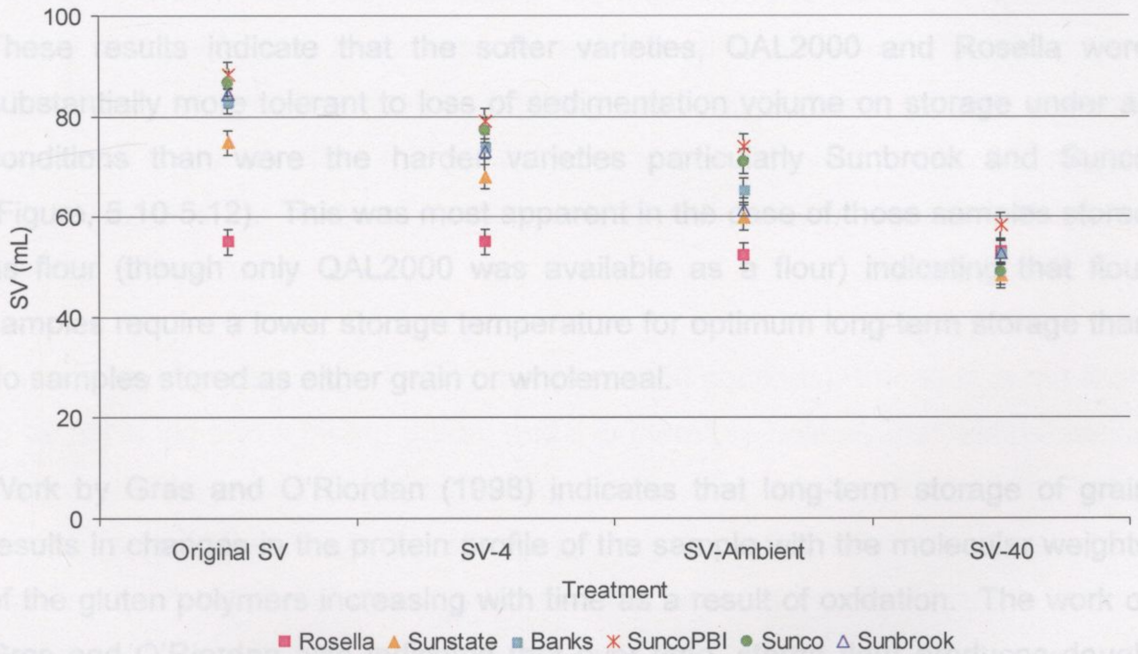

Figure 5.12: Effect of sedimentation volume (SV) of storage of flour samples for 12 months (SV-4, SV of samples stored at 4°C, SV-Ambient, SV of samples stored at ambient temperature, SV-40, SV of samples stored at 40°C).

This indicates that samples stored as whole grains are less prone to the effects of moderate temperatures than are wheatmeal samples. This is likely to be a result of the grain structure physically separating grain components and thus protecting against enzymic reactions whereas in the case of the wheatmeal samples, this structural protection is removed.

Samples stored as flour experienced the greatest level of deterioration of all the samples with many of the harder samples losing in excess of 40% of their original sedimentation volume when stored at 40°C and with five of the seven varieties experiencing severe deterioration at ambient temperature. In the case of these harder samples (i.e. all varieties except Rosella and QAL2000), results indicate a steady decrease in sedimentation volume as temperature increases. The softer samples however experience little change in sedimentation volume at either 4°C or at ambient temperature. This trend is also apparent to a lesser degree for both grain and wheatmeal with QAL2000 in particular experiencing little deterioration at lower temperatures.

These results indicate that the softer varieties, QAL2000 and Rosella were substantially more tolerant to loss of sedimentation volume on storage under all conditions than were the harder varieties particularly Sunbrook and Sunco (Figure, 5.10-5.12). This was most apparent in the case of those samples stored as flour (though only QAL2000 was available as a flour) indicating that flour samples require a lower storage temperature for optimum long-term storage than do samples stored as either grain or wholemeal.

Work by Gras and O'Riordan (1998) indicates that long-term storage of grain results in changes in the protein profile of the sample with the molecular weights of the gluten polymers increasing with time as a result of oxidation. The work of Gras and O'Riordan also indicated that over time, stored flour produces dough with increased mixing time and bread with reduced loaf volume. Further studies indicates that sedimentation volume is likely to provide a reliable indication of loaf volume and baking quality even for samples that have been stored for significant periods under non-ideal conditions (Greenaway et al., 1963; Zeleny, 1963). It is likely however, that genotypic variation influences the predictive value of these

relationships. Thus, the normal predictive value of sedimentation testing may be modified for samples following long-term storage.

5.3.6 Effect of sprouting on varieties differing in end-product use

Figure 5.13 illustrates the changes in sedimentation volume as a result of both modest and severe levels of sprouting, as gauged by changes in falling number value of wheatmeal samples. The three flours chosen for this study were selected to represent a range of sedimentation behaviours, with Rosella performing poorly, Sunstate achieving average results and Sunco performing strongly.

These results indicate that as the level of sprout damage increases, sedimentation volume also increases. This is particularly evident with the variety Sunstate for which a linear relationship exists between decreasing falling number and increasing sedimentation volume. This trend can also be observed for Rosella; however, the rise in sedimentation volume occurs only when the grain is heavily sprouted. This may be due to the original falling number of the weaker variety being relatively low compared with the other varieties. Sunco, on the other hand, showed no significant change in sedimentation volume, probably because it is already close to the maximum measurable volume (i.e. 100 mL). It is unclear what the cause of the increase in sedimentation volume with sprout damage is likely to be.

If sprouting is severe (e.g., Falling number < 250 seconds), the grain is not likely to be accepted into a milling grade, and it is therefore unlikely that sedimentation testing would be used to predict baking quality. For those samples with Falling Number over 250 seconds, the changes in sedimentation volume were small but significant. It can thus be concluded that the increase in sedimentation volume due to sprout damage is unlikely to be a serious problem in practice, assuming that sedimentation testing would only be warranted for sound grain.

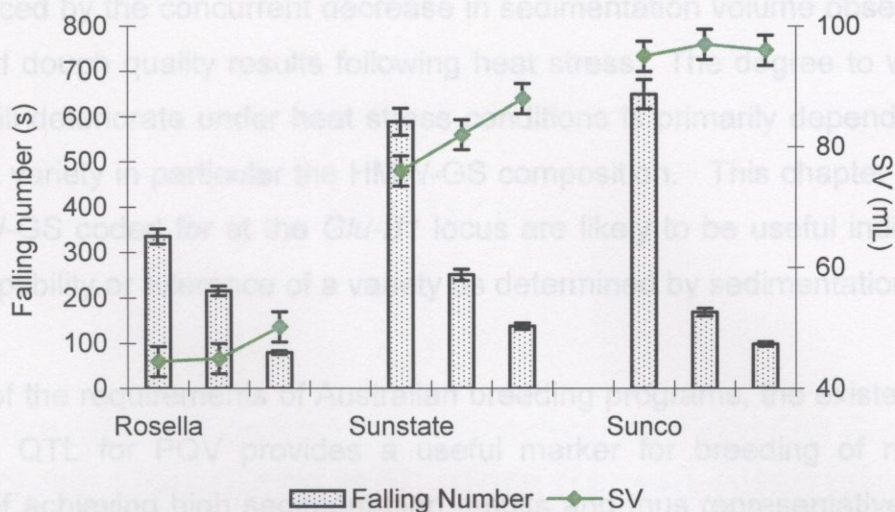

Figure 5.13: Effect of sprout damage on sedimentation volume (SV) for weak (Rosella), medium (Sunstate) and strong (Sunco) flours

5.4 Conclusions

Grain quality characteristics, as determined by sedimentation testing, are strongly influenced by both environmental and genetic factors. This is particularly evident with regard to environmental conditions, both pre- and post-harvest, with rainfall and, to a lesser degree, sprouting both resulting in increased sedimentation volume. Heat stress and storage conditions impacted negatively on quality as determined by sedimentation, though the degree to which these factors affected quality was dependent on the variety.

Zeleny sedimentation proved to be a useful tool for the testing of flour samples particularly as a predictive tool for the assessment of the quality of soft wheat samples, concurring with similar findings in Chapter 3.

The work presented in Chapter 5 indicates that sedimentation testing is still a reliable means of testing grain samples following modest sprout damage as changes in sedimentation results are only evident when levels of sprouting have exceeded those levels acceptable at receival for milling grades. Thus the predictive value of the sedimentation test is effectively unchanged.

The effects of heat stress on quality are detectable using sedimentation testing as evidenced by the concurrent decrease in sedimentation volume observed with decreased dough quality results following heat stress. The degree to which the sample will deteriorate under heat stress conditions is primarily dependent upon the wheat variety in particular the HMW-GS composition. This chapter indicates that HMW-GS coded for at the *Glu-B1* locus are likely to be useful indicators of the susceptibility or tolerance of a variety as determined by sedimentation testing.

In terms of the requirements of Australian breeding programs, the existence of at least one QTL for PQV provides a useful marker for breeding of new lines capable of achieving high sedimentation results and thus representative of good baking quality. Further, these results indicate that targeting of particular alleles, for example, those coded for at the abovementioned *Glu-B1* allele (i.e. the 20 and 7+9 alleles while avoiding the 17+18 and 13+16 alleles), could be of assistance in the selection of lines likely to result in grain with good breadmaking quality.

Overall, these results indicate the value of both the SDS and Zeleny sedimentation tests, as predictive tools in the grains industry in general and Australian breeding programs in particular, and highlight the need to take environmental factors into consideration when assessing grain quality and sedimentation results.

Chapter 6

Sedimentation Testing as a Predictive Tool for Grain Quality, as Compared with more Recent Alternatives

6.1 Introduction

As detailed in Section 1.4.2.5.1, the precursor to current sedimentation tests of wheat protein quality was developed by Lawrence Zeleny in the 1940's as a substitute for other more common protein tests such as the Kjeldahl test for protein content (rather than protein quality) and the Pelshenke test for breadmaking potential (Zeleny, 1947). Development of a new test method was required as the Kjeldahl test involves the heating of highly corrosive solutions whereas the Pelshenke test is time consuming and inaccurate. Therefore, a test involving hydration of flour samples in an acidified solution was developed and subsequently termed the Zeleny sedimentation test. Sedimentation testing was designed to take advantage of the tendency of gluten proteins to swell when dispersed in acidic solutions. Samples able to swell to the largest volume in a measuring cylinder were generally found to be of higher bread-baking potential than those resulting in a lower volume.

Sedimentation testing was first carried out in American laboratories using flour milled from American hard wheat varieties and resulted in strong correlations between the swelling capacity of the flour samples and the resulting volume of a loaf of bread baked with the same flour (Pinckney et al., 1957).

Sedimentation testing has subsequently been linked to wheat-quality attributes including allelic variation at the *Glu-A1* and *Glu-B3* loci (Ciaffi et al., 1995). Pinckney et al. (1957) rated the Zeleny sedimentation test as a more accurate means of determining baking quality than the Farinograph, while Dick and Quick (1983) used a microsedimentation test to predict cooked spaghetti firmness.

Industrially, sedimentation testing is predominantly used as a means of predicting loaf volume, the inference being that high sedimentation volume is a result of the same gluten proteins responsible for dough swelling during the bread making process and thus loaf volume (Adeyemi and Muller, 1975). Studies have shown that, in order to achieve high loaf volume when baking bread, the rheological properties of the flour must be considered. Optimal baking quality is achieved when the flour contains sufficient high-quality protein (particularly glutenin subunits) to result in a dough that is both strong and extensible (Bekes et al., 2001).

The SDS sedimentation test was developed many years ago and numerous new test methods have subsequently been developed including the swelling index of glutenin (SIG) test (Wang and Kovacs, 2002a) and the test for unextractable polymeric protein (%UPP) (Batey et al., 1991). Comparison of these predictive test methods with regard to wheat quality estimation is therefore required in order to optimise testing procedures.

The SIG test is not dissimilar in principle to sedimentation testing. That is both tests measure the extent of swelling of flour samples following hydration first in water followed by addition of a solution containing SDS and/or lactic acid. The SIG test employs low speed centrifugation in order to accelerate the rate of sedimentation and the resulting pellet is weighed rather than volume measured. The SIG test can thus be seen as a sedimentation test, accelerated by centrifugation in order to speed up the gravitational settling phase of the traditional test.

Two separate SIG test protocols have been used in this study, namely,

- SDS with lactic acid
- SDS

This chapter aims to examine the factors discussed in previous chapters with regard to optimisation of the sedimentation test for maximum reproducibility, and the potential for spurious correlations due, for example, to environmental factors. This information will be used to investigate the relationship between sedimentation testing and dough quality attributes as compared with other small scale quality tests particularly the swelling index of glutenin (SIG) test (Wang and Kovacs, 2002a, b). Other predictive tests investigated as alternatives to SDS sedimentation include the percentage of unextractable polymeric protein (%UPP), a recently developed rapid spectrophotometric test , as well as the Bean-propanol extraction method (Barker et al., 2005).

6.2 Methods and Materials

6.2.1 Sedimentation Testing

Sedimentation testing was carried out for wholemeal and flour samples as described in Sections 2.8.1 and 3.3.2.1, respectively.

6.2.2 Swelling Index of Glutenin (SIG) Test (SDS and SDS-lactic acid solutions)

This aspect of the project was performed in collaboration with Food Science Australia, North Ryde. Wholemeal samples (40 mg) were weighed into 1.5 mL Eppendorf tubes and 0.6 mL of distilled water added. Each tube was mixed using a single-tube vortex mixer for 5 seconds before being mixed on a thermomixer at 25°C and 1,400 rpm for 20 minutes. Tubes were vortexed again, separately, for 5 seconds at 10 and 20 minute intervals. Solutions containing either 3 % SDS or 3 % SDS/ 2 % lactic acid (0.6 mL) were added to the hydrated samples and tubes vortexed for 5 seconds. Thermomixing was repeated as above (including vortexing at 10 and 20 minute intervals) and tubes were centrifuged at 1000 x g for 5 minutes (or 300 x g for SDS-

lactic acid SIG test). The supernatant was discarded and the pellet centrifuged again at 1000 x g for 2 minutes (or 300 x g for SDS-lactic acid test). Any remaining supernatant was discarded, and the pellets and tubes weighed. The swelling index of glutenin was calculated as the weight of the swollen residue divided by the original sample weight (Wang and Kovacs, 2002a, b).

6.2.3 Bean Propanol-Extraction Method

The Bean propanol-extraction was carried out in collaboration with the Agriculture and Agrifood laboratory in Winnipeg, Canada. Flour samples (250 mg) were dispersed in 1 mL aqueous propan-1-ol and placed in a thermomixer for 5 minutes at 1400 rpm. The samples were centrifuged for 5 minutes at 8200 x g and the supernatant discarded. This procedure was carried out three times. The pellets were disrupted using a spatula and dried at 130°C for 1 hour. Nitrogen combustion analyses (Leco) were carried out on the dried pellet as described in section 2.3.1.2.

6.2.4 Rapid Spectrophotometric Assessment of Functional Wheat Proteins

This test was carried out in collaboration with the Agriculture and Agrifood laboratory in Winnipeg, Canada. Flour samples (10 mg) were extracted with 1.8 mL aqueous 50% (v/v) propan-1-ol for 30 minutes at 25°C and centrifuged at 13500 x g. The resulting supernatant is termed the 'gliadin rich' fraction.

Fresh flour samples (10mg) were extracted in 1.8 mL 50% (v/v) propan-1-ol and 0.2% dithiothreitol (DTT) for 30 minutes at 55°C and centrifuged at 13500 x g. The resulting supernatant is referred to as the 'total soluble protein' (TSP) fraction. Absorbances were taken using a UV-spectrophotometer at 280nm and the difference between the TSP and gliadin rich fractions were determined. This difference is termed the 'glutenin rich' fraction and is used for analyses.

6.3 Results and Discussion

All data presented in Chapter 6 is presented in full in Appendix E. For the purpose of analysis of sedimentation test results, hard and soft populations were grouped separately as each hardness type represents a different range of dough strength and is suited to different types of processing and food product. These different characteristics result in substantially different relationships between the quality data, as illustrated in Figure 6.1.

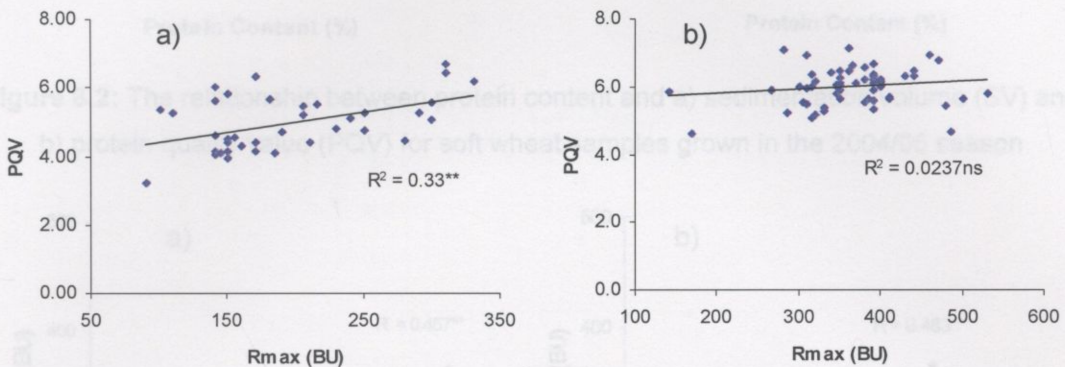

Figure 6.1: The opposing relationships between protein quality (PQV) and dough strength (Rmax) for a) soft and b) hard samples from the 2004/2005 season, ns, not significant, ** p>0.01

6.3.1 Sedimentation Testing

A range of hard and soft sample sets were obtained from three successive growth seasons and investigated by sedimentation testing. The results indicate that in the case of wholemeal samples, both sedimentation volume and PQV are related to protein content, with the exception of the soft wheats of the 2002/03 harvest. Sedimentation volume results in positive correlations with protein content while PQV results in a negative relationship (Figure 6.2).

Sedimentation volume and PQV are both correlated with Rmax and extensibility in the case of soft wheat samples (Figure 6.3). Results for hard varieties were less consistent than results for soft varieties with PQV correlated with extensibility though not with Rmax while sedimentation volume results in poor correlations with both Rmax and extensibility (Figure 6.4). These results are summarised in Table 6.1.

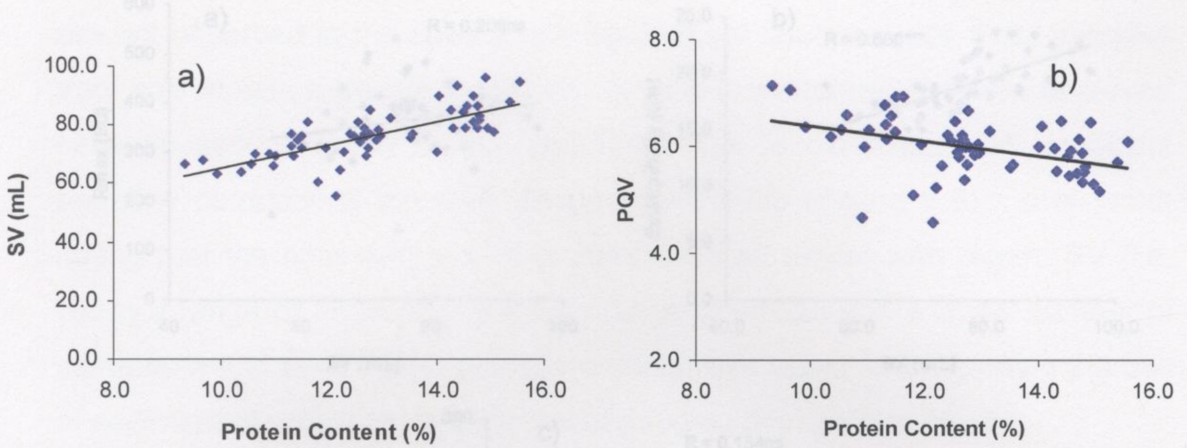

Figure 6.2: The relationship between protein content and a) sedimentation volume (SV) and b) protein quality value (PQV) for soft wheat samples grown in the 2004/05 season

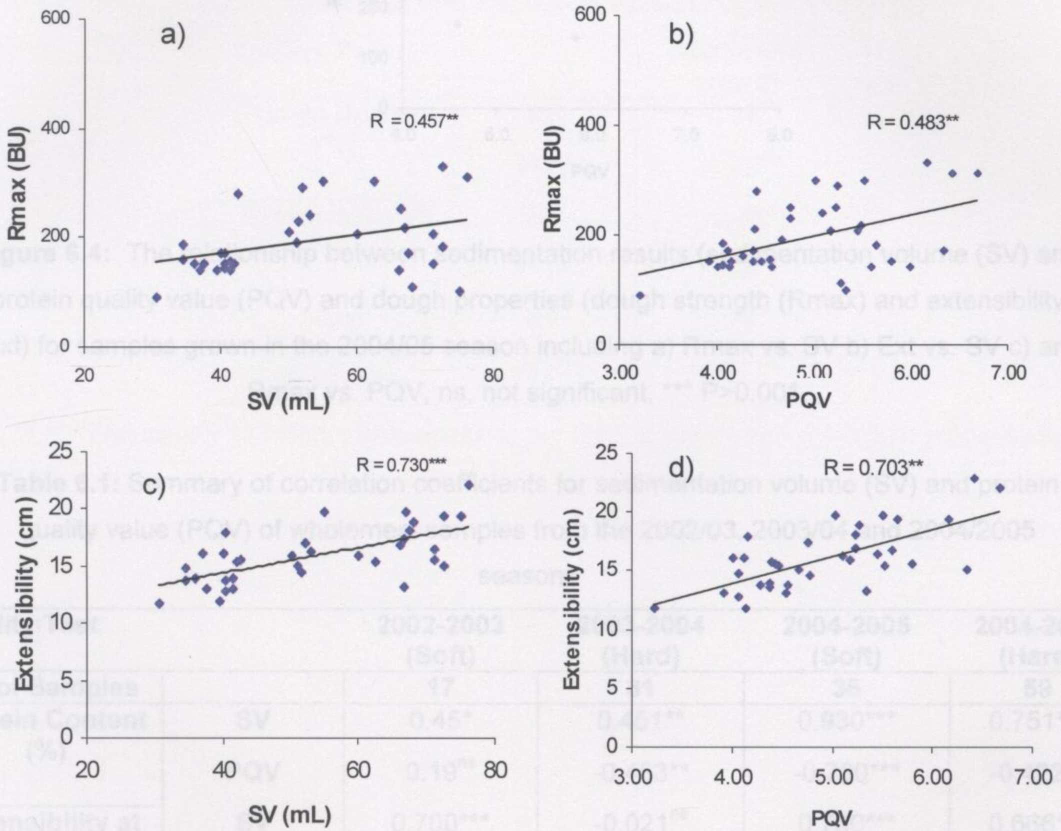

Figure 6.3: The relationship between sedimentation results (sedimentation volume (SV) and protein quality value (PQV) and dough properties (dough strength (Rmax) and extensibility (Ext) for soft samples grown in the 2004/05 season including a) Rmax vs. SV b) Rmax vs. PQV c) Ext vs. SV and d) Ext vs. PQV. ** P>0.01. *** P>0.001.

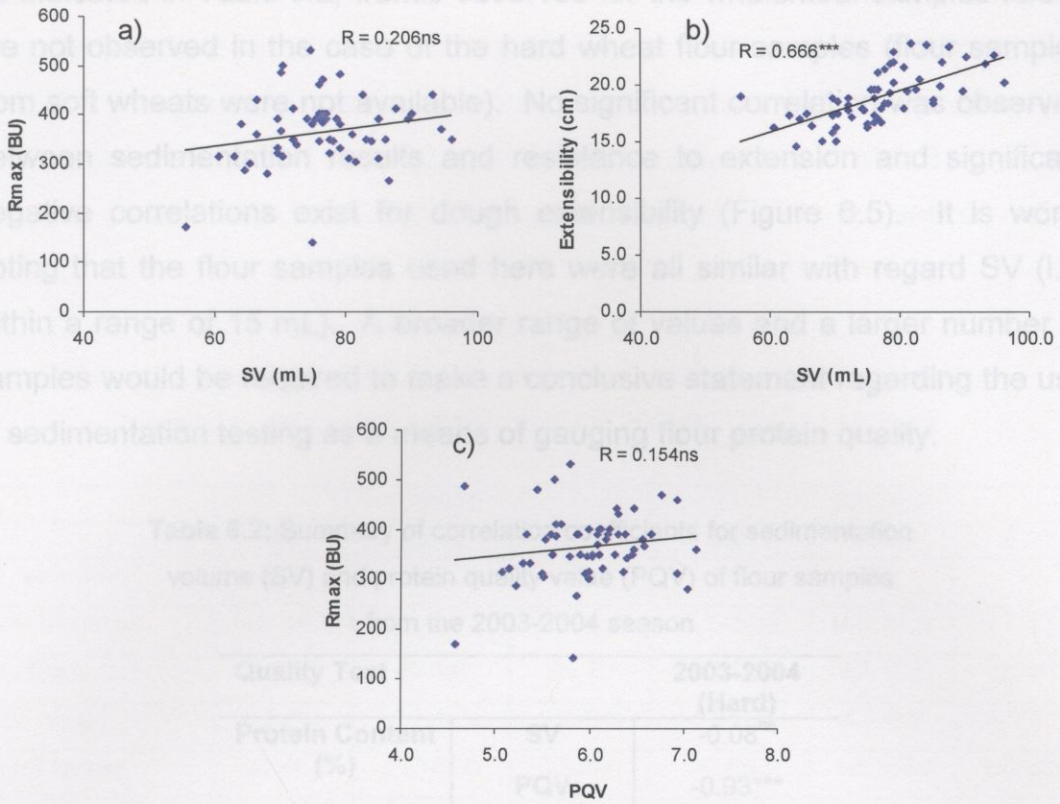

Figure 6.4: The relationship between sedimentation results (sedimentation volume (SV) and protein quality value (PQV) and dough properties (dough strength (Rmax) and extensibility (Ext) for samples grown in the 2004/05 season including a) Rmax vs. SV b) Ext vs. SV c) and Rmax vs. PQV, ns, not significant, *** P>0.001.

Table 6.1: Summary of correlation coefficients for sedimentation volume (SV) and protein quality value (PQV) of wholemeal samples from the 2002/03, 2003/04 and 2004/2005 seasons

Quality Test		2002-2003 (Soft)	2003-2004 (Hard)	2004-2005 (Soft)	2004-2005 (Hard)
No. of Samples		**17**	**31**	**36**	**59**
Protein Content (%)	**SV**	0.45*	0.451**	0.930***	0.751***
	PQV	0.19^{ns}	-0.453**	-0.780***	-0.422**
Extensibility at 45 min.	**SV**	0.700***	-0.021^{ns}	0.730***	0.666***
	PQV	0.724***	0.490*	0.703***	0.244*
Max. Resistance at 45 min.	**SV**	0.519*	0.370*	0.457**	0.206^{ns}
	PQV	0.451*	0.339^{ns}	0.483**	0.154^{ns}

*, **, *** Statistically significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. SV = sedimentation volume (mL), PQV = protein quality value

As indicated in Table 6.2, trends observed for the wholemeal samples above are not observed in the case of the hard wheat flour samples (flour samples from soft wheats were not available). No significant correlation was observed between sedimentation results and resistance to extension and significant negative correlations exist for dough extensibility (Figure 6.5). It is worth noting that the flour samples used here were all similar with regard SV (i.e. within a range of 15 mL). A broader range of values and a larger number of samples would be required to make a conclusive statement regarding the use of sedimentation testing as a means of gauging flour protein quality.

Table 6.2: Summary of correlation coefficients for sedimentation volume (SV) and protein quality value (PQV) of flour samples from the 2003-2004 season

Quality Test		2003-2004 (Hard)
Protein Content (%)	SV	-0.08^{ns}
	PQV	-0.93***
Extensibility @ 45 min.	SV	-0.35*
	PQV	-0.58**
Max. Resistance @ 45 min.	SV	0.28^{ns}
	PQV	0.37^{ns}

*, **, *** Statistically significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

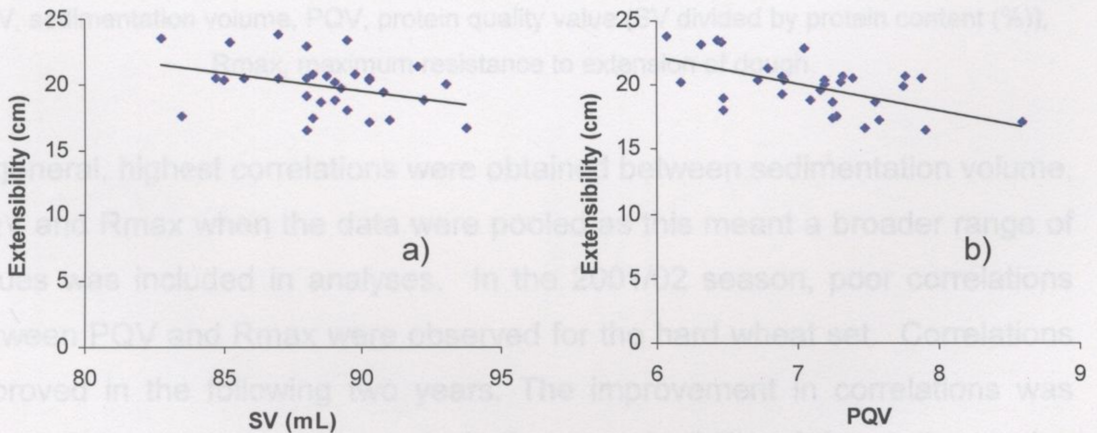

Figure 6.5: The relationship between a) sedimentation volume (SV) and b) protein quality value (PQV) and extensibility for flour samples from hard wheats grown in the 2003/04 season

Data for sedimentation volume and PQV were available for commercial grain samples received and tested by Allied Mills for the harvests of 2001/2 and 2002/3. Results from these seasons were combined with those described above and relationships to dough quality results calculated (Table 6.3). In the first two years, data were separated into hard and soft, as well as being pooled.

Table 6.3: Summary data and correlation coefficients for wheatmeal samples received by Allied Mills Australia from the 2001/02, 2002/03 seasons

	2001/02			2002/03		
	All	**Hard**	**Soft**	**All**	**Hard**	**Soft**
No. samples	78	60	18	88	70	18
Protein range	6.8-14.7	9.0-14.7	6.8-12.6	6.7-16.5	9.3-16.5	6.7-12.7
SV range	24-98	57-98	24-70	32-101	42-101	32-87
PQV range	3.3-8.1	5.7-8.1	3.3-6.9	4.2-8.0	4.2-8.0	4.2-7.1
Rmax range	50-460	210-460	50-330	60-685	180-685	60-280
Protein vs. SV	0.923***	0.763***	0.936***	0.769***	0.706***	0.828 ***
Protein vs. PQV	0.467***	-0.224 ns	0.577**	0.032 ns	0.173 ns	-0.209 ns
Protein vs. Rmax	0.742***	0.167 ns	0.524*	0.443***	0.316**	0.730***
SV vs. PQV	0.765***	0.456 ***	0.823***	0.652***	0.816***	0.363 ns
SV vs. Rmax	0.813***	0.273 *	0.643**	0.570***	0.313**	0.662**
PQV vs. Rmax	0.668***	0.200 ns	0.661***	0.328 ***	0.359***	-0.053 ns

*, **, *** Statistically significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.
SV, sedimentation volume, PQV, protein quality value (SV divided by protein content (%)), Rmax, maximum resistance to extension of dough.

In general, highest correlations were obtained between sedimentation volume, PQV and Rmax when the data were pooled as this meant a broader range of values was included in analyses. In the 2001/02 season, poor correlations between PQV and Rmax were observed for the hard wheat set. Correlations improved in the following two years. The improvement in correlations was presumably due to improvements in the reproducibility of the sedimentation and PQV methodology, as described in the earlier parts of this thesis. In accordance with results described in Chapter 5, samples likely to have been exposed to heat or drought stress were excluded from analyses wherever possible, though G x E effects may still have an impact on quality results.

Overall, sedimentation volume, without correction for protein content, explained 9-36% of variation in dough strength (Rmax) which, while not very strong made it a better predictor of Rmax than PQV. This is despite substantial seasonal effects on soft wheat quality, and demonstrates the promise of the improved sedimentation test to predict dough strength.

6.3.2 Swelling Index of Glutenin (SIG) Test

Tests for the swelling index of glutenin were carried out on the same sample sets as for the sedimentation tests described in section 6.3.1. Results indicate that SIG tests (using both the SDS and the SDS-lactic acid protocols) resulted in strong correlations with dough quality for three out of four sample sets. Significant correlations were observed between both SIG test protocols and protein content (Figure 6.6), extensibility (Figure 6.7) and Rmax (Figure 6.8) for hard flours, particularly in the 2004/05 season.

Taking into consideration the drought conditions prevalent in much of the wheat growing region during the 2002/2003 season and the subsequent effects these conditions may have had on protein quantity and quality, these results indicate that the swelling index of glutenin test shows promise as a test for the determination of dough quality. Results for SIG tests are summarised in Table 6.4.

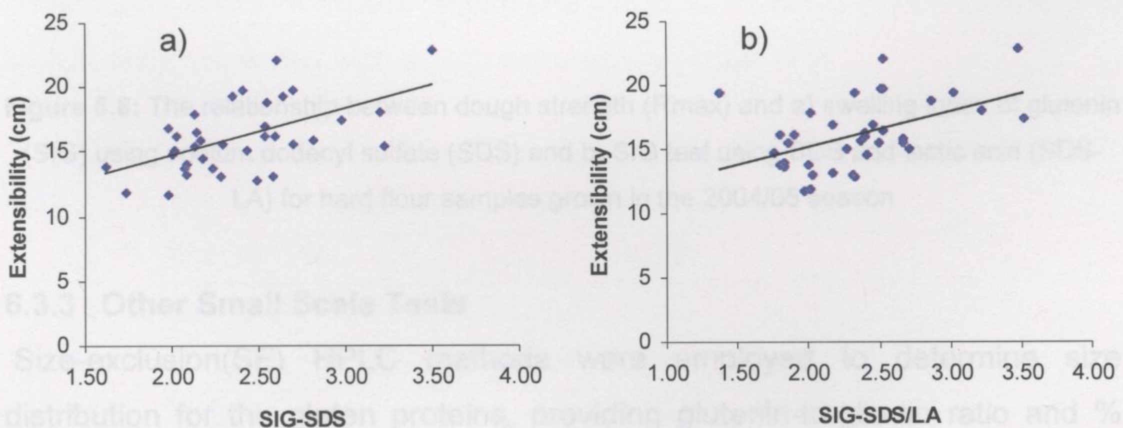

Figure 6.6: The relationship between protein content and a) swelling index of glutenin (SIG) using sodium dodecyl sulfate (SDS) and b) SIG test using SDS and lactic acid (SDS-LA) for hard flour samples grown in the 2004/05 season

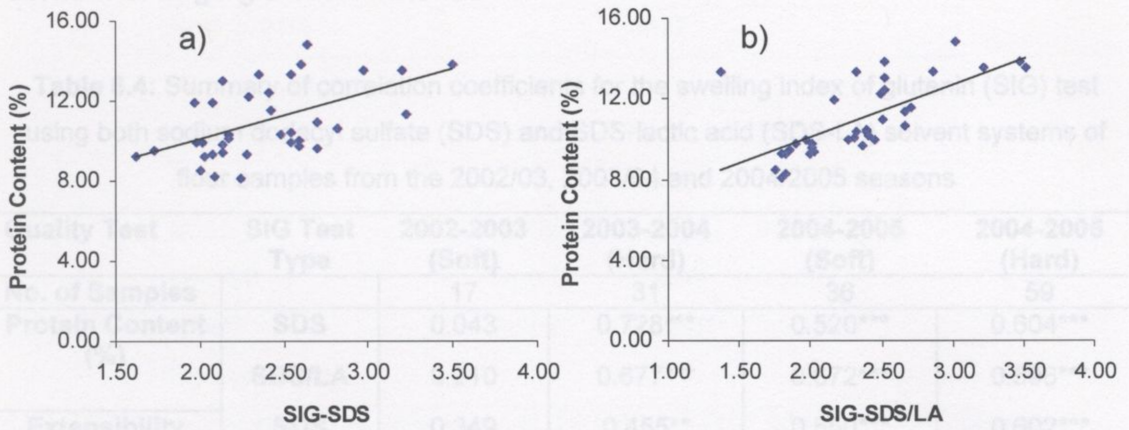

Figure 6.7: The relationship between dough extensibility and a) swelling index of glutenin (SIG) using sodium dodecyl sulfate (SDS) and b) SIG test using SDS and lactic acid (SDS-LA) for hard flour samples grown in the 2004/05 season

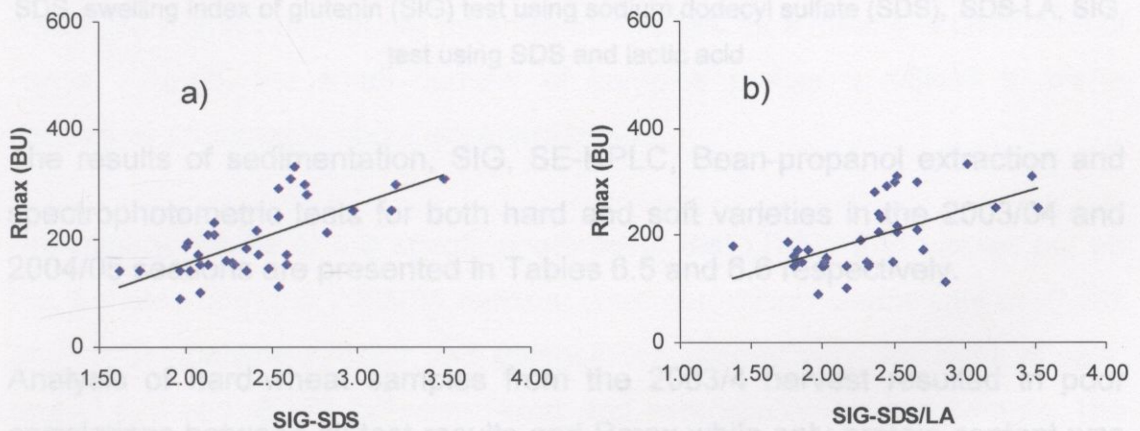

Figure 6.8: The relationship between dough strength (Rmax) and a) swelling index of glutenin (SIG) using sodium dodecyl sulfate (SDS) and b) SIG test using SDS and lactic acid (SDS-LA) for hard flour samples grown in the 2004/05 season

6.3.3 Other Small Scale Tests

Size-exclusion(SE) HPLC methods were employed to determine size distribution for the gluten proteins, providing glutenin-to-gliadin ratio and % UPP estimates; however, these procedures are considered too complex for routine testing, and thus hardly comparable to either sedimentation or SIG testing. Nevertheless, the SE-HPLC methods are considered to be useful for

examining the extent to which simplified methods might be useful to estimate content of large glutenin material.

Table 6.4: Summary of correlation coefficients for the swelling index of glutenin (SIG) test using both sodium dodecyl sulfate (SDS) and SDS-lactic acid (SDS-LA) solvent systems of flour samples from the 2002/03, 2003/04 and 2004/2005 seasons

Quality Test	SIG Test Type	2002-2003 (Soft)	2003-2004 (Hard)	2004-2005 (Soft)	2004-2005 (Hard)
No. of Samples		17	31	36	59
Protein Content (%)	**SDS**	0.043	0.728***	0.520***	0.604***
	SDS/LA	0.210	0.677***	0.672***	0.596***
Extensibility	**SDS**	0.349	0.455**	0.550***	0.602***
	SDS/LA	0.386	0.485**	0.491**	0.595***
Max. Resistance	**SDS**	0.004	0.098	0.682***	0.408***
	SDS/LA	0.604**	0.019	0.578***	0.424***

*, **, *** Statistically significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. SDS, swelling index of glutenin (SIG) test using sodium dodecyl sulfate (SDS), SDS-LA, SIG test using SDS and lactic acid

The results of sedimentation, SIG, SE-HPLC, Bean-propanol extraction and spectrophotometric tests for both hard and soft varieties in the 2003/04 and 2004/05 seasons are presented in Tables 6.5 and 6.6 respectively.

Analysis of hard-wheat samples from the 2003/4 harvest resulted in poor correlations between all test results and Rmax while only protein content was significantly correlated with dough extensibility (Table 6.5).

Table 6.5: Comparison of small-scale quality test results with dough properties for hard wheats from 2003/2004 harvest (wholemeal)

Hard 2003/04	n=30		
	Protein	**Rmax**	**Ext**
Protein		0.055	0.527**
Bean	-0.200	0.243	-0.205
Glu:Gli	-0.024	-0.216	-0.042
%UPP	0.241	0.287	-0.169
Spectroscopy	-0.329	-0.244	-0.170

*, **, *** Statistically significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Table 6.6: Comparison of small-scale test results with dough properties for soft wheats from 2004/2005 harvest (wholemeal)

Soft 2004/5	n=37		
	Protein	**Rmax**	**Ext**
Protein		0.388*	0.722***
Bean	-0.155	0.348*	0.219
Glu:Gli	0.204	-0.086	0.288
%UPP	0.005	0.636***	0.351*
Spectroscopy	-	-	-

*, **, *** Statistically significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Significant relationships were observed for 37 soft-wheat samples from the 2004/5 harvest (Table 6.5) with the Bean-propanol extraction test, %UPP and protein content correlating with Rmax. In the case of %UPP this correlation was more significant implying potential for this test as a means of prediction of Rmax. Again, the small number of samples makes it difficult to make conclusive statements about observed trends.

Preliminary results of the spectrophotometric test (Table 6.5) carried out on the samples from the 2003/04 season, gave correlation coefficients of -0.288 and -0.184 between Rmax and extensibility, respectively, for hard wheats, and -0.425 and -0.373 between Rmax and extensibility, for soft wheats. The test was not considered worth pursuing further due to the poor correlations obtained initially. In addition, the test procedure was found to be excessively time consuming for the purposes of providing a routine method comparable with sedimentation testing.

Overall none of the three alternative quality tests investigated in these analyses was indicated in preference to either sedimentation or the SIG test.

6.3.4 Comparison of PQV and SIG-SDS results

Comparison of correlation coefficients for sedimentation and for the swelling index of glutenin indicates that both tests are potentially useful predictors of dough quality. Correlations for the predictive tests were poor generally for the

2002/3 harvest (Table 6.4), possibly due to this having been a drought year. In the following year (2003/04), no relationship between SIG test results and Rmax was observed for hard wheats, although there were significant relationships with extensibility. Although r^2 values were still quite low, there were good correlations for both hard and soft wheats in the 2004/05 season with respect to both extensibility and resistance to extension (Table 6.4).

Sedimentation results on the other hand, correlated well with quality parameters across all three years (Table 6.3), although results were much stronger for soft-grained samples in both the 2002/03 and 2004/05 seasons (Table 6.1). In this case highly significant results were seen for both dough extensibility and resistance to extension, while the results for the hard samples (2003/04 and 2004/05) were inconsistent with poor correlations between sedimentation volume and extensibility and between PQV and resistance (Table 6.1).

The determination of correlation coefficients (Tables 6.1-6.6) is not necessarily the most affective approach to evaluating results for selection of flour samples with appropriate quality characteristics. A second approach is illustrated in Figures 6.9-6.10. These figures indicate the spread of PQV (Figure 6.9) and SIG-SDS (Figure 6.10) results compared with Rmax for 2003/4 hard wheat samples.

The horizontal lines at 350 BU for Rmax indicate the cut-off values often used by commercial bread manufacturers. In the case of PQV the value 6.0 can be considered as an acceptable value when analyzing wheat intended for bread making. Looking at Figure 6.10 it is evident that of the 28 samples with PQV>6, only four had Rmax<350BU but a larger number of samples (22) of acceptable Rmax would not be identified by PQV (top left quadrant) having values <6.

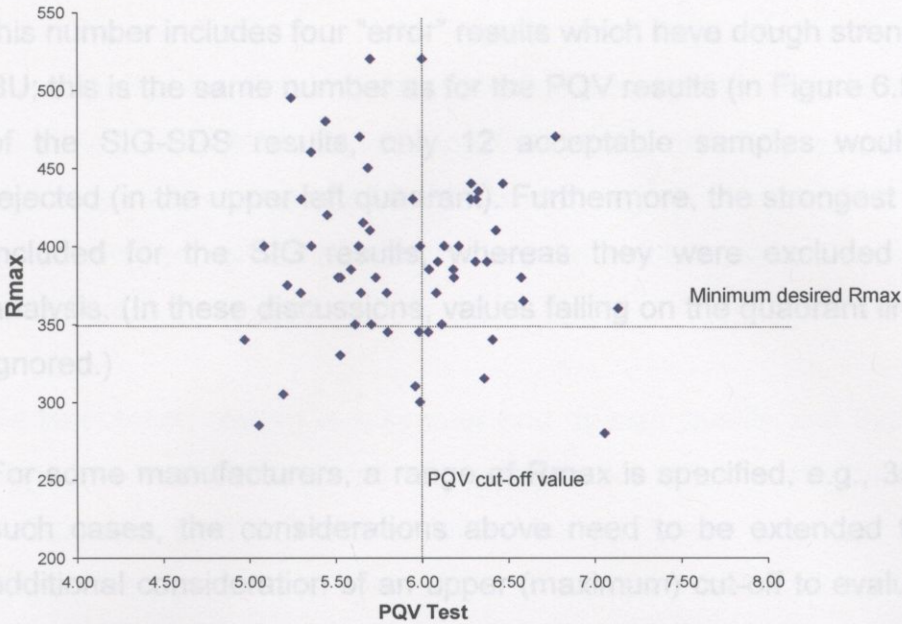

Figure 6.9: Relationship between protein quality value (PQV) and Rmax for hard wheat samples from the 2003 and 2004 harvests. Horizontal line represents minimum acceptable Rmax value; vertical line represents minimum acceptable PQV value.

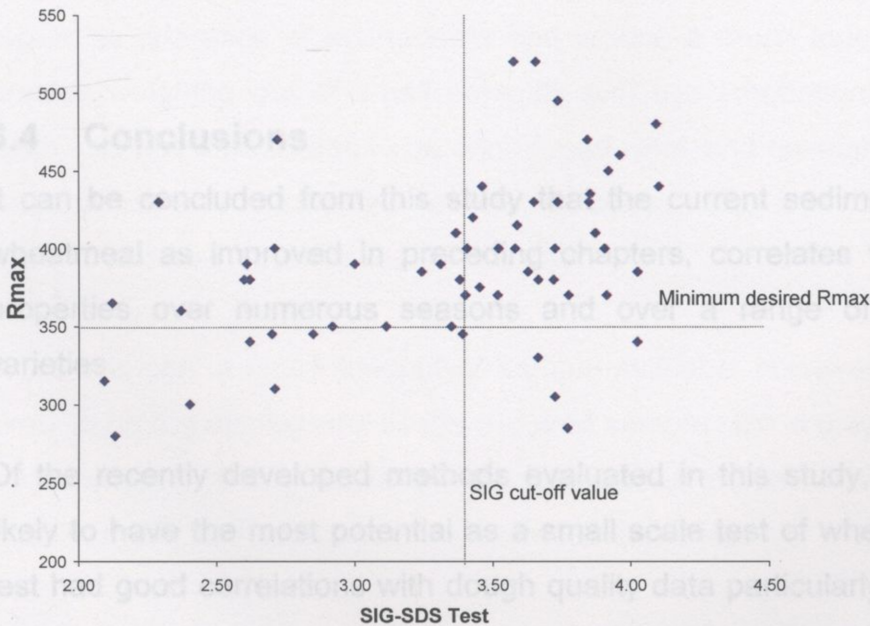

Figure 6.10: Relationship between the swelling index of glutenin (SIG-SDS) and Rmax for hard wheat samples from the 2003 and 2004 harvests. Horizontal line represents minimum acceptable Rmax value; vertical line represents minimum acceptable SIG-SDS value.

On the other hand, a larger number of acceptable samples (32) would be selected, based on the results of the SIG-SDS test expressed in Figure 6.10; this number includes four "error" results which have dough strength below 350 BU; this is the same number as for the PQV results (in Figure 6.9). In the case of the SIG-SDS results, only 12 acceptable samples would have been rejected (in the upper left quadrant). Furthermore, the strongest samples were included for the SIG results, whereas they were excluded for the PQV analysis. (In these discussions, values falling on the quadrant lines have been ignored.)

For some manufacturers, a range of Rmax is specified, e.g., 350-450 BU. In such cases, the considerations above need to be extended to include the additional consideration of an upper (maximum) cut-off to evaluate predictive usefulness.

These preliminary results, for a specific set of samples, suggest that the SIG-SDS test provides results that are more relevant to the requirements of this project, irrespective of correlation coefficients.

6.4 Conclusions

It can be concluded from this study that the current sedimentation test for wheatmeal as improved in preceding chapters, correlates well with dough properties over numerous seasons and over a range of hard and soft varieties.

Of the recently developed methods evaluated in this study, the SIG test is likely to have the most potential as a small scale test of wheat quality. This test had good correlations with dough quality data particularly for hard wheat varieties and proved a better predictor of commercially acceptable dough quality using the quadrant system than did SDS sedimentation for hard-grained samples grown in 2003/2004. In order to make a conclusive statement regarding the predictive value of the SIG and sedimentation tests

analyses would need to be determined over numerous seasons and include samples representing a broad range of quality characteristics.

In comparing the SIG test with sedimentation testing, several factors must be taken into consideration including the relative simplicity of the tests, sample size required, economy of the tests with regard to equipment and reagents and throughput of samples as well as the safety concerns applicable to each technique.

Sedimentation testing is a simpler and quicker quality test than the SIG test requiring almost no training of operators but rather a standard procedure is sufficient. Further, sedimentation testing takes only ten minutes (plus approximately 1 minute for addition of samples and shaking of cylinders) to test two samples in duplicate. As all of the preparation for each test can be completed while the previous test runs, this allows a throughput of up to 100 samples per day, in duplicate.

The SIG test on the other hand involves some level of operator training with regard to operation of instruments and is also a much longer test involving precise weighing out of small samples and two incubations of 20 minutes each. Samples also need to be centrifuged twice and reweighed. Despite the fact that numerous samples can be run concurrently it is unlikely that more than 60 samples could be tested in a day.

If there is only a small amount of sample available however the SIG test is likely to be the most useful as the required sample size is only 40 mg whereas sedimentation testing requires a sample size in excess of 5 g (6.3 g in the case of meal) and each sample is tested in duplicate.

With regard to expense, both tests require little by way of reagents with the only requirements being SDS powder and lactic acid both of which are relatively inexpensive though both an analytical balance and a centrifuge are required for SIG testing. These instruments are available already in many laboratories; however, if they needed to be purchased this could result in high

set-up costs for the SIG test (in excess of $3000). Sedimentation testing on the other hand can, if required, be carried out with only several capped glass measuring cylinders and an autopipette for rapid delivery of a consistent volume of reagent. Ideally however, an automated sedimentation apparatus would be required in order to maximise reproducibility. Commercial sedimentation apparatus cost approximately $4000; however, if resources permit, this type of instrument can be engineered on-site.

Both sedimentation and SIG testing are relatively safe test methods with neither requiring any harmful reagents or procedures. There is a minor risk with sedimentation that cylinders may smash; however, with careful handling, this is rarely an issue.

It can be concluded that both sedimentation and SIG testing procedures are safe, quick and potentially inexpensive procedures and both are likely to be useful predictors of dough quality. The decision as to which of the two tests should be implemented is likely to be made on the basis of equipment availability and/or sample size.

Chapter 7

General Discussion

Since the development of sedimentation testing in the 1940's (Zeleny, 1947) a number of studies have looked to modify the original test (Pinckney et al., 1957) or develop new, more effective sedimentation methods (Axford et al., 1978). Attempts have been made to describe the structure and composition of sediment (Eckert et al., 1993; Frazier et al., 1969), and numerous studies have compared the predictive nature of sedimentation testing with that of other quality tests (Dexter et al., 1980; Greenaway et al., 1963; Hewett and Redman, 1982; McDonald, 1985; Preston et al., 1982; Wang and Kovacs, 2002).

This study aimed to investigate sedimentation testing as a means of assessing wheat protein quality with regard to the sensitivity and reproducibility of the test, the manner by which the sediment forms, and the environmental and genetic factors capable of affecting sedimentation results. Further, this study looked to assess the overall value of the test as compared with other quality tests currently in use and to examine the potential for the development of novel applications for sedimentation testing within the grain industry. This study is distinctive in that it examines physical, chemical, environmental and genetic impacts on sedimentation testing, with a view to obtaining an overall indication of the usefulness, limitations and development potential of this type of testing.

7.1 Robustness and reproducibility of sedimentation testing

Many studies in the literature to date use sedimentation testing as a small scale quality test as a matter of course, however close investigation of the techniques employed indicates that substantial differences exist between the standard operating procedures of different laboratories (McDonald, 1985; Wang and Kovacs, 2002; Hewett and Redman, 1982; Adeyemi and Muller, 1975). Despite the differences in procedure, comparisons are often drawn between the findings of different studies. Limited information regarding the molecular basis of sedimentation test methods means that it is unclear how meaningful these comparisons are.

Results presented in Chapter 3 of this study seek to provide a clear indication of the factors capable of affecting sedimentation test results. Section 3.3 indicates that sedimentation test methods, particularly when an automated instrument is used, are robust methods capable of returning highly reproducible results. Small variations in most variable elements of the method, e.g. differences due to the operator, sample size, settling time, speed of rotation of mechanical stirrers and temperature during testing of samples, resulted in little or no change in sedimentation results.

Several factors, however, did influence results of the sedimentation test, for example, testing of samples using a manual procedure rather than a timed, automated instrument results in small differences in sedimentation volume. The use of instrumentation is preferable to manual performance of sedimentation procedures particularly with regard to the mixing and inversion phases of the sedimentation test. Small differences in instrument parameters (e.g. mixing speed) result in negligible differences between different samples and replicates of a single sample. Therefore, as with the effects on sedimentation due to the operator, differences in sedimentation results attributable to the type of instrument used can be minimised provided standard operating procedures are adhered to.

Alternatively, manual testing of flour samples results in mean variations between replicates of approximately 50% of experimental error and also results in different ranking of samples from that achieved using instrumentation.

The use of different grinders for preparation of wheatmeal samples prior to sedimentation testing proved to be the most critical of the factors tested with regard to achieving reproducible sedimentation results. The importance of grinding apparatus is a result of the subsequent particle size profile of the wheatmeal sample as presented in Chapter 3. Particle size profiles, and thus sedimentation results, differed substantially between different grinders regardless of whether the sample was soft-grained or hard-grained.

Operator differences, instrument availability and particle size profile are all likely to vary, to some degree, between different laboratories and are thus the most likely causes of interlaboratory variation in sedimentation results (Appendix B). Ideally grinding would be carried out using a protocol as close as possible to that of the recommended method (or for comparative studies, as close to that of the original study). Sieving of ground samples may be required in order to optimise particle size profile however it should be remembered that sieving will set an upper limit for particle size but allows for considerable variation in terms of percentages of smaller particles.

7.2 Sedimentation testing - a small scale test of protein quality

Many studies have highlighted the importance of protein quantity with regard to production of high quality baked products (Radovanovic, 2002; Wesley et al., 2001) however wheat varieties containing equal percentages of protein have also been found to have significantly different baking qualities (Cornish et al., 2001). These differences are attributable to differences in the quality of both the starch and protein of the sample though it is generally accepted that protein plays the more significant role in baking quality.

Numerous tests have been developed in order to gauge the quality of the protein present in small grain samples including Mixograph, 10g Farinograph, HPLC (Batey et al., 1991; Autran, 1994), SDS-PAGE (Gupta and Shepherd, 1990) and capillary electrophoresis (Uthayakumaran et al., 2005). While these techniques have major advantages including accuracy, quantification of protein fractions and the ability to identify variety as well as determine quality characteristics there are also limitations to these tests including capital cost (and subsequently availability), requirement for significant operator training and time consuming protein extraction methods. Thus a simple technique capable of rapid determination of protein quality at an affordable cost would be welcomed in the ongoing effort to balance price, speed and accessibility in quality testing. As sedimentation testing is rapid and economical it was an objective of this study to assess the value of sedimentation testing as a quality test as compared with other simple, small-scale tests.

As detailed in Chapter 6 sedimentation testing proved to be a useful predictor of wheat quality with results indicating that both sedimentation volume and PQV correlate with dough properties (Rmax and extensibility) over a number of seasons for wheatmeal samples whereas flour sedimentation results correlate negatively with Rmax and extensibility.

Sedimentation results were compared with a range of other small scale quality tests currently in use within the grain industry. These tests included the test for the swelling index of glutenin (SIG), the percentage of 'unextractable' polymeric protein' (%UPP), the Bean propanol-extraction test as well as a rapid spectrophotometric technique. Of these tests, sedimentation results proved the most consistent over numerous seasons and for testing of both hard and soft varieties. The SIG test resulted in comparable relationships with dough quality to those achieved with sedimentation testing for two of the three years analysed.

The other small scale tests returned few if any significant correlations with either Rmax or dough extensibility and as each of these tests involves significantly more preparation of samples as well as taking significantly more time to complete

analyses, either sedimentation or SIG testing would be preferable as a means of gauging wheat quality.

The interactions between wheat genotype and environmental conditions during growth and following harvest are the major factors in determination of wheat quality (Gianibelli et al., 2001). It is therefore likely that both these G x E interactions will influence the results of sedimentation testing as a test of protein quality.

The presence of HMW-GS in the genome of wheat samples proved, as expected, to be of significant importance with regard to sedimentation volume. Deletion of these subunits from the D genome, as in the case of the sequential removal in multi-null lines (Chapter 5) had the most significant effect on sedimentation results reducing sedimentation volume substantially. Deletion of the HMW-GS allele from the A genome also resulted in a decrease in sedimentation volume though not so great as that seen for deletion at the D genome. Deletion of the HMW-GS in the B genome resulted in minimal impact on sedimentation results.

When grain samples were exposed to an artificial heat shock during growth however, the HMW-GS coded for at the *Glu-B1* allele (i.e. the B genome) was responsible for conferring tolerance or susceptibility to heat stress, as determined by sedimentation testing. Similarly, subunits coded for in the A genome were involved in heat stress response while the subunits coded for in the D genome had no noticeable effect on sedimentation results. This result indicates that although the subunits coded for at the Glu-D1 allele (particularly the 5+10 and 2+12 subunits) are well documented to affect both dough strength and baking quality in a positive and negative fashion respectively (Antes and Wieser, 2001; Blumenthal et al., 1995b; Gupta et al., 1989; Khelifi and Branlard, 1992; Tohidifar et al., 2004), they have little impact on sedimentation results following heat stress.

In-field environmental conditions were important with regard to grain quality with increased rainfall resulting in a subsequent increase in quality as determined by

both sedimentation results and dough quality data. An increase in the number of days exceeding 35°C however, resulted in a negative impact on the same quality parameters. Several previous studies have also indicated that the quality of the grain is affected by the environmental conditions under which the grain is grown (Blumenthal et al., 1995a). Protein quality is particularly susceptible to climate conditions despite protein content often increasing under heat stress conditions.

Results presented in Chapters 3 and 5 indicate that Zeleny sedimentation testing (using isopropyl alcohol in place of SDS) often resulted in a broader range of sample values and stronger correlations with other quality data particularly for samples exposed to heat shock and samples containing sequentially nullified HMW-GS alleles. The stronger correlations between sedimentation results and quality characteristics occur despite the fact that in almost all studies carried out comparing the two sedimentation types (including throughout the course of this study), SDS sedimentation has proven the more effective of the two tests when assessing grain samples that have not been affected either by genetic manipulation or heat-stress. Thus Zeleny sedimentation testing may be particularly useful in assessing the quality of samples that have been exposed to extreme conditions.

It was observed in Chapter 5 that storage of grain over a twelve month period at varying temperature had a number of significant impacts on sedimentation behaviour and subsequent quality characteristics for grain, meal and flour samples. Samples underwent major changes with regard to protein quality particularly at high temperatures (40°C). Under these high temperature conditions, sedimentation volumes of all samples decreased substantially (i.e. noticeable changes were observed after 2 months) however all hard grain samples experienced decreases in sedimentation volume even when samples were refrigerated (4°C). Softer samples proved more resistant to decreases in sedimentation volume, particularly when stored as grain or meal; however all samples stored as meal decreased considerably from the results observed originally. The subsequent effect on sedimentation results is likely to be due to minor changes in the protein profile of the samples over time as described by

Gras and O'Riordan (1998). Their work indicated that gluten proteins oxidise over time causing a small but significant increase in the molecular weights of gluten polymers present in the samples resulting in a glutenin macropolymer that is less extensible and more resistant to extension (i.e. stronger). Doughs that are both extensible and strong often result in poor baking quality as a result of the protein material being too strong to expand well during proofing and baking. In turn, sediment volumes are likely to decrease as the proteins will be incapable of swelling to maximum volume in solution.

It can be concluded that while sedimentation testing is a useful predictor of dough quality, genomic characteristics such as variety and thus HMW-GS composition as well environmental conditions, particularly those related to increased temperature during growth and storage, strongly influence the resulting quality characteristics of the grain. The changes in grain protein quality as a result of G x E interactions are detectable by sedimentation testing regardless of whether the impact is positive (e.g. as a result of increased rainfall) or negative (e.g. as a result of heat stress).

7.3 Sediment composition and structure

A number of studies have reported that the large polymeric protein fraction is solely responsible for the formation of the floc during sedimentation testing of wheat samples, as the other protein components are soluble in solution (Eckert et al., 1993; Wang and Kovacs, 2002). The work presented in this study does not support this finding with fractionation of samples and subsequent testing of the freeze dried gluten (using SDS sedimentation methods) resulting in considerably reduced sedimentation volumes than those observed for whole flour samples (Chapter 4). Testing of gluten samples was carried out by reconstitution of gluten samples at both the level expected to be found in the flour samples (e.g. ~10%) and by testing using a gluten sample weight equivalent to that used for flour samples (i.e. 5 g). Where 5 g samples of gluten are tested this represents an approximate ten-fold increase in the level of gluten present in the flour sample.

Regardless of the level of gluten involved in the sedimentation, test volumes are still substantially lower than those of the corresponding flour.

This work further indicated that the interactions between the individual components of the flour strongly influence the outcome of the sedimentation test with no component, when taken in isolation, resulting in sedimentation volume equivalent to that of the whole flour. Considerable increases in sedimentation volume were observed when flour components were measured in combination particularly when both the gluten and water soluble components were tested together. This supports the idea that formation of the sediment relies on complex interactions between all fractions present in the whole flour. Sedimentation testing can be used as a means of testing individual flour components, for example the gluten test developed in Chapter 3, however these results may not necessarily concur with results for the corresponding whole flour samples as the gluten is not the only fraction under consideration.

While the literature indicates that fractionation and reconstitution of flour samples can result in test results equivalent to those observed for the whole flour (DuPont et al., 2005; Van Der Borght et al., 2005), substantial differences between fractionated and whole flour were observed throughout this study. In fact, following the fractionation and subsequent reconstitution of flour samples, very little of the functionality of the samples was retained. Similarly functionality was noticeably affected following even mild interference such as simple mixing of flour samples with water. The impact of fractionation procedures on sedimentation results indicates that sedimentation testing is sensitive to the hydration of the flour samples during the mixing of doughs/slurries.

An investigation of the relative importance of protein-protein interactions present in the sediment indicates that while the SDS present in solution is likely to cleave some of the bonds present in the native protein, bonding still occurs both within and between the protein subunits present in the sediment and is critical to the overall sediment volume. SDS is known to break the non-covalent (e.g. ionic) bonds partly responsible for the tertiary and quaternary protein structure that

gives native proteins their shape and facilitates the aggregation of subunits (Stryer, 1995). Hydrogen bonds, responsible for protein secondary structure, are also likely to be affected by the presence of SDS.
It is likely that the SDS present in the sedimentation solution results in widespread cleavage of the hydrogen bonds present in the proteinaceous material of the sediment. Thus, addition of a second agent capable of cleaving hydrogen bonds (e.g. urea) is unlikely to have any further effect on the material. It can thus be implied that hydrogen bonding is negligible with regard to stabilisation of the sediment structure as illustrated in Section 4.3.2.3.

Van der Waals forces (present in protein molecules in large numbers but with limited strength), occur between oppositely charged dipoles in protein molecules. As with hydrogen bonding, van der Waals forces play a comparatively minor role in the formation of the sediment as illustrated in Section 4.3.2.4. This may be a result of the water molecules in solution, which are polar in nature and therefore capable of dipole-dipole interactions, occupying binding sites that would otherwise be available for intramolecular van der Waals interactions

Disulphide linkages in protein molecules are essential to both the folding (tertiary structure) of proteins and also to the binding of protein subunits, as in the gluten macropolymer, to form large protein aggregates (Mathews et al., 2000; Stryer, 1995). Disulphide bonds usually form between two cysteine residues in the protein material. Addition of L-Cysteine to the sedimentation solution results in the cysteine residues present in the protein material of the sediment preferentially binding to freely available L-Cysteine, leading to unfolding of protein molecules and inhibition of protein aggregation. As illustrated in Section 4.3.2.1, protein denaturation is accompanied by considerable decreases in sedimentation volume the implication of which is that disulphide bonds are present during sedimentation testing causing protein aggregation and that disruption of these bonds critically destabilises the sediment.

The reliance of sedimentation volume on disulphide linkages emphasises the importance of the protein composition of the sample as protein subunits

containing a greater number of cysteine residues are likely to result in a greater number of disulphide linkages leading to increased sedimentation volume. As it has been observed that the presence of cysteine rich protein subunits results in stronger, more stable doughs this reaffirms the link between sedimentation testing and dough properties.

Section 4.3.2.2 indicates that despite the presence of SDS in sedimentation solution, ionic bonding remains an essential contributing factor to sediment structure and stability. Cleavage of the ionic bonds, as occurs in the presence of ammonium chloride, results in destabilisation of both tertiary and quaternary protein structure and to a lesser degree secondary protein structure.

It can be concluded that the sediment resulting from sedimentation testing involves complex interactions between all of the components of the flour or meal. The structure of the sediment is stabilised by ionic bonding and disulphide linkages and these interactions are responsible, at least in part, for the volume of sediment observed.

7.4 Novel applications of sedimentation testing

While the Zeleny sedimentation test was developed as a means of testing the quality of the protein material of wheat flour samples, since the development of the SDS sedimentation test, the majority of studies have focussed on wheatmeal samples. While this is useful as no milling is required prior to sample testing, it would also be useful to have a comparable SDS sedimentation test capable of testing flour samples as grain samples may not always be available or desirable.

The SDS sedimentation test for flour developed in the course of this study (Chapter 3) provides such a test. This test utilises a simple technique requiring only available equipment to provide a valid prediction of grain quality for flour samples and ranks samples in much the same order as the current test for wheatmeal. The test also uses the same reagents in solution as does the wheatmeal test.

Similarly, sedimentation testing has only been used to test the quality of wheat samples however the speed, economy and simplicity of these tests warrants further consideration with regard to other grain and sample types. For this reason, another important outcome of this study was the discovery that sedimentation techniques that are likely to be useful as a means of determination of the quality of other grain sample types including triticale and gluten samples (Chapter 3). Both of these sample types contain glutenin protein material (i.e. from the wheat/rye cross in Triticale samples) and as such would be expected to sediment to some degree as this material is a major factor in the formation of the sediment (Chapter 4).

While gluten samples initially proved problematic with regard to sample dispersion, once these issues were overcome, sedimentation could be carried out using both the same method and the same solution (3% SDS/2% LA) as used for testing wheat meal samples. Triticale samples on the other hand dispersed easily in solution; however, as the grain is a cross between wheat and rye genomes triticale is unlikely to contain all three HMW-GS in its genome (rather the genome is likely to contain subunits from the A and B genomes, but the rye (secalin) protein is likely to be present in place of the subunit coded for in the D genome (Lukaszewski and Gustafson, 1987)). As the glutenin fraction contributes a large portion of the sediment, it would thus be expected that triticale sedimentation results would be lower, on average, than those observed for wheat samples of similar protein content. It is also likely that development of a sedimentation test for rye and other grains not containing glutenin material would be complicated.

A key advantage of a sedimentation test for gluten is that, unlike various other gluten quality tests, the method does not involve long incubation or resting times for the gluten to be suitably relaxed and does not need uniform handling of sticky dough like samples as is the case for Keiffer rig analysis of gluten extensibility (Dunnewind et al., 2003). The limitation of this gluten test however, is that in order to carry out sedimentation, wet gluten samples need to be freeze dried and

ground. For this reason this type of sedimentation test is likely to be particularly useful for testing of commercially produced (dry) gluten samples.

Sedimentation testing proved to be a robust and reproducible test of grain protein quality returning correlations with dough quality equivalent to those observed for newer, more expensive and time consuming quality tests. Sedimentation testing can be used as a means of predicting changes in quality as a result of G x E interactions and has the potential for use as a quality test for a range of other sample types.

Sedimentation testing provides a useful alternative to other quality tests such as farinograph and small –scale baking tests as much smaller samples can be used and the time required to carry out testing is much reduced.

7.5 Further research

This research has raised a number a questions regarding sedimentation testing of wheat samples and thus several areas for further research can be identified.

i) It was not feasible in this study to do test baking and such data were not available from other laboratories using the samples available for this study. A study encompassing both dough and baking quality information along with sedimentation data would be important in furthering the current pool of information on sedimentation testing as it relates to Australian germplasm and clarifying the strength of the sedimentation test as a predictor of baking quality. Ideally such a study would also examine how the relationships between quality data are affected by G x E interactions

ii) Attempts to quantify protein composition throughout the sediment were inconclusive. A study comprising a larger range of single variety samples would be valuable with regard to information concerning the structure and composition of the flocculated material. Again the impact

of G x E interactions on the protein composition would be worthwhile as protein composition has been observed to change as a result of environmental conditions such as heat stress.

iii) This study focussed on the SDS sedimentation test as this sedimentation test currently enjoys the most widespread use. However, results presented here indicate that there are circumstances under which the Zeleny sedimentation test may still provide better predictions of grain quality. These results indicated that this is particularly the case for very soft varieties as well as for samples that have undergone some kind of degradation or deterioration, for example samples that have been heat stressed during growth. Development of a Zeleny-style sedimentation test for wholemeal samples may assist with this work

Chapter 8

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Appendix A

Effects due to the operator

MU, DN, Q, WE, MN, WW2, BO, GO, NE, WW – samples covering a range of sedimentation volumes; O1-O6 – Operator numbers; SV1-SV4 – Sedimentation volume replicates

		MU	DN	Q	WE	MN	WW2	BO	GO	NE	WW
O1	SV1	56.5	68	72	71	74	72	74	77	82	89
	SV2	57	68	70	71.5	73	73	74	78	87	89.5
	SV3	59	68.5	69	73	72	72.5	76	77	84	86
	SV4	59	67.5	69	72	70	73.5	75	74	79	88
O2	SV1	52	77	81	69	85	82	83	88	91	93
	SV2	54	76	81	69	83	82	79	86	86	93
O3	SV1	55	68	72	68	75	74	73	76	83	81
	SV2	55	68	73	70	73	72	74	74	81	81
O4	SV1	52	68	71	67	72.5	72	73	72.5	84	80
	SV2	54	67.5	69.5	70	76	72.5	76.5	76.5	82	79.5
O5	SV1	48.5	66.5	68	66	73	74	74	72	85	83
	SV2	49	67	68	67	72.5	74	72.5	72.5	82	83
O6	SV1	56	67	73.5	69.5	74	74	76	78	88.5	83
	SV2	56	67	73	71	75	73.5	76.5	77	81.5	82
O2(#2)	SV1	54	66	69.5	69	72.5	71.5	74	76	82	84.5
	SV2	55	68	70.5	69	75.5	72.5	76	76	84	83.5

Sample Preparation (Grinder) as compared with Particle Size Profile

Australian Hard (H2) grade grown in Gunnedah

SV – sedimentation volume; FN – Falling number, setting 1, 2 or 3 using either a 0.5mm or 0.8 mm screen; QC – Falling number mill, one setting, Udy – Udy cyclone mill, Perten – Perten laboratory scale mill.

	FN1(0.8)	FN2(0.8)	FN3 (0.8)	FN1(0.5)	FN2(0.5)	FN3(0.5)	QC	Udy	Perten
SV (mL)	71, 70	63, 65	62, 62	83, 80	77.5, 77.5	71.5, 73	56, 54.5	62, 64	44, 43.5
Particle Size (um)									
0.576	0.035	0.030	0.029	0.047	0.042	0.042	0.026	0.031	0.018
0.671	0.063	0.054	0.052	0.083	0.074	0.076	0.046	0.055	0.032
0.781	0.079	0.067	0.065	0.103	0.092	0.094	0.057	0.069	0.040
0.910	0.074	0.062	0.060	0.096	0.084	0.085	0.052	0.063	0.037
1.060	0.068	0.055	0.054	0.088	0.075	0.074	0.047	0.057	0.032
1.235	0.062	0.048	0.048	0.081	0.066	0.062	0.041	0.051	0.026
1.439	0.058	0.042	0.042	0.077	0.060	0.052	0.036	0.047	0.021
1.677	0.060	0.041	0.042	0.082	0.061	0.047	0.035	0.048	0.017
1.953	0.071	0.046	0.048	0.101	0.073	0.052	0.041	0.058	0.017
2.276	0.094	0.061	0.064	0.133	0.098	0.069	0.054	0.078	0.021
2.651	0.129	0.086	0.089	0.182	0.138	0.102	0.077	0.109	0.031
3.089	0.178	0.124	0.125	0.248	0.193	0.152	0.109	0.152	0.047

3.598	0.238	0.171	0.171	0.326	0.261	0.217	0.150	0.205	0.069
4.192	0.309	0.229	0.225	0.414	0.340	0.296	0.199	0.266	0.098
4.884	0.390	0.298	0.288	0.513	0.430	0.390	0.256	0.337	0.133
5.690	0.484	0.377	0.362	0.626	0.533	0.499	0.321	0.416	0.175
6.628	0.590	0.465	0.446	0.754	0.649	0.622	0.393	0.504	0.222
7.722	0.711	0.564	0.543	0.904	0.783	0.760	0.474	0.605	0.276
8.996	0.848	0.675	0.655	1.079	0.936	0.914	0.564	0.717	0.335
10.480	1.001	0.798	0.779	1.282	1.111	1.085	0.662	0.843	0.402
12.210	1.164	0.929	0.914	1.508	1.303	1.267	0.766	0.977	0.475
14.224	1.332	1.065	1.053	1.749	1.505	1.458	0.872	1.116	0.553
16.571	1.499	1.199	1.193	1.996	1.709	1.651	0.976	1.255	0.636
19.306	1.661	1.329	1.331	2.243	1.910	1.845	1.076	1.390	0.721
22.491	1.824	1.458	1.471	2.494	2.110	2.043	1.176	1.523	0.805
26.202	1.997	1.593	1.621	2.764	2.320	2.257	1.284	1.663	0.889
30.525	2.198	1.750	1.795	3.074	2.558	2.502	1.411	1.820	0.971
35.562	2.441	1.943	2.005	3.443	2.841	2.792	1.566	2.001	1.050
41.430	2.734	2.180	2.257	3.877	3.177	3.133	1.749	2.210	1.124
48.265	3.077	2.465	2.551	4.363	3.564	3.521	1.957	2.441	1.195
56.229	3.454	2.788	2.877	4.853	3.978	3.933	2.174	2.677	1.267
65.507	3.850	3.146	3.224	5.298	4.395	4.346	2.391	2.905	1.359
76.316	4.243	3.527	3.581	5.633	4.778	4.727	2.596	3.111	1.502
88.908	4.619	3.930	3.944	5.825	5.107	5.057	2.799	3.296	1.738
103.578	4.969	4.364	4.317	5.873	5.375	5.330	3.031	3.478	2.118
120.668	5.278	4.819	4.701	5.800	5.580	5.537	3.318	3.676	2.678
140.578	5.536	5.285	5.089	5.664	5.738	5.683	3.693	3.915	3.452
163.773	5.737	5.738	5.459	5.285	5.865	5.778	4.165	4.204	4.435
190.796	5.884	6.146	5.794	4.840	5.783	5.843	4.728	4.543	5.584
222.277	5.994	6.490	6.082	4.338	5.540	5.653	5.346	4.922	6.801
258.953	5.770	6.790	6.337	3.769	5.109	5.267	5.980	5.327	7.968
301.680	5.310	6.550	6.177	3.121	4.478	4.665	6.595	5.744	8.958
351.457	4.616	5.947	5.731	2.394	3.671	3.864	7.179	5.973	9.722
409.448	3.744	5.040	5.026	1.627	2.755	2.934	7.188	5.961	9.167
477.007	2.793	3.948	4.147	0.859	1.836	2.005	6.752	5.645	7.909
555.713	1.843	2.855	3.202	0.092	0.918	1.075	5.909	5.018	6.283
647.406	0.892	1.763	2.256	0.000	0.000	0.146	4.714	4.085	4.573
754.227	0.000	0.671	1.311	0.000	0.000	0.000	3.280	2.901	2.863
878.675	0.000	0.000	0.365	0.000	0.000	0.000	1.693	1.512	1.153

Australian Utility Hard (AuH2) grade grown in Moree

	FN1(0.8)	FN2(0.8)	FN3 (0.8)	FN1(0.5)	FN2(0.5)	FN3(0.5)	QC	Udy	Perten
SV (mL)	86, 83	71, 68.5	71, 70	92.5, 92.5	80, 79	77.5, 77.5	76, 77	70.5, 67.5	34.5, 35
Particle Size (um)									
0.576	0.034	0.031	0.027	0.049	0.039	0.042	0.036	0.031	0.000
0.671	0.061	0.055	0.049	0.087	0.069	0.076	0.064	0.056	0.000
0.781	0.077	0.069	0.061	0.108	0.086	0.094	0.080	0.070	0.000
0.910	0.072	0.064	0.057	0.100	0.079	0.085	0.074	0.065	0.000
1.060	0.066	0.058	0.051	0.090	0.071	0.075	0.067	0.059	0.000
1.235	0.060	0.052	0.045	0.081	0.063	0.063	0.060	0.053	0.000
1.439	0.056	0.046	0.040	0.074	0.056	0.053	0.055	0.048	0.000
1.677	0.058	0.045	0.038	0.076	0.056	0.049	0.056	0.048	0.000
1.953	0.069	0.051	0.042	0.090	0.065	0.056	0.066	0.055	0.000
2.276	0.091	0.066	0.054	0.119	0.085	0.076	0.087	0.072	0.021
2.651	0.124	0.091	0.076	0.163	0.119	0.110	0.121	0.100	0.026
3.089	0.170	0.128	0.106	0.225	0.167	0.162	0.168	0.138	0.036

3.598	0.226	0.176	0.146	0.301	0.227	0.229	0.227	0.185	0.050
4.192	0.291	0.234	0.194	0.387	0.297	0.310	0.296	0.241	0.067
4.884	0.366	0.303	0.252	0.486	0.379	0.406	0.376	0.306	0.089
5.690	0.452	0.384	0.319	0.599	0.474	0.514	0.468	0.379	0.116
6.628	0.549	0.476	0.398	0.726	0.583	0.635	0.572	0.463	0.144
7.722	0.662	0.581	0.489	0.873	0.710	0.768	0.691	0.559	0.177
8.996	0.792	0.701	0.594	1.045	0.858	0.916	0.826	0.668	0.215
10.480	0.940	0.835	0.712	1.246	1.028	1.078	0.975	0.792	0.254
12.210	1.102	0.981	0.841	1.472	1.217	1.253	1.134	0.929	0.298
14.224	1.272	1.134	0.979	1.719	1.420	1.439	1.294	1.076	0.342
16.571	1.446	1.290	1.121	1.978	1.631	1.634	1.449	1.231	0.388
19.306	1.618	1.447	1.268	2.246	1.847	1.841	1.594	1.393	0.437
22.491	1.790	1.609	1.425	2.525	2.070	2.069	1.730	1.565	0.491
26.202	1.972	1.782	1.600	2.828	2.311	2.330	1.866	1.755	0.552
30.525	2.176	1.978	1.805	3.174	2.584	2.640	2.014	1.969	0.623
35.562	2.414	2.204	2.047	3.579	2.900	3.007	2.182	2.211	0.701
41.430	2.692	2.464	2.328	4.041	3.262	3.427	2.373	2.477	0.783
48.265	3.009	2.754	2.645	4.541	3.662	3.883	2.581	2.753	0.857
56.229	3.349	3.061	2.982	5.023	4.066	4.331	2.791	3.015	0.913
65.507	3.697	3.375	3.325	5.433	4.450	4.734	2.992	3.246	0.945
76.316	4.037	3.689	3.663	5.708	4.777	5.051	3.179	3.431	0.963
88.908	4.362	4.005	3.996	5.824	5.036	5.268	3.364	3.575	0.997
103.578	4.673	4.336	4.335	5.794	5.231	5.401	3.572	3.702	1.099
120.668	4.964	4.682	4.683	5.648	5.367	5.465	3.823	3.837	1.330
140.578	5.230	5.042	5.043	5.444	5.457	5.487	4.135	4.012	1.756
163.773	5.467	5.398	5.394	5.057	5.511	5.488	4.497	4.241	2.426
190.796	5.675	5.726	5.718	4.640	5.546	5.381	4.890	4.526	3.377
222.277	5.861	6.011	6.005	4.191	5.398	5.173	5.281	4.853	4.621
258.953	5.775	6.266	6.263	3.691	5.086	4.820	5.648	5.208	6.136
301.680	5.464	6.088	6.135	3.111	4.582	4.292	5.992	5.574	7.841
351.457	4.910	5.604	5.730	2.443	3.887	3.594	5.909	5.733	9.597
409.448	4.148	4.851	5.068	1.716	3.051	2.776	5.518	5.651	11.273
477.007	3.257	3.917	4.223	0.988	2.216	1.958	4.845	5.283	11.464
555.713	2.366	2.935	3.297	0.261	1.380	1.141	3.980	4.640	10.544
647.406	1.475	1.953	2.371	0.000	0.544	0.323	3.004	3.736	8.664
754.227	0.584	0.971	1.444	0.000	0.000	0.000	2.001	2.627	6.137
878.675	0.000	0.000	0.518	0.000	0.000	0.000	0.997	1.361	3.250

Australian Standard White (ASW) grown in Gunnedah

	FN1(0.8)	FN2(0.8)	FN3 (0.8)	FN1(0.5)	FN2(0.5)	FN3(0.5)	QC	Udy	Perten
SV (mL)	63, 62	56, 54	50, 51	77, 75	64.5, 65	63, 66	57, 54.5	68, 64	36.5, 36.5
Particle Size (um)									
0.576	0.034	0.031	0.026	0.050	0.041	0.041	0.036	0.044	0.018
0.671	0.061	0.056	0.046	0.089	0.072	0.072	0.064	0.078	0.032
0.781	0.075	0.070	0.057	0.110	0.089	0.089	0.079	0.096	0.039
0.910	0.067	0.063	0.052	0.099	0.080	0.081	0.071	0.087	0.034
1.060	0.059	0.056	0.045	0.087	0.068	0.071	0.062	0.077	0.028
1.235	0.050	0.047	0.037	0.073	0.056	0.061	0.052	0.066	0.021
1.439	0.043	0.039	0.030	0.062	0.045	0.053	0.044	0.057	0.014
1.677	0.042	0.035	0.026	0.059	0.041	0.052	0.043	0.055	0.010
1.953	0.050	0.038	0.027	0.068	0.046	0.062	0.051	0.065	0.008
2.276	0.069	0.051	0.035	0.093	0.063	0.084	0.070	0.089	0.009
2.651	0.100	0.075	0.052	0.134	0.095	0.121	0.102	0.130	0.015
3.089	0.144	0.110	0.078	0.194	0.143	0.172	0.148	0.187	0.025

3.598	0.200	0.158	0.113	0.270	0.206	0.236	0.206	0.258	0.041
4.192	0.265	0.216	0.157	0.360	0.281	0.311	0.273	0.341	0.063
4.884	0.340	0.285	0.210	0.463	0.369	0.395	0.350	0.436	0.092
5.690	0.422	0.366	0.272	0.581	0.471	0.490	0.435	0.542	0.127
6.628	0.511	0.458	0.344	0.710	0.584	0.596	0.526	0.661	0.166
7.722	0.609	0.562	0.426	0.856	0.712	0.716	0.628	0.795	0.209
8.996	0.719	0.679	0.519	1.021	0.856	0.853	0.741	0.947	0.256
10.480	0.841	0.809	0.621	1.208	1.017	1.008	0.867	1.119	0.309
12.210	0.976	0.950	0.733	1.414	1.192	1.179	1.004	1.307	0.369
14.224	1.118	1.099	0.849	1.635	1.377	1.359	1.148	1.507	0.436
16.571	1.263	1.251	0.968	1.865	1.565	1.542	1.292	1.713	0.508
19.306	1.406	1.405	1.089	2.104	1.750	1.724	1.432	1.919	0.582
22.491	1.551	1.562	1.214	2.359	1.932	1.906	1.567	2.124	0.655
26.202	1.705	1.729	1.349	2.644	2.121	2.100	1.703	2.330	0.726
30.525	1.886	1.915	1.502	2.983	2.332	2.323	1.850	2.543	0.797
35.562	2.111	2.131	1.683	3.395	2.582	2.594	2.021	2.767	0.870
41.430	2.394	2.383	1.898	3.887	2.885	2.929	2.220	3.002	0.943
48.265	2.744	2.674	2.150	4.445	3.247	3.332	2.452	3.240	1.025
56.229	3.153	2.998	2.439	5.018	3.656	3.791	2.708	3.468	1.111
65.507	3.615	3.352	2.766	5.551	4.099	4.286	2.984	3.679	1.221
76.316	4.104	3.728	3.130	5.967	4.548	4.779	3.273	3.869	1.376
88.908	4.605	4.127	3.539	6.230	4.979	5.237	3.583	4.047	1.621
103.578	5.102	4.549	4.001	6.353	5.377	5.631	3.928	4.232	2.015
120.668	5.560	4.981	4.515	6.386	5.720	5.944	4.315	4.440	2.587
140.578	5.953	5.410	5.068	6.003	5.998	6.184	4.742	4.682	3.407
163.773	6.261	5.819	5.628	5.476	6.203	6.376	5.187	4.957	4.504
190.796	6.484	6.200	6.160	4.860	6.363	6.234	5.627	5.254	5.913
222.277	6.643	6.558	6.646	4.196	6.132	5.887	6.041	5.564	7.547
258.953	6.343	6.535	7.094	3.507	5.647	5.329	6.437	5.671	9.244
301.680	5.764	6.199	7.018	2.818	4.920	4.574	6.413	5.503	10.621
351.457	4.927	5.544	6.573	2.128	4.005	3.664	6.057	5.004	11.385
409.448	3.919	4.634	5.794	1.439	2.993	2.754	5.374	4.201	10.142
477.007	2.911	3.587	4.790	0.749	2.004	1.843	4.439	3.205	7.887
555.713	1.903	2.540	3.697	0.000	1.014	0.933	3.399	2.210	5.616
647.406	0.895	1.493	2.604	0.000	0.025	0.000	2.358	1.214	3.664
754.227	0.000	0.447	1.511	0.000	0.000	0.000	1.318	0.219	1.712
878.675	0.000	0.000	0.418	0.000	0.000	0.000	0.277	0.000	0.000

Effect of Sample Size on Sedimentation Volume

Sv – sedimentation volume; S1-S6 – samples covering a range of sedimentation volumes

Meal	SV-S1	SV-S2	SV-S3	SV-S4	SV-S5	SV-S6
5.8	47.5	58	65.5	72.75	79.5	89
5.9	48	58.25	66.75	73	79.5	91
6	48.5	58	67	73	80.75	91.5
6.1	48	58.5	67.5	73.75	81.5	91.75
6.2	48	59	68.25	74.75	82	91
6.3	49	59.5	68	75	82	91.5
6.4	49.5	59.75	68	74.5	82.5	91
6.5	50.25	60.25	68.75	76	82.25	91.25
6.6	51.5	61.5	69	75.5	82.75	91.75
6.7	51.25	61.75	69.5	76	83	92
6.8	51.75	62	70.25	76	83.75	92.5

Flour	SV-S1	SV-S2	SV-S3	SV-S4	SV-S5	SV-S6
4.5	44.5	60.5	67	76.5	82	91.75

4.6	45.75	61.75	68.5	77	83.25	92
4.7	46	62	69.5	78.25	84.5	93
4.8	48	62.75	70	78.5	85	93.5
4.9	50	63.5	70.5	79	85.5	93.5
5	50.25	64	70.5	79	86	94.5
5.1	51	64.5	70.25	79.5	86	94
5.2	52	65	71	79.5	86	94.5
5.3	52	66	71.25	80	87.5	95
5.4	52.5	66.75	72	81	88	95.5
5.5	52	67.5	73.25	81.75	88.75	96

Automation versus Manual Testing

R1-R4 – replicate sedimentation volume;

Automatic	R1	R2	R3	R4	Mean	Std. Dev.
Quambatook	72	70	69	68	69.75	1.707825
Manangatang	74	73	72	70	72.25	1.707825
Donald	68	68	68.5	67.5	68	0.408248
Wee Waa	89	89.5	86	86	87.625	1.887459
Goroke	77	78	77	74	76.5	1.732051
Murrayville	56.5	57	59	59	57.875	1.314978
Bordertown	74	74	76	75	74.75	0.957427
Weedallion	71	71.5	73	72	71.875	0.853913
Wee Waa 2	72	73	72.5	73.5	72.75	0.645497
Nea	86	83	87	85	85.25	1.707825
					Ave SDM	1.292305

Manual	R1	R2	R3	R4	Mean	Std. Dev.
Quambatook	68	66.5	67	64	66.375	1.701715
Manangatang	72	71	74.5	75	73.125	1.931105
Donald	64	68	67	69	67	2.160247
Wee Waa	74	72	71	71.5	72.125	1.314978
Goroke	73	72	72	78	73.75	2.872281
Murrayville	62	59	61	64	61.5	2.081666
Bordertown	67.5	67	70	67	67.875	1.436141
Weedallion	80	75	81	76	78	2.94392
Wee Waa 2	74.5	73	72.5	71.5	72.875	1.25
Nea	82	87	84	79	83	3.366502
					Ave SDM	2.105855

Effect of Mechanical Stirring on Sedimentation Volume

V1-V5 – Single variety samples each tested in quadruplicate; Ave. – Average of four replicates

Stirrer Speed (rpm)	V1	Ave	V2	Ave	V3	Ave	V4	Ave	V5	Ave
0	73, 72, 74, 73	73	59.5, 59, 60, 59.5	59.5	80, 80, 79.5, 80.5	80	72, 72, 72, 72	72	52.5, 52, 53, 52.5	52.5
200	75, 75, 74.5, 75.5	75	57, 57, 57.5, 56.5	57	82, 80.5, 83.5, 82	82	75, 75, 74, 76	75	49, 47, 50, 50	49
400	75, 75, 75, 75	75	58.5, 58, 59, 58.5	58.5	85, 84, 86, 85	85	75, 74.5, 74.5, 76	75	51,50, 50, 53	51
600	73, 75, 73, 74.25	73.75	59, 58, 58, 60	59	80.5, 80, 81, 80.5	80.5	75, 74, 76, 75	75	50, 50.5, 50.5, 51	50
800	74, 73, 73.5, 75.5	74	60, 60, 60, 60	60	81, 80.5, 81, 81.5	81	76, 76, 77, 75	76	51, 51, 51, 51	51
1000	73.5, 73, 74, 73.5	73.5	59, 61, 59, 61	60	82, 81, 82, 83	82	73, 73, 73.5, 72.5	73	50, 50, 49, 51	50

Effect of Hydration Time on Sedimentation Volume

V1-V5 – Single variety samples each tested in quadruplicate; Ave. – Average of four replicates

Time (s)	V1	Ave	V2	Ave	V3	Ave	V4	Ave	V5	Ave
30	73, 74	73.5	60, 60	60	82, 82	82	73.5, 72.5	73	50, 50	50
45	74, 74.5	74.25	61.5, 60.5	61	83, 84	83.5	74, 74	74	51, 53	52
60	76, 76	76	62, 63	62.5	83.5, 82.5	83	74, 74.5	74.25	52.5, 53	52.75
75	76.5, 77	76.75	63, 63	63	84.5, 85	84.75	75.5, 75.5	75.5	53, 53	53
90	77, 78	77.5	63, 63.5	63.25	85, 85	85	76.5, 75.5	76	53, 54	53.5
105	78, 78	78	64, 64	64	86.5, 87.5	87	76, 76	76	55, 55	55
120	78, 79	78.5	65, 66	65.5	87, 88	87.5	76, 77	76.5	55, 56	55.5
135	79, 79.5	79.25	66, 66.5	66.25	89, 89	89	77.5, 76.5	77	56, 57	56.5
150	80, 80	80	67, 67	67	90, 91	90.5	77, 77	77	57.5, 58	57.75

Effect of Reagent in Reaction solution

SDS versus CTAB

Sample ID	SDS/LA	CTAB/LA
7255	66.5	81
7254	68.25	63.5
7261	75.5	96.5
7257	75.75	91
7256	77	75
7260	77.5	67
7236	77.75	74.5
7259	77.75	67
7258	79	73.5
6905	82.5	97.5
6903	83	74
6926	84	84
Speed	84.5	91
6904	86.5	99
6915	86.75	80
6899	86.75	87
6911	88	98
6902	88.75	96
6914	90	100
6906	92	100

Lactic acid versus Formic acid

Sample ID	SDS/LA	SDS/FA
7254	62	75
7261	63.75	68
7258	64.25	73.5
7259 S	66	67
6903	66.25	74
7236	67	76
6915	68.25	68.5
7293	71	80
6926	72.25	74
7256	73.75	81
6899	74.5	71
7260	77.5	68
6911	79.5	84
6902	79.5	83.25
6905	80.5	87.25
6914	82	85
7259 W	82	81.5
6904	82.5	81
6906	83.5	79
7255	85	86

Effect of pH on Sedimentation Volume for Five Samples (1-5)

Meal	pH 1.7	Ave	pH 2.4	Ave	pH 2.7	Ave	pH 3.7	Ave
1	55, 55	55	66.5, 65.5	66	82, 83	82.5	91, 91	91
2	46.5, 47.5	47	55.5, 54.5	55	69, 69.5	69.25	86, 88	87
3	64, 64	64	81, 81	81	92, 93	92.5	99, 99	99
4	60, 62	61	74, 75	74.5	96, 96	96	98, 100	98
5	55, 55	55	69, 69	69	85, 87	86	101, 101	101

Flour	pH 1.7	Ave	pH 2.4	Ave	pH 2.7	Ave	pH 3.7	Ave
1	63, 63	63	75, 75	75	83, 83.5	83.25	93.5, 94.5	94
2	71, 71	71	89, 89.5	89.25	87, 87	87	96, 96	96
3	75.5, 74.5	75	91, 91.5	91.25	88, 89	88.5	95, 97	96
4	52, 52	52	60, 60	60	76, 76	76	90, 90	90
5	76, 78	77	92.5, 93	92.75	91, 92	91.5	96.5, 95.5	96

Effect of Temperature on Sedimentation Volume

SV(12) – Sedimentation volume at 12°C, SV (Ambient) – Sedimentation volume at ambient temperature; SV(37) – Sedimentation volume at 37°C.

Sample ID	SV (12)	Ave	SV(Ambient)	Ave	SV (37)	Ave
RO	49.5, 48.5	49	48, 48	48	47, 48	47.5
SF	48, 49	48.5	47, 49	48	48, 46	47
QU	50, 50.5	50.25	50.5, 51.5	51	49, 49	49
CO	60, 60	60	60, 60.5	60.25	57, 59	58
AN	69, 69.5	69.25	72, 72.5	72.25	69, 69.5	69.25
SS	74, 74	74	74, 74	74	73.5, 73	73.25
BA	78, 78.5	78.25	77, 79	78	77, 75	76
YE	82, 83	82.5	82.5, 83.5	83	80, 80.5	80.25
WW	81, 81	81	83, 83	83	80, 80	80
SU	93, 94	93.5	91, 93	92	89, 90	89.5

Modification of SDS sedimentation test for flour samples

Sample Number		*Grade/Variety*	Protein Content (%)	SDS Wholemeal Me	PQV Wholemeal	Flour Protein	1.5% SDS, 2%LA	*PQV*
6705	*2437*	*APW2*	10.8	59.5	5.51	9.64	72	7.47
6707	*2439*	*APW2*	10.8	65.5	6.06	9.40	77	8.19
6708	*2440*	*APW2*	10.6	59.5	5.61	9.35	68.5	7.32
6715	*2529*	*Banks*	14	59.5	4.25	12.13	57.5	4.74
6717	*2531*	Sun 36LB	14.2	67.5	4.75	12.32	74	6.01
6718	*2532*	Sunbri L3	12.8	62	4.84	11.92		
6719	*2533*	Sunbri I5	14.4	73.5	5.10	12.31	72.5	5.89
6720	*2534*	QAL2000	11.2	48	4.29	9.62	55	5.72
6721	*2535*	Sunstate/HYD19	13.9	62.5	4.50	12.03	55.5	4.61
6722	*2536*	Sunstate L3	14.5	70	4.83	12.65	72.5	5.73
6726	*2540*	Sun 413C	17	81.5	4.79	12.80	72	5.62
6727	*2541*	2001-13	13.2	56	4.24	10.80	60	5.56
6728	*2542*	Sun 361D	13.3	56.5	4.25	11.61	56.5	4.87
6729	*2543*	Sun 404F	14.1	65	4.61	12.14	60.5	4.98
6730	*2544*	Sun 37EW	13.4	59	4.40	12.08	58	4.80
6731	*2545*	Sun 409F	16.6	73.75	4.44	14.94	69	4.62
6732	*2546*	2001-15	14	57.5	4.11	11.93	57	4.78
6733	*2547*	Sun 409D	17.4	74	4.25	15.06	68	4.51
6734	*2548*	Sun376W	12.4	55	4.44	10.96	63	5.75
6735	*2549*	Banks L3	13.9	66	4.75	12.03	64.5	5.36
6736	*2550*	Sunsoft 98	14.4	71	4.93	12.43	71	5.71
6737	*2551*	*QT10380*	15	78.5	5.23	12.28	79	6.43
6738	*2552*	*QT10187*	13.8	79.5	5.76	12.29	81	6.59
6740	*2554*	*Sun 392A*	14.1	79.5	5.64	12.05	85.5	7.09
6742	*2556*	*Banks*	14.7	78.5	5.34	12.36	72	5.82
6745	*2559*	*Batavia*	14.7	71.5	4.86	12.61	78.5	6.22
6746	*2560*	*QT10162*	15	87	5.80	12.53	85	6.78
6748	*2562*	*QT10198*	14.8		0.00	13.52	82	6.06
6751	*2571*		15.5	78.5	5.06	13.31	62	4.66
6752	*2565*	QT8447	14.9	75.5	5.07	12.71	78	6.14
6753	*2566*	QT10776	14.1	74.5	5.28	12.37	82.5	6.67
6754	*2567*	QT10323	15	74.5	4.97	13.41	81	6.04
6756	*2568*	Hartog	14	73	5.21	12.51	80	6.39
6759	*2576*	Thornbill	14.7	67.5	4.59	12.72	66.5	5.23
6760	*2577*	Annuello	12.5	77	6.16	11.16	82.5	7.39
6761	*2578*	Chara	14.3	83.5	5.84	12.73	90	7.07

6779	*2590*	Annuello	13.1	76	5.80	11.70	85	7.27
6780	*2591*	Annuello	14.6		0.00	12.89	86.5	6.71
6781	*2592*	Chara	16.5	89.5	5.42	13.81	83	6.01
6782	*2593*	Lang	16.1	74	4.60	13.49	66.5	4.93
6783	*2594*	Leichhardt	14.7	71	4.83	12.79		
6786	*2613*	*Superb Bakers Blend*	11.4		0.00	10.51	67.5	6.42
6791	*2614*	Sun401D	13.8	68	4.93	11.59	62.5	5.39
6792	*2615*	Sun401E	13.9	65.5	4.71	12.44	60.5	4.86
6793	*2616*	Ref. Variety	15.4	74	4.81	13.95	64.5	4.62
6794	*2617*	Sun 376G	13.7	64	4.67	11.61	66	5.68
6795	*2618*	Sun 392A	13.4	72	5.37	12.02	79.5	6.61
6796	*2619*	Sun421D	13.5	71	5.26	11.85	65	5.48
6798	*2621*	Ref. Variety, Sunco	14.6	70	4.79	12.57	73.5	5.85
6799	*2622*		14.1	67	4.75	11.96	61	5.10

Development of a Sedimentation Test for Triticale Using Ten Triticale Samples

Meal	Protein Content	5% SDS, 5% LA	Ave	Flour	Protein Content	3% SDS/5% LA	Ave
	8.99	27, 28	27.5		8.567	44, 44	44
	9.02	27, 28	27.5		8.051	42, 43	42.5
	9.18	28, 28.5	28		8.265	48, 47	47.5
	9.22	28, 28	28		9.271	48, 48	48
	9.34	30, 31	30.5		9.097	47, 49	48
	9.48	31, 31	31		8.197	41, 43	42
	9.567	34, 36	35		7.979	39, 39	39
	9.822	36, 37	36.5		8.654	45.5, 46.5	46
	9.86	37, 38	37.5		8.365	42, 44	43
	9.97	38, 38	38		9.108	48, 49	48.5

Development of a Sedimentation Test for Gluten Using Fourteen Gluten Samples

VWG – Sample numbering system

Sample ID	Ext. (cm)	3% BuOH	5% BuOH
VWG038	5.5	54	43
VWG039	7.3	75	41
VWG040	7.7	75	53
VWG042	7.3	75	45
VWG043	7.8	76	47
VWG001	11	80	53
VWG034	8.1	81	54
VWG037	9.2	81	52
VWG036	6.7	82	51
VWG041	6.8	82	47
VWG032	10.1	83	50
VWG035	8.2	84	45
VWG003	10.7	89	61
VWG009	9.3	90	64

Appendix B

STANDARD OPERATING PROCEDURE

PROTEIN QUALITY ANALYSIS

Preparation of solution for sedimentation testing of wheatmeal

Requirements:

Distilled water

SDS (sodium dodecyl sulfate) powder

Lactic Acid (88% purity)

Dye (Toluidine blue stock solution)-

STOCK SOLN (0.03%)

- Weigh 0.3g± 0.01 of Toluidine blue.
- Transfer quantitatively to a 1litre volumetric flask.
- Dissolve by inverting flask fifteen times.

Preparation:

IN A CLEAR 3 LITRE BEAKER:

- **Weigh 2325g** ± 0.5g of distilled water, on Sartorius top pan balance.
- Place a magnetic stirrer bar in the distilled water, and place beaker on a magnetic stirrer inside a fume cupboard. Set the magnetic stirrer to stir vigorously
- Measure exactly 50mls of lactic acid using a 50 mL pipette and add this slowly to the water.
- Measure 75g ± 0.05g of SDS and add to the dilute lactic acid solution.
- Stir until the solution is completely clear and all SDS material is dissolved.

• Measure 100 mL of toluidine blue stock solution using a 100 mL measuring cylinder and add to solution, mix well.

•Transfer quantitatively to a large stock bottle.

Preparation of solution for sedimentation testing of flour

- This solution is made up as for the wheatmeal solution described above however 2367.5g ± 0.05g of distilled water is required and only 37.5g ± 0.05g of SDS is required.

STANDARD OPERATING PROCEDURE – as followed at Allied Mills Australia

PROTEIN QUALITY METHOD

• Adjust autopipettes to deliver exactly 50g ±0.1 g each of distilled water and reaction solution (as described above).

• Remove ~600g sample of known sedimentation volume (i.e. control sample) from freezer, defrost for at least 2 hours

• Grind defrosted wheat sample and determine SV as described below

NOTE: The control sample should be tested at the beginning of each day and each time a new bottle of solution is opened

GRINDING

• Use a sample divider to obtain samples of grain of approximately 100 g.

• Using stacked brass Endecott sieves of 2.36mm and 2.0mm remove all chaff, husks, seeds or other foreign material

• Shake gently until all small or cracked grains have passed through the sieve.

• Set falling number mill feeder (Perten 3170) to speed 1.

• Grind clean wheat until all grain have been ground, wait for 15 seconds before switching off the instrument.

• Mix ground wheat sample with spatula until homogenous and transfer into a sample bag – clean the grinder thoroughly between each sample

SEDIMENTATION TEST METHOD

1. Add 50.0ml of distilled water to a 100ml stoppered measuring cylinder,using an automatic pipette dispenser.

2. Weigh 6.30 ± 0.005 g of ground wheat sample using a Sartorius, top pan balance all samples should be tested in duplicate

3. Using a plastic funnel, add the weighed ground wheat sample to the measuring cylinder containing the 50mls of distilled water.

4. Repeat steps 1 to 3, four times i.e. once for each available stirrer.

5. Stopper each measuring cylinder the shake four times (i.e. four single movements), dispersing ground wheat in the distilled water. If any sample adhere to the stopper of the cylinder remove the stopper with a twisting motion, replace the stopper and shake once more. Repeat if necessary to ensure all sample is dispersed.

6. Place cylinder onto the mixing rack, remove stoppers

7. Turn instrument "on" at the main switch and then commence stirring cylinder contents at 900 revolutions per minute.

NOTE: Steps 3 to 7 should be completed within 40 seconds for maximum reproducibility

8. Let the cylinders stand for At 4mins 45 secs

9. Add reaction solution (as described above) using a calibrated serial dispenser, replace stoppers.

10. Invert cylinders 15 times and then stand for a further 5 mins exactly.

11. At 10mins total elapsed time, read the height of sediment in mL using the graduations on the measuring cylinder.

PROTEIN QUALITY VALUE (PQV)

To calculate protein quality value:

1. Determine Protein Content of sample using near infrared reflectance spectrocopy.

2. Calculation:

   PQV =Sedimentation volume (mL)/ % Protein content

Appendix C

Fractionation and Reconstitution of Whole and Defatted Flour Samples Using a Weaker (Annuello) and a Stronger (Yelta) Sample

SV- Sedimentation volume;

Flour	SV (flour)	Ave.	SV (starch + gluten)	Ave.	SV (starch + gluten + water solubles)	Ave.	SV (flour + excess water solubles)	Ave.
Annuello	69.5, 70.5	70	3.5, 4	3.75	17, 18	17.5	58, 60	59
Yelta	79, 80	79.5	6.5, 7	6.75	22, 23	22.5	62.5, 64.5	63.5

Defatted flour	SV (flour - lipids)	Ave.	SV (starch + gluten - lipids)	Ave.	SV (starch + gluten + water solubles - lipids)	Ave.	SV (flour - lipids + excess water solubles)	Ave.
Annuello	67.5, 69.5	68.5	10, 10	10	16.5, 17.5	17	59, 59	59
Yelta	78.5, 78	78.25	21, 23	22	30, 32	31	57.5, 58.5	58

Lipids	Flour	Ave.	Lipids Present	Ave.	Lipids Absent	Ave.
Annuello	69.5, 70.5	70	46.5, 47.5	47	41, 44	43.5
Yelta	79, 80	79.5	55, 57	56	52.5, 51.5	52

Gluten	Flour SV	Ave.	8% bran	Ave.	10% bran	Ave.	12% bran	Ave.	14% bran	Ave.
Annuello	69.5, 70.5	69	22, 24	23	28, 26	27	27.5, 26.5	27	30, 32	31
Yelta	79, 80	78	24.5, 25.5	25	29.5, 30.5	30	35.5, 38.5	37	45, 45	45

Individual Fractions	Gluten	Ave.	Starch	Ave.	Water solubles	Ave.
Annuello	49, 51	50	3, 3	3	0, 0	0
Yelta	56.5, 57.5	57	4, 6	5	0, 0	0

Bran	0% gluten	Ave.	5% gluten	Ave.	10% gluten	Ave.	15% gluten	Ave.
Annuello + bran	10, 11	10.5	18, 20	19	22, 24	23	42.5, 43.5	43
Yelta + bran	10, 11	10.5	20, 20	20	27.5, 28.5	28	45, 47	46
Annuello - bran	3.5, 4	3.75	18.5, 17.5	18	27, 27	27	32, 34	33
Yelta - bran	6.5, 7	6.75	17, 17	17	29.5, 30.5	30	45.5, 46.5	46

Effect of Bond Types on Sedimentation Behaviour

Rosella, QAL2000, Sunstate, SuncoPBI, Banks, Sunbrook – Australian wheat varieties

Meal Treatment	Concentration	Rosella	QAL2000	Sunstate	Sunco PBI	Banks	Sunbrook
Control		48, 49	50, 52	74, 74	83, 84	79.5, 78.5	74, 76
	Ave.	48.5	51	74	83.5	79	76
L-Cysteine	0.01M	30, 32	17.5, 18.5	34.5, 37.5	36, 37	44, 43	41.5, 43.5
	Ave.	31	18	36	36.5	43.5	42.5
	0.1M	12, 12	13.5, 13.5	20, 22	21, 22	22, 23	32, 34
	Ave.	12	13.25	21	21.5	22.5	33
Ammonium Chloride	0.1M	45, 47	41.5, 41	58, 58.5	70, 70	55, 56	58.5, 58
	Ave.	46	41.25	58.25	70	55.75	58.25
	1.0M	24.5, 25.5	25.5, 26	27, 30	28, 28	28, 30	28.5, 27.5
	Ave.	25	25.75	28.5	28	29	28
Urea	0.5M	46, 47	48, 51	77, 76	81, 82	80, 81	77, 76
	Ave.	46.5	49.5	76.5	81.5	80.5	76.5
	1.0M	49, 50	55, 54	76.5, 76	82, 81	77, 79	72.5, 73.5
	Ave.	49.5	54.5	76.25	81.5	78	73
Temperature	45C	37.5, 36.5	43, 42	75, 77	92, 91	77, 76.5	83, 83
	Ave.	37	42.5	76	91.5	76.75	83
CTAB	10mM	44, 46	52.5, 51.5	77, 79	90, 90	67, 68	79, 79
	Ave.	45	52	78	90	67.5	79
Flour							
Control		55, 55.5	52.5, 52	68, 70	81, 81	77, 76	74.5, 75.5
	Ave.	55.25	52.25	69	81	76.5	75
L-Cysteine	0.01M	7, 9	9, 9	17.5, 18	15.5, 15	13, 14	25, 24
	Ave.	8	9	17.75	15.25	13.5	24.5
	0.1M	8, 9	6.5, 6.5	11, 12	10, 10.5	11.5, 12	12, 11.5
	Ave.	8.5	6.5	11.5	10.25	11.75	11.75
Ammonium Chloride	0.1M	34.5, 34	21, 21.5	33, 33	38, 40	32, 35	32, 32
	Ave.	34.25	21.25	33	39	33.5	32
	1.0M	15, 17	11, 11	18, 19	18, 20	17, 17	17, 18
	Ave.	16	11	18.5	19	17	17.5
Urea	0.5M	53.5, 53	51, 51	70.5, 69.5	81, 81.5	77, 78	76.5, 76
	Ave.	53.25	51	70	81.25	77.5	76.25
	1.0M	50, 52	51, 51	71, 70	82, 81	73, 73.5	76, 77
	Ave.	51	51	70.5	81.5	73.25	76.5
Temperature	45C	52.5, 53.5	51, 53	70, 70	81, 82	73, 73	69, 68
	Ave.	53	52	70	81.5	73	68.5
CTAB	10mM	55.5, 56.5	48, 48	63, 63	78, 80	71.5, 70.5	66, 66
	Ave.	56	48	63	79	71	66

Protein Composition Throughout the Sediment

Rosella, Banks and Sunstate – Australian wheat varieties, UPP – Unextractable polymeric protein (analysis done with SE-HPLC)

		Rosella	Banks	Sunstate
Base of floc	%UPP1	50.4	57.9	77.1
	%UPP2	55	63.9	78.5
	Ave.	52.7	60.9	77.8
Centre of floc	%UPP1	59.3	49.7	74.2
	%UPP2	56.9	53.1	67.2
	Ave.	58.1	51.4	70.7
Top of floc	%UPP1	69.9	47.5	57.3
	%UPP2	75.3	41.3	63.7
	Ave.	72.6	44.4	60.5

Appendix D

Effects of HMW-GS alleles in a biscuit wheat background

Entry No. – Numbering system; F – Flour; FAR – Farinograph, EXT – Extensogrph, KR – Keiffer Rig;

Entry No	Glu-1 pattern	Glu-A1	Glu-B1	Glu-D1	Glu-A3	Glu-B3	Glu-D3	Meal Protein	Meal Moisture	Meal SDS-SV
100	+ + +	2*	7+8	2+12	f	d	A	7.869	11.824	23
88	+ + +	2*	7+8	2+12	f	d	A	7.88	11.055	12.75
36	+ + -	2*	7+8	N	f	d	A	9.033	11.083	12.5
40	+ + -	2*	7+8	N	f	d	A	9.113	11.017	16
36*	+ + -	2*	7+8	N	f	d	A	10.073	11.789	20.5
6*	+ + -	2*	7+8	N	f	d	A	9.438	13.777	12
2*	- + +	N	7+8	2+12	f	d	A	9.109	12.011	32.5
43	- + +	N	7+8	2+12	f	d	A	8.075	11.203	19
5	- + -	N	7+8	N	f	d	A	8.335	12.099	13
7*	- + -	N	7+8	N	f	d	A	9.47	12.985	10
31	- + -	N	7+8	N	f	d	A	8.735	10.99	15

Entry No	Meal PQV	F-Protein	F-Moisture	Leco Protein	Zeleny-SV	Zeleny-PQV	UPP-Whole	UPP-Flour	FAR Development Time	FAR Water Absorbtion
100	2.92	6.437	15.828	6.67	15.5	2.32	33.6	39.9	3	52.35
88	1.62	6.329	15.299	6.55	13	1.98	26.3	29.1	0.77	50.5
36	1.38	7.24	16.207	7.83	11	1.4	26.4	19.5	0.85	45.85
40	1.76	7.346	15.467	7.72	6	0.78	22.8	15.4	0.52	44.28
36*	2.04	8.001	12.773	9.07	13	1.43	44.3	24.4	0.77	52.63
6*	1.27	8.323	12.08	9.04	11	1.22	18.9	36.2	0.85	46.63
2*	3.57	6.853	13.162	8.21	18	2.19	56.2	36.2	0.55	46.95
43	2.17	6.005	15.948	6.65	9	1.35	27.7	25.8	0.59	44.88
5	1.56	6.672	14.731	7	5.5	0.79	14.8	12.3	0.55	48.23
7*	1.06	8.124	12.457	9.33	10	1.07	20.3	18.1	1.54	55.95
31	1.72	6.395	15.755	7	7	1	13.5	16.1	0.96	49.9

Entry No	FAR Stability	EXT Extensibility	EXT Resistance	KR Peak Force	KR Ext(mm)	DS Max Force	DS Area above curve	DS Distance on x-axis
100	1.57	15.1	9.1	13.2	50.2	28.9	1.622	0.125
88	0.87	16.4	8.2	8.3	85.6	14.5	0.5688	0.11
36	1.21	16.9	6.5	14.9	82.5	4.9	0.1345	0.05
40	0.55	14.3	7.9	8.35	67.5	22.6	1.23	0.115
36*	1.06	11.8	5.1	6.7	30	41.4	6.903	0.48
6*	1.5	6.6	3.5	6.75	30.1	29.8	2.385	0.185
2*	0.55	12.9	6.25	12.6	36.7	31.7	2.625	0.175
43	0.4	14.1	6.75	8.7	50.8	26.2	2.245	0.175
5	0.4	11.2	5.1	6.9	57.2	24	2.138	0.19
7*	1.83	9.5	6.5	10.8	41.6	39.3	5.684	0.3
31	1.35	12.5	7.8	7.5	55	26.2	2.405	0.21

Identification of QTL for sedimentation volume and protein quality value

J – Numbering system; SV - Sedimentation volume; PQV – Protein quality volume; 1, 2 – replicate samples

			2003-2004					2004-2005				
ID	REP.	Plot No	1	2	Ave SV	Protein %	PQV	1	2	Ave SV	Protein %	PQV
J-014	2	1212	63	64	63.5	11.5	5.52	62	63	62.5	10.5	5.95
J-014	1	421	64	62	63	11.9	5.29	65	67	66	11.0	6.00
J-027	1	121	88	86	87	12.9	6.74	81	79	80	13.9	5.76
J-027	2	702	85	85	85	13.4	6.34	72	75	73.25	10.2	7.18
J-031	1	418	87	87	87	11.6	7.50	76	75	75.5	9.6	7.86
J-031	2	1117	87	85	86	12.8	6.72	66	69	67.5	8.9	7.58
J-040	1	231	59	59	59	13.6	4.34	62.5	60	61.25	13.4	4.57
J-040	2	1129	53	53	53	13.9	3.81	50	52	51	9.1	5.60
J-044	2	1127	67	69	68	11.4	5.97	57	56	56.5	8.4	6.73
J-044	1	305	64	64	64	11.7	5.47	55	60	57.5	10.0	5.75
J-054	1	615	74	74	74	13.1	5.65	59	58	58.5	10.4	5.63
J-054	2	1020	68	69	68.5	12.5	5.23	52	52	52	8.2	6.27
J-082	2	703	74	74	74	12.2	6.07	62	60	61	9.2	6.63
J-082	1	124	76	76	76	11.9	6.39	63.5	63	63.25	10.4	6.08
J-083	1	525	89	90	89.5	11.7	7.65	71	71	71	12.0	5.92
J-083	2	713	91	90	90.5	11	8.23	65	66	65.5	8.6	7.62
J-095	2	1009	73	73	73	11.9	6.13	67	69	68	9.7	7.01
J-095	1	321	81	81	81	10.7	7.57	63	62	62.5	8.9	7.02
J-103	1	215	94	94	94	12.5	7.52	79	80	79.5	12.0	6.63
J-103	2	1010	90	90	90	12.8	7.03	80	84	82	10.3	7.96
J-117	1	205	64	64	64	11.7	5.47	66	65	65.5	12.4	5.28
J-117	2	1132	77	78	77.5	11.7	6.62	58.5	59	58.5	10.4	5.63
J-130	2	1021	82	82	82	13.1	6.26	61	59	60	8.3	7.23
J-130	1	222	80	80	80	12.7	6.30	70	69	69.5	10.8	6.44
J-132	1	614	94	93	93.5	12.8	7.31	57	56	56.5	9.8	5.77
J-132	2	1113	83	84	83.5	11.8	7.08	49	48	48.5	6.7	7.24
J-136	1	325	86	85	85.5	12	7.13	60	62	62	10.0	6.20
J-136	2	1224	87	87	87	11	7.91	61	62	61.5	8.9	6.91
J-161	1	624	82	85	83.5	12.6	6.63	62	59	60.5	10.6	5.71
J-161	2	1227	83	83	83	11.6	7.16	58	58	58	8.3	6.99
J-165	1	317	79	80	79.5	10.6	7.50	62	60	60.75	10.6	5.73
J-165	2	921	71	71	71	12.3	5.77	64	66	64.75	10.7	6.05
J-172	1	310	82	81	81.5	12.1	6.74	66	66	66	11.5	5.74
J-172	2	1128	83	86	84.5	11.8	7.16	54	53	53.5	7.6	7.04
J-188	1	324	95	96	95.5	12.1	7.89	86	86	86	11.5	7.48
J-188	2	1202	96	95	95.5	14.4	6.63	72	75	73.5	8.2	8.96
J-203	2	910	69	70	69.5	12.2	5.70	76	72	74	12.5	5.92
J-203	1	505	81	80	80.5	13.8	5.83	70	71	70.5	12.5	5.64
J-209	2	932	85	85	85	13.5	6.30	60.5	59	59.75	11.4	5.24
J-209	1	209	77	77	77	11.8	6.53	65.5	63	64.25	8.5	7.56
J-236	1	217	87	87	87	11.7	7.44	76	74	74.75	11.2	6.67
J-236	2	813	81	82	81.5	11.5	7.09	66	66	66	8.5	7.76
J-239	2	1007	84	85	84.5	11.2	7.55	64	64	64	9.0	7.11
J-239	1	632	80	80	80	12.9	7.20	68	65	66.25	9.6	6.90
J-241	1	327	84	83	83.5	12.3	6.79	79	79	79	10.3	7.67
J-241	2	1216	75	74	74.5	13.3	5.60	70	65	67.5	10.0	6.75
J-244	2	1210	74	75	74.5	12.6	5.91	57	58	57.5	8.2	7.01

J-244	1	602	71	70	70.5	13	5.42	60	61	60.25	10.4	5.79
J-252	2	710	83	83	83	12.5	6.64	60	59	59.5	7.8	7.63
J-252	1	203	90	94	92	11.8	7.80	77	78	77.5	11.8	6.57
J-253	2	1115	84	84	84	11.6	7.24	66	62	64	10.8	5.93
J-253	1	407	89	89	89	13.4	6.64	63	61	62	9.3	6.67
J-274	2	920	92	92	92	14.2	6.48	61	61	61	10.8	5.65
J-274	1	113	89	90	89.5	13.4	6.68	63	65	64	10.4	6.15
J-279	1	531	76	78	77	12.3	6.26	62	60	61	9.1	6.70
J-279	2	1024	79	81	80	11.1	7.21	60	60	60	9.4	6.38
J-298	2	1124	74	71	72.5	13.2	5.49	65	67	66	12.6	5.24
J-298	1	314	71	68	69.5	12.1	5.74	65.5	66	65.75	12.1	5.43
J-309	1	131	85	85	85	12	7.08	63	63	63	11.2	5.63
J-309	2	905	77	75	76	13.2	5.76	65	67	66	10.2	6.47
Janz	2	931	75	74	74.5	11.3	6.59	75	74	74.5	11.3	6.59
Janz	1	507	72	71	71.5	10.8	6.62	72	71	71.5	10.8	6.62
Kukri	1	207	89	87	88	11.6	7.59	89	87	88	11.6	7.59
Kukri	2	517	78	79	78.5	12.7	6.18	78	79	78.5	12.7	6.18

Effects of Heat-shock Versus Genotype for a Range of Quality Characteristics at Two Sites (Site A, Site B)

1-35 – Numbering system of single varieties

SITE A		**HMW-GS**		**NIR Control**		**NIR Heat shocked**		**SDS-sedimentation**	
	Glu-A1	Glu-B1	Glu-D1	Protein	Moisture	Protein	Moisture	Control 1	Control 2
35. Schomborsk	1.00	7+8, 7+9	2+12, 5+10	11.69	15.61	14.11	15.31	85.00	85.00
39. 6385			5+10	14.73	14.77	15.79	14.53	89.00	89.00
18. WW80			2+12	10.39	15.00	15.36	15.92	79.00	80.00
24. Machete	2*	17+18	2+12	11.17	16.17	15.04	14.73	74.00	76.00
06. Kite	2*	17+18	2+12	12.16	15.27	14.96	14.72	73.00	73.00
04. Vulcan	1.00	17+18	2+12	11.11	15.44	15.17	15.24	75.00	73.00
09. Oxley	2*	7+8	2+12	10.27	14.59	14.92	14.50	67.00	67.00
34. Kulin	2*	13+16, 17+18	2+12	10.95	15.11	13.08	14.61	90.00	89.00
42. ME71			5+10	11.82	15.40	12.38	14.07	70.00	71.00
49. Dollarbird	1.00	17+18	5+10	12.82	14.98	15.63	14.71	79.00	78.00
28. Ella			5+10	15.97	14.97	17.92	13.68	71.00	71.00
12. Tatiara	1.00	7+9	2+12	10.09	15.63	11.61	12.64	73.00	74.00
36. Janz	1.00	7+8	2+12	10.31	16.04	12.14	14.80	72.00	71.00
30. Kogat			5+10	14.14	15.20	16.31	14.93	63.00	n/a
08. Meering	2*	7+8	2+12	11.07	15.00	12.29	14.77	88.00	87.00
32. Scandia			2+12	14.20	14.86	16.39	14.07	83.00	83.00
48. Trigo			2+12	12.28	14.99	13.59	15.01	90.00	91.00
02. Hartog	1.00	17+18	5+10	11.57	15.31	14.50	14.47	67.00	65.00
03. Sunco	1.00	7+8	2+12	10.99	15.72	12.91	15.89	83.00	84.00
47. Lyallpur			5+10	13.99	15.14	15.28	14.24	79.00	79.00
38. 6384			2+12	11.70	14.93	14.57	14.45	78.00	78.00
25. Oligo			2+12	13.74	14.79	15.78	13.88	75.00	77.00
14. Molineux	1.00	7+8	5+10	13.50	14.42	14.47	14.65	71.00	71.00
16. Tincurrin	2*	7+8	2+12	9.96	14.99	11.48	15.94	63.00	64.00
33. Veery	1.00	7+9	5+10	12.29	14.77	14.93	14.47	69.00	70.00
31. Croesus			2+12	11.64	15.63	13.61	13.93	59.00	n/a
15. Dagger	1.00	7+9	5+10	12.24	15.33	11.95	15.08	67.00	67.00
43. Banks	2*	7+8	2+12	12.12	14.71	12.62	14.80	80.00	81.00
17.	1.00	7+8	2+12	11.70	14.96	14.66	14.88	78.00	79.00

Cunningham									
23. Kamilaroi	null	20.00		14.33	15.54	16.27	13.97	35.00	n/a
07. Condor	2*, null	7+8	2+12	11.17	15.03	13.20	14.81	79.00	77.00
13. Aroona	1.00	7+8, 7+9	2+12	14.79	15.19	14.89	14.55	79.00	76.00
50. Fang			5+10	9.58	14.81	10.69	14.85	52.00	52.00
11. Halberd	1.00	20, 7+9	5+10	10.20	14.86	10.60	15.14	48.00	49.00
46. Millewa	null	17+18	2+12	10.70	15.29	11.99	15.13	40.00	43.00
21. Miskle	2*	17+18, 7+8	2+12	11.43	15.53	13.78	15.39	69.00	70.00

SITE A	Control Ave	Control PQV	HS 1	HS 2	HS Ave	HS PQV	Control PQV - HS PQV	HS PQV - Control PQV
35. Schomborsk	85	7.2693064	75	n/a	75	5.3168864	1.95242	-1.95242
39. 6385	89	6.0425012	67	68	67.5	4.2748575	1.7676437	-1.7676437
18. WW80	79.5	7.6515881	90	91	90.5	5.89116	1.7604281	-1.7604281
24. Machete	75	6.7168189	75	77	76	5.0545358	1.6622831	-1.6622831
06. Kite	73	6.0018088	65	65	65	4.3449198	1.656889	-1.656889
04. Vulcan	74	6.6618653	77	75	76	5.0085673	1.653298	-1.653298
09. Oxley	67	6.5213159	73	73	73	4.8940735	1.6272425	-1.6272425
34. Kulin	89.5	8.1765028	87	87	87	6.652902	1.5236008	-1.5236008
42. ME71	70.5	5.9629536	56	56	56	4.5241558	1.4387978	-1.4387978
49. Dollarbird	78.5	6.1218124	75	75	75	4.7972368	1.3245756	-1.3245756
28. Ella	71	4.4447227	56	n/a	56	3.1243026	1.3204201	-1.3204201
12. Tatiara	73.5	7.28444	69	71	70	6.0313631	1.253077	-1.253077
36. Janz	71.5	6.9356873	69	69	69	5.6827541	1.2529332	-1.2529332
30. Kogat	63	4.4560758	53	n/a	53	3.2499387	1.2061371	-1.2061371
08. Meering	87.5	7.9013906	83	82	82.5	6.7138672	1.1875235	-1.1875235
32. Scandia	83	5.8442473	77	76	76.5	4.6683347	1.1759126	-1.1759126
48. Trigo	90.5	7.3727088	85	84	84.5	6.2168923	1.1558165	-1.1558165
02. Hartog	66	5.7053942	66	66	66	4.5517241	1.1536701	-1.1536701
03. Sunco	83.5	7.5971249	84	85	84.5	6.5463279	1.0507971	-1.0507971
47. Lyallpur	79	5.646487	70	71	70.5	4.6150825	1.0314045	-1.0314045
38. 6384	78	6.6683765	84	83	83.5	5.7301674	0.9382091	-0.9382091
25. Oligo	76	5.5325035	73	n/a	73	4.6264022	0.9061013	-0.9061013
14. Molineux	71	5.2612079	63	63	63	4.3553405	0.9058674	-0.9058674
16. Tincurrin	63.5	6.374862	64	65	64.5	5.620915	0.7539469	-0.7539469
33. Veery	69.5	5.6550041	74	75	74.5	4.990956	0.6640481	-0.6640481
31. Croesus	59	5.0709067	60	n/a	60	4.4088471	0.6620597	-0.6620597
15. Dagger	67	5.472068	57	58	57.5	4.8117155	0.6603525	-0.6603525
43. Banks	80.5	6.6446554	76	76	76	6.0240964	0.620559	-0.620559
17. Cunningham	78.5	6.7105488	90	90	90	6.1378981	0.5726507	-0.5726507
23. Kamilaroi	35	2.4417469	32	n/a	32	1.9671728	0.4745741	-0.4745741
07. Condor	78	6.9836154	87	87	87	6.5904098	0.3932055	-0.3932055
13. Aroona	77.5	5.2407357	73	74	73.5	4.9365303	0.3042054	-0.3042054
50. Fang	52	5.4257095	55	55	55	5.1459581	0.2797514	-0.2797514
11. Halberd	48.5	4.7563009	49	49	49	4.6208978	0.1354031	-0.1354031
46. Millewa	41.5	3.8803179	47	47	47	3.9192795	-0.0389616	0.0389616
21. Miskle	69.5	6.0815541	86	85	85.5	6.2028439	-0.1212898	0.1212898

SITE B	HMW-GS	NIR Control	NIR Heat schocked	SDS-sedimentation

	Glu-A1	Glu-B1	Glu-D1	Protein	Moisture	Protein	Moisture	Control 1	Control 2
03. Sunco	1.00	7+8	2+12	10.53	15.79	13.85	14.76	73.00	73.00
04. Vulcan	1.00	17+18	2+12	10.71	15.24	13.99	14.08	67.00	67.00
06. Kite	2*	17+18	2+12	12.78	15.09	14.55	14.93	79.00	79.00
07. Condor	2*, null	7+8	2+12	10.79	15.69	15.09	14.47	79.00	79.00
08. Meering	2*	7+8	2+12	10.54	14.49	14.60	14.63	75.00	75.00
09. Oxley	2*	7+8	2+12	10.81	14.45	15.49	14.47	68.00	68.00
12. Tatiara	1.00	7+9	2+12	11.61	12.69	12.05	15.68	71.00	72.00
13. Aroona	1.00	7+8, 7+9	2+12	13.00	14.99	14.79	14.62	77.00	78.00
16. Tincurrin	2*	7+8	2+12	10.04	14.96	10.73	14.99	66.00	68.00
17. Cunningham	1.00	7+8	2+12	11.10	15.17	13.68	15.13	77.00	78.00
18. WW80			2+12	11.31	16.44	15.16	14.46	86.00	n/a
21. Miskle	2*	17+18, 7+8	2+12	11.30	14.85	15.20	14.55	78.00	78.00
24. Machete	2*	17+18	2+12	10.98	14.73	15.01	14.36	76.00	77.00
25. Oligo			2+12	13.57	14.89	16.00	14.44	76.00	76.00
31. Croesus			2+12	11.54	15.23	14.85	15.17	63.00	n/a
32. Scandia			2+12	14.95	14.25	16.23	13.79	77.00	76.00
34. Kulin	2*	13+16, 17+18	2+12	10.68	15.22	13.50	14.75	89.00	89.00
36. Janz	1.00	7+8	2+12	10.10	15.29	12.24	14.59	74.00	75.00
38. 6384			2+12	13.02	14.46	15.04	15.69	86.00	87.00
43. Banks	2*	7+8	2+12	12.28	14.40	14.68	14.42	76.00	75.00
46. Millewa	null	17+18	2+12	10.33	15.43	11.68	15.04	45.00	45.00
48. Trigo			2+12	12.66	15.05	13.18	14.86	94.00	94.00
35. Schomborsk	1.00	7+8, 7+9	2+12, 5+10	11.45	15.30	14.49	15.69	77.00	77.00
02. Hartog	1.00	17+18	5+10	11.39	15.39	13.75	13.37	64.00	64.00
11. Halberd	1.00	20, 7+9	5+10	9.54	15.22	10.30	15.08	45.00	45.00
14. Molineux	1.00	7+8	5+10	12.88	14.80	14.50	14.53	69.00	69.00
15. Dagger	1.00	7+9	5+10	12.01	15.01	12.09	15.09	64.00	64.00
28. Ella			5+10	16.10	14.88	15.64	16.00	66.00	n/a
30. Kogat			5+10	14.38	14.86	15.80	15.73	63.00	n/a
33. Veery	1.00	7+9	5+10	12.46	14.87	14.72	15.27	75.00	75.00
39. 6385			5+10	15.07	14.62	15.34	14.09	71.00	71.00
42. ME71			5+10	12.52	15.63	13.69	13.57	66.00	66.00
47. Lyallpur			5+10	14.69	14.47	15.01	14.49	74.00	75.00
49. Dollarbird	1.00	17+18	5+10	12.17	15.43	15.67	13.83	69.00	68.00
50. Fang			5+10	10.00	14.82	11.33	15.36	54.00	55.00
23. Kamilaroi	null	20.00		14.55	15.11	16.06	13.63	29.00	n/a

SITE B

	Control Ave	Control PQV	HS 1	HS 2	HS Ave	HS PQV	Control PQV - HS PQV
03. Sunco	73	6.9345493	85	85	85	6.136298	0.7982512
04. Vulcan	67	6.2575885	70	71	70.5	5.0400343	1.2175542
06. Kite	79	6.18105	71	70	70.5	4.8446949	1.3363551
07. Condor	79	7.3209156	86	85	85.5	5.6663795	1.6545361
08. Meering	75	7.1157495	90	91	90.5	6.1999041	0.9158454
09. Oxley	68	6.2898899	86	86	86	5.551969	0.7379209
12. Tatiara	71.5	6.156363	72	72	72	5.9765917	0.1797713
13. Aroona	77.5	5.9633733	71	71	71	4.8011902	1.1621832
16. Tincurrin	67	6.6759665	59	60	59.5	5.5436504	1.1323161
17. Cunningham	77.5	6.982611	89	n/a	89	6.5063236	0.4762875
18. WW80	86	7.6045627	90	90	90	5.9351095	1.6694533
21. Miskle	78	6.9008228	89	90	89.5	5.8897078	1.011115

24. Machete	76.5	6.96531	79	80	79.5	5.2971748	1.6681352
25. Oligo	76	5.5989391	78	77	77.5	4.8449612	0.7539779
31. Croesus	63	5.4587991	27	n/a	27	1.8181818	3.6406172
32. Scandia	76.5	5.1180839	78	79	78.5	4.8355304	0.2825535
34. Kulin	89	8.3309932	87	89	88	6.5165877	1.8144055
36. Janz	74.5	7.3755074	66	66	66	5.3943604	1.9811469
38. 6384	86.5	6.643115	87	91	89	5.9191274	0.7239875
43. Banks	75.5	6.1482085	80	81	80.5	5.4851458	0.6630627
46. Millewa	45	4.3562439	54	53	53.5	4.5820486	-0.2258047
48. Trigo	94	7.4273072	84	83	83.5	6.334876	1.0924312
35. Schomborsk	77	6.7266533	79	n/a	79	5.4512835	1.2753698
02. Hartog	64	5.6194574	61	62	61.5	4.472402	1.1470554
11. Halberd	45	4.7150042	48	49	48.5	4.7096524	0.0053518
14. Molineux	69	5.3563111	57	57	57	3.9321192	1.4241919
15. Dagger	64	5.3284489	62	64	63	5.2100562	0.1183927
28. Ella	66	4.0986152	53	n/a	53	3.3889635	0.7096517
30. Kogat	63	4.381999	60	n/a	60	3.7977087	0.5842903
33. Veery	75	6.0182956	71	72	71.5	4.8563472	1.1619484
39. 6385	71	4.7122851	56	57	56.6	3.6892191	1.023066
42. ME71	66	5.2715655	47	47	47	3.4336645	1.837901
47. Lyallpur	74.5	5.0704417	75	78	76.5	5.0979608	-0.0275191
49. Dollarbird	68.5	5.6276701	60	n/a	60	3.8292169	1.7984531
50. Fang	54.5	5.4521809	58	60	59	5.2083333	0.2438475
23. Kamilaroi	29	1.9928532	31	n/a	31	1.929901	0.0629522

Effect of sprouting on varieties differing in end-product use

	FN1	FN2	FN3	FN Ave	SV1	SV2	SV3	SV4	SV Ave.
Rosella	355	323	333	337	44	45	45	44	44.5
	226	210	212	216	44.5	45	44	45.5	45
	88	76	76	80	49.5	51	50.5	50	50.25
Sunstate	572	594	601	589	74.5	77	76	76.5	76
	237	260	256	251	80	84	82	82	82
	149	122	143	138	88	88.5	87.5	88	88
Sunco	622	666	662	650	95.5	96	94.5	94	95
	152	189	166	169	97	97	98.5	95.5	97
	97	106	94	99	96.5	96	97	94.5	96

Effects of Long Term Storage Conditions on Sedimentation Testing at 4°C, ambient temperature and 40°C

MEAL	Original SV	Ave.	SV-4	Ave.	SV-Ambient	Ave.	SV-40	Ave.
QAL2000	51, 52	51.50	42, 43	42.50	48.5, 48	48.25	38, 40	39.00
Rosella	45, 45.5	45.25	41, 43	42.00	38, 38.5	38.25	33, 33	33.00
Sunstate	73, 74	73.50	61, 61.5	61.25	66, 66	66.00	61, 62	61.50
Banks	74.5, 74	74.25	67.5, 68.5	68.00	67.5, 67	67.25	62, 62	62.00
SuncoPBI	80, 80	80.00	73, 74	73.50	66, 65	65.50	58.5, 58	58.25
Sunco	95.5, 95	95.25	83.5, 83	83.25	72, 72.5	72.25	70, 70	70.00
Sunbrook	77.5, 78	77.75	63, 65	64.00	65, 65	65.00	60.5, 62.5	61.50

GRAIN	Original SV	Ave.	SV-4	Ave.	SV-Ambient	Ave.	SV-40	Ave.
QAL2000	50.5, 52.5	51.50	48.5, 49.5	49.00	51.5, 52	51.75	40, 41	40.50
Rosella	45.5, 45	45.25	31.5, 33.5	32.50	41, 40	40.50	28, 30	29.00
Sunstate	73, 74	73.50	73, 72	72.50	65.5, 63.5	64.50	57.5, 56.5	57.00
Banks	74, 74.5	74.25	69, 71	70.00	74, 73	73.50	56, 58	57.00
SuncoPBI	80, 80	80.00	73.5, 72.5	73.00	72, 73	72.50	69.5, 69	69.25
Sunco	95.5, 95	95.25	81, 80	80.50	72 ,72	72.00	71.5, 72.5	72.00
Sunbrook	77.5, 78	77.75	67, 67	67.00	71, 72	71.50	61, 63	62.00

FLOUR	Original SV	Ave.	SV-4	Ave.	SV-Ambient	Ave.	SV-40	Ave.
QAL2000	52, 54	53.00	50, 53	51.50	49, 50	49.50	45, 47	46.00
Rosella	55, 55	55.00	55, 55	55.00	52, 52.5	52.25	53, 53	53.00
Sunstate	75, 74.5	74.75	68.5, 67.5	68.00	59, 60.5	59.75	48, 48.5	48.25
Banks	82, 84	83.00	73, 75	74.00	65.5, 65	65.25	52.5, 53	52.75
SuncoPBI	88, 88.5	88.25	78, 80	79.00	73, 75	74.00	58, 58.5	58.25
Sunco	86, 87	86.50	77.5, 77	77.25	70.5, 71.5	71.00	48, 50	49.00
Sunbrook	84.5, 84	84.25	73, 73	73.00	61.5, 61	61.25	52, 53.5	52.75

Appendix E

SDS sedimentation versus dough quality and SIG – 2004

Sample No.	Wheat	Variety	SV ave	Wheat NIR Protein	Oven Moisture	Wheat %UPP	PQV	SIG test	E 45	R 45
NM417	Hard	Janz	86	13.61	9.01	53.20	6.32	4.58	20.2	435
NM418	Hard	Sun 376G	72.5	13.31	9.25	54.49	5.45	4.51	17.4	420
NM419	Hard	Sun 421T	82	13.80	9.23	58.66	5.94	4.57	16.6	430
NM420	Hard	Sun 421K	87.5	13.93	9.32	61.93	6.28	4.68	18.7	430
NM421	Hard	B406F-2	83.5	13.00	9.48	54.62	6.42	4.51	19.9	410
NM422	Hard	Sun 392A	81.5	14.47	9.22	56.22	5.63	4.48	18.8	470
NM423	Hard	Diamonbird	86	14.35	9.44	56.69	5.99	4.86	18	520
NM424	Hard	Sun 389A	78.25	13.74	9.29	58.62	5.69	4.8	19.3	520
NM425	Hard	Batavia	72	12.80	10.56	48.00	5.63	4.22	17.5	400
NM426	Hard	Ellison	69.5	13.13	10.81	60.87	5.30	4.56	18.6	430
NM427	Hard	Baxter	77.5	13.54	10.80	59.52	5.73	4.57	17.2	380
NM428	Hard	Carnamah	72.5	12.52	10.89	54.74	5.79	4.44	20.5	370
NM429	Hard	Sun 376G	66.5	12.07	10.25	52.56	5.51	4.3	16.5	380
NM430	Hard	Sun 421T	71	11.49	10.78	55.79	6.18	4.09	17.1	385
NM431	Hard	Janz	73	13.08	10.36	50.60	5.58	4.42	20.5	385
NM432	Hard	Sun 421T	72.5	13.70	10.06	54.55	5.29	4.54	19.6	370
NM433	Hard	B406F	72.5	12.72	10.02	52.61	5.70	4.14	20.7	350
NM434	Hard	Sun 421K	72	13.81	10.00	53.11	5.21	4.54	20.4	375
NM435	Hard	Sunco	77.25	13.60	10.39	53.16	5.68	4.46	20.6	450
NM436	Hard	Sun 389A	74.5	13.21	10.10	53.31	5.64	4.45	20.4	370
NM437	Hard	Sun 392A	78	13.80	10.36	58.91	5.65	4.65	20.1	415
NM438	Hard	Hartog	74.5	13.92	11.60	53.11	5.35	4.27	22.8	400
NM439	Hard	Sunco	75.5	14.55	11.20	52.16	5.19	4.6	20.2	305
NM440	Hard	Lang	72.5	14.36	11.64	52.44	5.05	4.39	23.5	285
NM441	Hard	QT 10580	77.5	14.04	11.40	55.96	5.52	4.93	20.7	330
NM442	Hard	Kennedy	77	15.34	10.98		5.02	5.09	23.8	370
NM443	Hard	Sunvale	76.25	15.00	11.59	56.18	5.08	4.94	23.2	400
NM444	Hard	Baxter	89	14.74	11.52	64.01	6.04	5.07	21.3	385
NM445	Hard	Sunco	75.5	15.20	11.51	53.80	4.97	5.03	23.3	340
NM446	Hard	QT 10776	73	13.94	11.71	53.09	5.24	4.74	20.4	495
NM447	Hard	QT 10198	78	14.57	11.77	55.45	5.35	4.65	19	460
NM448	Soft	VAW32	28.5	9.30	11.10	42.46	3.06	n/a	14.5	165
NM449	Soft	C1064	24	9.30	10.60	38.69	2.58	n/a	9	100
NM450	Soft	Sunsoft '98	30.75	9.80	11.20	43.07	3.14	n/a	15.5	235
NM451	Soft	Lorikeet	36.25	10.00	10.80	44.83	3.63	n/a	15	315
NM452	Soft	Snipe	32	9.60	10.80	42.67	3.33	n/a	13.8	120
NM453	Soft	WW3811	34	10.00	11.00	44.61	3.40	n/a	12.3	200
NM454	Soft	C1091	29	9.60	11.40	38.65	3.02	n/a	14.2	215

NM455	Soft	WW3362	31	10.40	10.80	40.16	2.98	n/a	13.9	210
NM456	Soft	Thornbill	32.75	9.50	10.50	45.67	3.45	n/a	14.2	270
NM457	Soft	Bowie	33	10.00	11.20	40.63	3.30	n/a	14.5	195
NM458	Soft	C1092	31.75	10.20	10.90	36.31	3.11	n/a	14.6	235
NM459	Soft	VAW3	31.75	9.3	11.30	45.90	3.41	n/a	16.1	165
NM460	Soft	C1032	24	9.70	10.30	33.99	2.47	n/a	8.9	160
NM461	Soft	C1149	25	9.70	10.80	40.58	2.58	n/a	12	100
NM462	Soft	WW3363	34.75	9.80	10.90	39.71	3.55	n/a	13.8	180
NM463	Soft	WW4033-5	36.5	9.90	10.60	47.52	3.69	n/a	14.5	150
NM464	Soft	DH3358	31	9.70	10.80	38.68	3.20	n/a	14	115

Sedimentation testing versus dough quality, SIG, %UPP, Bean-propanol extraction and rapid spectrophotometric test – 2003

n=30

TM No.	Wheat protein	SV ave	PQV	E 45	R 45	SIG-SDS	SIG-SDS/LAC	%UPP	Bean-Prop	Spectrophtotmetric
TM 6873	9.30	65.75	7.07	14.5	165	1.68	1.84	42.46	56.96	2.39
TM 6874	9.30	65.25	7.02	9	100	1.54	1.69	38.69	58.19	3.59
TM 6875	9.80	50	5.1	15.5	235	1.75	2.09	43.07	55.98	2.14
TM 6876	10.00	59	5.9	15	315	1.66	2.06	44.83	49.85	2.68
TM 6877	9.60	60.5	5.87	13.8	120	1.72	2.07	42.67	53.29	2.12
TM 6878	10.00	60.5	5.82	12.3	200	2.01	2.23	44.61	53.46	2.17
TM 6879	9.60	60	6.06	14.2	215	1.71	1.97	38.65	58.33	1.95
TM 6880	10.40	71.5	6.88	13.9	210	1.42	1.69	40.16	48.64	3.55
TM 6881	9.50	74	7.47	14.2	270	1.67	1.94	45.67	48.72	2.29
TM 6882	10.00	73	6.95	14.5	195	1.65	2.06	40.63	44.86	2.96
TM 6883	10.20	67.5	6.31	14.6	235	1.70	1.94	36.31	50.58	2.88
TM 6884	9.3	64.5	6.39	16.1	165	1.64	1.84	45.90	46.94	2.27
TM 6885	9.70	68	6.94	8.9	160	1.44	1.46	33.99	55.12	2.76
TM 6886	9.70	47.75	4.92	12	100	1.62	1.64	40.58	53.35	3.77
TM 6887	9.80	61.5	6.09	13.8	180	1.87	1.97	39.71	52.92	2.45
TM 6888	9.90	65	6.43	14.5	150	1.89	2.00	47.52	53.66	3.52
TM 6889	9.70	87	8.37	14	115	1.86	1.98	38.68	51.94	3.21
TM 7009	13.61	86.00	6.32	20.2	435	3.85	4.00	53.20	65.38	1.95
TM 7010	13.31	72.50	5.45	17.4	420	3.43	3.74	54.49	61.32	2.24
TM 7011	13.80	82.00	5.94	16.6	430	3.65	3.73	58.66	61.20	1.91
TM 7012	13.93	87.50	6.28	18.7	430	3.73	3.58	61.93	61.69	1.86
TM 7013	13.00	83.50	6.42	19.9	410	3.37	3.58	55.46	61.29	2.01
TM 7014	14.47	81.50	5.63	18.8	470	3.84	3.82	56.22	63.04	1.91
TM 7015	14.35	86.00	5.99	18	520	3.57	3.91	56.69	60.74	2.19
TM 7016	13.74	78.25	5.69	19.3	520	3.65	3.75	58.62	60.96	2.07
TM 7017	12.80	72.00	5.63	17.5	400	3.41	3.25	48.00	59.81	3.02
TM 7018	13.13	69.50	5.30	18.6	430	3.85	3.96	60.87	57.84	2.19
TM 7019	13.54	77.50	5.73	17.2	380	3.66	3.81	59.52	57.34	2.09
TM 7020	12.52	72.50	5.79	20.5	370	3.52	3.58	54.74	60.38	2.68
TM 7021	12.07	66.50	5.51	16.5	380	3.38	3.60	52.56	58.96	2.36
TM 7022	11.49	71.00	6.18	17.1	385	3.24	3.41	55.79	60.7	2.08
TM 7023	13.08	73.00	5.58	20.5	385	3.63	4.02	50.60	61.2	2.09
TM 7024	13.70	72.50	5.29	19.6	370	3.77	3.87	54.55	60.4	1.92
TM 7025	12.72	72.50	5.70	20.7	350	3.11	3.38	52.61	57.8	2.02
TM 7026	13.81	72.00	5.21	20.4	375	3.45	3.71	53.11	63.3	2.12

TM 7027	13.60	77.25	5.68	20.6	450	3.92	4.00	53.16	60.4	2.21
TM 7028	13.21	74.50	5.64	20.4	370	3.91	3.62	53.31		2.10
TM 7029	13.80	78.00	5.65	20.1	415	3.59	3.69	58.91	58.4	1.99
TM 7034	13.92	74.50	5.35	22.8	400	3.72	3.88	53.11	59.8	2.51
TM 7035	14.55	75.50	5.19	20.2	305	3.72	3.89	52.16		2.89
TM 7036	14.36	72.50	5.05	23.5	285	3.77	3.94	52.44	59.7	1.93
TM 7037	14.04	77.50	5.52	20.7	330	3.66	3.55	55.96	59.6	2.67
TM 7042	15.00	76.25	5.08	23.2	400	3.90	4.07	56.18	58.2	1.77
TM 7043	14.74	89.00	6.04	21.3	385	4.02	4.22	64.01	56.4	1.72
TM 7044	15.20	75.50	4.97	23.3	340	4.02	4.24	53.80	56.7	1.85
TM 7045	13.94	73.00	5.24	20.4	495	3.73	3.67	53.09	58.6	2.05
TM 7046	14.57	78.00	5.35	19	460	3.96	3.82	55.45	57.3	1.86

Sedimentation testing versus dough quality, SIG, %UPP and Bean-propanol extraction – 2005

DC No.	Protein	SV	PQV	Ext	Rmax	SIGSDS	SIGS-LAC	UPP	Bean
7646	9.40	42	4.47	15.4	150	2.13	1.86	39.92	55.1
7647	9.93	52	5.24	16.9	290	2.54	2.45	49.78	56.6
7648	10.41	53	5.09	16.2	240	2.54	2.39	48.23	57.9
7649	9.78	40.5	4.14	17.8	150	2.13	2.01	38.06	60.5
7650	11.30	62.5	5.53	15.4	300	3.22	2.67	51.60	61.2
7652	9.73	40	4.11	16.1	170	2.59	1.90	39.34	57.1
7653	10.94	55	5.03	19.7	300	2.69	2.51	46.69	55.9
7654	9.62	42.5	4.42	15.6	280	2.70	2.37	43.52	62.8
7655	9.26	40.5	4.38	13.6	170	2.07	1.82	37.43	58.0
7656	8.49	34.5	4.06	14.7	185	2.00	1.76	39.78	58.5
7657	8.08	34.5	4.27	13.7	160	2.24	1.80	38.85	59.5
7658	8.23	36	4.37	13.9	150	2.08	1.82	42.03	62.1
7659	9.23	37	4.01	16.1	140	2.02	1.80	37.50	58.3
7800	10.30	40.50	4.05	12.8	145	2.49	2.32	33.95	44.79
7801	10.00	37.50	3.91	13.0	150	2.59	2.31	34.12	48.43
7802	9.90	30.50	3.21	11.7	90	1.97	1.97	24.98	50.84
7803	9.30	41.50	4.54	13.0	155	2.27	2.03	26.85	51.92
7804	10.60	50.00	4.40	15.8	210	2.82	2.66	26.59	53.47
7805	10.10	51.50	4.76	14.5	230	2.17	2.41	34.57	45.60
7806	9.90	51.00	4.67	15.0	190	2.01	2.27	31.44	57.73
7808	9.20	41.50	4.56	13.8	140	1.62	2.00	29.28	42.44
7809	9.50	39.50	4.14	11.8	140	1.73	2.01	23.17	38.36
7810	10.30	60.00	5.18	15.9	205	2.17	2.40	23.27	45.54
7823	11.90	66.00	5.58	16.8	140	1.97	2.17	31.01	49.3
7824	13.50	66.50	4.75	17.4	250	2.97	3.52	42.98	52.1
7825	12.20	71.00	5.80	15.5	150	2.29	2.49	31.60	52.0
7826	13.00	71.00	5.45	16.4	205	2.14	2.52	31.72	54.8
7827	13.80	76.00	6.68	22.0	310	2.61	2.53	38.80	52.5
7828	12.40	67.00	5.49	19.7	215	2.41	2.53	32.14	51.9
7829	13.30	68.00	5.27	18.8	110	2.54	2.87	32.41	49.8
7830	13.80	89.00	6.42	22.8	310	3.50	3.48	42.30	53.3
7831	13.30	72.50	5.64	19.3	180	2.35	1.37	31.33	50.5
7832	13.30	75.00	5.99	19.3	140	2.35	2.33	32.63	53.3
7833	11.90	66.50	5.34	13.2	100	2.08	2.17	25.89	45.7
7834	13.50	67.50	5.23	18.0	250	3.19	3.21	40.18	51.6

7835	11.50	72.50	6.34	15.0	170	2.43	2.71	31.90	52.2
7836	14.80	88.00	6.16	19.3	330	2.63	3.02	38.72	58.2
7665	15.0	79.0	6.17	22.0	330	4.1	4.1	54.1	57.7
7666	13.0	76.0	4.81	18.8	390	3.7	3.7	53.0	60.0
7670	14.8	86.5	5.73	22.0	265	3.9	3.9	54.2	57.8
7671	12.7	78.5	4.11	19.3	390	3.2	3.3	52.3	57.0
7678	12.8	85.0	4.52	18.3	390	3.5	3.6	53.3	58.9
7679	14.8	81.5	5.88	23.6	305	4.0	4.0	52.9	57.9
7682	12.7	72.0	4.38	18.8	410	3.4	3.4	54.1	59.0
7683	14.8	78.5	5.85	21.8	390	4.0	3.9	52.9	55.3
7688	13.5	76.5	5.30	21.0	385	3.9	3.6	52.9	59.5
7689	12.6	74.0	4.47	17.9	390	3.6	3.2	51.8	60.0
7692	14.3	79.0	5.32	22.9	380	3.7	3.4	52.6	55.5
7693	14.5	79.0	5.94	20.1	480	4.1	3.7	53.2	61.0
7694	12.4	77.0	4.37	16.6	400	3.5	3.3	53.3	59.4
7695	12.5	76.0	4.20	16.6	400	3.4	3.2	53.7	60.0
7696	9.3	66.5	2.21	16.4	360	2.4	2.7	52.1	63.8
7697	14.3	85.0	3.87	18.6	310	2.7	3.6	37.9	55.6
7699	14.0	89.5	4.65	19.4	390	3.3	3.8	38.9	57.3
7700	9.6	68.0	2.05	15.0	280	2.1	2.7	31.4	61.3
7702	10.6	70.0	2.25	15.0	365	2.1	2.4	43.2	59.2
7703	14.8	83.0	4.96	20.4	350	3.4	3.6	43.4	54.5
7704	10.9	65.5	2.63	17.5	300	2.4	2.6	44.1	38.1
7705	15.4	87.5	5.95	23.3	410	3.9	4.1	50.0	66.6
7706	12.6	77.0	3.79	17.9	390	3.0	3.2	43.4	58.7
7707	11.4	75.0	2.99	16.5	380	2.6	2.9	44.4	47.6
7708	12.7	76.0	3.62	19.5	345	2.9	3.1	41.4	53.5
7709	10.4	64.0	2.69	14.6	380	2.6	2.7	36.9	59.6
7710	12.7	77.5	3.70	18.3	350	2.9	3.3	46.5	58.5
7711	14.5	84.0	4.91	23.5	345	3.4	3.8	46.6	60.1
7712	11.0	69.5	2.88	17.2	390	2.6	3.0	44.1	48.7
7713	14.7	90.0	5.26	22.5	400	3.6	4.0	45.9	57.7
7714	12.8	76.5	3.46	19.5	400	2.7	3.1	46.5	58.9
7715	10.5	66.5	2.41	16.4	430	2.3	2.6	43.0	53.6
7716	11.3	72.5	2.97	17.1	340	2.6	2.9	41.7	53.3
7717	9.9	63.0	2.07	17.3	315	2.1	2.2	38.0	48.7
7718	12.9	78.0	3.49	18.3	345	2.7	2.7	40.1	54.5
7719	15.6	94.5	5.29	22.6	370	3.4	3.6	40.1	51.8
7721	11.3	76.5	3.07	17.0	470	2.7	3.0	43.0	54.9
7722	13.1	82.5	4.55	19.6	440	3.5	3.7	46.0	50.7
7723	14.4	93.0	5.90	21.8	440	4.1	4.2	47.2	78.1
7725	12.5	81.0	3.68	19.4	360	2.9	2.7	45.0	47.8
7726	11.5	72.0	2.64	17.7	350	2.3	2.4	37.8	49.6
7727	14.7	80.5	3.93	21.5	310	2.7	2.9	36.1	44.9
7728	11.9	72.0	2.64	18.2	350	2.2	2.6	40.3	45.9
7730	11.6	80.5	2.52	17.9	310	2.2	2.4	47.1	50.3
7731	14.6	86.0	3.55	19.9	350	2.4	2.3	43.2	47.4
7732	15.1	77.5	3.79	21.4	320	2.5	2.7	37.0	45.5
7733	13.5	75.5	3.44	19.5	390	2.5	2.4	54.4	49.2
7734	11.3	69.5	2.41	15.8	320	2.1	2.3	44.9	48.1
7735	14.9	96.0	4.48	20.1	350	3.0	3.0	45.2	46.4
7736	12.1	55.5	2.52	19.0	170	2.1	2.0	33.5	47.0
7737	12.9	75.0	2.96	16.2	140	2.3	2.4	43.5	51.7
7811	10.9	70.0	4.32	16.2	485	4.0	3.7	55.6	41.7

7812	12.3	70.5	4.00	18.5	500	3.3	3.8	41.4	49.3
7813	12.6	74.5	3.50	17.7	530	2.8	3.1	49.7	66.2
7814	14.0	70.5	4.77	17.5	315	3.4	3.4	30.0	45.8
7817	11.5	76.0	3.77	17.3	460	3.3	3.2	49.6	65.0
7819	11.8	60.5	3.09	16.2	315	2.6	3.0	36.0	67.2
7821	12.7	69.5	3.62	18.8	330	2.9	3.3	36.8	53.9
7822	12.2	64.5	2.61	17.0	285	2.1	2.9	15.8	43.6