

Accepted Manuscript

Functional Properties and *in vitro* Digestibility of Buckwheat Protein Products:
Influence of Processing

Chuan-He Tang

PII: S0260-8774(07)00190-2
DOI: [10.1016/j.jfoodeng.2007.01.029](https://doi.org/10.1016/j.jfoodeng.2007.01.029)
Reference: JFOE 4878

To appear in: *Journal of Food Engineering*

Received Date: 16 May 2005
Revised Date: 11 December 2006
Accepted Date: 26 January 2007



Please cite this article as: Tang, C-H., Functional Properties and *in vitro* Digestibility of Buckwheat Protein Products: Influence of Processing, *Journal of Food Engineering* (2007), doi: [10.1016/j.jfoodeng.2007.01.029](https://doi.org/10.1016/j.jfoodeng.2007.01.029)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **TITLE.** Functional Properties and *in vitro* Digestibility of
2 Buckwheat Protein Products: Influence of Processing

3 **AUTHOR NAMES.** Chuan-He Tang*

4 **AUTHOR ADDRESS.**

5 *Department of Food Science and Technology, South China University of Technology, Guangzhou 510640,*
6 *P. R. China*

7 **AUTHOR EMAIL ADDRESS.** chtang@scut.edu.cn

8 **CORRESPONDING AUTHOR FOOTNOTE.** *To whom correspondence should be addressed. TEL:
9 86-20-87114262; FAX: 86-20-87114263; *E-mail:* chtang@scut.edu.cn.

Abstract.

The effect of processing on some functional properties and *in vitro* digestibility of buckwheat protein products (BWP) was investigated. In the spray-dried cases, the protein solubility (PS) of BWP was much greater than that of commercial soy protein isolates (SPI) at below pH 5 and above pH 6 ($P < 0.05$). At below pH 7, the PS of those BWPs by ultrasonic-assisted extraction was higher than that by mechanical extraction or by ultrasonic-assisted extraction and additional de-fatting treatment. The water-holding capacity of those BWPs obtained by freeze-drying was also significantly higher than that by spray-drying ($P < 0.05$). The fat adsorption capacity of freeze-dried BWPs was significantly higher than that of SPI and those spray-dried BWPs ($P < 0.05$). The emulsifying activity index (EAI) of some spray-dried BWPs was higher than that of SPI at below pH 4 and above pH 6, while the emulsion stability index (ESI) was lower than that of SPI at below pH 7 ($P < 0.05$). The de-fatting pretreatment could improve the EAI and ESI of BWP, especially at above pH 6. In simulated gastric fluid, the digestion pattern of BWP was evidently different from that of SPI. In the pepsin digestion, the % N release was affected by the processing. The BWPs with low lipid contents had higher % N release as compared to those with high lipid contents. These results suggested that the functional properties and *in vitro* digestibility of BWP be associated with its lipid and ash contents, which could be affected by processing. Thus, functional and nutritional BWPs, potential for food applications, can be produced through controlling the processing.

Keywords: Buckwheat protein products (BWP); functional property; *in vitro* digestibility; processing; ultrasonic

1. Introduction

Buckwheat (*Fagopyrum esculentum* Moench) is known as a valuable source of protein, and its amino acid composition is nutritionally superior to that of cereal grains (Pomeranz & Robbins, 1972). Although the digestibility of buckwheat protein is relatively low, it has many physiological activities which are potential for human health, such as hypocholesterolemic activity in rats, suppression in body fat, constipation, mammary carcinogenesis and colon carcinogenesis and in the formation of cholesterol gallstones in hamsters (Tomotake, Shimaoka, Kayashita, Yokoyama, Nakajoh & Kato, 2000, 2001; Kayashita, Shimaoka, Nakajoh & Kato, 1996; Kayashita, Shimaoka, Nakajoh, Yamazaki & Kato, 1997; Liu, Ishikawa, Huang, Tomotake, Kayashita, Watanabe & Kato, 2001). Furthermore, the buckwheat protein, as a vegetable protein, has some superior functional properties, such as higher nitrogen solubility index and higher water holding, emulsifying and foaming capacities (compared to soy protein isolate), contributing to its application in food formulations and processing (Bejosano & Harold, 1999; Zheng, Sosulski & Tyler, 1998; Tomotake, Shimaoka, Kayashita, Nakajoh & Kato, 2002).

To utilize the dietary protein as ingredients in food industry, it is necessary to investigate the functional and other properties of the proteins. There are several reports concerning the functional properties of buckwheat protein products (BWP) (Bejosano & Harold, 1999; Zheng *et al.*, 1998; Tomotake *et al.*, 2002), however, some functional properties of the proteins produced by different processing are inconsistent. For example, Zheng *et al.* (1998) indicated that the buckwheat protein concentrate spray-dried showed higher nitrogen solubility index at neutral pH, and higher water holding, emulsifying and foaming capacities, compared to a freeze-dried protein sample. However, in another study, the buckwheat protein concentrate, prepared by drying at 70 °C and then defatted with petroleum ether, showed poor foaming capacity (Bejosano & Harold, 1999). Additionally, poor water holding capacity and emulsifying stability of spray-dried buckwheat protein product were reported recently (Tomotake *et al.*, 2002). To the authors' knowledge, these differences in functional properties might be due to the diversity of preparation process, such as extraction method, drying mode and de-fatting treatment, since the differences of processing procedures will result in the differences of composition (e.g., protein content, fat content) and the extent of

protein thermal denaturation.

On the other hand, the commercial utilization of buckwheat proteins may be feasible, only when it is combined with the starch production from buckwheat. During the starch preparation, an alkaline extraction to remove the proteins is usually applied. In addition to the proteins, the lipids can also be effectively extracted together, if appropriate extraction process is chosen, since there may be the strong interactions between protein and lipid (Tomotake *et al.*, 2002). If the extraction technique of protein as well as lipid from buckwheat is resolved, it would greatly improve the production technology of the starch.

Thus, the first objective of this study was to prepare different buckwheat proteins with different compositions, using various processing. Then, the functional and nutritional (*in vitro* digestibility) properties of these proteins were investigated and compared, including the protein solubility, emulsifying properties, water holding and fat absorption capacities.

2. Materials and Methods

2.1. Materials

The buckwheat seeds were purchased in a retail outlet in Guangzhou (China), cultivated in Gansu Province (China). The soy protein isolates (SPI) was gifted from WonderfulTM Technol. Co. (China), with the 83% nitrogen content (determined by Kjeldahl method, $N \times 6.25$). Molecular weight (MW) marker kit (97 to 24 kDa) was from DingGuo Biol. Co. (China). The electrophoresis reagents and β -mercaptoethanol (2-ME) were purchased from Shanghai BOAO Biochemical Co. (China). All other chemicals were of analytical reagent or better grade.

2.2. Preparation of BWP

The buckwheat seeds were ground to pass through a 60-mesh screen to produce fine whole flour, in a

disintegrator. Various BWPs were prepared from the whole flour by different processing (e.g., extraction, drying, and/or de-fatting and dialyzing treatment) (**Figure 1**). Briefly, five hundred grams of buckwheat flour were dispersed in 5 liters of 0.05 M Tris-HCl buffer (pH 8.0), then extracted with usual mechanical stirring at ambient temperature for 4 h, or with ultrasonic-assisted treatment for 30 min (the ultrasonic frequency and power were 15 kHz and 500 W, respectively) (XingDongLi Ultrasonic Electron Equipment Co. Ltd., China). The resulting slurry was separated into the supernatant and residue by centrifugation at 9000 g for 20 min at 4 °C. The pH of the supernatant was adjusted to 4.5 with 0.1 N HCl, and at this pH, the protein precipitated isoelectrically. The resultant precipitate was obtained by centrifugation at 9000 g for 20 min. After a washing with an adequate amount of desalted water, the precipitates were dispersed and neutralized with 0.1 N NaOH. The dispersion was spray-dried, or freeze-dried to produce various BWPs. The spray-drying was carried out in a mini-spray drier (BUCHI Co., Swiss), with inlet and outlet temperatures of about 130 and 70 °C respectively. In the freeze-dried cases, the dispersions were dialyzed against de-ionized water (24 h, three changes, 4 °C).

In the experiments to investigate the effect of de-fatting treatment on the properties of BWP, the buckwheat flour was defatted with hexane (1:1 w/v, three changes). The chemical compositions (including protein, lipid, ash and moisture) of the buckwheat flour and protein products were determined according to AOAC procedures (1984).

2.3. Protein solubility (PS)

The PS was determined according to the modified method of Aluko & Yada (1995). Protein dispersions (0.1%, w/v) were prepared in 0.01 M Na₂HPO₄ adjusted to a specific value within the range of pH 2-10 with 0.1 N HCl or 0.1 NaOH. The dispersions were agitated with a magnetic stirrer for 1 h at ambient temperature, and then centrifuged (3000 g, 10 min) and further filtered with filter paper to obtain the filtrate. Protein contents of the filtrates were determined according to the Lowry method (Lowry, Rosebrough, Farr & Randall, 1951) using bovine serum albumin as a standard. Percent protein solubility

was calculated as $PS (\%) = (\text{protein content of sample} / \text{protein content of control}) \times 100$. The protein content of the dispersion (0.1%, w/v) in 0.1 N NaOH was used as the total protein content (or 100% protein solubility).

2.4. Water holding and fat absorption capacities (WHC and FAC)

WHC and FAC were determined using the method of Ahmedna, Prinyawiwatukul & Rao (1999) and Tomotake *et al.* (2002), with slight modification. Briefly, 5 g of sample was weighed into 50 mL centrifuge tubes pre-weighed. 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 (2000 g, 10 min). After the centrifugation, the amount of added distilled water resulting in the supernatant liquid in the test tube was recorded. WHC (grams of water per gram of sample) was calculated as $WHC = (W_2 - W_1) / W_0$, 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 FAC, 1 g of sample was weighed into 50 mL centrifuge tubes pre-weighed and thoroughly mixed with 4 mL of corn oil. The protein-oil mixture was centrifuged (3000 g, 20 min). Immediately after centrifugation, the supernatant was carefully removed, and the tubes were weighed. FAC (grams of oil per gram of protein) was calculated as $FAC = (F_2 - F_1) / F_0$, 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.

2.5. Emulsifying properties

The emulsifying properties of the samples were determined according to the method of Pearce & Kinsella (1978), with slight modification made by Tang, Yang, Chen, Wu & Peng (2005). For the emulsion formation, a volume of 3 mL of the 0.1% protein solution (in 0.05 M Tris-HCl buffer, pH 7.5) and 1 mL

of soybean oil were homogenized in FJ-200 High-Speed Homogenizer (Shanghai Specimen Model Co., China) for one minute at the maximum velocity. Immediately after the emulsion formation, 50 μ L of the emulsion was taken at 0 and 10 min from the bottom of the homogenized-emulsion, and diluted with a 0.1% sodium dodecyl sulphate (SDS) solution (1:100). Then, the diluted emulsion was shaken in a vortex mixer for a moment, and the absorbance at 500 nm was read in the spectrophotometer.

Emulsifying activity index (EAI) was calculated by Eq. (1), proposed by Cameron, Weber, Idziak & Cooper (1991), and adapted by Silva, Morais & Silvestre (2003) and Tang *et al.* (2005):

$$EAI = 2T / [(1-\theta)C] \quad (1)$$

where T is the turbidity; θ is the fraction of oil used to form the emulsion (0.25); and C the initial concentration of protein (0.1 g/100 mL). The turbidity was calculated by Eq. (2):

$$T = (2.203 \times A \times DF) / OP \quad (2)$$

where T is the turbidity, A is the absorbance, DF is the diluting factor (100) and OP is the optical path (0.01 m).

The emulsion stability index (ESI) was measured according to the method reported by Chobert, Bertrand-Hard & Nicolas (1988), and Tang *et al.* (2005), with slight modification. The $\Delta EAI\%$ was calculated by Eq. (3):

$$\Delta EAI\% = [(EAI_{\max} - EAI_{\min}) \times 100] / EAI_{\max} \quad (3)$$

where $EAI_{\max}$ is the highest value of EAI obtained for the diluted emulsions right after their formation, and $EAI_{\min}$ is the value obtained from the emulsion after storage for 10 min. The values of ESI were calculated by Eq. (4):

$$ESI = 1 / \Delta EAI\% \quad (4)$$

2.6. Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed on a discontinuous buffered system according to the method of Laemmli

(1970) using 12% separating gel and 4% stacking gel. The protein samples were solubilized in 0.125 M Tris-HCl buffer (pH 6.8), containing 1% (w/v) SDS, 2% (v/v) 2-mercaptoethanol (2-ME), 5% (v/v) glycerol and 0.025% (w/v) bromophenol blue, and heated for 5 min in boiling water before electrophoresis. For each sample, 10 µL was applied to each lane. After the electrophoresis, the gel was dyed in 0.25% Coomassie blue (R-250) in the 50% trichloroacetic acid and then destained in 7% acetic acid (methanol:acetic acid:water, 227:37:236 (v/v/v)).

2.7. Sequential *in vitro* protein digestion procedure

The *in vitro* digestibility of the proteins was evaluated by the method of Fu, Abbott & Hatzos (2002) and Nunes, Correia, Barros & Delgadillo (2004), with some modification. Pepsin (Sigma, P7000, 1:10000, 600-1000 units/mg) and trypsin (Genview, DH355-1, 1:250) were used for the *in vitro* digestion study. Briefly, 5 mL of 1% (w/v) BWP or SPI solutions in pH 1.5 HCl were pre-incubated in a water bath at 37 °C for 3-5 min. Then, an amount of pepsin (20 mg of pepsin per mL of 0.1 M KH₂PO₄, pH 2, buffer) was added, and the ratio of enzyme to protein substrate was 1:100 (w/w). The mixtures of pepsin and protein solution were mixed well and incubated at 37 °C. At the periods of time, e.g., 0, 1, 5, 10, 20, 30, 60 and 120 min respectively, aliquots of the mixtures were taken, and the pH was adjusted to 7.0 with 1.0 mol/L NaOH to stop the enzymatic reactions. Additionally, the final pepsin-digested hydrolysates (pH 7.0) were further digested by the addition of trypsin (20 mg of trypsin per mL of 0.1 M Tris-HCl buffer, pH 7.0) at 37 °C for 0, 1, 5, 10, 20, 30, 60 and 120 min, respectively. The applied amount of enzyme to initial protein substrate was 1:20 (w/w).

The digestion process was analyzed by SDS-PAGE as follows. The protein digestion samples (including those digested by pepsin or further by trypsin) were mixed with equal volumes of Laemmli sample buffer containing freshly added 2-ME (5%, v/v).

2.8. Nitrogen release during digestion

The % nitrogen release during digestion process was determined by the TCA-NSI method of Iwami, Sakakibara & Ibuki (1986), with some modifications. Ten milliliters of the digested mixtures were mixed with 10 mL of 10% (w/v) TCA (and the final concentration of TCA was 5%, w/v). The mixtures were then centrifuged (8000 g, 30 min) to obtain the precipitates. After washed with 10 mL of TCA (10%, w/v), the precipitates were obtained again by centrifugation at the same condition. The N content of the samples was determined by Kjeldahl method ($N \times 6.25$). The % N release during the digestion was calculated as % N release = $(N_0 - N_t) \times 100 / N_{\text{tot}}$, where t is the digestion time (min), N_t (mg) is the TCA-insoluble N after digestion for t (min), N_0 (mg) is the TCA-insoluble N in the protein sample, and N_{tot} (mg) is the total N of protein sample.

2.9. Statistical analysis

An analysis of variance (ANOVA) of the data was performed, and a least significant difference (LSD) test with a confidence interval of 95% was used to compare the means.

3. Results and Discussion

3.1. Compositions of BWP

The chemical composition of the buckwheat flour used in the present study was as follows (% w/w, wet basis): protein, 12.7; lipids, 2.95; moisture, 13.0; and ash, 1.59 (**Table 1**). These data were almost the same as those obtained by Tomotake *et al.* (2002). The compositions of various buckwheat protein products, produced by various processing, are shown in **Table 1**. The protein content of the various BWPs was 5 ~ 6.5 -fold higher as compared with that of the flour. In the ultrasonic-assisted extraction cases, the protein content of those BWPs (e.g., SU-BWP, FU-BWP) was significantly lower than that after usual mechanical stirring extraction ($P < 0.05$) (**Table 1**). The additional de-fatting treatment of the whole-meal flour with hexane could slightly increase the protein content of BWP (in both spray-drying and freeze-drying cases).

The lipid content of BWP was more dependent upon the extraction and de-fatting process. In the case of ultrasonic-assisted extraction, much more lipids (together with protein) ended up in the corresponding BWPs, than those resulting from mechanical extraction (**Table 1**). This enrichment with lipids of BWPs may be attributed to the mechanically induced emulsification of the lipids. Of course, the additional de-fatting pretreatment remarkably decreased the lipid content (**Table 1**). Additionally, it should be noted that the protein, as well as the lipid fraction, were much more efficiently extracted by ultrasonic-assisted extraction technique than by means of mechanical stirring extraction (data not shown). In fact, the lipid fraction in buckwheat flour may be associated with the protein fraction to some extent, possibly due to high affinity between lipid and protein (Tomotake *et al.*, 2002).

The effect of processing parameters on the moisture of BWP was less than that on the lipid content. The moisture content of BWP seemed to be negatively related with the lipid content (**Table 1**). The ash content of BWP was nearly unaffected by extraction method and de-fatting treatment. The differences of the ash content between the spray-drying and freeze-drying cases were caused by the application of dialyzing treatment in the freeze-drying cases (see: Section 2.2.).

The SDS-PAGE profiles of protein constituents of various BWPs in the presence of reducing agent 2-ME are shown in **Figure 2**. In all the cases, the pattern of protein constituents was almost identical, suggesting that the protein constituent pattern of BWP be unaffected by processing. The mobility of various polypeptides in the presence of 2-ME agrees with that of Fujino, Funatsuki, Inada, Shimono & Kikuta (2001), but is a bit different from that of Rout, Chrungoo & Rao (1997) and Milisavljević, Timotijević, Radović, Brkljačić, Konstantinović & Maksimović (2004).

3.2. Protein solubility

The PS of BWPs (spray-dried and freeze-dried) was minimum at pH 4-5 and gradually increased below

pH 4 and above pH 5 (**Figure 3**). In the spray-dried cases, the PS was significantly higher than that of commercial SPI at all pH values ($P < 0.05$), except at pH 5-6 (**Figure 3 A**). This agrees with the result of Tomotake *et al.* (2002). The PS profiles of various spray-dried BWPs were dependent upon the method of processing. For example, at below pH 6, the PS of SU-BWP was higher than that of DSU- and SM- BWPs, especially at near the isoelectric point (IEP) (close to pH 5). At above pH 7.0, however, the situation was on the contrary. The PS of SM-BWP was almost identical with that of DSU-BWP. Interestingly, these differences in PS were tightly related with the diversity of the lipids caused by processing method (**Table 1**). These results suggest that the presence of lipids in BWP might increase the thermal-stability of substrate protein, or suppress to some extent protein denaturation induced by heat treatment (e.g. spray-drying). However, the PS at above pH 7, at which the increased net negative charge on the protein dissociates the protein aggregates and the PS might increase (Tomotake *et al.*, 2002), seemed to be an exception. At alkaline pH, the presence of lipids on the contrary decreased the dissociation of protein aggregates.

In the freeze-dried BWPs, the PS was more markedly affected by the processing than that in the spray-dried cases. At above pH 6, the PS of FM- and DFU- BWPs (with lipids of 2.5% and 6.5% respectively) was significantly ($P < 0.05$) higher than that of FU-BWP (with lipids, 17.8%) (**Figure 3 B**). This PS profile difference at above pH 6 between the spray-dried and the freeze-dried BWPs may be caused by the difference in ash compositions (**Table 1**). In the FU-BWP, the agglomeration of lipid during freeze-drying, due to its high lipid content, may be another possible reason resulting in its poor PS at alkaline pH profile.

On the whole, the PS of spray-dried BWPs was higher than that of the freeze-dried, especially at pH 4-6. The data are in agreement with that of Zheng *et al.* (1998). The underlying mechanism may be attributed to the differences of particle size distribution between two kinds of BWPs (Cepeda, Villarán & Aranguiz,

1998). The PS of SU-BWP was significantly higher than that of commercial SPI at all pH values ($P < 0.05$), though it contained much higher lipid content (up to 18%). From the viewpoint of PS, the spray-dried BWP would be a good candidate to substitute commercial SPI in many food applications.

3.3. Water-holding and fat absorption capacities

The WHC of various BWPs was significantly lower than that of commercial SPI ($P < 0.05$), and at the same extraction condition, the WHC values of those BWPs obtained by freeze-drying were also significantly higher than that by spray-drying ($P < 0.05$) (**Table 2**). SPI had superior WHC in agreement with the result of a previous study (Tomotake *et al.*, 2002). The WHC of BWPs was relatively lower, though their PS (e.g., in SU-BWP) was much higher than that of SPI at pH 2~4 or 6-10, suggesting that there be no direct relationship between the PS and WHC of BWP. This result is also consistent with that of Tomotake *et al.* (2002). The WHC of various BWPs did not relate to its lipid content, and on the contrary, the additional de-fatting processing of the whole flour significantly decreased the WHC of BWP ($P < 0.05$). This may be due to the effect of the lipids on the protein stability of three-dimensional structure.

As expected, the FAC of BWP was higher than that of SPI, due to its higher affinity of protein for lipids. Tomotake *et al.* (2002) indicated that the higher amount of lipids of BWP might contribute to the higher FAC compared to that of SPI. However, in the present study, it was also shown that the FAC of BWP didn't relate to its lipid content, or extraction and drying processing (except the treatment of dialyzing). Interestingly, at the same extraction condition, the FAC of BWPs obtained by freeze-drying was much higher than that by spray-drying (**Table 1**). This result suggests that the FAC of BWP might be negatively related with the ash content, since in the freeze-dried cases, the BWP had low ash content due to the additional dialyzing treatment. However, further studies on the underlying mechanism will be required to verify the effect of the presence of ash on the FAC of BWP.

287

288 3.5. Emulsifying properties

289 The EAI of various BWPs was minimal at near IEP or pH 4-5, and increased at above pH 5 and below pH
 290 4 (**Figure 4**). The EAI profile as a function of pH was similar to that of PS, suggesting that the EAI be
 291 related to the PS. Similar EAI profile as a function of pH at above 4.0 and relationship between EAI and
 292 PS were observed in beach pea protein isolates (Chavan, McKenzie & Shahidi, 2001). The EAI values of
 293 spray-dried BWPs were, by and large, significantly higher than that of commercial SPI except at pH 4-5
 294 ($P < 0.05$) (**Figure 4 A**). The influence of processing or pretreatment on the EAI was inconsistent at
 295 different pH values. For example, SM-BWP and SU-BWP showed highest EAI at pHs 7 and 9,
 296 respectively, while the EAI of DSU-BWP was highest at below pH 4 ($P < 0.05$). A similar trend of EAI as
 297 function of pH was shown in the freeze-dried cases, however, DFU-BWP showed highest EAI at above
 298 pH 6 (**Figure 4 B**). This possibly suggested that in the absence of lipids, protein aggregates of BWP
 299 dissociated and dispersed in the O/W interface more easily at above the IEP (which was consistent with
 300 the PS results in the section 3.2.).

301

302 **Figure 5** shows ESI of various BWPs and SPI as a function of pH within pH range from 2 to 10. The ESI
 303 of spray-dried BWPs was more pH-dependent than that of freeze-dried samples. On the whole, the ESI of
 304 various BWPs showed a similar trend, which had a maximal value at a specific pH (e.g. pH 6 for
 305 DSU-BWP). As for SPI, there was a high 'platform' within the pH range from 3 to 6 in the ESI curve as
 306 function of pH (**Figure 5 A**). However, Tomotake *et al.* (2002) indicated that the spray-dried BWP and
 307 SPI showed lowest emulsion stability at near IEP (pH 4-6). The reason for this difference is yet unknown.

308 In the spray-dried BWPs, the ESI was significantly lower than that of SPI at below pH 5 ($P < 0.05$). At
 309 above pH 6, the ESI was inconsistent for various BWPs. However, DSU-BWP showed higher ESI values
 310 at pH 6-9 as compared to other BWPs and SPI (**Figure 5 A**). The data suggest that the presence of lipids
 311 might destabilize the emulsion of BWP.

3.6. *In vitro* digestibility

The *in vitro* digestibility of BWP and SPI was evaluated by TCA-soluble nitrogen release during digestion of pepsin and trypsin, in simulated gastric fluid. **Figure 6** shows a typical profile of the nitrogen release of FM-BWP and SPI during sequential pepsin and trypsin digestion, as a function of digestion time, and the *in vitro* digestibility (% N release) at the end of pepsin (and trypsin) digestion for various BWPs and SPI are summarized in **Table 2**. Since different BWP samples showed a similar digestion pattern in the present study, only FM-BWP was selected to illustrate the digestion pattern of BWP, and compared to that of SPI. As expected, BWP and SPI showed a different pattern of the % N release during pepsin and trypsin digestion (**Figure 6**).

BWP hydrolysis by pepsin remained linear for up to 10 min, and SPI hydrolysis for up to 20 min, while this linear slope of BWP was higher than that of SPI (**Figure 6**). The % N release of BWP increased much more slowly after 30 min, and kept nearly unchanged after more than 60 min. However, the % N release of SPI still kept higher increasing velocity after 30 min, and exceeded that of BWP after about 45 min. The difference in changes of hydrolysis velocity between BWP and SPI may be attributed to the difference in the susceptibility of their different protein constituents to pepsin digestion. SDS-PAGE analysis showed that, most of the protein constituents of buckwheat globulins (including 13S and 8S globulins) were rapidly and completely hydrolyzed by pepsin within 1 min to release oligo-peptides with MW lower than 20 kDa, while during the pepsin digestion of SPI, only the protein constituents of glycinin were easily digested (hydrolyzed within near 1 min), and the subunits (especially α' - and β - subunits) of β -conglycinin were much less digested (data not shown).

Upon further digestion by trypsin, the % N release of the pepsin-digested hydrolysates (with pH adjusted to 7.0) of BWP and SPI increased continuously, but developed in the different way (**Figure 6**). The % N

release of the BWP-hydrolysate increased much faster than that of SPI, especially in the initial trypsin digestion (~ 30 min). The presence of high activity of trypsin inhibitors in beans (Khokar & Chanhnan, 1986; Marquez, Fernandez & Alonso, 1998) may account for the differences of trypsin-digestion pattern between BWP and SPI.

After pepsin digestion for 120 min, the % N release of various BWPs was significantly ($P < 0.05$) less than that of commercial SPI (control), however, after further trypsin digestion (for another 120 min), on the contrary, the % N release of some BWPs (e.g., DSU-BWP, FM-BWP and DFU-BWP) was significantly ($P < 0.05$) higher than that of control (**Table 2**). The data suggest that the *in vitro* digestibility of buckwheat protein is dependent upon the choice of digestion model used for evaluating the protein digestibility. In this study, the *in vitro* digestibility of BWP can be considered to be less than that of commercial SPI (SPI has been well recognized as a kind of less digestible protein as compared to caseins), if only pepsin digestion is applied to evaluate the digestibility. However, when the sequential pepsin and trypsin digestion model is applied, the *in vitro* digestibility of BWP can be considered to be close, even better than that of SPI. Thus, the earlier viewpoint that the digestibility of buckwheat protein is relatively low should be reconsidered.

The *in vitro* pepsin digestibility of BWP was unaffected by the drying mode (spray-drying or freeze-drying) and the ash content, and tightly related to the lipid content (**Tables 1 and 2**). BWPs (e.g., DSU-BWP and DFU-BWP) with least lipid contents, obtained by de-fatting pretreatment, had highest *in vitro* pepsin digestibility (% N release) among all BWPs (**Table 2**). Whereas in freeze-dried cases, FU-BWP with high lipid content (17.8%) had least *in vitro* pepsin digestibility. Moreover, the *in vitro* pepsin and trypsin digestibility of BWP was also related to its lipid content, and BWPs with higher lipid contents had higher *in vitro* digestibility (**Tables 1 and 2**). These data suggest that the presence of lipids might decrease the *in vitro* pepsin and trypsin digestibility of BWP.

362

363 **4. Conclusions**

364 The influence of processing on the functional properties and *in vitro* digestibility of BWP was confirmed
 365 in this paper. The functional properties of BWP, including protein solubility, emulsifying properties and *in*
 366 *vitro* digestibility, were associated with its lipid content, and/or ash content which could be affected by
 367 processing. The drying mode, spray-drying or freeze-drying, also affected the functional properties.
 368 Therefore, functional and nutritional buckwheat protein products, potential for the application to food
 369 industry, could be obtained by selecting the processing.

370

371 **Abbreviations used.**

372 BWP, buckwheat protein products; SPI, soy protein isolate; PS, protein solubility (%); WHC, water
 373 holding capacity (grams of water gram of sample); FAC, fat absorption capacity (grams of oil per gram of
 374 protein); EAI, emulsifying activity index (m^2g^{-1}); ESI, emulsion stability index; SDS-PAGE, sodium
 375 dodecyl sulfate polyacrylamide gel electrophoresis; MW, molecular weight.

376

377 SM-BWP, the BWP after usual mechanical extraction and spray-drying processing; SU-BWP, the BWP
 378 after ultrasonic-assisted extraction and spray-drying processing; DSU-BWP, the BWP after
 379 ultrasonic-assisted extraction and spray-drying processing, plus the de-fatting pretreatment; FM-BWP, the
 380 BWP after usual mechanical extraction and freeze-drying processing; FU-BWP, the BWP after
 381 ultrasonic-assisted extraction and freeze-drying processing; DFU-BWP, the BWP after ultrasonic-assisted
 382 extraction and freeze-drying processing plus the de-fatting treatment.

383 **Acknowledgments.**

384 This work is part of the research projects of Chinese National Natural Science Fund (serial number:
385 20306008), sponsored by the NNSF of China. The author also gratefully acknowledges the financial
386 support from the Natural Science Fund of Guangdong Province, China (serial number: 05006525).

ACCEPTED MANUSCRIPT

References.

- Ahmedna, M., Prinyawiwatkul, W., & Rao, R. M. (1999). Solubilized wheat protein isolate: Functional properties and potential food applications. *Journal of Agricultural and Food Chemistry*, 47, 1340-1345.
- Aluko, R. E., & Yada, R. Y. (1995). Structure-function relationships of cowpea (*Vigna unguiculata*) globulin isolate: influence of pH and NaCl on physicochemical and functional properties. *Food Chemistry*, 53, 259-265.
- AOAC. (1980). *Official Methods of Analysis*. American Association of Analytical Chemists: Washington, DC.
- Bejosano, F. P., & Corke, H. (1999). Properties of protein concentrates and hydrolysates from Amaranthus and Buckwheat. *Industrial Crops and Products*, 10, 175-183.
- Cameron, D. R., Weber, M. E., Idziak, E. S., Neufeld, R. J., & Cooper, D. G. (1991). Determination of interfacial areas in emulsions using turbidimetric and droplet size data: correction of the formula for emulsifying activity index. *Journal of Agricultural and Food Chemistry*, 39, 655-659.
- Cepeda, E., Villarán, M. C., & Aranguiz, N. (1998). Functional properties of Faba bean (*Vicia faba*) protein flour dried by spraying drying and freeze drying. *Journal of Food Engineering*, 36, 303-310.
- Chavan, U. D., McKenzie, D. B., & Shahidi, F. (2001). Functional properties of protein isolates from beach pea (*Lathyrus maritimus* L.). *Food Chemistry*, 74, 177-187.
- Chobert, J. M., Bertrand-Hard, C., & Nicolas, M. G. (1988). Solubility and emulsifying properties of caseins and whey proteins modified enzymatically by trypsin. *Journal of Agricultural and Food Chemistry*, 36, 883-892.
- Fu, T.-J., Abbott, U. R., & Hatzos, C. (2002). Digestibility of food allergens and nonallergenic proteins in simulated gastric fluid and simulated intestinal fluid—a comparative study. *Journal of Agricultural and Food Chemistry*, 50, 7154-7160.
- Fujino, K., Funatsuki, H., Inada, M., Shimono, Y., & Kikuta, Y. (2001). Expression, cloning, and immunological analysis of buckwheat (*Fagopyrum esculentum* Moench) seed storage proteins. *Journal of Agricultural and Food Chemistry*, 49, 1825-1829.

- Iwami, K., Sakakibara, K., & Ibuki, F. (1986). Involvement of post-digestion 'hydrophobic' peptides in plasma cholesterol-lowering effect of dietary plant proteins. *Agricultural and Biological Chemistry*, 50, 1217-1222.
- Kayashita, J., Shimaoka, I., Nakajoh, M., & Kato, N. (1996). Feeding of buckwheat protein extract reduces hepatic triglyceride concentration, adipose tissue weight and hepatic lipogenesis in rats. *Journal of Nutrition Biochemistry*, 7, 555-559.
- Kayashita, J., Shimaoka, I., Nakajoh, M., Yamazaki, M., & Kato, N. (1997). Hypocholesterolemic effect of buckwheat protein extract in rats fed cholesterol enriched diets. *Nutrition Research*, 15, 691-698.
- Khokar, S., & Chanhan, B. M. (1986). Effect of domestic processing and cooking on in vitro protein digestibility of north bean. *Journal of Food Science*, 51, 1083-1085.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680-685.
- Liu, Z., Ishikawa, W., Huang, X., Tomotake, H., Kayashita, J., Watanabe, H., & Kato, N. (2001). A buckwheat protein product suppresses 1,2-dimethylhydrazine-induced colon carcinogenesis in rats by reducing cell proliferation. *Journal of Nutrition*, 131, 1850-1853.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin-phenol reagent. *Journal of Biological Chemistry*, 193, 265-268.
- Marquez, M. C., Fernandez, V., & Alonso, R. (1998). Effect of dry heat on the in vitro digestibility and trypsin inhibitor activity of chickpea flour. *International Journal of Food Science and Technology*, 33, 527-532.
- Milisavljević, M. D., Timotijević, G. S., Radović, S. R., Brkljačić, J. M., Konstantinović, M. M., & Maksimović, V. R. (2004). Vicilin-like storage globulin from buckwheat (*Fagopyrum esculentum* Moench) seeds. *Journal of Agricultural and Food Chemistry*, 52, 5258-5262.
- Nunes, A., Correia, I., Barros, A., & Delgadillo, I. (2004). Sequential in vitro pepsin digestion of uncooked and cooked sorghum and maize samples. *Journal of Agricultural and Food Chemistry*, 52, 2052-2058.
- Pearce, K. N., & Kinsella, J. E. (1978). Emulsifying properties of proteins: evaluation of a turbidimetric

- technique. Journal of Agricultural and Food Chemistry, 26, 716-723.
- Pomeranz, Y., & Robbins, G. S. (1972). Amino acid composition of buckwheat. Journal of Agricultural and Food Chemistry, 20, 270-274.
- Radovic, R. S., Maksimovic, R. V., Brkljacic, M. J., Gasic, I. E. V., & Savic, P. A. (1999). 2S albumin from buckwheat (*Fagopyrum esculentum* Moench) seeds. Journal of Agricultural and Food Chemistry, 47, 1467-1470.
- Rout, M. K., Chrungoo, N. K., & Rao, K. S. (1997). Amino acid sequence of the basic subunit of 13S globulin of buckwheat. Phytochemistry, 45, 865-867.
- Silva, J. G., Morais, H. A., & Silvestre, M. P. C. (2003). Comparative study of the functional properties of bovine globin isolates and sodium caseinate. Food Research International, 36, 73-80.
- Tang, C. H., Yang, X. Q., Chen, Z., Wu, H., & Peng, Z. Y. (2005). Physicochemical and structural characteristics of sodium caseinate biopolymers induced by microbial transglutaminase. Journal of Food Biochemistry, 29, 401-422.
- Tomotake, H., Shimaoka, I., Kayashita, J., Yokoyama, F., Nakajoh, M., & Kato, N. (2000). A buckwheat protein product suppresser gallstone formation and plasma cholesterol more strongly than soy protein isolate in hamsters. Journal of Nutrition, 130, 1670-1674.
- Tomotake, H., Shimaoka, I., Kayashita, J., Yokoyama, F., Nakajoh, M., & Kato, N. (2001). Stronger suppression of plasma cholesterol and enhancement of the fecal excretion of steroids by a buckwheat protein product than by a soy protein isolate in rats fed on a cholesterol-free diet. Bioscience, Biotechnology and Biochemistry, 65, 1412-1414.
- Tomotake, H., Shimaoka, I., Kayashita, J., Nakajoh, M., & Kato, N. (2002). Physicochemical and functional properties of buckwheat protein product. Journal of Agricultural and Food Chemistry, 50, 2125-2129.
- Zheng, G. H., Sosulski, F. W., & Tyler, R. T. (1998). Wet-milling, composition and functional properties of starch and protein isolated from buckwheat groats. Food Research International, 30, 493-502.

Figure Captions and Table legends.

Figure 1. Simplified flow chart for the production of buckwheat protein products.

Figure 2. SDS-PAGE profiles of various buckwheat protein products (BWPs) obtained by various processing. Lane 1, 2, 3, 4, 5 and 6 indicate FM-BWP, DFU-BWP, FU-BWP, SU-BWP, DSU-BWP and DSU-BWP, respectively; M indicates the protein markers.

Figure 3. Protein solubility profiles for spray-dried (A) and freeze-dried (B) buckwheat protein products and soy protein isolate as a function of pH. Vertical bars show standard deviations from three determinations.

Figure 4. Emulsifying activity index (EAI) of spray-dried (A) and freeze-dried (B) buckwheat protein products and soy protein isolate (SPI) as a function of pH. Vertical bars show the standard deviations from three determinations.

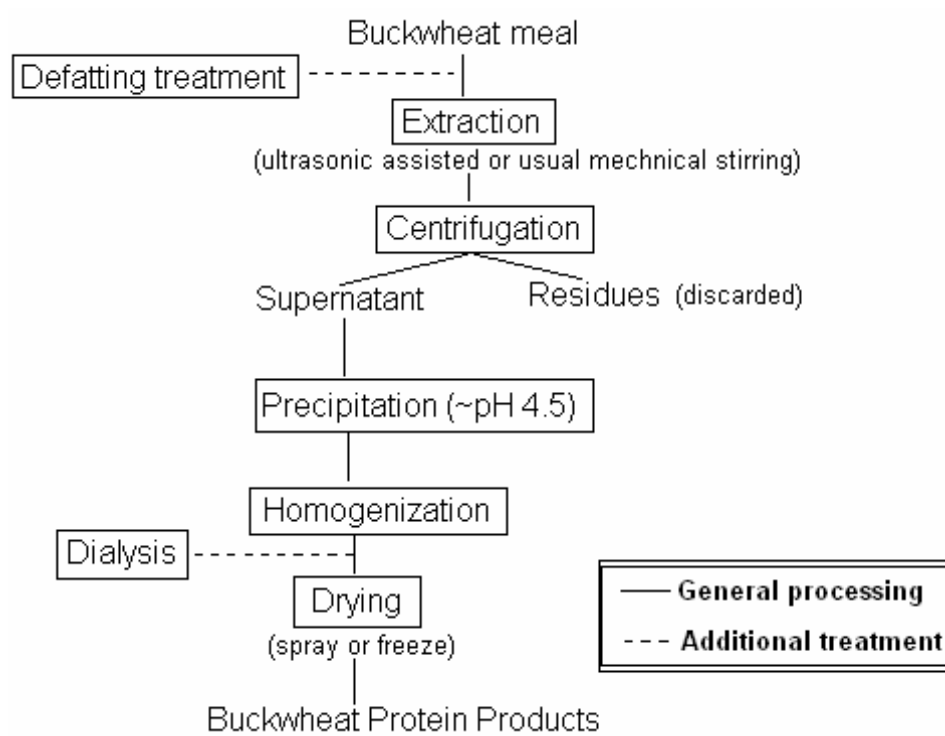
Figure 5. Emulsion stability index (ESI) of spray-dried (A) and freeze-dried (B) buckwheat protein products and soy protein isolate (SPI) as a function of pH. Vertical bars show the standard deviations from three determinations.

Figure 6. Typical profiles for the % nitrogen release during pepsin and trypsin digestion of FM-BWP and SPI.

Table 1. The chemical compositions of buckwheat flour and protein products obtained by different processing. Values are means and standard deviations ($n=2$).

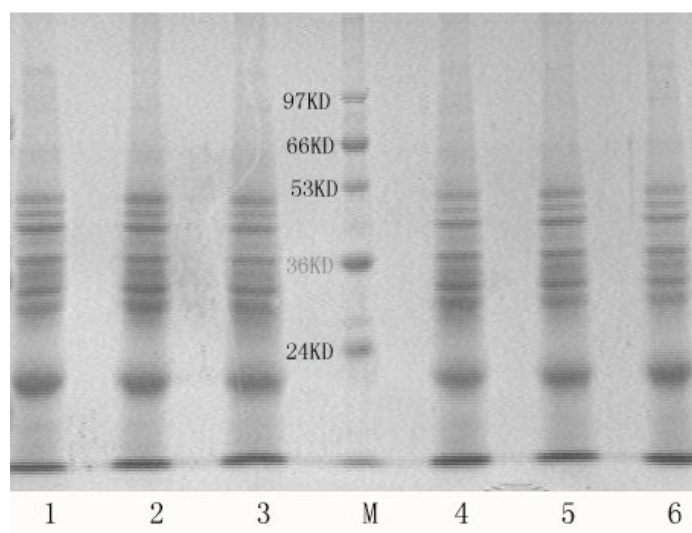
Table 2. Water holding and fat absorption capacities and *in vitro* pepsin (and trypsin) digestibility of buckwheat protein products and soy protein isolates. Values are means and standard deviations ($n=3$).

485 Fig. 1.



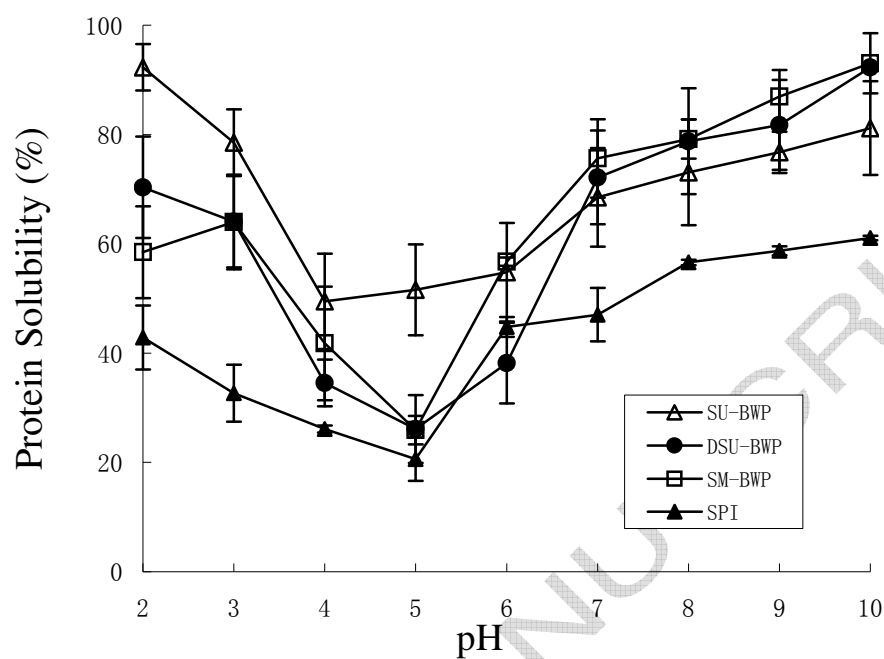
486

487 Fig. 2.

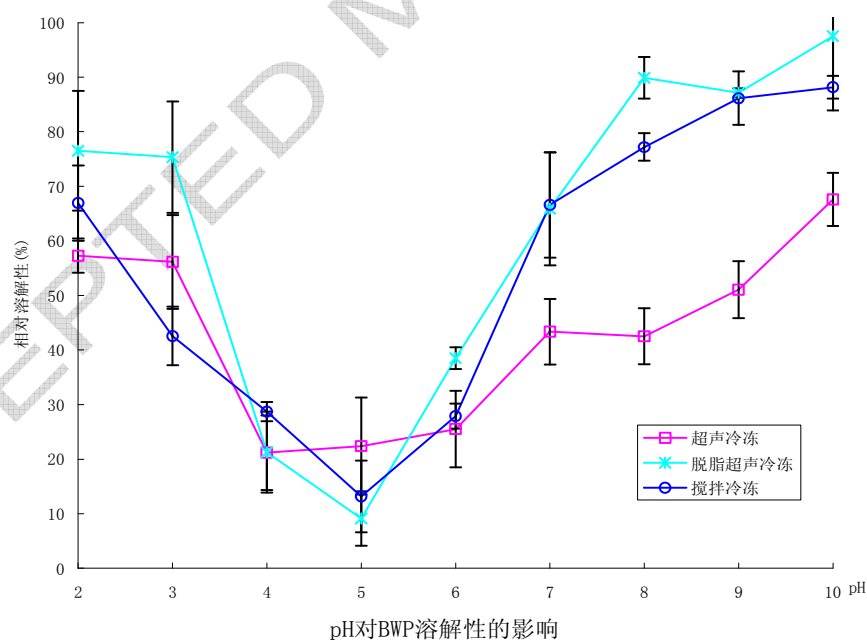


488

489 Fig. 3 (A)

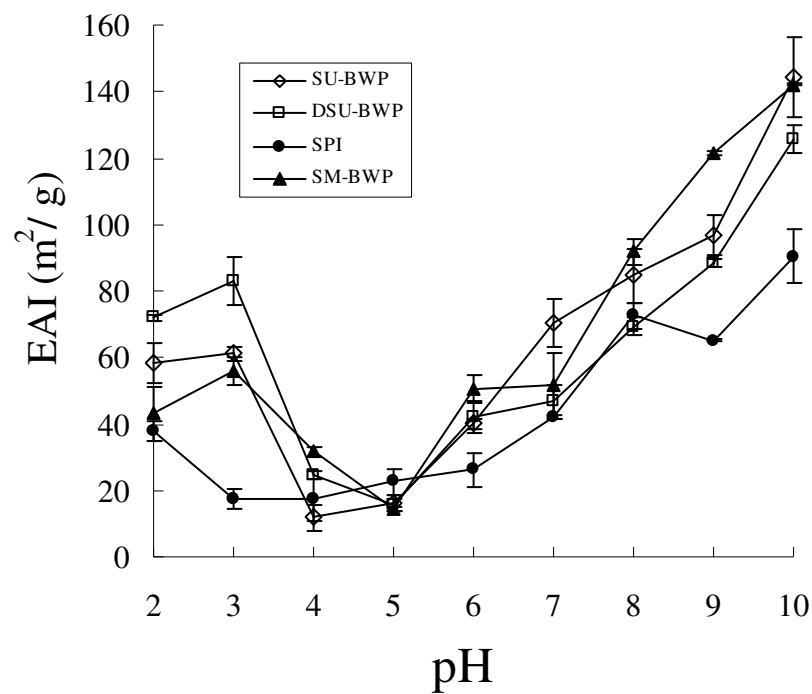


490
491 Fig. 3 (B)

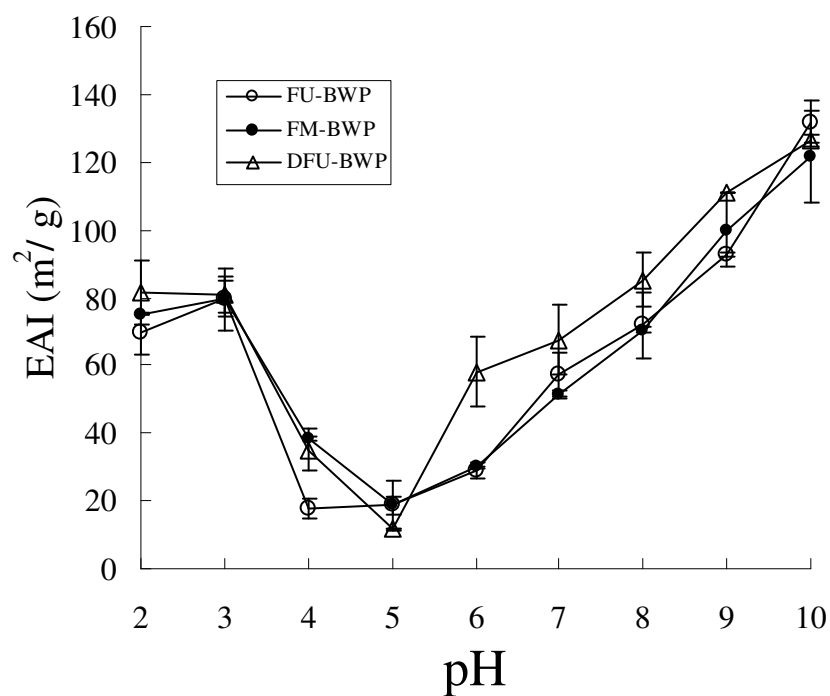


492

493 **Fig. 4 (A)**

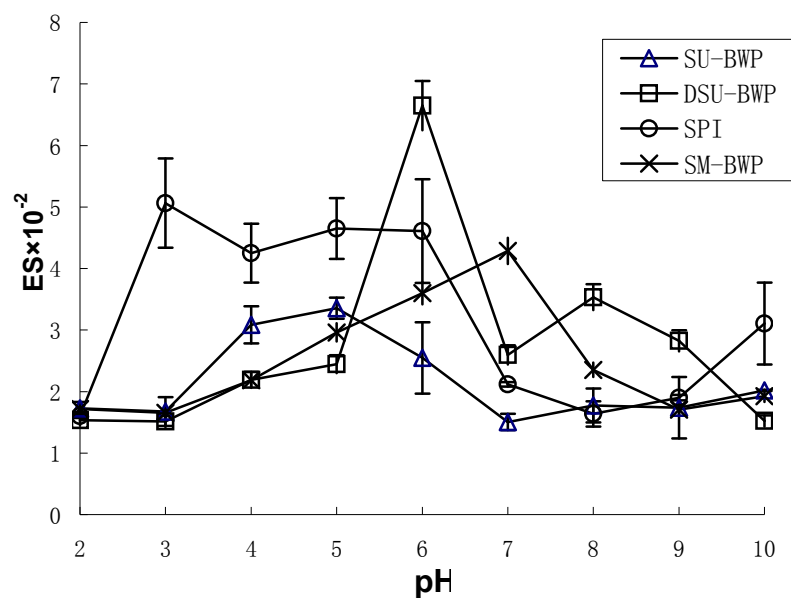


494 **Fig. 4 (B)**

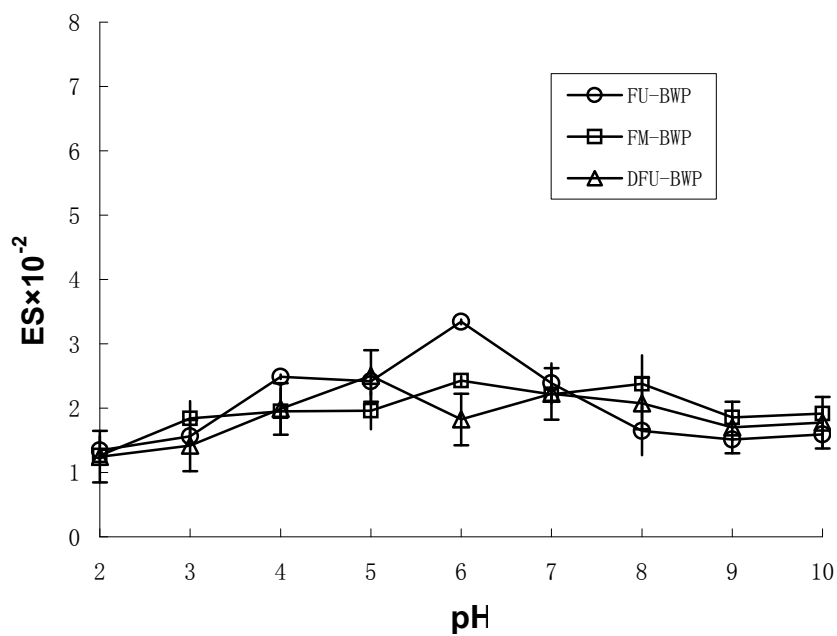


496

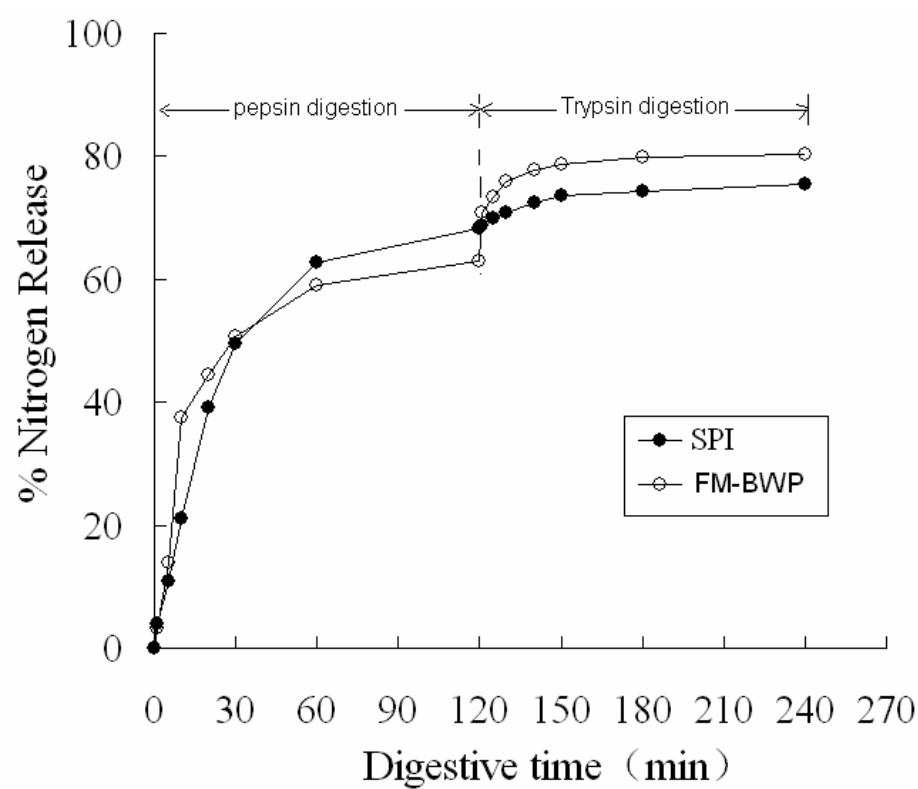
497 **Fig. 5 (A)**



498 **Fig. 5 (B)**
499



501 Fig. 6.



502

503 **Table 1.**

Processing/ Protein products ¹		Compositions of Buckwheat protein products (wet basis, % w/w)			
		Protein ²	Lipids	Moisture	Ash
The buckwheat flour		12.7±0.1	3.0±0.1	13.1±0.1	1.6±0.1
Spray-drying	SM-BWP	80.5±0.7 ^{bc}	5.5±0.5 ^c	6.3±0.1 ^a	2.6±0.2 ^a
	SU-BWP	65.1±0.2 ^e	17.9±2.1 ^a	4.2±0.1 ^d	2.4±0.1 ^a
	DSU-BWP	80.6±0.1 ^c	2.3±0.4 ^d	6.3±0.1 ^a	2.1±0.1 ^b
Freeze-drying	FM-BWP	81.4±0.3 ^b	6.5±0.2 ^b	4.5±0.1 ^c	1.0±0.1 ^c
	FU-BWP	66.1±0.4 ^d	17.8±0.2 ^a	3.9±0.1 ^d	0.8±0.2 ^c
	DFU-BWP	82.3±0.3 ^a	2.5±0.2 ^d	5.1±0.1 ^b	0.8±0.1 ^c

504 Different superscript characters (a-e) indicate significant ($P < 0.05$) difference in the same column.

505 ¹ SM-BWP, indicates the buckwheat protein product (BWP) after usual mechanical extraction and
 506 spray-drying processing; SU-BWP, the BWP after ultrasonic-assisted extraction and spray-drying
 507 processing; DSU-BWP, the BWP after ultrasonic-assisted extraction and spray-drying processing, plus the
 508 de-fatting pretreatment; FM-BWP, the BWP after usual mechanical extraction and freeze-drying
 509 processing; FU-BWP, the BWP after ultrasonic-assisted extraction and freeze-drying processing;
 510 DFU-BWP, the BWP after ultrasonic-assisted extraction and freeze-drying processing plus the de-fatting
 511 treatment.

512 ² Determined by Kjeldahl method, $N \times 6.25$.

513 **Table 2.**

Processing/ Protein products		WHC (g/g of sample)	FAC (g/g of sample)	<i>in vitro</i> Digestibility (% N release)	
				Pepsin ¹	Pepsin + trypsin ²
Spray-Drying	SM-BWP	2.98 ± 0.08 ^{bc}	1.63 ± 0.04 ^c	56.3 ± 2.1 ^c	73.5 ± 0.8 ^b
	SU-BWP	2.77 ± 0.01 ^c	1.60 ± 0.12 ^c	58.5 ± 1.7 ^b	74.2 ± 2.3 ^b
	DSU-BWP	2.27 ± 0.05 ^e	1.58 ± 0.08 ^c	64.6 ± 2.8 ^{ab}	81.3 ± 1.5 ^a
Freeze-Drying	FM-BWP	3.21 ± 0.05 ^b	5.41 ± 0.44 ^a	62.3 ± 2.2 ^b	80.3 ± 1.9 ^a
	FU-BWP	3.17 ± 0.20 ^b	4.09 ± 0.68 ^b	50.1 ± 1.3 ^d	72.5 ± 1.0 ^b
	DFU-BWP	2.42 ± 0.07 ^d	5.03 ± 0.49 ^b	62.6 ± 1.5 ^b	79.6 ± 1.2 ^a
Control (SPI)		4.09 ± 0.20 ^a	1.50 ± 0.06 ^{cd}	68.0 ± 1.2 ^a	75.2 ± 1.7 ^b

514 Different superscript characters (a-e) indicate significant ($P < 0.05$) difference in the same column.

515 ¹ The % N release of protein after pepsin digestion for 120 min.

516 ² The % N release of protein after pepsin digestion for 120 min and further trypsin digestion for another

517 120 min.