Influence of Germination on Wheat Quality. II. Modification of Endosperm Protein[1]

O. M. LUKOW[2] and W. BUSHUK, Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

Cereal Chem. 61(4): 340-344

ABSTRACT

Controlled laboratory germination of two wheat cultivars differing in mixing properties produced substantial differences between them in the distribution of protein in the modified Osborne solubility fractions. For all treatments, the content of residue protein in Glenlea flour was higher than in Neepawa flour. The residue (insoluble) fractions decreased as the gliadin and glutenin (soluble) fractions increased during germination for both cultivars. Gel filtration on Sephadex G-200 of the gliadin fractions from both cultivars produced profiles with five protein peaks (molecular weight >200,000, 105,000, 50,000, 16,800, and 5,000 daltons for peaks I–V, respectively) and two carbohydrate peaks (coeluting with protein peaks I and V). Soaking and germination affected the relative size of protein peak I and the two carbohydrate peaks. Qualitative and quantitative differences in sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of reduced and unreduced gliadin and glutenin were noted.

Although no single factor controls the breadmaking quality of wheat, protein is now considered the key flour component (Finney 1943, Khan and Bushuk 1979a). Functionality of wheat proteins in breadmaking is related to the relative amounts of Osborne protein fractions (Orth and Bushuk 1972). For best breadmaking performance, there is an optimum ratio of soluble protein to insoluble protein. The high proportion of insoluble protein in overly strong cultivars of wheat has been documented and correlated to their relatively poor baking quality (Butaki and Dronzek 1979, Mullen and Smith 1965, Orth and Bushuk 1972).

Breakdown of endosperm proteins occurs during germination of wheat. Hwang and Bushuk (1973) and Preston et al (1978) noted quantitative and qualitative changes in the flour proteins of germinated wheat that were associated with proteolytic activity. Attempts to correlate breadmaking functionality with the subunit composition of wheat proteins, whether from sound or germinated samples, have not been entirely successful. Accordingly, the proteins of flours milled from the grain of two wheat cultivars that had been subjected to various germination treatments were investigated in detail by means of protein fractionation, gel filtration, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). This study was undertaken to provide an explanation for the observed changes in breadmaking quality reported previously (Lukow and Bushuk 1984).

MATERIALS AND METHODS

Glenlea and Neepawa wheat samples were germinated and milled into flour as described previously (Lukow and Bushuk 1984).

Protein Fractionation

Flour protein was fractionated by the modified Osborne procedure of Chen and Bushuk (1970). Ten grams of flour was extracted sequentially with 0.5*M* sodium chloride, 70% aqueous ethanol, and 0.05*M* acetic acid. Albumins and globulins, both soluble in the salt solution, were determined as a single fraction. Extracts were freeze-dried and the nitrogen content determined by the micro-Kjeldahl procedure (AACC 1962). Reported values are averages of duplicate fractionations. Analysis of variance was conducted. Least significant differences were computed at the 5% level of significance.

Analytical

Gel-filtration chomatography was performed on a 88.0 × 2.6-cm column of Sephadex G-200 (Pharmacia Fine Chemicals). The solvent was a 0.1*M* acetic acid, 3*M* urea solution containing 20 m*M* potassium chloride. Potassium chloride was added to increase the ionic strength of the solvent and thereby minimize ionic interactions during chromatography (McMaster 1982). Protein content of the effluent was assayed by measuring the absorbance of individual fractions at 280 nm. Carbohydrate content was measured by the phenol-sulfuric acid method of Dubois et al (1956). The column was calibrated with standard proteins of known molecular weight.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed at pH 8.4 according to the method of Koenig et al (1970)

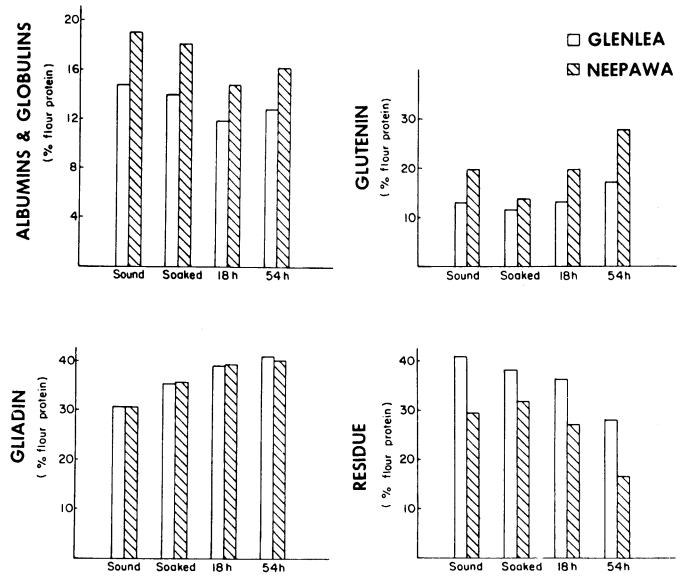

Fig. 1. Modified Osborne fractions (percent flour protein) obtained from Glenlea and Neepawa flours. Least significant differences of Glenlea fractions are: albumins and globulins, 1.6; gliadin, 2.0; glutenin, 3.2; and residue, 4.5. Least significant differences of Neepawa fractions are: albumins and globulins, 2.4; gliadin, 1.9; glutenin, 5.3; and residue, 4.1.

[1]Presented at the AACC 68th Annual Meeting, Kansas City, MO, November 1983. Publication No. 651, Department of Plant Science, University of Manitoba, with financial assistance from the Natural Sciences and Engineering Research Council of Canada, and publication No. 1117, Agriculture Canada, Research Station, Winnipeg, Manitoba.

[2]Present address: Agriculture Canada, Research Station, 195 Dafoe Rd., Winnipeg, Manitoba, Canada R3T 2M9.

as modified by Khan and Bushuk (1979b) for the EC470 vertical slab-gel electrophoresis apparatus. When reduction was required, 1% β-mercaptoethanol was added to the sample solvent. Molecular weights were estimated by the procedure of Weber and Osborn (1969).

RESULTS AND DISCUSSION

Protein Distribution

The proteins of the flours milled from four of the five treatments (sound, soaked, and 18- and 54-h germination) were fractionated, and the results are presented in the form of histograms for the albumins and globulins, gliadin, glutenin, and residue fractions (Fig. 1). For more accurate comparison, results were normalized to 100% protein recovery; actual recoveries varied from 85 to 95%.

The albumins and globulins fraction decreased slightly during germination for both cultivars. Of the gluten proteins, significant increases with germination were obtained for the gliadin fraction. Results for the two cultivars were essentially identical. The glutenin fraction increased slightly during germination. Neepawa samples contained more glutenin than the Glenlea samples for all treatments. This observation is consistent with the earlier results of Orth and Bushuk (1972). In addition, the rate of increase of glutenin during germination was greater for Neepawa. The residue fraction decreased substantially with progressive germination for both cultivars. Glenlea samples contained a greater proportion of residue protein than Neepawa. The rate of change in the solubility distribution of proteins was reasonably constant during germination. The soaked samples generally followed the same trend (either increasing or decreasing) for the various protein fractions as the germinated samples.

Gel Filtration of Gliadin Fractions

Two carbohydrate peaks and five protein peaks labeled I, II, III, IV, and V were present in all profiles obtained from gel filtration on Sephadex G-200 (Figs. 2 and 3). A high-molecular-weight (>200,000 daltons) gliadin fraction (peak I) eluted in the void volume of the column. Peak V proteins eluted at the total volume (V_t) of the column and hence are low-molecular-weight proteins (<5,000 daltons) or peptides. Carbohydrate was associated with peak I fractions. A relatively large amount of carbohydrate was associated also with the peak V fractions. Presumably this latter carbohydrate fraction is residual material that was not completely extracted with the albumins and globulins fraction. Its composition was not investigated further. Békés et al (1983) and McMaster (1982) did not find carbohydrate associated with the peak V fraction when gliadin was prepared from gluten that had been exhaustively washed to remove soluble material.

The molecular weights of the protein peaks, derived from a standard curve of log molecular weight versus partition coefficient

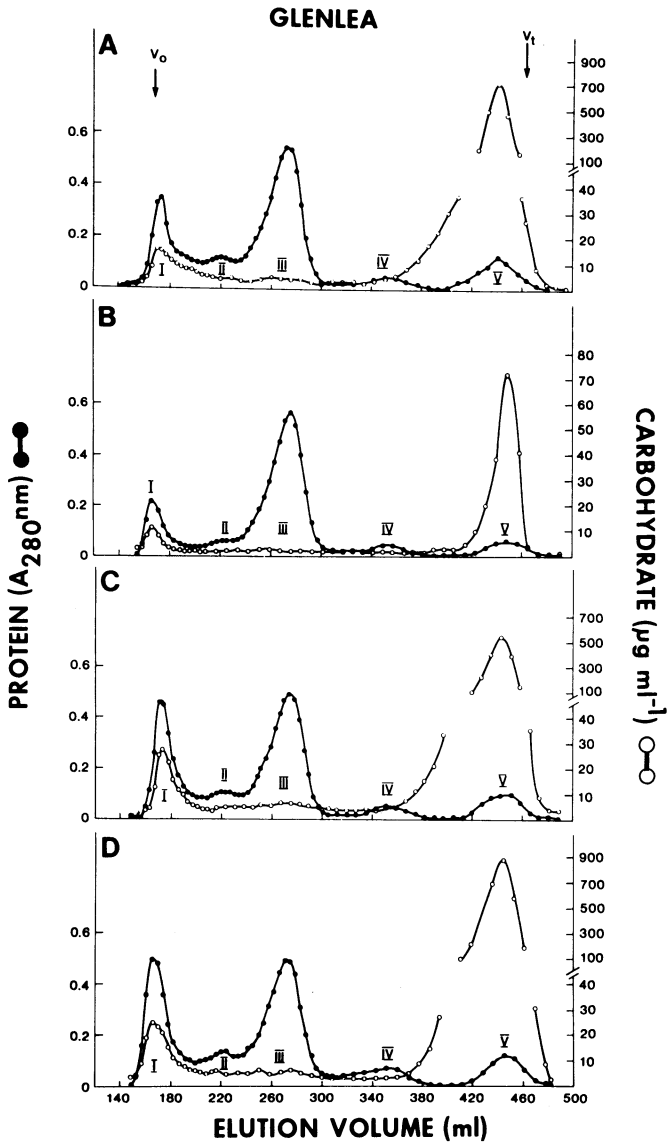

Fig. 2. Elution profile from gel-filtration chomatography of Glenlea gliadin fraction on Sephadex G-200. **A**, sound; **B**, soaked; **C**, 18 h; **D**, 54 h.

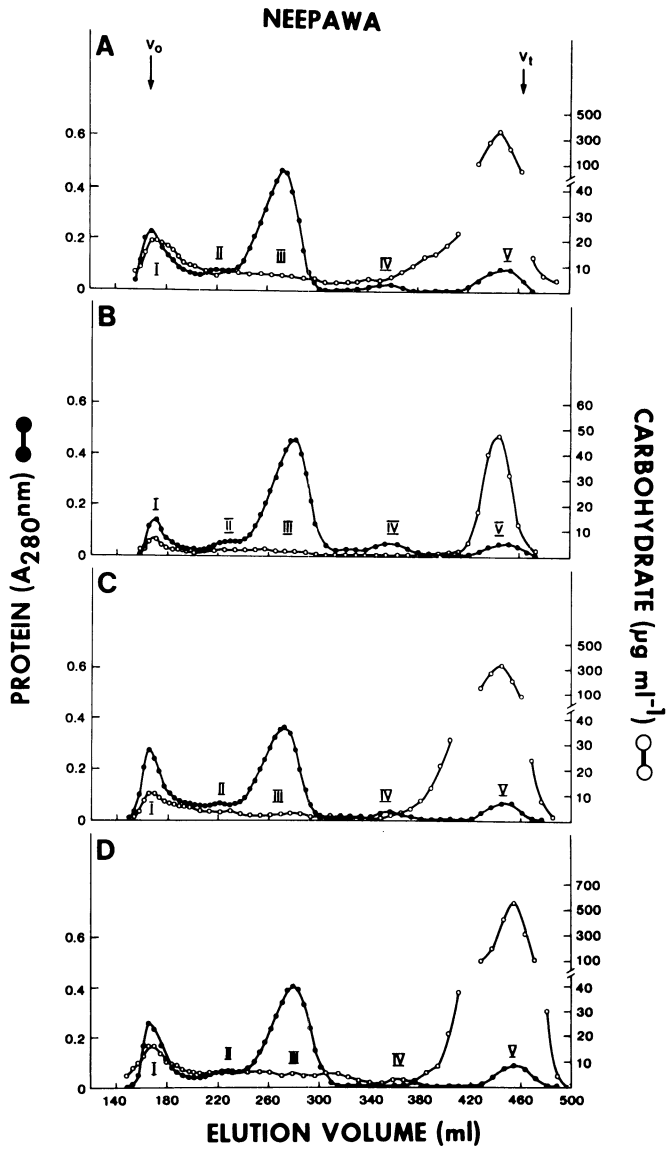

Fig. 3. Elution profile from gel-filtration chomatography of Neepawa gliadin fraction on Sephadex G-200. **A**, sound; **B**, soaked; **C**, 18 h; **D**, 54 h.

values, were as follows: peak I, >200,000; peak II, 105,000; peak III, 50,000; peak IV, 16,800; and peak V, 5,000. The presence of potassium chloride in the eluent produced symmetrical protein peaks with slightly lower elution volumes than previously reported by Békés et al (1983) and McMaster (1982).

The elution volumes of peaks I–V did not change after soaking or germination. For germinated samples, peaks II, IV, and V of Glenlea remained constant, while peak III decreased slightly. In the elution profile of the Glenlea soaked sample, peak I was substantially reduced compared to peak I of the sound sample. At 18 and 54 h, peak I was larger than the control sample and approached peak III in size (Fig. 2).

Concurrent with the modification of the protein peaks, the carbohydrate level in the gliadin fractions of Glenlea was altered by soaking and germination. The carbohydrate content of peak I declined after soaking and then increased after 18 and 54 h of germination. The most significant change in the amount of carbohydrate coeluting with peak V proteins occurred during the soaking step, during which the carbohydrate decreased by 90% compared to the sound sample. Presumably, these carbohydrates are of low molecular weight and are leached from the grain during soaking. During germination, this low-molecular-weight carbohydrate fraction increased to a level higher than that found in the sound samples. Apparently, the increased amylolytic activity produced low-molecular-weight dextrins and sugars that were extracted with the gliadin fraction.

Neepawa gliadin fractions showed the same trends in gel-filtration profiles as the Glenlea samples (Fig. 3). The area of Neepawa peak I was consistently smaller than peak I gliadin of the corresponding Glenlea samples.

SDS-PAGE

Gliadin, glutenin, and residue fractions were subjected to SDS-PAGE so that possible qualitative and quantitative changes in the molecular weights of protein subunits with germination could be examined.

The electrophoregrams of unreduced gliadin are shown in Fig. 4. A large number of subunits, ranging in molecular weight from 200,000 to 13,000 daltons, was observed. Some protein remained in the slot region; the amount appeared to be greater for the 18- and 54-h germinated samples (patterns 3, 4, 7, 8). In addition, there was increased streaking in the high-molecular-weight region of these

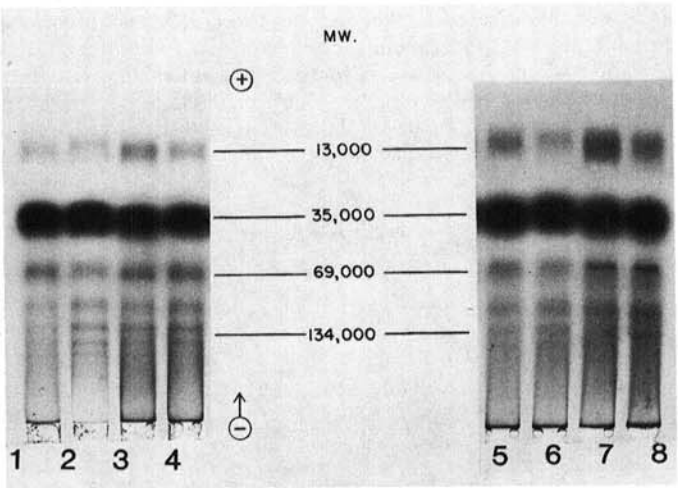

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis electrophoregrams of unreduced gliadin. Sample size: 1 mg of protein. Sample concentration: 10 mg of protein ml^{-1}. Neepawa: **1,** sound; **2,** soaked; **3,** 18 h; **4,** 54 h. Glenlea: **5,** sound; **6,** soaked; **7,** 18 h; **8,** 54 h.

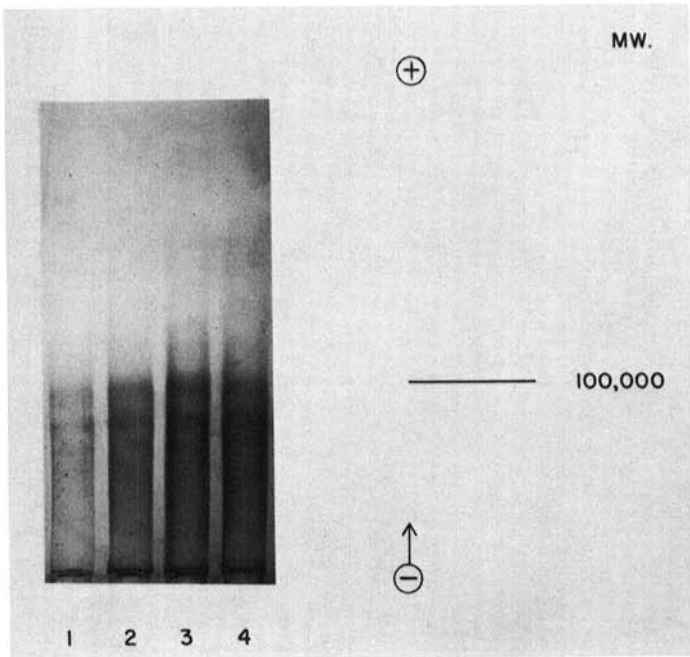

Fig. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis electrophoregrams of Sephadex G-200 peak I unreduced gliadin from Glenlea. Sample size: 400 μg of protein. Sample concentration: 10 mg of protein ml^{-1}. **1,** sound; **2,** soaked; **3,** 18 h; **4,** 54 h.

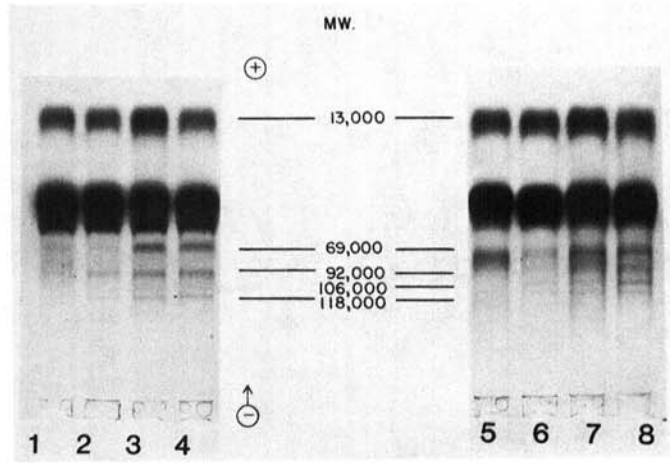

Fig. 6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis electrophoregrams of reduced gliadin. Sample size: 1 mg of protein. Sample concentration: 10 mg of protein ml^{-1}. Neepawa: **1,** sound; **2,** soaked; **3,** 18 h; **4,** 54 h. Glenlea: **5,** sound; **6,** soaked; **7,** 18 h; **8,** 54 h.

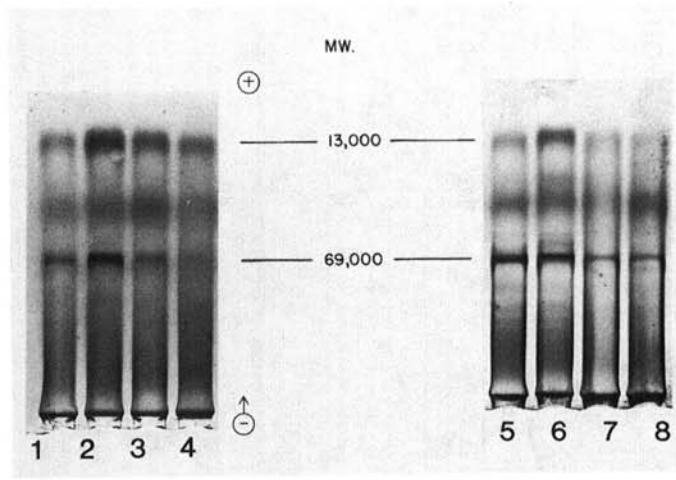

Fig. 7. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis electrophoregrams of unreduced glutenin. Sample size: 1 mg of protein. Sample concentration: 10 mg of protein ml^{-1}. Neepawa: **1,** sound; **2,** soaked; **3,** 18 h; **4,** 54 h. Glenlea: **5,** sound; **6,** soaked; **7,** 18 h; **8,** 54 h.

samples. For the Glenlea samples, the intensity of the 69,000-dalton band increased noticeably during germination (patterns 7, 8).

Substantial amounts of protein were found in the high-molecular-weight region. High-molecular-weight gliadin was a component of total gliadin and could be separated into the peak I fraction by gel filtration on Sephadex G-200. Unreduced peak I gliadin proteins of Glenlea were subjected to SDS-PAGE (Fig. 5). Large amounts of the preparations remained at the origin. The proteins that entered the gel covered a molecular weight range of approximately 200,000–100,000 daltons. The peak I fraction of the Neepawa samples behaved similarly on SDS-PAGE. These high-molecular-weight gliadin proteins may be the same proteins isolated by Békés et al (1983) and McMaster (1982) from gluten.

For the reduced-gliadin fractions of Glenlea, the 118,000-, 106,000-, 92,000-, and 69,000-dalton bands increased in intensity with increasing germination (Fig. 6). Of these four protein components, only the 69,000-dalton band is clearly evident in the pattern for the sound sample (pattern 5). After soaking, the three higher-molecular-weight protein components were faintly visible (pattern 6). Presumably, soaking and germination facilitate the solubilization of these components.

The reduced gliadin electrophoregram of the Neepawa sound sample (pattern 1) had faint bands in the molecular weight region 118,000–69,000 daltons. After soaking (pattern 2), 118,000-, 92,000-, and 69,000-dalton bands were more intense, and the intensity increased after 18 and 54 h of germination (patterns 3, 4). The 106,000-dalton component, present in the Glenlea electrophoregram, was absent in the Neepawa electrophoregram.

Electrophoregrams of unreduced glutenin are shown in Fig. 7. The patterns are characterized by extensive streaking of protein; only the 69,000- and 13,000-dalton bands were distinctly resolved. With germination, these bands decreased in intensity (patterns 3, 4, 7, 8). Heavy staining at the slots indicates that substantial amounts of high-molecular-weight material did not enter the gel; this behavior is typical of glutenin.

Approximately 17 subunits were observed in the electrophoregrams of reduced glutenin, in general agreement with results obtained by other workers (Bietz and Wall 1972, Hamauzu et al 1972, Khan and Bushuk 1977). The actual number of protein subunits obtained in previous studies depends on the method of glutenin preparation. Molecular weights of the subunits obtained in the present study ranged from 118,000 to 13,000 daltons. There were no qualitative or quantitative changes in reduced glutenin subunits during soaking or germination for both cultivars.

As with glutenin, very little unreduced residue protein entered the gel. Faint bands were visible with a predominant band of 150,000 daltons and extensive streaking of protein thoughout the gel. Upon reduction, subunit composition was very similar to that of reduced glutenin for both cultivars. No quantitative or qualitative changes in patterns of unreduced or reduced residue with soaking or germination were apparent for either cultivar.

CONCLUSIONS

Technologically, the shift in protein distribution during germination from high-molecular-weight (less soluble) to low-molecular-weight (more soluble) may be extremely significant. Wheat cultivars of good breadmaking quality are generally characterized by a specific distribution of protein. The most significant result vis-à-vis breadmaking quality, obtained for both Glenlea and Neepawa, was the decrease in the proportion of residue protein and the increase in gliadin and glutenin fractions with soaking and progressive germination. The improvement in baking quality of the 18-h Glenlea sample (Lukow and Bushuk 1984) may be directly related to this shift in the molecular-weight distribution of endosperm protein with concomitant modification of dough properties from overly strong to moderately strong. In this study, substantial increases of endo- and exoproteolytic activity were found during germination (Lukow and Bushuk 1984). Even low amounts of proteolytic activity may be sufficient to break a small number of critical peptide bonds and thereby produce a notable change in solubility distribution. Although peptide bond cleavage may have played a central role in changes in the solubility distribution of endosperm proteins, it may not be the sole factor. Degradation of carbohydrate and/or lipid associated with specific gluten proteins could disrupt the native gluten structure and generate smaller, more soluble protein aggregates. Further research is required to determine the precise role of flour carbohydrates and lipids on the solubility properties of endosperm proteins.

The increase in gliadin peak I in gel-filtration chomatography on Sephadex G-200 with germination of both cultivars is consistent with the shift from high-molecular-weight insoluble residue proteins into the ethanol-soluble fraction (Fig. 1). The peak I fraction of gliadin from Glenlea was consistently larger than the corresponding fraction from Neepawa for the different treatments; this may be one of several (as yet unknown) genotypic characters of gluten quality. The effect of varying proportions of high-molecular-weight gliadin (peak I) on the rheological and bread-making properties of wheat remains to be examined. Protein peaks II–V did not change significantly during germination; peak I protein increased preferentially at the expense of the less soluble (glutenin and residue) fractions.

The peak I fraction of gliadin decreased significantly (relative to other peak fractions) during the soaking treatment for both cultivars. Modification of the endosperm protein solubility occurred, possibly due to leaching of solubles from the kernels; high-molecular-weight gliadin proteins became insoluble in aqueous ethanol.

Qualitative and quantitative differences in protein components as indicated by SDS-PAGE were observed for the gliadin and glutenin fractions at various treatments for both cultivars. As a consequence of the soaking process, protein bands of relatively high molecular weight appeared in the SDS-PAGE patterns of reduced gliadin samples. The new bands increased in intensity during progressive germination. In sound samples, these high-molecular-weight components are not extracted in the gliadin fraction. The reasons for this change in solubility remain to be discovered.

A gliadin 69,000-dalton band, present in sound samples, increased in intensity with soaking and germination. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis results showed that unreduced glutenin of both cultivars contained protein bands of 69,000 and 13,000 daltons that decreased in intensity during germination. Further studies are required to establish whether the 69,000-molecular-weight component of glutenin is modified by the germination process and becomes soluble in the 70% ethanol solution used to extract the gliadins. However, we can conclude that, in addition to the general degradation of endosperm proteins, certain proteins are preferentially degraded to specific low-molecular-weight peptides.

ACKNOWLEDGMENTS

Financial support to O. M. Lukow from a Natural Sciences and Engineering Research Council Post-graduate Scholarship and a University of Manitoba Graduate Fellowship is gratefully acknowledged.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1962. Approved Methods of the AACC. Method 46-13, approved October 1976. The Association, St. Paul, MN.

BÉKÉS, F., ZAWISTOWSKA, U., and BUSHUK, W. 1983. Protein-lipid complexes in the gliadin fraction. Cereal Chem. 60:371.

BIETZ, J. A., and WALL, J. S. 1972. Wheat gluten subunits: Molecular weights determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chem. 49:416.

BUTAKI, R. C., and DRONZEK, B. 1979. Effect of protein content and wheat variety on relative viscosity, solubility and electrophoresis properties of gluten proteins. Cereal Chem. 56:162.

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.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., and

SMITH, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350.
FINNEY, K. F. 1943. Fractionating and reconstituting techniques as tools in wheat flour research. Cereal Chem. 20:381.
HAMAUZU, Z., ARAKAWA, T., and YONEZAWA, D. 1972. Molecular weights of glutenin- and gliadin-polypeptides estimated by SDS-polyacrylamide gel electrophoresis. Agric. Biol. Chem. 36:1829.
HWANG, P., and BUSHUK, W. 1973. Some changes in the endosperm proteins during sprouting of wheat. Cereal Chem. 50:147.
KHAN, K., and BUSHUK, W. 1977. Studies of glutenin. IX. Subunit composition of sodium dodecyl sulfate-polyacrylamide gel electrophoresis at pH 7.3 and 8.9. Cereal Chem. 54:588.
KHAN, K., and BUSHUK, W. 1979a. Structure of wheat gluten in relation to functionality in breadmaking. Page 191 in: Functionality and Protein Structure, ACS Symp. Ser., No. 92. A. Pour-El, ed. Am. Chem. Soc., Washington, DC.
KHAN, K., and BUSHUK, W. 1979b. 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.
KOENIG, R., STEGEMANN, H., FRANKSEN, H., and PAUL, H. L. 1970. Protein subunits in the potato virus x group. Determination of the molecular weights by polyacrylamide electrophoresis. Biochim. Biophys. Acta 207:184.
LUKOW, O. M., and BUSHUK, W. 1984. Influence of germination on wheat quality. I. Functional (breadmaking) and biochemical properties. Cereal Chem. 61:336.
McMASTER, G. J. 1982. Association of carbohydrate and protein in wheat gluten. Ph.D. thesis. University of Manitoba, Winnipeg, Manitoba.
MULLEN, J. D., and SMITH, D. E. 1965. Studies on short- and long-mixing flours. I. Solubility and electrophoretic composition of proteins. Cereal Chem. 42:263.
ORTH, R. A., and BUSHUK, W. 1972. A comparative study of the proteins of wheats of diverse baking quality. Cereal Chem. 49:268.
PRESTON, K. R., DEXTER, J. E., and KRUGER, J. E. 1978. Relationship of exoproteolytic and endoproteolytic activity to storage protein hydrolysis in germinating durum and hard red spring wheat. Cereal Chem. 55:877.
WEBER, K., and OSBORN, M. 1969. The reliability of molecular weight determination by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406.

[Received November 22, 1983. Revision received February 8, 1984. Accepted February 10, 1984]