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Functional Properties of Protein Isolates from *Caragana korshinskii* Kom. Extracted by Three Different Methods

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ABSTRACT: Seeking cheap, sustainable protein sources greatly facilitates in alleviating the dependence upon expensive animal-based protein in many developing countries. *Caragana korshinskii* Kom. offers a good alternative feedstock because of its high-content of protein, low fertilizer and pesticide requirements, excellent stress (high salty and less water) tolerance, wide adaptability, etc. The functional properties of *C. korshinskii* Kom. protein isolates by three different extraction methods were investigated. The extraction processes greatly influenced the physiological characteristics of protein isolates. *C. korshinskii* Kom. protein isolate by traditional alkaline extraction (Al-CPI) exhibited good performance on emulsifying activity index, oil and water absorption capacity, and foaming property compared to A-CPI (*C. korshinskii* Kom. protein isolate by the acetone precipitation method) and TCA-CPI (*C. korshinskii* Kom. protein isolate by trichloroacetic acid−acetone precipitation). The water and oil adsorption capacities of Al-CPI were observed at 4.99 and 3.45 g/g, respectively, even much higher than those of soy protein isolate (SPI) (3.94 and 2.95 g/g, respectively). The highest foaming capacity was observed by Al-CPI at 185.0%, followed by A-CPI (177.5%), TCA-CPI (142.5%), and SPI (141.9%), respectively. It has to be noted that A-CPI showed good solubility at acidic pH and excellent *in vitro* digestibility. After sequential pepsin−trypsin digestion, the percentage of N release of A-CPI reached up to 83.7%, which was 1.63 times that of Al-CPI (51.2%), 1.19 times that of TCA-CPI (70.1%), and slightly higher than that of the commercial SPI (82.5%). These results indicate that *C. korshinskii* Kom. holds great potential for application in the animal feed and food additive industry.

KEYWORDS: *Caragana korshinskii Kom., functional properties, in vitro digestibility, protein extraction, protein isolate, alkaline extraction*

INTRODUCTION

With the increasing demand for animal feed and food industry, plant proteins have received continuous concerns in the recent couple of years.[1] As one of the vital sources for human beings and animals, plant-based protein guarantees necessary nutrition demands because it offers many physiological activity potentials for their health promotion. It is generally supplemented to food and animal feed to improve certain functional characteristics of the final products.[2] As a kind of leguminosae shrubs, *Caragana korshinskii* Kom. offers an alternative option for animal feeds or food additives because of several favorable characteristics: high-content of protein, low fertilizer and pesticide requirements, excellent stress (high salty and less water) tolerance, and wide adaptability. Generally, it needs to be harvested every 3 years to make it flourish.[3] In China, *C. korshinskii* Kom. is widely grown in the arid or semi-arid regions in the northwest of China to prevent water and soil erosion, as well as control desertification. Recently, a large amount of this shrub was artificially planted as sand binders on desertified sandy land in Inner Mongolia, which greatly contributed to the ecological and economic value.[4] The residues of this shrub are generally used as firewood, green fertilizer, livestock forage, etc. Because of the abundant cellulosic polysaccharides in *C. korshinskii* Kom., Sun et al. suggested that it needed to be fractionized by converting cellulose, hemicellulose, and lignin into valuable chemical products.[3] Futhermore, a recent study[5] reported that the content of free amino acids in *C. korshinskii* Kom. was up to 176.6 g/kg of leaves, with aspartic acid, glutamic acid, praline, and serine accounting for more than 70% of the total amino acids.

For use of protein as food ingredients in the food industry, it is quite necessary to investigate their functional properties, including emulsifying and foaming properties, water- and oil-binding capacity, and protein digestibility.[2,6,7] These intrinsic physicochemical characteristics affect their behavior in food systems during processing, manufacturing, storage, and preparation.[8] Previous studies have reported the functional properties of plant protein isolates, including rice bran protein isolate,[9] buckwheat protein isolate,[6] soy protein isolate,[8] etc. Although many species of *Caragana* have been widely used for sand dune fixation and livestock forage, protein-based ingredients of *Caragana* are not yet well-used in food applications. This is probably due to limited information on the structural and functional properties of protein isolated from *Caragana*.

Various extraction processes have been investigated to improve protein extractability from green plant stems, fruits,

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and leaves. Alkaline extraction is widely used for soybean isolate protein recovery from defatted soy flakes after pressing and hexane extraction. Some researchers also demonstrated that trichloroacetic acid−acetone (TCA−acetone) precipitation gave the highest yield of total protein and the greatest number of protein spots for the following two-dimensional gel electrophoresis (2-DE) analysis.[10] However, protein functional properties are greatly dependent upon their preparation processes, which may affect protein structure along with amino acid composition. For example, protein three-dimensional (3D) structures as well as protein functionalities undergo a significant change during isoelectric precipitation. Rawdkuen et al.[11] reported that solubility of tilapia protein was found to be highest using the conventional washing process (3.91 mg/g), followed by an alkaline-aided process (0.57 mg/g) and acid-aided process (0.23 mg/g). Yang et al.[12] suggested that the *in vitro* digestibility and bile acid binding of rice protein were primarily controlled by the degree of the alkaline extraction method.

Therefore, the objective of the current study was to investigate the effect of various extraction methods on the functional properties of *C. korshinskii* Kom. protein isolates. The variances in protein solubility, water and oil adsorption capacities, emulsifying property, and foaming functionality of protein isolates were compared. *In vitro* digestibility was also conducted to evaluate its potential as food ingredients, aiming toward a better understanding of the functional property for their possible applications in the food industry.

MATERIALS AND METHODS

Materials. *C. korshinskii* Kom. was collected in LiangCheng county of Inner Mongolia, China. Air-dried leaves and tissues were milled and passed through a 20-mesh screen and then stored at −20 °C until further use. The defatted soy protein isolates (SPIs) were kindly gifted from Yu Wang Group (Shandong province, China). Pepsin from porcine gastric mucosa (≥250 units/mg) and trypsin from porcine pancreas [1000−2000 benzyl arginine ethyl ester (BAEE) units/mg of solid] were purchased from Sigma (St. Louis, MO). β-Mercaptoethanol (β-ME) were purchased from Shanghai BOAO Biochemical Co., China. All other chemicals used were analytical-grade.

Preparation of *C. korshinskii* Kom. Protein Isolates (CPIs). CPIs were prepared from milled materials by the alkali extraction,[13] acetone precipitation, and TCA−acetone precipitation[14] methods.

For the alkaline extraction method, briefly, 100 g of milled material was dispersed in 2 L of 0.12 M NaOH and then extracted with stirring at 37 °C for 1 h. The resulting slurry was separated into the supernatant and residue by centrifugation at 8000*g* for 20 min at 4 °C. The pH of the supernatant was adjusted to 4.0 with 0.1 N HCl to isoelectrically precipitate protein. The resultant precipitate was obtained by centrifugation at 8000*g* for 20 min at 4 °C again and freeze-dried to obtain Al-CPI.

For the acetone precipitation method, 100 g of milled material was dispersed in 2 L of extraction buffer [62.5 mmol L^{-1} Tris-HCl (pH 6.8), 0.5% sodium dodecyl sulfate (SDS), 10% glycerol, and 5% β-ME]. The mixture was incubated at 4 °C for 7 h and centrifuged at 8000*g* for 20 min at 4 °C. Precooled acetone was then added to the protein supernatant and incubated at −20 °C overnight. After centrifugation at 8000*g* for 20 min at 4 °C, the precipitate was then rinsed 3 times with precooled acetone and dried under nitrogen to obtain A-CPI.

For the TCA-acetone precipitation method, the protein supernatant was obtained by the same procedure with acetone precipitation method. However, the supernatant was mixed with cold acetone containing 10% TCA (w/v) and 0.07% β-ME (v/v). Proteins were allowed to precipitate overnight at −20 °C, then collected by centrifugation at 8000*g* for 20 min at 4 °C. After discarding the supernatant, the precipitate was rinsed three times with precooled acetone containing 0.07% β-ME, and dried under nitrogen to obtain TCA-CPI.

Protein Solubility (PS). PS was determined according to the method by Aluko and Yada,[15] with some modifications. Briefly, 1% (w/v) protein dispersions were prepared in 0.01 M Na_2HPO_4. Then, the pH of the solutions was carefully adjusted from pH 1.0 to 10.0 with either 0.1 N HCl or 0.1 N NaOH. After 1 h of stirring at 25 °C, the dispersions were centrifuged at 4000*g* for 20 min. For total soluble protein content (control), the protein samples were dispersed in 0.1 N NaOH with magnetic strirring and then centrifuged. The protein content in the supernatants was determined by the Braford method using bovine serum albumin (BSA) as the standard. Protein solubility was calculated as eq 1.

$$\text{PS}\ (\%) = \frac{\text{protein content of sample}}{\text{protein content of control}} \times 100\% \quad (1)$$

Emulsifying Properties. Emulsifying properties were determined according to the method by Pearce and Kinsella,[16] with some modifications. Protein dispersions [0.1% (w/v), 3 mL] were prepared in 0.05 M Tris-HCl buffer (pH 7.5). A total of 1 mL of pure corn oil was then added to this dispersion. The emulsions were obtained by homogenizing the mixture for 5 min using an ultrasonic disrupter (Ningbo Scienta Biotechnology Co., Ltd., Zhejiang province, China). A total of 50 μL of the emulsions was taken at 0 and 10 min from the bottom of the homogenized emulsion and diluted with a 0.1% SDS solution (1:100). The absorbance at 500 nm at specific intervals was used to calculate the emulsifying activity index (EAI) and the emulsifying stability index (ESI) as eqs 2 and 3

$$\text{EAI} = \frac{2(2.203A\text{DF})}{\text{OP}[(1-\theta)C]} \quad (2)$$

where A is the absorbance at 500 nm, DF is the diluting factor (100), OP is optical path (0.01 m), θ is the fraction of oil used to form the emulsion (0.25), and C is the initial protein concentration (0.1 g/mL)

$$\text{ESI} = \frac{\text{EAI}_{\max}}{[(\text{EAI}_{\max} - \text{EAI}_{\min}) \times 100]} \quad (3)$$

where $\text{EAI}_{\max}$ and $\text{EAI}_{\min}$ mean the EAI obtained at 0 and 10 min, respectively.

Water Absorption Capacity (WAC). WAC was determined using the method by Tomotake et al.,[17] with some slight modifications. A total of 2 g of protein isolates was dispersed in 20 mL of distilled water and mixed for 30 s every 10 min using a glass rod with 4 times of repetition. The mixture was centrifuged at 4000*g* for 20 min. The supernatant was carefully discarded. WAC was calculated as the increased percentage of the sample weight.

Oil Absorption Capacity (OAC). OAC was determined by the method from Sze-Tao and Sathe.[18] Briefly, 0.5 g of protein isolates and 3.0 mL of corn oil were mixed and stirred for 1 min. After a holding period of 30 min, the mixture was centrifuged at 3000*g* for 20 min. OAC was expressed as the percentage of oil trapped by the protein isolate.

Foaming Capacity (FC) and Foam Stability (FS). FC and FS were determined by the method described by Sze-Tao et al.[18] A total of 1 g of protein isolates was dissolved in 100 mL of distilled water and homogenized for 1 min at high speed. The blend was immediately transferred to a 100 mL graduated cylinder. FC was calculated as the percentage ratio of the volume increase to that of the original volume. For the determination of FS, the changes of foam volume in the graduated cylinder were recorded at intervals of 15, 30, and 60 min.

***In Vitro* Digestibility.** The *in vitro* digestibility of protein was evaluated by the method from Nunes et al.,[19] with some modifications. A total of 15 mL of 1% (w/v) CPI or SPI solutions in 0.1 M HCl was preincubated in a water bath at 37 °C for 5 min. Then, an amount of pepsin (20 mg of pepsin/mL of 0.1 M KH_2PO_4 buffer at pH 2) was added, and the ratio of enzyme/protein substrate was 1:100 (w/w). The mixture was gently shaken at 37 °C for 0, 1, 5, 10, 20, 30, 60, 120, 150, and 180 min, and the pH was adjusted to 7.0 with 1.0 M NaOH

to stop the enzymatic reactions. When pepsin hydrolyzation was finished, the protein hydrolyzate was further digested by the addition of trypsin (20 mg of trypsin/mL of 0.1 M Tris-HCl buffer at pH 7.0) at 37 °C for 1, 5, 10, 20, 30, 60, 120, 150, and 180 min. The ratio of enzyme/protein substrate was 1:20 (w/w).

The digested hydrolysates were mixed with 10% (w/v) TCA, with a final concentration of TCA of 5% (w/v), and centrifuged at 4000*g* for 20 min. The nitrogen content of the sample was determined by the Kjeldahl method (N × 6.25). The percentage of N release during the digestion was calculated by Iwami et al.[20]

$$\text{percentage of N release} = \frac{(N_0 - N_t) \times 100}{N_{tot}} \times 100\% \quad (4)$$

where t is the digestion time (min), N_t (mg) is the TCA-insoluble N after digestion for t (min), N_0 (mg) is the TCA-insoluble N in the protein sample, and N_{tot} (mg) is the total N of the protein sample.

Statistical Analysis. The results are presented as the mean ± standard deviation (SD). All experiments were performed in triplicate. Significant differences between means ($p < 0.05$) were determined by a two-tailed t test.

RESULTS AND DISCUSSION

PS. The PS profiles of protein isolates from various extraction methods as a function pH were shown in Figure 1.

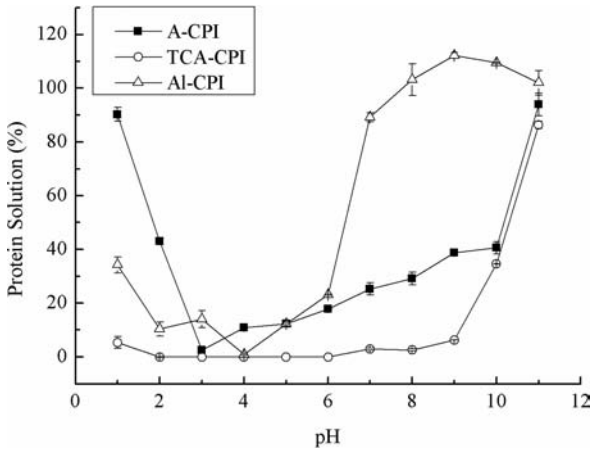

Figure 1. Protein solution profiles (PS) of *C. korshinskii* Kom. protein isolates as a function of pH. Vertical bars show SDs from three determinations.

The PS of A-CPI and Al-CPI showed a typical bell-shaped curve. The PS increased on either side of a specific pH value, including acidic and alkaline, with the minimum solubility of A-CPI (2.56%) and Al-CPI (1.01%) observed at pH 3.0 and 4.0, respectively. However, the PS of Al-CPI increased significantly from pH 4.0 to 9.0 compared to that of A-CPI. Generally, insoluble protein aggregate was generated around the isoelectric point, thus resulting in the lowest solubility.[2] Singh et al.[21] indicated that the loss of electrostatic repulsive forces, high bulk density, and large diameter of the aggregates could cause the formation of protein aggregates. Similar results were also observed in *Ginkgo biloba* seed protein isolates[22] and *Akebia trifoliata* var. australis seed protein isolates.[13] In contrast, PS of TCA-CPI was not significantly affected by pH below 9.0. It exhibited relatively low solubility (below 6.27%) over this wide pH range, suggesting severe protein denaturation during the extraction process. Besides, polymeric contaminants co-precipitated with TCA-CPI may also hinder protein resuspension and affect its PS.[23] However, it exerted a substantial increase between pH 9.0 and 11.0. In the range of pH 6.0−11.0, the pH−PS curves of TCA-CPI and A-CPI showed similar trends and the PS of Al-CPI was much higher than that of A-CPI and TCA-CPI. It has to be noted that high solubility (above 100%) was observed in all CPIs when pH was above 11.0.

As an important physicochemical property of protein, PS has a strong influence on the texture, color, and sensory properties.[24] However, the PS is dependent upon the protein resources, extraction methods, pH value, etc. At acidic pH below 3.0, PS of A-CPI was significantly higher than that of Al-CPI. On the contrary, Al-CPI showed a higher value of PS than A-CPI at basic pH of 7.0−10.0. It may be attributed to the exposed surface amino acid composition of protein isolates with different levels of carboxyl and amino groups.[25]

Emulsifying Property. Both EAI and ESI were investigated to study the variances in emulsifying properties of CPIs by three different extraction methods. EAI expresses the interfacial area stabilized per unit weight of protein, characterizing the ability of a protein to absorb to the oil−water interface. As shown in Figure 2, the lowest EAI values of A-CPI (9.64 m^2/g),

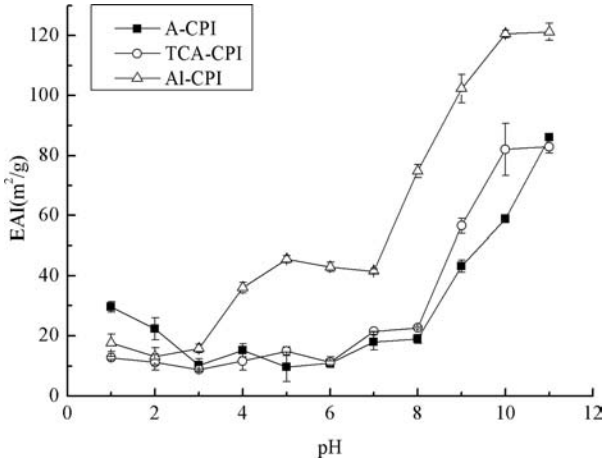

Figure 2. Effects of pH on EAI properties of *C. korshinskii* Kom. protein isolates. Vertical bars show SDs from three determinations.

TCA-CPI (8.71 m^2/g), and Al-CPI (13.15 m^2/g) were obtained at near isoelectric point, which is similar to the trends of PS−pH profiles. Tang speculated that EAI was well-correlated with PS in buckwheat protein isolates.[6] In addition, surface charge and surface hydrophobicity were also found to affect EAI of protein isolates. Karaca et al.[25] presented a multiple regression predictive model for EAI, in which these factors were all taken into consideration. EAI of CPIs at alkaline pH was found to be significantly higher than that at acidic pH. pH exerted its effects on emulsification properties primarily by altering the charge distribution on protein molecules. Du et al.[13] inferred a higher surface hydrophobicity of protein and hydrophobic−hydrophilic balance on the protein-surface-restrained protein−water interaction, resulting in a lower EAI in acidic environment. With the increase of pH above 7.0, EAI of CPIs showed a remarkable enhancement and reached the highest value at pH 11.0. ESI describes the stability of an emulsion formed by protein isolates to resist changes in its physiochemical property over a defined time period. As shown in Figure 3, the ESI of various CPIs showed a pH dependence over a wide range of pH values, which is similar to the result of buckwheat protein products.[6] Several physiochemical character-

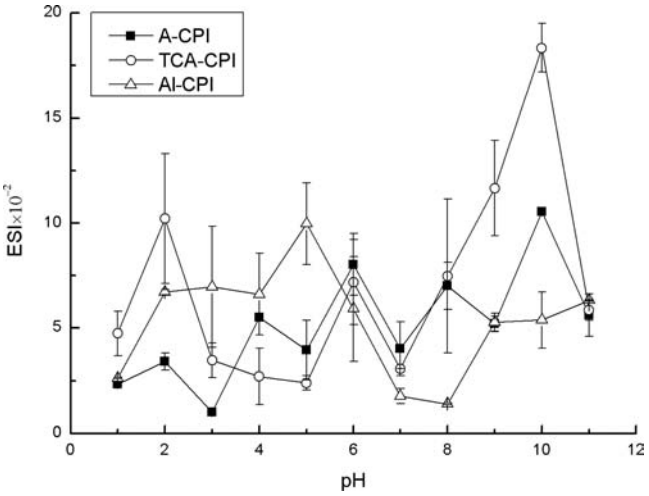

Figure 3. ESI of *C. korshinskii* Kom. protein isolates over a wide range of pH values. Vertical bars show SDs from three determinations.

istics, including surface charge, solubility, and surface hydrophobicity, determine the ESI property of a specific protein isolate. Furthermore, the concentration of sodium chloride in protein isolates affects ESI because of the salting-in and salting-out mechanism.[21]

WAC. WAC of a protein isolate describes its ability to bind water molecules under limited water conditions. As illustrated in Table 1, the WAC of protein isolates by various extraction methods was compared to commercial SPI. The highest WAC was obtained by Al-CPI (4.99 g/g), followed by commercial SPI (3.94 g/g), A-CPI (3.76 g/g), and TCA-CPI (2.79 g/g). Several intrinsic characteristics (protein conformation, hydrophilic–hydrophobic balance of amino acids, etc.) and environmental parameters (pH, ionic strength, temperature, etc.) have been considered to be responsible for the water-binding capacity of protein isolates.[7] It was noteworthy that A-CPI showed lower WAC than TCA-CPI, which was prepared by the same extraction buffer. It might be due to the difference in protein diversity and solubility (as shown in Figure 1). Low WHC of protein isolates makes it less susceptible to heat denaturation. Different preparations of protein isolates lead to various polar amino acids of the protein–water interface, having a significant effect on the water absorption properties. In addition, PS of A-CPI was significantly higher than that of TCA-CPI at neutral pH. As reported earlier, protein with high solubility exhibited low WAC.[26]

Table 1. Water Absorption and Fat Absorption Capacities[a] of *C. korshinskii* Kom. Protein Isolates and Soy Protein Isolates

	WAC (g/g)	OAC (g/g)
Al-CPI	4.99 ± 0.54	3.45 ± 0.08
TAC-CPI	3.76 ± 0.02	3.30 ± 0.11
A-CPI	2.79 ± 0.13	2.42 ± 0.60
SPI	3.94 ± 0.41	2.95 ± 0.53

[a]Data are given as the mean value ± SD (n = 3).

The WAC of TCA-CPI was much higher than field pea protein isolates (2.05 g/g),[27] lower than freeze-drying buckwheat protein (3.21 g/g),[6] and comparable to beach pea protein isolates (2.57–2.88 g/g).[7]

OAC. It indicated that OAC of CPIs by various extraction methods ranged from 2.42 to 3.45 g/g (Table 1). OAC of CPI was higher than that of buckwheat protein isolates (1.58–1.63 g/g)[6] and comparable to that of *Lupinus* protein isolates (1.53–3.06 g/g).[28] It was noted that the OAC of Al-CPI and TCA-CPI was even much higher than that of commercial SPI (2.95 g/g). It is suggested that lipid–protein complexes, protein components, and protein conformational characteristics contributed to the difference in OAC of protein isolates.[8,17] The protein structure has both hydrophilic and hydrophobic groups and, thereby, interacts with water and oil simultaneously in the food system.[29] In a previous study, Lin et al.[30] suggested that higher binding of oil to proteins was owing to the availability of lipophilic groups. The high OAC of protein isolates could be used in the food industry, such as ground meal formulation, meat substitutes and extenders, donghnuts, and soups. However, a relatively lower value of OAC (2.42 g/g) was observed at A-CPI, which may also be desirable in deep-fat-frying food.

FC and FS. It was shown from Table 2 that the foaming capacities of Al-CPI, A-CPI, TCA-CPI, and SPI were 185.0, 177.5, 142.5, and 141.9%, respectively. The FC of Al-CPI was markedly higher than that of beach pea (128–143%)[7] and *G. biloba* seeds (42.4–84.6%).[22] The differences of FC of protein isolates were determined by the diffusion rates of protein at the air–water interface to unfold its structure as well as the capability to encapsulate air particles.[31] High PS is a prerequisite to achieve better FC and FS.[22] It is clear from Figure 1 that the highest PS was obtained by Al-CPI at pH 7.0, followed by A-CPI and TCA-CPI, which matched well with the trend of FC. Furthermore, more interaction at the air–water interface and flexibility of the protein surfactant molecules facilitated the stable formation of air bubbles, suggesting that a high FC was obtained.[9] FS indicates the percentage of foam remaining after a given period of time, which is dependent upon the formation of a thick cohesive layer around the air bubble . As seen in Table 2, foams from Al-CPI, A-CPI, and TCA-CPI were all quite stable after 30 and 60 min. Similar trends were observed for legume seeds[31] and beach pea isolates.[7] The high FS could be attributed to the unfolding of the protein structure, which facilitated surface hydrophobic

Table 2. FC and FS[a] of *C. korshinskii* Kom. Protein Isolates and Soy Protein Isolates

property	Al-CPI	TCA-CPI	A-CPI	SPI
foam expansion	185.0 ± 7.1	142.5 ± 10.6	177.5 ± 3.5	141.9 ± 6.2
FS (%)				
15 min	132.5 ± 10.6	131.0 ± 8.5	165.0 ± 7.1	141.3 ± 6.7
30 min	123.0 ± 2.8	127.0 ± 9.9	157.5 ± 3.5	135.6 ± 8.0
60 min	119.0 ± 1.4	127.5 ± 3.5	154.5 ± 2.1	119.4 ± 2.7

[a]Data are given as the mean value ± SD (n = 3).

association as well as reduced air leakage, therefore preventing rupture and coalescence.[32]

***In Vitro* Digestibility.** *In vitro* digestibility characterizes the digestion capability of protein isolates by pepsin and trypsin in a simulated gastric solution, indicating an outstanding property to evaluate the physiological function. *In vitro* digestibility of CPIs by three different methods and SPI were investigated by sequential pepsin−trypsin digestion. A typical profile of the nitrogen release of CPIs and SPI was obtained in Figure 4. At

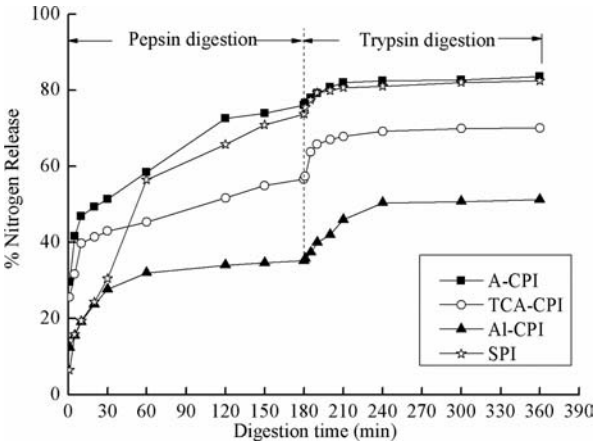

Figure 4. Typsin profiles for the percentage of nitrogen release during pepsin and trypsin digestion of *C. korshinskii* Kom. protein isolates. Initial protein concentrate, 1% (w/v) in 0.1 M HCl; pepsin solution, 20 mg of pepsin/mL of 0.1 M KH_2PO_4 buffer (pH 2); trypsin solution, 20 mg of trypsin/mL of 0.1 M Tris-HCl buffer (pH 7.0); the ratio of pepsin/protein substrate, 1:100 (w/w); the ratio of trypsin/protein substrate, 1:20 (w/w); temperature, 37 °C.

the beginning of pepsin digestion, the enzymatic hydrolysate of TCA-CPI and A-CPI showed a marked liner increase of the percentage of N release. After 20 min, the percentage of N release increased slowly before leveling off at 180 min. With the similar tendency of pepsin digestion, the enzymatic hydrolysate of SPI and Al-SPI exhibited a significant increase of the percentage of N release before 60 and 30 min, respectively. The percentage of N release of SPI kept a steady increase until the end of pepsin digestion, with that of Al-CPI being kept almost unchanged after 30 min in contrast. At the end of pepsin digestion, the percentage of N release of A-CKP was 73.9%, which is higher than those of SPI (70.9%), Al-CPI (35.5%), and TCA-CPI (54.9%). During the period of trypsin digestion, the percentage of N release of CPIs and SPI showed a steady increase during the first 60 min. At the end of sequential pepsin−trypsin digestion, the percentage of N release of A-CPI reached up to 83.7%, which was 1.63 times that of Al-CPI (51.2%), 1.19 times that of TCA-CPI (70.1%), and slightly higher than that of the commercial SPI (82.5%). Tang[6] speculated that the difference in protein hydrolysates was attributed to the susceptibility of their different protein constituents to pepsin−trypsin digestion. It has to be noted that protein digestibility of A-CPI was even higher than those of the whole meals for cowpea (73%) and pigeonpeas (59%).[33] A previous study reported similar results with the digestibility of protein isolates from Finale pea and Frijaune pea ranging from 83.7 to 85.4%.[34]

In summary, the functional properties of *C. korshinskii* Kom. protein isolates by three different extraction methods were investigated. In comparison to protein isolates from other legumes, Al-CPI exhibited good solubility over a wide range of pH values as well as high absorption capability and foaming functionality. The extraction processes greatly influenced the physiological characteristics of protein isolates. Al-CPI exhibited good performance on emulsifying activity index, oil and water absorption capacities, and foaming property compared to A-CPI and Al-CPI. However, A-CPI showed good solubility at acidic pH and excellent *in vitro* digestibility. After sequential pepsin−trypsin digestion, the percentage of N release of A-CPI reached up to 83.7%, which was 1.63 times that of Al-CPI (51.2%), 1.19 times that of TCA-CPI (70.1%), and slightly higher than that of the commercial SPI (82.5%). Therefore, *C. korshinskii* Kom. holds great potential for application of its protein isolates because of its good functional properties, suggesting a possible application as additives in the animal feed and food additive industry.

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Notes

The authors declare no competing financial interest.

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