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Physicochemical and functional properties of protein isolate obtained from cottonseed meal

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ABSTRACT

To investigate the effect of preparation methods of cottonseed meals on protein properties, the physicochemical and functional properties of proteins isolated from hot-pressed solvent extraction cottonseed meal (HCM), cold-pressed solvent extraction cottonseed meal (CCM) and subcritical fluid extraction cottonseed meal (SCM) were investigated. Cottonseed proteins had two major bands (at about 45 and 50 kD), two X-ray diffraction peaks (8.5° and 19.5°) and one endothermic peak (94.31 °C–97.72 °C). Proteins of HCM showed relatively more β-sheet (38.3%–40.5%), and less β-turn (22.2%–25.8%) and α-helix (15.8%–19.5%), indicating the presence of highly denatured protein molecules. Proteins of CCM and SCM exhibited high water/oil absorption capacity, emulsifying abilities, surface hydrophobicity and fluorescence intensity, suggesting that the proteins have potential as functional ingredients in the food industry.

1. Introduction

Cottonseed, which is available in many temperate and tropical countries, is one of the richest sources of oilseeds mostly processed to extract oil that is used as edible fat (Zhou, Zhang, Gao, Wang, & Qian, 2015). Cottonseed meal is a co-product of the cottonseed oil processing industry. With processing, typical yields from cottonseed are 50% meal, 22% hulls, 16% oil and 7% linters, with a 5% loss (Hinze et al., 2015). The commonly used methods of lipid extraction from oil seeds are pressing and extraction with organic solvents (cold or hot). Pressing is the process of mechanically pressing liquid out of liquid containing solids, whereas extraction refers to the process of separating a liquid from a liquid-solid system (Anderson et al., 2016). Subcritical fluid extraction is one of the newly emerging clean and environment-friendly technologies for food products (Zheng, Ren, Su, Yang, & Zhao, 2013). During the oil extraction from cottonseeds, a portion of free gossypol binds with the epsilon amino group of lysine, thereby reducing the availability of lysine. Free gossypol in cottonseed meal depends on the variety of cultivars, methods of oil extraction and proportion of kernel to husk (Nagalakshmi, Rama Rao, & Panda, 2007). According to the Food and Drug Administration (FDA), a protein food product made from cottonseed is considered edible if it contains less than 0.045% free gossypol (FDA regulations, 1974). Several edible products have been developed, and cottonseed flours and protein concentrates have been accepted as functional and nutritional additives for meat products, baked goods, and cereals (Zhuge, Posner, & Deyoe, 1988). The use of cottonseed as protein source for humans does not depend only on the nutritional value of cottonseed, but also on their ability to be used as, or to be incorporated into, foods. Therefore, the functional properties of proteins rather than their nutritional value largely determine their acceptability as ingredients in various foods (Tsaliki, Pegiadou, & Doxastakis, 2002).

The functional attributes of food proteins depend on their molecular size, charge distribution, and three-dimensional structure. The structure-function relationships of proteins determine their interactions with themselves and with other ingredients in complex food systems (Joshi et al., 2012). The important functional properties of proteins in foods include hydration, water/oil combination, gelling, emulsification, foaming formation and rheological behaviours. These properties are influenced by environmental factors and processing conditions (Shevkani, Singh, Kaur, & Rana, 2015). Some of the physicochemical and functional properties of these proteins have already been reported (Mohan & Narasinga Roa, 1988; Tsaliki, Pegiadou, & Doxastakis, 2004; Tsaliki et al., 2002; Zhou et al., 2015). However, few studies have explored the influence of preparation methods of cottonseed meals in protein physicochemical and functional properties. The present work mainly aims to compare the physicochemical and functional properties of proteins isolated from hot-pressed solvent extraction cottonseed meal

(HCM), cold-pressed solvent extraction cottonseed meal (CCM) and subcritical fluid extraction cottonseed meal (SCM). The results can be used to further enhance the use of cottonseed proteins and investigate the methods used to prepare cottonseed meals, ultimately broadening the applications of cottonseed meals.

2. Materials and methods

2.1. Materials

HCM (TianKang, XinLiang, JingGu, and YiHai), CCM (Colour cotton, Insect-resistant cotton) and SCM (Colour cotton) were provided by the Institute of Cotton Research of Chinese Academy of Agricultural Sciences. Cottonseeds undergo a series of suitable pretreatment for hot pressing, such as cleaning, conditioning, decorticating, cracking, flaking, cooking (at 160–180 °C for 20 min), extruding, and drying to optimal moisture content of 9%. TianKang cottonseed meal (protein content: 56.23%), XinLiang cottonseed meal (protein content: 58.26%), JingGu cottonseed meal (protein content: 59.62%), and YiHai cottonseed meal (protein content: 60.86%) were respectively obtained from cottonseed oil extracted via hot pressing at 110–115 °C and then leached with hexane overnight. Colour cottonseed meal (protein content: 65.19%), insect-resistant cottonseed meal (protein content: 61.89%), peanut meal (protein content: 57.87%), and soybean meal (protein content: 47.45%) were obtained from seed oil extracted via cold pressing below 60 °C by LH188 oil pressing machine (Foshan Nanhai Lihua Electronic Technology Co., Ltd, China) and then leached with hexane overnight. Subcritical fluid extraction cottonseed meal (protein content: 66.65%) was obtained from colour cottonseed oil extracted by butane four times at 47 °C for 36 min for each round by using a CBE-5L subcritical fluid extraction equipment (Henan province subcritical extraction biological technology Co., Ltd, China). The free gossypol content of all cottonseed meal was lower than 0.012%. All samples were crushed to pass through a 40-mesh screen. All chemicals were reagent grade and obtained from Sigma-Aldrich Co (St. Louis, USA).

2.2. Preparation of protein isolate

Protein isolate was extracted and purified from defatted meal according to a reported method with some modifications (Timilsena, Adhikari, Barrow, & Adhikari, 2016). Meal (2 kg) added to petroleum ether (4 l) was stirred at room temperature for 60 min, then left standing, until natural sedimentation of the meal and organic solvent separation occurred. Recovery of organic solvents was then completed, and the precipitated cottonseed meal added to petroleum ether, this being repeated three times. The meal was subsequently placed in a fume hood at room temperature for 12 h. The protein isolate was obtained from the defatted meal. The defatted meal was dispersed in alkaline water (pH 11.0) by using a meal-to-water ratio of 1:20 to extract the protein. The slurry was treated at 45 °C for 20 min by using a KQ-700DE CNC ultrasonic device (Ultrasonic Instrument Co., Ltd, Kunshan, China, 650 W). The slurry was centrifuged at 1500 × *g* for 30 min, and the supernatant containing the dissolved protein was collected. This supernatant was acidified to a pH of 4.5 to induce precipitation of the dissolved protein. The protein precipitate was recovered through centrifugation and then resuspended in alkaline water to a pH of 7.0. This protein solution was vacuum freeze-dried and then the protein samples were stored at 4 °C until further tests.

2.3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Protein profile was determined using SDS-PAGE according to the modified Laemmli method (Arogundade, Mu, & Akinhanmi, 2016). SDS-PAGE test was performed on a gel slab comprising 4% stacking gel and 12.5% separating gel in a SDS-Tris-glycine discontinuous buffer system. The protein solution (2 mg/ml) was mixed with sample buffer (1:4, v/v) containing either 0% or 10% β-mercapto-ethanol and then boiled for 5 min. Each lane of the gel was loaded with 8 μl of sample. Following electrophoresis, the gels were stained with 0.1% Coomassie Brilliant Blue to reveal the protein bands. After removing the free dye, the gels were photographed using a Gel Doc XRTM System (Bio-Rad Laboratories, Hercules, CA).

2.4. Protein solubility (PS)

PS was determined according to the method described by Wu, Wang, Ma, and Ren (2009). In brief, 500 mg of proteins were dispersed in 50 ml of deionized water maintained at different pH values (pH 3.0, 5.0, 7.0, 9.0, and 11.0). The mixture was stirred for 1 h and then centrifuged at 1500 × *g* for 30 min. The protein contents of the supernatants were measured using the Bradford method with bovine serum albumin as standard. PS was expressed as percentage ratio of supernatant protein content to the total protein content.

2.5. Water absorption capacity (WAC) and oil absorption capacity (OAC)

WAC and OAC were determined using the method described by Ajibola, Malomo, Fagbemi, and Aluko (2016) with some modifications. Protein samples (0.5 g) were dispersed in distilled water (or soybean oil) (5 ml) in a 10 ml pre-weighed centrifuge tube. The dispersions were vortexed for 1 min, allowed to stand for 30 min, and centrifuged at 1400 × *g* for 30 min at room temperature. The supernatant was decanted, excess water (or oil) in the upper phase was drained for 10 min, and tube containing the protein residue was weighed again to determine the amount of water or oil retained per gram of sample.

2.6. Foaming capacity (FC) and foam stability (FS)

FC and FS were determined according to the method described by Timilsena et al. (2016). Protein samples (0.5 g) were dispersed in distilled water (50 ml) previously adjusted to the specified pH (4.0, 5.0, and 7.0). Foam was formed using XHF-D H-SPEED homogenizer (Ningbo Xinzhi Inc., China) at 10,000 × *g* for 2 min.

2.7. Emulsifying activity index (EAI) and emulsion stability index (ESI)

EAI and ESI were measured according to the method described by Zhang, Yang, Zhao, Hua, and Zhang (2014) with some modifications. Soybean oil (2 ml) and 1% (w/v) proteins (6 ml) were mixed. The mixture was homogenized using XHF-D H-SPEED homogenizer (Ningbo Xinzhi Inc., China) at 10,000 × *g* for 1 min. An aliquot of the emulsion (50 μl) was pipetted from the bottom of the container at 0 and 10 min after homogenization and then mixed with 0.1% SDS solution (5 ml). The absorbance of the diluted solution was measured at 500 nm by using a spectrophotometer (UV-1200, Instrument Co., Ltd, Shanghai, China).

2.8. Measurement of surface hydrophobicity (H_0)

H_0 was measured according to Kato and Nakai's method (Kato & Nakai, 1980) with some modifications. Protein samples were prepared through serial dilution with phosphate buffer solution (10 mM, pH 7.0) to obtain protein concentrations ranging from 1.0 mg/ml to 0.02 mg/ml. For each measurement, 5 ml of diluted sample was placed into 10 ml test tubes, in which 25 μl of 8 mM ANS solution was added. The tube contents were mixed and incubated in the dark for 15 min at ambient temperature. Fluorescence intensity (FI) was measured using a fluorescence spectrometer (LS55, PE Inc., USA) at 390 nm (excitation) and 470 nm (emission), with a constant excitation and emission slit of 5 nm. The slope of FI versus protein concentration linear regression plot (r = 0.99) was used as H_0 index.

2.9. Intrinsic fluorescence

Intrinsic fluorescence analysis was performed as described by Arogundade et al. (2016) with modifications. Samples were diluted with phosphate buffer solution up to 0.2 mg/ml protein content. Intrinsic fluorescence emission spectra were obtained by a fluorescence spectrophotometer (LS55, PE Inc., USA). Sample solutions were excited at 295 nm, and emission spectra were recorded from 300 nm to 400 nm (both with a slit width of 5 nm) to minimize the effect of tyrosine residues.

2.10. Fourier-transform infrared (FTIR) spectroscopy and protein secondary structure

Infrared spectra and protein secondary structure were measured according to Shevkani et al. (2015). All the spectra recorded using FTIR spectrometer (Vertex 70, BRUKER Inc., Germany) and were the average of 16 scans from 4000 cm^{-1} to 400 cm^{-1} and were acquired at a resolution of 4 cm^{-1}.

2.11. Thermal properties

Thermal properties of protein were analyzed using a differential scanning calorimeter (TA Q2000-DSC, NewCastle, DE, USA). Protein samples (3.0 mg) were accurately weighed into pans and then 0.1 M phosphate buffer solution (pH 7.0, 10 μl) was added. The pans containing the protein samples and buffers were sealed and equilibrated at 25 °C for 2 h. Peak denaturation and enthalpy change of denaturation were computed from the thermograms by using the Universal Analysis 2000 software (V3.8B, TAInc., USA).

2.12. Protein crystallinity measurement

The X-ray diffraction (XRD) patterns were measured with diffractometer (D8ADVANCE Bruker, Germany) using copper anode with k_α ratio of 0.5. The diffractograms were taken between 5° and 55° with a step size of 0.02°.

2.13. Statistical analysis

The data were in duplicate, and subjected to statistical analysis, using analysis of variance (ANOVA) to determine significant differences between the samples ($p < 0.05$). Differences between the treatment means were separated using Duncan's multiple range tests.

3. Results and discussion

3.1. SDS-PAGE

The protein profile of cottonseed protein isolates (CPIs) determined by SDS-PAGE in reduced and non-reduced conditions was shown in Fig. 1. Under non-reducing condition (Fig. 1A), major high-intensity bands were found at approximately 50 and 45 kDa for CPIs, indicating that these two protein fractions could be the main components of CPIs. In the presence of β-mercaptoethanol (reducing condition) (Fig. 1B), two main bands were also observed in CPIs, and many additional minor bands appeared between 14 and 35 kDa. The emergence of these minor bands under reducing condition suggests the presence of intermolecular disulfide bond in CPIs. Under reducing condition, TKCPI and YHCPI (lane 1 and 4) showed a set of bands similar to those in non-reduced protein, suggesting the minimal level of disulfide bonds in TKCPI and YHCPI. Moreover, the SDS-PAGE profile showed that the intensity of a 14 kDa band was higher in lanes 1–4 than in lanes 5 and 7, whereas the intensity of 20–50 kDa bands were higher in lanes 5 and 7 than in lanes 1–4. This result suggested that CPIs (TKCPI, XLCPI, JGCPI, and YHCPI) of HCM contain many low-molecular-weight protein subunits, and this phenomenon may be attributed to high-temperature thermal processing (Zhang et al., 2014). It probably indicated that the disulfide-bond of CPIs subunit was broken and the larger protein was degraded during hot-pressing treatment.

3.2. PS

Solubility at various pH values serves as an indicator of how well protein isolates will perform when they are incorporated into food systems, as well as the extent of protein denaturation due to heat or chemical treatment (Horax, Hettiarachchy, Chen, & Jalaluddin, 2004). All CPIs presented different solubility profiles at pH 3.0–11.0, the maximum solubility for protein isolates was observed at pH 11.0, and in all cases, the minimum solubility was at pH 5.0 (Fig. 2). The solubility profiles revealed that solubility decreased with the increase in pH; the minimum solubility was reached at pH 5.0, and the solubility increased with further increase in pH. Similar results were reported for the minimum solubility of PPI at pH 5.0 (Li, Xue, Chen, Ding, & Wang, 2014). This result was due to the reduced interaction between protein and water, and this phenomenon enhances protein-protein interactions, resulting in the protein aggregation and precipitation (Ivanova, Chalova, Koleva, & Pishtiyski, 2013). CPIs, PPI, and SPI displayed different degrees of solubility at pH 7.0–11.0, and SBECPI and TKCPI showed a higher solubility than SPI. CPIs extracted from HCM also showed different degrees of solubility at pH 7.0–11.0, in which TKCPI displayed the highest solubility. This observation suggested that protein denaturation can cause improvement in certain functional properties (Ghribi et al., 2015).

3.3. WAC and OAC

WAC is an index of the ability of proteins to absorb and retain water. OAC is the ability of fat to bind the non-polar side chains of proteins. WAC and OAC influence the texture and mouthfeel characteristics of foods and food products, such as comminuted meats, extenders or analogues, and baked dough (Adebowale, Adeyemi, & Oshodi, 2005). The WAC and OAC significantly differed among different protein isolates, and ranges of WAC and OAC values of CPIs were 1.6–2.9 g/g and 3.0–5.4 g/g, respectively (Table 1). The WAC and OAC values of CPIs (TKCPI, XLCPI, JGCPI, and YHCPI) from HCM were lower than those of CPIs (CCPI, IRCPI, and SBECPI) and SPI, which might have destroyed both hydrophilic and hydrophobic groups of CPIs from HCM during thermal processing. The WAC values of all CPIs were higher than those of PPI. This result indicated that the less of soluble proteins and lower availability of polar amino acids in PPI. CCPI, IRCPI, and SBECPI had higher WAC and OAC values than PPI and SPI, in which SBECPI had the highest values. The present WAC and OAC values were consistent with the reported values for peanut and soybean proteins (Wu et al., 2009; Zhang et al., 2013). It could be concluded based from the results that CPIs of CCM and SCM demonstrated a desirable ability to absorb and retain the water and oil of some foods, especially comminuted meat and baked doughs (Adebowale et al., 2005).

3.4. EAI and ESI

The ability of food proteins to form and stabilize emulsions is critical to their role as food ingredients in a wide range of applications (Ghribi et al., 2015). EAI and ESI significantly differed among CPIs ($p < 0.05$) (Table 1). The EAI and ESI values of CPIs were 13.3–23.1 m^2/g and 17.3–29.6 min, respectively (Table 1), in which TKCPI had the highest EAI and IRCPI had the highest ESI. Emulsifying properties was mainly dependent on lowering the tension at the oil-water interfaces and controlling diffusion and aggregation of oil droplets by forming an adsorption layer (Mcclements, 2007). The high ESI of IRCPI indicated that it can be preferably used as emulsifier in oil-in-water emulsions. The EAI and ESI values of all CPIs were higher than

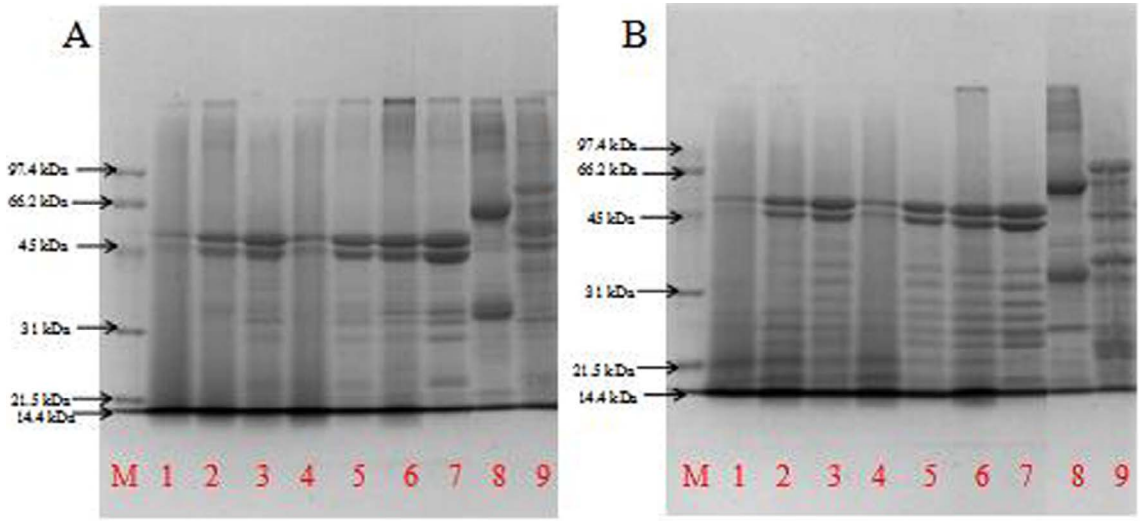

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of protein under non-reducing (A) and reducing (B) conditions. Lane 1–9: TKCPI; XLCPI; JGCPI; YHCPI; CCPI; IRCPI; SBECPI; PPI; and SPI, respectively. TKCPI, XLCPI, JGCPI, YHCPI, CCPI, IRCPI and SBECPI are protein isolate from TianKang cottonseed meal, XinLiang cottonseed meal, JingGu cottonseed meal, YiHai cottonseed meal, colour cottonseed meal, insect-resistant cottonseed meal, and subcritical fluid extraction cottonseed meal, respectively; PPI and SPI are protein isolate from cold-pressed solvent extraction meals of peanut and soybean; M: Molecular weight marker.

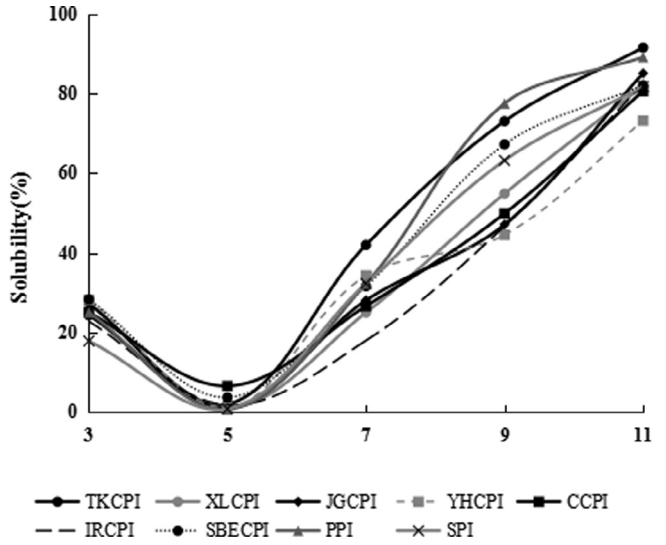

Fig. 2. Solubility profiles of protein isolates. TKCPI, XLCPI, JGCPI, YHCPI, CCPI, IRCPI and SBECPI are protein isolate from TianKang cottonseed meal, XinLiang cottonseed meal, JingGu cottonseed meal, YiHai cottonseed meal, colour cottonseed meal, insect-resistant cottonseed meal, and subcritical fluid extraction cottonseed meal, respectively; PPI and SPI are protein isolate from cold-pressed solvent extraction meals of peanut and soybean.

those of PPI, which indicated CPIs can be used in emulsified foods, such as sweetmeats, bakery products and sausages. These results were slightly lower than those reported for PPI, but higher than those reported for SPI (Gong et al., 2016; Zhang et al., 2013). CPIs with high EAI showed high solubility (Fig. 2 and Table 1), which exhibited a positive correlation of EAI with solubility and were in agreement with reported of Ghribi et al. (2015).

3.5. FC and FS

Foaming properties (FC and FS) are desired in many instances and are utilized in food systems for aeration and whipping (Timilsena et al., 2016). Table 1 showed the FC and FS values for CPIs. The lowest FC and FS values were observed at pH 5.0 (15.1%–31.1% and 38.8%–89.0%), and this result was attributed to pH values close to the isoelectric points of proteins. Moreover, FC and FS increased when pH values were lower or higher than 5.0 and peaked at pH 7.0 (50.0%–81.5% and 73.3%–96.9%). This result was consistent with previous reports that FC and FS of CPIs was highest at pH 7.0 (Tsaliki et al., 2002). This observation was similar with the solubility profile of these CPIs, indicating that the higher protein solubility was correlated with the higher FC and FS of the dispersion. FC was closely related to the concentration of soluble proteins, because soluble proteins can reduce surface tension at the interface between air bubbles, increasing the FC of proteins (Adebowale & Lawal, 2004). TKCPI showed the highest FS value and solubility at pH 7.0 (Table 2 and Fig. 2). Thus, a high solubility increases the viscosity of a solution, resulting in stiffer foam structure. The high FC and FS value were observed in SBECPI at pH 5.0 and 7.0. SBECPI could be considered to be a suitable foaming ingredient for use in different food products, such as cakes, bread, whipped cream, ice cream and some confectionery products.

3.6. H_0

H_0 indicates the number of hydrophobic groups on the surface of a protein and which are in contact with water; H_0 is closely related to the solubility and emulsifying properties of proteins (Zhao et al., 2013). Table 1 shows the extent of the exposure of hydrophobic groups in proteins as predicted by H_0; the result indicated that H_0 of the CPIs (561.7 for CCPI; 616.7 for IRCPI and 727.5 for SBECPI) of CCM and SCM was significantly higher than that of the CPIs (103.7 for TKCPI, 128.1 for XLCPI, 110.5 for JGCPI, and 120.5 for YHCPI) of HCM. These findings implied that the CPIs of CCM and SCM contain more hydrophobic groups that were in contact with the polar environment. Moreover, CPIs of HCM with lower H_0 values show partial denaturation and subsequent aggregation of hydrophobic groups (He et al., 2014). The unfolded cottonseed protein aggregated due to hot pressing treatment of cottonseed meal. Based on the changes between the H_0 index of CPIs from HCM, CCM and SCM, different preparation methods of cottonseed meals can lead to the conformational change of CPI. The oil-water interface is dominated by hydrophobic interactions, and exposure of non-polar hydrophobic groups at the interface greatly affects the emulsifying properties (Kato & Nakai, 1980). H_0 of the CPIs of CCM was significantly higher than PPI (162.1), but lower than SPI (746.4). This difference may be attributable to the distinct tertiary conformations of the proteins. SPI showed a high H_0, EAI, and ESI (Table 1); thus, H_0 is apparently correlated with EAI and ESI. A comparison of H_0 with EAI and ESI indicates that H_0 values are positively correlated with enhanced

Table 1
Functional properties and surface hydrophobicity (H_0) of protein isolates.[a]

Sample	Protein (%)	WAC/g/g	OAC/g/g	EAI m^2/g	ESI min	pH 4.0		pH 5.0		pH 7.0		H_0
						FC (%)	FS (%)	FC (%)	FS (%)	FC (%)	FS (%)	
TKCPI	91.28	1.8 ± 0.3^{c}	3.7 ± 0.1^{e}	23.1 ± 0.6^{b}	21.5 ± 0.8^{d}	44.4 ± 0.4^{bcd}	83.2 ± 0.1^{c}	22.2 ± 0.2^{bcd}	79.8 ± 0.2^{abc}	57.4 ± 0.2^{cd}	96.9 ± 0.3^{a}	103.7 ± 0.0^{g}
XLCPI	92.08	1.6 ± 0.3^{d}	3.5 ± 0.5^{f}	21.1 ± 0.3^{c}	18.7 ± 0.2^{f}	35.2 ± 0.2^{d}	84.2 ± 0.1^{b}	21.1 ± 0.1^{bcd}	62.8 ± 0.7^{de}	55.6 ± 0.4^{cd}	86.6 ± 0.1^{bc}	128.1 ± 3.2^{f}
JGCPI	96.54	1.9 ± 0.3^{c}	3.0 ± 0.4^{g}	16.5 ± 0.8^{e}	20.7 ± 0.1^{de}	53.7 ± 0.2^{ab}	86.2 ± 0.0^{a}	23.3 ± 0.3^{abcd}	56.9 ± 0.1^{e}	60.2 ± 0.3^{cd}	88.9 ± 0.5^{abc}	110.5 ± 0.3^{g}
YHCPI	96.56	1.9 ± 0.6^{c}	4.0 ± 0.0^{d}	13.3 ± 0.8^{g}	24.4 ± 0.6^{c}	40.7 ± 0.0^{cd}	86.4 ± 0.0^{a}	15.1 ± 0.3^{d}	38.8 ± 0.1^{f}	59.4 ± 0.4^{cd}	80.9 ± 0.1^{cd}	120.5 ± 0.6^{fg}
CCPI	90.98	2.4 ± 0.8^{b}	4.7 ± 0.0^{b}	21.6 ± 0.7^{c}	17.3 ± 0.4^{g}	53.7 ± 0.2^{ab}	72.4 ± 0.1^{g}	21.0 ± 0.1^{bcd}	77.5 ± 0.3^{abcd}	59.3 ± 0.0^{cd}	87.5 ± 0.0^{abc}	561.7 ± 1.7^{d}
IRCPI	84.76	2.4 ± 0.04^{b}	4.7 ± 0.1^{b}	18.5 ± 0.9^{d}	29.6 ± 0.3^{b}	38.9 ± 0.2^{d}	80.95 ± 0.3^{d}	20.0 ± 0.1^{cd}	72.1 ± 0.2^{bcde}	50.0 ± 0.6^{de}	73.3 ± 0.7^{d}	616.7 ± 4.6^{c}
SBECPI	89.82	2.9 ± 0.1^{a}	5.4 ± 1.1^{a}	18.6 ± 0.9^{d}	25.0 ± 0.8^{c}	57.4 ± 0.2^{a}	74.2 ± 0.1^{f}	31.1 ± 0.2^{a}	89.0 ± 0.4^{a}	81.5 ± 0.0^{a}	88.6 ± 0.2^{abc}	727.5 ± 1.6^{b}
PPI	94.48	1.4 ± 0.9^{e}	3.9 ± 0.7^{d}	14.8 ± 0.7^{f}	15.2 ± 0.1 h	43.5 ± 0.5^{bcd}	76.6 ± 0.0^{e}	25.6 ± 0.3^{abc}	73.5 ± 0.3^{abcd}	64.8 ± 0.2^{bc}	94.3 ± 0.0^{ab}	162.1 ± 3.6^{e}
SPI	83.05	2.3 ± 0.1^{b}	4.5 ± 0.3^{c}	31.2 ± 0.5^{a}	32.5 ± 0.6^{a}	42.6 ± 0.2^{cd}	80.7 ± 0.6^{d}	28.9 ± 0.4^{ab}	87.6 ± 0.6^{ab}	65.7 ± 0.5^{bc}	90.2 ± 0.1^{abc}	746.4 ± 5.9^{a}

[a] Results are mean ± standard deviations of duplicate analysis. Values followed by different letter in the same column are significantly different ($p < 0.05$). WAC, water absorption capacity; OAC, oil absorption capacity; EAI, emulsifying activity index; ESI, emulsifying stability index; FC, foaming capacity; FS, foam stability.

emulsifying properties. When a high number of hydrophobic groups is exposed (i.e., H_0 is high), the stronger the binding between emulsifier and oil droplet will be in emulsions (Kato & Nakai, 1980). High H_0 values in the CPIs of CCM and SCM improved the molecular arrangement at oil-water interface, thereby enhancing the emulsifying property during processing (Wong et al., 2012).

3.7. Intrinsic fluorescence

The intrinsic fluorescence of tryptophan residues primarily reflects the environment of the protein; this parameter is a sensitive means of characterizing protein conformation, and thus can be employed to investigate alteration in tertiary structures of proteins (Antonov, Zhuravleva, Cardinaels, & Moldenaers, 2015). As shown in Fig. 3, SBECPI of SCM had lower emission maximum (λ_{max}) values, which indicated that the tryptophan residues in protein were surrounded by a more hydrophobic environment (Li et al., 2014). Fig. 3 shows that the CPIs (TKCPI, XLCPI, JGCPI, and YHCPI) of HCM had low FI, an indication of the presence of highly denatured protein molecules. Therefore, denaturation probably led to extensive exposure of the tryptophan residues to a hydrophilic (aqueous) environment, and this phenomenon could have contributed to fluorescence quenching. By contrast, the results suggested a less denatured and more folded conformation for the CPIs (CCPI and IRCPI) of CCM; these characteristics reduced the exposure of tryptophan to the hydrophilic environment and hence led to a higher FI compared to the protein extracted from HCM (Ajibola et al., 2016). The SBECPI of SCM showed the highest FI (Fig. 3), demonstrating a more folded conformation than that of other CPIs, and hence a closer packing of tryptophan residues within the hydrophobic pocket (Ajibola et al., 2016). Different preparation methods of cottonseed meals could lead to the change of tertiary structures of cottonseed proteins.

3.8. FTIR spectrum analysis

FTIR spectroscopy, an important tool used to estimate the secondary structure of proteins, can be used to obtain information on the structural composition of proteins (Kong & Yu, 2007). Amide I bands, which are used to study the secondary structure of proteins, demonstrated that 1615–1638, 1638–1645, 1645–1662, and 1662–1682 cm^{-1} corresponded to the β-sheet structure, random coil, α-helix structure and β-turn structure, respectively (Hou et al., 2017). Table 2 shows the structure of CPIs in amide I band after peak shape fitting and area calculation. The secondary structure of CPIs consisted mostly of β-structure. The results are similar to those reported for cottonseed protein (Mohan & Narasinga Roa, 1988). The result showed that the dominant secondary structure in soybean protein is β-structure, and a conclusion similar to that of Song and Zhao (2014) was drawn. CPIs (TKCPI, XLCPI, JGCPI, and YHCPI) of HCM showed higher amount of β-sheet structure and lower amounts of α-helical structure and β-turn structure than CPIs (CCPI, IRCPI, and SBECPI) of CCM and SCM; this result demonstrated a phenomenon wherein α-helix and β-turn structures of CPI transform into β-sheet structure due to denaturation. Alteration of the secondary structure of most globular proteins is commonly due to the loss of α-helix (Kong & Yu, 2007). Timilsena et al. (2016) reported that a decrease in β-sheet structure accompanied by an increase in α-helix, β-turn, and random coil structures in chia seed protein is due to denaturation. The alteration of secondary structure indicated that under high temperature and low pH, the random coil portion was converted into β-turn and β-sheet through self-assembly of proteins (Diniz et al., 2014).

3.9. Thermal properties

Differential scanning calorimetry reveals the structural and conformational changes in proteins. Table 2 shows the onset temperature

Table 2
Relative content of the secondary structural features and thermal properties of protein.[a]

Sample	β-Sheet (%)	Random coil (%)	α-Helix (%)	β-Turn (%)	T_o (°C)	T_d (°C)	ΔH (J/g)
TKCPI	39.3 ± 0.8^{a}	19.2 ± 2.3^{a}	17.4 ± 1.1^{cd}	24.1 ± 2.7^{cd}	85.65 ± 0.14^{cde}	97.55 ± 0.40^{bc}	3.51 ± 0.06^{d}
XLCPI	40.5 ± 1.0^{a}	17.9 ± 1.9^{ab}	15.8 ± 1.4^{d}	25.8 ± 1.1^{bcd}	85.23 ± 0.38^{de}	95.24 ± 0.02^{de}	7.76 ± 0.22^{a}
JGCPI	38.3 ± 0.3^{ab}	16.8 ± 0.0^{ab}	19.5 ± 0.2^{bcd}	25.4 ± 0.1^{bcd}	83.47 ± 0.40^{e}	96.55 ± 0.00^{bcd}	7.45 ± 0.71^{a}
YHCPI	38.7 ± 0.8^{a}	20.9 ± 0.8^{a}	18.2 ± 0.6^{bcd}	22.2 ± 3.0^{d}	85.58 ± 0.06^{cde}	97.72 ± 0.03^{b}	6.24 ± 0.07^{ab}
CCPI	29.1 ± 1.1^{cd}	12.4 ± 0.9^{a}	27.8 ± 0.1^{a}	30.7 ± 0.6^{ab}	86.29 ± 0.16^{cd}	94.31 ± 0.00^{e}	5.57 ± 0.14^{bc}
IRCPI	30.2 ± 0.3^{cd}	17.4 ± 0.3^{ab}	22.7 ± 0.3^{b}	29.7 ± 0.3^{abc}	85.59 ± 0.12^{cde}	95.36 ± 0.45^{cde}	4.45 ± 1.06^{cd}
SBECPI	30.4 ± 1.1^{cd}	18.4 ± 0.4^{ab}	21.3 ± 0.4^{bc}	29.9 ± 0.6^{ab}	87.81 ± 0.63^{c}	95.68 ± 0.14^{bcde}	7.87 ± 0.15^{a}
PPI	25.0 ± 3.5^{d}	22.2 ± 2.4^{a}	19.2 ± 2.8^{bcd}	33.6 ± 0.4^{a}	84.29 ± 0.09^{de}	87.70 ± 0.75^{f}	0.98 ± 0.39^{e}
					98.81 ± 1.28^{a}	104.61 ± 0.81^{a}	7.40 ± 0.14^{a}
SPI	33.1 ± 0.2^{bc}	16.6 ± 2.1^{ab}	20.0 ± 0.3^{bcd}	30.3 ± 0.6^{ab}	74.64 ± 0.96^{f}	77.04 ± 0.43^{g}	0.21 ± 0.05^{e}
					94.00 ± 0.81^{b}	96.11 ± 1.28^{bcde}	1.15 ± 0.18^{e}

[a] Results are mean ± standard deviations of duplicate analysis. Values followed by different letter in the same column are significantly different ($p < 0.05$). T_o, onset gelatinization temperature; T_d, peak denaturation; ΔH, enthalpy change of denaturation.

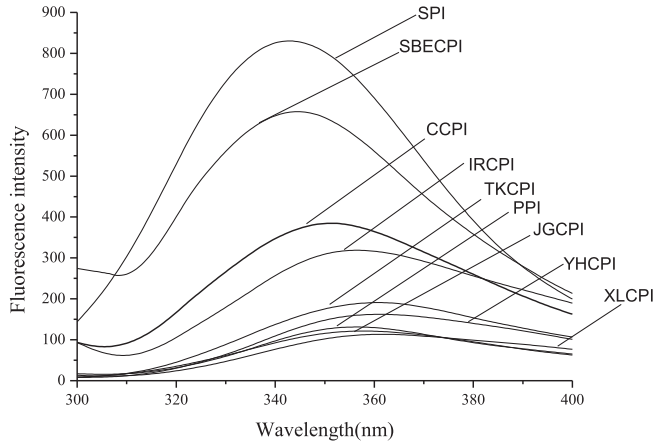

Fig. 3. Intrinsic fluorescence spectra of protein isolates with excitation at 295 nm (for tryptophan). TKCPI, XLCPI, JGCPI, YHCPI, CCPI, IRCPI and SBECPI are protein isolate from TianKang cottonseed meal, XinLiang cottonseed meal, JingGu cottonseed meal, YiHai cottonseed meal, colour cottonseed meal, insect-resistant cottonseed meal, and subcritical fluid extraction cottonseed meal, respectively; PPI and SPI are protein isolate from cold-pressed solvent extraction meals of peanut and soybean.

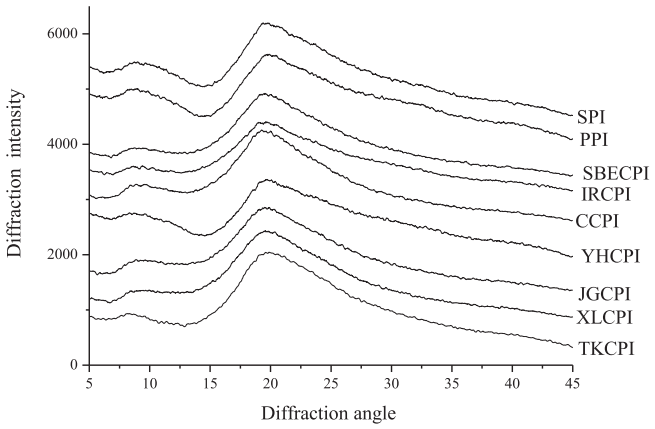

Fig. 4. X-ray diffraction (XRD) pattern of proteins isolate. TKCPI, XLCPI, JGCPI, YHCPI, CCPI, IRCPI and SBECPI are protein isolate from TianKang cottonseed meal, XinLiang cottonseed meal, JingGu cottonseed meal, YiHai cottonseed meal, colour cottonseed meal, insect-resistant cottonseed meal, and subcritical fluid extraction cottonseed meal, respectively; PPI and SPI are protein isolate from cold-pressed solvent extraction meals of peanut and soybean.

(T_o), peak denaturation (T_d) and enthalpy change of denaturation (ΔH). T_d reflects the thermal stability of the proteins, whereas ΔH depicts the proportion of undenatured proteins and the extent of ordered structures. In addition, the thermal properties can reflect the extent of the tertiary conformation of proteins (Shevkani et al., 2015; Tang & Sun, 2011). Different CPIs differed significantly ($p < 0.05$) in terms of their T_o, T_d, and ΔH. Protein gelatinization parameters, such as T_o, T_d, and ΔH, were 83.47 °C–87.81 °C, 94.31 °C–97.72 °C, and 3.51–7.87 J/g, respectively (Table 2). CPIs showed only one endothermic peak, and T_o, T_d, and ΔH for all CPIs were higher than 83 °C, 94 °C, and 3.5 J/g, respectively. This result is consistent with previous findings (Zhou et al., 2015). CCPI and IRCPI of CCM and SBECPI of SCM showed high T_o, indicating that the structures of the proteins became less stable during the heating process. CPIs (TKCPI, XLCPI, JGCPI, and YHCPI) of HCM showed high T_d, which was possibly due to high proportion of β-sheet conformations (Shevkani et al., 2015). Two endothermic peaks were observed in PPI and SPI, consistent with previous results (He et al., 2014; Hua, Cui, Wang, Mine, & Poysa, 2005). TKCPI of HCM showed a low ΔH, indicating a low content of ordered secondary structures. It could be speculated that the presence of highly denatured protein molecules in TKCPI. In addition, it has been reported that the structural conformations of proteins during extraction may be closely related to ΔH and that an organic solvent can induce the denaturation of protein structures, so they should still be taken into consideration (Tang, Wang, Liu, & Wang, 2009).

3.10. Protein crystalinity

Food materials in solid states may be crystalline, semi-crystalline, or amorphous. Fig. 4 shows the XRD patterns of the protein isolates. CPIs showed two diffraction peaks at approximately 8.5° and 19.5°, whereas PPI and SPI showed two diffraction peaks at approximately 9.0° and 20.0°, respectively. The diffraction profile showed partial crystallinity of the CPIs. This XRD result is slightly different from the XRD pattern of soy protein, wherein the XRD pattern shows three peaks at 8.5°, 19.5°, and 24.4° (Wang et al., 2006). Two similar peaks and the same diffraction angles (8.5° and 19.5°) were observed for African yam bean protein isolate as previously reported (Arogundade et al., 2016).

4. Conclusions

This study demonstrated the preparation methods of cottonseed meal influenced the physicochemical and functional properties of CPIs from meals. It was found that the intermolecular interaction and subunit of HCM protein was changed, which resulted in depolymerisation of protein and broken disulfide-bond of the subunit. The analysis of surface hydrophobicity and intrinsic fluorescence spectra in CPIs of CCM and SCM with high H_0 and FI showed a less denatured and more folded conformation. The observations in FTIR spectroscopy demonstrated a phenomenon whereby α-helix and β-turn structures of CPI transform into β-sheet structure due to denaturation. CPIs had two X-ray diffraction peaks (8.5° and 19.5°) and one endothermic peak (94.31 °C–97.72 °C). Proteins of CCM and SCM displayed high WAC,

OAC, and emulsifying abilities, indicating that proteins of cold-pressed solvent extraction and subcritical fluid extraction cottonseed meals can be used effectively in the food industry.

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References

Adebowale, K., & Lawal, O. (2004). Comparative study of the functional properties of bambarra groundnut (*Voandzeia subterranean*), jack bean (*Canavalia ensiformis*) and mucuna bean (*Mucuna pruriens*) flours. *Food Research International*, *37*(4), 355–365.

Adebowale, Y. A., Adeyemi, I. A., & Oshodi, A. A. (2005). Functional and physicochemical properties of flours of six *Mucuna* species. *African Journal of Biotechnology*, *4*(12), 1461–1468.

Ajibola, C. F., Malomo, S. A., Fagbemi, T. N., & Aluko, R. E. (2016). Polypeptide composition and functional properties of African yam bean seed (*sphenostylis stenocarpa*) albumin, globulin and protein concentrate. *Food Hydrocolloids*, *56*, 189–200.

Anderson, A. D., Alam, M. S., Watanabe, W. O., Carroll, P. M., Wedegaertner, T. C., & Dowd, M. K. (2016). Full replacement of menhaden fish meal protein by low-gossypol cottonseed flour protein in the diet of juvenile black sea bass *centropristis striata*. *Aquaculture*, *464*, 618–628.

Antonov, Y. A., Zhuravleva, I. L., Cardinaels, R., & Moldenaers, P. (2015). Structural studies on the interaction of lysozyme with dextran sulfate. *Food Hydrocolloids*, *44*, 71–80.

Arogundade, L. A., Mu, T. H., & Akinhanmi, T. F. (2016). Structural, physicochemical and interfacial stabilisation properties of ultrafiltered african yam bean (*sphenostylis stenocarpa*) protein isolate compared with those of isoelectric protein isolate. *LWT–Food Science and Technology*, *69*, 400–408.

Diniz, R. S., dos Reis Coimbra, J. S., de Carvalho Teixeira, Á. V. N., da Costa, A. R., Santos, I. J. B., Bressan, G. C., et al. (2014). Production, characterization and foamability of α-lactalbumin/glycomacropeptide supramolecular structures. *Food Research International*, *64*, 157–165.

Ghribi, A. M., Gafsi, I. M., Blecker, C., Danthine, S., Attia, H., & Besbes, S. (2015). Effect of drying methods on physico-chemical and functional properties of chickpea protein concentrates. *Journal of Food Engineering*, *165*, 179–188.

Gong, K. J., Shi, A. M., Liu, H. Z., Liu, L., Hu, H., Adhikari, B., et al. (2016). Emulsifying properties and structure changes of spray and freeze-dried peanut protein isolate. *Journal of Food Engineering*, *170*, 33–40.

He, X. H., Liu, H. Z., Liu, L., Zhao, G. L., Wang, Q., & Chen, Q. L. (2014). Effects of high pressure on the physicochemical and functional properties of peanut protein isolates. *Food Hydrocolloids*, *36*, 123–129.

Hinze, L. L., Horn, P. J., Kothari, N., Dever, J. K., Frelichowski, J., Chapman, K. D., et al. (2015). Nondestructive measurements of cottonseed nutritional trait diversity in the U.S. National cotton germplasm collection. *Crop Science*, *55*(2), 771–782.

Horax, R., Hettiarachchy, N. S., Chen, P., & Jalaluddin, M. (2004). Functional properties of protein isolate from cowpea (*Vigna unguiculata* L. Walp.). *Journal of Food Science*, *69*, 119–121.

Hou, F., Ding, W., Qu, W., Oladejo, A. O., Xiong, F., Zhang, W., et al. (2017). Alkali solution extraction of rice residue protein isolates: Influence of alkali concentration on protein functional, structural properties and lysinoalanine formation. *Food Chemistry*, *218*, 207–215.

Hua, Y., Cui, S. W., Wang, Q., Mine, Y., & Poysa, V. (2005). Heat induced gelling properties of soy protein isolates prepared from different defatted soybean flours. *Food Research International*, *38*(4), 377–385.

Ivanova, P., Chalova, V., Koleva, L., & Pishtiyski, I. (2013). Amino acid composition and solubility of proteins isolated from sunflower meal produced in bulgaria. *International Food Research Journal*, *20*(6), 2995–3000.

Joshi, M., Adhikari, B., Aldred, P., Panozzo, J. F., Kasapis, S., & Barrow, C. J. (2012). Interfacial and emulsifying properties of lentil protein isolate. *Food Chemistry*, *134*, 1343–1353.

Kato, A., & Nakai, S. (1980). Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. *Biochimica et Biophysica Acta (BBA)–Protein Structure*, *624*(1), 13–20.

Kong, J., & Yu, S. (2007). Fourier Transform infrared spectroscopic analysis of protein secondary structures. *Acta Biochimica et Biophysica Sinica*, *39*(8), 549–559.

Li, C., Xue, H., Chen, Z., Ding, Q., & Wang, X. (2014). Comparative studies on the physicochemical properties of peanut protein isolate-polysaccharide conjugates prepared by ultrasonic treatment or classical heating. *Food Research International*, *57*, 1–7.

Mcclements, D. J. (2007). Critical review of techniques and methodologies for characterization of emulsion stability. *Critical Reviews in Food Science and Nutrition*, *47*, 611–649.

Mohan, R. I., & Narasinga Roa, M. S. (1988). Physicochemical properties of gossypin (11s protein) and congossypin (7s protein) of glanded cottonseed. *Journal of Agricultural & Food Chemistry*, *36*(2), 241–245.

Nagalakshmi, D., Rama Rao, S. V., & Panda, A. K. (2007). Cottonseed meal in poultry diets: A review. *Journal of Poultry Science*, *44*(2), 119–134.

Shevkani, K., Singh, N., Kaur, A., & Rana, J. C. (2015). Structural and functional characterization of kidney bean and field pea protein isolates: A comparative study. *Food Hydrocolloids*, *43*, 679–689.

Song, C. L., & Zhao, X. H. (2014). Structure and property modification of an oligochitosan-glycosylated and crosslinked soybean protein generated by microbial transglutaminase. *Food Chemistry*, *163*, 114–119.

Tang, C. H., & Sun, X. (2011). A comparative study of physicochemical and conformational properties in three vicilins from *Phaseolus* legumes: Implications for the structure–function relationship. *Food Hydrocolloids*, *25*, 315–324.

Tang, C. H., Wang, X. Y., Liu, F., & Wang, C. S. (2009). Physicochemical and conformational properties of buckwheat protein isolates: Influence of polyphenol removal with cold organic solvents from buckwheat seed flours. *Journal of Agricultural and Food Chemistry*, *57*, 10740–10748.

Timilsena, Y. P., Adhikari, R., Barrow, C. J., & Adhikari, B. (2016). Physicochemical and functional properties of protein isolate produced from Australian chia seeds. *Food Chemistry*, *212*, 648–656.

Tsaliki, E., Pegiadou, S., & Doxastakis, G. (2002). Evaluation of the foaming properties of cottonseed protein isolates. *Food Hydrocolloids*, *16*, 645–652.

Tsaliki, E., Pegiadou, S., & Doxastakis, G. (2004). Evaluation of the emulsifying properties of cottonseed protein isolates. *Food Hydrocolloids*, *18*, 631–637.

Wang, Q., Du, Y., Hu, X., Yang, J., Fan, L., & Feng, T. (2006). Preparation of alginate/soy protein isolate blend fibers through a novel coagulating bath. *Journal of Applied Polymer Science*, *101*, 425–431.

Wong, B. T., Zhai, J., Hoffmann, S. V., Aguilar, M.-I., Augustin, M., Wooster, T. J., et al. (2012). Conformational changes to deamidated wheat gliadins and β-casein upon adsorption to oil–water emulsion interfaces. *Food Hydrocolloids*, *27*, 91–101.

Wu, H., Wang, Q., Ma, T., & Ren, J. (2009). Comparative studies on the functional properties of various protein concentrate preparations of peanut protein. *Food Research International*, *42*(3), 343–348.

Zhang, Y., Yang, R., Zhao, W., Hua, X., & Zhang, W. (2014). Physicochemical and emulsifying properties of protein extracted from soybean meal assisted by steam flash-explosion. *Innovative Food Science & Emerging Technologies*, *23*(3), 131–137.

Zhang, Y., Zhao, W., Yang, R., Ahmed, M. A., Hua, X., Zhang, W., et al. (2013). Preparation and functional properties of protein from heat-denatured soybean meal assisted by steam flash-explosion with dilute acid soaking. *Journal of Food Engineering*, *119*(1), 56–64.

Zhao, Q., Xiong, H., Selomulya, C., Chen, X. D., Huang, S. F., Ruan, X., et al. (2013). Effects of spray drying and freeze drying on the properties of protein isolate from rice dreg protein. *Food and Bioprocess Technology*, *6*, 1759–1769.

Zheng, L., Ren, J., Su, G., Yang, B., & Zhao, M. (2013). Comparison of *in vitro* digestion characteristics and antioxidant activity of hot- and cold-pressed peanut meals. *Food Chemistry*, *141*, 4246–4252.

Zhou, J. Z., Zhang, H., Gao, L., Wang, L., & Qian, H. F. (2015). Influence of pH and ionic strength on heat-induced formation and rheological properties of cottonseed protein gels. *Food & Bioproducts Processing*, *96*, 27–34.

Zhuge, Q., Posner, E. S., & Deyoe, C. W. (1988). Production study of a low-gossypol protein product from cottonseed meal. *Journal of Agricultural & Food Chemistry*, *36*(1), 153–155.