A PHYSICOCHEMICAL STUDY ON GLUTEN OF WHEAT VARIETIES OF DIFFERENT BREADMAKING PROPERTIES

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Reuben Cherukut Butaki

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ABSTRACT

Butaki, Reuben Cherukut. M.Sc., The University of Manitoba, May, 1977. A Physicochemical Study on Gluten of Wheat Varieties of Different Breadmaking Properties. Major Professor: Dr. B. L. Dronzek.

Several physicochemical techniques have been applied to study the properties of the glutens of four wheat varieties known to differ in mixing strength and breadmaking potential. Solubility in dilute acetic acid, viscosity, electrophoresis, amino acid analysis, reconstitution studies and the stretching properties of these glutens, were used to determine the basis for the intrinsic differences in the glutens. The effect of flour protein on gluten properties within the three Manitou and three Glenlea samples was investigated.

The 0.05N acetic acid soluble fraction for the weak variety Talbot contained 90.8% of the total gluten protein. The corresponding values for the other varieties arranged in the known order of increasing mixing strength were: Manitou 79.8% - 84.1%, Norquay 80.1% and Glenlea 68.7% - 70.2%.

The relative viscosity number of the gluten proteins dissolved in AUC (0.1M acetic acid, 3M urea, 0.01M cetyltrimethyl ammonium bromide) for the samples of the strong variety Glenlea ranged from 19.9 - 22.5. The relative viscosity numbers for the other varieties arranged in the known order of decreasing mixing strength were: Norquay 18.3, Manitou 11.6 - 12.8 and Talbot 6.3 Treatment with 2-mercaptoethanol lowered the

relative viscosity values to the following: Glenlea 5.2, Norquay 4.5, Manitou 4.3 and Talbot 3.2

The relative viscosity values of the acetic acid soluble fraction for Talbot and Glenlea were nearly the same.

Adding small amounts of freeze-dried gluten to a weak flour greatly improved the mixograph curve of the flour. Mixing tolerance index of the flour was improved most by the varieties in the following order of decreasing ability: Glenlea, Norquay, Manitou and Talbot.

Disc-electrophoresis of the alcohol soluble protein and Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis of the reduced gluten and reduced acetic acid soluble fraction, revealed differences in the subunit number and pattern between varieties. However, electrophoretic results could not be linked directly with breadmaking potential or mixing properties of the flour.

The four varieties showed similar amino acid composition at each level of sample: grain, flour and gluten. The minor differences in amino acid distribution were insufficient to explain the gross differences in the rheological properties of the flours.

Stretching of the glutens under a constant load revealed the following order of increasing resistance to stretch: Talbot, Manitou, Norquay and Glenlea.

This investigation revealed that for the two varieties Manitou and Glenlea, the acetic acid soluble gluten increased slightly with increase in flour protein. However, viscosity, electrophoresis, reconstitution and stretching studies showed little change in any measured gluten parameter with flour protein. This lead to the conclusion that the

gluten properties were characteristic of the variety and were not affected qualitatively by flour protein.

I. INTRODUCTION

The importance of wheat as a human food cannot be overemphasized. Wheat was one of the main crops of China five thousand years ago. Bread containing a raising agent (leavened) was known in Egypt in 2000 B.C. Today wheat is one of the principal food crops in the world and is consumed in Europe, North and South America, Australasia and Africa.

Wheat is consumed in many different forms in different parts of the world. However, many studies on wheat utilization for breadmaking have been based on the production of the well-risen loaf of bread. This product has been adapted to high technological production. Wheat protein has been found to be mainly responsible for the technological success of wheat in bread production. Consequently, studies on wheat utilization have centred on protein content and protein characterization. The higher the protein content of a flour, the larger the loaf of bread produced from it. This relationship can also apply in comparisons of flours from a single variety but having different protein contents.

Two varieties may have the same protein content, yet one will yield a larger loaf of bread than the other for the same weight of flour. Differences in protein type (gluten quality) between varieties have been cited as the basis for these differences in loaf volume potential. Mixing and other rheological properties between wheat varieties displaying different loaf-volume potential are usually different.

Using the prevalent North American baking processes in comparing pure wheat varieties, it is observed that flours with weak farinograph

mixing curves yield small loaves of bread. Wheat varieties whose flours have strong mixing curves give large loaves of bread. However, flours of some varieties which give very strong farinograph mixing curves also yield bread with low volume and poor crumb texture. The flour of such cultivars is characterized by long mixing requirements and extremely elastic glutens. Inadequate dough development results in the poor loaf volume potential of these flours. In Canada wheat varieties of overly strong and weak type are marketed under the utility class.

When a stiff dough is gently washed under tap water, an elastic insoluble pellet of protein known as gluten results. Glutens, isolated from flours of different protein contents, contain essentially the same protein content. Therefore, protein content differences between flours are eliminated by washing the gluten. Consequently, any physicochemical differences or similarities between glutens reflect the type of protein without the influence of protein content.

This thesis was initiated to study physicochemical differences between glutens of wheat varieties known to differ in mixing properties and breadmaking potential. The varieties and the classification on the farinograph were: Talbot (weak), Manitou (medium strong), Norquay (strong) and Glenlea (very strong). For each of two varieties (Manitou and Glenlea) grain was selected at three different protein contents. Within each cultivar gluten from the three derived flour samples was compared to determine if flour protein affected gluten properties. Consequently, the objectives of this investigation were: to examine any characteristic properties associated with glutens of weak, medium strong, strong and very strong varieties and to determine if these properties were affected qualitatively by flour protein content.

II. LITERATURE REVIEW

A. Flour Protein and Breadmaking Quality

Wheat flour protein is the single most important component that has maintained the technological superiority of common wheat (*T. aestivum*) over other cereals for making bread. The protein content of flour commonly used for bread production is around 12.0% on 14.0% moisture basis. However, the viscoelastic and gas-retention properties of dough during fermentation, resulting in a light soft-textured bread, are due to the protein (Pomeranz, 1968).

Highly significant positive correlations between loaf volume and flour protein is well established. Examples of early work in this field include those by Larmour (1931) and Harris and Bailey (1937). The higher the protein content, the larger the loaf produced from the flour. Sandstedt and Ofelt (1940) reported wide differences in loaf volume at any protein level within pure varieties. These differences increased with increasing protein content. Protein-loaf volume regression lines within pure hard winter wheat varieties were found (Bayfield *et al.*, 1941) to be characteristic of the variety. They concluded that differences in regression lines inferred differences in gluten quality.

Protein content of the flour for a single variety had a large influence on the mixing characteristics (farinograph curves) and loaf volume (Bushuk *et al.*, 1969). In the same study, the loaf volume by the remix baking procedure, was highest for the medium strong variety

and decreased with decreasing or increasing strength as judged by the farinograph test. Finney and Barmore (1948) found that protein content was the major factor accounting for variation in loaf volume within a pure variety. They found that despite the linear relation between these two factors, certain varieties had distinctly different regression lines. These were interpreted to represent differences in protein quality.

The first comprehensive study of wheat flour proteins was made by Osborne (1907). Using sequential fractionation for the classification of proteins, Osborne identified four main fractions on the basis of solubility characteristics. These were albumin (water-soluble and heat coagulable); globulin (salt-soluble); gliadin (soluble in 70% ethanol); and glutenin (insoluble in alcohol but soluble in dilute acid or dilute alkali). The contribution of the different flour protein fractions to breadmaking has received much attention in recent years.

1. Albumins and Globulins

The albumins and globulins have been prepared by various techniques. This soluble flour protein fraction has been extracted from wheats of widely varying baking quality. Studies have been made in an attempt to identify its role in rheological properties of dough.

Pence and Elder (1953) prepared albumins from flour by extraction with a dilute phosphate buffer and separately extracted globulins using 1M sodium chloride solution. The purified albumin consisted of at least six individual components of similar molecular size but different in electrophoretic properties. Three individual components were found in the purified globulin fraction. Bell and Simmonds (1963) differentially

extracted flour proteins from twenty-six wheat samples, ranging in nitrogen content from 1.49 - 2.92%. The solvents used were 0.01M sodium pyrophosphate pH 7.0 and 0.05M formic acid pH 3.5. A negative correlation was found between total nitrogen and the sodium pyrophosphate soluble fraction. In contrast the formic acid soluble fraction and total nitrogen were both positively correlated with baking score.

Mullen and Smith (1965) obtained similar amounts of albumins and globulins from the short and long mixing flours. Characteristic differences in relative amounts of individual electrophoretic components were reported by Pence *et al.* (1954a) between albumins of durum wheat flours and club and common wheat flours. Among the common wheat and club wheat flours, only minor varietal differences were observed. Pence *et al.* (1954b) reported that the water-soluble protein contents of thirty flours of widely varying type and baking quality ranged from 13 - 22% of the total flour protein. Neither the content nor the ratio of soluble: gluten protein was correlated to baking quality.

Several studies have been made on the effects of the water and salt-soluble flour proteins on the baking performance of glutens or flours. Exclusion of the water-soluble fraction from reconstituted doughs caused no reduction in loaf-volume or bread quality (Finney, 1943). However, the soluble flour components were found to be required for maximum performance of all the glutens except that from the durum wheat (Pence *et al.*, 1951). The water-soluble fractions from wheat varieties of widely different flour quality, had similar effects on reconstituted flours (Hoseney *et al.*, 1969a). They concluded that despite its necessity

for optimum bread volume, this fraction was not involved in flour quality differences.

2. Gluten Proteins

Beccari is reputed to have performed the first investigation on gluten in 1728 (Bailey, 1941). He found no glutenous material in bean or barley flour. Ewart (1968) studied solubility properties of proteins of wheat and several cereals. He found gluten (0.1N acetic acid soluble fraction) and the overall solubility of the proteins to be correlated with baking quality. Oats and maize were devoid of the acetic acid soluble protein. These cereals are incapable of forming a viscoelastic dough.

Bushuk (1962) was the first to adapt the farinograph to produce a successful or normal curve for dried vital gluten. Doguchi and Hlynka (1967) rated the farinograph mixing curve of the gluten of a hard red spring wheat, stronger than that of a soft winter wheat. They concluded that, their results supported the view that the physical properties of dough were in large measure attributed to its gluten.

B. Gluten Composition and Structure

The physicochemical behaviour of gluten is dependent on its composition and the three dimensional interaction of its components. Crude gluten has been reported (Abbot, 1959) to contain: 85% protein, 8.3% lipid, 6% starch and 0.7% ash.

Pence *et al.* (1950) studied the amino acid composition of glutens from wheats of diverse baking characteristics and different flour protein. The amino acid compositions of these glutens were essentially the same.

The proportions of amino acids for pure varieties of spring and winter wheats at similar protein contents, were found to be nearly constant (Hepburn and Bradley, 1965). Glutamic acid, phenylalanine and proline tended to be higher in high protein samples. The remaining amino acids showed the reverse trend to varying degrees. Woychik *et al.* (1961) stated that high glutamic acid and proline characterized gluten proteins. Since the two amino acids together accounted for 50% of the peptide bound amino acids, it was suggested that these amino acids were of particular structural importance in gluten proteins. In this study, the α-component (mainly glutenin) contained more glycine than the other gluten components (gliadin). However, no major differences in amino acid composition could explain the gross property differences. Recently Bietz and Rothfus (1971) stated that glycine occurred more frequently in glutenin and proline was more frequent in gliadin sequences. In the same study glutamine was reported to be positioned differently in glutenin and gliadin.

Studies of hydrogen ion equilibria of wheat gluten lead Wu and Dimler (1963) to conclude that glutamic acid and aspartic acid were present in the amide forms. Glutamic acid and proline were found to be most numerous in gluten. Later Wu *et al.* (1967) found that the decrease in intrinsic viscosities of glutenin with increasing ionic strength was larger than for gliadin. They concluded that the glutenin molecule was held together more loosely.

The effect of ascorbic acid on gluten was found (Zentner, 1968) to be similar to that of reducing or sulfhydryl blocking agents. However, no reduction of disulfide bonds was detected. It was concluded that

ascorbic acid displaced some "bound" water from the gluten structure. The softening effect of ascorbic acid on gluten resulted from the lubricating action of this water.

Hoseney *et al.* (1970) reported that free polar lipids (principally glycolipids) interacted with gliadin proteins through hydrophilic bonds and with glutenin through hydrophobic bonds. Consequently, they postulated that in crude gluten, the lipid was bound to both protein groups simultaneously. The gluten gas-retaining complex could be viewed to be gliadin and glutenin units linked by polar lipids.

Further structural characterization of gluten proteins has been achieved by electron miscroscopy. Seckinger and Wolf (1970) observed that gliadin spread on water formed a film, whereas glutenin formed strands. Moreover, the protein particles from hard wheats were more compact and resistant to disruption. Those from soft wheats were expanded and easily disrupted. Orth *et al.* (1973) used the scanning electron microscope to examine bread wheat flour proteins that had been separated by gel filtration. The high molecular-weight fraction (glutenin-like) had disc-shaped particles joined together by stringy fibrous strands. The lower molecular weight fraction (gliadins) contained uniform small aggregates of amorphous protein particles. Compared with purified glutenin, the high molecular-weight fraction appeared highly heterogenous.

C. Viscoelasticity of Gluten Proteins

Despite similarity in the amino acid composition, glutens of various classes and varieties of wheat are known to display great diversity in their stretching properties. Muller (1973) has suggested some prerequisites for high or rubber-like elasticity in materials. The molecules should be long chain, with free rotating links, weak forces between molecules, and firm cross-links at a few points in the long chain. Wheat gluten has been demonstrated (Muller, 1969) to possess high elasticity. In the same study a strong flour was shown to be more heavily cross-linked than a soft flour.

Covalent cross-linking of the flour proteins (gluten proteins) has been shown to be due to the disulfide bonds. The number of disulfide groups per unit weight of flour showed an increase with protein content among flours of single varieties and a mixed grist (Axford *et al.*, 1962). However, disulfide bond value and protein content were inversely related. Similar results were reported by Tsen and Anderson (1963) who measured the sulfhydryl and disulfide contents of flours from one variety each of: hard, soft and durum wheat. Results showed that on the weight basis, the hard wheat flour contained the highest numbers of disulfide bonds. The soft wheat flour contained the least disulfide and sulfhydryl bonds. In general, an inverse relation between disulfide bonds value and protein content was obtained for the three types of flour.

The rate of stretching, under constant load, of gluten washed from air-classified flour fractions was greatest for the gluten from 35-63μ fraction (Kaminski and Halton, 1964). Conversely, that from fractions containing particles less than 35μ had the least rate of stretching.

Jelaca and Hlynka (1972) reported that pentosans produced a decrease in the rate of stretching of gluten in the Kosmina test. The pentosan effect was similar to that of the iodate flour improver.

Prihoda *et al.* (1971) found the rheological properties of the dough as measured by the Hoeppler consistometer to be related to flour quality. The flow curves obtained showed that at constant velocity of deformation the stress increased or at constant stress, the velocity of deformation decreased with flour strength. These conclusions are in agreement with the observation that glutens from weak wheats stretch at a faster rate than glutens from strong wheats.

Incubation of gluten with proteolytic enzymes (Kruger, 1971) resulted in marked changes in stretching characteristics of the gluten. Increasing concentration of the enzymes increased the mean rate of stretching in a stretching test; decreased farinograph consistency and reduced the volume of the bubble on the alveograph.

Ponte *et al.* (1967) reported that organic solvents decreased gluten extensibility to varying degrees. Within the alkane family, the C_6 - C_{10} paraffins conferred the greatest decrease in extensibility in a gluten stretching test. This "strengthening" effect was postulated to be due to the ability of the solvents to generate high molecular weight aggregates in the gluten.

Measurements of the viscosity of flour-water mixtures as criteria for flour quality, has interested cereal chemists for many years. The basis of these viscosity measurements is the swelling of gluten proteins and the resultant increase in the viscosity of the flour water-mixture.

Williams and Williams (1967) have stated that of the hydrodynamic

properties of macromolecules, viscosity is the most intimately related to shape. Intrinsic viscosity of randomly coiled macromolecules are much larger than those for compact structures (Van Holde, 1971).

Sollars (1969) compared the flour viscosities of one hard red spring wheat, three soft winter wheats and a club wheat, all having similar protein contents. Viscosity differences were found to be varietal. The gluten fraction was shown to be responsible for the varietal differences in viscosity.

Cluskey and Wu (1971) demonstrated the effect of various solvents on the conformation of gluten proteins, glutenin and gliadin. Urea solutions unfolded the gluten molecule to the completely unordered conformation. A decrease in pH enhanced the effect of urea solutions. At pH 3-4 the positive charges on glutenin repelled each other thereby disrupting α-helices.

Pence and Olcott (1952) reported that reducing agents lowered the relative viscosity of the gluten proteins by fragmenting these proteins. Reduction of disulfide bonds was found to be responsible for the lowering of the viscosity. Nielsen *et al.* (1962) showed that glutenin had a much higher intrinsic viscosity than gliadin. The viscosity of glutenin was reduced markedly by disulfide bond cleaving agents, whereas that of gliadin was not affected. It was concluded that the disulfide bond linked glutenin polypeptide resulted in the elastic and cohesive protein present in whole gluten.

The rate constant, k, of aggregation of gluten proteins, as determined by a turbidity time curve, was found by Arakawa and Yonezawa (1975) to be greater for the strong flour than for the weaker flours. Gliadins

from strong, medium and weak flours exhibited similar aggregation rates. It was concluded that the aggregation behaviour of the gluten was mainly determined by the nature of its glutenin fraction.

Matsuo and McCalla (1964) examined the viscosity of glutens dispersed in 8% sodium salicylate and in lactic acid-sodium chloride solutions. Intrinsic viscosity values fell in the following decreasing order: hard, soft and durum wheats. Reduction of the gluten proteins with excess sodium sulfite or performic acid, resulted in low intrinsic viscosities for all glutens. However, the order of the values remained the same.

D. Solubility of Gluten Proteins and Baking Quality

The study of gluten proteins has been hampered by the insolubility of these proteins in most solvents. Several solubility characteristics of the gluten proteins have come to light as a result of efforts to solubilize these proteins. Such solubility properties have been associated with good or poor quality gluten.

Jones *et al.* (1959) showed that the characteristic properties of gluten were associated with their least soluble fraction α. This fraction was mainly composed of glutenin. In the same study no significant differences were observed in the electrophoretic patterns of the fraction α for bread wheats of different baking quality.

Studies on short and long mixing flours (Mullen and Smith, 1965) revealed major solubility differences in gluten proteins. The short-mixing flour gluten was more readily soluble in water or dilute acid than the long-mixing flour gluten. In a subsequent study (Smith and

Mullen, 1965) showed that the protein-starch residues (glutenins) had long-mixing requirements in the farinograph. Addition of water solubles (gliadins) markedly shortened the mixing requirements. They concluded that the mixing differences of the weak and strong flour were determined by characteristics of the protein-starch residues and the quantity and molecular weight distribution of the gliadins.

Tsen (1967) reported that the weak flour contained a higher proportion of acetic acid soluble protein than the strong flour. He concluded that the largest protein aggregates of the soft wheat flour were smaller and more liable to disaggregation. Conversely, the strong flour contained large protein aggregates whose structure was less liable to disaggregation. Cluskey and Dimler (1967) reported that the acetic acid insoluble protein in wheat gluten had high molecular weight and had glutenin-like properties. However, the amino acid composition was akin to the water-soluble wheat proteins than to glutenin.

More stability to mixing in the farinograph was imparted to a dough by crude gluten than by gluten dispersed in dilute acetic acid (Mecham *et al.*, 1965). When the large particle size fraction from this dispersion was removed, the remainder of the dispersion resulted in further decrease in stability to mixing.

Pomeranz (1965) dispersed flour proteins in 3M urea in 0.01M pyrophosphate buffer pH 7.0. High quality flours had low protein dispersibility. Conversely, low quality flours had high dispersibility in this solvent. He postulated that the ease of dispersibility and disaggregation of proteins in urea was related to the structural lability of wheat proteins during mechanical processing of wheat dough. The

residue insoluble in 6M urea solution of a gluten and a lipid treated storage protein, were reported by Simmonds and Wrigley (1972) to contain additional material. The additional material was found to be a high molecular weight protein with glutenin characteristics. It was concluded that interaction between lipid and protein occurred in wheat flour during dough-formation, leading to the formation of high molecular weight material with characteristics of glutenin.

Orth and Bushuk (1972) studied twenty-six wheat cultivars of diverse baking quality grown at four locations. They showed that the proportion of the acetic acid soluble protein (glutenin) was negatively correlated with loaf volume. The residue protein was positively correlated with loaf volume per unit flour protein. Tanaka and Bushuk (1972) reported that of the two varieties they examined, the one with the longer farinograph dough development time contained less acetic acid soluble protein and more insoluble protein. Insignificant change was observed in gliadin and a slight increase in acetic acid soluble protein with increasing protein content, within the two varieties.

Dronzek *et al.* (1970) determined protein solubility distribution of three common bread wheat (AABBDD) varieties and their derived AABB tetraploid progeny. Two of the common wheats had both better baking quality and a higher proportion of residue protein than their related tetraploids. They concluded that the protein solubility distribution differences were related to breadmaking quality. The proteins of the AABB (durum) contain more gliadins than those of the AABBDD (common bread wheat). This results in a more extensible gluten for the durum and a more elastic gluten for the hexaploid or common wheat.

E. Gliadin, Glutenin and Baking Quality

The insoluble protein mass known as gluten consists mainly of the alcohol soluble protein (gliadin) and the acetic acid or dilute alkali soluble protein (glutenin) as originally defined by Osborne (1907). Glutenins prepared by a variety of methods have turned out to be qualitatively similar although differences have been noted (Kasarda *et al.*, 1976). The specific contribution of glutenin or gliadin or of their components, to breadmaking quality has received much attention in recent years.

Reconstitution studies with various flour constituents led Finney (1943) to conclude that the recognized differences in breadmaking quality of wheat varieties were entirely accounted for by differences in their gluten fractions. Guess (1900) reported that gliadin and glutenin of wheat flour were the chief determining factors in breadmaking quality. The same study showed that the elastic quality of the gluten was improved in proportion as the ratio of gliadin to glutenin increased.

Hoseney *et al.* (1969b) determined the gliadin to glutenin ratio for four wheat varieties of widely different breadmaking quality and found an almost constant ratio of 53:47. Reconstitution experiments in the same study established that gliadin proteins controlled loaf volume, while the glutenin fraction controlled the mixing requirements of a wheat flour. Shogren *et al.* (1969) further characterized the role of gliadin and glutenin proteins. The decrease in glutenins and increase in gliadins resulted in a large decrease in mixing time baking absorption and an increase in the oxidation requirements of a flour.

Lee and MacRitchie (1971) extracted flour proteins from wheat

varieties with urea solutions. Early urea extracts of all the wheat cultivars reduced mixogram tolerance and mixing stability and markedly increased alveograph extensibility. On the contrary, the later urea and sodium hydroxide extracts all had the opposite effect. Gel-filtration of the flour proteins confirmed that the early urea extracts contained low molecular weight proteins. The later extracts consisted of high molecular weight proteins. High molecular weight gluten proteins consists mainly of glutenin and the low molecular weight consists mainly of gliadins. MacRitchie (1973) separated gluten proteins of a weak over-extensible flour by gel-filtration chromatography. Addition of whole gluten or high molecular weight protein fraction to the flour, yielded doughs which closely matched a strong, well-balanced flour in the physical-dough testing characteristics. Hence, by altering the proportion of high molecular weight gluten proteins in the flour, it was possible to convert a weak flour into a strong one.

Preston *et al.* (1975) reported that a synthetic dough consisting of gliadin, glutenin and starch gave a strong mixing curve in an electronic recording dough mixer. Replacement of the gliadin by high molecular weight gliadin improved the mixing curve. Conversely, lower molecular weight gliadins, acetic acid soluble glutenin and albumins gave weaker mixing curves. The proteins from several wheat flours varying in baking quality were fractionated on a column of 4% agarose by Huebner and Wall (1976). The elution profiles showed significant differences in the glutenin region. The ratio of the high molecular weight glutenin I to the lower molecular weight glutenin II was greater for flours exhibiting long mixing times and strong doughs. The weak flours possessed lesser

contents of both glutenin I and the unextracted protein. The work of Huebner and Wall (1976) confirmed earlier studies by Orth and Bushuk (1972) who found the acetic acid insoluble (residue) protein to be positively correlated with loaf volume.

Electrophoretic pattern and polypeptide subunits of gluten proteins have been investigated within cultivars of widely differing baking quality. Attempts have been made to link electrophoretic pattern and/or subunits to dough rheology or baking quality.

Elton and Ewart (1960) first applied the starch gel electrophoresis technique to wheat proteins. They found patterns of similar electrophoretic mobilities for gluten proteins of four varieties of diverse baking quality. Only slight differences were observed in distribution of intensities. Coulson and Sim (1964) performed starch gel electrophoresis on aqueous extracts of ground grain for thirty-four different wheat varieties. They noted major compositional differences in the gliadin fraction. However, no obvious correlation was found between protein pattern and physical characteristics of the flour. Using the same technique, Coulson and Sim (1965), later showed that the "wedge" proteins were gliadins. The "adhering" proteins consisted of the slowest moving endosperm fractions together with albumins. Electrophoretic comparison of the gluten of a hard and a soft wheat (Cluskey *et al.*, 1961) showed that the former contained more α, less beta and identical amounts of gamma and omega components to the later.

Gluten proteins of eight wheat varieties showed significant differences in electrophoretic patterns (Elton and Ewart, 1962). The slowest moving bands were concluded to control the rheological properties of the dough. Barley, maize and oats lacked these bands and it was less marked

in rye. Acetic acid extracts of flours of five hard red winter wheats grown at six locations were examined electrophoretically by Koenig *et al.* (1964). Differences were found in the distribution of protein components and the protein content for locations of growth as well as for varieties. The long-mixing flours had less glutenin-gliadin than the short-mixing flours. Orth and Bushuk (1972) observed large intervarietal differences in the gliadin polyacrylamide gel electrophoretic patterns. However, these could not be related to differences in baking quality.

Patterns and relative concentrations of electrophoretic components of gluten proteins, from hard and soft wheats in aluminium lactate-lactic acid pH 3.1, showed no significant differences (Jones *et al.*, 1959). The characteristic properties of gluten were associated with the least soluble fraction α (mainly glutenin). Chromatography on sulfoethyl cellulose and starch gel electrophoretic patterns of gliadins from common, durum and club wheats showed few differences (Huebner and Rothfus, 1968). In this study, only minor differences were obtained for a good quality bread wheat as compared to a poor quality wheat.

Polyacrylamide gel electrophoretic patterns of gliadin proteins of two pure varieties were not affected by flour protein content (Tanaka and Bushuk, 1972). Varietal differences in the electrophoretic patterns of the long-mixing and the medium strong wheat were observed. However, these differences could not be related to baking quality.

Elton and Ewart (1966) performed starch gel electrophoresis of reduced glutenins of four wheat varieties. They observed components with similar mobility with the corresponding gliadins. They supported the view that glutenins were built from gliadin proteins.

The interaction of the amphiphile sodium dodecyl sulfate with proteins has been shown to result in rod-like molecules. The length of these particles are a unique function of their molecular weight (Reynolds and Tanford, 1970). Sodium dodecyl sulfate polyacrylamide gel electrophoresis has been used successfully to determine molecular weight of gluten protein components (Bietz and Wall, 1972; Orth and Bushuk, 1973). The molecular weight of the largest glutenin component of hexaploid wheats has recently been revised downward (Khan and Bushuk, 1976).

Danno *et al.* (1974) extracted protein with sodium dodecyl sulfate from flours of a wide range of nitrogen content and baking quality. Sodium dodecyl sulfate electrophoresis of this fraction did not reveal any characteristic patterns. The insoluble fraction, believed to be glutenin, was further characterized. Isoelectric focusing, amino acid composition and electrophoretic patterns of subunits of the reduced insoluble fraction showed close similarities with purified glutenin (Danno *et al.*, 1976).

III. MATERIALS AND METHODS

A. Wheat and Flour Samples

The parentage of the wheat varieties selected for this study is shown in Table 1.

The wheat samples used in the study were grown as check varieties in yield trials at the University of Manitoba during 1974. After harvest the protein content of samples from the various plots was determined and found to cover a fairly wide range. For each of two of the varieties (Manitou and Glenlea) grain samples were placed in any one of high, medium or low protein category. The protein content of a category within either Manitou or Glenlea, depended on the range of protein content encountered. The grain for the varieties Norquay and Talbot were pooled to form a single sample for each variety.

The grain was analyzed for test weight, thousand kernel weight and protein content. The grain was milled into flour using the Buhler experimental mill according to the approved method 26-20 of the American Association of Cereal Chemists (A.A.C.C., 1969). The following flour and dough characteristics were determined according to the approved (A.A.C.C., 1969) methods: [farinogram (54-21), extensigram (54-10), mixogram (54-40), sedimentation (54-60)]. The breadmaking potential of each flour sample was determined according to the "remix" procedure described by Irvine and McMullan (1960).

TABLE 1. Parentage of the Wheat Varieties

Variety	Pedigree
Manitou	(Thatcher^{7}-Frontana x Thatcher^{6}-Kenya Farmer) x Thatcher^{6}-P.I.170925
Glenlea	Pembina^{2} x Bage x CB100 CB100 = (Sonora 64 x Tezanos Pintos Precoz) x Nainari 60
Norquay	(Lerma Rojo x Sonora 64) x Justin
Talbot	Trumbull-Hope-Hussar x F_1 (Dawsons G.C.^{2}-Ridit x Cornell 595)

B. Determination of Protein Content

Protein contents of the grain, flour, wet gluten, dry gluten, and gluten extracts were determined in the course of this study.

The protein content of the flour and dry gluten (freeze-dried or oven-dried) were determined according to the macro-Kjeldahl procedure. The protein contents of the acetic acid extracts of gluten were determined using the micro-Kjeldahl procedure.

It was not possible to determine the protein content of the AUC (0.1M acetic acid, 3M urea, 0.01M cethyltrimethyl ammonium bromide) extracts by the Kjeldahl method. High concentration of nitrogen in the form of urea made the Kjeldahl procedure unsuitable for protein content determination. Consequently, the biuret procedure using the modified biuret reagent as suggested by Noll *et al.* (1974) was used for protein determination of the AUC extracts.

A calibration curve for protein determination of the AUC extracts, in a spectrophotometer was constructed by determining absorbance of increasing amounts of freeze-dried gluten at 550 nm. An aliquot of the AUC extract was withdrawn, usually 0.3 ml, and 10.0 ml of the biuret reagent added. Subsequently, steps in the procedure were the same as those outlined by Noll *et al.* (1974). The amount of protein was read from the calibration curve and the total protein in the sample computed.

C. The Gluten Washing Procedure

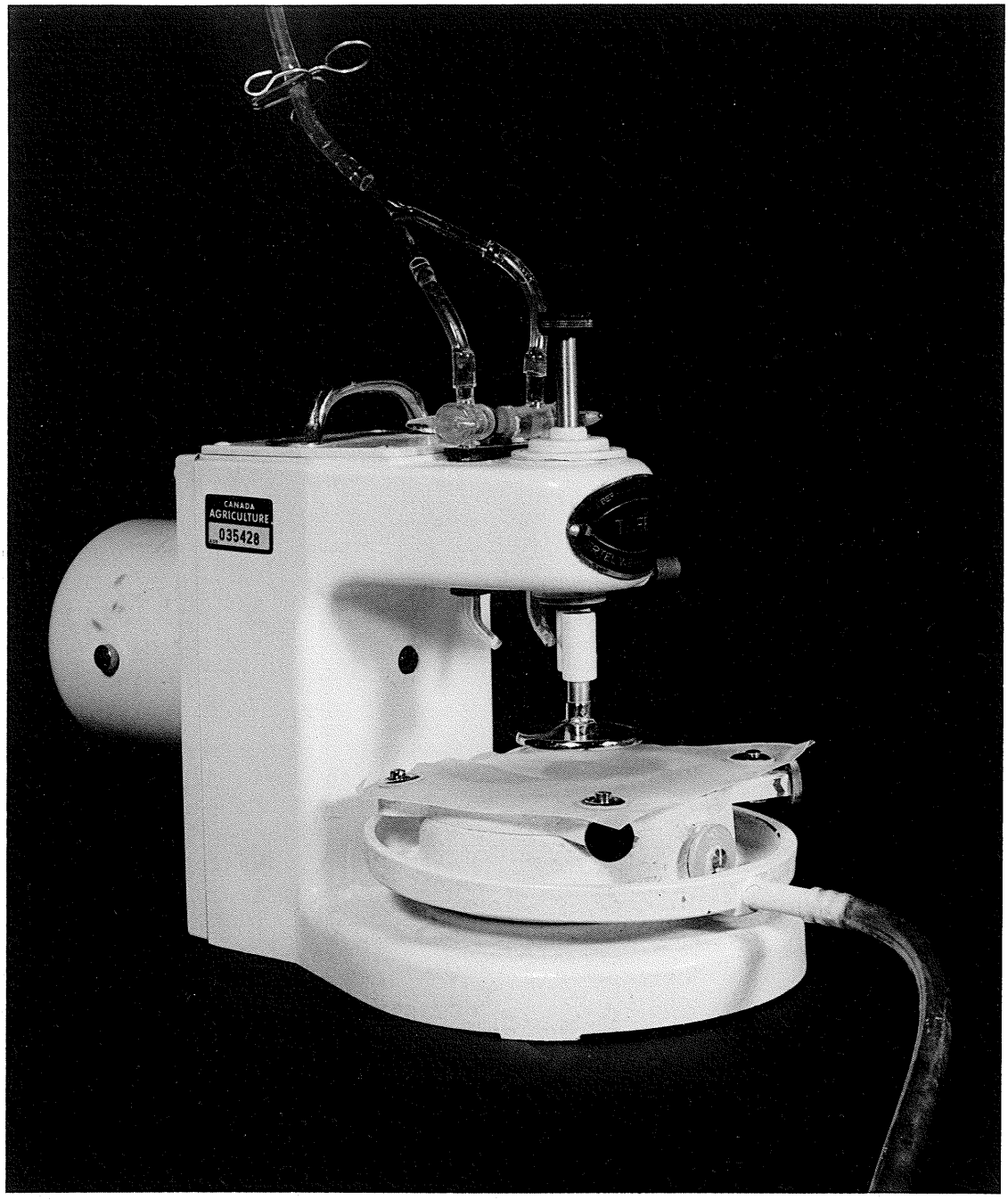
Ten grams of flour on "as is" basis was weighed into a suitable container. A minimal amount of distilled water was added to the flour and kneaded into a dough. Gluten was recovered from the dough by washing with a dilute sodium chloride solution buffered to pH 6.8 with phosphate and using the Theby washer (Fig. 1) according to method 38-11 of the American Association of Cereal Chemists (A.A.C.C., 1969).

Briefly, the dough was placed on the silk cloth screen and a regulated constant drip of the buffered salt-solution started. The gluten washer was started and the starch was washed out of the gluten by the gentle kneading action of the gluten washer pad. A plastic spatula was used to gather separated pieces of dough or gluten in order to form a single coherent mass. After twelve minutes, the gluten ball was removed and washed under cold tap water until no milkiness was visible in the wash water.

Excess water was removed from the gluten by rolling the gluten between the hands until a particular consistency had been reached. When the gluten piece felt "tacky", the gluten was moulded by folding the edges inwards until it formed a uniform ball. This was the wet gluten as referred to in subsequent parts of this thesis.

The wet gluten ball was weighed and then dried in an oven at $100^{o}C$ for twenty-four hours. The dry gluten was removed, placed in a desiccator to cool and then the weight of the cool dry gluten determined.

Figure 1. The Theby gluten washing machine.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D. Effect of Gluten on Mixograph Properties of Weak Flour

The glutens washed from flour samples were freeze-dried, ground using a pestle and mortar and stored in a cold room at 0-4^{o}C until required.

Two days prior to use, the gluten samples were removed and placed on a bench at room temperature. This allowed the samples to equilibrate with the ambient conditions of temperature and moisture. The Talbot flour used in the other parts of this thesis was used as the base flour (or control).

The mixograms of the base flour and all other composited flours were determined following the approved method 54-40 of the American Association of Cereal Chemists (A.A.C.C., 1969). Increasing amounts of gluten were used to replace the appropriate weight of flour and maintain the same weight. Three levels of such composited flour was prepared for each gluten sample. These levels were 0.5 g, 1.0 g and 2.0 g added gluten. The composited flour was thoroughly blended and a mixograph curve determined.

The effect of the various glutens and different levels of the glutens, on the mixogram characteristics of the control or base flour was determined. Dough development time, mixing tolerance index (M.T.I.) and maximum resistance to mixing were measured.

E. Amino Acid Analysis

Amino acid compositions of whole wheat grain, flour and gluten for all the samples were determined. The amino acid analyses were performed on the Beckman Model 121 Automatic amino acid analyzer.

An appropriate quantity of the protein sample (6 mg) was placed in culture tubes and 6N HCl added. Nitrogen was bubbled into the solution in order to remove all oxygen from the solution. The sample tubes were tightly closed and hydrolysis of the sample was done at 105-110^{o}C for 24 hours. After removal from the oven, the cool samples were dried under vacuum in a desiccator over sodium hydroxide. The samples were made to suitable volume and insoluble material removed by centrifugation. The amino acid analysis was carried out according to the standard procedure of Spackman *et al* (1958).

F. The Gluten Stretching Test

A two-gram portion of the wet gluten was cut off. It was moulded with the fingers by folding the edges inwards until a uniform round ball was formed. The gluten ball was rested for twenty minutes in a beaker containing distilled water maintained at 30^{o}C in a water bath.

After twenty minutes, the gluten ball was subjected to stretch at constant load in a similar method to that of Kaminski and Halton (1964). A wire hook attached to a rubber stopper was threaded through the centre of the gluten ball. A weighted wire hook was threaded contiguous with the first one through the gluten ball. The weighted hook plus its load weighed 9.9 g. The whole assembly, except the stopper, was carefully lowered into a one liter measuring cylinder filled with distilled water. The measuring cylinder remained immersed in a water bath maintained at 30^{o}C throughout the experiment. The level of the bottom of the weighted hook, relative to a scale attached to the outside of the measuring cylinder, was noted at the beginning and after time intervals.

G. Extraction of Gluten Proteins with 0.05N Acetic Acid

A one gram portion of the freshly washed wet gluten was cut and placed in a glass centrifuge tube. Five ml of 0.05N acetic acid were added into the gluten-containing vessel. The gluten proteins were extracted using the Potter and Elvejhem tissue grinder for ten minutes at 0-4^{o}C (ice slush). The suspension was centrifuged at 20,000 g, in a Sorval centrifuge maintained at 0-4^{o}C, for ten min. The supernatant was placed in a measuring cylinder. The precipitate was re-extracted twice, each time with a further five ml of the 0.05N acetic acid. All the supernatants were combined and placed in a 25 ml volumetric flask and made up to volume using the 0.05N acetic acid.

The precipitate or residue, after the third consecutive extraction, was uniformly dispersed in 0.1M sodium hydroxide and made up to a convenient volume with the same solution.

The protein contents of the wet gluten, acetic acid dispersed gluten (supernatant), and the residue were determined. Four complete extractions were performed on gluten from each flour sample.

H. Viscosity Determination of Gluten Proteins

The gluten proteins were dissolved in the highly dissociating AUC (AUC = 0.1M acetic acid, 3M urea, 0.01M cethyltrimethyl ammonium bromide) solvent of Meredith and Wren (1966). The viscosity of this protein solution was determined in an Ostwald type viscometer.

Preliminary experiments on the length of time of extraction, showed that fifteen minutes were adequate for the extraction of almost all of the gluten proteins for all the varieties.

A two gram portion of the freshly washed wet gluten was cut off and placed in a homogenizing vessel. Ten ml of the AUC solvent was placed in the vessel containing the gluten. The gluten proteins were extracted using the Potter and Elvejhem homogenizer set at the speed of five on a scale of 0 - 10. The homogenate was centrifuged at 27,000 g for twenty minutes, at room temperature, 18-20^{o}C. The supernatant was filtered through glass wool into a graduated cylinder. The filtration removed a small scum (probably lipid) floating on the surface of the supernatant. The total volume of the filtrate was recorded. The filtrate was a clear viscous liquid.

The temperature of the sample was brought to 25^{o}C by incubating in a water bath. Three ml of the solution were pipetted into the wide arm of the viscometer which was secured by a clamp in an upright position inside a water bath maintained at 25^{o}C. After a temperature equilibration of about one minute, and using a rubber bulb, the solution was sucked up the narrow viscometer arm past the top marking A on that arm. The rubber bulb was removed allowing the solution to freely flow down the viscometer by force of gravity. When the meniscus of the solution reached the top marking A on the small arm of the viscometer a stop watch was started. The stop watch was stopped when the meniscus of the solution reached the lower marking B on the narrow arm of the viscometer. The time difference in seconds taken by the solution to flow from the top marking to the lower marking was recorded. The determination of this time difference for each sample was repeated thrice and the average was recorded as the time of flow in seconds. The times of flow of the reference AUC solvent solution was similarly determined.

Three separate gluten extracts per flour sample were made and their times of flow determined. The average of these determinations was used to calculate the Relative Viscosity Number. Derivation of the Relative Viscosity Number:

$$\text{Relative Viscosity Number} = \frac{ts}{tr}$$

where ts = time in seconds for the sample solution to flow between the markings A and B, and

tr = time in seconds for the reference AUC solution to flow between the markings A and B.

The relative viscosity of the 0.05N acetic acid soluble gluten, extracted under the same conditions as used for AUC was determined for two varieties only (Glenlea, very strong and Talbot, weak).

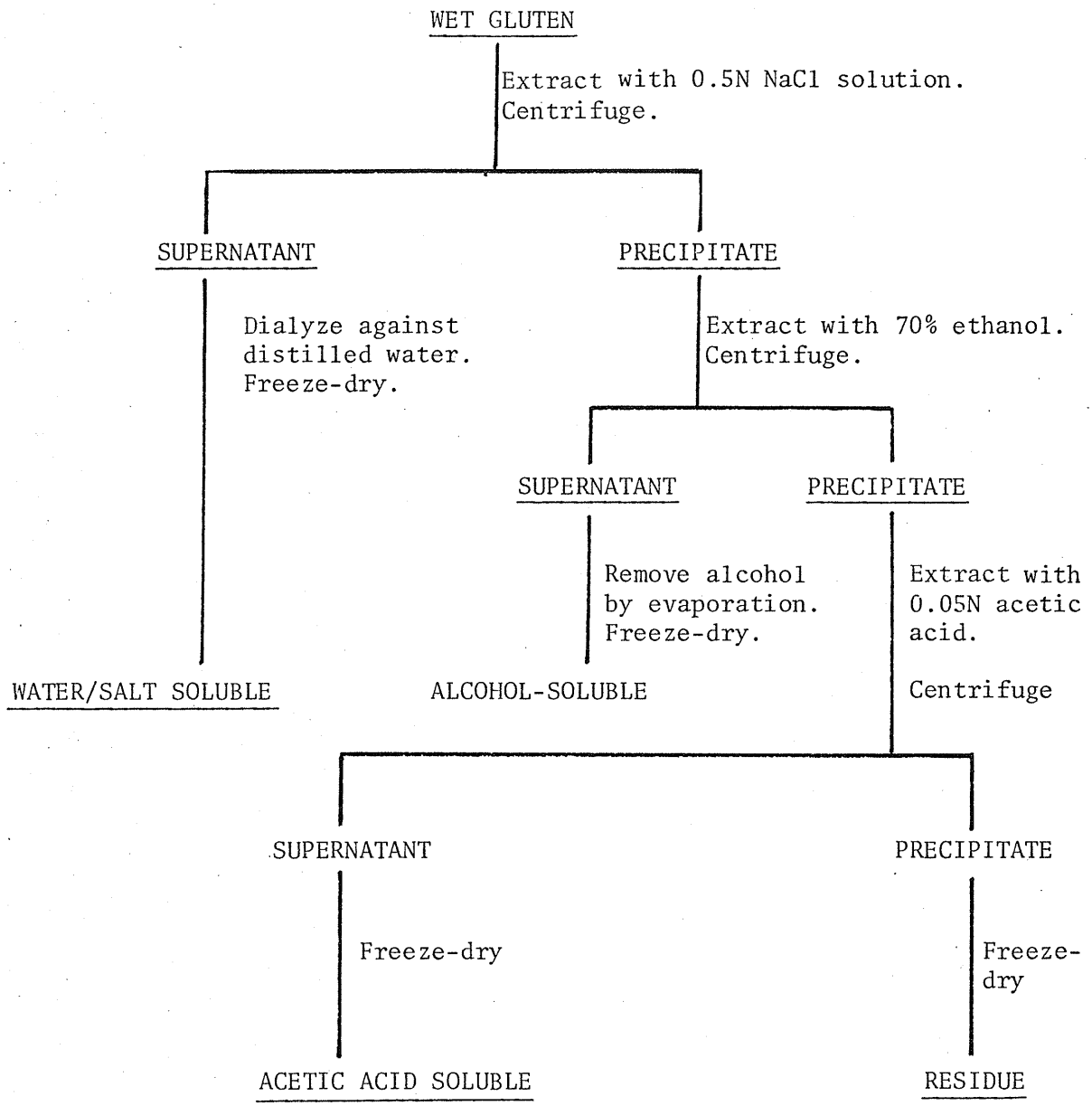
I. Protein Fractionation Procedure

The classical procedure of Osborne (1907) as modified by Chen and Bushuk (1970) developed primarily for fractionating flour proteins was used to fractionate the proteins of wet gluten. The water and salt-soluble flour proteins were expected to have been removed during the gluten preparation. Nevertheless, the procedure was adhered to from the start. This procedure is presented schematically in Fig. 2.

J. Disc Electrophoresis

Disc electrophoresis was performed on the 70% ethanol soluble, acetic acid soluble and dilute salt-soluble protein fractions of gluten, of all the samples. Methyl green was used as the visual tracking dye

Figure 2. Summary of protein fractionation procedure.



during electrophoresis. Disc electrophoresis was performed according to the following steps:

1. Sample Preparation

A protein sample was dissolved in 4M dimethylformamide in 0.1M acetic acid at a concentration of 10 mg protein per ml. The mixture was agitated in a Vortex shaker until all the solids had dissolved. The protein solution was clarified by centrifugation on a bench centrifuge at high speed for ten minutes. To the protein solution was added sucrose so that it formed 10% (w/v). This aided in the layering of the proteins on the surface of the gel.

2. Gel and Electrode Buffer Solutions

The reagents (and the correct proportions of the solutions) necessary for the preparation of disc gels and the electrode buffers are shown in Tables 2 and 3, respectively.

3. Preparation of Gels

The acrylamide/bisacrylamide and the potassium hydroxide/glacial acetic acid/Temed, solutions were prepared and stored in brown bottles. This minimized any deterioration through photochemical reactions. However, riboflavin and ammonium persulfate solutions were prepared fresh each time as required.

The lower gel solutions were mixed in the correct proportions as indicated in Table 2. Sufficient quantities to prepare eight or twelve discs were measured out. The gel solution was placed in a buchner flask and degassed using a water pump. Clean oven-dried small glass tubes were sealed at one end with parafilm paper. The air-free gel solution

TABLE 2. Solutions for the Preparation of Disc-gels

Reagent	Upper gel conc./100 ml	Parts in gel	Lower gel conc./100 ml	Parts in gel
Acrylamide	10.0 g	1	30.0 g	1
N,N,methylene-bisacrylamide	0.8 g		0.8 g	
Ammonium persulfate	60 mg	2	120 mg	1
Riboflavin	1 mg		2 mg	
1N Potassium hydroxide	48 ml	1	24.0 ml	2
Glacial acetic acid	3.6 ml		53.2 ml	
TEMED*	0.2 ml		0.4 ml	

*TEMED = N,N,N',N'-Tetramethylethylenediamine.

TABLE 3. Reagents for the Preparation of Electrode buffers for Disc gel Electrophoresis

Reagent	Concentration per liter	Remarks
Upper Electrode Buffer		pH 4.0
Glycine	28.1 g	Dilute by 10
Glacial acetic acid	3.05 ml	before use.
Lower Electrode Buffer		pH 4.3
Glacial acetic acid	43.0 ml	Dilute by 10
1N Potassium hydroxide	120.0 ml	before use.

was carefully decanted into the glass tubes to a predetermined height. Distilled water was carefully introduced on to the top of the gel solution. This layer of water excluded any air and ensured a flat gel surface. The rack containing the tubes was placed between two fluorescent lamps. Complete polymerization of the gels took forty-five minutes. The layer of water was removed using a strip of blotting paper.

The upper gel was prepared according to Table 2. The degassed upper gel was placed on the surface of the lower gel. After layering with distilled water it was polymerized for thirty minutes. The layer of water was removed.

The seal of parafilm paper was removed and the tube placed in the upper compartment of the electrophoresis apparatus. The lower and upper running buffers were placed in their respective compartments. Air bubbles were removed from the gel surfaces before electrophoresis. The apparatus was cooled by tap water throughout.

4. Sample Application and Electrophoresis

Twenty micro-liters (20 µl) of protein solution was carefully applied on to the surface of the upper gel using a micropipette. Electrophoresis was started. A power of four (4) mA per tube was maintained throughout the process. Electrophoresis was stopped after three hours.

5. Gel-retrieval and Processing

The gels were removed from the glass tubes using a plastic syringe filled with distilled water. The needle was carefully inserted between the gel and glass tube. The gel was released from the glass surface by simultaneously rotating the glass tube and introducing the water into

the interphase. The gel was cut at the dye front and placed in a test tube. The tubes were filled with a fixing/staining solution containing 0.025% Coomassie Brilliant Blue R in 10% trichloroacetic acid. The tubes were sealed and placed in a rotary shaker for 2-3 hr. The gels were destained using a solution containing glacial acetic acid:methanol: water at 1:2.5:9 ratio, until the bands were clearly visible. The gels were photographed for record.

K. Sodium Dodecyl Sulface Polyacrylamide Gel Electrophoressis (SDS PAGE) of Gluten, Gliadin and Glutenin

1. Sample Preparation

The protein content of the freshly washed gluten was determined by the micro-Kjeldahl procedure. A small portion of the gluten (0.1 g) was cut off and placed in a test tube. The amount of protein solvent was added so that the protein concentration was 10 mg per ml. The test tube was sealed. The mixture was incubated at $40^{o}C$ in a water bath overnight (about 16 hr). The suspension was agitated on a Vortex mixer until all solids had dissolved. The solution was centrifuged on a bench centrifuge at maximum speed for twenty minutes. The clear supernatant was decanted into another clean oven-dried glass tube. Only a thin layer of sediment remained after centrifugation.

Two different samples were prepared from this protein solution: unreduced and reduced gluten protein. The unreduced sample was simply obtained by storing a portion of the protein solution in a separate vial. This sample was used for sodium dodecyl sulfate polyacrylamide gel electrophoresis directly without further treatment. The reduced sample

was obtained by the addition of 2-mercaptoethanol at 1% (v/v) to another portion of the protein solution. The reduced samples of glutenin and gliadin were prepared in the same way. Sucrose was added to each sample (reduced and unreduced) to achieve 10% sucrose (w/v) in the final solution. Bromophenol blue was used as the tracking dye during electrophoresis.

2. The Molecular Weight Markers

The molecular weight markers used for the estimation of the molecular weight of the gluten protein subunits are shown in Table 4. These marker proteins were complexed with sodium dodecyl sulfate by dissolution at 2 mg per ml in the protein solvent solution. Reduced and unreduced molecular weight marker samples were prepared. Both these two sets of standard proteins were used to estimate the molecular weights of the gluten protein subunits.

3. Solutions for Electrophoresis

The protein solvent was prepared by making a 2% w/v SDS solution in the stock buffer.

The stock buffer (0.125M Tris-borate, pH 8.9) was prepared by weighing 15.1 g Tris and 7.7 g boric acid into a one liter volumetric flask and making it to the mark with distilled water.

The electrode buffer, pH 8.9, was prepared by making the stock buffer 1% (w/v) with sodium dodecyl sulfate.

TABLE 4. List of SDS-PAGE Molecular Weight Markers

Protein	Molecular weight daltons	Source
Cytochrome C (Equine heart)	12,400	Calbiochem
Myoglobin (Horse skeletal muscle)	17,000	Calbiochem
Pepsin	35,000	Sigma
Albumin (Bovine)	65,400	Calbiochem
γ Globulin (Human)	153,100	Calbiochem

4. Preparation of Gels

Five percent acrylamide gels were prepared by dissolving 10.5 g acrylamide, 0.273 g bisacrylamide cross-linking agent, 0.21 g sodium dodecyl sulfate, 75 mg sodium sulfite in a portion of the electrode buffer. Dimethyl propionitrile (0.525 ml) was added to this solution and all was throughly mixed. The solution was made up to 207 ml with electrode buffer in a graduated measuring cylinder. The solution was dearated thoroughly and 4.2 ml of 2% ammonium persulfate was added to act as the catalyst for polymerization. The whole gel solution was mixed quickly to achieve uniformity. The gel was polymerized on an EC vertical gel electrophoresis unit using an eight slot thick gel mold.

After polymerization the excess gel, external to the slot former was cut away. The electrophoresis unit was placed in the vertical

position. The electrode buffer was placed in both the lower and upper chambers of the apparatus. The slot former was removed and the whole unit cooled by circulating tap water.

5. Electrophoresis

The gel was pre-run for 30 min at 100V (50 mA). 50µl of the sample was applied into the wells cast by the slot former. Electrophoresis was started and run at 100V (50 mA). When the bromophenol blue tracking dye reached a predetermined distance (7-8 cm) the electrophoresis was stopped. This took about three hours.

6. Gel-removal and Processing

The gel was removed and cut at the tracking dye front. The distance between the origin and the dye front was measured and the gel placed in 12% trichloroacetic acid solution for 30-45 min or until the dye turned yellow. The gel was removed and rinsed with distilled water for one min. The proteins were stained with Coomassie Brilliant Blue according to the method of Koenig *et al.* (1970). The gels were destained until the protein bands were clearly visible then photographed.

This procedure was applied to molecular weight markers, gluten proteins, reduced gliadins and reduced glutenins. The molecular weights of the gluten protein components were determined by comparison with the mobility of the standard proteins.

IV. RESULTS AND DISCUSSION

A. Technological Properties of Grain and Flour Samples

1. Grain Samples

The technological properties of the grain samples used in this study are shown in Table 5. The moisture content of the grain was between 12.8% and 13.4%.

The protein content of Manitou samples varied from 10.9% to 14.1%. Glenlea grains had protein content between 13.3% and 15.4%. Norquay protein was 12.9% while the Talbot grain had protein content of 11.6%.

The other properties indicated that these grains were normal well-filled kernels with adequate flour yield properties.

TABLE 5. Technological Properties of the Grain Samples of Four Wheat Varieties

	Manitou			Glenlea			Norquay	Talbot
% Protein (N x 5.7) (14% m.b.)	10.9	12.4	14.1	13.3	14.7	15.4	12.9	11.6
* Test weight kg/hl	83.8	83.8	83.9	78.2	77.7	76.8	78.6	83.3
* 1000 Kernel weight (g)	31.4	34.2	36.1	39.5	39.4	38.8	36.5	37.3
% Flour yield (14% m.b.)	70.7	70.8	72.6	73.2	71.6	71.4	71.3	72.9

* On "as is" moisture basis.

2. Flour Samples

The technological properties of the flour samples used in this study, and derived from the grain samples previously described, are depicted in Table 6.

The wet gluten, dry gluten and sedimentation values showed progressive increase with flour protein within Manitou samples. A similar trend was observed with Glenlea samples. Sedimentation values for Glenlea samples were greater than those for Manitou samples. Talbot had the lowest sedimentation value and that for Norquay was comparable to those of Glenlea. Protein content of the flour is included in the figures of farinograms, mixograms and extensigrams beside the respective curves.

The farinograms of the flours are shown in Fig. 3. Figure 3 and Table 6 show that for the Manitou samples percent water absorption, dough development time and stability increased with flour protein. Only water absorption increased with flour protein within Glenlea samples. Development time and stability of Glenlea samples were identical. However, Glenlea has what is generally referred to by cereal technologists as long-mixing requirements. Norquay had mixing properties similar to Glenlea flours. Talbot had a short development time and stability.

The mixograms of the flours are depicted in Fig. 4. The mixogram curve peak and general resistance to mixing breakdown showed that Glenlea flours were stronger than all other flours. Talbot flour was the weakest. Norquay was stronger than Manitou flours.

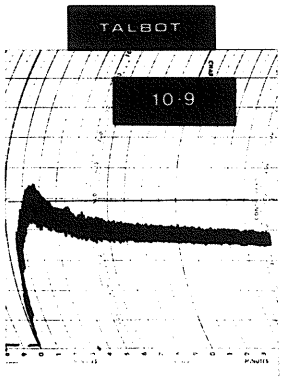
The effect of protein content on the Glenlea flour properties was shown by a greater maximum resistance at the peak for the high protein flour and a low one for the low protein flour. The mixogram of the high

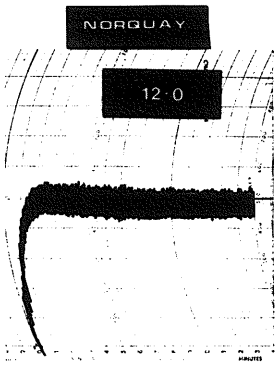
TABLE 6. Technological Properties of the Flour Samples of Four Wheat Varieties

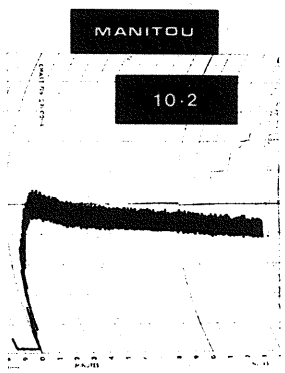
	Manitou			Glenlea			Norquay	Talbot
% Protein (N x 5.7) (14% m.b.)	10.2	11.6	13.4	12.6	13.9	14.6	12.0	10.9
% Wet gluten	27.0	31.7	40.1	32.3	35.5	37.8	33.0	36.9
% Dry gluten	9.1	11.3	14.0	12.4	13.7	14.3	11.8	11.4
% Ash (14% m.b.)	0.50	0.47	0.46	0.46	0.44	0.48	0.54	0.48
Sedimentation values	38	43	50	65	66	69	62	26
Farinograph								
Absorption, %	63.2	63.9	65.1	58.5	59.8	60.8	60.9	54.7
Development time (min)	2.0	2.5	5.5	3.0	3.0	3.0	4.0	1.8
Stability (min)	4.5	9.0	10.0	13.5	13.5	13.5	13.0	2.0
Extensigraph								
Length, cm	17.0	16.5	16.3	25.8	29.0	26.2	27.1	16.5
Max. height B.U.	485	570	570	805	825	900	685	75
Area, cm^2	122.0	142.2	133.2	294.0	331.2	323.8	264.7	23.8
Remix loaf Vol. cc	730	750	860	605	645	655	890	510

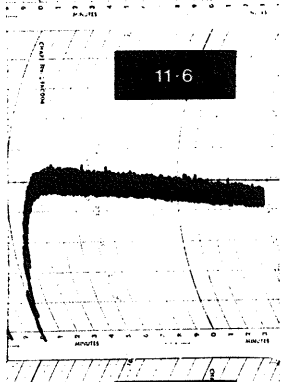
Figure 3. Farinograms for flour samples of four wheat varieties.

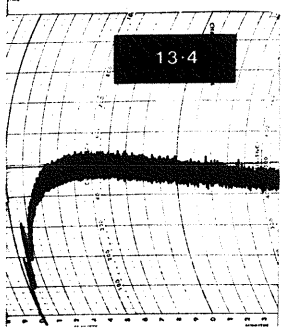
FARINOGRAMS

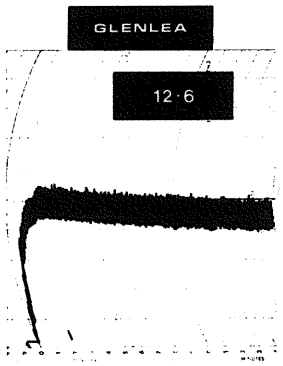


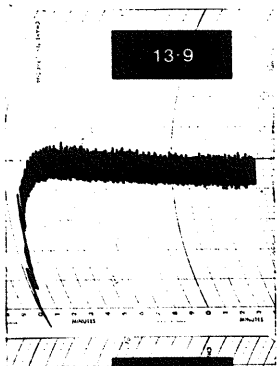












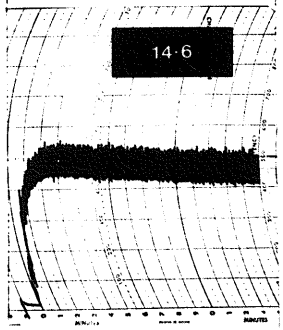
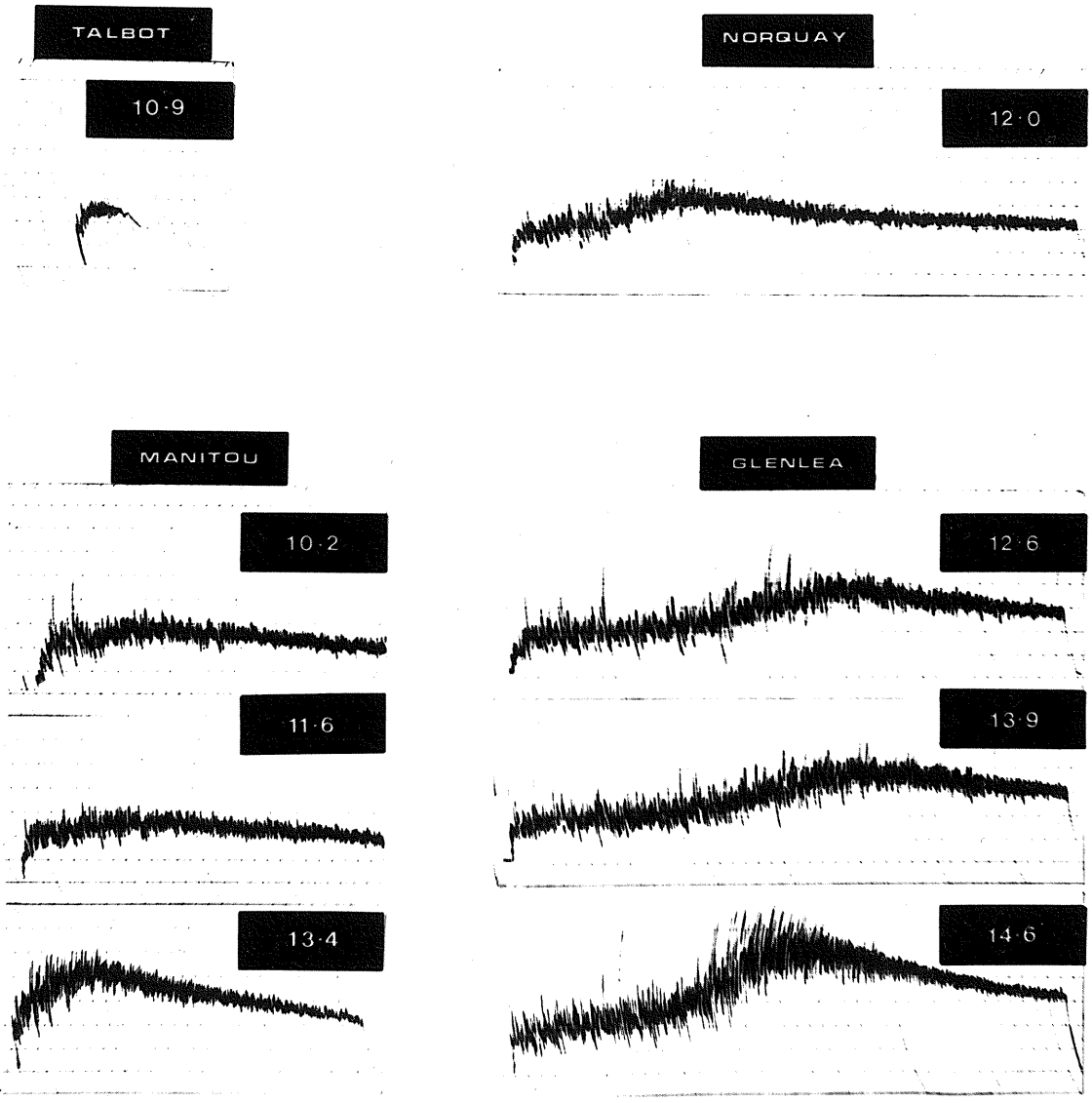


Figure 4. Mixograms for flour samples of four wheat varieties.

MIXOGRAMS


TALBOT
10·9
NORQUAY
12·0
MANITOU
10·2
11·6
13·4
GLENLEA
12·6
13·9
14·6

protein Manitou showed stronger features than that for the low protein flours.

The extensigrams of the flours are shown in Fig. 5. The extensibility of the doughs of all varieties showed some small differences. However, maximum resistance (measure of elasticity) and area under the curve (general strength) showed that Glenlea flours were superior to all other varieties examined. Other varieties showed the following order of decreasing strength: Norquay, Manitou and Talbot.

The Loaf Volume and other baking results are shown in Table 6 and Fig. 6. Norquay flour gave the largest loaf. This was followed in order of decreasing loaf-volume potential by: Manitou, Glenlea and Talbot flours. The effect of flour protein within Manitou and Glenlea samples was to improve breadmaking potential.

The physical dough tests and breadmaking potential revealed that Glenlea had long-mixing requirements (farinograph and mixograph) and greater general strength (extensigraph). However, due to inadequate dough development in mixing during baking the loaf volumes were low. Manitou had medium-strong mixing properties and good breadmaking potential. Talbot was weak, with poor breadmaking potential.

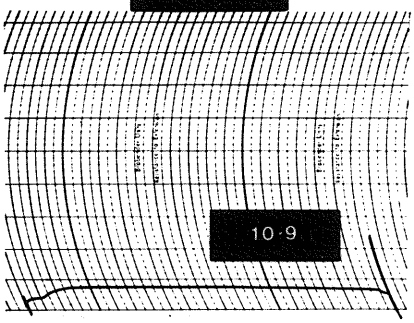
In spite of long-mixing requirements in the farinograph, Norquay gave a large loaf of bread. These varieties could therefore be classified in the following way: Glenlea (very strong), Norquay (strong), Manitou (medium strong) and Talbot (weak).

It was therefore instructive to investigate further, the properties of the glutens of these varieties in order to identify the basis for the differences in mixing behaviour and breadmaking potential. Using

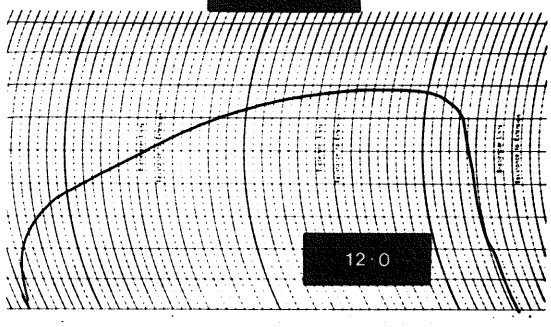
Figure 5. Extensigrams for flour samples of four wheat varieties.

EXTENSIGRAMS

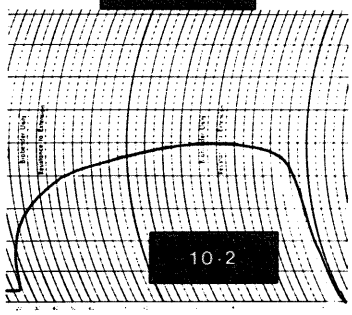
TALBOT

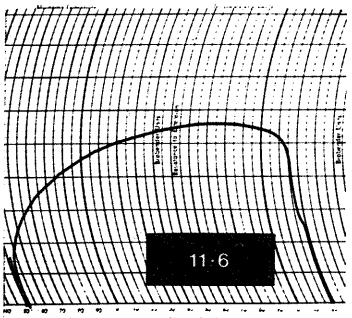


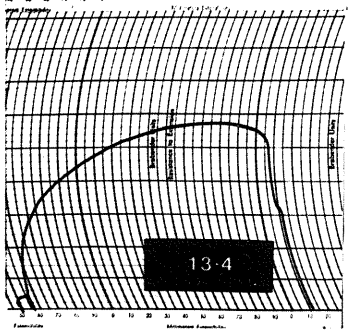
NORQUAY



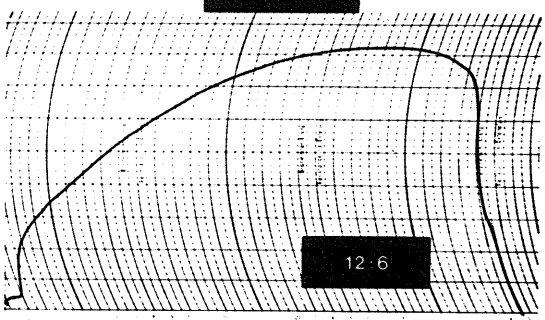
MANITOU

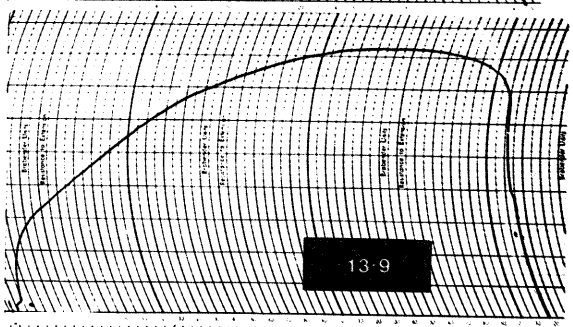






GLENLEA





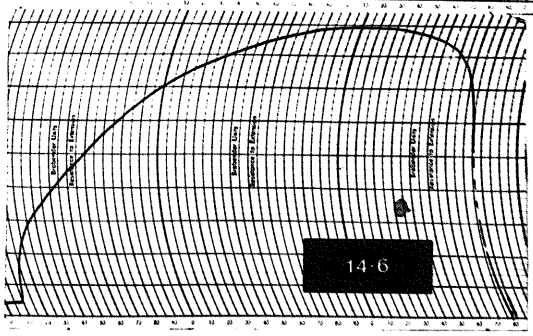
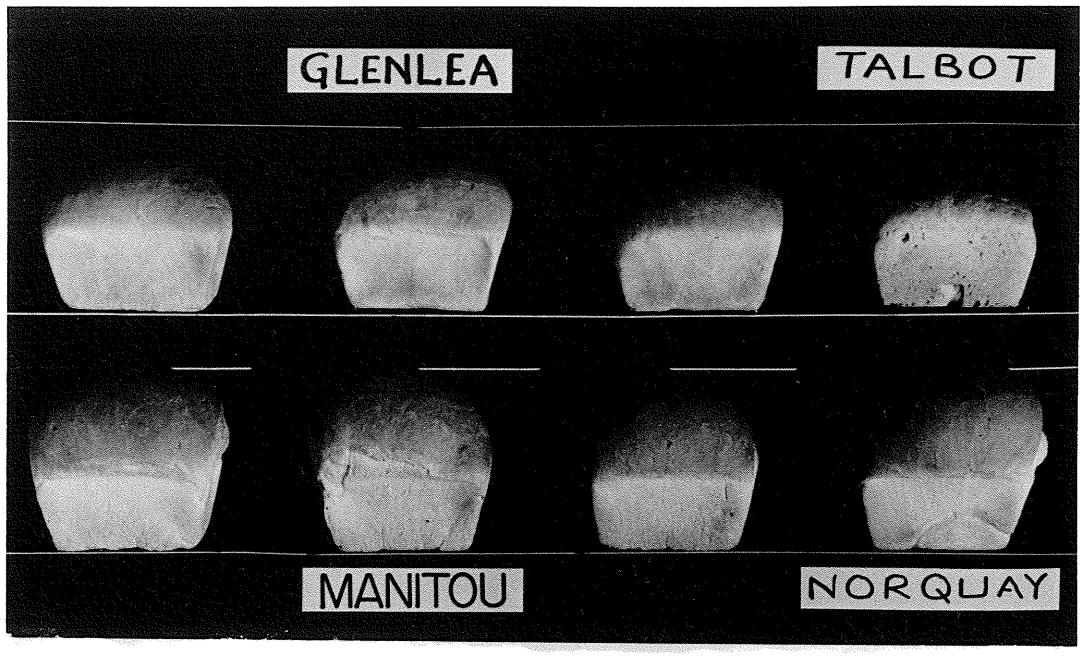
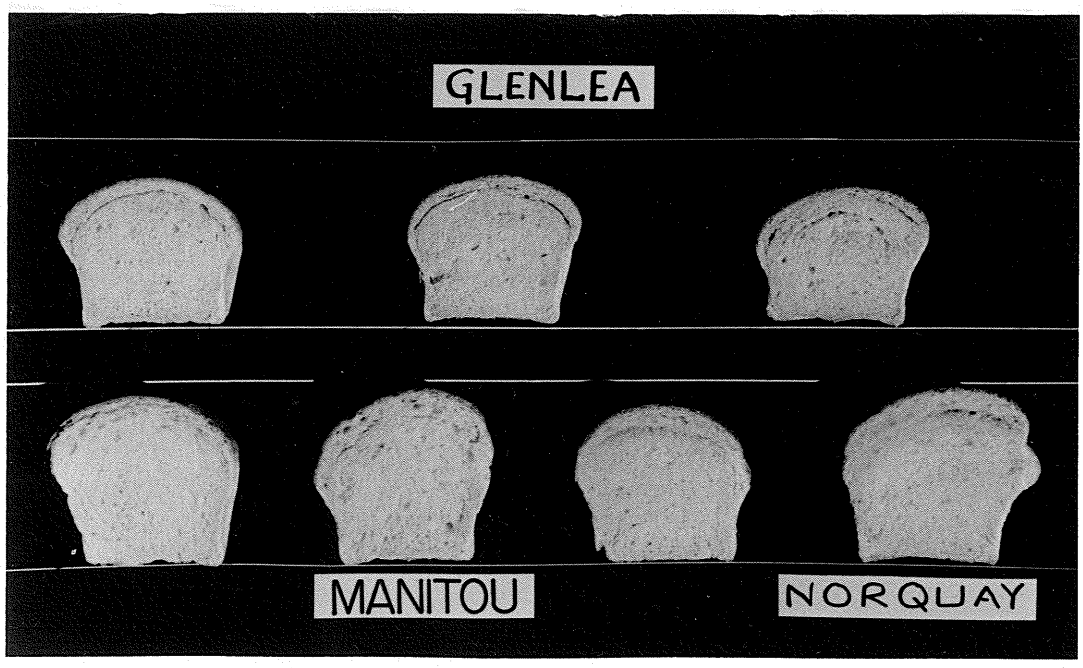


Figure 6. Bread from flour samples of four wheat varieties. Glenlea and Manitou loaves arranged from left to right in order of decreasing flour protein.

Top: External features.

Bottom: Internal features.


GLENLEA
TALBOT
MANITOU
NORQUAY


GLENLEA
MANITOU
NORQUAY

gluten as the basic material for investigation eliminated most of the effect of differences in protein content.

Since gluten has been shown to control flour properties (Finney, 1943) it was necessary to determine the amount of gluten obtained from the same weight of flour of the four varieties.

B. Gluten Content of the Flour of Four Wheat Varieties

Table 7 shows the weight of the wet and dry gluten and the protein content of the dry gluten for all the flours studied.

TABLE 7. Weight of Wet Gluten and Dry Gluten Washed from 10 g Flour and Protein Content of Dry Gluten for Four Wheat Varieties

	Manitou			Glenlea			Norquay	Talbot
Flour protein (N x 5.7) (14% m.b.)	10.2	11.6	13.4	12.6	13.9	14.6	12.0	10.9
Wet gluten, g	2.70	3.17	4.01	3.23	3.55	3.78	3.30	3.69
Dry gluten, g	0.97	1.13	1.40	1.24	1.37	1.43	1.18	1.14
Gluten protein (N x 5.7), % (0% m.b.)	83.5	84.8	83.4	87.9	87.0	87.6	83.9	79.9

The weight of the wet gluten obtained from a flour increased with increase in flour protein. Flours with high protein content yielded larger glutens than those with low protein. However, this generalization applied only in some cases. Talbot, which had a flour protein of 10.9%, gave a wet gluten of 3.69 g. Glenlea flour with a protein content of 14.6% yielded only 3.78 g wet gluten, while the Manitou flour with 13.4%

protein gave 4.01 g wet gluten.

The weight of the dry gluten followed the same trend. The dry and wet gluten expressed a percentage of the flour weight are shown graphically in Figs. 7 and 8. Results in Fig. 7 show a greater rise in the amount of wet gluten with increasing flour protein content for the variety Manitou than in Glenlea. Although Glenlea flours had generally higher protein than Manitou flours, the former yielded proportionately lower amounts of gluten.

The same general trend was observed in Fig. 8, showing the results of percentage dry gluten of flours of four varieties. The amount of water present in the wet gluten was determined by differences in the weights of the wet and dry glutens. In all cases the water level was approximately two-thirds of the weight of the wet gluten.

The effect of the same weight of gluten on the mixograph properties of a weak flour was the next logical step in the characterization of these wheat varieties.

C. Effect of Glutens on Mixograph Properties of Weak Flour

Reconstitution studies have established gluten to be responsible for the recognized differences in breadmaking of wheat varieties (Finney, 1943). The effect of small quantities of freeze-dried gluten derived from varieties widely differing in mixing properties, on the mixograph properties of a weak flour, was therefore instructive.

Freeze-dried gluten from four different wheat varieties was added in increasing amounts to a weak control flour. The mixograph curves of these composited flours were determined. The effects of the added gluten

Figure 7. Proportion of wet gluten (% flour solids) washed from 10 g of flour for four wheat varieties.

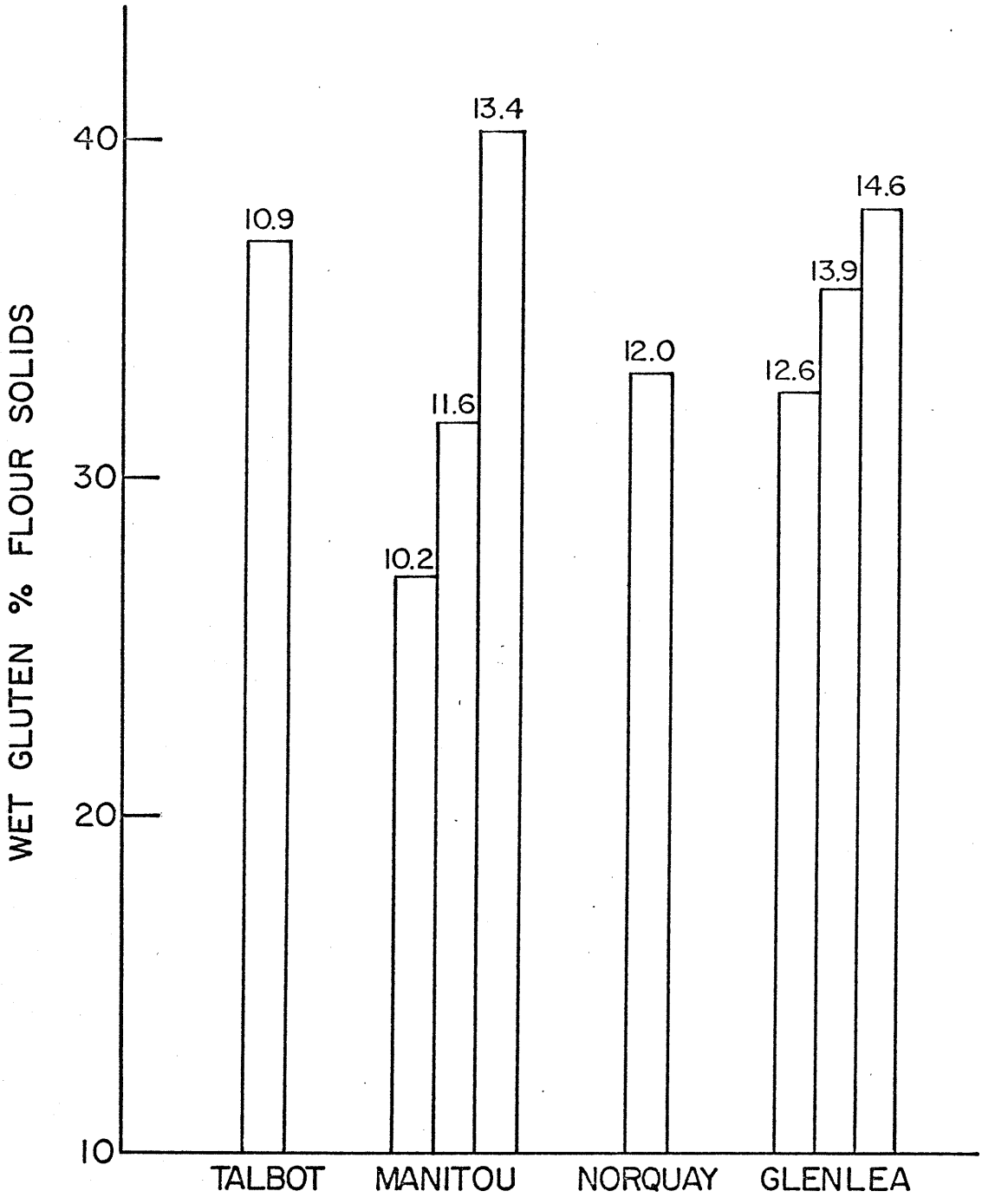
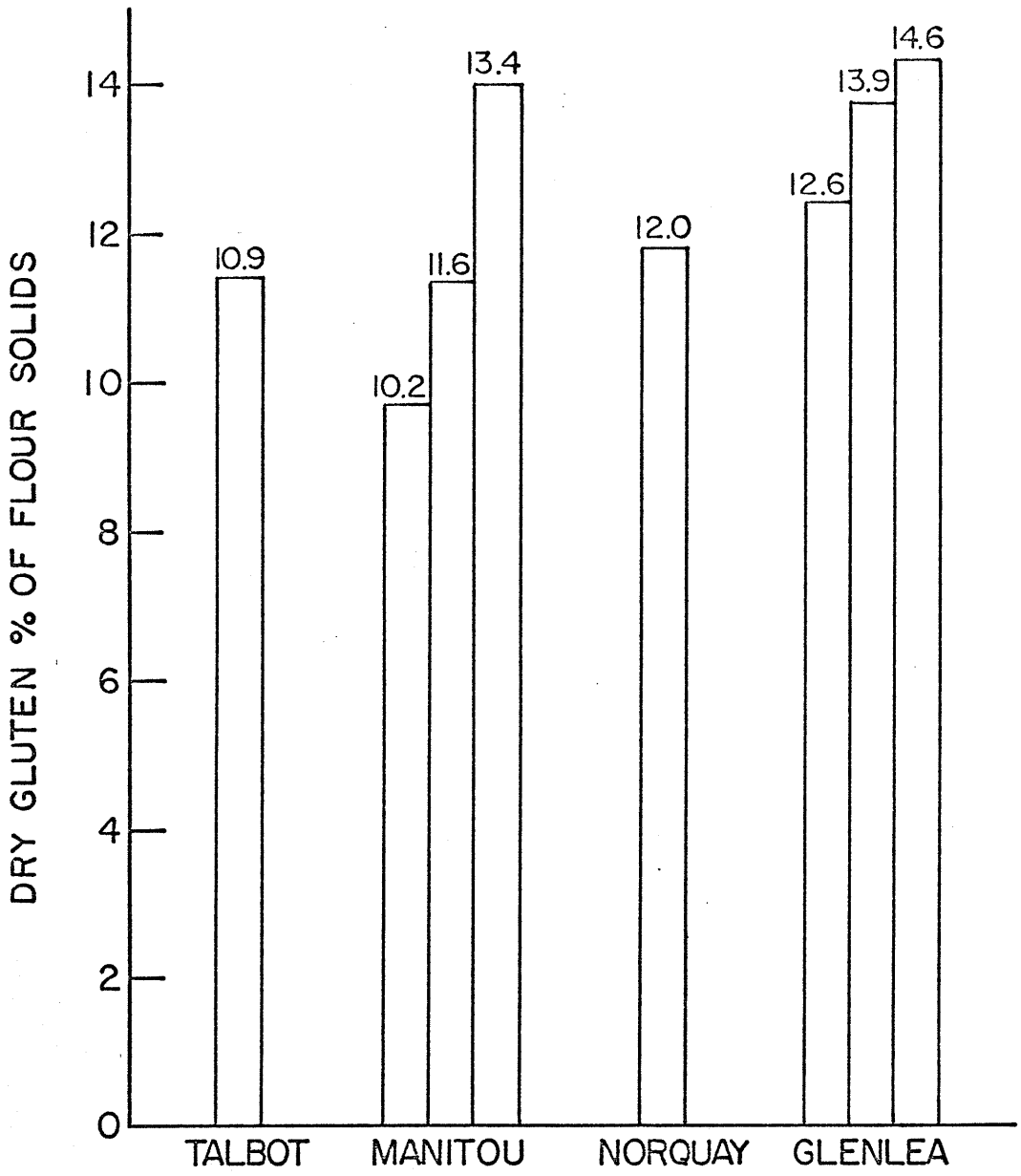

WET GLUTEN % FLOUR SOLIDS
40
30
20
10
10.9
10.2
11.6
13.4
12.0
12.6
13.9
14.6
TALBOT
MANITOU
NORQUAY
GLENLEA

Figure 8. Proportion of dry gluten (% flour solids) washed from 10 g of flour for four wheat varieties.


DRY GLUTEN % OF FLOUR SOLIDS
0
2
4
6
8
10
12
14
10.9
10.2
11.6
13.4
12.0
12.6
13.9
14.6
TALBOT
MANITOU
NORQUAY
GLENLEA

on dough development time, mixing tolerance and maximum resistance were observed.

Glutens from low, medium and high protein flour of the varieties Manitou and Glenlea were used as separate samples. Effects of the three Manitou glutens on mixograph properties of the control flour revealed no differences among them. Similar results were obtained within Glenlea glutens. Consequently, measurements of the effects of Manitou glutens were averaged and treated as a single sample in the graphical representation of mixing tolerance and maximum resistance. Glenlea glutens were treated in a similar manner. The effects of Talbot and Norquay glutens were also examined.

Although the effects of three levels of added gluten were determined, the discussion will be limited to the low and high level of addition only.

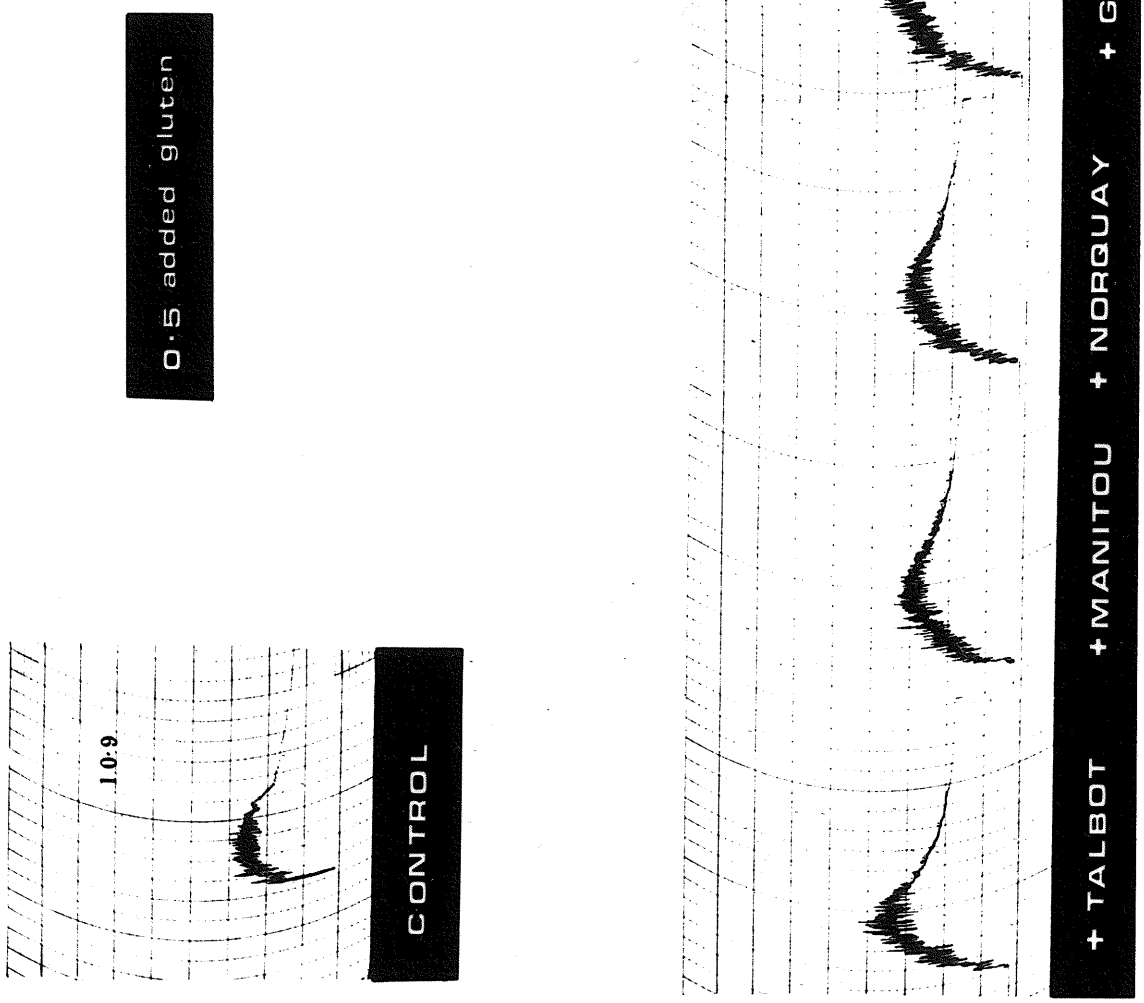
1. Effect of 0.5 g Added Gluten

Mixograms of the control flour containing 0.5 g freeze-dried gluten from four different varieties is shown in Fig. 9. The mixogram of the control flour is included for comparison.

Glenlea and Norquay gluten increased development time more than Manitou and Talbot glutens. Manitou gluten was slightly better than Talbot gluten. On the whole, development time increase was minimal.

Fig. 9 also shows that tolerance to mixing (M.T.I.) and maximum resistance in the mixogram were substantially increased by glutens from all four cultivars. Glenlea gluten imparted more tolerance to mixing than all other glutens. The other glutens increased this parameter in the following order of decreasing ability: Norquay, Manitou and Talbot. Glutens derived from all the four cultivars increased maximum consistency.

Figure 9. Effect of the addition of 0.5 g gluten from four different wheat varieties on the mixogram of a weak flour.


CONTROL
0.5 added gluten
10:9
+ TALBOT
+MANITOU
+ NORQUAY
+ GLENLEA

However, only small differences were observed between different varieties. But Glenlea and Norquay glutens increased maximum resistance slightly more than Manitou and Talbot glutens. Generally, the base flour (Talbot) mixogram properties were still predominant.

2. Effect of 2.0 g Added Gluten

Mixograms of control and control flour containing 2.0 g freeze-dried gluten from the four indicated varieties are shown in Fig. 10. Glutens derived from all the cultivars greatly improved the mixogram properties of the control flour.

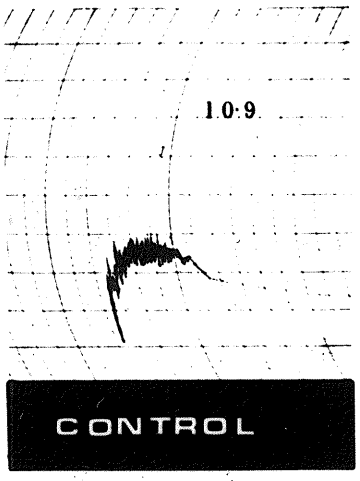
Dough development time and tolerance to mixing breakdown were increased by the glutens of the four varieties in the following order of decreasing ability: Glenlea, Norquay, Manitou and Talbot. Glenlea gluten increased these properties most while Talbot increased them least.

The highest maximum resistance to mixing was imparted by Talbot gluten. This was followed respectively by Glenlea, Norquay and Manitou. The relative position of Talbot gluten in the improvement of this parameter was a departure from all previous determinations of the other parameters.

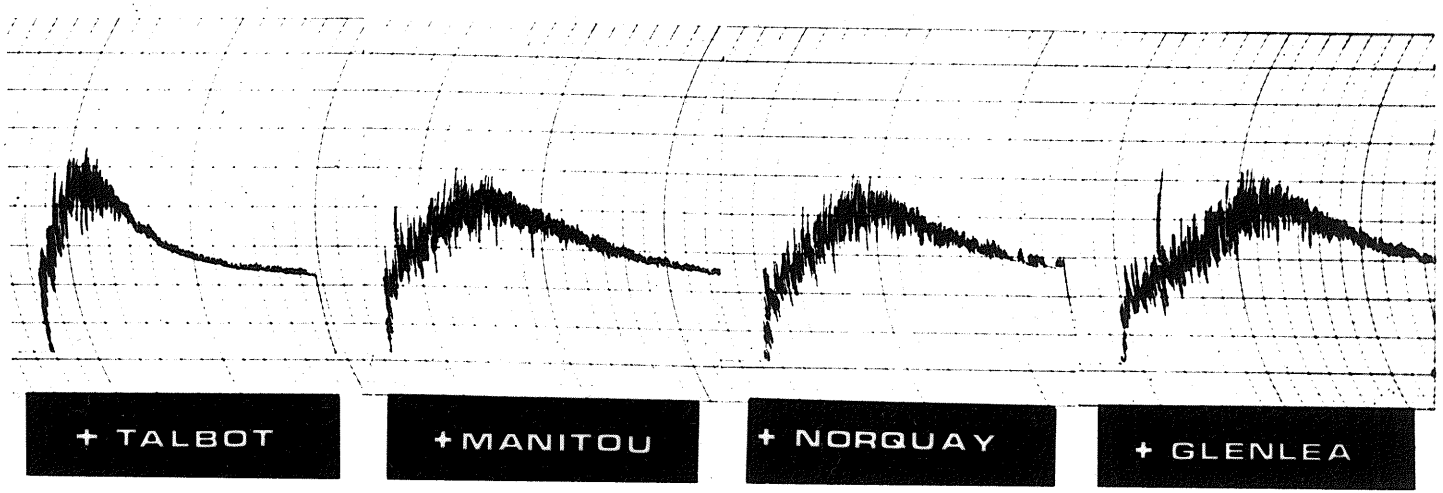
Generally, Glenlea gluten improved the overall mixogram properties of the base flour more than all other cultivars.

The rate of increase in the mixing tolerance index and maximum resistance of the Talbot flour with increasing amounts of added gluten was examined. Mixing tolerance index is the ability of a flour to withstand breakdown during mixing. This parameter was determined as the consistency of the curve (mixogram units) after two minutes of mixing.

Figure 10. Effect of the addition of 2.0 g gluten from four different wheat varieties on the mixogram of a weak flour.



2·0 added gluten



Maximum resistance (mixogram units) measures the point of the highest consistency of the mixogram.

3. Effect of Added Gluten on Mixing Tolerance Index

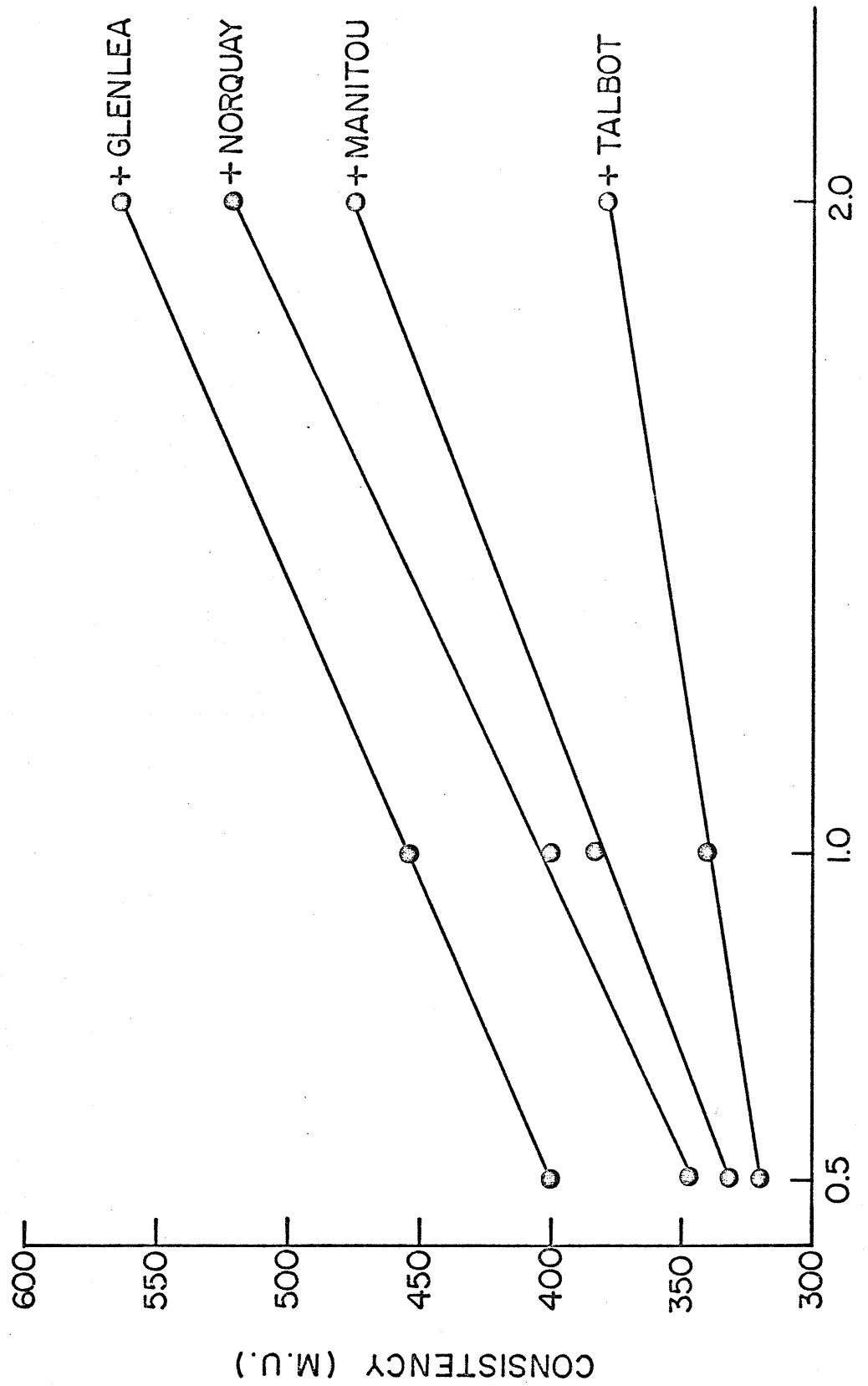
Fig. 11 shows the dough consistency increase with the added gluten content of the four flour-gluten composites.

The increase in consistency of the dough at each level of added gluten was greatest for Glenlea gluten. This was followed respectively by Norquay, Manitou and Talbot gluten. Differences in consistency of doughs containing different varieties was small at the lower level of added gluten. These differences became greater as more gluten was added to the control flour. The different rates of increase in mixing tolerance in doughs containing glutens from different varieties resulted in a fan-shaped family of curves. The curve for each variety had a different slope. Visual inspection indicated that, Glenlea and Norquay gluten yielded the highest slopes, followed by Manitou gluten. Talbot gluten-containing dough had the lowest slope.

The curves were vertically displaced to different positions. In the vertical displacement of consistency curves, the following order from top to bottom was established: Glenlea, Norquay, Manitou and Talbot.

Results in Fig. 11 indicated that the glutens from Glenlea, Norquay, Manitou and Talbot imparted different magnitudes of mixing tolerance to a weak flour. The addition of the same quantity of Glenlea gluten protein to the flour, yielded a dough with greater tolerance to mixing than those containing Manitou, Norquay or Talbot gluten. Talbot gluten had the smallest effect on mixing tolerance of the base flour. Norquay and Manitou glutens were intermediate between the aforementioned varieties.

Figure 11. The mixogram consistency (M.U.) measured two minutes from the onset of mixing, of doughs containing increasing amounts of glutens from four wheat varieties.


+ GLENLEA
+ NORQUAY
+ MANITOU
+ TALBOT
600
550
500
450
400
350
300
CONSISTENCY (M.U.)
0.5
1.0
2.0
WEIGHT OF ADDED GLUTEN (g)

The rate of increase of mixing tolerance with added gluten (slope) showed that Glenlea gluten imparted the highest strength per g protein. This was followed respectively by Norquay, Manitou and Talbot glutens. These slopes may be taken to indicate quality differences between these glutens. In this respect Glenlea gluten was stronger than Norquay, Manitou or Talbot gluten in that decreasing order of strength.

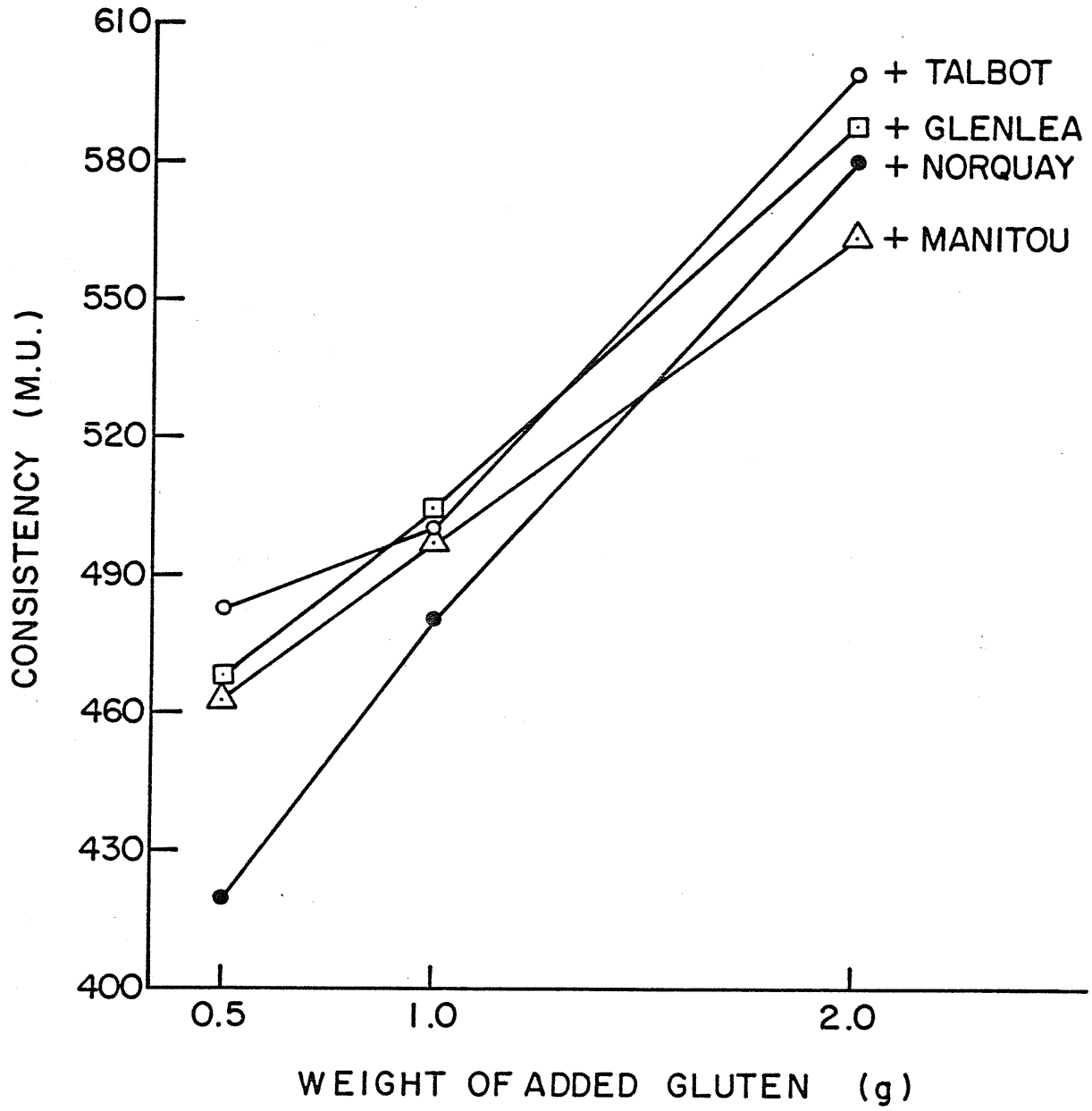
4. Effect of Added Gluten on Maximum Resistance

Fig. 12 compares the maximum resistance (mixograph units) of doughs containing gluten from four wheat varieties at different levels of added gluten. The different levels were 0.5 g, 1.0 g, 2.0 g added gluten.

At the low level of added gluten Talbot increased maximum resistance most, while Norquay increased this property least. Glenlea and Manitou gluten were intermediate in their effects. In the medium level of added gluten, there were virtually no differences between the effects of Talbot, Glenlea and Manitou gluten. Norquay gluten was still low in its improving effects.

At the highest level of added gluten, maximum resistance was increased most by Talbot gluten, followed in decreasing order by Glenlea, Norquay and Manitou. Furthermore, the slopes of these varietal curves were different. The following order of decreasing magnitude in slope was observed: Norquay, Glenlea and Manitou. The results for the effect of Talbot gluten were difficult to interpret. Talbot gluten appeared to be more superior to the "strong" wheat varieties in those properties measured by the maximum resistance in the mixograph. This was more apparent at the high level of added gluten.

Figure 12. Maximum resistance (M.U.) of doughs containing increasing amounts of glutens from four wheat varieties.


610
580
550
520
490
460
430
400
CONSISTENCY (M.U.)
+ TALBOT
+ GLENLEA
+ NORQUAY
+ MANITOU
0.5
1.0
2.0
WEIGHT OF ADDED GLUTEN (g)

The reconstitution studies showed that the glutens from low, medium and high protein Manitou samples gave identical effects on mixograph properties of the weak base flour. Comparable results were observed within Glenlea samples. Consequently, flour protein had no qualitative effect on the properties of the glutens derived from them.

Addition of small quantities of gluten proteins resulted in large changes in mixograph properties of the weak base flour. This indicated that gluten proteins were one of the most important components in the mixing behaviour of a flour dough.

Doughs containing glutens from different varieties resisted mechanical breakdown in the following order of decreasing ability: Glenlea, Norquay, Manitou and Talbot. Thus, the dough containing Glenlea gluten possessed greater resistance than that containing Talbot gluten.

Therefore, Glenlea gluten could be taken to be stronger than those of all other varieties examined and Talbot may be taken to be the weakest. Manitou and Norquay were intermediate in strength. Alternatively, mixing strength per unit protein was greatest for Glenlea gluten and least for Talbot gluten.

Mixing characteristics imparted by a gluten to the weak base flour were governed by variety. Strong wheats imparted more mixing strength; weak wheats imparted less mixing strength.

The findings in the present study are in agreement with those of Doguchi and Hlynka (1967) who reported the farinogram curve of a hard red spring wheat to be stronger than that of a soft winter wheat.

The twenty common amino acids are the building blocks of the flour proteins. The study of the amino acid distribution in the grain, flour

and gluten was considered relevant in the attempt to identify the differences in rheological properties of the four wheat varieties.

D. Amino Acid Analysis

The amino acid compositions of the grain, flour and glutens of the varieties Manitou, Glenlea, Norquay and Talbot, used in this thesis were determined. All amino acid values are expressed in grams of amino acid N per 100 gram of total Kjeldahl N.

1. Amino Acid Composition of Grain

The results of the amino acid composition of whole grain for three samples each of Manitou and Glenlea and also for Talbot and Norquay are presented in Table 8.

The most abundant amino acids in the grain were glutamic acid and proline. Furthermore, for Manitou and Glenlea, glutamic acid increased with increase in protein content. The Glenlea sample which had the highest protein content also had the highest amount of glutamic acid. And the Manitou grain sample with the lowest protein among all the samples, also contained the least amount of glutamic acid.

In general, the results of amino acid composition of the grains are consistent with findings by others. Hepburn and Bradley (1965) found the proportions of amino acids for pure varieties of spring and winter wheats at similar protein contents to be nearly constant.

2. Amino Acid Composition of Flour

The amino acid composition of the flour of the four wheat varieties: Manitou, Glenlea, Norquay and Talbot are shown in Table 9.

TABLE 8. Amino Acid Composition of Whole Grain of Four Wheat Varieties (g N/100 g Total Kjeldahl N)

	Manitou			Glenlea			Norquay	Talbot
Grain protein (Nx5.7)*	10.9	12.4	14.1	13.3	14.7	15.4	12.9	11.6
Amino acid								
Lysine	3.3	3.0	3.0	2.6	2.7	2.8	3.1	3.2
Histidine	3.4	3.4	3.5	3.1	3.2	3.7	3.5	3.5
Ammonia	17.1	17.8	18.4	18.1	18.5	20.7	17.5	17.3
Arginine	8.0	8.2	8.2	7.3	7.9	7.7	8.0	8.3
Aspartic acid	3.0	2.9	3.0	2.7	2.9	3.0	3.4	3.3
Threonine	2.0	1.9	1.9	1.8	1.9	2.1	2.1	2.0
Serine	3.3	3.4	3.4	3.4	3.6	3.8	3.6	3.5
Glutamic acid	17.8	18.9	19.3	19.3	20.2	21.9	18.7	18.3
Proline	7.5	8.0	8.2	7.6	7.7	8.5	7.7	7.9
Glycine	4.5	4.5	4.4	4.3	4.6	5.0	4.7	4.4
Alanine	3.1	2.9	3.0	2.8	3.0	3.2	3.2	3.3
Valine	2.9	2.9	2.9	2.7	2.9	3.2	3.1	3.1
Methionine	0.8	0.7	0.7	0.7	0.7	0.6	0.8	0.8
Isoleucine	2.1	2.1	2.1	2.0	2.1	2.4	2.2	2.2
Leucine	4.2	4.2	4.2	4.0	4.2	4.6	4.4	4.5
Tyrosine	1.2	1.3	1.2	1.3	1.4	1.2	1.0	1.3
Phenylalanine	2.3	2.5	2.5	2.3	2.3	2.4	2.4	2.4
% N Recovery	86.5	88.6	89.9	86.0	89.8	96.8	89.4	89.3

* 14% moisture basis.

TABLE 9. Amino Acid Composition of Flours of Four Wheat Varieties (g N/100 g Total Kjeldahl N)

	Manitou			Glenlea			Norquay	Talbot
Flour protein (Nx5.7)*	10.2	11.6	13.4	12.6	13.9	14.6	12.0	10.9
Amino acid								
Lysine	2.4	2.2	2.2	2.1	2.1	2.1	2.3	2.5
Histidine	3.1	3.0	3.2	3.1	3.1	3.3	3.1	3.3
Ammonia	19.3	18.8	21.3	21.2	21.6	22.2	19.6	19.6
Arginine	6.3	5.8	6.1	5.8	5.9	6.0	6.4	6.8
Aspartic acid	2.5	2.3	2.6	2.3	2.4	2.4	2.6	2.7
Threonine	1.9	1.7	1.8	1.8	1.8	1.9	1.9	1.9
Serine	3.6	3.4	3.7	3.8	3.7	3.9	3.6	3.5
Glutamic acid	20.9	20.5	22.8	22.7	23.0	24.0	21.2	20.9
Proline	9.0	8.7	9.5	8.9	9.2	9.2	8.6	8.8
Glycine	3.9	3.6	3.9	4.2	4.3	4.3	3.9	3.8
Alanine	2.6	2.4	2.6	2.4	2.5	2.6	2.6	2.8
Valine	2.8	2.6	2.7	2.8	2.7	3.0	2.9	3.0
Methionine	0.8	0.7	0.6	0.7	0.7	0.7	0.8	0.9
Isoleucine	2.2	2.1	2.2	2.3	2.2	2.4	2.2	2.3
Leucine	4.4	4.2	4.4	4.4	4.4	4.6	4.4	4.6
Tyrosine	1.3	1.2	1.4	1.1	1.4	1.4	1.4	1.3
Phenylalanine	2.5	2.5	2.7	2.4	2.4	2.5	2.4	2.5
% N Recovery	89.5	85.7	93.7	92.0	93.4	96.5	89.9	91.2

* 14% moisture basis.

Glutamic acid and proline were the most abundant amino acids and accounted for over 28% of the amino acids present in the flours. Glutamic acid showed an increase with increase in flour protein for the Manitou and Glenlea samples.

Lysine showed a slight decrease with increase in flour protein within Manitou samples. No trend could be detected among Glenlea flours. Talbot flour had the highest lysine content among all the flour samples.

In spite of the differences in mixing properties and breadmaking potential, the amino acid composition of the flours of the four wheat varieties was very similar. The amino acid composition of the flour was not related to the mixing properties or breadmaking potential of the wheats examined. These results are in general agreement with the findings of Tkachuk (1966) who reported that hard red spring wheat flours of good and poor breadmaking quality showed little variation in amino acid composition.

3. Amino Acid Comoposition of Gluten

The amino acid composition of the glutens from the samples of the four varieties studied is shown in Table 10. Better recoveries of N were obtained for the glutens as compared to wheat and flour samples.

Generally, the amino acid compositions of the glutens from the four varieties were similar. Glutamic acid and proline formed over 30% of all the amino acids present in the glutens.

The limiting amino acid lysine was present in lower amounts in Glenlea gluten as compared to the other three varieties. Within Manitou and Glenlea, lysine decreased and showed no change, respectively, with increasing flour protein.

TABLE 10. Amino Acid Composition of Glutens of Four Wheat Varieties (g N/100 g Total Kjeldahl N)

	Manitou			Glenlea			Norquay	Talbot
Flour protein (Nx5.7)*	10.2	11.6	13.4	12.6	13.9	14.6	12.0	10.9
Amino acid								
Lysine	1.9	1.8	1.7	1.4	1.4	1.4	1.6	1.8
Histidine	3.6	3.3	3.3	3.2	3.2	3.1	3.2	3.4
Ammonia	22.8	22.2	22.1	22.1	23.1	22.0	21.7	21.5
Arginine	6.2	5.7	5.5	5.5	5.5	5.3	5.6	6.1
Aspartic acid	2.1	2.0	2.0	1.9	1.6	1.6	1.8	2.0
Threonine	1.9	1.8	1.8	1.8	1.8	1.7	1.9	1.8
Serine	4.2	4.2	4.1	4.3	4.4	4.2	4.3	4.2
Glutamic acid	24.3	24.7	24.9	25.1	25.3	24.4	23.9	22.9
Proline	10.4	11.0	10.9	10.1	10.3	9.8	10.0	9.9
Glycine	4.1	3.9	3.8	4.1	4.1	4.0	3.9	3.7
Alanine	2.4	2.3	2.2	2.1	2.1	2.0	2.2	2.3
Valine	2.8	2.7	2.7	2.5	2.6	2.5	2.8	2.9
Methionine	0.8	0.8	1.0	0.6	0.7	0.7	0.8	0.8
Isoleucine	2.5	2.5	2.4	2.3	2.3	2.3	2.4	2.4
Leucine	4.9	4.8	4.7	4.6	4.6	4.4	4.6	4.7
Tyrosine	1.7	1.7	1.6	1.8	1.8	1.7	1.6	1.6
Phenylalanine	2.9	2.9	3.0	2.6	2.6	2.5	2.6	2.7
% N Recovery	99.5	98.3	97.7	96.0	97.4	93.6	94.9	94.7

* 14% moisture basis.

Glenlea had the lowest value of methionine among all the samples studied. Manitou glutens contained slightly larger amounts of proline than all the other varieties.

These differences were minor and could not be directly used to account for differences in rheological properties of the flours. These results support those of Pence *et al.* (1950) who found that the amino acid compositions of glutens from wheats of diverse baking characteristics were essentially the same.

Stretching of doughs is commonly used by flour technologists as a rough test to assess flour strength. Stretching glutens from the four varieties under carefully controlled conditions was therefore, considered the next useful test in the comparison of these varieties.

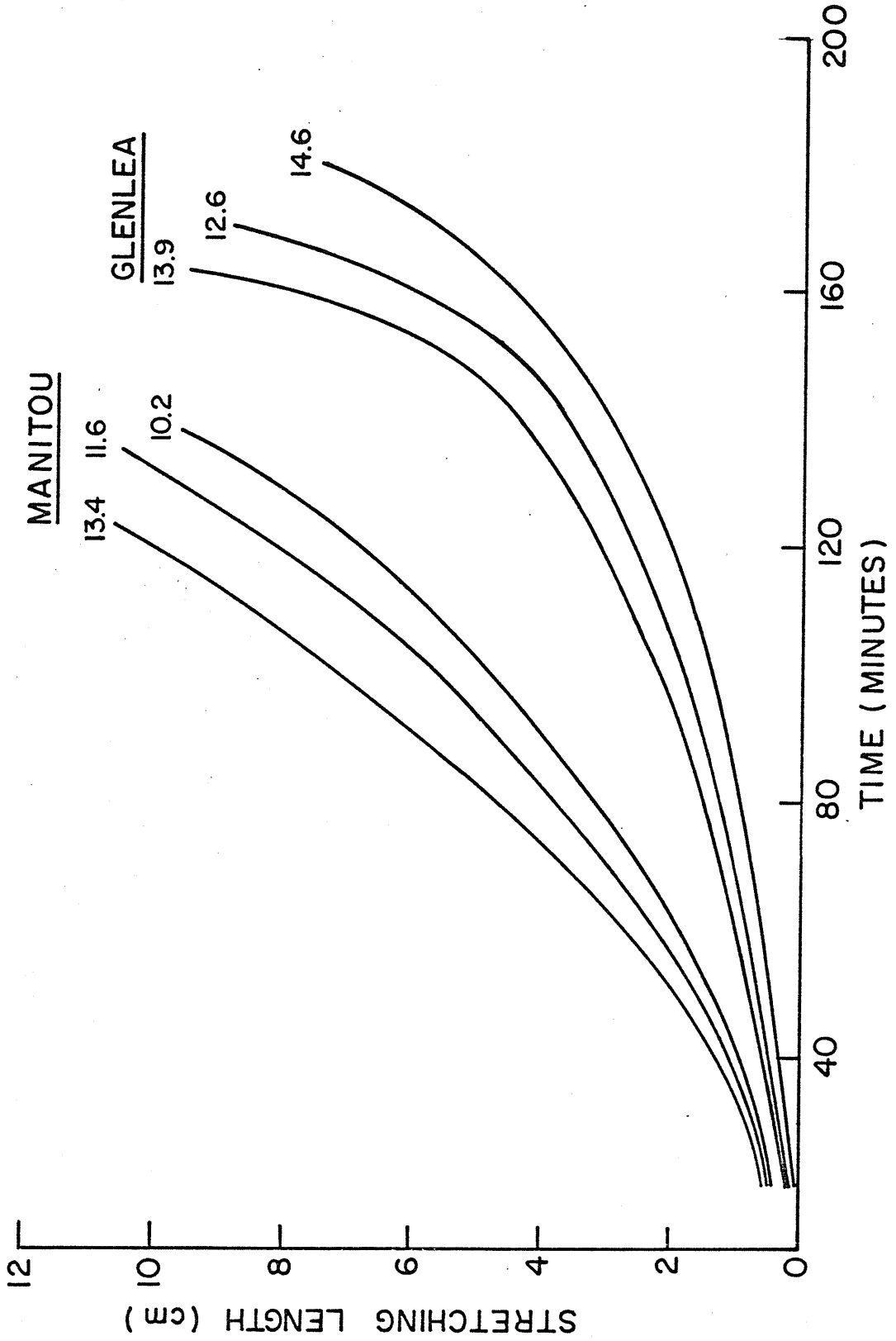
E. Stretching Properties of Glutens of Four Wheat Varieties

1. Stretching Properties of Manitou and Glenlea Glutens

Flour samples were available at three different protein contents for two varieties only: Manitou and Glenlea. Fig. 13 shows the rate of elongation of glutens washed from three flours of different protein content for each of the two varieties. The rate of elongation of the Manitou glutens as a group was greater than that of Glenlea glutens.

Among the Manitou glutens, that from the high protein flour had slightly greater rate of stretch, followed respectively, by those from medium and the low protein flour. The differences were considered insignificant and were attributed entirely to the experimental error of the method used.

Figure 13. Increase in length with time of stretch of glutens from two wheat varieties.


MANITOU
13.4
11.6
10.2
GLENLEA
13.9
12.6
14.6
STRETCHING LENGTH (cm)
0
2
4
6
8
10
12
40
80
120
160
200
TIME (MINUTES)

The rate of stretch of Glenlea glutens was much slower than those of Manitou glutens. Among the Glenlea glutens, the one recovered from the medium protein flour (13.9%) had a greater rate of elongation, followed in order by that from low protein and high protein flour. The differences were small and insignificant.

These results showed that within a variety the stretching properties of glutens derived from flours of different protein contents were similar. Flour protein content from which a gluten was washed did not affect the stretching properties of the gluten significantly.

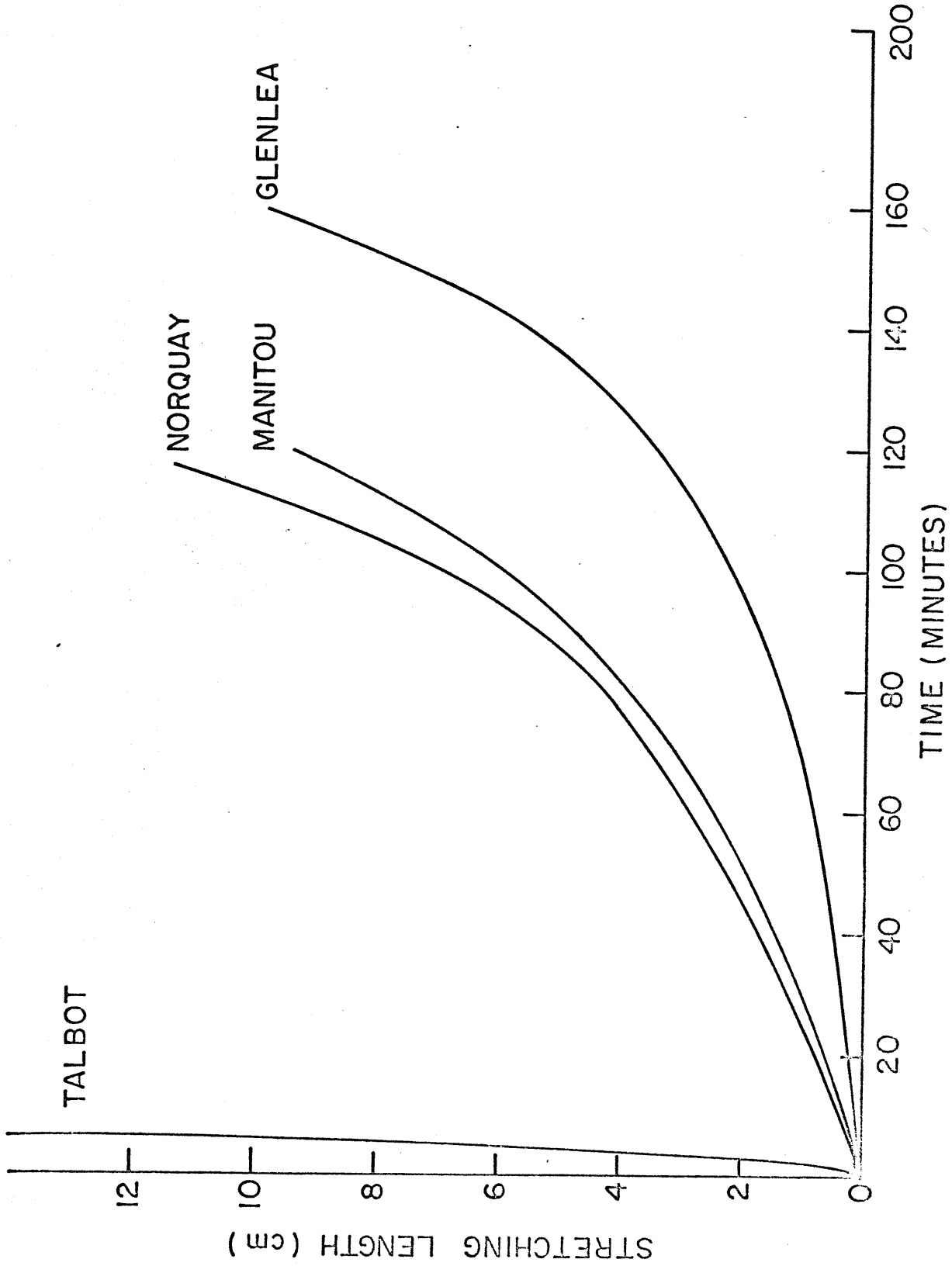
The greater rate of stretching of Manitou glutens as compared to Glenlea glutens suggested that stretching properties were related to flour strength. The stronger variety, Glenlea, had a more elastic gluten than the medium-strong variety, Manitou. These findings support those of Prihoda *et al.* (1971) who found that at constant stress, the velocity of deformation decreased with flour strength for doughs measured on a Hoeppler Consistometer.

2. Stretching Properties of Manitou, Glenlea, Norquay and Talbot Gluten

The curves in Fig. 14 show, the increase in the length of stretch with time, of glutens washed from the four varieties. The curve for Talbot gluten is on the extreme left, followed by Norquay, Manitou and Glenlea.

The foregoing section of this thesis had established that no significant differences existed in the stretching properties of glutens within Manitou or Glenlea samples. Consequently, each curve of either Manitou or Glenlea in Fig. 14 is an average of the curves of glutens derived from the three flours within each variety.

Figure 14. Increase in length with time of stretch of glutens from four wheat varieties.


TALBOT
NORQUAY
MANITOU
GLENLEA
STRETCHING LENGTH (cm)
0
2
4
6
8
10
12
20
40
60
80
100
120
140
160
200
TIME (MINUTES)

The gluten of the weak variety Talbot had the most rapid rate of stretch among the varieties studied. The gluten of the strong variety Glenlea had the lowest rate of stretch. Norquay and Manitou glutens were almost identical in their stretching characteristics. The rate of stretch was slightly greater for the Norquay gluten than for Manitou gluten.

In comparisons of the stretching characteristics of the gluten of an English wheat and a Manitoba wheat and glutens of their air-classified fractions, Kaminski and Halton (1964) found that, the English wheat gluten stretched at a greater rate than that of the Manitoba wheat. The English wheat was weak and the Manitoba wheat was strong.

The results of the present study indicate that, the gluten of the weak variety Talbot had the fastest rate of stretch. Glenlea gluten had the slowest rate of stretch. Muller (1969) suggested that a strong flour was more heavily cross-linked than a weak flour. On the basis of the results in the present study, it may be concluded that, Glenlea gluten may be heavily cross-linked. Conversely, Talbot gluten may be less heavily cross-linked. Norquay and Manitou glutens fall in between these two.

The results in the present study could be interpreted in another way. Tsen (1967) suggested that, the largest protein aggregates of the soft wheat flour were smaller and more liable to disaggregation. He suggested the opposite for the protein of the strong flour. The stretching properties of the glutens as determined in this thesis, suggest that gluten of the weak flour is formed of smaller aggregates of protein of less compact nature and hence can stretch easily. The gluten of the

strong flour is formed of larger aggregates which are more compact and not liable to rapid stretch.

The stretching properties of the glutens suggested that the bonds between the gluten proteins of the weaker flour may be more susceptible to scission than those of the stronger flours. Dissolution of the glutens in dilute acetic acid was, therefore, a logical extension of the comparative study of the four varieties.

F. Solubility of Gluten Proteins in 0.05N Acetic Acid

The solubility characteristics of the gluten proteins are presented in two ways: the soluble and insoluble fractions. The recovery of the extraction ranged from 93.0% to 97.6% and this was considered adequate for the present study.

In the histograms showing the soluble and insoluble proteins (Figs. 15 and 16, respectively) the varieties are arranged from left to right in the order of increasing flour strength, as determined by dough mixing characteristics. Within Manitou and Glenlea, the samples are arranged from left to right in order of increasing flour protein of the parent flours. The results for each sample represent means of four complete independent extractions. The standard deviations arranged for Manitou and Glenlea samples in order of increasing flour protein were: Manitou (1.4, 0.6, 2.7), Glenlea (1.1, 2.5, 1.8), Norquay 3.5 and Talbot 3.2

Solubility of flour proteins in dilute acetic acid has been studied by several workers. For example, Mullen and Smith (1965) showed that the main difference between short (weak) and long mixing (strong) flours,

was that the latter contained less acetic acid soluble protein. The present study was undertaken to examine whether a similar trend applied in comparisons of isolated glutens from flours widely differing in mixing properties.

1. Soluble Fraction

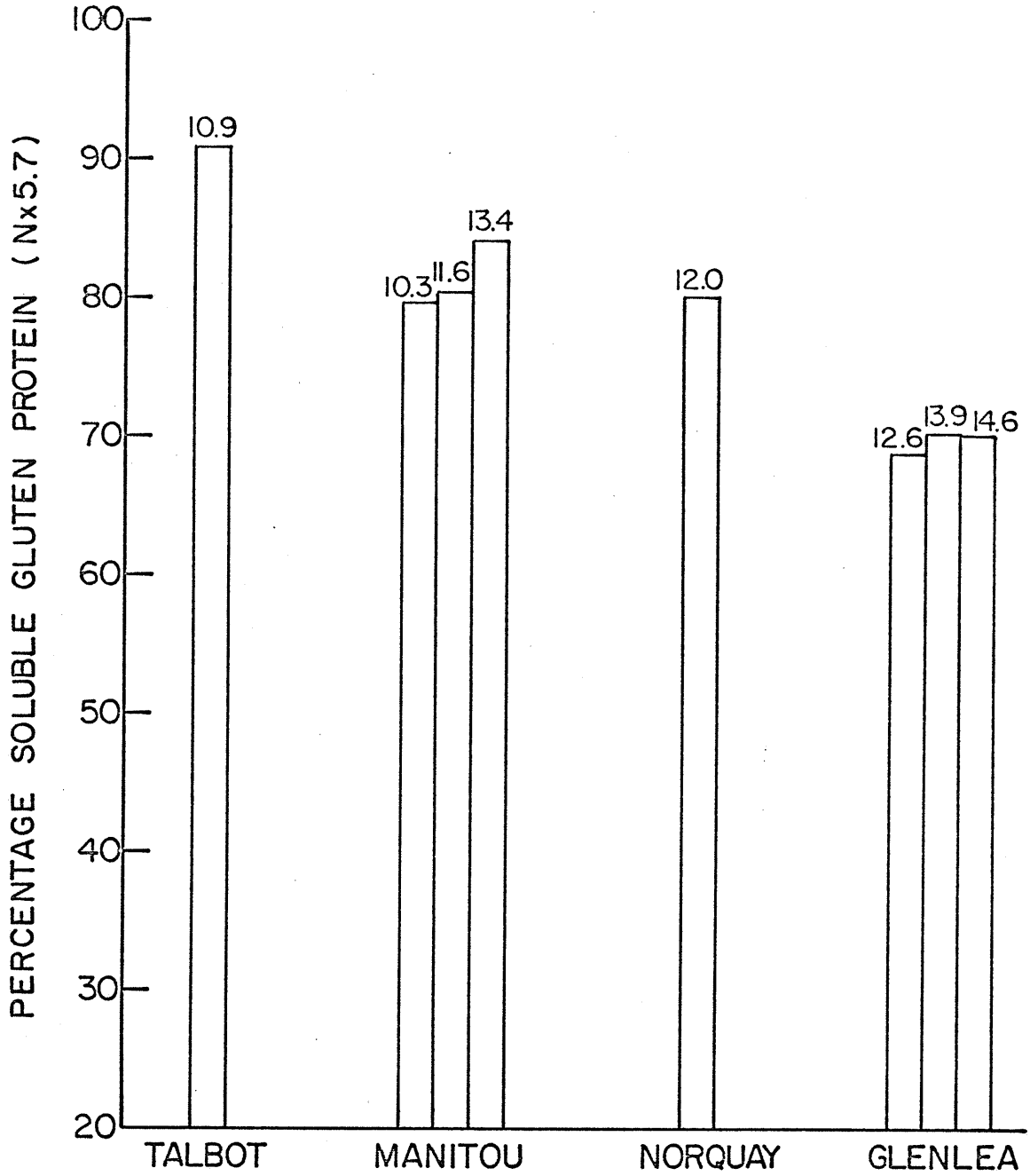
Fig. 15 shows the proportions of gluten proteins soluble in 0.05N acetic acid, as a percentage of the total gluten protein, for the four varieties studied. Above each bar in the histogram is shown the protein content of the parent flour.

Results in Fig. 15 indicate that Talbot contained the highest protein (90.8%) in the soluble fraction. The other varieties contained lower amounts in the following decreasing order: Manitou, Norquay and Glenlea.

Manitou samples, arranged in the order of increasing flour protein, contained 79.8%, 80.4% and 84.1% of the total protein in the soluble fraction. The gluten of the low protein flour had less soluble fraction, and the high protein flour had more soluble gluten. Consequently, this variety showed an increase in the acetic acid soluble fraction with increasing flour protein. However, the increase was small.

Glenlea samples arranged in order of increasing flour protein contained: 68.7%, 70.2% and 70.2% of the total gluten proteins in the soluble fraction. The soluble fraction of the low protein flour was slightly less than those of the two higher protein samples. The soluble fractions for the gluten of the two higher protein flours were identical. A trend for increase in the soluble fraction with increase in flour protein was observed. However, the difference between the higher protein

Figure 15. 0.05N Acetic acid soluble gluten proteins of four wheat varieties arranged from left to right in order of increasing flour strength.


PERCENTAGE SOLUBLE GLUTEN PROTEIN (Nx5.7)
100
90
80
70
60
50
40
30
20
10.9
10.3
11.6
13.4
12.0
12.6
13.9
14.6
TALBOT
MANITOU
NORQUAY
GLENLEA

flours and the low protein flour was small.

Tanaka and Bushuk (1972) obtained a similar increase of solubility with protein content, of the acetic acid extractable protein of flours, of two varieties widely different in mixing characteristics.

Norquay contained 80.1% of the total gluten protein in the soluble fraction. This placed Norquay close to Manitou, in the quantity of the soluble fraction, among the four varieties examined in the present study.

2. The Insoluble Fraction

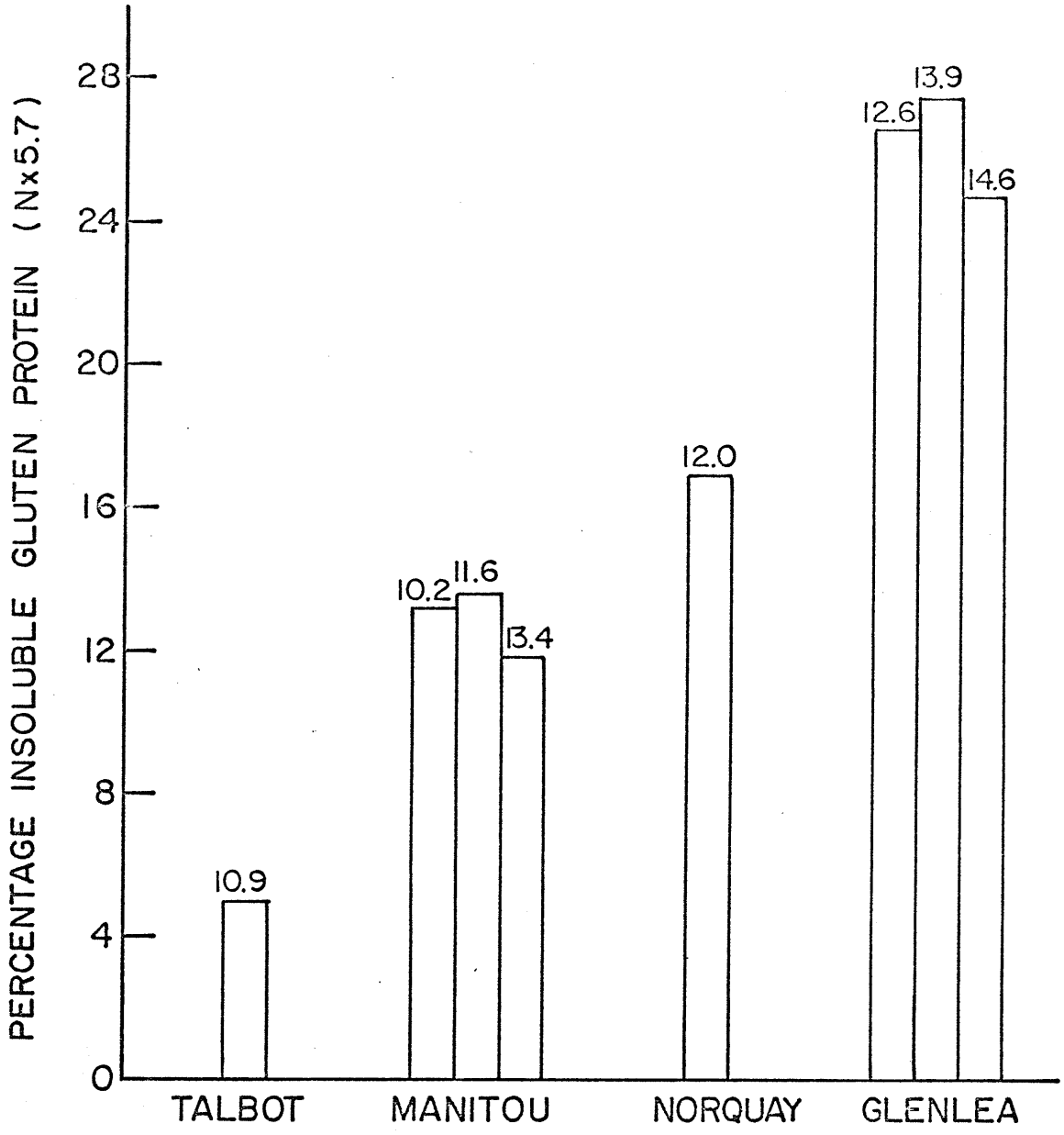
Results of the amount of protein contained in the insoluble fraction expressed as a percentage of the total gluten protein for all the varieties studied is presented in Fig. 16.

The weak variety Talbot contained the lowest (5%) while the long mixing variety Glenlea contained the highest amount of protein in the insoluble fraction. Glenlea samples arranged in the order of increasing flour protein, contained the following percentage of the total gluten protein, in the insoluble fraction: 26.5, 27.4 and 24.7. The corresponding values for Manitou glutens arranged in order of increasing flour protein were: 13.2%, 13.6% and 11.8%. Norquay had 16.9% of the total protein in the insoluble fraction.

Dimler (1967) reported that the acetic acid insoluble protein in wheat gluten had high molecular weight and had glutenin-like properties. The present study is consistent with the work of Tsen (1967) who reported that, weak flour contained a higher proportion of acetic acid soluble protein than the strong flour.

The varieties in the present study could be classified in decreasing amount of the insoluble fraction thus: Glenlea, Norquay, Manitou and

Figure 16. 0.05N Acetic acid insoluble gluten proteins of four wheat varieties arranged from left to right in order of increasing flour strength.


PERCENTAGE INSOLUBLE GLUTEN PROTEIN (N x 5.7)
0
4
8
12
16
20
24
28
10.9
10.2
11.6
13.4
12.0
12.6
13.9
14.6
TALBOT
MANITOU
NORQUAY
GLENLEA

Talbot. Thus, the weak variety (Talbot) contained less and the stronger varieties contained more of the acetic acid insoluble fraction to varying degrees. The acetic acid insoluble fraction was directly proportional and the soluble fraction inversely proportional to the flour strength as determined by dough mixing tests. The findings in this study are in agreement with those of Orth and Bushuk (1972). These workers found that the acetic acid soluble protein was negatively correlated with loaf volume, while the residue protein was positively correlated with loaf volume per unit flour protein.

The solubility studies of glutens in acetic acid in this thesis, have further indicated that for the two varieties Manitou and Glenlea, gluten properties were not significantly affected by flour protein.

Solubility of the glutens in dilute acetic acid clearly indicated differences between weak and strong varieties. Therefore, it was of interest to establish whether the proteins dissolved in acetic acid for the weak variety (Talbot) and the strong variety (Glenlea) had the same or different viscosity properties.

G. Relative Viscosity of the 0.05N Acetic Acid Soluble Gluten

The relative viscosity number of the acetic acid soluble gluten of the varieties Talbot and Glenlea are shown in Table 11. The flour protein of the samples are also shown. The relative viscosity number of the acetic acid soluble gluten of only two varieties was determined in order to ascertain whether there existed differences between the glutens of the weak and the strong varieties.

TABLE 11. Relative Viscosity Number of Glutens in 0.05N Acetic Acid for Two Wheat Varieties

	Talbot	Glenlea
Flour protein % (N x 5.7) (14% m.b.)	10.9	14.6
Relative viscosity number (0.05N HAC = 1)	5.1	4.9

The relative viscosity number of the weak variety, Talbot, was slightly greater than that of the strong variety, Glenlea. However, the differences were insignificant. These results demonstrated that there existed very little difference in the relative viscosity of the acetic acid soluble gluten of the weak and the strong flour. In fact, in absolute values the weak flour had greater viscosity number than the strong flour. The acetic acid soluble protein molecules from the weak flour had the same properties, as measured by the relative viscosity, as those of the strong flour.

These results ruled out any hope of distinguishing the weak variety from the strong variety by determination of the relative viscosity number, of the acetic acid soluble gluten fraction. Therefore, the relative viscosity differences of the acetic soluble gluten protein were probably unrelated to flour strength.

The solubility results indicated that the strong variety contained more insoluble fraction than the weaker flours. The viscosity of the acetic acid soluble gluten indicated that there was little difference

between the weak variety and the strong one. Viscosity in the highly dissociating solvent AUC was investigated next, in order to determine the properties of the total gluten proteins.

H. Viscosity Studies of Gluten Proteins in AUC

The highly dissociating AUC solvent has been claimed to dissolve 95% of the flour proteins by breaking hydrogen and hydrophobic bonds (Meredith and Wren, 1966). In the same study this solvent was used to determine the gel-filtration properties of flour proteins and these were found to retain their average molecular weights.

Owing to the absence in the literature of viscosity studies of the AUC gluten extracts, and in an attempt to elucidate the nature of the gluten particles in this highly dissociating medium, this study was undertaken to compare glutens of four varieties.

1. Extracted Proteins

The results in Table 12 showed that the amount of protein contained in the same weight (two grams) of wet gluten was nearly constant for all the samples and varieties examined. Wet gluten contains about two-thirds water and of the remainder weight only 80% is protein. This explains the apparent low values of protein contained in the gluten.

In the results in Table 12, neither the effect of variety nor flour protein could be detected in the amount of protein obtained from the same weight of wet gluten.

These gluten extracts were next used for the determination of the relative viscosity number.

TABLE 12. Total AUC Soluble Protein (g) from Two Grams of Wet Gluten for Four Wheat Varieties

		Manitou			Glenlea			Norquay	Talbot
Flour protein (N x 5.7) (14% m.b.)	%	10.2	11.6	13.4	12.6	13.9	14.6	12.0	10.9
Gluten proteins (N x 5.7)	(g)	0.497	0.473	0.476	0.464	0.510	0.527	0.521	0.462

2. Relative Viscosity Number

The relative viscosity number of the gluten extracts, reduced gluten extracts and the protein content of the flours, are depicted in Table 13. The results show wide differences in the relative viscosity number of the gluten extracts from the varieties Manitou, Glenlea, Norquay and Talbot. The relative viscosity number of Glenlea samples were the highest, followed in decreasing order by Norquay, Manitou and Talbot glutens.

TABLE 13. Relative Viscosity Number of Glutens and 2-Mercaptoethanol Treated Glutens of Four Wheat Varieties in AUC

	Manitou			Glenlea			Norquay	Talbot
Flour protein (Nx5.7) % (14% m.b.)	10.2	11.6	13.4	12.6	13.9	14.6	12.0	10.9
Relative viscosity number (AUC = 1)	12.3	12.8	11.6	19.9	21.5	22.5	18.3	6.3
Relative viscosity number (reduced glutens)	-	4.3	-	-	5.2	-	4.5	3.2

The relative viscosity number of Manitou glutens varied from 11.6 for the high protein flour to 12.8 for the medium protein flour. The gluten of the low protein flour had a relative viscosity number of 12.3. Thus, the gluten of the high protein flour had a slightly lower viscosity than that from the medium protein flour. However, no trend of viscosity of gluten extracts with flour protein was detected in Manitou.

The gluten of the strong variety Glenlea had the highest relative viscosity number among the varieties examined. Within Glenlea the relative viscosity number of the low protein flour was lower and that of the high protein flour was higher. These results indicated a trend of increasing relative viscosity number with increase in flour protein among Glenlea glutens.

Norquay gluten had a relative viscosity number almost as high as those of the Glenlea samples. Talbot gluten had the lowest relative viscosity number among all the varieties studied.

On the basis of these results, the relative viscosity numbers of these glutens could be classified into low, medium or high categories. On such a scale gluten of the weak variety Talbot would have (low); those of Manitou (medium) and those of the long-mixing flours Norquay and Glenlea, high viscosity numbers.

The results in the present study are in general agreement with those of Matsuo and McCalla (1964). These workers reported that gluten dispersed in sodium salicylate and in lactic acid-sodium chloride solutions yielded intrinsic viscosity values in decreasing order: hard, soft and durum wheats. However, the work presented in this thesis adds a new dimension by considering viscosity of the total gluten proteins and using a highly dissociating medium.

3. Relative Viscosity Number Per Gram Protein

Fig. 17 shows a graphical representation of the relative viscosity number of gluten per gram protein for the four varieties studied. The total protein in the AUC extract was determined and then divided by the measured relative viscosity number in order to obtain the relative viscosity per gram protein. This abolished the viscosity differences due to small differences in the gluten protein present. The varieties in Fig. 17 are arranged from left to right in the known order of increasing mixing strength as determined by physical dough tests.

The gluten of the weak variety Talbot had the lowest relative viscosity per gram protein (13.6). The relative viscosity per gram protein values for Glenlea glutens ranged from 42.0 to 42.9. These were the highest values among all the varieties examined. Norquay gluten had a value of 35.1.

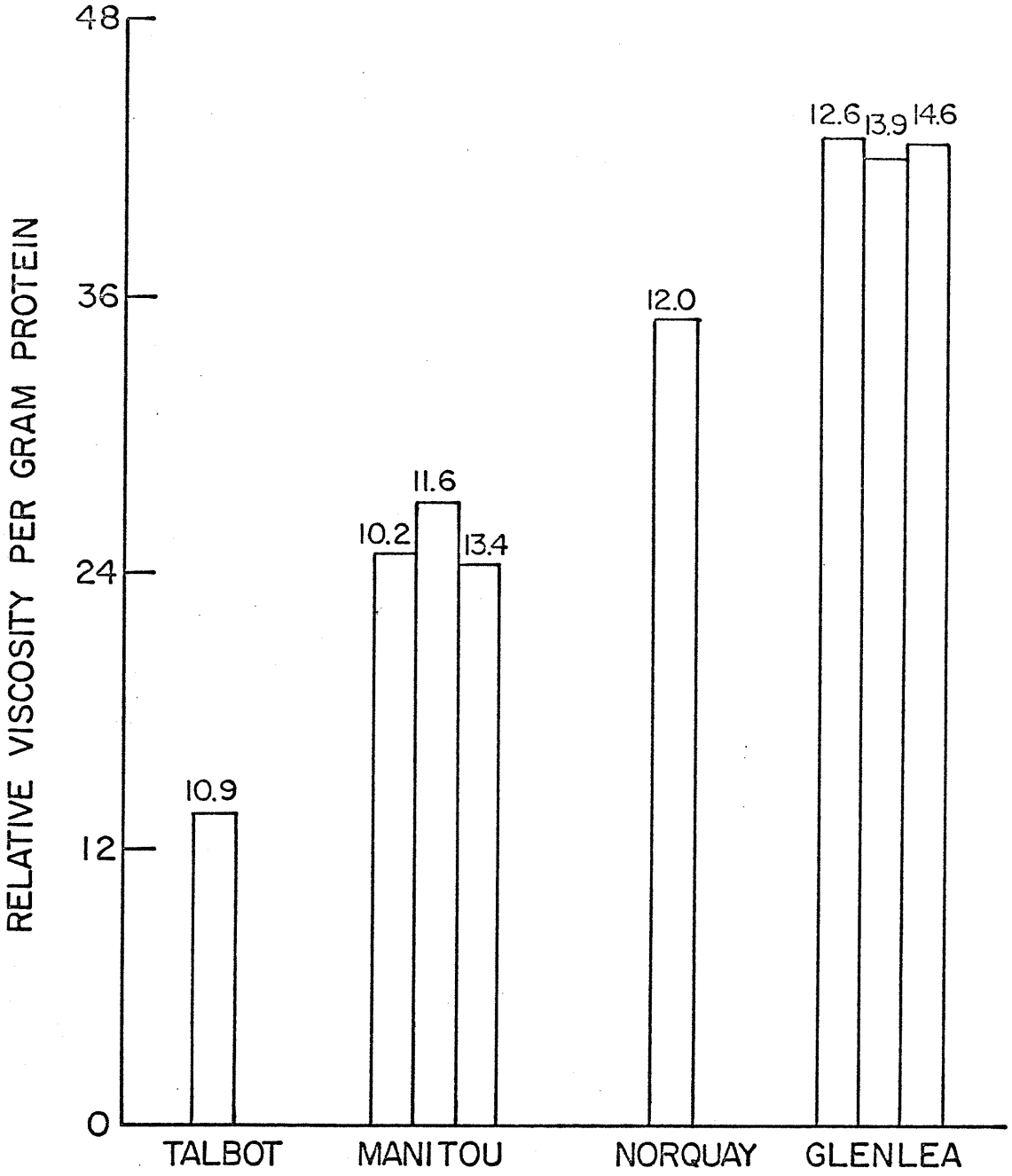
Among the Manitou glutens, the relative viscosity per gram protein fell between 24.3 and 27.0. The gluten of the medium protein flour had a slightly higher value than the other two samples.

The Glenlea glutens derived from three different flours had virtually the same values of viscosity per gram protein.

The viscosity results further supported the postulate that flour protein had no detectable influence in the quality of the gluten proteins as measured by viscosity. The three glutens derived from three different flours of Manitou had very close viscosity properties. The same was true for glutens of the variety Glenlea.

Furthermore, the same weight of gluten proteins derived from varieties of widely differing mixing strength had different relative viscosity.

Figure 17. Relative viscosity of gluten per gram protein in AUC for four wheat varieties arranged from left to right in order of increasing flour strength.


RELATIVE VISCOSITY PER GRAM PROTEIN
48
36
24
12
0
10.9
10.2
11.6
13.4
12.0
12.6
13.9
14.6
TALBOT
MANITOU
NORQUAY
GLENLEA

The strong flours had greater relative viscosity per gram protein than the weaker flours.

The differences detected in the relative viscosity of these glutens is a reflection of the properties of the molecules contained therein. Recently, Huebner and Wall (1976) showed that, the ratio of the high molecular weight glutenin I to the lower molecular weight glutenin II was greater for flours exhibiting long mixing times and strong doughs. In the same study the weak flours possessed lesser contents of both glutenin I and the unextracted protein.

The findings in this thesis are in keeping with the gel filtration results of Huebner and Wall (1976). In the present study the relative viscosity numbers of the four varieties fell in the following order of decreasing value: Glenlea, Norquay, Manitou and Talbot.

It is, therefore, suggested that the viscosity differences between these varieties is in part a reflection of the proportions of the high molecular weight glutenin present in the glutens.

4. Relative Viscosity Number of Reduced Gluten

The relative viscosity number of reduced gluten extracts are shown in Table 13.

The relative viscosity values of reduced glutens were significantly lower than those of corresponding unreduced samples in all varieties examined. However, differences between varieties were still discernible. These results are in agreement with those of Matsuo and McCalla (1964) who found that the intrinsic viscosity values of the reduced glutens fell in the same order as the corresponding unreduced glutens.

Reduced Talbot gluten had the lowest relative viscosity among the

varieties examined in the present study. The reduced gluten of Glenlea had the highest relative viscosity. The differences between varieties were small. The reduced gluten of Manitou and Norquay had relative viscosity values intermediate between those of Talbot and Glenlea.

Excess mercaptoethanol cleaved all disulfide bonds of the glutenin molecule resulting in the subunits of glutenin. This lowered the relative viscosity substantially. These results underline the importance of disulfide bonds in the structure of flour protein molecules.

The findings in this thesis agree with earlier work by Pence and Olcott (1952) who reported that reducing agents lowered the relative viscosity of the gluten proteins by fragmenting these proteins.

The differences in the relative viscosity values of reduced glutens from different varieties in the present study are interpreted to suggest the occurrence of greater momentary reassociation of the glutenin molecules in Glenlea than in Talbot. Alternatively, the particle size of the glutenin subunits were large in the following decreasing order: Glenlea, Norquay, Manitou and Talbot.

The operation of either or both of these phenomena could result in greater viscosity for Glenlea glutens and progressively lower values for Norquay, Manitou and Talbot.

The drop in viscosity between reduced and the unreduced glutens was greatest for Glenlea and decreased progressively in the following order: Norquay, Manitou and Talbot. If this lower viscosity is due primarily to disulfide bond cleavage (Pence and Olcott, 1952) then Glenlea had a greater number of disulfide bonds and Talbot gluten had the least number of these bonds.

Gluten proteins are made up of polypeptide subunits which can be separated by electrophoresis. Comparison of the electrophoretic properties of the gluten proteins of the four wheat varieties, was considered a useful technique in further characterizing the gluten proteins.

I. Disc Electrophoresis of Gluten Proteins

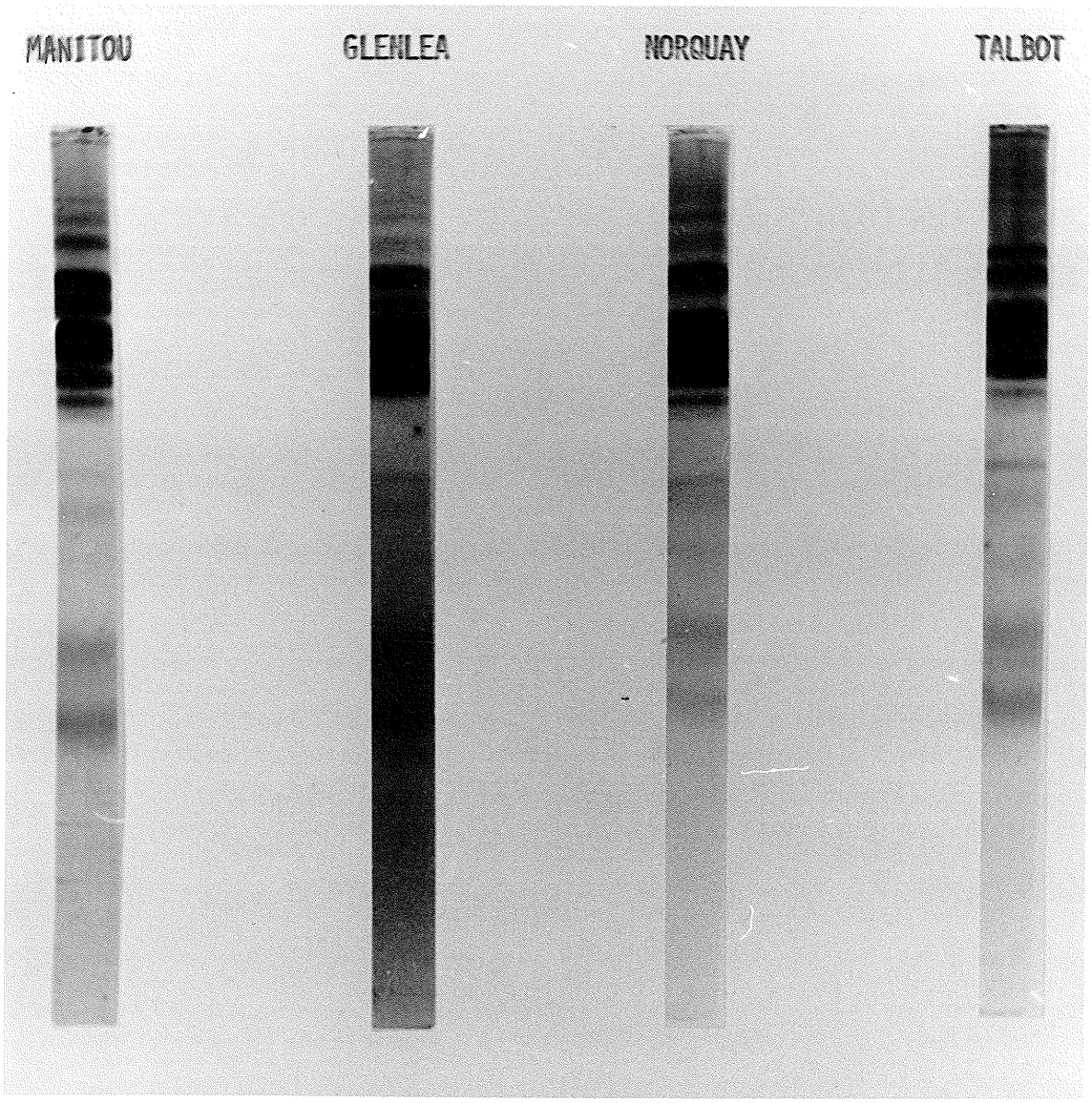
Jones *et al.* (1959) showed that gluten consisted of at least five electrophoretically distinct components. Five of these were found in the gliadin preparations, but glutenin appeared to consist of only one. Other studies have used starch-gel (Huebner and Rothfus, 1968) and polyacrylamide gel (Orth and Bushuk, 1972) techniques in the study of gliadin proteins from wheat varieties of diverse mixing characteristics. These workers attempted to identify any link between electrophoretic results with the rheological characteristics of the flours, without success.

The purpose of the present study was to examine the electrophoretic characteristics of four wheat varieties and determine whether there may be small, but important differences which could be associated with mixing characteristics.

1. The Alcohol-Soluble Fraction

The results of the disc-electrophoresis of the alcohol-soluble proteins from the four wheat varieties are shown in Fig. 18. The results for the four varieties are arranged from left to right, thus: Manitou, Glenlea, Norquay and Talbot.

Figure 18. Disc-electrophoretic patterns for the alcohol-soluble proteins of Manitou, Glenlea, Norquay and Talbot glutens.


MANITOU
GLENLEA
NORQUAY
TALBOT

The number of heavily stained protein (or major) bands were different among the four varieties. Talbot had eight, Manitou six, Norquay seven and Glenlea had five major gliadin subunits. These subunits were considered major, since they contained most of the proteins. The mobilities of these bands were the same where present among the four varieties.

In addition to the major bands, each variety had some faint or minor fast bands. Talbot had four minor subunits. Manitou, Norquay and Glenlea, each had five minor gliadin subunits. All these bands were faster than their corresponding major subunits.

The number and pattern of the gliadin subunits were different in each of the four varieties examined. These differences could not be correlated directly with mixing properties or breadmaking potential of the varieties. The differences in number, pattern and protein distribution among the subunits were considered varietal.

The results in this thesis are in agreement with those of Coulson and Sim (1964) who noted major compositional differences among varieties in the electrophoresis of the gliadin fraction. But these workers found no obvious correlation between the pattern and the physical characteristics of the flour.

In the present study, electrophoresis was also performed on three Manitou gliadin samples extracted from glutens derived from flours containing different protein contents. No differences in pattern, number or protein distribution in the subunits was observed.

Tanaka and Bushuk (1972) reported that flour protein had no effect on the polyacrylamide gel electrophoretic patterns of gliadin proteins

for flours of two pure varieties. The results of the present study confirm this finding.

2. The Alcohol-Soluble and Salt-Soluble Fractions

The results of disc-electrophoresis of the dilute salt-soluble fraction of Glenlea gluten and the corresponding alcohol-soluble fraction are depicted in Fig. 19. The dilute salt-soluble fraction is on the left and the alcohol-soluble fraction is on the right. The subunit pattern and number of these two gels were identical. However, more protein was present in the alcohol-soluble fraction than in the dilute salt-soluble fraction.

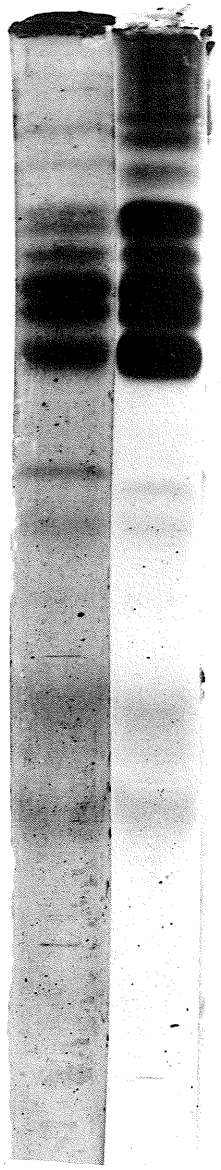
These results lead to the conclusion that the dilute salt-soluble proteins were the same as the alcohol-soluble proteins and not albumins or globulins.

The acetic acid soluble fraction was subjected to disc-electrophorsis. These results are not shown. But it was observed that most proteins did not enter the gel. It was considered that useful information might be gained by comparison of the sodium dodecyl sulfate polyacrylamide gel electrophoretic patterns of the four wheat varieties.

J. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis of Gluten Proteins

SDS-polyacrylamide gel electrophoresis has been used successfully for the determination of molecular weight of the reduced gluten protein components (Bietz and Wall, 1972; Orth and Bushuk, 1973). Furthermore, Orth and Bushuk (1973) attempted, without success, to determine if any relationship existed between breadmaking quality and the glutenin subunits.

Figure 19. Disc-electrophoretic patterns for the alcohol-soluble (1) and the salt-soluble (2) proteins of Glenlea.


1
2

The objective of the present study was to examine an additional number of cultivars and attempt to identify any subtle differences in gluten protein subunit number, pattern or protein distribution which may have a profound effect on the gluten properties.

Although SDS-PAGE is commonly performed on reduced gluten proteins, some information could be gained from electrophoresis of unreduced glutens.

1. Unreduced Glutens

The electropherogram of unreduced gluten complexed with sodium dodecyl sulfate is shown in Fig. 20. Arranged from left to right are patterns for Talbot, Norquay, Glenlea and Manitou glutens. Most protein did not enter the gel. The samples within Manitou and Glenlea are arranged from left to right in order of increasing flour protein.

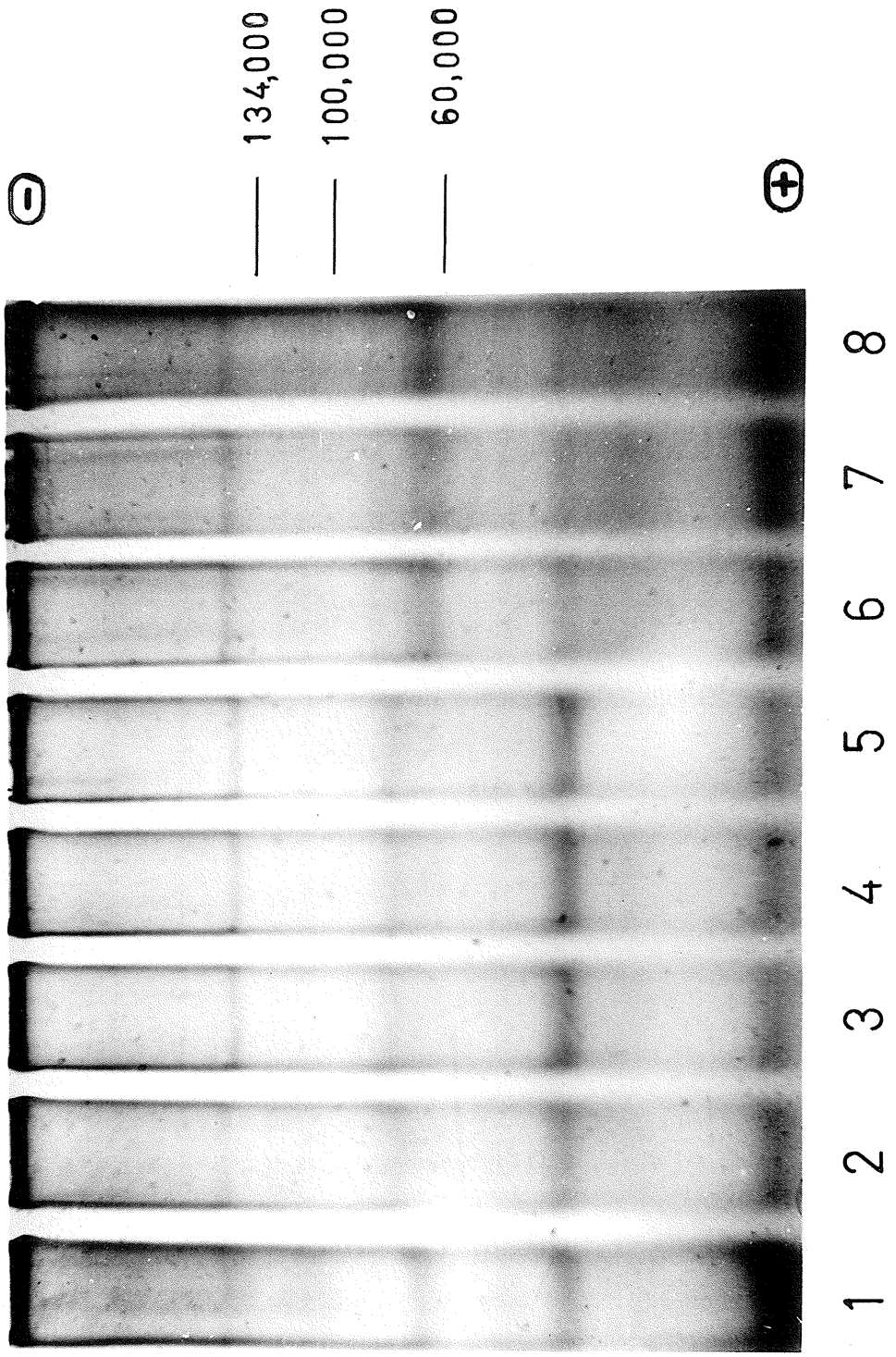
a. The number and pattern of the protein bands. Four main protein bands were common to the gluten patterns of the four varieties examined. The fastest band and the two slowest protein bands absorbed little protein stain. The second fastest protein band stained most strongly. This indicated that most of the protein which entered the gel was located in the second fastest band.

Talbot gluten contained two more protein bands between the slowest band and the third fastest common band.

The patterns for the three Manitou glutens were identical. Similarly, those for Glenlea yielded the same patterns to one another.

b. The protein distribution among subunits. One high molecular weight band was clearly visible among the glutens of all the four varieties. This was a thin band which, though common to all varieties,

Figure 20. SDS-polyacrylamide gel electrophoretic patterns for the unreduced glutens of Talbot (1), Norquay (2), Glenlea (3, 4, 5) and Manitou (6, 7, 8).


134,000
100,000
60,000
1
2
3
4
5
6
7
8

appeared more distinctly in Manitou and Glenlea glutens than in Talbot and Norquay glutens. According to the intensity of the protein stain, this band contained little protein.

The second slowest band was more diffuse. The Manitou glutens contained more protein in this band than the glutens of Glenlea, Norquay and Talbot. The intensity of the stain in this band for glutens of Glenlea, Norquay and Talbot was equally light.

The third slowest band contained most proteins that entered the gel. This band was heavily, but equally stained in glutens of all the four varieties examined.

The fastest protein band showed a slight difference between the Talbot gluten and glutens from the other varieties. In this band Talbot gluten was heavily stained. The glutens from the other varieties were less heavily stained. There were no differences among these varieties.

These results indicated that, during the sodium dodecyl sulfate polyacrylamide gel electrophoresis of the gluten proteins from the four varieties, most protein did not enter the gel. Of those proteins that entered the gel, there were four common major bands present in all cultivars examined. In addition, Talbot gluten contained two more minor bands.

Most proteins, that entered the gel, were present in the second fastest band. In addition, the fastest band of Talbot was heavily stained. This indicated that Talbot, in contrast to all the other cultivars, contained more protein in the fastest band. Manitou glutens differed from the others by having more protein in the third fastest band. Glutens from Glenlea, Norquay and Talbot had less protein in this band.

One high molecular weight band was observed in all gluten samples examined. This subunit entered the gel although no reduction of the gluten proteins had been performed. This gluten protein subunit was presumed to have dissolved in the SDS-buffer used.

Slight varietal differences were noted in the protein content of the bands. Furthermore, Talbot contained two more bands than the other varieties. However, these differences could not be related to the mixing characteristics of the wheats. Moreover, the differences were minor and could not be used to characterize these cultivars.

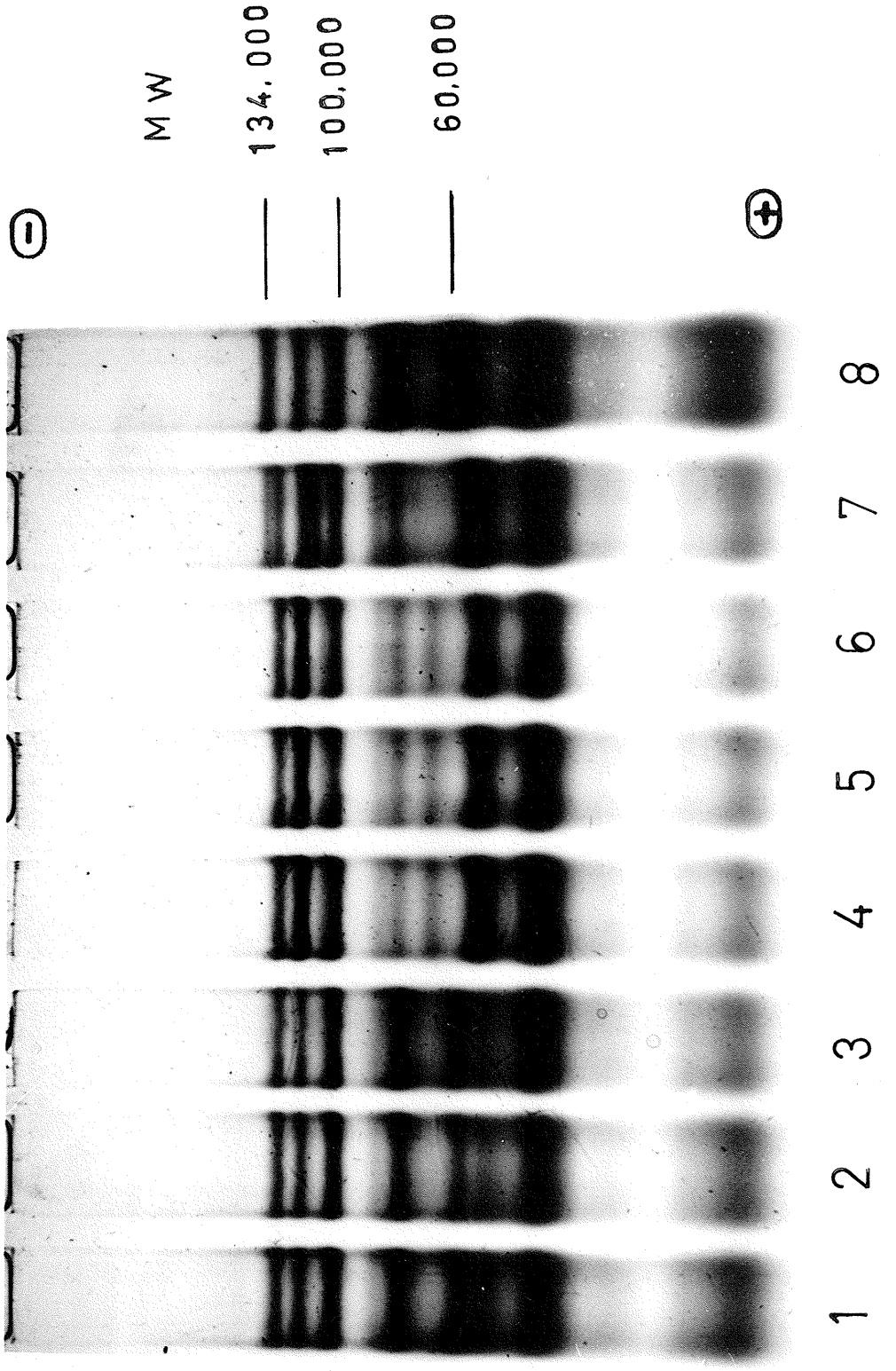
Proteins from flours possessing a wide range of baking quality were dissolved in SDS (Danno *et al.*, 1974). Electrophoresis of these proteins did not reveal any characteristic patterns that could be related to baking quality. The findings in this thesis are in general agreement with the observation of Danno *et al.* (1974).

2. Reduced Glutens

The anionic detergent SDS imparts a uniform charge on protein molecules and the size of the protein particles are a unique function of their molecular weight (Reynolds and Tanford, 1970).

The results of sodium dodecyl sulfate polyacrylamide gel electrophoresis of the reduced gluten proteins from four wheat varieties are depicted in Fig. 21. The quantity of protein applied to the gel was the same for each sample. In each case, all the protein entered the gel. The numbers and pattern of subunits, and the protein distribution among the subunits in the various cultivars was observed.

Figure 21. SDS-polyacrylamide gel electrophoretic patterns for the reduced glutens of Manitou (1, 2, 3), Glenlea (4, 5, 6), Norquay (7) and Talbot (8).


MW
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a. Subunit number and pattern. The three Manitou glutens had identical subunit number and pattern regardless of the flour protein. Glenlea samples also gave identical patterns in spite of the differences in flour protein from which the glutens were derived.

The results are arranged by variety from left to right thus: Manitou, Glenlea, Norquay and Talbot. Within Manitou and Glenlea samples, the results are arranged from left to right in order of increasing flour protein.

Manitou gluten had a total of three subunits in the high molecular weight region: 100,000 daltons or over. Between molecular weight 60,000 and 100,000 daltons, Manitou had three bands. The pattern of the three later bands was such that there were two fast bands and one slow ones. Therefore, in the region of molecular weight above 60,000 daltons, Manitou gluten possessed six distinct bands.

Glenlea gluten had a total of seven distinct subunits (or bands) in the region of molecular weight greater than 60,000 daltons. Three bands were present in the molecular weight region above 100,000 and four between 60,000 and 100,000. Of the latter four subunits (or bands) the fastest one was more heavily stained than the other three which were all equally lightly stained. The pattern for Glenlea gluten subunits in the molecular weight region between 60,000 and 100,000 daltons, was characterized by three slow bands, followed by a doublet and then two single subunits.

Norquay gluten possessed eight distinct bands in the molecular weight region above 60,000 daltons. The subunit pattern for Norquay gluten was unique compared to the other varieties examined. There were

five subunits of molecular weight above 100,000. Starting from the origin, these comprised of two doublets and a singlet. Below this molecular weight, followed a doublet and a singlet. The middle doublet absorbed less stain than all the other subunits within this cultivar.

Talbot gluten had five bands of molecular weight 60,000 daltons and above. Above 100,000 daltons, Talbot had three subunits. These were followed by two singlets. The singlets both absorbed the protein stain heavily. This probably indicated that these subunits contained more protein than the higher molecular weight bands.

The molecular weights of the glutenin subunits in the present study are consistent with the recently revised figures (Khan and Bushuk, 1976) for hexaploid wheats.

b. Protein distribution among subunits. The three bands (Manitou, Talbot and Glenlea) or five (Norquay) of molecular weight above 100,000 daltons, all absorbed similar quantities of protein stain. These bands were distinct and the results probably indicated that they contained equal amounts of protein. The middle three bands of Glenlea or two in the case of Norquay were lightly stained. Comparable subunits in Manitou and Talbot glutens were equally more stained than those of either Glenlea or Norquay. The significance of this protein distribution was not clear. However, Glenlea and Norquay are characterized by having long mixing requirements. The two observations could not be directly correlated. The other subunits had a similar distribution of protein in all the four varieties.

c. Subunit pattern and baking quality. Characteristic patterns for each variety were observed among the reduced gluten proteins of the

four wheat varieties. In the region of molecular weight 60,000 daltons and above: Manitou contained six, Glenlea seven, Norquay eight and Talbot five protein bands. These varieties have weak (Talbot), medium strong (Manitou) and very strong (Norquay, Glenlea) mixing characteristics. However, the protein subunit number and pattern could not be correlated with rheological properties of the flours. These results are consistent with those of Orth and Bushuk (1973).

d. Flour protein and subunit pattern. Three Manitou reduced gluten samples were examined by electrophoresis. Each gluten sample was isolated from a flour of different protein content. These glutens had identical sodium dodecyl sulfate polyacrylamide gel electrophoretic patterns. Glenlea gluten samples were also identical to one another in spite of the flour protein differences. These results indicated that flour protein had no qualitative effect on the subunit pattern or protein distribution among the reduced proteins of these two varieties.

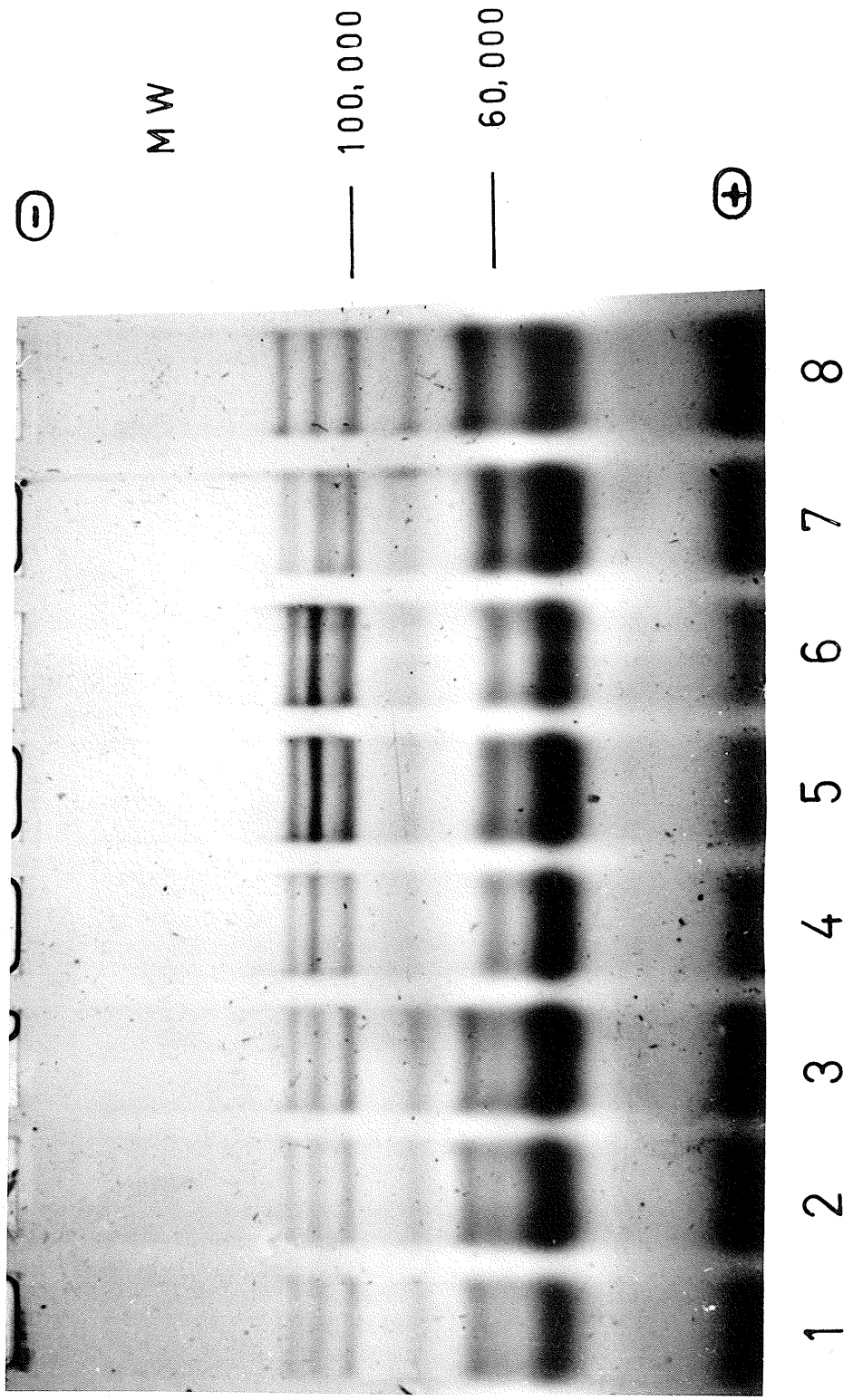
3. Reduced Alcohol-Soluble Gluten Proteins

The results of the sodium dodecyl sulfate polyacrylamide gel electrophoresis of the reduced alcohol-soluble proteins are depicted in Fig. 22.

The results for the four varieties are arranged from left to right in the following order: Manitou, Glenlea, Norquay and Talbot.

The major feature of these results was the prominence of the fast moving bands. Almost all the protein was contained in the fast moving protein bands. These bands contained similar amounts of protein among all the varieties examined. The high molecular weight subunits were faintly stained. This showed that the alcohol soluble proteins were mainly made of the fast moving bands.

Figure 22. SDS-polyacrylamide gel electrophoretic patterns for the reduced alcohol-soluble proteins of Manitou (1, 2, 3), Glenlea (4, 5, 6), Norquay (7) and Talbot (8) glutens.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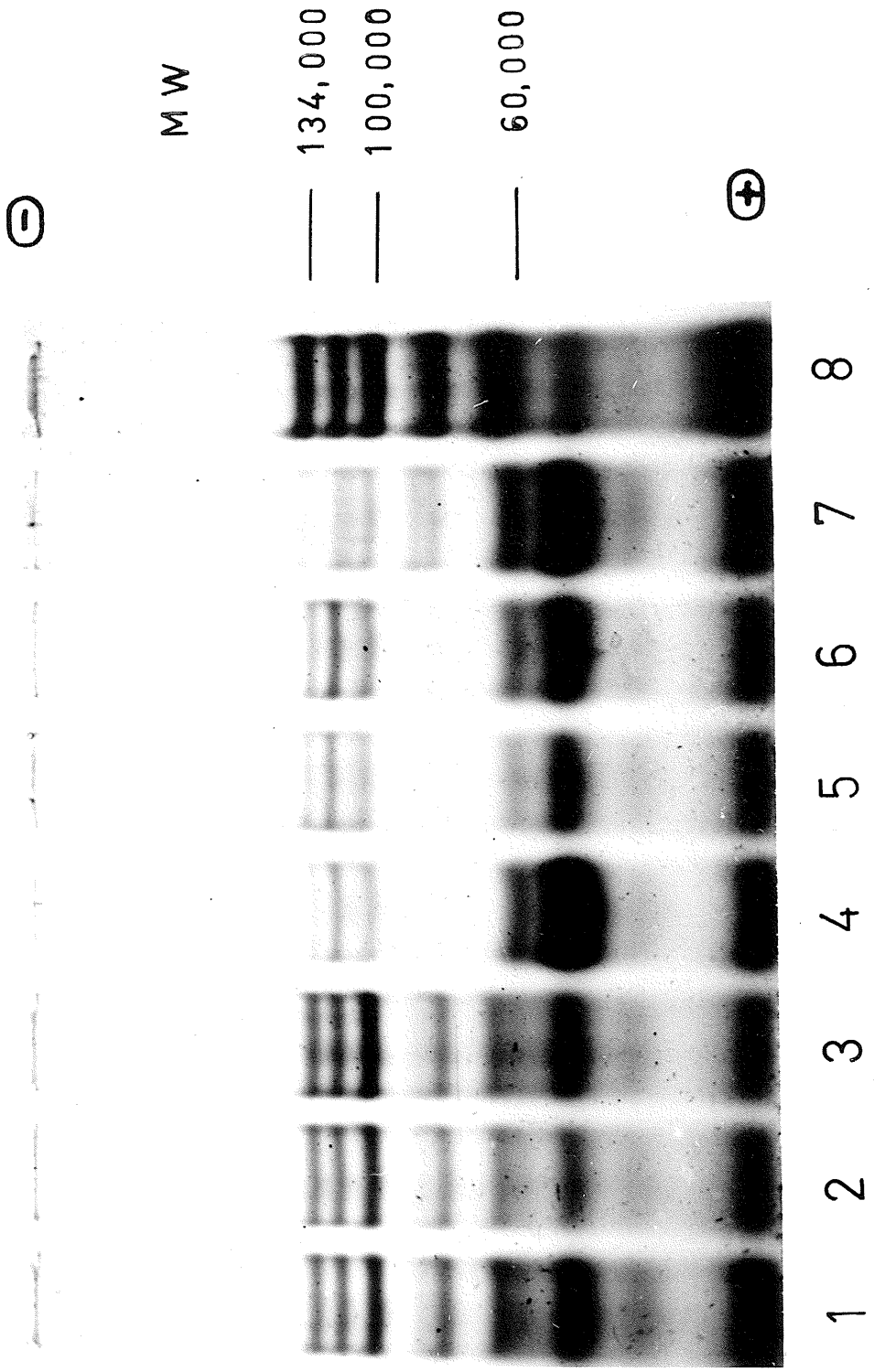
4. Reduced Acetic Acid Soluble Gluten Proteins

The results of the sodium dodecyl sulfate polyacrylamide gel electrophoresis of reduced glutenins for the four varieties is depicted in Fig. 23. These results were very similar to those of reduced whole gluten. In the molecular weight above 60,000, Manitou had six subunits, Glenlea seven, Norquay eight and Talbot five. Manitou, Glenlea and Talbot gluten had three protein subunits each in the molecular weight region above 100,000 daltons. Norquay gluten had five subunits in this region.

The protein distribution among the subunits was similar to that obtained with the corresponding whole glutens. The fast-running bands stained more deeply than the slow bands. This indicated that the fast bands contained more protein than the slow moving ones. Between varieties, the first six slowest moving bands of Glenlea and seven of Norquay, were less deeply stained than the corresponding bands of Manitou and Talbot glutenin. In addition the sixth subunit, from the origin, of Glenlea almost disappeared.

Glenlea and Norquay had long mixing requirements in the rheological tests. Manitou and Talbot were weaker than either of the foregoing varieties in mixing properties. The significance of the protein distribution among the subunits could not be adequately correlated with the mixing properties of these flours. However, it seemed that the long mixing flours contained less acetic acid soluble protein in the high molecular weight or slowest moving protein subunits.

Figure 23. SDS-polyacrylamide gel electrophoretic patterns for the reduced acetic acid soluble proteins of Manitou (1, 2, 3), Glenlea (4, 5, 6), Norquay (7) and Talbot (8) glutens.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100,000
60,000
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V. GENERAL DISCUSSION

Protein is the most important factor controlling the mixing properties and baking potential of a wheat flour. The mixing properties of a flour-water dough are governed by the protein content of the flour and the intrinsic properties of its gluten proteins. When gluten is separated from the starch by washing a flour dough, the protein content of the gluten within a variety will be the same regardless of flour protein. Results in this thesis showed that within different bread wheat varieties, the weak wheat contained slightly less protein in the dry glutens than the stronger varieties (Table 7). However, the effect of protein content is largely abolished and the properties of the glutens reflect the type of protein present.

This thesis was undertaken to compare the physicochemical properties of glutens of four wheat varieties, known to differ in mixing strength. The objective was to identify the basic differences in intrinsic properties of gluten proteins that might explain differences in mixing strength. The wheat varieties selected ranged in mixing strength, according to the farinograph test, from weak to very strong.

The weight of wet and dry gluten, obtained from the same weight of flours of equivalent protein contents, showed no large differences between varieties. Generally, the higher protein flours gave correspondingly larger amounts of wet and dry gluten.

The effect of the same weight of freeze-dried glutens, from the four different varieties, on the mixogram of a weak flour was examined. Small

quantities of gluten resulted in large changes in mixograph properties of the weak base flour. This finding supported the view that gluten was one of the most important components affecting mixing properties of a dough.

Doughs containing glutens from different varieties resisted mixing dough breakdown in the following order of decreasing ability: Glenlea, Norquay, Manitou and Talbot. This showed that Glenlea gluten had greater resistance to mixing breakdown as compared to glutens from the weaker varieties. MacRitchie (1973) converted a weak flour into a strong one by increasing the proportion of its high molecular weight gluten protein. The results of the present study suggest that Glenlea had a higher proportion of the high molecular weight gluten protein than the weaker flours. Furthermore, these results suggest that the distribution of this fraction among the glutens of the other varieties studied is in the following order of decreasing amount: Norquay, Manitou and Talbot.

The amino acid composition of the grain, flour and gluten of all the samples of the four varieties was determined. It was found that at every level of sample (grain, flour and gluten) the varieties showed similar amino acid composition. The minor differences in specific amino acids were not sufficient to explain the gross differences in the rheological properties of the flours.

Stretching the glutens under a constant load revealed that, Talbot gluten stretched more rapidly and Glenlea gluten less rapidly, than the glutens of the other varieties studied. These results support the view that, glutens from strong flours possess more elastic glutens than those derived from weaker flours. The stretching properties of the glutens are consistent with the mixing properties of the parent flours. Muller

(1969) suggested that a strong flour was more heavily cross-linked than a soft flour.

The solubility of glutens in 0.05N acetic acid was examined. This solvent dissolved 90.8% of the gluten protein of the weak variety, Talbot. On the other hand, only 68.7 - 70.2% of the Glenlea (the strongest variety) dissolved under the same conditions. The corresponding values for Manitou were between 79.8 - 84.1% and for Norquay 80.1%. A small increase in gluten solubility with flour protein content for Manitou and Glenlea samples was observed. However, this trend was not conclusive. Accordingly, it was concluded that flour protein was not related to the solubility of the glutens derived from the flours. These solubility results are consistent with similar studies on flour by Mullen and Smith (1965) and Tsen (1967).

Viscosity has been of great interest in the physicochemical studies of macromolecules. The relative viscosity number of the 0.05N acetic acid soluble gluten protein was determined for the weak variety, Talbot and the long mixing variety, Glenlea. The results showed that Talbot gluten had a slightly larger relative viscosity number than Glenlea gluten. The differences were insignificant. These results suggested that the viscosity of the acetic acid soluble gluten was not related to flour strength.

The viscosity of the gluten proteins in the highly dissociating solvent AUC was determined in order to examine if the dissolved glutens showed any differences in viscosity properties.

There were no significant differences in the quantity of protein extracted by AUC from two-gram portion of gluten from each flour. Yet,

the relative viscosity number of the three Glenlea glutens ranged from 19.9 - 22.5, while the corresponding values for the other varieties were: Norquay 18.3, Manitou 11.6 - 12.8 and Talbot 6.3. When these results were expressed as relative viscosity number per gram protein, it was established that flour protein had no effect on the viscosity of the glutens within the varieties Manitou and Glenlea. The viscosity properties determined were entirely a reflection of the type of gluten. The strong gluten had significantly higher viscosity than the weak gluten. The gluten properties were the same for any single variety irrespective of the protein content of the parent flour.

Treatment of the gluten extracts with 2-mercaptoethanol, greatly lowered the relative viscosity number of all the glutens. However, the relative positions of the varieties were still maintained. The relative viscosity numbers of the reduced glutens were: Glenlea 5.2, Norquay 4.5, Manitou 4.3 and Talbot 3.2. There was a larger drop in the relative viscosity of the stronger varieties than the weaker one.

The differences in the relative viscosity number of the reduced glutens was taken to infer differences in the average molecular weight of the glutenin polypeptide subunits. The results obtained in this thesis suggest that the sizes of the reduced glutenin polypeptide chains are larger in the long mixing flours as compared to the weaker flours. Accordingly, the average molecular weight of the glutenin polypeptide subunits for the four varieties decreased progressively in the following order: Glenlea, Norquay, Manitou and Talbot.

The properties of gluten are largely governed by the properties of the individual polypeptide chains of the alcohol-soluble (gliadin), the acetic acid soluble glutenin and the acetic acid insoluble (residue)

glutenin proteins. Polyacrylamide gel electrophoresis was considered a useful technique to study the number, distribution and pattern of the polypeptides, of the gluten proteins and subsequently establish whether there was any relationship with flour strength.

Polyacrylamide gel electrophoresis of the alcohol-soluble fraction showed differences in the number and pattern of the polypeptide subunits between the varieties. There were no differences in protein distribution (content) among the corresponding subunits for all the varieties studied, as judged by visual inspection of the intensity of the protein band. Consequently, the differences in number and pattern of the subunits between the varieties was considered varietal and had no relation with the mixing properties of the flour.

The unreduced whole gluten was studied using the SDS-PAGE technique. Most proteins did not enter the gel. The proteins that entered the gel, were concentrated in the fast moving bands. However, there was one high molecular weight subunit, common to all the varieties studied, which entered the gel. These results showed that most of the gluten proteins were too large to enter the gel. However, gluten also contained some small molecular weight proteins which were found in the fast moving bands. The SDS-PAGE of unreduced whole gluten did not reveal any differences between the varieties studied.

SDS-PAGE of the reduced whole gluten and the reduced acetic acid soluble (glutenin) fraction showed wide differences in the number and pattern of the polypeptide subunits of the four varieties. SDS-PAGE of the alcohol-soluble fraction revealed that most protein was found in the fast moving bands.

For the reduced glutens, no differences were noticed in the distribution of protein among the subunits, as judged by visual inspection of the intensity of the protein bands. However, the reduced glutenins showed that for the long mixing varieties, Norquay and Glenlea, the high molecular weight subunits contained less protein than the corresponding Manitou and Talbot subunits. These electrophoretic results revealed no direct relationship with mixing properties of the flour or breadmaking quality.

SUMMARY AND CONTRIBUTIONS TO KNOWLEDGE

1. The highly dissociating solvent AUC has been demonstrated to be useful in relative viscosity studies of gluten proteins. Relative viscosity of glutens dissolved in AUC have demonstrated that there are distinct differences between glutens from weak and strong wheat varieties. Strong varieties have larger relative viscosity values than weak varieties.

2. Extracting of wet glutens, from weak and strong varieties, with dilute acetic acid, showed that the gluten from a strong variety contained more insoluble protein than the same weight of the gluten from a weak variety. The glutens had similar amounts of total protein.

3. Electrophoresis of the gluten proteins revealed differences between varieties. These could not be associated directly with the mixing properties of the flours.

4. Amino acid analysis of the grains, flours and glutens showed similarities between four wheat varieties widely differing in mixing characteristics.

5. Stretching of glutens under a constant load revealed that, the strong variety, had a low rate of stretch and the weaker varieties had faster rates of stretching, to varying degrees.

6. Small amounts of freeze-dried gluten improved the mixogram of a weak flour greatly. The extent of improvement depended on the mixing strength of the added gluten. Gluten from a strong flour improved the mixogram more than that from a weak flour.

7. Studies made in the course of this thesis have confirmed that the properties of the gluten are characteristic of a variety and are not affected by flour protein.

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