The Effect of Protein Content on the Quality of a Winter Wheat Cultivar, Norstar and the Factors Affecting the Absorption Properties of Norstar Flour

by

Hamit Koksel

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THE EFFECT OF PROTEIN CONTENT ON THE QUALITY OF A WINTER WHEAT CULTIVAR, NORSTAR AND THE FACTORS AFFECTING THE ABSORPTION PROPERTIES OF NORSTAR FLOUR

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

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Five composite samples of Norstar, a Canada Western Winter wheat with a range of protein content, from each of two locations, Minto and Portage were used to study winter wheat quality. Samples of Neepawa (hard red spring), Glenlea (utility class) and Frankenmuth (soft white winter) were also included for comparison. The quality of a winter wheat cultivar, Norstar in terms of milling, rheological and technological properties and baking quality was examined. Further studies were undertaken to determine the factors which might contribute to low water absorption of Norstar flour.

The test weight values of the Norstar samples were not affected by protein content. Kernel weights of Norstar samples decreased with increasing protein content. No significant correlation coefficients were found between the hardness test results and wheat protein content. The milling quality of the Norstar samples in terms of flour yield and flour ash content were maintained at both locations over the range of protein contents studied.

The water holding capacities of the isolated Norstar glutens were lower than the Neepawa gluten but higher than the Glenlea gluten. The Zeleny sedimentation values of the highest protein content Norstar samples were higher than the sedimentation value for the Neepawa sample of higher protein content which indicated that Norstar had stronger gluten properties. The pentosan and starch damage levels of Norstar flours were lower than in the Neepawa flour.

Regression analysis on the combined data of Norstar-Portage and Norstar-Minto samples showed that flour protein content gave a closer estimate of remix loaf volume than other quality tests (R^2 = 0.9480). The comparison of Norstar samples with the Neepawa sample (control) and the composite red spring wheat samples indicated that the winter wheat Norstar is of comparable quality to the hard red spring wheats in terms of remix loaf volume.

Regression analysis on the combined data of Norstar-Portage and Norstar-Minto samples showed that flour protein gave a closer estimate of farinograph absorption than other quality tests (R^2 = 0.9212). The comparison of Norstar samples with the Neepawa sample (control) and the Eastern Prairie composite samples of the 1985 crop of Canada Western Red Spring wheat samples indicated that the farinograph absorption responses of the winter wheats were significantly lower when compared to the red spring wheats at the same protein levels.

The farinograph studies using the Norstar and Neepawa samples with a wide range of starch damage levels indicated that the difference in farinograph absorption was too great to be explained only by the compositional differences of these samples.

Neepawa gluten when added to a base flour at the same concentration as Norstar gluten resulted in a significantly higher increase in farinograph absorption as compared to Norstar gluten. The insoluble fraction of each variety when added to a base flour at various levels resulted in significantly greater increases in farinograph absorption compared to soluble fraction of the same variety. The water holding capacity of the gluten, the reconstituted gluten and acetic acid-insoluble fractions of Neepawa were greater than the respective glutens and acetic acid-insoluble fraction from Norstar. The bound water values (obtained by DSC) of gluten and gluten fractions of Neepawa were considerably higher as compared to the gluten and corresponding fractions of Norstar.

Gel filtration profiles of acetic acid-soluble and acetic acid-insoluble fractions of Norstar gluten were very similar to corresponding fractions of Neepawa. The acetic acid-insoluble fraction of Norstar had a greater portion of its carbohydrates coeluted with the proteins which eluted with the void volume. However, the corresponding fraction of Neepawa had relatively greater portion of its carbohydrates coeluted with the lower molecular weight proteins.

Norstar gluten had a slightly greater proportion of the amino acids which had a potential negative charge. The Norstar glutens also had a higher sodium content than the Neepawa gluten.

It was possible to increase the baking absorption of a Norstar flour by 5% over the farinograph absorption without a decrease in loaf volume and dough handling properties. With Uptake 80 (a vegetable fiber) in the baking formula it was possible to increase the baking absorption of the Norstar flour by 7% over the original farinograph absorption and still maintain the loaf volume and dough handling properties.

Scanning electron microscopy studies on the freeze dried and critical point dried gluten and gluten fractions of Norstar and corresponding fractions of Neepawa were very similar.

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Chapter I

INTRODUCTION

Winter wheat is produced and consumed in almost all of the wheat growing countries in the Northern Hemisphere. The wheats grown in the Southern Hemisphere do not go through a complete dormancy and should not be classed as winter wheats. However, even with these wheats excluded, winter wheat still makes up over two thirds of the wheat grown around the world and accounts for roughly 60% of the wheat traded annually (Suderman, 1983).

Winter wheat has been grown in southwestern Alberta for almost a century (Hay et al., 1950). Southern Alberta has been the traditional winter wheat production area in the Canadian prairies (Grant et al., 1974). The expansion of winter wheat area into Saskatchewan and Manitoba in recent years is also very encouraging (Suderman, 1983) with the area seeded to winter wheat in western Canada in 1985 almost doubled compared to the previous year (Grain Research Laboratory, 1985).

The quality of Canada western red winter wheat has been studied to some extent. Prairie grown winter wheats are suitable for the production of a wide variety of cereal products including French-type breads, certain type of noodles, flat breads, steamed breads and related products (Grain Research Laboratory, 1985). Hence, its quality meets the requirements of some local products in wheat importing countries. However, the milling and baking properties and gluten characteristics of these winter wheats require further investigation. Therefore the first part of this study was undertaken to assess the potential quality of

Canada Western Red Winter wheat in terms of milling, rheological and technological properties and baking quality. The variety Norstar represents almost all of the Canada Western Red Winter class comprising 94.1% of the winter wheat seeded area in Western Canada in 1985 (Grain Research Laboratory, 1985). Therefore Norstar was selected for this study.

The Norstar samples were obtained from a fertilizer study conducted at two different locations. The samples from each location were bulked separately into five composite samples with a range of protein contents. These samples were used in milling, baking and other tests to assess the effect of protein content on these quality parameters.

Another objective of this study was derived from the preliminary observations that the variety Norstar had acceptable breadmaking quality but low farinograph and baking absorption. Good breadmaking quality is characterized by baking properties which include, moderate mixing time, good mixing tolerance and dough handling properties, high loaf volume with good crumb and crust characteristics as well as high water absorption. Therefore the various factors which might contribute to low water absorption of Norstar wheat were also studied.

The water absorption values are usually affected by several factors at the same time. Unfortunately, an assessment of the influence of the various factors such as protein content, starch damage, pentosan content, amylolytic and proteolytic activities on the water absorption (all at the same time) is very complicated. Therefore as a practical experimental approach some of the variables which determine the water absorption were held constant while the influence of other variables were determined.

This research was then extended to study the effect of glutens and acetic acid-soluble and acetic acid-insoluble gluten fractions of Norstar and Neepawa samples on farinograph absorption. The water binding capacities of these glutens and gluten fractions were also determined by differential scanning calorimetry. Amino acid analysis was performed on these samples to investigate the possible relationship between amino acid composition and bound water content. The gluten and gluten fractions were also examined by scanning electron microscopy and gel filtration.

Chapter II

LITERATURE REVIEW

2.1 THE QUALITY AND PRODUCTION OF WINTER WHEAT IN WESTERN CANADA

2.1.1 Introduction

Winter wheat is produced in almost all the wheat growing countries in the Northern hemisphere, and makes up over two thirds of the wheat grown around the world annually (Suderman, 1983). Winter wheat also has been grown successfully in southern Alberta which has been the traditional winter wheat production area in the western Canadian prairies (Grant et al., 1974). Winter wheat was grown in southern Alberta at the turn of the century before spring wheat became popular. Later with the introduction of the variety Marquis the spring wheats began to replace winter wheats in Southern Alberta (Grant, 1983). It might be speculated that plant diseases such as root rot or wheat streak mosaic may have been some of the factors which resulted in a shift to hard red spring wheat in the early 1900's (Grant, 1983).

The recent expansion of winter wheat acreage in western Canada is encouraging. Statistics Canada estimates (as at October 4, 1985) that the area seeded to winter wheat in western Canada in 1985 was 0.49 million hectares representing an increase from 0.26 million hectares sown in 1984 (Grain Research Laboratory, 1985).

Winter wheat is planted in late summer or fall to germinate and develop a young plant prior to onset of cold weather. The plant stays

dormant during the winter and growth is resumed in early spring. Plant maturity is attained in early summer. Spring wheat is grown in regions where the winters are too severe for winter wheat to survive.

Winter wheat production is favored over spring wheat because winter wheat can utilize fall moisture for germination, make effective use of early summer rainfall and can compete better with weeds. Winter wheat also protects the soil against wind and water erosion in the fall. Winter wheat matures earlier than spring wheat and has higher yield compared to spring wheat (Grant et al., 1974). Despite all of the advantages of winter wheat further development of winter wheat varieties with improved winter hardiness and disease resistance is required in order to expand the production of winter wheat in western Canada (Grant, 1983).

2.1.2 The Quality of Prairie Grown Winter Wheats

The winter wheat cultivars grown in southern Alberta in the first half of the century had poor milling and baking qualities (Grant, 1983). With the release of the variety Winalta from the Research Station at Lethbridge, Alberta in 1961 (Grant, 1964), the milling and baking quality of Canadian western winter wheat has improved by the shift to the variety Winalta (Grant et al., 1974). In milling and baking quality Winalta was superior to the other winter wheats, and approached the hard red spring wheats (Grant et al., 1974). However, the winter wheat in western Canada has about two percent lower protein content than hard red spring wheats (Grant, 1983).

Following the introduction of Winalta the winter wheat breeding program at Lethbridge has released two improved varieties of winter wheat; Sundance in 1971 (Grant et al., 1974) and Norstar in 1977 (Grant, 1980).

2.1.3 Effects of High Protein Content Obtained by High Rates of Nitrogen Fertilizer on Quality

Protein content and quality are the most significant factors influencing baking performance. For a specific cultivar, higher protein content means higher loaf volume and water absorption and also higher blending potential or the ability to carry weak, low protein wheats (Tipples et al.,1977). However, some high protein wheats give loaf volumes that are lower than the normal performance of that cultivar at equivalent protein content (Bushuk et al.,1978).

When soil moisture is not limiting, nitrogen is the major nutrient that influences protein content. Protein content is usually increased with increasing increments of nitrogen (Dubetz, 1972). Some studies have shown that the wheat protein can be increased substantially (up to 21%) by applying nitrogen fertilizer to the soil and by subjecting the plants to a soil moisture stress (Dubetz, 1977) or by late spraying with urea (Finney et al., 1957).

Tipples et al. (1977) reported that Neepawa wheat grown under irrigation in nitrogen fertilizer trials, showed a marked weakening of physical dough characteristics and a deterioration in baking quality at very high protein content. Bushuk et al. (1978) found a decrease in bread-making quality with increasing protein for samples at the top of the protein range. The proportion of acetic acid-soluble glutenin increased with increasing protein content. Bushuk et al. (1978) suggested that

the increase in the ratio of soluble glutenin to insoluble glutenin might be an explanation of the decrease in quality (per unit protein) with increasing protein content.

Studies by Tanaka and Bushuk (1972) showed that quantitative distribution of flour proteins among solubility fractions was not affected by protein content; neither were the electropheretic patterns of the albumins, globulins and gliadins. Dubetz et al. (1979) reported that Neepawa wheat showed changes in amino acid composition with increased levels of nitrogen fertilizer, and proposed that the changes in amino acid composition might be a reflection of shifts in the proportions of certain protein fractions.

Unlike the marked weakening in the mixing and baking quality exhibited by the spring wheats (Tipples et al., 1977; Bushuk et al., 1978; Dubetz et al., 1979), there was only a small change in the quality of winter wheats with increasing protein content (Pittman and Tipples, 1978). Winalta, which has strong dough characteristics maintained good milling and baking quality over a range of protein contents with no weakening at higher protein contents. The softer cultivar Kharkov 22 MC, which had shorter dough mixing characteristics, appeared to reach its limit in baking quality at around 14% protein content, and became weaker with increasing protein content (Pittman and Tipples, 1978). Tipples et al. (1977) reported that a pronounced weakening in mixing and baking quality at high protein content might be caused by a combined interaction of high fertilizer with location, cultivar and conditions of irrigation, rather than a straightforward effect of high protein content.

In another study with winter wheats of different protein contents produced in a fertilizer study, the breadmaking quality of the flours increased as protein content was raised from the lowest to the intermediate level. The flours of intermediate and highest protein were of equivalent breadmaking quality (Timms et al., 1981). The authors suggested that this result was due to an effect of the relative levels of nitrogen to sulphur available to the plants grown on that particular soil. Analysis of glutens indicated that the ratio of sulphur to nitrogen fell as grain protein content increased and this correlated with lower proportion of sulphur amino acids. They concluded that late application of high levels of nitrogen fertilizer in the absence of sulphur fertilizer led to a change in the level of available sulphur such that it became insufficient for normal grain development.

2.2 THE FACTORS THAT INFLUENCE THE WATER ABSORPTION CAPACITIES OF WHEAT FLOURS

2.2.1 Introduction

Water is the second most important ingredient next to flour in most baked products and appears to be almost unique among liquids in its ability to form a dough from flour (Bushuk and Winkler, 1957). Water is a normal constituent of flour (about 14%), dough (40 to 46%) and baked products (over 35%) (MacRitchie, 1980; Pomeranz, 1985). Water content can affect the stability of flour and the keeping quality of bread.

When flour and water are mixed together, finely granulated flour changes into a mass possessing elastic, viscous and plastic properties (Pyler, 1973), and water plays an important role in this process. The mobility of a dough is directly related to its water content (Hlynka, 1959). At low water contents, doughs are stiff and lack cohesion. As

the water content increases, doughs become softer and more extensible suggesting that one of the functions of water is to impart the property of mobility to the dough and to act as a lubricant. Eventually at higher ratios of water to flour a batter or a flour suspension is obtained (Bloksma, 1971).

2.2.2 Water in Flour, Dough and Bread

2.2.2.1 Definition and Measurement of Water Absorption

The amount of water that flour requires to yield a dough or batter of optimum consistency or viscosity is called absorption and is usually expressed as a percentage based on flour weight (Bushuk and Hlynka, 1964; Pyler, 1973). Flour absorption has long been recognized as an important factor in baking quality with high absorption values being generally preferred. High water absorption values will increase the yields of baked products and also exert a favorable effect on the shelf life of yeast-leavened dough products and batter cakes (Pyler, 1973).

A number of instruments have been developed to measure water absorption. The Simon "Research" water absorption meter and the Brabender Farinograph are frequently used to determine water absorption. The values obtained by these instruments are used as a guide in the bakery for determining the water absorption of doughs (Kent-Jones and Amos, 1967; Pyler, 1973).

The amount of water is a critical factor to ensure a dough with proper handling and machining properties and further to ensure optimum performance in baking. Problems may arise at different stages of a baking process because of incorrect water addition or inappropriate mixing time and speed (MacRitchie, 1980).

Some of the frequently encountered problems during dough processing are that the dough is either too dry and lacks coherence, or is very soft and sticky. These problems during processing can cause the quality of the final product to be impaired. Insufficient water in the dough can lead to dough portions being lost during processing while excess water content can cause adhesion to rollers and conveyor belts with consequent disruption of processing (MacRitchie, 1980).

The water retention capacity of wheat flour a property related to water absorption is measured by determining the amount of water held by a flour against a mild centrifugal force (Finney and Yamazaki, 1946; Yamazaki, 1953; Larsen, 1964). Sollars (1972) modified the alkaline-water retention capacity method of Yamazaki (1953) and developed a distilled-water retention capacity test. This test was used in combination with fractionation and reconstitution techniques to locate the flour fractions causing the water retention capacity differences between wheat classes (Sollars, 1973a, b). The water retention capacities of gluten and starch from the hard red winter wheat flours were found to be moderately higher than those from soft white winter and club wheat flours. The tailings fraction from hard wheat flours had a much higher water retention capacity than tailings from soft wheat flour (Sollars, 1973b).

2.2.2.2 Forms of Water in Flour and Dough

Flour normally contains about 14% moisture. This is the equilibrium moisture content at an average relative humidity of about 70%. At this level of moisture, flour stores well without the growth of microorganisms and without significant changes in chemical properties (Bushuk and

Hlynka, 1964). This water can be regarded as an integral part of the flour composition.

Water is associated with the specific chemical groups in the constituents of flour. Therefore, the degree of water binding is determined by the amount of water bound by each of the various flour components (Bushuk and Hlynka, 1964). The polysaccharides, starch and pentosans, found in flour have hydroxyl groups, and oxygen in the ring structure of their sugar units and also oxygen in the bridges between sugar units. All of these chemical groups are capable of interaction with water through hydrogen bonds. The peptide bonds and side chains of some amino acids of proteins also have the ability to interact with water (Bushuk and Hlynka, 1964).

It has long been recognized that all the water in hydrophilic colloids like dough is not held by the solid material with equal firmness and the same degree of attraction. Some of the water is bound tightly to the solid surfaces in flour (also in dough) and does not possess the properties of free water. These observations led to a need for differentiation of the types of water in such systems based on the firmness in which the water is held (Skovholt and Bailey, 1935). Hence the concepts of free and bound water were introduced.

Although quite frequently a distinction has been made between free and bound water in food systems, there is no agreement on a definition, and no standard method for the determination of bound and free water (Toledo et al., 1968). Whenever any measurements are made, the definition rests entirely on the method of determination (Davies and Webb, 1969). Most definitions in the literature are in essence the same.

Bound water has been defined as the water in the vicinity of macromolecules whose properties differ detectably from those of bulk water in the same system. This is a very general definition and offers several problems, as each technique measures different properties of the system and it is unlikely to have a sharp boundary between bound and free water (Kuntz and Kauzman, 1974). In principle all the results on bound and free water can be interrelated, but in practice large discrepancies may arise.

Different methods were developed to study the state of water in flour-water systems. Newton and Cook (1930), Skovholt and Bailey (1935), and Kuhlmann and Golossowa (1936) determined the amount of bound water in flour-water systems. Newton and Cook (1930) tried to quantitate the bound water in flour-water suspensions in an attempt to obtain an index of flour quality. They were unable to find any significant difference in degree of hydration between strong and weak flours.

Skovholt and Bailey (1935) were among the early workers to consider the state of water in dough rather than in a flour-water suspension. The researchers used a freezing point depression technique to determine the bound water in dough and the percentage of bound water held by the solids of different flours. An attempt was made to determine if bound water values differed with variable mechanical treatment. The results suggested that bound water values of doughs produced with different mixing speeds were not significantly different while the percentage of bound water held by the different flours were slightly different and ranged from 42.1 to 45.4%. Vail and Bailey (1940) also used a freezing point depression technique and found that the average calculated bound water was 28.6% when calculated as bound water held per unit weight of dry matter.

Lee (1970) defined bound water in dough as "water held by the flour which cannot contribute to hydrolytic enzyme action". In this study, the bound water value of flour was reported as 29.4 g/100 g dry flour solids.

Toledo et al. (1968) used nuclear magnetic resonance (NMR) spectroscopy to determine the amount of bound water. They found that not all of the bound water was held with the same force because there was a gradual decrease in liquid water as the temperature decreased. Therefore, it was suggested that the temperature at which the NMR readings are taken must be specified along with the bound water readings. Toledo et al. (1968) reported that the bound water content of wheat flour at $-18^{o}C$ as 0.29 ± 0.01 g water/g dry flour.

A commonly used definition of bound water is that fraction of total water of a system which will not freeze when the material is cooled to a subzero temperature. Various methods have been used for the determination of freezable water (free water). The proportion of unfreezable (or bound) water is estimated by subtraction from the known total moisture level.

Davies and Webb (1969) used a calorimetric method, differential scanning calorimetry for determination of bound water. Since DSC was used for this thesis project, literature related to this technique will be reviewed in some detail.

Differential scanning calorimetry (DSC) belongs to a group of experimental techniques called thermal analysis. These techniques have similar operational principles. As a sample is heated or cooled according to a predetermined programme, some physical property of the

sample is recorded as a function of temperature on a thermal analysis curve (Daniels, 1973; Simatos et al., 1975). When a material undergoes a change in physical state such as a crystallization, melting or vaporization, heat is either absorbed or liberated. Measurement of the amount of heat involved in the process can provide valuable qualitative and quantitative information. The instrumental technique used to study this phenomena is referred to as differential scanning calorimetry (Lund, 1983).

The measuring principle of DSC is to compare the rate of heat flow to the sample and a reference material which are subjected to identical temperature changes (heating or cooling) at a programmed rate. The changes in the sample that are associated with absorption or evolution of heat cause a change in the differential heat flow which is then recorded as a peak. The area under the peak is proportional to the enthalpic change, the direction of which indicates whether the thermal event is exothermic or endothermic (Biliaderis, 1983; Simatos et al., 1975).

DSC is a useful tool for studying the various heat related phenomena in foods. It has been employed to study the heat denaturation and thermal stabilities of various proteins (Arntfield and Murray, 1981; Kaisersberger and Munzing, 1983), gelatinization of starch (Biliaderis et al., 1980) and physical state and properties of water in food systems (Davies and Webb, 1969; Ross, 1978). Davies and Webb (1969) used DSC in calorimetric determination of free (freezable) and bound (unfreezable) water within the range of 0.6 - 0.9 g total water/g dry flour. They found that the mean value for bound water was 0.33 ± 0.016 g/g dry flour in the water content range studied.

Bushuk and Mehrotra (1977a, b) used differential thermal analysis (DTA) in the boiling and melting modes to obtain information on the binding of water in dough. Since in the boiling mode of DTA the endothermic process at 100°C removes both bound and free water, the method only gives a measure of energy of water binding but cannot be used to determine the amount of bound water. It was shown that, in the boiling mode, strong-mixing flour bound water more strongly than weaker mixing flour. Binding energies increased with increased level of starch damage and pentosan levels. Water binding energy of proteins was lower than starch (Bushuk and Mehrotra, 1977a).

In the melting mode DTA data can be used to calculate the amount of bound water. Bushuk and Mehrotra (1977b) found that, below a water content of 0.3 mg/mg dry flour, all of the water was of the bound type. This increased linearly with increasing moisture. The level of starch damage and protein content had no effect on the amount of bound water as determined in the melting mode of DTA (Bushuk and Mehrotra, 1977b).

2.2.2.3 Interpretation of Water Sorption Isotherms in Relation to the State of Water in Foods

Another measurement of water binding in food systems that has been used is the water sorption isotherms (Labuza, 1975). An isotherm is a sigmoid curve that describes, at specified moisture levels the equilibrium relation of the amount of water adsorbed by food components and vapor pressure or relative humidity (Pomeranz, 1985).

The isotherm can be divided into three regions. The low humidity range of the isotherm is concave in relation to the humidity axis and called the monolayer region. This region represents a strong association of the first layer of water molecules with the specific sites on

the surface of the adsorbing food and it is not available for solvent action or as a lubricant. In the intermediate range, the isotherm has a region of inflection that is basically linear. This region represents the deposition of a second layer of water molecules. The degree of binding is decreased in this region as measured by NMR (Labuza, 1975). Finally, in the high humidity range the isotherm is concave in relation to moisture content axis. This portion represents the adsorption of additional layers of water. The water in this region is usually referred as the free water and has a vapor pressure equal to that of bulk water. It retains its usual physical properties, i.e. freezing easily, and serving as a dispersing agent for the colloid substances and as a solvent for crystalline compounds (Labuza, 1975; Pomeranz, 1985) and can contribute to hydrolytic enzyme action (Lee, 1970). As more and more layers of water are superimposed on each other, as in the case of dough mixing, the new water molecules are farther removed from the adsorbing surface and are held with progressively less strength to the adsorbing surface. A point is reached when the ultimate layer of water is relatively free to penetrate into the damaged starch granules along the ruptured surfaces and into the protein particles along the capillary interfaces to act as a solvent and as a lubricant between flour particles (Pyler, 1973).

2.2.3 Effect of Flour Proteins on Water Absorption

Protein content is one of the most important factors which influence the absorption capacities of flours. The magnitude of actual flour absorption is to a great extent dependent upon the protein content. Merritt and Stamberg (1941) used twelve commercially milled flours

ranging in protein content from 7.3 to 15.5% (14% m.b.) and found that the absorption as determined by farinograph, increased about 1.5 percent for each 1 percent increase in flour protein content.

Bayfield et al. (1941), used several hard also soft winter wheats also found that absorption values showed a definite trend to increase as the amount of protein increased. But the extent of the increase was not the same for different varieties. These studies (Bayfield et al., 1941 Merritt and Stamberg, 1941) also showed that the rate of change in relation to protein content was not as great with low protein flours as compared to high protein flours, and below 9% protein there was little or no further decrease in absorption.

Finney and Yamazaki (1946) determined the water retention capacities of hard red winter wheat flours by a centrifuge method. They found that the water retention capacity of the samples was essentially a linear function of protein content within a variety. However, each variety had distinctly a different regression line, and the slopes of the lines increased as water retention capacity increased. From these studies it could be concluded that the variations in water-imbibing capacity of proteins from different wheat varieties could be partly responsible for the differences in water absorption capacities of their flours. However, in these earlier studies the combined effect of other factors such as starch damage, pentosans and enzyme activities were not considered.

2.2.4 Effect of Starch on Water Absorption

Quantitatively starch is the principal constituent of the wheat endosperm and, hence of the flour prepared from it. The starch content of wheat flours generally ranges between 65 to 71% at 14% moisture basis and it is inversely related to protein content (D'Appolonia, 1971).

Starch is a natural polymer of D-glucose units in two basic forms. One of these, amylose, consists of glucose units combined into a linear chain by alpha-1,4-glycosidic linkages and makes up 20-30% of the total starch. In contrast, the other starch component, amylopectin has a highly branched structure. In amylopectin alpha-1,4-linkages also predominate but branching is introduced by alpha-1,6-glycosidic bonds.

In wheat endosperm, starch occurs as discrete granules embedded in a protein matrix, and it exists in two types. The larger lenticular granules have a diameter between 20 to 40 μm, and small spherical granules in the range of 1 to 10 μm (MacRitchie, 1980). The size of the starch granule also depends on the variety of wheat and the conditions under which it was grown (Sandstedt, 1961).

During milling, the grinding action of the mill rolls may cause a portion of the wheat starch granules to undergo mechanical damage i.e. starch granules may be cracked, fractured, or otherwise damaged. The starches of different wheat types differ in susceptibility to physical damage. More damage to starch occurs generally during the milling of hard wheats. The relation between starch damage and kernel hardness will be discussed later in more detail.

For commercially milled flours of all types, starch damage ranges between 0-45% as determined by the method of Farrand (1964). The Farrand method measures starch damage in arbitrary units, which are expressed on a percentage scale. Most bread wheat flours have between 15 to 30% of damaged starch. Damaged starch absorbs 2.5 times more water than granular starch.

The extent of starch damage is one of the major factors which influences the water absorption capacity of a flour. Sandstedt (1955) has shown that the water-imbibing capacity of undamaged starch is about 35% of the starch weight. The water-imbibing capacity is constant for undamaged starch, but increases as the level of starch damage in a given flour increases. Therefore, more water is required to produce a dough of standard consistency. Damaged starch offers a potential way to increase water absorption of a flour and hence the yield of bread that is produced from it.

Increasing the level of starch damage causes a linear increase in water absorption at initial mixing. However, the water absorption value at this stage does not necessarily relate to baking absorption, which is normally judged by the handling properties of dough at panning and not at the initial mixing stage. The reason for this is that the dough softens during fermentation and has a lower consistency than the initial dough. An important part of this softening is caused by the action of proteolytic and amylolytic enzymes, with the more pronounced effect of the latter.

Although undamaged starch is relatively resistant to attack by alpha- and beta-amylase at the fermentation temperature, damaged starch is

readily accessible to water and enzyme penetration and eventually becomes liquefied with sufficient amylase and reaction time. The water initially held by the damaged granules is released and the dough becomes softer.

2.2.5 Kernel Hardness

2.2.5.1 Introduction

It has long been recognized that wheat cultivars vary in kernel hardness. Further, it is generally accepted that hard kernel cultivars have better milling and baking characteristics compared to softer wheats. Kernel hardness exerts a direct influence on starch damage, water absorption, gassing power, and diastatic properties of flour milled from the wheat. Therefore, hardness is an important characteristic on which wheat is valued in the market place. In many wheat growing countries, vitreousness is used as a means of segregating hard wheats by visual appearance (Simmonds, 1974), and there is a tendency of using the terms hard and vitreous as synonyms. However, kernel hardness and vitreosity are not due to the same fundamental property. Hard wheats are usually more vitreous than soft wheats which are opaque in visual appearance but some varieties of soft wheat show changes in vitreousness under certain conditions.

Since the early attempts to correlate hardness to quality, a number of methods have developed for measuring grain hardness. These can be classified into several groups depending on whether they are based on grinding, crushing, cutting, abrasion or indentation by a stylus. Some researchers have also used energy required or time required to grind wheat kernels as a measure of wheat hardness (Kosmolak, 1978). Although

these methods are not necessarily measuring the same parameter, they are all more or less capable of classifying wheats into hard and soft types.

2.2.5.2 The Relation between Kernel Hardness and Damaged Starch

The starches of different wheat types vary in their susceptibility to physical damage. More damage to the starch granule occurs during hard wheat milling than during soft wheat milling. The starch granules in soft wheats do not appear to be tightly held together in endosperm cells. During the early stages of soft wheat milling the fracture of the endosperm tends to take place through the cells and starch granules are easily released (MacRitchie, 1980). In contrast, starch granules in hard wheats are much more firmly bound in a stronger protein matrix. These granules are released at a later stage in the milling process. This results in more physical damage to the starch granules (MacRitchie, 1980).

2.2.5.3 Measurement of Grain Hardness

Biffen (1908) developed a hardness test based on crushing wheat kernels on an iron plate. This test gave a visual separation of soft and hard wheats since soft wheat kernels are broken down to form fine soft powder. In constrast hard grains are crushed to angular fragments or a gritty powder. This method did not attempt to measure the size of the particles. Therefore, it provided only a qualitative evaluation of hardness and was not sensitive enough to differentiate two samples which are similar in hardness. Cutler and Brinson (1935), followed Biffen' s principle and developed a procedure to obtain a numerical value of granularity. Wheat was ground and sieved on two different sieves. The granulation number (or index obtained for fineness) was obtained from

calculations involving the amount obtained for all three fractions obtained and an estimation of the mean size of the particles in each fraction. With this method granulation of the wheat meal was a highly stable varietal characteristic (Cutler and Brinson, 1935).

Symes (1961) showed that wheat meal granularity, as measured by particle size index, was a varietal characteristic and varied with changes in moisture and protein content. Symes (1965) using particle size index as a measure of hardness suggested that a major gene was responsible for difference in hardness between two wheat varieties.

Katz et al.(1959) reported the use of a commercial portable hardness tester called the Barcol impressor. The tester consisted mainly of a spring-loaded stylus, a case, and a dial micrometer. Hardness is measured by the distance the stylus is displaced into the case when the tester is pressed against a test object. Readings of the micrometer are referred to as hardness numbers. With this tester Katz et al. (1959) determined the hardness numbers in three different sections: the germ end, the center section, and the brush end of the wheat kernel. The periphery of the kernels was found to be harder than the region around the crease.

Katz et al. (1961) used the same hardness tester to show that the hardness of hard wheats decreased with increasing moisture content while soft wheats showed no significant change in hardness up to a moisture content of 13%; above this moisture content soft wheats declined in hardness.

Hardness tests based on crushing or indentation of single kernels are both tedious and labor intensive. However, these tests are valuable for

certain research purposes (Simmonds, 1974) but have been found to be unsuitable for quality control procedures.

The pearling test was a test used in grading barley for malting purposes and was adapted as a hardness test (McCluggage, 1943). In this test the sample fraction which has been pearled off in a laboratory-scale barley pearler is used as a hardness index. McCluggage (1943) found that as wheat increased in hardness the amount of material removed in pearling decreased. McCluggage (1943) reported that the amount of wheat pearled off was not affected by temperature and variations in moisture content between 7 and 15%. However, Kramer and Albrecht (1948) found that the pearling index especially for soft wheats decreased as the moisture content of the grain increased. These workers suggested that the moisture content of the wheat should be comparable when conducting the pearling tests. Chesterfield (1971) found a high correlation between pearling resistance and particle size index ($r = 0.94$) with a modified Strong-Scott barley pearler. The moisture content of the grain did not affect the results significantly.

The Brabender Hardness Tester is another instrument used to measure hardness. Anderson et al. (1966) used Brabender Hardness Tester and a pin mill to obtain kernel hardness or friabilitity indices. The studies suggested that the flour yield or flour fraction surface area could be used to rate wheats according to kernel hardness and friability.

The Brabender Hardness Tester was also used by Greenaway (1969) on commercial wheat samples. He suggested that the wheat hardness index (peak height/percent flour yield) is a more sensitive measure of wheat hardness than the hardness peak. The wheat hardness index was highly

correlated to protein content per sq.m of flour (0.93) and a correlation coefficient of -0.85 was found between the pearling index and the wheat hardness index. The wheat hardness index was inversely proportional to moisture content, and directly proportional to protein content.

Obuchowski and Bushuk (1980) used a number of commonly used hardness methods. In this study the wheat hardness index, flour yield, and grinding torque, all obtained with the two-step Brabender Hardness Tester, gave the same ranking of the cultivars examined. Particle size index, obtained with Brabender Quadrumat mill, also ranked the cultivars in the same order. However, pearling resistance index did not give the same ranking as the other methods did. The moisture content of maximum discrimination also varied somewhat among the methods investigated.

2.2.5.4 Theories of Grain Hardness

Starch Granule-Protein Matrix Adhesion: Barlow et al. (1973) separated storage protein from the starch by air classification and floatation in mixtures of chloroform and benzene. With a micropenetrometer the hardness of individual particles, which were embedded in a resin, was determined. The average values for the wheat samples tested were 33.1 and 27.5 kg/mm^2 for protein and starch respectively. There was no significant difference between starch or protein from different varieties. On the basis of these results it was concluded that the properties of protein matrix and starch granules were not related to hardness. They postulated that grain hardness is related to the adhesion of starch and protein, being greater in hard than soft wheats (Barlow et al., 1973).

Simmonds (1971) and Barlow et al. (1973) observed that the starch granules prepared from harder wheats had more protein adhering to them compared to softer ones. A high proportion of the protein associated with the starch granules was buffer-soluble with more soluble material in harder wheats (Simmonds et al., 1973). It was further suggested that water-soluble material was responsible for adhesion of protein matrix to starch granules.

Continuity of Protein Matrix: Stenvert and Kingswood (1977) reported that the differences in hardness in wheat was determined by the continuity of protein matrix and the strength with which it physically entrapped starch granules. From the scanning electron microscopy studies, they suggested that a tight physical entrapment of starch granules in a continuous protein phase resulted in the contents of endosperm cells attaining their maximum strength. This rigid cell structure resulted in the granular nature and high starch damage of hard wheat flours. Whereas, a discontinuous protein matrix with air spaces would result in a weak structure and would allow the ready release of starch granules without damage in soft wheats (Stenvert and Kingswood, 1977).

2.3 EFFECTS OF PENTOSANS ON WATER ABSORPTION

2.3.1 Structure and Properties of Pentosans

Pentosans constitute a minor component of wheat flour but these carbohydrates exert a measurable effect on the oxidation requirements, mixing and hydration properties of wheat flour doughs (Pomeranz, 1985; Pyler, 1973). It is well established on the basis of reconstitution studies that in the absence of pentosans, doughs are soft, slack, and moist. Addition of the pentosan fraction restores to the doughs the normal boldness and dryness (Shellenberger et al., 1966).

Pentosans are polysaccharides consisting primarily of pentose sugars, xylose and arabinose. However, the crude pentosan preparations also contain starch, small amounts of bound ferulic acid, and polysaccharide fractions containing D-galactose as building units (D'Appolonia and Kim, 1976). Pentosans are classified on the basis of solubility which is based on the degree of arabinose branching on the xylose backbone. Water-insoluble pentosans have a greater degree of branching than water-soluble pentosans (Shelton and D'Appolonia, 1985).

Water-soluble pentosans form extremely viscous solutions. Baker et al. (1943) showed that the purified water-soluble pentosans, when dissolved in water gave a clear solution which was highly viscous at a concentration of 1%. Udy (1956) reported that the intrinsic viscosity of water-soluble pentosans was 15 to 20 times greater than soluble proteins.

Another important property of water-soluble pentosans is their gel forming ability. The capacity of aqueous extracts of wheat flour to form gels in the presence of oxidizing agents was first observed by Durham (1925). This phenomena has been studied by several groups and reviewed by D'Appolonia and Kim (1976), Neukom and Markwalder (1978) and Shelton and d'Appolonia (1985).

2.3.2 The Role of Pentosans on Water Absorption Capacity

Both water-soluble and -insoluble pentosans are extremely hydrophilic (Shelton and D'Appolonia, 1985). These pentosans constitute a branched structure in which hydroxyl groups are ideally placed for the attachment of water molecules (Pyler, 1973). Kulp (1968) studied the increase in

water absorption of flour by the addition of water-soluble and -insoluble pentosans preparations. The water-soluble and water-insoluble pentosans absorbed about 10 times their weight of water when the calculations were based on the pentosan contents of these fractions (Kulp, 1968). Jelaca and Hlynka (1971) confirmed the results obtained by Kulp (1968) on water absorption. Using the constant-dough weight farinograph method, Jelaca and Hlynka (1971) found that water-soluble pentosans of a wheat flour absorbed 9.2 times their weight of water while the water-insoluble pentosans absorbed 8.0 times their weight of water.

Tao and Pomeranz (1967) extracted pentosans from flours of various types of wheats, and studied the effect of adding water-soluble pentosans on farinograph absorption. The pentosans from hard red winter, hard red spring, soft red winter, durum and club wheats increased the farinograph absorption of a composite hard red winter wheat flour substantially. Adding pentosans, except for durum in the presence of excess potassium iodate increased water absorption additionally.

Chapter III

METHODS AND MATERIALS

3.1 WHEAT SAMPLES

The main set of wheat samples used for this study was from a fertilizer study of a hard red winter wheat cultivar, Norstar. Norstar was developed at the Agriculture Canada Research Station, Lethbridge, Alberta. It is currently the major variety of the red winter wheat class, grown in Western Canada, and it is the hardiest among all licensed varieties of winter wheat. The Norstar samples were grown under various levels of N-fertilizer and at two different locations, Portage and Minto in 1983-84 season. The samples from each location were bulked separately according to protein contents into five composite samples to obtain a wide range of protein contents. Another sample of Norstar at 11.9% protein content, grown in Homewood location in 1983 was obtained and used in milling studies.

A Neepawa sample was obtained from United Grain Growers Limited, Winnipeg, Manitoba. It was grown in 1984 and certified seed grade No.1, and was used as a control for all quality tests. Another sample of Neepawa (1.5 kg) at a low protein content was provided by Dr. A. B. Campbell of Agriculture Canada Research Station, Winnipeg, Manitoba. This sample was used in the starch damage studies.

One sample of Glenlea (Canada Utility class) and one sample of Frankenmuth (Canada Eastern Soft White Winter class) were also included

for comparison purposes. The Glenlea sample was a bulked sample obtained from the Agro-Man test which was grown at several Manitoban locations in 1982. The Frankenmuth sample was certified seed grade No.1 and received from King Grain Limited, Chatham, Ontario. Where necessary samples were cleaned on a Carter Dockage Tester before use.

3.2 TESTS ON GRAIN SAMPLES

3.2.1 Test Weight

The hectoliter weight was determined on a dockage free sample using an Ohaus Test Weight apparatus equipped with a half-liter container. The weight in grams of the measured 0.5 L of wheat was multiplied by 200 to obtain the equivalent kilograms per hectoliter. The average of three determinations was reported on an "as is" moisture basis.

3.2.2 Thousand Kernel Weight

The weight per thousand kernels was determined by counting the number of seeds in 20 g of grain. Before weighing all broken and foreign material was first removed by hand-picking. The 20 g sample was then weighed. All tests were done in duplicate and the average result is reported on a 14% moisture basis.

3.2.3 Moisture Content

The moisture content of the whole grain samples was determined with an electronic moisture meter (Canadian Aviation Electronic Ltd. model CAE 919). The test was performed on 250 g of wheat according to the approved method 44-11 (AACC 1983).

3.2.4 Kernel Hardness

The grain samples were stored in open containers at 22°C and 64% relative humidity to eliminate the possible effects of different moisture level on kernel hardness. After the equilibriation process the moisture contents of the samples were 12.3 ± 0.2%. Three different methods were used to determine kernel hardness: particle size index, pearling resistance test, and grinding time test.

3.2.4.1 Particle Size Index

Approximately 11 g of sample were ground on a Falling Number Lab mill model 3303. Ten grams of this meal were weighed and seived through a 200 mesh brass cloth (opening 74 μm). Approximately 10 grams of wheat kernels were placed on each sieve together with the meal to prevent clogging of the sieve. The weight of the material which passed through the sieve was determined and expressed as a percentage.

3.2.4.2 Grinding Time

The grinding time method of Kosmolak (1978) was used to determine grain hardness. The grinding time was the time required to grind 5 g of ground meal which had passed through a burr mill.

3.2.4.3 Pearling Resistance Test

Twenty grams of wheat were pearled for 20 sec in a Strong Scott barley pearler. The weight of the remaining pearled grain was reported in grams as the pearling resistance index.

3.3 MILLING AND TESTS ON FLOUR AND SEMOLINA SAMPLES

3.3.1 Flour Milling

The hard red winter and red spring wheats were tempered overnight to 15.5% moisture content unless otherwise specified. The soft wheat sample was tempered to 14.5% moisture content. All wheats were milled into flour on a Buhler pneumatic laboratory mill (model MLU 202). The flour was rebolted through a 70 GG (236 μ) screen and blended before use.

3.3.2 Semolina Milling and Preparation of the Samples for Studying the Effect of Starch Damage on Water Absorption

Samples of Neepawa and Norstar wheat were milled according to the method of Dexter et al. (1982) into semolina (coarse endosperm middlings) on a Buhler lab mill which was designed for durum milling. The samples were tempered overnight to 16.5% moisture content. After purification on a laboratory purifier, the flour was removed by sieving through a 10XX screen. The granular product, namely coarse endosperm middlings from each wheat sample, was then subdivided into four sub-samples. Three of these sub-samples were passed through the reduction rolls of the Buhler lab mill. The gap settings were 0.06-0.02 mm (wider than regular setting), 0.04-0.01 mm (regular setting), and 0.02-0.01 mm (closer than regular setting) for the production of low, medium and high starch damage levels, respectively.

3.3.3 Moisture Content

Moisture content of flour and semolina was determined by using a Brabender Rapid Moisture Tester according to the approved method 44-15A (AACC 1983).

3.3.4 Ash Content

The ash content of flour was determined according to the approved method 8-1 (AACC 1983). Five grams of flour were weighed into a silica dish and incinerated at 575°C overnight. The results are presented as a percent on a 14% moisture basis.

3.3.5 Gluten Content

The wet gluten content was determined using an automatic gluten washing instrument, Glutomatic 2100 (Falling Number, Sweden). Ten grams of flour and an appropriate volume of 2% NaCl solution were mixed for 20 sec to form a dough and then washed for 5 min with 2% NaCl. The excess water was removed and the washed gluten weighed. The results are expressed as a percentage on 14% moisture basis. For the dry gluten determination, the wet gluten balls were dried in air oven at 130°C to a constant weight and weighed.

3.3.6 Zeleny Sedimentation Test

The Zeleny sedimentation test was carried out according to the approved method 44-11 (AACC 1983).

3.3.7 Farinograph Test

Farinograph curves were obtained by the constant flour weight approved method 54-21 (AACC 1983). Fifty grams of flour (14% moisture basis) were used with the small mixing bowl and water was added to obtain a curve with maximum consistency centered on the 500 B.U. line.

3.3.8 Extensigraph Test

Extensigraph curves were obtained according to approved method 54-10 (AACC 1983) as modified by Holas and Tipples (1978). Doughs were made by mixing 200 g of flour (14% moisture basis), 4 g of salt (dissolved in water) and water equal to the farinograph absorption less 2% to compensate for the effect of salt. The doughs were mixed in the farinograph mixer to the dough development time. A 150 g dough piece was weighed, rounded, shaped and placed into a humidified chamber until testing. The dough pieces were stretched to obtain extensigrams after 45 and 135 min rest periods with rounding and shaping at 90 min. The measurements are reported for the 135 min curves.

3.3.9 Amylograph Test

Amylograms were obtained by using the Brabender visco-amylograph according to the approved method 22-10 (AACC 1983). The amylograph curves were obtained using 65 g of flour (14% moisture basis) dispersed in 460 ml of water at 35°C. The temperature was increased at a rate of 1.5°C per minute to 95°C. Maximum viscosity in Brabender Units (B.U.) at the center of peak was reported as the malt index or amylograph peak value.

For the rapid amylograph test 25 g of flour (14% moisture basis) and 100 ml of distilled water was used. The thermoregulator was set at a constant temperature of 90°C.

3.3.10 Baking Procedure

The baking performance of the samples was evaluated by Grain Research Laboratory (GRL) Remix experimental baking procedure of Irvine and McMullan (1960) as modified by Kilborn and Tipples (1981). The baking formula included 100 grams of flour (14% moisture basis), yeast (3%), salt (1%), sucrose (2.5%), potassium bromate (15 ppm), monobasic ammonium phosphate (0.1%) and 60° Lintner malt syrup (0.6%). Baking absorption for each flour sample was obtained by subtracting 2% from the farinograph absorption. Later, in the study of the effect of different water absorption levels, the baking absorption was increased at 2% increments until the dough lost its handling properties.

3.3.11 Alpha-amylase Activity

The method of Campbell (1980) as modified by Kruger and Tipples (1981) was used to determine alpha-amylase activity. All readings were obtained on a Perkin-Elmer model 191 Grain Amylase Analyzer.

3.3.12 Exoprotease (hemoglobinase) Activity

The exoprotease activity was determined using the method of Bushuk et al. (1971) as modified by Macri (1985). The substrate consisted of 1% bovine hemoglobin (Sigma type II) in 0.2 M sodium acetate buffer (pH 4.5). Flour samples (100 mg, 14.0% moisture basis) were suspended in

5.0 ml of substrate solution and incubated for 2 h at 37°C with vortexing at half hour intervals. The reaction was stopped by adding 5.0 ml of 5% trichloroacetic acid (TCA). The mixture was clarified by centrifugation (27,000 x g for 10 min). Aliquots of 200 µl of supernatant were analyzed for TCA-soluble nitrogen according to the method of Moore and Stein (1954). The aliquots were diluted to 2.0 ml with 0.2 M sodium acetate buffer followed by the addition of 2.0 ml of ninhydrin solution. The samples were placed in a boiling water bath for 20 min, cooled, and then diluted with 5.0 ml of aqueous ethanol. The absorbance was read at 570 nm. Glutamate (0-30 µg/2 ml acetate buffer) was used to obtain the standard curve. The exoprotease activity was expressed as µg glutamate/g flour/hr. The determinations were carried out in duplicate and the average values reported.

3.3.13 Endoprotease (azocaseinase) Activity

The endoprotease activity was determined by the method of Preston et al. (1978) with some modifications. The azocasein solution (1.4%) was prepared in 0.05 M citric acid-disodium phosphate buffer (pH 6.0) and dialyzed overnight at 4°C against the buffer (10 x the volume of the substrate solution). Flour samples (2 g, 14% moisture basis) were extracted in 7.0 ml of 0.05 M sodium acetate buffer (pH 5.5) at 4°C for 1 hr on a variable speed multipurpose rotator (10 rpm). Suspensions were filtered through a glass microfiber filter (Whatman GF/C). Aliquots (0.5 ml) of the filtrate were incubated with 3.5 ml of azocasein solution at 40°C for 4 h with vortexing at 1 hr intervals. The reaction was terminated by the addition of 5 ml of cold 10% TCA followed by filtration to remove precipitated proteins. After adding 2.0 ml of

cold 0.5 N sodium hydroxide to an equal volume of filtrate, the resulting solution was allowed to sit for 20 min. The absorbance was determined at 440 nm. The azocaseinase activity was expressed as the change in absorbance at 440 nm per hr/g of dry sample. Kruger (1973) reported that this assay was linear up to 0.1 absorbance unit.

3.3.14 Determination of Pentosans

The pentosans were determined by the method of Cerning and Guilbot (1973). The principle of the method is the hydrolysis of pentosans and the dehydration of the resulting pentoses into furfural by 4.15 N hydrochloric acid. The furfural was separated by steam distillation and determined colorimetrically at 530 nm.

3.3.15 Starch Damage

Starch damage was determined on 5 g flour samples according to the method of Farrand (1964).

3.4 TESTS ON GLUTEN AND GLUTEN FRACTIONS

3.4.1 Preparation of Gluten Samples

The gluten samples were prepared by mixing 200 g of flour with 120 ml of distilled water into a dough for 2 min in a GRL mixer. After 15 min of resting the dough was placed on a 70 mesh screen and washed gently by hand kneading, first with tap water and later with distilled water. The partly washed dough mass was allowed to relax for 1/2 h in cold distilled water (4°C). The washing operation was continued until the gluten was formed and the washing solution was clear. After the washing

was completed, the gluten samples were freeze-dried, weighed, crushed in a Carver press and ground in a coffee grinder to a particle size of less than 149 μm. The moisture, protein and pentosan contents of the gluten samples were determined and the samples were stored at 4°C.

3.4.2 Fractionation of Gluten Samples

The freeze-dried gluten samples (20 g) were extracted with 1 L of 0.05 N acetic acid. The samples were stirred with a magnetic stirrer at a slow speed for 2 h at 4°C to prevent mechanical degradation of the gluten. The slurry was centrifuged for 30 min at 20,000 g. The supernatant and precipitate (after dispersing in deionized water) were dialyzed for 24 h at 4°C against deionized water. Both the residue and the soluble fractions were freeze-dried, weighed, ground using a coffee grinder to a particle size of less than 149 μm and then stored at 4°C. The moisture, protein and pentosan contents were determined in all fractions.

3.4.3 Amino Acid Analysis

Thirty mg of freeze-dried gluten and gluten fractions were weighed into hydrolysis tubes. After the addition of 4.0 ml of 6 N HCl (triple distilled) the contents of the hydrolysis tube were frozen. The tube was attached to a vacuum rack, and evacuated to a pressure less than 10 microns of mercury. The contents of the tube were allowed to thaw (under vacuum) and refrozen to remove the entrapped air. The evacuated hydrolysis tubes were placed in a forced-air oven at 110°C for 24 hours. The hydrolysed sample was then frozen and the HCl was evaporated to dryness in a vacuum desiccator over sodium hydroxide. The dried samples

were dissolved in 10 ml of 0.2 N sodium citrate buffer, pH 2.2, and filtered (Whatman No. 40) to remove the insoluble material. The filtrate was refiltered through a 0.22 μm filter (Millipore Co.) before amino acid analysis.

The amino acid analyses were performed with LKB model 415 Alpha Plus amino acid analyzer using a three step buffer system. The first two buffers were 0.2 M citrate buffers at pH 3.20 and 4.25 respectively. The last buffer was a 1.2 M citrate buffer, pH 6.45. The sample (20 μl) was injected on a column (4.6 x 200 mm) which was packed with LKB Ultropac 8 cation exchange resin in the sodium form. The amino acids were quantitated using a ninhydrin reagent after separation on the column.

3.4.4 Determination of Sodium

The gluten or flour samples (1 g) were ashed overnight using 1.5% alcoholic magnesium acetate. The ash was dissolved in 15 ml of 3 N HCl by heating the crucibles. The solution was filtered through ashless filter paper (Whatman No. 40). The ash was washed from the filter paper with the 3 N HCl and then with water. The volume of the filtrate was made up to 100 ml. A Perkin Elmer 403 atomic absorption spectrophotometer equipped with a hollow cathode sodium lamp was used for the determination. The instrument was standardized with 0.5 ppm of sodium chloride and all samples were determinated at 589 nm.

3.4.5 Water Holding Capacity of Gluten Samples

The procedure described by Sollars (1972) was used with some modification. Gluten (1 g, as is basis) was weighed into a tared centrifuge tube and 5 ml distilled water was added. The tube was slowly shaken by using a tube shaker to suspend the sample and allowed to stand for 20 min with shaking every 5 min. The tube was centrifuged for 15 min at 2,000 x g. The supernatant was decanted, and the tube was drained for 5 min and then weighed. The water holding capacity of the gluten samples was expressed as a percent and corrected for moisture content.

3.4.6 Water Holding Capacity of Acetic Acid-Insoluble Gluten Fractions

The water holding capacity of the acetic acid-insoluble gluten fractions (0.5 g) was determined according to the approved method 88-04 (AACC, 1983).

3.4.7 Water Holding Capacity of Reconstituted Gluten

After the water holding capacity of acetic acid-insoluble gluten fraction was determined, it was mixed with the respective soluble fraction in a tube. Both fractions were in the hydrated form. The tube was shaken by using a tube shaker and allowed to stand for 20 min with shaking every 5 min. The tube was centrifuged for 15 min at 2,000 x g. The supernatant was decanted and the tube weighed. For determination of water holding capacity the tube contents were oven dried to the constant weight.

3.4.8 Differential Scanning Calorimetry (DSC)

Gluten or gluten fraction samples were weighed (0.9 ± 0.1 mg) into DSC pans. The samples were wetted by adding 5-10 µl of water. The DSC pans were hermetically sealed and reweighed for the determination of the water content of the sample. The samples were placed in the standard DSC cell with an empty pan used as a reference. The unit was cooled to -70°C with liquid nitrogen and then allowed to equilibrate at -15°C. The DSC cell was heated at a rate of 1°C/min to 15°C. The equipment used was a Dupont Model 9900 Thermal Analyzer with a standard DSC cell. Enthalpies were calculated using DSC 1.1 Standard Analysis software which was supplied with the machine.

3.4.9 SDS Sedimentation Test for Gluten and Gluten Fractions

The method described by McDermott (1983) was used with slight modification. Gluten or a gluten fraction (750 mg, dry basis) was weighed into a 100 ml stoppered, calibrated cylinder. Three ml ethyl alcohol (95%) and 50 ml of dilute lactic acid (0.25%) were added. Tubes were placed on the shaker used for the Zeleny sedimentation test (AACC method 44-11, 1983) and mixed for 15 min. Fifty ml of SDS/lactic acid reagent (1.5% SDS, 0.25% lactic acid) was added and the tubes were mixed for 10 min. The tube contents were allowed to sediment for 1 h for the gluten samples while the acetic acid insoluble gluten fractions were allowed to sediment for 4 h and 16 h. The volume of the sediment after the standing period was read and reported.

3.4.10 Gel Filtration Chromatography

Hydrated Sephadex G-200 matrix was equilibrated in AUC solvent (0.1 M acetic acid, 3 M urea and 0.01 M cetyl trimethylammonium bromide) and deaerated by aspirating at reduced pressure. The column was packed as outlined in the booklet "Gel Filtration Theory and Practice" supplied by Pharmacia Fine Chemicals, Uppsala, Sweden (1982). The column was packed at a 15 cm head pressure and was operated using a downward flow technique and a 11 cm operating pressure. Freeze-dried sample (35 mg insoluble fraction or 25 mg soluble fraction) was extracted in 5 ml AUC overnight at 4^{0}C and centrifuged at 20,000 x g for 20 min. The supernatant was applied to the column. The column was eluted with AUC buffer. Five ml fractions were collected from the column with a fraction collector. The protein content of the fractions was measured at 280 nm. The carbohydrate content was determined by the phenolsulfuric acid method of Dubois et al. (1956). The excluded (void) volume (V_0) was determined as the elution volume of blue dextran. The total volume (Vt) was determined by chromatographing tryptophan. The column was calibrated for molecular weight using the following standards according to the method of Whitaker (1963).

	Molecular weight (daltons)	Source
Blue dextran	2,000,000	Pharmacia
Gamma-globulin	160,000	Sigma
Bovine serum albumin	68,000	Sigma
Alpha-chymotrypsinogen	23,000	Sigma
Cytochrome c	13,000	Calbiochem
D-L-tryptophan	204	Matheson, Coleman & Bell

3.5 SCANNING ELECTRON MICROSCOPY (SEM)

3.5.1 Materials used in SEM

SEM was used to examine the gluten samples, the acetic acid-soluble and acid-insoluble gluten fractions, the semolina and flour. The flour for the SEM studies was obtained by remilling the semolina (as described in 2.3.2) to produce samples with a range of starch damage.

3.5.2 Preparation of Flour Samples

In preliminary examination of the flour samples with the scanning scope studies it was not possible to observe the degree of starch damage on the granules. Therefore, specimens were prepared for SEM by treating the starch damaged flour with a hydrolytic enzyme in an attempt to accentuate the fractures on the starch granules. One gram (14% moisture basis) of damaged flour was weighed into a 15 ml centrifuge tube. Five ml of 100 µg/ml amylase solution (barley malt alpha-amylase, Sigma type VIII-A) was added. The enzyme was prepared in a 100 mM sodium acetate buffer (pH 5.5) which contained 1 mM $CaCl_2$. The tubes were placed on a shaker in a water bath at 35°C and hydrolyzed for 1.5, 3, 6, 9 and 20 h. The flour suspension was centrifuged for 10 min at 10,000 g (4°C) and the supernatant decanted. The precipitate was washed with the same sodium acetate buffer, centrifuged, and the supernatant decanted. The washing procedure was repeated three times. The precipitate was then frozen and freeze dried.

3.5.3 Preparation of Gluten and Gluten Fractions

The same gluten samples, acetic acid-soluble and acetic acid-insoluble gluten fractions which were prepared for the water holding studies were also used for SEM studies. These samples were freeze dried. Another set of gluten samples were prepared by critical point drying (Porter et al., 1972).

3.5.4 Examination of Specimens

The dried samples were attached to circular stubs with double-sided tape. All the mounted specimens were then coated with gold to a thickness of 10-20 nm with a Balzar sputter coater. The coated specimens were viewed in a Cambridge "Stereoscan" MK IIa scanning electron microscope at an accelerating potential of 10 kv, and photographed on 35 mm Kodak Panatomic X film (ASA 32).

Chapter IV

RESULTS AND DISCUSSION

4.1 TECHNOLOGICAL CHARACTERISTICS OF WHEAT GRAIN SAMPLES

The results of the quality tests performed on the wheat grain samples are shown in Table 1. Simple correlation coefficients between the various quality parameters for the Norstar-Minto samples, for the Norstar-Portage samples and for the combined quality data for the Norstar-Minto and Norstar-Portage samples were determined. The correlation coefficients for each data set are presented in Table 2, Table 3 and Table 4, respectively.

4.1.1 Test Weight

The test weights for the Norstar-Minto samples were slightly higher than those for the Norstar-Portage samples (Table 1). Test weight was not affected by the protein content in the Norstar samples. All samples met the test weight requirements for grade No.1 Canada Western Red Winter wheats except for the highest protein content sample from the Portage location. The test weight of 77.3 kg/kl for this sample was slightly lower than the grade requirement of No.1 Canada Western Red Winter wheat of 78 kg/hl.

The correlation coefficients between test weight and other quality parameters including wheat protein content were not significant for Norstar-Minto and Norstar-Portage samples (Tables 2 and 3). For the combined data of the Norstar-Minto and -Portage samples (Table 4), test

weight was inversely correlated with the particle size index but positively correlated with pearling resistance with both being significant at the 1% level. Although the test weight for Neepawa, Glenlea and Frankenmuth samples which were chosen as controls, were lower than the Norstar samples, all met the test weight requirements for No.1 Canada Western Red Spring, No.1 Canada Utility and No.1 Canada Eastern White Winter wheat respectively.

TABLE 1

Tests on Whole Grain Samples.

Sample[1]	Grinding time (min)	Particle size index (%)	Pearling index (%)	1000 Kernel weight[2] (g)	Test weight[3] (kg/hl)
Norstar-Minto (8.8)	0.89	16.2	12.6	33.2	80.1
" (9.7)	0.90	16.0	12.9	32.1	81.1
" (11.5)	0.90	15.9	13.2	31.1	81.1
" (12.4)	0.84	15.5	13.3	31.1	80.8
" (13.2)	0.84	15.6	13.3	30.8	81.0
Norstar-Portage (10.0)	1.00	18.8	9.4	34.9	79.2
" (11.3)	0.99	18.6	10.4	33.1	79.2
" (11.8)	0.99	18.2	10.8	33.5	79.4
" (12.8)	0.89	17.9	10.4	29.0	79.1
" (13.8)	0.90	18.1	10.8	30.6	77.3
Neepawa (15.0)	0.64	15.6	12.9	28.8	77.6
Glenlea (14.5)	0.58	11.2	11.1	41.0	75.0
Franken-muth (8.7)	2.99	28.1	5.4	36.0	78.8

1 The figures in parenthesis represent % wheat protein content (N x 5.7, 14% moisture basis).
2 14% moisture basis
3 "as is" basis

TABLE 2

Simple Correlation Coefficients between Various Quality Parameters of Norstar-Minto Samples.

	GT	PSI	PRI	MKW	HW	FY	ASH	FP
Wheat protein	-0.7753	-0.9321	0.9609	-0.9490	0.5588	0.4466	-0.6046	0.9966
Grinding time (GT)		0.8649	-0.6337	0.5622	-0.0456	-0.3001	0.2318	-0.7753
Particle size index (PSI)			-0.9163	0.8647	-0.4827	-0.4592	0.6003	-0.9347
Pearling resistance index (PRI)				-0.9906	0.7093	0.4653	-0.7241	0.9619
1000 kernel weight (MKW)					-0.7708	-0.5144	0.7655	-0.9511
Hectoliter weight (HW)						0.7429	-0.9598	0.5733
Flour yield (FY)							-0.8705	0.4717
Flour ash (ASH)								-0.6233

r > 0.878 significant at 5% level

r > 0.959 significant at 1% level

TABLE 3

Simple Correlation Coefficients between Various Quality Parameters of Norstar-Portage Samples.

	GT	PSI	PRI	MKW	HW	FY	ASH	FP
Wheat protein	-0.8699	-0.8692	0.7920	-0.8515	-0.7256	-0.9442	-0.1102	0.9973
Grinding time (GT)		0.8310	-0.4290	0.9613	0.6185	0.7581	-0.0837	-0.8366
Particle size index (PSI)			-0.7265	0.8777	0.3394	0.8694	0.4231	-0.8759
Pearling resistance index (PRI)				-0.5173	-0.3783	-0.8995	-0.5622	0.8330
1000 kernel weight (MKW)					0.4432	0.8296	0.1529	-0.8274
Hectoliter weight (HW)						0.5158	-0.5465	-0.7006
Flour yield (FY)							0.3860	-0.9558
flour ash (ASH)								-0.1655

r > 0.878 significant at 5% level

r > 0.959 significant at 1% level

TABLE 4

Simple correlation Coefficients between Various Quality Parameters of Norstar Samples from Both Locations, Minto and Portage.

	GT	PSI	PRI	MKW	HW	FY	Ash	FP
Wheat protein	-0.3325	0.0636	-0.0348	-0.6894	-0.3348	-0.0532	-0.5263	0.9958
Grinding time (GT)		0.8280	-0.7779	0.7277	-0.4315	0.8052	-0.3050	-0.3826
Particle size index (PSI)			-0.9827	0.3583	-0.8144	0.8399	-0.3960	-0.0141
Pearling resistance index (PRI)				-0.3343	0.7909	-0.8700	0.3583	0.0476
1000 kernel weight (MKW)					-0.0209	0.5363	0.1918	-0.7018
Hectoliter weight (HW)						-0.4681	0.1744	-0.2619
Flour yield (FY)							-0.4745	-0.1153
Flour ash (ASH)								-0.5180

r > 0.632 significant at 5% level

r > 0.765 significant at 1% level

4.1.2 Thousand Kernel Weight

The kernel weights of the Norstar samples from Minto and Portage decreased with increasing protein content (Table 1). All Norstar samples had higher kernel weight values compared to the Neepawa sample but lower kernel weights compared to the Glenlea and Frankenmuth samples. The inverse correlation between wheat protein content and thousand kernel weight was significant for Norstar-Minto samples at the 5% level (Table 2) but not significant for Norstar-Portage samples (Table 3). For the combined data of the Norstar-Minto and -Portage samples a negative correlation between wheat protein content and thousand kernel weight was significant at the 5% level (Table 4). Pittman and Tipples (1978) also found that kernel weight decreased with increasing protein content for several hard red winter wheat varieties which were grown under various fertilizer levels in Alberta.

4.1.3 Kernel Hardness

Three different tests, particle size index, pearling resistance index and grinding time were used to determine kernel hardness. The results of these tests are presented in Table 1. All Norstar samples were softer than the Neepawa and Glenlea samples as determined by grinding time and particle size index but had comparable hardness as determined by pearling resistance index. The Frankenmuth wheat sample was the softest sample as determined by all three hardness tests. There was a negative correlation between wheat protein content for the Norstar-Minto samples and particle size index significant at the 5% level and a positive correlation between wheat protein content and pearling resistance index significant at the 1% level (Table 2). The correlation between wheat protein content and grinding time was not significant. The corre-

lation coefficients between wheat protein content for the Norstar-Portage samples and the three hardness test results were high (Table 3) but none were significant at the 5% level. For the combined data of the Norstar-Minto and -Portage samples no significant correlation coefficients were found between the three hardness tests and wheat protein content. For the combined data, all of the hardness test results were highly correlated to each other and significant at the 1% level (Table 4).

4.1.4 Grain Protein Content

All Norstar samples had lower protein contents compared to Neepawa (Table 1). It was not possible to find low protein Neepawa samples in the same range as the Norstar samples, to use as a control in all the quality tests. Neepawa was the predominant Canada Western Red Spring wheat variety grown in the Prairies in 1985 with an estimated 43.7% of Canada Western Red Spring wheat being seeded to Neepawa. Therefore, the quality data from the Grain Research Laboratory for eastern prairie composite samples of No.1 Canada Western Red Spring wheat is given in Appendix A (Grain Research Laboratory, 1985) and will be referred to when comparing the wheat samples.

4.2 MILLING CHARACTERISTICS

In a preliminary study to determine the optimum conditioning level for winter wheat one sample of Norstar wheat (from Homewood location) was conditioned (at 14%, 15.0%, 15.5% and 16%) and allowed to stand overnight. The milling yields and flour ash contents were found to be similar for all conditioning levels (Table 5). Therefore, in subsequent

experiments all Norstar samples were conditioned at 15.5% moisture. This was the same conditioning level used for the Neepawa and Glenlea samples.

TABLE 5

Milling Yield and Ash Content of Norstar Wheat (Homewood) at Various Conditioning Levels.

Moisture Level (%)	Milling Yield (%)	Ash Content[1] (%)
14.5	77.2	0.403
15.0%	77.6	0.408
15.5	77.3	0.396
16.0%	77.4	0.401
Range	77.4 ± 0.2	0.402 ± 0.006

1 14% moisture basis

The data for the milling characteristics of the wheat samples are presented in Table 6. All of the Norstar samples had a higher flour yield than the Neepawa, Glenlea and Frankenmuth samples. The correlation coefficients between flour yield and wheat protein or test weight for the Norstar-Minto samples and for the combined data from both locations were found to be not significant (Tables 2 and 4). However, for Norstar-Portage samples the correlation coefficient between flour yield and wheat protein was significant at the 5% level (Table 3). Pittman and Tipples (1978) reported that flour yield had little relationship with either protein content or test weight.

TABLE 6

Milling Characteristics of the Wheat Samples.

Sample[1]	Flour yield (%)	Flour ash content[2] (%)	Flour protein[3] (%)	Protein loss on milling (%)
Norstar-Minto (8.8)	74.3	0.418	7.7	1.1
" (9.7)	74.9	0.377	8.8	0.9
" (11.5)	74.5	0.356	10.6	0.9
" (12.4)	74.6	0.356	11.6	0.8
" (13.2)	74.9	0.343	12.5	0.7
Norstar-Portage (10.0)	77.1	0.350	8.7	1.3
" (11.3)	75.9	0.333	10.2	1.1
" (11.8)	75.8	0.323	10.9	0.9
" (12.8)	75.4	0.331	11.7	1.1
" (13.8)	75.2	0.347	12.8	1.0
Neepawa (15.0)	71.2	0.374	14.5	0.5
Glenlea (14.5)	72.4	0.438	13.7	0.8
Franken-muth (8.7)	74.2	0.434	8.0	0.7

1 The numbers in parenthesis represent % wheat protein content (N x 5.7, 14% moisture basis).
2 14% moisture basis
3 N x 5.7, 14% moisture basis

4.2.1 Ash Content

All milled samples had an acceptable ash content. The correlation coefficients between protein content and ash content were not significant for all Norstar samples (Tables 2, 3 and 4). Pittman and Tipples (1978) working with winter wheat samples grown in Alberta found that neither wheat ash nor flour ash content appeared to be markedly affected by protein content. However, Tipples et al. (1977) found that flour ash tended to fall with increasing protein content of Neepawa wheat. All

Norstar flour samples except Norstar-Minto 8.8 and 9.7% protein content samples were lower in ash content than Neepawa. Both Glenlea and Frankenmuth flour samples were higher in ash than the Norstar and Neepawa samples.

4.2.2 Protein Loss on Milling

The protein loss on milling was higher for the Norstar samples compared to the other wheat samples (Table 6). This might be due to the differences in protein distribution in the kernels between endosperm and bran layers. Despite the higher protein loss on milling, the Norstar samples had good milling characteristics with low ash and high milling yields.

4.3 COMPOSTION AND TECHNOLOGICAL CHARACTERISTICS OF FLOUR

4.3.1 Flour Protein Content

Flour protein content and quality are the most significant factors influencing baking performance in terms of loaf volume. Flour protein is also one of the most important factors which influence the water absorption capacity, and blending potential of wheat flours.

The protein contents of the flour samples are presented in Table 7. As expected all Norstar samples had a lower flour protein content than the Neepawa and Glenlea samples due to their lower wheat protein contents. Highly significant correlation coefficients were found between the protein content of the Norstar samples and the various quality parameters (Tables 8, 9 and 10).

TABLE 7

Gluten Characteristics and Sedimentation Values of the Flour Samples.

Sample[1]	Flour protein[2] (%)	Wet gluten[3] (%)	Dry gluten[3] (%)	WHC[4] (%)	Zeleny sedimentation (c.c.)
Norstar-Minto (8.8)	7.7	17.4	6.3	176.2	36
" (9.7)	8.8	20.4	7.4	175.7	49
" (11.5)	10.6	28.0	10.2	174.5	60
" (12.4)	11.6	30.1	10.9	176.2	68
" (13.2)	12.5	32.6	11.8	176.3	68
Norstar-Portage (10.0)	8.7	22.3	8.2	172.0	42
" (11.3)	10.2	27.6	10.1	173.3	55
" (11.8)	10.9	28.8	10.4	176.9	59
" (12.8)	11.7	31.9	11.5	177.4	68
" (13.8)	12.8	33.7	12.3	174.0	70
Neepawa (15.0)	14.5	40.5	13.9	191.4	66
Glenlea (14.5)	13.7	33.1	13.0	154.6	61
Franken-muth (8.7)	8.0	20.5	7.4	177.0	20

1 The numbers in parenthesis represent % wheat protein content (N x 5.7, 14% moisture basis).
2 N x 5.7, 14% moisture basis
3 14% moisture basis
4 Water holding capacity = (wet gluten - dry gluten) x 100/dry gluten

TABLE 8

Simple Correlation Coefficients between Various Quality Parameters of Norstar-Minto Samples.

	FP	WG	DG	SED	FA	DDT	EXT	MAXR	ENER
Remix loaf volume	0.9766	0.9620	0.9592	0.9611	0.9882	0.9273	0.9551	0.8559	0.9759
Flour protein (FP)		0.9945	0.9934	0.9756	0.9882	0.9612	0.8949	0.9192	0.9792
Wet gluten (WG)			0.9999	0.9747	0.9718	0.9838	0.8533	0.9416	0.9686
Dry gluten (DG)				0.9747	0.9690	0.9850	0.8483	0.9448	0.9675
Zeleny sedimentation (SED)					0.9459	0.9462	0.8957	0.9595	0.9952
Farinograph absorption (FA)						0.9293	0.9298	0.8500	0.9626
Development time (DDT)							0.7792	0.9384	0.9267
Extensibility (EXT)								0.7360	0.9327
Maximum resistance (MAXR)									0.9299

r > 0.878 significant at 5% level

r > 0.959 significant at 1% level

TABLE 9

Simple Correlation Coefficients between Various Quality Parameters of Norstar-Portage Samples.

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	FP	WG	DG	SED	FA	DDT	EXT	MAXR	ENER
Remix loaf volume	0.9870	0.9631	0.9720	0.9398	0.9897	0.9450	0.3682	0.9329	0.9328
Flour protein (FP)		0.9905	0.9921	0.9801	0.9871	0.9574	0.4344	0.9010	0.9535
Wet gluten (WG)			0.9989	0.9962	0.9789	0.9302	0.4824	0.8355	0.9185
Dry gluten (DG)				0.9912	0.9863	0.9297	0.4670	0.8453	0.9137
Zeleny sedimentation (SED)					0.9625	0.9285	0.4827	0.8105	0.9154
Farinograph absorption (FA)						0.9478	0.3413	0.8963	0.9086
Development time (DDT)							0.2016	0.9469	0.9672
Extensibility (EXT)								0.1324	0.3737
Maximum resistance (MAXR)									0.9394

r > 0.878 significant at 5% level

r > 0.959 significant at 1% level

TABLE 10

Simple Correlation Coefficients between Various Quality Parameters of Norstar Samples from both Locations, Minto and Portage.

	FP	WG	DG	SED	FA	DDT	EXT	MAXR	ENER
Remix loaf volume	0.9737	0.9346	0.9326	0.9519	0.9820	0.9202	0.7705	0.8682	0.9291
Flour protein (FP)		0.9847	0.9825	0.9746	0.9598	0.9189	0.7628	0.8822	0.9586
Wet gluten (WG)			0.9995	0.9608	0.9119	0.8826	0.7745	0.8355	0.9358
Dry gluten (DG)				0.9560	0.9081	0.8784	0.7706	0.8370	0.9317
Zeleny sedimentation (SED)					0.9395	0.9174	0.7557	0.8644	0.9424
Farinograph absorption (FA)						0.9352	0.7166	0.8382	0.8804
Development time (DDT)							0.5549	0.9019	0.8713
Extensibility (EXT)								0.4650	0.7290
Maximum resistance (MAXR)									0.9150

r > 0.632 significant at 5% level
r > 0.765 significant at 1% level

4.3.2 Gluten Content

The gluten content of flour is related to the protein content of flour and widely used to predict bread-making quality. Wet gluten and dry gluten contents of the samples are presented in Table 7. The wet gluten and dry gluten contents for all of the Norstar samples increased with increasing protein content. The Norstar samples were lower in wet gluten and dry gluten compared to the Neepawa and Glenlea samples. As expected the correlation coefficients between remix loaf volume and gluten content for the Norstar samples were significant at the 1% level (Tables 8, 9 and 10). Highly significant correlation coefficients were found between gluten content and most quality parameters.

4.3.3 Water Holding Capacity

Water holding capacities of glutens from all the Norstar samples and the Frankenmuth sample were similar (Table 7). The water holding capacities of all Norstar glutens were lower than that of the Neepawa gluten but higher than the water holding capacities of the Glenlea gluten.

4.3.4 Zeleny Sedimentation Test

The Zeleny sedimentation is used widely for assessment of wheat quality especially in wheat breeding programs. The sedimentation values of the Norstar samples from Minto and Portage (Table 7) increased with increasing flour protein content. The correlation coefficients between the sedimentation value and the flour protein content were significant at the 1% level for the Norstar samples (Tables 8, 9 and 10). Highly significant correlation coefficients were found between the sedimentation value and the other quality parameters.

4.3.5 Starch Damage

The starch damage values of Norstar samples from both locations varied slightly but did not appear to be affected by protein content (Table 11). The level of damaged starch in Norstar wheat flours were lower than in the Neepawa and Glenlea flours and higher than in the Frankenmuth flour.

TABLE 11

Damaged Starch and Pentosan Contents of the Flour Samples.

Sample[1]	Starch damage (F.U.)[2]	Pentosan content (%)
Norstar-Minto (8.8)	18	1.0
" (9.7)	20	1.0
" (11.5)	17	1.1
" (12.4)	16	1.1
" (13.2)	17	1.1
Norstar-Portage (10.0)	15	1.1
" (11.3)	17	1.0
" (11.8)	17	1.1
" (12.8)	17	0.9
" (13.8)	17	1.0
Neepawa (15.0)	20	1.2
Glenlea (14.5)	30	1.2
Franken-muth (8.7)	4	1.3

1 The numbers in parenthesis represent % wheat protein content (N x 5.7, 14% moisture basis).
2 F.U. = Farrand Units

4.3.6 Pentosan Content

Kulp (1968) reported that the water-soluble and acetic acid-insoluble pentosans absorbed 11 times and 10 times their weight of water respectively. Therefore, the pentosans are an important flour constituent regulating water absorption capacity of flours. Bushuk (1966) reported that about one quarter of the water in dough is associated with pentosans.

The pentosan contents of all of the flour samples were determined and presented in Table 11. The pentosan contents of the Norstar flour samples from Minto and Portage were similar and not affected by protein content (Table 11). The pentosan content of the Norstar flours were lower than the pentosan content of the Neepawa, Glenlea and Frankenmuth flours.

4.3.7 Amylolytic Activity

Amylase activity has an important effect on the baking performance of flours because a considerable amount of water is held by the damaged starch granules during dough mixing. Increasing the level of starch damage causes a parallel increase in the water absorption capacity of a flour at the initial mixing. However, damaged starch is very susceptible to amylolytic attack. Excessive alpha-amylase will dextrinize a considerable portion of starch during fermentation and early oven stages in the baking process, releasing the water initially held by damaged granules. This will result in a wet, gummy crumb and highly colored loaf upon baking.

The amylolytic activities as determined by falling number, amylograph peak viscosity and alpha-amylase activity are presented in Table 12. The Norstar-Portage samples had a higher alpha-amylase activity, lower falling number values and lower amylograph peak viscosity than the Norstar-Minto samples. However, the amylolytic activity of these samples were not extreme and would probably not affect the flour quality detrimentally. Alpha-amylase activity increases dramatically during sprouting and germination. Lukow and Bushuk (1984) found that alpha-amylase activity increased 1600-fold and 3000-fold for Glenlea and Neepawa samples, respectively after 54 h of germination. From these observations, all of the Norstar samples were considered sound.

TABLE 12

The Properties of the Flour Samples Related to Proteolytic and Amylolytic Activities.

Sample[1]	Falling number (sec)	Amylograph peak viscosity (B.U.)[5]	Exoprotease activity[2]	Endoprotease activity[3]	Alpha-amylase activity[4]
Norstar-Minto (8.8)	337	580	545	0.0224	1.5
" (9.7)	366	800	530	0.0224	0.3
" (11.5)	375	820	485	0.0326	0.3
" (12.4)	380	800	510	0.0326	0.3
" (13.2)	374	800	420	0.0326	0.3
Norstar-Portage (10.0)	329	570	510	0.0285	1.7
" (11.3)	303	280	480	0.0295	4.8
" (11.8)	282	220	510	0.0315	6.8
" (12.8)	289	320	430	0.0326	4.4
" (13.8)	327	450	380	0.0366	2.9
Neepawa (15.0)	427	780	430	0.0285	0.3
Glenlea (14.5)	333	290	510	0.0336	5.5
Franken-muth (8.7)	321	530	640	0.0326	1.3

1 The numbers in parenthesis represent % wheat protein content (N x 5.7, 14% moisture basis).
2 µg glutamate/g flour/hr
3 ΔOD_{440}/g dry flour/hr
4 mg maltose/min/g x 10^{-3}
5 Brabender Units (viscosity)

4.3.8 Proteolytic Activities

The gluten is the unique viscoelastic protein of dough which determines its technological properties. Excessive proteolytic activity can have a detrimental effect on its quality. Redman (1971) presented evidence that softening of gluten was due to proteolytic hydrolysis of gluten proteins. Protease activity can lower the consistency and hence affect farinograph characteristics and baking absorption. Johnson and

Miller (1953) reported that, there was a linear relationship (r = 0.99) between Rhozyme P-11 proteinase concentrations freed of alpha-amylase and decrease in dough consistency after 4 h of digestion at pH 4.7 and 30°C.

The proteolytic activities as determined by exoprotease and endoprotease activity are presented in Table 12. The endoprotease activity increased slightly in the Norstar-Portage samples as the protein content increased. The lowest exoprotease activity was found in the highest protein Norstar samples. Lukow and Bushuk (1984) reported that protease activity increased gradually during germination. After 54 h of germination, the exoprotease activity and endoprotease activity increased around 2-fold for both Neepawa and Glenlea samples. Both hemoglobin and azocasein are different from indigenous wheat proteins and it is not known if the exo- and endoproteolytic activities determined using these substrates are the true measures of the in vivo proteolytic activity of wheat.

4.3.9 Rheological Properties of Flour Samples

4.3.9.1 Farinograph Test

All flour samples were subjected to the Brabender farinograph test which is a test widely used for the evaluation of mixing properties of flour. The farinograms of the Norstar-Minto samples and the Norstar-Portage samples along with the control samples are shown in Figures 1 and 2 respectively.

The farinograph parameters of the flour samples are presented in Table 13. With increasing protein content, farinograph absorption,

dough development time and stability values for all Norstar samples increased while mixing tolerance index values decreased. There were high correlation coefficients between farinograph absorption and flour protein content which were significant at the 1% level for all Norstar samples at both locations and for the combined data from both locations (Tables 8, 9 and 10). The correlation coefficients between the dough development time and protein content were significant at the 1% and 5% levels for Norstar samples from the Minto and Portage locations, respectively (Tables 8 and 9). For the combined data from the two locations the correlation coefficient between dough development time and flour protein content was also significant at the 1% level (Table 10). Significant correlation coefficients were also found between dough development time and the other quality parameters in the Norstar samples. Farinograph absorptions of all Norstar samples were lower than Neepawa and Glenlea but higher than Frankenmuth. Stability values of the Norstar samples with the high protein content from both locations were between Neepawa and Glenlea. All these samples had stability values higher than Frankenmuth. At the high protein range Norstar samples had a longer dough development time than Neepawa and Glenlea. Dough development time of Frankenmuth was the lowest among all samples (Table 13).

Figure 1: Farinograms of the Norstar-Minto Flour Samples together with the Varieties which are used as Control.

1 Norstar-Minto (12.5)*

2 Norstar-Minto (11.6)

3 Norstar-Minto (10.6)

4 Norstar-Minto (8.8)

5 Norstar-Minto (7.7)

NP Neepawa (14.5)

GL Glenlea (13.7)

FR Frankenmuth (8.0)

* The numbers in parenthesis represent % flour protein content (N x 5.7, 14% moisture basis).

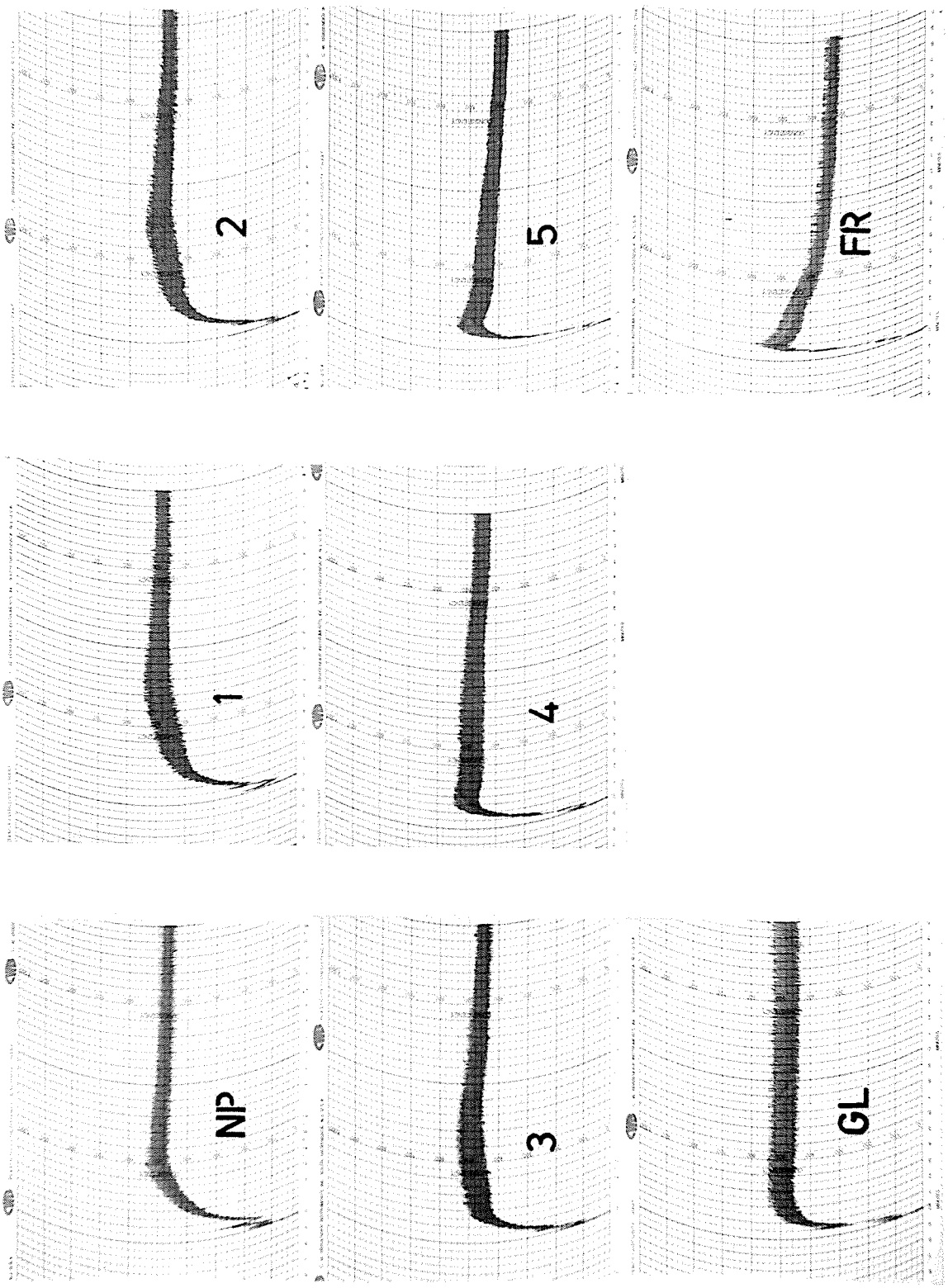

NP
1
2
3
4
5
GL
FR

Figure 2: Farinograms of the Norstar-Portage Samples together with the Varieties which are used as Control.

1 Norstar-Portage (12.8)*

2 Norstar-Portage (11.7)

3 Norstar-Portage (10.9)

4 Norstar-Portage (10.2)

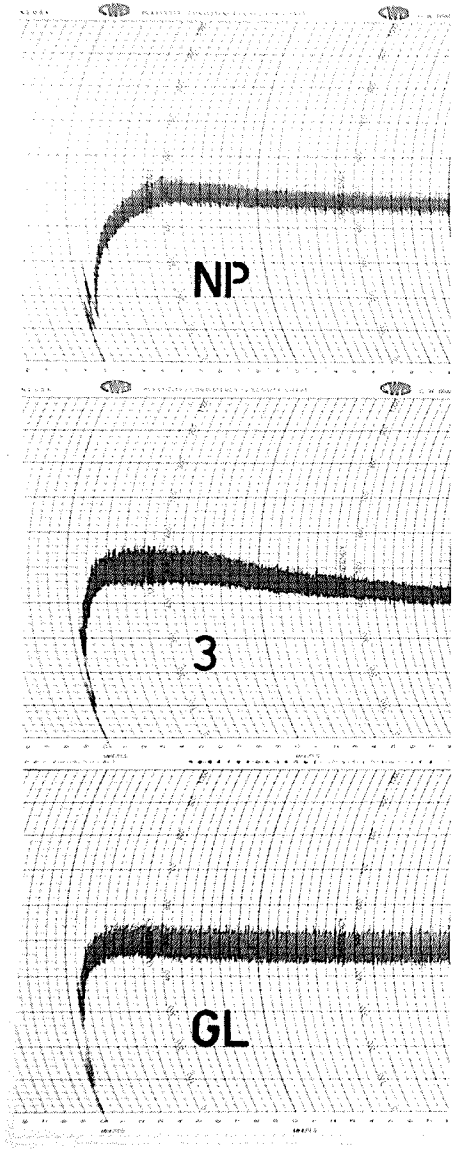
5 Norstar-Portage (8.7)

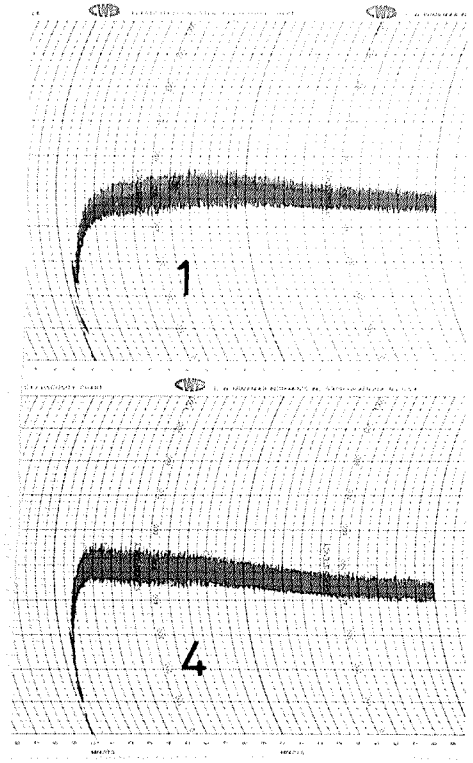
NP Neepawa (14.5)

GL Glenlea (13.7)

FR Frankenmuth (8.0)

* The numbers in parenthesis represent % flour protein content (N x 5.7, 14% moisture basis).


NP
3
GL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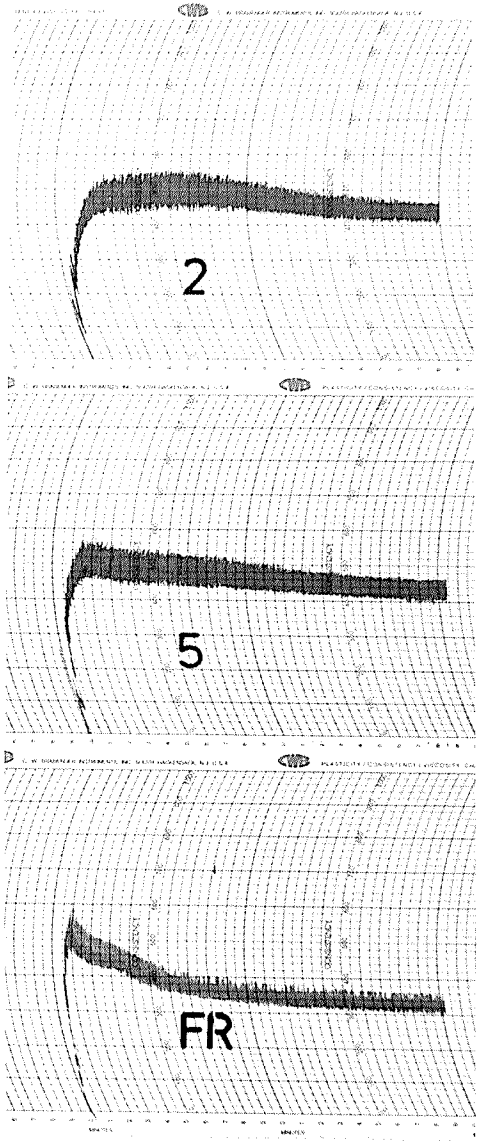
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FR

TABLE 13

Farinograph Parameters of the Flour Samples.

Sample[1]	Farinograph absorption (%)	Dough development time (min)	Mixing tolerance index[2] (B.U.)	Stability (min)
Norstar-Minto (8.8)	52.7	1.5	60	2.5
" (9.7)	53.4	2.0	20	11.0
" (11.5)	54.8	7.0	30	11.5
" (12.4)	56.4	7.5	25	12.5
" (13.2)	57.3	8.0	20	15.5
Norstar-Portage (10.0)	52.8	2.0	35	9.5
" (11.3)	54.3	2.5	30	9.5
" (11.8)	54.4	4.5	40	9.5
" (12.8)	55.6	6.5	40	10.5
" (13.8)	56.8	8.0	30	15.5
Neepawa (15.0)	62.0	5.0	25	10.0
Glenlea (14.5)	60.2	4.0	10	>20.0
Franken-muth (8.7)	49.8	1.0	160	1.8

1 The numbers in parenthesis represent % wheat protein content (N x 5.7, 14% moisture basis).
2 Brabender Units (consistency)

4.3.9.2 Extensigraph Test

The results of the extensigraph test are presented in Table 14. With increasing protein content, all extensigraph parameters (extensibility, maximum resistance and energy) increased for both the Norstar-Minto and the Norstar-Portage samples. For the Norstar-Minto samples the correlation coefficients between flour protein content and two extensigraph parameters (extensibility and maximum resistance) were significant at the 5% level. The correlation coefficient between flour protein content and energy value was significant at the 1% level (Table 8).

TABLE 14

Extensigraph Parameters of the Flour Samples.

Sample[1]	Extensibility (mm)	Resistance to extension at 5 cm (B.U.)[3]	Maximum resistance (B.U.)[3]	Rm/E[2]	Energy (cm^2)
Norstar-Minto (8.8)	160	333	455	2.84	100
" (9.7)	175	345	570	3.26	133
" (11.5)	172	450	705	4.10	152
" (12.4)	202	380	688	3.41	178
" (13.2)	200	400	700	3.50	180
Norstar-Portage (10.0)	176	315	530	3.01	126
" (11.3)	194	310	520	2.68	136
" (11.8)	198	400	643	3.25	178
" (12.8)	188	425	668	3.55	182
" (13.8)	188	515	863	4.59	207
Neepawa (15.0)	198	318	485	2.45	132
Glenlea (14.5)	168	585	830	4.94	185
Franken-muth (8.7)	160	138	150	0.94	34

1 The numbers in parenthesis represent % wheat protein content (N x 5.7, 14% moisture basis).
2 Rm/E = Ratio of maximum resistance to extensibility
3 B.U. = Brabender Units (resistance to extension)

For the Norstar-Portage samples the correlation coefficient between flour protein content and extensibility was not significant, but the correlation coefficients between flour protein content and the other two parameters (maximum resistance and energy) were significant at the 5% level (Table 9). For the combined data of both locations the correlation coefficients between flour protein content and maximum resistance and energy were significant at the 1% level, and between flour protein content and extensibility at the 5% level (Table 10). There were also

significant correlations between extensigraph parameters and the other quality parameters (Table 8, 9, 10).

The extensigrams of Norstar samples had high resistance and ratio values (Table 14). This could be due to a low farinograph absorption. Shuey (1975) reported that a dough with a high resistance is tough, bucky and difficult to machine while high ratio values are indicative of a large resistance compared to its extensibility. Therefore, these doughs on fermentation tend to be short. The high resistance values of Norstar samples may cause problems in automated baking systems. However, this problem is not of major concern, since a slight increase in absorption will lower the resistance.

Extensibility values of all Norstar samples were higher than the Glenlea and Frankenmuth samples except for the Norstar-Minto sample with the lowest protein content. The Norstar samples with 11.8 and higher flour protein content had an extensibility value comparable to the Neepawa sample. Maximum resistance and energy values of all Norstar samples were lower than the Glenlea samples except for the Norstar-Portage sample with the highest protein content. However, all Norstar samples had maximum resistance values higher than the Neepawa sample except for the Norstar-Minto sample with the lowest protein content. Energy values of all Norstar samples were higher than Neepawa except for the Norstar samples with the lowest protein content from both locations. The ratio values of all Norstar samples were lower than Glenlea and higher than Neepawa and Frankenmuth. All extensigraph parameters of the Frankenmuth sample were lower than the corresponding values of all other samples (Table 14).

4.4 BAKING PERFORMANCE

There are many methods for predicting the baking quality of a flour which are based on physical, chemical, or biochemical properties of flour. However, in some cases the results of the prediction tests might be misleading in terms of baking quality. It is generally accepted that there is no real substitute for an actual baking test. Therefore, the baking performance of the Norstar flour samples was evaluated using a GRL remix baking test and a GRL remix blend test.

4.4.1 GRL Remix Baking Test

The external and internal loaf characteristics of the baked samples are shown in Figures 3 and 4. At the higher protein content range the Norstar samples from both locations had good crumb characteristics. They had good, uniform cell structure comparable to the Neepawa sample. At the lower protein end the cell structure of the Norstar samples were close and not uniform and comparable to the Glenlea sample. The Frankenmuth sample had extremely coarse cell structure and a very dense texture.

The baking test results are presented in Table 15. Remix loaf volume values increased with increasing protein content for both the Norstar-Minto and Norstar-Portage samples. For the Norstar samples, there was a high correlation coefficient between remix loaf volume and flour protein content, significant at the 1% level for the both locations (Tables 8 and 9) and also significant at the same level for the combined data from both locations (Table 10).

Figure 3: External and Internal Loaf Characteristics of the Norstar-Minto Samples together with the Varieties used as Control (Remix Baking Process).

1 Norstar-Minto (12.5)*

2 Norstar-Minto (11.6)

3 Norstar-Minto (10.6)

4 Norstar-Minto (8.8)

5 Norstar-Minto (7.7)

NP Neepawa (14.5)

GL Glenlea (13.7)

FR Frankenmuth (8.0)

* The numbers in parenthesis represent % flour protein content (N x 5.7, 14% moisture basis).

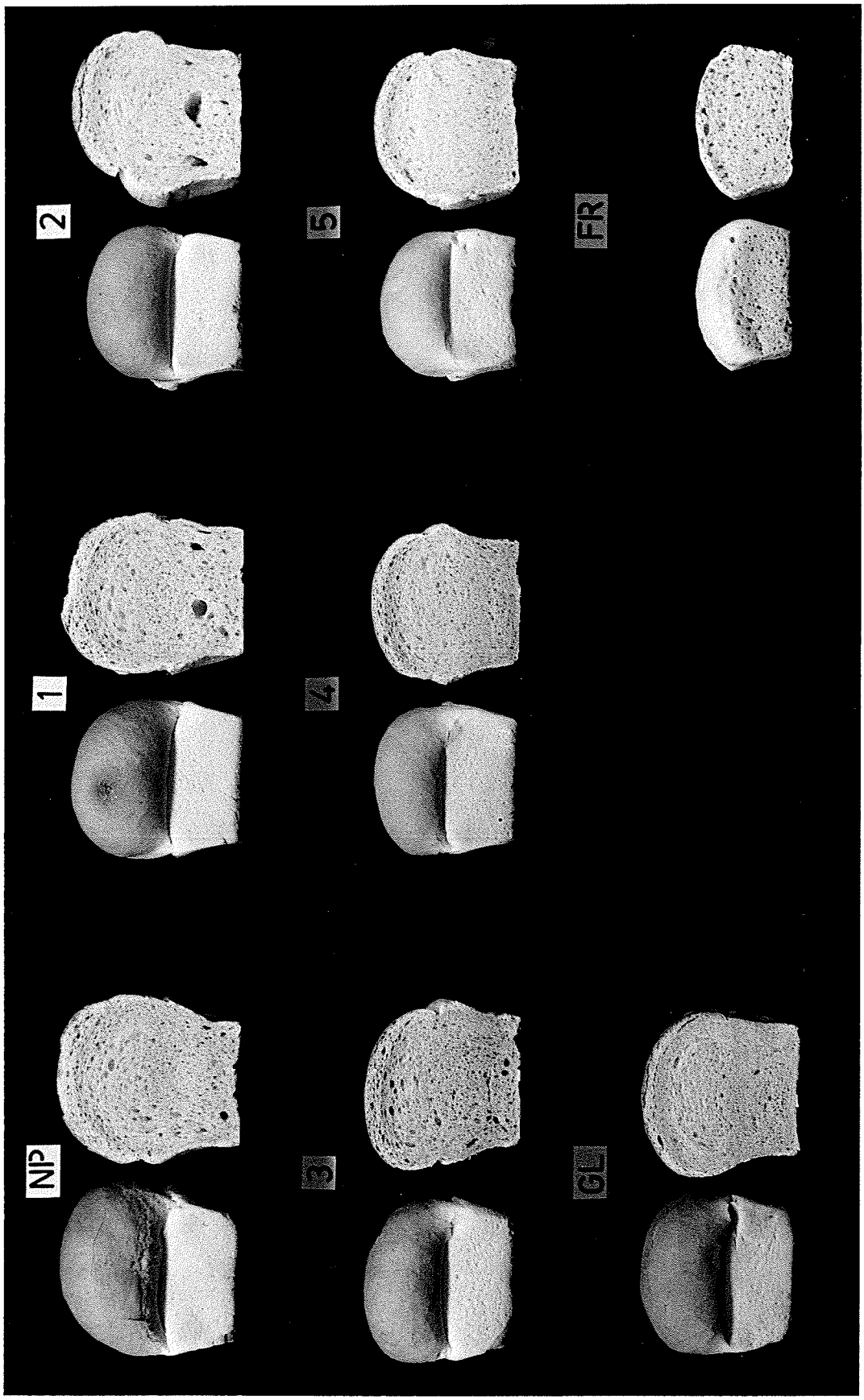

NP
1
2
3
4
5
GL
FR

Figure 4: External and Internal Loaf Characteristics of the Norstar-Portage Samples together with the Varieties used as Control (Remix Baking Process).

1 Norstar-Portage (12.8)*

2 Norstar-Portage (11.7)

3 Norstar-Portage (10.9)

4 Norstar-Portage (10.2)

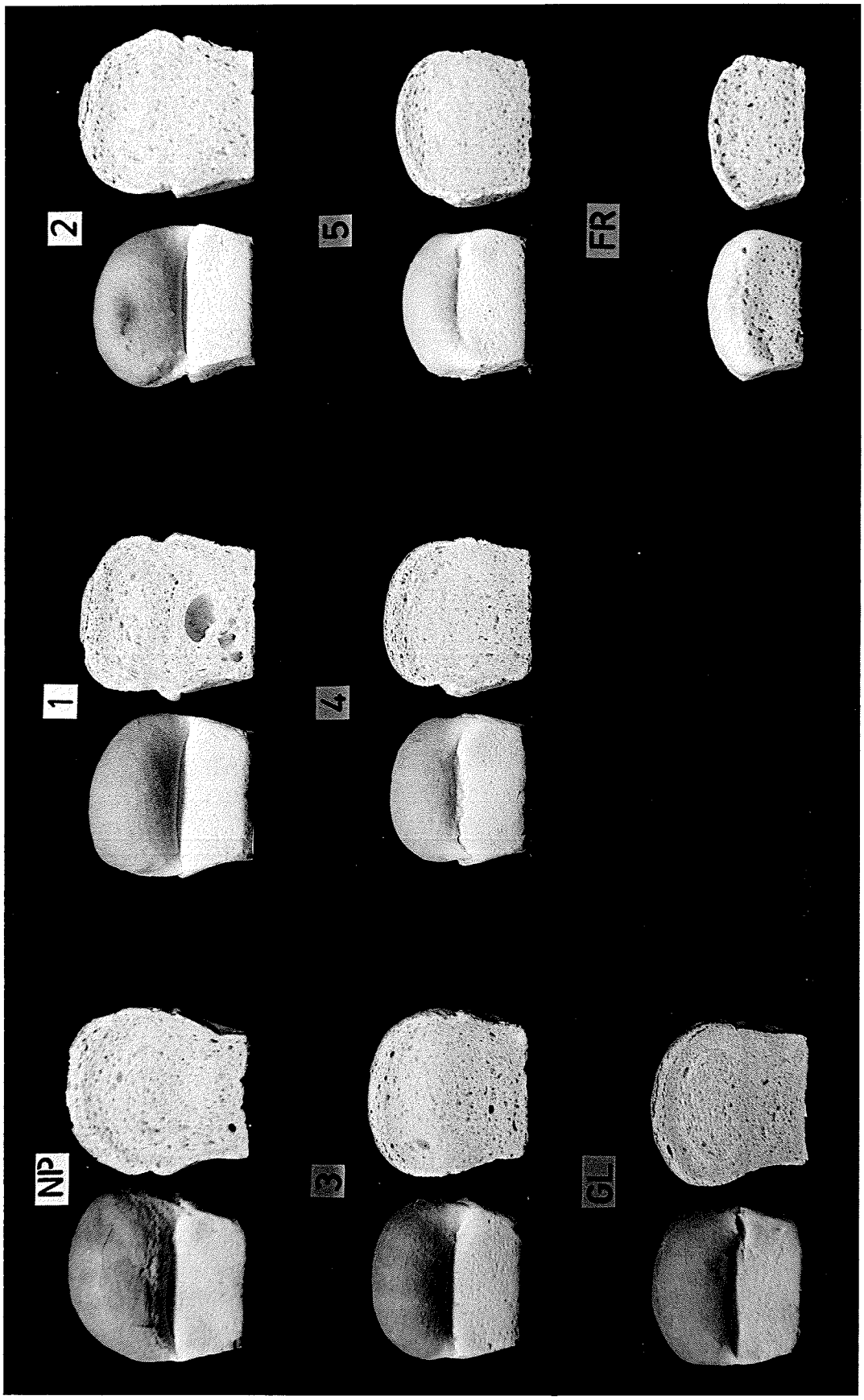
5 Norstar-Portage (8.7)

NP Neepawa (14.5)

GL Glenlea (13.7)

FR Frankenmuth (8.0)

* The numbers in parenthesis represent % flour protein content (N x 5.7, 14% moisture basis).


NP
1
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3
4
5
GL
FR

TABLE 15

Absorption and Baking Characteristics of the Flour Samples.

Sample[1]	Farinograph absorption (%)	Baking absorption (%)	Remix loaf volume (c.c.)	Unit loaf volume[2] (c.c./%)	Remix blend loaf volume (c.c.)
Norstar-Minto (8.8)	52.7	50.7	540	70.1	425
" (9.7)	53.4	51.4	598	68.0	440
" (11.5)	54.8	52.8	688	64.9	495
" (12.4)	56.4	54.4	833	71.8	500
" (13.2)	57.3	55.3	838	67.0	530
Norstar-Portage (10.0)	52.8	50.8	580	66.7	430
" (11.3)	54.3	52.3	675	66.2	460
" (11.8)	54.4	52.4	705	64.7	470
" (12.8)	55.6	53.6	750	64.1	495
" (13.8)	56.8	54.8	865	67.6	525
Neepawa (15.0)	62.0	60.0	918	63.3	595
Glenlea (14.5)	60.2	58.2	685	50.0	605
Franken-muth (8.7)	49.8	47.8	350	43.8	---

1 The numbers in parenthesis represent % wheat protein content (N x 5.7, 14% moisture basis).
2 Loaf volume per unit protein.

The unit loaf volumes of Norstar and Neepawa were higher than Glenlea and Frankenmuth with Frankenmuth having the lowest unit loaf volume value (Table 15). A true comparison of all samples is not possible due to the differences in protein content of these samples. However, the Neepawa sample will be compared to the Norstar samples later in the thesis using regression analysis.

4.4.2 GRL Remix Blend Test

The remix blend baking procedure determines the ability of a flour to carry a soft wheat flour, and supplements the results obtained from the standard remix baking procedure (Kilborn and Tipples, 1981). A variety which requires a very long dough development time might result in a low loaf volume in the standard remix test due to undermixing. If it performs well in the remix blend method, this shows that its protein is of good quality but requires longer mixing for proper development.

None of the Norstar samples performed well in remix blend procedure (Table 15). The samples with lower protein content from both locations were not acceptable in terms of crumb and crust characteristics while high protein content Norstar samples were not comparable to Neepawa in terms of crumb and crust characteristics. Even the samples with the highest protein content were not comparable to Neepawa in terms of crumb and crust characteristics. Considering the fact that the baking quality is affected by both protein quality and quantity, the relatively lower protein content of the Norstar samples might be the reason for their unsatisfactory baking performance in remix blend procedure.

4.5 COMPARISON OF THE NORSTAR AND NEEPAWA SAMPLES BY USING REGRESSION ANALYSIS

4.5.1 Baking Quality

The baking quality of the Norstar samples as determined by remix loaf volume was affected by various quality parameters (Tables 8, 9 and 10). To study the effect of these parameters on the variation in remix loaf volume, the coefficients of determination (R^2 values) were determined (Table 16). In a simple or multiple regression analysis R^2 value meas-

ures the contribution of the independent variables to the variation in the dependent variable.

TABLE 16

R^2 Values Between Remix Loaf Volume and Various Flour Quality Parameters.

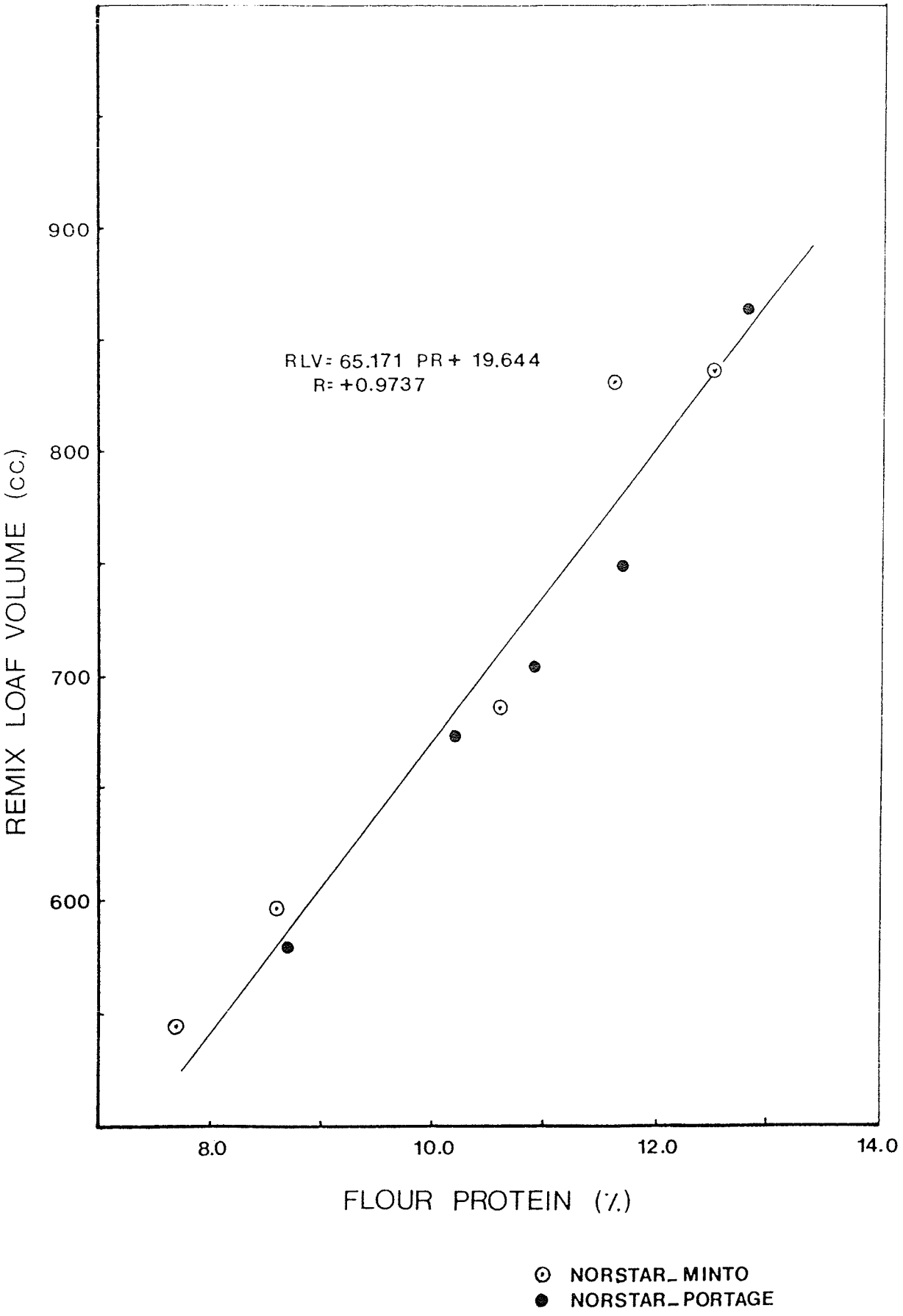
Independent Variable	R^2
Flour protein	0.9480
Zeleny sedimentation	0.9061
Energy of Extension	0.8633
Dough development time	0.8467
Maximum resistance	0.7538
Extensibility	0.5936
Starch damage	0.0768

Table 16 shows that flour protein gives a closer estimate of remix loaf volume than the other quality tests, and 94.8% of the total variation in remix loaf volume can be accounted for by a linear function of flour protein content. The linear regression of remix loaf volume on flour protein content is presented in Figure 5. If Neepawa samples with a comparable protein content range were available, it would be possible to compare the linear response of remix loaf volume to changes in flour protein content in these two cultivars by simply comparing the slopes of the corresponding regression equations. Unfortunately in this study, it was not possible to obtain Neepawa samples with a protein range comparable to the Norstar samples. The Neepawa sample selected for this study was not in the protein range of the Norstar samples. One method that could be used to compare the Neepawa sample with the Norstar samples was to extrapolate the regression line for the Norstar samples presented in Figure 5. Another method was to compare the quality of

Norstar samples with Canada Western Red Spring wheat class which is mainly composed of Neepawa. The quality data on several composite samples of No.1 Canada Western Red Spring wheat produced by the Grain Research Laboratory (1985) is presented in Appendix A.

It was also decided to compare the Norstar samples with the set of Neepawa samples used by Rodriguez-Bores (1976). These Neepawa samples were grown in Burdett, Alberta in 1973 in a fertilizer-irrigation study. Their protein content, loaf volume and farinograph absorption values are presented in Appendix B. In this set of samples, loaf volumes showed a decrease with increasing protein for samples at the top of the protein range (Bushuk et al., 1978). Therefore, only the samples with low protein content were used to calculate a regression equation. These samples had a comparable protein content range to the Norstar samples.

Figure 5: The Linear Regression of Remix Loaf Volume against Flour Protein Content for the Norstar Samples from Portage and Minto Locations.


900
800
700
600
REMIX LOAF VOLUME (cc.)
RLV= 65.171 PR + 19.644
R= +0.9737
8.0
10.0
12.0
14.0
FLOUR PROTEIN (%)
NORSTAR_MINTO
NORSTAR_PORTAGE

The prediction intervals of the individual responses of remix loaf volume for the corresponding flour protein content values of the Neepawa sample and several composite samples of No.1 Canada Western Red Spring wheat were calculated and presented in Table 17. The remix loaf volume value of the Neepawa sample and the remix loaf volume values of the spring wheat samples reported by the Grain Research Laboratory (1985) are not excluded from the calculated 95% confidence interval of the regression line of Norstar samples. This indicates that the qualities of the Norstar samples and the composite samples of No.1 Canada Western Red Spring wheat listed in Table 17 are comparable in terms of remix loaf volume. The comparison of the regression line of Norstar samples (RLV = 65.171 PR + 19.644) with the one (RLV = 63.996 PR - 78.153) calculated by using the data presented by Rodriguez-Bores (1976) also confirms this conclusion. The slopes of the two regression lines were not significantly different at the 5% level. Therefore, it can be concluded that there is no significant difference in the linear response of remix loaf volume to changes in flour protein between Norstar and Neepawa on the basis of the samples compared.

A number of factors affect remix loaf volume at the same time (Tables 10 and 16). For an assessment of the influence of all these factors on remix loaf volume multiple regression analysis was used to find equations that best predicted the relationship between various quality parameters and remix loaf volume. The regression equations were derived by using the "Stepwise" procedure from the SAS package (Statistical Analysis System, SAS Institute Inc.) which is available on the University of Manitoba computer (AMDAHL 580).

TABLE 17

The Remix Loaf Volume Values and 95% Confidence Intervals for some Spring Wheat Samples.

	Protein content (%)	Remix loaf volume (c.c.)	95% Confidence interval[4]
Neepawa	14.5	918	893 - 1036
EPC[2] No.1 C.W. HRS (1984)[1]	13.3	910	817 - 956
" " " " (1985)[1]	13.4	880	823 - 963
" " " " (1985)[1]	12.1	780	740 - 877
EPC No.1 C.W. HRS[3]	13.1	855	804 - 943

1 From Agriculture Canada Crop Bull. No.166 (Grain Research Laboratory, 1985).
2 Eastern prairie composite.
3 10-year average.
4 Calculated from the regression equation of Norstar samples (RLV = 65.171 PR + 19.644).

Using the data from Tables 11, and 13, 14 and 15 multiple regressions were constructed in which remix loaf volume was expressed as a function of flour protein, sedimentation value, energy, dough development time, maximum resistance, extensibility and starch damage. Some of the regression equations are presented in Table 18. The evaluation of the regression equations showed that the model involving the three variables (flour protein content, dough development time and extensibility) was significant at the 1% level, and 96.2% of the total variation in remix loaf volume could be accounted for by a linear function of these three variables. The introduction of more independent variables into the equation did not improve the model to a significant extent.

TABLE 18

Regression Equations of RLV as a Function of Other Quality Parameters.

Regression formula	R^2
RLV = 65.171 PR + 19.664	0.948
RLV = 55.114 PR + 6.826 DDT + 91.965	0.952
RLV = 35.106 PR + 14.033 DDT + 1.502 EXT - 10.877	0.962
RLV = 24.801 PR + 12.954 DDT + 2.022 EXT + 0.141 RM - 82.776	0.964

RLV = Remix loaf volume
PR = Flour protein content
DDT = Farinograph dough development time
EXT = Extensigraph extensibility value
RM = Extensigraph maximum resistance value

4.5.2 Water Absorption Capacity

The farinograph absorptions of the Norstar flours were affected by a number of factors (Tables 8 and 9 and 10). The effect of these factors on the variation of farinograph absorption was studied by determining the R^2 values (Table 19). Flour protein content gave a closer estimate of farinograph absorption than all other tests. Over 92% of the total variation in farinograph absorption could be accounted for by a linear function of flour protein.

Starch damage and pentosan content which are also known to affect farinograph absorption were found to be less significant. Obviously, these results cannot be generalized, and were simply due to lack of variation in such parameters in this study. The very narrow ranges of factors such as pentosan content were a result of the fact that all of the samples used in the regression analysis were from a single wheat cultivar, Norstar.

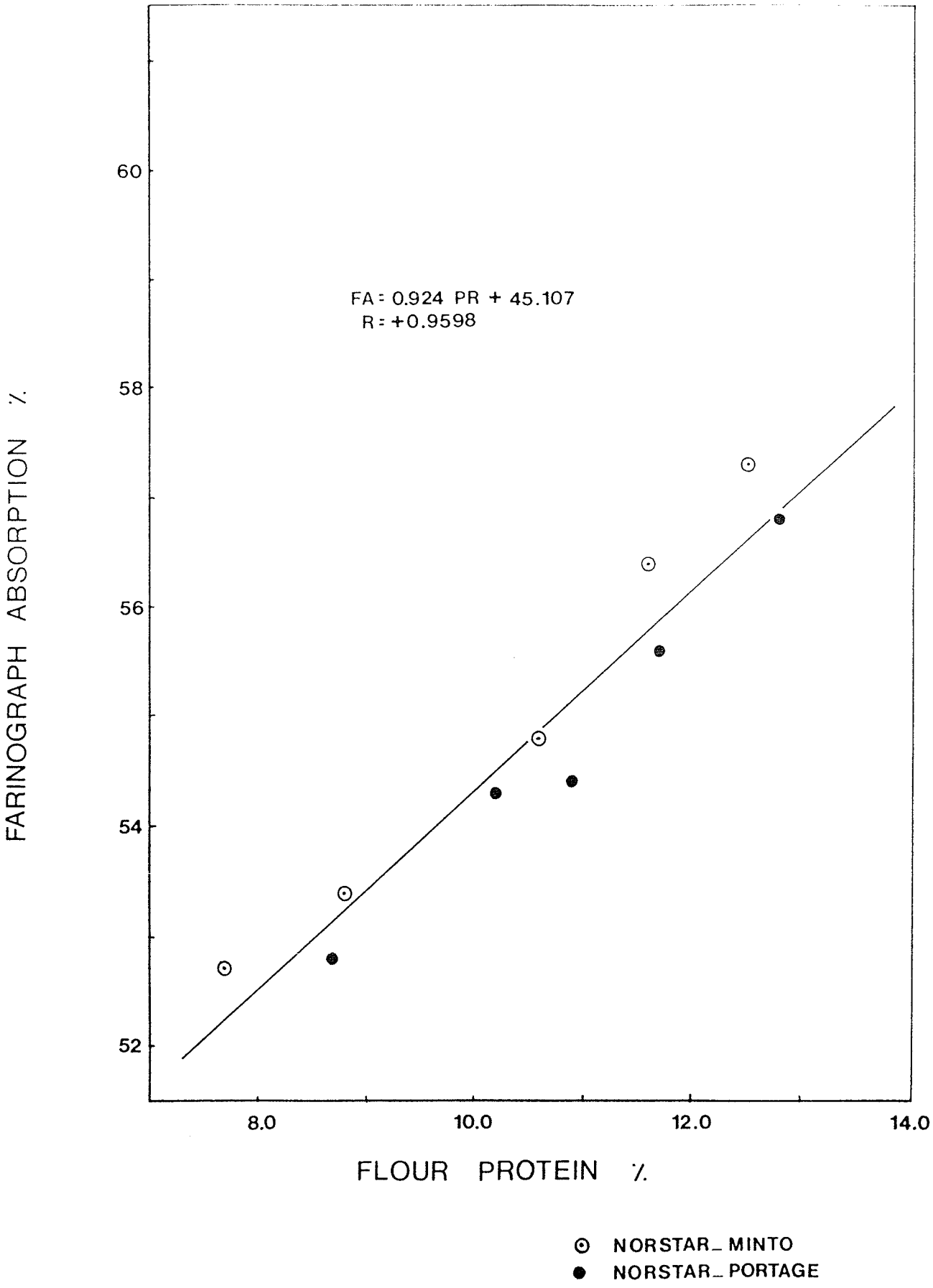
TABLE 19

R^2 Values Between Farinograph Absorption and Various Flour Characteristics.

Independent variable	R^2
Flour protein	0.9212
Endoprotease activity	0.6583
Exoproteolytic activity	0.6225
Starch damage	0.0433
Alpha-amylase activity	0.0063
Pentosan content	0.0046

The linear regression of farinograph absorption on flour protein content is presented in Figure 6. The prediction intervals of individual responses of farinograph absorption for the corresponding flour protein content values were calculated and presented in Table 20 for a Neepawa sample and for the same set of composite samples of red spring wheats as in Table 17.

Figure 6: The Linear Regression of Farinograph Absorption against Flour Protein Content for the Norstar Samples from Portage and Minto Locations.


FA = 0.924 PR + 45.107
R = +0.9598
60
58
56
54
52
8.0
10.0
12.0
14.0
FARINOGRAPH ABSORPTION %
FLOUR PROTEIN %
NORSTAR_MINTO
NORSTAR_PORTAGE

The farinograph absorptions of the Neepawa sample and the red spring wheat samples reported by the Grain Research Laboratory (1985) are excluded from the calculated 95% confidence interval of the regression line of Norstar samples (Table 20). This indicates that there is some difference between Norstar samples (Table 13) and the red spring wheat samples listed in Table 20 in terms of farinograph absorption. The slope of the regression line of the Norstar samples (FA =0.92 PR + 45.11) was not significantly different from the one (FA = 0.80 PR + 52.28) calculated by using the data presented by Rodriguez-Bores (1976). This indicates that the rate of increase in farinograph absorption is comparable between these two sets of samples. However, the intercept values of these regression lines were significantly different at the 1% level indicating that the Neepawa samples start with a higher absorption value and have a comparable rate of increase as the Norstar samples with increasing protein content. The significantly higher intercept value results in significantly higher farinograph absorption for Neepawa at each protein content level compared to Norstar.

For an assessment of the influence of other factors on farinograph absorption, multiple regression analyses were performed as mentioned earlier. Multiple regressions were constructed in which farinograph absorption was expressed as a function of flour protein, endoprotease and exoprotease activities, starch damage, alpha-amylase activity and pentosan content by using the data from Tables 7, 11, 12 and 13. Some of these equations are presented in Table 21. The evaluation of the regression equations showed that the model involving the three variables (flour protein content, alpha-amylase activity and endoproteolytic activity) was significant at the 1% level, and 98.2% of the total varia-

TABLE 20

The Farinograph Absorptions and 95% Confidence Intervals for some Red Spring Wheat Samples.

	Protein content (%)	Farinograph absorption (%)	95% Confidence interval
Neepawa	14.5	62.0	57.2 - 59.8
EPC[2] No.1 C.W. HRS (1984)[1]	13.3	63.1	56.2 - 58.6
" " " " (1985)[1]	13.4	65.5	56.2 - 58.7
" " " " (1985)[1]	12.1	65.3	55.1 - 57.5
EPC No.1 C.W. HRS[3]	13.1	65.5	56.0 - 58.4

1 From Agriculture Canada Crop Bull. No.166 (Grain Research Laboratory, 1985).
2 Eastern prairie composite.
3 10-year average.
4 Calculated from the regression equation of Norstar samples (FA = 0.924 PR + 45.107).

tion in farinograph absorption could be accounted for by a linear function of these three variables. Introduction of more independent variables into the regression equation did not improve the model to a significant extent.

Moss (1961) studied the influence of variety and milling system on milling damage. In this study multiple regression analysis was used to determine the influence of some flour characteristics on farinograph absorption. However, only two independent variables (starch damage and diastatic activity) were used in the calculations compared to six independent variables tested in our study.

TABLE 21

Regression Equations of Farinograph Absorption as a Function of Some Quality Parameters.

Regression formula	R^2
FA = 0.924 PR + 45.107	0.921
FA = 0.956 PR - 0.160 AML + 45.141	0.971
FA = 1.171 PR - 0.144 AML - 88.901 ENDO + 45.513	0.982
FA = 1.327 PR - 0.138 AML - 165.552 ENDO - 0.148 SD	0.986

FA = Farinograph absorption
PR = Flour protein content
AML = Alpha-amylase activity
ENDO = Endoproteolytic activity
SD = Starch damage

4.6 FURTHER STUDIES ON THE FACTORS CONTRIBUTING TO THE DIFFERENCES IN FARINOGRPAH ABSORPTION BETWEEN NORSTAR AND NEEPAWA WHEATS

The first section of the thesis showed that the quality of the winter wheat variety, Norstar selected for this study was acceptable and comparable to Canada Western Red Spring wheats in terms of milling, rheological properties and baking quality. However, the water absorption capacities of Norstar flours were significantly lower than the Neepawa flour. Therefore, a number of studies on the factors which might contribute to lower water absorption of Norstar wheat were undertaken.

The water absorption values of flours are determined by a complex interaction of protein content and quality, starch damage level, proteolytic and amylolytic activities and pentosan content (Bushuk and Hlynka, 1964; Tipples, 1969). Unfortunately, an assessment of the influence of all these factors on the water absorption at the same time is very complicated. For this reason, as a practical experimental approach some of the factors which influence water absorption were held constant while the influence of the others factors were determined.

4.6.1 Comparison of Norstar and Neepawa Varieties at Various Starch Damage Levels

Table 11 showed little variation in starch damage in Norstar samples. To study the effect of starch damage on water absorption it was necessary to have a wider range of starch damage. Therefore, a composite sample of Norstar was prepared by blending the grain samples from Minto location to obtain a Norstar sample with equal protein content to the low protein Neepawa sample obtained from the Agriculture Canada Research Station in Winnipeg.

In this study the wheat from each variety was milled into semolina (or coarse endosperm middlings). The semolinas were subdivided into four flour subsamples, three of these subsamples were passed through the reduction rolls of a Buhler Lab mill at different setting for each sample to produce a range of starch damage levels as described in section 2.3.2.

Table 22 showed that the milling conditions did not severely affect the flour properties except starch damage of the Norstar or Neepawa samples. Comparing the two varieties, the protein contents were the same but the pentosan contents were slightly different. The reproducibility of the methods for the proteolytic enzyme activities, (exoprotease and endoprotease activity) was determined by analyzing one sample of Norstar five times. The data were used to calculate the standard deviation for each procedure. The standard deviations were 7.9 and 7.4×10^{-4} for exoprotease and endoprotease activites respectively. The differences in proteolytic activity between the lowest and highest starch damaged samples are within the limits of experimental error for both the Norstar and Neepawa samples. Although there is some difference

in terms of alpha-amylase activity between the two varieties, both samples are sound.

TABLE 22

The flour properties of Two Samples of Norstar and Neepawa Used in Starch Damage Study.

Sample[1]		Protein content[2] (%)	Pentosan content (%)	Alpha-amylase activity[3]	Exoprotease activity[4]	Endoprotease activity[5]
Norstar	(0)	11.7	1.15	0.2	445	0.0348
	(48)			0.4	460	0.0334
Neepawa	(0)	11.7	1.30	1.6	490	0.0356
	(64)			1.5	475	0.0368

1 The numbers in parenthesis represent the starch damage in Farrand Units.
2 N x 5.7, 14% moisture basis
3 mg maltose/min/g x 10^{-3}
4 µg glutamate/g flour/hr
5 ΔOD_{440}/g dry flour/hr

The quality characteristics of the Norstar and Neepawa flour samples produced with a range of starch damage are presented in Table 23. In both varieties as the starch damage increased there was a parallel increase in farinograph absorption. The amylograph peak viscosity decreased as the starch damage increased in both varieties. There was also a slight decrease in wet and dry gluten contents with increasing starch damage. The decrease in amylograph peak viscosity is probably because the damaged starch granules are very susceptible to amylolytic attack. The susceptibility of the damaged granules will result in more starch hydrolysis especially at the early stages of the amylograph test and lowers the viscosity. Holas and Tipples (1978) used various flour streams which ranged widely in starch damage level (15-89 Farrand

Units). They found that amylograph peak viscosity values tended to decrease with increasing starch damage.

To investigate the relationship between farinograph absorption and the level of starch damage, the linear regression of farinograph absorption on starch damage was calculated for both varieties (Figure 7). It is apparent from the figure that the regression lines are almost parallel. In other words there is no significant difference between these two samples in linear response of farinograph absorption to incremental changes in starch damage within the range studied. However, there was a difference in intercept values. A t-test on the intercept values showed that they differ significantly at the 1% level. The protein contents of these two samples were equal, and pentosan contents were only slightly different. Both samples were sound, and comparable in terms of proteolytic activities. Hence, the comparison of the intercepts of the two regression lines at "zero" starch damage level will eliminate the effect of protein content and starch damage. Only a small portion of the difference in farinograph absorption might be attributed to the differences in amylolytic activity and pentosan content. However, the difference in farinograph absorption (4.6% at zero starch damage level) is too large to be explained only by the differences in amylolytic activity and pentosan content. Some of the differences in farinograph absorption are probably due to qualitative differences between the proteins of the two varieties. This will be investigated in the next section.

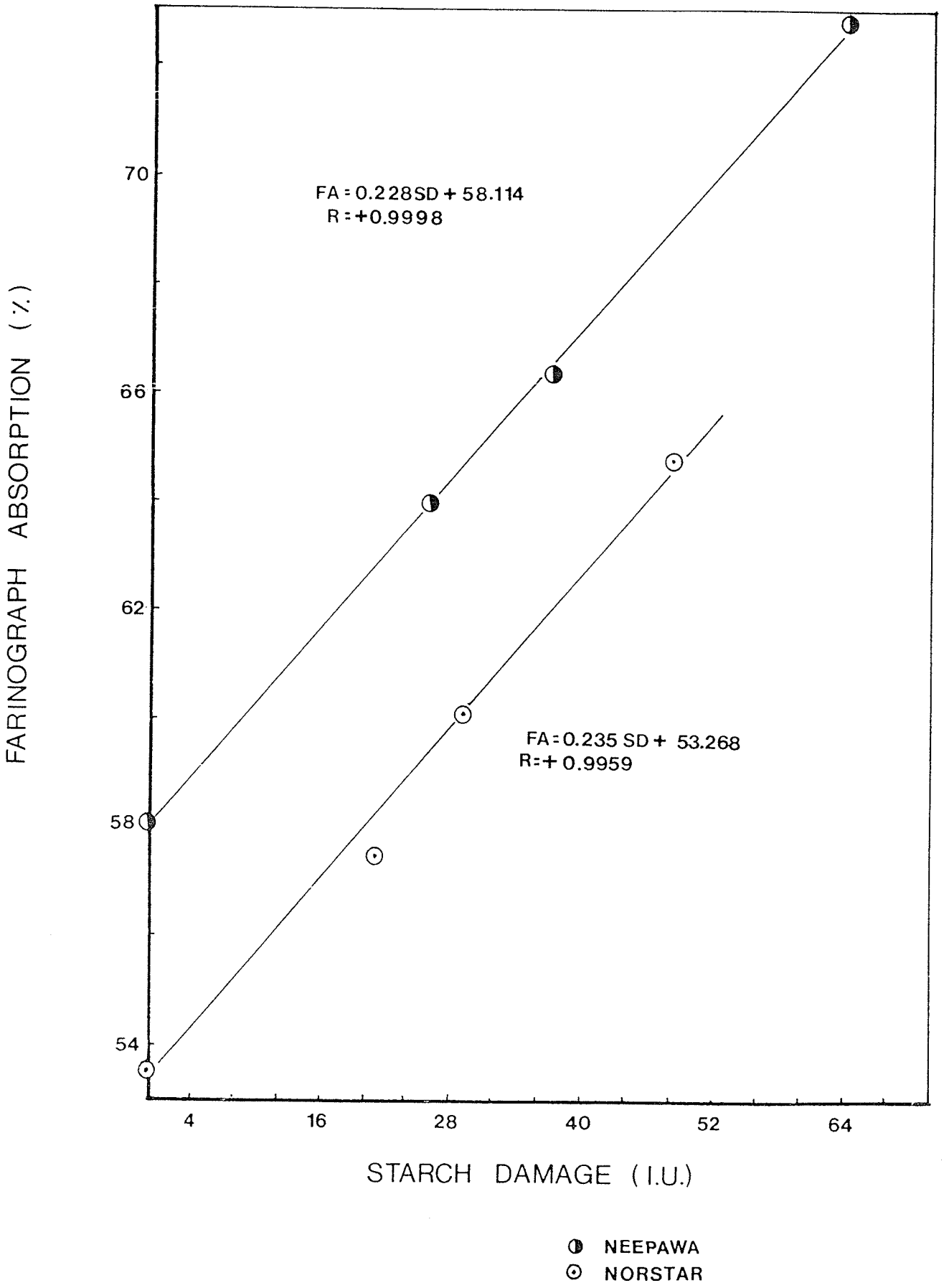
TABLE 23

The Effect of Starch Damage on Some Quality Characteristics of Norstar and Neepawa Samples.

Sample	Starch damage (F.U.)[1]	Farinograph absorption (%)	Rapid amylograph peak height (B.U.)[2]	Wet gluten content (%)	Dry gluten content (%)	WHC[3]
Norstar	0	53.6	1615	--	--	---
	21	57.6	1315	30.1	11.1	171.2
	29	60.1	1145	29.8	11.0	170.9
	48	64.8	1110	29.5	10.9	170.6
Neepawa	0	58.2	1390	--	--	---
	26	64.0	1180	30.9	11.1	178.4
	37	66.4	1110	30.3	10.9	178.0
	64	72.8	985	29.8	10.7	178.5

1 Farrand Units
2 Brabender units
3 Water holding capacity = (wet gluten - dry gluten) x 100/dry gluten

Figure 7: The Linear Regressions of Farinograph Absorption against Starch Damage for Norstar and Neepawa Samples.


FARINOGRAPH ABSORPTION (%)
70
66
62
58
54
FA = 0.228SD + 58.114
R = +0.9998
FA = 0.235 SD + 53.268
R = + 0.9959
4
16
28
40
52
64
STARCH DAMAGE (I.U.)
NEEPAWA
NORSTAR

4.6.2 The Influence of Norstar and Neepawa Glutens on Farinograph Characteristics

The qualitative difference between the proteins of the Norstar and Neepawa flours were studied by isolating the glutens from the flour and adding the glutens back to a base flour at different gluten levels. The farinograph characteristics of the base flour (Norstar at 7.7% protein content) and the farinograph characteristics of the samples produced by adding Norstar or Neepawa gluten to the base flour are presented in Table 24. There were differences in farinograph absorption, dough development time, mixing tolerance and stability responses between the glutens of the two varieties at the same protein content with Neepawa gluten having a greater effect on farinograph characteristics than Norstar glutens.

TABLE 24

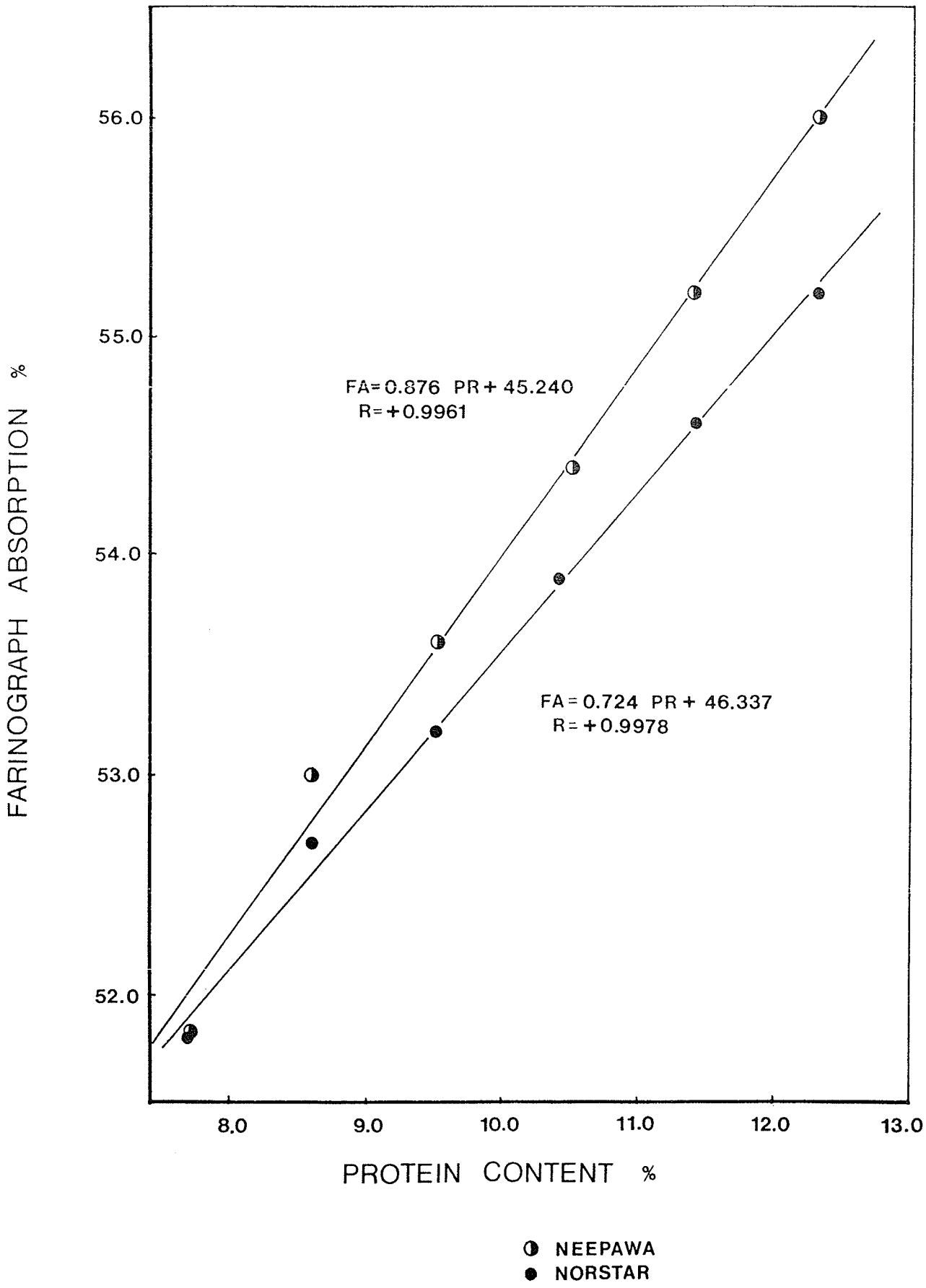
Effect of Added Neepawa and Norstar Glutens on Farinograph Characteristics.

	Protein content[1] (%)	Farinograph absorption (%)	Dough development time (min)	Mixing tolerance index (B.U.)	Stability (min)
Base flour (Norstar)	7.7	51.8	1.75	70	3.5
+Norstar gluten	8.6	52.7	1.75	50	4.5
" "	9.5	53.2	1.75	40	12.0
" "	10.4	53.9	2.00	35	13.5
" "	11.4	54.6	2.50	15	17.5
" "	12.3	55.2	3.00	5	>20.0
+Neepawa gluten	8.6	53.0	1.75	40	6.0
" "	9.5	53.6	2.25	35	8.0
" "	10.5	54.4	2.50	30	10.5
" "	11.4	55.2	3.00	15	16.5
" "	12.3	56.0	3.50	10	>20.0

1 N x 5.7, 14% moisture basis

The effect of Norstar and Neepawa glutens on farinograph absorption at various protein contents was investigated by determining the regression of farinograph absorption on flour protein (Figure 8). A t-test on the slopes of the two regression lines showed that the slopes were significantly different at the 5% level. Therefore, the rate of increase in farinograph absorption due to changes in flour protein was significantly higher by the addition of Neepawa gluten than Norstar gluten.

Figure 8: The Linear Regressions of Farinograph Absorption against Protein Content (Increased by Added Glutens) for Norstar and Neepawa.


FARINOGRAPH ABSORPTION %
56.0
55.0
54.0
53.0
52.0
FA=0.876 PR+45.240
R=+0.9961
FA=0.724 PR+46.337
R=+0.9978
8.0
9.0
10.0
11.0
12.0
13.0
PROTEIN CONTENT %
NEEPAWA
NORSTAR

4.7 PROTEIN SOLUBILITY FRACTIONS

The qualitative difference between the proteins of the two varieties was investigated by fractionating the glutens isolated from Neepawa and Norstar flour into acetic acid-soluble and acetic acid-insoluble fractions. The fractionation of the gluten was undertaken on the ground freeze-dried glutens by dissolving the gluten in 0.05 M acetic acid with mild magnetic stirring followed by centrifugation to separate the soluble and insoluble fractions. A very mild fractionation procedure was adopted because the fractions were to be used in further studies on protein functionality. Analytical tests such as amino acid analysis, pentosan content, sodium content, SDS sedimentation and gel filtration were also performed on these fractions and will be discussed in the following sections.

The percent recoveries of 0.05 N acetic acid-soluble and acetic acid-insoluble gluten fractions from Norstar and Neepawa glutens are presented in Table 25. The recovery of the acetic acid-insoluble fraction of Neepawa gluten was slightly greater while the recovery of its acetic acid-soluble fraction was slightly less as compared to Norstar gluten.

TABLE 25

Percent Recoveries[1] of 0.05 N Acetic Acid-Soluble and Acetic Acid-Insoluble Gluten Fractions from Glutens of Norstar and Neepawa Flours.

	Neepawa	Norstar
% Acetic Acid-Soluble Protein	45.2 (48.7)[2]	46.4 (52.3)
% Acetic Acid-Insoluble Protein	47.7 (51.3)	42.3 (47.7)
Total Recovery	92.9 (100.0)	88.8 (100.0)

1 Percent of total gluten protein
2 The figures in parenthesis represent the recoveries normalized to 100%

4.7.1 Composition of Gluten, Acetic Acid-Soluble and Acetic Acid-Insoluble Gluten Fractions

The protein, moisture, sodium and pentosan contents of the gluten and gluten fractions from both varieties are presented in Table 26. The protein and pentosan contents of the gluten, acetic acid-soluble and acetic acid-insoluble fractions of the Norstar were comparable to gluten and corresponding fractions of Neepawa.

Although the sodium contents of the Norstar and Neepawa flours were comparable, the sodium content of the Norstar gluten was more than twice the sodium content of Neepawa gluten. The sodium content of the tap water and distilled water used to isolate the gluten were determined, and found to be 2 ppm and 0.2 ppm respectively. It is well known that sodium and calcium ions found in salt and yeast food will reduce hydration capacity of gluten by causing its structure to tighten up leaving fewer binding sites for water (Meredith, 1969). Hydration studies on flour, starch and dry gluten have indicated that the effect of salt was mainly on gluten (Bushuk, 1963). As determined using a centrifugation technique, the amount of water held decreased for gluten and flour, and

TABLE 26

Composition of Gluten, Acetic Acid -Soluble and Acetic Acid-Insoluble Gluten Fractions from Norstar and Neepawa Samples.

	Protein content[1] (%)	Moisture content (%)	Pentosan content (%)	Sodium content (ppm)
Neepawa				
Total gluten	82.3	4.5	0.38	115 (34)[2]
Soluble fraction	94.8	4.4	0.15	
Insoluble fraction	69.3	4.8	0.55	
Norstar				
Total gluten	82.3	4.0	0.40	332 (30)[2]
Soluble fraction	93.7	4.9	0.18	
Insoluble fraction	68.9	6.3	0.58	

1 N x 5.7, dry basis
2 The figures in parenthesis represent sodium content of the flours which were used for preparing corresponding glutens.

remained constant for starch as the ionic strength of salt increased from 0 to 0.5. At a 2% salt level, the hydration capacity of gluten decreased by eight percent. In a salted dough, the amount of water associated with gluten would be somewhat less than in a salt-free dough (Bushuk, 1966). Norstar gluten appeared to bind a greater amount of sodium ions as compared to Neepawa gluten, under exactly the same conditions. This might effect their bound water and water holding capacities and will be discussed later.

4.7.2 Water Holding Capacity of Gluten, Gluten Fractions and Reconstituted Gluten from Norstar and Neepawa Samples

Preliminary studies of water holding capacity against a mild centrifugal force showed that a portion of acetic acid-insoluble fraction was soluble in water. Quinn and Paton (1979) also reported that a centrifu-

gation method which uses an excessive amount of water would result in a portion of water-soluble proteins being decanted with the supernatant. Therefore, this method with excessive amount of water was not suitable for acetic acid-insoluble fractions and was used only for gluten samples. With the acetic acid-soluble fraction even the smallest amount of water which was barely enough to wet the sample resulted in a very thick, viscous solution. Therefore, it was not possible to determine water holding capacity of the acetic acid-soluble fraction.

The water holding capacities of acetic acid-insoluble gluten fractions were determined according to the approved method 44-11 (AACC, 1983). In this method, only enough water was added to saturate the sample with no separate liquid phase. Quinn and Paton (1979), using this method found that the water holding capacity measurement was not affected by the solubility of the material.

The water holding capacities of total glutens, the acetic acid-insoluble fractions and reconstituted gluten are presented in Table 27. The water holding capacities of total and reconstituted glutens and acetic acid-insoluble fractions of Neepawa were greater than the same Norstar fractions. However, the water holding capacities of the insoluble fractions from both varieties were about 5-times greater than the water holding capacities of the original glutens. The hydrated insoluble fractions did not resemble the original glutens in terms of viscoelastic properties, but had the consistency of a soft gel.

TABLE 27

Properties of Gluten, Acetic Acid-Soluble and Acetic Acid-Insoluble Gluten Fractions from Norstar and Neepawa Samples.

	DSC bound water[1]	Water holding capacity (%)	SDS sediment-ation[2]
Neepawa			
Total gluten	0.53	169.2	82
Soluble fraction	0.61	---	---
Insoluble fraction	0.31	869.0	61; 19
Reconstituted gluten		377.8	
Norstar			
Total gluten	0.37	155.6	92
Soluble fraction	0.40	---	---
Insoluble fraction	0.27	838.2	75; 21
Reconstituted gluten		321.9	

1 g bound water per gram dry sample
2 60 min sedimentation time for gluten samples, 4 hr and 16 hr sediment-ation times for acetic acid-insoluble residue

4.7.3 Bound Water Contents of Gluten and Gluten Fractions Determined by DSC

The principle of DSC measurement is to compare the rate of heat flow to the sample and a reference material which are subjected to identical temperature changes. The phase changes in the sample that are associated with absorption or evolution of heat (such as crystallization or melting) causes a change in the differential heat flow which is then recorded as a peak. The area under the peak is related to the energy involved in the phase change.

The DSC thermal analyzer used in this study was equipped with a microcomputer and software which integrated the area under the curve and gave the calculated enthalpy values. The water was calculated using the

enthalpy values for pure water and water-sample mixture which was corrected for moisture content. Typical thermograms of the distilled, deionized water and water-gluten mixture are shown in figures 9 and 10 respectively.

Figure 9: Typical DSC Thermogram of Distilled Deionized Water.

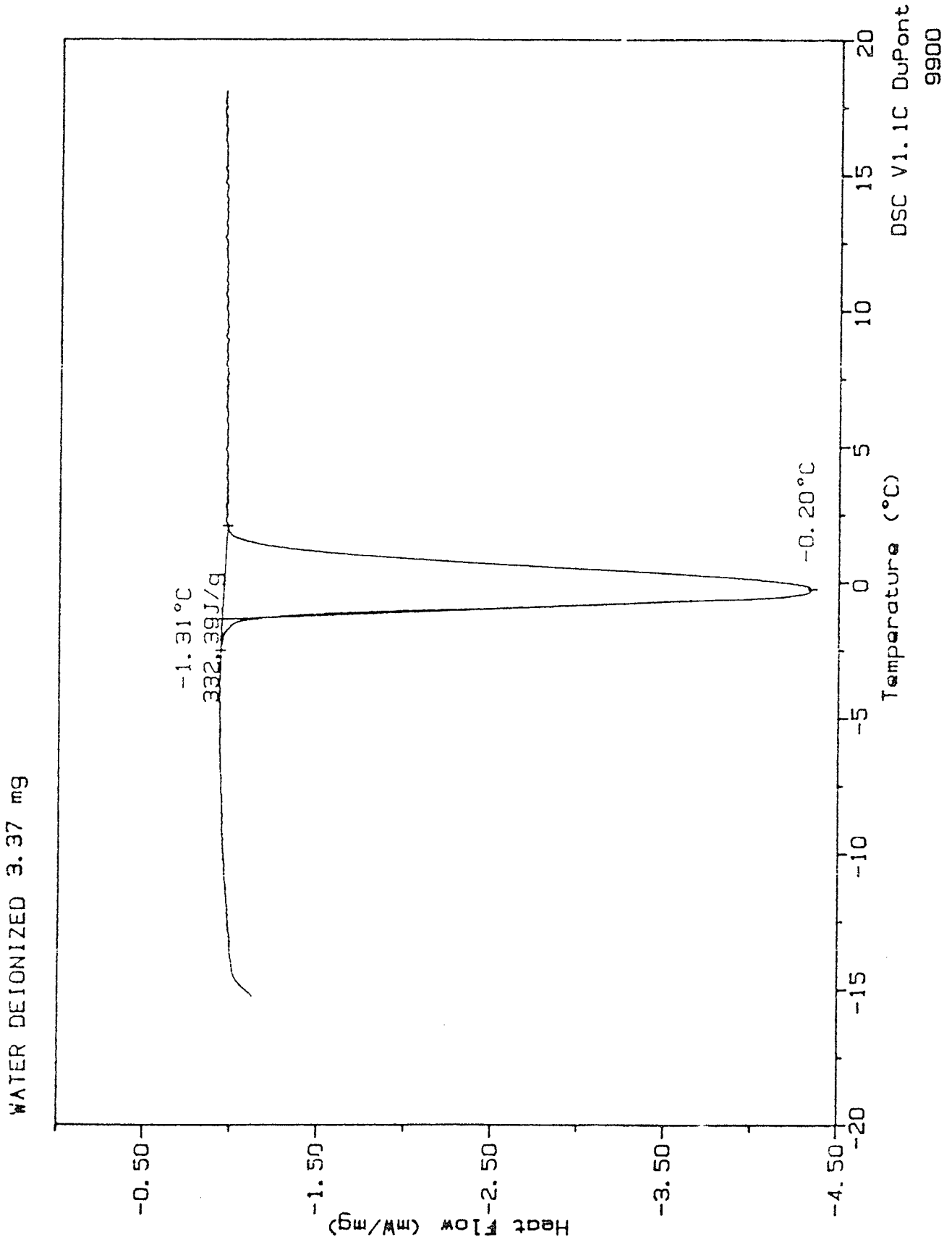
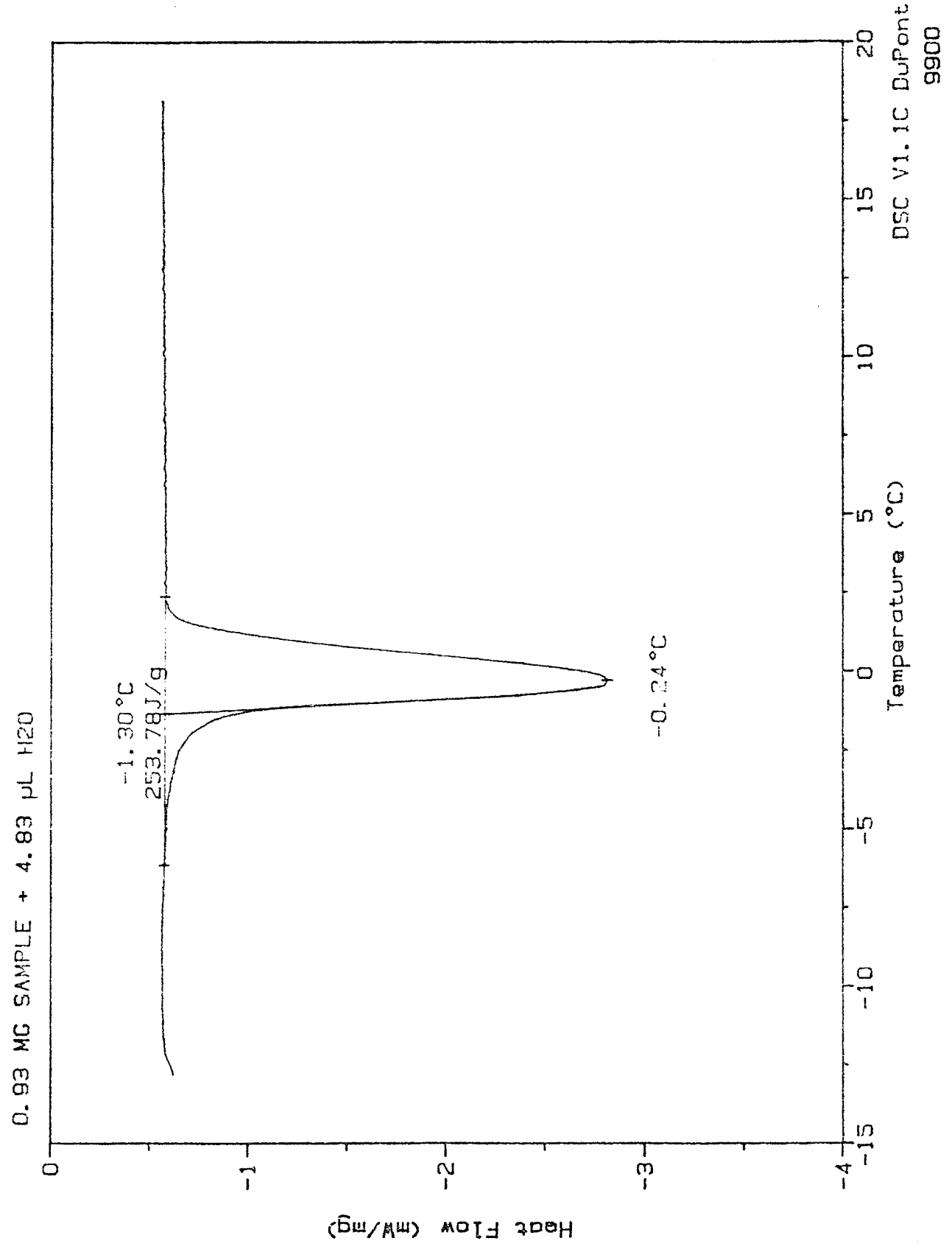

WATER DEIONIZED 3.37 mg
-1.31°C
332.39J/g
-0.20°C
Heat Flow (mW/mg)
-0.50
-1.50
-2.50
-3.50
-4.50
-20
-15
-10
-5
0
5
10
15
20
Temperature (°C)
DSC V1.1C DuPont
9900

Figure 10: Typical DSC Thermogram of Water-Gluten (Neepawa) Mixture.


0.93 MG SAMPLE + 4.83 µL H20
-1.30°C
253.78J/g
-0.24°C
Heat Flow (mW/mg)
Temperature (°C)
DSC V1.1C DuPont
9900

Preliminary studies were undertaken on wet gluten ball samples which were prepared by mixing freeze dried gluten and water. With this procedure it was not possible to obtain reproducible results because of the nonhomogeneous and sticky nature of the wet gluten which caused problems in weighing. Therefore, it was decided to weigh dry samples directly into the DSC pans and add water on top of the dry sample. Although this method gave more reproducible results, in some cases it resulted in a 10% variation between duplicate determinations. Since no mixing of gluten (or gluten fraction) took place; it might be argued that first mixing them into a wet gluten mass could possibly affect the results. In this study an assumption was made that mixing might affect the water holding capacity considerably but would not affect bound water content to a great extent. The DSC methodology used for the determination of bound water in gluten and gluten fractions requires further improvement.

The bound water values for gluten and gluten fractions are presented in Table 27. Neepawa gluten and its acetic acid-soluble and acetic acid-insoluble fractions had higher bound water contents compared to the Norstar gluten and the corresponding fractions. For each variety the soluble fraction had more bound water while the insoluble fraction had less bound water compared to the original glutens.

From these results it can be concluded that, if equal amounts of water were mixed with the same amount of Neepawa and Norstar glutens, Norstar gluten will have more free water than Neepawa gluten. This extra free water certainly will have a lubricating effect between the particles and therefore increase the mobility. This discussion can be extended to include gluten and gluten fractions which are incorporated into a dough system.

4.7.4 SDS Sedimentation Test

The SDS sedimentation test developed by McDermott (1983) to study the properties of commercial vital glutens was employed in the present study with some modification. The acetic acid-soluble fractions of both varieties were completely dissolved in SDS and resulted in a clear solution with no sediment. The glutens and acetic acid-insoluble gluten fractions of both varieties were turbid and it was not possible to get a reading after ten minutes of sedimentation as suggested by McDermott (1983). However, it was possible to get sedimentation readings for the glutens of both varieties after 60 min and for the insoluble fraction after 4h. Therefore, it was decided to take a reading at 4h and also allow the insoluble fraction to sediment another 12 h before taking the second reading.

The SDS sedimentation values for the glutens and the gluten fractions from both varieties are presented in Table 27. The Norstar gluten had a higher sedimentation value than Neepawa gluten. The insoluble fraction of Norstar had higher sedimentation value than the one from Neepawa after both sedimentation periods. The Zeleny sedimentation values of the Norstar-Minto and Norstar-Portage samples with the highest protein content were also higher than the Neepawa sample (Table 7) although their protein contents were lower than the Neepawa sample. Both sedimentation test results suggest that Norstar gluten can be classified as the strong type.

4.7.5 The Influence of Adding Soluble and Insoluble Gluten Fractions of Norstar and Neepawa on Farinograph Characteristics

To study the effects of the gluten fractions on farinograph parameters these fractions were added back to the same base flour to obtain flour samples with a range of protein contents. The blending of the base flour with the gluten fractions was done in the mixing bowl of the farinograph for three minutes prior to the actual farinograph test. The results are presented in Table 28. There was difference between farinograph absorption and dough development time responses between the corresponding gluten fractions of the two varieties at each protein content. The fractions obtained from the Neepawa gluten had a greater farinograph absorption than corresponding fractions of Norstar. There were also differences in the farinograph responses of the soluble and insoluble fractions of each variety. The insoluble fraction of each variety resulted in a higher farinograph absorption than its soluble counterpart. This observation together with the water holding capacity studies on gluten fractions suggested that acetic acid-insoluble proteins are more important to water absorption capacities of flours.

These results also suggested that acetic acid-soluble proteins had considerably more effect on dough-strength as measured by dough development time compared to the acetic acid-insoluble proteins. The addition of the insoluble fraction resulted in either a slight increase in development time or no increase. Previous studies by Preston and Tipples (1980) on gluten fractions have also shown similar results. These workers reported that acetic acid-soluble fraction had dough strengthening effect whereas the acetic acid-insoluble gluten fraction at higher levels had a slight dough weakening effect.

TABLE 28

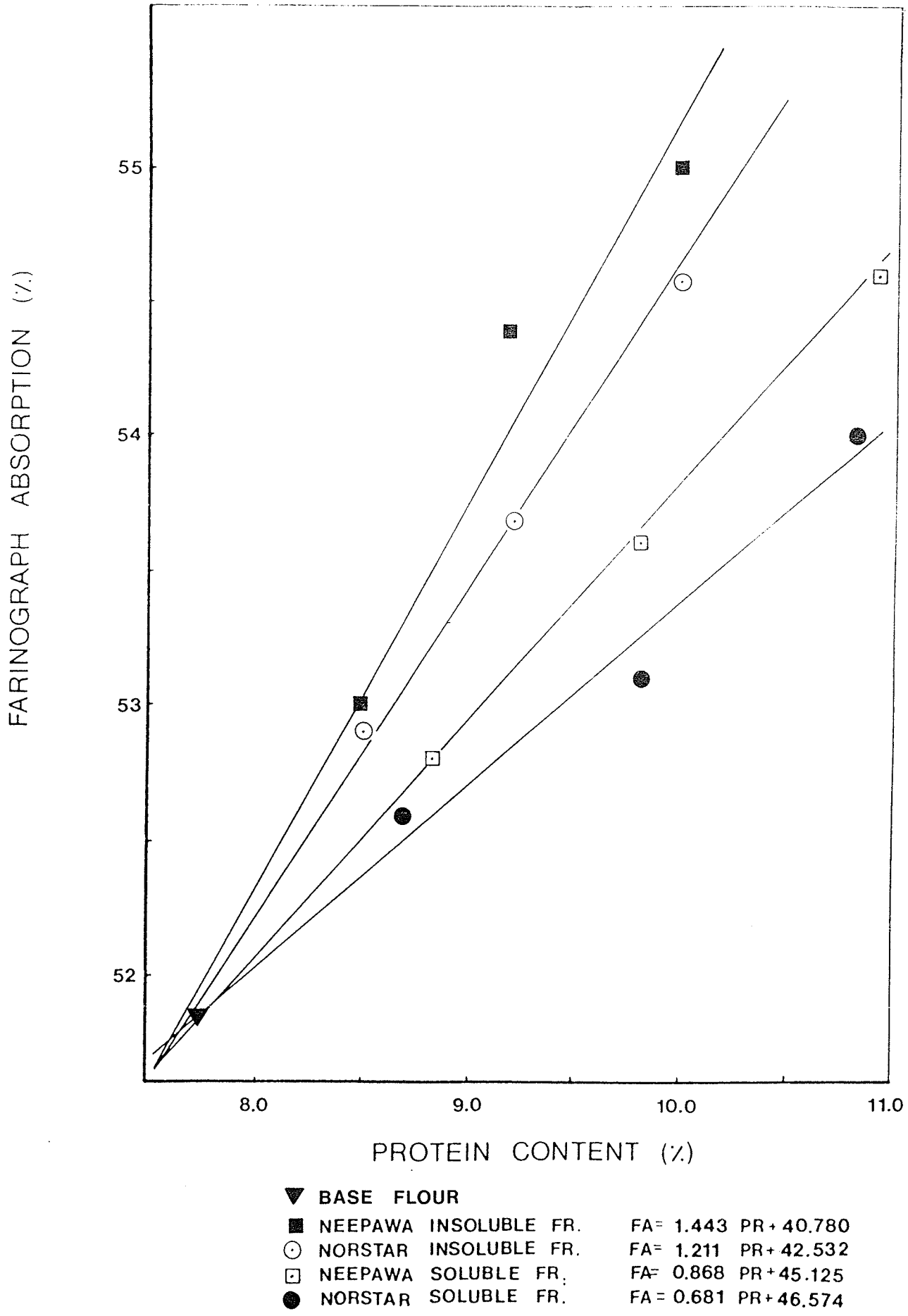
Effect of Added Acetic Acid-Soluble and Acetic Acid-Insoluble fractions of Neepawa and Norstar Gluten on Farinograph Absorption and Dough Development Time.

	Protein content[1] (%)	Farinograph absorption (%)	Dough development time (min)
Base flour (Norstar)	7.7	51.8	1.75
+Norstar soluble fraction	8.7	52.6	1.75
	9.8	53.1	2.25
	10.8	54.0	4.00
+Norstar insoluble fraction	8.5	52.9	1.75
	9.2	53.7	2.00
	10.0	54.6	2.00
+Neepawa soluble fraction	8.8	52.8	1.50
	9.8	53.6	2.50
	10.9	54.2	4.50
+Neepawa insoluble fraction	8.5	53.0	2.00
	9.2	54.4	1.75
	10.0	55.0	2.00

1 N x 5.7, 14% moisture

To examine the increased rate of farinograph absorption due to the addition of these fractions, the regression of farinograph absorption on flour protein is presented in Figure 11 for soluble and insoluble fractions of both varieties.

Figure 11: The Linear Regressions of Farinograph Absorption against Protein Content (Increased by Added Acetic Acid-Soluble and Acetic Acid-Insoluble Fractions) for Norstar and Neepawa.


FARINOGRAPH ABSORPTION (%)
55
54
53
52
8.0
9.0
10.0
11.0
PROTEIN CONTENT (%)
BASE FLOUR
NEEPAWA INSOLUBLE FR. FA= 1.443 PR + 40.780
NORSTAR INSOLUBLE FR. FA= 1.211 PR + 42.532
NEEPAWA SOLUBLE FR. FA= 0.868 PR + 45.125
NORSTAR SOLUBLE FR. FA = 0.681 PR + 46.574

A regression analysis and a t-test on the slopes of these regression lines showed that the slopes of some of the regression lines were different at the 5% level of significance. The rate of increase in farinograph absorption due to increasing protein content was significantly higher with addition of insoluble fractions than for soluble fractions for both Neepawa and Norstar. The slopes of the regression lines of Neepawa soluble and Norstar soluble fractions were significantly different but the slopes of the regression lines of Neepawa insoluble and Norstar insoluble fractions were not significantly different.

4.7.6 Gel Filtration of Acetic Acid-Soluble and Acetic Acid-Insoluble Gluten Fractions

Acetic acid-soluble and acetic acid-insoluble gluten fractions were examined for possible differences in molecular weight distribution by gel filtration chromatography on Sephadex G-200 using AUC as the solvent. The highly dissociating solvent AUC was selected because it was shown to solubilize as much as 98% of the flour protein in some bread wheats (Bushuk and Wrigley, 1971). Orth and Bushuk (1973) used a shorter extraction time and obtained a 93% extractability. Protein levels of all fractions were monitored as absorbance at 280 nm. Effluents of the acetic acid-insoluble fractions were assayed also for carbohydrate levels by the phenol-sulfuric acid method (Dubois et al., 1956).

The gel filtration profiles of the acetic acid-insoluble fractions of Norstar and Neepawa glutens are presented in Figures 12 and 13 respectively. The elution profiles of the insoluble fractions of the two varieties were very similar. They both had a major protein peak eluted with the void volume. The acetic acid-insoluble fraction of Norstar

gluten had a greater portion of its carbohydrates coeluting with the major peak compared to the corresponding fraction of Neepawa. However, Neepawa had a relatively larger portion coeluting with the lower molecular weight proteins. There might be some difference between these two cultivars in the molecular sizes of carbohydrates and proteins which are associated to form different glycoproteins. These different glycoproteins might affect the water absorption or other quality characteristics. This possibility could be worthy of further investigation but no further investigations were performed on this phenomena due to time restriction.

Figure 12: Elution Profile of the Acetic Acid-Insoluble Gluten Fraction of Norstar on Sephadex G-200.

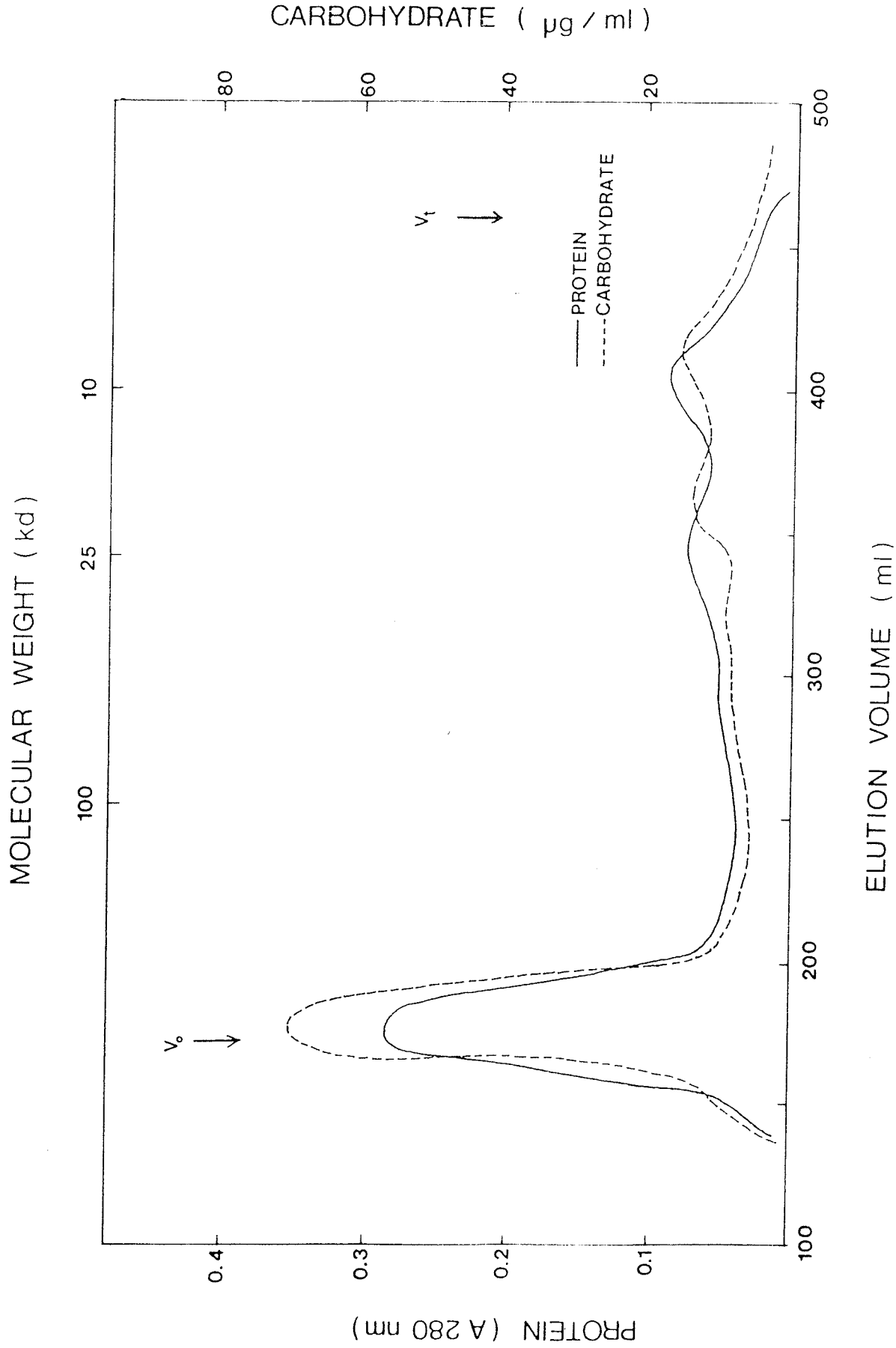
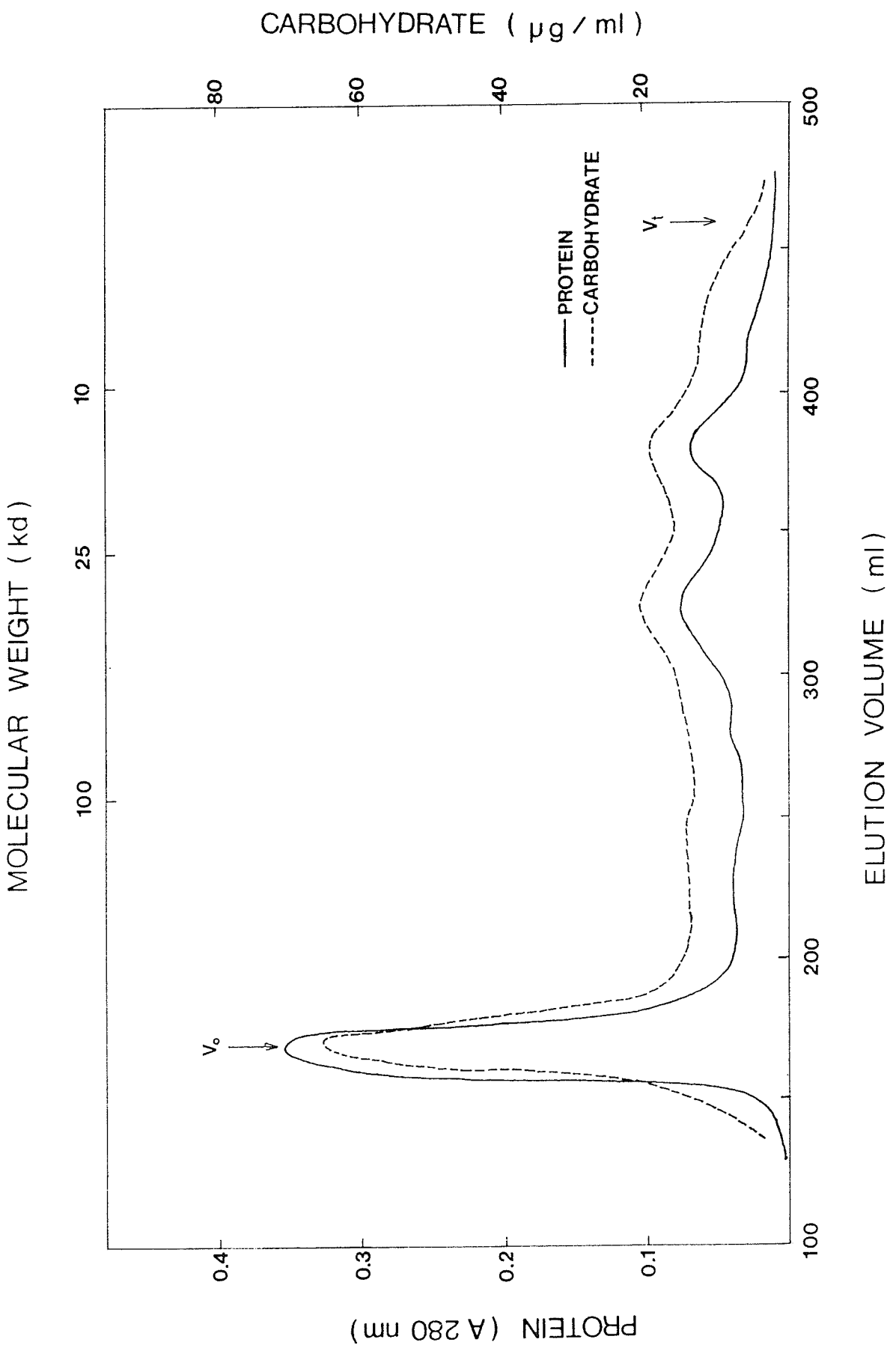

CARBOHYDRATE (µg / ml)
80
60
40
20
PROTEIN
CARBOHYDRATE
V_t
V_o
MOLECULAR WEIGHT (kd)
10
25
100
ELUTION VOLUME (ml)
500
400
300
200
100
PROTEIN (A 280 nm)
0.4
0.3
0.2
0.1

Figure 13: Elution Profile of the Acetic Acid-Insoluble Gluten Fraction of Neepawa on Sephadex G-200.


CARBOHYDRATE (µg / ml)
80
60
40
20
MOLECULAR WEIGHT (kd)
100
25
10
PROTEIN
CARBOHYDRATE
V_t
V_o
ELUTION VOLUME (ml)
500
400
300
200
100
PROTEIN (A 280 nm)
0.4
0.3
0.2
0.1

The gel filtration profiles of acetic acid-soluble fractions of Norstar and Neepawa glutens are presented in Figures 14 and 15 respectively. The elution profiles of the soluble fractions of the two varieties were quite similar. Both profiles had three major peaks. One peak eluted around the void volume, one around 25,000 and another around 10,000 daltons.

Figure 14: Elution Profiles of Acetic Acid-Soluble Fractions of Norstar Gluten on Sephadex G-200.

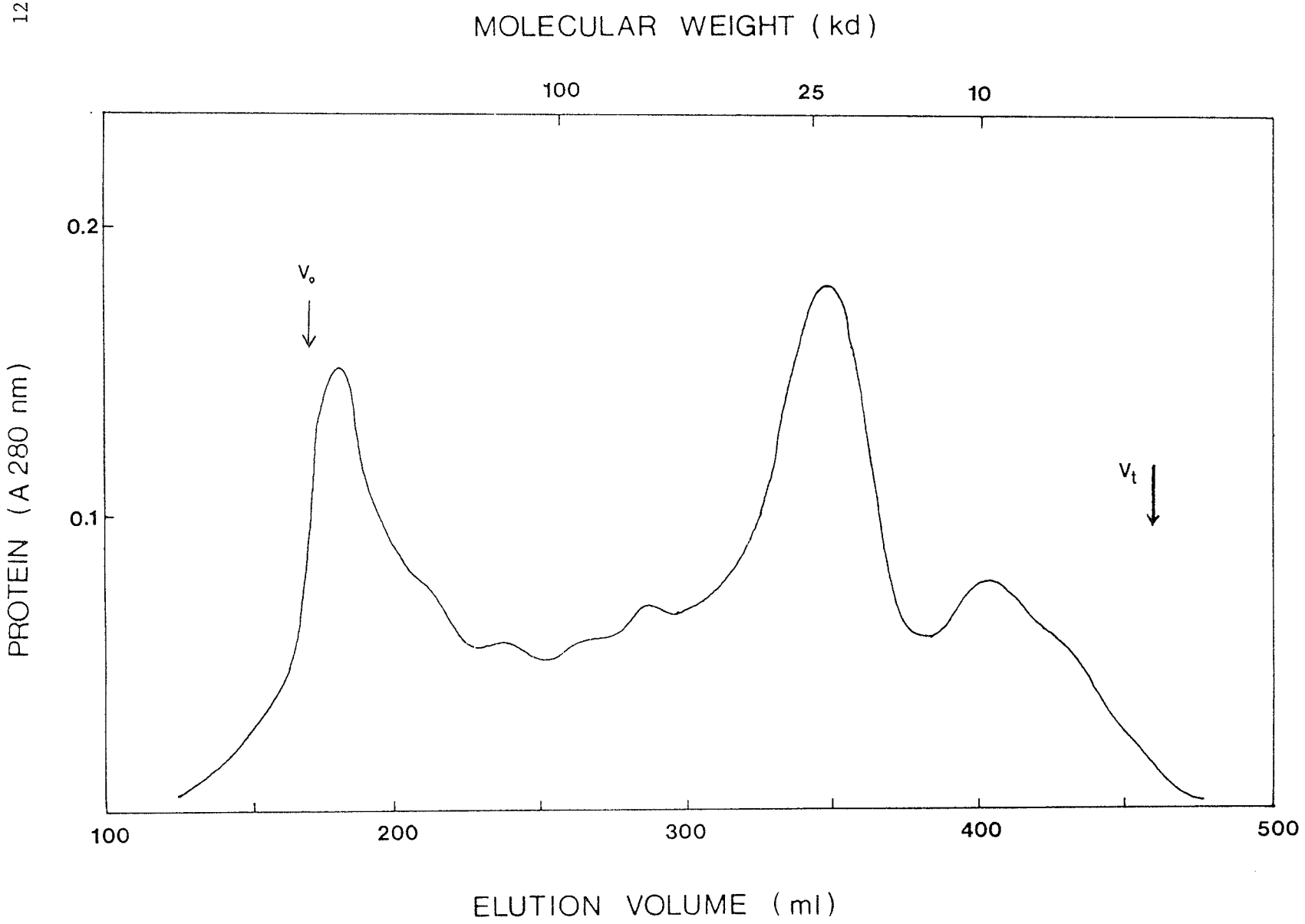
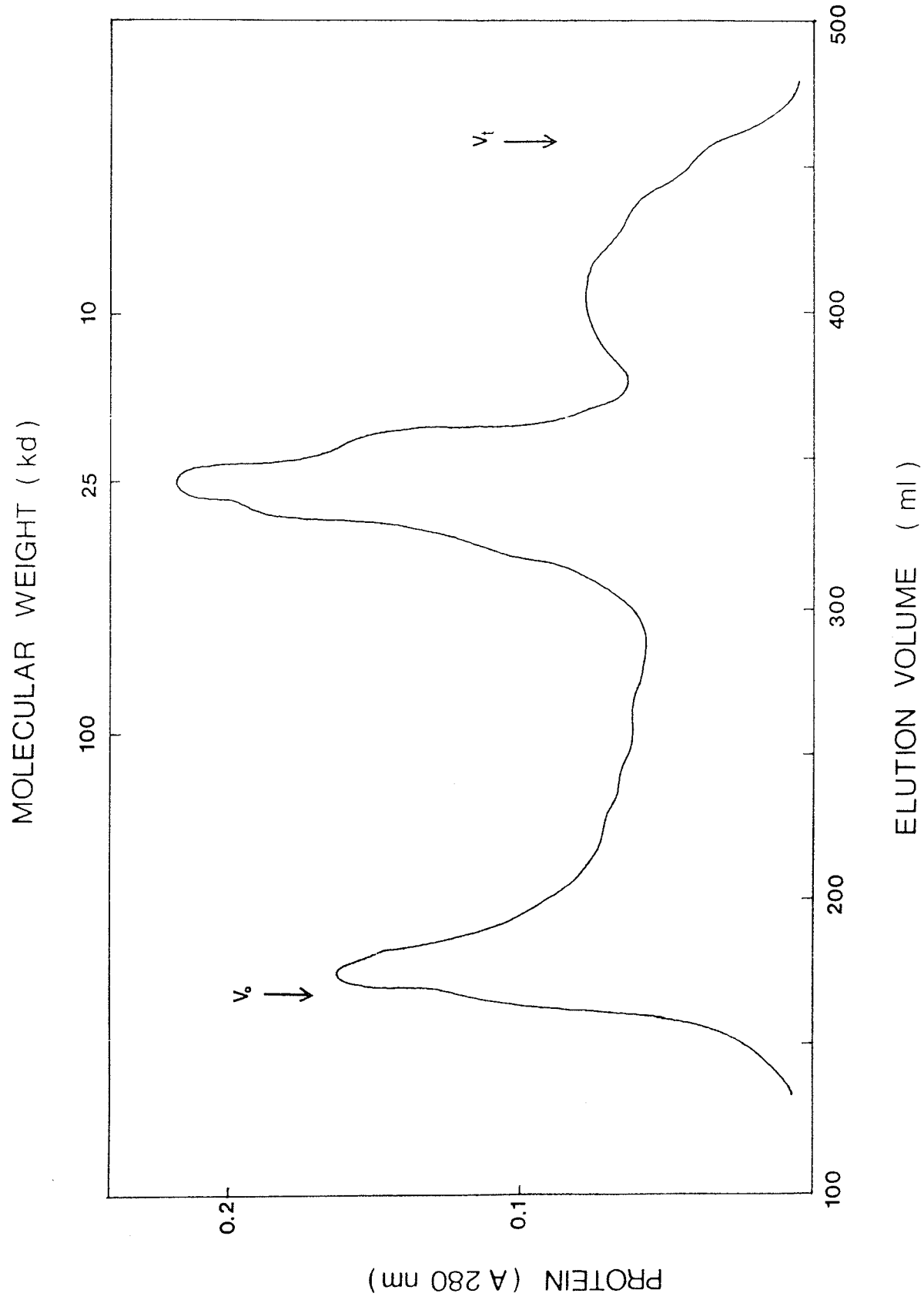

MOLECULAR WEIGHT (kd)
100
25
10
0.2
0.1
PROTEIN (A 280 nm)
V_o
V_t
100
200
300
400
500
ELUTION VOLUME (ml)

Figure 15: Elution Profiles of Acetic Acid-Soluble Fraction of Norstar Gluten on Sephadex G-200.


MOLECULAR WEIGHT (kd)
10
25
100
V_o
V_t
0.2
0.1
PROTEIN (A 280 nm)
100
200
300
400
500
ELUTION VOLUME (ml)

4.7.7 Amino Acid Composition of the Glutens and Solubility Fractions of Norstar and Neepawa

The glutens and acetic acid-soluble and acetic acid-insoluble gluten fractions of Norstar and Neepawa were subjected to amino acid analysis to determine if amino acid composition had any relationship to the bound water content and other characteristics. The amino acid analysis results are presented in Table 29.

TABLE 29

Amino Acid Composition* of Gluten and 0.05 N Acetic Acid-Soluble and Acetic Acid-Insoluble Fractions from Neepawa and Norstar.

	Neepawa			Norstar		
	Gluten	Soluble gluten	Insoluble gluten	Gluten	Soluble gluten	Insoluble gluten
Lysine	1.80	1.00	2.22	1.70	1.07	2.52
Histidine	2.11	2.03	2.01	2.07	2.04	2.11
Arginine	3.24	2.63	3.48	3.20	2.58	3.73
Aspartic acid	3.51	2.78	3.81	3.42	2.84	4.22
Threonine	2.25	1.91	2.51	2.25	1.91	2.59
Serine	3.69	3.44	4.18	3.78	3.38	4.15
Glutamic acid	35.38	38.34	34.89	35.37	37.80	32.97
Proline	12.74	13.61	11.01	12.22	14.30	11.04
Glycine	3.14	2.36	4.19	3.33	2.25	4.16
Alanine	3.18	2.89	3.51	3.29	2.79	3.61
Cysteine	1.67	1.83	1.35	1.81	1.67	1.40
Valine	3.86	3.62	3.95	3.97	3.55	4.15
Methionine	1.55	1.42	1.51	1.60	1.52	1.71
Isoleucine	3.21	3.35	3.02	3.34	3.34	3.16
Leucine	6.62	6.50	6.74	6.73	6.43	6.91
Tyrosine	2.94	3.04	3.35	3.23	2.69	3.22
Phenylalanine	5.33	5.24	4.64	4.93	5.84	4.82
Ammonia	3.78	4.00	3.63	3.76	4.01	3.54

* g amino acid/100 g protein, Tryptophan not determined, cysteine was partly destroyed but included in calculations.

Although the amino acid compositions of glutens and corresponding gluten fractions of the two varieties, Norstar and Neepawa were quite similar, there were some minor differences in certain amino acids. Glutamic acid and proline accounted for almost 50% of the amino acids of the gluten and the gluten fractions.

The acetic acid-soluble fraction from each cultivar was composed of higher amounts of glutamic acid, proline, isoleucine and phenylalanine and lower amounts of lysine, arginine, aspartic acid, threonine, serine, glycine, alanine and valine compared to its acetic acid-insoluble counterpart from the same variety. As expected, the amino acid composition in the glutens of each cultivar was intermediate to that of the acetic acid-soluble and acetic acid-insoluble fractions.

A comparison of the amino acid composition of gluten and its fractions from each variety (Table 29) was undertaken with the bound water values (Table 27). Although in each variety there appeared to be a relationship between amino acid composition and bound water values, when the data from both cultivars were combined, none of the correlation coefficients between amino acids and bound water values were significant at the 5% level. The highest correlation coefficient was between the serine content and bound water ($r = -0.745$, significant at the 8.9% level). The highest positive correlations were found between the bound water and ammonia content ($r = +0.729$, significant at 10.0% level) and between bound water and glutamic acid content ($r = +0.727$, significant at 10.2% level). From these results it seems that the degree of amidization may have an influence on bound water contents.

To investigate the relation between amino acid composition and bound water contents; average hydrophobicity, positive and negative charge potentials, charge ratio, frequency of charged groups and degree of amidization were calculated as described by McMaster (1982) and Lukow (1983). Amino acid side chain hydrophobicities which were listed by Bigelow (1967) were used in the calculation of average hydrophobicities of the gluten and the gluten fractions. Tryptophan was not determined and cysteine was partially destroyed but the value obtained for the latter was used in the calculations. Therefore, the calculated parameters can be considered only as approximations, but could be a useful supplement to the other results obtained.

Calculations were made as follows:

$$\text{a) Average hydrophobicity} = \frac{\text{Total hydrophobicity (TH)}}{\text{Total moles of amino acids/100 kg protein}}$$

TH = (moles of amino acid/100 kg protein) x (hydrophobicity/residue)

b) Negative charge potential (NCP) = Total moles of glu+asp/100 kg prot.

NCP less amide groups = NCP - (moles of amide groups)/100 kg protein

c) Positive charge potential (PCP) = (total moles of arg + lys + his)/ 100 kg protein

$$\text{d) Ratio of positive to negative charge} = \frac{\text{PCP}}{\text{NCP less amide groups}}$$

$$\text{e) Frequency of charged groups} = \frac{\text{PCP + NCP less amid groups}}{\text{Total moles of amino acids/100 kg prot.}}$$

$$\text{f) degree of amidization (\%)} = \frac{\text{Moles of amide groups/100 kg protein}}{\text{(Total moles of glu + asp)/100 kg protein}} \times 100$$

Although frequency of charged groups were equal for both the Neepawa and the Norstar glutens, positive to negative charge potential of the Norstar gluten was lower than the Neepawa gluten implying that a higher proportion of charged amino acids in the Norstar gluten was negatively charged. Negative charge potential less the amide groups gives an indication of the relative amount of the amino acids with a potential of having negative charge. The pH of dough is around 5 to 6. At this pH the carboxyl group of glutamic acid and aspartic acid are predominantly in the ionized form i.e. negatively charged. Table 30 shows that this parameter (negative charge potential less amide groups) was slightly greater in the Norstar gluten than in the Neepawa gluten. The sodium content of the Norstar gluten (Table 26) was also greater than in the Neepawa gluten. From this observation amino acids of the Norstar gluten apparently may interact with the charged groups (such as sodium) to a greater extent and thereby lower its water binding capacity.

The degree of amidization of the Norstar gluten was greater than that of the Neepawa gluten. Bull and Breese (1968) suggested that amide groups do not bind water, and also inhibit the binding of water by other polar groups because amides form hydrogen bonds with the groups which normally bind water. This would provide less opportunity for the water to bind with the protein.

TABLE 30

Average Hydrophobicities, Charge Potentials, and Amidization of Gluten and 0.05 N Acetic Acid-Soluble and Insoluble Fractions from Neepawa and Norstar.

	Neepawa			Norstar		
	Gluten	Soluble Gluten	Insoluble Gluten	Gluten	Soluble Gluten	Insoluble Gluten
Average hydrophobicity (Kcal/residue)	1.040	1.055	0.987	1.031	1.078	1.000
Positive charge potential (moles/100 kg)	44.5	35.0	48.1	43.3	35.2	52.2
Negative charge potential (moles/100 kg)	266.9	281.6	265.8	266.1	278.3	255.9
Negative charge potential less amide groups (moles/100 kg)	45.1	46.5	52.7	45.3	42.9	48.1
Positive to negative charge ratio	0.99	0.75	0.91	0.96	0.82	1.09
Frequency of charge groups (groups/residue)	0.12	0.11	0.14	0.12	0.11	0.14
Amidization (%)	83.1	83.5	80.2	83.0	84.6	81.2

The studies reported in this part of the thesis have been performed on the gluten and gluten fractions derived from only two wheat cultivars. Similar studies using wheat cultivars of diverse genotype and quality (especially in terms of water absorption) are required to extend the findings of this study to bread wheats, in general.

4.8 EFFECT OF INCREASED BAKING ABSORPTION ON REMIX LOAF VOLUME AND DOUGH HANDLING PROPERTIES

The fact that Norstar had good baking quality but low farinograph absorption raised a question on whether it is possible to increase baking absorption for this variety without significantly affecting baking quality. Two different approaches were taken to answer this question. First, more water was added into baking formula until a substantial deterioration on the loaf volume or dough handling properties had occurred.

The remix loaf volume and dough handling properties of Norstar and Neepawa samples at increasing absorption levels are presented in Table 31. For the Norstar sample it was possible to increase baking absorption by 5% over the farinograph absorption without a decrease in loaf volume. At this point an increase in water absorption caused a rapid decrease in both loaf volume and dough handling properties. In normal baking procedure, baking absorption was determined to be -2% of farinograph absorption. However, optimum baking absorption for the Norstar was 3% more than the farinograph absorption as judged by loaf volume and dough handling properties. For the Neepawa sample a baking absorption which was 4% higher than the farinograph absorption did not cause a substantial deterioration in baking quality. At this point it is necessary to mention that the Norstar and the Neepawa flours used in this

study were not of similar protein content and it was not possible to find a Neepawa sample in the same range as the Norstar samples. Therefore, a true comparison of the Norstar and Neepawa cultivars in terms of their perfomance at increased baking absorption was not possible.

TABLE 31

Remix Loaf Volume and Dough Handling Properties of Norstar and Neepawa Samples at Increased Absorption Values.

	Flour protein (%)	Farinograph absorption (%)	Baking absorption (%)	Remix loaf volume (c.c.)	Dough handling properties
Norstar	12.6	57.0	55.0	860	dry and stiff
"			58.0	875	good
"			60.0	905	good
"			62.0	870	soft but manageable
"			64.0	445	difficult to handle
Neepawa	14.5	62.0	60.0	925	satisfactory
"			62.0	950	good
"			64.0	900	good
"			66.0	810	soft but manageable

The second approach which was followed in this study was the use of additives to increase the baking absorption. The studies with sodium stearoyl lactylate (SSL) showed that it did not improve the farinograph absorption. Later a vegetable fiber called Uptake 80 (Woodstone Foods, Portage la Prairie, Manitoba) was used. It was reported by the company that in a typical analysis Uptake 80 had 7% protein, 7% crude fiber and 2% ash. The studies with Uptake 80 (2%) showed that it increased the farinograph absorptions of the Norstar and Neepawa samples by 3% and 2% respectively. A combination of SSL (1%) and Uptake 80 (2%) did not improve the farinograph absorption.

Remix loaf volume and dough handling properties of the Norstar and Neepawa samples with 2% Uptake 80 at increasing absorption levels are presented in Table 32. It was possible to increase the baking absorption of Norstar 7% over the original farinograph absorption value without a significant deterioration in dough handling properties. A baking absorption of over 7% over the farinograph absorption resulted in a large decrease in loaf volume and a sticky dough. For the Neepawa sample a baking absorption which was 4% higher than the original farinograph absorption did not cause a substantial deterioration in loaf volume and the dough handling properties were acceptable.

TABLE 32

Remix Loaf volume and Dough Handling Properties of Norstar and Neepawa Samples Including 2% Pea Fiber at Increased Absorption Values.

	Farinograph absorption (%)	Baking absorption (%)	Remix loaf volume (c.c.)	Dough handling properties
Norstar				
+ 2% pea fiber	60.0	60.0	830	good
"		62.0	905	good
"		64.0	850	soft but manageable
"		66.0	450	difficult to handle
Neepawa				
+ 2% pea fiber	64.0	62.0	890	good
"		64.0	850	good
"		66.0	745	good

4.9 SCANNING ELECTRON MICROSCOPY

4.9.1 The Comparison of the Milling Characteristics of Norstar and Neepawa Using SEM

Table 23 (section 4.6.1) showed that Norstar and Neepawa samples resulted in different starch damage levels, with more starch damage in the Neepawa samples. Therefore, it was decided to investigate the milling properties of these two cultivars by SEM. The same set of samples which was used in the starch damage study (section 4.6.1) was also utilized in the SEM studies. The scanning electron micrographs of Norstar and Neepawa samples are presented in Figures 16 and 18 and Figures 17 and 19 respectively. In each figure the plates A to D represent an increasing order of starch damage.

Figure 16: Scanning Electron Micrographs of Various Milling Products of Norstar.

A Coarse endosperm middlings

B Flour produced at 0.06-0.02 mm reduction roll gap setting

C Flour produced at 0.04-0.01 mm reduction roll gap setting

D Flour produced at 0.02-0.01 mm reduction roll gap setting

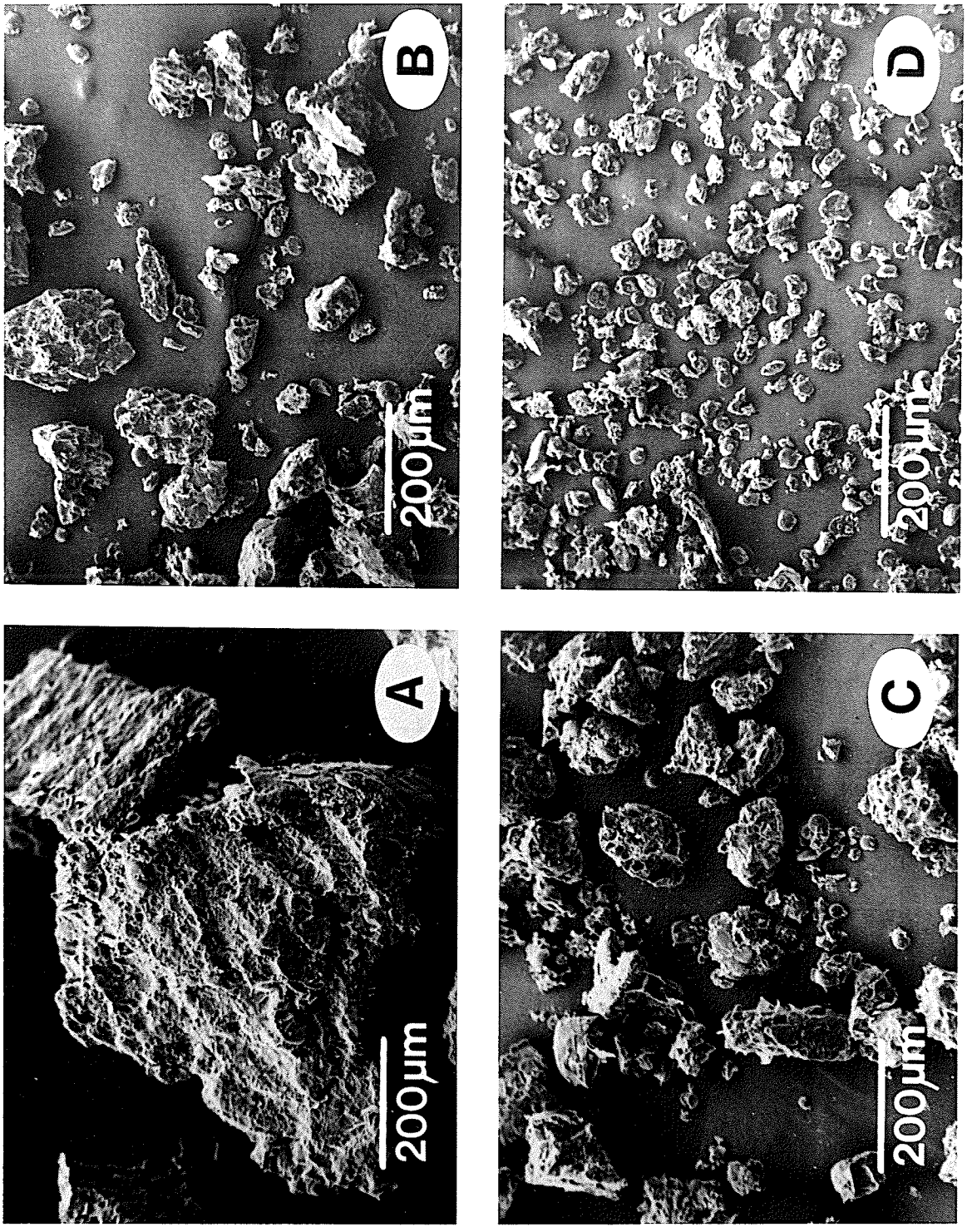

A
200µm
B
200µm
C
200µm
D
200µm

Figure 17: Scanning Electron Micrographs of Various Milling Products of Neepawa.

A Coarse endosperm middlings

B Flour produced at 0.06-0.02 mm reduction roll gap setting

C Flour produced at 0.04-0.01 mm reduction roll gap setting

D Flour produced at 0.02-0.01 mm reduction roll gap setting

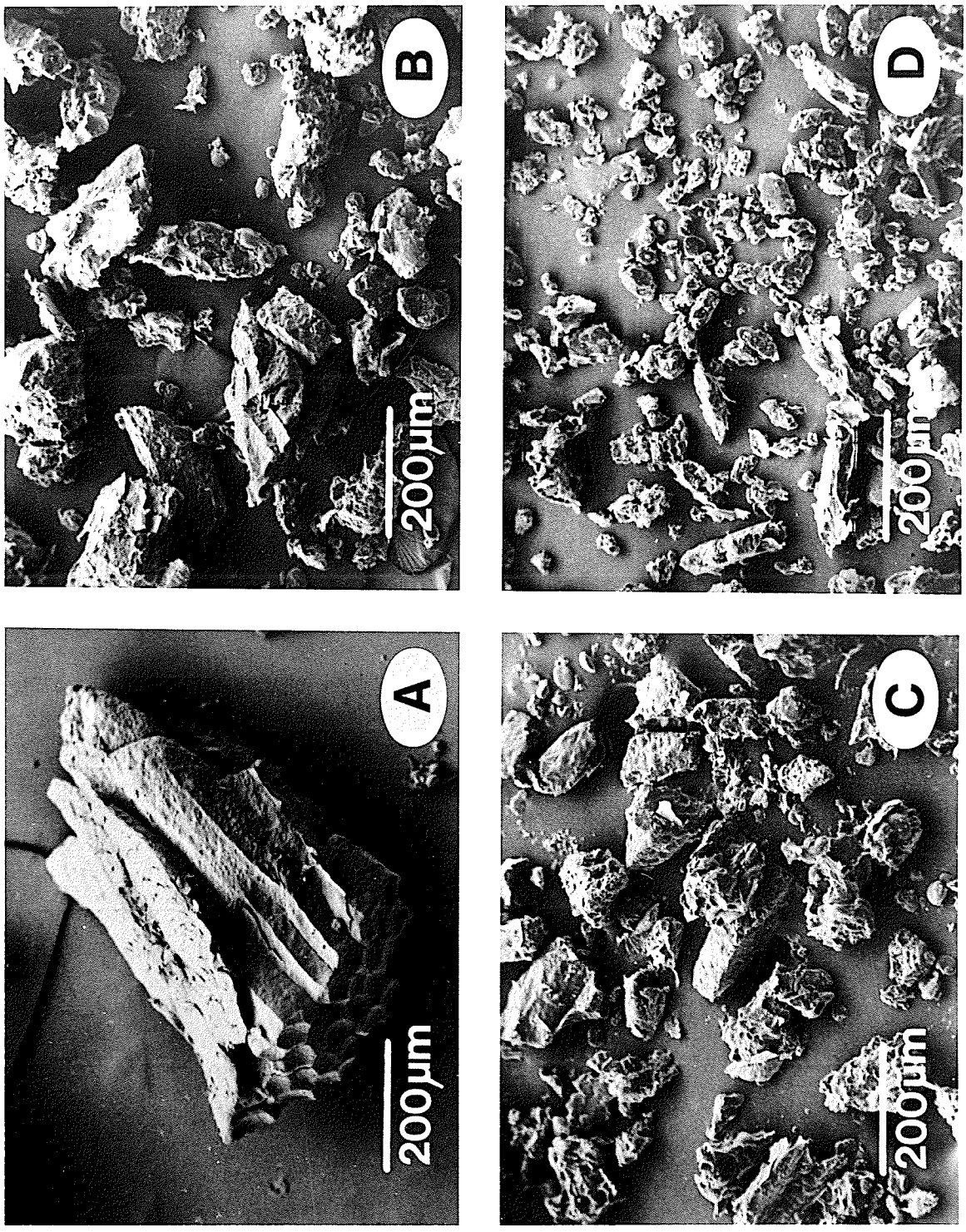

A
200µm
B
200µm
C
200µm
D
200µm

Figure 18: Scanning Electron Micrographs of Various Milling Products of Norstar.

A Coarse endosperm middlings

B Flour produced at 0.06-0.02 mm reduction roll gap setting

C Flour produced at 0.04-0.01 mm reduction roll gap setting

D Flour produced at 0.02-0.01 mm reduction roll gap setting

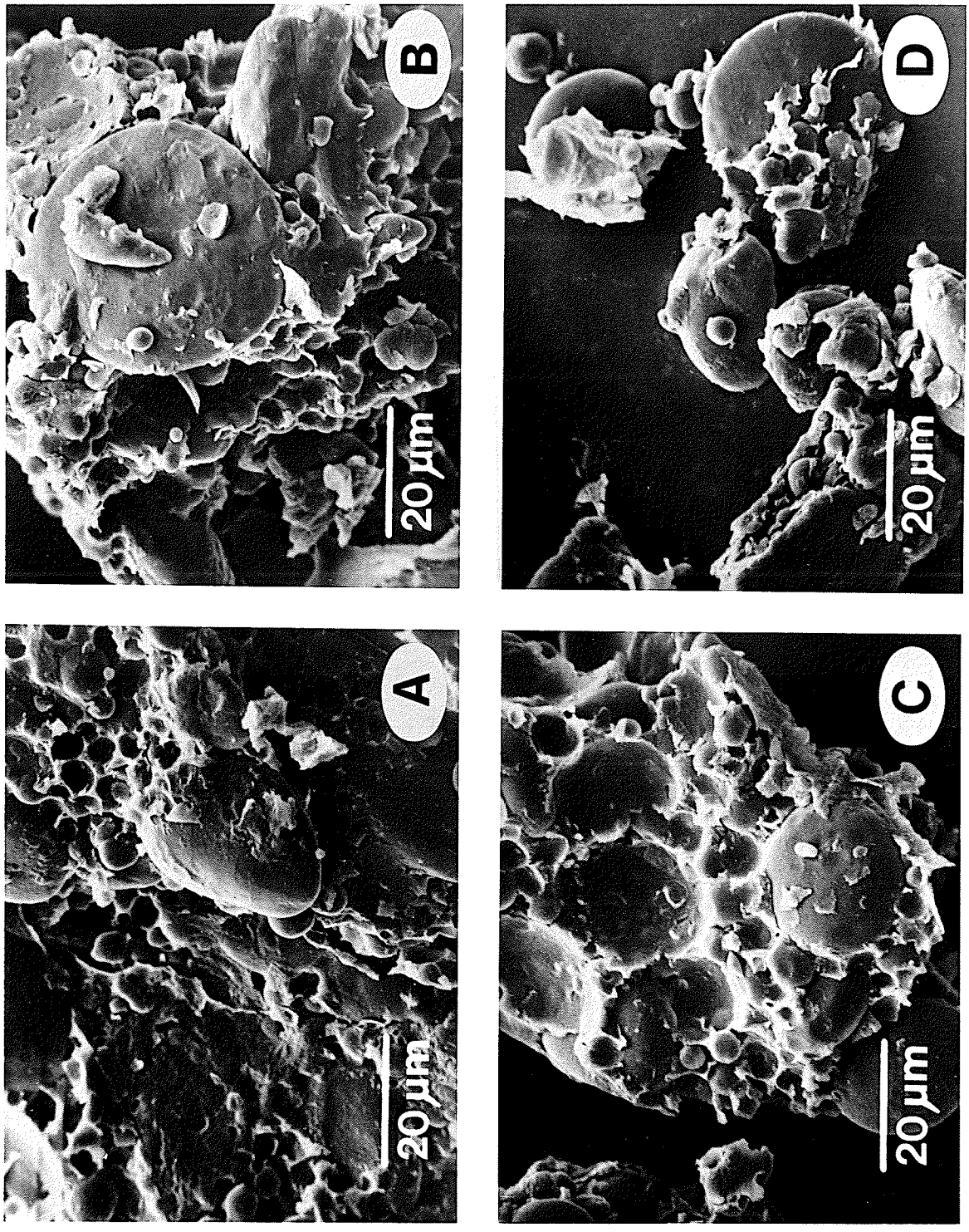

A
20 µm
B
20 µm
C
20 µm
D
20 µm

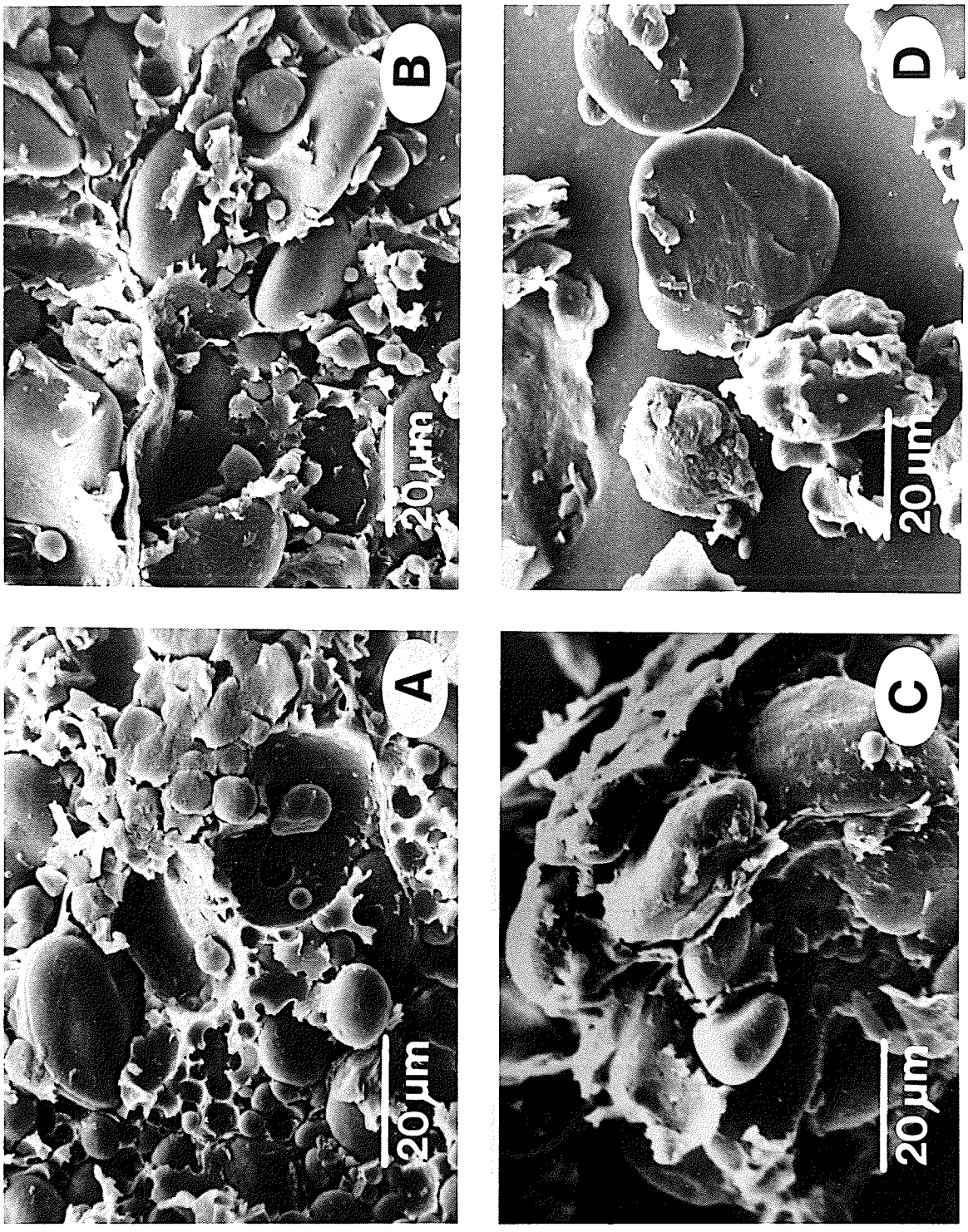
Figure 19: Scanning Electron Micrographs of Various Milling Products of Neepawa.

A Coarse endosperm middlings

B Flour produced at 0.06-0.02 mm reduction roll gap setting

C Flour produced at 0.04-0.01 mm reduction roll gap setting

D Flour produced at 0.02-0.01 mm reduction roll gap setting


A
20 µm
B
20 µm
C
20 µm
D
20 µm

Figures 16 and 17 illustrate that for both the cultivars as expected the particle size of the milled products decreased as the gap setting between the reduction rolls was decreased. However, for each milling roll-setting the particle size of Neepawa flour produced was slightly larger compared to corresponding Norstar flour. The particles of Neepawa were also more angular with sharper edges and corners. Both of these observations are consistent with their kernel hardness.

At a higher magnification it was possible to observe a few damaged starch granules with scanning scope and they are presented in Figures 18 and 19. However, it was not possible to observe an increased degree of starch damage on granules as previously determined by the method of Farrand (1964). Therefore, specimens were prepared for SEM by treating the same set of samples with alpha-amylase with an attempt to observe the fractures on starch granules, since the damaged portion of a starch granule is more susceptible to amylase action.

Flour samples were treated with the enzyme for different time intervals as described in section 3.5.2. An examination with scanning scope showed that 20 h of hydrolysis was sufficient to observe the milling damage on starch granules. The SEM micrographs of the Norstar samples are presented in Figures 20 and 22 and of the Neepawa samples in Figures 21 and 23. In each figure the plates A to D represent increasing level of starch damage.

Figure 20: Scanning Electron Micrographs of the Enzyme-Treated Milling Products of Norstar.

A Coarse endosperm middlings

B Flour produced at 0.06-0.02 mm reduction roll gap setting

C Flour produced at 0.04-0.01 mm reduction roll gap setting

D Flour produced at 0.02-0.01 mm reduction roll gap setting

A
20 µm
B
20 µm
C
20 µm
D
20 µm

Figure 21: Scanning Electron Micrographs of the Enzyme-Treated Milling Products of Neepawa.

A Coarse endosperm middlings

B Flour produced at 0.06-0.02 mm reduction roll gap setting

C Flour produced at 0.04-0.01 mm reduction roll gap setting

D Flour produced at 0.02-0.01 mm reduction roll gap setting

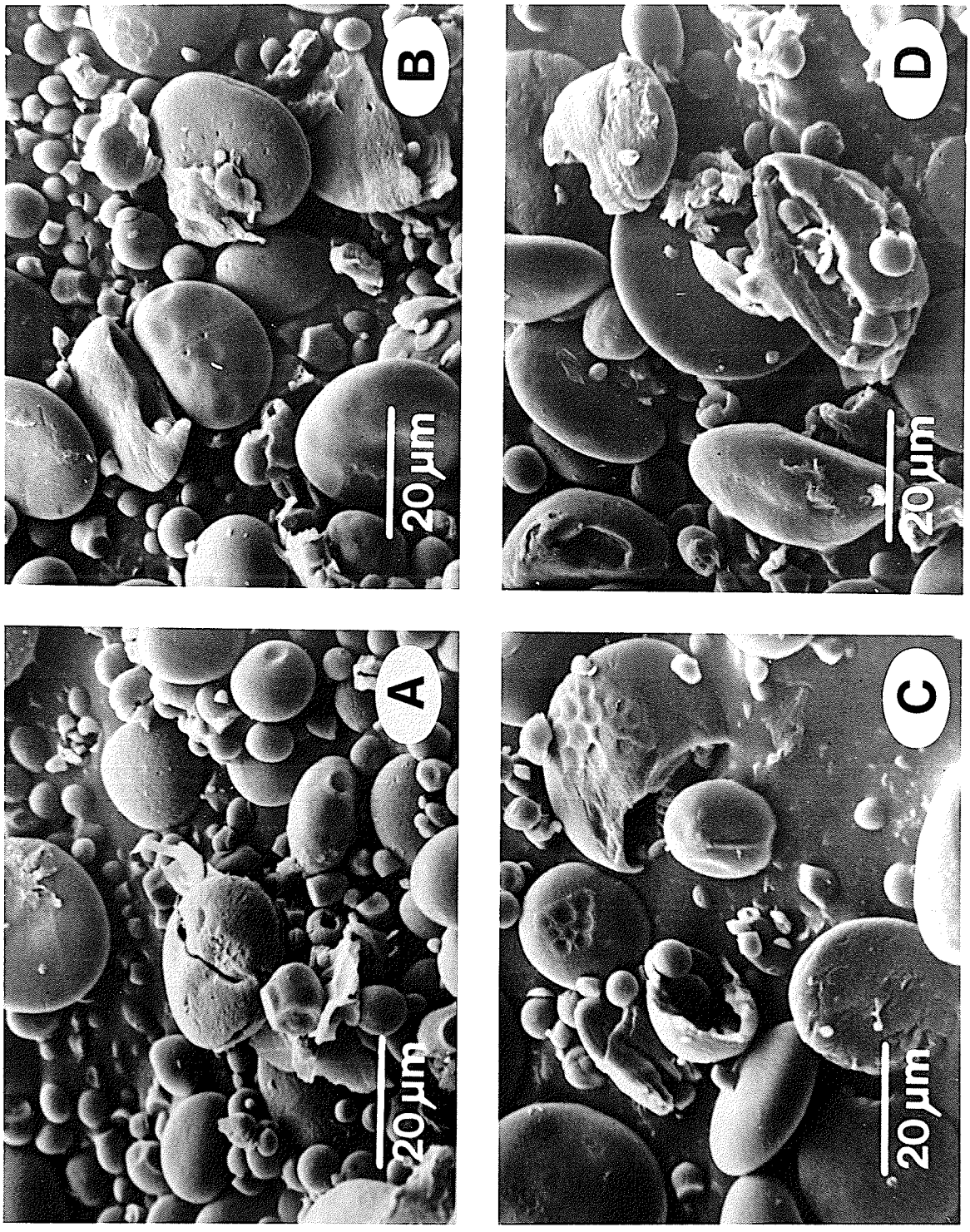

A
20 µm
B
20 µm
C
20 µm
D
20 µm

Figure 22: Scanning Electron Micrographs of the Enzyme-Treated Milling Products of Norstar.

A Coarse endosperm middlings

B Flour produced at 0.06-0.02 mm reduction roll gap setting

C Flour produced at 0.04-0.01 mm reduction roll gap setting

D Flour produced at 0.02-0.01 mm reduction roll gap setting

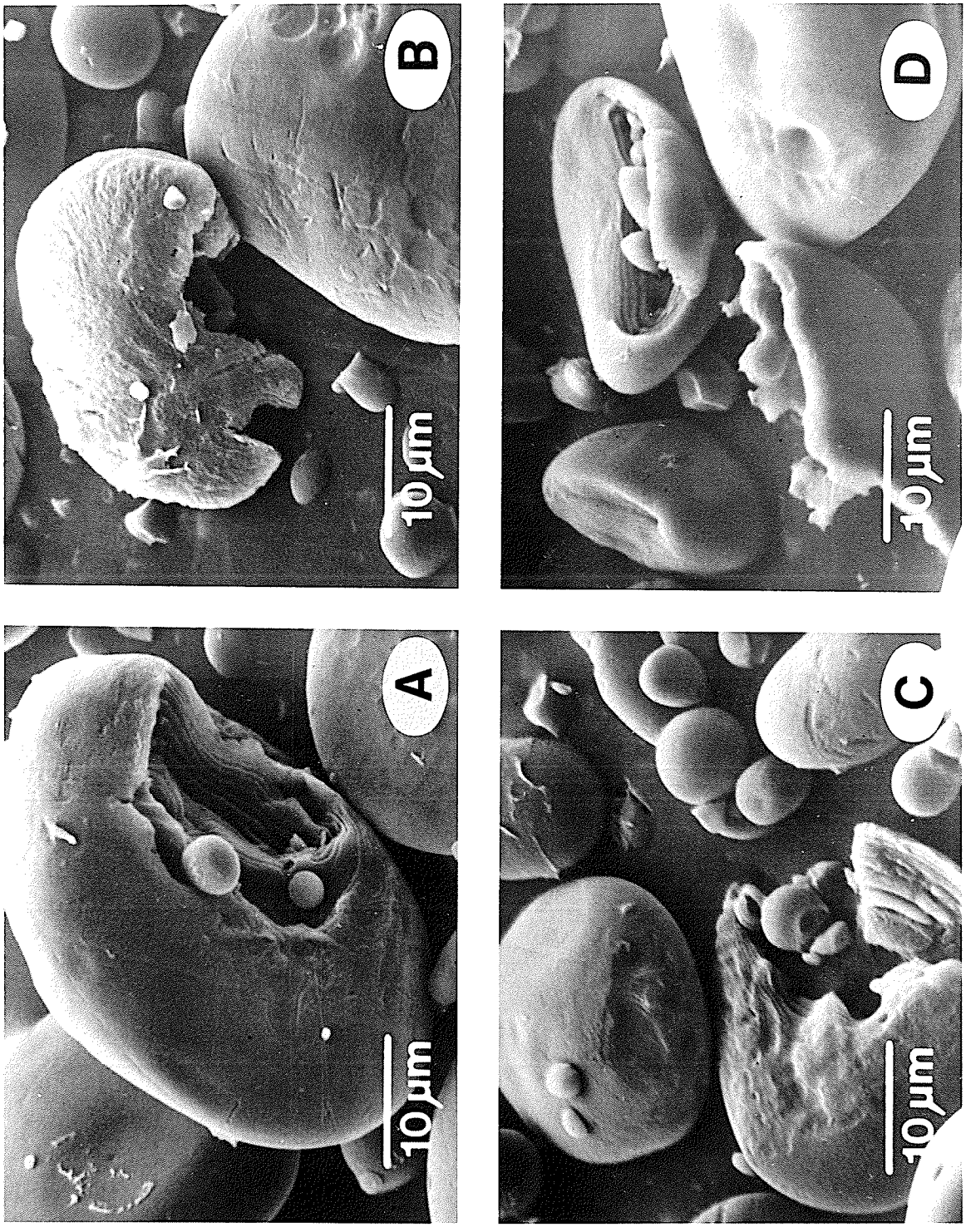

A
10 µm
B
10 µm
C
10 µm
D
10 µm

Figure 23: Scanning Electron Micrographs of the Enzyme-Treated Milling Products of Neepawa.

A Coarse endosperm middlings

B Flour produced at 0.06-0.02 mm reduction roll gap setting

C Flour produced at 0.04-0.01 mm reduction roll gap setting

D Flour produced at 0.02-0.01 mm reduction roll gap setting

A
10 µm
B
10 µm
C
10 µm
D
10 µm

After the enzyme treatment it was possible to observe an increased level of starch damage within each variety. The number of damaged granules and the extent of the damage occurred increased progressively. However, there were no clear differences between the samples from each variety which were milled under the same conditions.

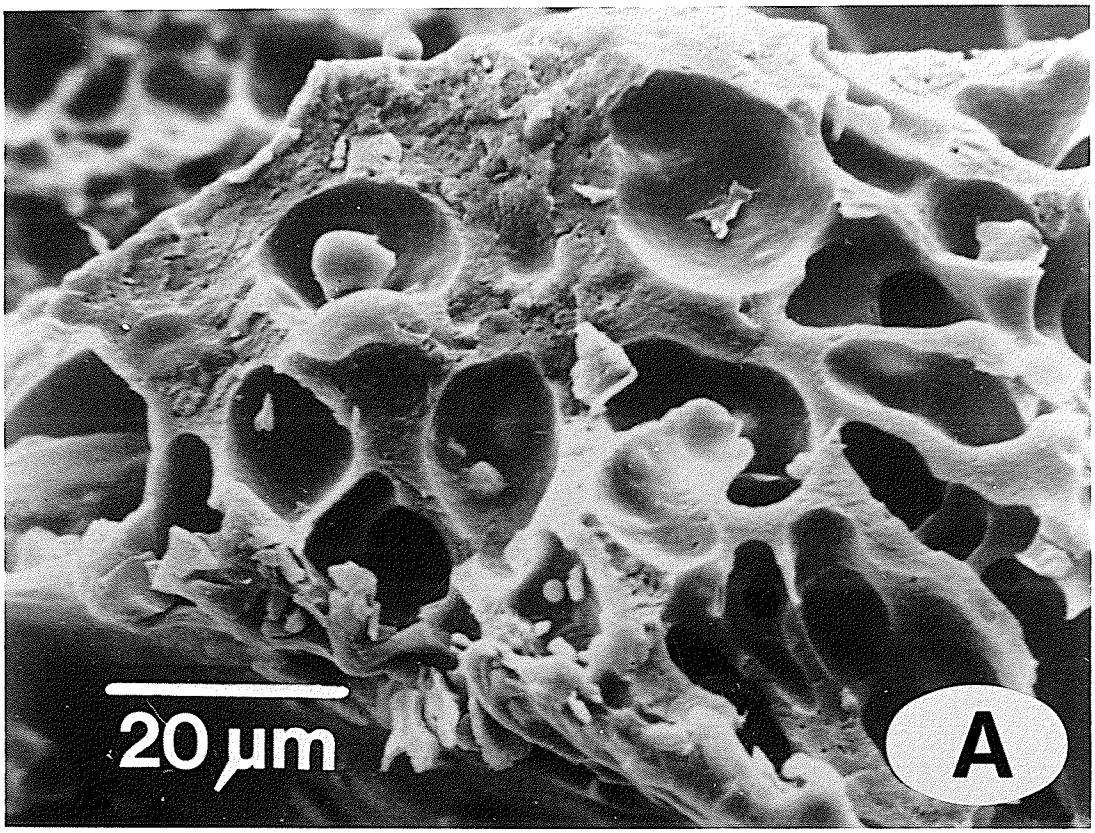
4.9.2 SEM Studies of the Glutens, Acetic Acid-Soluble and Acetic Acid-Insoluble Gluten Fractions of Norstar and Neepawa

The freeze dried gluten and gluten fractions of Norstar and Neepawa were used in SEM studies with an attempt to relate their microscopic structure to their properties. The SEM micrographs of the freeze dried gluten and gluten fractions are presented in Figures 24, 25 and 26.

Figure 24: Scanning Electron Micrographs of the Freeze Dried Gluten Samples.

A Neepawa

B Norstar


20 µm
A

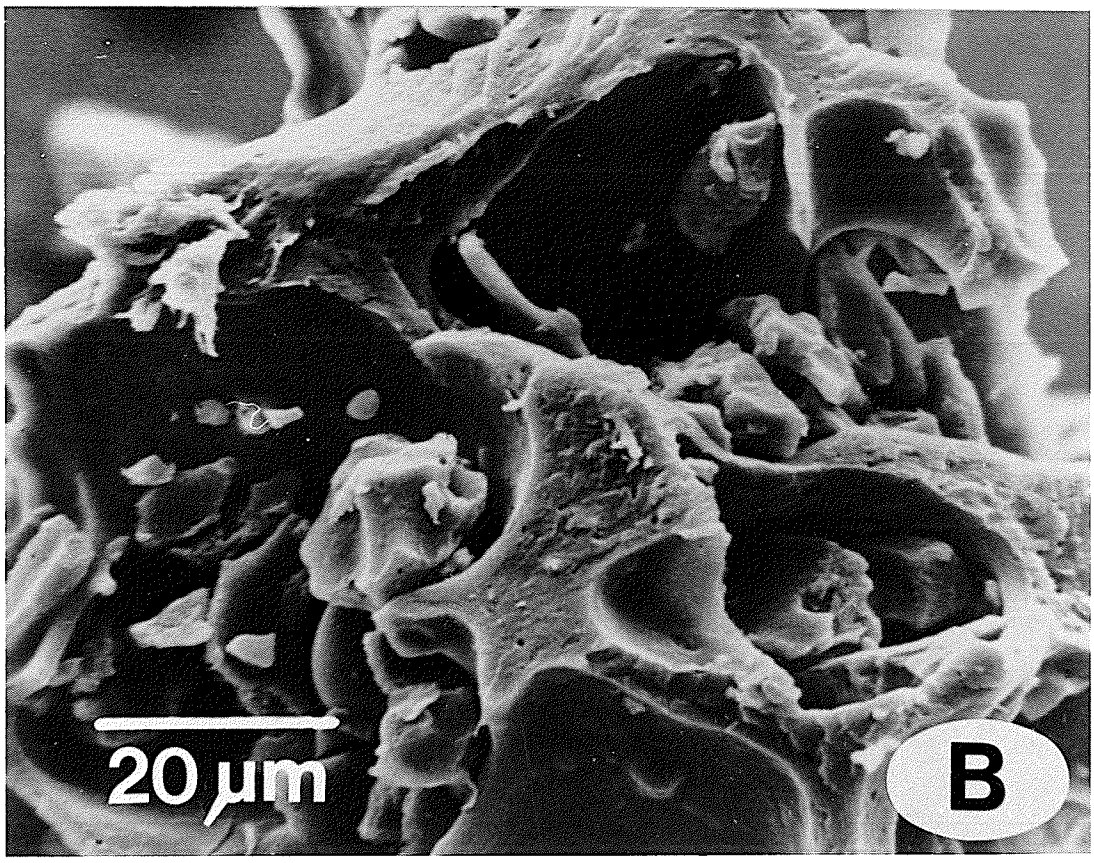
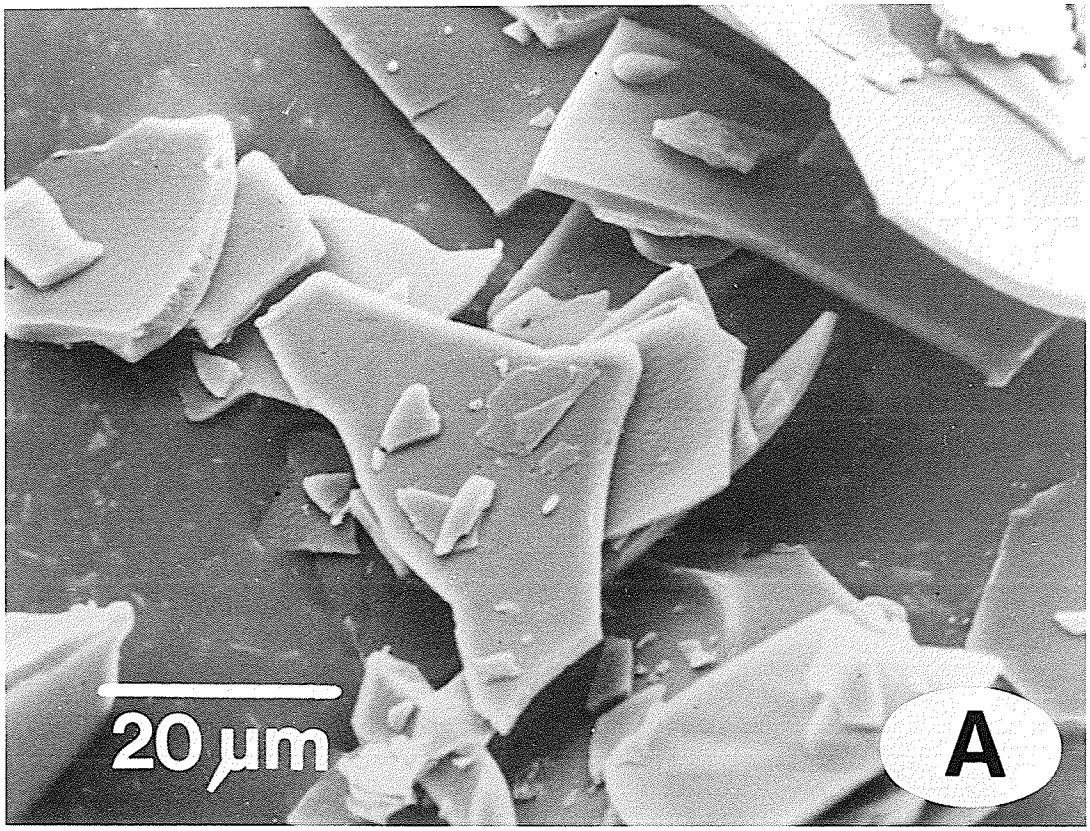

20 µm
B

Figure 25: Scanning Electron Micrographs of the Freeze Dried Soluble Gluten Fractions.

A Neepawa

B Norstar


20 µm
A

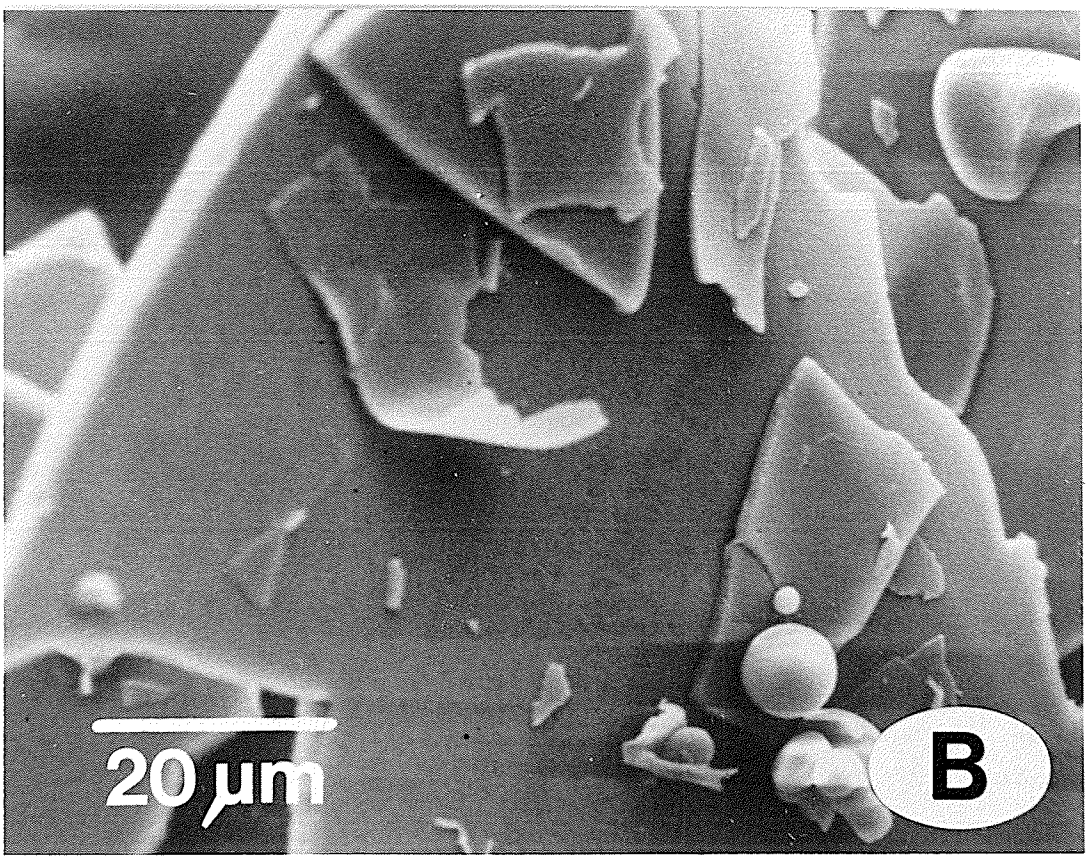
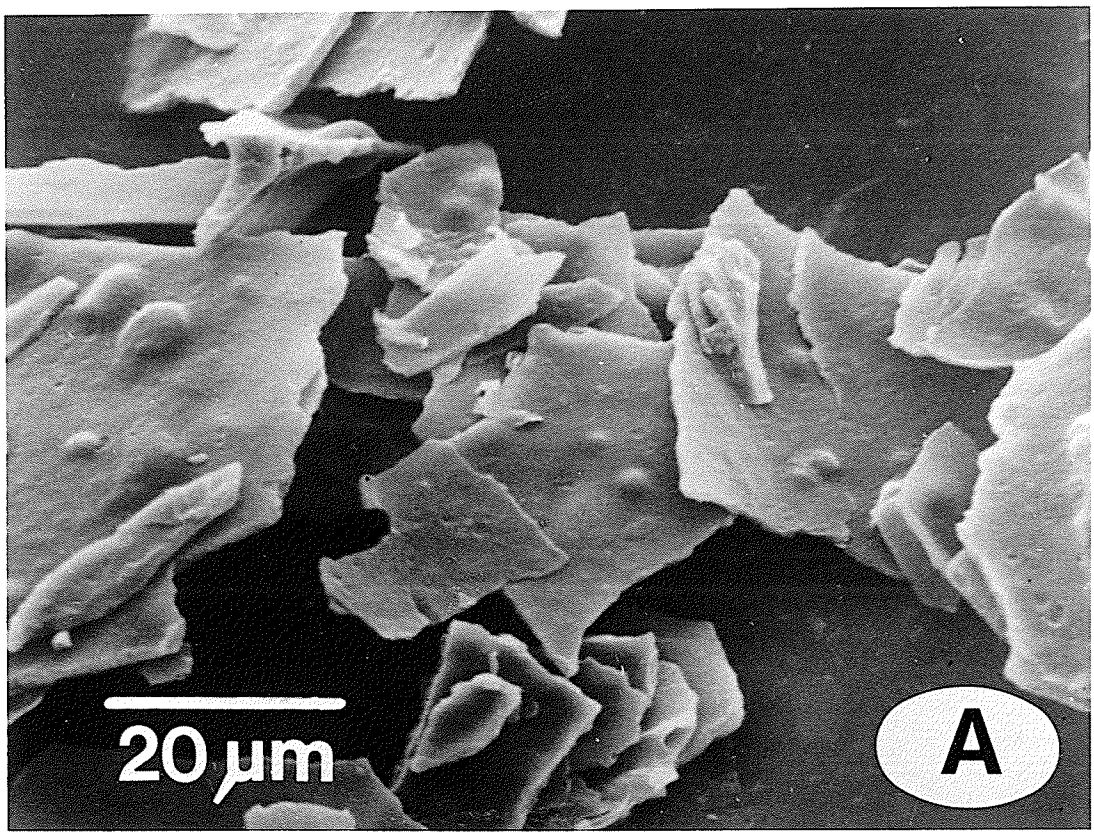

20 µm
B

Figure 26: Scanning Electron Mirographs of the Freeze Dried Insoluble Gluten Fractions.

A Neepawa

B Norstar


20 µm
A

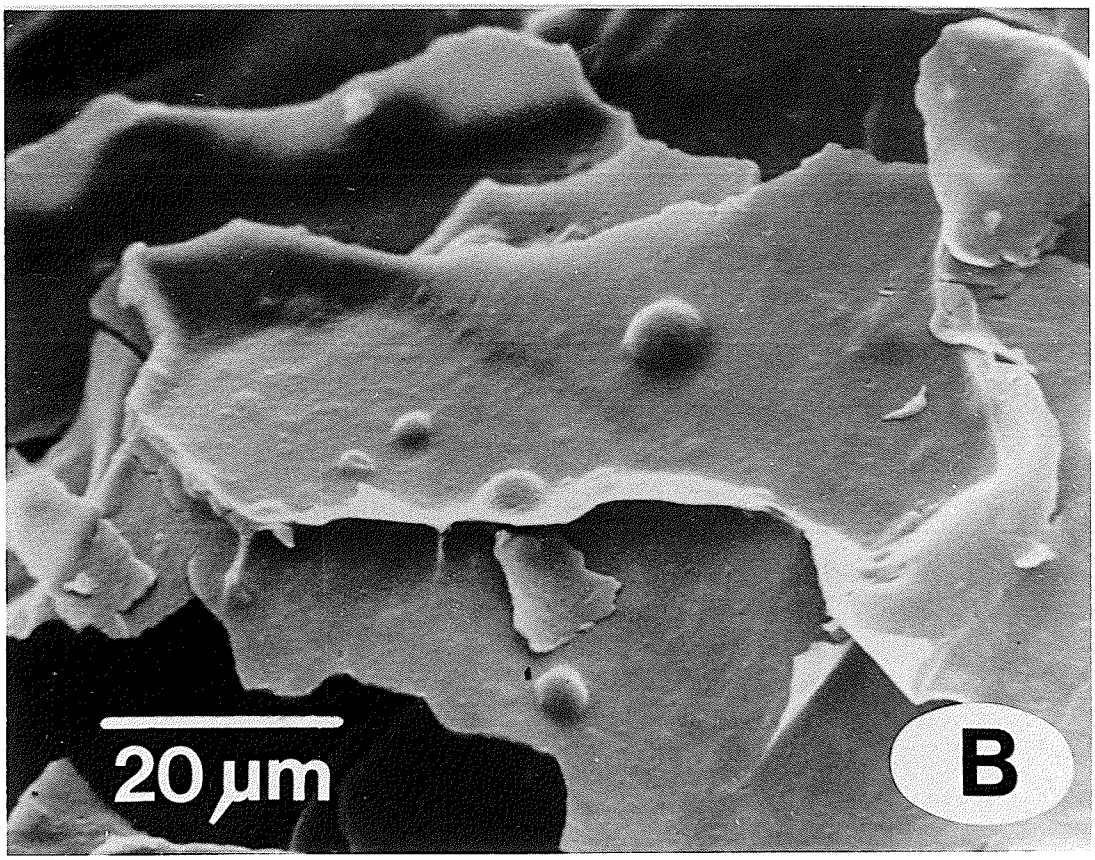

20 µm
B

Figure 24 shows typical SEM micrographs of total gluten from Norstar and Neepawa. A sponge-like structure was evident for each variety, both having air cells of approximately equal size. The matrices surrounding the air cells had an even texture but were marked with tiny pits.

A collection of thick film-like pieces characterize the SEM micrographs of the soluble gluten fractions from Norstar and Neepawa (Figure 25). As with the total gluten, there was no appreciable difference between the two varieties. The insoluble gluten fractions from the two varieties also appeared as film-like pieces (Figure 26). However, these were considerably thinner than those seen for the soluble fractions. The gluten fractions of figures 25 and 26 had been subjected to grinding in a commercial coffee grinder. The edges of the fragments of the insoluble fractions were noticeably more jagged than those seen on the fragments of the soluble fractions (Figure 25).

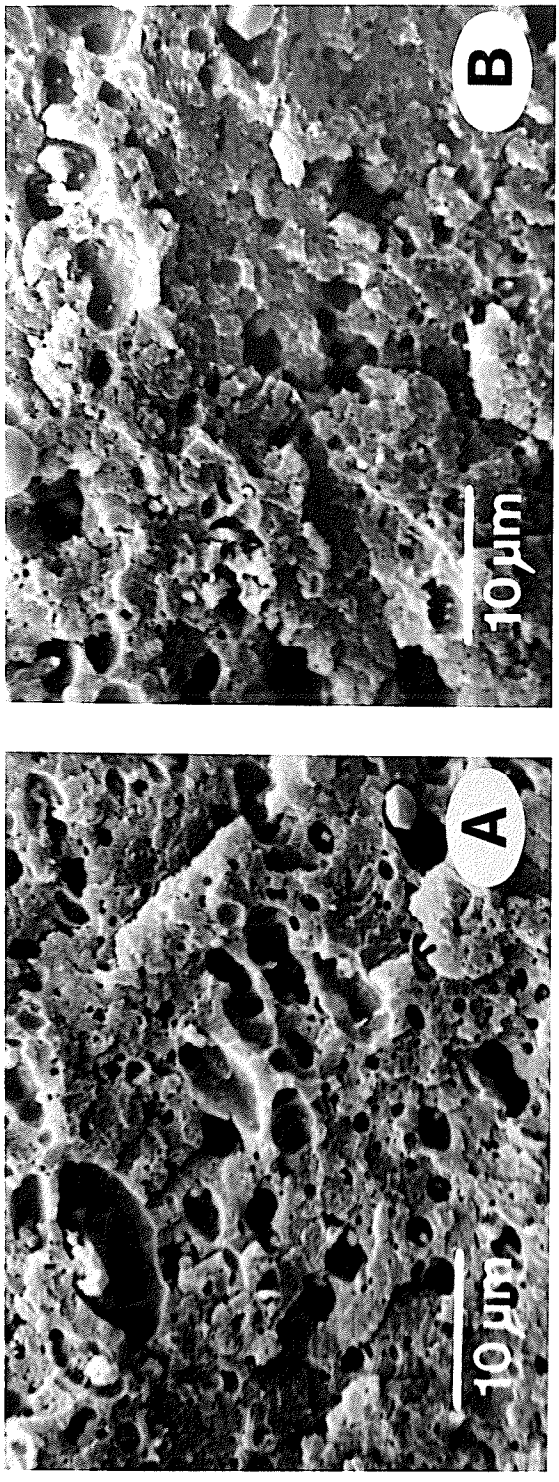
It was reported that during air or vacuum drying the specimens are grossly damaged by surface tension forces, and important surface details are often badly distorted or completely destroyed by forces measured in tons per square inch (Anderson, 1951). Critical point drying is recognized as a desirable procedure for avoiding the destructive action of surface tension in drying from water (Porter et al., 1972). Therefore, the glutens of Norstar and Neepawa were critical point dried for SEM studies. Another gluten from a soft wheat, Frankenmuth was also used in this study. The SEM micrographs of these three glutens are presented in Figure 27.

Figure 27: Scanning Electron Micrographs of the Critical Point Dried Gluten Samples.

A Neepawa

B Norstar

C Frankenmuth


A
10 µm
B
10 µm

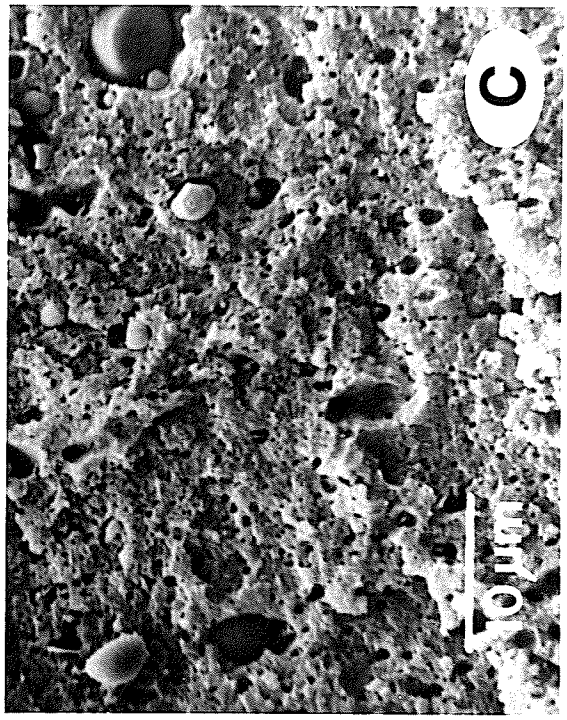

C
10 µm

Figure 27 showed that critical point dried gluten samples of Norstar and Neepawa were very similar. They both had sponge-like structure having air cells with approximately equal size. Both Norstar and Neepawa glutens had air cells larger than Frankenmuth gluten. In the scanning electron microscopy studies it was not possible to observe differences in gluten structure that could be related to gluten quality.

Chapter V

SUMMARY AND CONCLUSIONS

The objectives of the thesis were to study the quality of Canada Western Red Winter wheats, and to examine the factors which influence the low water absorption capacities of winter wheats. In the first section of the study the winter wheat variety Norstar, the predominant Canadian variety of hard red winter wheat class, was selected for the quality studies. Five composite samples of Norstar with wheat protein ranges of 8.8-13.2% and 10.0-13.8% from Minto and Portage respectively were used in the quality studies. One Canada Western Red Spring wheat (cv. Neepawa), one Canada Utility wheat (cv. Glenlea) and one soft white winter wheat (cv. Frankenmuth) were also included for comparison.

The test weight values of the Norstar samples were not affected by protein content. The test weight values of Neepawa, Glenlea and Frankenmuth were lower than the Norstar samples but all met the test weight requirements of Canada No.1 grade for the respective wheat class. The kernel weights of Norstar samples decreased with increasing protein content. All Norstar samples had higher kernel weight values compared to the Neepawa sample but lower kernel weights compared to Glenlea and Frankenmuth. For the combined data of all of the Norstar samples a negative correlation was found between wheat protein and thousand kernel weight which was significant at the 5% level.

The kernel hardness increased with increasing protein content for all Norstar samples at both locations. However, the combined data from both

locations showed no significant correlation coefficients between the three hardness test results and wheat protein content. All Norstar samples were softer than the Neepawa and Glenlea samples. The Frankenmuth sample was the softest sample as determined by all three tests. The milling quality of Norstar samples in terms of flour yield and flour ash content was maintained at both locations over the range of protein contents studied.

The pentosan content and starch damage values of the Norstar flour samples were not affected by the protein content and were all lower than the Neepawa flour sample. The lower pentosan and starch damage levels of Norstar samples could be a possible explanations for some but not all of the difference in absorption between Norstar and Neepawa. All flour samples had low amylolytic and proteolytic activities and were considered sound. The amylolytic and proteolytic activites were also not affected by protein content.

The wet and dry gluten contents of the Norstar samples increased with increasing protein content. Highly significant correlations were found between the gluten content and most quality parameters. The water holding capacities of the Norstar glutens were lower than the Neepawa gluten but higher than the Glenlea gluten. The Zeleny sedimentation values of the highest protein content Norstar samples from both locations were higher than the Neepawa sample, although the protein contents were lower than Neepawa. This indicates that Norstar has stronger gluten properties.

The farinograph absorption, dough development time and stability values increased while the mixing tolerance index values decreased for all Norstar samples as the protein content increased. The extensigraph

parameters (extensibility, maximum resistance, energy and ratio values) for all Norstar samples also increased with increasing protein content. The extensigraph ratio values of the Norstar sampes were higher than Neepawa and were comparable to Glenlea at the high protein content range. There were significant correlations between protein content and most of the farinograph and extensigraph parameters.

The remix loaf volume values for the Norstar samples increased with flour protein content. Simple regression analysis on the combined data of the Norstar-Portage and the Norstar-Minto samples showed that flour protein gave a closer estimate of remix loaf volume than the other quality tests and 94.8% of the total variation in remix loaf volume can be accounted for by a linear function of flour protein content. The comparison of the Norstar samples with the Neepawa sample (control) and the Eastern Prairie composite Canada Western Red Spring wheat samples of the Grain Research Laboratory (1985) indicated a comparable quality in terms of remix loaf volume. Multiple regression analysis showed that a model involving the three variables (flour protein content, dough development time, and extensibility) was significant at the 1% level with an R^2 value of 0.962.

Simple regression analysis on the combined data of Norstar-Portage and Norstar-Minto samples showed that flour protein gave a closer estimate of farinograph absorption than all other tests. Over 92% of the total variation in farinograph absorption could be accounted for by a linear function of flour protein content. Pentosan content and starch damage values were found to be not significant due to a lack of variation of these parameters in the samples studied. A comparison of the Norstar samples with the Neepawa (control) and the Eastern Prairie

composite Canada Western Red Spring wheat samples of the Grain Research Laboratory (1985) showed that farinograph absorption responses at the same protein level were significantly different. A multiple regression analysis showed that a model involving the three variables (flour protein content, alpha-amylase, and endoproteolytic activities) was significant at the 1% level with an R^2 value of 0.982.

The first section of the thesis showed that the quality of Canada Western Winter wheat was comparable to Canada Western Red Spring wheats in terms of milling, rheological properties and baking quality. However, the water absorption capacities of Norstar flours were significantly lower than that of Canada Western Red Spring wheat flours. Therefore, the various factors which might contribute to the low water absorption capacities of Norstar flours were investigated in the second section of the thesis.

The effect of starch damage on water absorption was examined with one grain sample from Norstar and one from Neepawa (at the same protein content) which were milled at various reduction roll-settings to produce samples with a range of starch damage levels. For both varieties as the starch damage increased there was a parallel increase in farinograph absorption and a decrease in amylograph peak heights. The linear regression lines of farinograph absorption against starch damage for both varieties were almost parallel. A t-test on the intercept values showed that they were significantly different. These wheat samples were of similar protein content, comparable in terms of proteolytic activities and slightly different in pentosan content and amylolytic activity. However, the difference in farinograph absorption (4.6% at zero starch damge level) was too large to be explained only by the differences in amylolytic activity and pentosan content. Therefore, further work was

undertaken to study the possible qualitative differences between proteins of the two varieties.

The glutens from the Norstar and Neepawa flours were isolated and added back to a base flour at various levels. In the farinograph studies, the Neepawa gluten resulted in greater responses on farinograph characteristics than the Norstar gluten. The linear regressions of farinograph absorption against the flour protein content were calculated for both varieties. The slopes of the regression lines were significantly different i.e. the rate of increase in farinograph absorption due to the changes in flour protein content was significantly different by addition of Neepawa gluten than by addition of the Norstar gluten.

The glutens of the two varieties were further fractionated into acetic acid-soluble and acetic acid-insoluble fractions for further investigation and various tests were performed on these gluten and gluten fractions. The protein and pentosan contents of the gluten and the gluten fractions of Norstar were comparable to gluten and corresponding fractions of Neepawa. The sodium contents of the glutens isolated from Norstar and Neepawa were significantly different. Norstar gluten had more than twice the sodium content of the Neepawa gluten. The sodium levels in the Norstar and Neepawa flours were similar.

The water holding capacities of the total and reconstituted glutens and acetic acid-insoluble fractions of Neepawa were higher than the water holding capacity of those from Norstar. The hydrated insoluble fraction from Norstar and Neepawa had a water holding capacity about 5-times greater than the respective gluten. The bound water values of gluten and gluten fractions of Neepawa, as determined by DSC, were

considerably higher compared to the gluten and corresponding fractions of Norstar.

SDS sedimentation values of gluten and the acetic acid-insoluble fraction from Norstar were higher than the SDS sedimentation values of the gluten and corresponding fraction of Neepawa. The Zeleny sedimentation values of the Norstar samples were also relatively high. Both sedimentation tests indicated that Norstar had a stronger gluten structure.

The gluten fractions from both Norstar and Neepawa were added to the same base flour at various levels in a farinograph study. Neepawa gluten fractions resulted in greater farinograph absorption responses as compared to Norstar gluten fractions. The regression analysis of farinograph absorption against flour protein content showed that the slopes of the regression lines of the insoluble fractions were significantly higher than that of the soluble fractions for both varieties. Therefore, the rate of increase in farinograph absorption which was due to increased protein content was significantly higher with addition of the insoluble fraction than with the soluble fraction.

The slopes of the regression lines of the soluble fractions of Neepawa and Norstar were significantly different while the slopes of the regression lines of the insoluble fractions were not significantly different. The acetic acid-soluble fraction of both varieties caused a considerable increase in dough development time while the insoluble fractions resulted in either a slight increase or no increase in dough development time.

The gel filtration experiments showed that the protein elution profiles of the respective gluten fractions (acetic acid-insoluble and acetic acid-soluble fractions) of both varieties were very similar. The acetic acid-insoluble fraction of Norstar gluten had a greater portion of its carbohydrates coeluted with the proteins which eluted in the void volume. However, the corresponding fraction of Neepawa had a larger portion of its carbohydrates coeluted with the lower molecular weight proteins. It is possible that in these two cultivars the carbohydrates and proteins of different molecular sizes interacted through secondary forces or covalent bonds to form glycoproteins. The nature of the interactions between carbohydrates and proteins and their interactions with lipids might affect the flour quality. Further research is required to study the interactions of flour proteins with carbohydrates and lipids in relation to quality characteristics of wheat cultivars.

The parameter, "negative charge potential less amide groups" calculated from the amino acid analysis data was slightly greater in the Norstar gluten than in the Neepawa gluten. This could indicate that Norstar gluten has a slightly greater proportion of its amino acids which have a potential negative charge. Norstar gluten had a higher sodium content than Neepawa gluten. Apparently, Norstar gluten due to its amino acid composition may interact more with charged groups such as sodium. This interaction could result in lower water binding capacity. However, more comprehensive studies are necessary to support the statement.

All Norstar samples had relatively low farinograph absorption. However, baking studies showed that baking absorption could be increased considerably without a substantial deterioration of loaf volume and dough handling properties. For the Norstar sample it was possible to

increase the baking absorption by 5% over the farinograph absorption without a decrease in loaf volume.

The farinograph studies with Uptake 80 (a vegetable fiber) showed that when added at a 2% level it increased the farinograph absorptions of the Norstar and Neepawa flours by 3% and 2% respectively. A combination of Uptake 80 (2%) and sodium stearoyl lactylate (1%) did not improve farinograph absorption. Baking studies showed that it was possible to increase the baking absorption of the Norstar flour by 7% over the original farinograph absorption value without a significant deterioration in loaf volume and dough handling properties. These studies suggested that lower farinograph absorptions of the winter wheats are not of major concern because it was possible to increase the baking absorption of winter wheat to a considerable extent over the farinograph absorption. Unfortunately, a true comparison of Norstar and Neepawa flours at increased baking absorption was not possible due to the unavailability of a low protein Neepawa sample.

Scanning electron microscopy was undertaken on the flour produced for the starch damage studies. The microscopy studies showed that for each milling roll-setting the particle size of the Neepawa flour produced was slightly larger, and the particles of Neepawa were more angular with sharper edges and corners than the Norstar flour. However, it was not possible to observe with the scanning scope the degree of starch damage on the starch granules. After the enzyme treatment of the flour samples it was possible to observe an increased level of enzyme attack on the starch granules within each variety. However, there was no clear difference between the corresponding samples of each variety.

In conclusion, the attempts to determine some of the factors which might contribute to lower water absorption capacity of Norstar flour and lower water holding capacity of Norstar gluten revealed that the abilities of Norstar gluten and gluten fractions to increase the farinograph absorption of a base flour were significantly lower than the gluten and corresponding fractions of Neepawa. This indicated a qualitative difference between the Norstar and Neepawa proteins. The sodium content of Norstar gluten was higher than in Neepawa gluten. The proportion of the amino acids with a potential negative charge and degree of amidization were slightly higher in the Norstar gluten than in the Neepawa gluten. Furthermore, bound water values of Norstar gluten and gluten fractions were lower than the Neepawa gluten and gluten fractions. All of these findings indicated that Neepawa flour bound and absorbed more water than Norstar flours.

CONTRIBUTIONS TO KNOWLEDGE

This study was carried out to investigate the quality of a winter wheat cultivar, Norstar (Canada Western Red Winter wheat) and the factors affecting the absorption properties of Norstar flour. Contributions to knowledge from this research are listed below:

1. The quality of the winter wheat variety, Norstar was acceptable and comparable to Canada Western Red Spring wheats in terms of milling, rheological properties and baking quality. However, the water absorption capacities of Norstar flours were significantly lower than the Neepawa flour.
2. The farinograph study using the Norstar and Neepawa samples with wide ranges of starch damage showed that the difference in their farinograph absorption was probably too large to be explained only by the difference in their composition i.e. protein content, pentosan content, starch damage level etc.
3. The Neepawa gluten resulted in significantly greater increases in farinograph absorption compared to Norstar gluten when added to a base flour at various levels.
4. Although the sodium contents of the Norstar and Neepawa flours were similar, the sodium contents of Norstar and Neepawa glutens were 332 and 115 ppm respectively. The Norstar gluten also had a slightly greater proportion of its amino acids which have a potential negative charge.
5. The bound water contents of gluten and gluten fractions of Neepawa as determined by DSC were considerably greater than the gluten and corresponding fractions of Norstar.

6. The water holding capacities of gluten, reconstituted gluten and acetic acid-insoluble gluten fractions of Neepawa were greater than the corresponding glutens and the acid-soluble fraction of Norstar.
7. The acetic acid-soluble fraction of the Neepawa gluten resulted in significantly greater increases in farinograph absorption compared to corresponding fraction of Norstar when added to a base flour at various levels. The insoluble fraction of each variety resulted in a significant increase in farinograph absorption compared to the soluble fraction of corresponding variety when added to a base flour at various levels.
8. The acetic acid-insoluble fraction of Norstar gluten had a greater portion of its carbohydrates coeluting with the proteins which eluted in the void volume. However, the acetic acid-insoluble fraction of Neepawa had relatively larger portion of carbohydrates coeluting with the lower molecular weight proteins.
9. It was possible to increase the baking absorption of Norstar flour by 7% over the farinograph absorption of this flour when a vegetable fiber product was included in the baking formula.

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Appendix A

QUALITY DATA FOR EASTERN PRAIRIE GRADE COMPOSITE SAMPLES OF 1985 CROP NO.1 CANADA WESTERN RED SPRING WHEAT.

	Minimum protein level			No.1 C.W. 13.5	
Quality Parameters	14.5	13.5	12.5	1984	Mean[1]
Wheat					
Test weight (kg/hl)	79.5	80.3	80.9	80.6	81.9
1000 kernel weight (g)	26.6	27.8	30.0	27.7	30.2
Protein content (%)	14.7	13.8	12.6	13.9	13.7
Flour yield (%)	75.5	75.8	75.6	76.1	75.4
Flour					
Protein content (%)	14.3	13.4	12.1	13.3	13.1
Wet gluten (%)	41.6	38.4	34.8	40.1	39.3
Ash content (%)	0.49	0.49	0.48	0.47	0.47
Alpha-amylase activity	1.4	2.0	9.3	0.5	---
Amylograph peak height (B.U.)	640	610	585	770	715
Starch damage (F.U.)	30	31	34	30	28
Bread					
Loaf volume (c.c.)	940	880	780	910	855
Blend loaf volume (c.c.)	785	725	680	730	700
Rheological Tests					
Farinograph absorption (%)	65.9	65.5	65.3	63.1	65.5
Dough development time (min)	5.75	5.00	4.75	4.50	5.00
Extensibility (mm)	230	240	220	240	220
Maximum resistance (B.U.)	470	450	410	435	415
Energy (cm^2)	150	145	125	140	130

1 Mean values for the 10-year period 1974 to 1983 (Canadian Grain Commission, 1985).

Appendix B

THE QUALITY DATA ON THE NEEPAWA SAMPLES REPORTED BY RODRIGUEZ-BORES (1976).

Flour protein (%)	Farinograph absorption (%)	Remix loaf volume (c.c.)
9.3	60.1	500
10.7	60.4	645
12.7	62.0	705
14.1	63.5	820
14.7	64.3	875
15.5	64.7	850
15.9	64.4	845
15.8	64.5	860
16.1	64.5	950
16.2	64.7	900
16.4	64.3	852

Note: The first five samples which had the lower protein contents were used in regression analysis.