Procedure for Isolating Monomeric Proteins and Polymeric Glutenin of Wheat Flour[1]

B. X. FU and H. D. SAPIRSTEIN

Cereal Chem. 73(1):143–152

ABSTRACT

A new method for fractionation of monomeric (albumins, globulins, and gliadins) and polymeric (native unreduced) glutenin proteins of wheat flour has been developed. Proteins were first separated into 50% (v/v) 1-propanol soluble (50PS) and insoluble (50PI) fractions. The 50PI protein was essentially free of monomeric proteins and comprised mainly glutenin; 50PS protein was a mixture of monomeric proteins and polymeric glutenin. Polymeric glutenin in 50PS protein was isolated under nonreducing conditions by precipitation with 1-propanol to a concentration of 70%. Polyacrylamide gel electrophoresis at pH 3.1 showed that the precipitated glutenin fraction (70PI) contained some monomeric proteins, mainly ω-gliadins. Sodium dodecyl sulfate polyacrylamide gel electrophoresis showed that no polymeric glutenin remained soluble in aqueous 70% 1-propanol. The fractionation procedure was therefore highly selective. This fractionation procedure, in conjunction with reversed-phase high-performance liquid chromatography was then used to examine the flour proteins of two Canadian wheat cultivars (Glenlea and Katepwa) of diverse dough strength. While the amounts of total polymeric glutenin (≈50% of flour protein) and the proportions of ω-gliadins in 70PI glutenin (≈30%) were comparable for both cultivars, flour of the very strong mixing Glenlea contained 21% more insoluble (50PI) glutenin and 30% less soluble (70PI) glutenin as determined by Kjeldahl analysis. The ratios of 50PI to 70PI glutenin (4.5 and 2.8 for Glenlea and Katepwa, respectively) were directly proportional to the mixograph dough development times. Results showed that 50PI and 70PI glutenins had the same subunit composition and similar high molecular weight to low molecular weight subunit ratios. The difference in solubility of the polymeric glutenin in 1-propanol is probably due to a difference in molecular size. The results obtained in this study confirmed the importance of both the soluble and insoluble polymeric glutenin in determining flour strength. The protein isolation procedure should be useful for physicochemical characterization of soluble and insoluble glutenin fractions, and for isolating pure glutenin from gliadin-glutenin mixtures.

Recent research on the biochemical basis of breadmaking quality of wheat flour has intensified the need for an accurate and reliable method for separating the polymeric (unreduced or native) glutenin from the monomeric or single polypeptide chain wheat flour proteins (albumins, globulins, and gliadins). The rationale for such a separation is twofold. First, the relative amount of polymeric protein in a flour appears to be strongly related to the functionality of the flour in breadmaking (Mecham et al 1962, Tsen 1967, Orth and Bushuk 1972, Tanaka and Bushuk 1973, Huebner and Wall 1976, Field et al 1983, MacRitchie 1987, Gupta et al 1993). Second, after reduction of polymeric glutenin, its subunit composition can be used to predict the breadmaking potential of a wheat cultivar (Payne et al 1979, 1981; Ng and Bushuk 1988; Gupta et al 1989, 1991a).

Many fractionation procedures have been reported to separate the glutenins from the other classes of wheat proteins. Physicochemical approaches have almost invariably been based on the distinction of the very large molecular size of polymeric glutenin. Methods have included ultracentrifugation (Hoseney et al 1969a, Goforth and Finney 1976), gel filtration (Meredith and Wren 1966, Huebner and Wall 1976, Payne and Corfield 1979, Bottomley et al 1982), and size-exclusion high-performance liquid chromatography (SE-HPLC) (Lundh and MacRitchie 1989, Dachkevitch and Autran 1989, Singh et al 1990, Batey et al 1991, Gupta et al 1993). More widely used fractionation methods, because of their apparent simplicity and low cost, have been procedures based on the differential solubility of polymeric glutenin and the monomeric proteins in various solvents and pH levels. Some of the more frequently used methods, which also vary in the type of starting material used (i.e., flour, dough, or gluten), include modified Osborne sequential fractionation (Chen and Bushuk 1970, Bietz and Wall 1975), pH precipitation (Jones et al 1959, Orth and Bushuk 1973), and various other solvent fractionation approaches (MacRitchie 1978, Danno 1981, Kruger et al 1988, Burnouf and Bietz 1989). Some workers have combined different approaches to study particular fractions such as modified Osborne fractionation followed by size-exclusion chromatography to separate gliadins from ethanol-soluble low molecular weight glutenin (Huebner and Bietz 1993), and size-exclusion chromatography followed by ion-exchange chromatography to isolate and purify glutenins (Lew et al 1992). Comparative analysis of some of these methods (Orth and Bushuk 1973, Khan and Bushuk 1979, Chakraborty and Khan 1988) and overall results have indicated that the success of these techniques to produce consistent yields of relatively pure gliadin and glutenin fractions has been elusive. The problematic nature of separation of wheat proteins based on solubility has been reviewed (Miflin et al 1983).

A more direct approach to isolate polymeric glutenin is to avoid gluten formation entirely and first quantitatively remove the monomeric proteins by direct extraction of flour with nonreducing solvents such as dimethyl sulfoxide (Burnouf and Bietz 1989, Gupta and MacRitchie 1991) or 50% 1-propanol (Byers et al 1983, Singh et al 1991), thus leaving a starchy residue containing the polymeric glutenin that can be subsequently extracted under reducing conditions. While this type of procedure appears to be successful in obtaining relatively pure glutenin in essentially one step, it also results in the extraction of some polymeric protein. The nature and amount of this soluble glutenin, which can be directly extracted from flour using nonreducing solvents, has been largely ignored in the scientific literature.

While much useful information on the composition and functionality of wheat flour proteins has been accumulated, there still is no simple method for obtaining polymeric protein that is free of contamination by the monomeric proteins and vice versa. This article describes a new relatively straightforward procedure for preparing soluble and insoluble polymeric glutenin and monomeric proteins of high purity. The procedure is based on the dif-

[1]Publication 257 of the Department of Food Science, University of Manitoba, Winnipeg, MB Canada R3T 2N2.

Publication no. C-1996-0115-03R.

ferential solubility of these proteins in aqueous 50 and 70% 1-propanol. The procedure was tested by fractionating the proteins of flour milled from two bread wheat cultivars of widely different strength.

MATERIALS AND METHODS

Wheat Cultivars

Samples of two Canadian wheat cultivars, Glenlea and Katepwa, were selected for this study. Glenlea is a cultivar of the Canada Western Extra Strong Red Spring wheat class, which is noted for its extra strong dough mixing characteristics. Katepwa is a Canada Western Red Spring wheat cultivar. The wheats were milled on a Buhler experimental mill into straight-grade flour. The protein (N × 5.7, 14% mb) contents of the flours were 13.7 and 13.3% for Glenlea and Katepwa, respectively.

Extraction of Wheat Proteins Using an Osborne-type Fractionation

Glenlea and Katepwa flours (2.5 g) were extracted at room temperature (23 ± 1°C) with 12.5 ml of 0.5*M* NaCl solution in a 30-ml centrifuge tube for 1 hr with brief vortexing every 15 min. Each suspension was centrifuged for 10 min at 3,000 × *g*, and the supernatant was collected. To remove residual salt, the residue was washed two times with 7.5 and 5.0 ml of deionized and distilled water for 2 min each, followed by centrifugation. The three supernatants were pooled. The remaining residue was then extracted three times with 12.5, 7.5, and 5.0 ml of 50% (v/v) 1-propanol (BDH, HiPerSolv HPLC-grade) for 1, 0.5, 0.5 hr, respectively, with brief vortexing every 15 min. The three 50% 1-propanol supernatants were pooled. Aliquots of each fraction and freeze-dried residue were analyzed by acid polyacrylamide gel electrophoresis (A-PAGE) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Fractionation and Quantification of Wheat Proteins Based on Solubility in 1-Propanol Solutions

Flour samples (2.5 g) were sequentially extracted at room temperature with 12.5, 7.5, and 5.0 ml of 50% 1-propanol for 1.0, 0.5, and 0.5 hr, respectively, with brief vortexing every 15 min. After centrifugation (10,000 × *g*, 10 min) and pooling of the three supernatants, hereafter referred to as the 50% 1-propanol soluble (50PS) fraction, the protein content was determined by micro-Kjeldahl method (AACC 1983). As with other fractions described below, samples for Kjeldahl analysis were prepared in triplicate. The residue was freeze-dried, and its protein content was determined.

Glutenin was precipitated from a 5.0-ml aliquot of the pooled 50PS fraction by the addition of 3.4 ml of 1-propanol to bring the final 1-propanol concentration to 70% (v/v). The mixture was vortexed and allowed to stand at room temperature for 1 hr. After centrifugation (20,000 × *g*, 10 min), a 1.0-ml aliquot of the supernatant was used for protein determination by micro-Kjeldahl method. The concentration of protein in the 70% 1-propanol precipitate was calculated by difference. Three protein fractions were obtained through the fractionation procedure as described above: 50% 1-propanol insoluble residue (50PI), and two subfractions of the 50PS fraction; 70% 1-propanol insoluble (70PI) and soluble (70PS) material.

To analyze the protein composition of the 70PI and 70PS fractions by electrophoresis or RP-HPLC, the scale of the 70% 1-propanol precipitation procedure was reduced using 0.5 ml of the 50PS fraction. After centrifugation (15,000 × *g*, 10 min), the supernatant and precipitate were evaporated to dryness in a Speed Vac concentrator (Savant Instruments, Farmingdale, NY).

A-PAGE

The conditions of A-PAGE were as described by Sapirstein and Bushuk (1985). To check the presence of monomeric proteins of various fractions, a short-run (≈100 min) procedure was employed to retain the gliadins and the higher mobility albumins and globulins within the gel.

The 50PS fraction (0.5 ml) was mixed with 0.25 ml of 0.25% (w/v) aluminum lactate buffer (pH 3.1) solution containing 40% (w/v) sucrose and 0.5% (w/v) methyl green dye (extract dilution solution). The dried 70PI and 70PS fractions were redissolved in 0.5 ml of 50% 1-propanol and mixed with 0.25 ml of extract dilution solution. To check 50PI glutenin for the presence of monomeric proteins, 50 mg of freeze-dried sample was extracted with 0.2 ml 50% 1-propanol at room temperature for 1 hr with intermittent vortexing. After centrifugation (15,000 × *g*, 10 min), an aliquot of 0.1 ml of clear supernatant was mixed with 0.05 ml of extract dilution solution.

SDS-PAGE

SDS-PAGE was performed according to Ng and Bushuk (1987), with some modifications. The stacking and separating acrylamide gel concentrations were 3.5 and 14%, respectively. Electrophoresis was performed for 4 hr at 25 mA. Dried 70PS and 70PI fractions (from 0.5 ml of the 50PS fraction) were redissolved in 1.0 ml of buffer solution, pH 6.8, containing 0.063*M* Tris-HCl, 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 20% (v/v) glycerol, and 0.01% (w/v) pyronin Y (SDS extraction buffer). The 50PS fraction (0.5 ml) was mixed with 0.5 ml of SDS extraction buffer. The 50PI fraction (50 mg) was extracted with 0.5 ml of 50% 1-propanol containing 1% (w/v) dithiothreitol (DTT), and an aliquot (0.3 ml) was mixed with 0.3 ml of SDS extraction buffer.

RP-HPLC

Samples were analyzed using a Hewlett-Packard 1090M liquid chromatograph incorporating a DR5 solvent delivery system, autosampler, and heated column compartment maintained at 50°C for analyses. A Zorbax 300 SB-C8 (Rockland Technologies, Inc., Newport, DE) column (300 Å pore size, 5 µm particle size, 15 cm × 4.6 mm, i.d.) was used in conjunction with a Zorbax 300 SB-C8 cartridge guard column (1.25 cm × 4 mm, i.d.). Solvents for RP-HPLC were: A, distilled water deionized, purified, filtered (0.2 µm) using a Milli-Q system (Millipore Ltd.) and deaerated; and B, acetonitrile (ACN, HPLC grade, 0.45-µm filtered, Burdick and Jackson). Both solvents contained 0.1% (v/v) trifluoroacetic acid (HPLC-grade, Sigma). Solvents were continuously and slowly sparged with helium during analyses. Solvent flow rate was maintained at 1.0 ml/min. After sample injection and an initial 3-min isocratic condition at 23% solvent B, proteins were eluted in an 82-min linear gradient from 23–44% solvent B. The column was equilibrated at 23% solvent B for 8 min between runs. The column eluent was monitored at 214 nm using a Hewlett Packard 1090 diode array detector-series II incorporating a 6-mm path length, 8-µl flow cell, and 4-nm slit assembly. Control of the chromatograph and data quantitation was provided by Hewlett-Packard HPLC Chemstation DOS series software implemented on a 486/50 MHz personal computer.

Sample Preparation for RP-HPLC

The method used to prepare samples for RP-HPLC was partly adapted from procedures described by Singh et al (1991) and Marchylo et al (1989). Four stock solutions are required: A, 50% (v/v) 1-propanol; B, 0.08*M* Tris-HCl, pH 7.5, containing 50% (v/v) 1-propanol; C, solution B containing 1% (w/v) DTT; D, solution B containing 14% (v/v) 4-vinylpyridine. Control samples for RP-HPLC comprised reduced and alkylated solution A-insoluble flour protein; i.e., mainly glutenin prepared as follows. Flour (50 mg) was extracted twice with 1 ml of 50% (v/v) 1-propanol for 30 min at room temperature with intermittent vortexing and centrifuged for 3 min at 2,200 × *g* in a Heraeus-Christ Biofuge A microcentrifuge. The residue was washed with 1 ml of 50% 1-propanol for ≈1 min, centrifuged, and the supernatant was

discarded. The remaining solvent was removed by careful aspiration using a Pasteur pipette. The residue, or alternatively 70PI from 0.5 ml 50PS, or freeze-dried 50PI fractions (50 mg), was reduced with 0.1 ml of freshly prepared solution C for 1 hr at 60°C. This was followed by alkylation with 0.1 ml of solution D at 60°C for 15 min just before RP-HPLC analysis. The samples were vortexed briefly during reduction and alkylation. Following alkylation, samples were centrifuged for 5 min at 15,000 × *g*, and supernatants were carefully removed and syringe-filtered (0.45 µm Millex HV) into microvials which were subsequently sealed for RP-HPLC analysis.

Collection of RP-HPLC Peaks for SDS-PAGE

To collect sufficient protein of RP-HPLC peaks, a multiple sample injection procedure was used (Marchylo and Kruger 1988). The chromatogram was divided into three regions based on retention times to separate ω-gliadins, high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). Peaks in each region were collected manually and evaporated to dryness in a Speed Vac concentrator. Samples were redissolved in 200 µl of SDS extraction buffer and 20 µl aliquots were analyzed by SDS-PAGE.

RESULTS

Composition of Osborne-type Wheat Protein Fractions

The modified Osborne protein fractionation scheme (Chen and Bushuk 1970) first extracts albumin and globulin proteins using a 0.5*M* salt (NaCl) solution. Protein in the first residue, which is extractable with aqueous alcohol, has been termed gliadin, while the remaining residue has been widely referred to as glutenin, which is partly soluble in dilute acid or dissociating agents. A-PAGE patterns of Osborne-type extracts (Fig. 1) shows that the 50% 1-propanol soluble fraction (lane 3) and the insoluble residue (lane 4) were contaminated with albumin and globulin proteins. It is particularly noteworthy that the 50% 1-propanol insoluble fraction (lane 4), which was previously assumed to contain only glutenin protein, was highly contaminated with gliadins. Furthermore, there appeared to be a noticeably smaller amount of gliadins in the residue fraction of Glenlea compared to Katepwa. Similar results have been obtained in a related publication (Dupuis et al 1996) involving different samples of Katepwa and Glenlea wheats. In that study, it was found that the Osborne acetic acid-soluble protein fraction contained substantial quantities of gliadin proteins; the actual values were 2.0 and 4.6% (total flour protein basis) in Glenlea and Katepwa, respectively.

Using an analogous sequential fractionation procedure, Singh et al (1991) reported some difficulty in resuspending the residue (glutenins) remaining after the initial salt and propanol extractions. The difficulty in the extraction of gliadins by aqueous alcohol after salt extraction may be due to the partial formation of dough in the centrifuged salt-insoluble residue. Furthermore, the salt in the first extract may induce aggregation of the gliadins with glutenin and thereby decrease their subsequent solubility in

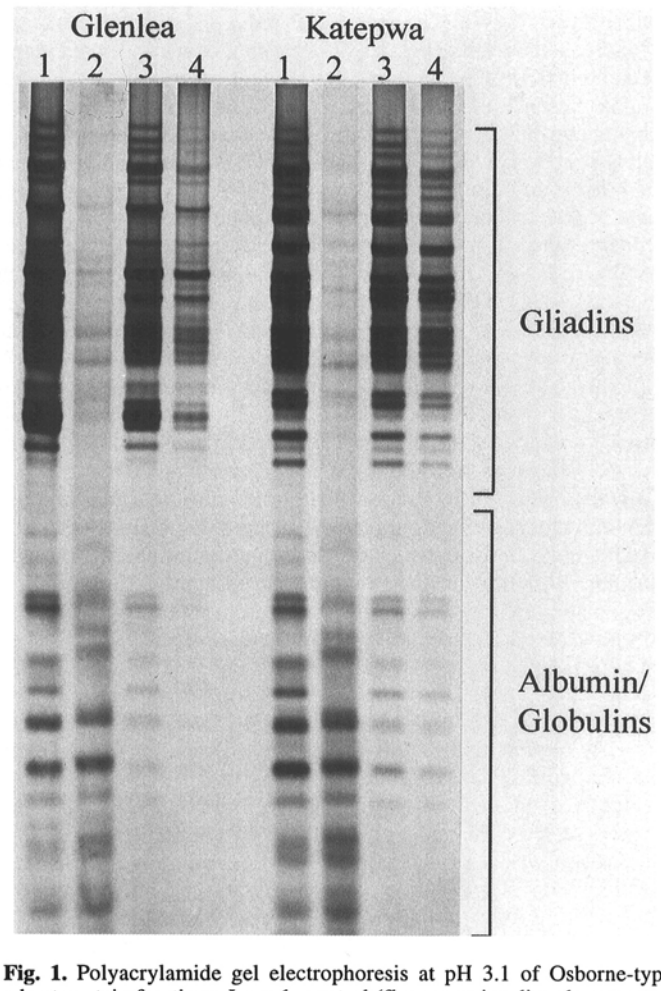

Fig. 1. Polyacrylamide gel electrophoresis at pH 3.1 of Osborne-type wheat protein fractions. Lane 1, control (flour proteins directly extracted in 50% 1-propanol); lane 2, flour proteins soluble in 0.5*M* NaCl; lane 3, residue of NaCl extraction soluble in 50% 1-propanol; lane 4, 50% 1-propanol insoluble residue.

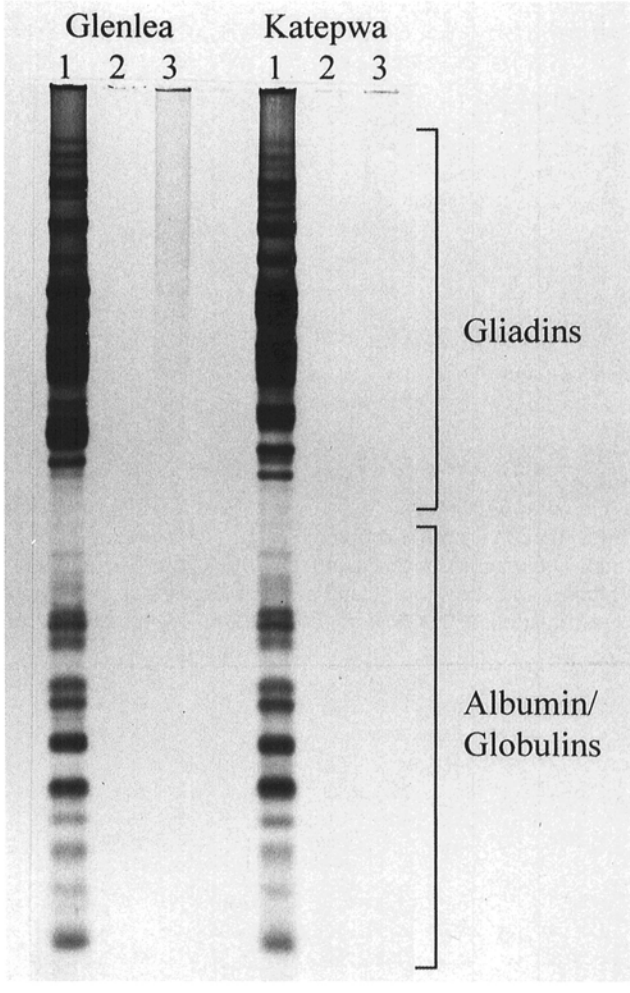

Fig. 2. Polyacrylamide gel electrophoresis at pH 3.1 of lane 1, 50% 1-propanol soluble flour proteins; lane 2, residue of 50% 1-propanol extract soluble in 0.5 M NaCl; lane 3, freeze-dried residue reextracted with 50% 1-propanol.

aqueous alcohol. Conversely, the alcohol-soluble Osborne fraction, when examined by SDS-PAGE under reducing conditions, clearly revealed the presence of all the HMW glutenin subunits for samples of both Katepwa and Glenlea (results not shown).

Fractionation of Proteins into 50% 1-propanol Soluble and Insoluble Fractions

Because of the relative ineffectiveness of 0.5*M* salt solution to completely extract the albumin and globulin proteins from wheat flour, and the subsequent difficulties in extracting the gliadins from the residual polymeric glutenins using 50% 1-propanol, we reversed the extraction sequence; proteins were first extracted with 50% 1-propanol followed by 0.5*M* NaCl extraction of the remaining residue to examine the efficiency of the alcohol extraction. The A-PAGE patterns of the resulting fractions (Fig. 2) clearly showed that 50% 1-propanol appears to extract essentially all of the monomeric proteins in the Katepwa and Glenlea flours. Subsequent extracts of the alcohol-insoluble residue with either salt solution (Fig. 2, lane 2) or additional 1-propanol (Fig. 2, lane 3) failed to reveal any bands by A-PAGE. However, as HMW-GS were clearly evident by SDS-PAGE of the reduced 50% 1-propanol soluble protein (Fig. 3, compare lanes 2 and 3), a substantial amount of polymeric glutenin was also extracted with the monomeric proteins in this fraction. The next step was to isolate the glutenin from the 50PS protein fraction.

One additional observation on the SDS-PAGE separation of the reduced 1-propanol insoluble residue is in regard to the presence in Glenlea (Fig. 3, lane 3) of a single band in a region between the HMW- and LMW-GS. The relative mobility of this band in the 50% 1-propanol insoluble residue separated in the presence of a reducing agent, and its complete absence in 50% 1-propanol extracts of flour separated by SDS-PAGE under nonreducing conditions (result not shown) are consistent with its identity as a D-group LMW-GS (Jackson et al 1983, 1985). While HMW albumins in bread wheat have been shown to have electrophoretic mobility in SDS-PAGE similar that of D-glutenin subunits, these albumins were also found not to be extractable from flour using aqueous alcohol (Gupta et al 1991b). The presence or absence of D-glutenin subunits, which are coded by genes on the short arm of group 1 chromosomes, has been indirectly associated with negative and positive contributions to mixing and baking quality, respectively (Masci et al 1993). These workers also pointed out that this may be the case only for cultivars without excessive strength and elasticity. As Glenlea can be classified as an extra strong dough mixing cultivar, the significance of its D-glutenin subunit in relation to technological quality is unknown.

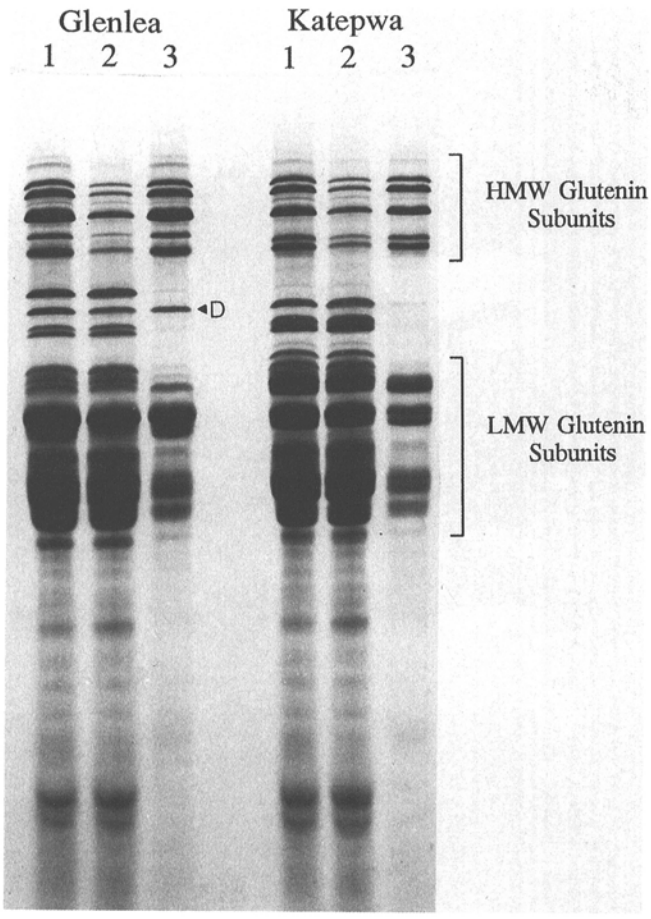

Fig. 3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of lane 1, reduced total flour proteins; lane 2, reduced 50% 1-propanol soluble flour proteins; lane 3, reduced 50% 1-propanol insoluble residue. D = D-glutenin subunit in Glenlea (lane 3).

Isolation of Glutenin from 50PS Fraction

Preliminary experiments showed that an increasing amount of protein can be precipitated from the 50PS fraction by raising the 1-propanol concentration above 50%. Therefore, we analyzed the effect of the 1-propanol concentration on the type and amount of precipitated proteins. In these experiments, pure 1-propanol was added to freshly prepared 50PS extracts to achieve concentrations of 65.0, 67.5, 70.0, 72.5, and 75.0%. After a 1-hr rest at room temperature, followed by centrifugation (15,000 × *g*, 10 min), the respective residues and supernatants were evaporated to dryness, and analyzed by A-PAGE and SDS-PAGE.

SDS-PAGE results (Fig. 4) showed that 1-propanol concentrations below 70% left a significant amount of glutenin as evident by the presence of HMW-GS in the SDS-PAGE patterns of the supernatant fractions of both flours. By contrast, the 70PS supernatant protein contained no HMW-GS (Fig. 4), indicating that all the polymeric glutenin present in the 50PS fraction was precipitated at 70% aqueous 1-propanol. The effect of increasing the 1-propanol concentration above 70% was effectively shown by A-PAGE. The A-PAGE composition of the same fractions (Fig. 5) analyzed by SDS-PAGE showed that the 70% 1-propanol fractionation produced the greatest differentiation between the soluble and precipitated fractions. A small amount of monomeric proteins, mainly ω-gliadins and some albumins and globulins, coprecipitated with the polymeric glutenin in 70% 1-propanol. However, as the 1-propanol concentration increased to 75%, considerably more of the monomeric proteins was precipitated with glutenin. These results indicate that, based on visual examination of the electrophoretic results, 70% appears to be the optimum concentration of 1-propanol to precipitate the polymeric glutenin with the least contamination of monomeric proteins.

Composition of 1-propanol Soluble and Insoluble Fractions by RP-HPLC

The highly complex and heterogeneous nature of wheat protein composition is well illustrated by RP-HPLC (Fig. 6). While the presence of glutenin subunits was not evident in the 50PS fraction chromatograms (Fig. 6A), they were clearly revealed upon precipitation of this fraction with 70% 1-propanol (Fig. 6C). Compared with the chromatograms of the 50PI glutenin fraction (Fig. 6B), which was essentially free of monomeric proteins, the 70PI fraction (Fig. 6C) contained several additional components that eluted earlier than the HMW-GS. These peaks represent mainly ω-gliadins (Bietz and Burnouf 1985, Kruger et al 1988, Marchylo et al 1989). Collection of these peaks (in addition to those of the HMW- and LMW-GS) and analysis by SDS-PAGE (Fig. 7, lane 5) confirmed their identity with ω-gliadins given their electrophoretic mobility between the HMW-GS and LMW-GS (Fig. 7, lanes 1, 2, and 3). It can be seen that only a faint trace of the D-glutenin

subunit in the 50PI fraction of Glenlea flour was visible by SDS-PAGE (Fig. 7, lane 2). We suspected that this subunit corresponded to the minor RP-HPLC peak in the 50PI fraction eluting at ≈22 min (Fig. 6B), i.e., just outside the HMW-GS boundary (for peak collection) before HMW-GS 10. This was confirmed by SDS-PAGE analysis of the peak isolated from the 50PI RP-HPLC separation (results not shown). Whether the soluble glutenin (70PI) fraction of Glenlea flour contained any significant quantity of the D-glutenin subunit was not investigated in this study, although this seems likely.

As RP-HPLC was effective in separating the 70PI glutenin from the co-precipitated monomeric proteins, the amount of glutenin that was soluble in 50% 1-propanol could be easily quantified (see below). The RP-HPLC result showed that the 50PI glutenin (Fig. 6B) and 70PI glutenin (Fig. 6C) have virtually the same subunit content. These two fractions were also essentially identical in the ratio of HMW to LMW glutenin subunit composition. For the 50PI fraction, the HMW-to-LMW GS ratios for Glenlea and Katepwa were 0.57 ± 0.03 and 0.47 ± 0.02, respectively, based on triplicated determinations. The corresponding ratios for 70PI glutenin were 0.54 ± 0.04 and 0.48 ± 0.03.

Quantification of Wheat Protein Fractions

The relative amounts of the various 1-propanol soluble and insoluble fractions that were isolated are presented in Table I. As the protein contents of both Glenlea and Katepwa flours were comparable, these data represent a close approximation of the difference in the absolute amount of glutenin and monomeric protein content in the two wheats. We found that ≈50% of the total flour protein in both cultivars comprised glutenin; Glenlea had a moderately higher content of glutenin than Katepwa (52 vs. 48%). For the 50PI glutenin, the difference increased to 7.5 percentage points, likewise in favor of Glenlea. In absolute terms, Glenlea had ≈21% more (50% 1-propanol) insoluble glutenin when compared with Katepwa, and more than 30% less soluble glutenin that was extractable in the 50PS fraction (subsequently precipitated by aqueous 70% 1-propanol). About 20% of the Glenlea glutenin and 25% of the Katepwa glutenin was soluble in 50% 1-propanol extracts of flour. This quantity of soluble glutenin does not include the ω-gliadins that co-precipitates in the 70PI fraction (Fig. 6C). The amounts of these ω-gliadins, as a proportion of total protein in the 70PI fraction were comparable at 30.3 ± 1.4% and 32.8 ± 0.4% for Glenlea and Katepwa, respectively.

The results presented above indicate that the main difference in the flour protein of the two wheat cultivars is in the relative quantities of the two types of polymeric glutenin that were isolated, soluble, and insoluble in aqueous 50% 1-propanol. Accordingly, we evaluated the ratio of 50PI to 70PI glutenin (Table I) as a potential index of functionality of the flour protein for breadmaking. The parameter so obtained will be referred to as glutenin solubility index (GSI). The GSI for Glenlea and Katepwa were 4.5 and 2.8, respectively. As Glenlea is known to be much stronger than Katepwa, both the magnitude and direction of difference in GSI values seem plausible. The mixograph dough development times (a commonly used index of flour strength) for the two wheats (4.7 ± 0.1 and 2.9 ± 0.02 min for Glenlea and Katepwa, respectively) were directly proportional to the GSI values. The protein fractionation procedure reported in this study requires evaluation with many more wheat samples of diverse genotype and breadmaking quality before any robust conclusions can be drawn regarding the direct relationship between GSI and

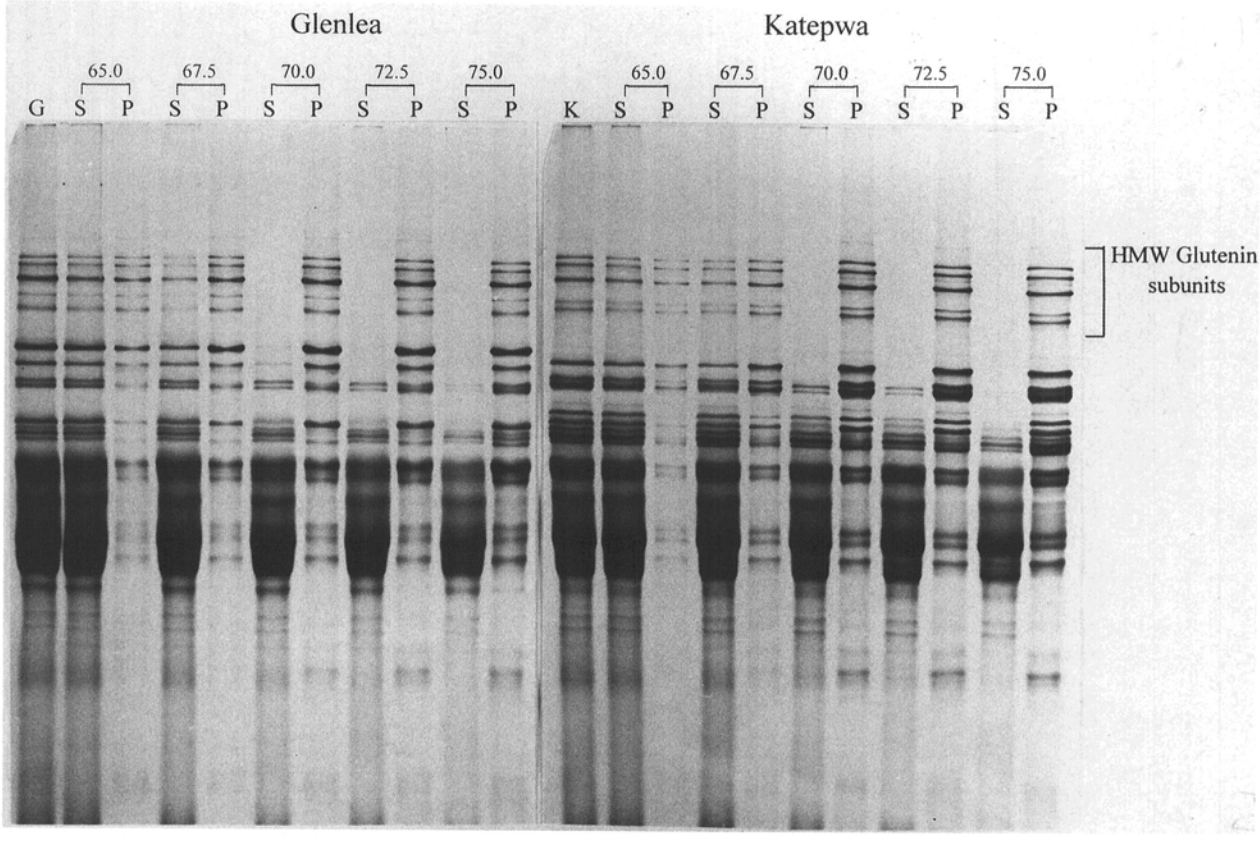

Fig. 4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of reduced supernatants (S) and precipitates (P) of 50% 1-propanol soluble flour proteins obtained by adding 1-propanol to concentrations of 65.0, 67.5, 70.0, 72.5, and 75.0%. Lanes G and K are reduced 50% 1-propanol soluble proteins of Glenlea and Katepwa, respectively.

dough mixing properties. However, the similarity between GSI and mixing time values obtained in this study is compelling and points to the importance of obtaining an accurate quantitation of glutenin in flour and the potential practical benefits of separating total glutenin into soluble and insoluble fractions.

One further observation regarding the 50PI glutenin fraction follows in relation to its quantification by RP-HPLC after reduction and alkylation. In this case, the magnitude of the difference between Glenlea and Katepwa was substantially higher than that obtained by Kjeldahl protein analysis of the 50PI starchy residue. Replicated quantitation of the integrated areas of the chromatograms of the 50PI fraction (as in Fig. 6B) showed that Glenlea contained ≈42% more glutenin when compared to Katepwa. It appeared that the quantification accuracy of the relative protein determination by RP-HPLC may be suspect. In general, the quantitation accuracy by HPLC, when polymeric glutenin is involved, depends on many factors. Among the more important of these are the requirements for complete protein extraction (reduction), efficient and homogeneous filtering of the reduced and alkylated glutenin subunits, and complete elution of the glutenin subunits in the aqueous ACN gradient. Preliminary investigation of the first of these factors yielded an interesting result. Following our standard reduction and alkylation steps involving the 50PI protein, we repeatedly extracted the remaining residue with the 50% 1-propanol/1% DTT reducing solution C. Subsequently, we reduced the dried starchy residue with an aqueous solution of 2% SDS containing 1% DTT, thus essentially replacing 1-propanol with SDS in the reducing solvent solution. SDS-PAGE of the resulting extracts of both Katepwa and Glenlea samples revealed a substantial number of protein bands of varying intensity. The bands represented some, but not all of the HMW- and LMW-GS, as well as some novel subunits of unknown identity. Based on a subjective assessment of band staining intensity, there also appeared to be a greater quantity of this protein in the Katepwa sample than in the Glenlea sample, which is consistent with the RP-HPLC integration results that were obtained. This indicates that a significant amount of polymeric glutenin is insoluble in 50% 1-propanol solutions containing reducing agent, and that the quantity of this residue protein is not related to breadmaking quality. This unexpected result warrants further investigation.

DISCUSSION

The challenges in obtaining a sharp and accurate separation of polymeric and monomeric proteins of wheat flour using differential solubility techniques have been known by cereal chemists for a long time (Osborne 1924). Cross-contamination of the major wheat endosperm protein classes, monomeric proteins in glutenin preparations and polymeric glutenin in gliadin preparations, represents the main problem in preparing relatively pure protein fractions for analysis and study.

While overlapping solubility of highly complex and heterogenous wheat protein fractions may partly explain the difficulties encountered, results reported in the scientific literature suggest that a principal factor contributing to cross contamination of some wheat protein fractions is the noncovalent chemical interactions that exist in the flour or are induced by the solvents added. These interactions are probably increased in procedures where dough or gluten replaces flour as the starting material for protein fractionation. Also, it appears that interactions can be accentuated by flour defatting at the outset of the fractionation used in many studies;

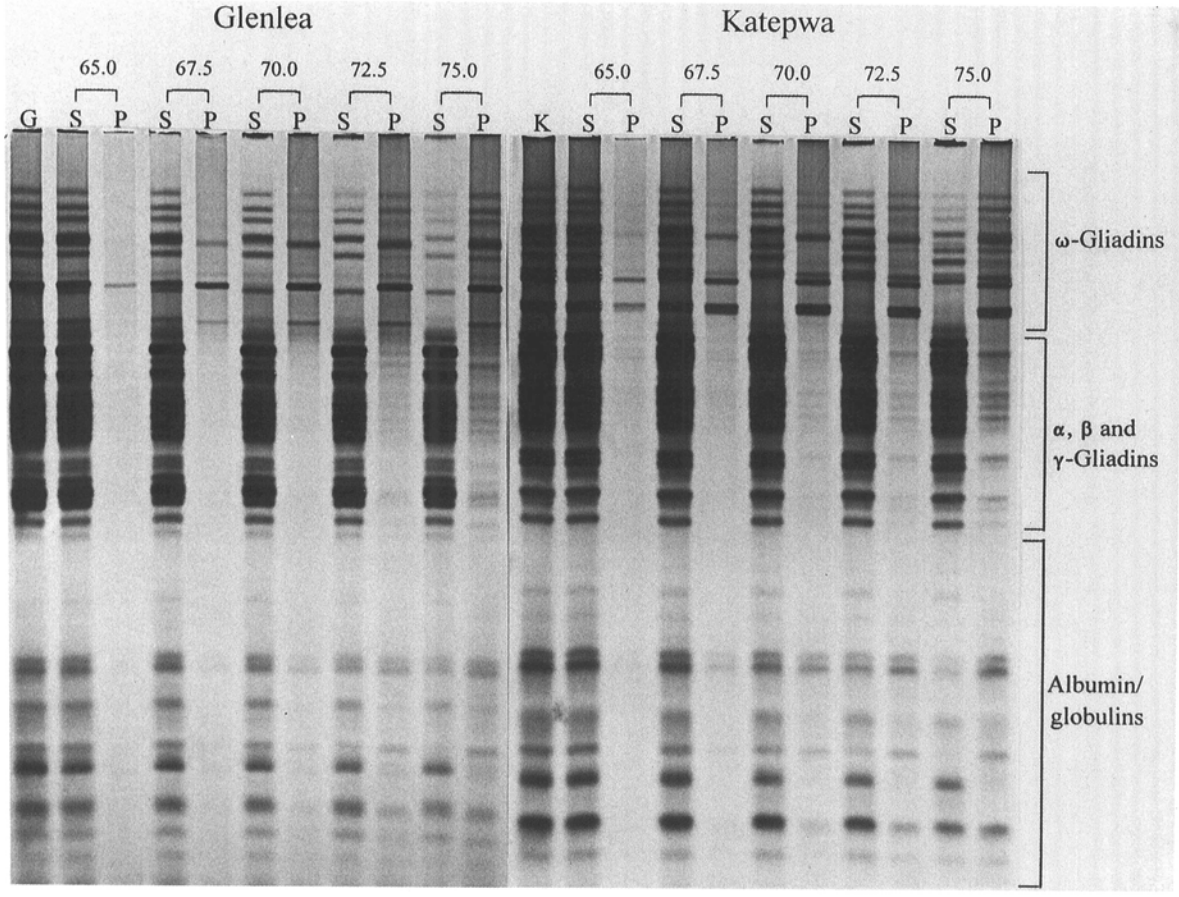

Fig. 5. Polyacrylamide gel electrophoresis at pH 3.1 of supernatants (S) and precipitates (P) of 50% 1-propanol soluble flour proteins obtained by adding 1-propanol to make various concentrations as in Fig. 4.

this results in the insolubilization of some albumins and globulins (Miflin et al 1980, Byers et al 1983).

Additional aggregation of monomeric and polymeric proteins may be induced by centrifugation used for separating the solubles. Centrifugation of protein-starch dispersions, as in Osborne type fractionations which begin by water or salt solution extraction of flour, concentrates monomeric and polymeric proteins in the insoluble residues. It seems plausible under these preparative conditions that noncovalent interactions between monomeric proteins and polymeric glutenin would be considerable. Moreover, in the modified Osborne procedure of Chen and Bushuk (1970), a flour sample is extracted initially in a 0.5*M* salt solution to specifically accentuate the insolubility of the gliadins (which are quite soluble in deionized water), thus optimizing their separation from the albumins and globulins. Consequently, it should not be surprising to find considerable amounts of monomeric proteins, most notably the gliadins apparently insoluble in, or more correctly unextractable with, aqueous ethanol (Orth and Bushuk 1973, Bietz and Wall 1975, Dupuis et al 1996) or 1-propanol as shown in this study. As a result, a varying quantity of gliadins, an amount which seems to be genotype-specific and inversely related to dough strength (Dupuis et al 1996), will then be extractable in the subsequent acetic acid fractionation of glutenin. Accordingly, the identity of acetic acid soluble flour protein with glutenin (Orth and Bushuk 1972) appears to be inaccurate.

In addition to difficulties in gliadin protein solubility created by using salt solutions to extract albumin and globulin proteins from flour, it appears that glutenin solubility is similarly affected. Polymeric glutenin, in particular, is known to be adversely affected by the presence of salt in the extraction solution (Hoseney et al 1969b, Huebner 1970, Kim and Bushuk 1995). Moreover the glutenin of stronger wheat cultivars appears to be more sensitive to this effect than weaker cultivars (Huebner 1970, Kim and Bushuk 1995). Accordingly, prior exposure of flour proteins to salt solutions, as in an Osborne-type fractionations, probably renders glutenin less soluble in acetic acid or similar solvents used for subsequent extraction and may even affect the subunit composition of extracted glutenin as was recently observed (Kim and Bushuk 1995).

This study has shown that better flour protein fractionation is

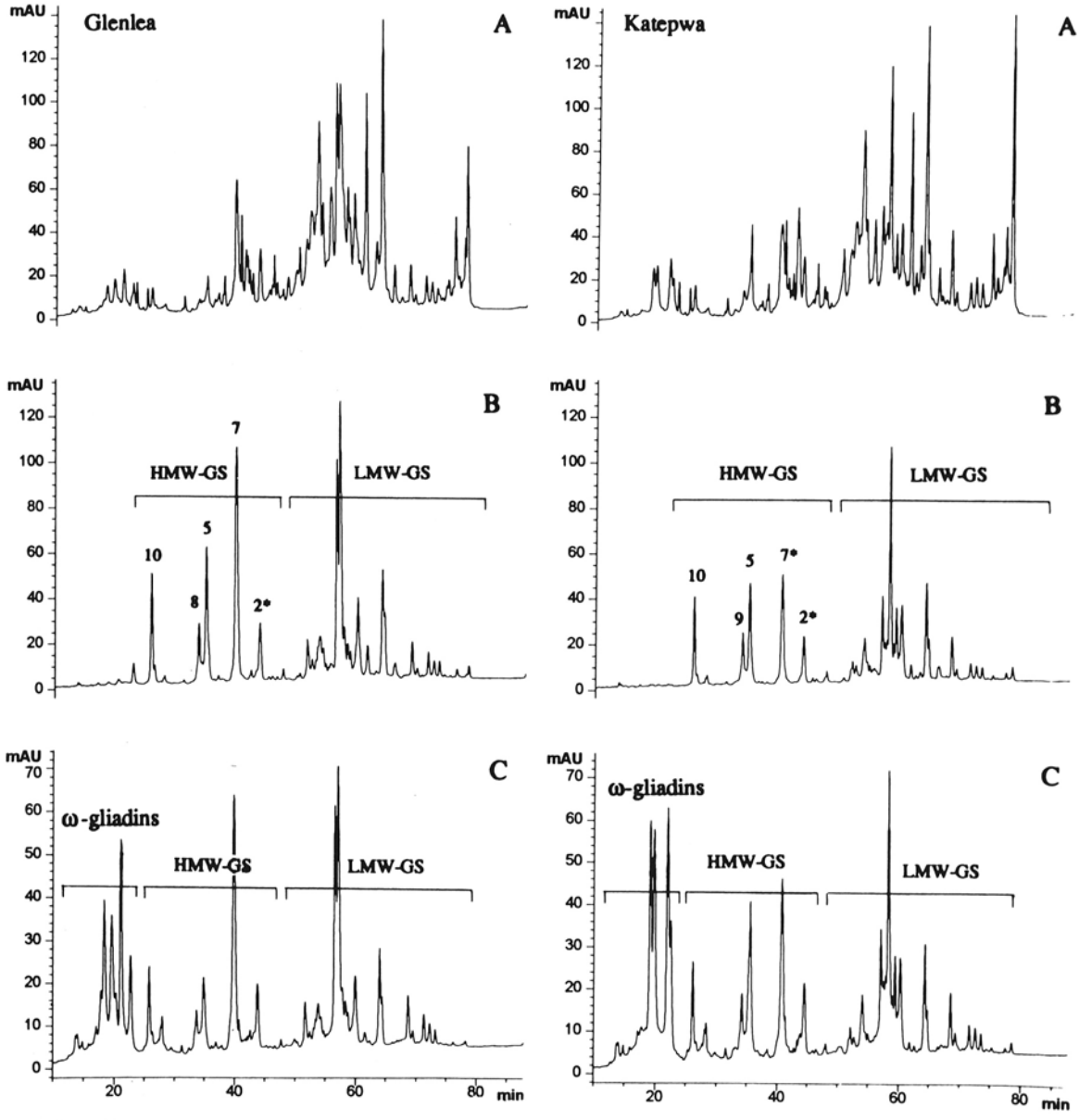

Fig. 6. Reversed-phase high-performance liquid chromatograms of reduced and alkylated flour proteins of Glenlea and Katepwa wheats. A, 50% 1-propanol soluble fractions. B, 50% 1-propanol insoluble residue. C, precipitate of 50% 1-propanol soluble fraction obtained by increasing the 1-propanol concentration to 70% (70PI fraction in text).

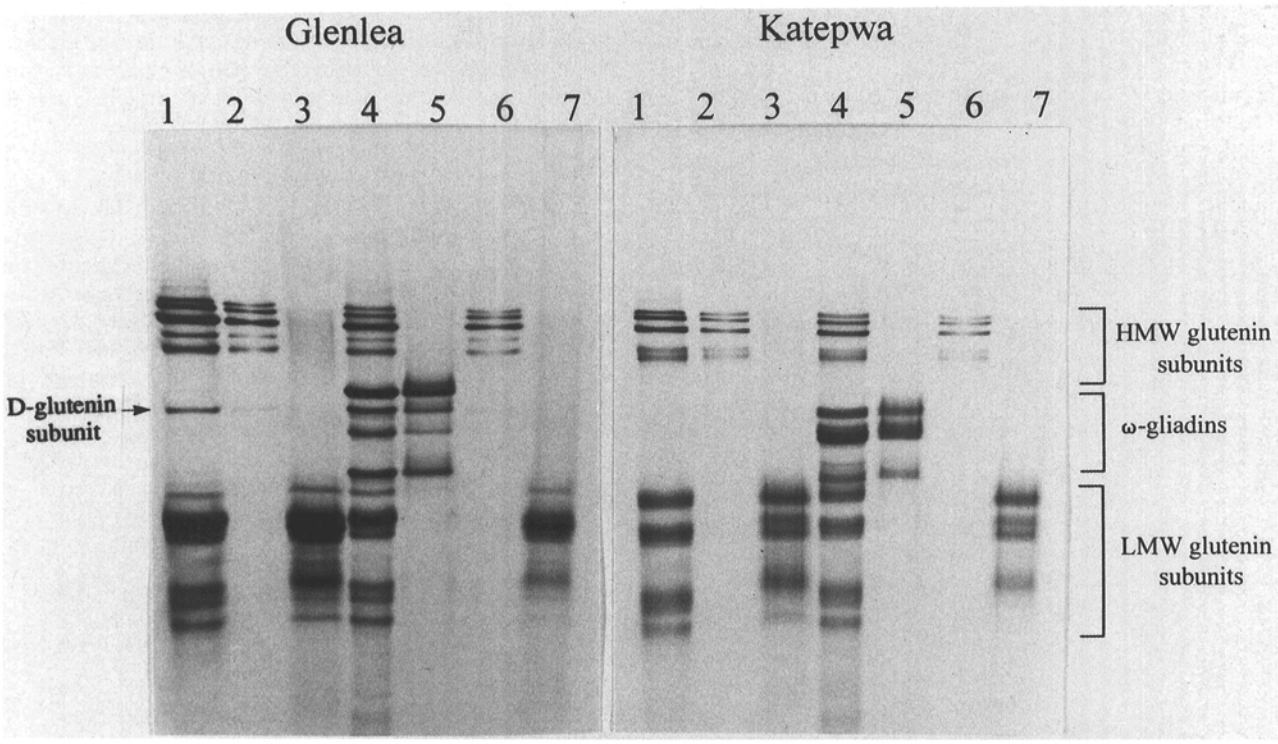

Fig. 7. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of reduced Glenlea and Katepwa wheat flour proteins: lane 1, 50% 1-propanol insoluble residue; lanes 2 and 3, collected peaks by reversed-phase high-performance liquid chromatograms (RP-HPLC) corresponding to high molecular weight and low molecular weight (HMW and LMW) glutenin subunits, respectively of Fig. 6B; lane 4, precipitate of 50% 1-propanol soluble fraction obtained by increasing the 1-propanol concentration to 70% (70PI fraction in text); lanes 5, 6, and 7: collected RP-HPLC peaks of 70PI fraction corresponding to regions of ω-gliadins, HMW- and LMW-glutenin subunits, respectively in Fig. 6C.

TABLE I
Amount of 1-Propanol Fractionated Protein in Katepwa and Glenlea as a Percentage of Total Flour Protein[a]

Cultivar	Monomeric Protein[b]	70PI Glutenin[c]	50PI Glutenin[d]	Total Glutenin	Ratio of 70PI to Total Glutenin
Glenlea	47.9 ± 1.4	9.4 ± 0.4	42.7 ± 1.0	52.1 ± 0.8	18.0 ± 0.6
Katepwa	52.0 ± 1.6	12.8 ± 0.2	35.2 ± 1.2	48.0 ± 0.7	26.6 ± 0.5

[a] Means of triplicate determinations ± standard deviations.
[b] 50% 1-propanol soluble protein less 70PI glutenin.
[c] Glutenin in 50% 1-propanol soluble fraction precipitated in 70% 1-propanol. Values corrected according to reversed-phase high-performance liquid chromatography quantitation to exclude ω-gliadins; determined by difference.
[d] 50% 1-propanol insoluble glutenin.

achievable by reversing the order of the Osborne solvents by using aqueous alcohol in the first step to remove monomeric proteins that would otherwise interact and aggregate with polymeric glutenin if salt solution is used first. While it is possible to fractionate monomeric flour proteins and polymeric glutenin according to their differential solubility in 50% 1-propanol, this separation is not complete; a significant quantity of polymeric glutenin, an amount which appears to be genotype-specific (18 and 26% of total glutenin in Glenlea and Katepwa, respectively) is extracted in this solvent. However, the glutenin soluble in 50PS can be easily separated from the monomeric proteins by selective precipitation in 70% 1-propanol. The supernatant of this fractionation contains albumins, globulins, and most of the gliadin proteins.

It was interesting to note that the selective precipitation of 70PI glutenin also resulted in the co-precipitation of a considerable amount of some ω-gliadins. The 70PI ω-gliadins, as quantified by RP-HPLC, represented almost one-third of the 70PI glutenin protein fraction. These ω-gliadins were distinguished from the other ω-gliadins in both Glenlea and Katepwa wheats by their relatively higher electrophoretic mobility in A-PAGE. On this basis, it would appear that these ω-gliadins are coded by genes at the *Gli-A1* or *Gli-B1* loci as the slow-moving ω-gliadins are known to be coded by genes on the short arm of chromosome 1D (Shepherd 1968, Wrigley and Shepherd 1973, Sozinov and Poperelya 1982). The significance of this finding in regard to glutenin functionality is unclear. The reasons for the similarity in solubility of the 70PI glutenin and some ω -gliadins, as well as the possibility of a specific interaction between these proteins, remain to be investigated.

CONCLUSIONS

A new method for fractionation of monomeric and polymeric proteins of wheat flour has been developed. Proteins were first separated into 50% 1-propanol soluble and insoluble fractions. The 50PI protein was essentially free of monomeric proteins and comprised mainly glutenin, while 50PS protein was a mixture of monomeric proteins and polymeric glutenin. Polymeric glutenin in 50PS protein was isolated under nonreducing conditions by precipitation with 1-propanol to a concentration of 70%. The RP-HPLC and SDS-PAGE results showed that the 50PS and 70PI glutenin had the same subunit composition, and similar HMW-to-LMW subunit ratio. Thus, we found no qualitative difference between the soluble and insoluble polymeric fractions. On the other hand, quantitative differences were substantial. The proportion of total polymeric glutenin that was soluble in aqueous 1-propanol was much less in Glenlea wheat than in Katepwa. Glenlea also contained significantly more 50PI glutenin. These results, and in particular the ratio of 50PI to 70PI glutenin, are consistent with the relative breadmaking quality characteristics of the two cultivars used in this study. The precise reason for the difference in solubility of the polymeric glutenin in aqueous 1-propanol remains to be elucidated, although a difference in glu-

tenin molecular size is probably a principal factor. It seems likely that the very strong mixing characteristics of Glenlea flour derive from a greater percentage of larger sized polymers than is present in Katepwa (Gupta et al 1993, MacRitchie and Gupta 1993). The results obtained in this study confirm the importance of both the soluble and insoluble polymeric glutenin in determining dough strength. The protein isolation procedure described herein should be useful for physicochemical characterization of soluble and insoluble glutenin fractions in future studies of the biochemical basis of breadmaking quality of wheat cultivars.

ACKNOWLEDGMENTS

We gratefully acknowledge financial assistance from the Natural Sciences and Engineering Research Council of Canada. We also thank Walter Bushuk for his helpful comments and review of the manuscript.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC, 8th ed. Method 46-11A. The Association: St. Paul, MN.

BATEY, I. L., GUPTA, R. B., and MacRITCHIE, F. 1991. Use of size-exclusion high performance liquid chromatography in the study of wheat flour proteins: An improved chromatographic procedure. Cereal Chem. 68:207-209.

BIETZ, J. A., and WALL, J. S. 1975. The effect of various extractants on the subunit composition and association of wheat glutenin. Cereal Chem. 52:145-155.

BIETZ, J. A., and BURNOUF, T. 1985. Chromosomal control of wheat gliadin: Analysis by reversed-phase high-performance liquid chromatography. Theor. Appl. Genet. 70:599-609.

BOTTOMLEY, R. C., KEARNS, H. F., and SCHOFIELD, J. D. 1982. Characterization of wheat flour and gluten proteins using buffers containing sodium dodecyl sulfate. J. Sci. Food Agric. 33:481-491.

BURNOUF, T., and BIETZ, J. A. 1989. Rapid purification of wheat glutenin for reversed-phase high-performance liquid chromatography: Comparison of dimethyl sulfoxide with traditional solvents. Cereal Chem. 66:121-127.

BYERS, M., MIFLIN, B. J., and SMITH, S. J. 1983. A quantitative comparison of the extraction of protein fractions from wheat grain by different solvents, and of the polypeptide and amino acid composition of the alcohol-soluble proteins. J. Sci. Food Agric. 34:447-462.

CHAKRABORTY, K., and KHAN, K. 1988. Biochemical and breadmaking properties of wheat protein components. I. Compositional differences revealed through quantitation and polyacrylamide gel electrophoresis of protein fractions from various isolation procedures. Cereal Chem. 65:333-340.

CHEN, C. H., and BUSHUK, W. 1970. Nature of proteins in Triticale and its parental species. I. Solubility characteristics and amino acid composition of endosperm proteins. Can. J. Plant Sci. 50:9-14.

DACHKEVITCH, T., and AUTRAN, J. C. 1989. Prediction of baking quality of bread wheats in breeding programs by size-exclusion high-performance liquid chromatograph. Cereal Chem. 66:448-456.

DANNO, G. 1981. Extraction of unreduced glutenin from wheat flour with sodium dodecyl sulfate. Cereal Chem. 58:311-313.

DUPUIS, B., BUSHUK, W., and SAPIRSTEIN, H. D. 1996. Characterization of acetic acid soluble and insoluble fractions of glutenin of bread wheat. Cereal Chem. 73:131.

FIELD, J. M., SHEWRY, P. R., and MIFLIN, B. J. 1983. Solubilisation and characterisation of wheat gluten proteins: Correlations between the amount of aggregated proteins and baking quality. J. Sci. Food Agric. 34:370-377.

GOFORTH, D. R., and FINNEY, K. F. 1976. Separation of glutenin from gliadin by ultracentrifugation. Cereal Chem. 53:608-610.

GUPTA, R. B., and MacRITCHIE, F. 1991. A rapid one-step one dimensional SDS-PAGE procedure for analysis of subunit composition of glutenin in wheat. J. Cereal Sci. 14:105-109.

GUPTA, R. B., SINGH, N. K., and SHEPHERD, K. W. 1989. The cumulative effect of allelic variation in LMW and HMW glutenin subunits on physical dough properties in the progeny of two bread wheats. Theor. Appl. Genet. 77:57-64.

GUPTA, R. B., BEKES, F., and WRIGLEY, C. W. 1991a. Predicting values of LMW glutenin alleles for dough quality of bread wheat. Pages 615-621 in: Gluten Proteins 1990. W. Bushuk and R. Tkachuk, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.

GUPTA, R. B., SHEPHERD, K. W., and MacRITCHIE, F. 1991b. Genetic control and biochemical properties of some high molecular weight albumins in bread wheat. J. Cereal Sci. 13:221-235.

GUPTA, R. B., KHAN, K., and MacRITCHIE, F. 1993. Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. J. Cereal Sci. 18:23-41.

HOSENEY, R. C., FINNEY, K. F., SHOGREN, M. D., and POMERANZ, Y. 1969a. Functional (breadmaking) and biochemical properties of wheat flour components. III. Characterization of gluten protein fractions obtained by ultracentrifugation. Cereal Chem. 46:126-135.

HOSENEY, R. C., FINNEY, K. F., SHOGREN, M. D., and POMERANZ, Y. 1969b. Functional (breadmaking) and biochemical properties of wheat flour components. II. Role of water solubles. Cereal Chem. 46:117-125.

HUEBNER, F. R. 1970. Comparative studies on glutenins from different classes of wheat. J. Agric. Food Chem. 18:256-259.

HUEBNER, F. R., and WALL, J. S. 1976. Fractionation and quantitative differences of glutenin from wheat varieties varying in baking quality. Cereal Chem. 53:258-269.

HUEBNER, F. R., and BIETZ, J. A. 1993. Improved chromatographic separation and characterization of ethanol-soluble wheat proteins. Cereal Chem. 70:506-511.

JACKSON, E. A., HOLT, L. M., and PAYNE, P. I. 1983. Characterisation of high molecular weight gliadin and low molecular weight glutenin subunits of wheat endosperm by two-dimensional electrophoresis and the chromosomal localisation of their controlling genes. Theor. Appl. Genet. 66:29-37.

JACKSON, E. A., HOLT, L. M., and PAYNE, P. I. 1985. *Glu-B2*, a storage protein locus controlling the D group of LMW glutenin subunits in bread wheat (*Triticum aestivum*). Genet. Res. Camb. 46:11-17.

JONES, R. W., TAYLOR, N. W., and SENTI, F. R. 1959. Electrophoresis and fractionation of wheat gluten. Arch. Biochem. Biophys. 84:363-376.

KHAN, K., and BUSHUK, W. 1979. Studies of glutenin. XII. Comparison by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of unreduced and reduced glutenin from various isolation and purification procedures. Cereal Chem. 56:63-68.

KIM, H. R., and BUSHUK, W. 1995. Salt sensitivity of acetic acid-extractable proteins of wheat flour. J. Cereal Sci. 21:241-250.

KRUGER, J. E., MARCHYLO, B. A., and HATCHER, D. 1988. Preliminary assessment of a sequential extraction scheme for evaluating quality by reversed-phase high-performance liquid chromatography and electrophoretic analysis of gliadins and glutenins. Cereal Chem. 65:208-214.

LEW, E. J.-L., KUZMICKY, D. D., and KASARDA, D. D. 1992. Characterization of low molecular weight glutenin subunits by reversed-phase high-performance liquid chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and N-Terminal amino acid sequencing. Cereal Chem. 69:508-515.

LUNDH, G., and MacRITCHIE, F. 1989. Size exclusion HPLC characterisation of gluten protein fractions varying in breadmaking potential. J. Cereal Sci. 10:247-253.

MacRITCHIE, F. 1978. Differences in baking quality between wheat flours. Food Technol. 13:187-194.

MacRITCHIE, F. 1987. Evaluation of contributions from wheat protein fractions to dough mixing and breadmaking. J. Cereal Sci. 6:259-268.

MacRITCHIE, F., and GUPTA, R. B. 1993. Functionality-composition relationships of wheat flour as a result of variation in sulfur availability. Aust. J. Agric. Res. 44:1767-1774.

MARCHYLO, B. A., and KRUGER, J. E. 1988. The effect of injection volume on the quantitative analysis of wheat storage proteins by reversed-phase high-performance liquid chromatography. Cereal Chem. 65:192-198.

MARCHYLO, B. A., KRUGER, J. E., and HATCHER, D. W. 1989. Quantitative reversed-phase high-performance liquid chromatographic analysis of wheat storage proteins as a potential quality prediction tool. J. Cereal Sci. 9:113-130.

MASCI, S., LAFIANDRA, D., PORCEDDU, E., LEW, E. J.-L., TAO, H. P., and KASARDA, D. D. 1993. D-glutenin subunits: N-terminal sequences and evidence for the presence of cysteine. Cereal Chem. 70:581-585.

MECHAM, D. K., SOKOL, H. A., and PENCE, J. W. 1962. Extractable protein and hydration characteristics of flours and doughs in dilute acid. Cereal Chem. 39:81-93.

MEREDITH, O. W., and WREN, J. J. 1966. Determination of molecular-weight distribution in wheat flour proteins by extraction and gel filtration in a dissociating medium. Cereal Chem. 43:169-186.

MIFLIN, B. J., BYERS, M., FIELD, J. M., and FAULKS, J. A. 1980. The isolation and characterisation of proteins extracted from whole milled seed, gluten and developing protein bodies of wheat. Ann. Technol. Agric. 29:133-147.

MIFLIN, B. J., FIELD, J. M., and SHEWRY, P. R. 1983. Cereal storage proteins and their effects on technological properties. Pages 255-319 in: Seed Proteins. J. Daussant, J. Mosse, and J. Vaughan, eds. Academic Press: New York.

NG, P. K. W., and BUSHUK, W. 1987. Glutenin of Marquis wheat as a reference for estimating molecular weights of glutenin subunits by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chem. 64:324-327.

NG, P. K. W., and BUSHUK, W. 1988. Statistical relationships between high molecular weight subunits of glutenin and breadmaking quality of Canadian-grown wheats. Cereal Chem. 65:408-413.

ORTH, R. A., and BUSHUK, W. 1972. A comparative study of the proteins of wheats of diverse baking qualities. Cereal Chem. 49:268-275.

ORTH, R. A., and BUSHUK, W. 1973. Studies of glutenin. I. Comparison of preparative methods. Cereal Chem. 50:106-114.

OSBORNE, T. B. 1924. The Vegetable Proteins, 2nd ed. Longmans Green and Co.: London.

PAYNE, P. I., and CORFIELD, K. G. 1979. Subunit composition of wheat glutenin proteins, isolated by gel filtration in a dissociating medium. Planta 145:83-88.

PAYNE, P. I., CORFIELD, K. G., and BLACKMAN, J. A. 1979. Identification of a high-molecular-weight subunit of glutenin whose presence correlates with bread-making quality in wheats of related pedigree. Theor. Appl. Genet. 55:153-159.

PAYNE, P. I., CORFIELD, K. G., HOLT, L. M., and BLACKMAN, J. A. 1981. Correlations between the inheritance of certain high-molecular-weight subunits of glutenin and bread-making quality in progenies of six crosses of bread wheat. J. Sci. Food Agric. 32:51-60.

SAPIRSTEIN, H. D., and BUSHUK, W. 1985. Computer-aided analysis of gliadin electrophoregrams. I. Improvement of precision of relative mobility determination by using a three reference band standardization. Cereal Chem. 63:372-377.

SHEPHERD, K. W. 1968. Chromosomal control of endosperm proteins in wheat and rye. Pages 86-96 in: Proc. 3rd Int. Wheat Genet. Symp. Aust. Acad. Sci.: Canberra.

SINGH, N. K., SHEPHERD, K. W., and CORNISH, G. B. 1991. A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. J. Cereal Sci. 14:203-208.

SINGH, N. K., DONOVAN, G. R., BATEY, I. L., and MacRITCHIE, F. 1990. Use of sonication and size-exclusion high performance liquid chromatography in the study of wheat proteins. I. Dissolution of total proteins in the absence of reducing agents. Cereal Chem. 67:150-161.

SOZINOV, A. A., and POPERELYA, F. A. 1982. Polymorphism of prolamins and variability of grain quality. Qual. Plant. Foods Hum. Nutr. 31:243.

TANAKA, K., and BUSHUK, W. 1973. Changes in flour proteins during dough mixing. I. Solubility results. Cereal Chem. 50:590-596.

TSEN, C. C. 1967. Changes in flour proteins during dough mixing. Cereal Chem. 44:308-317.

WRIGLEY, C. W., and SHEPHERD, K. W. 1973. Electrofocusing of grain proteins from wheat genotypes. Ann. N.Y. Acad. Sci. 209:154-162.

[Received April 24, 1995. Accepted October 23, 1995.]