

## Preparation and Functional Properties of a Protein Isolate from Defatted Wheat Germ

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### ABSTRACT

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A protein isolate (90% protein) was prepared from defatted wheat germ by alkaline extraction at pH 9.5 and isoelectric precipitation at pH 4.0. Nitrogen solubility of wheat germ protein isolate (WGPI) was 72% at pH 6.0. WGPI compared well with bovine serum albumin (BSA) in its emulsifying properties. Foaming capacity of WGPI was similar to that of egg white standard while the foam stability was significantly lower ( $P <$

0.05). Surface hydrophobicity of WGPI as measured by a fluorescence method followed the same trend as that of emulsifying properties (WGPI  $<$  BSA). Electrophoresis of WGPI revealed a wide band at  $\approx 55$  kDa range and another one at 35 kDa. Several polypeptides were observed at  $>21$  kDa MW. Wheat germ offers a potential source of functional protein isolate for possible food applications.

Wheat germ is a by-product of wheat milling industry that has the potential as a food ingredient. Wheat germ is reported to be an excellent source of vitamins, minerals, dietary fiber, and proteins (Garcia et al 1972, Morrison et al 1982, Jensen and Martens 1983). Defatted wheat germ contains  $\approx 30\%$  protein, most of which is albumins and globulins (Pomeranz et al 1970). Wheat germ has been shown to be useful as an ingredient in several food products including bread (Vitti et al 1979, Zaitsev and Khomets 1983, Gudunova et al 1986), biscuits (Markianova et al 1984), muffins (Turnbough and Baldwin 1986), and frankfurters (Gnanasambandam and Zayas 1992, 1994). Vani and Zayas (1995) reported the solubility and water retention of wheat germ protein flour. However, studies have not been reported on wheat germ protein isolate for food use. The objective of this study was to investigate protein isolate produced from defatted wheat germ, and study its functional properties.

### MATERIALS AND METHODS

Defatted wheat germ (VIOBIN wheat germ no. 1) was obtained from Viobin Corporation, Monticello, IL. Other chemicals used in the study were of reagent grade.

#### Preparation of Protein Isolate

Protein isolate from defatted wheat germ was prepared by alkaline extraction and subsequent isoelectric precipitation (Fig. 1). Defatted wheat germ was dispersed in 5 volumes of 1.0M NaCl solution, stirred for 15 min at ambient temperature followed by adjusting to pH 9.5 using 1.0M NaOH, and stirring for 30 min. The pH was maintained at pH 9.5 throughout the extraction period. After 30 min of extraction, the suspension was centrifuged at  $15,000 \times g$  for 20 min at ambient temperature. The supernatant was filtered through glass wool to remove insoluble material, adjusted to pH 4.0 with 1.0N HCl to precipitate the proteins and centrifuged again at  $15,000 \times g$  for 20 min at ambient temperature. The precipitate was dispersed in 50% ethyl alcohol (1:5) stirred for 30 min, and centrifuged at  $15,000 \times g$  for 20 min at ambient temperature. The precipitate was washed several times with distilled deionized water (pH 4.0) and dispersed in a small amount

of distilled deionized water, and adjusted to pH 7.0. The dispersed product denoted as wheat germ protein isolate (WGPI) was freeze-dried and stored at 5°C until further analysis.

#### Nitrogen Solubility

Nitrogen solubility of WGPI was determined by a modified method of Betschart (1974). Protein solutions (1%, on protein basis) in distilled deionized (dd) water were made by weighing equivalent amounts of WGPI directly into 50-ml centrifuge tubes. Two tubes per replicate were prepared and adjusted to six different levels (pH 2.00, 4.00, 6.00, 8.00, 10.00, and 12.00) using either 1.0, 0.1, or 0.01N NaOH or HCl. The suspensions were shaken (Lab-Line Environ-Shaker, Lab-Line Instrument, Inc., Melrose Park, IL) at 250 rpm for 30 min and centrifuged at  $5,000 \times g$  for 15 min at ambient temperature. The supernatants were filtered (Whatman no. 4) and analyzed for nitrogen (AOAC 1984), and percent nitrogen solubility was calculated.

#### Emulsifying Properties

Emulsifying properties (emulsifying capacity and emulsion stability) were determined by a modified method of Yasumatsu et al (1972). Protein solutions (25 ml, 1% based on protein content) in dd water of WGPI and BSA were adjusted to pH 7.0 and mixed with 25 ml of soybean oil (100% soy oil, Associated Wholesale Grocers, Kansas City, KS). The mixture was sonicated for 30 sec at setting 3 of Branson Sonifier 450 (Branson Ultrasonics, Danbury, CT). The emulsion formed was centrifuged at  $2,500 \times g$  for 30 min (CRU-5000 Centrifuge, International Equipment, Needham Hts, MA). Emulsifying capacity was calculated by measuring the height of the layer separated in the centrifuge tube, expressed as percent of total height of the liquid. To measure emulsion stability, the emulsions prepared were taken into test tubes in duplicate and held at 70°C in a water bath for 45 min. Emulsion separation was calculated as described for emulsifying capacity and expressed as emulsion stability.

#### Foaming Properties

Foaming properties (foaming capacity and foam stability) of WGPI were determined by an air-purge method as described by Kato et al (1983). Egg white (80% protein, National Egg Products, Social Circle, GA) was used as a standard. Aqueous protein solutions (2 ml, 5%) of both standard and WGPI were prepared and were adjusted to pH 7.0. The solutions were placed into 15-ml glass test tubes (9 in.  $\times$  1.5 in.), and air was introduced at 1 psi from an air compressor for 15 sec. The height of foam formed was measured at 0, 2, 5, 10, and 20 min. The height in millimeters of

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foam formed at 0 time was expressed as foaming capacity, and foam stability was calculated from the equation:

$$\text{Foam stability} = V_0 \times t/V$$

where  $V$  is the change in the volume of foam occurring during the time interval  $t$ , and  $V_0$  is the volume of foam at 0 time.

### Hydrophobicity Determination

Surface hydrophobicity of WGPI was determined by a hydrophobic fluorescence probe, 1-anilino-8-naphthalene sulfonate (ANS) method (Hayakawa and Nakai 1985). Protein solutions ranging in concentrations from 0.0015 to 0.015% were prepared by serially diluting from a 0.015% protein solution in 0.01M phosphate buffer (pH 7.0). ANS (10  $\mu$ l) in 0.01M phosphate buffer were added to 2 ml of protein solution. Fluorescence intensity of ANS-protein conjugates were measured with a spectrofluorometer (SF23/B, Kontron) at excitation and emission wavelengths of 390 nm and 470 nm, respectively. Linear regression of fluorescence intensity on y-axis vs. protein concentration on x-axis was calculated and the  $x$  coefficient value of the regression equation was used as an index of protein hydrophobicity. Bovine albumin (BSA) (96–99% albumins, Sigma Chemical Co., St. Louis, MO) was used as a standard protein sample to compare with WGPI.

### Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the samples was performed according to Laemmli (1970). A vertical slab gel electrophoresis unit (Protean, Bio-Rad Laboratories, Richmond, CA) and power supply (3000 Xi, Bio-Rad) were used. The stacking and separating gels were 8 and 12% polyacrylamide respectively.

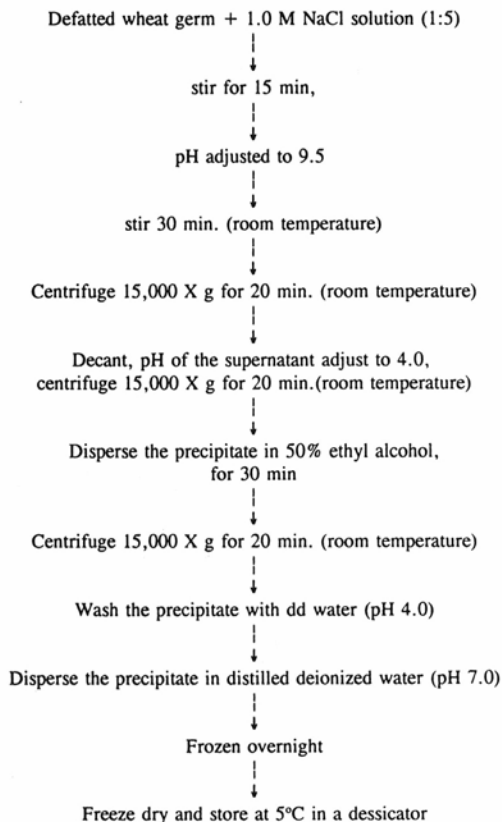


Fig. 1. Flow diagram of preparation of protein isolate.

### Statistical Analyses

Four replicates of the extractions were performed. A completely randomized design was used. Least square means procedures were used to separate means, and differences reported are significant ( $P < 0.05$ ) (SAS 1988).

## RESULTS AND DISCUSSION

### Protein Isolate

Alkaline extraction procedure resulted in a sample with a mean protein content of 90.2% ( $N \times 5.8$ ). Yield (on protein basis) of protein isolates were in the 18–28% range. A pH of 9.5 was used to solubilize the proteins, and proteins were precipitated at pH 4.0, where the proteins of wheat germ were the least soluble (apparent pI). Addition of 1M NaCl improved the protein extractability, and as a result, an increase in protein content of the sample from 82 to  $\approx 90.0\%$  was observed. Proteins of wheat germ are free from gluten proteins, the major component of wheat endosperm proteins (Pomeranz 1970). Protein quality of wheat gluten in terms of amino acid composition is lower than that of wheat germ. Glutamic acid and proline are two major amino acids of gluten proteins comprising  $\approx 45\%$  of total amino acids. Studies reported that protein quality of wheat germ in terms of amino acid composition is superior to that of wheat gluten (Shurpalekar and Rao 1977). Most of the proteins in wheat germ are albumins and globulins and have relatively higher amounts of essential amino acids, including lysine, methionine, and threonine (Pomeranz 1970). Protein extractability generally increases with increase in pH. At pH  $> 10.0$ , nonprotein components that interfere with protein extraction are dissolved, resulting in improved protein recovery at isoelectric precipitation (Chen and Houston 1970). However, a higher extraction pH may denature the proteins and form lysino-alanine complexes, resulting in a decreased nutritional value, and possibly form toxic compounds (DeGroot and Slump 1969). Apart from carotenes, wheat germ also reportedly contains pigments including xanthophylls and glycoflavones (King 1962). In the present study, dispersion of the protein precipitate in 50%

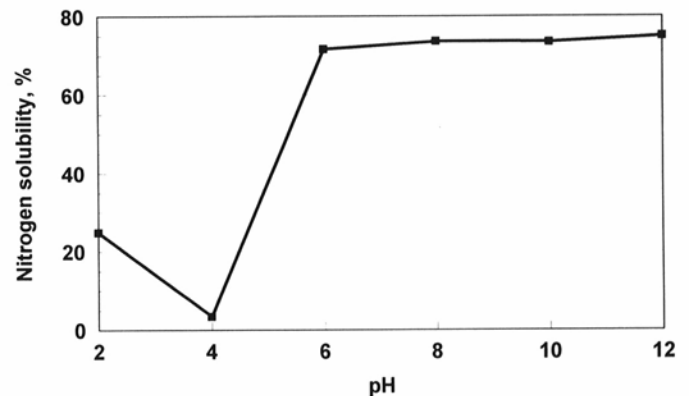


Fig. 2. Nitrogen solubility of wheat germ protein isolate.

TABLE I  
Emulsifying Properties of Wheat Germ Protein Isolates (WGPI)  
as Compared to Bovine Serum Albumin (BSA)<sup>a</sup>

Sample	No. of Observations	Emulsifying Capacity, %	Emulsion Stability, %
WGPI	12	61.9a	61.2
BSA	12	67.4b	66.4
LSD <sup>b</sup>		5.5	8.6

<sup>a</sup> Mean values in the same column with different letters are significantly different ( $P < 0.05$ ).

<sup>b</sup> Least significant difference.

ethyl alcohol improved the protein content and resulted in a lighter tan color of the sample, probably due to removal of alcohol-soluble pigments in the samples.

### Nitrogen Solubility

Nitrogen solubility of WGPI is presented in Figure 2. WGPI showed the lowest nitrogen solubility at pH 4.0 and highest at pH 6.0. The solubility did not increase beyond pH 6.0. Nitrogen solubility is the most important functional property that influences other properties such as emulsification, gelation, and foaming, and, thus, determines the behavior of the proteins in food products. Proteins with a higher solubility generally have a better emulsifying properties. Denaturation of proteins that occurs during processing and storage is the major extrinsic factor that affects the solubility and, thereby, the functional properties of proteins. Preparation of WGPI resulted in a higher nitrogen solubility, perhaps due to minimal denaturation during extraction process. A higher nitrogen solubility of WGPI (>70%) at pH 6.0, would make it a suitable candidate for food applications.

### Emulsifying Properties

WGPI showed an emulsifying capacity (EC) of 62% as compared to 67% for BSA standard (Table I). A similar trend was observed in emulsion stability of the samples. An EC higher than that of BSA can be explained by its homologous nature (96–99% albumins), a higher solubility, and a higher emulsifying capacity. Factors that affect emulsifying properties are adsorption kinetics, interfacial load, decrease of interfacial tension, rheology of the interfacial film, and surface hydrophobicity of the interfacial film (Das and Kinsella 1990). Unfolding of proteins at oil and water interfaces plays a significant role in the formation and stability of emulsion. WGPI had an EC and ES of 61.8 and 61.2%, respectively, as compared to BSA, which had an EC and ES of 67.4 and 66.4%, respectively. No significant reduction in emulsifying capacity was observed upon heat treatment for 30 min at 45°C. The EC of WGPI was ≈90% of that of BSA, thus, WGPI may be a useful functional protein additive in emulsion-based food products.

### Foaming Properties

Foaming properties of WGPI and egg white standard are pre-

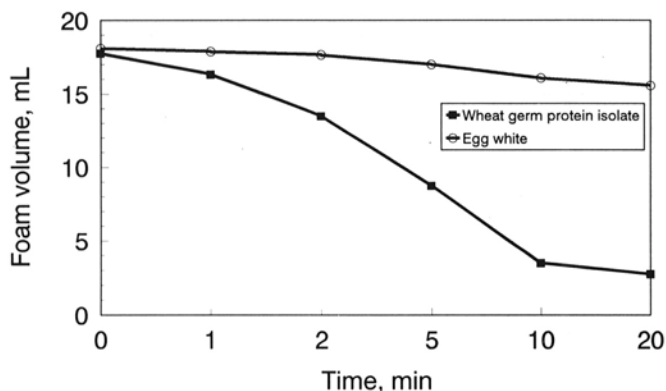


Fig. 3. Foaming properties of wheat germ protein isolate as compared to egg white.

TABLE II  
Hydrophobicity of Wheat Germ Protein Isolate (WGPI) as Compared to Bovine Serum Albumin (BSA)<sup>a</sup>

Sample	No. of Observations	X Coefficients	Standard Error of Coefficient
BSA	16	7980.0a	206.2
WGPI	16	1014.7b	96.8

<sup>a</sup> Mean values in the same column with different letters are significantly different ( $P < 0.05$ ).

sented in Figure 3. The foaming capacity of WGPI was similar to that of egg white standard (17.8 and 18.1 ml, respectively). However, after 20-min standing time, the stabilities of foams from WGPI were significantly lower than that of the standard, as indicated by a foam volume of 2.8 ml for WGPI and 15.6 ml for egg white standard. Protein solubility is the important criterion for foam formation and the stability of foams formed depends on the physical properties of the membrane film, including mechanical strength, viscoelasticity, diffusivity of component molecules in the film, and restorative properties. Townsend and Nakai (1983) reported that hydrophobicity, viscosity, and dispersibility are important factors that determine foaming properties of proteins. Solubility and molecular flexibility of proteins important for foam formation do not always guarantee a stable foam, unless such proteins are also capable of intermolecular interactions resulting in desired cohesiveness for the foam formation (Kinsella et al 1985). The ability of WGPI to form foams can be attributed to a highly soluble nature. Lack of sufficient foam stability of WGPI could be due to several factors, including inability to form a continuous intermolecular polymer and lack of sufficient viscosity. Viscosity of the foam also depends on presence of soluble compounds in the sample, such as sucrose, that would increase the viscosity. Damodaran (1994) observed that the ability of protein to reduce the surface tension upon adsorption is critical for foamability. The ability of WGPI to form stable emulsions denotes a capacity to effectively reduce the interfacial tension and favorable orientation at the oil and water interface. Hence, inability of WGPI to form a relatively stable foam, as compared to egg white, might be due to lack of sufficient intermolecular (protein-protein) interaction, and thus a reduced cohesion. However, this problem can possibly be overcome by modifying the WGPI by physical, chemical, or enzymatic methods to promote intermolecular interactions and impart sufficient viscosity to form stable foams.

### Hydrophobicity

Determination of hydrophobicity of protein samples is an important criterion to evaluate the functional property of foods. Hence, hydrophobicity of WGPI was determined using a fluores-

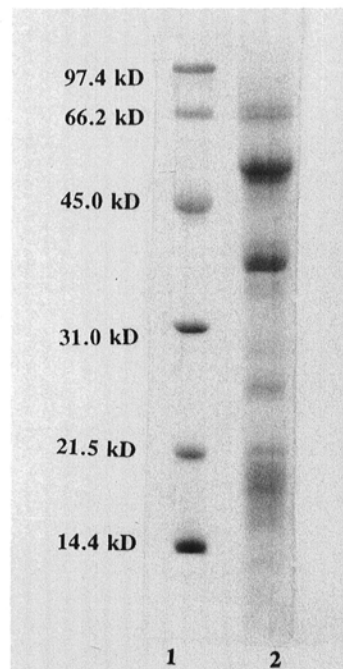


Fig. 4. Electrophoretic pattern of wheat germ protein isolate.

cence-probe method. Hydrophobicity of WGPI as compared to BSA is presented in Table II. Values for hydrophobicity of BSA in the present study were significantly higher ( $P < 0.05$ ) than those of WGPI. Studies strongly indicate a relationship between hydrophobicity and other functional properties of proteins (Nakai et al 1980, Townsend and Nakai 1983, Voutsinas et al 1983, Hayakawa and Nakai 1985, Aluko and Yada 1993). Surface hydrophobicity is also strongly correlated with emulsifying activity (Kato and Nakai 1980, Nakai 1983). Results from the present study indicate a similar relationship, because samples with higher hydrophobicity had higher emulsifying properties: BSA > WGPI. Higher emulsifying properties of BSA in this study might be due to higher hydrophobicity.

### Electrophoresis

Electrophoresis (SDS-PAGE) of WGPI was performed to obtain information on the molecular weight and distribution pattern of the protein components (Fig. 4). A wide band was observed at  $\approx 55$  kDa and another at 35 kDa. Several polypeptides were noticed at  $< 21$  kDa. Gluten is composed mainly of two groups of proteins: gliadins (a prolamin) and glutenin (a glutelin). Prolamins are soluble in 70% alcohol, while glutenins are soluble in dilute acids or bases. Glutenin proteins vary from 100 kDa to several million (Hoseney 1986). Absence of bands higher than 66 kDa in WGPI samples suggest that the wide band in the sample may be a globulin or albumin. Further, solubility of  $> 70\%$  in aqueous system at pH 6.0 suggest that WGPI samples prepared in the present study might be almost free of gluten.

### CONCLUSIONS

Preparation of functional and nutritional protein ingredients from unconventional sources has become important due to increasing cost of production of animal proteins. Defatted wheat germ presents an additional source of protein isolates for food use. Protein isolates prepared from defatted wheat germ had a nitrogen solubility of  $> 70\%$  at pH 6.0, and compared well with BSA in emulsifying properties. Foaming capacity of WGPI was comparable to that of egg white. However, foams made of WGPI were not as stable as those from egg white. A high nitrogen solubility, favorable emulsifying properties, foaming capacity, and hydrophobicity, make WGPI a useful ingredient for several food products, including processed meats, cereal and baked foods, and beverages. WGPI also might be useful in food formulations for persons allergic to gluten. Preparation of WGPI is another potential avenue for utilization of wheat germ, a by-product of wheat milling industry.

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