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ORIGINAL ARTICLE

Chemical composition, molecular weight distribution, secondary structure and effect of NaCl on functional properties of walnut (*Juglans regia L*) protein isolates and concentrates

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Abstract Chemical composition, molecular weight distribution, secondary structure and effect of sodium chloride concentration on functional properties of walnut protein isolates, concentrates and defatted walnut flour were study. Compared with walnut protein concentrates (75.6%) and defatted walnut flour (52.5%), walnut protein isolates contain a relatively high amount of protein (90.5%). The yield of walnut protein isolates and concentrates was 43.2% and 76.6%, respectively. In molecular weight distribution study, Walnut protein isolates showed one peak with molecular weight of 106.33 KDa (100%) and walnut protein concentrates showed four peaks with molecular weight of 16,725 KDa (0.8%),104.943 KDa(63.9%), 7.3 KDa (11.4%), 2.6 KDa (23.9%). The secondary structure of walnut protein isolates was similar to that of walnut protein concentrates, but was differ from that of defatted walnut flour. The addition of sodium chloride (0~1 M) could improve the functionality of walnut protein concentrates, isolates and defatted walnut flour. The maximum solubility, water absorption capacity, emulsifying properties and foaming properties of walnut protein isolates, concentrates and defatted walnut flour were at sodium chloride solutions of 1.0 M, 0.6 M, 0.4 M, 0.6 M, respectively. The solubility of walnut protein concentrates (32.5%) in distilled water with 0 M sodium chloride was lower than that of walnut protein isolates (35.2%). The maximum solubility of walnut protein isolates, concentrates and defatted walnut flour in solution were 36.8%, 33.7% and 9.6% at 1.0 M sodium chloride solutions, respectively. As compared with other vegetable proteins, walnut protein isolates and concentrates exhibited better emulsifying properties and foam stability.

Keywords Walnut protein isolates · Walnut protein concentrates · Functional properties · NaCl concentration

Abbreviations

DWF	Defatted walnut flour
Isolates	Walnut protein isolate
Concentrates	Walnut protein concentrate
EC/ES	Emulsion activity/emulsion stability
FC/FS	Foaming capacity/foaming stability
WAC	Water absorption capacity
NSI	Nitrogen solubility index

Introduction

Plant proteins play a significant role in human nutrition, particularly in developing countries where average protein intake is less than that required. Plant protein products are gaining increased interest as ingredients in food systems throughout many parts of the world and the final success of utilizing plant proteins as additives depends greatly upon the favor characteristics that they impart to foods. The production of plant protein isolates is of growing interest to industry because of the increasing applications of plant proteins in food markets. In recent years, major and minor oilseeds such as soybean (Kinsella 1979; Kumar et al. 2011), cowpea (Aluko and Yada 1995), lupin seed (Pozani et al. 2002), cashew nut (Ogunwolu et al. 2009) and faba

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bean (Krause et al. 1996; Guzel and Sayar 2012) have attracted a great deal of interest as a source of protein to supplement human diets. In oilseed proteins, numerous studies on protein functionality have been reported (Molina Ortiz et al. 2004; Yu et al. 2007; Yoshie-Stark et al. 2008; González-Pérez and Vereijken 2007; Sze-Tao and Sathe 2000a; Igene et al. 2005; Lawal et al. 2007).

Walnuts (*Juglans regia L.*) are widely distributed all over the world, and they are common in China. On a global basis, walnuts rank second behind almonds in tree nut production. In 2010, global production of walnuts was 1,500,000 t. China leads the world production of walnuts, followed by the US. In 2010, China accounted for 33.33% of global walnut production. Moreover, walnut is not only an agricultural commodity, but its leaves, barks, stems, pericarps, fruits, flowers and ligneus membranes are all applied for different medicinal uses in China. These fruits are receiving increasing interest as a healthy foodstuff because their regular consumption has been reported to decrease the risk of coronary heart disease (Blomhoff et al. 2006; Davis et al. 2007; Albert et al. 2002; Sabate et al. 1993; Lavedrine et al. 1999). In view of the increasing production of walnut globally, there is a need for an increased utilize of the walnut, especially the nutritious walnut kernel. The walnut kernel is of high food value with 65% oil and 18–24% protein contents, and it can be used as an adjunct in chocolate and chicken feeds, and also can be utilization as ingredient of many foodstuffs such as bakery products to enhance the nutrition value of products (Mexis et al. 2009). Walnuts oil is major product of walnut production and is one of the important special oils used for salad dressing and cooking (Oliveira et al. 2002). Besides walnut oil and protein, Walnut contain 12~16% carbohydrates, 1.5~2.0% cellulose, 1.7~2.0% mineral (Lavedrine et al. 2000; Prasad 2003; Savage 2001; Sze-Tao and Sathe 2000b; Wardlaw 1999; Gharibzahedi et al. 2011). As a by-product of oil production, walnut protein products are therefore being considered as an additional source of plant protein for use in human food products. Walnuts nutrient composition has been investigated by several investigators (Sze-Tao and Sathe 2000b; Amaral et al. 2003; Savage 2001; Pereira et al. 2008). However, chemical composition and functional properties of walnut protein isolates and concentrates have not been investigated. Functional properties are characteristics of the protein, which are determined by a number of factors: source; procedure employed to obtain seed flours, concentrate and isolates and physiochemical factor like pH, salt and temperature. Factors, such as pH and salts, affect physicochemical properties and interaction between proteins and in turn alter functional properties (Kinsella 1979; Mwasaru et al. 2000; Phillips et al. 1991). NaCl is a principal ingredient in food formulation due to its flavor, preservative and protein-solubilizing properties (Gimeno et al. 1999; Seman et al. 1980). The objective of this study was to determine chemical composition, molecular weight distribution, secondary structure and effect of NaCl on functional properties of walnut protein concentrates and isolates. The investigated functional properties include the water solubility, emulsifying activity, emulsifying stability, foaming capacity and foam stability, water absorption capacity.

Materials and methods

Preparation of defatted walnut flour The defatted walnut flour (DWF) was produced according to the method of Sze-Tao and Sathe (2000b). Walnuts (*Juglans regia L.*) were purchased from Xinjiang supermarket in China. Walnut was ground in a Waring Blender. The flour was defatted with hexane [flour/hexane ratio of 1:10 (w/v)] under constant magnetic stirring for 3 h. The slurry was vacuum filtered through filter paper and the residue was used for subsequent extraction. Hexane extractions were repeated until the filtrate was clear. Residue from the last extraction and filtration step was air dried in a fume hood. DWF was ground to 150 meshes with Waring Blender and stored at −20 °C until further use.

Preparation of protein isolates The walnut protein isolates was prepared according to the process described by Wolf (1970) with minor modifications. Defatted walnut flour (DWF) was extracted by stirred for 2 h at room temperature (about 25 °C) with de-ionized water adjusted to pH 11.0 with 1 M NaOH [flour: water ratio, 1:20(w/v)]. The slurry was centrifuged at 10,000 g for 30 min at 4 °C in a CR22G centrifuge (Hitachi Koki Co.,76 Hitachinake, Japan). The insoluble walnut protein pellet was re-slurried with pH-adjusted de-ionized water as above and centrifuged again. The supernatants mixed together were adjusted to pH 4.5 (isoelectric point) kept for 2 h at 4 °C with 1 M HCl and subsequently centrifuged at 10,000 g for 30 min at the same temperature. The precipitate was washed with de-ionized water, resolubilized in de-ionized water, neutralized to pH 7 with 1 M NaOH at room temperature, then dialyzed against water and lyophilized. All lyophilized protein samples were stored in airtight plastic bottles at −20 °C until further use.

Preparation of protein concentrates The walnut protein concentrate was prepared according to the process described by Wolf (1970) with minor modifications. The first procedure was washing defatted walnut flour with 95% aqueous alcohol (1:20, w/v) and stirred for 1 h at ambient temperature (about 25 °C). The suspension was vacuum filtered through filter paper and the residue was air dried in a fume hood. Then the residues were dispersed in de-ionized water

(1:20, w/v) at room temperature and the pH of the dispersion was adjusted to 4.5 (isoelectric point) by the addition of 1 N HCl and was stirred using a magnetic stirrer for 2 h. The slurry was then centrifuged (10,000*g*, 30 min, 4 °C). The precipitate was washed with de-ionized water, resolubilized in de-ionized water, neutralized to pH 7.0 with 1 M NaOH at room temperature, then dialyzed against water and lyophilized. All lyophilized protein samples were stored in airtight plastic bottles at −20 °C until further use.

Analytical methods Moisture, fat and ash contents were determined according to the methods of AOAC (2000), numbers 950.46, 960.39 and 920.153, respectively. The protein content of sample was determined by the micro-Kjeldhal method (AOAC 2000) through the use of the protein-nitrogen coefficient of 5.30 (Sze-Tao and Sathe 2000b). Carbohydrates were determined according to the method of Zhu et al. (2006). The contents were expressed on a dry weight basis.

Molecular weight distribution by SEC-HPLC The molecular weight distribution was determined by High performance size exclusion chromatography (SEC-HPLC). Walnut protein isolates (5 mg mL^{-1}) were extracted by sodium phosphate buffer (0.05 M, pH 8.0) containing sodium chloride (0.3 M) for 4 h at 25 °C under constant magnetic stirring and then were centrifuged at 10,000 g for 10 min (25 °C). The supernatant was filtered through a cellulose acetate membrane with a pore size of 0.45 μm (Sartorius Co, Ltd, Gottingen, Germany). A Waters 2690 liquid chromatogram system (Waters Chromatography Division, Milford, MA, USA) equipped with a Shodex protein KW-804 column (Shodex Separation and HPLC Group, Tokyo, Japan) and a Waters 996 photodiode array detector was used to determine the molecular weight distribution. The flow rate was 1 mL min^{-1} using phosphate buffer (0.05 mol L^{-1}, 0.3 mol L^{-1} NaCl, pH 7.0) as the mobile phase. About 10 μL protein solutions were injected into the column and the eluent was monitored at 280 nm. All samples were measured in triplicate and the representative examples were selected for discussion. A calibration curve of 10 standard proteins was used for interpreting the results.

Circular dichroism (CD) spectra measurement CD spectra were scanned at the far-UV range (200–250 nm) with a CD spectropolarimeter (Jasco J-715, Jasco Corp., Tokyo, Japan) in a 0.1 cm quartz CD cuvette (Hellma, Muellheim, Baden, Germany) at 25 °C. The protein concentration for CD analysis was 50 mg mL^{-1}. Distilled water that was used to dissolve walnut protein isolates was used as blank solution for all of the samples. The values of scan rate, response, bandwidth, and step resolution were 100 nm min^{-1}, 0.25 s, 1.0 nm, and 0.2 nm, respectively. Five scans were averaged to obtain one spectrum. The CD data were expressed in terms of mean molar ellipticity [θ] (mdeg).

Protein solubility in water This was determined according to the modified methods of Rodriguez-Ambriz et al. (2005). In summary, protein sample (200 mg) were dispersed in 20 mL of de-ionized water and the mixture was also prepared in different concentrations (0~1.0 M) of sodium chloride solutions. The solution was stirred at room temperature for 30 min and centrifuged at 8,000 g for 20 min. Protein contents in the supernatant were determined using the Bradford method (1976) (Bradford 1976). All experiments were performed as triplicate determinations and the mean values and corresponding standard deviations were reported. Protein solubility was then calculated as follows:

$$\text{Solubility } \% = \frac{\text{protein content in supernatant}}{\text{total protein content in sample}} \times 100$$

Emulsifying properties Emulsifying activity(EA)was determined according to Pedroche et al. (2004) with modifications. Samples (1.0 g) were dispersed in 25 mL deionised water at room temperature (about 25 °C). And the mixture was also prepared in different concentrations (0~1.0 M) of sodium chloride solutions. This protein solution was mixed with 25 mL of soybean oil and then was homogenised at a speed of 8,000 g for 1 min. The emulsion was then centrifuged at 1,300 g for 5 min. Emulsifying activity was expressed as follows:

$$\text{Emulsifying activity } \% = \frac{\text{Height of emulsified layer}}{\text{Height of the contents of the tube}} \times 100$$

Emulsion stability (ES) was measured by re-centrifugation following heating at 80 °C for 30 min and was expressed as follows:

$$\text{Emulsion stability } \% = \frac{\text{Height of remaining emulsion layer}}{\text{Height of original emulsified layer}} \times 100$$

Foaming properties Foaming capacity (FC) and stability (FS) were based on the method described by Ogunwolu et al. (2009) with minor modification. Protein samples (500 mg) were dispersed in 50 mL of de-ionized water. And then the mixture was also prepared in different concentrations (0~1.0 M) of sodium chloride solutions. The solutions were stirred at a speed of 10,000 g for 2 min. The blend was immediately transferred into a 100 mL graduated cylinder. The volume was recorded before and after stirring. FC was expressed as the volume (%) increased due to stirring. For the determination of FS, foam volume changes in the graduated cylinder were recorded at 30 min of

storage. Foam capacity and foam stability were then calculated according to the following formulae:

$$\text{Foam capacity \%} = \frac{(\text{volume after whipping} - \text{volume before whipping})\text{ml}}{(\text{volume before whipping})\text{ml}} \times 100$$

$$\text{Foam stability \%} = \frac{(\text{volume after standing} - \text{volume before whipping})\text{ml}}{(\text{volume before whipping})\text{ml}} \times 100$$

Water absorption capacity Water absorption capacity (WAC) was determined using the method described by Rodriguez-Ambriz et al. (2005) with minor modification. Sample (1 g) was weighed into 15 mL pre-weighed centrifuge tube. Then sodium chloride solutions (10 mL, 0–1.0 M NaCl dissolved in de-ionized water) were added in small increments to the tube under continuous stirring with a glass rod. After being held at room temperature (about 25 °C) for 30 min, the tube was centrifuged at 2,000 g for 20 min. In the end, the amount of added de-ionized water resulting in the supernatant liquid in the test tube was recorded. WAC (grams of water per gram of sample) was calculated as:

$$\text{WAC} = \frac{W_2 - W_1}{W_0}$$

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).

Statistical analysis All analyses were done in triplicate, and data are reported as means ± standard deviation. Where appropriate, data were analyzed for significance using analysis of variance and Fisher's least significant difference (LSD at a 5% significance level) by General Linear Model of SPSS (Software version 11.0, SPSS, Chicago, Illinois).

Results and discussion

Chemical characterization of walnut protein isolates and concentrates The proximate composition of defatted walnut flour (DWF), walnut protein isolates and concentrates are showed in Table 1. DWF contained 1.8% fat, indicating that defatted procedure could reduce the fat content of samples effectively. Protein content of DWF, walnut protein isolates and concentrates were significantly different ($p < 0.05$). Compared with walnut protein concentrates (75.6%) and DWF (52.5%), walnut protein isolates contain a relatively high amount of protein (90.5%). The difference in protein content for walnut protein isolates and concentrates may be attributed to the extraction method used. The yield of walnut protein isolates and concentrates was 43.2% and 76.6%, respectively. Results indicated that the alkaline extraction-isoelectric precipitation method can improve the protein content of walnut protein products better than isoelectric precipitation process. It was suggested that walnut protein concentrates and isolates could be considered as an additional source of plant protein in food products.

High performance size exclusion chromatography The size exclusion chromatogram using a high-performance liquid chromatogram system was used to study molecular weight distribution of walnut proteins and the results are shown in Fig. 1a–d. Molecular weight was estimated from the calibration curve of standard protein for the column (Fig. 1a). DWF showed four peaks with the retention time around 5.82 min,9.83 min,12.29 min,19.72 min, corresponding to the molecular weight of 18,824 KDa (2.1%), 96.99 KDa (48.6%), 3.83 KDa (43.8%) (Fig. 1b). Compared with walnut protein polypeptides molecular weights reported by Sze-Tao and Sathe (2000b), the peak of 18,824 KDa may be due to the protein aggregation. In addition, extensive disulfide cross-linking may lead to aggregation (Hamada 1997). Walnut protein isolates showed one peak with molecular weight of 106.33 KDa (100%) (Fig. 1c). Walnut protein concentrates showed four peaks with molecular weight of 16,725 KDa (0.8%),104.943 KDa(63.9%), 7.3 KDa (11.4%), 2.6 KDa (23.9%),respectively (Fig. 1d). Compared with walnut protein isolates, the protein composition of walnut protein concentrates and DWF were more complex with the protein aggregation. Under alkali conditions, sulfhydryl and disulfide bond interchange is more favored and NaOH must have broken some of hydrogen and disulfide bonds that might have reduced its molecular size and aggregation. So, the high molecular weight fraction is absent in walnut protein isolates.

Circular dichroism spectra CD spectra are remarkably sensitive to the secondary structures of proteins. Far-UV CD spectra of the DWF, walnut protein isolates and concentrates are shown in Fig. 2a–c, respectively. Far-UV CD spectra of DWF are different from walnut protein isolates and concentrates. DWF was calculated composed of 80.4% α-helix, 4% β-sheet, 5.7% β-turn and 15.3% random coil by the computer program. And the secondary structure estimation of walnut protein isolates revealed 34.9% α-helix, 11% β-sheet, 23.3% β-turn and 32% random coil, which is similar to that of walnut protein concentrates. Walnut protein isolates and concentrates contained more β-turn, random coil and less α-helix than DWF, which suggested that walnut protein isolates and concentrates have less ordered secondary structure during the extraction process. The nitrogen solubility index (NSI) of walnut protein isolates and concentrates DWF

Table 1 Chemical composition of walnut protein isolates, concentrates and defatted walnut flour (DWF)

Materials	Protein (%)	Fat (%)	Ash (%)	Moisture (%)	Carbohydrate (%)	NSI[b] (%)	Yield (%)
DWF[a]	52.5±0.33c	1.8±0.12a	1.9±0.02	9.2±0.02a	34.6±0.16a	4.9±0.12c	
Isolates	90.5±0.45a	0.23±0.04b	2.3±0.03	4.5±0.03b	2.5±0.03c	29.1±0.23a	43.2
Concentrates	75.6±0.35b	0.27±0.06b	2.0±0.02	4.5±0.04b	17.7±0.07b	28.2±0.18b	76.6

Results represent the average of three determinations ±SD, values in the same column with different letters are significantly different ($p< 0.05$) ($n=3$)

[a] *DWF* defatted walnut flour

[b] *NSI* nitrogen solubility index

were significantly different ($p< 0.05$) from one another. So, the higher NSI of walnut protein isolates and concentrates may be another reason responsible for the less ordered second structure.

Water solubility Effects of NaCl concentration on protein solubility of walnut protein isolates and concentrates and walnut defatted flour (DWF) are presented in Fig. 3a. For three walnut protein samples, the results showed that there was a decrease in protein solubility as NaCl concentration increased from 0 M to 0.1 M. However, beyond this concentration, the protein solubility of these three protein samples was increased. The maximum solubility of walnut protein isolates, concentrates and DWF in solution were 36.8%, 33.70% and 9.6% at 1.0 M sodium chloride solutions, respectively. Among these three protein samples, walnut protein isolates showed the highest solubility at different sodium chloride concentrations. Protein solubility is known to increase with moderately increasing salt concentrations, due to the salting-in effect. The effective mechanisms of

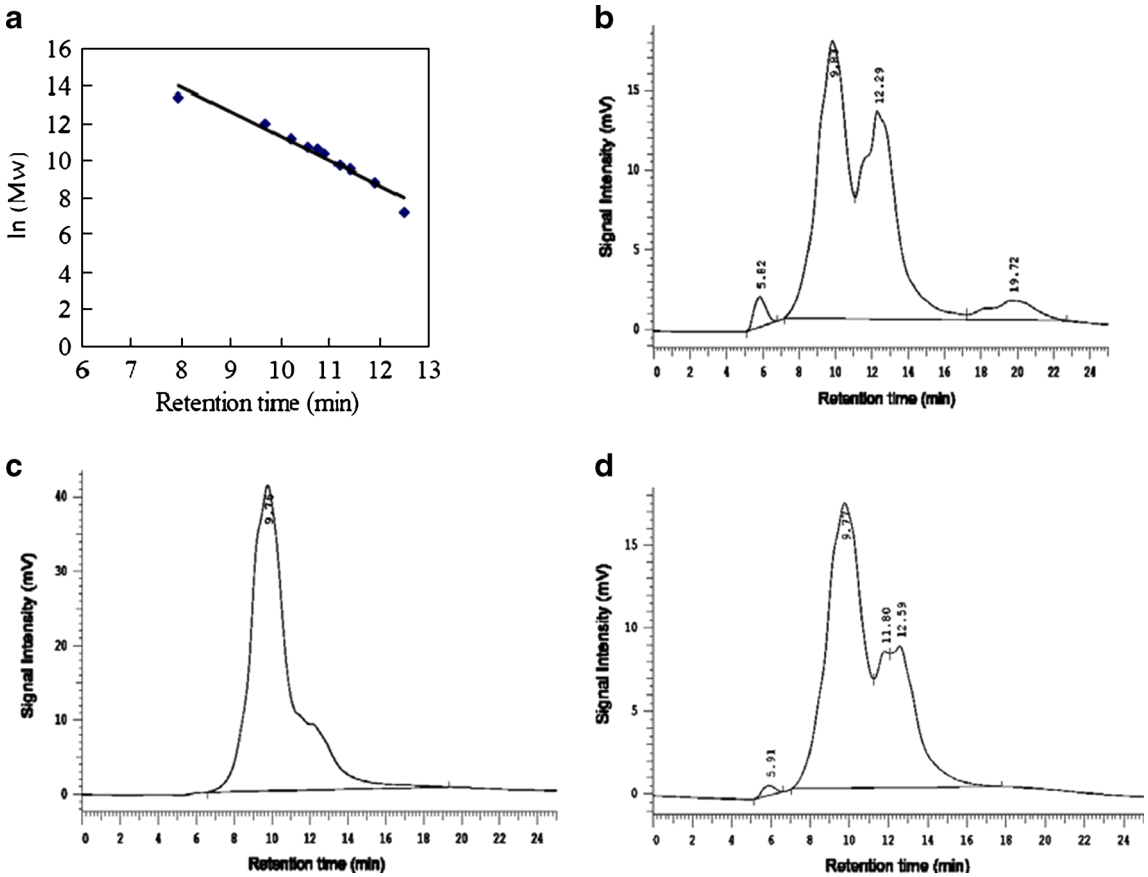

Fig. 1 High performance size exclusion chromatography (SEC-HPLC) profiles of defatted walnut flour (DWF), Isolates and Concentrates. **a** The calibration curve of standard proteins; **b** DWF; **c** Isolates; **d** Concentrates; A calibration curve of 10 standard proteins was used for interpreting the results. Ten standard proteins were thyroglobulin (MW: 669, 000), aldolase (MW: 158,000), BSA (MW: 67,000), ovalbumin (MW: 43,000), peroxidase (MW: 40,200), adenylate kinase (MW: 32, 000), myoglobin (MW: 17,000), ribonuclease A (MW: 13,700), aprotinin (MW: 6500), and vitamin B12 (MW: 1350), respectively. *(n=3)*

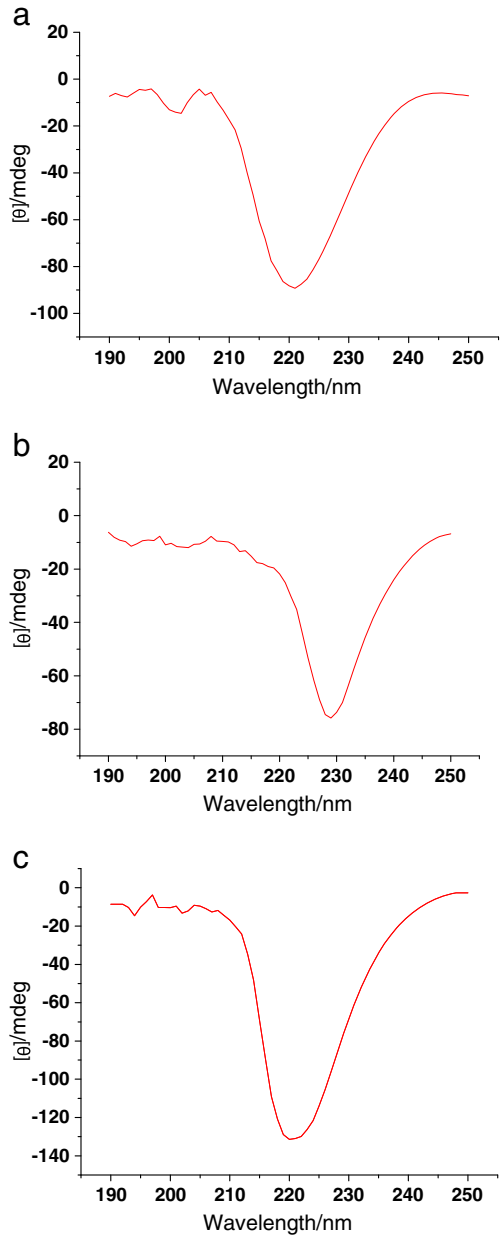

Fig. 2 **a** Far-UV circular dichroism spectra of DWF. **b** Far-UV circular dichroism spectra of Isolates. **c** Far-UV circular dichroism spectra of Concentrates. (n=3)

ionic strength on protein solubility include electrostatic interactions, solvent effect, salting-in and salting-out effect (Davis et al. 2007). In comparison with other research reports, the solubility of walnut protein isolates (35.2%) in distilled water with 0 M sodium chloride was higher than that of *Brassica carinata* protein isolates (32%) (Pedroche et al. 2004), sesame protein isolate (33%) (Khalid et al. 2003) and cowpea protein isolate (32%) (Ragab et al. 2004). However, the solubility of walnut protein concentrates (32.5%) in distilled water with 0 M sodium chloride was lower than that of walnut protein isolates. The chemical composition and isolated method contributed to the difference of walnut protein isolates and concentrates on solubility.

Emulsifying properties Another factor that plays a role in protein-emulsifying properties is salt presence. Sodium chloride affected the protein emulsifying properties mainly by two mechanisms: (1) salts reduce the electrostatic repulsion between droplets through electrostatic screening and (2) high concentrations of electrolytes alter the structural organization of water molecules, which alters the strength of the hydrophobic interactions between non-polar groups (McClements 1999). The effects of NaCl concentration on emulsifying activity (EA) and emulsion stability (ES) of walnut protein isolates, concentrates and walnut defatted flour (DWF) are shown in Fig. 3c–d. The results showed that EA and ES of walnut protein isolates, concentrates and DWF increased in the range 0–0.4 M sodium chloride. Beyond this salt concentration (0.4–1.0 M), EA and ES gradually decreased due to the salting effect of sodium chloride. Chobert et al. (1987) reported similar results. In 0.4 M sodium chloride, the highest EA values of walnut protein isolates, concentrates and DWF were observed (60.2%, 63.6% and 60.8%, respectively), which increased by 9.3%, 11.3% and 7.1% compared with that at 0 M sodium chloride. With 0 M sodium chloride, EA values of walnut protein isolates, concentrates and DWF was 50.9%, 52.3% and 52.7%, respectively, which were comparable to cowpea protein isolate (Ragab et al. 2004), sesame protein isolate (Khalid et al. 2003). This is in agreement with Kinsella et al. (1985), who stated that the emulsifying capacity of proteins tends to decrease as protein concentration is increased, and this is also consistent with the similar reported observations on winged bean protein concentrate (Sathe et al. 1982), and sunflower protein isolate (Lin et al. 1974). The change in ES was similar to that of EA, except that ES value of walnut protein isolates was higher than that of walnut protein concentrates, which was higher than that of DWF at different sodium chloride concentrations (0–1.0 M). This was in agreement with the general correlation between ES and nitrogen solubility found in previous studies (Crenwelge et al. 1974; Hung and Zayas 1991). Various factors, including pH, droplet size, net charge, interfacial tension, viscosity and protein conformation, could affect the values of ES (Hung and Zayas 1991). And the effective concentration of protein was positively related to emulsion stability; while the sodium chloride had salting-in and salting-out effects on protein, the effective concentrations of protein at both high and low sodium chloride concentrations were completely opposite, resulting in similar effects on ES. The increase in ES resulting from a low sodium chloride concentration might have been achieved through formation of charged layers around the fat globules, resulting in mutual repulsion and/or formation of a hydrated

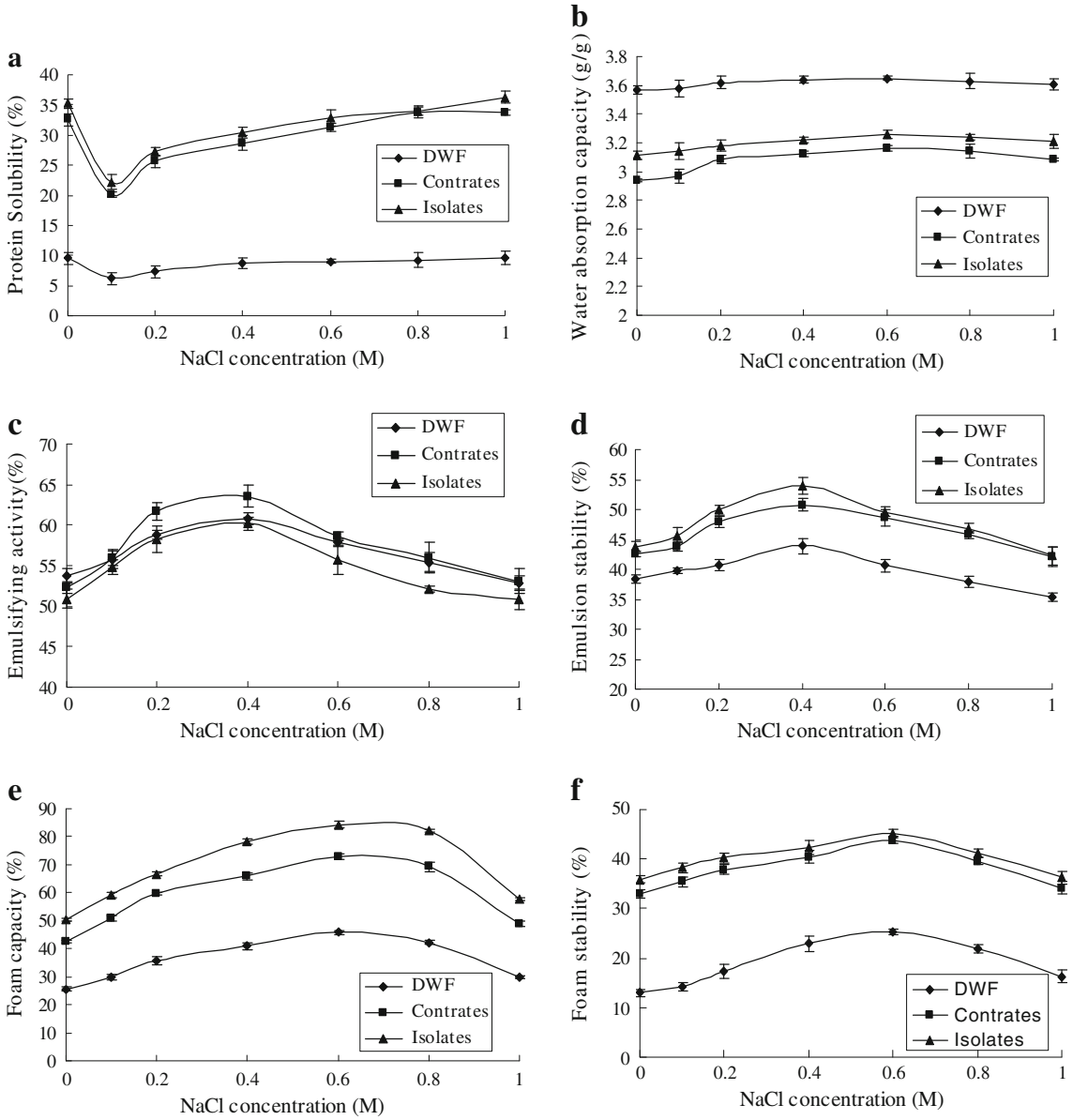

Fig. 3 Effects of NaCl concentration on physico-chemical characteristics of walnut protein isolates, concentrates and defatted walnut flour (DWF) (n=3)

layer around the interfacial material, factors which lower interfacial energy and retard droplet coalescence (Albert et al. 2002).

Foaming properties The effects of salt concentration on foam capacity(FC)and foam stability(FS)of three walnut proteins (walnut protein isolates, concentrates and DWF) are shown in Fig. 3e–f. The results showed that FC and FS of three walnut proteins increased as the salt concentration increased from 0 to 0.6 M, and then decreased from 0.6 to 1.0 M. Among these three protein samples, FC and FS of walnut protein isolates was higher than that of walnut protein concentrates, which was higher than that of DWF, and they were all significantly different ($p< 0.05$) from one another. Also, the initial increase in salt concentration, up to 0.6 M, enhanced FC and FS of walnut protein isolates and concentrates and DWF, after which further increase in salt concentration from 0.6 M to 1.0 M reduced FC and FS progressively. By contrast, walnut protein isolates showed higher FC and FS values in distilled water than that of sesame protein isolate (Khalid et al. 2003) and cowpea protein isolate (Ragab et al. 2004). Initial increase in foaming properties might be attributed to increase in protein solubility at these salt concentrations. Meanwhile, this may be attributed to the fact that addition of sodium chloride, at a concentration up to 0.6 M, enhances the protein solubility by weakening the hydrophobic interaction of the protein while high salt concentration had an adverse effect on FC

due to the salting effect of sodium chloride. To exhibit good foaming, a protein must be capable of migrating at the air–water interface, unfolding and rearranging at the interface (Hailing and Walstra 1981). The foam capacity and stability were enhanced by greater protein concentration, because this increases the viscosity and facilitates the formation of a multilayer, cohesive protein film at the interface (Damodaran 1997).

Water absorption capacity The effects of salt concentration on water absorption capacity (WAC) of three walnut proteins (walnut protein isolates, concentrates and DWF) are shown in Fig. 3b. The water absorption capacity (WAC) of three walnut proteins showed that DFW was found to possess the highest WAC of 3.6 $g\,g^{-1}$ compared with that of Isolate-B (2.9 $g\,g^{-1}$) and with that of Isolate-A (3.1 $g\,g^{-1}$) in distilled water (with 0 M NaCl). It suggested that there was no direct correlation between solubility and WAC of protein. High protein solubility did not necessarily mean high WAC and this result was consistent with the other studies (Hermansson 1979; Prinyawiwatkul et al. 1997). Carbohydrates contain hydrophilic parts, such as polar or charged side chains, which can enhance WAC (Jitngarmkusol et al. 2008). Moreover, the differences in WAC between Isolate-A and Isolate-B can be attributed to the denatured proteins which can bind more water through exposure of hydrophilic groups (Davis et al. 2007). WAC value of DWF (3.57 $g\,g^{-1}$) was higher than 3.55 $g\,g^{-1}$ of commercial soy protein isolate (SPI) reported by Ke-Xue Zhu et al. (2010). High WAC of DWF makes it a potential ingredient in meat, bread, and cakes industries.

WAC values of walnut protein isolates, concentrates and DWF increased with sodium chloride concentration in the range 0–0.6 M, and then decreased from 0.6 M to 1.0 M. Among these three protein samples, WAC of DWF was higher than that of walnut protein isolates, which was higher than that of walnut protein concentrates, and they were all significantly different ($p < 0.05$) from one another. At low salt concentration, hydrated salt ions binding weakly to charged groups of protein did not affect the hydration shell of charged groups of the protein; at high salt concentration, much of the existing water was bound to salt ions; meanwhile the intermolecular interactions of proteins were strengthened and this caused dehydration of protein and reduction in WAC (Lawal et al. 2005).

Conclusion

This study has shown that the molecular weight distribution of walnut protein isolates, concentrates and defatted walnut flour are differently, which was due to the differ physicochemical characteristics of walnut protein concentrates, isolates and defatted walnut flour. Because of alkali conditions extraction, molecular weight distribution of walnut protein isolates was single relatively without protein aggregation than walnut protein concentrates and defatted walnut flour. The secondary structure of walnut protein isolates and concentrates were similar and different from that of defatted walnut flour, which suggested that walnut protein isolates and concentrates have less ordered secondary structure during the extraction process. In addition, the higher NSI of walnut protein isolates and concentrates may be another reason responsible for the less ordered second structure. The addition of sodium chloride could improve the functionality of walnut protein concentrates, isolates and defatted walnut flour. The maximum solubility, water absorption capacity (WAC), emulsifying properties and foaming properties of walnut protein isolates, concentrates and defatted walnut flour were at sodium chloride solutions of 1.0 M, 0.6 M, 0.4 M, 0.6 M, respectively. As compared with other vegetable proteins, walnut protein isolates and concentrates exhibited better emulsifying properties and foam stability. By contrast, walnut protein isolates had higher values of ES, FC, FS, WAC than that of walnut protein concentrates, which was significantly correlated with the chemical components and extracted method. The results revealed that walnut protein isolates and concentrates with suitable functional properties could be produced from the defatted walnut flour as a good protein ingredient in food systems. Meanwhile, it suggested that walnut protein isolates and concentrates can be considered as an additional source of protein concentrates and isolates for use in human food products.

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References

Albert CM, Gaziano JM, Willett WC, Manson JAE (2002) Nut consumption and decreased risk of sudden cardiac death in the Physicians' Health Study. Arch Intern Med 162(12):1382–1387

Aluko R, Yada R (1995) Structure-function relationships of cowpea (Vigna unguiculata) globulin isolate: influence of pH and NaCl on physicochemical and functional properties. Food Chem 53 (3):259–265

Amaral JS, Casal S, Pereira JA, Seabra RM, Oliveira BP (2003) Determination of sterol and fatty acid compositions, oxidative stability, and nutritional value of six walnut (Juglans regia L.) cultivars grown in Portugal. J Agric Food Chem 51(26):7698–7702

AOAC (2000) Official methods of analysis of AOAC International, 17th edn. AOAC International, Gaithersburg

Blomhoff R, Carlsen MH, Andersen LF, Jacobs DR (2006) Health benefits of nuts: potential role of antioxidants. Br J Nutr 96(S2):S52–S60

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72(1–2):248–254

Chobert JM, Bertrand-Harb C, Nicolas MG, Gaertner HF, Puigserver AJ (1987) Solubility and emulsifying properties of caseins chemically modified by covalent attachment of L-methionine and L-valine. J Agric Food Chem 35(5):638–644

Crenwelge D, Dill C, Tybor P, Landmann W (1974) A comparison of the emulsification capacities of some protein concentrates. J Food Sci 39(1):175–177

Damodaran S (1997) Food proteins and their applications. In: Damodaran S, Paraf A (eds) Food proteins: an overview. Marcel Dekker, New York, pp 1–21

Davis L, Stonehouse W, du Loots T, Mukuddem-Petersen J, van der Westhuizen FH, Hanekom SM, Jerling JC (2007) The effects of high walnut and cashew nut diets on the antioxidant status of subjects with metabolic syndrome. Eur J Nutr 46(3):155–164

Gharibzahedi SMT, Mousavi SM, Hamedi M, Khodaiyan F (2011) Determination and characterization of kernel biochemical composition and functional compounds of Persian walnut oil. Journal of Food Science and Technology http://www.springerlink.com/content/07532j13544rn811/ (Accessed on 13th, November, 2011)

Gimeno O, Astiasarán I, Bello J (1999) Influence of partial replacement of NaCl with KCl and CaCl2 on texture and color of dry fermented sausages. J Agric Food Chem 47(3):873–877

González-Pérez S, Vereijken JM (2007) Sunflower proteins: overview of their physicochemical, structural and functional properties. J Sci Food Agr 87(12):2173–2191

Guzel D, Sayar S (2012) Effect of cooking methods on selected physicochemical and nutritional properties of barlotto bean, chickpea, faba bean, and white kidney bean. J Food Sci Technol 49(1):89–95

Hailing PJ, Walstra P (1981) Protein stabilized foams and emulsions. Crit Rev Food Sci 15(2):155–203

Hamada J (1997) Characterization of protein fractions of rice bran to devise effective methods of protein solubilization. Cereal Chem 74(5):662–668

Hermansson AM (1979) Methods of studying functional characteristics of vegetable proteins. J Am Oil Chem Soc 56(3):272–279

Hung S, Zayas J (1991) Emulsifying capacity and emulsion stability of milk proteins and corn germ protein flour. J Food Sci 56(5):1216–1218

Igene F, Oboh S, Aletor V (2005) Effects of some processing techniques on the functional properties of winged bean seed flours. Int J Food Agric Environ 3(2):28–31

Jitngarmkusol S, Hongsuwankul J, Tananuwong K (2008) Chemical compositions, functional properties, and microstructure of defatted macadamia flours. Food Chem 110(1):23–30

Khalid E, Babiker E, El Tinay A (2003) Solubility and functional properties of sesame seed proteins as influenced by pH and/or salt concentration. Food Chem 82(3):361–366

Kinsella JE (1979) Functional properties of soy proteins. J Am Oil Chem Soc 56(3):242–258

Kinsella J, Damodaran S, German B (1985) Physicochemical and functional properties of oilseed proteins with emphasis on soy proteins. New Protein Foods (USA) 5:107–179

Krause JP, Mothes R, Schwenke KD (1996) Some physicochemical and interfacial properties of native and acetylated legumin from faba beans (Vicia faba L.). J Agric Food Chem 44(2):429–437

Kumar SS, Balasubramanian S, Biswas A, Chatli M, Devatkal S, Sahoo J (2011) Efficacy of soy protein isolate as a fat replacer on physico-chemical and sensory characteristics of low-fat paneer. J Food Sci Technol 48(4):498–501

Lavedrine F, Zmirou D, Ravel A, Balducci F, Alary J (1999) Blood cholesterol and walnut consumption: a cross-sectional survey in France. Prev Med 28(4):333–339

Lavedrine F, Ravel A, Villet A, Ducros V, Alary J (2000) Mineral composition of two walnut cultivars originating in France and California. Food Chem 68(3):347–351

Lawal O, Adebowale K, Ogunsanwo B, Sosanwo O, Bankole S (2005) On the functional properties of globulin and albumin protein fractions and flours of African locust bean (Parkia biglobossa). Food Chem 92(4):681–691

Lawal O, Adebowale K, Adebowale Y (2007) Functional properties of native and chemically modified protein concentrates from bambarra groundnut. Food Res Int 40(8):1003–1011

Lin M, Humbert E, Sosulski F (1974) Certain functional properties of sunflower meal products. J Food Sci 39(2):368–370

McClements DJ (1999) Emulsion stability. In: Food emulsions: principles, practices, and techniques. CRC Press, Boca Raton, London, New York, Washington, DC, pp 185–233

Mexis SF, Badeka AV, Riganakos KA, Karakostas KX, Kontominas MG (2009) Effect of packaging and storage conditions on quality of shelled walnuts. Food Control 20(8):743–751

Molina Ortiz SE, Puppo MC, Wagner JR (2004) Relationship between structural changes and functional properties of soy protein isolates–carrageenan systems. Food Hydrocoll 18(6):1045–1053

Mwasaru MA, Muhammad K, Bakar J, Che Man YB (2000) Influence of altered solvent environment on the functionality of pigeonpea (Cajanus cajan) and cowpea (Vigna unguiculata) protein isolates. Food Chem 71(2):157–165

Ogunwolu SO, Henshaw FO, Mock HP, Santros A, Awonorin SO (2009) Functional properties of protein concentrates and isolates produced from cashew (Anacardium occidentale L.) nut. Food Chem 115(3):852–858

Oliveira R, Fátima Rodrigues M, Gabriela Bernardo-Gil M (2002) Characterization and supercritical carbon dioxide extraction of walnut oil. J Am Oil Chem Soc 79(3):225–230

Pedroche J, Yust M, Lqari H, Giron-Calle J, Alaiz M, Vioque J, Millan F (2004) Brassica carinata protein isolates: chemical composition, protein characterization and improvement of functional properties by protein hydrolysis. Food Chem 88(3):337–346

Pereira JA, Oliveira I, Sousa A, Ferreira IC, Bento A, Estevinho L (2008) Bioactive properties and chemical composition of six walnut (Juglans regia L.) cultivars. Food Chem Toxicol 46 (6):2103–2111

Phillips L, Yang S, Kinsella J (1991) Neutral salt effects on stability of whey protein isolate foams. J Food Sci 56(2):588–589

Pozani S, Doxastakis G, Kiosseoglou V (2002) Functionality of lupin seed protein isolate in relation to its interfacial behaviour. Food Hydrocoll 16(3):241–247

Prasad RBN (2003) Walnuts and pecans. In: Benjamin C, Trugo LC, Finglas PM (eds) Encyclopedia of food sciences and nutrition. Academic, London, pp 6071–6079

Prinyawiwatkul W, Beuchat LR, McWatters KH, Phillips RD (1997) Functional properties of cowpea (Vigna unguiculata) flour as affected by soaking, boiling, and fungal fermentation. J Agric Food Chem 45(2):480–486

Ragab DDM, Babiker EE, Eltinay AH (2004) Fractionation, solubility and functional properties of cowpea (Vigna unguiculata) proteins as affected by pH and/or salt concentration. Food Chem 84 (2):207–212

Rodriguez-Ambriz S, Martinez-Ayala A, Millan F, Davila-Ortiz G (2005) Composition and functional properties of Lupinus campestris protein isolates. Plant Food Hum Nutr (Formerly Qualitas Plantarum) 60(3):99–107

Sabate J, Fraser GE, Burke K, Knutsen SF, Bennett H, Lindsted KD (1993) Effects of walnuts on serum lipid levels and blood pressure in normal men. New Engl J Med 328(9):603–607

Sathe S, Deshpande S, Salunkhe D (1982) Functional properties of lupin seed (Lupinus mutabilis) proteins and protein concentrates. J Food Sci 47(2):491–497

Savage G (2001) Chemical composition of walnuts (Juglans regia L.) grown in New Zealand. Plant Food Hum Nutr (Formerly Qualitas Plantarum) 56(1):75–82

Seman D, Olson D, Mandigo R (1980) Effect of reduction and partial replacement of sodium on bologna characteristics and acceptability. J Food Sci 45(5):1116–1121

Sze-Tao K, Sathe S (2000a) Functional properties and in vitro digestibility of almond (Prunus dulcis L.) protein isolate. Food Chem 69 (2):153–160

Sze-Tao KWC, Sathe SK (2000b) Walnuts (Juglans regia L): proximate composition, protein solubility, protein amino acid composition and protein in vitro digestibility. J Sci Food Agric 80(9):1393–1401

Wardlaw G (1999) Perspective of nutrition. McGraw-Hill, New York

Wolf WJ (1970) Soybean proteins. Their functional, chemical, and physical properties. J Agric Food Chem 18(6):969–976

Yoshie-Stark Y, Wada Y, Wäsche A (2008) Chemical composition, functional properties, and bioactivities of rapeseed protein isolates. Food Chem 107(1):32–39

Yu J, Ahmedna M, Goktepe I (2007) Peanut protein concentrate: production and functional properties as affected by processing. Food Chem 103(1):121–129

Zhu KX, Zhou HM, Qian HF (2006) Proteins extracted from defatted wheat germ: nutritional and structural properties. Cereal Chem 83 (1):69–75

Zhu KX, Sun XH, Chen ZC, Peng W, Qian HF, Zhou HM (2010) Comparison of functional properties and secondary structures of defatted wheat germ proteins separated by reverse micelles and alkaline extraction and isoelectric precipitation. Food Chem 123 (4):1163–1169