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Original article

Water-soluble myofibrillar proteins prepared by high-pressure homogenisation: a comparison study on the composition and functionality

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Summary To expand utilisation of meat in various products, the characterisation and functionalities of water-soluble myofibrillar proteins (WSMP) induced by high-pressure homogenisation (HPH) were determined by comparison with those of soy protein isolate (SPI) and whey protein isolate (WPI). WSMP had high contents of protein (87.40%), which was mainly composed of myosin, actin and tropomyosin. The essential amino acids of WSMP achieved the FAO/WHO/UNO (2007) standards for preschool children, and the contents of lysine and sulphur-containing amino acids of WSMP were higher than those of SPI, making it desirable for children formulations. WSMP showed higher surface hydrophobicity while its water solubility was similar to that of SPI, but lower than that of WPI. WSMP demonstrated superior water/oil absorption capacities and emulsifying properties. The fibrous structure and high hydrophobic activity characteristics of WSMP were able to stabilise oil droplets with submicron droplet size, consequently responsible for its excellent emulsifying properties.

Keywords Functional property, high-pressure homogenisation, soy protein isolate, water-soluble myofibrillar protein, whey protein isolate.

Introduction

Proteins are being increasingly used to facilitate the engineering for fabrication of novel food products, such as protein beverages and therapeutic powder foods. The effectiveness of proteins utilisation in food production depends on their functional characteristics, which can be tailored to satisfy the various demands of food product manufacturers. These functional properties are influenced by both the intrinsic factors (e.g. the protein resource and structure) and extrinsic elements (e.g. ionic strength and food technologies) (Siddique *et al.*, 2016).

Globally, about 40% of humans' total protein consumption is accounted by proteins derived from animal, and it is predicted to grow substantially by 2050 (Boland *et al.*, 2013). The total consumption of meat was projected to rise by 102% (an additional 233 M tones of meat) between 2000 and 2050 by the United Nation's Food and Agricultural Organisation (FAO) (FAO 2006). These figures clearly depict the increasing consumption of muscle food and its global significance in replenishing human nutritional needs for protein. It is unequivocal that meat containing high-quality proteins has a vital role to play as an ideal protein source for human supply. Meat protein distinguished itself owing to the presence of abundant essential amino acids with its high digestibility, but with no limiting amino acids when comparing to that of beans and whole wheat (Pereira & Vicente, 2013). However, for extraction of component, meat has not been exploited as a supplementary protein ingredient to the same extent as milk or soybean. One major limitation is that myofibrillar proteins (MP, comprised almost 50% of muscle proteins) display inferior functional properties at low ionic strength, such as poor water solubility and low emulsifying properties (Zhou *et al.*, 2015; Chen *et al.*, 2016b). To enlarge the application scope of meat in various products, we recently proposed the potential application of high-pressure homogenisation (HPH) to selectively modify the structure of MP for improved solubility in water without obvious hydrolysis of individual protein polypeptides (Chen *et al.*, 2016b). As the rules for food formulation and processing are mainly consisted of the characterisation and functional properties of food

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proteins, these properties of HPH-induced water-soluble MP (WSMP) must be elucidated to utilise WSMP as an effective protein supplement.

To enable the application of protein extracts as functional ingredient in food formulation and prolong their stable storage, it is common to convert them into a dry powder form (Huda *et al.*, 2001; Sun *et al.*, 2002; Aguilera *et al.*, 2003). So far, there is still limited knowledge available regarding the characterisation and functionality of WSMP and these properties have been scarcely compared to those of such frequently used food proteins as whey protein isolate (WPI) and soy protein isolate (SPI). Therefore, the objective of this study was to (1) analyse the composition (chemical composition, protein composition, amino acid profile and surface hydrophobicity) of the WSMP and (2) compare the functionalities (solubility, water holding and fat absorption capacity, emulsion activity index, emulsion stability index, emulsion droplet size and charge) of WSMP with those of commonly used proteins (WPI and SPI) (Fig. 1a). Attempts taken towards understanding the functional properties of MP at low ionic strength can facilitate the innovation of meat products and be beneficial to the application of muscle proteins as food ingredients in formulated delivery system at low ionic strength.

Materials and methods

Materials

The frozen chicken breast (stored for 4 days after slaughter) obtained from Sushi Food Co., Ltd. (Nanjing, China), was used in this research. Soy protein isolate (SPI, RP0034) and whey protein isolate (WPI, Hilmar™ 9490) were purchased from Shanghai Ryon Biological Technology Co., Ltd. (Shanghai, China), and Hilmar Cheese Company Inc. (Hilmar, CA, USA), respectively. Soybean oil (Jinlongyu, Shanghai, China) was obtained from a local supermarket for the preparation of oil-in-water emulsions.

Preparation of WSMP

Water-soluble chicken breast MP dispersions with HPH were prepared as previously reported (Chen *et al.*, 2016b). In brief, the minced meat (100 g) was homogenised and washed four times with cold (4 °C) ultrapure water (pH 7.0). Then, the washed myofibrils were suspended in water and treated by 15,000 psi (103 MPa) HPH for two passes. The HPH was carried out by a high-pressure homogeniser (Mini DeBee, Bee International, South Easton, MA, USA) equipped with a single-pressure intensifier and a 75-μm opening Y-type diamond nozzle (Genizer™, Los Angeles, CA, USA) and implemented with a rapid cooling system for controlling the outlet temperature blow 20 °C. Finally, the HPH-treated dispersions were lyophilised for 48 h using a freeze dryer (Alpha 2–4 LSCplus; Martin Christ, Landkreis Osterode, Lower Saxony, Germany) at −80 °C compressor temperature and 0.1 mbar vacuum pressure. The freeze-dried powders were milled and sieved using a screen mesh (0.3 mm in aperture). The obtained samples were used as WSMP for further analysis.

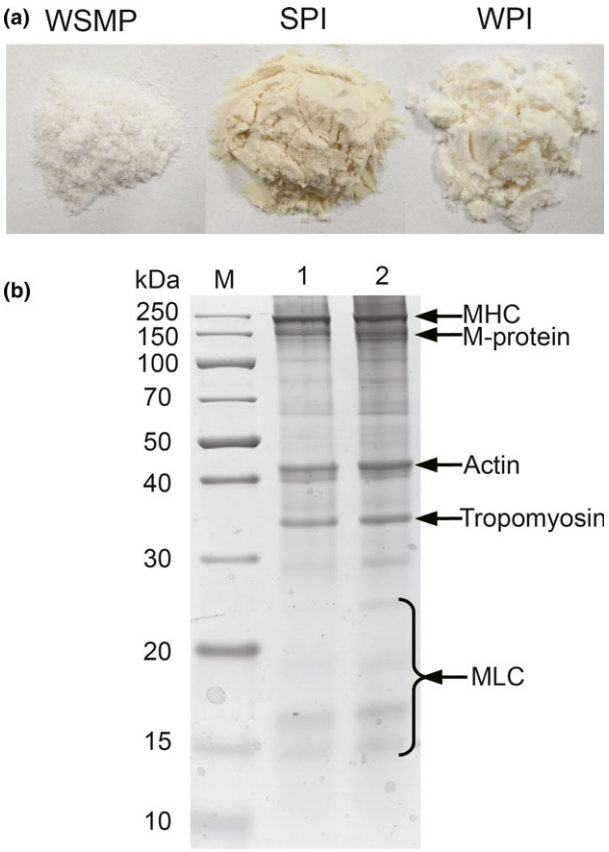

Figure 1 (a) Photographic representations of water-soluble myofibrillar protein (WSMP), soy protein isolate (SPI) and whey protein isolate (WPI). (b) SDS–PAGE profiles of (1) raw myofibrils and (2) WSMP at concentrations of 2 mg mL^{-1}. M: maker, MHC: myosin heavy chain, MLC: myosin light chain.

Determination of chemical compositions

WSMP, SPI and WPI samples were analysed according to the standard procedures (AOAC. 2005) for crude protein (N × 6.25) (Method No. 920.87), moisture (Method No. 925.1) and ash content (Method No. 923.03).

Determination of protein profile

Raw myofibril or WSMP were mixed with a sample buffer (20% glycerol, 5% β-mercaptoethanol, 4%

SDS, 0.125 M Tris, pH 6.8) to reach a final protein concentration of 2 mg mL^{-1}. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was conducted with a 4% acrylamide stacking gel and a 12% separating gel to observe the myofibrillar profiles following the method previously described (Chen *et al*., 2016a).

Determination of amino acid profile

Amino acid compositions of all samples were determined by an amino acid analyser (L-8900, Hitachi, Japan) basing on the procedure of Deng *et al*. (2015). Firstly, samples (30 mg) were hydrolysed in 3 mL 6 M HCl solution with drops of phenol for 24 h at 110 °C after 60 min of under a stream of nitrogen. Then, 1 mL hydrolysate was centrifuged at 6000 g for 5 min and 200 μL of supernatant was evaporated under a stream of nitrogen at 50 °C. Finally, the residual material was suspended in 1.5 mL of 0.2 M HCl solution and filtered through a 0.22-μm membrane for analysis. The results of amino acid composition were expressed as mg/g protein.

Determination of protein surface hydrophobicity

As described by Chen *et al*. (2014) with some modifications, 8-anilino-1-naphthalene sulphonic acid (ANS) was used to measure the surface hydrophobicity. 10 μL of 15 mM ANS solution (0.1 M sodium phosphate, pH 7.0) was added into 2 mL of 1 mg mL^{-1} WSMP, SPI and WPI dispersions (10 mM sodium phosphate, pH 7.0), respectively. After reaction for 20 min at 25 °C, the fluorescence was determined by a spectrophotometer (SpectraMax M2; Molecular Devices Limited, Sunnyvale, CA, USA) at an excitation wavelength of 380 nm, an emission wavelength in the range of 410–570 nm and a scanning speed of 300-nm/min.

Determination of the functional properties

Water solubility
Protein samples were completely dispersed (5 mg mL^{-1}) in aqueous solution (10 mM sodium phosphate, pH 7.0). Then, the protein solutions were centrifuged at 8000 g for 20 min (Liu *et al*., 2015). The solubility was defined as the ratio of protein content in the supernatant relative to that of protein suspension before centrifugation.

Water holding and fat absorption capacities
The water holding capacity (WHC) and fat absorption capacity (FAC) were measured using the method of Kaushik *et al*. (2016).

Emulsifying properties
The emulsifying activity index (EAI) and emulsion stability index (ESI) were evaluated to determine the emulsifying properties on the basis of a previous report (Pearce & Kinsella, 1978) with a slight modification. Soy oil (2 mL) and 6 mL of 0.5% protein solution (5 mM sodium phosphate buffer, pH 7.0) were initially homogenised using a high-speed mechanical shear unit (T25, IKA, Staufen, Germany) at 15 000 rpm for 2 min. Fifty microlitres of the emulsion was pipetted from the bottom of the emulsion into 5 mL of SDS solution (0.1%, w/v) at 0 and 10 min after homogenisation. After shaking the diluted emulsions using a vortex mixer for a few seconds, the absorbance of these diluted emulsions were detected at 500 nm wavelength using a UV–vis spectrophotometer (U-3010, Hitachi, Japan). The absorbance measured at 0 min (A0) and 10 min (A10) was used to calculate the EAI and ESI according to Xu & Liu (2016).

Droplet size and zeta potential
The *Z*-average size and zeta potential of droplets in oil-in-water emulsions stabilised by WSMP, SPI and WPI were evaluated using a Zetasizer (ZS-90, Malvern instruments Ltd., Worcestershire, UK) according a method previously reported (Kaushik *et al*., 2016).

Statistical analysis

All data are presented as mean ± standard deviation (SD) values of three or four independent experiments. The analyses of variances, means and SDs were analysed with the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA). A $P < 0.05$ significance level was used to determine the differences between each treatment.

Results and discussion

Chemical compositions of WSMP

The chemical composition of WSMP is listed in Table 1. The main component of WSMP was protein (87.40%), followed by moisture (5.12%) and ash (4.99%). No remarkable differences ($P > 0.05$) were shown in the protein contents of WSMP, SPI and WPI, except in the moisture and ash content. The differences in moisture and ash content may be due to the dissimilarity in water retention ability of the proteins and the extraction processes (Chavan *et al*., 2001; Tang *et al*., 2006; Ghribi *et al*., 2015).

Protein profile of WSMP

Individual protein compositions of WSMP were visualised through SDS–PAGE (Fig. 1b). WSMP showed

Table 1 Proximate analysis of WSMP, SPI and WPI*

Samples	Protein (%)	Moisture (%)	Ash (%)	Other (%)
WSMP	87.40 ± 1.55a	5.12 ± 0.43a	4.99 ± 0.73a	2.50 ± 2.24a
SPI	89.37 ± 1.53a	4.29 ± 0.33b	4.31 ± 0.51a	2.04 ± 0.72a
WPI	88.31 ± 1.38a	4.83 ± 0.57ab	3.05 ± 0.14b	3.82 ± 0.93a

[a–b]Different letters in the same column indicate significant differences at $P < 0.05$.
*Values were mean of values ± SD, $n = 4$.

a typical polypeptide composition of MPs: myosin heavy chain (MHC, 225 kDa), M-protein (160 kDa), actin (43 kDa), tropomyosin (37 kDa) and myosin light chain (MLC1-3, 25 kDa, 17 kDa and 15 kDa) were consistent with previous reports (Eppenberger *et al*., 1981; Xiong, 1994). The intensity of bands appeared much stronger at the positions of MHC, actin and tropomyosin bands, suggesting that they are the dominant components in WSMP.

SPI is a mixture of various protein subunits, and the major components are 7S ß-conglycinin and 11S glycinin, representing more than 80% of the total (Nishinari *et al*., 2014). The 7S globulin consists of three subunits α (67 kDa), α′ (71 kDa) and ß (50 kDa), while the 11S globulin (a hexamer) is composed of an acidic subunit A (35 kDa) and a basic subunit subunit B (20 kDa) (Nishinari *et al*., 2014). In the case of WPI, α-lactalbumin (14 kDa) and ß-lactoglobulin (18 kDa) are the dominant protein subunit, constituting about 70% of the total protein (Jambrak *et al*., 2014). The protein constituents of SPI and WPI are distinctly different from those of WSMP, which is usually composed of multisubunits having high molecular weight (Fig. 1b). Thus, it is conceivable that the properties of WSMP might be markedly distinct from those of SPI and WPI.

Amino acid profile of WSMP

The amino acid compositions of WSMP, SPI and WPI are given in Table 2. WSMP was found to be abundant in glutamic acid, lysine, aspartic acid, leucine,

Table 2 Amino acid profiles, E/T (%)*, total sulphur amino and aromatic acids of WSMP, SPI and WPI†

	Amino acids content (mg/g)			
Amino acids	WSMP	SPI	WPI	Requirements of preschool child
Histidine‡	30.73 ± 0.70a	28.08 ± 1.33b	16.14 ± 0.95c	18**
Isoleucine‡	39.97 ± 0.27c	44.73 ± 0.90b	57.22 ± 0.03a	31**
Leucine‡	72.13 ± 1.93b	71.63 ± 1.15b	102.45 ± 0.38a	63**
Lysine‡	78.61 ± 0.40b	51.93 ± 1.95c	87.46 ± 0.96a	52**
Methionine‡	21.87 ± 1.35b	8.48 ± 0.78c	35.66 ± 0.45a	
Phenylalanine‡	53.42 ± 1.65a	52.20 ± 1.91a	29.25 ± 0.38b	
Threonine‡	39.62 ± 1.42b	40.33 ± 1.56b	63.56 ± 1.05a	27**
Valine‡	42.30 ± 0.49b	41.74 ± 1.98b	59.74 ± 0.86a	42**
Tyrosine‡	30.30 ± 0.90b	36.56 ± 0.76a	36.07 ± 1.93a	
Cysteine‡	4.67 ± 0.86b	0.58 ± 0.08c	21.87 ± 1.55a	
Arginine	56.47 ± 2.48b	75.46 ± 2.06a	24.26 ± 2.25c	
Alanine	44.86 ± 1.72b	37.45 ± 0.84c	49.10 ± 0.68a	
Aspartic acid	73.69 ± 3.53c	118.58 ± 0.74a	102.69 ± 2.46b	
Glutamic acid	134.24 ± 7.03c	212.71 ± 1.88a	174.08 ± 3.28b	
Glycine	25.39 ± 1.11b	38.07 ± 1.07a	17.76 ± 1.07c	
Proline	31.18 ± 0.33c	51.28 ± 1.11b	57.88 ± 1.46a	
Serine	30.72 ± 0.84c	53.38 ± 2.85a	45.10 ± 1.52b	
Total sulphur amino acids§	26.55 ± 2.21b	9.06 ± 0.86c	57.53 ± 2.00a	26**
Total aromatic amino acids¶	83.72 ± 2.55b	88.76 ± 2.68a	65.32 ± 2.32b	46**
E/T (%)*	51.05 ± 2.14a	39.06 ± 2.31b	51.97 ± 2.78a	36††

*The proportion of essential amino acids (E) to the total amino acids (T) of the protein concentrate.
†All the data are expressed as mean ± SD and are the mean of three replicates. Means with the different letters within the same row are significantly different ($P < 0.05$).
‡Essential amino acids; §methionine + cysteine.; ¶tyrosine + phenylalanine; **data from FAO/WHO/UNO (2007); ††data cited from Ghribi *et al*. (2015).

arginine and phenylalanine, which was in accordance with the results for these amino acids of chicken muscles listed by Wattanachant *et al.* (2004). Comparing to SPI, WSMP contained higher levels of histidine, lysine, methionine, cysteine and alanine ($P < 0.05$), where lysine is likely to be the limiting amino acid in SPI (Table 2), hemp protein isolates (Tang *et al.*, 2006), buckwheat protein (Tomotake *et al.*, 2002) and in rice bran protein isolates (Wang *et al.*, 1999). Thus, WSMP may be a reliable lysine source and be used to complement those proteins that are short of lysine in food formulation. In addition, histidine, phenylalanine, arginine and glycine contents of WSMP were higher than those of WPI ($P < 0.05$) (Table 2).

Infants or preschool children have very critical nutritional requirements, and it is identified that nine amino acids are necessary for infants: threonine, leucine, isoleucine, lysine, valine, tryptophan, phenylalanine, methionine and histidine (Tang *et al.*, 2006). Regarding the preschool children (1–2 years old), all the essential amino acids in WSMP, SPI and WPI are sufficient for the FAO/WHO/UNO (2007) recommended requirements, except for the contents of sulphur-containing amino acids and lysine in SPI as well as the content of histidine in WPI which were slightly lower than the recommendations (Table 2). The sulphur-containing amino acids were also found to be limited in hemp protein isolate (Tang *et al.*, 2006), *Akebia trifoliata* var. *australis* seed protein isolate (Du *et al.*, 2012), beach pea protein isolate (Chavan *et al.*, 2001) and chickpea protein concentrate (Ghribi *et al.*, 2015). It seems that WSMP is an effective alternative for the supplementation of sulphur-containing amino acids in preschool children. Significantly higher levels ($P < 0.05$) of the essential amino acid to the total amino acid ratios (E/T, %) were found in WSMP (51.05%) and WPI (51.97%) than in SPI (39.06), with all the E/T values being well above 36.00% (Table 2), a level considered to be adequate for an excellent protein formulation (Ghribi *et al.*, 2015). The results indicated that WSMP had ideal compositions of essential amino acids appropriate for preschool children. Thus, the quality of WSMP was superior to that of SPI when serving as a desirable protein source for nutritional formulas.

Surface hydrophobicity of WSMP

The surface hydrophobicity manifests the exposure extent of hydrophobic groups in protein molecules, which plays an important role on affecting the interfacial tension and emulsifying property of a protein (Du *et al.*, 2012). The fluorescence intensity is positively correlated with surface hydrophobicity (Chen *et al.*, 2014). As displayed in Fig. 2, the WSMP exhibited the highest surface hydrophobicity, demonstrating that a higher extent of hydrophobic clusters appeared at the surface of WSMP when compared to SPI and WPI. WPI showed superior surface hydrophobicity than SPI (Fig. 3), suggesting greater hydrophobic sites exposed to the exterior of WPI. The difference among the three proteins was consistent with results in the FAC and emulsifying properties (Fig. 3b and 4a).

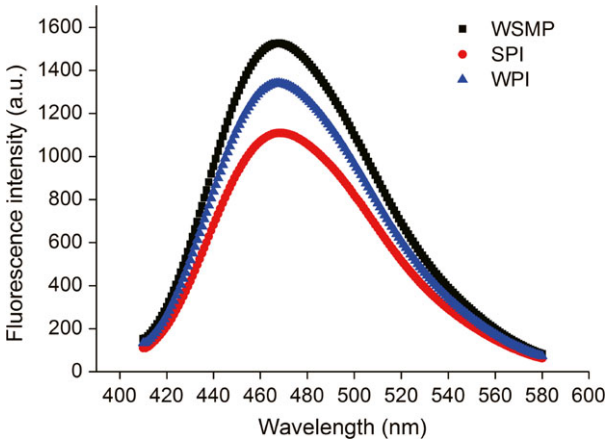

Figure 2 Surface hydrophobicity of WSMP, SPI and WPI in water (pH 7.0). The values represent the average of three determinations.

Functional properties of WSMP

Water solubility

As one of the most critical functional properties, water solubility is a vital characteristic of a protein in the acceptability of beverages, additives and fortifier (Ito *et al.*, 2004; Zhao *et al.*, 2013; Zhang *et al.*, 2015). The solubility of WSMP, SPI and WPI is illustrated in Fig. 3a. Both SPI and WPI had relatively high water solubilities, 75.32% and 90.41%, respectively. Similar findings of high solubility for SPI and WPI at neutral pH were obtained by Jiang *et al.* (2009) and Siddique *et al.* (2016), respectively. The solubility of WPI was the highest, while the WSMP displayed the lowest solubility (72.97%) among the three powders (Fig. 3a). Without specific processing procedures, the native MPs, consisting of intact myofibril structure, had very low solubility in water (Ito *et al.*, 2004). Therefore, it was of interest to note the high water solubility (72.97%) of WSMP, which was even comparable to that of SPI (Fig. 3a).

The protein structure, molecular size and exposed ionisable amino and carboxyl groups influence the sensitive balance between repulsive and attractive intermolecular forces, which regulated the solubility of proteins (Chen *et al.*, 2012; Shilpashree *et al.*, 2015a). Muscle myofibril structure mainly consists of thin and thick myofilaments (Pearce *et al.*, 2011). By the

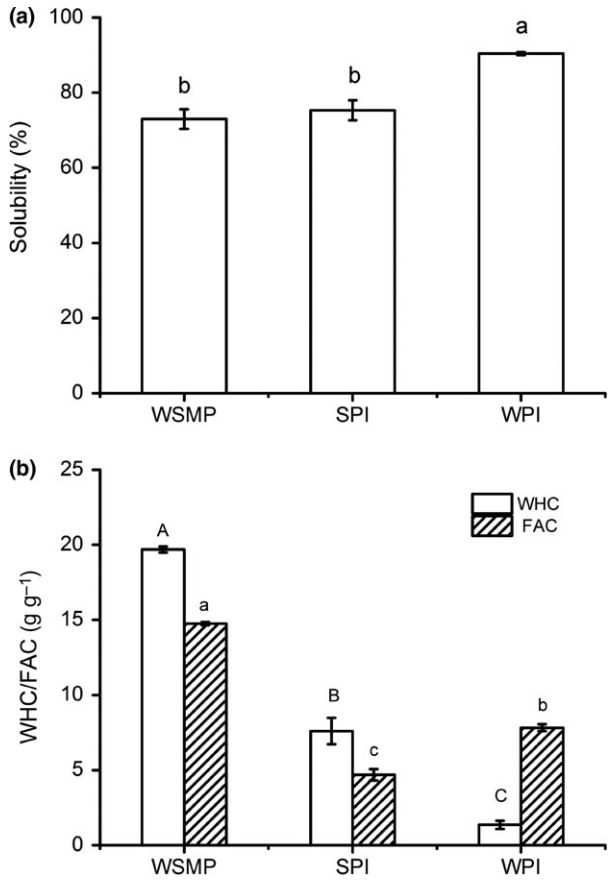

Figure 3 (a) Solubility and (b) water holding capacity (WHC) and fat absorption capacity (FAC) of WSMP, SPI and WPI in water (pH 7.0). Values are means ± SD (n = 3). The values with different letters are significantly different ($P < 0.05$).

modification of HPH, the complex macromolecular structure can be dissociated and fragment to small particles of submicron size in water. Following lyophilisation, the nano/submicron size particles in protein powder would undergo strong Brownian motion in aqueous suspensions, preventing them from centrifugal aggregation (Chen *et al*., 2016b). Probably owing to the fibrillar protein structure of WSMP having a higher molecular weight (Fig. 1b) and higher surface hydrophobicity (Fig. 2), its solubility was significant lower compared to that of WPI ($P < 0.05$). Extremely high water solubility of WPI might be attributed to the low molecular weight of α-lactalbumin (14 kDa) and ß-lactoglobulin (18 kDa), as discussed above. The high solubility of WSMP in water at neutral pH is a useful characteristic for potential beverage applications.

WHC and FAC

The WHC and FAC are expressed as the capacity of a protein to hold an amount of added water or oil. As shown in Fig. 3b, a lower FAC for SPI, in comparison with the other proteins, indicated its hydrophilic characteristic (Tomotake *et al*., 2002; Tang *et al*., 2006; Kaushik *et al*., 2016), whereas WPI showed the lowest WHC, indicating the presence of more lipophilic side chains in its structure compared with other proteins (Kaushik *et al*., 2016). The WHC and FAC of WSMP were significantly higher than those of SPI and WPI ($P < 0.05$).

The hydration of proteins can be affected by several intrinsic factors in its solution environment, such as protein conformation (shape and size), steric factors and polarity (hydrophilic–hydrophobic balance). Although WSMP had a low solubility compared to WPI and SPI (Fig. 3a), its WHC was high (Fig. 3b). This indicated that WHC might not have direct relationship with water solubility, which was consistent with the results from buckwheat protein (Tomotake *et al*., 2002) and for lentil protein isolates (Joshi *et al*., 2011). The polar amino groups of protein molecules are the major sites responsible for protein–water interactions (Chavan *et al*., 2001), the higher water absorption in WSMP might be caused by the higher accessibility of polar amino acids to the bulk solvent. In addition, the difference in WHC of proteins can be due to variations in conformational characteristics. It was demonstrated that fibres in the protein samples may have a function in improving the WHC of protein isolates (Zhao *et al*., 2013). Compared with the compact globular structures of subunit proteins in SPI (Li *et al*., 2007) and WPI (Siddique *et al*., 2016), the fibrous structures of filament proteins (Chen *et al*., 2016b) having relatively high molecule weights in WSMP (Fig. 1b) might be more flexible and favourable for water binding as water is more likely to penetrate fibrillar structures and be immobilised by capillary forces.

The capacity of protein to bind fat is controlled by several parameters such as hydrophobicity, size, surface area and flexibility of the protein (Tomotake *et al*., 2002). It was suggested that the fat-binding ability of protein relies on nonpolar side chains (hydrophobic characteristics) that bind hydrocarbon chains, hence making a contribution to increased oil absorption (Tomotake *et al*., 2002; Ghribi *et al*., 2015). The strong FAC in WSMP (Fig. 3b) might be due to the enhanced hydrophobic characteristics of proteins (Fig. 2) and the excellent fat-binding function of nonpolar amino acid side chains. Also, the physical structural feature of the WSMP may have greater porosity allowing more entrapment of fat compared to SPI and WPI. With high fat absorption, WSMP is appropriate to be used in food products where fat retention is required.

Emulsifying activity and emulsion stability

Emulsifying properties relate to the ability of the protein to form films at the oil/-water interface. In fact,

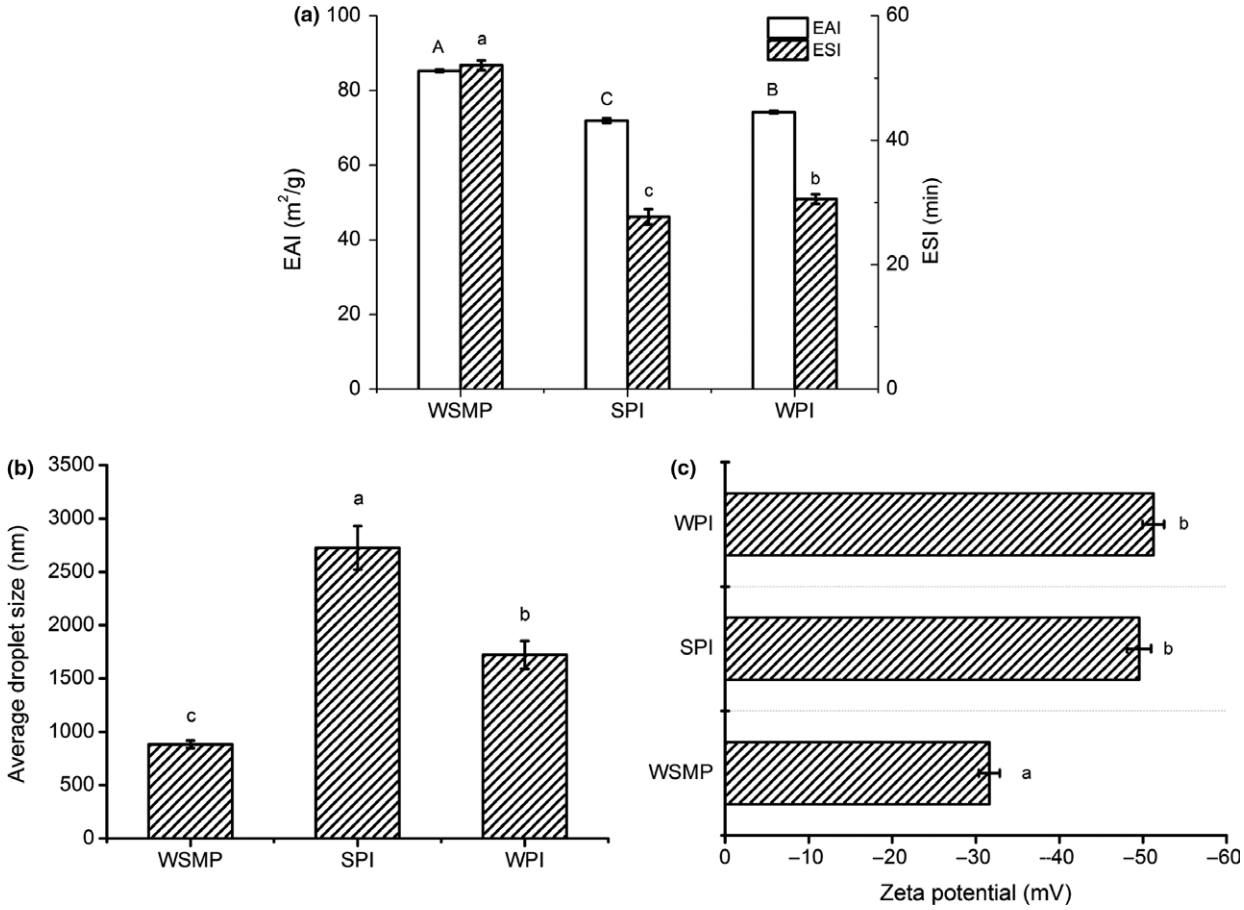

Figure 4 (a) Emulsifying activity index (EAI) and emulsion stability index (ESI) of WSMP, SPI and WPI as well as (b) average size and (c) zeta potential of oil-in-water emulsions stabilised by WSMP, SPI and WPI. Values are means ± SD ($n = 4$); the bars with different letters are significantly ($P < 0.5$).

they are important functional characteristics for determining the applications of proteins in the areas of foods, cosmetics and medicine (Xu & Liu, 2016). As depicted in Fig. 4a, WSMP demonstrated the highest EAI and ESI of stabilised emulsions among three proteins. The emulsifying activity and emulsion stability of WPI were significantly stronger than those of SPI ($P < 0.05$). Likewise, the Z-average size of WSMP-stabilised emulsion droplets was 883.54 nm, which was smaller than the droplet size of emulsions prepared with SPI and WPI (Fig. 4b). The larger droplet size of SPI emulsion (2726.41 nm) (Fig. 4b) is in line with its lower EAI and ESI, compared to that of the other proteins (Fig. 4a). Overall, these results indicated that WSMP was more capable of forming an interfacial membrane, thus promoting the dispersion of oil droplets and stabilising emulsion droplets when compared with SPI and WPI.

The emulsifying properties of proteins are strictly affected by the solubility, surface charge, hydrophilic–hydrophobic balance and conformational flexibility (Chen *et al*., 2012; Shilpashree *et al*., 2015a; Xu & Liu, 2016). Increased protein solubility can contribute to the increase in EAI as proteins with high solubility can move rapidly and diffuse to the interface for adsorption (Shilpashree *et al*., 2015a). However, for WSMP, the solubility was slightly lower than that of the other proteins (Fig. 3a), which contrasts with the EAI results (Fig. 4a). These results suggested that solubility might not be the only factor determining protein adsorption at the oil-/water interface. It had been indicated that surface hydrophobicity of a protein was a critical factor in affecting the emulsifying properties (Wang *et al*., 1999; Ghribi *et al*., 2015; Zhang *et al*., 2015). The increased hydrophobic groups exposed on protein surface would promote the interaction between the protein and lipid phase (Fig. 3b), resulting in an enhancement of emulsifying properties (Lee *et al*., 2009). Thus, the higher EAI value of WSMP compared with SPI and WPI might be resulted from

stronger surface hydrophobic activity as observed in Fig. 2. The characteristics of the droplets contained in a food emulsion, such as the charge, the interfacial properties and interactions, strongly influenced its physical stability (McClements, 2007). Generally, proteins can stabilise emulsions by forming a film over oil droplets and, thereby preventing coalescence and flocculation through steric hindrance or electrostatic repulsion (Taherian *et al*., 2011). The high 'net' charge in proteins can generate strong interparticle repulsion and led to a more stable cohesive interface, thus retarding emulsion coalescence (Chen *et al*., 2012; Shilpashree *et al*., 2015b). An electrostatically stabilised emulsion usually has a minimum zeta potential of ±30 mV (Kaushik *et al*., 2016). As shown in Fig. 4c, all emulsions presented absolute zeta potential values higher than 30 mV. However, emulsion droplet of WSMP had a lower surface charge than SPI and WPI (Fig. 4c), which was not in agreement with the results of EAI and ESI (Fig. 4a). The flexible fibrillar structure of WSMP with high molecular weight as early mentioned may have strong steric hindrance adsorption at the droplet interface, contributing to the high stability of emulsions. Besides, filament subunits in the WSMP with high WHC could initiate a swelling state to increase the interfacial thickness and the viscosity of continuous phase (data not shown), which might be the determinant for the high emulsifying stability of WSMP (McClements, 2007), even though the surface charge was low (Fig. 4c).

Conclusions

This study determined the characterisation and functional properties of WSMP prepared by HPH. WSMP was found to contain 87.40% protein in which MHC, actin and tropomyosin were the predominant subunits. WSMP contained higher levels of lysine and total sulphur amino acids compared to SPI, and all the essential amino acids were sufficient to meet the FAO/WHO/UNO (2007) standard needs, which was considered to be an ideal protein source for preschool children. Higher surface hydrophobicity was also detected in WSMP when compared with those of SPI and WPI. Probably due to this, the WSMP showed lower water solubility than that of WPI (90.41%); however, it displayed comparably high solubility (72.97%) relative to SPI. Moreover, WSMP had the highest WHC and FAC among the tested proteins, indicating its superior amphipathic properties. As a consequence, WSMP showed stronger surface activity and were more capable of stabilising emulsion droplets of small size, leading to higher emulsifying properties such as EAI and ESI, even though WSMP emulsified oil droplet with lower surface charge. Therefore, WSMP may have practical applications in food formulations at low ionic strength for paediatric and adult nutrition.

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