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Physicochemical and Functional Properties of Buckwheat Protein Product

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This study was conducted to compare the physicochemical and functional properties of buckwheat protein product (BWP), soy protein isolate (SPI), and casein. BWP was prepared from buckwheat flour by the method including alkaline extraction and isoelectric precipitation. The amino acid composition of BWP was very similar to that of buckwheat flour. The protein solubility (PS) of BWP was much greater than that of SPI at all pH levels (pH 2−10) but lower than that of casein at pH 7−10. The isoelectric point of BWP was around pH 4. The higher aromatic hydrophobicities (ARH) of BWP, SPI, and casein were obtained at lower pH levels (pH 2−3). The emulsifying stability (ES) of BWP was lower than those of SPI and casein at high pH levels (pH 7−10). At all pH levels, BWP formed a thin emulsion. Regression analysis showed that the ARH of BWP was significantly associated with the ES. Although the water holding capacity of BWP was quite lower than that of SPI, its fat absorption capacity was slightly higher than those of SPI and casein. These results indicated that the physicochemical properties of BWP were different from those of SPI or casein. Thus, BWP is a potential source of functional protein for possible food application.

KEYWORDS: Buckwheat protein product; soy protein isolate; casein; physicochemical properties; functional properties

INTRODUCTION

Buckwheat (*Fagopyrum esculentum* Moench) is mostly consumed in the form of flour, which is used as a material for bread, pancakes, noodles, and other food items. One of the notable features of buckwheat is the high biological value of its protein, although its digestibility is relatively low (*1*). Our previous study demonstrated that a buckwheat protein product (BWP) from buckwheat flour has a potent hypocholesterolemic activity in rats (*2*, *3*) and the activity of BWP was far stronger than that of soy protein isolate (SPI) (*4*). Our studies have further indicated that consumption of BWP caused suppression in body fat (*5*), constipation (*6*), mammary carcinogenesis (*7*), and colon carcinogenesis (*8*) in rats and in the formation of cholesterol gallstones in hamsters (*9*).

Some food proteins have been shown to possess nutritional, physiological, and functional properties. A vegetable protein, soy protein, also has some nutritional and physiological properties, and its functional properties contribute to its application in food formulations and processing. To develop dietary protein for utilization as ingredients in the food industry, it is necessary to determine the physicochemical and functional properties of the proteins (*10*). Some plant protein isolates have been already shown to possess some functional properties (*11*−*13*). Although the physiological properties of BWP have recently attracted considerable attention (*14*), there is no report on the physicochemical properties of BWP. The objective of the present study was to evaluate the physicochemical and functional properties of BWP. The properties of BWP were compared with those of SPI and casein.

MATERIALS AND METHODS

Materials. Buckwheat flour (Manjyu A, third flour) was purchased from Nikkoku Flour Mill (Nagano, Japan). A BWP was prepared from buckwheat flour according to the process described elsewhere (*2*). Briefly, buckwheat flour was suspended in distilled water and the pH was adjusted to ∼8 by the addition of 0.1 N NaOH. This suspension was mixed well with a homomixer to extract buckwheat protein. The resulting slurry was separated into the supernatant and residue by a continuous centrifuge (7500*g* for 20 min). The pH of the supernatant was adjusted to 4.5 by the addition of 0.1 N HCl to precipitate the protein isoelectrically. A continuous centrifuge (7500*g* for 20 min) recovered most of the isoelectric precipitate formed. After a washing with an adequate amount of distilled water, the isoelectric precipitates

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were neutralized and dissolved with 0.1 N NaOH and sterilized at 90 °C for 10 min. The solution was spray-dried to produce BWP. SPI and casein were purchased from Nissin Oil Manufacture (Tokyo, Japan) and Wako Pure Chemicals (Osaka, Japan), respectively. The chemical compositions of BWP, buckwheat flour, SPI, and casein were determined according to AOAC (*15*) procedures. For amino acid analysis, the hydrolysis of the samples was performed in the presence of 6 N HCl at 110 °C for 22 h. The amino acid compositions of BWP, buckwheat flour, SPI, and casein were determined by an amino acid analyzer (L-8500, Hitachi, Tokyo, Japan).

Protein Solubility (PS). PS was determined according to the modified method of Aluko and Yada (*12*). Protein dispersions (0.1% w/v) were prepared in 0.01 M Na_2HPO_4 adjusted to pH 2−10. For total soluble protein content (control), the samples were dispersed in 0.1 N NaOH. The protein dispersions were stirred at 23 °C for 1 h, centrifuged (3000*g* for 10 min), and filtered through filter paper. Protein contents of the filtrate were determined according to the Lowry method using bovine serum albumin as a standard (*16*). Percent protein solubility was calculated as PS (%) = (protein content of sample/protein content of control) × 100.

Aromatic Hydrophobicity (ARH). ARH was determined by using a modified method of Iwami et al. (*17*). The protein solution (2 mL), which is filtrates from above and contained soluble proteins, was diluted to give protein concentrations at 0.005% (w/v). ARH was then determined using 1-anilino-8-naphthalenesulfonate (ANS) as a probe. Ten microliters of ANS (8.0 mM in 0.1 M phosphate buffer, pH 7.0) solution was added. Fluorescence intensity (FI) was measured with a spectrofluorometer (RF-5000, Shimadzu, Tokyo, Japan) at wavelengths (λ_{ex}, λ_{em}) of 390 and 470 nm. The FI reading was standardized by adjusting the reading of the fluorometer to 30% full scale in methanol.

Emulsion Stability (ES). ES was determined by measuring the amount of retained water in the emulsions (*13*). Two milliliters of pure corn oil was added into the 2 mL of protein dispersion (0.1% w/v), and emulsions were formed in a centrifuge tube by using the high-speed blender. The emulsions were stored for 12 h at room templeture. ES was expressed as the percent water retained (%) = [(total mL of water in emulsion − mL of water released)/total mL of water in emulsion] × 100.

Emulsion Viscosity (EV). Emulsions for viscosity measurements were prepared according to the procedure described above. Each emulsion was measured using a viscometer (BL, Tokimec, Tokyo, Japan). EV was expressed in centipoises (cP).

Water Holding (WH) and Fat Absorption (FA) Capacities. WH and FA capacities were determined using the method of Ahmedna et al. (*11*). Five grams of sample was weighed into 50 mL centrifuge tubes preweighed. For each sample, distilled water was added in small increments to a series of tubes under continuous stirring with a glass rod. After the mixture was thoroughly wetted, samples were centrifuged (1000*g* for 5 min). After the centrifugation, the amount of added distilled water resulting in the supernatant liquid in the test tube was recorded. WH (grams of water per gram of sample) was calculated as WH = $(W_2 - W_1)/W_0$, where W_0 is the weight of the dry sample (g), W_1 is the weight of the tube plus the dry sample (g), and W_2 is the weight of the tube plus the sediment (g). Triplicate samples were analyzed for each sample.

For FA capacity, 1 g of sample was weighed into 50 mL centrifuge tubes preweighed and thoroughly mixed with 10 mL of corn oil. The protein−oil mixture was centrifuged (2000*g* for 5 min). Immediately after centrifugation, the supernatant was carefully removed, and the tubes were weighed. FA (grams of oil per gram of protein) was calculated as FA = $(F_2 - F_1)/F_0$, where F_0 is the weight of the dry sample (g), F_1 is the weight of the tube plus the dry sample (g), and F_2 is the weight of the tube plus the sediment (g). Triplicate samples were analyzed for each sample.

Statistical Analysis. All data represent a mean value of at least duplicate analyses. The statistical significance of the difference between values was analyzed by a one-way analysis of variance and then by Duncan's multiple-range test (*18*) or Student's *t* test. Results were considered to be significant at $p < 0.05$. Stepwise multiple regression was done according to the statistical analysis system package (SPSS). ES was used as the dependent variable, while PS, ARH, and EV served as independent variables. Variables left in the regression models were significant at $p < 0.05$.

Table 1. Amino Acid Composition of Buckwheat Flour, Buckwheat Protein Product, Soy Protein Isolate, and Casein[a]

amino acid	mmol/g of N			
	buckwheat flour	BWP	SPI	casein
Asp	4.74	4.67	5.39	3.45
Thr	2.14	1.98	1.59	2.61
Ser	3.14	3.14	2.53	2.84
Glu	7.44	7.46	8.54	10.58
Gly	5.01	4.50	3.05	1.79
Ala	3.18	3.00	2.49	2.49
Cys-	1.39	0.98	0.12	0.13
Val	2.82	2.96	2.48	3.83
Met	0.93	0.91	0.45	1.40
Ile	1.81	1.93	2.17	3.03
Leu	3.31	3.48	3.40	4.67
Tyr	0.93	1.04	1.11	2.21
Phe	1.75	2.01	1.84	2.26
Lys	2.73	2.33	2.31	3.45
His	1.04	1.01	0.90	1.41
Arg	3.49	3.93	2.43	1.38
Pro	2.06	2.14	2.65	6.21
total	47.91	47.46	43.46	53.74

[a] Duplicate analysis.

RESULTS AND DISCUSSION

Chemical and Amino Acid Compositions. The composition of BWP was as follows (% w/w): protein, 65.8; lipids, 22.0; nonfiber carbohydrate, 5.9; and water, 3.1. The composition of original buckwheat flour was as follows (% w/w): protein, 12.1; lipids, 3.1; nonfiber carbohydrate, 68.5; and water, 13.5. The protein concentration of BWP was increased ∼5-fold compared with that of buckwheat flour. The lipid concentration of BWP was also increased ∼7-fold. Although the reason for this elevation in the lipid concentration is unknown at present, the lipid fraction might have been coprecipitated with BWP proteins in the process of acid precipitation, possibly due to high affinity between the lipids and proteins. The composition of SPI was as follows (% w/w): protein, 85.4; lipids, 2.1; nonfiber carbohydrate, 1.2; and water, 8.2. The composition of casein was as follows (% w/w): protein, 83.3; lipids, 2.4; nonfiber carbohydrate, 0; and water, 11.6. The amino acid composition of BWP was very similar to that of starting buckwheat flour (**Table 1**). Buckwheat protein has been believed to be of good quality because of its well-balanced amino acid composition (*19*). Casein and soybean protein-based formulas have been used as the source of nutrition for infants due to their good amino acid composition (*10*). BWP had higher levels of glycine and arginine in comparison to those of casein or SPI. Other amino acids in BWP were similar to those of SPI and casein. The results indicated that BWP also had profiles of essential amino acids required for infants similar to those of casein and SPI. In our previous study, a defatted BWP was prepared by removing the lipid fraction of BWP by the treatment with organic solvent (chloroform/methanol, 2:1, v/v) and added to the experimental diet. Food intake in the growing rats fed the defatted BWP diet (201 ± 5 g/2 weeks) was significantly lower than that in the rats fed BWP (225 ± 6 g) at dietary level of 200 g/kg of protein. Treatment with the organic solvent might suppress the digestibility of the BWP protein by altering its three-dimensional structure. The defatted BWP appears to be not suitable for utilization as a functional food ingredient.

Protein Solubility. The PS of BWP was minimum at pH 4 and increased gradually below pH 3 and above pH 5 (**Figure**

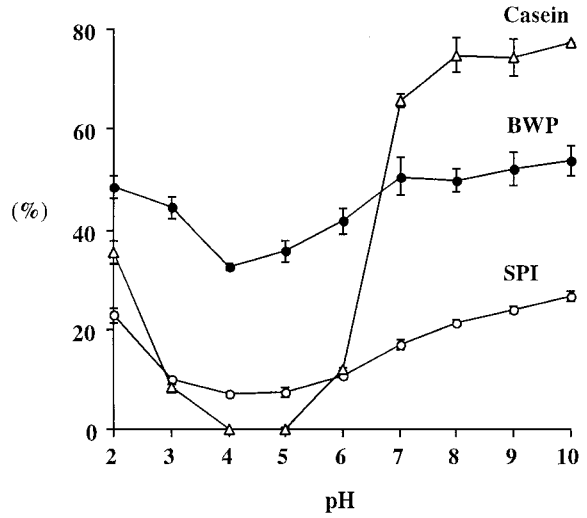

Figure 1. Protein solubility of buckwheat protein product, soy protein isolate, and casein (0.1% suspensions, w/v) in the pH range of 2–10. Values are means ± SD ($n = 3$).

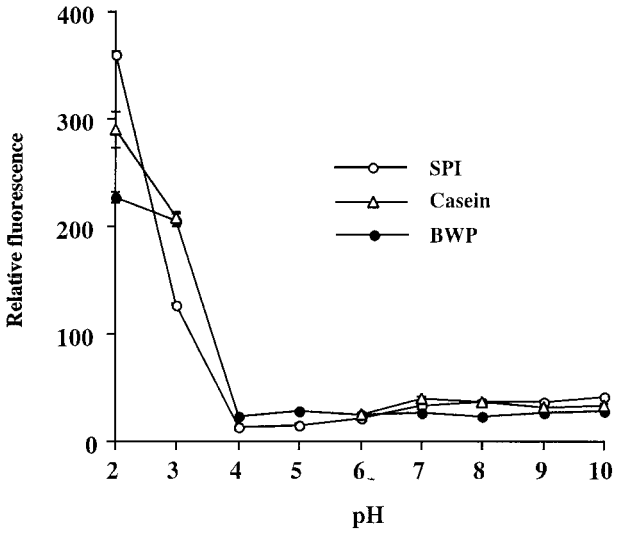

Figure 2. Aromatic hydrophobicity (ARH) of buckwheat protein product, soy protein isolate, and casein (0.005% solution, w/v) in the pH range of 2–10. Error bars are too small to show. ARH values of casein at pH 4–5 were not shown because the protein solubility of casein was 0% at those pH values.

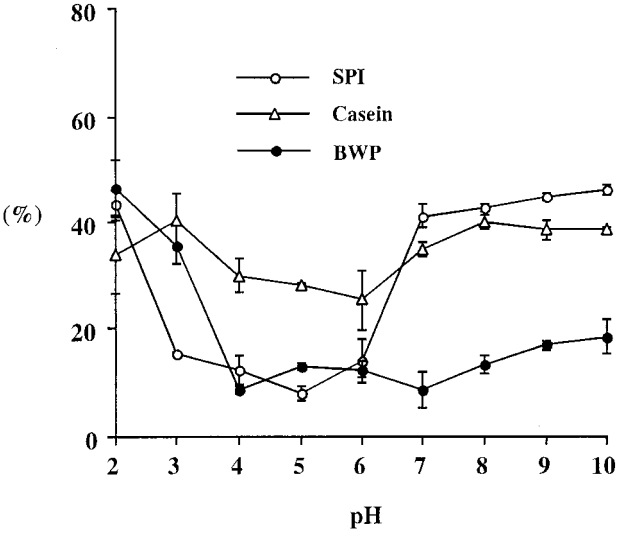

Figure 3. Emulsion stability of buckwheat protein product, soy protein isolate, and casein (0.1% suspensions, w/v) in the pH range of 2–10. Values are means ± SD ($n = 3$).

1). The BWP protein had an isoelectric point at pH 4. SPI had a similar PS profile in comparison to BWP, but the PS of SPI was quite lower than that of BWP at all pH values ($p < 0.05$). An earlier paper indicated that 80% of protein from buckwheat flour was water-soluble and that the protein−protein interaction was immediately induced by heat treatment at >90 °C (*20*). However, the results in this study showed that the sterilization step of BWP did not cause much loss of solubility. The lipids in BWP might disturb protein denaturation induced by heat treatment. Although the PS of casein was 0% at pH 4−5, this was much higher than that of BWP at pH 7−10. The PS of BWP, SPI, and casein increased with increasing pH from pH 5. At higher pH values, the increased net negative charge on the protein dissociates the protein aggregates, and the solubility might increase (*12*). At lower pH values, the increased net positive charge contribute to the solubility. The total contents of positively charged amino acids (Lys, His, and Arg) of BWP, SPI, and casein were 7.27, 5.64, and 6.24 mmol/g of N, respectively (**Table 1**). The residues of these ionic amino acids might be at least partially responsible for the PS at low pH levels. In this study, the PS of casein was greater than that of SPI at pH 7−10 (**Figure 1**). Belozerski et al. (*21*) reported that buckwheat protein contains globulin (40−45%), albumin (20−25%), and glutelin (10−13%). Thus, the greater part of BWP protein is considered to be derived from globulin, the main component of buckwheat protein. Addition of 1.0 M NaCl elevated the PS of BWP regardless of pH: The PS values of BWP in 1.0 M NaCl at pH 2, 3, 4, 5, 6, 7, 8, 9, and 10 were 41, 47, 53, 59, 61, 64, 65, 65, and 62%, respectively. Because globulin appears to be rich in BWP, NaCl may have a significant effect on the solubility of this protein. In general, superior functional attributes for most applications in food processing are associated with the solubility of proteins. Because solubility is the main characteristic of protein for use in beverages, BWP may offer a protein source for fortified beverages.

Aromatic Hydrophobicity. The ARH of BWP was lower than those of SPI and casein at pH 2 and higher at pH 3 when compared to that of SPI ($p < 0.05$) (**Figure 2**). Generally, the higher ARH values of BWP, SPI, and casein were obtained at lower pH values. A similar result was obtained for cowpea protein isolate by Aluko and Yada (*22*). Previous study suggested that the exposure of hydrophobic groups and the subsequent aggregation of the unfolded protein molecules cause a decrease in solubility (*23*). In this study, however, there was no significant relationship between the PS and ARH in any samples. The reason for this lack of relationship is unknown at present.

Emulsion Stability and Emulsion Viscosity. The ES of BWP was not different from that of SPI at pH 4−6 ($p > 0.05$) (**Figure 3**). However, the ES values of BWP were significantly lower than those of SPI at pH 7−10 ($p < 0.05$) (**Figure 3**). The ES values of casein were significantly higher than those of BWP at pH 4−10 ($p < 0.05$). Although the PS values of SPI were quite lower than those of casein at pH 7−10, the ES values of SPI were slightly higher than those of casein. A similar result was obtained by Ahmedna et al. (*11*). They reported that although the solubility of sodium caseinate was greater than that of SPI at pH 7.5, they exhibited similar ES values. The EV of BWP was quite lower than those of SPI and casein at pH 6−10 ($p < 0.05$) (**Figure 4**). The EV of casein was significantly higher than that of SPI at pH 6−10 ($p < 0.05$).

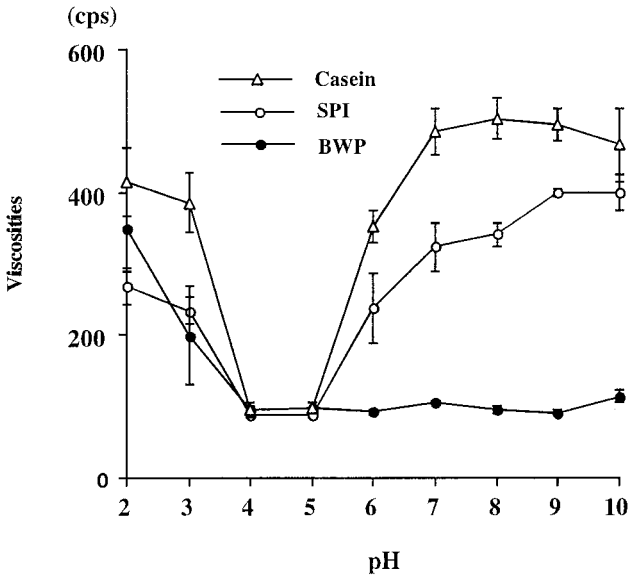

Figure 4. Emulsion viscosity of buckwheat protein product, soy protein isolate, and casein (0.1% suspensions, w/v) in the pH range of 2–10. Values are means ± SD ($n = 3$).

The roles of ARH, PS, and EV in determining the ES of BWP, SPI, or casein were investigated using backward-stepwise multiple-regression analysis. The following regression equations were obtained for BWP, SPI, or casein:

$$\text{(BWP) ES} = 9.249 + 0.149\ \text{ARH} \qquad (R^2 = 0.920)$$

$$\text{(SPI) ES} = -3.700 + 2.062\ \text{PS} \qquad (R^2 = 0.925)$$

$$\text{(casein) ES} = 26.022 + 0.023\ \text{EV} \qquad (R^2 = 0.467)$$

In general, the ES can be affected by the presence of soluble proteins. The PS of SPI also had a significant effect on the ES ($p < 0.05$). Although the PS of BWP was much greater than that of SPI at pH 7−10, the ES of BWP was lower than that of SPI. Aluko and Yada (*12*) suggested that formation of a charged layer around the fat globules and/or the formation of a hydrated layer around the interfacial material lowered interfacial energy and retarded droplet coalescence. Polar lipids in BWP might affect the formation of a film around the droplet because of its high surface tension, resulting in poor rheological properties of the interfacial film. SPI and casein foamed high emulsion viscosity at high pH levels (Figure 4). The high viscosity and/or gelation of a hydrated layer around the droplet might relate to the ES of SPI and casein. The ARH had a positive effect for the ES of BWP ($p < 0.05$). Nakai (*24*) had indicated that proteins possessing larger numbers of nonpolar regions at their surface have a greater activity to form and stabilize emulsions. Thus, the samples with the highest ARH may form the most stable emulsion. Our results obtained here showed that the ARH of BWP had a significant effect on the ES ($p < 0.05$). Another regression model showed that the EV of casein made a positive contribution to the ES ($p < 0.05$). The results were different from those of BWP or casein. Relatively high values of the ES of BWP at low pH (pH 2−3) might be useful for emulsified foods such as sausages, meat products, and mayonnaise.

Water Holding and Fat Absorption Capacities. The WH of BWP was slightly higher than that of casein ($p < 0.05$), but significantly lower than that of SPI ($p < 0.05$) (**Table 2**). SPI had superior WH in agreement with the results of the previous study (*11*). The WH of BWP was low despite its high PS at pH 6−7. There was no relationship between the PS and WH of BWP. Ahmedna et al. (*11*) suggested that high protein solubility did not necessarily relate to high WH. The FA of BWP was slightly higher than those of SPI and casein ($p < 0.05$). The ability of protein to bind fat depends on several parameters such as hydrophobicity, degree of denaturation, and the size and flexibility of the protein. Lin and Zayas (*25*) suggested that the ability of protein to bind fat depends on nonpolar side chains that bind hydrocarbon chains, thereby contributing to increased oil absorption. BWP can bind slightly higher fat than SPI despite its high PS. Because BWP contains a higher amount of lipids (22%) than SPI (2.1%) and casein (2.4%), the BWP lipids might contribute to higher FA.

Table 2. Water Holding and Fat Absorption Capacities of Buckwheat Protein Product, Soy Protein Isolate, and Casein[a]

	g/g of sample	
	WH	FA
BWP	3.32 ± 0.02^b	2.88 ± 0.13^a
SPI	7.25 ± 0.26^a	2.61 ± 0.09^b
casein	1.47 ± 0.05^c	0.88 ± 0.03^c

[a] Values are means ± SD ($n = 3$) Within a row, means with different superscript letters are significantly different by Duncan's multiple-range test ($p < 0.05$).

The results of the present study provided significant information on the functional aspects of BWP. Because the functional properties of BWP were different from those of SPI and casein, BWP has potential applications for fortified products by virtue of its higher water solubility, higher fat absorption capacity, etc. The physicochemical properties and physiological and nutritional functions of BWP make this protein a valuable ingredient for food industries.

ABBREVIATIONS USED

BWP, buckwheat protein product; SPI, soy protein isolate; PS, protein solubility; ARH, aromatic hydrophobicity; ES, emulsifying stability; EV, emulsion viscosity; WH, water holding capacity; FA, fat absorption capacity.

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