

FULL ARTICLE

Comparison of wheat, soybean, rice, and pea protein properties for effective applications in food products

Hefei Zhao¹ | Chun Shen¹ | Zijian Wu¹ | Zhong Zhang² | Changmou Xu¹

¹Food Processing Center, Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE, USA

²Department of Food Science, Cornell University, Ithaca, NY, USA

Correspondence

Changmou Xu, Food Processing Center, Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE 68588, USA.
Email: cxu13@unl.edu

Zhong Zhang, Department of Food Science, Cornell University, Ithaca, NY 14853, USA.
Email: zz79@cornell.edu

Funding information

University of Nebraska Foundation, Grant/Award Number: 1024460

Abstract

Pea and rice proteins are promising to substitute allergenic proteins, and increasingly, play important roles in the food industry because of their hypoallergenic characteristics and nutritional value. However, manufacturers generally provide limited functionality information on these proteins. Therefore, this study comprehensively compared functional properties of wheat, soybean, rice, and pea proteins for their industrial applications and illustrated correlation among various functionalities. Results showed that protein solubility (PS) was highly related to its water absorption (WA) capacity, emulsifying activity index (EAI), and emulsion stability index (ESI). The overall functionality of pea protein was close to that of soybean protein while rice protein cannot match with all other proteins. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis indicated the composition of each protein was unique. While the deconvolution of the amide I band of the Raman spectra indicated soybean and pea proteins that shared similar features, but they were different from that of wheat and rice proteins.

Practical applications

Due to the allergenicity of wheat and soybean proteins, food manufacturers are looking for alternative protein sources. Rice and Pea proteins are promising substitutes because of their “allergen-friendly” as well as their emergence in the food market. This study provided a comprehensive comparison of the functionality of commercially available wheat, soybean, rice, and pea proteins. The information presented in this study would be helpful to food scientists, scholars, or engineers when they develop appropriate application of various proteins in food products.

KEYWORDS

functionality, protein, Raman spectrum, SDS-PAGE, secondary structure

1 | INTRODUCTION

Commercially available protein products are mainly extracted from milk whey, egg white, soybeans, and wheats (Brand, Pichler, & Kulozik, 2014; Shevkani, Singh, Kaur, & Rana, 2015), because these materials have relatively high protein content, good cost performance, and are widely accepted by most of consumers. However, all these proteins contain allergens, belonging to major food allergenic concern

in the United States, defined as “The Big Eight,” including milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheats, and soybeans (Koeberl, Clarke, & Lopata, 2014). If replacing them with other proteins, the functionalities to keep the sensory and texture that have been generally accepted by customers should not be sacrificed.

The proteins derived from various sources usually have different physicochemical characteristics and functionalities, which influence their practical applications in the food industry. Besides the

consideration of tastiness and nutrition, maintaining the functionalities of the protein, which is going to be replaced, is equally important for the success of the project. Therefore, it is important to know that protein functionalities or compare the functionalities of one protein with another as a reference if food science researchers and engineers were going to either apply a protein into a new food or substitute one protein in an original receipt with another protein.

Currently, the plant proteins used by the food industry are mostly derived from soybeans and wheats. However, because of the allergenicity, food manufacturers are looking for alternative protein sources (Shevkani et al., 2015). Pea and rice are two promising plant protein sources that have “allergen-friendly” characteristic, well-balanced profile of amino acids (Nunes, Raymundo, & Sousa, 2006), higher digestibility, and biological value (Amagliani, O'Regan, Kelly, & O'Mahony, 2017).

On the nutritional aspect, peas not only contain about 20% proteins with higher levels of lysine and tryptophan than cereal grains, but also contain 5%–20% lesser trypsin inhibitors than soybeans (Shevkani et al., 2015). The nutritional quality of rice protein is evaluated to be equivalent or higher than that of other cereals but significantly lower than proteins derived from animal sources and legumes/oilseed crops (Day, 2013). People can have pea and rice as substitutes of soybean and wheat as protein sources, but should not replace them as food ingredients solely based on the nutritional aspect. It is crucial to maintain their functional properties as well as the reason mentioned previously. Commercial pea and rice proteins increasingly play crucial roles in foodstuffs because of their allergen-friendly characteristics and well-balanced profile of amino acids. The organic rice protein market is estimated at USD 41.7 Million in 2016 and is projected to reach USD 96.5 Million by 2021 (Marketsandmarkets.com, n.d.). The global pea protein market revenue was USD 24.8 million in 2016 (Mordorintelligence.com, 2017), and it is also predicted to reach a value of 34.8 million USD by the year 2020 (Researchandmarkets.com, 2017). However, limited functionality information on these proteins is available, especially when they compared with the most used wheat and soybean proteins. It is very necessary to address this gap as functionality maybe even more important than the hypoallergenic characteristics and nutritional value of proteins in their food applications. Joshi, Liu, and Sathe (2015) assessed selected functional properties of seed flours; nevertheless, there is a lack of literature on a comparative study of the physical and functional properties of commercial protein products under identical experimental conditions. Although the functionalities of some proteins have been reported, due to the experimental conditions that they are usually not consistent, and some functionalities which are shown in one article are not exhibited in another article, it is not very meaningful to compare data among different studies.

Therefore, the objective of this work is to compare various physical and functional properties of commercial wheat, soybean, rice, and pea proteins in exactly the same conditions. Most valuable functionality parameters were selected from three main groups including properties associated with hydration, protein structure/rheological characteristics, and surface prosperities based on a literature review (Pietrysiak,

Smith, Smith, & Ganjyal, 2018; Sharma, Su, Joshi, Roux, & Sathe, 2010). The correlation of various protein properties was also investigated.

2 | MATERIALS AND METHODS

2.1 | Materials and chemicals

As plant proteins used in food products mainly are sourced from the market, four popular commercial protein powders (wheat protein isolates, soybean protein isolates, rice protein concentrates, and pea protein concentrates) were selected for the comparison of various physical and functional properties (Table S1). Sodium dodecyl sulfate (SDS) was purchased from Thermo SCIENTIFIC (Rockford USA). Mini-PROTEAN® TGX™ Pre-cast sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gel for Any kD™ (#456-9033), protein ladder (#161-0363), bromophenol blue (#161-0404), QC Colloidal Coomassie Stain(#161-0803), and 2-Mercaptoethanol (#1610710EDU) were all from Bio-Rad (Hercules, USA). Potato standard test starch was from Perten Instrument (Hägersten, Sweden). All other chemicals were obtained from Fisher Scientific (New Jersey, USA).

2.2 | Proximate composition and physical properties

Protein content was determined with a LECO TruMac® N/CN nitrogen analyzer according to the method of Gamlath, Leong, Ashokkumar, and Martin (2018). The moisture content of plant protein powder was measured by Mettler Toledo HB43-S Halogen moisture analyzer based on the method of Zhang, Kim, Yokoyama, & Kim, (2018). Ash and fat content was determined by the method of Mudalal, Babini, Cavani, and Petracci (2014). The particle size $D_{[4,3]}$ of plant protein powder was measured by static light scattering on a Mastersizer 3000 (Malvern Instruments Ltd., Westborough, USA), based on the method of Murphy et al. (2015). Bulk density was determined by the method of (Muzaffar & Kumar, 2016). The sample color was measured by Minolta Colorimeter CR-300 (Minolta Camera Co., Osaka, Japan) in on the Hunter L^* , a^* , and b^* scale, based on the method of Sharma et al. (2010).

2.3 | Functional properties

Adapted from methods of Boye et al. (2010), protein solubility (PS) was determined at various pH values between 2 and 12 by using a LECO TruMac® N nitrogen analyzer as described previously. Water and lipid absorptions (LA) were measured by the method of Sharma et al. (2010). The emulsifying properties of protein powder were expressed as EAI and ESI based on the turbidimetric method (Akasha, Campbell, Lonchamp, & Euston, 2016), and were calculated by following equations:

$$\text{EAI} \left(\text{m}^2/\text{g} \right) = \frac{2 \times T \times A_0 \times \text{dilution factor}}{C \times \varphi \times 10^3} \quad (1)$$

$$\text{ESI (min)} = \frac{A_0}{A_0 - A_{10}} \times 100\% \quad (2)$$

where C is the weight of protein per unit volume (g/ml), φ is oil volumetric fraction, T is 2.303, A_0 is absorbance immediately after the homogenization, the dilution factor is 100 here, A_{10} is absorbance of sample at the 10 min after the homogenization.

Foaming capacity (FC) and stability (FS) were studied by the method of Sharma et al. (2010). FC was computed as the percentage of increment in the volume of the suspensions at the initial point just after mixing, while FS was estimated as the percentage of foam remaining at the time of 30 min (Shevkani et al., 2015). Pasting property was determined by a Micro Visco-Amylo-Graph®-U (C.W. Brabender® Instrument, INC. South Hackensack, USA). A 12 g potato standard test starch was added by 11.50% (protein to starch) net protein then both materials were evenly dry-mixed before testing; 12 g of potato standard test starch, 14 g of wheat flour (Gold Medal brand), and gluten-free flour (Kind Arthur brand) was also applied for curve references. The peak viscosity, final viscosity, breakdown, and setback (the difference between final viscosity and peak viscosity) were determined as the description in work of Marcoa and Rosell (2008). Least Gelation Concentration (LGC) from 2% to 20% (w/v) was determined by the method of Boye et al. (2010). LGC was the lowest concentration at which the sample did not fall after inversion of the tube and 10 slow taps with an index finger.

2.4 | Protein profiling and fractionation of SDS-PAGE

A 1.0 mg of each kind of protein powder was dispersed in 1ml of reducing buffer and analyzed according to the method of Amagliani, O'Regan, Kelly, and O'Mahony (2017). Bio-Rad Mini-PROTEAN® TGXTM Pre-cast SDS-PAGE gel for Any kD™ was used to run protein samples.

2.5 | Raman spectra

Raman spectra of samples were based on the work of Zhang, Zhang, Lin, and Vardhanabhuti (2012) with some modification. Raman spectra detection was implemented by an XploRA ONE™ 785 nm Raman Spectrometer System (HORIBA, Ltd., Kyoto, Japan) with a 785 nm near-infrared diode laser. A tiny portion of protein powder was placed on a glass slide, and generally compressed by another glass slide, then the upper slide was removed. Five times points were collected for each of the six different spots, which were tantamount to 30 times determinations in total. Each collection accumulated 10 times shot and the average value was acquired for one single report. Spectral data were baseline corrected and standardized according to the protein phenylalanine peak at $1003 \pm 1 \text{ cm}^{-1}$ by a MATLAB R2017a software (MathWorks®, Inc., Natick, USA). Locating overlapping peaks and deconvoluting Raman bands in Amide I ($1600\text{--}1700 \text{ cm}^{-1}$) region

were undertaken by means of PeakFit Version 4.12 software (SPSS Inc., Chicago, IL) (Sun et al., 2011).

2.6 | Statistical analysis

Triplicate data were analyzed by one-way analysis of variance (ANOVA) and multiple comparison tests with Fisher's least significant difference (LSD) method integrated into SAS® Version 9.4 (SAS Institute Inc., Cary, USA). Significant differences ($p < .05$) between means were identified using the LSD multiple range test. Principle component analysis (PCA) was applied for giving an overall profile of protein functionalities that differentiate protein by its functionalities. Raman spectral data were analyzed by a MATLAB R2017a software (MathWorks®, Natick, USA) for baseline correction and standardization the intensity of protein phenylalanine peak at $1003 \pm 1 \text{ cm}^{-1}$.

3 | RESULTS AND DISCUSSION

3.1 | Proximate composition and physical properties

Although all studied protein powders look similar, their proximate composition and physical properties varied significantly (Table 1 and Figure S1). The protein content of these powders ranged from 76.69% (Rice powder) to 86.98% (wheat powder). Soybean and pea powder had higher moisture contents, 8.06% and 8.60%, respectively, than wheat (5.89%) and rice (3.96%) powder. Soybean and pea protein powders contain more ashes than rice and wheat powders by around 20-fold. No fat content was detected in all powders, except pea powder (0.58%). The $D_{[4,3]}$ of wheat powder was the highest among all protein samples, which indicated the largest size of this powder. As shown in Figure S1, although the size class of the highest volume densities of four proteins was comparable to each other, the wheat had the widest size distribution which rendered the wheat had the highest $D_{[4,3]}$ value among proteins. The bulk densities of rice, pea, and wheat powder were close to each other, while that of soybean powder was about half value as compared to those three proteins. The wheat protein had the highest bulk density which means applying the same amount of protein in weight, wheat protein would provide less volume than other proteins. In terms of the color, pea and rice proteins were in beige while soybean and wheat proteins were in white pale (Table 1). The L^* values of soy and wheat proteins were close to each other and higher than the equivalents of pea and rice proteins. Based on the a^* and b^* values, rice and pea proteins were far more in redness and yellowness than soybean and wheat proteins. The lowest L^* and highest a^* and b^* values of pea made the pea protein looked the darkest among the four samples in general. Usually, the white powders such as soybean and wheat protein in this study are welcomed because it minimizes the impact on the appearance of foods. If rice or pea protein was applied to foods, the color issue should be considered.

TABLE 1 Proximate compositions and physical properties of selected commercial wheat, soybean, rice, and pea protein materials

Source	Protein (%)	Moisture (%)	Ash (%)	Fat (%)	$D_{[4,3]}$ μm	Bulk density g/mL	Color			Picture
							L^*	a^*	b^*	
Wheat	86.98 $\pm$ 0.15 ^a	5.89 $\pm$ 0.07 ^c	0.12 $\pm$ 0.03 ^b	NA	82.57 $\pm$ 1.83 ^a	0.609 $\pm$ 0.020 ^a	87.19 $\pm$ 0.07 ^b	0.11 $\pm$ 0.03 ^d	11.25 $\pm$ 0.12 ^c	
Soybean	86.36 $\pm$ 0.08 ^a	8.06 $\pm$ 0.12 ^b	4.01 $\pm$ 0.16 ^a	NA	48.43 $\pm$ 0.20 ^c	0.351 $\pm$ 0.013 ^c	89.28 $\pm$ 0.22 ^a	0.47 $\pm$ 0.02 ^c	11.33 $\pm$ 0.17 ^c	
Rice	76.69 $\pm$ 0.73 ^c	3.96 $\pm$ 0.08 ^d	0.24 $\pm$ 0.05 ^b	NA	48.07 $\pm$ 0.49 ^c	0.555 $\pm$ 0.010 ^b	83.68 $\pm$ 0.06 ^c	2.23 $\pm$ 0.02 ^b	19.62 $\pm$ 0.12 ^b	
Pea	78.01 $\pm$ 0.10 ^b	8.60 $\pm$ 0.10 ^a	3.83 $\pm$ 0.17 ^a	0.58 $\pm$ 0.10 ^a	69.77 $\pm$ 1.43 ^b	0.520 $\pm$ 0.044 ^b	78.74 $\pm$ 0.04 ^d	4.34 $\pm$ 0.04 ^a	21.57 $\pm$ 0.21 ^a	

Note: Results are expressed as means of triplicate determinations $\pm$ SE; different letters within each column indicate significant difference in that group ($p < .05$). Abbreviations ($D_{[4,3]}$ = means diameter of volume; NA = means non-detectable).

3.2 | Functional properties

Functionality is defined as “any property of a food or food ingredient, except its nutritional ones, that affects its utilization” (Kim, Perera, Rajapakse, & Kim, 2016; Kligner, Mangino, Fligner, & Mangino, 1991). Protein functionality is usually cataloged into three main groups (Moure, Sineiro, Domínguez, & Parajó, 2006): (a) properties associated with hydration such as PS, lipid/water absorption (LA/WA); (b) properties related with the protein structure and rheological characteristics such as viscosity, elasticity, adhesiveness, aggregation, & gelatinization; and (c) properties related with the protein surface characteristics such as FC, FS, emulsifying properties (emulsion activity index, EAI and emulsion stability index, ESI). Here, the functionalities were compared among commercial protein powders of wheat, soybean, rice, and pea.

3.2.1 | pH solubility

Solubility is one of the most crucial protein functionalities because other protein functionalities, such as emulsifying, forming, and gelatinization are usually directly associated with it (Shevkani et al., 2015). Usually, native proteins and low denaturation can be indicated by high solubility (Ghribi et al., 2015). It was clearly observed in Figure 1a that isoelectric points (pI) were about pH 4–5, 4–6, and 6–7 for soybean, pea, and wheat protein, respectively, at which allowed these proteins to exhibit a minimum solubility that is close to zero. Our results agreed with the reports that pea proteins extracted by ultrafiltration and isoelectric precipitation had the lowest solubility between pH 4 and 6 (Boye et al., 2010), soybean protein fractions showed isoelectric points in the range from 5.1 to 5.6 (Chove, Grandison, & Lewis, 2001), and gluten protein showed an isoelectric pI (PI) at 6.4 (Majzoobi & Abedi, 2014). Pea and soybean protein performed a typical “U shape” in solubility-pH curve but it is not entirely perfect in acid range that is lower than pH 5. The wheat protein showed a perfect “U shape” in PS crossing the pH range. At the acidic range (pH 2–5), wheat protein obtained about 40%–60% solubility which was remarkably higher than other proteins and indicated that it is a promising candidate for applying in acidified protein beverages, although it is unsuitable for the gluten-allergic group. But for the other three proteins, stabilizers would be required to maintain a homogeneous beverage system at this acidic range. Different from all the other proteins, the rice protein had the lowest solubility almost through the entire pH range, and even at extreme pH value, the solubility was still lower than 10%. This coincided with the rice dreg protein reported in literature (Li et al., 2012). Day (2013) also pointed out that glutelins are account for 80% of the total rice protein, and rice glutelins are water-insoluble because of their extensive aggregation, disulfide bond cross-linking and glycosylation except in dilute alkali.

3.2.2 | Foaming capacity

Soybean and pea proteins showed relatively high FC as the initial volume of form, and they were very close to each other during the

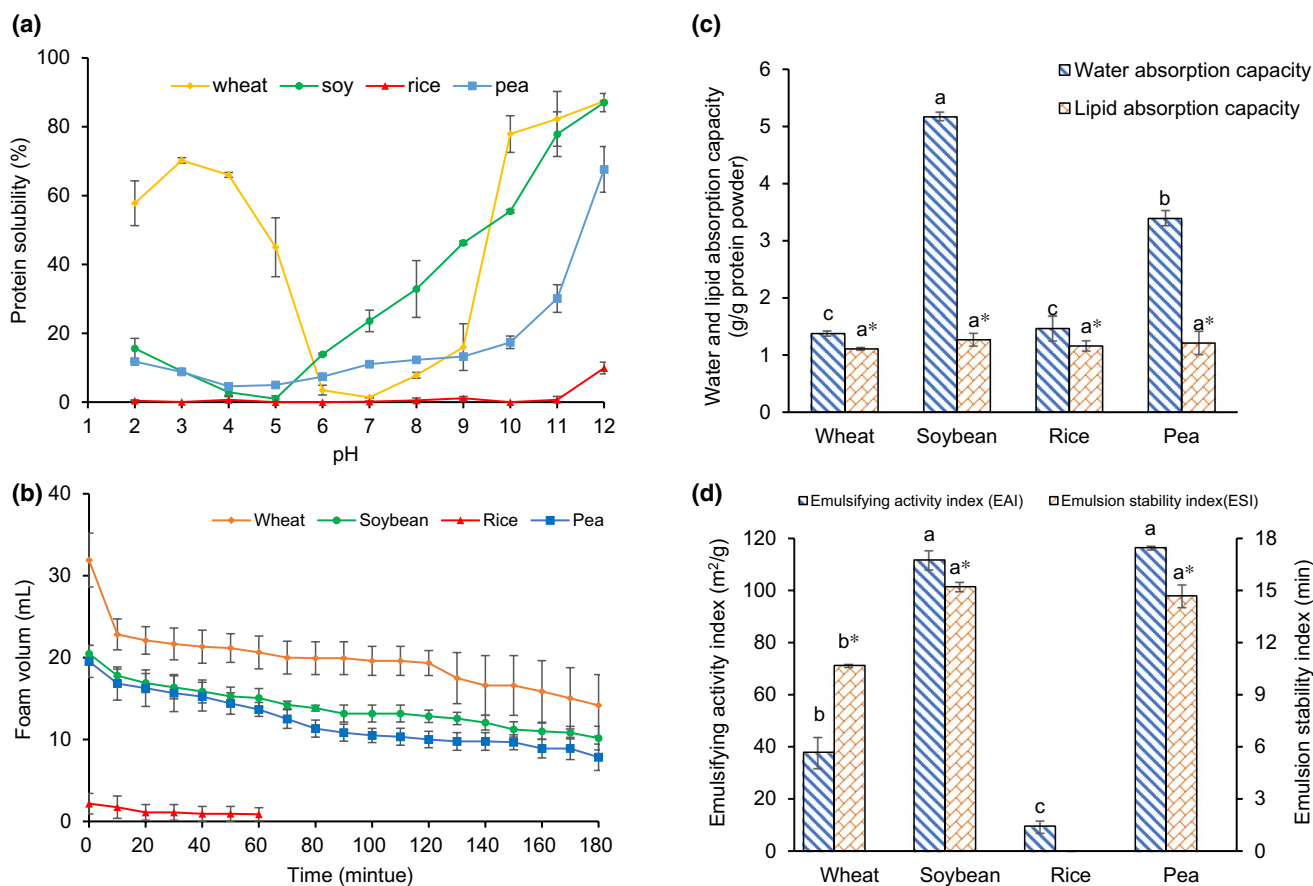


FIGURE 1 Functional properties of wheat, soybean, rice, and pea protein. EAI and ESI of proteins were determined at pH 7. Since rice had very low EAI value, the ESI of rice protein was not detectable. Different letters in each group indicate significant difference ($p = .05$), $n = 3$

period of measurement (Figure 1b). Although wheat protein had a low solubility at pH 7, it showed the highest FC which explained why wheat flour is used for making bread foam. While rice protein presented a very poor foaming ability. Foam stability was estimated at 68.03%, 82.44%, 89.74%, 50.00% for wheat, soybean, pea, and rice proteins, respectively, by the percentage of foam remaining at the time of 30 min. Wheat protein did not perform the highest stability because the volume of wheat foam dropped down drastically at the very beginning in about 10 min. The foam volume of soybean and pea were stable although their capacity cannot complete with that of wheat. The rice protein showed a stability up to 50%, however, it is meaningless due to its extremely low foam capacity. To acquire good foaming ability, the protein should have a high solubility in the water phase; therefore, enable it to decrease interfacial tension and to shape strong elastic films encompassing the dispersed air bubbles (Murray, 2007). It is not reasonable for the high foamability of wheat protein because of its low solubility. And it is the same argument as its emulsifying properties that is the sharing force changed the protein secondary structure. In addition, as oil can be used as antifoaming agents (Kougias, Boe, Einarsdottir, & Angelidaki, 2015), the fat content also should be taken into consideration. As the fat contents in all four proteins were little, the forming ability of proteins was almost not influenced by the fat.

3.2.3 | Water and LA capacity

Soybean protein had the highest WA capacity (5.168 g water/g protein), which was almost 1.5 times of the capacity of pea protein (3.389 g/g) and three times of rice (1.46 g/g), and wheat (1.376 g/g) proteins (Figure 1c). But all proteins had a similar oil absorption capacity that was around 1.2 g oil/g protein, and the values of rice and wheat were close to their WA capacity. The mechanism of oil absorption is attributed mostly to physical entrapment of the oil between particles; in support of this, a correlation coefficient of 0.95 between bulk density and fat absorption by alfalfa leaf protein was found by a previous study (Wang & Kinsella, 1976). This also indicates that of the water was only physically entrapped into space among the wheat and rice protein particles, and the mechanism can be explained by their low PS, and very limited hydration was contributed to their water holding. The less bulk density, the more total volume that powders can provide by the same amount of weight. But for wheat protein, although it had the highest bulk density which provided it the least total volume by the comparison at the same weight, it had almost the same oil absorption to other proteins. A possible explanation is that wheat protein had bigger particles as the curve of particle size distribution shown in Figure S1, which indicated it had more space among particles, therefore, enable it to entrap more oil.

3.2.4 | Emulsifying properties at pH 7

For each protein, EAI and ESI were positively related to each other (Figure 1d). The emulsifying capacity of proteins was consistent with their solubility at pH 7, ranked as soybean protein > pea protein > wheat protein > rice protein. The EAI and ESI of soybean protein were insignificantly different from the equivalents of pea protein, although the solubility of soybean protein was as about 1.5 times as that of pea protein at pH 7 (Figure 1a). Therefore, it is very possible that pea protein can substitute soybean protein in meat and sausage products. High surface hydrophobicity and high solubility of protein were both reported as a crucial condition for optimum emulsifying properties in terms of EAI (Nakai, 1983). Liu et al. (2014) also reported that a higher proportion of larger molecular weight (MW) peptides and higher surface hydrophobicity played a dominant role in the emulsifying properties if the solubility of the sample reached a certain value that is high enough. Therefore, it is reasonable that pea and soybean proteins had a similar emulsifying property while their solubility was different. Due to the solubility of wheat and rice proteins were extremely low at pH 7, their emulsifying properties were not comparable with soybean and rice proteins. However, the result shows the emulsifying ability of wheat protein was not very low as compared to that of rice protein although both wheat and rice had extremely low solubility at pH 7. The possible reason is that the shearing force denatured the wheat protein, then let wheat protein had a higher surface activity.

3.2.5 | Brabender pasting property

Brabender curves can provide information on pasting properties and viscosities of starches, proteins, and flours (Figure 2). The important information is the maximum viscosity around 10

min (point A) and the distance between the maximum viscosity and minimum viscosity around 18 min (point B), which defined as breakdown (A–B). The measurement of commercial wheat flour and gluten-free flour was used as references, and proximate composition analysis of potato starch standard, wheat flour (Gold Medal), and gluten-free flour (Kind Arthur) listed as: protein content $0.224\% \pm 0.047\%$, $12.24\% \pm 0.05\%$, and $4.75\% \pm 0.04\%$; moisture $11.99\% \pm 0.12\%$, $10.24\% \pm 0.24\%$, $9.86\% \pm 0.01\%$, respectively. As can be seen in Figure 2, adding pea or soybean protein increased the maximum viscosity of potato standard starch (1,260 mPas) to that of commercial gluten-free product (1801 mPas), but they returned to the point B very close to its original point of pure potato standard starch, although the break-down of adding pea protein (1,089 mPas) was slightly smaller than that of adding soybean protein (1,176 mPas). This was probably at a high temperature (95°C), the proteins were denatured and the viscosity after that mainly depended on the character of potato starch. While the maximum viscosity only increased slightly by adding rice or wheat protein into potato standard starch. This could be attributed to the low solubility of rice or wheat proteins at neutral pH. As the same reason of protein denatured, the breakdown of adding rice or wheat protein was almost the same as pure potato standard starch. The overall pasting property of commercial wheat flour was lower than all the other measurements, even if its protein content $12.24\% \pm 0.24\%$ was slightly higher than the added protein 11.50%. These results showed that the commercial pea and soybean proteins were promising ingredients for blending wheat flour due to the increment effect on maximum viscosity. Marcoa and Rosell (2008) reported that by adding 5% (w/w, flour-protein blend basis) of the different protein isolates into rice flour, the maximum viscosity was decreased due to the dilution effect on the starch concentration. On the contrary, Liang and King (2003)

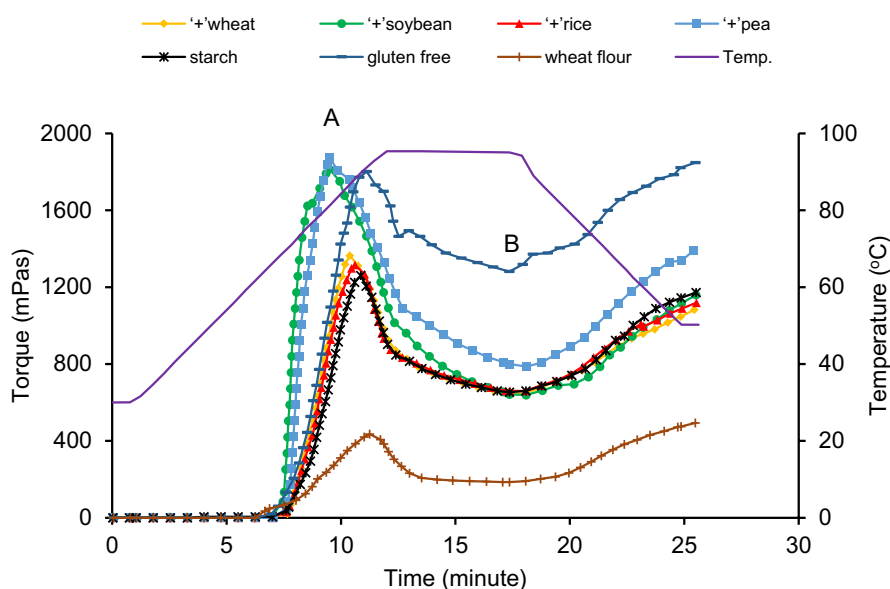


FIGURE 2 Effect of proteins incorporation on the pasting properties of standard starch. A 12 g of potato standard starch was added by 11.50% net protein (starch + protein-total 14 g); 14 g of wheat flour and gluten free flour were applied as references

found that protein removal by alkaline protease digestion from white flour greatly reduced the maximum viscosity. These findings coincide with this study that adding proteins could increase the viscosity of food matrix, but the viscosity of proteins is weaker than the equivalent starches.

3.2.6 | Least gelation concentration

In our study, wheat protein can be gelatinized at 8% (w/v), which was lower than soybean protein (12%) and pea protein (14%). However, the gelatinization of rice protein was not observed the entire concentration range from 2% to 20%. The LGC determines the lowest amount of proteins that can build a firm gel network, without any collapse, therefore, a lower LGC value indicated a higher gelatinization ability. Sun and Arntfield (2010) reported that salt extraction enabled pea protein isolates to perform gelatinization as low as 5.5% of LGC which was considered as a high gelatinization ability. The LGC of pea protein was 14% in this study which was very close to O'Kane, Vereijken, Gruppen, and Van Boekel (2005) report that 16% (w/v) for pea protein isolates was the minimum requirement for heat-induced gel formation at neutral pH 7.1, and hydrophobic interaction is a crucial factor in the formation of protein gel networks. Rice protein contains glutelin with a high molecular weight which constitutes up to 80% of the total rice protein (Day, 2013). Rice glutelins are extensively aggregated with disulfide bond cross-linking and contain glycosylation that makes them

generally insoluble except in dilute alkali (Day, 2013). Therefore, it is possible that the synergistic effect of the larger amount of unhydrolyzed protein and very limited exposed hydrophobic polypeptides inhibited the gelatinization ability of rice protein.

3.3 | Principle component analysis

The PCA was implemented to reduce the multidimensional data of protein functionalities to lower dimensions while retaining most of the information. PCA cataloged the data of protein functionalities into different clusters; therefore, differentiate similarities/differences based on these properties. From the score plot in Figure 3, the first and second components explained 83.11% of all variances of functionalities in total. The functionalities of four proteins were cataloged into four different groups of clusters, and the overall functionalities of soybean and pea proteins were close to each other. As shown in the eigenvector matrix in Table S2, the first component (PC1) was mainly represented by PS, WA capacity, and emulsifying properties (EAI and ESI) in view of their greater values. The PS highly related to WA capacity, EAI and ESI based on correlation coefficients of 0.990, 0.847, and 0.730, respectively. Soybean protein obtained the highest score on first component, followed by pea protein, wheat protein, and rice protein, which was consistent with their solubility at pH 7 (Figures 1a and 3). The second component (PC2) was represented by foaming ability as the same argument. Therefore, the wheat protein had the highest value in the

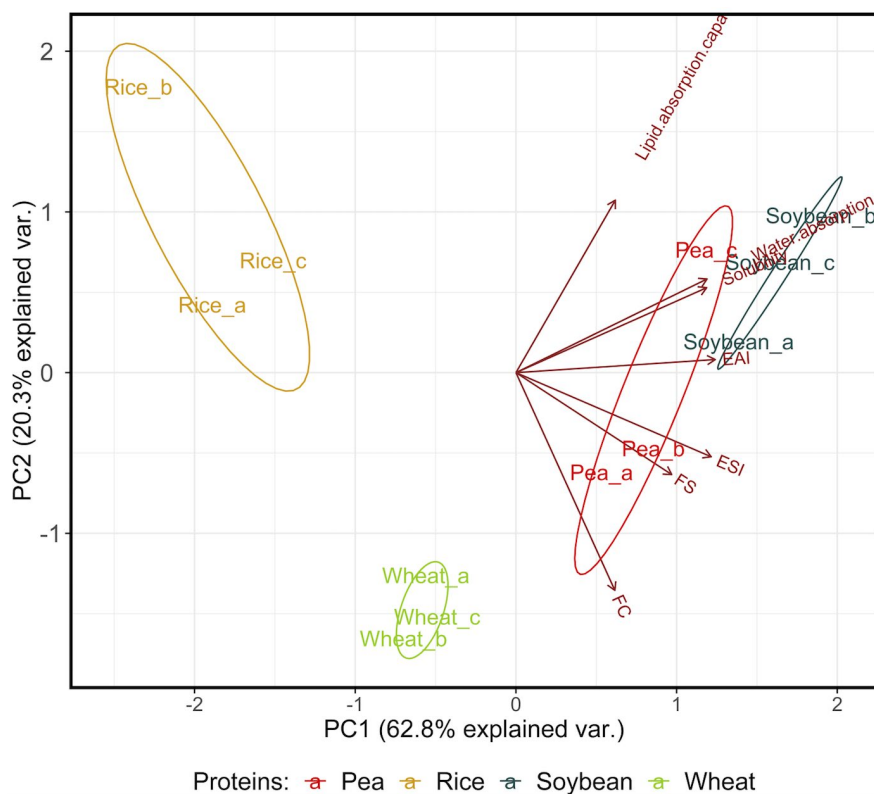


FIGURE 3 Score plot of PCA to differentiate overall functionalities. The component 1 represent for protein solubility at pH7 (PS), water absorption capacity (WC), emulsifying properties (EAI & ESI); The component 2 represent for lipid absorption capacity (LC), foaming capacity (FC), and foaming stability (FS)

second component because of its excellent foaming ability (Figures 1b and 3). In sum, PCA offered an overall profile classification of protein functionalities and clearly differentiated them into different groups. Also, the PC1 and PC2 can be explained by eigenvectors in Table S2 and coincided with all previous functional property data.

3.4 | SDS-PAGE analysis

Protein profiles by SDS-PAGE in four types of plants varied greatly (Figure 4). Under reducing condition, 30, 22, and 15 kDa fractions were observed in soybean protein sample. The 30 kDa referred to α -glutelin, the 22 kDa band indicated a subunit of 66 kDa glutelin (Van Der Borght et al., 2006), and the 15 kDa polypeptide was probably globulin (Kawakatsu, Hirose, Yasuda, & Takaiwa, 2010). According to previous study, 7S (b-conglycinin) and 11S (glycinin) generally account for 80% of protein in soybean (Nishinari, Fang, Guo, & Phillips, 2014). As shown in the gel, the 7S globulin, which is reported exhibiting high functionality in terms of solubility, foaming, and emulsifying properties (Chove, Grandison, & Lewis, 2007), is composed of three subunits defined as α (67 kDa), α' (71 kDa), and β (50 kDa). Also, the 11S globulin is a hexamer having five different subunits, each subunit contains an acidic subunit. Acidic subunit A (acidic pI) has about 35 kDa molecular mass and basic subunit B (basic pI) has about 20 kDa molecular mass (MW). A and B are linked by a disulfide

bond. As reducing reagent was served as breaking down disulfide bond, subunit A (35 kDa) and subunit B (20 kDa) MW can be clearly observed in the lane of soybean.

For the rice protein, the clear lane indicated that the condition contained high concentrations of reducing reagent (Amagliani et al., 2017) employed in this work was strong enough to break down rice protein structuring although 80% of rice protein was highly cross-linked glutelins. The bands 110, 25, 19, and 13 kDa were clearly observed, respectively. The 25 and 19 kDa bands were reported (Amagliani et al., 2017) as the glutelin subunits including α -glutelin, β -glutelin, but the glutelin precursor (52 kDa) was ambiguously observed. The predominant 13 kDa was reported (Amagliani et al., 2017) as prolamin. Rice was reported to contain 10% of alcohol-soluble proteins (prolamin), 10% of salt-soluble proteins (globulin and albumin), and 90% of alkali-soluble proteins (glutelin) (Liu et al., 2018). Therefore, the predominant prolamin band indicated the reducing condition was more efficient for solubilizing prolamin since there were still proteins can be seen on the top of the stacking gel.

In pea protein, subsequent bands of 70–100 kDa, 28–48 kDa were clearly observed, which were reported (Adal et al., 2017) as convicillin, vicillin, and α -subunits of legumin. However, the β -subunit of legumin, 22.3–23.1 kDa (Barac et al., 2010) was not found in this study. The α -subunits of legumin indicated that primary storage proteins in pea, 60 kDa legumin (11S), which contains two subunits fractions cross-linked by disulfide bond can be reduced under experiment conditions (Shand, Ya, Pietrasik, & Wanasundara, 2007), therefore, the 60 kDa band totally disappeared in the lane. A 19 kDa was found as a significant bond, and this bond was also reported by Adal et al. (2017), but in their SDS-PAGE graph, 19 kDa was a weak bond. In wheat protein, three bands around 100 kDa can be clearly observed under reducing conditions. These bands indicated high molecular weight (HMW) glutenin (Gao et al., 2010). The bands with a molecular weight between about 30 and 50 kDa should indicate α -, β -, and γ -subunits of gliadin, which were consistent with previous studies (Žilić, Barac, Pešić, Dodig, & Ignjatović-Micić, 2011). In sum, our study illustrated a clear SDS-PAGE profile for all the four plant proteins and compared them on the same page.

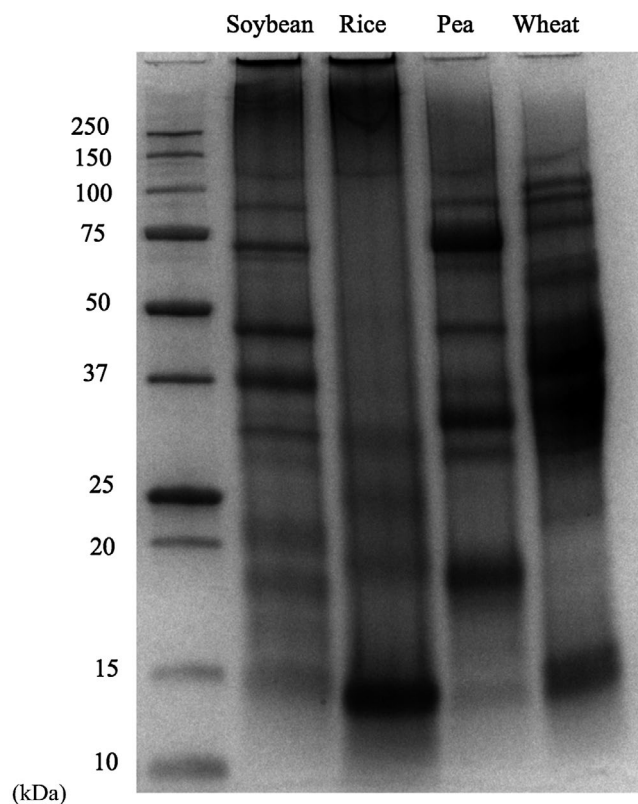


FIGURE 4 SDS-PAGE analysis of soybean, rice, pea, and wheat proteins under reducing condition

3.5 | Raman measurement

The wavenumbers of major Raman bands of each protein in the region of 450–2000 cm^{-1} were recognized according to literature references (Rygula et al., 2013; Sun et al., 2011; Zhang et al., 2012), including S-S stretching at around 507 cm^{-1} , C-S stretching at around 640 cm^{-1} , tyrosine(Tyr) at doublet 860/833 cm^{-1} , phenylalanine (Phe) at around 620 and 1003 cm^{-1} , $-\text{CH}_2$, or $-\text{CH}_3$ bend 1449 cm^{-1} , amide I from 1600–1700 cm^{-1} (Figure 5). However, the pattern of Raman spectra did not show a significant difference among proteins. The connection between the intensity of disulfide bonds and protein molecular weight was

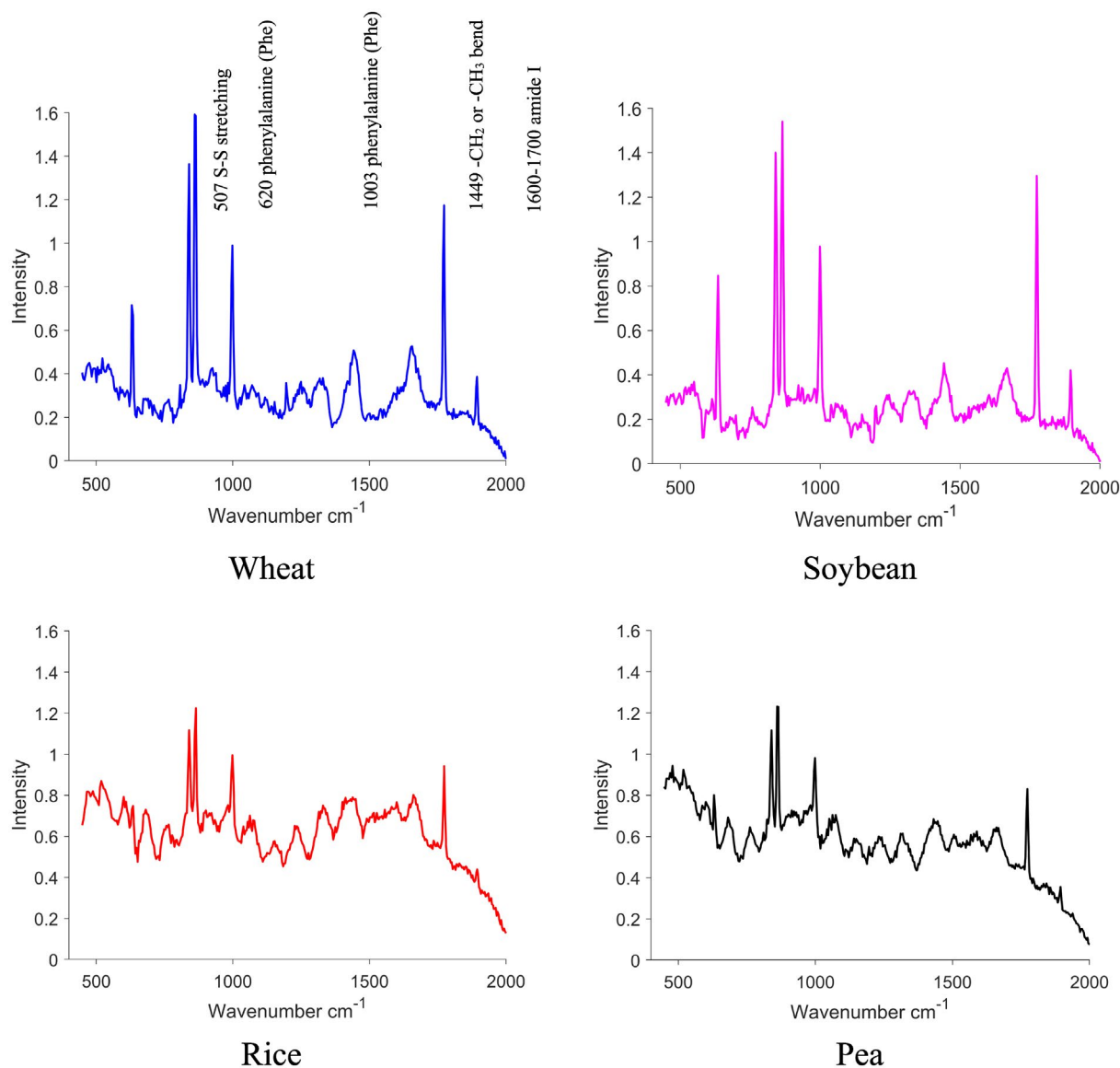


FIGURE 5 Raman Spectrum of soybean, rice, pea, and wheat proteins. Each sample was determined 30 times at 6 different points

not observed, either. For instance, the rice protein is mainly constituted by glutelins, which is ranging from 45 to 150 kDa and expected as high disulfide bond cross-linking (Day, 2013), but the intensity of disulfide bonds of rice protein was not observed stronger than other proteins.

Deconvolved and curve-fitted Raman bands of amide I of all proteins were presented in Figure S2. Structure components were distributed in all the range of amide I from 1600 to 1700 cm^{-1} for wheat and rice proteins, while these were generally found from 1630 to 1700 cm^{-1} for soybean and pea proteins. This interesting finding might indicate some agreement with the group of functionalities as mentioned in PCA analysis (Figure 3). Further, the primary feature of the amide I band was centered at 1657, 1668, 1660, and 1668 cm^{-1} for wheat, soybean, rice, and pea, respectively. As can be seen, the central band of soybean and pea were the same, while that of rice was

close to the wheat. The connection between the wavenumber of components and secondary structure was explained in Table S3 based on published work (Byler & Susi, 1988; Sun et al., 2011). Deconvolution of the amide I band has been successfully applied to monitoring the denaturing process of proteins. Zhang et al. (2012) found that heating whey protein took α -helix component at 1656 cm^{-1} and β -sheet component at 1674 cm^{-1} of whey protein away which indicated thermal denaturation. Bertram, Kohler, Böcker, Ofstad, and Andersen† (2006) identified nine bands in the amide I region on meat myofibrillar, and study showed that these bands had either negative or positive correlation to the temperature applied for the heat treatment. Li, Bomser & Zhang (2005) found that heating bovine immunoglobulin G (IgG) at 82°C for 120 s converted β -sheet into random coils. Various treatments such as heat, enzyme hydrolysis can modify or enhance protein functionalities (Panyam, 1996). And almost all of treatments are

associated with changing in secondary structure, and the change can be observed by using deconvolution of the amide I band as mentioned above. However, to the best of our knowledge, these observations depended upon the same kind of protein before and after treatment. And there is currently a lack of literature to build up the correlation among different proteins with secondary structure difference. Further study will be required to establish connections among a large amount of different kind of proteins, their secondary structures, and protein functionalities.

4 | CONCLUSIONS

This study demonstrated the overall functionality of pea protein was very close to that of soybean protein, which indicates that pea protein is a promising soybean protein substitute, especially in the aspects of functionalities associated with hydration. It may replace soybean protein in protein shakes, sausages, and reconstruction meats, etc. The overall functionality of rice protein cannot match with all the other three proteins; the foaming ability of wheat protein cannot be substituted by all the other three proteins. PS was highly associated with other functionalities, and it is recommended that manufacturers would better to provide this information with products. Although raw data of Raman spectra did not offer a connection between functionalities and protein structure based on the method applied, deconvolution of the amide I band did indicate differences between the group of pea and soybean protein and the group of wheat and rice protein. This finding was consistent with the results of SDS-PAGE, as well as the results of PCA analysis on protein functionalities, which indicates the Raman spectra still could be a useful tool to differentiate proteins. Overall, the information presented in this study would be helpful for food scientists, scholars, and engineers in the appropriate application of various proteins in food products.

ACKNOWLEDGMENTS

This work was supported by the Layman Seed Award program of the University of Nebraska Foundation for developing rapid detection methods of food components using Raman Spectroscopy (No. 1024460). The authors thank Ms. Wanying Cao and Mr. Lei Wang for their helping in SDS-PAGE experiment.

CONFLICT OF INTEREST

The authors declare no competing financial interest.

AUTHOR CONTRIBUTIONS

Hefei Zhao designed the study, performed the experiments, interpreted the results, wrote, and edited the manuscript. Chun Shen performed the protein functionality experiment. Zijian Wu designed SDS-PAGE experiments, interpreted the results. Zhong Zhang designed the study and helped the Raman spectra experiment. Changmou Xu designed the study, interpreted the results, wrote, and edited the manuscript.

ORCID

Hefei Zhao  <https://orcid.org/0000-0003-1988-7230>

Changmou Xu  <https://orcid.org/0000-0002-8115-8445>

REFERENCES

- Adal, E., Sadeghpour, A., Connell, S., Rappolt, M., Ibanoglu, E., & Sarkar, A. (2017). Heteroprotein complex formation of bovine lactoferrin and pea protein isolate: A multiscale structural analysis. *Biomacromolecules*, 18(2), 625–635. <https://doi.org/10.1021/acs.biomac.6b01857>
- Akasha, I., Campbell, L., Lonchamp, J., & Euston, S. R. (2016). The major proteins of the seed of the fruit of the date palm (*Phoenix dactylifera* L.): Characterisation and emulsifying properties. *Food Chemistry*, 197, 799–806. <https://doi.org/10.1016/j.foodchem.2015.11.046>
- Amagliani, L., O'Regan, J., Kelly, A. L., & O'Mahony, J. A. (2017a). Composition and protein profile analysis of rice protein ingredients. *Journal of Food Composition and Analysis*, 59, 18–26. <https://doi.org/10.1016/j.jfca.2016.12.026>
- Amagliani, L., O'Regan, J., Kelly, A. L., & O'Mahony, J. A. (2017b). The composition, extraction, functionality and applications of rice proteins: A review. *Trends in Food Science & Technology*, 64, 1–12. <https://doi.org/10.1016/j.tifs.2017.01.008>
- Barac, M., Cabrilo, S., Pesic, M., Stanojevic, S., Zilic, S., Macej, O., & Ristic, N. (2010). Profile and functional properties of seed proteins from six pea (*Pisum sativum*) genotypes. *International Journal of Molecular Sciences*, 11(12), 4973–4990. <https://doi.org/10.3390/ijms11124973>
- Bertram, H. C., Kohler, A., Böcker, U., Ofstad, R., & Andersen, H. J. (2006). Heat-induced changes in myofibrillar protein structures and myowater of two pork qualities. A combined FT-IR spectroscopy and low-field NMR relaxometry study. *Journal of Agricultural and Food Chemistry*, 54(5), 1740–1746.
- Boye, J. I., Aksay, S., Roufik, S., Ribéreau, S., Mondor, M., Farnworth, E., & Rajamohamed, S. H. (2010). Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. *Food Research International*, 43(2), 537–546. <https://doi.org/10.1016/j.foodres.2009.07.021>
- Brand, J., Pichler, M., & Kulozik, U. (2014). Enabling egg white protein fractionation processes by pre-treatment with high-pressure homogenization. *Journal of Food Engineering*, 132, 48–54. <https://doi.org/10.1016/j.jfoodeng.2014.02.012>
- Byler, D. M., & Susi, H. (1988). Fourier deconvolution of the Amide I Raman band of proteins as related to conformation. *Applied Spectroscopy*, 42(5), 819–826.
- Chove, B. E., Grandison, A. S., & Lewis, M. J. (2001). Emulsifying properties of soy protein isolate fractions obtained by isoelectric precipitation. *Journal of the Science of Food and Agriculture*, 81(8), 759–763. <https://doi.org/10.1002/jsfa.877>
- Day, L. (2013). Proteins from land plants – Potential resources for human nutrition and food security. *Trends in Food Science & Technology*, 32(1), 25–42. <https://doi.org/10.1016/j.tifs.2013.05.005>
- Chove, B. E., Grandison, A. S., & Lewis, M. J. (2007). Some functional properties of fractionated soy protein isolates obtained by microfiltration. *Food Hydrocolloids*, 21(8), 1379–1388. <https://doi.org/10.1016/j.foodhyd.2006.10.018>
- Gamlath, C. J., Leong, T. S. H., Ashokkumar, M., & Martin, G. J. O. (2018). The inhibitory roles of native whey protein on the rennet gelation of bovine milk. *Food Chemistry*, 244, 36–43. <https://doi.org/10.1016/j.foodchem.2017.10.029>
- Gao, L., Ma, W., Chen, J., Wang, K. E., Li, J., Wang, S., ... Yan, Y. (2010). Characterization and comparative analysis of wheat high molecular weight glutenin subunits by SDS-PAGE, RP-HPLC, HPCE, and MALDI-TOF-MS. *Journal of Agricultural and Food Chemistry*, 58(5), 2777–2786. <https://doi.org/10.1021/jf903363z>

- 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. <https://doi.org/10.1016/j.jfoodeng.2015.06.021>
- Joshi, A. U., Liu, C., & Sathe, S. K. (2015). Functional properties of select seed flours. *LWT - Food Science and Technology*, 60(1), 325–331. <https://doi.org/10.1016/j.lwt.2014.08.038>
- Kawakatsu, T., Hirose, S., Yasuda, H., & Takaiwa, F. (2010). Reducing rice seed storage protein accumulation leads to changes in nutrient quality and storage organelle formation. *Plant Physiology*, 154(4), 1842–1854. <https://doi.org/10.1104/pp.110.164343>
- Kim, S. K., Perera, U., Rajapakse, N., & Kim, S. (2016). *Seafood processing by-products*. NY: Springer-Verlag.
- Kligner, K. L., Mangino, M. E., Fligner, K. L., & Mangino, M. E. (1991). Relationship of composition to protein functionality. In *ACS Symposium series-American Chemical Society (USA)* (pp. 1–12).
- Koeberl, M., Clarke, D., & Lopata, A. L. (2014). Next generation of food allergen quantification using mass spectrometric systems. *Journal of Proteome Research*, 13(8), 3499–3509. <https://doi.org/10.1021/pr500247r>
- Kougiass, P. G., Boe, K., Einarsdottir, E. S., & Angelidaki, I. (2015). Counteracting foaming caused by lipids or proteins in biogas reactors using rapeseed oil or oleic acid as antifoaming agents. *Water Research*, 79, 119–127. <https://doi.org/10.1016/j.watres.2015.04.034>
- Li, S.-Q., Bomser, J. A., & Zhang, Q. H. (2005). Effects of pulsed electric fields and heat treatment on stability and secondary structure of bovine immunoglobulin G. *Journal of Agricultural and Food Chemistry*, 53(3), 663–670.
- Li, X., Xiong, H., Yang, K., Peng, D., Peng, H., & Zhao, Q. (2012). Optimization of the biological processing of rice dregs into nutritional peptides with the aid of trypsin. *Journal of Food Science and Technology*, 49(5), 537–546. <https://doi.org/10.1007/s13197-011-0303-6>
- Liang, X., & King, J. M. (2003). Pasting and crystalline property differences of commercial and isolated rice starch with added amino acids. *Journal of Food Science*, 68(3), 832–838. <https://doi.org/10.1111/j.1365-2621.2003.tb08251.x>
- Liu, C.-K., Chen, C.-A., Lee, T.-Y., Chang, H.-H., Liao, H.-F., & Chen, Y.-J. (2018). Rice protein prolamin promotes anti-leukemia immunity and inhibits leukemia growth in vivo. *Food and Chemical Toxicology*, 112, 435–440. <https://doi.org/10.1016/j.fct.2017.09.043>
- Liu, Y., Li, X., Chen, Z., Yu, J., Wang, F., & Wang, J. (2014). Characterization of structural and functional properties of fish protein hydrolysates from surimi processing by-products. *Food Chemistry*, 151, 459–465. <https://doi.org/10.1016/j.foodchem.2013.11.089>
- Majzoobi, M., & Abedi, E. (2014). Effects of pH changes on functional properties of native and acetylated wheat gluten. *Journal Homepage*, 21(3), 1219–1224.
- Marcoa, C., & Rosell, C. M. (2008). Effect of different protein isolates and transglutaminase on rice flour properties. *Journal of Food Engineering*, 84(1), 132–139. <https://doi.org/10.1016/j.jfoodeng.2007.05.003>
- Marketsandmarkets.com. (n.d.). *Organic rice protein market by type*. <https://www.marketsandmarkets.com/Market-Reports/organic-rice-proteinmarket-122246113.html>
- Mordorintelligence.com. (2017). *Pea protein market - Growth, trends and forecasts (2018- 2023)*. <https://www.mordorintelligence.com/industry-reports/north-america-pea-proteinmarket>
- Moure, A., Sineiro, J., Domínguez, H., & Parajó, J. C. (2006). Functionality of oilseed protein products: A review. *Food Research International*, 39(9), 945–963. <https://doi.org/10.1016/j.foodres.2006.07.002>
- Mudalal, S., Babini, E., Cavani, C., & Petracci, M. (2014). Quantity and functionality of protein fractions in chicken breast fillets affected by white striping. *Poultry Science*, 93(8), 2108–2116. <https://doi.org/10.3382/ps.2014-03911>
- Murphy, E. G., Roos, Y. H., Hogan, S. A., Maher, P. G., Flynn, C. G., & Fenelon, M. A. (2015). Physical stability of infant milk formula made with selectively hydrolysed whey proteins. *International Dairy Journal*, 40, 39–46. <https://doi.org/10.1016/j.idairyj.2014.08.012>
- Murray, B. S. (2007). Stabilization of bubbles and foams. *Current Opinion in Colloid & Interface Science*, 12(4–5), 232–241. <https://doi.org/10.1016/j.cocis.2007.07.009>
- Muzaffar, K., & Kumar, P. (2016). Comparative efficiency of maltodextrin and protein in the production of spray-dried tamarind pulp powder. *Drying Technology*, 34(7), 802–809. <https://doi.org/10.1080/07373937.2015.1080724>
- Nakai, S. (1983). Structure-function relationships of food proteins: With an emphasis on the importance of protein hydrophobicity. *Journal of Agricultural and Food Chemistry*, 31(4), 676–683. <https://doi.org/10.1021/jf00118a001>
- Nishinari, K., Fang, Y., Guo, S., & Phillips, G. O. (2014). Soy proteins: A review on composition, aggregation and emulsification. *Food Hydrocolloids*, 39, 301–318. <https://doi.org/10.1016/j.foodhyd.2014.01.013>
- Nunes, M. C., Raymundo, A., & Sousa, I. (2006). Rheological behaviour and microstructure of pea protein/κ-carrageenan/starch gels with different setting conditions. *Food Hydrocolloids*, 20(1), 106–113. <https://doi.org/10.1016/j.foodhyd.2005.03.011>
- O'Kane, F. E., Vereijken, J. M., Gruppen, H., & Van Boekel, M. A. J. S. (2005). Gelation behavior of protein isolates extracted from 5 cultivars of *Pisum sativum* L. *Journal of Food Science*, 70(2), 132–137.
- Panyam, D. (1996). Enhancing the functionality of food proteins by enzymatic modification. *Trends in Food Science & Technology*, 7(4), 120–125. [https://doi.org/10.1016/0924-2244\(96\)10012-1](https://doi.org/10.1016/0924-2244(96)10012-1)
- Pietrysiak, E., Smith, D. M., Smith, B. M., & Ganjyal, G. M. (2018). Enhanced functionality of pea-rice protein isolate blends through direct steam injection processing. *Food Chemistry*, 243, 338–344. <https://doi.org/10.1016/j.foodchem.2017.09.132>
- Researchandmarkets.com. (2017). *Pea protein market by type- global trends & forecast to 2020*. <https://www.researchandmarkets.com/reports/3733842/organic-pea-proteinmarket-by-type-application>
- Rygula, A., Majzner, K., Marzec, K. M., Kaczor, A., Pilarczyk, M., & Baranska, M. (2013). Raman spectroscopy of proteins: A review. *Journal of Raman Spectroscopy*, 44(8), 1061–1076.
- Shand, P. J., Ya, H., Pietrasik, Z., & Wanasundara, P. K. J. P. D. (2007). Physicochemical and textural properties of heat-induced pea protein isolate gels. *Food Chemistry*, 102(4), 1119–1130.
- Sharma, G. M., Su, M., Joshi, A. U., Roux, K. H., & Sathe, S. K. (2010). Functional properties of select edible oilseed proteins. *Journal of Agricultural and Food Chemistry*, 58(9), 5457–5464.
- 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.
- Sun, W., Zhao, Q., Zhao, M., Yang, B., Cui, C., & Ren, J. (2011). Structural evaluation of myofibrillar proteins during processing of cantonese sausage by Raman spectroscopy. *Journal of Agricultural and Food Chemistry*, 59(20), 11070–11077.
- Sun, X. D., & Arntfield, S. D. (2010). Gelation properties of salt-extracted pea protein induced by heat treatment. *Food Research International*, 43(2), 509–515.
- Van Der Borgh, A., Vandeputte, G. E., Derycke, V., Brijs, K., Daenen, G., & Delcour, J. A. (2006). Extractability and chromatographic separation of rice endosperm proteins. *Journal of Cereal Science*, 44(1), 68–74. <https://doi.org/10.1016/j.jcs.2006.03.005>
- Wang, J. C., & Kinsella, J. E. (1976). Functional properties of novel proteins: Alfalfa leaf protein. *Journal of Food Science*, 41(2), 286–292. <https://doi.org/10.1111/j.1365-2621.1976.tb00602.x>

- Zhang, S., Kim, N., Yokoyama, W., & Kim, Y. (2018). Effects of moisture content on mechanical properties, transparency, and thermal stability of yuba film. *Food Chemistry*, 243, 202–207. <https://doi.org/10.1016/j.foodchem.2017.09.127>
- Zhang, S., Zhang, Z., Lin, M., & Vardhanabhuti, B. (2012). Raman spectroscopic characterization of structural changes in heated whey protein isolate upon soluble complex formation with pectin at near neutral pH. *Journal of Agricultural and Food Chemistry*, 60(48), 12029–12035. <https://doi.org/10.1021/jf303780c>
- Žilić, S., Barać, M., Pešić, M., Dodig, D., & Ignjatović-Micić, D. (2011). Characterization of proteins from grain of different bread and durum wheat genotypes. *International Journal of Molecular Sciences*, 12(12), 5878–5894. <https://doi.org/10.3390/ijms12095878>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Zhao H, Shen C, Wu Z, Zhang Z, Xu C. Comparison of wheat, soybean, rice, and pea protein properties for effective applications in food products. *J Food Biochem*. 2020;00:e13157. <https://doi.org/10.1111/jfbc.13157>

Figure S1. Particle size distribution of protein powders of wheat, soybean, rice, and pea

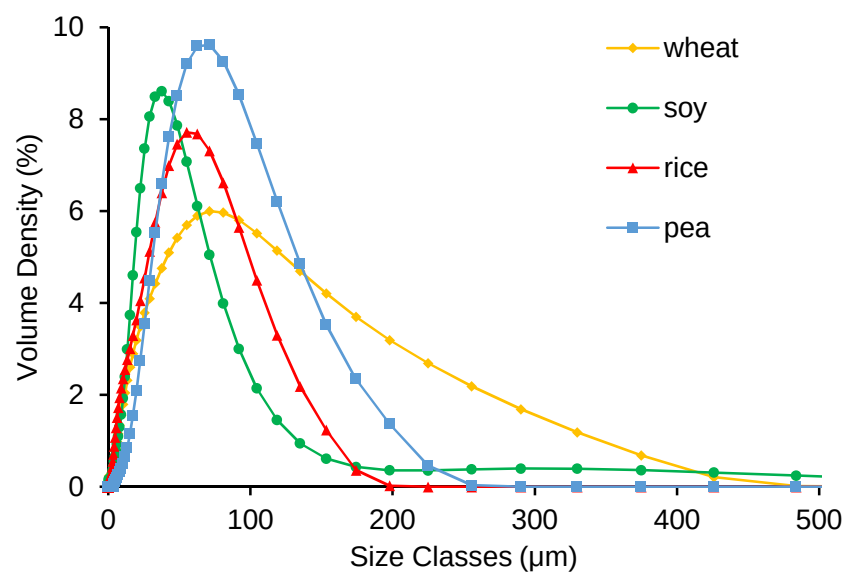


Figure S2. Deconvolved and curve-fitted Raman bands of amide I of proteins

Note: Each sample was determined 30 times at 6 different points

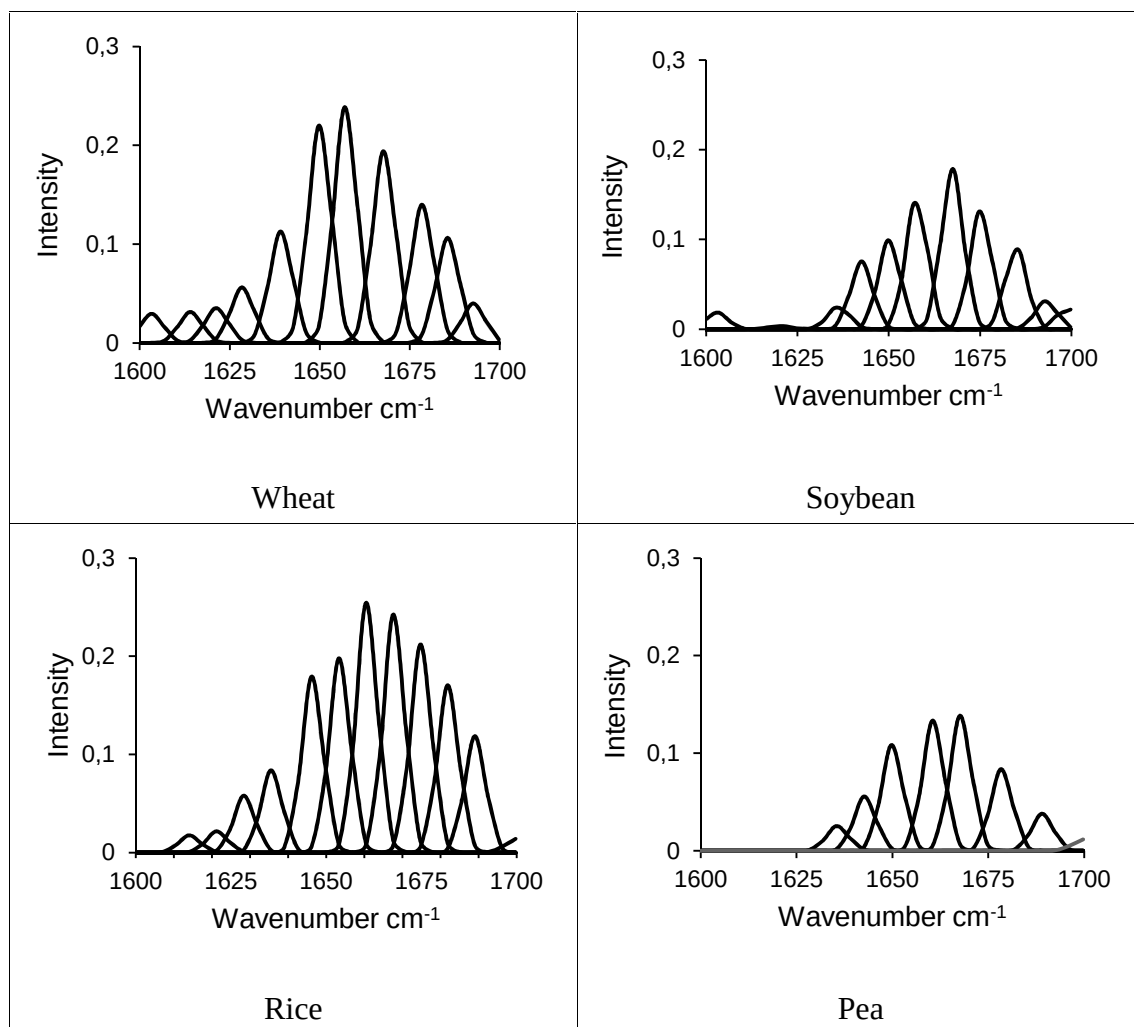


Table S1. Scientific, brand names and manufacturers of selected commercial wheat, soybean, rice, and pea protein materials

Protein powders	Scientific Name	Brand Name	Manufacturers
Wheat	<i>Triticum aestivum</i>	Gem Pro TM HPG	MANILDRA Milling Co., Hamburg, USA
Soybean	<i>Glycine max</i>	ProFam [®] 066-974	ADM Milling Co., Decatur, USA
Rice	<i>Oryza sativa</i>	Organic Oryzatein [®] Ultra 80	AXIOM Foods, Los Angeles, USA
Pea	<i>Pisum sativum</i>	Pro-Tex1485PP	ProFood International, Naperville, USA

Table S2. Eigenvector & correlation of different protein properties

	Component 1	Component 2	Correlation of Protein Solubility at pH 7
Protein Solubility at pH 7(PS)	0.432*	-0.255	1.000
Water absorption capacity (WC)	0.433*	-0.281	0.990*
Lipid absorption capacity (LC)	0.225	-0.518	0.497
Foaming capacity (FC)	0.224	0.654*	0.182
Foaming stability (FS)	0.353	0.306*	0.515
Emulsifying activity index (EAI)	0.452*	-0.039	0.845*
Emulsion stability index (ESI)	0.442*	0.253	0.730*

* Indicate significant difference at $P < 0.05$.

Table S3. Determined frequencies of Amide I bands and relative structures

	Wheat protein	Soybean protein	Rice protein	Pea protein
other	1603	1603	-	-
other	1614	-	1614	-
β -sheet	1621	1621	1621	-
β -sheet	1628	-	1628	-
turn	1639	1636	1636	1636
random coil	-	1643	1646	1643
α -helix	1650	1650	-	1650
α -helix	1657	1657	1654	-
β -sheet	-	-	1661	1661
β -sheet	1668	1668	1668	1668
turn	1678	1675	1675	1678
turn	1686	1686	1682	-
turn	1693	1693	1689	1689

Note: As reported, α -helix, band at 1650-1652 cm^{-1} ; β -sheet, bands at 1620-1627, 1628-1632, 1663-1665, or 1670-1673 cm^{-1} ; turns, band at 1635-1639 cm^{-1} ; random coil, band at 1642-1646 cm^{-1} ; other (amino acid side chains), bands at 1604-1607 and 1609-1619 cm^{-1} .