

# Proteins Extracted from Defatted Wheat Germ: Nutritional and Structural Properties

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## ABSTRACT

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The main by-product of the wheat germ oil extraction process is a defatted wheat germ meal, which has a relatively high protein content, making it an attractive and promising source of vegetable proteins. Four protein fractions (albumin, globulin, prolamine, and glutelin) and protein isolate from defatted wheat germ flour (DWGF) were fractionated and then characterized by amino acid analysis, SDS-PAGE, and differential scanning calorimetry (DSC). Albumin was the major fraction (34.5%) extracted, followed by globulin (15.6%), glutelin (10.6%), and prolamine (4.6%). Protein isolate was mainly composed of albumin and globulin. These protein fractions and protein isolate showed an excellent balance of all essential amino acids, with a relatively high level of glutamic acid, arginine, leucine, and glycine, whereas cystine was lacking. All the estimated nutritional quality parameters based on amino acids composition showed that defatted wheat germ proteins had good nutritional quality. Nonreduced and reduced SDS-PAGE analyses showed that S-S bonds

were deficient in the structure of wheat germ proteins. The albumin fraction consisted of 19 major polypeptide bands with  $M_r$  14,000–84,000. The globulin fraction showed four distinct polypeptides or polypeptide group bands with  $M_r$  55,000, 37,000–43,000, 24,000, and 12,000–20,000, which may be the components of the 8S-type and 11S-like proteins. The prolamine fraction showed a predominant doublet-like band at  $M_r$  17,000–16,000, while the glutelin fraction showed five major polypeptide bands with  $M_r$  39,000, 20,000, 18,000, 17,000, and 14,000. Protein isolate and DWGF showed very similar SDS-PAGE patterns. Except for prolamine and glutelin fractions without detectable calorimetric response, the globulin fraction possessed the highest thermal stability ( $T_d = 83.80^\circ\text{C}$ ,  $\Delta H = 1.36 \text{ J/g}$ ), followed by protein isolate ( $T_d = 80.05^\circ\text{C}$ ,  $\Delta H = 0.76 \text{ J/g}$ ), while the albumin fraction was lowest ( $T_d = 69.72^\circ\text{C}$ ,  $\Delta H = 0.53 \text{ J/g}$ ). The findings on defatted wheat germ proteins are important for their potential application as functional food ingredients.

Wheat germ, one of the main by-products from the flour milling industry, is the most nutritious part of the wheat kernel (Haridas et al 1980). It is a rich source of vitamin E, B group vitamins, proteins, dietary fiber, and minerals (Amado and Arrigoni 1992). It also contains some relatively low-cost functional phytochemicals such as flavonoids, sterols, octacosanols, and glutathione (Sullivan and Howe 1937; Pietrzak and Collins 1996; Ai-Hooti et al 2002). Therefore, wheat germ has been considered a healthy food that can help people to prevent certain cancers and other health problems.

Raw wheat germ containing as much as 10% oil is mainly used in food, medical, and cosmetic industries as a source of oil (Kahlon 1989). The main by-product of the oil extraction process is a defatted wheat germ meal, which has a relatively high protein content ( $\approx 30\%$ ) and a high amount of essential amino acids (Ge et al 2001), making the defatted wheat germ meal one of the most attractive and promising sources of vegetable proteins.

Considering the current rapid growth in world population, there is bound to be an increase in demand for protein consumption. However, animal proteins are expensive and not available in many countries and regions. Therefore, in recent years, the search for new alternatives and cheap sources of good-quality proteins has become an important research trend.

Annually there is a high production of wheat germ in the world. However, human consumption of this precious wheat germ is limited; the majority is used for animal feeding purposes. For this reason, efforts are being made to devise efficient methods for the recovery of proteins from defatted wheat germ meal and to prepare acceptable products for human consumption.

Grewe and LeClerc (1943) reported the proportion of Osborne protein fractions from commercial wheat germ. Hettiarachchy et al (1996) and Ge et al (2000) have reported the functional and physicochemical properties of protein isolate prepared from defatted

wheat germ. Vani and Zayas (1995) reported the solubility and water retention of wheat germ protein flour. Denaturation of wheat germ proteins (5% NaCl soluble proteins) during drying was also reported by Lupano and Añón (1986). However, detailed and comprehensive information on fractionation, nutritional quality, and physicochemical properties of defatted wheat germ meal has not been reported.

The purpose of this work was to study classification of defatted wheat germ proteins by amino acid composition, predicted nutritional quality, molecular weight distribution of subunits, and thermal properties.

## MATERIALS AND METHODS

Raw wheat germ (RWG) was a gift from Huaian Xinfeng Flour Mill (Jiangsu, China). The electrophoretic chemicals were purchased from Sigma-Aldrich Chemical (St. Louis, MO). Molecular weight markers were purchased from Shanghai Institute of Biochemistry (Shanghai, China). All other chemicals used in the experiments were of analytical grade.

### Defatted Wheat Germ Flours (DWGF) Preparation

Raw wheat germ was selected and cleaned to remove contaminants. Wheat germ was defatted with *n*-hexane for 8 hr and air-dried at room temperature ( $\approx 20^\circ\text{C}$ ). The defatted wheat germ meal was milled using a laboratory-scale hammer mill and the resulting flour (DWGF) was sieved through a 60-mesh screen. The flour was kept in sealed glass jars at  $4^\circ\text{C}$  until used.

### Chemical Analysis

The proximate composition of RWG and DWGF were determined according to official methods (AOAC 1984). The moisture content was determined by drying in an oven at  $105^\circ\text{C}$  until a constant weight was obtained. Ash was determined by weighing the incinerated residue obtained at  $525^\circ\text{C}$  after 4 hr. Crude fat was extracted by Soxhlet system with petroleum ether. Finally, crude protein was determined by the micro-Kjeldahl method and a conversion factor of 5.45 was used to quantify the crude protein content (Tkachuk 1969). The carbohydrate content was estimated by subtracting the sum of percentage of moisture, crude fat, crude protein, and ash contents from 100%.

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**TABLE I**  
Proximate Chemical Composition of Raw Wheat Germ (RWG) and Defatted Wheat Germ Flour (DWGF) (g/100 g, wb)<sup>a</sup>

	Moisture	Ash	Fat	Protein (N × 5.45)	Carbohydrate (by difference)
RWG	12.72 ± 0.07a	4.34 ± 0.39a	10.03 ± 0.19a	27.62 ± 0.66b	45.29
DWGF	10.07 ± 0.08b	4.78 ± 0.36a	0.84 ± 0.11b	31.42 ± 1.03a	52.89

<sup>a</sup> Values are mean ± standard deviation of four determinations. Mean values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

**TABLE II**  
Distribution and Protein Content of Protein Fractions of Defatted Wheat Germ Meal<sup>a</sup>

	% of Total Protein <sup>b</sup>	Protein Content % <sup>c</sup> (db)
Albumin	34.5 ± 1.10a	33.4 ± 0.60c
Globulin	15.6 ± 0.53b	82.7 ± 1.51a
Prolamine	4.6 ± 0.20d	11.6 ± 0.85d
Glutelin	10.6 ± 0.20c	75.6 ± 1.22b
Insoluble residues <sup>d</sup>	34.7	–
Protein isolate	–	88.5 ± 1.14

<sup>a</sup> Values are mean ± standard deviation of three replicates. Means followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

<sup>b</sup> % of total protein = [total proteins (g) of each fraction extracted from 100 g of meal/total proteins (g) of 100 g of defatted wheat germ meal] × 100.

<sup>c</sup> Protein content % = g of proteins in 100 g of extracted solids.

<sup>d</sup> Insoluble residues = 100 – (albumins + globulins + prolamins + glutelins).

### Protein Fractionation

Protein fractionation was performed according to the classical method of Osborne (1924) with minor modifications. Suspensions of flour and water (1:10, w/v) were stirred for 2 hr at room temperature and centrifuged at 8,000 rpm for 20 min at 4°C. The supernatant was recovered and stored. The residues were reextracted one time with the same solvent, and recovered supernatants were combined and designated the albumin fraction, which was concentrated and freeze-dried. The pellet was resuspended in a solution of 50 mM Tris-HCl, pH 8.0, containing 0.5M NaCl and stirred as before; the pellet was reextracted again with the same solvent. The resulting supernatants were combined and dialyzed against deionized water for 72 hr, the dialyzates were freeze-dried, and the resulting protein powders were designated the globulin fraction. The pellet was resuspended with 70% (v/v) aqueous 2-propanol (2PrOH), extracted twice under stirring for 2 hr, and centrifuged at 8,000 rpm for 20 min at 4°C. The resulting supernatants were concentrated and freeze-dried and these products were designated the prolamine fraction. The pellet was resuspended in a solution of 0.05M NaOH after centrifugation. The supernatants were dialyzed against deionized water for 72 hr and the dialyzates were freeze-dried; the resulting protein powders were designated the glutelin fraction. A micro-Kjeldahl method was used to determine protein content in the protein fractions (N × 5.45).

### Protein Isolate Preparation

Defatted wheat germ protein isolate was prepared according to the procedure described by Ge et al (2000) with some modifications. DWGF was dispersed in 0.5M NaCl solution (1:8, w/v) and stirred for 30 min at ambient temperature. The suspension was adjusted to pH 9.5 with 1M NaOH. After stirring for 30 min, the suspension was centrifuged at 3,000 rpm for 20 min at ambient temperature. The supernatant was adjusted to pH 4.0 with 1.0M HCl to precipitate the proteins and centrifuged again at 3,000 rpm for 20 min at ambient temperature. The precipitates were washed three times with deionized water (pH 4.0), dispersed in a small amount of deionized water, and adjusted to pH 7.0 using 0.1M NaOH. The dispersed product was freeze-dried.

### Amino Acid Analysis

For the determination of the amino acids, samples of protein fractions and protein isolate (150 mg) were subjected to acid hydrolysis with 5 mL of 6M HCl under nitrogen atmosphere for 24 hr at

110°C. Each hydrolyzate was washed into a 50-mL volumetric flask and made up to the mark with distilled water. The amino acids were subjected to RP-HPLC analysis (Agilent 1100) after pre-column derivatization with *o*-phthalaldehyde (OPA) (Jarret et al 1986) or with 9-fluorenylmethyl chloroformate (FMOC) (Näsholm et al 1987).

Methionine and cysteine were determined separately by oxidation products according to the performic acid procedure of Moore (1963) before hydrolysis in 6M HCl. Tryptophan was determined after alkaline hydrolysis by isocratic ion-exchange chromatography with *o*-phthalaldehyde derivatization followed by fluorescence detection (Ravindran and Bryden 2005). Amino acid composition was reported as g of amino acid/100 g of protein.

### Parameters of Nutritional Quality

The nutritional parameters of wheat germ proteins and their fractions were calculated using their amino acid composition including 1) proportion of essential amino acids (E) to the total amino acids (T) of the protein; and 2) amino acid score (AAS) = (mg of amino acid /g of test protein/mg of amino acid/g of FAO/WHO/UNU standard pattern) × 100.

The FAO/WHO reference pattern of essential amino acid requirements (g/100 g of protein) (FAO/WHO 1973) was used as the standard. The AAS indicated the most limiting amino acid of the protein compared to a reference protein.

3) Predicted protein efficiency ratio (PER) values. The predicted PER values of wheat germ proteins and their fractions were estimated by three regression equations developed by Alsmeyer et al (1974):

$$\text{I. PER} = -0.684 + 0.456 (\text{Leu}) - 0.047 (\text{Pro})$$

$$\text{II. PER} = -0.468 + 0.454 (\text{Leu}) - 0.105 (\text{Tyr})$$

$$\text{III. PER} = -1.816 + 0.435 (\text{Met}) + 0.780 (\text{Leu}) + 0.211 (\text{His}) - 0.944 (\text{Tyr})$$

4) Predicted biological values (BV). The predicted BV values were calculated using the regression equation of Mørup and Olesen (1976).

$$\text{BV} = 10^{2.15} \times q_{\text{Lys}}^{0.41} \times q_{\text{Phe+Tyr}}^{0.60} \times q_{\text{Met+Cys}}^{0.77} \times q_{\text{Thr}}^{2.4} \times q_{\text{Trp}}^{0.21}$$

where  $q_i = a_i \text{ sample}/a_i \text{ reference}$  for  $a_i \text{ sample} \leq a_i \text{ reference}$  and  $q_i = a_i \text{ reference}/a_i \text{ sample}$  for  $a_i \text{ sample} \geq a_i \text{ reference}$ ;  $a_i$  represented mg of amino acid/g of total essential amino acids.

### SDS-PAGE

Nonreduced (without β-mercaptoethanol) and reduced (with β-mercaptoethanol) SDS-PAGE of the sample were conducted using the discontinuous system described by Laemmli (1970) with 4% stacking and 12% separating gel. The separating gel was run at a constant current of 20 mA for ≈3 hr. The gels were stained with Coomassie brilliant blue R-250. Subunit molecular weight was estimated by using a LMW calibration kit (Shanghai Institute of Biochemistry, China) consisting of phosphorylase ( $M_r$  97,400), bovine serum albumin ( $M_r$  66,200), rabbit actin ( $M_r$  43,000), bovine carbonic anhydrase ( $M_r$  31,000), trypsin inhibitor ( $M_r$  20,100), and hen egg white lysozyme ( $M_r$  14,400).

### Differential Scanning Calorimetry (DSC)

The thermal denaturation of the protein fractions was examined with a Perkin-Elmer Pyris-1 DSC. Lyophilized samples (1 mg each)

**TABLE III**  
**Comparative Amino Acid Profiles of Defatted Wheat Germ Flour (DWGF), Four Protein Fractions, and Protein Isolate (g/100 g of protein)**

	DWGF	Albumin	Globulin	Prolamine	Glutelin	Protein Isolate	FAO/WHO/UNU <sup>a</sup>	
							Child	Adult
Essential amino acids								
Isoleucine (Ile)	2.92	3.17	3.88	3.31	4.26	4.20	2.80	1.30
Leucine (Leu)	6.64	6.43	7.23	8.04	7.64	7.80	6.60	1.90
Lysine (Lys)	6.69	7.86	6.53	3.69	6.88	6.72	5.80	1.60
Methionine (Met)	1.64	2.10	1.43	1.42	2.07	2.11	2.50 <sup>b</sup>	1.70 <sup>b</sup>
Met + Cys	2.41	3.00	2.04	2.32	2.44	2.73		
Phenylalanine (Phe)	4.06	4.07	4.98	5.28	4.70	4.89	6.30 <sup>c</sup>	1.90 <sup>c</sup>
Phe + Tyr	6.45	6.77	8.02	7.91	8.41	8.13		
Threonine (Thr)	4.50	4.31	3.46	3.57	3.61	4.13	3.40	0.90
Valine (Val)	4.53	4.98	6.22	4.67	5.84	6.37	3.50	1.30
Histidine (His)	2.47	2.59	3.35	2.72	2.82	2.82	1.90	1.60
Tryptophan (Trp)	1.15	1.48	0.78	1.55	1.21	0.68	1.10	0.50
Nonessential amino acids								
Alanine (Ala)	7.89	4.45	6.58	2.86	3.68	9.81		
Arginine (Arg)	6.43	13.24	10.91	11.97	11.32	6.11		
Aspartic acid (Asp) <sup>d</sup>	9.43	11.08	7.90	4.68	6.96	9.34		
Cysteine (Cys) <sup>e</sup>	0.77	0.90	0.61	0.90	0.37	0.62		
Glutamic acid (Glu) <sup>f</sup>	15.19	15.28	17.29	23.40	18.96	15.08		
Glycine (Gly)	5.64	6.14	5.26	5.66	6.06	5.93		
Serine (Ser)	4.46	4.66	4.93	4.54	4.54	4.80		
Tyrosine (Tyr)	2.39	2.70	3.04	2.63	3.71	3.24		
Proline (Pro)	6.45	3.95	4.48	8.66	6.53	5.15		

<sup>a</sup> FAO/WHO/UNU energy and protein requirements (1985).

<sup>b</sup> Requirements for methionine + cysteine.

<sup>c</sup> Requirements for phenylalanine + tyrosine.

<sup>d</sup> Aspartic acid + asparagine.

<sup>e</sup> Cysteine + cystine.

<sup>f</sup> Glutamic acid + glutamine.

were directly weighed into the aluminum pans and 10  $\mu$ L of 0.01M pH 7.5 phosphate buffer was added. An empty pan was used as a reference. Scanning was done at 30–120°C at a heating rate of 10°C/min. Indium standards were used for temperature and energy calibrations. Thermal denaturation temperature ( $T_d$ ) and denaturation enthalpy ( $\Delta H$ ) were calculated from thermograms.

### Statistical Analysis

Data were analyzed by ANOVA using SAS statistical software package (v. 8.1, SAS Institute, Cary, NC). Least square differences (LSD) were used to determine significant difference at the 5% level. Each value was determined by at least three replicates. Results were given as mean  $\pm$  SD.

## RESULTS AND DISCUSSION

### Proximate Chemical Composition of RWG and DWGF

The moisture, ash, fat, protein, and carbohydrate contents of RWG and DWGF are shown in Table I. Defatting resulted in a significant ( $P < 0.05$ ) reduction of the fat of wheat germ (10.03 to 0.84%). Compared with RWG, DWGF contains a relatively high amount of protein (31.42%, wb). RWG and DWGF contain a large amount of carbohydrates (45.29 and 52.89%, respectively). Other authors found that the carbohydrates were mainly composed of sugars (sucrose and raffinose), fibers, pentosans, and starch (Dubois et al 1960; Amado and Arrigoni 1992).

### Fractionation of Defatted Wheat Germ Proteins

The distribution and protein content of protein fractions in DWGF are presented in Table II. The globulin fraction had the highest protein content followed by glutelin, albumin, and prolamine.

A large amount of carbohydrates and pigments were extracted into the albumin and prolamine fractions causing their low protein content. The water-soluble albumin was the predominant protein fraction, accounting for 34.5% of the total proteins, which was higher than that in wheat flour and rice (Padhye and Salunkhe

1979; Wrigley and Bietz 1988). The salt-soluble and alkali-soluble fractions were 15.6 and 10.6%, respectively. However, the alcohol-soluble fraction (prolamine) was only 4.6% lower than that in wheat flour (Wrigley and Bietz 1988). The other 34.7% of total protein were insoluble residues, which show that the sequential extraction with four solvents did not extract all of the protein in DWGF. The ratio of protein fractions of commercial wheat germ reported by Grewe and LeClerc (1943) was 30.2, 18.9, 14.0, 0.37, and 30.2, respectively, for albumin, globulin, prolamine, glutelin, and residue. When compared with the reported values, the relative proportion of glutelin was higher, while prolamine was lower. These differences may be due to wheat cultivars, extracting procedures, and meal preparation methods.

On the other hand, the protein isolate obtained indicated a recovery yield in the range of 24.0–37.0% with relatively high protein content (88.5%). Albumin and globulin, the predominant protein fractions in DWGF, would also be the main protein fractions of the protein isolate. Therefore, the preparation of protein isolate would be an effective method for recovering proteins from defatted wheat germ meal.

### Amino Acid Composition

DWGF, its four protein fractions, and protein isolate were analyzed for amino acid composition and the results are presented in Table III. In general, glutamic acid, arginine, leucine, and glycine were all abundant in wheat germ flour protein fractions. In addition, aspartic acid and lysine were high in DWGF, albumin, globulin, glutelin, and protein isolate. DWGF, its four protein fractions, and protein isolate had a well-balanced amino acid composition. Moreover, most of the essential amino acids in wheat germ protein fractions and protein isolates were at a higher level than in the reference pattern (FAO/WHO/UNU 1985). The most striking was the abundance of lysine, which is the first limiting amino acid in the grain. However, cysteine was lacking in wheat germ proteins. This fact indicates that S-S bonds would be absent in the structure of wheat germ proteins. The results agree with those previously reported by Tömösközi et al (1998).

**TABLE IV**  
**Distribution of Amino Acid Classified According to Similar Chemical Properties in Defatted Wheat Germ Flour (DWGF),**  
**Four Protein Fractions, and Protein Isolate (g/100 g of protein)**

Group	DWGF	Albumin	Globulin	Prolamine	Glutelin	Protein Isolate
Hydrophobic (nonpolar) <sup>a</sup>	40.93	36.78	40.84	41.44	41.99	46.93
Uncharged polar <sup>b</sup>	12.12	12.57	12.04	11.65	12.23	12.78
Basic <sup>c</sup>	15.59	23.69	20.79	18.39	21.02	15.65
Acidic <sup>d</sup>	24.62	26.36	25.19	28.08	25.92	24.41
Sulfur-containing <sup>e</sup>	2.41	3.00	2.04	2.31	2.44	2.73
Aromatic <sup>f</sup>	7.60	8.25	8.80	9.46	9.62	8.81

<sup>a</sup> Gly, Ala, Val, Leu, Pro, Met, Phe, Trp, and Ile.

<sup>b</sup> Ser, Thr, Cys, and Tyr.

<sup>c</sup> Lys, Arg, and His.

<sup>d</sup> Asp and Glu.

<sup>e</sup> Cys and Met.

<sup>f</sup> Phe, Tyr, and Trp.

**TABLE V**  
**Nutritional Evaluation of Defatted Wheat Germ Flour (DWGF), Four Protein Fractions, and Protein Isolate**

Parameters <sup>a</sup>	DWGF	Albumin	Globulin	Prolamine	Glutelin	Protein Isolate
E/T, %	40.50	40.84	41.99	37.95	42.62	43.67
Amino acid score	68.5	79.3	58.0	65.7	69.3	70.6
Limiting amino acids						
First	Met + Cys (68.5)	Ile (79.3)	Met + Cys (58.0)	Met + Cys (65.7)	Met + Cys (69.3)	Trp (70.6)
Second	Ile (73.0)	Met + Cys (85.2)	Trp (80.9)	Lys (67.8)	Thr (90.3)	Met + Cys (77.5)
Estimates of PER						
I	2.04	2.06	2.40	2.58	2.49	2.63
II	2.29	2.17	2.50	2.91	2.61	2.73
III	2.34	2.11	2.28	3.16	2.14	2.72
Estimates of BV	64.2	89.6	42.9	55.2	47.7	66.9

<sup>a</sup> E/T, proportion of essential amino acids (E) to total amino acids (T); PER, predicted protein efficiency ratio; BV, biological values.

The amino acid composition of wheat germ flour had been reported by several investigators (Pomeranz et al 1970; Amado and Arrigoni 1992; Garcia et al 1972; Miladi and Hegsted 1972). Compared with previous findings, DWGF had a relatively lower content of isoleucine, methionine, valine, and arginine, and a higher content of alanine and proline. These differences may be attributed to wheat cultivars and analytical techniques.

Protein isolate was prepared by alkaline water extraction and isoelectric precipitation from DWGF. Compared with DWGF, isoleucine, valine, tyrosine, methionine, alanine, phenylalanine, leucine, and histidine were extracted in great proportion, while a loss of the content of tryptophan, cystine, threonine, arginine, and proline was observed in protein isolate. The loss of tryptophan is probably due to mild acid treatment in the preparation process of protein isolate, whereas the lower contents of cystine, threonine, and arginine may have been caused by alkaline processing, which can cause destruction of cystine, threonine, arginine, serine, and lysine (Provansal et al 1975). Therefore, effectively controlling the pH value is very important during protein isolate preparation. The amino acid composition of protein isolate is generally in accordance with previous publications (Ge et al 2000), except for the higher contents of methionine, leucine, alanine, and aspartic acid, and the lower level of arginine, isoleucine, lysine, and glutamic acid in our isolate product.

Among the four protein fractions, globulin contained the lowest amount of tryptophan and sulfur-containing amino acids, whereas prolamine had the lowest amount of lysine. The albumin and glutelin fractions had better-balanced amino acid patterns when compared with the globulin and prolamine fractions. Therefore, albumin, the predominant fraction of DWGF, was the best for healthy food formulation.

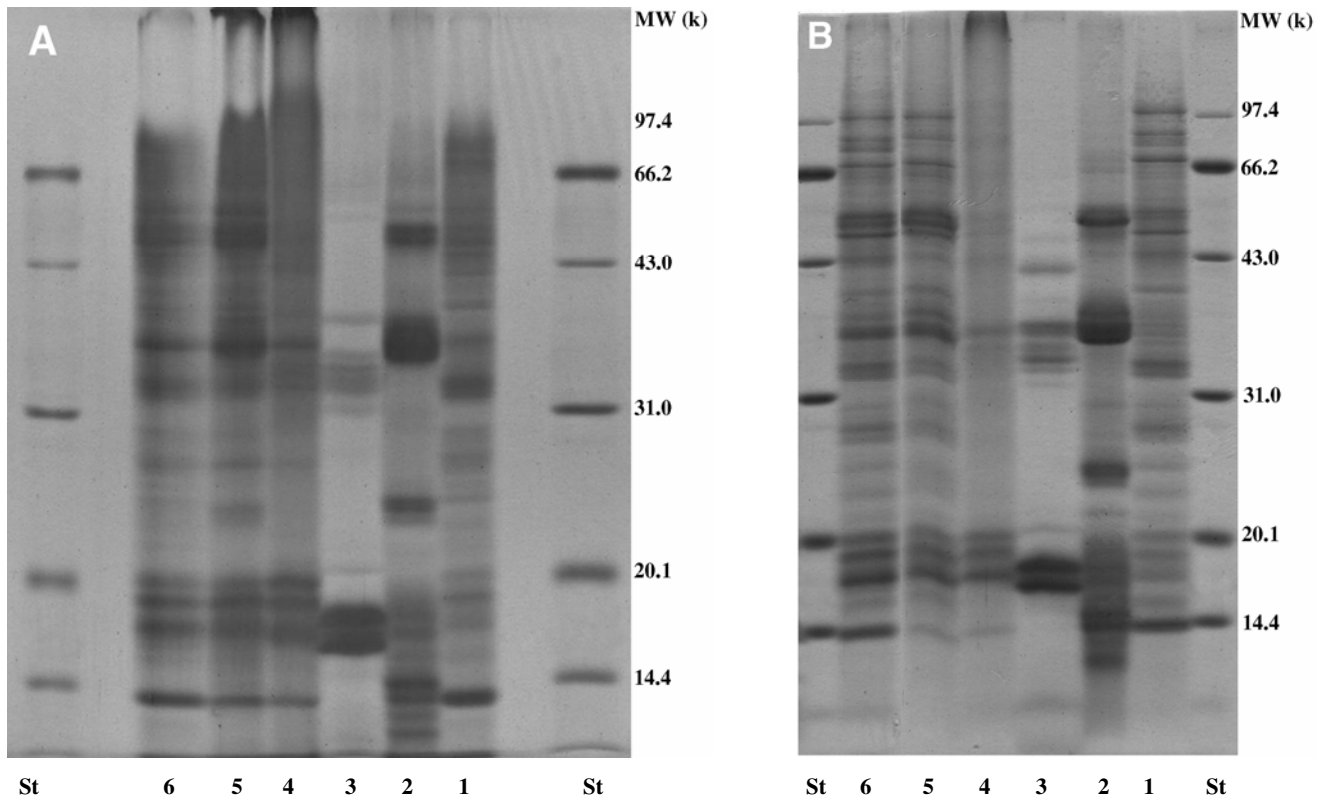
Classification of amino acids in different groups according to chemical properties are shown in Table IV. Among the four protein fractions and protein isolate, the albumin fraction contained the highest amount of sulfur-containing amino acids and basic amino acids, whereas hydrophobic and aromatic amino acids

content were the lowest, while the sulfur-containing amino acids in globulin fraction were the lowest. The prolamine fraction contained the highest amount of acidic amino acids, whereas the content of uncharged polar amino acids was the lowest. The glutelin fraction had the highest total aromatic amino acids. Compared with the four protein fractions, the protein isolate had the highest amounts of hydrophobic amino acids and uncharged polar amino acids, while the basic and acidic amino acids contents were the lowest.

#### **Estimated Nutritional Quality Based on Amino Acids Composition**

Protein is one of the essential nutrients in the human diet. Both the amount and quality of protein provided by a food are important. The protein quality, also known as the nutritional or nutritive value of a food, depends on its amino acid content and on the physiological utilization of specific amino acids after digestion, absorption, and minimal obligatory rates of oxidation. Because direct assessment of protein nutritional value in human subjects is impractical for regulatory purposes, methods based on in vitro (chemical) and animal bioassays for assessment of protein quality have been developed. Herein, a case is made for the use of amino acid data as a basis for estimation of nutritional quality of wheat germ proteins. The ratio of essential to total amino acids, amino acid score, limiting amino acids, PER, and BV of different protein fractions and protein isolate of DWGF are shown in Table V.

Four protein fractions and protein isolate had a higher ratio of essential to total amino acids than the pattern recommended by WHO (at least 36%). As would be expected, protein isolate with the ratio of 43.67% ranked the highest. Among the four protein fractions and protein isolate, the albumin fraction had the highest AAS values followed by protein isolate, glutelin, and prolamine, while the score of globulin fraction was the lowest. Sulfur-containing amino acids were the limiting amino acids of wheat germ proteins and protein fractions. In addition, isoleucine was the limiting amino acid in albumin and DWGF. Tryptophan and sulfur-containing amino acids were the first and second limiting



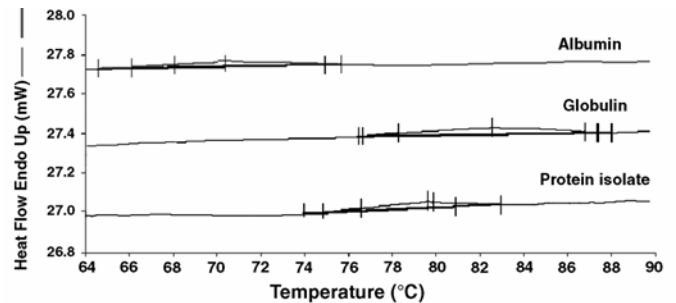
**Fig. 1.** SDS-PAGE of defatted wheat germ proteins and protein fractions. **A**, Nonreduced (without  $\beta$ -mercaptoethanol); **B**, reduced (with  $\beta$ -mercaptoethanol). Standard (St), albumin (1), globulin (2), prolamine (3), glutelin (4), protein isolate (5), defatted wheat germ flour (DWGF) (6).

amino acids of protein isolate, respectively. Predicted PER values of defatted wheat germ protein fractions and protein isolate all exceeded 2.00, which describes a protein of good to high quality (Friedman 1996). The prolamine fraction and protein isolate had the highest PER value, while that of albumin fraction was lowest. The PER values of the protein isolate and prolamine fraction were rather satisfactory compared with a standard casein PER of 2.5 (Friedman 1996). Predicted BV suggested that albumin had the highest value (89.6), followed by prolamine, glutelin, and globulin. Protein isolate and flour were intermediate. PER and BV values of rice (Padhye and Salunkhe 1979) and beach pea proteins (Chavan et al 2001) were calculated according to the same regression equations. The predicted PER values for rice and beach pea proteins were reported as  $-6.26$  to  $1.2$  and  $1.12$  to  $2.99$ , respectively, while their BV values were reported as  $3.1$  to  $74.5$  and  $26.77$  to  $52.21$ , respectively. Compared with earlier observations, defatted wheat germ proteins possess a high nutritional quality.

### SDS-PAGE

The nonreduced (without  $\beta$ -mercaptoethanol) and reduced (with  $\beta$ -mercaptoethanol) SDS-PAGE patterns for defatted wheat germ proteins and protein fractions are presented in Fig. 1. No distinct differences were found comparing nonreduced with reduced patterns, which showed that disulfide bonds were deficient in the structure of wheat germ proteins. The result is consistent with the previous amino acid analysis.

As observed in Fig. 1B, the protein subunits distribution profiles for the albumin fraction (Lane 1) were very complicated, 19 major polypeptides bands listed according to the molecular weight were  $M_r$  84,000, 78,000, 76,000, 71,000, 60,000, 57,000, 55,000, 51,000, 46,000, 35,000, 34,000, 28,000, 24,000, 22,000, 20,000, 18,000, 17,000, 15,000 and 14,000. Compared with the albumin fraction, the globulin fraction (Lane 2) possessed fewer polypeptide bands. There were four distinct polypeptide or polypeptide group bands at  $M_r$  55,000, 37,000–43,000, 24,000, and 12,000–20,000.



**Fig. 2.** Differential scanning calorimetry thermograms of albumin, globulin, and protein isolate from defatted wheat germ.

The globulins from wheat seed contained only two components with the sedimentation constants  $\alpha$  and  $\gamma$ . Furthermore, in wheat germ, the  $\gamma$  component exists as a pure state at  $M_r$  213,000 (Danielson 1956). Danielson (1949) identified globulins with sedimentation coefficients of 7-8S in barley, wheat, and oats. In addition, the 8S globulin of wheat is enriched in the embryo. The major 8S globulin component in wheat primarily consists of polypeptides of  $M_r$  50,000–55,000 and 40,000, and the subunits do not appear to be associated by disulfide bonds (Burgess and Shewry 1986). Therefore, the two polypeptides at  $M_r$  55,000 and 37,000–43,000 may be the components of 8S globulin of wheat germ. In addition, 11S-like globulins, called triticins, were present in the starchy endosperm of wheat (Singh et al 1988). Triticins consist of large ( $M_r \approx 40,000$ ) and small ( $M_r \approx 22,000$ – $23,000$ ) polypeptide chains. The subunit at  $M_r$  24,000 as well as polypeptide at  $M_r$  37,000–43,000 probably comprise 11S-like proteins. However, our observations of subunits of the wheat germ globulin fraction differ from the observations that two globulins purified from wheat seed comprised two subunits at  $M_r$  35,000 and 49,000 that were homologous to 11S-like globulins (Marccone and Yada 1995; Marccone et al 1998a).

Prolamine fraction (Lane 3) showed a predominant doublet-like band at  $M_r$  17,000–16,000 and some minor bands at  $M_r$  48,000, 39,000, 37,000, 35,000, 34,000, and 20,000. In addition to some HMW protein components that did not enter the gel, the glutelin fraction (Lane 4) showed five major polypeptide bands with estimated molecular weights at  $M_r$  39,000, 20,000, 18,000, 17,000, and 14,000.

In cereals, prolamine, and glutelin are the storage proteins, while albumin and globulin are generally considered to be metabolic or structural proteins (Wrigley and Bietz 1988). In wheat germ, albumin and globulin proteins are enzymes or enzyme inhibitors that have the function related to wheat germination. There are no less than 46 kinds of enzymes reported in wheat germ (Pomeranz 1988).

The SDS-PAGE pattern of protein isolate (Lane 5) was similar to that of DWGF (Lane 6). The results demonstrate that most protein fractions in wheat germ are extractable by alkaline extraction followed by acidic precipitation. The molecular weight distribution of protein isolate generally corresponds to earlier reported data (Ge et al 2000).

### Differential Scanning Calorimetry (DSC)

DSC is a rapid, easy, and capable technique for supplying both thermodynamic (heat capacity, enthalpy, and entropy) and kinetic data (reaction rate and activation energy) on protein denaturation, and has been used extensively in various food systems (Myers 1990). The DSC thermograms of albumin, globulin, and protein isolate are presented in Fig. 2. The albumin fraction showed an endothermic peak at  $69.72 \pm 0.17^\circ\text{C}$  with the enthalpy of  $0.53 \pm 0.03 \text{ J/g}$ . The globulin fraction possessed significantly ( $P < 0.05$ ) higher denaturation temperature ( $T_d = 83.80 \pm 0.22^\circ\text{C}$ ) and transition enthalpy ( $\Delta H = 1.36 \pm 0.09 \text{ J/g}$ ) than the albumin fraction. Our observations about wheat germ albumin and globulin fractions disagree with earlier reports (León et al 2003) that wheat albumin and globulin gave endothermic peaks at  $50\text{--}55^\circ\text{C}$  with  $\Delta H$  of  $1\text{--}2 \text{ J/g}$ , which might be due to different extraction procedures and cultivars. However, the denaturation temperature of the wheat germ globulin fraction was generally consistent with previous findings ( $84\text{--}96$ ,  $77$ , and  $88^\circ\text{C}$ ) for wheat globulin (Harwalkar and Ma 1987; Marcone et al 1998b). In addition, Lupano and Añón (1986) studied the denaturation of wheat germ proteins extractable in 5% NaCl during drying using DSC. Their results showed one endothermic peak at  $\approx 83^\circ\text{C}$  with enthalpy of  $1.43 \text{ J/g}$  (dry matter). Our results for the globulin fraction are similar to reported values. Compared with the other cereal proteins, the albumin of wheat germ was more heat-sensitive than rice ( $73.3^\circ\text{C}$ ) and oat ( $87^\circ\text{C}$ ) albumins, whereas the enthalpy of wheat germ globulin was lower than those ( $3.14 \text{ J/g}$  and  $5.39 \text{ cal/g}$ ) of rice and oat globulins (Ma and Harwalkar 1984; Ju et al 2001). Compared with leguminous proteins, wheat germ globulin had a considerably lower  $\Delta H$  value, which may be due to the formation of leguminous proteins with a compact and ordered conformation (Casey 1999). The prolamine and glutelin fractions did not show any detectable thermal response.

It is rather difficult to obtain endothermic peaks with the major wheat proteins, gliadins and glutenins, which in some cases show little or no calorimetric response (Ma 1990). Four endothermic peaks were observed when wheat gluten was heated from  $30$  to  $115^\circ\text{C}$  in a differential scanning calorimeter (Eliasson and Hegg 1980). The peaks at  $64.6$  and  $111.8^\circ\text{C}$  were assigned to thermal transitions in starch, whereas the peaks at  $88.4$  and  $101.4^\circ\text{C}$  were attributed to gluten protein transitions. However, the apparent enthalpies of the protein transitions were very small. Arntfield and Murray (1981) studied the heat denaturation of several plant proteins using DSC; only wheat gluten did not show a characteristic thermogram. They suggested that the thermal denaturation of wheat gluten involved both endothermic and exothermic events that cancelled each other. However, Hosney et al (1986) proved that wheat gluten has essentially no long-range order, which can

explicitly explain the absence of a denaturation peak in the DSC thermogram. Protein isolate from defatted wheat germ showed a significantly ( $P < 0.05$ ) higher denaturation temperature ( $80.05 \pm 0.34^\circ\text{C}$ ) than the albumin fraction but was not significantly ( $P < 0.05$ ) lower than the globulin fraction. Furthermore, protein isolate showed a  $\Delta H$  value ( $0.76 \pm 0.03 \text{ J/g}$ ) between those of albumin and globulin fractions, which can be interpreted as the protein isolate being mainly composed of albumin and globulin fractions (Hettiarachchy et al 1996). In addition, the degree of protein denaturation increased in either the extremely acid or alkaline region during preparation which consequently decreased the  $\Delta H$  value (Arntfield and Murray 1990).

### CONCLUSIONS

Defatted wheat germ contains a relatively high amount of protein (34.9%, db) and has a well-balanced amino acid profile, which can make DWGF a good vegetable protein supplement for cereal-based diets. Furthermore,  $\approx 40\%$  of proteins can be effectively extracted as protein isolate, which mainly comprised albumin and globulin. Defatted wheat germ protein fractions and protein isolate show an excellent balance of all essential amino acids and have a good nutritional quality. Disulfide bonds were deficient in the structure of wheat germ proteins. The albumin fraction exhibited 19 major polypeptide bands in the SDS-PAGE pattern, and the globulin fraction showed four distinct polypeptides or polypeptide group bands at  $M_r$  55,000, 37,000–43,000, 24,000, and 12,000–20,000, which may be the components of the 8S-type and 11S-like proteins. Prolamine fraction showed a predominant doublet-like band at  $M_r$  17,000–16,000, while the glutelin fraction showed five major polypeptides bands with  $M_r$  39,000, 20,000, 18,000, 17,000, and 14,000. Protein isolate and DWGF showed very similar SDS-PAGE patterns. Except for prolamine and glutelin fractions without detectable calorimetric response, the globulin fraction possessed the highest thermal stability, while the albumin fraction was lowest. These results will be useful for wheat germ protein extraction, drying, storage, and processing.

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