PHYSICOCHEMICAL AND STRUCTURAL STUDIES OF GLUTENIN IN RELATION TO BREADMAKING QUALITY

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by

Lei Gao

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

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To my parents and my wife

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ABSTRACT

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Physicochemical and Structural Studies of Polymeric Glutenin in Relation to Breadmaking Quality

Major Professor: Dr. W. Bushuk

Glutenin preparations from glutens of four Canadian wheat cultivars of diverse breadmaking quality were tested for protein solubility in 6M urea/6% (w/v) sodium dodecyl sulfate (SDS) solution at 50°C. Essentially all of the protein from the glutenin preparations was dissolved by dispersing 12 mg of the glutenin preparation in 5 ml of the solvent and incubating for 24 hr. The rate of solubilization was indirectly related to the "dough strength" of the wheat cultivar. The results by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) showed that for three of the wheat cultivars, the high molecular weight (HMW) subunit composition of the solubilized reduced glutenin was the same as that of the respective flours. For the cultivar Katepwa, subunits 9 and 10 were not resolved in the pattern of the solubilized glutenin. No HMW subunits were observed in the SDS-PAGE pattern for the solubilized but unreduced glutenin. The solubilization procedure should be useful for further studies on characterization of unreduced glutenin of different wheat cultivars in the context of the functionality of this protein in breadmaking.

Gel filtration on Sephacryl S-500 monitored at 280 nm of flours, glutens, or glutenin preparations were carried out to examine the possible relationship of molecular weight distribution of wheat proteins, with an emphasis on glutenin, to breadmaking potential of the flours. The gel filtration profiles showed a continuous range of molecular weight from millions (excluded from the column) down to the size of gliadins (single polypeptide chain). The change in absorbance of

the proteins from flour to gluten and further to glutenin generally confirmed the removal of albumins/globulins, and gliadins at different stage of the Osborne fractionation procedure. The excluded peak from the Sephacryl S-500 column in all cases did not contain as much protein as indicated by the magnitude of the corresponding absorbance. The major glutenin molecules in all samples seem to range approximately from several hundred thousand to several million daltons (near the void volume). The molecular weight distribution of proteins, as measured either by absorbance or by the appearance of the HMW glutenin subunits as seen in SDS-PAGE of the collected fractions was found to be generally similar and the difference of the profile such as the size of the excluded peaks did not consistently correlate with breadmaking quality potential of the original flours. No significant change was observed by gel filtration in profile for glutenin solutions maintained at 50°C for different time intervals.

The gel filtration studies of partially-reduced doughs mixed with 20, 80, and 500 μmol dithiothreitol (DTT) showed that the absorbance of the fractions containing most of the HMW glutenin subunits, as demonstrated by SDS-PAGE under reduced conditions, was decreased drastically upon the addition of the lowest level (20 μmol) of DTT, which is consistent with the marked decrease in farinograph resistance. The drop in absorbance continued progressively with addition of more reducing agent but to a lesser extent. In contrast to the drastic drop in farinograph consistency, the distribution of the HMW glutenin subunits in the fractions from gel filtration did not change significantly upon the addition of the low level of DTT, and shifted to a lower molecular weight range (larger elution volume) upon the addition of very high level (500 μmol) of DTT. The gel filtration results indicated that the difference among the wheat varieties of diverse breadmaking quality was not large enough in molecular weight distribution to be detected by gel filtration chromatography used in this study. Perhaps, the difference may, to a large extent as shown by the difference in the rate of solubilization, lie in the secondary forces in which the difference would be eliminated by the solubilization process and therefore would be undetectable by techniques like gel filtration. This conclusion was further supported by the results of the eight Australian *Glu-1* null wheat lines whose HMW glutenin subunits ranged in number

from five to none but whose molecular weight distributions of glutenin by gel filtration were all similar.

Studies on the Australian *Glu-1* null wheats revealed that the classical Osborne glutenin fraction (unreduced) is composed of two distinct entities, *i.e.* low molecular weight (LMW) glutenin and HMW glutenin, with the former exceeding the latter in proportion. The LMW glutenin comprised polymeric molecules containing only LMW glutenin subunits and no HMW glutenin subunits. The subunits of the LMW glutenin contained mainly two groups of polypeptides of approximate molecular weights of 44 and 36 kDa, respectively, as shown by SDS-PAGE under reduced conditions. The LMW glutenin had a similar molecular weight distribution to that of the HMW glutenin which contained HMW subunits or/and LMW subunits, as reflected by gel filtration profile. It was also found that the HMW glutenin molecules were always in the large polymers regardless of the number and type of HMW subunits present in a wheat variety. Amino acid analysis indicated that the amino acid composition of the LMW glutenin was similar to that of the ethanol-soluble HMW gliadins. It is postulated that the LMW glutenin may play an important role in the rheological properties of dough and breadmaking quality of wheat flours along with the HMW glutenin.

An attempt was made in this study to obtain information on the molecular structure of HMW glutenin in relation to its functionality in doughs during breadmaking. In this context, doughs were mixed in a farinograph from the flour of the Canadian bread wheat Katepwa in the absence and presence of varying amounts of DTT. The glutenin of the control and the partially-reduced doughs was examined by SDS-PAGE with and without reduction during electrophoretic analysis. At low concentration of DTT added to dough (20 μmol/50 g flour), the farinograph properties were markedly affected but no HMW subunits were liberated as indicated by SDS-PAGE without reduction. At higher concentration of DTT (80 μmol-3,000 μmol/50 g flour), several types of glutenin subunit oligomers (partially-reduced glutenin) and all of the HMW subunits (2*, 5, 7, 9, and 10) were liberated gradually with increasing DTT concentration. HMW glutenin oligomers, appearing as three close-moving bands in SDS-PAGE, with molecular weights higher than that

of the largest HMW subunit (2^*), were observed for doughs treated with the higher concentrations of DTT. Based on their apparent molecular weights, the three oligomers appeared to be dimers composed of HMW subunits as follows: 2^*+7, 2^*+9, and $5+10$. The results of this study are consistent with a "block" model of the molecular structure of glutenin which is proposed on the basis of the results of this study.

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I. INTRODUCTION

Wheat is one of the major cereals in the world for food production and, the most important cereal in the western world for baking breads because of its unique properties of forming a viscoelastic dough which is ideal for producing an expanded porous loaf.

In contrast to the important role of wheat in modern life, the quality of wheat for end-uses has been mostly an intricate mystery despite the extensive research on this cereal around the world. For the purpose of quality improvement, an understanding of how the responsible component molecules of the wheat kernel contribute to the quality of wheat is required before anything can be done with regards to the improvement of the molecules, for example, by biotechnology.

Wheat quality for breadmaking has been studied more extensively than for other end-uses. After the discovery that breadmaking quality of wheat varieties is controlled by their protein quality and quantity (Finney and Barmore 1948), it has been established that protein content of wheat kernels is largely an environmentally-controlled factor and can be increased by agronomic practices. However, high protein content does not suffice as a substitute for protein quality (Bushuk *et al.* 1969) which is mainly a genetically-controlled factor. Intensive research has been undertaken and progress has been admirably achieved on the relationship between the functionality of wheat proteins and breadmaking potential of flours. A important advance in research was that of Orth and Bushuk (1972) who showed that, on the basis of statistical results from twenty-six wheat varieties, the glutenin fraction of wheat storage proteins was the key component responsible for the intervarietal differences in breadmaking quality of flours. Probably because of the immense interactions between wheat flour components involved in breadmaking quality, all wheat samples do not fit in this statistical relationship. Nevertheless, that study identified the component responsible for quality, and has stimulated research in a specific

direction. Considerable progress has since been made in the study of gluten proteins in relation to wheat quality.

In the last decade, research on the breadmaking quality of wheats has been concentrated on the high molecular weight (HMW) glutenin subunits. The HMW subunits have been studied intensively with regards to their genetic, chemical and conformational characteristics, and ultimately the relationship of their structure to breadmaking quality. Our knowledge of the structure, genetics, chemistry, and functionality of HMW glutenin subunits has expanded greatly as reviewed recently by Shewry *et al.* (1992).

The most important progress after the pioneering work of Orth and Bushuk (1972) has been the work of Payne and co-workers on the relationship of HMW glutenin subunits to breadmaking quality of European wheats (Payne *et al.* 1979; Payne *et al.* 1981; Payne *et al.* 1984; Payne 1987; Payne *et al.* 1987). The HMW glutenin subunits are so called because they are of relatively lower mobility (higher molecular weight) than other subunits as shown by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) for glutenin subunits obtained after complete reduction of glutenin by disulfide bond reducing agents. The relationship between the presence of certain HMW subunits and breadmaking quality of wheat flours has been generally confirmed by a number of studies. One of the corroborative results is that of Ng and Bushuk (1988) who used a statistical approach and showed a correlation between the presence of certain HMW glutenin subunits and breadmaking quality of wheat flours. Because most of the breadmaking quality differences among wheat varieties for breadmaking can be accounted for by the number and type of the HMW glutenin subunits in wheat, the practical significance of the progress of this type of research has been that it can provide plant breeders with a powerful means for efficient selection of bread wheats in wheat breeding.

Recent studies of the HMW subunits of glutenin indicated that quantitative and qualitative effects may contribute to breadmaking quality difference associated with specific HMW subunit alleles (Halford *et al.* in press). Their results indicated that some quality differences may result from the effects on the total amount of HMW subunit protein. Based on statistical analysis

between percentage of HMW subunits and mixograph time to peak or loaf volume, Lawrence *et al.* (1988) concluded that the HMW subunits appeared to be similar in terms of quality, and that the different gene loci effects are due to their ability to produce different amount of HMW subunits. However, other reports suggested that certain HMW subunits are associated with good quality while some others with poor quality (Payne *et al.* 1987; Pogna *et al.* 1987; Rogers *et al.* 1990) or unrelated to quality (Pogna *et al.* 1987). The qualitative difference between the HMW subunits seems much more difficult to explain at the subunit level. According to the studies of Payne and co-workers, the HMW subunit composition can only account for *ca.* 47-60% variation in breadmaking quality for 79 British-grown wheats (Payne *et al.* 1987) and *ca.* 43% of the intervarietal variability in the Zeleny sedimentation volume, and 71% in the Alveograph W value for 33 Spanish-grown varieties (Payne *et al.* 1988). The variation in the HMW subunit composition accounted for 67.5% of the variation in loaf volume per unit protein content in the statistical equation of Ng and Bushuk (1988). Furthermore, wheat varieties of theoretically good combinations of HMW glutenin subunits for breadmaking can have quite different rheological and breadmaking properties (Ng and Bushuk 1988). Therefore, the progress in the studies of HMW glutenin subunits has produced only part of the answer to the intervarietal difference in breadmaking quality, and therefore the elucidation of the polymeric (molecular) structure of glutenin must be achieved for a complete understanding of the role of this constituent in breadmaking quality.

In the studies of polymeric glutenin, an important aspect of the structure/functionality of glutenin is whether or not there is a relationship between the molecular weight distribution of the proteins and breadmaking quality of the flours. This question is not new; it has been raised by many researchers although most studies seem to have had problems with the extreme insolubility of the gluten proteins, especially that of the glutenin fraction. Much effort has been made in the literature on the solubilization of gluten proteins from wheat flour, dough or gluten. Much inconsistency exists in the literature on the effectiveness of the solvents and the procedures used for the solubilization of gluten proteins. However, there is no report on the solubilization and

molecular weight distribution of glutenin preparations despite the commonly-recognized importance of this fraction in determining breadmaking quality. The reason for this is the difficulty in solubilizing this fraction. Many reports (for review, see section "Solubilization of Glutenin" under "Literature Review") indicated that a good solvent for solubilization of proteins of one type of flour product (*e.g.* dough) may be unsuitable for the solubilization of the proteins of another type. Therefore, the prerequisite for a study of molecular weight distribution of glutenin and other major properties of this fraction is its solubilization. In this study, the solubilization of the Osborne glutenin fraction has been successfully achieved and the results are presented in the first part of this thesis.

After the solubilization procedure was developed, it was subsequently used for the preparation of glutenin samples for the study of molecular weight distribution of this fraction by gel filtration chromatography. For comparative purpose, flours of Canadian wheat samples from which the glutenin fractions were prepared were also chromatographed under the same conditions. Two gluten preparations from flour samples were prepared and analyzed on the same column for further comparison.

All the HMW glutenin subunits, which are believed to play a key role in functional polymeric glutenin, are coded by the *Glu-1* gene loci on the long arms of the group one chromosomes, 1A, 1B, and 1D of common wheat (Payne *et al.* 1980; Payne 1987). Each locus consists of two genes, each encoding a low relative mobility x-type subunit and a high relative mobility y-type subunit (Payne *et al.* 1981). In fact, only three, four, or five subunits are possible in bread wheats, resulting from the silencing of some genes (Shewry *et al.* 1992). In the present study, wheat lines with *Glu-1* alleles null were examined by gel filtration and compositional analysis. Because the *Glu-1* null wheat samples lack certain HMW glutenin subunits, they are unique samples for the study of the effect of the presence of certain HMW subunits in the polymeric glutenin molecules on the molecular weight distribution of this protein. As in the gel filtration study of the Canadian wheat varieties, the glutenin preparations from the *Glu-1* null wheat samples were chromatographed to determine if the molecular weight distribution is related to breadmaking

potential. The results strongly suggested that there is no direct relationship between the two. This conclusion logically suggests that the quality of glutenin proteins of wheat flours lies in the inherent structure of the glutenin molecules, rather than simply in their size or subunit composition. Therefore, knowledge of glutenin structure at the molecular level could be the ultimate answer to the quality differences arising from the differences in the structure of polymeric glutenin molecules, which in turn determines the breadmaking quality of dough.

In the study of the subunit structure, a number of approaches have been used to obtain a detailed structure of glutenin subunits. The amino acid sequences have been the basis for research on the conformational structure of HMW subunits. A striking characteristic of the HMW glutenin subunits is that the cysteine residues are generally on the two ends of the polypeptides while only on one side are the cysteine residues in low molecular weight (LMW) glutenin subunits (for review, see Kasarda 1989). The secondary structure of the subunits has been predicted from the amino acid sequences and determined directly by circular dichroism and Fourier transform-infrared spectrometry of the whole proteins by the group of Shewry and co-workers (Tatham *et al.* 1984; Tatham *et al.* 1985; Field *et al.* 1987). The overall dimensions and shape in solution determined by intrinsic viscosity (Field *et al.* 1987) and the hydrated solid in a recent study by scanning tunnelling microscopy (Miles *et al.* 1991) has suggested a model of an extended rod-shaped protein. Recent studies (Field *et al.* 1987) suggested that the repetitive domain of the rod-shaped protein is a beta-spiral, indicated by the repetitive beta-reverse turns in a central domain with the cysteine residues predominantly at either end of the HMW glutenin subunit polypeptides. It has been suggested that the beta-spiral structure, formed by the repetitive central domain, contributes to gluten elasticity (Shewry *et al.* 1992), but there is no direct evidence for this hypothesis.

The molecular basis for viscoelasticity of dough has to be elucidated at the molecular level, and the understanding of the fundamental properties of HMW glutenin subunits has provided an initial basis (Shewry *et al.* 1992). The establishment of the molecular structure of glutenin would

facilitate further research on the functionality of subunits, and form the basis of improvement of breadmaking quality by biotechnology.

In contrast to the rich collections of studies on glutenin subunits, there is relatively little information on the molecular structure of polymeric glutenin. The study on glutenin structure in this thesis represents a renewed attempt to probe the structure of polymeric glutenin using modern techniques such as solubilization, gel-filtration, rheology (farinography), and electrophoresis.

In summary, the goal and scope of this study were: 1) to develop a procedure for solubilization of the whole glutenin fraction without using reducing agents or vigorous mechanical stirring; 2) to compare the gel filtration profiles of glutenins from wheats of diverse breadmaking quality, and to examine their possible relationship to breadmaking quality; 3) to probe the molecular structure of glutenin in relationship to its functionality in breadmaking.

II. LITERATURE REVIEW

A. Introduction

In the continuing and persistent research on the relationship between the molecular structure and functionality of wheat proteins in breadmaking and other end-use processes, a tremendous amount of work has been published on wheat proteins especially since the introduction of gel filtration and electrophoresis techniques. However, many questions still remain and much work is needed before the relationship is elucidated between structure and functionality.

This project encompasses several problems in the search for the relationship between the structure and functionality of wheat proteins. This review will accordingly cover these aspects in detail. The first part will deal with the solubilization of glutenin which formed the basis for the approach of gel filtration to obtaining an understanding of the relationship of molecular weight distribution to functionality of wheat proteins. Since documentation is scarce in the literature (see section B) on the solubilization of glutenin from glutenin preparations, most of the research work cited in this part will be on the solubilization of proteins in wheat flours and glutens both in dough and separated gluten balls. All other parts of the review will be concentrated on publications on the respective problems in this study.

B. Solubilization of Glutenin

The importance of glutenin in breadmaking quality is well documented in the literature since the publication by Orth and Bushuk (1972). However, it has been very difficult to solubilize the whole glutenin fraction from Osborne fractionation except using strong acid or alkaline solution

as originally used by Osborne (1907) as the solvent for classification of wheat proteins. It is known that the use of the solvents causes breakdown of covalent bonds of wheat proteins (Donovan 1967; Goforth *et al.* 1977).

It is well-documented that wheat proteins in different forms, although they are all derived from wheat flour, are quite different in terms of solubility properties. More proteins become soluble with dough mixing (Tsen 1967; Mamaril and Pomeranz 1966; Danno and Hoseney 1982a,b) and/or the presence of oxidizing agents during mixing (Tsen 1969); it is much easier to solubilize glutenin from gluten than from glutenin preparations (Ewart 1980; Huebner and Wall 1980). It is not clear why glutenins in such different forms as mentioned above differ in solubility (Ewart 1980; Danno *et al.* 1990).

In contrast to the lack of publications on solubilization of the glutenin fraction, there have been many reports on the solubilization of wheat proteins from flours, doughs, or glutens.

Cook and Rose (1934) reported that the gluten prepared from a high quality Canadian grown wheat could be dispersed into urea or sodium salicylate solutions by shaking the mixture for six hours. The proportion of solubilization was determined by analyzing the filtrate and the retainant for protein following filtration.

Jones *et al.* (1959) used acetic acid (HAc) (0.01N) to solubilize in a Waring blendor glutens made from flour defatted with n-butanol; the solubilized gluten was further fractionated into glutenin and gliadins. The proportion of solubilization was about 73% of total flour protein (or 85% of the gluten protein). Chen and Bushuk (1970) used acetic acid to prepare acetic acid soluble fractions from flours in the procedure of sequential fractionation, based on the conventional Osborne procedure (1907). Other fractionation procedures in which organic acids were used include the one by Hoseney *et al.* (1969) in which 0.005N lactic acid was used and the those used by MacRitchie (1978) and by Danno *et al.* (1974). In such procedures as these, however, a significant amount of protein is often left undissolved even with vigorous mechanical stirring involved in the procedure, despite the relatively strong acidity of the solvent.

Jankiewicz and Pomeranz (1965b) reported that complete extraction of wheat proteins into 3M urea-0.01M pyrophosphate buffer (pH 7.0) was achieved by using a Waring blendor which apparently contributed to the increased extractability of proteins. It is, however, suspected that the true extractability in that study might be overestimated due to the possible presence of urea in the dialysed sample which was used for nitrogen determination of the solubilized fraction.

Meredith and Wren (1966) reported a procedure for dissolving up to 98% of wheat flour proteins with the help of vigorous homogenization. In that procedure, the solvent contained 0.1M acetic acid, 3M urea and 0.01M cetyltrimethylammonium bromide (AUC). Some other researchers achieved much lower extractions, however, with this solvent and somewhat different mechanical stirring; as low as 72% by Danno *et al.* (1974) under gentle stirring, and 75% by Payne and Corfield (1979) with a magnetic stirrer. Among the solvents used by Shearer *et al.* (1975), AUC achieved the maximum yield of *ca.* 79% while all other solvents used were lower in extractability. Field *et al.* (1983) achieved an extraction of between 70-80% in their procedure with several solvents including AUC.

Cluskey and Dimler (1967) used a mixture of hydrochloric acid and 2-chloroethanol in their fractionation procedure, with even stronger acidity (0.1N HCl) than that of AUC. In another study, Inamine *et al.* (1967) used 0.1M aqueous dimethylaminoethanol to solubilize gluten by homogenization and reported that about 85% of the protein was solubilized. It was concluded in that study that the exposure to the pH 10.5 condition employed in the study was not harmful to the integrity of gluten proteins as compared with chloroethanol/HCl solvent.

Jennings and Watt (1967) used phenol-HAc-water (2:1:1, w/v/v) as a solvent and achieved almost complete solubilization of wheat flour proteins by homogenization, and later by sonication (Gallus and Jennings 1968). The solvent was rarely used by other researchers probably because of the suspicion that it broke disulfide bonds since the solvent could disperse flour proteins into the subunit level without using a reducing agent (Stanley *et al.* 1968).

Danno *et al.* (1974) used 1% SDS solution (pH 6.8) for the solubilization of wheat proteins. The extraction was carried out by making a suspension of the flour (defatted with n-butanol) and

by gentle stirring for half an hour followed by centrifugation. Extraction was made with the same procedure with another eleven solvents. The SDS solution gave the highest extraction (75%), slightly better than the AUC (72%) under the conditions used. Gel filtration studies showed that most of the proteins extracted with the SDS solution are of low molecular weights (< 75 kDa). The residue protein after the first extraction can be extracted almost completely by another extraction with SDS in combination with 2-mercaptoethanol (ME)/mercuric chloride. Graveland *et al.* (1979) used a 1.5% SDS solution and obtained similar extraction (65-67%) from flours.

Bietz and Wall (1975) showed that more than one solvent was required for almost complete solubilization of glutenin from flour under mild mechanical stirring, based on the experiment using a series of solvents to fractionate wheat proteins. After sequential extraction by NaCl, ethanol, and HAc, much glutenin remained undissolved known as either gel or residue protein. It is known that the residue protein could be solubilized by the solvent of 0.01N HAc with 0.2 mM $HgCl_2$ (Mecham *et al.* 1972), or reducing agents. However, the mercury salt was found to disrupt disulfide bonds of the protein by Danno *et al.* (1975), which was confirmed by Khan and Bushuk (1979a).

Kobrehel and Bushuk (1977) found that almost all of the glutenin, which was prepared by AUC-pH precipitation procedure of Orth and Bushuk (1973), could be dissolved into fatty acid solutions under alkaline conditions (soap) by rotating the mixture overnight. The solubilization was attributed to the ability of the fatty acids to disrupt hydrophobic interactions in the glutenin aggregates. Later, Kobrehel and Matignon (1980) extended the use of soap for flours and found that about 90-95% of the proteins could be extracted from wheat flour by stirring overnight into soap solutions of certain concentration which depended on the type of soap and the properties of wheat varieties.

Ewart (1979) successfully dispersed gluten from mechanically developed dough into a solvent of 5% SDS-8M urea-0.5M HAc, by using a so-called "M.S.E. Atomix mixer", for the purpose of viscosity measurement. A follow-up study (Ewart 1980) showed that up to 98% of the proteins in gluten were dissolved into the solvent. In this study, however, gluten was used rather than

glutenin in the determination of intrinsic viscosity change of glutenin upon exposure to reducing agents because the presence of gliadin in gluten was found to have improved the solubility of glutenin (Ewart 1980).

Danno (1981) reported another approach to solubilizing glutenin by extracting the flour twice with a phosphate buffer (pH 7.0) containing 0.5% sodium dodecyl sulfate (SDS), and taking the second extraction as the glutenin obtained by stirring with a Waring blendor. The glutenin in this procedure, however, accounted only for about 20% of the flour protein, or about half of that from the classical Osborne fractionation (Chen and Bushuk 1970; Orth and Bushuk 1972).

Bottomley *et al.* (1982) showed that 0.069M SDS-0.1M Tris/HCl buffer (pH 8.0) extracted wheat flour proteins (*ca.* 95%) more efficiently than any other solvents including the AUC. The use of this solvent also gave better resolution of the extracted proteins during gel filtration chromatography. The extractions from glutens, which were prepared from mechanically-developed doughs, however, were equally high for all solvents, suggesting improved extractability of glutenin from dough than from flours. Danno and Hoseney (1982a,b) showed that *ca.* 95% of proteins could be solubilized into 1% SDS (pH 7.0) solution by gentle stirring for 2 hr from freeze-dried doughs which had been mixed for 15 min in a mixograph while the extent of solubilization was about 60-70% for the flours under the same conditions.

Field *et al.* (1983) developed a modified AUC solvent, 0.01M acetic acid-6M urea-0.055M cetyltrimethylammonium bromide, lower acid and higher detergent concentrations than those of the AUC of Meredith and Wren (1966), for the solubilization of glutens. Among the five solvents tested, the modified AUC proved the best for the solubilization of the proteins from glutens prepared from eleven wheat varieties. All other solvents, *i.e.* 1% SDS-0.01M HAc, 0.2% SDS-3M urea-0.01M HAc, 1% SDS-6M urea-0.01M HAc, and the AUC of Meredith and Wren (1966), only solubilized between 70-80% of the gluten proteins.

Singh *et al.* (1990a) successfully extracted by sonication almost all of the proteins from strong or weak flours into 2% SDS-0.05M sodium phosphate buffer (pH 6.9). The increased solubility by sonication was believed to be similar to dough-mixing in a mixograph, but at much higher

efficiency. In that study it was concluded that the solubilization involved cleavage of covalent (mostly disulfide) bonds as well as non-covalent forces of glutenin molecules so that very large glutenin molecules became smaller and more soluble but were still larger than gliadin molecules.

He *et al.* (1991) reported that a near complete extraction of proteins from wheat flour could be obtained with 1% SDS (pH 7.0) solution, for a period of ten and twenty four hours for the poor and the good breadmaking varieties, respectively.

Generally, it has been the experience of most researchers that solubilization of wheat storage proteins from flour or any other forms is not easy. Many studies included vigorous mechanical stirring which has long been suspected to cause cleavage of covalent bonds, and was confirmed by a recent report (Singh *et al.* 1990a). Early in 1935, Rose and Cook (1935) demonstrated that in urea solution within the pH range of 6.1 and 9.2, the viscosity of gluten was independent of the pH value. However, on both sides of this range the viscosity of the gluten solution dropped rapidly after an initial increase, suggesting instability of gluten proteins under both lower and higher pH conditions. More recently, it was reported (Goforth *et al.* 1977) that wheat proteins could be hydrolyzed even with low concentration of acids, including acetic acid and lactic acid which were used in their functional studies of glutenin. Therefore, a solubilization procedure without mechanical stirring and a solvent at or near neutral pH would be desirable for studying the physicochemical properties of glutenin in its polymeric or native form.

In summary, the best solvents which have been reported for solubilization of flour or gluten proteins without using reducing agents are, therefore, AUC introduced by Meredith and Wren (1966) and sodium dodecyl sulfate (SDS) buffer by Danno (1981), both used for flours with vigorous mechanical stirring, and also urea solutions by Cook and Rose (1934) for gluten by shaking and Jankiewicz and Pomeranz (1965a,b) for flour by homogenization and shaking, urea-SDS-acetic acid by Ewart (1979) for preparing gluten and glutenin dispersions, SDS buffer with gentle stirring used by Bottomley *et al.* (1982) for flour and gluten and by Danno and Hoseney (1982a,b) for dough, modified AUC for gluten by stirring overnight by Field *et al.* (1983), SDS solution for flour by He *et al.* (1991), and SDS buffer by sonication by Singh *et al.* (1990a).

Additional solvents reported are soap solutions for glutenin from AUC-pH precipitation by Kobrehel and Bushuk (1977) and soap solution for flour by Kobrehel and Matignon (1980). None of these reports investigated the solubilization of total Osborne glutenin fraction.

C. Gel Filtration Studies of Wheat Proteins

Studies on the molecular weight distribution of wheat proteins and their fractions have been an important part of the overall attempt to elucidate the relationship between protein structure and breadmaking quality. Research in this area has been closely related to the progress in the development of new media and techniques of gel filtration for the study of molecular weight distribution, and also to the progress in solubilizing wheat storage proteins. Although gluten proteins are extremely complex and very difficult to study, progress has been made on wheat proteins with the help of gel filtration and electrophoretic techniques.

1. Gel Filtration and Protein Composition

Jones *et al.* (1963) were among the first to use the gel filtration technique to fractionate wheat proteins. The samples, which represented *ca.* 73% of total flour proteins as reported earlier by Jones *et al.* (1959), were prepared by extraction with 0.01N HAc of a gluten ball made from defatted flour, and fractionated on Sephadex G-75. Six fractions were generated from the elution. The fractions were compared by starch gel electrophoresis (pH 3.1) under unreduced conditions, and the first two were found to contain mostly glutenin, which remained at the point of application, and slight amount of alpha- and beta-gliadins. A subsequent study (Wright *et al.* 1964) used Sephadex G-100, which was found to have a better separation of glutenin from gliadins than did Sephadex G-75.

Proteins extracted by urea solution from wheat flour were also fractionated on Sephadex G-200 in an acetate buffer (Jankiewicz and Pomeranz 1965a). The results indicated that wheat proteins

could exist in several conformations, and aggregation and disaggregation took place under the conditions of isolation and fractionation (Jankiewicz and Pomeranz 1965b). High heterogeneity was observed in fractions from the chromatography of urea extracts.

Huebner and Wall (1974) used gel filtration for the preparation of glutenin subunits. The gel filtration of reduced and alkylated glutenin subunits was run on Sephadex G-200 in 4M urea/0.03N HAc. The SDS-PAGE patterns revealed that the first peak from the gel filtration did not contain HMW glutenin subunits, and nor the third (last). The first peak in this system was rechromatographed in 6M GnHCl/0.01N HAc, and separated into roughly three new peaks, which produced different subunit composition from those of the second and third peaks in the first gel filtration profile. Khan and Bushuk (1979b) also found that the amount of protein represented by each peak from the gel filtration chromatography in dissociating solvents of reduced and alkylated glutenin depended on the solvent used. In that study, the excluded peak did not contain HMW glutenin subunits on SDS-PAGE, despite the type of the solvent, suggesting the extraordinarily strong association between the glutenin subunits which were eluted at the void volume as large aggregates. These results indicate that chemical reduction of disulfides does not necessarily break all the associative forces between the glutenin subunits which can aggregate by strong secondary forces, and also suggest that the strong non-covalent interactions may have played a role in the molecular weight distribution of wheat proteins in some studies.

Wrigley (1965) reported that an improved ion-exchange chromatographic separation of wheat storage proteins could be obtained by increasing the operating temperature of chromatography from room temperature to 40°C. The improvement was believed to have been derived from the reduced non-ionic interactions. Later, it was reported (Wasik *et al.* 1979) that glutenin dissolved in sodium stearate solution tended to become turbid if left undisturbed at 21°C. Elevated temperature was necessary and adequate for maintaining the glutenin in solution since the turbid suspension could be clarified almost instantaneously if heated to 45°C. This may explain why the chromatography by Wrigley (1965) was improved in resolution upon elevated temperature.

Meredith and Wren (1966) introduced the famous AUC solvent for the solubilization and chromatography of wheat flour proteins. The chromatography on Sephadex G-200 produced four peaks, representing glutenin, gliadins, albumin and a non-protein fraction, respectively. It was found that addition of 2-mercaptoethanol eliminated the glutenin peak and shifted the molecular weight distribution to lower values. This again suggested the polymeric nature of glutenin fraction of wheat storage proteins. In that study, it was found that neither sulfhydryl-blocking agents nor deliberate aeration caused observable change in molecular weight distribution of glutenin and gliadin peaks. Extraction or mere wetting of flour did not induce any change in molecular weight distribution through sulfhydryl-disulfide interchange.

Another gel filtration medium, Bio-gel P-300 in 8M urea was used (Crow and Rothfus 1968) to fractionate reduced and cyanoethylated glutenin, and glutenin and gliadins prepared from wheat flour by the procedure of Jones *et al.* (1959). The glutenin fraction emerged continuously from the column of Bio-gel P-300 over a range from exclusion volume to half of the fractionation volume. Based on starch gel electrophoresis of the fractions after reduction, they suggested that glutenin molecules differed only in the degree to which the same subunits were repeated in their composition (Crow and Rothfus 1968). The cyanoethylated glutenin was separated into three fractions, each of which contained a different mixture of polypeptides as resolved by starch gel electrophoresis. The first fraction of reduced glutenin eluted in the excluded volume from the column; this result suggested the presence of high molecular weight aggregates even in 8M urea.

Huebner and Rothfus (1971) investigated the stability of glutenin toward various dissociating forces. Extraction with, and exposure to urea solution, phenol-acetic acid-water solvent (pH 1.4), and sonication used in their study did not seem to have altered the molecular weight distribution of glutenin as detected by gel filtration on Sephadex G-100 and on Bio-gel P-300, or by starch gel electrophoresis. This observation suggests that the aggregation or disaggregation of gluten proteins as reported in the literature is affected by preparation procedures in the respective studies.

Shearer *et al.* (1975) reported that gliadin and glutenin were stable for up to 20 years of ambient temperature storage, and only marginal change occurred in the glutenin molecular weight

distribution, based on a gel filtration study of extracts of wheat proteins by eight separate solvent systems.

Payne and Corfield (1979) investigated the subunit composition by SDS-PAGE of wheat protein fractions isolated by gel filtration in the AUC solvent. They concluded that the glutenin fractions of different molecular size from gel filtration were made up from the same subunits but in different proportion, based on the subunit composition resolved by SDS-PAGE of the fractions from gel filtration.

Danno *et al.* (1990) investigated the molecular weight distribution of a glutenin preparation, which was obtained with SDS buffer by a Waring blendor from a blended flour and which represented *ca.* 20% of wheat proteins. The gel filtration profile had two peaks, a small peak which contained no glutenin at the void volume, and a broad peak eluting from near the void volume to the elution volume of the column corresponding to that of gliadins. Only a slight difference was observed among the subunit compositions of different glutenin fractions eluted from the column. It was suggested that polymerization of glutenin by sufhydryl-disulfide interchange occurred during the purification and separation procedures. This study used a single sample of commercial flour (not a pure wheat variety) and therefore, it is not possible to relate their results to possible inter-varietal differences in breadmaking potential.

2. Molecular Weight Distribution and Breadmaking Quality

The relationship of molecular weight distribution to breadmaking quality has been a long-lasting challenge to cereal chemists. Several studies have been published on this subject although the results are not always consistent with each other.

Huebner (1970) used a gel filtration approach to estimate the glutenin/gliadin ratios of wheat glutens and their relationship to breadmaking quality. In this study, glutens were extracted with 0.05N acetic acid by vigorous stirring from each of eleven varieties of wheats representing five classes of wheat grown in the United States, and chromatographed on a column of Sephadex G-

100 in 0.1M HAc-2M urea. The glutenin/gliadin proportions were similar for all varieties investigated, ranging from 46-50% gliadin and 50-54% glutenin. The extent of extraction for the gluten proteins, however, were not known from that study.

Lee and MacRitchie (1971) used gel filtration to prepare gluten protein fractions and then studied the effect on dough of these gluten protein fractions through reconstitution studies. In their procedure, the protein components of flours from four Australian wheats were fractionated by a successive extraction procedure using water, various urea solutions and 0.1M NaOH solution. The gel filtration profiles of the fractions on Bio-gel P-150 in 0.01M HAc-2M urea (pH 4.0), showed that the early urea extracts contained proteins of small size, and the late extracts contained almost exclusively proteins of large size. The proportion of high molecular weight protein components increased with each successive extraction. The addition to dough of the protein in the early urea extracts decreased mixing stability and gave weaker, more extensible doughs while that of the late extracts increased mixing stability and gave stronger doughs.

Huebner and Wall (1976) reported that there was a quantitative difference between flours of different baking quality in molecular weight distribution, as measured by absorbance at 280 nm, by gel filtration chromatography on Sepharose 4B of the flour proteins, with the good breadmaking flour having a larger peak eluted at the void volume. The proteins for application on the gel filtration column were the combined extracts of three successive extractions with AUC solvent of defatted flours that were originally different in mixing time and dough strengths. The combined extracts after dialysis and lyophilization were dissolved and chromatographed in 5.5M GnHCl solution. In that study, the amount of residual protein, which comprised *ca.* 10 to 20% of total flour proteins, was not represented in the profile of the gel filtration although wheat varieties with larger excluded peak seem also to have a larger proportion of proteins in their respective residue. Nevertheless, this study stimulated much interest in the relationship between molecular weight distribution and breadmaking quality of wheat proteins.

Bottomley *et al.* (1982) used sodium dodecyl sulfate (SDS) buffer (0.069M SDS-0.1M Tris/HCl, pH 8.0) for extraction and gel filtration of proteins from flour or gluten. It was found that the ratio

between the areas under the peak excluded from the column and the following flat peak correlated with breadmaking quality of the flours. Both of the peaks were glutenins based on amino acid composition and SDS-PAGE patterns, and their polymeric nature as reflected by a change in the profile of gel filtration of the sample upon chemical reduction.

Danno and Hoseney (1982b) compared the chromatograms on Sepharose CL-4B of wheat flour and dough proteins solubilized in 1% SDS (pH 7.0) solution. It was found that the fraction eluted at and near the void volume of flour extracts contained no protein while for dough extracts, glutenin was eluted in the void volume and through a flat region to the second peak. However, the SDS extracts from overmixed doughs did not show any difference in elution profile even though the extracts from those doughs had a lower viscosity, as compared to those from normally mixed doughs (Danno and Hoseney 1982a).

A modified AUC solvent (0.01M HAc-6M urea-0.055M cetyltrimethylammonium bromide) was used for solubilization and fractionation of the extracts of glutens (Field *et al.* 1983a). Two peaks were resolved in the chromatography of the gluten proteins on columns of controlled pore glass (a gel filtration medium). The first peak, eluted at the void volume, contained a large number of components with a wide range of molecular weights including almost all of the HMW glutenin subunits as resolved by SDS-PAGE under reduced conditions while the second peak contained mainly single polypeptide chains (gliadins) as indicated by the shifting of the first peak upon chemical reduction to the volume corresponding to the second peak of an unreduced sample. The relative amount of material (glutenin/gliadin) present in the two peaks estimated on the basis of the measurement of absorbance at 280 nm was found to be correlated to the breadmaking quality.

Singh *et al.* (1990b) used size-exclusion HPLC to fractionate wheat flour proteins which were solubilized by using sonication into SDS buffer (pH 6.9). Three peaks were obtained in the profile, corresponding to glutenin which was excluded, gliadins, and albumin/globulin, respectively. The percentage of area of the glutenin peak was found to be positively correlated to breadmaking potential of the flour (Singh *et al.* 1990b). This finding is generally consistent with the results of

Orth and Bushuk (1972) because sonication brought the otherwise insoluble glutenin into solution in the former study.

D. Studies on Low-Molecular-Weight Glutenin

Wheat proteins can be classified into albumin, globulin, gliadin and glutenin, based on the classical Osborne (1907) system, which is still the most widely used one in the studies of wheat proteins. It is traditionally accepted that the aqueous alcohol-soluble gliadins and the insoluble glutenin are different classes of wheat storage proteins in that glutenin is in chemical terms, a group of high-molecular-weight polymers composed of many polypeptide subunits linked with intermolecular disulfide bonds, while gliadin is a group of single polypeptides. However, there is a general lack of knowledge about how so many polypeptides as observed in SDS-PAGE under reduced conditions are organized in the polymeric glutenin molecules.

On the other hand, gel filtration chromatography has revealed that there are both single polypeptide gliadins and a considerable amount of polypeptide oligomers in the aqueous ethanol-soluble wheat proteins (Beckwith *et al.* 1966; Nielson *et al.* 1968). This group of polymeric molecules has been called high molecular weight gliadins (Beckwith *et al.* 1966; Bietz and Wall 1973; Bietz and Wall 1980), aggregated gliadins (Shewry *et al.* 1983), or low molecular weight (LMW) glutenin (Nielson *et al.* 1968; Kanazawa and Yonezawa 1973; Autran *et al.* 1987; Feillet *et al.* 1989). It was found that this group of oligomers occur in common wheats as well as in durum wheats (Autran *et al.* 1987). It is generally unknown how this peculiar group of protein functions in the wheat kernel or in breadmaking process.

There are reports that suggest that glutenin originates from monomeric gliadins (Beckwith *et al.* 1965; Yoshida *et al.* 1980) as well as those negating this possibility (Lee 1968a; Lee 1968b; Redman 1973). Another system for the classification of wheat storage proteins (Shewry *et al.* 1986) was therefore proposed in an attempt, among other purposes, to overcome the confusion derived from the Osborne classification by classifying proteins by their subunit composition.

Bietz and Wall (1973) studied the amino acid composition of glutenin subunits prepared by ethanol precipitation from the reduced and alkylated glutenin preparations. The amino acid analysis of ethanol- soluble and insoluble glutenin subunits showed that they differed markedly and the former is much more similar to high molecular weight gliadin or purified gliadin. Approximately 62% of subunits by weight were soluble as gliadin in neutral 70% ethanol. It was postulated that glutenin contained both ethanol-soluble and -insoluble subunits in the same polymeric molecule.

Bietz and Wall (1980) further characterized the high molecular weight gliadin and the ethanol soluble glutenin subunits of wheat. Based on the results of acid-PAGE and N-terminal sequence analysis, and earlier amino acid composition analysis (Bietz and Wall 1973), they concluded that many LMW glutenin subunits and ethanol-soluble reduced glutenin subunits were identical. It was postulated that these polypeptides could, *in vitro* or *in vivo*, combine with each other to form high molecular weight gliadin, or interact with the ethanol-insoluble HMW glutenin subunits to form polymeric glutenin. However, it was not known if the LMW glutenin subunits, which are of approximate molecular weight of 44 and 36 kDa, occur *in situ* as high-molecular-weight, ethanol-insoluble polymers by themselves, in addition to their presence as alcohol soluble high molecular weight gliadins in gluten (Bietz and Wall 1980).

Shewry *et al.* (1983) isolated a fraction of aggregated gliadins from the excluded peak of gel filtration on Sephacryl S-300 of the aqueous ethanol-extracted gliadins. Upon reduction, this fraction comprised two major components with molecular weights of about 44 kDa and 36 kDa. They also found that the amino acid composition of these aggregated gliadins was similar to those of monomeric alpha-, beta-, and gamma- gliadins. Amino acid sequencing from the N-terminal also showed the presence of sequence types of alpha- and gamma-type gliadins, but the major sequence type was not related to any of the monomeric gliadins. Although this study confirmed the presence of high molecular weight gliadins in aqueous ethanol extracts as reported by Bietz and Wall (1973), it did not support the notion that the high molecular weight gliadins are formed from the monomeric gliadins.

Structural studies showed that the circular dichroism spectra of the aggregated gliadin and the LMW glutenin subunits were more similar to those of the alpha-, beta-, and gamma- gliadins than to those of the omega-gliadins or HMW glutenin subunits (Tatham *et al.* 1987). This conclusion is consistent with their amino acid composition and sequence similarities.

Field *et al.* (1983b) studied the 50% aqueous propan-1-ol extracts of ground wheat grain by gel filtration chromatography on column of controlled-pore glass in a modified AUC solvent. SDS-PAGE analysis of the fractions under unreduced and reduced conditions demonstrated that the HMW subunits of glutenin and some of subunits of the high molecular weight gliadin (S-rich prolamins) were present predominantly or partially in large aggregates (*ca.* 1 million Da) stabilized by disulfide bonds. Other S-rich prolamins (including alpha-, beta-, and gamma- gliadins) by the nomenclature of Shewry *et al.* (1986) and all the S-poor prolamins (omega-gliadins) were present predominantly or only as monomers. It was shown that the large molecules from the aqueous propanol extracts contained not only LMW glutenin subunits but also HMW subunits. It is not known whether the presence of the large aggregates was because of the presence of the HMW glutenin subunits as traditionally perceived in the polymeric glutenin.

Autran *et al.* (1987) prepared gliadin extracts by 70% ethanol from semolina (a durum wheat product). It was found that in this extract the LMW glutenin as called in that study, was of a smaller size than that of HMW glutenin which was also extracted and excluded from the chromatographic column. The LMW glutenin contributed 14.4% and 27.1% of the extracted protein from a medium and a good pasta-making durum wheat, respectively, based on densitometry of the electrophoretic patterns under reduced condition. It was concluded but not substantiated by evidence that the LMW glutenin exists as an independent entity (without HMW glutenin subunits in the polymeric molecule) in the ethanol-insoluble fraction of wheat proteins, because the LMW glutenin in that study was an aqueous ethanol extract and therefore actually a preparation equivalent to the high molecular weight gliadin of Beckwith *et al.* (1966).

There is, therefore, no clear evidence indicating the presence of glutenin composed of only LMW subunits in the aqueous ethanol-insoluble protein fraction of wheat protein although it

seems that the validity of this notion has been assumed by some researchers. The presence of the LMW glutenin or its subunits has been correlated to the quality of pasta products (Feillet *et al.* 1989 and references therein).

It was recently reported (Huebner and Bietz 1991) that the amount of the high molecular weight gliadins correlated negatively with loaf volume, mixing times, and overall baking quality of flours. This report suggests the technological importance of high molecular weight gliadins in the breadmaking process.

E. Structure of Polymeric Glutenin

In the last decade, the understanding of the HMW glutenin subunits has been greatly advanced in genetics, chemistry, and structure, and their relationship to breadmaking quality (for review see Bushuk and Tkachuk 1991; Shewry *et al.* 1992). In the continuing search for a structure/functionality relationship, several models have been proposed for the structure of polymeric glutenin. However, the fine details of the structure remain a challenging subject of research.

Ewart (1968) was the earliest to report a structural model of polymeric glutenin. The proposed model has been modified in a series of papers based on new evidence (Ewart 1972; Ewart 1977; Ewart 1979; Ewart 1988). In the latest model of Ewart (1988), glutenin was described as a linear polymer composed of structural units in concatenation linked with one disulfide bond. The rheological properties of dough are dependent on the presence of rheologically-effective and ineffective disulfide bonds, thiol groups and on the secondary forces in the concatenation.

Based on the results of extraction with SDS solution and fractionation by precipitation/re-dissolution and gel filtration techniques, Graveland *et al.* (1985) proposed a model for the structure of glutenin, in which the polymeric glutenin (glutenin I) is composed of glutenin structural units called glutenin II molecules linked through their constituent HMW glutenin subunits, each glutenin II being an oligomer of three HMW glutenin subunits and four LMW clusters called

glutenin IIIa molecules which are attached to one of the HMW subunits. Each IIIa oligomer is composed of three LMW subunits. In their model, therefore, glutenin is essentially a linear polymer linked by disulfide bonds between the HMW glutenin subunits as the backbone, with several LMW glutenin subunit clusters each covalently bonded by disulfides to every other two HMW glutenin subunits of the backbone in the polymer.

Kasarda (1989) proposed a model for the linkage of HMW and LMW glutenin subunits of the structural units of glutenin, based on the proposed beta-spiral structure of HMW glutenin subunits. It was assumed that the gliadins and the glutenin subunits all have regions of beta-spiral structure, because they all have substantial parts of their primary structure in the form of repeating sequences. In the model, a typical glutenin molecule is composed of structural units with several LMW glutenin subunits aligned in a plane and linked by disulfide bonds at their ends and to a HMW glutenin subunit. The beta-spiral region of the adjacent polypeptides can interact with each other in a plane to stabilize the planar structure.

The marked effect of disulfide reducing agents on the rheological properties of dough in the farinograph has been generally explained on the basis of reduction of "rheologically-effective" disulfide bonds of gluten proteins (for review, see Bloksma and Bushuk 1988). Ng *et al.* (1991) proposed a model for glutenin structure based on the drastic effect of a small amount of disulfide reducing agent, which did not release any HMW glutenin subunits, on the rheological properties of dough as measured by the farinograph. It was suggested that glutenin is composed of an intermediate structure, called partially-reduced glutenin oligomers, between the polymeric level and the single chain polypeptide subunits. It was postulated that each partially-reduced glutenin oligomer contains most or all of the glutenin subunits of native polymeric glutenin. However, no details were given about the nature of the partially-reduced glutenin oligomers. Furthermore, the drastic drop of the farinograph consistency and no release of glutenin subunits upon addition of a small amount of reducing agent could simply be explained as the result of random but insufficient breakdown of the large polymeric glutenin molecules, making it unnecessary to

propose the presence of the partially-reduced glutenin oligomers as a unique structural unit in the polymeric glutenin.

The major characteristic described for structural models of glutenin is the presence of inter-polypeptide disulfide bonds in polymeric structures. This type of model is based on the well-known effect of disulfide reducing agents on the rheological properties of dough and on the insolubility of glutenin in strongly dissociating solvents which would usually break secondary forces among protein subunits. Although it has been popularly considered that HMW polymeric glutenin is formed through intermolecular disulfide bonds by glutenin subunits, Kasarda *et al.* (1976) proposed an alternative model for the glutenin structure in which the subunits of glutenin aggregate through strong secondary bonding forces such as hydrogen bonds, ionic attractions, and hydrophobic interactions with considerable specificity and cooperativity derived from the conformational structure of the protein subunits. Disulfide bonds exist only within glutenin subunits, and function to stabilize the conformation of the subunits so that the secondary forces could direct the protein subunits into linear aggregates.

Khan and Bushuk (1979a) examined by SDS-PAGE both crude and purified glutenin preparations in reduced and unreduced forms, and proposed a model for the structure of functional glutenin, based on the results that unreduced glutenin, although being purified, contained many protein components that entered the gel. These components corresponded, in mobility, to many of the subunits of reduced glutenin identified by SDS-PAGE. In this model, the polymeric glutenin comprised two types of structural units, the first type (GluI) being held in the complex by strong non-covalent bonds and some of them entering the gel in SDS-PAGE, and the second one (GluII) consisting of large molecules linked by interpolypeptide disulfide bonds. Non-covalent interactions between the two structural units provided for the mobility required for viscous flow under stress, and the disulfide crosslinks of GluII contribute the elastic component of glutenin, gluten and dough.

Although substantive indirect evidence exists for each of the models, the evidence is not yet conclusive beyond doubt for any one model. Further research on the exact nature of the structural

units and glutenin subunits in the structural units, along with other aspects reflecting the characteristics of glutenin structure, is necessary in order to elucidate the structure of glutenin, and thereby its relationship to breadmaking quality. Some new results in this context are presented and discussed in this thesis.

III. MATERIALS AND METHODS

A. Materials

1. Canadian Wheat Cultivars

Grain of four Canadian wheat cultivars was selected for this study: Glenlea (HMW glutenin subunits composition 2*, 7+8, and 5+10), a Canada Utility wheat cultivar with overly strong dough strength for breadmaking (under Canadian baking conditions), HY320 (1, 7+8, and 2+12), a Canada Prairie Spring wheat of weak dough strength, Katepwa (2*, 7+9, and 5+10), a Canada Western Red Spring wheat with strong dough characteristics and optimum breadmaking quality, and Norstar (1, 7+8, and 5+10), a Canada Western Red Winter wheat with strong dough characteristics but somewhat weaker than those of Katepwa. Each wheat sample was tempered (15.5% moisture) and milled into straight flour on a Buhler pneumatic experimental mill. Flour extraction ranged from 67%-76%. Each wheat has physical and baking characteristics typical of its respective wheat class upon characterization (for characterization data, see Appendix I). The nomenclature of Payne and Lawrence (1983) was used for the HMW glutenin subunits throughout this thesis.

2. Australian *Glu-1* Null Wheat Samples

Seed of eight *Glu-1* null wheat lines used in this study, named from A1 (HMW glutenin subunits composition 1, 17+18, and 5+10) through A8 (all *Glu-1* loci are null), was provided by Dr. F. MacRitchie of C.S.I.R.O. Grain Quality Research Laboratory, Sydney, Australia. The lines

were grown on an experimental field of the University of Manitoba to produce the grain required for the study. The lines were originally derived from crosses between a mutant line of the cultivar Olympic null at the *Glu-B1* locus due to a deletion of part of the long arm of chromosome 1B, and an isogenic line of the cultivar Gabo null at the *Glu-A1* and *Glu-D1* loci (Lawrence *et al.* 1988). The *Glu-A1* null is found frequently in wheat cultivars, and the *Glu-D1* null is associated with a chromosome translocation, either lack of expression is due to a position effect or a deletion associated with the translocation.

Listed in Table 1 is the composition of the HMW subunits of glutenin for the *Glu-1* null lines, according to the report by Lawrence et al. (1988). The characterization data of these wheat lines are in Appendix II.

TABLE 1. The HMW Glutenin Subunit Composition of the Australian *Glu-1* Null Wheat Lines

Wheat line/ Sample name	HMW glutenin subunits[a]		
	Glu-A1	*Glu-B1*	*Glu-D1*
A1	1	17+18	5+10
A2	1	----	5+10
A3	1	17+18	---
A4		17+18	5+10
A5	--	----	5+10
A6	--	17+18	---
A7	1	----	---
A8	--	----	---

[a] According to Lawrence *et al.* (1988)

3. Chemicals

The urea used was from Sigma (Sigma Chemical Co., St. Louis, MO) and used without further purification. Sodium dodecyl sulfate (SDS), electrophoresis purity, was obtained from Bio-Rad Laboratories (Richmond, CA).

Dithiothreitol (DTT), a disulfide reducing agent, and N-ethylmaleimide (NEMI), a sulfhydryl blocking agent, were used in farinograph mixing studies; both were obtained from Sigma Chemical Co. (St. Louis, MO).

All chemicals used were of reagent grade.

B. Methods for Chemical Analysis

1. Protein Determination

Total protein was determined by the micro-Kjeldahl method 46-13 (AACC 1983) for nitrogen. The protein contents of the flours, glutens, or glutenin preparations, were obtained by multiplying the nitrogen content by the conversion factor of 5.7 (Tkachuk 1969). The protein contents of flours are 12.1, 9.8, 12.7, and 9.8% for Glenlea, HY320, Katepwa, and Norstar, respectively. For the Australian *Glu-1* null wheats labelled as A1 through A8, the protein contents of the flours are 9.3, 9.4, 9.6, 8.2, 10.0, 7.8, 10.0, and 10.4%, respectively. The fluctuation in protein content of the samples was caused by the variability in vitality of the lines due to the null form of some genes (Lawrence *et al.* 1988)

2. Amino Acid Analysis

The hydrolysis procedure for amino acid composition analysis of the glutenin preparations was performed under vacuum for 24 h in 6N HCl with 1% phenol addition as a protectant for amino acids. A sample of the original glutenin was hydrolyzed at the same time for comparison in the case of residue protein assay. The amino acid composition was determined on an automated LKB analyzer which uses the ion exchange separation procedure and ninhydrin detection and quantization method (Andrews and Baldar 1985).

C. Preparation and Solubilization of Glutenins

1. Preparation of Glutenins by Osborne Fractionation Procedure

Wet glutens were prepared from the flours according to AACC method 38-10. The wet gluten balls were immediately used for the preparation of glutenin, according to the Osborne fractionation procedure. The cohesive mass was cut into small pieces in each extraction. After sequential extraction with 0.5M NaCl and 70% ethanol (Chen and Bushuk 1970), the residual proteinaceous mass, termed total glutenin fraction or glutenin hereafter unless otherwise indicated, was freeze-dried, ground to pass a 16 mesh sieve (1.19 mm opening), and stored in a refrigerator in sealed containers.

The yields of glutenin protein (Nx5.7) from the Canadian wheats as a proportion (%) of the total protein content of the flours were 63.0, 57.2, 53.0, and 45.6% for Glenlea, HY320, Katepwa and Norstar, respectively. The protein (Nx5.7; d.b.) contents of the four glutenin fractions were 70.0, 66.7, 60.9, 77.4%, respectively.

The yields of glutenin protein as a proportion of total flour protein from the Australian cultivars from A1 through A6 were 55.0, 53.2, 45.6, 53.7, 41.8, and 38.2%, respectively. The yields of gluten, and glutenin, for A7 and A8 samples were extremely variable under normal washing conditions. The glutenin protein yields were 16.97 and 7.77% for A7 and A8 with the normal procedure (AACC method 38-10), and 41.6 and 32.3% for A1 and A8 with a more careful operation, respectively. The protein (Nx5.7; d.b.) contents of the final glutenin preparations from the Australian flours were quite close to each other, ranging from 68.7% (lowest) for A8 to 77.2 % (highest) for A5.

The nature of the non-protein constituent of these crude glutenin preparations was not investigated.

The glutenin protein yields from wet gluten of all the wheats used in the preparation of glutenin are slightly higher than the values obtained by direct fractionation of flour proteins (Chen

and Bushuk 1970). It appears that some non-glutenin proteins are "aggregated" with the glutenin during dough-making and gluten-washing in the fractionation procedure used in the present study. The term "aggregated or aggregation" as used in this thesis refers to formation of larger particles through non-covalent interactions.

2. Solubilization of Glutenin in Urea/SDS Solvent

a. Solvent Preparation

The solvent, 6M urea containing 6% (w/v) SDS, was prepared by making a solution of urea and then adding the SDS.

b. Solubilization Procedure

In the solubilization procedure, glutenin (*e.g.* 12 mg) was weighed into 15 ml centrifuge tubes and the solvent (5 ml) was added. The tubes were then vortexed for 25 s on a Vortex-Genie mixer (Scientific Industries, Inc. Bohemia, NY) at a setting of 10 to disperse any lumps, sealed, and placed in a water bath at 50°C and allowed to stand for the desired solubilization time.

After incubation the tubes were vortexed again and centrifuged at 20,000xg at 40°C for 30 min to remove a small amount of insoluble material. The clear supernatant and the presence of a firm pellet in the centrifuge tube was taken as an indication that the centrifugal speed was sufficient to separate undissolved material from the solution. The supernatant was pipetted into a test tube and allowed to cool to room temperature. No cloudiness was observed after this step. An aliquot of the cooled supernatant was taken for measurement of absorbance at 280 nm for protein determination from the standard curve (see below).

c. Development of Standard Curve for Protein Determination

The standard curve for determination of solubilized protein as a function of time was prepared by plotting the absorbance at 280 nm of supernatants containing 5, 10, 15, 20, or 25 mg of

glutenin solubilized for 24 hr at 50°C as described above. The 24 hr solubilization time was adopted on the basis of kinetic measurements to be described later.

The amount of protein solubilized for each data point in the standard curve was calculated by subtracting the amount of protein in the insoluble residue (see below) after centrifugation. Because of small but significant intervarietal differences, a separate standard curve was prepared for each cultivar. Each point in the standard curves represents the average of three determinations. The r^2 value of the linear regression of absorbance versus protein concentration for the curves was equal to or greater than 0.99.

The amount of protein in the insoluble residue (which contained urea and SDS) after centrifugation was determined using amino acid analysis. The residue was washed with distilled water (3x2 ml) in an attempt to remove the solvent and the dissolved but entrapped protein. The conditions for vortexing (once every washing) and subsequent centrifugation in the washing procedure were the same as those for solubilization except that no incubation was involved and every step was at room temperature (22-25°C). After washing, the residue was carefully transferred into hydrolysis tubes for protein hydrolysis. A glutenin sample control of known protein content (Glenlea glutenin) without any treatment was hydrolyzed with the residue samples as a control. It was assumed that the washing procedure did not dissolve any of the previously undissolved proteins. The amount of proteins in the residue was used to calculate the rate of solubilization in the development of the standard curve.

d. Determination of Solubilized Proteins as a Function of Time

Glutenin (12 mg) was used for the determination of the amount of protein solubilized as function of time, following the procedure under the section "Solubilization Procedure". The amount of solubilized glutenin protein for a specific set of experimental conditions was estimated from the "standard curve", which was calibrated by the assay of residue protein obtained from the solubilization procedure.

D. Gel Filtration Chromatography

1. The Gel Filtration Conditions

Sephacryl S-500, in dispersed form (Pharmacia, Uppsala, Sweden), was equilibrated with the solvent of 6M urea/6% SDS by repeated change of the solvent. The gel slurry was then poured into a column (2.6x100 cm). The same solvent was used for elution. The column for gel filtration chromatography was maintained at 40 °C by circulating water through the jacket around the column.

The column was operated by downward flow and the elution was monitored continuously at 280 nm. The elution rate was *ca.* 14 ml/hr for optimum resolution, according to the recommended value in the manual titled "Gel Filtration, Theory and Practice" (Laboratory Separation Division, Pharmacia, 1987). Fractions were collected with an LKB 2211 Superrac automatic fraction collector.

Before and after the gel filtration of the samples from flour, the column was calibrated with three standards, thyroglobulin (bovine), of MW 669 kDa, apoferritin (horse spleen), of MW 443 kDa, and albumin (bovine serum), of MW 66 kDa. No observable change in elution volume was observed for duplicate runs. At the end of the experiment, the column was washed with water and sodium hydroxide solution; no protein was detected by SDS-PAGE in the eluate collected from the two times of consecutive washing.

2. Sample Preparation for Chromatography

In sample preparation for application on the gel filtration column, flour or freeze-dried dough (100 mg), or gluten or glutenin (30 mg Kjeldahl protein) was weighed into a 15 ml centrifuge tube, and the following steps were carried out as under the section "Solubilization of Glutenin", with slight modification. After the vortexing following the standing in water bath for 24 hr and prior

to centrifugation, the sample intended for application on the column was overlaid in the centrifuge tube with a layer of hexane (Payne and Corfield 1979). After centrifugation, a syringe was used to penetrate the hexane layer into the supernatant, and all of the supernatant was taken and immediately the same amount of supernatant was applied onto the column for chromatography.

3. Fraction Collection and Purification

The eluate was collected on a drop basis (200) from the elution of gel filtration. The portion of the eluate collected in the tubes was pooled according to the profile of the chromatogram (on which the recorder strikes a mark each time the collection tube changes), as indicated in the figures under the section "Results and Discussion". The pooled fractions were concentrated with an ultrafiltration apparatus (molecular weight cut-off, 5 kDa, from Amicon Corporation, Lexington, MA) to remove most of the urea, and dialyzed (molecular weight cut-off, 5 kDa, from Medical Industries, Inc., Los Angeles) against distilled water. The dialysis was carried out in the first two days at room temperature, and then in a refrigerator until no foam was formed when the dialyzate was poured into the sink. The contents of the dialysis bag were transferred into plastic containers for freeze drying. The freeze-dried material was carefully transferred into containers, sealed, and stored in a freezer until used for SDS-PAGE analysis.

E. Electrophoretic Analysis

1. The General Procedure of SDS-PAGE

SDS-PAGE was carried out on an LKB 2001 unit according to a modified Laemmli procedure of Ng and Bushuk (1987) under both unreduced and reduced conditions (with 5% 2-mercaptoethanol, in extracting buffer) where applicable. The stacking and the separating gel concentrations were 3.03% and 17.3%, respectively. Electrophoresis was carried out initially for

45 min at 35 mA followed by 18 hr at 15 mA. Electrode buffer was circulated during operation of electrophoresis, and maintained at 20°C by the use of circulating water.

2. Sample Preparation for SDS-PAGE

a. Solubilized Protein by Urea/SDS Solvent

In the SDS-PAGE for the protein in the solubilization of glutenin experiment, all samples applied to the gel were aliquots of the supernatant from the solubilization of 5 mg glutenin protein in 1 ml of the urea/SDS solvent. Analyses were carried out with and without reduction. SDS-PAGE patterns of the flour extract of each wheat cultivar (controls) were obtained with the procedure of Ng and Bushuk (1987).

b. Residue Protein after Solubilization by Urea/SDS Solvent

For SDS-PAGE of proteins in the residue after extraction with 6M urea/6% SDS, glutenin (30 mg Kjeldahl protein) was weighed into centrifuge tubes, and the solvent (5 ml) was added for solubilization as described in "Solubilization of Glutenin". The residue obtained from this solubilization was washed once as described above for the amino acid analysis, and subsequently an appropriate amount of SDS-PAGE extracting buffer (with 2-mercaptoethanol) was added so that a protein concentration of 6 mg/ml (based on the solubilization rate of *ca.* 95% from preliminary chemical protein assay) was obtained for each slurry which was formed by vortexing from the residue. This was followed by the normal extraction procedure except that the tubes were centrifuged under 20,000xg to obtain the supernatant for electrophoretic analysis. Flour controls were prepared according to the normal procedure except that the amount of the flour used was adjusted so that the same protein concentration (6 mg/ml) in the slurries was obtained. In the SDS-PAGE analysis, 6 μl of supernatant of each residue or control flour sample was applied onto the gel. Electrophoresis was terminated when the tracking dye just came off the gel so that the

protein components of low molecular weight remained on the gel. The following steps remaining for SDS-PAGE are the same as in the "General Procedure".

c. Subunit Composition of Dimeric Bands

For the determination of subunit composition of the dimeric bands, the region containing the bands was excised with reference to a flanking lane which had been previously cut out and stained as in the conventional SDS-PAGE procedure. The gel piece containing the bands was subsequently ground in a mortar and pestle, extracted with SDS-PAGE extracting buffer (with ME), and centrifuged at 20,000xg for 30 min. The supernatant was analyzed by SDS-PAGE.

d. Two-Step SDS-PAGE

Separation of proteins by two-step SDS-PAGE was performed as described by Singh and Shepherd (1985, 1988). In the first step, unreduced protein extracts were loaded on a 1.0 mm thick gel for electrophoretic analysis. To determine the subunit composition of resolved proteins, a gel lane of the unreduced extracts was cut out from the gel of the first run. The gel piece cut out for the second run was equilibrated in a solution containing 2% (v/v) 2-mercaptoethanol for one and a half hour at 37°C, and subsequently loaded as the stacking gel onto a thicker separating gel (1.5 cm) for the second step. Electrophoresis of the reduced proteins in the gel piece was carried out as described in the "General Procedure" section above.

e. Fractions from Gel Filtration

Sample preparation of fractions collected from the gel filtration column followed "General Procedure", with the size of the sample adjusted so that the subunits in the fractions gave bands of reasonable intensity for visibility and resolution in SDS-PAGE, based on subjective judgement. When same amount of material from the freeze-dried fractions was used for SDS-PAGE, the materials from the excluded peaks were found in preliminary experiment to have quite low protein content (based on visually observed intensity of the bands), and accordingly the volume of the

extracts of the SDS-PAGE extracting buffer applied onto the gel well was doubled. The samples from the second and third fractions had higher protein content and the volume of extracts used for electrophoresis was halved, relative to the ordinary size.

F. Farinograph Mixing for Structural Studies of Glutenin

1. Mixing Under Air

Fifty grams of flour (cultivar Katepwa) and 32.3 ml of water were mixed into a dough with 0 (control), 20, 80, 500, and 3,000 μmol DTT. Immediately after mixing for 20 min, subsamples of the doughs were collected, frozen, freeze-dried, and ground manually in a mortar and pestle. The ground samples were stored in sealed containers in a refrigerator for SDS-PAGE analysis.

2. Mixing Under Nitrogen (N_2)

Fifty grams of flour and 3,000 μmol DTT were placed in a farinograph mixing bowl, gently flushed with nitrogen (N_2) with frequent stirring for 20 min, and then water (32.3 ml) was added and mixing was continued under nitrogen for 20 min. Analogous doughs were prepared to which 500 or 2,000 μmol NEMI was added after 3 min of mixing. Doughs with or without NEMI were collected immediately, sealed in a container which had been flushed with nitrogen, frozen, and freeze-dried. After freeze-drying, the freeze-drier was brought to atmospheric pressure by adding nitrogen through the bleeding valve. Samples were sealed and removed from the freeze-drier, and stored in a freezer for SDS-PAGE analysis.

IV. RESULTS AND DISCUSSION

A. Solubilization of Glutenin

1. Extraction Based on Residue Protein Assay

The amount of protein extracted in the supernatant by 6M urea/ 6% (w/v) SDS solvent, after 24 hr at 50°C (Table 2) comprised essentially all of the protein in the crude glutenin preparations. This level of solubility was considered sufficient to provide soluble (unreduced) glutenin that was representative of the total glutenin in the wheat cultivar.

TABLE 2. Solubilization of Protein from Glutenin Fraction by 6M Urea/6% SDS after Incubation for 24 hr at 50°C

Wheat cultivar	Extraction[a] (%)
Glenlea	>99
HY320	>99
Katepwa	>99
Norstar	>99

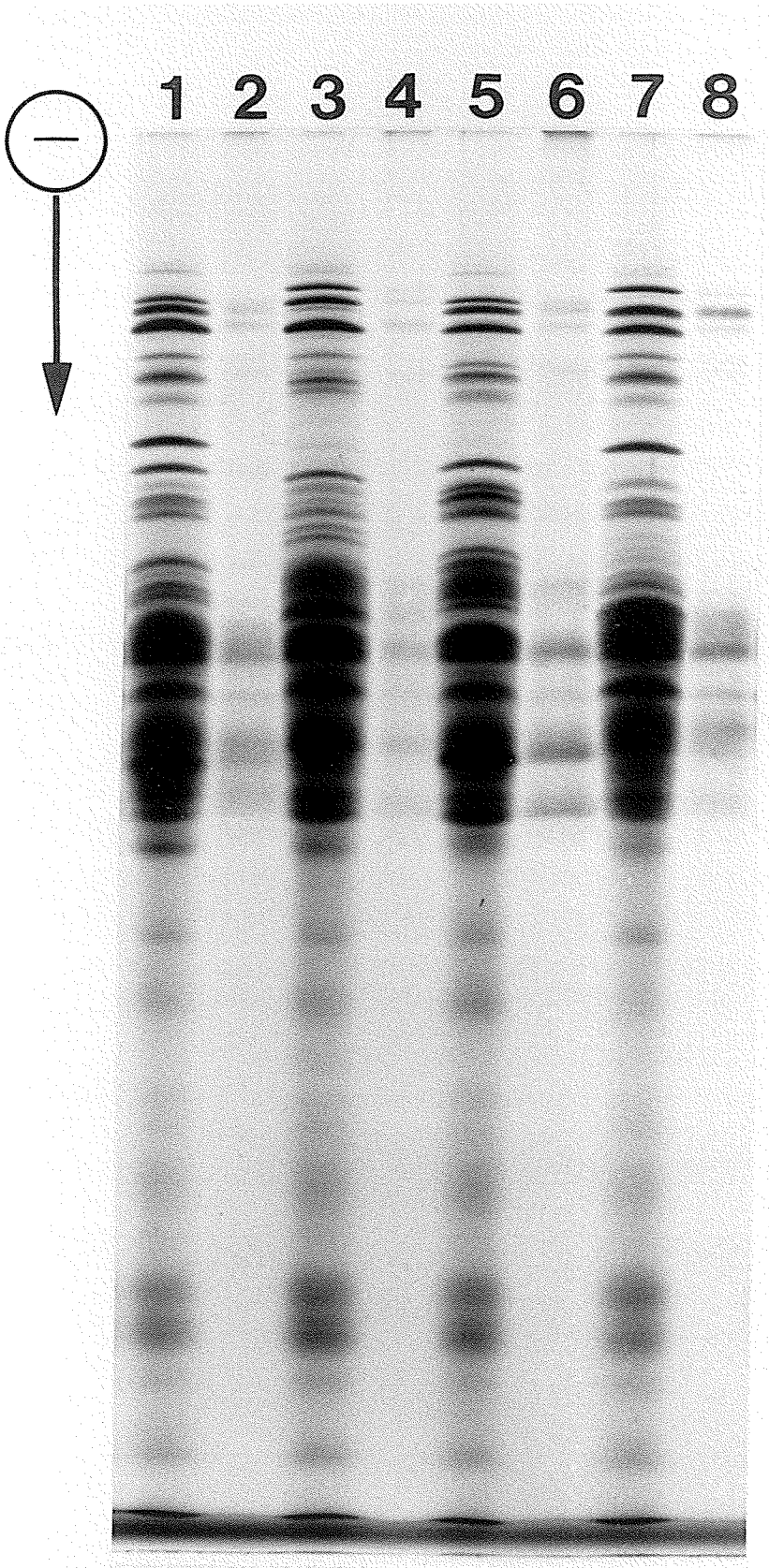
[a] Based on the sum of the amino acids determined by an amino acid analyzer for the residue after solubilization by the urea/SDS solvent. The glutenin control had a calculated recovery of 97%

When it was assumed (based on preliminary assay for protein in the residue) that *ca.* 95% of the protein was extracted and SDS-PAGE was used to examine the protein type and approximate amount with equivalent amount of flour proteins, the results (Fig. 1) indicated that much less glutenin was found for the residue, which is generally consistent with the amino acid assay of the

Figure 1. SDS-PAGE patterns of proteins (reduced) in the residues after the solubilization procedure of glutenins. The same volume (6 μl) of supernatant was applied onto each lane.

Lanes 1, 3, 5, and 7, flour control of Glenlea, HY320, Katepwa, and Norstar, respectively;

Lanes 2, 4, 6, and 8, proteins in residue of glutenin from Glenlea, HY320, Katepwa, and Norstar, respectively.


1 2 3 4 5 6 7 8
−

residue (Table 2). It was found, however, that the slight amount of protein in the residue was in the form of both HMW and presumably LMW glutenin subunits. This is consistent with the amino acid composition of the residue protein which contains a high proportion of glutamic acid and glycine as well as proline (data in Appendix III). These results are generally in agreement with those of Bottomley *et al.* (1982) who reported that the residue of gluten and flour after extraction by a SDS buffer (pH 8.0) contained glutenin as indicated by SDS-PAGE results under reduced conditions.

2. Effect of Duration of Extraction at 50°C

The solubilization of glutenin protein at 50°C as a function of time for the four wheat cultivars (Fig. 2) showed significant intercultivar differences that appear to be related to "dough strength" of the wheat flours (see Appendix I for characterization data). This relationship needs to be confirmed by further experiment with more wheat samples of known baking characteristics, which is beyond the scope of this study and so was not carried out here. This observation is generally consistent with the result of He *et al.* (1991) who showed that the protein of a poor breadmaking quality flour was more readily soluble in 1% SDS solution than that of a good quality flour; the solubility increased with extraction time for both flours. It should be noted that maximum solubilization for all four cultivars was obtained after a 24 hr solubilization period.

3. The Extraction Procedure

Lower concentration of SDS in 6M urea and various concentrations of SDS in solutions without urea and lower temperature conditions were investigated. The solubility results varied substantially for glutenins of different wheat cultivars. Solubilizations obtained with the milder conditions were generally inferior to those obtained with 6% SDS in the 6M urea solution at 50°C. At room temperature, this solvent solubilized only 30% of the protein in the glutenin of Glenlea

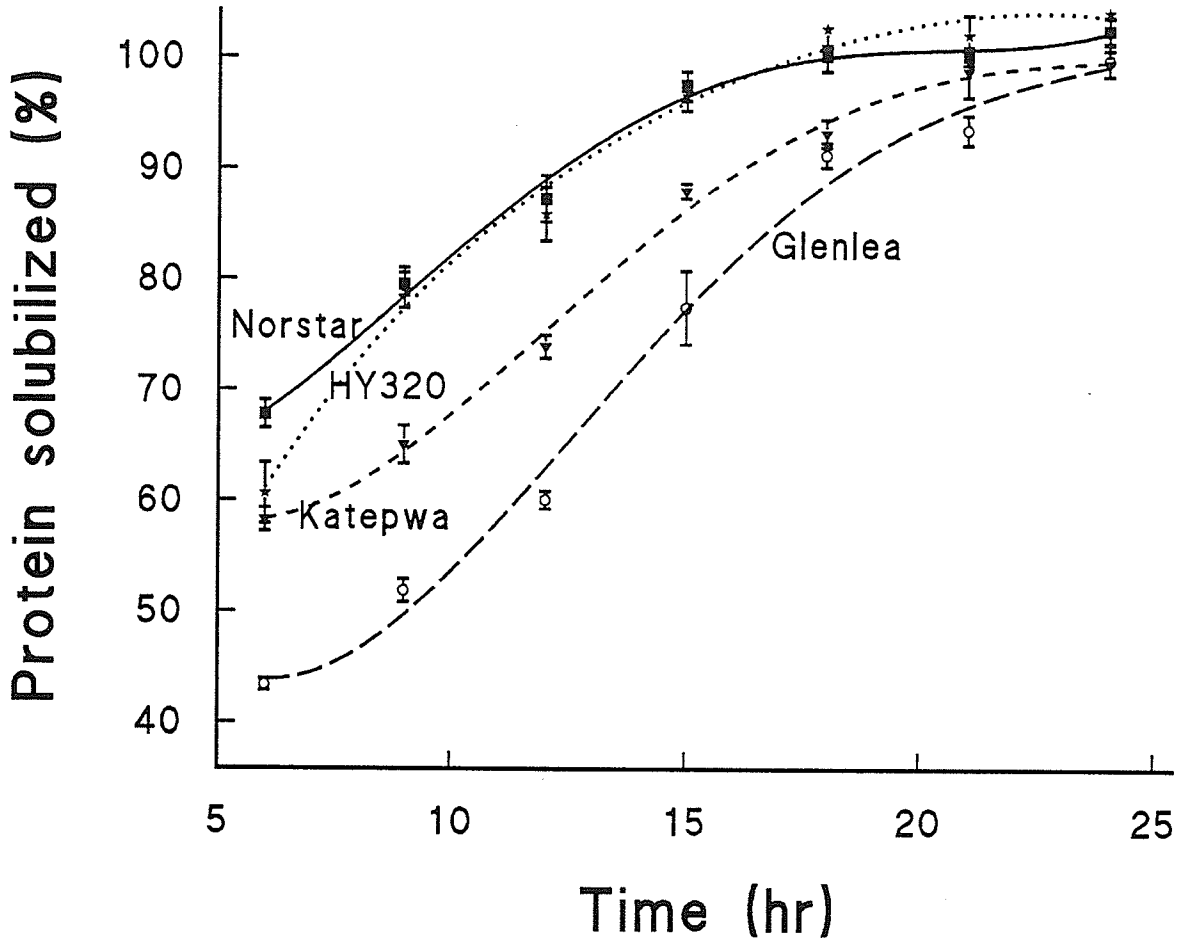


Figure 2. Solubilization of the protein of the Osborne glutenin fraction in urea/SDS solvent as a function of time of incubation at 50°C.

compared with almost complete solubilization at 50°C. Accordingly, for the comparative study of wheat samples and kinetics, the 6M urea/6% SDS solvent at 50°C was used for solubilization of glutenin. Vigorous mechanical stirring was avoided for solubilization in this study since it has been reported (Singh *et al.* 1990a) that the use of high energy dispersion forces such as sonication can cause the scission of covalent bonds in wheat protein (and presumably in glutenin).

The conditions for solubilization used in this study are probably more vigorous than those used by some other researchers who succeeded in solubilizing virtually all of the protein in flour or in gluten (*e.g.* Bottomley *et al.* 1982). However, those results are not directly comparable with the results reported here because the starting material from which gluten proteins were solubilized are different. It is known that it is more difficult to solubilize glutenin from a glutenin fraction than from glutens (Ewart 1980; Huebner and Wall 1980).

4. SDS-PAGE Results of Solubilized Glutenin

To investigate the possibility of disulfide (S-S) bond scission during solubilization over prolonged periods at 50°C, the solubilized glutenin was analyzed by SDS-PAGE for possible liberation of glutenin subunits. Accordingly, electrophoresis was carried out on unreduced and reduced solubilized glutenin. Emphasis was on the high molecular weight (HMW) subunits which can be easily identified by SDS-PAGE.

The results indicated that there were no free HMW glutenin subunits in unreduced solubilized glutenin, as in Fig. 3 for two of the four wheat cultivars (lanes 1 and 2) that were analyzed. Some bands corresponding to the gliadins (Fig. 3, lane 1) are considered to be the carryover of gliadins due to incomplete extraction by 70% ethanol in the preparation procedure; extracts by regular SDS-PAGE extracting buffer (no ME) showed the same pattern (hence not shown). The results showed no evidence by SDS-PAGE of S-S scission to the extent that monomeric subunits were liberated. However, this evidence does not preclude the possibility of S-S scission and liberation of glutenin oligomers of lower average molecular weight (and hence higher solubility) than that

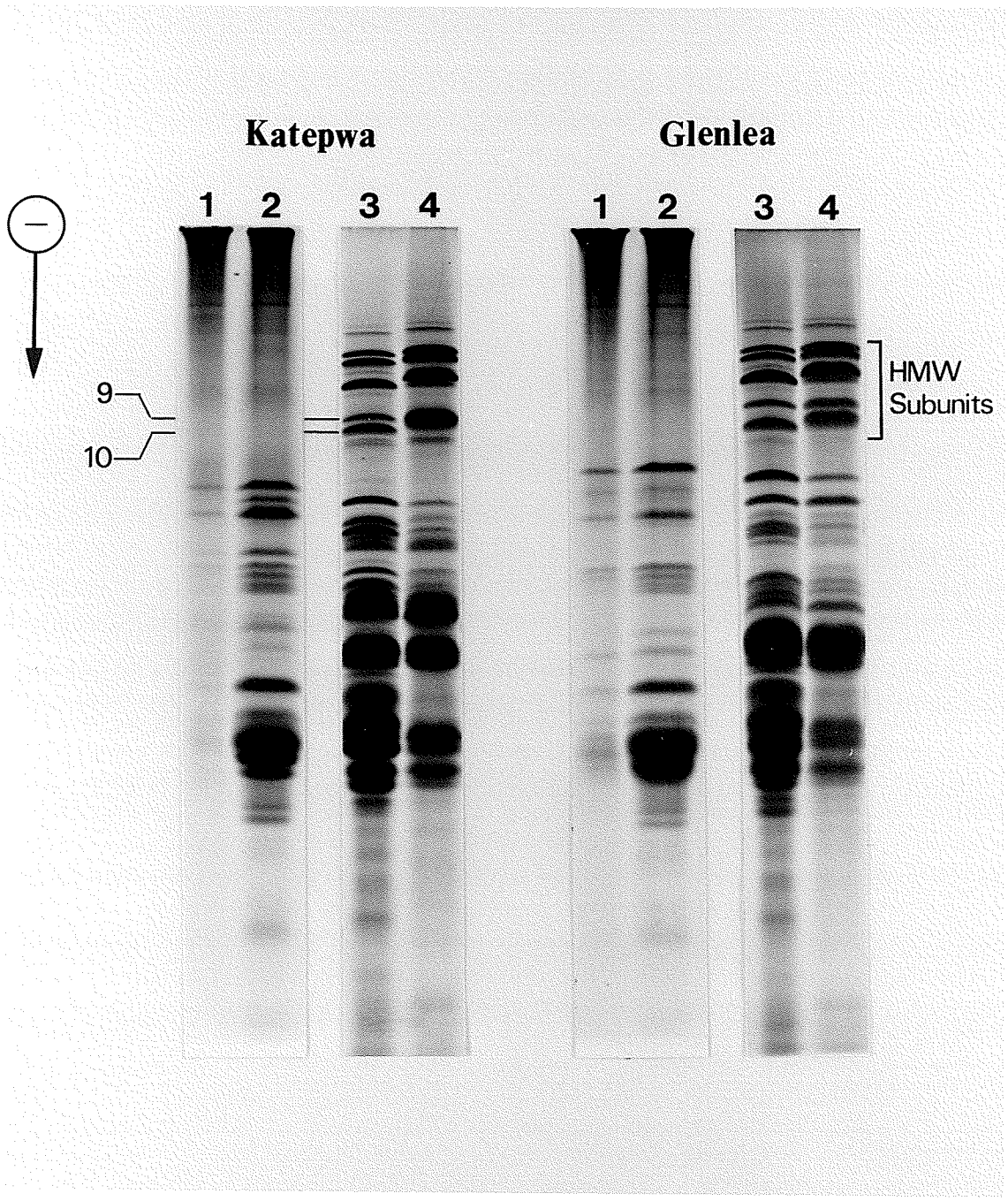
Figure 3. SDS-PAGE patterns of two wheat cultivars:

1, unreduced solubilized glutenin;

2, unreduced extract of flour;

3, reduced extract of flour;

4, reduced solubilized glutenin.


Katepwa
Glenlea
1
2
3
4
1
2
3
4
9
10
HMW
Subunits

of native (unreduced) glutenin. This possibility will be investigated by gel filtration chromatography in the following part of the project. The possibility that other covalent bonds are broken under conditions used in this study seems unlikely although it is possible that some covalent modification of the amino groups of the proteins could have occurred by the cyanate impurities in urea as suggested by the slight change of the mobilities of the glutenin subunits in SDS-PAGE (see below). This possibility remains to be confirmed by experiment.

SDS-PAGE results for reduced glutenin (Fig. 3, lanes 3 and 4) showed the same subunit composition as obtained for the solubilized glutenin and control flours for three of the four cultivars examined (results for one cultivar of this group, Glenlea, are shown in Fig. 3). However, for all cultivars, there was an overall slightly lower mobility for the reduced proteins from the solubilized sample in the SDS-PAGE, which is probably caused by carbamylation of amino groups in the presence of urea at the elevated temperature as suggested by Cole and Mecham (1966). For some unknown reason, the pattern of Katepwa glutenin, in contrast to that of the control flour, did not show a clear resolution of subunits 9 and 10, which are highlighted in Fig. 3. These two subunits are not present together in any of the other three cultivars used in this study.

5. Discussion on Solubilization of Glutenin

It has been shown that essentially all of glutenin protein prepared from wet gluten can be solubilized with a solvent comprising 6M urea and 6% sodium dodecyl sulfate by prolonged still incubation (about 24 hr) at an elevated temperature (50°C). There was no evidence from SDS-PAGE results that the increased solubilization involved scission of disulfide bonds. Scission of other covalent bonds under the conditions used also appears unlikely. Gel filtration in the following part of this research did not show significant changes in profiles of samples incubated for additional one or two days.

Kinetic experiments showed that the rate of glutenin solubilization appears to be related to "dough strength" of the wheat cultivar. This observation is consistent with the hypothesis that

dough strength, which derives directly from "gluten strength", in turn, depends significantly on the non-covalent aggregative forces between glutenin molecules (see Bloksma and Bushuk 1988 for general reference).

The SDS-PAGE HMW subunit composition of the solubilized glutenin was the same as that of the native glutenin of flour for three of the four wheat cultivars investigated. In the case of the cultivar Katepwa, subunits 9 and 10 were not resolved in the pattern of the solubilized glutenin by the SDS-PAGE procedure used in this study, but were resolved in the pattern of glutenin extracted directly from flour.

The solubilization procedure described in this part of the project should be useful for comparative studies and characterization of glutenin from different wheat cultivars in relation to its role in breadmaking quality.

B. Gel Filtration Chromatography Studies of Flours

It has long been suspected that the molecular weight distribution of flour proteins may be related to the breadmaking potential of the flours (Huebner and Wall 1976). However, contradictory results exist in the literature. This confusion is further aggravated in some studies by the fact that there was always some protein which was left unextracted and remained in the residue so that the molecular weight distribution of the proteins may not be representative of the proteins as a whole in wheat flour or dough. This part of the present study was an attempt to add more information to the current understanding of the molecular weight distribution/functionality since a solubilization procedure, which can solubilize essentially all of the glutenin fraction, was successfully developed in the first part of this project.

The gel filtration chromatography of solubilized protein from flour samples was carried out in an attempt to compare with results reported in the literature, especially with the results of Huebner and Wall (1976), and with those of glutenin in the present study.

1. Gel Filtration Profiles of Flour Extracts

Despite the diverse breadmaking quality of the flours used in this study, the profiles for the flours are generally all similar in shape, with an excluded peak followed by an initial continuous range of molecules up to the shoulder of the peak with highest absorbance in the profile (Fig. 4). After the highest peak, there seemed to be a relatively large peak composed of a broad range of molecules.

The dough strength of Glenlea is known to be much greater than that of Katepwa (Appendix I), but the extracted protein from the Katepwa flour seemed to have larger average molecular weight than that from the former, since the peak with the highest absorbance for Katepwa was eluted earlier than that of Glenlea (Fig. 4). however, the flour of Glenlea seems to have its highest peak eluted at about the same volume as those of Norstar and HY320, which are much weaker than Glenlea in terms of dough strength. Therefore, the distribution of protein (absorbance) did not seem to be consistent with the hypothesis that glutenin from stronger flours have more large molecules than the glutenin from weaker flours in the profile of molecular weight distribution.

According to Kasarda (1989) the first peak from gel filtration might be deceptive because the turbidity of the eluting solution at the void volume contributed to light absorption, and therefore gave the appearance of increased amount of protein. In this study, the fractions for the excluded peak did not look turbid to any extent. Therefore, the absorbance of the excluded peak did not seem to be from the turbidity of the eluate, but from protein or other ultraviolet light absorbing substances contained therein.

2. SDS-PAGE of Fractions from Gel Filtration

The SDS-PAGE results for fractions from the gel filtration of the varieties were also similar in the general distribution of the HMW glutenin subunits (Figs. 5, 6, 7, 8. Numbers 1-7 identify the fraction collected). It can be seen that the first fractions which corresponded to the excluded

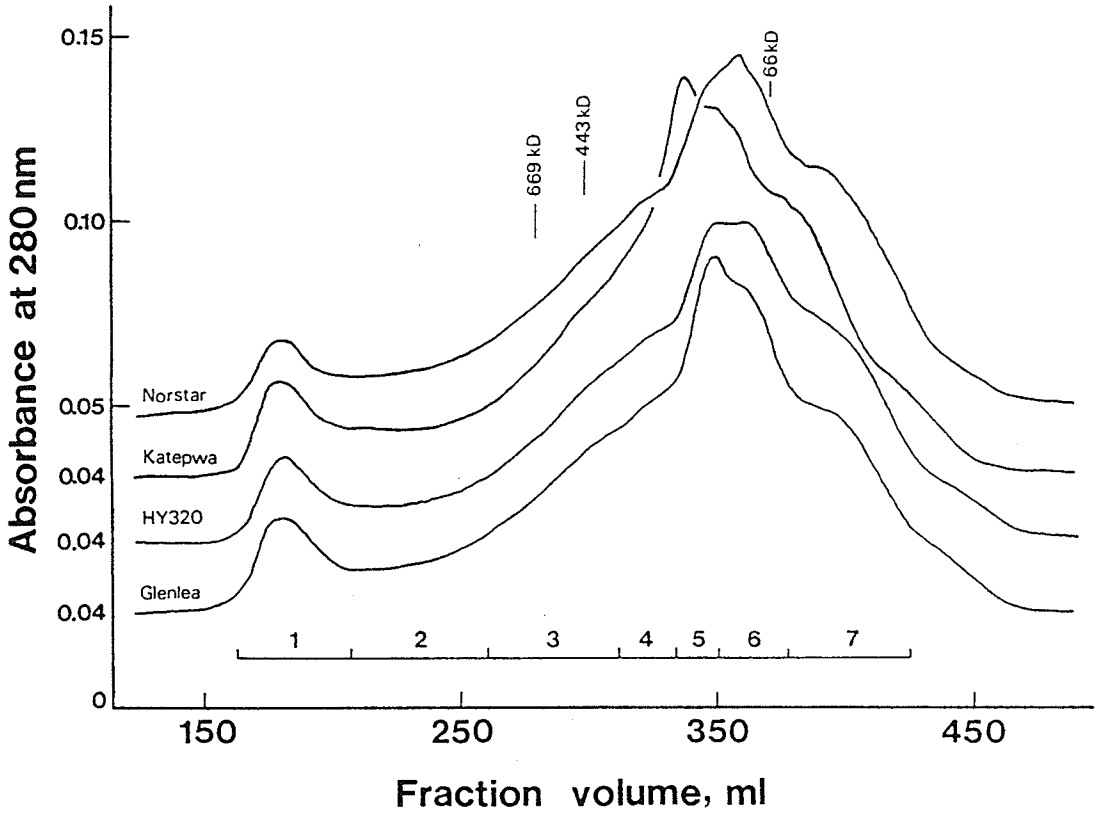


Figure 4. Gel filtration chromatography elution profiles of the Canadian flour samples (100 mg) on Sephacryl S-500 in 6M urea/6 % SDS solution.

Figure 5. SDS-PAGE patterns of the fractions (1-7) from gel filtration of Glenlea flour and of the flour control (Fl) under unreduced (A) and reduced (B) conditions.

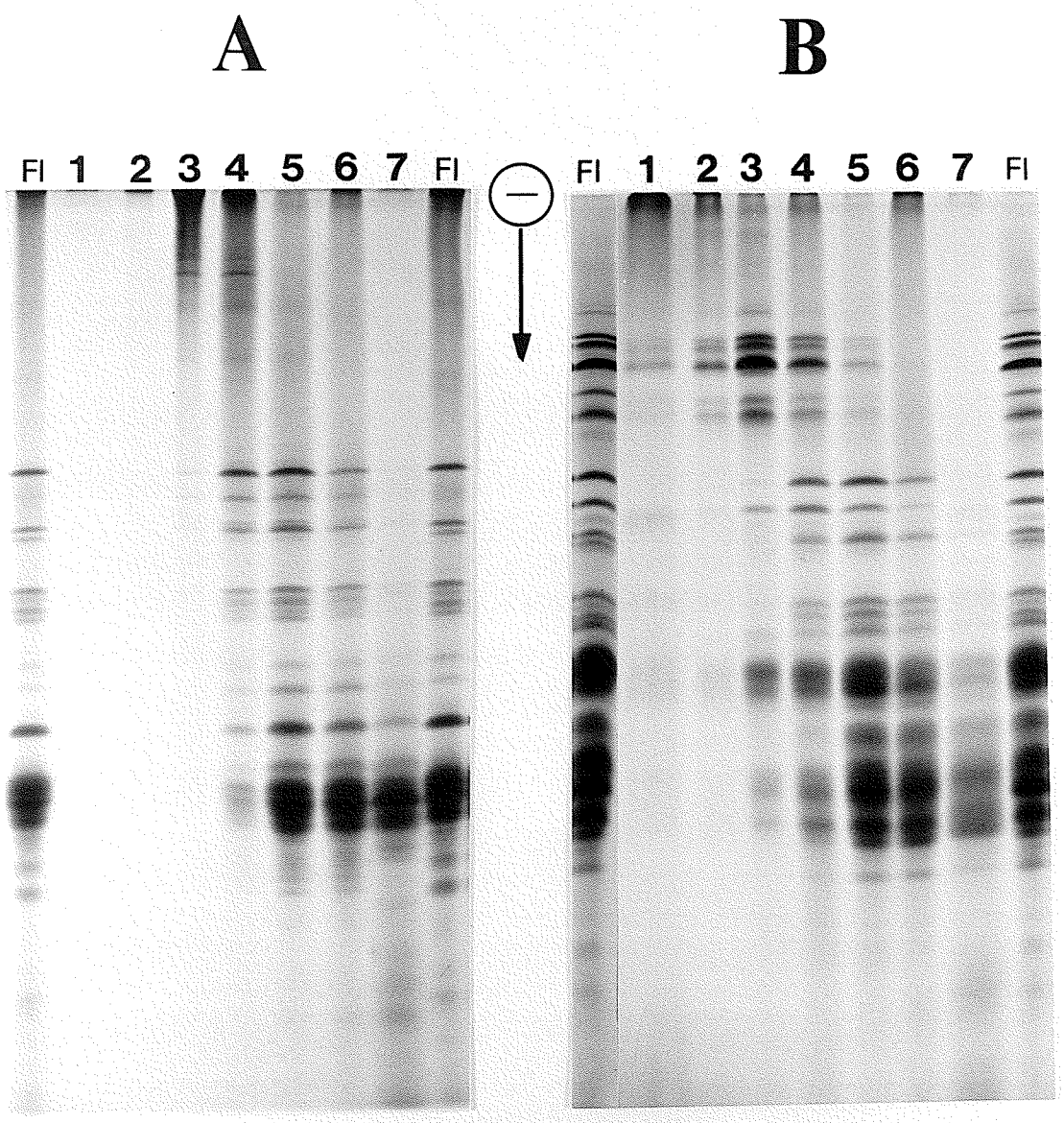

A
B
Fl 1 2 3 4 5 6 7 Fl
Fl 1 2 3 4 5 6 7 Fl

Figure 6. SDS-PAGE patterns of the fractions (1-7) from gel filtration of HY320 flour and of the control flour (Fl) under unreduced (A) and reduced (B) conditions.

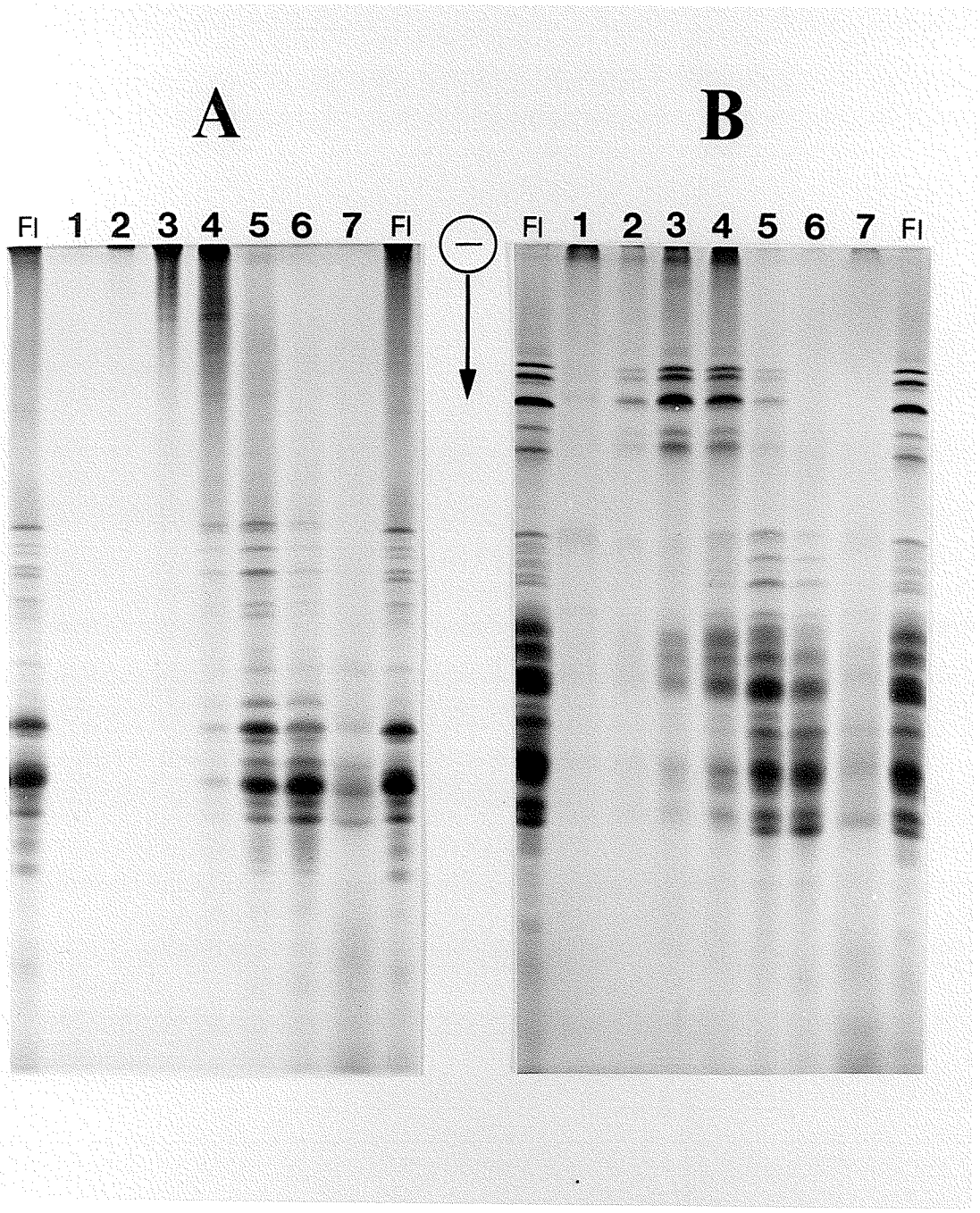

A
B
Fl 1 2 3 4 5 6 7 Fl
Fl 1 2 3 4 5 6 7 Fl

Figure 7. SDS-PAGE patterns of fractions (1-7) from gel filtration of Katepwa flour and of the control flour (Fl) under unreduced (A) and reduced (B) conditions.

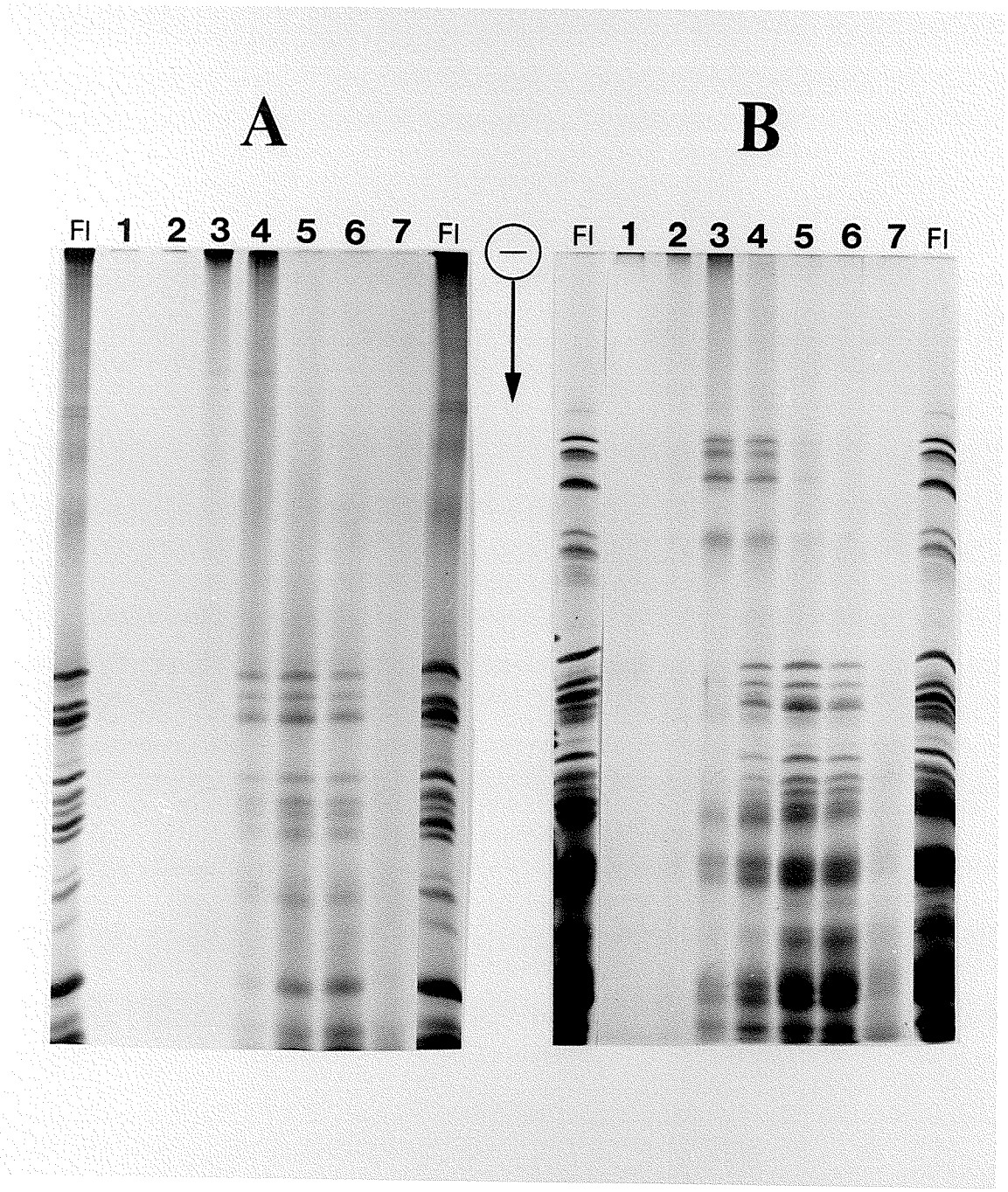
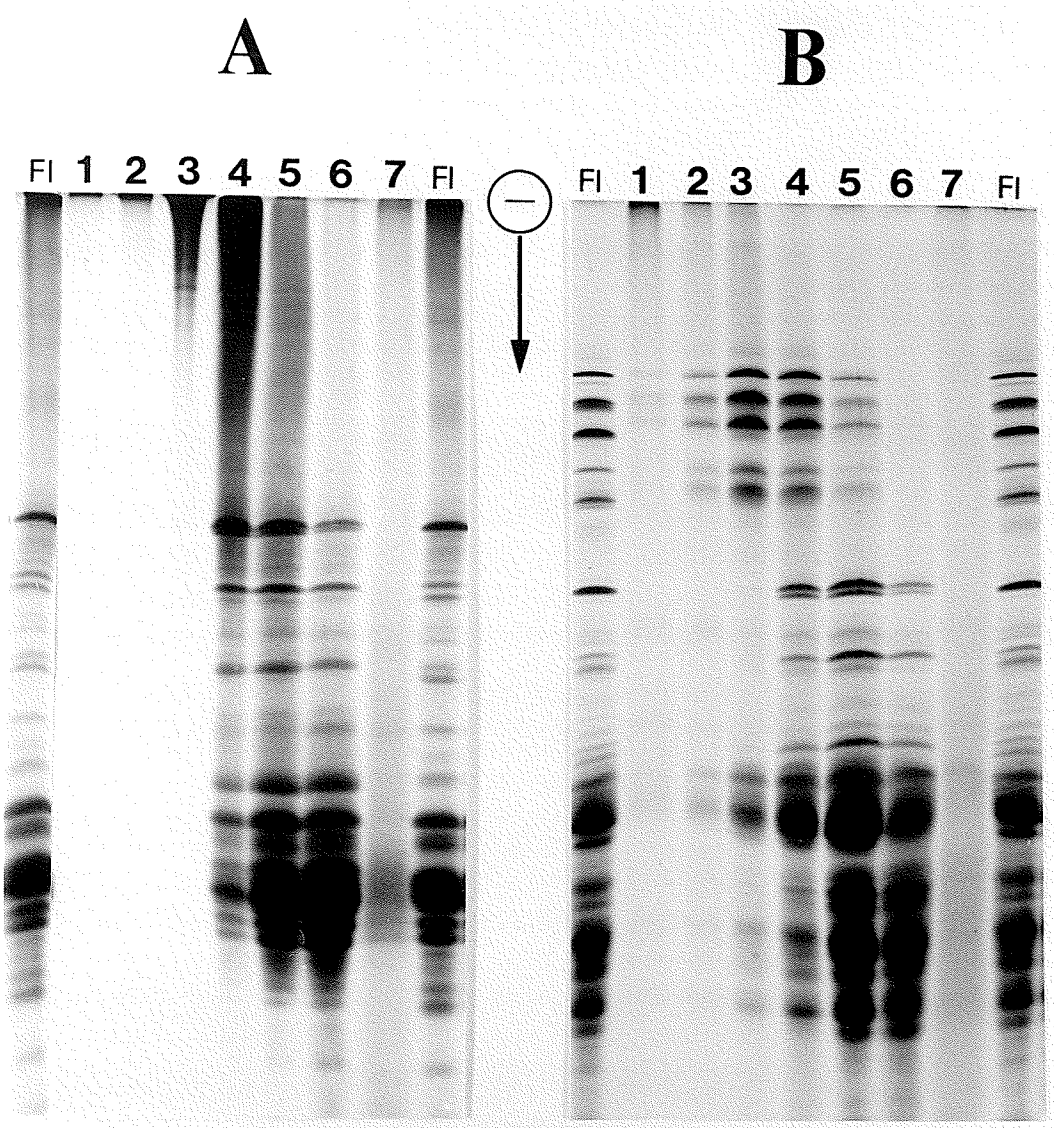

A
B
Fl 1 2 3 4 5 6 7 Fl
Fl 1 2 3 4 5 6 7 Fl

Figure 8. SDS-PAGE patterns of fractions (1-7) from gel filtration of Norstar flour and of the control flour (Fl) under unreduced (A) and reduced (B) conditions.


A
B
Fl 1 2 3 4 5 6 7 Fl
Fl 1 2 3 4 5 6 7 Fl

peak generally contained little protein as visually observed in the SDS-PAGE under reduced conditions even though the amount of sample applied onto the gel was twice the amount of the normal level. Most HMW glutenin subunits were mainly found in fractions 3 and 4, while the gliadins were in fractions 4, 5, and 6.

The distribution of the HMW glutenin subunits does not seem to be consistent with regard to its relationship to breadmaking potential of the flours. The first fraction from Glenlea, which is the strongest flour used in this study, contained the most HMW glutenin subunits as compared with the first fractions of other varieties (subjectively), which seems to be in a line with the idea that a strong variety has larger molecules of glutenin containing HMW glutenin subunits. However, the second fraction from Katepwa, which is an excellent breadmaking variety, contained the least amount of HMW glutenin subunits of all the varieties tested in this part of the study.

The fact that most of the HMW glutenin subunits were in fractions 3 and 4 indicated that the polymeric glutenin containing HMW subunits ranged from a few hundred thousand to about a few million daltons in terms of molecular weight, with the average in the order of half a million daltons. The fractions were eluted before the highest peak in the profile. Flours of wheat varieties of diverse breadmaking potential have quite similar molecular weight distribution of total proteins based on the elution profiles of gel filtration. Accordingly, gel filtration chromatography does not appear to be a useful technique for the detection of intervarietal differences in molecular weight distribution of glutenin in the context of its role in breadmaking potential. Perhaps columns with better resolution and monitored more specifically for proteins are required.

3. Discussion on Gel Filtration of Flour Extracts

It is known that wheat storage proteins can interact with each other and form aggregates and precipitate even in a detergent solution (Wasik *et al.* 1979). Increased temperature was found effective for improving the resolution of chromatography (Wrigley 1965), probably by decreasing the non-ionic interactions between the proteins. In this study, elevated temperature (40^{o}C) was

used in an attempt to keep the proteins from aggregating. Aggregation would result in a decreased resolution in gel filtration and cause small molecules to be eluted in the fractions for larger molecules. However, it is clear that none of the proteins in the first three fractions entered the SDS-PAGE gel under unreduced conditions (Figs. 5A, 6A, 7A, and 8A). This indicates that the non-covalent aggregation of small molecules did not take place in the solvent during gel filtration; this was probably achieved by the use of the elevated temperature (40°C) and the SDS detergent in urea solution in this study. However, the resolution was insufficient to separate protein fractions adequately to obtain individual peaks as reported by Huebner and Wall (1976). The major reason for lack of resolution is probably because this study used a medium of much higher exclusion limit which would have low resolution for the relatively small molecules like gliadins. Another possible reason for this could derive from the nature of wheat storage proteins, especially glutenins, which may comprise a range of large molecules. The molecular weight distribution of glutenin reported here is similar to that of Danno *et al.* (1990) in terms of the wide range of sizes.

It is interesting to note that the first fraction did not contain as much protein as suggested by the magnitude of the absorbance. This fraction was excluded from the column which in many studies, has been correlated with breadmaking quality. Therefore, when the first peaks were compared from one variety to another, what was compared was not only protein, but also (may be mostly) other flour constituents. Unfortunately, information on the amount of protein contained in the first peak has not been stipulated in most of the studies for the relationship between the molecular weight distribution and breadmaking quality (Field *et al.* 1983a; Bottomley *et al.* 1982; Huebner and Wall 1976). The actual protein content of the excluded peaks might differ from one study to another because of the difference in the exclusion limit of the gel filtration media used. The finding of the present study that the first fraction contained little protein is consistent with the results of Danno *et al.* (1990), and Danno and Hoseney (1982b).

When the dissolved glutenin sample was incubated for an additional one or two days, no differences in the gel filtration profiles were observed (results not shown). Therefore, it was concluded that protein disulfide bonds were not cleaved during solubilization or chromatography.

Because the excluded peak from gel filtration was implicated in breadmaking quality, it is necessary to examine how much and what type of protein was present in the fraction represented by this peak for samples of glutenin fractions and glutens. This part of the project is discussed in the following section.

C. Gel Filtration Chromatography of Glutenin Preparations and Glutens

1. Gel Filtration of Glutenins

The gel filtration profiles for the glutenin preparations showed two prominent peaks (Fig. 9), one excluded and the other, a broad peak centered at about 350 ml of elution volume. The shape of the second peak here is quite different from those of the profiles for the flours, which is, perhaps, due to the removal of the non-gluten proteins and most of the gliadins in the process of glutenin preparation.

The excluded peak appears to be significantly different in size from one variety to another, unlike those in the profiles of their flour. The glutenin from HY320 had the largest peak, followed closely by Glenlea and Katepwa. Norstar had the smallest excluded peak of all in Fig. 9. Therefore, the size of the excluded peaks does not seem to be consistently related to the dough strength of the varieties, and therefore, nor to their breadmaking potential.

In the elution profiles, the large broad peaks centered at about 350 ml of elution volume showed relatively small differences in terms of shape among varieties. Interestingly, on the shoulder of the broad peak in the profile of Katepwa glutenin, there seems to be an extra peak corresponding to fraction 7. Similarly, glutenin of Norstar also seemed to contain more protein (if judged by the higher absorbance) at the same elution volume as in that of Katepwa after the largest peak in the profile of Glenlea or HY320.

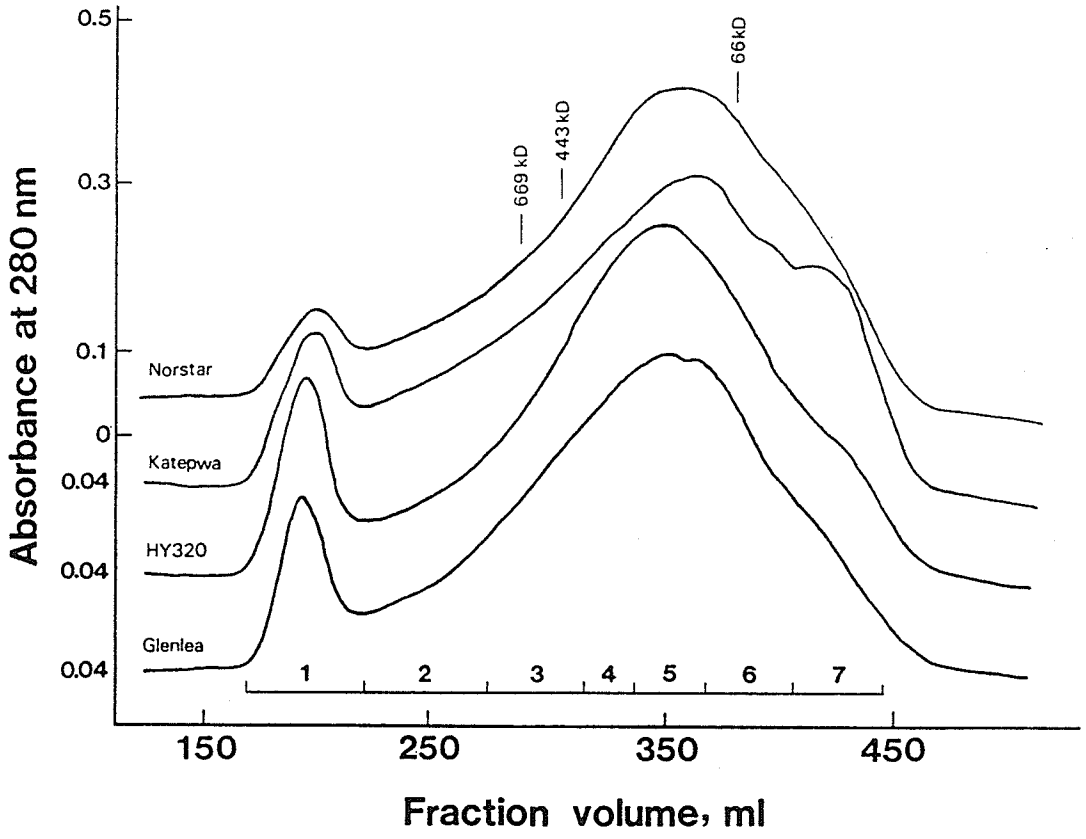


Figure 9. Gel filtration chromatography elution profiles of the glutenin preparations (30 mg Kjeldahl protein) from the Canadian flours on Sephacryl S-500 in 6M urea/6 % SDS solution.

2. SDS-PAGE Results

The electrophoretic results (Figs. 10, 11, 12, 13) showed different patterns from those for the fractions obtained from the elution of flour extracts (Fig. 4). Firstly, the HMW glutenin subunits distribution in the fractions from elution of glutenin samples seemed to have expanded over more fractions, from fraction 2 (fraction 1 for HY320) to fraction 6. Secondly, most of the HMW glutenin subunits seem to be concentrated in fractions 2, 3, 4, and 5, in contrast to only in fractions 3 and 4, for flour samples.

For wheat varieties HY320 and Katepwa, fraction 1 contained a considerable amount of HMW glutenin subunits in the SDS-PAGE under reduced conditions (Figs. 11B, 12B). while the same fractions from Glenlea and Norstar contained little HMW subunits (Fig. 10B, 13B). These results indicate that differences among the cultivars do exist in the molecular weight distribution of components of the glutenin fraction. The presence of HMW glutenin subunits in the first fraction collected from the elution of glutenin of a variety like Katepwa indicated that the polymeric glutenin is of higher molecular weight than the glutenin of such varieties whose first fraction does not contain any HMW glutenin subunits. The glutenin of HY320 also had HMW subunits in its first fraction while that of the varieties Norstar and Glenlea did not. Considering the breadmaking quality differences among these cultivars, it can be concluded that the presence of HMW glutenin subunits in the first fraction is not consistently correlated to the breadmaking potential of the flours.

It is interesting to note that for fractions 1, 2 and 3 collected from gel filtration of any of the glutenins, there were no bands corresponding in mobility to gliadins in the SDS-PAGE patterns under unreduced conditions (Figs. 10A, 11A, 12A, 13A). This result suggests that there does not seem to be aggregation of small molecules in the early (high MW) fractions, and is consistent with the result for the flours shown previously. These fractions showed many bands under reduced conditions (Figs. 10B, 11B, 12B, 13B), which is consistent with the idea of Payne and Corfield (1979) who concluded that glutenin polymers contain various subunits but in different proportions.

Figure 10. SDS-PAGE patterns of fractions (1-7) from gel filtration of Glenlea glutenin preparation and of the control flour (Fl) under unreduced (A) and reduced (B) conditions.

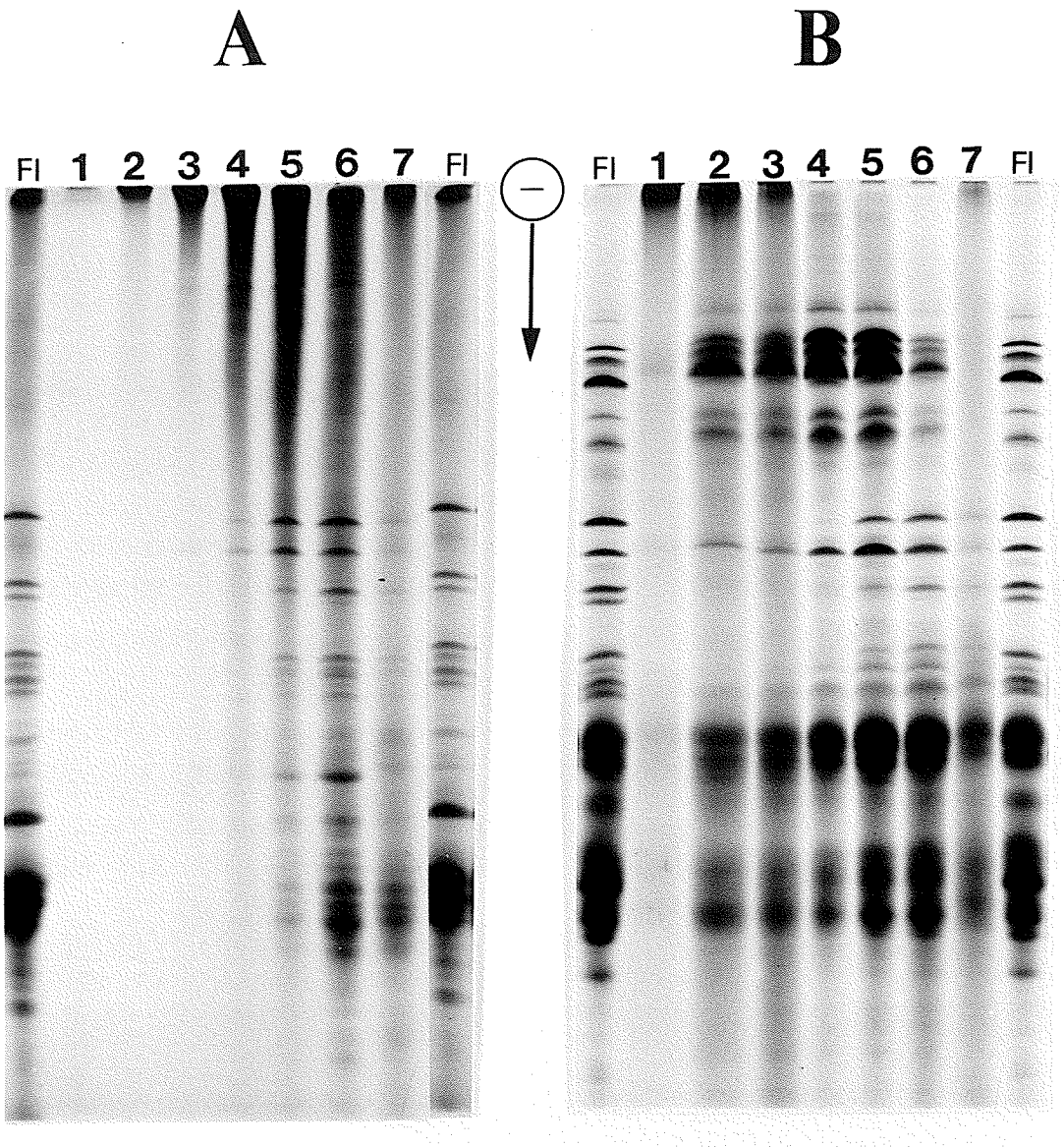

A
B
Fl 1 2 3 4 5 6 7 Fl
Fl 1 2 3 4 5 6 7 Fl

Figure 11. SDS-PAGE patterns of fractions (1-7) from gel filtration of HY320 glutenin preparation and of the control flour (Fl) under unreduced (A) and reduced (B) conditions.

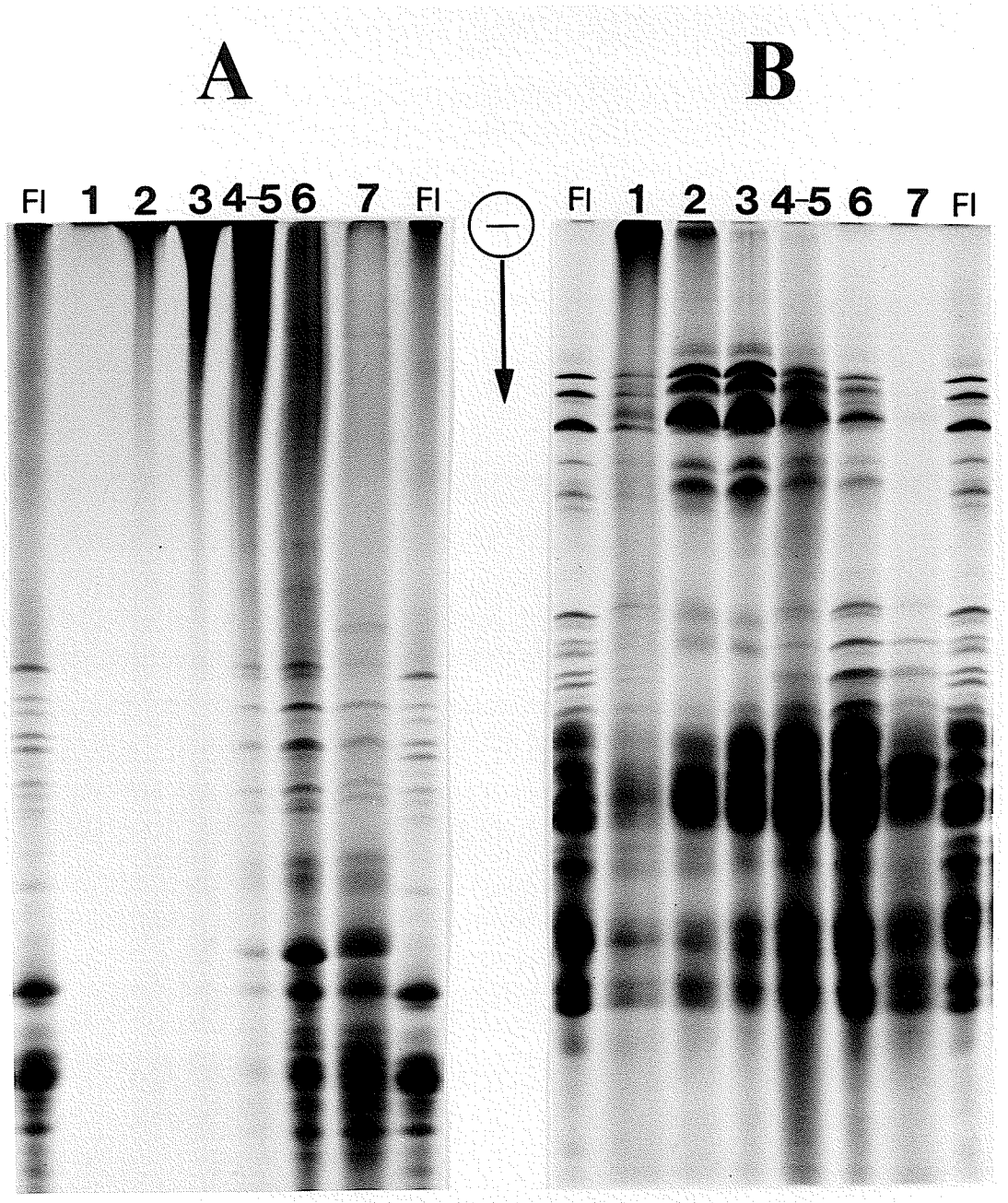

A
B
Fl 1 2 3 4 5 6 7 Fl
Fl 1 2 3 4 5 6 7 Fl

Figure 12. SDS-PAGE patterns of fractions (1-7) from gel filtration of Katepwa glutenin preparation and of the control flour (Fl) under unreduced (A) and reduced (B) conditions.

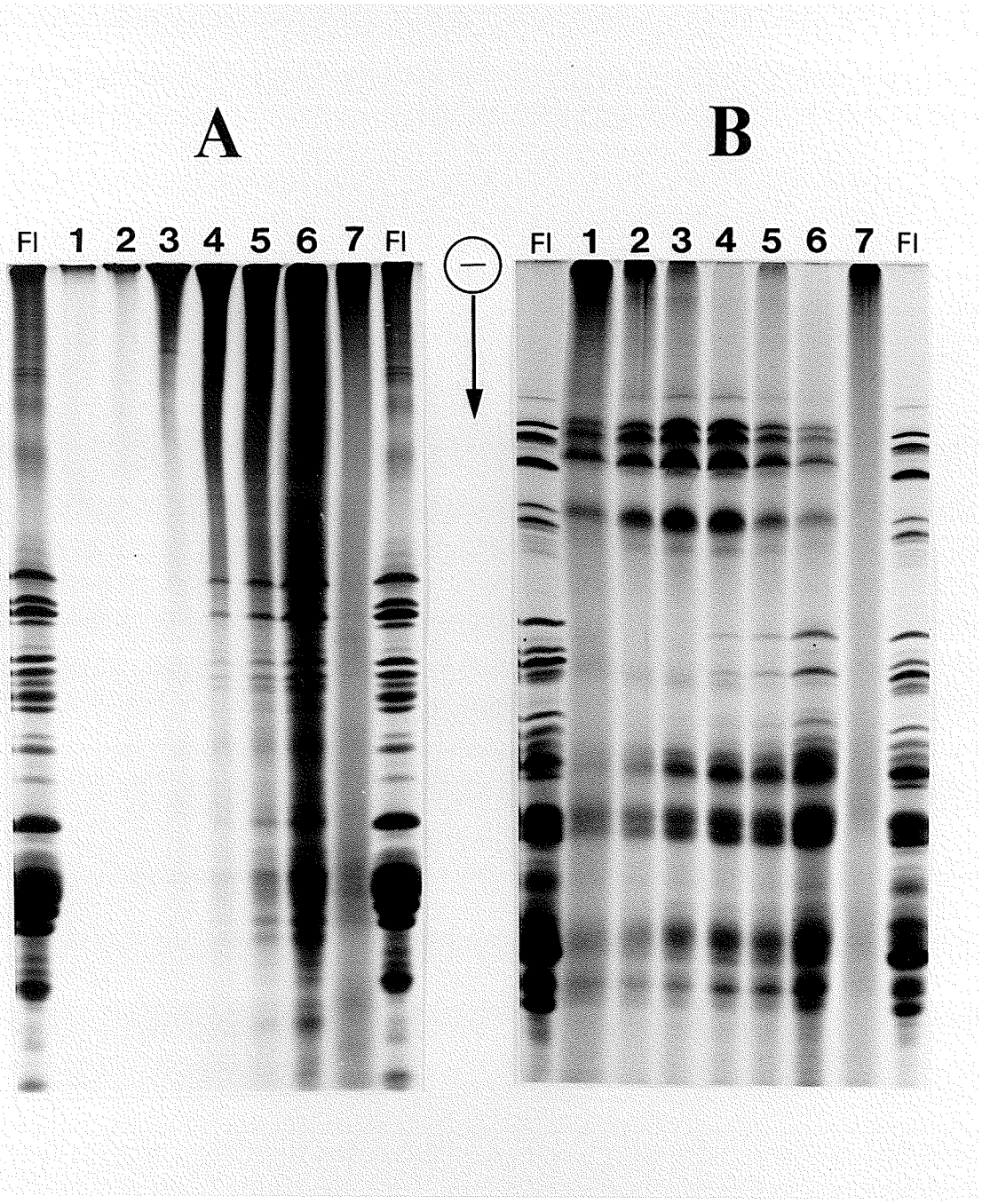
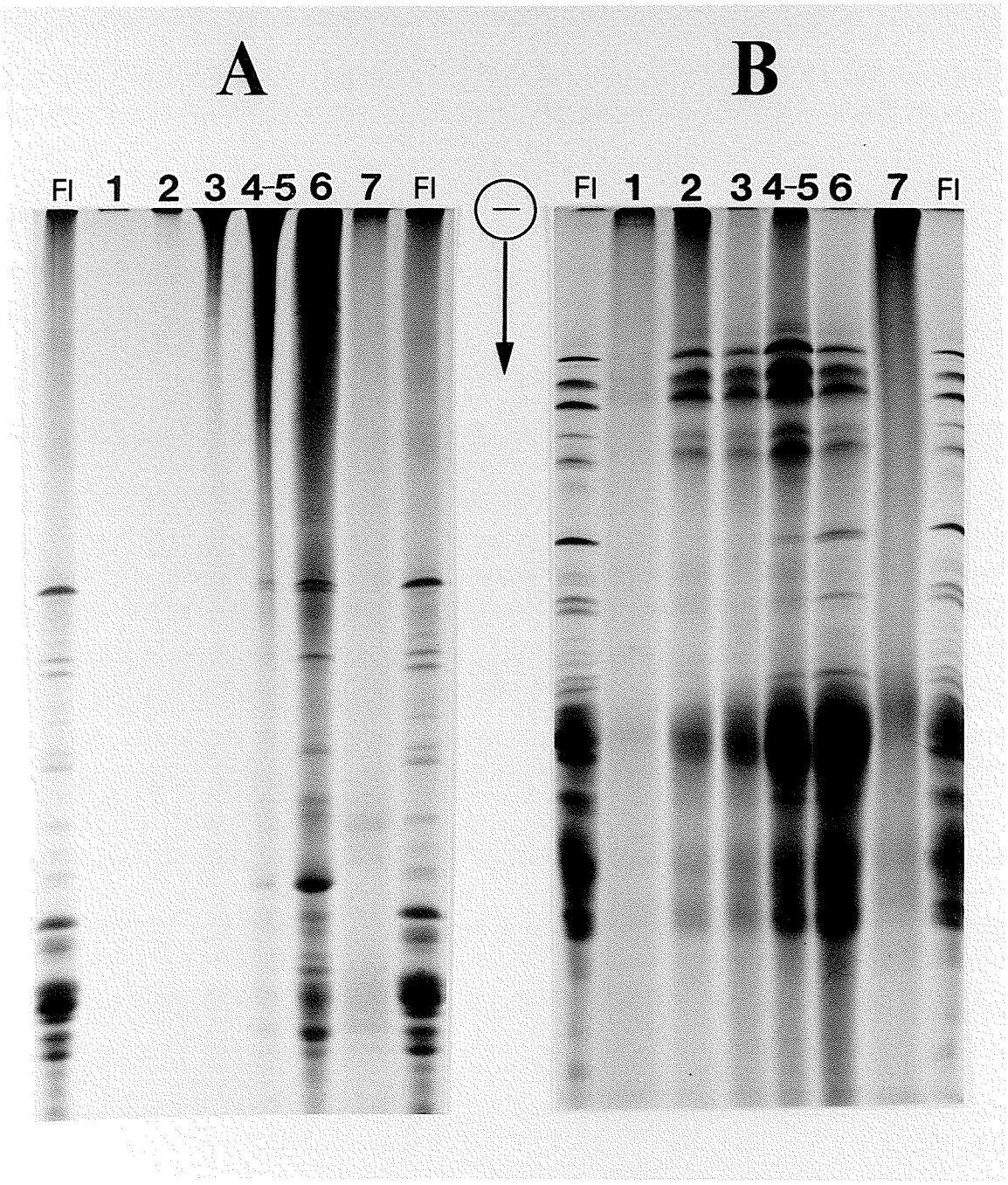

A
B
Fl 1 2 3 4 5 6 7 Fl
Fl 1 2 3 4 5 6 7 Fl

Figure 13. SDS-PAGE patterns of fractions (1-7) from gel filtration of Norstar glutenin preparation and of the control flour (Fl) under unreduced (A) and reduced (B) conditions.


A
B
Fl 1 2 3 4-5 6 7 Fl
Fl 1 2 3 4-5 6 7 Fl

SDS-PAGE results of gel filtration fractions of Glenlea showed that a gliadin-like protein was aggregated with the polymeric glutenin as indicated by its presence in fractions 2 and 3 under reduced conditions and its presence (based on mobility) in fractions 4 through 7 under unreduced conditions (Fig. 10B). Similar results were obtained for the glutenin of HY320 (Fig. 11B). It is not clear at this stage whether it is an LMW subunit of glutenin.

Based on the elution volume for the standards, fractions 6 and 7 were eluted at the volume for smaller molecules like gliadins. The presence of polymeric protein containing HMW subunits in the late fraction was probably caused by the overlapping of the large peak of polymeric proteins due to their predominant proportion of the proteins in each sample.

It is interesting to note that the fraction 7 of Katepwa and Norstar glutenins did not contain any protein in the SDS-PAGE patterns (Fig. 12, 13) although their profiles showed an extra peak on the shoulder of the large broad peak (Fig. 9). Only some smearing and streaking was observed on SDS-PAGE gel (Fig. 12B, lane 7). The staining as smearing and streaking in the lane for this fraction was probably caused by the presence of lipids which, due to their small size, should have been fractionated into this fraction by gel filtration. Coomassie Brilliant Blue dyes are known to be also excellent stains for lipids (Nakamura and Handa 1984). However, the true identity of these components and their association with glutenin remains to be elucidated.

3. Gel Filtration of Gluten

The gel filtration studies of flour extracts and glutenin preparations gave suggestions on the complexity and difficulty in the interpretation of the gel filtration profiles in the context of breadmaking potential. In order to examine if there was any difference in the distribution of polymeric proteins of glutens among the varieties, a preliminary study was carried out by gel filtration for glutens from varieties Katepwa and HY320 (Fig. 14).

It can be seen from the profiles for the two glutens, that the shape of the profiles appear to be between those for flours and those for glutenins. As expected, the profiles for glutens contained

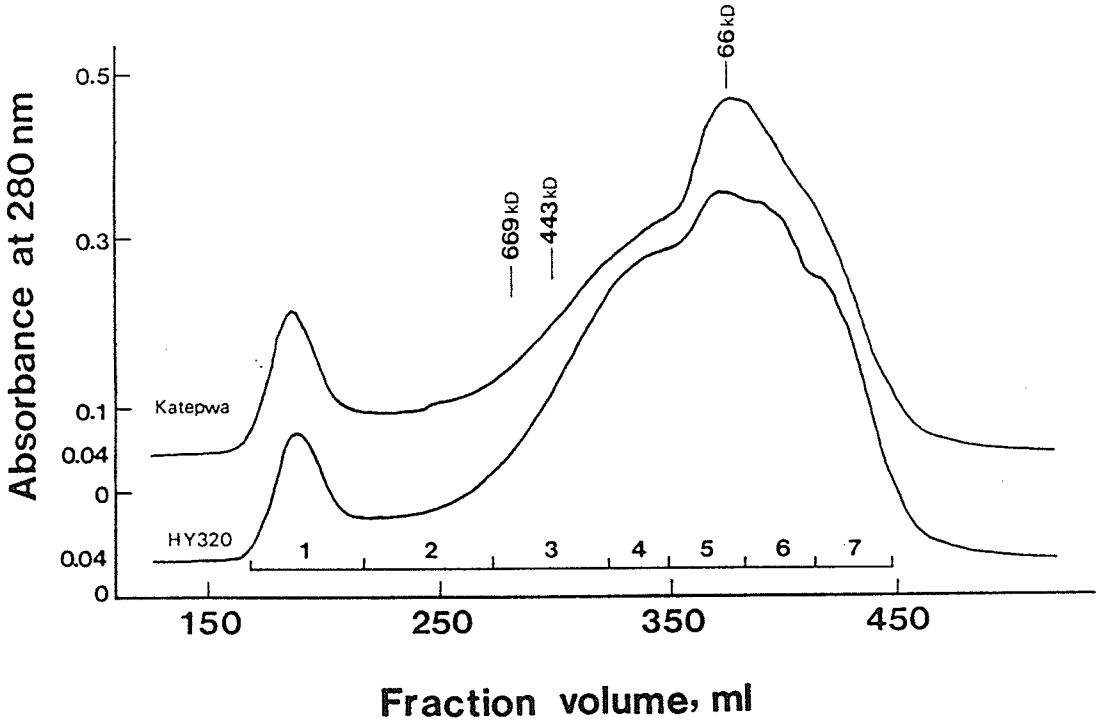


Figure 14. Gel filtration chromatography elution profiles of the gluten preparations (30 mg Kjeldahl protein) from HY320 and Katepwa on Sephacryl S-500 in 6M urea/6 % SDS solution.

large peaks, corresponding to fraction 6 and the later half of fraction 5 (Fig. 14), which contained gliadins as indicated by the electrophoretic results (Fig. 15). These large peaks are not visible in the profiles for glutenins (Fig. 9), except for the profile of Katepwa in which there is a much smaller peak which corresponds to the large peaks of the gluten profiles and, which was found to contain probably lipids (see previous section).

As in the profiles of the flour extracts, fraction 1 of the glutens showed little protein as indicated in the SDS-PAGE pattern under reduced conditions. Unlike the fraction 4 of flours, fraction 4 of glutens contained a large amount of polymeric protein containing HMW glutenin subunits. Fraction 6 of glutens did not contain any HMW subunits while the corresponding fractions from elution of glutenins did. These results generally indicated that the polymeric glutenin containing HMW subunits expanded into larger elution volume from flour to gluten and further to glutenin.

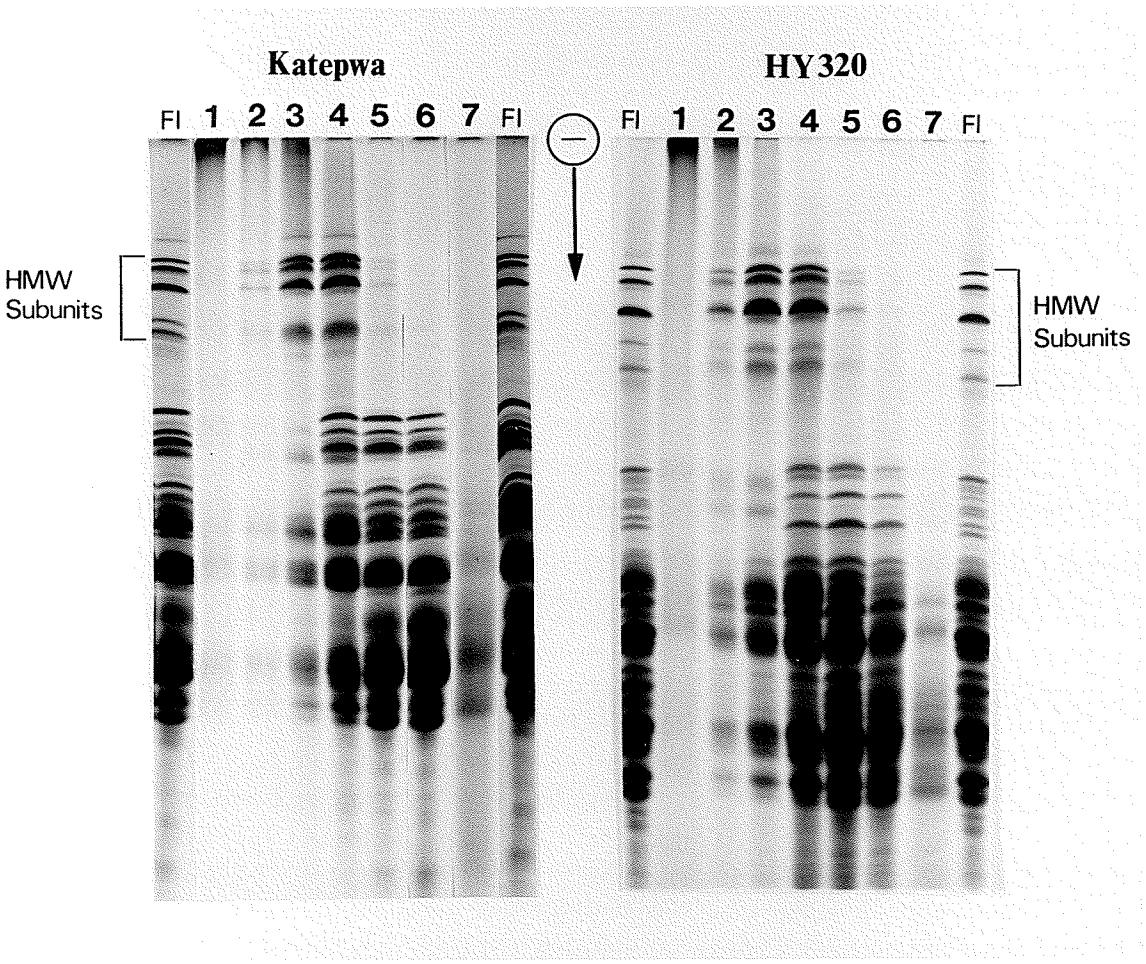
It is not clear why the HMW glutenin subunit-containing molecules expanded into more fractions. It is possible that this was caused by the greater percentage of HMW glutenin in the samples due to the stepwise removal of other LMW protein components during sequential fractionation.

4. Discussion on Gel Filtration Results of Glutenins and Glutens

The gel filtration study of glutenins and glutens showed some similar results to those obtained from the gel filtration of flours in terms of the wide range of molecular size, and of the position of the major HMW glutenin subunit-containing fractions in the fractionation range of the column. Nevertheless, the chromatography of the glutenins and glutens revealed some additional information.

The profiles of the gel filtration of glutenins and glutens, as compared with those of the flours, showed that the size of the later fractions were progressively reduced from flour to gluten and finally to glutenin, apparently arising from the gradual removal of albumins/globulins, and

Figure 15. SDS-PAGE patterns of fractions (1-7) from gel filtration of Katepwa (A) and HY320 (B) glutens and of the control flour (Fl) under reduced conditions.


Katepwa
HY320
Fl 1 2 3 4 5 6 7 Fl
Fl 1 2 3 4 5 6 7 Fl
HMW Subunits
HMW Subunits

gliadins, respectively. However, the removal of the gliadins was not complete in the glutenin preparations, as shown by the SDS-PAGE patterns of the collected gel filtration fractions.

The HMW subunit-containing glutenin molecules were found in broader fraction volumes although the major HMW subunit-containing fractions were eluted at about the same fraction volumes. The expansion seems to be caused by the increased content of glutenin in the progression from flour to gluten, and from gluten to glutenin.

The molecular weight distribution of gluten or glutenin as obtained by measuring the absorbance at 280 nm of eluate of gel filtration did not seem to be consistently related to breadmaking quality of the wheat varieties. These results, along with those from the gel filtration studies of flour extracts, indicate that there is probably no obvious relationship between the molecular weight distribution of the flour protein and breadmaking quality of the flour.

D. Gel Filtration Chromatography of Partially-Reduced Proteins of Doughs

It is well-known that a small amount of reducing agent causes a drastic change in the rheological properties of dough (Bloksma and Bushuk 1988). Gel filtration is an effective technique for examining the molecular weight distribution change with reduction of the proteins which would explain the drastic rheological changes.

1. Gel Filtration Results

Figure 16 shows the overlaid chromatograms for extracts of the partially-reduced doughs containing 0, 20, 80, and 500 μmol DTT and mixed in a farinograph for 20 min under air. Fractions 1 to 7 from the gel filtration chromatography were collected as indicated.

It can be seen clearly that the gradual increase in the amount of reducing agent progressively and consistently reduced the amount of protein of fraction one material. However, the change did not seem to correspond proportionally (determined subjectively) to the amount of reducing agent

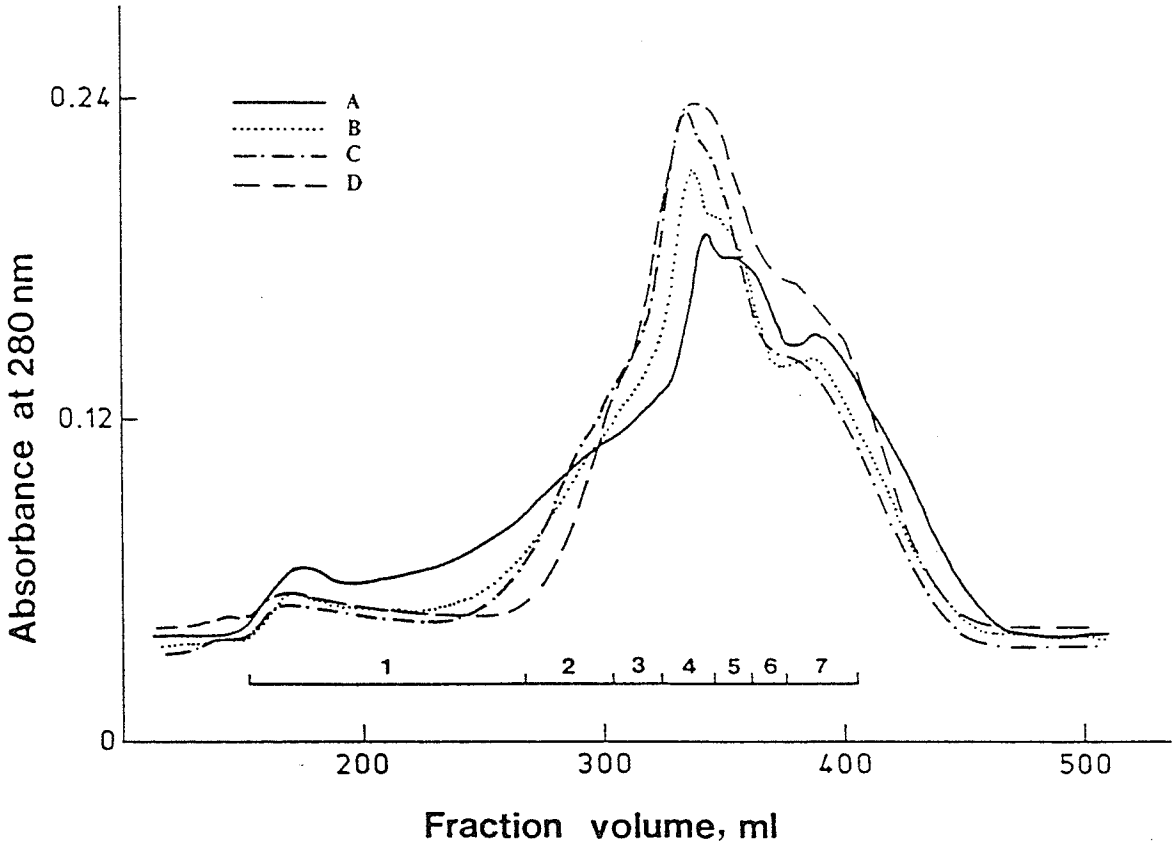


Figure 16. Gel filtration chromatography elution profiles of the partially-reduced doughs (100 mg, d.b., cultivar Katepwa) on Sephacryl S-500 in 6M urea/6 % SDS solution. A, control dough (flour [50 g] and water); B, dough with 20 μmol dithiothreitol (DDT); C, dough with 80 μmol DDT; D, dough with 500 μmol DDT.

added to the dough in farinograph mixing. The most drastic change took place upon the initial addition of the 20 μmol DTT. From Fig. 16, it can be seen that the initial 20 μmol DTT drastically reduced the amount of protein in the first segment (by elution) of the profile (compare curves A and B). This result is consistent with the hypothesis that the initial reduction is of glutenin with a drastic reduction of molecular weight (Ewart 1977). However, the excluded peak was not completely eliminated upon the addition of the reducing agent, even at higher level, suggesting that it may be non-protein in nature. However, the nature of this excluded peak was not investigated in this study.

Interestingly, in addition to the maximum response of farinograph resistance to the initial dose of reducing agent, the absorbance of fraction 7 seemed to decrease slightly, along with the decrease of the excluded peak. The only group of molecules which increased upon reduction at this stage was the one that eluted slightly earlier than the highest peak. This suggests that the small molecules in fraction 7 participated in the formation of the molecules in the increased peaks of the profile.

The results represented here are generally consistent with the trend of profile changes upon the exposure to reducing agent as reported by Belitz *et al.* (1987). However, the results presented in that study were not fully discussed with regard to the change in molecular weight distribution, and no details of the experimental conditions were reported such as the ratio of reducing agent to glutenin and whether the glutenin was completely solubilized by the urea/acetic acid solvent.

Additional amounts of DTT (*e.g.* with the 80 μmol level, Dough C) did not cause as much further change in the gel filtration profile as by the initial 20 μmol DTT (Dough B, Fig. 16). The addition of 500 μmol DTT caused the greatest drop in the amount of large molecules eluted before the highest peak in the profile (Dough D, in Fig. 16) and a simultaneous rise in the vicinity of the highest peak. An increase in the amount of protein in fraction 7 in the profile was also observed, in contrast to the decrease observed for this fraction for the dough treated with smaller amount of DTT (Fig. 16). It is speculated that the small molecules corresponding to fraction 7 was used for the formation of the molecules which were eluted slightly before the highest peak to couple

the free sufhydryl groups released by the initial addition of the reducing agent in the farinograph mixing. Subsequently, those small molecules were released by the increased amount of the added reducing agent. However, this remains to be confirmed by experiment.

It is interesting to note that for the control dough of Katepwa (Fig. 16, line A), the chromatographic profile is generally similar to the profile of Katepwa flour (Fig. 4). However, there are some small but significant differences. The most obvious is that the size of the excluded peak for the control dough is smaller whereas that of the peak corresponding to the fraction 7 is larger than the respective peaks in the profile for the flour (Fig. 4). The difference was probably caused by farinograph mixing, which may cause breakdown of large protein molecules into smaller ones (Tsen 1967; Mamaril and Pomeranz 1966; Danno and Hoseney 1982a,b). Another difference between the profiles for dough and flour is the relatively larger amount of protein (by absorbance) for dough between the excluded peak and the highest peak (control dough in Fig. 16) than that in Fig. 4. From these results, it seems that the farinograph mixing increased the amount of protein which eluted between the excluded peak and the highest peak in the profile and decreased the amount of protein (absorbance) in both the excluded peak and the peak corresponding to fraction 7 in the profile of Katepwa flour.

2. SDS-PAGE Results

SDS-PAGE results for the fractions collected from the gel filtration chromatography of the control dough and of the partially-reduced doughs are shown in Fig. 17 (unreduced) and Fig. 18 (reduced). Some of the adjacent fractions were combined in the preparation for electrophoresis as indicated in the figure. The purpose of SDS-PAGE analysis of the fractions was to examine the effect of limited amount of reducing agent on the possible re-distribution of HMW glutenin subunit-containing molecules in the fractions.

It can be seen from Figs. 17 and 18 that most of the HMW glutenin subunits in all dough samples from A through D are in the fractions 2 and 3. Fraction 1 also contained some HMW

Figure 17. SDS-PAGE patterns of fractions (1-7) from gel filtration of the partially-reduced doughs and of the control flour (Fl) under unreduced conditions.

A, control dough (flour and water);

B, dough mixed with 20 μmol dithiothreitol (DTT);

C, dough with 80 μmol DTT;

D, dough with 500 μmol DTT

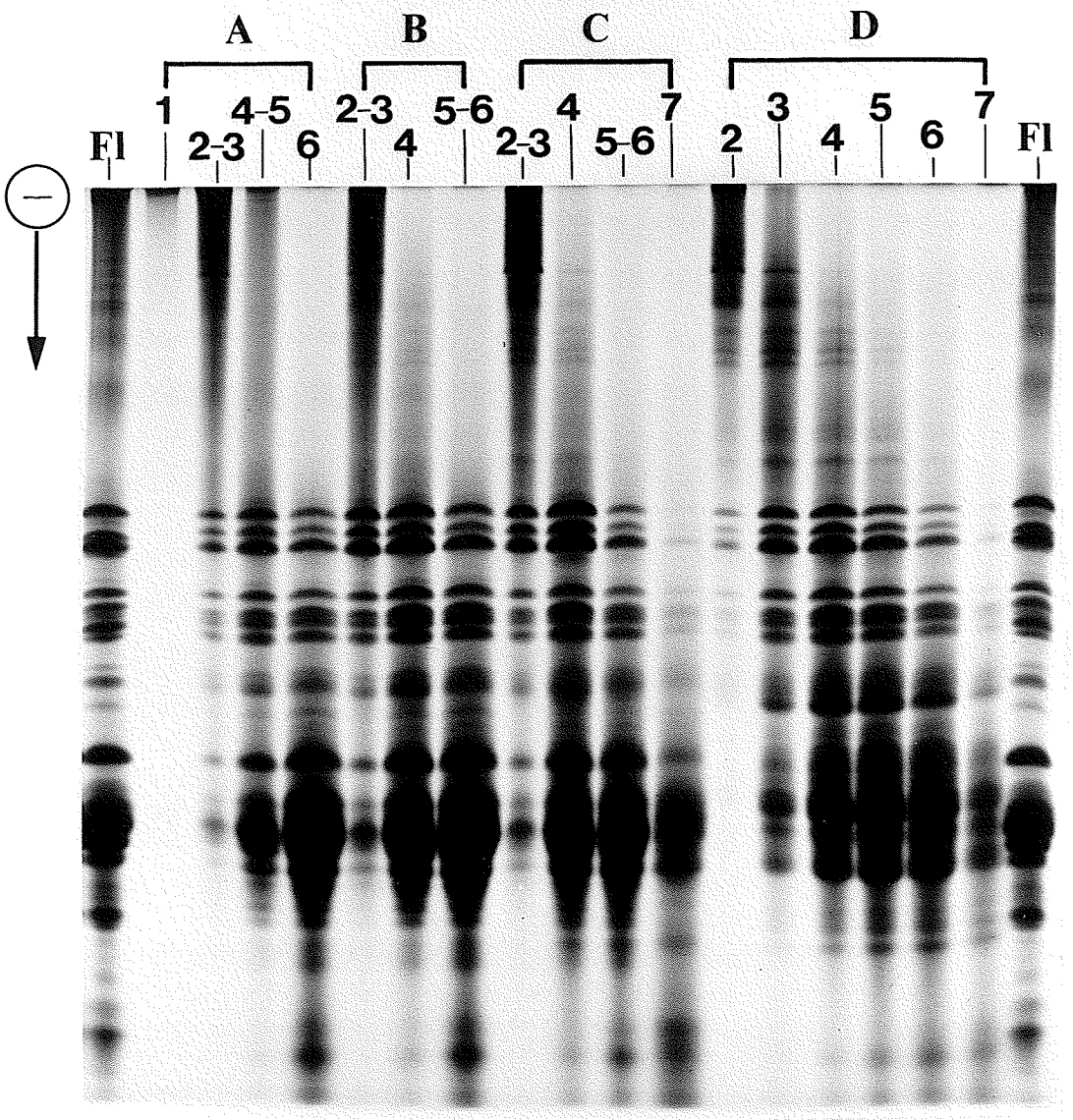

A
B
C
D
1
4-5
2-3
5-6
4
7
3
5
Fl
2-3
6
2-3
5-6
2
4
6
Fl
−

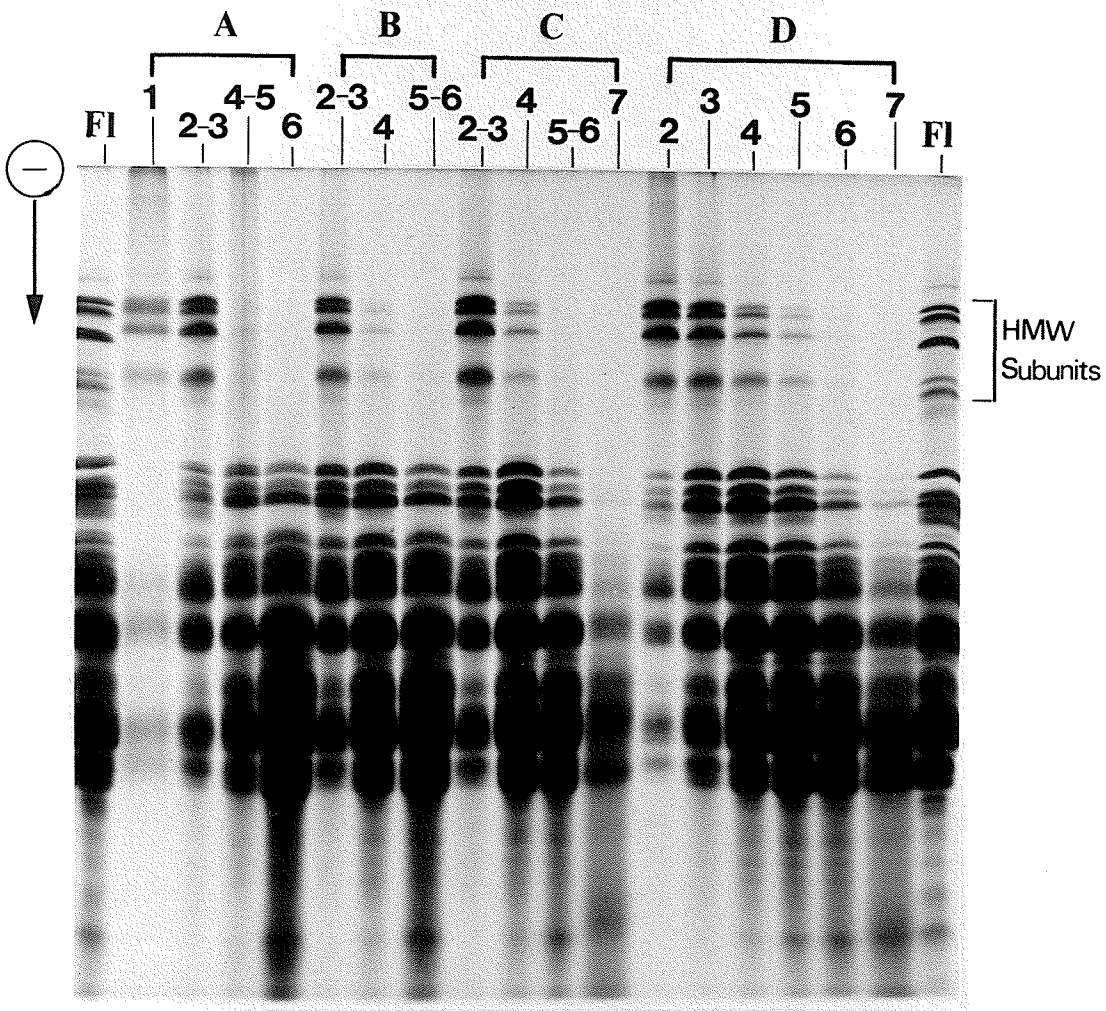
Figure 18. SDS-PAGE patterns of fractions (1-7) from gel filtration of the partially-reduced doughs and of the control flour (Fl) under reduced conditions.

A, control dough (flour and water);

B, dough mixed with 20 μmol dithiothreitol (DTT);

C, dough with 80 μmol DTT;

D, dough with 500 μmol DTT


A
B
C
D
Fl
1
2-3
4-5
6
2-3
4
5-6
2-3
4
5-6
7
2
3
4
5
6
7
Fl
HMW
Subunits

glutenin subunits as polymeric molecules which are too large to enter the gel under unreduced condition (Fig. 17).

The lowest amount of DTT (20 μmol) did not change the distribution of the HMW glutenin subunits to any substantive extent. Fraction 4 of dough mixed with 20 μmol DTT (see B in Figs. 16, 18) contained only slight amount of HMW subunits although it apparently contains polymeric glutenin which caused streaking in the unreduced gel (Fig. 17). These polymers were composed of only LMW glutenin subunits which entered the gel of SDS-PAGE under reduced conditions and moved to the region for LMW subunits and the gliadins (see Fig. 18). The proteins seem similar to the high molecular weight gliadins (Beckwith *et al.* 1966) in terms of their polymeric nature under unreduced conditions and the mobility of their subunits under reduced conditions.

Upon the addition of larger quantities of DTT (80 μmol), the glutenin was apparently depolymerized (reduced) into small oligomers some of which were fractionated down to fraction 4, as seen from pattern in lane 4 for sample C (Fig. 17). For the dough treated with 500 μmol DTT, the HMW subunits are clearly visible in fraction 4, and even evident in fraction 5 (Fig. 18).

3. Discussion of Gel Filtration Results of Partially-Reduced Doughs

The marked drop of the absorbance of the fractions containing large glutenin molecules (the fraction 1 in Fig. 16 contained much more material than usual) explains well the corresponding drastic decrease in farinograph consistency as shown in Fig. 25 in section " Structure of Glutenin". This underscores the importance of the glutenin fraction in the determination of the farinograph consistency and, therefore, the rheological properties of dough.

After reduction by a small amount of reducing agent, the dough retained very little of its normal viscoelastic properties. This is logical because it is the glutenin fraction that imparts the unique viscoelastic properties to wheat dough. However, even with the addition of 80 μmol DTT to dough, the distribution of the HMW subunits across glutenin fractions from gel filtration did not change as markedly as does the farinograph consistency (Fig. 18). These results suggest that

the relatively small differences in rheological properties (dough strength) among the flours of varying breadmaking quality as compared to the drastic change by addition of reducing agent may be derived from some more subtle differences which are not detectable by gel filtration chromatography used in this study. In general, the results obtained here are consistent with those of Meredith and Wren (1969) and Danno and Hoseney (1982a); both groups showed that there was no observable difference between optimally-developed dough and extensively-overmixed dough in their gel filtration chromatographic profiles.

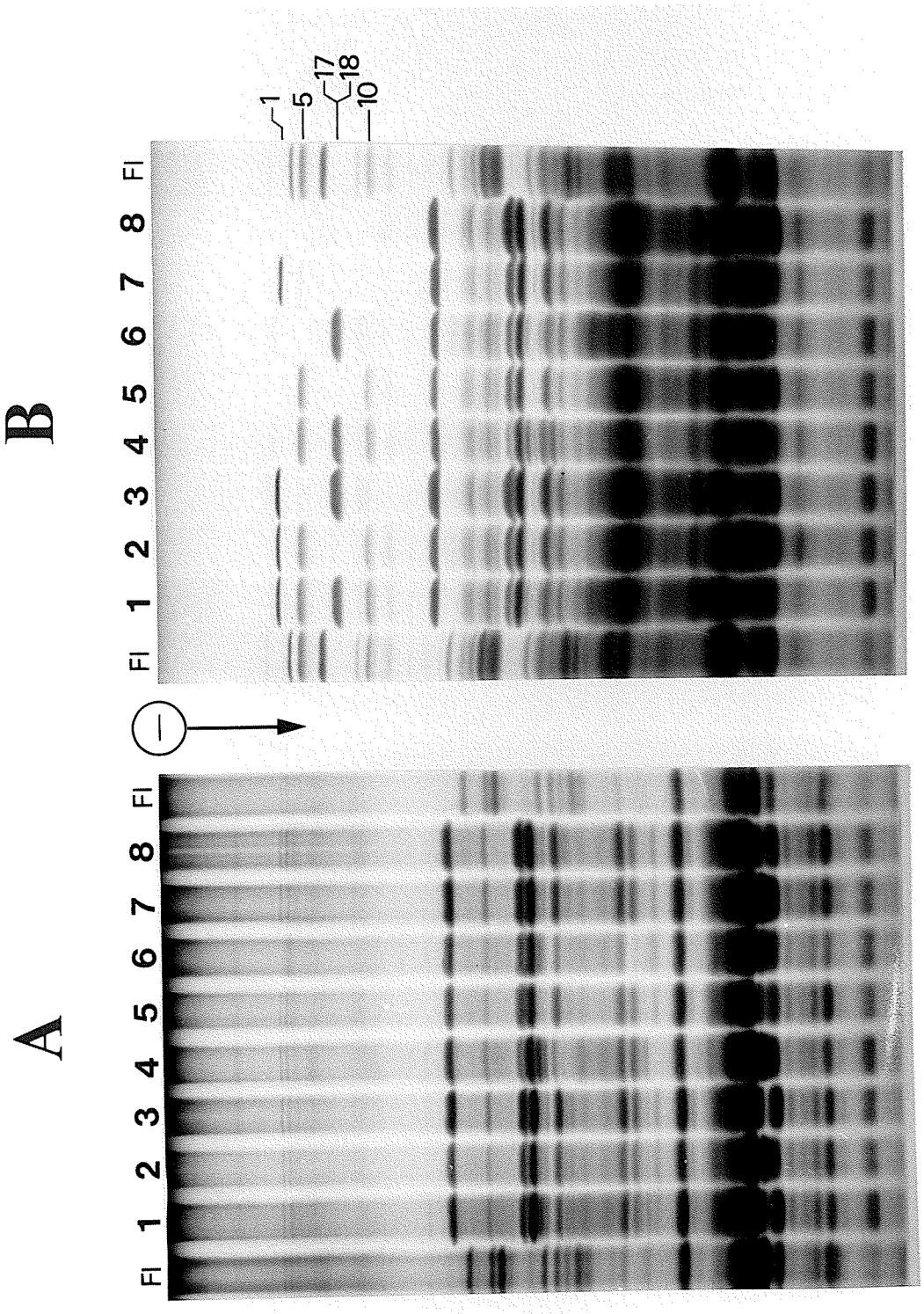
In addition, the gel filtration profile of the control dough (no reducing agent), when compared to that of the flour, showed that the largest glutenin molecules, as indicated by the HMW glutenin subunits in SDS-PAGE, seem to have been re-distributed. The smallest and the largest glutenin molecules became somewhat medium-sized glutenin polymers which are still much larger than gliadins. This result suggests that dough mixing exerted an effect on glutenin molecules similar to that of sonication (Singh *et al.* 1990a). The results of this study are generally consistent with those of several reports (Tsen 1967; Mamaril and Pomeranz 1966; Danno and Hoseney 1982a,b).

F. Studies on Australian *Glu-1* Null Samples

1. Characterization of the *Glu-1* Null Wheats

Figure 19 shows the standard SDS-PAGE patterns under unreduced (A) and reduced (B) conditions of the eight wheat lines with varying number of HMW weight glutenin subunits. The control flour (Fl) was used as the standard reference. The patterns under unreduced conditions showed little difference among the samples (Fig. 19A), while the patterns under reduced conditions (Fig. 19B) confirmed the expected differences in their HMW glutenin subunit composition, ranging from 5 (1, 5, 17, 18, 10) in A1, in the order of increasing mobility in SDS-PAGE, according to the nomenclature of Payne and Lawrence (1983), to none (in A8). The HMW glutenin subunit composition of these samples is the same as reported by Lawrence *et al.* (1988).

Figure 19. SDS-PAGE patterns of the eight Australian *Glu-1* null wheat samples (lanes 1-8 for A1-A8, respectively) and of the Neepawa control flour (Fl) under unreduced (A) and reduced (B) conditions. Numbers indicate the HMW subunits in normal (euploid) wheat line. The subunit composition of the Neepawa standard is 2*, 5, 7, 9, and 10.


A
B
Fl 1 2 3 4 5 6 7 8 Fl
Fl 1 2 3 4 5 6 7 8 Fl
1
5
17
18
10

Patterns of some samples under reduced conditions contained some faint bands in the position of the HMW subunits that had been removed. It is not known whether they are the regular HMW subunits, or some other polypeptides such as the E-bands as reported by Gupta and Shepherd (1987). Their complete identity was not investigated.

As expected from their HMW glutenin subunit composition, the chemical, rheological, and breadmaking test results showed marked differences among the eight wheat samples (see Appendix II). Generally, removal of the HMW glutenin subunits shortened the farinograph dough development time and increased the mixing tolerance index. Deletion of the HMW subunits did not cause any significant change in farinograph absorption. From the extensigraph results (Appendix II-D), it can be seen that the removal of the HMW subunits caused drastic decrease in dough resistance, while the extensibility remained essentially unchanged. All these results are consistent with the general decrease of Zeleny sedimentation values and of the loaf volumes of the wheat samples. The decreases in the sedimentation value and loaf volume were generally parallel. The results obtained here are generally consistent with previous reports (Payne *et al.* 1987; Ng and Bushuk 1988) that different HMW subunits contribute a different increment to the breadmaking potential.

In the preparation of glutens from the wheat lines with varying number of HMW subunits by hand-washing procedure, it was very difficult to obtain any gluten from A7 and A8. The doughs were very plastic but not elastic. The gluten was eventually obtained by the hand-washing procedure (AACC method 38-10); it had the consistency of chewed "gum". Gluten from all other samples was "normal" in the hand-washing procedure for glutens.

2. Gel Filtration Chromatography Results of Glutenins

The gel filtration chromatography of glutenin preparations from the *Glu-1* null samples (Fig. 20) showed a remarkable similarity in their profiles in spite of the marked difference in HMW glutenin subunit composition and in the rheological and baking properties. In each profile there

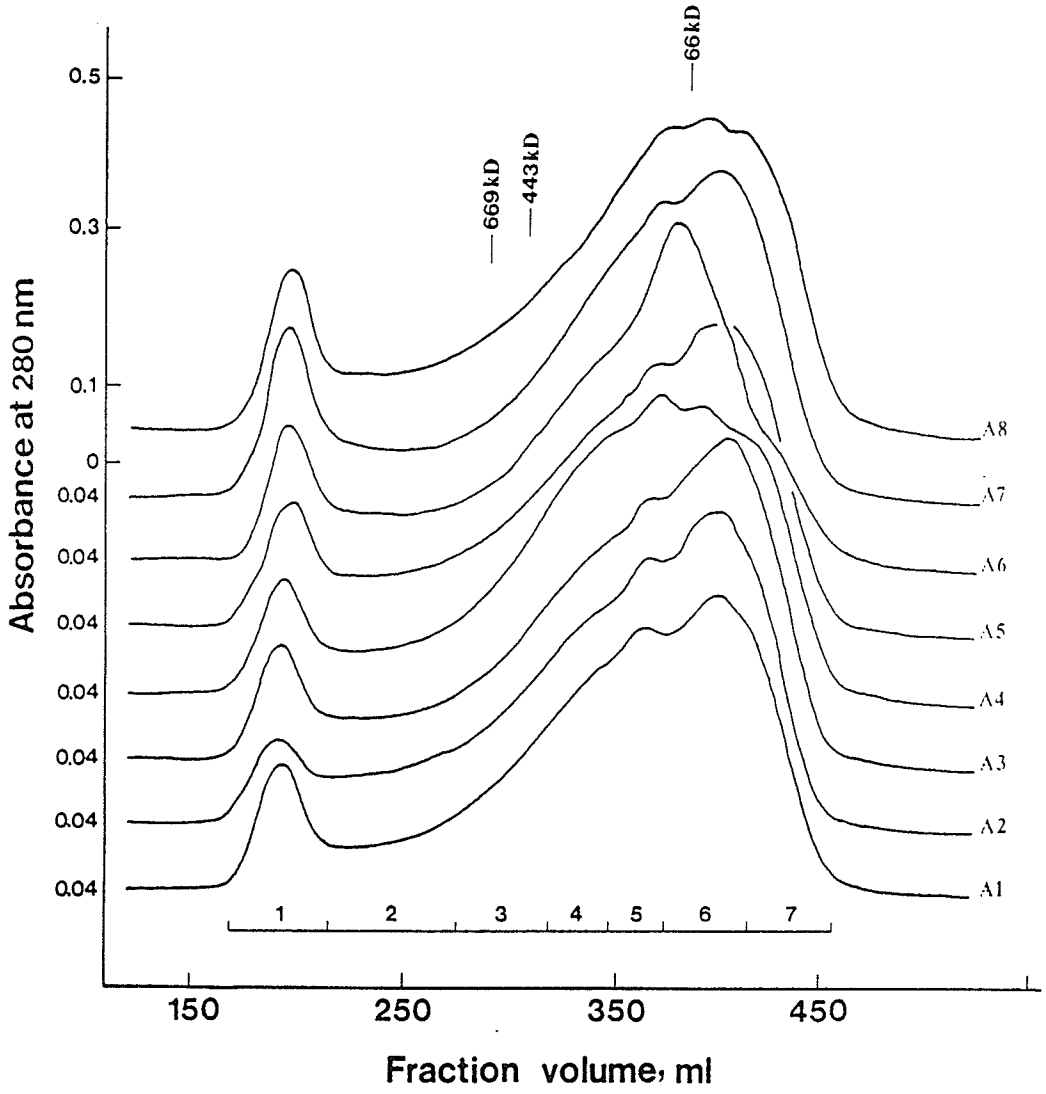


Figure 20. Gel filtration chromatography elution profiles of glutenin preparations from the Australian *Glu-1* null wheat samples on Sephacryl S-500 in 6M urea/6 % SDS solution. Numbers 1-7 identify the fractions that were collected for SDS-PAGE.

is one excluded peak and also one group of peaks which seems to form a broad peak centred at about 375 ml elution volume. The profiles are generally similar to those for the glutenins from the Canadian wheats (Fig. 9).

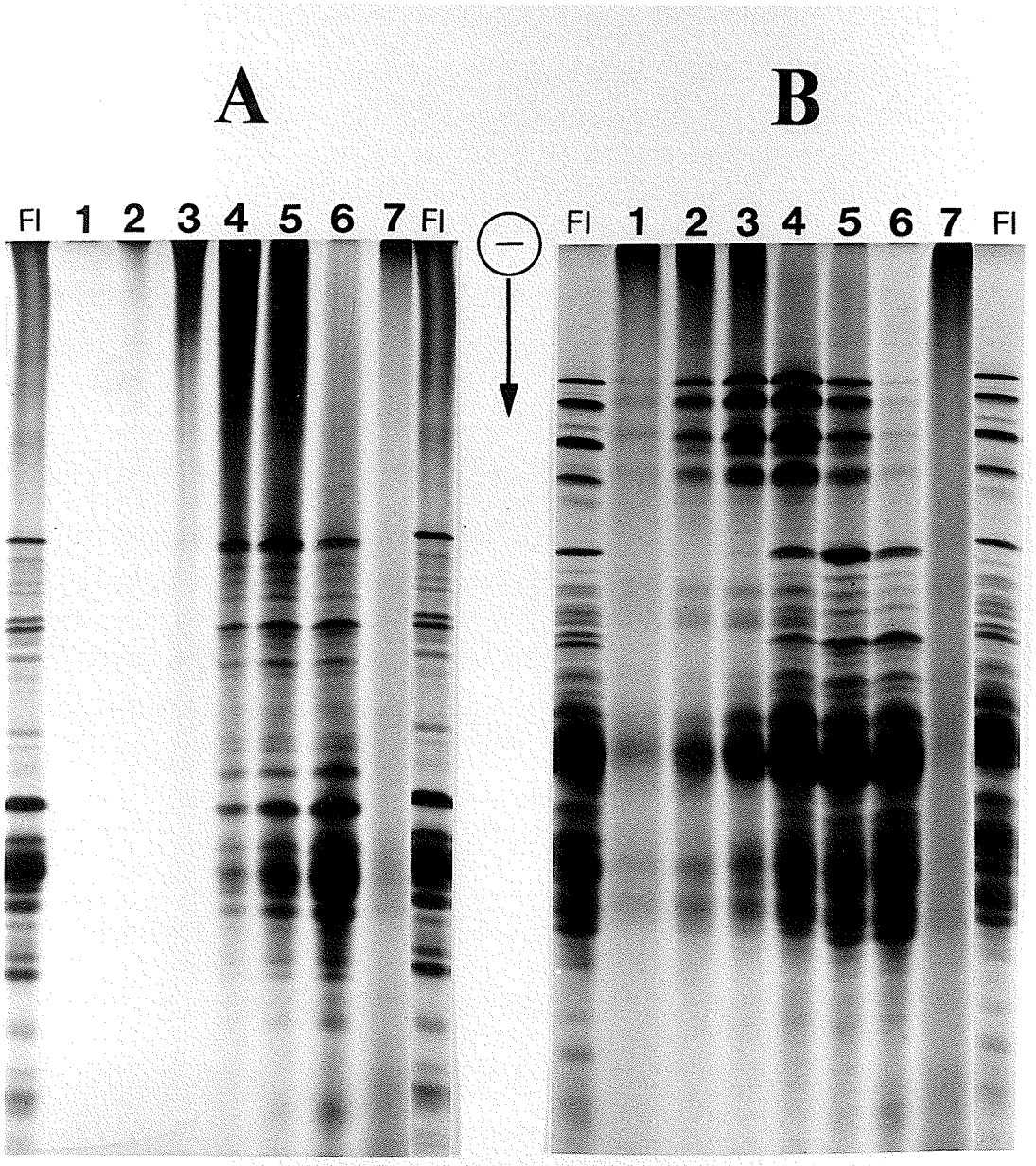
The size of the excluded peak varied slightly from one sample to another; the range of variation was small (as judged visually). However, there was no relationship between the size of the peak and breadmaking potential of the flours.

The shape of the broad peak of the profiles varied much more among the samples than did the excluded peak although no obvious relationship of the broad peak (size or shape) to rheological or baking properties can be drawn from these profiles. The broad peaks were eluted slightly later than the analogous peak of the glutenin preparations from the Canadian wheats (Fig. 9) but the difference was not as large as might be expected from the large differences in rheological properties, Zeleny sedimentation volume and test baking loaf volume. Accordingly, it was concluded that for the wheats examined herein, relationship between the molecular weight distribution, as measured by absorbance at 280 nm, for flours or glutenin preparations and breadmaking quality is not obvious. This conclusion, however, does not invalidate the relationship between the amount and the solubility of glutenin and breadmaking quality for wheat varieties of diverse quality as observed by Orth and Bushuk (1972) because in the present study the same amount of glutenin was applied onto the column. Another possible explanation is that the variation in breadmaking quality is mostly derived from non-covalent forces, which could affect solubility but would not be detected by gel filtration chromatography because they would be destroyed in the process of solubilization.

3. SDS-PAGE of Fractions from Gel Filtration

The SDS-PAGE results for A1, the wheat with all the HMW glutenin subunits, showed that the first peak contained both HMW and LMW subunits (Fig. 21). As observed for the Canadian

Figure 21. SDS-PAGE patterns of fractions (1-7) from gel filtration of A1 (all five HMW subnunits) glutenin preparation and of the control (A1) flour (Fl) under unreduced (A) and reduced (B) conditions.


A
B
Fl 1 2 3 4 5 6 7 Fl
Fl 1 2 3 4 5 6 7 Fl

wheats, the HMW subunits of A1 seemed to be concentrated in the fractions collected in the middle of the elution volume (fractions 2, 3, 4, and 5). Fraction 6 contained a small amount of HMW subunit protein, unlike those of the Canadian wheats, probably because of the slightly later start of collection of this fraction in the elution of the *Glu-1* null samples.

The SDS-PAGE results (Fig. 22) for the glutenin A7 (null for all except HMW subunit 1) showed overall distribution of the HMW subunit across the fractions similar to that of A1, although there is only one present in A7. The first peak contained only a slight amount of the subunit; most of the HMW subunit was concentrated in fractions 2, 3, 4, and 5. It is very interesting to note that although there is only one HMW subunit in A7, still, it is in the form of large polymeric molecules, giving the same distribution pattern across the fractions as obtained for A1 which has all five HMW subunits. The rheological and breadmaking properties of the two lines are quite different (Appendix II). The distribution pattern of the HMW subunits for all other *Glu-1* null lines A2 through A6 was essentially the same (hence the SDS-PAGE patterns not shown). These results strongly suggest that the intervarietal difference in glutenin or/and gluten quality does not seem to lie in the size of polymeric molecules of glutenin, but in several other aspects including the polymeric structure and non-covalent interactions of glutenin which ma be collectively called the "quality" of glutenin. However, it should be kept in mind that the resolution of gel filtration in this study was rather low for the large range of molecules.

Under reduced conditions, the faint bands which were only marginally discernable in the flours (Fig. 19B) showed much stronger band intensity in the SDS-PAGE patterns of the fractions from gel filtration of glutenin (Fig. 23). This is probably because of the concentration effect of gel filtration and the higher loading of proteins onto SDS-PAGE gel. Nevertheless, the fact that these faint bands were in the earlier fractions of gel filtration indicates that these bands are probably closely related or linked to the HMW glutenin subunits in the polymeric glutenin.

For the null line A8 (no HMW subunits), fractions from gel filtration contained large polymeric proteins, which are composed of only LMW glutenin subunits as shown in SDS-PAGE under reduced conditions (Fig. 23B, most clearly shown in fractions 1 and 2). The LMW subunits

Figure 22. SDS-PAGE patterns of fractions (1-7) from gel filtration of A7 (one [1] HMW subunit) glutenin preparation and of the control (A7) flour (Fl) under unreduced (A) and reduced (B) conditions.

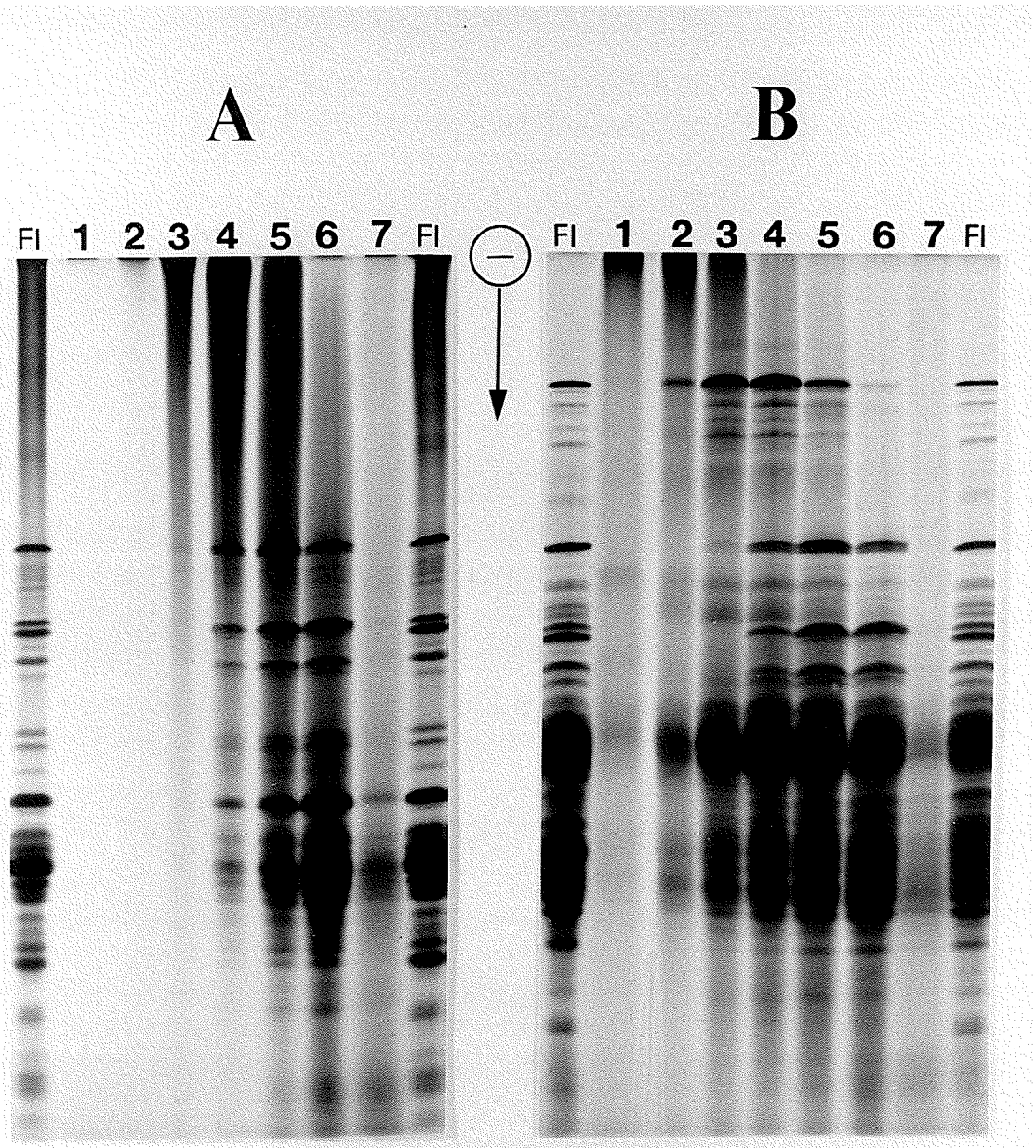
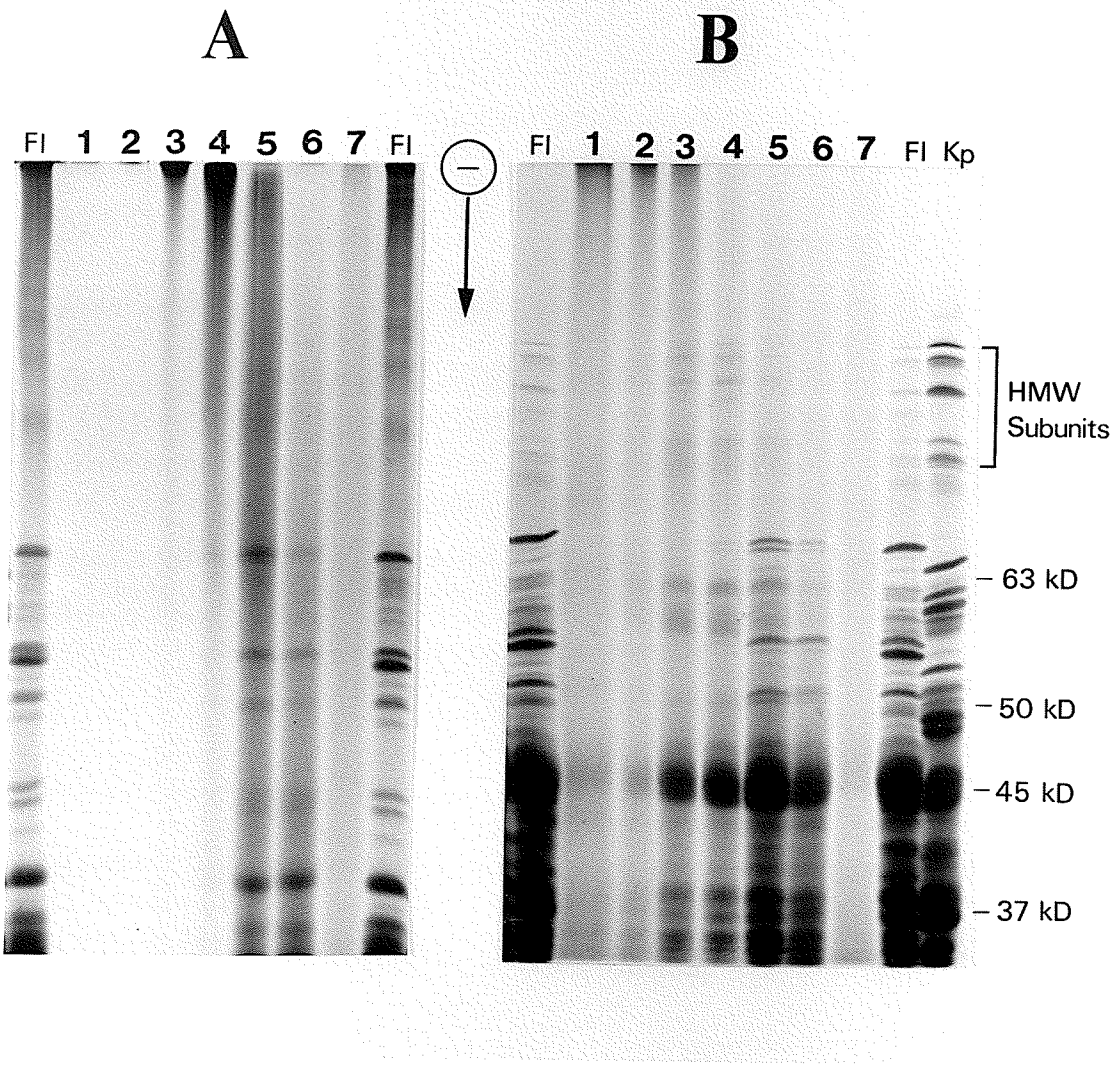

A
B
Fl 1 2 3 4 5 6 7 Fl
Fl 1 2 3 4 5 6 7 Fl

Figure 23. SDS-PAGE patterns of fractions (1-7) from gel filtration of A8 (no HMW subunits) glutenin preparation and of the control (A8) flour (Fl) under unreduced (A) and reduced (B) conditions.


A
B
Fl 1 2 3 4 5 6 7 Fl
Fl 1 2 3 4 5 6 7 Fl Kp
HMW
Subunits
63 kD
50 kD
45 kD
37 kD

are mainly two groups of polypeptides of approximate molecular weights of 44 and 36 kDa (the latter ranging from 33 to 39 kDa). There is also a smaller proportion of proteins with molecular weights of 58 to 63 kDa, as shown in the SDS-PAGE patterns (Fig. 23B). This indicates that LMW glutenin subunits without the HMW subunits can form large polymeric molecules which are too large to enter the gel (Fig. 23A). These large molecules appear to be stabilized by inter-molecular disulfide bonds because they survived the solubilization condition in the preparation of the samples for gel filtration and the SDS-PAGE separation under unreduced conditions.

The LMW glutenin polymer which is insoluble in aqueous ethanol and was not removed in the preparation of glutenin was probably also present in other fractions such as fraction 3 or 4 although this is not clear because of the presence of some faint bands which are probably E-bands described by Gupta and Shepherd (1987). However, the predominant presence of LMW glutenin subunits in these fractions as revealed by SDS-PAGE under reduced conditions (Fig. 23B) strongly indicates the existence of polymeric molecules comprising only LMW subunits.

The presence of large polymeric molecules of LMW poses the question with regard to the identity of glutenin in wheat flours or doughs. Traditionally, glutenin has been described as polymers composed of both HMW and LMW subunits. It has been reported that oligomers composed of only LMW glutenin subunits are present in the Osborne gliadin fraction (Beckwith *et al.* 1966). These oligomers were called high molecular weight gliadin (Bietz and Wall 1980) and LMW glutenin (Nielson *et al.* 1968; Kanazawa and Yonezawa 1973). It is not known whether or not there are, in Osborne glutenin, large molecules composed of only LMW glutenin subunits as glutenin (Bietz and Wall 1980). The evidence presented here confirmed the existence of glutenin composed of LMW subunits only, in the glutenin fraction obtained by Osborne fractionation, along with those composed of HMW subunits only and those containing both HMW and LMW glutenin subunits. The aqueous ethanol-insoluble glutenin, composed of only LMW subunits will be tentatively named LMW glutenin although it is different in solubility from the LMW glutenin extracted in the Osborne gliadin fraction. Similarly, the polymeric glutenin composed of HMW subunits only and those of both HMW and LMW subunits will be termed as HMW glutenin.

4. Amino Acid Analysis

Removal of the HMW glutenin subunits showed a considerable effect on the amino acid composition of the glutenin preparations of the *Glu-1* null lines (Fig. 24).

In the glutenin preparations, the content of the glutamic acid/glutamine (Glx) and proline showed gradual and considerable decrease with the removal of increasing number of HMW subunits (Fig. 24). Glx decreased in molar percentage from 33% for A1 to 27% for A8, and proline from 13% for A1 to 11% for A8, respectively. The tyrosine content also decreased, although to a lesser extent, from 2.5% for A1 to 2.2% for A8. These decreases are consistent with the removal of the HMW subunits which contain higher proportion of these amino acids than other endosperm proteins.

Several amino acids increased with the removal of HMW subunits. Most significant increases were obtained for alanine (from 3.9 for A1 to 5.3% for A8), aspartic acid (from 3.4% for A1 to 5.2 for A8), and for lysine (from 1.8 for A1 to 2.9% for A8). These increases are generally consistent with the decrease in the number of HMW subunits in the Osborne glutenin.

The amino acid composition (Table 3) of the glutenin from A8 flour, the line with no HMW subunits, is generally in agreement with the published composition of the LMW subunits (*e.g.* glutenin peak 4 by Belitz *et al.* 1987). It should be noted, however, that the amino acid composition of A8 glutenin is significantly different from that of the aqueous ethanol-soluble glutenin (fraction 2 of Bietz and Wall 1973). Interestingly, the composition of A8 glutenin, although similar to, is not the same as that of the high molecular weight gliadin (fraction 4 in Table 3). These results suggest that the glutenin of line A8 differs from the high molecular weight gliadin (fraction 4 from Bietz and Wall 1973), not only in size and solubility, but also in amino acid composition. However, it is not known if the difference is due to the presence of other constituents as contaminants in the glutenin of A8. On the other hand, the composition of the A8 glutenin is

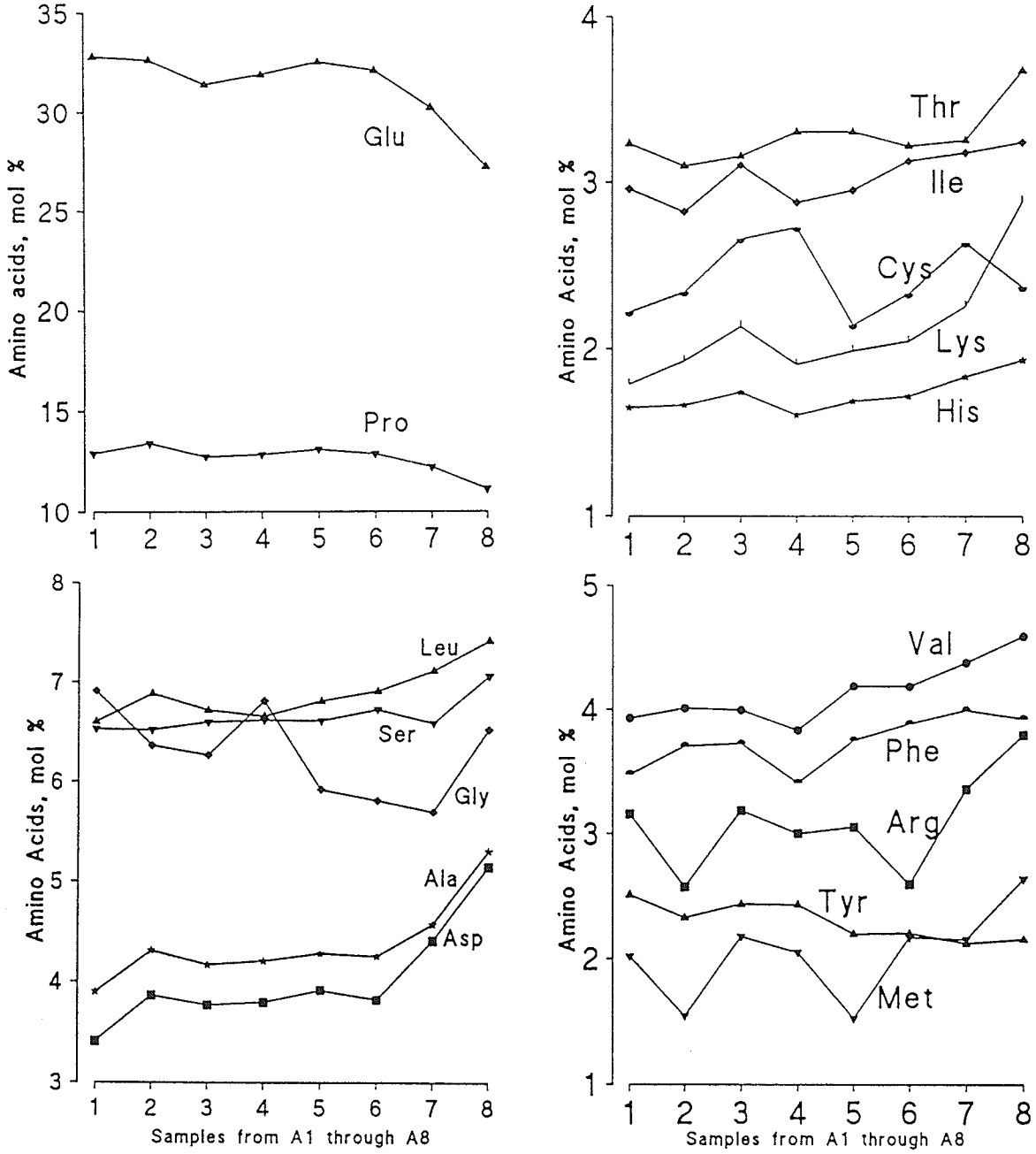


Figure 24. Variation of content of amino acids of glutenin fractions of the *Glu-1* null lines.

quite similar to that of the ethanol-insoluble glutenin of Bietz and Wall (1973), except that the glycine content is significantly lower in A8, which is probably due to the presence of the HMW

TABLE 3. The Amino Acid Composition (mol %) of A8 Glutenin as Compared with those of Relevant Fractions in Literature

	A8[a]	Glutenin Peaks[b]				Gluten Fractions[c]				
		1	2	3	4	1	2	3	4	5
Asx	5.2	4.1	2.8	2.7	4.5	3.2	1.4	0.9	2.0	2.7
Thr	3.7	4.8	3.2	2.7	3.6	3.2	2.4	2.6	2.6	3.1
Ser	7.1	8.2	6.5	7.6	6.8	6.6	7.2	7.1	7.0	5.9
Glx	27.2	20.9	32.3	35.3	29.2	32.7	39.4	41.6	37.5	32.8
Pro	11.1	10.5	12.4	13.6	11.6	11.0	15.0	15.4	15.1	13.5
Gly	6.5	10.9	12.8	4.2	5.6	12.9	3.3	7.4	4.9	9.2
Ala	5.3	5.1	4.1	3.7	5.3	4.1	2.4	2.0	2.7	4.0
Val	4.6	7.3	4.2	4.9	5.8	3.5	4.3	3.1	4.0	1.4
Met	1.6	1.5	1.1	1.6	1.7	0.8	1.3	0.9	1.3	4.8
Ile	3.2	5.8	2.8	4.4	4.9	2.3	3.7	2.9	3.4	3.3
Leu	7.4	6.7	5.9	7.8	7.8	6.1	7.5	5.6	7.4	6.7
Tyr	2.2	3.7	3.8	1.7	2.1	4.2	1.5	2.6	1.7	2.7
Phe	3.9	2.7	2.3	4.2	3.9	2.5	4.5	3.9	4.1	3.2
His	1.9	1.6	1.4	1.6	1.6	1.3	1.3	2.3[d]	1.4	1.5
Lys	2.9	2.4	1.6	1.3	1.9	2.8[d]	2.7[d]	0.4	1.0	1.4
Arg	3.8	3.8	2.8	2.7	3.7	2.7	2.2	1.3	2.6	2.2
Cys	2.4					0.0	0.0	0.0	1.3	1.4

a The glutenin fraction by the procedure of Osborne (Chen and Bushuk 1970) from A8 *Glu-1* null flour

b The major glutenin subunit peaks fractionated by gel filtration in the order of elution of the reduced Osborne glutenin by Belitz *et al.* (1987)

c From Bietz and Wall (1973). The gluten fractions: 1, Ethanol insoluble aminoethyl (AE) -glutenin; 2, Ethanol soluble AE-glutenin; 3, The 44 kDa AE-glutenin fraction from 2 after reduction; 4, HMW-gliadin; 5, Purified gliadin

d Data including AE-Cys

subunits in the glutenin of Bietz and Wall (1973). Tatham *et al.* (1987) also reported the amino acid composition of high molecular weight gliadins (aggregated gliadins). The Glx and Pro contents in their study were higher (*ca.* 35% and 16%, respectively), and the Gly content was lower (*ca.*

3.5%) than those of A8 obtained in this study. The results of the amino acid analyses suggest that the LMW glutenin from A8 is different from the high molecular weight gliadin or the ethanol-soluble glutenin. It seems to be a separate entity which is co-present with the HMW glutenin in the ethanol-insoluble residue of the Osborne fractionation.

5. Discussion of the Aqueous Ethanol-Insoluble LMW Glutenin

Besides the evidence from the SDS-PAGE results, other results in this study apparently also support the presence of LMW glutenin both in terms of the large molecular size and in terms of considerable proportion in the Osborne glutenin fraction, most notably in the fraction from A8. The gel filtration profile of A8 glutenin which is largely the same as that of glutenin from A1, suggests that the size distribution of the LMW glutenin of A8 is similar to that of the HMW glutenin in glutenin preparations of other wheat lines.

It is known that the HMW subunits represent only about 10% of the total flour protein (Halford *et al.* in press). The remaining glutenin (*ca.* 40% or more of total protein) is generally considered to be comprised of LMW subunits which are presumably covalently linked with the HMW subunits in the polymeric glutenin molecules. This hypothesis, however, requires at least that the HMW subunits be linearly linked with each other (Graveland *et al.* 1985) based on the number of sulfhydryl groups on the HMW and the LMW subunits. As shown later under the section "Structural Studies of Glutenin", the results here suggest that the HMW glutenin subunits are, however, probably linked in blocks with rheologically-ineffective disulfide bonds inside the block and rheologically-effective ones between the blocks. In such a case, it seems less likely that the HMW glutenin subunit blocks have enough sufhydryl groups available for all of the LMW subunits in the glutenin fraction. The assumption that the glutenin fraction of normal wheat lines contain both HMW and LMW glutenin offers an explanation for the problem of stoichiometry between HMW and LMW subunits. The yield of glutenin from A8 (no HMW subunits), which may be taken as the yield of LMW glutenin, was about 32% of total flour protein.

The presence of LMW glutenin may help to explain why the molecular weight distribution of flour proteins or glutenin fractions does not appear to be related to dough strength or breadmaking quality. In the preparation of glutenins from the *Glu-1* null wheats, the glutens from A7 and A8 were quite different from the others in terms of rheological properties. Those glutens were much softer, smoother, and more extensible. Accordingly, the presence of variable amounts of LMW glutenin in different wheat varieties may account for variations in rheological properties. Further work is needed to confirm this hypothesis.

In summary, the results obtained in this part of the study indicate that the Osborne glutenin fraction comprises at least two different entities: 1) a group of polymeric molecules consisting of LMW subunits only, and 2) a group of molecules of HMW subunits only or both HMW and LMW glutenin subunits. These two types of glutenin polymers have been tentatively named LMW glutenin and HMW glutenin, respectively, based on the absence and presence of HMW subunits. The two types of glutenin polymers appear to be of similar size as shown by gel filtration chromatography on the column used in the present study. Both types of glutenin play a substantive role in the functional properties of doughs from flours of common (hexaploid) wheats.

G. Structure of Glutenin

In part B of this section, the gel filtration profiles of protein extracts of the flours of the four Canadian wheat varieties of diverse breadmaking quality were reported and the glutenin preparations from these flours did not show any obvious differences under the conditions used in this study. Similarly, it was found that the profiles of the *Glu-1* null wheat flours, which also differed widely in baking quality, showed remarkable similarities in terms of the shape and overall distribution of the absorbance. Based on these results, it seems logical to presume that breadmaking quality and the relevant rheological properties of dough must depend on the inherent "quality" of glutenin molecules, and most likely their interactions with each other and with protein molecules belonging to other fractions, and with other constituents of the flour. It is possible,

however, that the resolving power of the gel filtration column used in this study was not sufficient enough to demonstrate any differences in molecular weight distribution.

The inherent quality of glutenin molecules cannot be understood unless the total molecular structure of the molecules is elucidated. This part of the study was an attempt to contribute to the work in the elucidation of the structure of glutenin, based on the electrophoretic analysis and rheological properties as measured by the farinograph.

1. Dough Mixing in Farinograph

It can be seen from the farinograms in Fig. 25 that a small amount of disulfide reducing agent (*e.g.* 20 μmol DTT) caused a drastic drop in dough consistency. The effect on dough consistency seemed to be saturated at about 80 μmol DTT. The farinogram for 500 μmol DTT was essentially the same as that for the dough treated with 80 μmol DTT. This observation is generally consistent with that of Jones and Carnegie (1969) who used the farinograph to determine the proportion of the so-called "rheologically-effective" disulfide groups in wheat flour.

For the doughs treated with 3,000 μmol DTT and mixed under nitrogen, the change in consistency was similar to that for analogous doughs mixed under air, except that there was a gradual, albeit small, increase in consistency beyond 5 min of mixing (Fig. 26), probably due to the loss of dough moisture caused by nitrogen flushing. NEMI, a sulfhydryl blocking agent, had no discernible effect on the farinograms of doughs containing 3,000 μmol DTT and mixed under nitrogen (Fig. 26).

2. SDS-PAGE of Partially-Reduced Doughs

The electrophoregrams for the extracts of the partially-reduced doughs (Fig. 27) showed that there was no release of HMW glutenin subunits, in spite of the drastic drop in farinograph

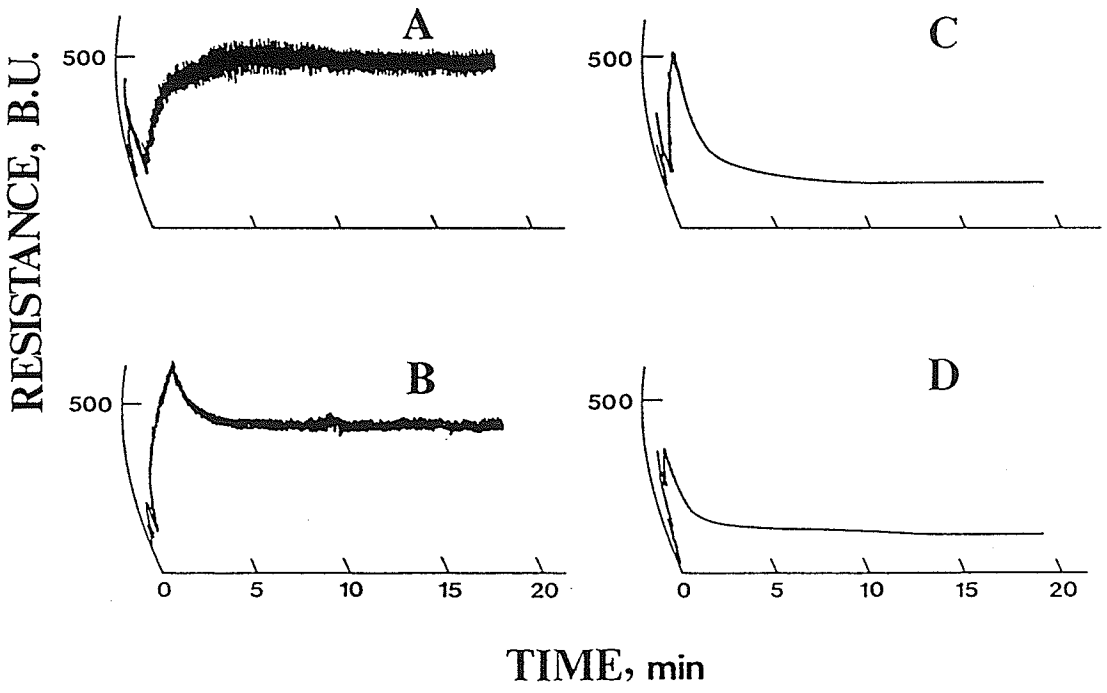


Figure 25. Farinograph curves for partially-reduced doughs mixed under air. A, flour (50 g) and wat er (control dough); B, dough mixed with 20 μmol dithiothreitol (DTT); C, dough mixed with 80 μmol DTT; D, dough mixed with 3,000 μmol DTT.

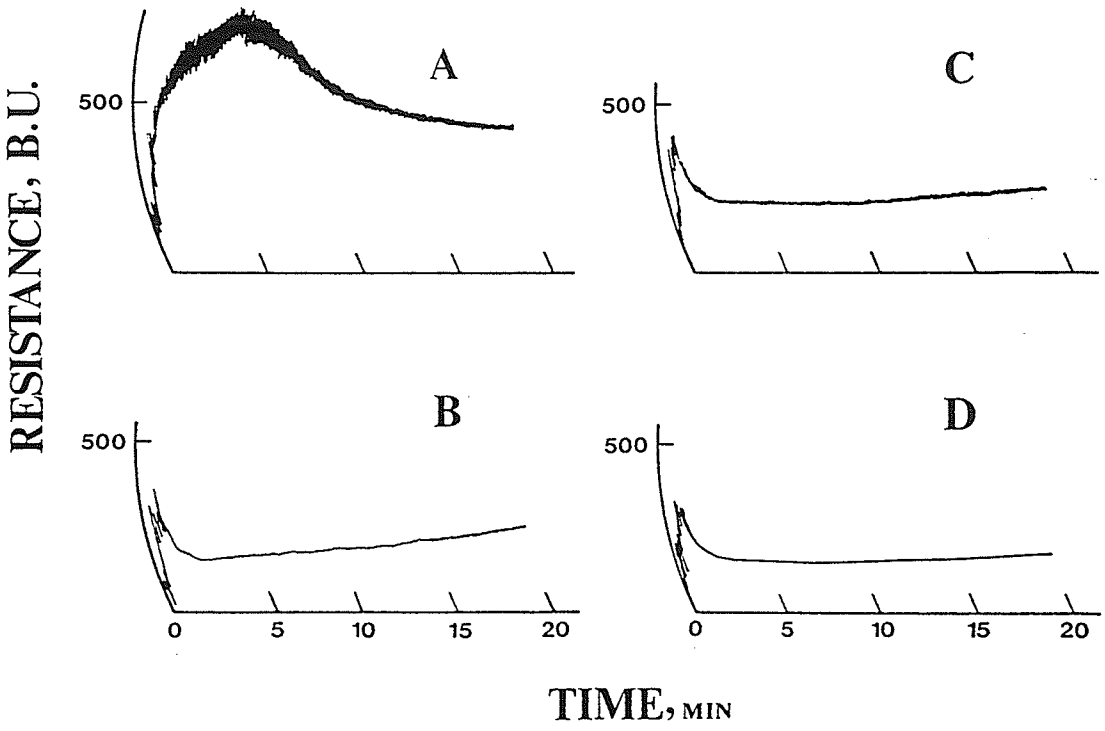


Figure 26. Farinograph curves of partially-reduced doughs mixed under nitrogen. A, dough mixed with 500 μmol N-ethylmaleimide (NEMI); B, dough mixed with 3,000 μmol dithiothreitol (DTT); C, dough mixed with 3,000 μmol DTT and 500 μmol NEMI; D, dough mixed with 3,000 μmol DTT and 2,000 μmol NEMI.

Figure 27. SDS-PAGE patterns of the partially-reduced doughs. Lanes 2-6 for dough mixed under air; Lanes 7-10 for doughs mixed under nitrogen.

Lanes 1 and 11, control (Katepwa) flour;

Lane 2, flour (50 g) and water (control dough) mixed under air;

Lane 3, dough mixed with 20 μmol dithiothreitol (DTT);

Lane 4, dough mixed with 80 μmol DTT;

Lane 5, dough mixed with 500 μmol DTT;

Lane 6, dough mixed with 3,000 μmol DTT;

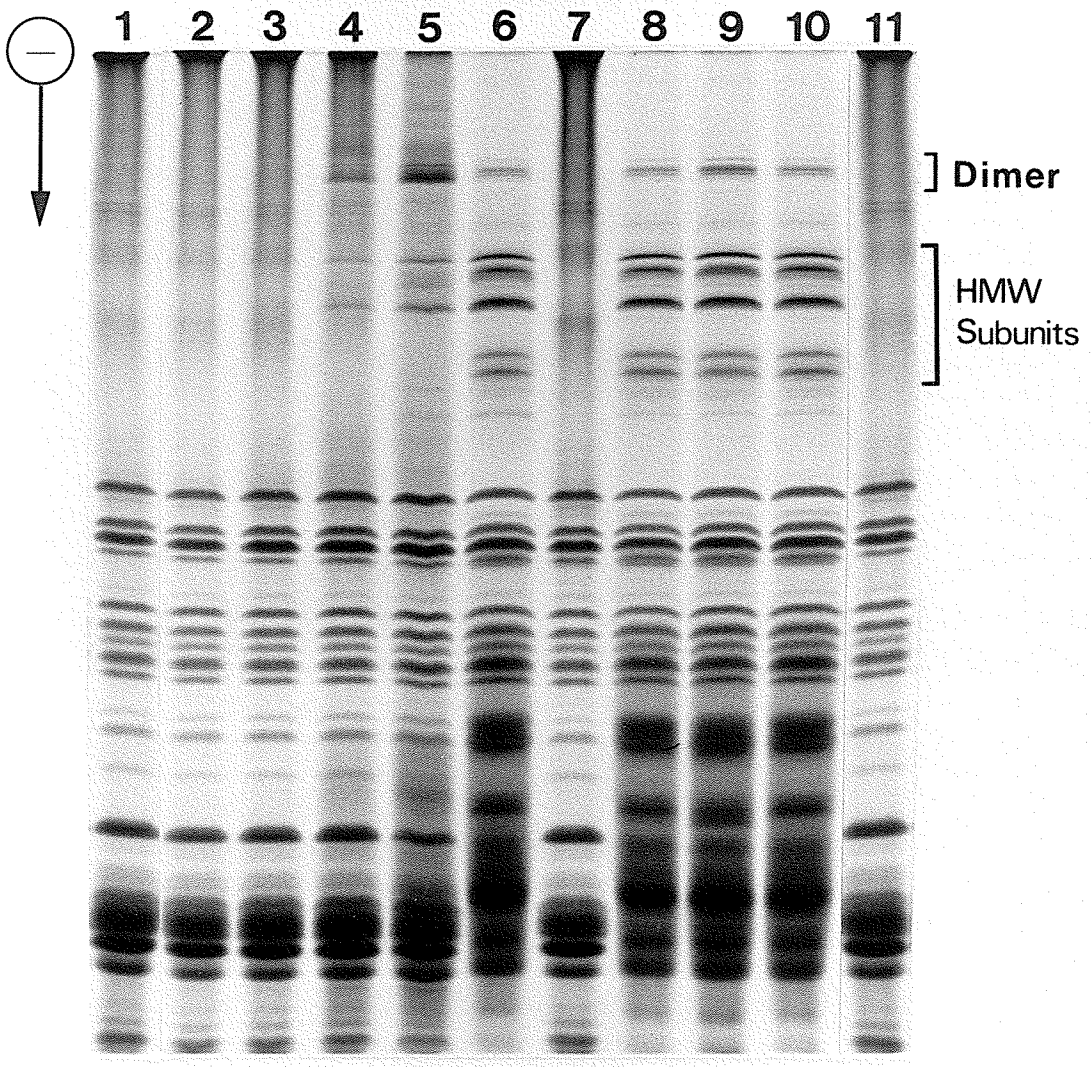
Lane 7, dough mixed with 500μmol N-ethylmaleimide (NEMI) under nitrogen;

Lane 8, dough mixed with 3,000 μmol DTT;

Lane 9, dough mixed with 500 μmol NEMI+3,000 μmol DTT;

Lane 10, dough mixed with 2,000 μmol NEMI+3,000 μmol DTT;

"Dimer", position indicating the region with dimeric bands (see text).


1
2
3
4
5
6
7
8
9
10
11
Dimer
HMW
Subunits

consistency, upon addition of 20 μmol of DTT. This observation is consistent with the earlier findings of Ng *et al.* (1991).

For the samples mixed with higher levels of DTT, the HMW subunits appeared gradually at differential rates with increasing DTT concentration. The SDS-PAGE pattern and the band intensity of the HMW subunits of the partially-reduced doughs were quite different from those of completely-reduced sample (*e.g.* lane 5 in Fig. 28). The observed difference in susceptibility of glutenin disulfides to DTT suggests variability in spacial arrangement of subunits in the micro environment of the polymeric glutenin molecules. Matsumura *et al.* (1984) suggested that some interpolypeptide disulfide bonds of glutenin are less reactive than others since different subunits were released to a different extent upon addition of a low concentration of 2-mercaptoethanol based on the assumption that the HMW subunits are linked linearly one after one in glutenin.

The electrophoretic results showed that even at a relatively high concentration of reducing agent when the HMW subunits are almost completely released, many of the LMW glutenin subunits seemed to be still bound in polymers or oligomers, as indicated by the relatively low intensity of the region for LMW glutenin subunits (compare Fig. 27 with lane 4 or 5 in Fig. 28 on page 110). This result is consistent with the gel filtration result which showed that the profile segment containing the HMW subunits was reduced and the segment for the LMW glutenin subunits was not increased by the initial addition of 20 μmol DTT (Fig. 18).

At higher levels of DTT (80 μmol to 3,000 μmol, lanes 4-6 in Fig. 27), it was observed at least three closely-migrating bands, with mobilities slightly lower than that of the largest HMW glutenin subunit (2^* for cv. Katepwa). The nature of these bands is discussed below.

First of all, it is noteworthy that the closely-migrating low-mobility bands migrated into the gel under conditions where a large amount of protein was retained at the front of the separating gel (Fig. 27). This result suggests that the low-mobility bands are intermediate products from the reduction of partially-reduced glutenin oligomers upon exposure to additional DTT.

Experiments were undertaken to exclude oxygen and to block the free sulfhydryl groups, which were produced during mixing under partial reduction, to investigate the possibility of the

low-mobility bands being produced during mixing by oxidation or by reassociation of glutenin subunits produced by reduction with DTT. The dough samples were analyzed by SDS-PAGE without reduction for these closely-migrating low-mobility bands. As shown in Fig. 27 (lanes 7, 8, 9, 10) these bands appeared in the electrophoregrams of doughs mixed in air or nitrogen in the presence of DTT with or without the sulfhydryl blocking agent (NEMI). Accordingly, it was concluded that the low-mobility bands exist as part of the structure of the glutenin polymer and are not an experimental artifact formed by reoxidation of sulfhydryl groups of subunits during dough mixing. Recent work by Werner *et al.* (in press) also suggested the concept that HMW subunits are present in polymeric glutenin in certain unique combinations.

3. SDS-PAGE of the Low-Mobility Bands

In order to determine the subunit composition of the low-mobility bands, the bands were analyzed by SDS-PAGE after their complete reduction with mercaptoethanol (Fig. 28). The results showed that these bands contained all of the five HMW and no LMW subunits of glutenin. Two-step electrophoresis produced the same evidence (data not shown).

Based on the relative molecular weights (by SDS-PAGE) of the low-mobility bands and of the HMW glutenin subunits, it was estimated that the following dimeric combinations of the HMW subunits fit the molecular weights of the three low-mobility bands: $2^{*}+7$, $2^{*}+9$, and $5+10$. These combinations remain to be verified by other analytical methods.

Lawrence and Payne (1983) reported analogous closely-migrating bands, named "O" bands, which resulted from partial reduction of ground wheat endosperm prior to electrophoresis. Whether or not the bands described here, obtained by a different procedure, are the same as the "O" bands remains to be established. Results of Matsumura *et al.* (1984) and those of Graveland *et al.* (1985) also suggested the presence of HMW glutenin oligomers in partially-reduced glutenin, based on their lower mobilities than those of the HMW subunits in SDS-PAGE analysis.

Figure 28. SDS-PAGE patterns of the dimeric bands.

Lane 1, control flour;

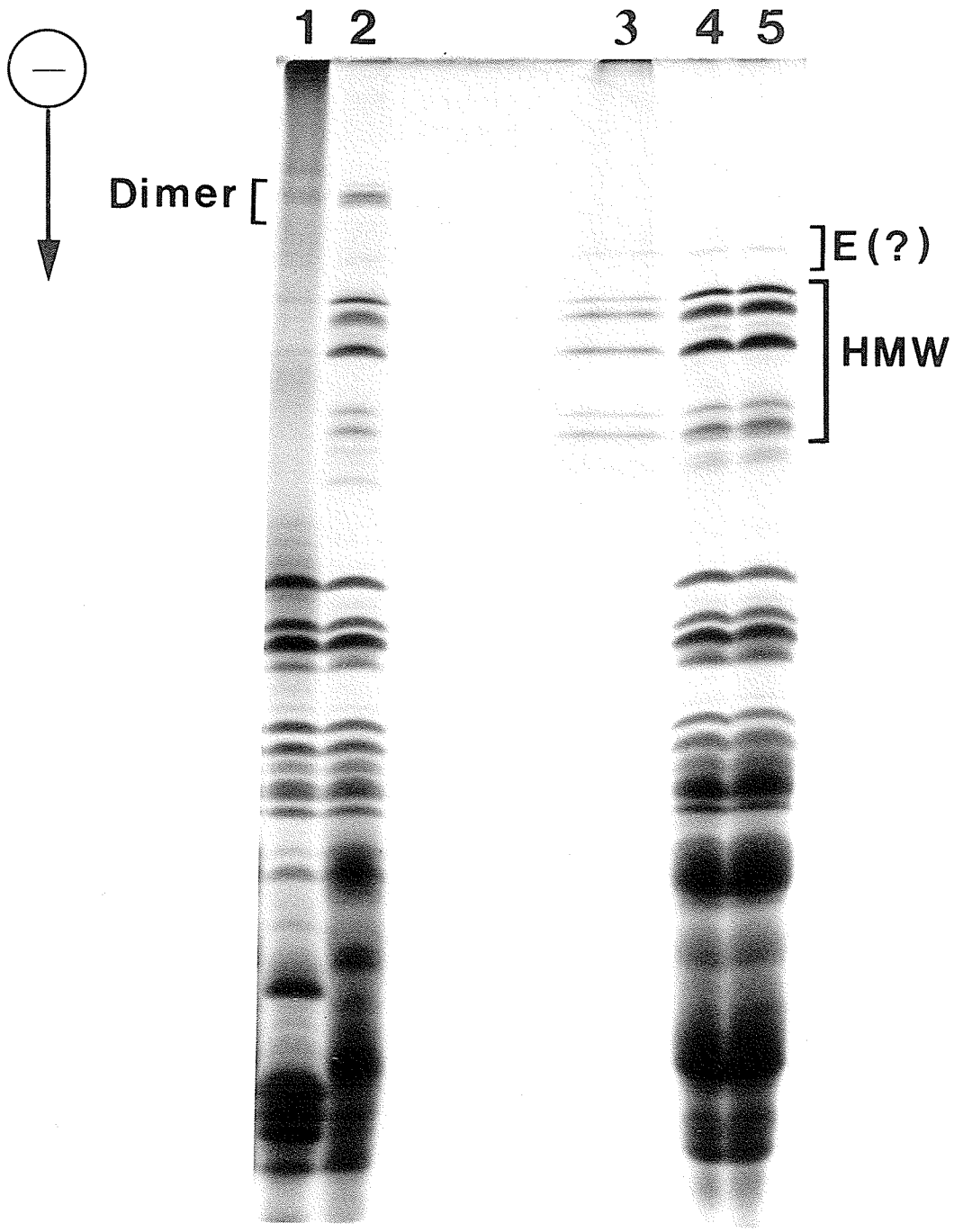
Lane 2, dough mixed with 3,000 μmol dithiothreitol;

Lane 3, extract of gel portion containing dimeric bands;

Lane 4, sample in lane 2 with 2-mercaptoethanol (ME) in extracting buffer;

Lane 5, sample in lane 1 with ME in extracting buffer;

"E(?)", position indicating the region with E-bands as reported by Gupta and Shepherd (1987).


1 2
3 4 5
Dimer [
]E(?)
HMW

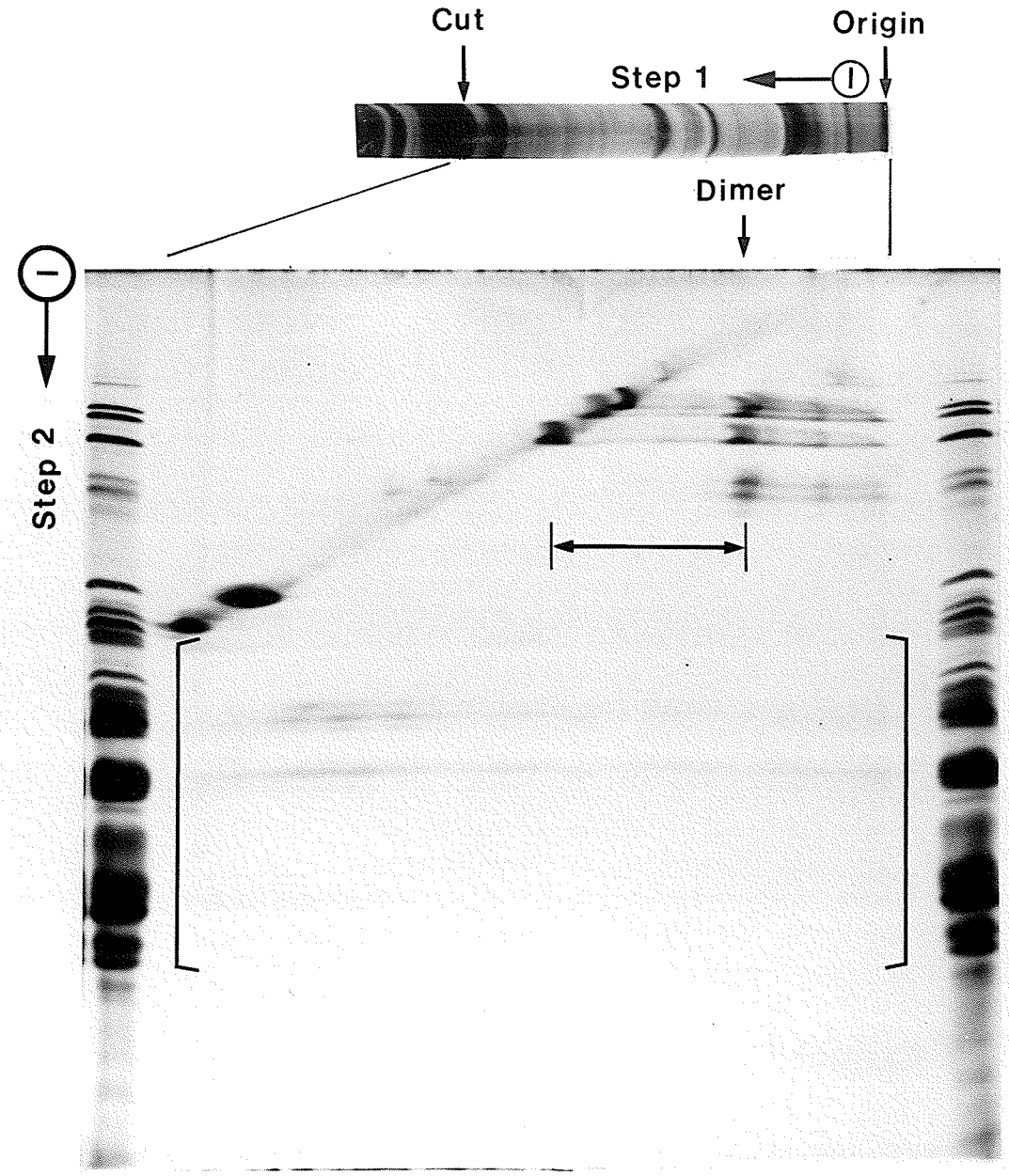
Also illustrated in Fig. 28 (lane 3), is another band with a slightly lower mobility than that of the largest HMW glutenin subunit (2^*) but of similar band intensity in the electrophoregram. It was derived from the protein in the piece of gel that contained the dimers. It seems to be closely associated with the HMW subunits and/or with their dimers. Based on mobility in SDS-PAGE, this band may be one of the E-type bands reported by Gupta and Shepherd (1987). The nature of its relationship to the HMW glutenin subunits remains to be determined.

Finally, it is interesting to note that the accumulation of the dimeric bands as shown by SDS-PAGE seems to be related to the small drop in farinograph consistency beyond the marked decrease upon first addition of small amount of DTT. At low levels of DTT, the dimers were not seen. After the consistency reached a plateau at about 80 μmol DTT, the bands appeared and were present at all higher levels of DTT used in the present study (up to 3,000 μmol). This result suggests that the disulfide bonds that are reduced to produce dimeric bands are of the rheologically-ineffective type.

4. Results of Two-Step SDS-PAGE

The purpose of the two-step SDS-PAGE was to examine the subunit composition of other oligomers released from the partial reduction of dough. From the bottom part of the two-step gel for the dough mixed with 500 μmol DTT (bracket in Fig. 29), it can be seen that there are a few faint, but clearly visible, bands corresponding to the region of mobility of the LMW glutenin subunits. These bands are not the artifacts reported by Tasheva and Dessev (1983) since parallel experiments for doughs which were reduced with 3,000 μmol DTT and analyzed under the same conditions repeatedly showed that only the dough reduced with 500 μmol DTT had these bands. This result may explain why there were no LMW glutenin subunits detected by Graveland *et al.* (1985) who used a higher concentration of reducing agent.

Figure 29. Two-step electrophoretic pattern for partially-reduced dough with 500 μmol dithiothreitol. "Origin", position indicating the front the separating gel in the first step; "Dimer", position indicating the closely-migrating dimeric bands in the first step. The two flanking lanes are for control flour (with 2-mercaptoethanol).


Cut
Origin
Step 1
Dimer
Step 2

Besides the LMW glutenin subunits, the two-step gel also showed bands corresponding to the HMW glutenin subunits. The bands in the first step gel, with relative molecular weights larger than those of the dimeric bands, appear to be oligomers composed predominantly of HMW glutenin subunits. The production of these oligomers suggests that the disulfide bonds that link HMW glutenin subunits in polymeric glutenin are more resistant to reduction than the disulfides between the LMW and HMW subunits or between the LMW subunits at higher level of DDT. Differential susceptibility of disulfides to reduction would lead to a differential release of LMW subunits with increasing concentration of reducing agent.

Additionally, the region between the dimeric bands and the HMW subunits in the first-step gel (arrow range in Fig. 29), showed bands of oligomers comprising LMW subunits and a single HMW subunit. Obviously, some LMW subunits appear to be tightly linked to a single HMW subunit in the polymeric structure of glutenin.

5. A Block Model for the Structure of Polymeric Glutenin

SDS-PAGE results for doughs reduced with low concentrations of DTT (20 μmol DTT/50 g flour) showed that there were no reduced proteins that can be visualized by this analytical method. However, farinograph results for the same doughs showed a marked drop in dough consistency, suggesting a marked decrease in molecular size of the component(s) responsible for the consistency of dough. The key component in this regard is presumed to be the glutenin.

At higher concentrations of DTT (80 or more μmol/50 g flour), a group of unique dimeric bands was observed of known HMW subunits and another protein which may be equivalent to one of the E-bands reported by Gupta and Shepherd (1987); the concomitant additional drop in farinograph consistency was small.

The saturation of the decrease in farinograph consistency supports the hypothesis of the presence of rheologically-effective disulfide bonds, and thus the presence of unique structural blocks in polymeric (molecular) glutenin. The further release of the dimeric bands after saturation

of the rheological effect suggests that these bands were released from a structural unit (*e.g.* partially-reduced glutenin oligomer). The depolymerization of these oligomers did not contribute substantially to the decrease of farinograph consistency.

The accumulation of the dimeric bands occurred at much higher concentrations of DTT than that of other oligomers which entered the SDS-PAGE gel in the unreduced procedure. This differential appearance of breakdown oligomers suggests that there are, in polymeric glutenin, relatively stronger or inaccessible disulfide linkages between the two specific HMW glutenin subunits which appeared as dimers.

The two-step electrophoresis (see Fig. 29) showed the presence of two other oligomers: 1) those composed predominantly of HMW subunits and, 2) those of LMW subunits and a single HMW subunit.

Results of this study suggest that polymeric glutenin comprises unique submolecular structural units which can be identified as distinct partially-reduced glutenin oligomers. The type of the oligomer produced apparently depends on the concentration of reducing agent added. The first group of distinct oligomers (blocks), is produced by the reduction of the so-called rheologically-effective disulfide groups. These oligomers are of high molecular weights and contain all or most of the subunits of total glutenin. Further reduction, at higher concentrations of DTT, produced other unique oligomers of lower molecular weight. The oligomers that have been identified in the present study include dimers of specific HMW subunits, oligomers of all five HMW subunits, and oligomers of several LMW subunits and only one HMW subunit.

The breakdown of glutenin and its relationship to rheological properties of doughs, along with the results from other sections of this thesis, suggests that the glutenin of HMW subunits (HMW glutenin) is responsible for the toughness and elasticity of gluten (dough) while the glutenin of LMW glutenin subunits (LMW glutenin) is probably responsible for its viscosity, along with the gliadins as suggested in the literature (Belitz *et al.* 1987; MacRitchie 1978; MacRitchie 1987).

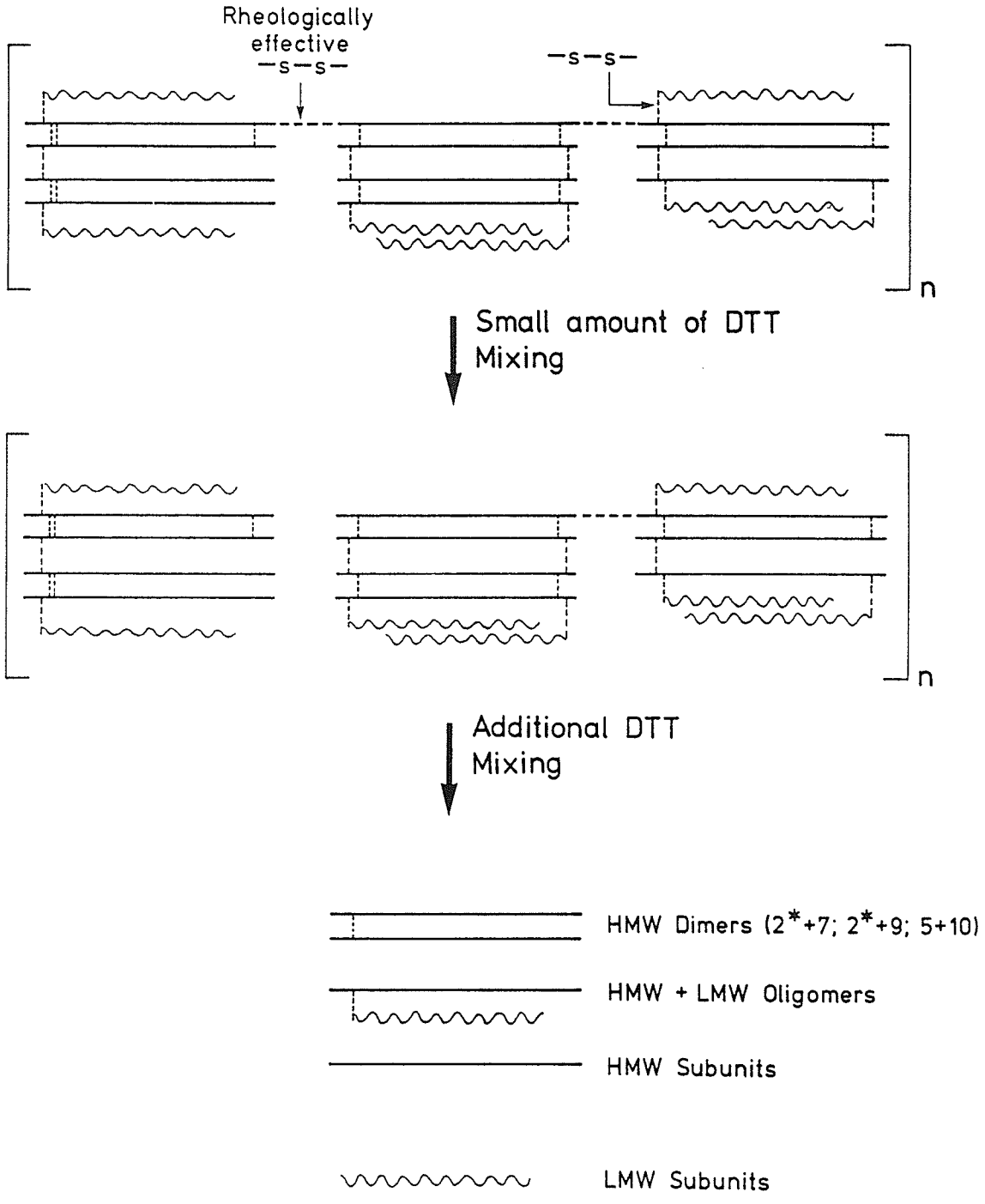


Fig. 30. A hypothetical model for the polymeric structure of glutenin and its breakdown upon exposure to increasing amounts of dithiothreitol under dough mixing condition.

The new evidence presented in this part of the study is incorporated into a modification of the model for glutenin of Ng *et al.* (1991) shown in Fig. 30. In this modification, the HMW subunits are co-linked by disulfide bonds at the ends of the polypeptide chains as suggested by the known evidence that the cysteine residues are mostly located on the ends of the subunits (see Kasarda 1989 and references therein). This model is somewhat different from that of Graveland *et al.* (1985). It can better explain: 1) the drastic drop in farinograph consistency upon reduction of a relatively small number of rheologically-effective disulfide bonds without producing monomeric subunits, 2) differential release of HMW glutenin subunits with further reduction and, 3) the production of glutenin oligomers. Further work is required to determine the precise details of the proposed model at the molecular level of structure.

V. GENERAL DISCUSSION

A. Solubilization of Glutenin

Glutenin (unreduced) was successfully solubilized in 6M urea/6% SDS, without the involvement of vigorous mechanical stirring. The solubilization was more than 99% complete as shown by the amino acid analysis and SDS-PAGE of the protein in the residue after solubilization and removal of the dissolved protein.

There was no evidence from SDS-PAGE or gel filtration chromatography indicating that the disulfide bonds were cleaved during solubilization. The HMW glutenin subunit compositions of the reduced and solubilized glutenin were the same as those of the respective flours although there were slight variations in their relative mobilities of the subunits. The variation in mobility is believed to be caused by the presence of cyanate impurities in the urea. The solubilization procedure should be an important advance in the study of the glutenin fractions, or glutenin of gluten, dough or flours. The solubilization procedure made possible the subsequent study of the molecular weight distribution of the polymeric (molecular) glutenin.

B. Gel Filtration Chromatography of Flours and Glutenins

Gel filtration chromatography of extract of flours and glutenin preparations on Sephacryl S-500 showed a spectrum of proteins from polymeric (some larger than the exclusion volume) to monomeric polypeptides. Most glutenin molecules that contained HMW subunits ranged in relative molecular weight from several hundred thousand (elution volume of *ca.* 350 ml) to several million daltons (near the excluded peak).

The shape of the elution profiles generally confirmed the differential solubilization in fractionation of Osborne (1907) in which the albumin/globulin are essentially completely removed as in gluten washing, followed by almost complete removal of the gliadins in the preparation of glutenins, resulting in a glutenin fraction comprising mainly high molecular weight polymeric glutenin.

It has been proposed by some researchers that glutenin polymers of wheat varieties differ in their molecular weight distribution (for review see Kasarda 1989). In the present study, the gel filtration chromatographic results did not confirm this hypothesis. However, it is possible that the reason for this is the low resolution of Sephacryl S-500 for the wide range of molecular weight of the molecules encountered in this study. Some other substances in flours, gluten, or glutenin seem to have strong absorption at 280 nm, most noticeably in the excluded peak since SDS-PAGE showed that these fractions contained very little protein. The nature of these substances was not investigated. The size of the excluded peak varied with wheat variety, but it did not seem to be consistently correlated with breadmaking potential of the varieties used in the study. This peak probably included non-protein substances which would invalidate some of the published correlations between the size of the excluded peak and breadmaking potential of flours of diverse breadmaking quality. In the literature, absorbance at 280 nm has been almost exclusively used for the estimation of protein in gel filtration chromatography fractionation. A more accurate procedure for estimation of protein in gel filtration chromatography is required for the study of molecular weight distribution of wheat proteins in relation to their functionality in the breadmaking process.

The major purpose of this study was to obtain information on the molecular weight distribution of polymeric glutenin; accordingly a gel filtration medium of very large exclusion limit was selected. The resolution for glutenin and gliadins could be improved if a medium of smaller exclusion limit is used.

Results of this study suggest that the differences of the glutenins relevant to protein quality of wheat varieties of diverse breadmaking qualities may lie to a large extent in the physical or non-

covalent intermolecular interactions which, however, would be disrupted by the solubilization procedure and therefore not reflected by the gel filtration chromatography results. The strength of the interactions was evidenced by the different rates of solubilization of glutenins of different wheat varieties as shown in the first part of this thesis. The UV method for estimating the degree of solubilization is inexpensive, convenient and relatively fast, but at the cost of accuracy. It is suggested that more intensive studies involving more wheat varieties would be beneficial to the understanding of the intervarietal differences in the molecular weight distribution of glutenin in the context of breadmaking quality.

While the gel filtration profiles did not confirm a relationship between the molecular weight distribution of glutenin and breadmaking quality of flours or glutenin preparations, the profiles for doughs treated with a small amount of reducing agent showed a progressive shift in molecular weight distribution, especially the decrease in the amount of molecules of the largest size. The change of the elution profile of the doughs mixed with different amounts of reducing agent accounts for the drastic drop in farinograph resistance on the addition of a small amount of reducing agent, and for the subsequently more gradual decrease on addition of larger amounts of reducing agent. The farinograph results are consistent with the hypothesis that the glutenin molecules are composed of "blocks" of subunits joined by "rheologically-effective" disulfide bonds. This hypothesis is generally consistent with the suggestion of Ewart (1977) that reducing agents tend to first reduce the disulfide bonds in the middle of glutenin molecules, which would result in a drastic drop in average molecular weight and hence a decrease in farinograph consistency.

C. Results of *Glu-1* Null Wheat Samples

1. Gel Filtration Studies

Glutenin preparations were obtained by Osborne fractionation from wheat samples with partially or completely null *Glu-1* loci. The yield of glutenin in terms of protein quantity ranged

from 32% (the lowest) for A8 (no HMW subunits) to 55% from A1 (all five HMW subunits). The yield of glutenin from A8 was the lowest probably because of the difficulties encountered in collecting the gluten particles in the process of gluten washing; some physical loss of glutenin is suspected.

Gel filtration chromatography showed similar profiles among the glutenins of the eight samples in spite of the marked differences in HMW subunit composition and baking qualities of the flours. The similarity of the gel filtration profiles of these wheat samples indicates that molecular weight distribution, as reflected by the technique used, did not seem to be correlated to dough strength or breadmaking quality (*e.g.* loaf volume) of flours. These results are somewhat contradictory to the published information which suggests that a greater content of glutenin of higher molecular weight is related to higher dough strength.

As observed for glutenin preparations of the Canadian wheats, SDS-PAGE results of the gel filtration fractions of glutenin preparations of *Glu-1* null wheats showed that the HMW subunits are always present in the polymeric glutenin molecules of the largest size. The molecular weight distribution was not related to the number or type of HMW subunits present. Accordingly, it appears that the "quality" of glutenin molecules, not their molecular weight in the normal range, is the key to their contribution to differences in breadmaking quality of diverse wheat varieties.

2. Aqueous Ethanol-Insoluble LMW Glutenin

This part of the study demonstrated unequivocally that glutenin molecules comprising only LMW glutenin subunits are present in a high proportion in the aqueous ethanol-insoluble glutenin preparations. It can be concluded from the results of the present study that the Osborne glutenin fraction is composed of mainly two groups of polymeric glutenin molecules, tentatively named HMW glutenin, and LMW glutenin.

The amino acid composition showed that the LMW glutenin is different from the high molecular weight gliadins (ethanol-soluble) although both contain LMW polypeptides of the same

relative molecular weight. The similarity and extent of identity of the LMW glutenin and high molecular weight gliadin remain to be elucidated.

The molecular weight distribution of the LMW glutenin appears to be similar to that of the HMW glutenin as indicated by gel filtration chromatography on Sephacryl S-500 medium. However, the results of the experiments on the *Glu-1* null lines suggest that the roles of the two glutenins in the rheological properties (and hence breadmaking quality) are different.

Results of amino acid analyses showed variations in amino acid compositions that were generally consistent with the number and type of HMW subunits present in the sample.

D. Model of Polymeric Glutenin

The study on the structure of glutenin based on farinograph and SDS-PAGE results led to a new model for the structure of polymeric glutenin. This model is somewhat different from those already published and reviewed in the Literature Review section. Particularly, it explains the marked drop in farinograph consistency on the addition of a small amount of disulfide-reducing agent, and the differential release of HMW subunits and the production of their dimers.

According to the postulated model, polymeric glutenin is composed of "structural blocks" comprising tightly linked glutenin subunits joined by disulfide bonds. The blocks are inter-linked by disulfide bonds which contribute consistency more to the change in rheological properties of dough when reduced than do the intra-block disulfides. The inter-block disulfides are presumed to be equivalent to the "rheologically-effective" disulfides reported in the literature.

A preliminary pictorial representation of the model has been developed. This model incorporates both the HMW and LMW subunits.

Both glutenins contribute to rheological properties of dough and hence to breadmaking potential. Preliminary results suggest that the LMW glutenin may be another important factor responsible for intervarietal variations in breadmaking quality.

VI. CONTRIBUTION TO KNOWLEDGE

1. A procedure was developed for the solubilization of glutenin. This procedure can solubilize more than 99% of the glutenin protein in Osborne glutenin fraction; no disulfide or other covalent bonds are cleaved during solubilization.

2. The molecular weight distribution of glutenin extracts of flours, glutens and glutenin fraction by gel filtration chromatography was found to be a continuous spectrum of molecular weights. The size of the excluded peak was not related to breadmaking potential of diverse wheat varieties.

3. No correlation was found between breadmaking quality of flour and the molecular weight distribution of glutenin as estimated by gel filtration chromatography of extracts of flour, gluten, or Osborne glutenin fractions under the conditions used in this study. It is impossible to conclude if the lack of resolution is real, or if the resolution of the column was too low to detect small differences in molecular weight distribution.

4. The results of studies reported in this thesis indicate that glutenin of bread wheats comprises at least two types of polymers, those comprising HMW and LMW subunits and those comprising only LMW subunits. The HMW glutenin seems to be responsible for the elasticity of the dough, and the LMW glutenin probably for the viscosity.

5. The marked drop in farinograph consistency on addition of small amounts of reducing agents involves the so-called rheologically-effective disulfide groups of the HMW glutenin. No HMW

glutenin subunits are produced at this level of reduction; the early eluted material in the gel filtration profile showed a significant shift toward the lower molecular weight direction.

6. The HMW subunits were found to be in certain combinations in the molecules of the HMW glutenin. The HMW subunits could be differentially released from the HMW glutenin after the addition of the reducing agent reached a certain level.

7. LMW glutenin subunits can form polymeric molecules; the molecular weight distribution of those polymers appear to be similar to that of polymeric glutenin comprising both HMW and LMW subunits.

8. The LMW glutenin is a major storage protein in proportion and probably an important contributor to breadmaking quality, especially to inter-varietal variations.

9. A "block" model for polymeric HMW glutenin was proposed.

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APPENDIX I. Characterization of the Canadian Wheats

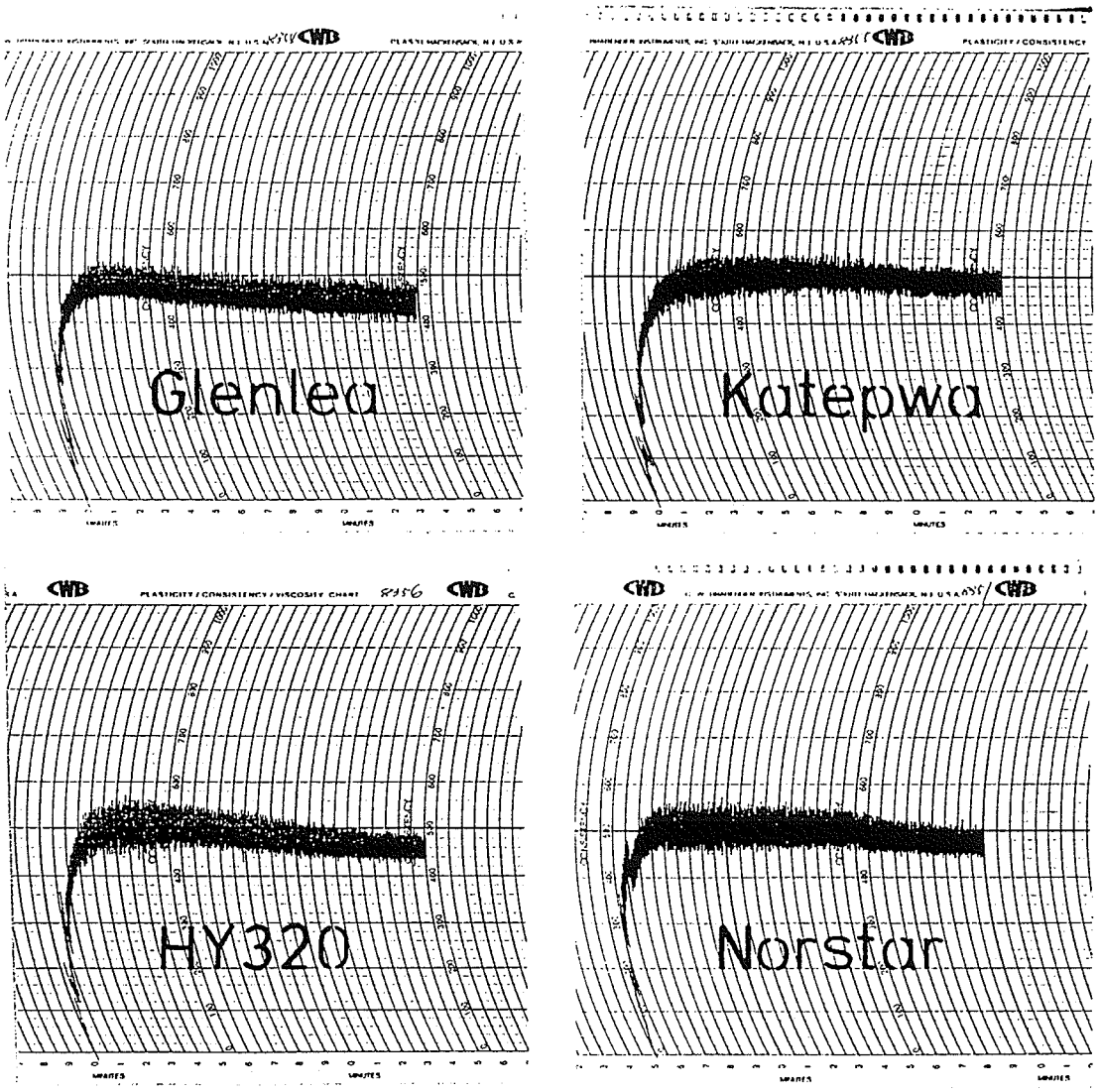
A. Chemical and Baking Characterization[a]

WHEAT/ FLOUR SAMPLE	WHEATS				FLOURS								BAKING	
	HWt	PSI	Prot	FN	Yld	Prot	Ash	WetG	Sed	Amg	FN	SD	BAbs	LV
Glenlea	75.1	49.9	12.8	308	69.9	12.1	0.490	29.7	60	340	334	29.7	62	695
HY320	77.2	72.1	11.6	392	67.1	9.8	0.401	27.7	54	965	445	10.0	57	630
Katepwa	77.3	72.1	13.1	392	67.1	12.7	0.425	34.1	64	690	493	21.3	62	800
Norstar	78.5	63.3	11.2	317	76.0	9.8	0.385	26.9	48	645	392	17.7	56	700

a. The Canadian wheat cultivars used in this study. The abbreviations: HWt, hectare weight (kg); PSI, particle size index (%); Prot, protein content (14% m.b., AACC Method 46-12 or 39-10); FN, Hagberg falling number (sec, AACC Method 56-81B); Yld, flour yield (%, AACC Method 26-20); Ash, flour ash content (%, 14% m.b., AACC Method 08-01); WetG, flour wet gluten content (%, 14% m.b., ICC Method 137); Sed, Zeleny sedimentation value (ml, AACC Method 56-60); Amg, amylograph peak viscosity (B.U., AACC Method 22-10); SD, starch damage (%, AACC Method 76-30A), BAbs, baking absorption (%, 14% m.b.); LV, loaf volume of the remix test (ml).

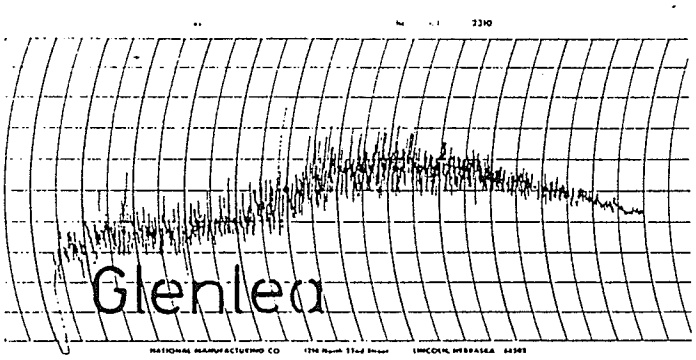
APPENDIX I. Characterization of the Canadian Wheats

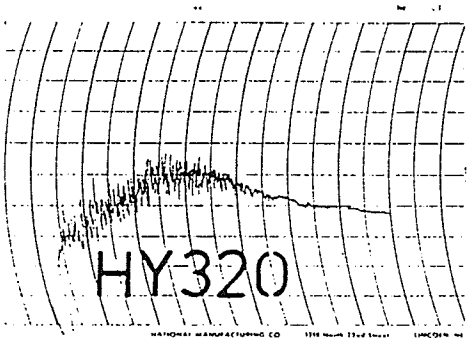
B. Farinograph Curves

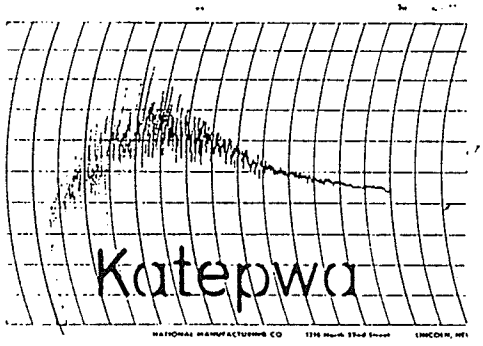


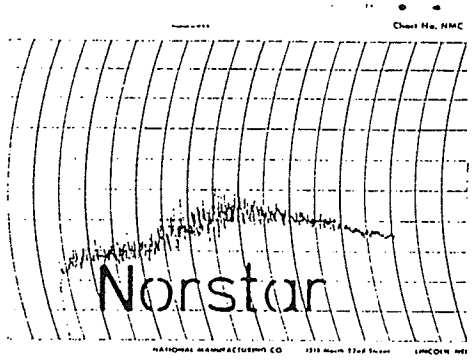
APPENDIX I. Characterization of the Canadian Wheats

C. Mixograph Curves



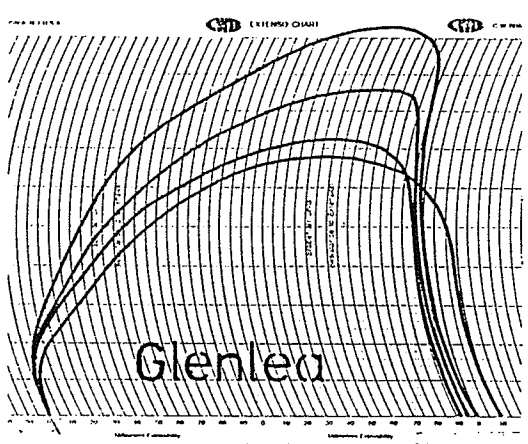


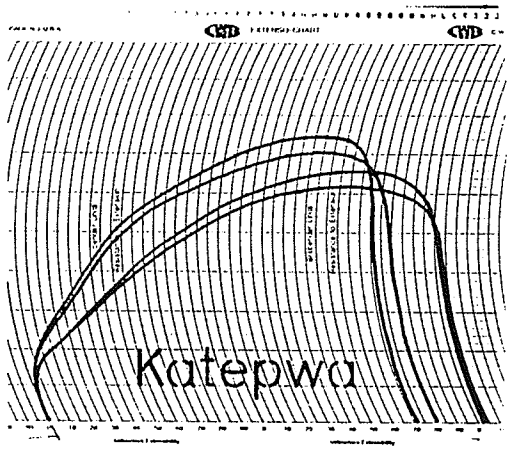


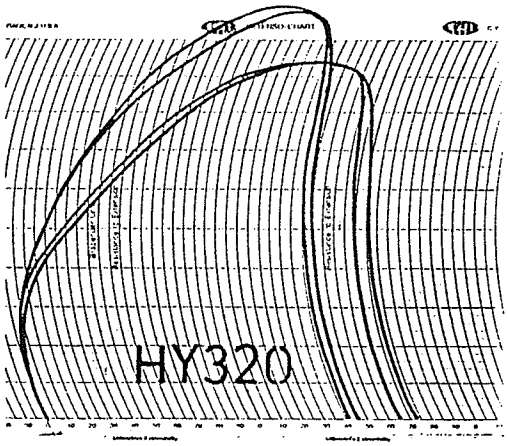


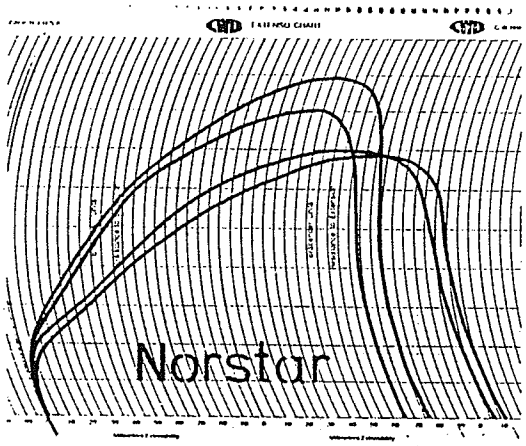
APPENDIX I. Characterization of the Canadian Wheats

D. Extensigraph Curves









APPENDIX II. Characterization of the Australian Glu-1 Null Wheats

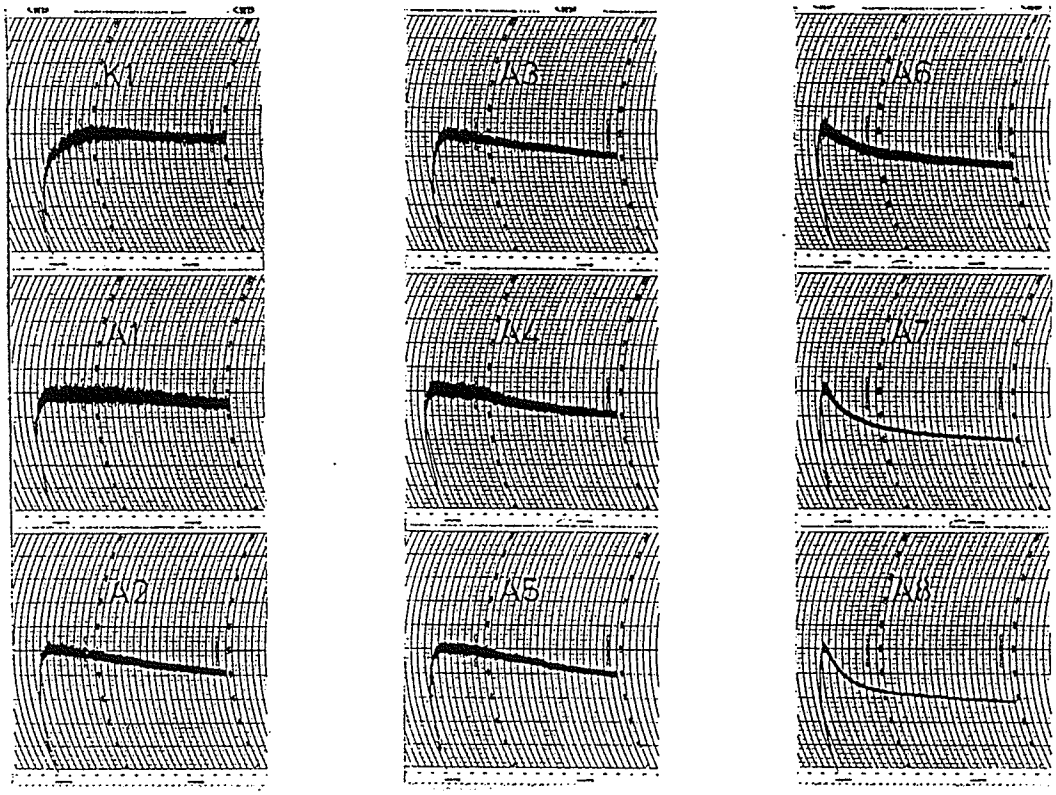
A. Chemical and Baking Characterization[a]

WHEAT/ FLOUR SAMPLE	WHEATS					FLOURS						BAKING	
	HWt	PSI	Ash	Prot	FN	Yld	Prot	Ash	WetG	Sed	Amg	BAbs	LV
K1[b]	79.1	55	1.51	14.4	460	71.6	13.9	0.35	39.1	67	920	64	745
A1	77.3	72	1.53	9.9	395	70.7	9.3	0.41	19.0	42	950	55	560
A2	74.4	66	1.79	10.7	420	68.2	9.4	0.45	23.7	35	1020	56	495
A3	76.2	61	1.67	10.6	430	74.0	9.6	0.43	28.2	27	930	59	510
A4	79.2	63	1.49	9.5	310	71.2	8.2	0.41	17.4	35	440	56	490
A5	76.3	56	1.72	11.1	425	69.2	10.0	0.44	27.2	33	920	60	525
A6	77.5	74	1.53	10.1	395	64.2	7.8	0.40	21.0	12	920	52	395
A7	72.6	56	1.79	11.4	445	68.9	10.0	0.50	-	12	950	55	420
A8	74.4	58	1.73	11.3	420	69.9	10.4	0.48	-	11	840	55	440

a. The grain of the Australian Glu-1 wheat lines used in this study. The abbreviations are the same as used in Appendix I-C.
b. A Canadian bread wheat of excellent breadmaking quality, which was used as a control.

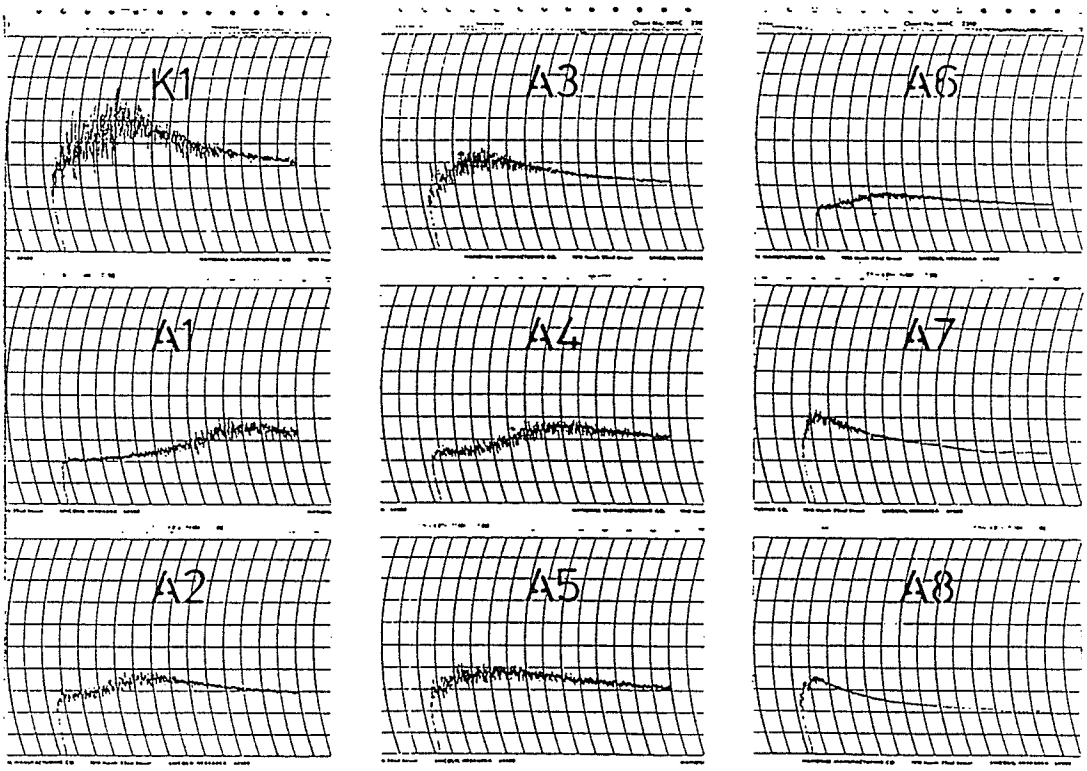
APPENDIX II. Characterization of the Australian <u>Glu-1</u> Null Wheats

B. Farinograph Curves



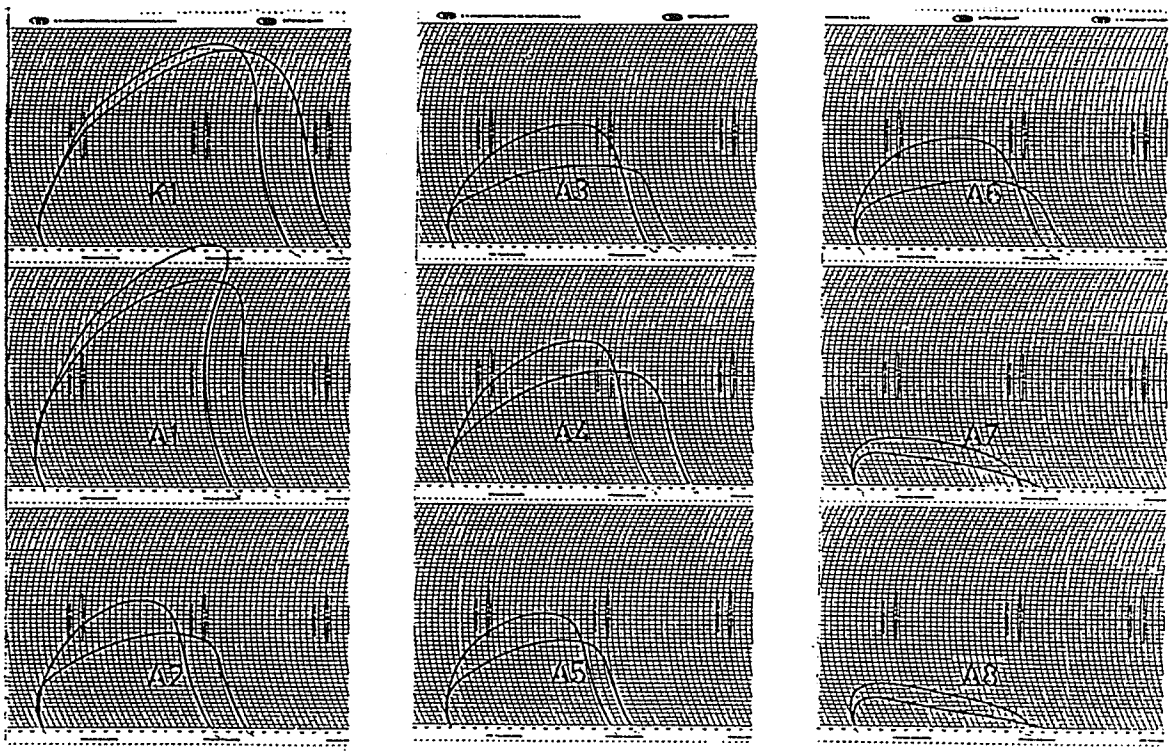
APPENDIX II. Characterization of the Australian <u>Glu-1</u> Null Wheats

C. Mixograph Curves



APPENDIX II. Characterization of the Australian <u>Glu-1</u> Null Wheats

D. Extensigraph Curves



APPENDIX III. The Amino Acid Composition[a] (mol%) of the Residue Protein after the Solubilization Procedure

Amino Acid	Residue source from solubilization			
	Glenlea	HY320	Katepwa	Norstar
Asx	2.3	7.8	8.7	5.0
Thr	-[b]	4.5	6.3	3.3
Ser	12.4	9.0	10.4	8.3
Glx	16.1	23.3	20.2	37.2
Pro	-	7.0	-	9.1
Gly	14.4	14.9	22.0	9.8
Ala	17.6	7.9	11.5	5.1
Val	11.2	5.2	5.8	4.8
Met	-	-	-	-
Ile	-	-	-	3.7
Leu	7.5	3.8	-	3.6
Tyr	5.0	-	-	-
Phe	1.5	3.0	-	1.9
His	6.6	4.4	2.8	2.2
Lys	5.4	4.0	3.6	1.8
Arg	-	5.2	8.7	4.3
Cys	-	-	-	-

a The signal for amino acid was not significantly higher than noise in analysis, and therefore recorded as naught in the composition