# Some Nutritional and Functional Properties of Defatted Wheat Germ Protein

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Defatted wheat germ protein (DWGP) was isolated by alkaline extraction at pH 9.5 and subsequent isoelectric precipitation at pH 4.0, and its nutritional and functional properties were studied. The results showed that the amino acid content of defatted wheat germ was as high as 26.793 g/100 g, and the contents of eight essential amino acids were all relatively high. The isoelectric point of DWGP was 4.0. When pH >6.0, the DWGP had high solubility with a nitrogen solubility index of 70%. The emulsifying activity and emulsifying stability of DWGP were similar to those of bovine serum albumin and a little higher than those of casein. DWGP had good foaming capacity, but its foaming stability (FS) was not very good. However, the FS of DWGP can be improved through physical, chemical, or enzymatic methods. Moreover, DWGP had excellent water retention (WR); especially at pH 8.0 and a temperature of 70 °C, the WR of DWGP was the highest at 229.4%. DWGP offers is a potential source of functional protein isolate for possible food applications.

**Keywords:** *Functional properties; defatted wheat germ; protein*

## INTRDUCTION

Wheat germ, being a byproduct of the flour milling industry, is reported to be one of the potentially most excellent sources of much-needed vitamins, minerals, dietary fiber, calories, proteins, and some functional microcompositions at a relatively low cost. In view of its high nutritive value and palatability, wheat germ is praised as "the natural nutrient treasure-house and life source of mankind". However, the presence of wheat germ adversely affects the keeping quality as well as the reprocessing quality of the flour. In recent years, with the development of food industry technology and the improvement of people's living standards, most commercial milling of wheat into flour aims at the maximum extraction of the endosperm with the minimum possible contamination by germ. As a result, there is a rich annual deposit of wheat germ in the world. However, the transformation and application of wheat germ begins very late, and most of it is generally used in animal feed formulations. Hitherto, the overall level of synthetic utilization of wheat germ has been still at the initial stage, not having made the jump to a new level, and the precious wheat germ source has not been amply, rationally, and efficiently utilized (Shurpalekar and Rao, 1977; Ge et al., 1999).

After extration of the wheat germ oil for further processing, defatted wheat germ is a kind of highly nutritive protein material, which contains >30% protein. Wheat germ protein has been classified with superiorly effective animal protein, and it is rich in 17 amino acids, especially the essential amino acids lysine, methionine, and threonine, in which many of the cereal grains are deficient. Therefore, wheat germ is potentially a nutritious food supplement; particularly, defatted wheat germ is a kind of natural high-grade protein and amino acid fortification substance (Ge et al., 1999).

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A new supercritical carbon dioxide (SC-$CO_2$) method has been used for extracting the wheat germ oil with good effect to human health (Liu et al., 1992). Use of wheat germ protein in bakery products, especially in breads (Gudunova et al., 1986; Girard, 1990; Cakmakli et al., 1995), cookies (Bajaj et al., 1991), biscuits (Markianova et al., 1984), and muffins (Turnbough and Baldwin, 1986), and in comminuted meat products (Gnanasambandam and Zayas, 1992, 1994) as an extender has been reported. There have been some studies to report on wheat germ protein for its individual functionality (Amado and Arrigoni, 1992; Vani and Zayas, 1995). However, very little work has been done to evaluate the properties of defatted wheat germ protein (DWGP), especially DWGP obtained by SC-$CO_2$.

The purpose of this study was to isolate the DWGP from the remainder after the extraction of the wheat germ oil rich in natural vitamin E by SC-$CO_2$. A further objective was to study the functional properties of DWGP, such as the nitrogen solubility index (NSI), emulsifying activity (EA), and water retention (WR). Data on the functional behavior of DWGP would be useful for predicting food applications, design of machines, and development of operations for its industrial processing.

## MATERIALS AND METHODS

**Raw Materials and Treatments.** Wheat germ of *Triticum aestivum* was purchased from a flour mill and then was cleaned and selected; enzymes were killed. After wheat germ oil was extracted by SC-$CO_2$, defatted wheat germ, the remainder in the extractor, was obtained.

Six standard protein markers obtained from Sigma Chemical Co. were used as references, Their molecular masses were 97.4, 66.2, 43.0, 31.0, 20.1, and 14.4 kDa, respectively.

**Preparation of DWGP.** Protein isolate from defatted wheat germ was prepared by alkaline extraction and subsequent isoelectric precipitation. Defatted wheat germ was dispersed in 5 volumes of 1.0 M NaCl solution and stirred for 15 min at ambient temperature; this was followed by adjust-

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ment of the pH to 9.5 using 1.0 M 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 15000*g* for 20 min at ambient temperature. The supernatant was filtered through glass wool to remove insoluble material, adjusted to pH 4.0 with 1.0 N HCl to precipitate the proteins, and centrifuged again at 15000*g* for 20 min at ambient temperature. The precipitate was dispersed in 50% ethyl alcohol (1:5), stirred for 30 min, and centrifuged at 15000*g* for 20 min at ambient temperature. The precipitates were washed several times with distilled deionized water (pH 4.0), dispersed in a small amount of distilled deionized water, and adjusted to pH 7.0. The dispersed product denoted DWGP was freeze-dried and stored at 5 °C until further analysis.

**DWGP Masses and Amino Acid Determination.** Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) of the sample was performed on a PROTEAN II apparatus produced by Bio-Rad Laboratories, Richmond,CA, and staining was done with improved silver staining (Ng and Bushuk, 1987). The stacking and separating gels were 8 and 12% polyacrylamide, respectively.

Electrode buffer (1000 mL): Tris, 30.3 g; Gly, 144.2 g; SDS, 10 g; pH 8.3.

Sample buffer (10 mL): SDS, 500 mg; $\beta$-mercaptoethanol, 1 mL; glycerine, 3 mL; bromophenol blue, 4 mg; 1 mol/L Tris-HCl, 2 mL; pH 6.8.

Molecular weights of subunits were calculated according to the relative mobility ($m_R$) of protein bands from standards and sample in the same gel.

$$m_R = \text{distance of sample/distance of stainer}$$

An amino acid autoanalytic apparatus was used to determine the content of DWGP amino acids according to the method of Yu et al. (1994).

**Tests of DWGP Functionality.** *Nitrogen Solubility.* Nitrogen solubility at various pH values was determined for DWGP. Protein solutions (1%, on protein basis) in distilled deionized water were made by weighing equivalent amounts of DWGP directly into 50 mL centrifuge tubes. Two tubes per replicate were prepared and adjusted to six different levels (pH 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0) using either 1.0, 0.1, or 0.01 N NaOH or HCl. The suspensions were shaken at 250 rpm for 30 min and centrifuged at 5000*g* for 15 min at ambient temperature. The supernatants were filtered and analyzed for nitrogen (AOAC, 1984), and percent nitrogen solubility was calculated.

*Emulsifying Properties.* Emulsifying properties [emulsifying activity (EA) and emulsifying stability (ES)] were determined by using a modified method of Lin and Zayas (1987). Protein solutions (25 mL, 1% based on protein content) in distilled deionized water of DWGP and bovine serum albumin (BSA) were adjusted to pH 7.0 and mixed with 25 mL of soybean oil. The mixture was sonicated for 30 s at setting 3 of a Branson 450 sonifier. The emulsion formed was centrifuged at 2500*g* for 30 min. EA 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 ES, 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 EA and expressed as ES.

*Foaming Properties.* Foaming properties [foaming capacity (FC) and foaming stability (FS)] of DWGP were determined by an air-purge method as described by Kato et al. (1983). Egg white (80% protein, GA) was used as a standard. Aqueous protein solutions (2 mL, 5%) of both standard and DWGP were prepared and adjusted to pH 7.0. The solutions were placed into 15-mL glass test tubes (9 in. × 1.5 in.), and air was introduced at 1 psi from an air compressor for 15 s. The height of foam formed was measured at 0, 2, 5, 10, and 20 min. The height in millimeters of foam formed at 0 time was expressed as FC, and FS was calculated from the equation

$$FS = V_0 t / V$$

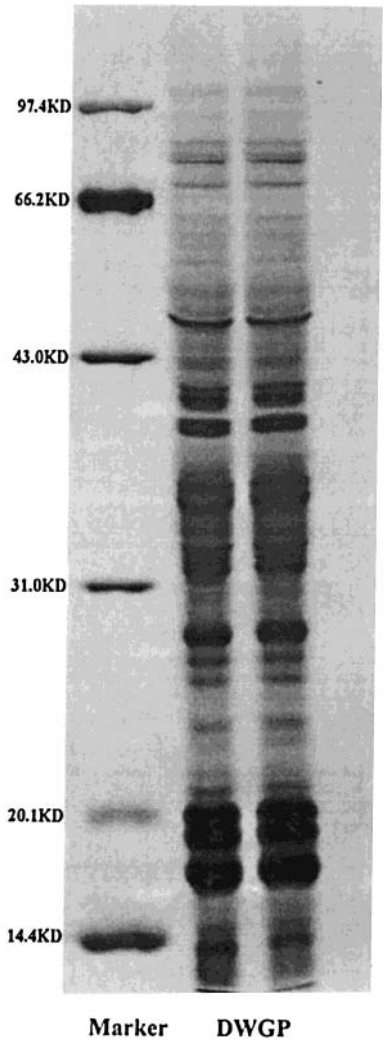


**Figure 1.** SDS−PAGE pattern of DWGP.

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.

*Water Retention.* The method of Vani and Zayas (1995) was used for WR determination. A 2.5 g sample was dispersed into 47.5 mL of distilled deionized water. The solution then was mixed by magnetic stirrer, and the pH was adjusted to 4.0, 5.0, 6.0, 7.0, or 8.0. Solutions were incubated at 5, 15, 30, or 70 °C for 30 min and then centrifuged for 30 min at 1200*g*. The precipitate was weighed. WR was calculated as the difference between hydrated weight and original weight per gram of sample.

**Statistical Analyses.** The data from all runs represented a mean value of three replicates and were evaluated statistically (SAS, 1988).

## RESULTS AND DISCUSSION

**Molecular Weights of DWGP.** Electrophoresis (SDS−PAGE) of DWGP was performed to obtain information on the molecular weight and distribution pattern of the protein components (Figure 1 and Table 1). Twenty-seven bands were observed distinctly between 13.2 and 91.3 kDa.

**Amino Acid Content of DWGP.** The amino acid content of DWGP wais shown in Table 2. The amino acid varieties of DWGP were inclusive, and it was especially rich in essential amino acids and limited amino acids for the human body. Furthermore, the composition ratio of essential amino acids of DWGP, comparable to egg and milk proteins, was basically close

**Table 1. Molecular Weights of DWGP Determined by SDS−PAGE**

| no. | $m_R$ | mol wt, kDa | no. | $m_R$ | mol wt, kDa |
|---|---|---|---|---|---|
| 1 | 0.1500 | 91.3 | 15 | 0.5417 | 36.1 |
| 2 | 0.1833 | 84.4 | 16 | 0.5667 | 34.0 |
| 3 | 0.2083 | 79.6 | 17 | 0.5958 | 31.7 |
| 4 | 0.2250 | 76.5 | 18 | 0.6083 | 30.8 |
| 5 | 0.2500 | 72.1 | 19 | 0.6750 | 26.3 |
| 6 | 0.2625 | 69.9 | 20 | 0.6917 | 25.3 |
| 7 | 0.2833 | 66.6 | 21 | 0.7250 | 23.4 |
| 8 | 0.3375 | 58.6 | 22 | 0.7583 | 21.6 |
| 9 | 0.3583 | 55.7 | 23 | 0.7792 | 20.6 |
| 10 | 0.3667 | 54.6 | 24 | 0.8500 | 17.4 |
| 11 | 0.4250 | 47.6 | 25 | 0.8750 | 16.4 |
| 12 | 0.4417 | 45.7 | 26 | 0.9000 | 15.4 |
| 13 | 0.4833 | 41.5 | 27 | 0.9667 | 13.2 |
| 14 | 0.5167 | 38.3 | | | |

**Table 2. Amino Acid Content of DWGP (Grams per 100 g)**

| amino acid | content | amino acid | content |
|---|---|---|---|
| tryptophan | 0.216 | valine | 1.429 |
| threonine | 0.943 | arginine | 4.779 |
| isoleucine | 1.739 | histidine | 0.858 |
| leucine | 1.128 | alanine | 1.676 |
| lysine | 2.314 | aspartic acid | 1.643 |
| methionine | 0.265 | glutamic acid | 5.052 |
| phenylalanine | 1.041 | glycine | 1.482 |
| tyrosine | 0.794 | serine | 1.434 |
| total | | | 26.793 |

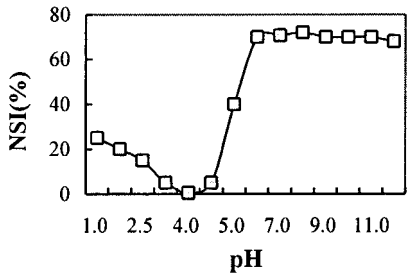


**Figure 2.** Nitrogen solubility of DWGP.

to the model value issued by the FAO/WHO with good amino acid equilibrium, which was greatly significant from the nutritional point of view.

**Nitrogen Solubility of DWGP.** As a kind of food protein material, the nitrogen solubility of DWGP is the most critical factor in its protein functionality because it affects the emulsifying, foaming, whippinh, and gelling capacities and further affects the stability, texture, color, and sensory properties of products. The nitrogen solubility profile is an excellent index of protein functionality.

Nitrogen solubility changes with the pH value of DWGP are presented in Figure 2. The lowest nitrogen solubility was found at pH 4.0 and the highest at pH 6.0. In the range of pH 1.0−4.0, the solubility decreased from 25 to 0.5% alongside the growth of pH value, but in the range of pH 4.0−6.0, the solubility increased with the growth of pH value and reached its highest value of 70% at pH 6.0. Moreover, the solubility did not increase beyond pH 6.0, which maintained ~70%.

A high nitrogen solubility of DWGP (>70%) meant that it was an excellent food protein material, which would make it a suitable candidate for food applications.

**Emulsifying Properties of DWGP.** Using casein and BSA standards compared to DWGP, the comparison of their emulsifying properties is presented in Figure 3. It was shown that EA and ES of DWGP were a little higher than those of casein and close to those of BSA.

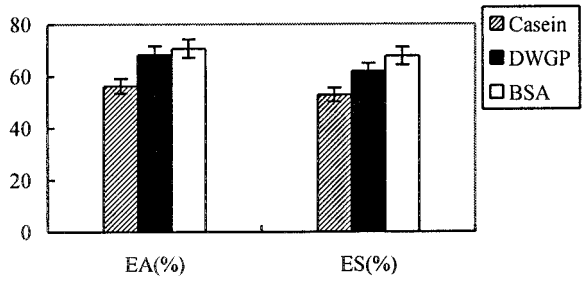


**Figure 3.** Emulsifying properties of DWGP.

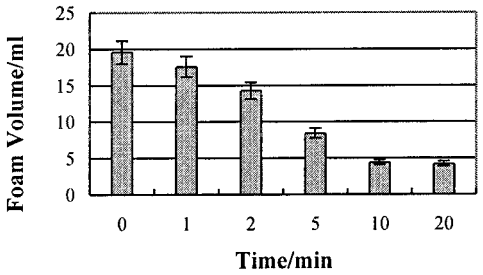


**Figure 4.** Foaming properties of DWGP.

No significant reduction (9.38%) in EA was observed upon heat treatment for 30 min at 45 °C. Therefore, DWGP had strong emulsifying properties, making it a good functional protein additive for application in emulsified foods.

**Foaming Properties of DWGP.** With hydrophilic and hydrophobic groups, DWGP had good surface activity, which could lower the surface tension of water and form foams during vigorous stirring. It is presented in Figure 4 that DWGP possessed good FC. The foam volume was 19.6 mL at 0 min, which was similar to that of egg white (Townsend and Nakai, 1983). However, the FS of DWGP was not good; the foam volume was only 4.2 mL after 20 min.

Nitrogen solubility is an important criterion for foam formation, and the stability of foams depends on the physical properties of viscoelasticity, interaction among molecules, etc. Therefore, the high FC of DWGP was due to its high NSI, and its relative bad FS could be overcome by adjusting its viscoelasticity and molecule interaction through physical, chemical, or enzymatic methods.

**Water Retention of DWGP.** WR was an important factor that influences basic quality characteristics and yield of protein products because it is important in the determination of mechanical strength, elasticity, plasticity, and flow of food materials and was critical for desirable functions of plant protein materials, such as swelling, wettability, water holding capacity, gelation, and surface properties (Vani and Zayas, 1995).

The factors influencing the WR of proteins were pH value and temperature. It is shown in Figure 5 that the highest WR (229.4%) was obtained at pH 8.0 and 70 °C and the lowest WR (156.2%) at pH 4.0 and 5 °C. Proteins were capable of binding large quantities of water because of their ability to form hydrogen bonds between water molecules and polar groups of polypeptide (Jones and Tung, 1983). The isoelectric point of DWGP was pH 4.0, so, lowering the pH to 4.0 probably converted carboxyl groups toward nonionized forms, which damaged the formation of hydrogen bonds, thereby reducing the water binding of DWGP. With the increase of temperature, there was an increase of WR of DWGP, which could be attributed to the water imbibing capacity

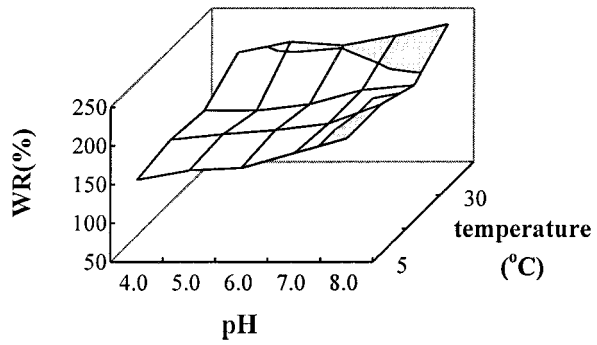


**Figure 5.** Water retention of DWGP,

of DWGP because the slight heat denaturation did not lower its water imbibing capacity but instead improved this property. In addition, the viscoelastic increase and conformational changes of DWGP caused by heat denaturation transformed the characteristic hydrogen bonds and then probably improved the WR of DWGP.

**Conclusions.** It is a current trend to explore functional and nutritional protein ingredients from unconventional sources at relatively low cost. DWGP isolated from wheat germ, a byproduct of the wheat milling industry, is abundantly nutritious, with high contents and equilibrium pattern of amino acids. Moreover, the desirable functional properties, including favorable nitrogen solubility, emulsifying properties, foaming properties, and water retention, make DWGP a useful protein ingredient for foods. All of these properties provide an effective way to utilize wheat germ. Further studies are needed for the comprehensive utilization of wheat germ, a natural nutrient source for mankind.

## ABBREVIATIONS USED

DWGP, defatted wheat germ protein; NSI, nitrogen solubility index; EA, emulsifying activity; ES, emulsifying stability; BSA, bovine serum albumin; FC, foaming capacity; FS, foaming stability; WR, water retention; SDS−PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SC-$CO_2$, supercritical carbon dioxide.

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