Effect of Processing pH on the Properties of Peanut Protein Isolates and Oil[1]

K. C. RHEE, C. M. CATER, and K. F. MATTIL, Protein Chemistry Laboratory, Soil and Crop Sciences Department, and Food Protein Research and Development Center, Texas Engineering Experiment Station, Texas A&M University, College Station 77843

ABSTRACT

Peanut protein isolates and oil were prepared from raw peanuts using an aqueous system at several different pH values for protein extraction and precipitation. Some of their physical and chemical properties, as well as the extent of their recoveries, were determined in order to compare the effects of pH during preparation of isolates on the quality and yield of the products. The maximum recoveries of both protein and oil from raw peanuts were obtained at pH 8.0, and extraction pH values higher than 8.0 considerably reduced the protein solubility of the isolates with no significant increases in the recovery of the proteins. Higher pH values also increased the loss of oil through saponification. All four isolates remained stable when heated; no protein coagulation occurred even if isolates were heated at 95°C. for 30 min. Solution viscosities were quite low at all protein concentrations as compared to other vegetable proteins, and the pH of extraction and precipitation of the isolate significantly influenced viscosity characteristics.

In the last two decades, much interest has been shown in peanuts, along with other oilseeds, as potential sources of supplementary protein for human foods. This interest has resulted from the concern over the lack of adequate protein to provide needed nourishment for large segments of the world's population now and in the years ahead.

The yearly world production of peanuts in the early 1970s is expected to exceed 17 million metric tons (1). This is equivalent to more than three million metric tons of crude proteins, of which only a negligible portion is presently being utilized for human food consumption. The major proportion of peanuts is converted to peanut oil. Commerical processes for extracting peanut oil commonly utilize expellers at high temperature and/or pressure, or organic solvents, which may denature the proteins. To prevent this undesirable effect during processing, several new methods for simultaneously recovering proteins and oil from raw peanuts have been developed using aqueous media (2-6) with varying degrees of success.

Most of the previous studies on the properties of peanut protein isolates were done with solvent-extracted peanut meal as the protein source (7,8). Very little information is available concerning the effects of various processing conditions during the wet-processing of peanuts on the physiochemical and functional properties of peanut protein isolates and oil. In this report, the effects of pH on the yields and quality of peanut protein isolates and oil during aqueous processing are compared. Isolates were prepared using neutral or alkaline pH values during extraction and acidic pH values during protein precipitation.

MATERIALS AND METHODS

The materials and detailed conditions for the preparation of peanut protein isolates have been described previously (6). Peanut kernels which had had the skins

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removed (blanched) mechanically without heat [moisture 4.5%; oil 49.5%; protein (N X 5.46) 27.5%] were ground with an Urschel mill (cutting space of 0.25 mm.). One part (100 g.) of the ground sample was then dispersed in six parts (600 ml., w./v.) of warm deionized water (65°C.) by stirring constantly with a magnetic stirrer.

The pH of the dispersion was adjusted to the desired value (7.0, 8.0, 9.0, or 10.0) by adding 1N NaOH solution. After pH adjustment, extraction was continued 1 hr. in a water bath (60°C.) with occasional stirring. The dispersion was then centrifuged at 4,000 X g for 30 min. using a swinging bucket rotor. Centrifugation resulted in separation of a fibrous residue from the oil-protein aqueous emulsion. The pH of the aqueous phase was then adjusted to 3.5, 4.5, or 5.5 by adding 1N HCl in order to precipitate the protein and to destabilize the emulsion. A second centrifugation at 4,000 X g for 30 min. yielded a solid fraction (peanut protein isolate) and an oil fraction. The peanut protein isolates were dried by lyophilization without further purification.

The recovery of protein isolates was measured by weighing the dried products. Nitrogen content was determined by the micro-Kjeldahl method. A factor of 5.46 was used to convert the nitrogen content to the amount of protein (9). The amount of free oil obtained was determined by weighing, whereas solvent extraction with petroleum ether was employed to determine the oil content of solid materials.

Peanut proteins in the isolate were classified according to their solubility characteristics by the Osborne method (10). Amino acid analyses were performed according to the method of Moore et al. (11) with Beckman Model 120C amino acid analyzers. Samples were hydrolyzed in constant-boiling HCl for 24 hr. under a nitrogen flush. Tryptophan was determined by a separate basic hydrolysis with barium hydroxide (12) and cystine by the performic acid oxidation method as described by Schram et al. (13).

Protein solubility was determined by suspending the appropriate amounts of peanut protein isolates in distilled water, adjusting the pH of aliquots to the desired value, and then agitating 30 min. at room temperature. After readjustment of

TABLE I. EFFECT OF PROTEIN EXTRACTION AND PRECIPITATION pH CONDITIONS ON THE RECOVERY OF PROTEIN ISOLATE AND OIL

Extraction pH	Precipitation pH	Recovery (g./100-g. sample)[a]	
		Isolate	Oil
7.0	3.5	28.9 ± 0.7	42.4 ± 1.5
	4.5	28.5 ± 1.3	43.4 ± 1.3
	5.5	30.3 ± 0.9	41.7 ± 1.3
8.0	3.5	29.0 ± 1.2	42.9 ± 1.1
	4.5	28.1 ± 1.5	45.4 ± 1.2
	5.5	29.9 ± 1.4	43.0 ± 1.3
9.0	3.5	28.5 ± 1.3	42.8 ± 1.3
	4.5	28.0 ± 1.2	45.8 ± 1.5
	5.5	28.9 ± 1.5	42.5 ± 1.4
10.0	3.5	28.0 ± 1.6	42.6 ± 1.4
	4.5	27.5 ± 0.9	45.6 ± 1.5
	5.5	28.5 ± 1.3	42.4 ± 1.4

[a]Moisture-free basis.

suspension pH, as required, the suspension was centrifuged, and the dissolved protein content of the aqueous phase measured.

In heat coagulation experiments, the protein solutions were heated for 30 min., then cooled to room temperature and centrifuged. The amount of protein remaining in solution was taken as a solubility index of heat-treated protein.

Viscosity was measured in a standard Ubbelohde dilution suspended level type ASTM D 445 viscometer, with the time recorded to the nearest tenth of a second on a stopwatch. The average of at least ten measurements was used. The standard deviation in viscosity measurement was 0.1% at the water bath temperature of 30°C.

Free fatty acid value, saponification number, peroxide value, iodine value (Wijs' method), and unsaponifiable matter were determined by standard AOAC procedures (14) and the refining loss by the standard AOCS laboratory cup method (15). Unless otherwise specified, the data shown represent the average of four replicate analyses with the standard deviation from the mean for each value.

RESULTS AND DISCUSSION

The effects of both protein extraction and precipitation pH values in an aqueous medium on the recovery of peanut protein isolates and oil are presented in Table I. Under the experimental conditions, extraction pH values higher than 8.0 did not show significant effects on the yields of either the protein isolates or oil when the protein was precipitated at the same pH. A slightly lower recovery (about 2% each) of both products was observed using an extraction pH of 7.0. Extraction pH values higher than 10.0 caused a gradual decrease in the yields of both protein isolates and oil if the pH used during the protein precipitation step was identical (i.e., 4.5) in each test.

On the other hand, the pH during the protein precipitation significantly affected the recovery of both protein isolates and oil. For both products, the maximum recovery was obtained at a precipitation pH of 4.5. Subsequent studies showed that the optimum precipitation pH would be 4.00 ± 0.25 (6). Although the precipitation pH values of 4.5 to 5.0 were previously reported to be optimal (4,16), it was observed that a shift of one unit in the precipitation pH toward the alkaline side (from 4.5 to 5.5) not only reduced the recovery of both protein and oil significantly, but also increased the formation and stability of the oil-in-water emulsion. Similar observations were made at precipitation pH values below 3.5. It was therefore concluded that the pH of protein precipitation must be kept as close as possible to 4.00 ± 0.25 in order to ensure maximum recovery of both products.

As shown in Table II, the protein and oil contents of the protein isolates also were affected by both protein extraction and precipitation pH values. The protein content of the isolates increased gradually as the extraction pH increased, whereas the oil content of the isolates decreased under the same conditions. Within a fixed pH for protein extraction, the protein content of isolates decreased as the precipitation pH departed further from the point of minimum solubility of the peanut proteins, pH 4.00 ± 0.25. This decreasing recovery of the protein content was more pronounced toward alkaline than acidic pH values. However, the oil content of the isolates decreased as the precipitation pH decreased from 5.5 to 3.5. This suggests that the selection of proper pH values for protein extraction and precipitation is very important in obtaining the desired type of product. For

TABLE II. PROTEIN AND OIL CONTENT OF PROTEIN ISOLATES PREPARED AT DIFFERENT pH CONDITIONS OF PROTEIN EXTRACTION AND PRECIPITATION

Extraction pH	Precipitation pH	Composition (%)[a]	
		Protein	Oil
7.0	3.5	88.5 ± 1.4	6.0 ± 1.1
	4.5	90.6 ± 0.9	7.4 ± 1.2
	5.5	83.5 ± 1.3	9.5 ± 0.9
8.0	3.5	88.4 ± 1.8	5.4 ± 0.9
	4.5	91.7 ± 2.2	6.2 ± 1.0
	5.5	85.1 ± 1.8	8.3 ± 1.7
9.0	3.5	89.7 ± 1.4	4.2 ± 1.7
	4.5	92.0 ± 1.5	5.5 ± 1.2
	5.5	87.6 ± 1.0	6.0 ± 1.1
10.0	3.5	91.5 ± 1.2	3.8 ± 1.0
	4.5	93.9 ± 1.1	4.4 ± 1.4
	5.5	88.7 ± 0.9	5.6 ± 1.0

[a]Moisture-free basis.

example, if one desires to produce a product low in oil content, one may have to use relatively lower precipitation pH even though recovery of protein will be lower.

The classical Osborne classification of proteins (10), as applied to peanut protein isolates, confirms the solubility data obtained in aqueous solution. Table III indicates that over 90% of the proteins in the isolates prepared at an extraction pH of 7.0, 8.0, or 9.0 was soluble in CO_2-free deionized water at pH 6.7. With the exception of 7.0/4.5 isolates (extracted at pH 7.0 and precipitated at pH 4.5), the water-soluble protein fraction decreased and the salt- and alkaline-soluble protein fractions increased as the extraction pH increased. The sodium tungstate test indicated that there was little nonprotein nitrogen in the peanut protein isolates. An exceptionally high level of insoluble residue was present in isolates prepared at an extraction pH of 10.0 or higher.

TABLE III. CLASSIFICATION OF PROTEINS IN PROTEIN ISOLATES ACCORDING TO SOLUBILITY[a]

Fractions Soluble in	Percentage of Total Nitrogen			
	7.0/4.5[b]	8.0/4.5[b]	9.0/4.5[b]	10.0/4.5[b]
CO_2 free water, pH 6.7	91.0 ± 0.2	98.6 ± 0.2	92.4 ± 0.8	84.5 ± 1.2
1 M NaCl solution, pH 7.0	0.9 ± 0.1	0.5 ± 0.2	0.8 ± 0.2	2.0 ± 0.7
70% Aqueous ethanol	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.2	0.1 ± 0.1
0.2% NaOH solution	7.9 ± 0.1	0.8 ± 0.1	4.4 ± 0.1	6.7 ± 0.7
Insoluble residue	Negligible[c]	Negligible[c]	Negligible[c]	6.7 ± 1.0
Nonprotein nitrogen	Negligible[c]	Negligible[c]	Negligible[c]	Negligible[c]

[a]Osborne classification (10).
[b]Protein extraction pH/protein precipitation pH.
[c]Less than 0.1%.

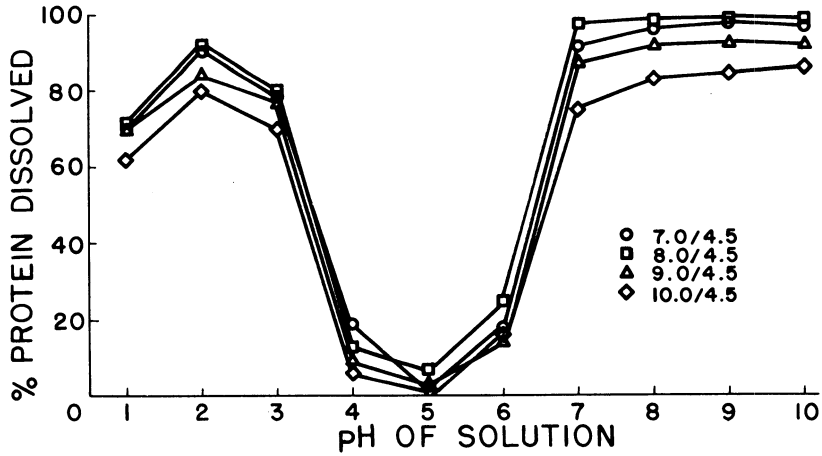

Fig. 1. Effect of extraction pH values on the protein solubility of isolates in water at various pH values.

The influence of the isolate preparation pH values on the solubility characteristics of the proteins as a function of solution pH are reported in Fig. 1. In all cases, the minimum protein solubility was observed at pH 5.0. Approximately 98% of the protein present in the isolates prepared at an extraction pH of 8.0 was soluble in water at pH 7.0. Corresponding values for isolates prepared at pH 7.0, 9.0, and 10.0 are 92, 87, and 75%, respectively. Solubility of the proteins increased with increasing solution pH values. For example, at pH 10.0, the protein solubility was 97% for isolates prepared at pH 7.0; 99% for pH 8.0 isolates; 92% for pH 9.0 isolates; and 86% for pH 10.0 isolates.

Figure 2 summarizes the solubility characteristics of the protein comprising each of the protein isolates over a range of protein concentrations in neutral deionized water. The relative solubilities of the pH 8.0 isolate were higher than those of the other isolates at all of its concentrations tested. The next highest solubilities were exhibited by the pH 7.0 isolate followed by the pH 9.0 isolate while the pH 10.0 isolate was least soluble at all five of the concentrations which were tested.

Nearly 100% of the pH 8.0 isolate was soluble at a concentration of 50 mg./ml. or lower, whereas it was 98% at a concentration of 100 mg./ml. Solubilities of the pH 7.0 and 9.0 isolates decreased markedly with increasing concentration. For example, at 10.0 mg./ml. concentration level, the solubilities were 100% for pH 7.0 isolates and 97% for pH 9.0 isolates; whereas at 25 mg./ml. concentration level the corresponding values were 96 and 94%. At 100 mg./ml. protein concentration, 92% of the pH 7.0 isolate and only 87% of the pH 9.0 isolate were soluble. For pH 10.0 isolates, however, only 80% was soluble even at 5.0 mg./ml. protein concentration and the protein solubility also decreased slightly as the concentration increased, i.e., 75% soluble at 100 mg./ml.

The heat stability of the proteins in the isolates was determined by measuring the amount of protein left in the solution after heat treatment. These experiments were carried out at neutral pH (7.0) and without added salts, in order to minimize the protective effects of charge and salts on the proteins during heating. As

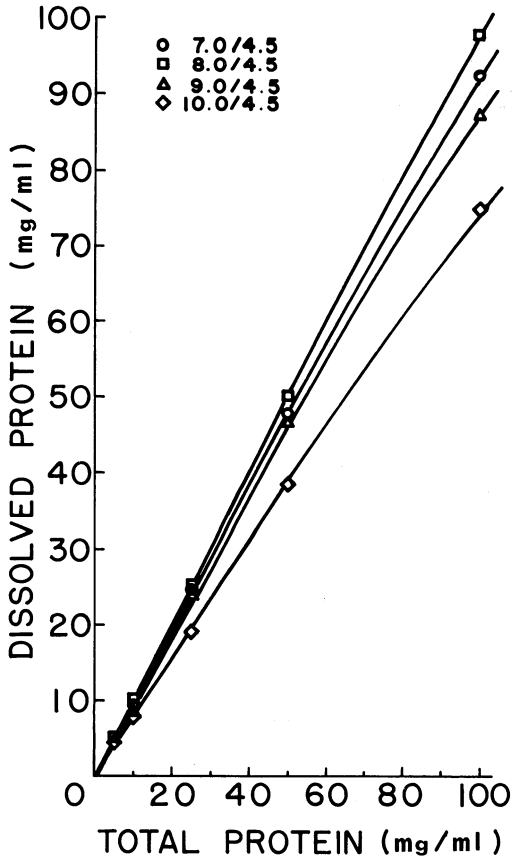

Fig. 2. Effect of extraction pH values on the protein solubility of isolates in water at various protein concentrations (pH 7.0).

summarized in Fig. 3, peanut proteins comprising all four isolates were very stable to heat treatment even under these adverse conditions. Heating at 95°C. for 30 min. did not significantly alter the solubility characteristics of the proteins. As might be expected, however, boiling the protein solution increased the protein denaturation rapidly. After 30 min. boiling under reflux, less than 10% of the proteins remained in the solution.

The viscosity of peanut protein isolates was determined at various protein concentrations in deionized water at neutral pH, and the results are shown in Fig. 4. The intrinsic viscosity of 0.82 centipoise indicates that peanut proteins have the low viscosity characteristics expected of globular proteins. The apparent viscosities of the protein solutions were dependent upon protein concentrations and the pH of the protein preparations. The changes in solution viscosity were linear for pH 7 and 8 isolates, ranging from 0.85 centipoise at 1% concentration to 1.25 and 1.35 respectively at 10% protein concentration. As the protein preparation pH increased, the viscosity profile became an exponential function of the protein concentration.

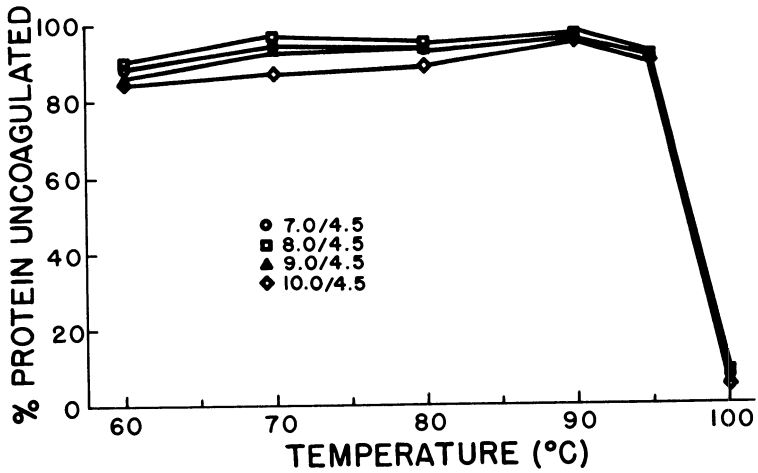

Fig. 3. Effect of extraction pH values on the heat stability of protein isolates in water at pH 7.0 (1% protein solution).

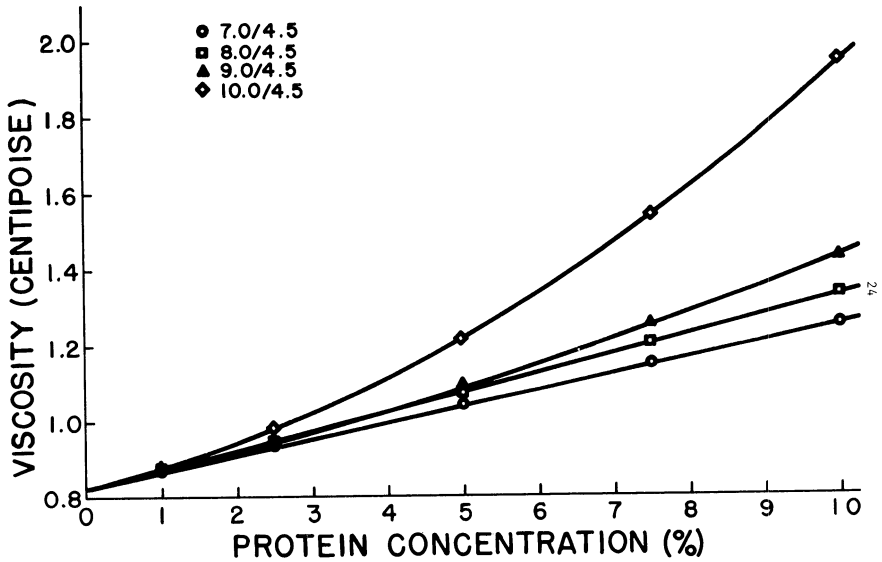

Fig. 4. Effect of extraction pH values on the viscosity of protein isolates in water at various protein concentrations (pH 7.0). All measurements were made at 30.0 ± 0.1°C.

For example, for pH 10 isolates, the viscosity increased from 0.87 centipoise at 1% to 1.96 centipoises at 10%. These low viscosity values are comparable with those of milk, about 1.3 to 1.6 centipoises (17,18), and are substantially less than those of soybeans, which show 0.2 poises or higher at 25°C. for a 16% protein concentration (19).

TABLE IV. AMINO ACID COMPOSITION OF PROTEIN ISOLATES

Amino Acid	g. Amino Acid/16 g. Nitrogen				
	7.0/4.5	8.0/4.5	9.0/4.5	10.0/4.5	Avg. Standard Deviation
Lysine	2.9	3.0	3.0	2.8	± 0.1
Histidine	2.0	2.4	2.5	2.3	± 0.1
Arginine	12.8	12.8	12.8	12.3	± 0.7
Aspartic acid	12.9	12.3	12.4	12.6	± 0.2
Threonine	2.5	2.5	2.9	2.6	± 0.1
Serine	4.9	5.1	4.8	4.9	± 0.1
Glutamic acid	21.1	21.4	20.2	20.6	± 0.5
Proline	4.9	4.8	4.8	4.8	± 0.3
Glycine	4.1	4.1	4.1	4.5	± 0.1
Alanine	3.9	3.9	3.9	4.0	± 0.2
Cystine	1.4	1.4	1.3	1.4	± 0.2
Valine	4.7	4.4	4.5	4.6	± 0.2
Methionine	1.1	1.0	1.2	1.0	± 0.1
Isoleucine	3.5	3.4	3.9	3.5	± 0.3
Leucine	6.8	6.6	7.1	6.7	± 0.7
Tyrosine	4.1	4.3	4.2	4.6	± 0.1
Phenylalanine	5.5	5.6	5.3	5.7	± 0.2
Tryptophan	0.9	1.0	1.1	1.1	± 0.1

TABLE V. PHYSICOCHEMICAL PROPERTIES OF OIL

Parameter	7.0/4.5	8.0/4.5	9.0/4.5	10.0/4.5
Free fatty acid value	1.2 ± 0.5	1.3 ± 0.2	1.4 ± 0.1	1.5 ± 0.2
Saponification, number	191.0 ± 1.2	191.5 ± 0.9	192.0 ± 1.1	194.0 ± 1.0
Peroxide number[a]	0.8 ± 0.1	0.8 ± 0.3	0.9 ± 0.5	0.8 ± 0.3
Iodine value	93.2 ± 0.8	92.8 ± 0.2	92.1 ± 0.9	92.4 ± 0.6
Unsaponifiable matter (%)	0.2 ± 0.3	0.2 ± 0.2	0.5 ± 0.3	0.5 ± 0.2
Refining loss (%)	1.8 ± 0.5	2.1 ± 0.8	2.9 ± 0.2	3.9 ± 0.4

[a]Expressed as milliequivalent peroxides per kg. of oil.

Amino acid profiles of the protein isolates prepared at various pH values are reported in Table IV along with the average standard deviation for each amino acid. Among the isolates, there were no significant differences in amino acid content. Methionine and tryptophan contents were quite low in all isolates. Histidine, lysine, and threonine contents were also considered low to be a well-balanced food ingredient (20).

The effect of protein extraction pH values on the physicochemical properties of the recovered oil is summarized in Table V. With the exception of refining losses, physicochemical properties were quite similar. Refining loss increased substantially as the extraction pH increased, from 1.8% for the isolates prepared at pH 7.0 to about 4% for pH 10.0 isolates. Free fatty acid value, saponification number, and unsaponifiable matter increased slightly with increasing extraction pH. Normally, the free fatty acid content of the oil reflects the refining loss; but for some reason refining loss became significantly greater as the extraction pH increased, and little correlation to the free fatty acid values was found.

In summary, careful consideration must be taken in choosing proper pH conditions when using an aqueous system for the recovery of both peanut oil and protein isolates for the following reasons: 1) Too high a protein extraction pH tends to considerably reduce the protein solubility without any significant increases in recovery of the protein. This also reduces the quality of the oil as evidenced by greater refining loss; and 2) too low a protein extraction pH leaves a considerable amount of protein in solution unrecovered. The pH of protein precipitation should be kept as close as possible to 4.00 ± 0.25 in order to destabilize the oil-in-water emulsion and to ensure the maximum recovery of both protein and oil. The functional properties of peanut protein isolates prepared by an aqueous processing method (such as low solution viscosity and high solubility even at 90° to 95°C.) should have significant implications in considering potential food uses.

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