COMPREHENSIVE EXAMINATION OF PROTEIN DISTRIBUTION PROFILE IN WHEAT GRAIN

Toshio MITSUNAGA\(^1\) and Hisateru MITSUDA\(^2\)

\(^1\)Department of Food Science, Faculty of Home Economics, Kyoto Women's University, Kyoto
\(^2\)Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto

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Summary A wheat grain was divided into three portions, endosperm, germ and bran, and the protein profile of each was examined comparatively after sequential extraction with isopropyl alcohol, sodium chloride, lactic acid and KOH solutions. Recovery of the protein in the extracts was 93–96\%. The relative protein concentrations of the four soluble fractions of endosperm were very similar to those of bran, but germ showed a distinct distribution of the soluble proteins. Soluble protein fractions of endosperm, germ and bran, particularly their NaCl soluble fractions, exhibited distinct individuality upon examination by polyacrylamide gel electrophoresis and gel filtration. Indeed, it was suggested that the gel electrophoretic profile could serve as a criterion for the quality evaluation of wheat flour. On the other hand, the KOH soluble proteins of endosperm, germ and bran, which gave indiscrete electrophoretic patterns, existed predominantly in highly aggregated forms which disintegrated upon exposure to 1% SDS or by reduction with 2-mercaptoethanol.

The distribution spectrum of wheat proteins has not been fully established due to the lack of definitive procedures for extraction and fractionation. Wheat proteins are in general fractionated according to their solubilities by sequential extraction of ground samples (1–3). A problem encountered in this classical method arises from low recovery of proteins. MAES (4) devised an effective method in which a sample thoroughly ground with sand is packed in a column and progressively extracted with appropriate solvents. This method has been recently modified by MATTERN et al. (5).

While proteins of endosperm have been extensively investigated in relation to its processing properties (6), the protein distribution profiles of germ and bran are

\(^1\) 光永俊郎，\(^2\) 滝田久輝
yet ill-defined. Indeed, no overall description of the protein spectra of germ and bran has been reported since Grewe (7) and Teller (8) carried out a series of analysis in which a classical extraction method was employed. Accordingly, a detailed description of the protein profiles of wheat, which undoubtedly has important implications in nutritional studies, need be provided. This communication describes the protein fractionation of endosperm, germ and bran by a recently developed method and some properties of the fractions, including amino acid compositions, electrophoretic and gel filtration behaviours. Also, the use of the gel electrophoretic profile as a criterion for the quality evaluation of wheat flour is discussed.

MATERIALS AND METHODS

The wheat sample is Canada Western (harvested in 1973) supplied by the Nisshin Flour Milling Co., Ltd. All reagents used are analytical reagent grade.

Progressive extraction of the wheat protein. Extraction of the wheat proteins was performed by the modified procedure of Maes method (5). Briefly, five grams of wheat sample (endosperm, germ and bran) were first ground with 5 g of previously washed and dried celite (No. 503) in a mortar and then with 100 g sea sand. A small amount of 40% isopropyl alcohol was added to this mixture. The resultant paste was packed into a column (3 × 30 cm), which was subsequently eluted sequentially with 200 ml each of 40% isopropyl alcohol, 2% NaCl solution, 3.85% lactic acid and 0.1% KOH. After exhaustive dialysis against 0.01 N CH₃COOH solution, the fractions were lyophilized.

Determination of protein. In order to quantify the recovery and relative amount of the protein in each extract, nitrogen contents of wheat samples (endosperm, germ and bran) and the protein extracts before dialysis against 0.01 N CH₃COOH solution were determined by the Kjeldahl method (9).

Polyacrylamide gel electrophoresis in 8 M urea. The lyophilized protein samples of the isopropyl alcohol and lactic acid soluble fractions were readily soluble in 8 M urea solution. The NaCl and KOH soluble fractions, which were hardly soluble in 8 M urea solution, were first dissolved in 6 M guanidin hydrochloride and then dialyzed against 8 M urea. Solutions thus prepared were found to be sufficiently stable. Gel electrophoresis was performed on 3.5–7% acrylamide gel in the presence of 8 M urea using the buffer system described by Ornstein and Davis (10). The KOH soluble fraction was also run on 7% acrylamide gel in 0.2% KOH solution containing 1% SDS. After electrophoresis, gels were stained with 1% Amido Black 10 B in 20% methanol solution containing 7% acetic acid for protein and with periodic acid-Schiff reagent (PAS) for carbohydrate as described by Kessler (11).

Gel electrophoresis of reduced and alkylated protein fractions. The KOH soluble fraction containing huge aggregates not admittted to 7% gel was arbitra-
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rily divided into two subfractions by gel filtration on a Sepharose 4B column and subjected to gel electrophoresis after reduction with or without alkylation (12). To a solution of the subfraction (1 mg protein) in 0.11 M Tris-HCl buffer (pH 8.5) containing 8 M urea 2-mercaptoethanol was added to a final concentration of 0.11 M, and the mixture was allowed to stand at room temperature for 24 hr. Thirty milligrams of iodoacetamide was then added to the reaction mixture. Electrophoresis was carried out on these samples after dialysis against appropriate buffers.

Analysis of the amino acids. The amino acid composition was determined using a Hitachi model KLA-5A amino acid analyzer. Samples (3 mg) were hydrolyzed in 6 M HCl (6 ml) in vacuum sealed tubes at 110°C for 22 hr. No corrections were made for possible destruction of certain amino acids that might occur during acid hydrolysis of carbohydrate containing proteins.

RESULTS AND DISCUSSION

Distribution profiles and amino acid compositions of proteins of endosperm, germ and bran

The distribution profiles of the soluble proteins in endosperm, germ and bran are presented in Table 1. Endosperm contains a high proportion of isopropyl alcohol and KOH soluble fractions which total to 83.6%, but the content of NaCl soluble fraction is low. This is contrasted by the protein profile of germ in which the proteins are more or less evenly distributed among the extracts. The profile of bran proteins is much similar to that of endosperm except that the order of the minor fractions (NaCl soluble and lactic acid soluble fractions) is reversed.

Table 1. Relative concentrations of the four soluble protein fractions of endosperm, germ and bran.

|          | Isopropyl alcohol soluble (%) | NaCl soluble (%) | Lactic acid soluble (%) | KOH soluble (%) | Total (%) |
|----------|-----------------------------|-----------------|------------------------|-----------------|----------|
| Endosperm| 47.1                        | 2.4             | 10.1                   | 36.5            | 96.1     |
| Germ     | 22.9                        | 14.5            | 20.1                   | 35.7            | 93.1     |
| Bran     | 43.3                        | 9.2             | 3.0                    | 37.4            | 92.9     |

Table 2 shows the amino acid profiles of the all fractions of endosperm, germ and bran. The corresponding fractions of germ and bran exhibit general similarity in their amino acid composition except for appreciably high proportion of cystine in the NaCl soluble fraction of germ. Other prominent differences worth noting are that all the fractions of endosperm are composed of high proportions of glutamic acid and proline while glycine and alanine contents of germ
Table 2. The amino acid compositions (mole%) of the soluble protein fractions of endosperm, germ and bran.

| Amino acid   | IPA* | Endosperm | Germ | Bran |
|--------------|------|-----------|------|------|
|              | NaCl | Lactic | KOH  | NaCl | Lactic | KOH  | NaCl | Lactic | KOH  |
| Lysine       | 1.16 | 3.61    | 4.68 | 3.30 | 4.80    | 7.68 | 8.10 | 6.15    | 2.08 | 4.60    | 6.89 | 4.30 |
| Histidine    | 1.87 | 2.86    | 2.44 | 1.75 | 2.35    | 2.75 | 2.67 | 2.19    | 1.70 | 3.90    | 3.81 | 2.20 |
| Arginine     | 2.23 | 5.33    | 4.71 | 2.59 | 5.00    | 8.83 | 8.37 | 6.12    | 1.83 | 9.54    | 6.49 | 5.40 |
| Aspartic acid| 2.19 | 5.92    | 0.80 | 4.25 | 7.07    | 6.97 | 8.02 | 8.85    | 3.79 | 8.30    | 7.59 | 5.76 |
| Threonine    | 2.10 | 4.15    | 4.32 | 3.55 | 4.79    | 5.08 | 4.74 | 4.76    | 3.02 | 3.86    | 4.79 | 3.85 |
| Serine       | 4.44 | 6.71    | 6.97 | 5.50 | 5.45    | 0.40 | 6.13 | 6.00    | 5.47 | 5.80    | 7.16 | 6.34 |
| Glutamic acid| 39.18| 24.67   | 26.68| 32.00| 21.96   | 13.83| 13.98| 13.12   | 36.04| 16.07   | 14.17| 22.28|
| Proline      | 16.20| 7.05    | 9.68 | 11.36| 7.32    | 2.68 | 2.79 | 3.81    | 15.55| 3.55    | 6.04 | 10.42|
| Glycine      | 3.88 | 9.45    | 6.28 | 6.80 | 10.90   | 14.69| 10.81| 11.22   | 4.92 | 13.37   | 14.46| 10.85|
| Alanine      | 2.77 | 3.24    | 5.92 | 5.22 | 8.62    | 9.74 | 9.57 | 9.32    | 4.70 | 7.74    | 9.05 | 6.62 |
| Cystine      | 2.38 | 4.81    | 6.44 | 1.79 | 1.54    | 11.89| trace| trace   | 1.00 | 3.40    | 4.15 | 0.51 |
| Valine       | 3.64 | 5.54    | 5.30 | 3.48 | 5.27    | 5.24 | 7.20 | 7.31    | 3.87 | 5.49    | 4.79 | 4.83 |
| Methionine   | 1.01 | 1.63    | 1.68 | 1.58 | 1.41    | 2.64 | 1.13 | 2.00    | 0.47 | 0.88    | 0.46 | 1.78 |
| Isoleucine   | 3.64 | 3.47    | 4.37 | 3.42 | 3.19    | 2.95 | 4.12 | 4.61    | 3.68 | 3.35    | 2.93 | 3.41 |
| Leucine      | 5.30 | 6.14    | 3.08 | 7.16 | 5.28    | 0.16 | 6.93 | 7.72    | 6.15 | 4.90    | 4.63 | 6.25 |
| Tyrosine     | 1.77 | 2.07    | 2.70 | 2.86 | 2.37    | 2.00 | 2.24 | 3.01    | 1.49 | 2.44    | 1.13 | 1.96 |
| Phenylalanine| 6.24 | 3.31    | 3.95 | 3.39 | 2.68    | 2.43 | 3.20 | 3.81    | 4.24 | 2.81    | 1.46 | 3.24 |

* Isopropyl alcohol.
Fig. 1. Electrophoresis of the protein components of isopropyl alcohol soluble fractions of endosperm (a), germ (b) and bran (c). Electrophoresis was carried out on 7% polyacrylamide gel in the presence of 8M urea using buffer system described by Ornstein and Davis.

Fig. 2. Electrophoresis of the protein components of 2% NaCl soluble fractions of endosperm (a), germ (b) and bran (c). The experimental conditions are as described for Fig. 1.

and bran are much higher than that of endosperm. Among the fractions of germ the NaCl soluble fraction is distinct from the others for its low serine and leucine contents.

*Gel electrophoretic patterns of the soluble proteins of endosperm, germ and bran*

Figure 1 shows electrophoretic profiles of isopropyl alcohol soluble fractions of
endosperm (a), germ (b) and bran (c). At least 15 components are detectable in the endosperm extract, 16 components in the germ extract and 10 components in the bran extract. All the fractions, particularly isopropyl alcohol soluble fraction of endosperm, contain aggregates which are not admitted to the concentrating gel (2.5%) and resolving gel (7%). There is no apparent similarity in the protein compositions of the three fractions.

The electrophoretic patterns of NaCl soluble fractions of endosperm (a), germ (b) and bran (c) are shown in Fig. 2 which clearly indicates marked individuality of each fraction. On the contrary, the electrophoretic profiles of lactic acid soluble fractions of germ and bran are very similar (Fig. 3). Lactic acid soluble fraction of endosperm is distinct in that it exhibits several eminent bands at the advancing front. The KOH soluble fractions comprised high proportions of aggregates which upon gel electrophoresis accumulated at the upper region of the gel columns giving anomalously diffused patterns. It was thought that removal of the large aggregates, which retard migration of other protein components, may improve electrophoretic pattern. Accordingly, the KOH soluble fractions were subfractionated by gel filtration on Sepharose 4 B and subjected to gel electrophoresis. The result, as shown in Fig. 4 for endosperm, was not with success yielding indistinct pattern. Gel filtration profiles, however, can provide some information regarding molecular weight distribution of the KOH soluble fractions. As shown in Fig. 5, the elution profiles indicate apparent diversity among the protein fractions of endosperm, germ and bran. The proportion of large aggregates eluted at the void volume of the column is appreciably higher in the KOH soluble fraction of endosperm than in the germ and bran fractions.
Fig. 4. Gel filtration patterns of KOH soluble fractions of endosperm (a), germ (b) and bran (c). The electrophoretic pattern on the left (Fig. 4a) is that obtained with the protein fraction eluted at the void volume of the column and the pattern on the right were obtained with the pooled fraction eluted between the elution volume marked by the two arrows. Gel filtration was carried out on a Sepharose 4B column (2 × 30 cm) in 8 M urea solution.

Disaggregation of the KOH soluble proteins by treatment with SDS and by reduction with 2-mercaptoethanol

There may be two possible reasons for the appearance of the indistinct electrophoretic patterns of the KOH soluble fractions, i.e., progressive aggregation through disulfide bond formation and reversible polymerization of physical nature or both. In the experiments that follow, these possibilities were examined.

In the presence of SDS, the KOH soluble proteins underwent disintegration
leading to the formation of several smaller units. Figure 6 shows electrophoretic profiles of KOH soluble proteins of endosperm (a), germ (b) and bran (c) obtained in 0.2% KOH solution containing 1% SDS. The disintegration patterns of the three fractions are very similar, each showing the presence of the smallest unit with molecular weight of 10,000 order. The results seem to indicate an important role of hydrophobic interaction in the aggregation of the KOH soluble fractions. It
should be noted that not all of the proteins comprised in the KOH soluble fraction are susceptible to SDS, since considerable amounts of protein still remained at the top of the gel. However, these aggregates disappeared by reduction which also led to disintegration of the aggregates (Fig. 6). It seems that the three KOH soluble fractions respond diversely to reduction. In conclusion, the KOH soluble protein occur predominantly in aggregated forms which disintegrate into several smaller units upon exposure to SDS or by reduction.

The use of the gel electrophoretic profile as a criterion of the quality evaluation of wheat flour

The electrophoretic profiles of the soluble proteins of wheat so far presented do not provide a means that allows indisputable differentiation of endosperm proteins from the proteins of germ and bran. The only difference worth noting is in the electrophoretic profiles of NaCl soluble fractions of the three portions (endosperm, germ and bran) (Fig. 2). In order to find the electrophoretically distinct components in the proteins of the three portions, the NaCl soluble fraction of endosperm was mixed with those of germ and bran separatory, and the two mixtures were subjected to gel electrophoresis. The resultant profiles are presented in Fig. 7, in which E+G and E+B respectively represent endosperm-germ and endosperm-bran mixtures. Comparison of the composite patterns with the corresponding

![Fig. 7](image)

**Fig. 7.** Electrophoresis of the mixtures of NaCl soluble fractions of endosperm and germ (E+G), and endosperm and bran (E+B). The experimental conditions are as described for Fig. 1.

![Fig. 8](image)

**Fig. 8.** Electrophoresis of isopropyl alcohol soluble fractions of endosperm (a), germ (b) and bran (c). Electrophoresis was carried out on 3.5% polyacrylamide gel and the patterns were obtained by staining with PAS reagent.
individual patterns (Fig. 2) can reveal contamination of endosperm. However, it requires a close examination to identify the contaminants, germ or bran.

In search for a more simple criterion, it was found that isopropyl alcohol soluble fractions of germ and bran, but not endosperm, contained PAS positive components which migrated into 3.5% polyacrylamide gel (Fig. 8). Since only endosperm does not contain this component, single electrophoretic run on a given flour sample is required to examine its purity. Thus, this procedure can serve as a criterion which permit a rapid evaluation of flour quality. It is of interest to note that NaCl soluble fraction of endosperm, but not those of germ and bran, contained PAS positive components which migrated into 7% polyacrylamide gel.

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