

Physico-Chemical and Functional Properties of Protein Concentrate from Lima Beans (*Phaseolus lunatus*)

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ARTICLE INFO

Received: 21/3/2022
Revised: 21/4/2022
Accepted: 27/5/2022
Published: 28/6/2022

KEYWORDS

Protein;
Lima bean;
Phaseolus lunatus;
Protein concentrate;
Functional properties.

ABSTRACT

The purpose of this study was to characterize physico-chemical properties (including chemical composition, molecular weight) and functional properties (including oil and water absorption capacities, emulsifying activity and emulsifying stability indexes, foaming ability, rheological properties) of protein concentrate from Lima beans (*Phaseolus lunatus*) (PPC). The PPC recovery and the protein yields were 11.56 and 67.66%, respectively. The PPC has the moisture, protein, fat, ash, and total carbohydrate contents of 9.50, 71.77, 0.22, 6.23, and 11.94%, respectively. This PPC has fractions with molecular weight ranging from 16 to 72 kDa which correspond to the 7S globulin (43 – 75 kDa) and 11S globulin (21 – 38 kDa) subunits and other polypeptides. Besides, the oil-absorption capacity of PPC (2.86 mL/g) was higher than that of soy protein isolate (2.29 mL/g), while their water-absorption capacity was insignificant different ($p > 0.05$). Emulsification capacity, foamability of PPC increased with the increasing of its concentrations, its acidity or alkalinity. The obtained PPC can be used as an alternative to traditional sources of proteins for various applications in the food industry.

Doi: <https://doi.org/10.54644/jte.70B.2022.1180>

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1. Introduction

The primary sources of animal proteins are casein, whey protein, fish protein, pig and chicken muscle, hemoglobin, blood plasma protein, gelatin and egg yolks [1]. Normally, proteins from animal origin are expensive and relatively difficult to obtain, which leads to the development of studies to obtain protein from plants sources. The primary plant proteins come from legume seeds such as soybean, mung bean, pea and chickpea [2]. With high protein content and low cost, legume has become an important source of protein in researches and applications. After the acquisition process, the plant proteins are improved in functional characteristics to be used in foods for different purposes such as improving the texture, balancing the plant and animal proteins, etc...

Protein concentrate and protein isolate products are popular on the market today. The main difference between them is a result of the slight differences in the production process. Protein concentrates often contain 65–70% protein or can reach 80%, while protein isolates can contain more than 90% protein due to its process of dismissing any non-protein components [3].

In addition to the common bean, soybeans, the previous studies showed that Lima beans (*Phaseolus lunatus*) are a natural source of plant-based protein with a wide variety of essential amino acids [4,5]. Its seeds contain proteins (10.1% to 20.7%), carbohydrate (30.7 – 62.4%), low fat (0.1 – 1.2%), dietary fiber (2.1 – 4.9%), and total ash (1.7-3.7 %) [6]. Protein from Lima beans may be useful in some food formulations. However, there is limited information on physico-chemical and functional properties such as emulsifying capacity, foamability, viscosity, water-absorption capacity, oil-absorption capacity, rheological properties, molecular weights, etc...

Therefore, the aim of this study is to describe physico-chemical and functional properties of *Phaseolus* protein concentrate (PPC) that are suitable for food applications.

2. Materials and Methods

2.1. Materials

Lima beans were purchased at Phu Minh Tam Trading and Service Co., Ltd, Ho Chi Minh City, Vietnam. Soy protein isolate (SPI) was adopted from Lime Vietnam Co., Ltd, Ho Chi Minh City, Vietnam. Sodium hydroxide (NaOH), hydrochloric acid (HCl), sulfuric acid (H₂SO₄), ethanol (C₂H₆O), were purchased from Xilong Ltd. with purity ≥ 99%.

Phaseolus protein concentrate was obtained following the procedure of Betancur-Ancona *et al.* with some modifications (Figure 1) [5]. Lima beans were cleaned of impurities, soaked in water at a ratio of 1:2 (w:v) for 3 h, then peeled. The peeled beans were dried (50°C, 12 h) to attain a final moisture content of less than 10%. Dried beans (100 g) were finely ground and sieved (100 mesh) using a Seka Z10 mill (power of 2980 W, for 10 min). Next, this flour (150 g) was soaked in 96% ethanol at a ratio of 1:3 (w:v) for 24 h and then centrifuged using a Rotanta 460 Hettich centrifuge (1100×g, 20 min, in twice replications) for lipid removal. The defatted flour was dried (50°C, 6 h). Then, the flour was mixed with water at a ratio of 1:6 (w:v), adjusted to pH 11 (1 N NaOH solution) and stirred continuously for 1 hour. The mixture was then centrifuged (1100×g, 20 min). The supernatant containing proteins was collected and adjusted to pH of 4.5 using 1N HCl solution. The suspension was allowed to settle for 2 h at 4°C, then centrifuged at (1100×g, 20 min). The centrifuged solid was convectively dried at 50°C for 6 h, and then milled to obtain a 100-mesh size PPC powder.

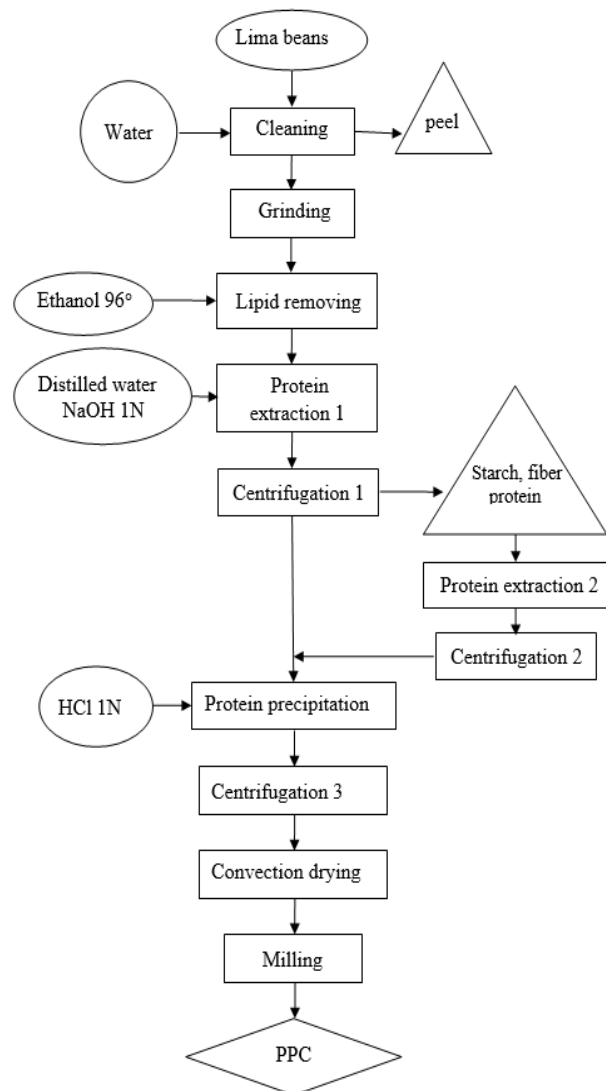


Figure 1. Flowchart of *Phaseolus* protein concentrate (PPC) production

2.2. Analytical methods

PPC Yield (H, %) was determined by formula (1):

$$H = \frac{m_1}{m_0} \times 100 \quad (1)$$

Where, m_1 was the weight of PPC (g); m_0 was the weight of lima beans (g).

Protein yield (PY, %) is the ratio of the amount of protein in PPC to the initial total weight of proteins in the beans. PY was calculated by formula (2):

$$PY = \frac{H \times C_p}{C_0} \quad (2)$$

Where, H was the yield of protein production (%); C_p - protein content in PPC (%); C_0 - protein content in initial beans (%).

Determination of protein molecular weights was conducted using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) based on the method of Laemmli et al. with 4% stacking gel and 12.5% resolving gel [7].

Water-absorption capacity (WAC) of protein samples (PPC and SPI) was determined according to a published method of Yu et al. [8]. Accordingly, 1.0 g of protein was mixed with 10 mL of distilled water by the Velp ZX3 shaker at 3000 rpm for 2 minutes. The mixture was then allowed to rest at room temperature for 30 min before centrifugation (1100×g, 20 min) using a Hermle Z366 centrifuge. The centrifuge tube was then inverted for 10 minutes to remove any excess water. The remaining powder residue in the centrifuge tube was weighed. The WAC was calculated according to the formula (3):

$$WAC = \frac{W_2 - W_1}{W_0} \quad (3)$$

Where, W_0 (g) was the mass of the dry protein; W_1 (g) was the mass of the centrifuge tube containing the dry protein; W_2 (g) was the mass of the centrifuge tube containing the wet protein residue after centrifugation.

Oil-absorption capacity (OAC) was determined by the method proposed by Yu et al. with some modifications [8]. Centrifuge tubes containing 1.0 g of protein powder (W_0) with 10 mL (V_1) olive oil (Olivioilà, Italy) were shaken at 3000 rpm for 2 min. The mixture was left at room temperature for 30 min, then centrifuged (1100×g, 20 min). The volume of oil supernatant was measured (V_2). The OAC (mL/g) was calculated according to the formula (4):

$$OAC = \frac{V_1 - V_2}{W_0} \quad (4)$$

Emulsifying activity index (EAI) and emulsifying stability index (ESI) were determined according to Pearce & Kinsella [9]. Accordingly, 20 mL of 0.1% (w/v) protein solution at different pH (2,4,6,8 and 10) was mixed with 6.6 mL of olive oil (Olivioilà, Italy) and homogenized with the IKA T18 digital Ultra Turrax homogenizer for 1 min at 9000 rpm. For each sample, 50 μL of emulsion at 0 min and 10 min of homogenization was pipetted and diluted with 5 mL of 0.1% sodium dodecyl sulfate solution. The absorbance of the diluted emulsion was determined at 500 nm using a Hitachi UH5300 spectrophotometer.

Emulsifying activity index (EAI, m²/g) and emulsifying stability index (ESI, min) were determined according to formula (5) and (6), respectively:

$$EAI = \frac{2 \times 2.303 \times A_0 \times F}{C \times \Phi \times 10000} \quad (5)$$

Where, A_0 was the absorbance of emulsion immediately after homogenization ($t = 0$ min); $F = 100$ was the dilution factor; C - concentration of the protein solution (g/mL); Φ - first fraction in the emulsion system.

$$ESI = \frac{A_0 \times t}{A_0 - A_{10}} \quad (6)$$

Where, A_0 and A_{10} were the absorbances of emulsions measured immediately after 0 min and 10 min of homogenization, respectively.

Foamability (FA, %) was determined according to the method of Coffman and Garcia [10]. PPC solutions (2% w/v) were mixed with 50 mL of distilled water and adjusted to pH from 2 to 10 with a step of 2. The mixture was whipped with a Bosch MSM66130 Blender at a maximum speed of 14500 rpm for 5 minutes. Volumes were recorded before (V_1) and after (V_2) whipping and FA was calculated as the percentage volume increase, following the formula (7):

$$FA = \frac{V_2 - V_1}{V_1} \times 100 \quad (7)$$

The rheological properties of PPC solutions were evaluated using a rheometer (Haake Rheostress 1). The PPC solutions (1 mL) with concentrations of 3% (PPC3), 5% (PPC5), 7% (PPC7), 10% (PPC10), 12 % (PPC12) (w/v) were placed in the sample position. Samples were stabilized at 30°C for 5 min. The shear stress (τ , Pa) and viscosity (η , Pa.s) was measured when the shear rate ($\dot{\gamma}$, 1/s) run from 0 to 300 s⁻¹ (first cycle) and from 300 to 0 s⁻¹ (second cycle). The Herschel-Bulkley model (8) was using for determine the rheological type of PPC solutions: probe P35 TiL ($\varnothing = 35$ mm) was used, distance between probe and base was 1 mm, temperature of 30 °C.

$$\tau = k (\dot{\gamma})^n + \tau_0 \quad (8)$$

Where, k was the consistency coefficient, n was the flow behavior index, and τ was the shear stress.

Chemical analyses including protein, carbohydrate, fat, moisture and ash contents were estimated according to Kjeldahl method (AOAC 979.09), Phenol – sulfuric acid method (AOAC 988.12), Soxhlet method (AOAC 4.5.01), AOAC 925.09, and AOAC 923.03, respectively.

Statistical analysis. Each experiment was done in triplicate. Data were expressed as means \pm standard deviation (SD). IBM SPSS Statistics Version 26.0 was used to analyze the variance, and the Duncan's test was performed to determine the statistical significance between samples at a level of 0.05.

3. Results and Discussion

3.1. Production yield and chemical composition

The analytical results (Table 1) showed that the PPC yield (H) was 11.56 % and the protein yield (PY) was 67.66%.

Table 1. Yield production and chemical compounds of protein samples

	Sample	Protein flour	PPC	SPI	PPI*
Chemical compounds, %	Protein	25.65 \pm 1.18 ^a	71.77 \pm 2.72 ^b	90.06 \pm 0.82 ^c	69.90 \pm 0.96
	Carbohydrate	63.56 \pm 1.31 ^a	11.94 \pm 0.42 ^b	3.52 \pm 0.01 ^c	26.60 \pm 0.47
	Lipid	0.98 \pm 0.01 ^a	0.22 \pm 0.01 ^b	0.20 \pm 0.01 ^b	0.67 \pm 0.20
	Moisture	5.50 \pm 0.01 ^a	9.50 \pm 0.01 ^b	1.26 \pm 0.01 ^c	8.60 \pm 0.34
	Ash	3.83 \pm 0.01 ^a	6.23 \pm 0.01 ^c	4.75 \pm 0.01 ^b	2.82 \pm 0.10
	H (%)		11.56 \pm 0.48		
	PY (%)		67.66 \pm 1.36		

The values in the table are compared per each row and different top letters (a,b,c) indicate a significant difference ($p < 0.05$). *Adapted from [5].

The protein, carbohydrate, lipid, moisture, and ash (%) in peeled lima beans were 25.65, 63.56, 0.98, 5.50 and 3.83, respectively. The protein content of PPC (71.77 %) swas slightly higher than that of protein isolate (69.9 %) from lima beans (PPI) in the work of Ancona et al. but lower than that of the SPI sample (90.06 %). Meanwhile, the carbohydrate content in our PPC (11.94 %) was lower than that of the PPI sample (26.60 %). The ash content in the PPC sample was high (6.23 %), possibly due to the presence of amount of salt after protein precipitation. The PPC sample contains almost no fat (0.22 %).

3.2. Protein molecular weight

To determine the molecular weight of protein, the SDS-PAGE gel imagine of PPC and SPI samples were taken (Figure 2). The results showed that SPI contained high (92, 84 kDa), medium (60, 57, 47

kDa) and low (35, 15 kDa) molecular weight fractions. The PPC sample contained several bands: medium (72, 49, 44, 40 kDa) and more low molecular weight fraction (32, 19, 16 kDa). The bands in these protein samples corresponded to the 7S globulin (43 – 75 kDa) and 11S globulin (21 – 38 kDa) subunits and other polypeptides [1].

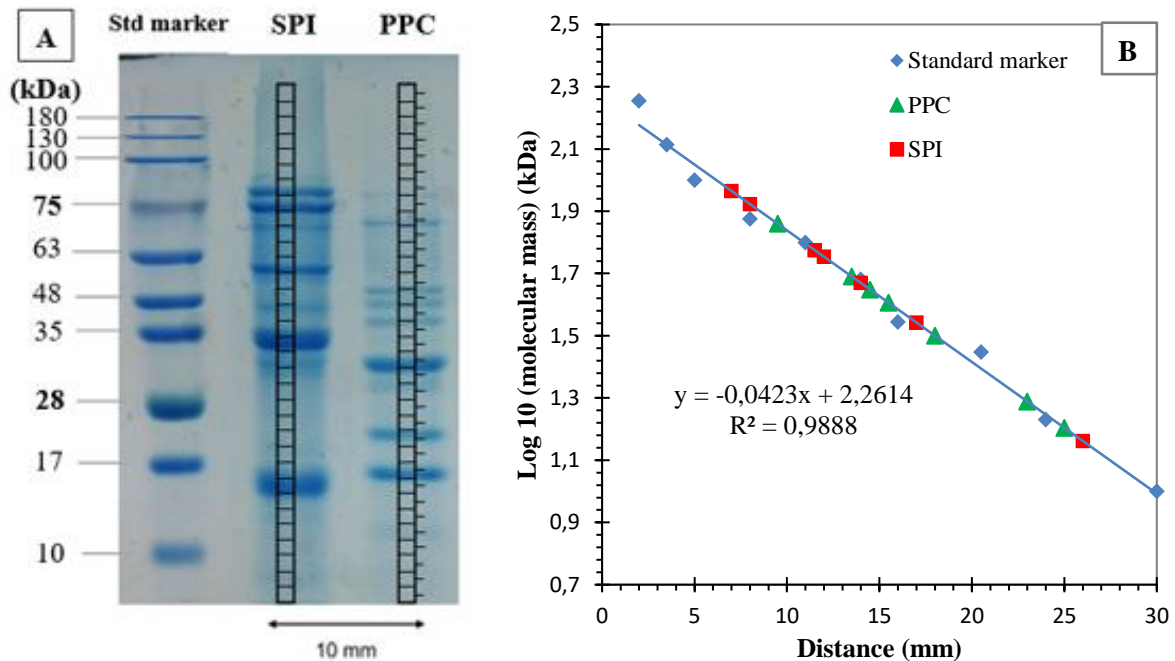


Figure 2. SDS-PAGE measurement results (A) and Calibration curve between logarithm of molecular weight and travel distance of protein (B)

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

Protein has both hydrophilic and hydrophobic properties, therefore can interact with water and oil in foods. Water and oil absorption capacities of PPC (Table 2) were 3.01 g/g and 2.86 mL/g, respectively. According to Chagas and Sanroto [11] and Makeri et al. [12], the albumin content in the lima beans accounted for 9.8–12.2 % and the globulins ranged from 45.5–51%, while the soy protein contains albumin and globulin levels of 32.82 and 34.95, respectively. Theoretically, total albumin and globulin content (proteins with high water holding capacity) in PPC and SPI were insignificantly different, so that lead to the same values of WAC. The fact that PPC had a relatively high WAC (3.01 g/g) compared with the WAC value of wheat protein (1.04 g/g) [13] makes PPC can be used to reduce the amount of free water present in the dough.

Oil absorption capacity of PPC is higher than that of SPI, which indicates that PPC contains more nonpolar fragments than SPI does [14]. With an OAC of 2.86 mL/g, the PPC could be used as an additive in processing some food products such as sausages, sponge cakes, chiffon, mayonnaise and salad sauces [14].

Table 2. Water and oil absorption capacities of protein samples

Sample	WAC, g/g	OAC, mL/g
PPC	3.01 ± 0.09 ^a	2.86 ± 0.17 ^a
SPI	3.08 ± 0.08 ^a	2.29 ± 0.09 ^b

Means in the same column with different letters (a-b) indicate a significant difference ($p < 0.05$)

3.4. Emulsifying activity index (EAI) and emulsifying stability index (ESI)

Figures 3 and 4 showed that pH influenced on EAI and ESI of PPC and SPI suspensions. Both EAI and ESI reached their minimum at pH 4 (isoelectric point, pI) and maximum at pH 10.

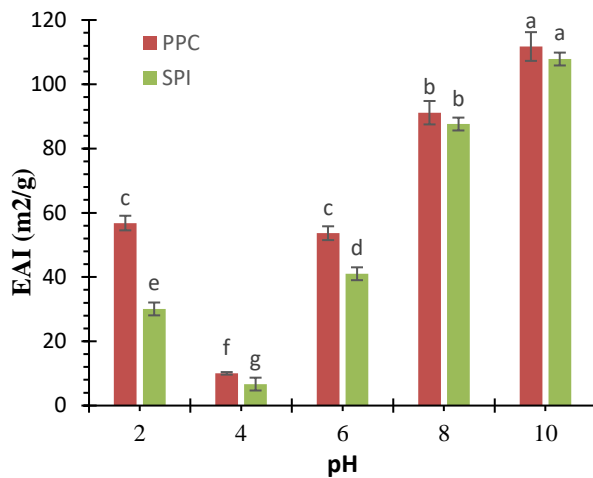


Figure 3. EAI of PPC and SPI at different pH

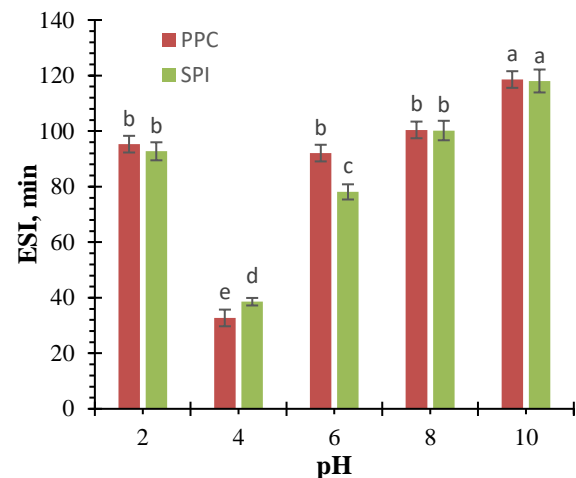


Figure 4. ESI of PPC and SPI at different pH

When oil droplets were formed in the PPC solution, the hydrophobic parts of the protein molecules were absorbed on the droplet surface, while the hydrophilic parts (which include amino $-NH_2$ and carboxyl $-COOH$ groups) were in the aqueous environment. The $-COOH$ group was converted to $-COO^-$ at $pH > pI$ and the $-NH_2$ group was converted to $-NH_3^+$ at $pH < pI$, therefore making the droplets surfaces electrically charged and repulse each other, hence stabilizing the emulsion and increasing ESI [15], [16]. At pH near to pI, the charges of droplets surface are close to zero and hence the repulsive forces between droplets are weak, therefore lowering ESI. Moreover, at this pH, the protein molecules were less mobile to move to the droplets surfaces due to their low electrical charges, resulting in lower EAI [17].

3.5. Foamability

Figure 5 showed that foamability of PPC suspension increased when increasing its concentration from 2 to 10%. The order of foamability was the same over a period of 8 h. The general improvement of foamability with concentration of PPC is similar to that of other protein isolates and is the result of the effect of decreasing surface tension of the air-liquid interface by the protein molecules absorbed on the liquid surface [18].

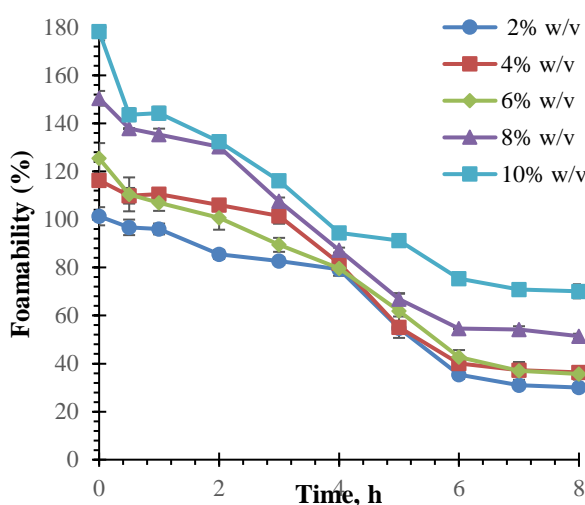


Figure 5. Influence of PPC concentration on foamability

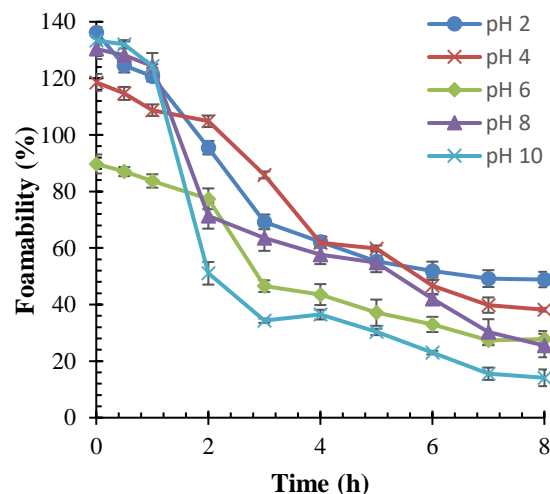


Figure 6. Influence of pH of PPC suspension on foamability

Figure 6 showed that pH significantly affected the foamability of PPC suspensions. At $t = 0$, the foamability of PPC suspension in the order of pH was $6 < 4 < 2 \approx 8 \approx 10$. This result was in accordance with the report of Betancur-Ancona *et al.* [19], in which the foamability of PPC was highest at acidic and alkaline conditions. At these conditions, the proteins are electrically charged, hence repulse each other. Therefore, the protein molecules become more mobile and quicker diffuse to the air-liquid interface to wrap the air pockets, thus increasing the foamability [20]. In the opposite, the low foamability at pH of 6 and 4 was possibly due to the low solubility and low mobility of PPC near its isoelectric point [21].

After 8 h, the order of foam stability was different from that of foamability with respect to pH: $10 < 6 \approx 8 < 4 < 2$ ($p < 0.05$) (Figure 6). This result indicated that despite the foamability at pH of 4 and 6 was lowest, the foams were relatively stable, compared to that formed at pH of 10 and 8. The reason was that at pH near the isoelectric point, electrostatic repulsive forces between protein molecules were small, hence facilitating the formation of a thicker protein layer at the air-liquid interface.

3.6. Rheological properties

Rheological measurements were done in order to evaluate food behaviors. Knowing the rheological properties is important for the food manufacturing industry. It can help to determine the processability of food materials in the manufacturing pipeline, the stability of manufactured liquid and semisolid food products under different storage conditions [22]. The regression equations showed the relationships between shear stress (τ) and shear rate ($\dot{\gamma}$) of PPC solutions according to the Herschel-Bulkley model in first cycle (gradually increasing the shear rate from 0 to 300 s^{-1}) and second cycle (gradually reducing the shear rate from 300 to 0 s^{-1}) are shown in Tables 3 and Figure 7.

Table 3 showed that all the regression equations of PPC solutions at different concentrations had a flow behavior index of $0 < n < 1$. According to Steffe, if $0 < n < 1$, the fluid is shear thinning; if $1 < n < \infty$, the fluid is shear thickening [23]. This proved that all protein samples have a pseudoplastic nature, that is, as the shear rate increases, the viscosity decreases [22]. There is no threshold stress for pseudoplastic, which means that when a force is applied it flows immediately. Furthermore, we suggest that at the beginning of the first rheological measurement cycle, the protein solution was partially in a gel state. After the first cycle, this gel state was possibly destroyed and not completely recovered, thus lowering the shear stress observed in the second cycle.

The viscosity curves in the samples (Figure 8) were almost similar. The viscosity in the shear rate range $1\text{--}50 \text{ s}^{-1}$ decreased sharply, then tended to stabilize until the end. However, increasing and decreasing the shear rate did not cause significantly changes of the shear stress and viscosity values. That means when we use a shear force equivalent to a shear rate below 300 s^{-1} , it does not deform the structure of the protein solution.

Table 3. Regression equations according to Herschel-Bulkley model for PPC solutions.

Sample	1-st cycle ($0 \rightarrow 300, \text{ s}^{-1}$)	R^2	2-nd cycle ($300 \rightarrow 0, \text{ s}^{-1}$)	R^2
PPC3	$\tau = 0.0879 \dot{\gamma}^{0.3279}$	0.8788	$\tau = 0.2148 \dot{\gamma}^{0.1998}$	0.9532
PPC5	$\tau = 0.1609 \dot{\gamma}^{0.3499}$	0.9201	$\tau = 0.214 \dot{\gamma}^{0.3019}$	0.9110
PPC7	$\tau = 0.0993 \dot{\gamma}^{0.4625}$	0.9387	$\tau = 0.208 \dot{\gamma}^{0.3353}$	0.9320
PPC10	$\tau = 0.1168 \dot{\gamma}^{0.5009}$	0.9802	$\tau = 0.4783 \dot{\gamma}^{0.265}$	0.9697
PPC12	$\tau = 0.1124 \dot{\gamma}^{0.5989}$	0.9746	$\tau = 0.2754 \dot{\gamma}^{0.4404}$	0.9709

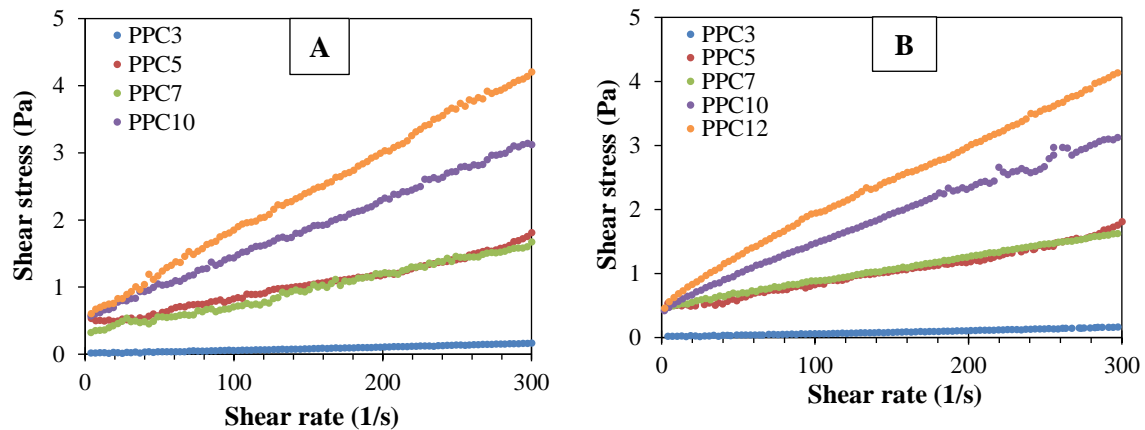


Figure 7. Changes of shear stress with shear rate of PPC solutions at the first (A) and the second (B) cycles.

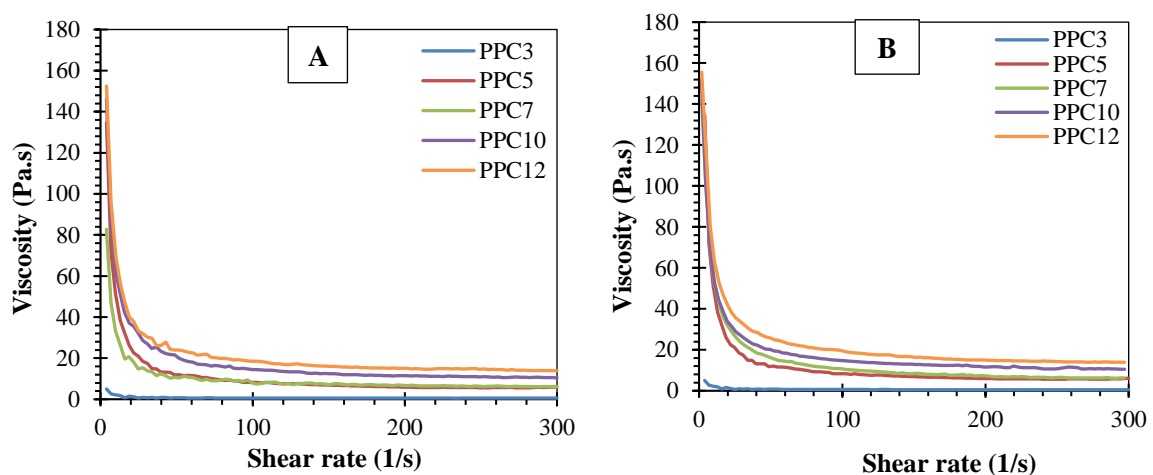


Figure 8. Changes of viscosity with shear rate of PPC solutions at the first (A) and the second (B) cycles.

4. Conclusions

With advantages in the functional properties, such as high water and oil absorption capacity, good foaming ability, the protein concentrate from Lima beans can be applied in food industry. For example, PPC can be used in sausages production, manufacture of dairy and dairy related products and confectionery products. PPC might not only improve the nutritional value but also the texture of the product. Moreover, PPC can be a source of protein supplements for vegetarians, and a structural improvement additive for gluten-free bakery products. Using a shear force equivalent to a shear rate below 300 s^{-1} does not deform the structure of the protein solution. However, the protein recovery efficiency (PY of 67.66 %) and the protein content in PPC preparations (71.77 %) were still not very high. That poses a mission for us to improve these values of the product.

Acknowledgments

This work belongs to the project in 2022 funded by Ho Chi Minh City University of Technology and Education, Vietnam.

We sincerely thank the following students for their contributions in conducting the experiments and for their honesty in obtaining these valuable results: Nguyen Dang Chung (Student ID: 17116055), Nguyen Thi Bac (Student ID: 17116052), Nguyen Van Minh Ha (Student ID: 17116064), Le Phuc Nguyen (Student ID: 17116101).

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