



## Comparative study on the Chemical Composition and Functional Properties of some Legume Protein Preparations

Ayat A. A. Kheder<sup>1</sup>, Fatma M. M. Salama<sup>2</sup>, Mohamed A.A. Azzam<sup>2</sup>, Hala A.A. Abd El-Rahman<sup>1</sup>

<sup>1</sup>Dairy Research Department, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.

<sup>2</sup>Dairy Department, Faculty of Agriculture, Cairo University.



CrossMark

### Abstract

Pea, beans-phaseolus, and cowpea seeds were used in preparing protein preparations (PP, BPP, and CP, respectively). The chemical composition and functional properties (protein solubility, water/fat binding capacity, foaming ability and stability, viscosity, and emulsifying activity and stability) were examined. In comparison between the three protein preparations, PP was the highest in moisture, protein, and fat while the lowest in fiber and carbohydrates. No significant difference between the ash content of the three preparations. PP, CP, and BPP covered the needs daily recommended total indispensable amino acids for old children 3-10y and adults and can cover the total sulfur amino acids by more than 61% and the aromatic amino acids by more than 135%. PP showed higher solubility in acidic pH regions, whereas, CP showed the opposite. BPP was the highest water-binding capacity. The oil-binding capabilities of PP, BPP, and CP were similar. At pH 7.0, the foaming capacity of CP solution was double that of PP and BPP solutions. However, the foam of the BPP solution was the highest stability. The BPP was the highest in emulsifying activity and emulsion stability. From that PP, BPP and CP could be good sources of indispensable amino acids for old children 3-10 y and adults, especially aromatic amino acids. Also, they showed satisfactory functional properties as required in the manufacture of alternative food products.

**Keywords:** pea; beans-phaseolus; cowpea; chemical composition; amino acids; functional properties

### Introduction

The problem of high prices of raw materials globally and the consequent high cost of production of many food products made a large segment of consumers unable to buy these products because of low income, especially in developing countries [1], [2]. Therefore, there is an urgent need to search for more nutritionally good and cheap food to meet the consumer's daily needs of nutrients. Legume seeds are cheap, readily available, and rich sources of nutrients, especially proteins, fats, and fibers [3], [4], [5], [6]. Although plant proteins may appear to be deficient in the consumer's supply of some essential amino acids, many researchers have reported the suitability of legume seeds as cheaper sources of plant proteins and as alternatives to expensive animal proteins in many food formulations [7], [8], [9], [10], [11], [12]. Nutritionists and food manufacturers need accurate information about the characteristics of these

proteins, to enable them to develop new food products. Of course, these characteristics are largely responsible for the quality and acceptability of the finished product [13]. Hence, this study aimed to separate and evaluate the proteins of local varieties of legumes (peas, beans-phaseolus, and cowpeas) in terms of chemical composition and functional properties in preparation for their use in the manufacture of alternative food products. The functional properties examined included protein solubility, water/fat absorption capacity, foaming capacity, and foam stability, viscosity, emulsifying activity, and emulsifying stability.

### Materials and Methods

#### Materials

Legumes seeds "Peas, (*Pisum Sativum*) beans-phaseolus (*Phaseolus Vulgaris*), and cowpeas (*Vigna Sinensis*)" were obtained from Crops Research Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt.

\*Corresponding author e-mail: [cheesemilk1@gmail.com](mailto:cheesemilk1@gmail.com). (Ayat Ahmed kheder)

Receive Date: 13 November 2021, Revise Date: 25 December 2021, Accept Date: 27 December 2021

DOI: [10.21608/ejchem.2021.105806.4873](https://doi.org/10.21608/ejchem.2021.105806.4873)

©2022 National Information and Documentation Center (NIDOC)

### Preparation of pea, beans-phaseolus, and cowpea proteins:

Peas (PP), beans-phaseolus (BPP), and cowpeas (CP) proteins were prepared from whole seeds to extract as shown in Fig.1 following the method described by [14] [with slight modifications].

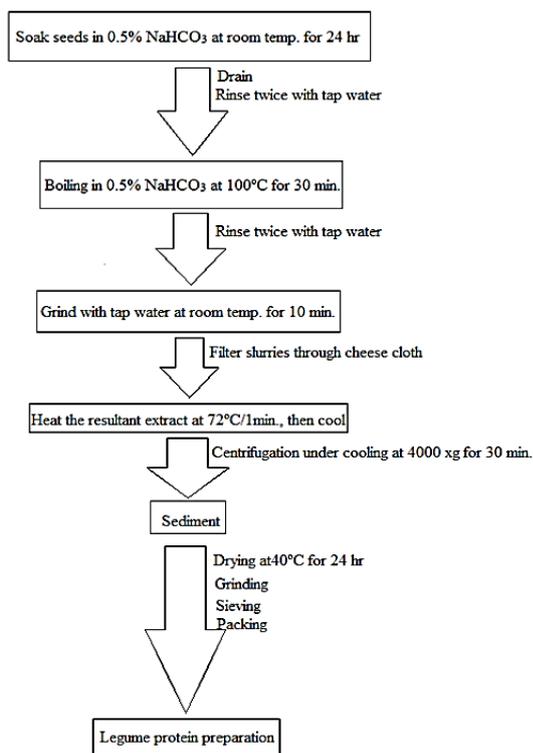


Fig. 1. Flowchart for preparing the legume protein preparations

### Chemical Analysis

Moisture, fat, crude fiber, and ash contents were determined according to the methods of [15], numbers 950.46, 960.39, 985.29, and 920.153, respectively. The protein content of PP, BPP, and CP samples was determined by the micro-Kjeldahl method [15], number 950.46. Carbohydrate were calculated by difference, according to [16] using the following equation: % Carbohydrate = % Total solids - % (fat + protein + ash + fibre). **Amino acids (AA)** were analyzed by a reverse-phase HPLC (model L 7400, HITACHI, Japan) fitted with a Denali C185 micron column (4.6 × 150 mm). The flow rate was 1 ml min<sup>-1</sup> with a fluorescence detector. The cysteine content of the protein sample was separately obtained by the method of [17]. In order to determine the tryptophan content of the proteins, aliquots containing known amounts of proteins were dispersed into glass ampoules together with 1 ml 5M NaOH. The ampoules were flame sealed and incubated at 110°C for 18 hours. The tryptophan contents of the alkaline hydrolysates were determined calorimetrically using the methods of [18] as

modified by [19]. Other amino acids were determined via measurement on hydrolysates using an amino acid analyzer (Sykam-S7130) based on high-performance liquid chromatography technique. Sample hydrolysates were prepared following the method of [20], in [21]. Two hundred mg of sample were placed in a hydrolysis tube. Then 5 ml 6M HCl were added to the sample into the tube, tightly closed, and incubated at 110°C for 24 hours. After incubation, the solution was filtered and 200 ml of the filtrate was evaporated to dryness at 140°C for an hour. Each hydrolysate after dryness was diluted with one ml of 0.12 M, pH 2.2 citrate buffers, the same standard applied to amino acids. An aliquot of 150 µL of sample hydrolysate was injected into a cation separation column at 130°C. Ninhydrin solution and an eluent buffer (the buffer system contained sodium acetate 90% and acetonitrile 10%) were delivered simultaneously into a high-temperature reactor coil (16 m length) at a flow rate of 0.7 ml/min. The buffer/ninhydrin mixture was heated in the reactor at 130°C for 2 minutes to accelerate the chemical reaction of amino acids with ninhydrin. The products of the reaction mixture were detected at wavelengths of 570 nm and 440 nm on a dual-channel photometer. The amino acid composition was calculated from the areas of standards obtained from the integrator and expressed as g of amino acid/100 g of test protein. Several methods to determine the quality of dietary protein, one is to compare the indispensable amino acid (IAA) pattern of a test protein to the indispensable amino acid (IAA) pattern of a reference protein. This is called indispensable amino acid score (IAAS). IAAS was calculated using the [22] reference pattern and using this equation [23]:

$$\text{IAAS} = \text{IAA in g/100g of test protein} / \text{IAA in g/100g of reference protein} \times 100$$

The indispensable amino acid showing the lowest IAAS was called the “limiting indispensable amino acid” [24]. The indispensable amino acid index (IAA index) was calculated using this equation [25]:

$$\text{Where } \text{LogIAA} = 0.1[\log(a_1/a_s \times 100) + \log(a_2/a_s \times 100) + \dots + \log(a_n/a_{ns} \times 100)]$$

$a_1 \dots a_n$  = indispensable amino acids content of the sample

$a_s \dots a_{ns}$  = indispensable amino acids values from the protein reference. Recommended indispensable amino acid scoring patterns for 3 to 10 y old children and adults were: Ile = 30; Leu = 61; Lys = 48; SAA = 23; Trp. = 6.6; Val = 40; AAA = 41; Thr. = 25; and His = 16 mg/g protein [22].

Biological Value (BV) was estimated from IAA index

$$\text{BV} = 1.09 (\text{IAA index}) - 11.73 \text{ [25]}$$

## Functional Properties

### Protein solubility:

To determine the solubility of a protein, a protein sample (200 mg) was distributed in 20 ml of de-ionized water. Then adjust the pH of the mixture to 2, 4, 6, 8, 10 with 1 N HCl and 1 N NaOH. The mixture was stirred at room temperature for 30 minutes and centrifuged at  $8000 \times g$  for 20 minutes. The nitrogen content of the supernatant was determined by the micro-Kjeldahl method. Nitrogen solubility was expressed as a percent of nitrogen in the supernatant to that of the total nitrogen in the sample [26].

Solubility % = [nitrogen content in supernatant / Total nitrogen original sample]  $\times$  100

### Water/Oil binding capacity:

Water binding capacity (WBC) is determined by mixing approximately 2.5 g of sample with 25 ml distilled water in a pre-weighed centrifuge tube (50 ml); the tube is stirred with a glass rod and stands at room temperature for 30 min. The tube is then centrifuged at  $2000 \times g$  for 10 min. The supernatant is carefully decanted and the centrifuge tube containing the protein sample is re-weighed. WBC is expressed as the number of grams of water absorbed per gram of protein and calculated according to the following equation [26]:

$$WBC = (W3 - W2) / W1$$

where W1 is the weight of the dry sample (in gr), W2 is the weight of the tube plus the dry sample (in gr), and W3 is the weight of the tube plus the sediment (in gr).

Oil binding capacity (FBC) is determined by mixing approximately 2.5 g of sample with 12.5 ml corn oil in a pre-weighed centrifuge tube (50 ml); the sample was gently stirred and stood at room temperature for 30 min. The tube is then centrifuged at  $5000 \times g$  for 20 min. The supernatant is carefully removed and the centrifuge tube containing the protein sample is re-weighed. FBC is expressed as the number of grams of oil absorbed per gram of protein and calculated according to the following equation [27]:

$$FBC = (W3 - W2) / W1$$

where W1 is the weight of the dry sample (in gr), W2 is the weight of the tube plus the dry sample (in gr), and W3 is the weight of the tube plus the sediment (in gr).

### Foaming capacity (FC) and Foam stability (FS):

Foaming capacity (FC) and stability (FS) were based on the method described by [28]. A 2.3 g protein sample was dispersed in 35 ml of "de-ionized

water using an electric vortex (Hoidolph REAX top) at a "1" speed setting for 1 min. The pH of the resulting protein solution was adjusted to 7.5 with either 0.1 M HCl or 0.1 M NaOH. The protein solution was heated at 60°C for 15 min and cooling to 15°C, then stirred using an electric stirrer (Matest) at a "3" speed setting for 2 min. The stirred solution was transferred immediately to a 100 ml graduated cylinder and stand at room temperature for 40 min. The volume of foam was recorded before and after stirring. FC was expressed by the percentage of increase in foam volume as a result of stirring. For FS estimation, the foam volume change was recorded over 40 min at equal time intervals. All analysis was performed in triplicate. FC and FS were then calculated according to the following equations:

$$FC \% = [(Foam\ volume\ after\ stirring - Foam\ volume\ before\ stirring) / Foam\ volume\ before\ stirring] \times 100$$

$$FS \% = [Foam\ volume\ after\ standing\ time / Foam\ volume\ after\ stirring] \times 100$$

### Viscosity

The viscosity of protein solution (pH =7) was measured using a Brookefield viscometer (Brookefield Engineering Laboratories Inc., Middleboro, Mass., U.S.A.) and a ULA spindle No.2. Viscosity values are expressed in centipoise (cP). Measurements were made at 100 rpm for 30 s and 25°C. [29].

### Emulsifying activity and stability:

To determine the emulsifying activity (EA), a protein sample (3.5 g) was dispersed in 50 ml of "de-ionized" water using an electric vortex (Hoidolph REAX top) at a "1" speed setting for 1 min. Add corn oil (50 ml) to the mixture. The sample was intensively vortexed for 30 min. The resulting emulsion was divided into four equal volume aliquots, two were centrifuged at  $1100 \times g$  for 5 min, while the others were centrifuged under the same conditions after heating in a water bath at 85°C for 15 min and cooling to 15°C. The height of the emulsion and the height of the total layer were measured in unheated tubes to calculate the EA. The same measurements for the heated tubes were taken to calculate emulsion stability (ES). EA and ES were then calculated according to the following equations [30]:

$$EA \% = [height\ of\ emulsion\ (cm) / height\ of\ total\ layer\ (cm)] \times 100$$

$$ES \% = [height\ of\ emulsion\ (cm) / height\ of\ total\ layer\ (cm)] \times 100$$

### Statistical analysis

All analyses were done in triplicate, and data were reported as means  $\pm$  standard deviation. Where appropriate, data were analyzed for significance using analysis of variance and Fisher's least significant difference (LSD at a 5% significance level) by Analytical Software. 2009. Statistics 9 [31]

### Results and discussion

#### Chemical composition

The chemical composition of the three preparations (PP, BPP, and CP) is shown in Table 1. CP was distinguished by its lower moisture content than other preparations (BPP and PP). Ash amount was significantly low (<1%) in all preparations. This is due to the process of blanching the seeds, which led to the loss of a large number of salts in the boiling water. As to the protein content, there were statistically significant differences between PP and BPP and CP in crude protein (31.1, 20.43, and 20.38%, respectively). CP contained the lowest fat content. There was a slightly significant difference between the carbohydrate content of CP and BPP (56.24 and 52.27%, respectively), while PP had the lowest carbohydrate content (41.59%). When comparing the three preparations in terms of fiber content, BPP was the highest.

#### Amino acid profile

The results obtained from the analysis of indispensable amino acids (Table 2) showed that each of PP, CP, and BPP can provide adult and old children- 3-10 years with about 100.9, 81.74, and 62.2% of recommended sulfur amino acids (Methionine and Cysteine), respectively. It was also noted that PP covered 179.51% of total aromatic amino acids (Phenylalanine and Tyrosine), while both BPP and CP covered 142.68 and 135.61%, respectively from these amino acids. PP, BPP, and CP were very poor in valine and isoleucine and rich in lysine. PP, CP, and BPP covered histidine, tryptophan, threonine, and leucine with rates ranging from 50% to more than 80%. In general, any of the three preparations (PP, BPP, and CP) can cover more than 70% of the total indispensable amino acids

recommended by [22] the FAO for old children- 3-10 y and adults (Table 2). In this study, BPP has a higher content of phenylalanine and methionine (2.61, and 0.70 g/100 g protein, respectively) when compared to lima bean (*Phaseolus Lunatus*) protein concentrate which contains 0.52 g phenylalanine and 0.35 g methionine /100 g protein as reported by [32]. The non-indispensable amino acids profile of the three preparations (PP, BPP, and CP) (Table 2) showed that PP was significantly higher in glycine, arginine, and alanine (5.48, 5.23, and 2.37 g/100 g protein, respectively), while BPP contained the highest amount of proline, glutamic acid, aspartic acid and tyrosine (27.42, 20.62, 10.97 and 3.24 g/100 g protein, respectively).

From Table 3 it is clear that all the indispensable amino acids showed a high score in the three preparations, and the least of them were valine in PP and then isoleucine in BPP and CP, as limiting amino acids. (I/T, %) showed a marked decrease in BPP (23.21 %) compared to the same ratio in PP and CP (32.34 and 30.17 %, respectively). The reported I/T ratio for PP and CP approaches 36% and which is sufficient to characterize these proteins as an ideal [22]. Indispensable amino acids index (IAAI) and biological value (BV) for PP were higher (55.59 and 48.86, respectively) than that of CP and BPP (44.77 & 37.07 and 37.93 & 29.62, respectively).

#### Protein solubility

Table 4 shows the protein pH-solubility profiles of PP, BPP, and CP. PP, CP, and BPP had a minimum solubility at pH 5.5 (isoelectric point) with values of 16.0, 14.62, and 7.9 %, respectively. This is similar to what [33] mentioned when studying the solubility of lentil protein. The highest solubility values for CP and BPP were 34.87 and 23.80% at pH 10, respectively, and for PP was 77.9% at pH 2. PP showed higher solubility in acidic pH regions than alkaline pH regions, whereas, CP showed the opposite. BPP was the least soluble in acidic, neutral, and alkaline pH regions. The high solubility of CP in alkaline media suggests that it can be used in the formulation of plant-based beverages [34] [35].

Table 1: Chemical composition of legumes protein preparations<sup>1</sup> (g/100 g powder)

Chemical constituents	Preparations <sup>1</sup>			L.S.D
	PP	BPP	CP	
Moisture	13.10 <sup>a</sup> ±0.36	12.25 <sup>b</sup> ±0.56	11.48 <sup>c</sup> ±0.48	0.2352
Protein <sup>2</sup>	31.10 <sup>a</sup> ±2.20	20.43 <sup>b</sup> ±0.37	20.38 <sup>b</sup> ±0.56	2.2772
Fat	11.65 <sup>a</sup> ±1.10	10.55 <sup>a</sup> ±0.47	8.34 <sup>b</sup> ±0.37	1.7871
Ash	0.95 <sup>a</sup> ±0.08	0.90 <sup>a</sup> ±0.075	0.98 <sup>a</sup> ±0.06	0.1976
Crude fiber	1.60 <sup>c</sup> ±0.30	3.60 <sup>a</sup> ±0.42	2.58 <sup>b</sup> ±0.42	0.1605
Carbohydrate	41.59 <sup>c</sup> ±0.410	52.27 <sup>b</sup> ±0.56	56.24 <sup>a</sup> ±0.41	1.1942

<sup>1</sup>Preparations: pea protein (PP), beans- phaseolus protein (BPP) and cowpeas protein (CP); <sup>2</sup>protein: N%  $\times$  6.25. Superscripts a, b, c: the same letters in the row mean that the results are not significantly different ( $P < 0.05$ ).

Table 2: Amino acids profile of legumes protein preparations<sup>1</sup> (g/100g protein).

Amino acids	Preparations <sup>1</sup>				RIAA <sup>7</sup>	IAAS <sup>8</sup> %		
	PP	BPP	CP	<i>L.S.D</i>		PP	BPP	CP
Methionine <sup>+</sup>	1.45 <sup>a</sup> ±0.06	0.70 <sup>c</sup> ±0.08	1.13 <sup>b</sup> ±0.04	0.0453				
Cysteine <sup>+</sup>	0.87 <sup>a</sup> ±0.05	0.73 <sup>b</sup> ±0.04	0.75 <sup>ab</sup> ±0.04	0.1213				
<b>Total SAA<sup>2</sup></b>	<b>2.32<sup>a</sup> ±0.05</b>	<b>1.43<sup>c</sup> ±0.11</b>	<b>1.88<sup>b</sup> ±0.09</b>	<b>0.2464</b>	<b>2.3</b>	<b>100.87</b>	<b>62.17</b>	<b>81.74</b>
Phenylalanine <sup>+</sup>	4.65 <sup>a</sup> ±0.06	2.61 <sup>c</sup> ±0.06	4.47 <sup>b</sup> ±0.03	0.0431				
Tyrosine <sup>+</sup>	2.71 <sup>b</sup> ±0.19	3.24 <sup>a</sup> ±0.10	1.09 <sup>c</sup> ±0.10	0.1180				
<b>Total AAA<sup>3</sup></b>	<b>7.36<sup>a</sup> ±0.14</b>	<b>5.85<sup>b</sup> ±0.11</b>	<b>5.56<sup>c</sup> ±0.08</b>	<b>0.2796</b>	<b>4.1</b>	<b>179.51</b>	142.68	135.61
Lysine	7.76 <sup>b</sup> ±0.06	5.51 <sup>c</sup> ±0.06	8.25 <sup>a</sup> ±0.05	0.1642	4.8	161.67	114.79	171.88
Isoleucine <sup>+</sup>	1.04 <sup>a</sup> ±0.07	0.34 <sup>b</sup> ±0.03	0.22 <sup>c</sup> ±0.03	0.0460	3.0	34.67	11.33	7.33
Leucine <sup>+</sup>	3.62 <sup>a</sup> ±0.06	3.31 <sup>b</sup> ±0.03	3.02 <sup>c</sup> ±0.07	0.1379	6.1	59.34	54.26	49.51
Threonine	2.34 <sup>b</sup> ±0.06	1.85 <sup>c</sup> ±0.06	2.97 <sup>a</sup> ±0.07	0.0131	2.5	93.60	74.00	118.80
Tryptophan <sup>+</sup>	0.84 <sup>a</sup> ±0.02	0.56 <sup>b</sup> ±0.08	0.56 <sup>b</sup> ±0.07	0.1354	0.66	127.27	84.85	84.85
Valine <sup>+</sup>	0.76 <sup>a</sup> ±0.07	0.78 <sup>a</sup> ±0.03	0.73 <sup>a</sup> ±0.05	0.1511	4.0	19.00	19.50	18.25
Histidine	3.33 <sup>b</sup> ±0.17	1.29 <sup>c</sup> ±0.10	4.05 <sup>a</sup> ±0.15	0.3992	1.6	208.13	80.63	253.13
<b>Total IAA<sup>4</sup></b>	<b>29.37<sup>a</sup> ±0.07</b>	<b>20.92<sup>c</sup> ±0.08</b>	<b>27.24<sup>b</sup> ±0.10</b>	<b>0.2172</b>	<b>29.06</b>	<b>101.10</b>	72.00	93.74
Aspartic acid	6.70 <sup>c</sup> ±0.20	10.97 <sup>a</sup> ±0.03	9.22 <sup>b</sup> ±0.03	0.2688				
Glutamic acid	10.80 <sup>c</sup> ±0.18	20.62 <sup>a</sup> ±0.08	13.88 <sup>b</sup> ±0.08	0.3044				
Serine	3.11 <sup>a</sup> ±0.14	1.85 <sup>b</sup> ±0.10	3.30 <sup>a</sup> ±0.10	0.3022				
Arginine	5.23 <sup>a</sup> ±0.11	1.68 <sup>c</sup> ±0.06	4.16 <sup>b</sup> ±0.04	0.0838				
Glycine	5.48 <sup>a</sup> ±0.11	2.36 <sup>c</sup> ±0.01	4.39 <sup>b</sup> ±0.07	0.1511				
Alanine	2.37 <sup>a</sup> ±0.08	1.06 <sup>c</sup> ±0.10	2.20 <sup>b</sup> ±0.05	0.0625				
Tyrosine	2.71 <sup>b</sup> ±0.13	3.24 <sup>a</sup> ±0.09	2.09 <sup>c</sup> ±0.10	0.0521				
Proline	25.04 <sup>b</sup> ±0.12	27.42 <sup>a</sup> ±0.11	23.82 <sup>c</sup> ±0.07	0.2807				
<b>Total NIAA<sup>5</sup></b>	<b>61.44<sup>c</sup> ±0.08</b>	<b>69.20<sup>a</sup> ±0.10</b>	<b>63.06<sup>b</sup> ±0.07</b>	<b>0.0288</b>				
<b>Total AA<sup>6</sup></b>	<b>90.81<sup>a</sup> ±0.04</b>	<b>90.12<sup>c</sup> ±0.11</b>	<b>90.30<sup>b</sup> ±0.10</b>	<b>0.2515</b>				

<sup>1</sup>Preparations: pea protein (PP), beans- phaseolus protein (BPP) and cowpeas protein (CP); <sup>2</sup>Total SAA: Total sulfur amino acids; <sup>3</sup>Total AAA: Total aromatic amino acids; <sup>4</sup>Total IAA: Total Indispensable amino acids; <sup>5</sup>Total NIAA: non-Indispensable amino acids; <sup>6</sup>Total AA: Total amino acids; <sup>7</sup>RIAA: recommended Indispensable amino acid scoring patterns for old children 3-10y and adults (FAO, 2013); <sup>8</sup>IAAS: Indispensable amino acids score. + Non-polar amino acids. Superscripts a, b, c: the same letters in the row mean that the results are not significantly different ( $P < 0.05$ ).

Table 3: Nutritional quality of legumes protein preparations<sup>1</sup> based on their amino acids content.

Preparations <sup>1*</sup>	Protein <sup>2</sup> , %	I/T, % <sup>3</sup>	IAAI <sup>4</sup>	LIAA <sup>5</sup>	CS <sup>6</sup>	BV <sup>7</sup>
PP	31.10	32.34	55.59	valine	19.00	48.86
BPP	20.43	23.21	37.93	isoleucine	11.33	29.62
CP	20.38	30.17	44.77	isoleucine	7.33	37.07

<sup>1</sup>Preparations: pea protein (PP), beans- phaseolus protein (BPP) and cowpeas protein (CP); <sup>2</sup>protein, %:  $N \times 6.25$ ; <sup>3</sup>I/T, %: total indispensable amino acids / total amino acids  $\times 100$ ; <sup>4</sup>IAAI: Indispensable amino acids index; <sup>5</sup>LIAA: limiting indispensable amino acid; <sup>6</sup>CS: chemical score; <sup>7</sup>BV: biological value.

Table 4: Effect of pH on solubility of legume protein preparations<sup>1</sup>

pH	Preparations <sup>1</sup>			<i>L.S.D</i>
	PP	BPP	CP	
2	77.90 <sup>Aa</sup> ±0.10	17.81 <sup>Bc</sup> ±0.09	20.74 <sup>Db</sup> ±0.11	0.0307
4.5	61.97 <sup>Ba</sup> ±0.03	13.67 <sup>Cc</sup> ±0.05	17.33 <sup>Eb</sup> ±0.12	0.0590
5.5	16.00 <sup>Fa</sup> ±1.00	7.90 <sup>Fc</sup> ±0.10	14.62 <sup>Fb</sup> ±0.08	1.1845
7	18.50 <sup>Eb</sup> ±0.50	10.32 <sup>Ec</sup> ±0.12	23.06 <sup>Ca</sup> ±0.08	0.4094
8	21.00 <sup>Db</sup> ±1.00	12.75 <sup>Dc</sup> ±0.12	31.50 <sup>Ba</sup> ±0.10	1.5021
10	52.31 <sup>Ca</sup> ±0.09	23.80 <sup>Ac</sup> ±0.10	34.87 <sup>Ab</sup> ±0.09	0.2215
<i>L.S.D</i>	0.8266	0.0505	0.1633	--

<sup>1</sup>Preparations: pea protein (PP), beans-phaseolus protein (BPP), and cowpeas protein (CP) Superscripts A, B, F: the same letters in the column mean that the results are not significantly different ( $p < 0.05$ ). Superscripts a, b, c: the same letters in the row means that the results are not significantly different ( $p < 0.05$ ).

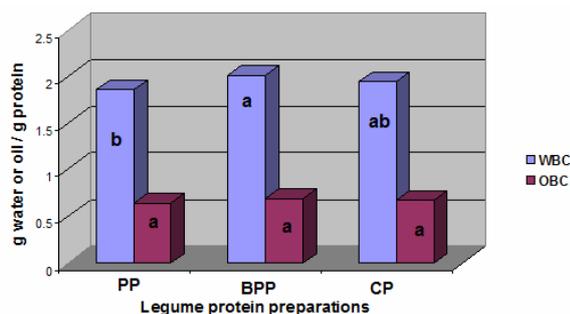


Fig.2 Water and oil binding capacity legume protein preparations. Columns with different letters indicate statistical differences ( $P < 0.05$ ).

### Water binding capacity

As shown in Fig.2 PP, BPP and CP had a water-binding capacity (WBC) of 2.02, 1.95, and 1.86 g H<sub>2</sub>O/g protein for BPP, CP, and PP respectively.

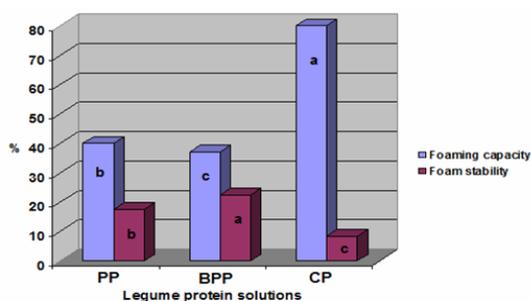


Fig.3 Foaming capacity and foam stability legume protein solutions. Columns with different letters indicate statistical differences ( $P < 0.05$ ).

### Foaming properties

Foaming capacity (FC) and foam stability (FS) of PP, BPP, and CP at pH 7 are shown in Fig.4. CP had the highest FC (80.0 %), followed by PP (40.0%), then BPP (37.14%). This is due to the high solubility of CP at pH 7 than that of PP and BPP (Fig. 2). [39] Reported that when the net charge of the protein increases, the hydrophobic interaction weakens, and thus the solubility of the protein increases, allowing the protein to diffuse to the air-water interface more quickly, encapsulating air molecules and thus increasing foam formation.

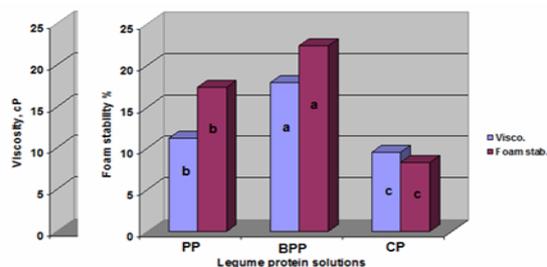


Fig.4 Relationship between viscosity and foam stability in legume protein solutions. Columns with different letters indicate statistical differences ( $P < 0.05$ ).

Higher protein solubility does not necessarily mean higher WBC [36].

The high WBC of BPP, CP, and PP is due to their fiber content (Table 1). Fibers contain hydrophilic parts, such as polar or charged side chains, which can enhance WBC [37]. The data obtained was similar to WBC values for lentil protein isolates (1.9 – 2.0 ml H<sub>2</sub>O/g protein) as reported by [38].

### Oil binding capacity

Fig.2 shows the oil binding capacity (OBC) of PP, BPP, and CP. There was no significant difference between OBC values of BPP, CP, and PP (0.69, 0.67, and 0.64 g oil/g protein, respectively). OBC is due to the protein's content of non-polar amino acids (Table 2). [38] Reported that OBC of lentil protein isolates was increased due to the presence of several non-polar side chains that associate with lipid hydrocarbon chains.

The highest FS was observed after whipping the BPP solution for 40 minutes, followed by PP then CP. This is due to the high viscosity of the BPP solution (Fig.4). These results indicate that there is a direct relationship between the viscosity of the protein solution and foam stability [40]. A balance between the solubility of the protein in water and the viscosity of the resulting solution is required to achieve better foaming properties, which is observed in BPP and PP.

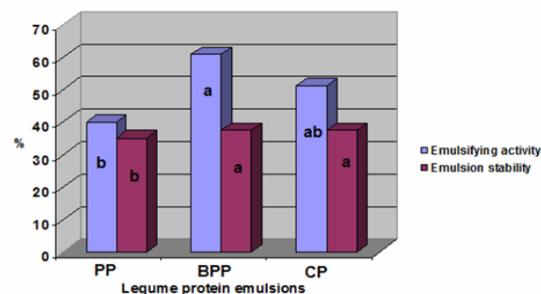


Fig.5 Emulsifying activity and emulsion stability of legume protein emulsions. Columns with different letters indicate statistical differences ( $P < 0.05$ ).

### Emulsifying properties

Fig.5 shows emulsifying activity (EA) (60.97, 51.30 and 40.00%) and emulsion stability (ES) (37.50, 37.50 and 35.13 %) for BPP, CP and PP, respectively. It is noticeable that EA decreases with increasing protein content. For example, PP (31.1% protein) showed the least EA (40.00%), while BPP (20.43% protein) had the highest EA (60.97%). The increase in the solubility of BPP and CP in alkaline medium enhances the interaction between the oil phase and the aqueous phase and thus the EA and ES increase (Table 4 & Fig.5). This result was in

agreement with [36], who stated that the emulsifying capacity of proteins tends to increase as protein solubility is increased.

### **Conclusion**

The results of this study indicate that pea, bean-phaseolus, and cowpea protein preparations can be considered good sources of dietary fiber and indispensable amino acids, especially sulfur and aromatic amino acids. Also, their good functional properties qualify them for use in various alternative food products.

### **Compliance with the ethical statement**

All authors of this paper have no conflict of interest with one or organization.

### **Acknowledgment**

We thank God Almighty for our success in writing this research, then we thank the workers of the Field Crops Department at the Agricultural Research Center for providing raw materials for the research experience.

### **References**

- Schönfeldt, H. C., and Hall, N. G., Dietary protein quality and malnutrition in Africa. *British Journal of Nutrition*, 108(S2), S69-S76 (2012).
- Ghosh, S., Assessment of protein adequacy in developing countries: *Quality matters. Food Nutrition. Bull. Suppl.*, 34, 244-246 (2013).
- Emire, S.A. and Tizazu, H., Chemical composition, physicochemical and functional properties of Lupin (*Lupinus albus*) seed grown in Ethiopia. *Afr. J. Food Agriculture. Nutrition. Dev.*, 10, 3029-3045 (2010).
- Sharma, S., Yadav, N., Singh, A. and Kumar, R., Nutritional and antinutritional profile of newly developed chickpea (*Cicer arietinum L*) varieties. *International. Food Research. Journal.* ,20, 805- 810 (2013).
- Liyanage, R., Perera, O.S., Wethasinghe, P., Jayawardana, B.C., Vidanaarachchi, J.K. and Sivakanesan, R., Nutritional properties and antioxidant content of commonly consumed cowpea cultivars in Sri Lanka. *Journal of Food Legumes.*, 27, 215-217 (2014).
- Simsek, S., Herken, E.N. and Ovando-Martinez, M., Chemical composition, nutritional value and in vitro starch digestibility of roasted chickpeas. *Journal of the Science of Food and Agriculture*, 96, 2896-2905 (2015).
- Sun, L., Chen, W., Liu, Y., Li, J., and Yu, H., Soy protein isolate/cellulose nanofiber complex gels as fat substitutes: Rheological and textural properties and extent of cream imitation. *Cellulose*, 22, 2619– 2627 (2015). <https://doi.org/10.1007/s10570-015-0681-4>
- Sangita, B., Manisha, M., Sharma, S.K., Yadav, D.N., Gupta, R.K., Optimization of fermentation conditions for probiotic soy yogurt using response surface methodology. *Journal of Food Processing and Preservation*doi:10.1111/J.pp.12415 (2016).
- Yadav, D. N., Bansal, S., Jaiswal, A. K. and Singh, R., Plant-Based Dairy Analogues: An Emerging Food. *Agricultural Research and Technology: Open Access J*, 10(2): 555781, 1- 4 (2017).
- Dabija, A., Codină, G. G., Gâtlan, A., Sănduleac, E. T. and Rusu, L., Effects of some vegetable proteins addition on yogurt quality. *Scientific Study and Research Chemistry and Chemical Engineering, Biotechnology, Food Industry*, 19 (2), pp.181 – 192. (2018).
- Chen, W., Liang, G., Li, X., He, Z., Zeng, M., Gao, D., Qin, F., Goff, H. D., and Chen, J. Effects of soy proteins and hydrolysates on fat globule coalescence and meltdown properties of ice cream. *Food Hydrocolloids*, 94, 279–286. (2019). <https://doi.org/10.1016/j.foodhyd.2019.02.045>
- Guler-Akin, M. B., Avkan, F. and Akin, M. S. A novel functional reduced fat ice cream produced with pea protein isolate instead of milk powder. *Journal of Food Processing and Preservation.* 15901, 1-11. (2021).
- Queirós, R.P., Saraiva, J.A., da Silva, J.A.L., Tailoring structure and technological properties of plant proteins using high hydrostatic pressure. *Critical Reviews in Food Science and Nutrition journal* 58, 1538–1556 (2018).
- Johnson, K. W., & Snyder, H. E. Soymilk: a comparison of processing methods on yields and composition. *Journal of food science*, 43(2), 349-353 (1978).
- AOAC *Official Methods of Analysis of Association of Official Analytical Chemists* 19th ed., Published by AOAC International, Gaithersburg, Maryland, USA. (2012).
- Pellet, P. L., and Shadarevian, S. *Food composition. Tables for use in the Middle East. Food composition. Tables for use in*

- the Middle East.*, No.Edn. 2 pp.126 pp. ref.40. (1970).
17. Liddell, H.F. and Seville, B. *Colorimetric determination of cysteine. Analyst.*, 84: 133 - 137 (1959).
  18. Spies, J.R. and Chamber, D.C., Chemical determination of tryptophan in proteins. *Analytical Chemistry journal.*, 21: 1249-1266 (1949).
  19. Rama Rao, M.V., Tara, M.R. and Krishnan, C.K., Colourimetric estimation of tryptophan content of pulses. *Journal of Food Science and Technology*, 11: 13-216. (1974).
  20. Moore, S. and Stein, W.H., *Chromatographic amino acids determination by the use of automatic recording equipment. Methods Enzyme.*, 16: 819-831 (1963).
  21. Ijarotimi, O.S. and Olopade, A.J. Determination of amino acid content and protein quality of complementary food produced from locally available food materials in Ondo State, Nigeria. *Malaysian Journal of Nutrition*. 15(1): 87-95 (2009).
  22. *Food and Agriculture Organization of the United Nations (FAO) Dietary Protein Quality Evaluation in Human Nutrition.* FAO Food and Nutrition Paper No. 92, p. 29. *Food and Agriculture Organization of the United Nations Viale Delle Terme di Caracalla, Rome,* (2013). <https://www.fao.org/3/i3124e/i3124e.pdf>
  23. Ukpene, A.O. and I made F.N., Amino acid profiles of seven cowpea varieties grown in Agbor. *Nigerian Annals of Natural Sciences*, 15(1): pp 72 -78 (2015). <https://www.researchgate.net/publication/297745895>
  24. Block, R. J., and Mitchell, H. H., The correlation of the amino acid composition of proteins with their nutritive value. *in nutrition abstracts reviews journal.* Vol. 16, No. 2, pp. 249-278 (1946).
  25. Oyedeji, A. O., Odeyemi, B., Adewuyi, S. and Azeez, L., Physical Properties, Chemical Composition, and Nutritional Quality Potentials of *Lonchocarpus sericeus* (Cube root) Seeds and Seed Oil. *walailak journal science and technology.*, 18(2): 7025, 1- 15. (2021). <https://doi.org/10.48048/wjst.2021.7025>.
  26. Rodriguez-Ambroz, S.; Martinez-Ayala, A.; Millan, F. and Davila-Ortiz, G., Composition and functional properties of *Lupinus campestris* protein isolates. *Plant Foods for Human Nutrition*, 60, 99–107 (2005).
  27. Barbut, S. Determined water and fat holding In *Methods of Testing Protein Functionality*, (G.M. Hall, ed.) *Blackie Academic and Professional, and in print of Chapman and Hall*, 2-6 Boundary Row, London SE1 8 HN. 186. (1996).
  28. Phillips, L.G., W. Schulman and J. E. Kinsella., pH and heat treatment effects on foaming of whey protein isolates. *Journal of Food Science*, 55: 1116-1119 (1990).
  29. Benoit, S. M., Afizah, M. N., Ruttarattanamongkol, K., & Rizvi, S. S. H., Effect of pH and temperature on the viscosity of texturized and commercial whey protein dispersions. *International Journal of Food Properties*, 16(2), 322-330 (2013).
  30. Naczka, M., Diosady, L. and Rubin, L.J., Functional properties of canola meals produced by two-phase solvent extraction system. *Journal of Food Science*. 50, 1685-1688, 1692 (1985).
  31. Boyd, C., Petersen, S., Gilbert, W., Rodgers, R., Fuhlendorf, S., Larsen, R. and Geaghan, J. P., Analytical Software. 2009. Statistics 9. Tallahassee, Florida, USA. Evaluation of Methods Used to Improve Grasslands as *Ring-Necked Pheasant (Phasianus colchicus) Brood Habitat*, 72, 82 (2018).
  32. Chel-Gurrero. Mario, D. M., and Martinez-Ayala, A.L., Lima bean (*Phaseolus lunatus*) protein hydrolysates with ACE-I inhibitory activity. *Food and Nutrition Sciences*, 3, 511-521 (2012).
  33. Olalekan, A. J., & Bosede, B. F., Comparative study on chemical composition and functional properties of three Nigerian legumes (jack beans, pigeon pea, and cowpea). *Journal of Emerging Trends in Engineering and Applied Sciences*, 1(1), 89-95 (2010).
  34. Wang, S., Chelikani, V., Serventi, L., Evaluation of chickpea as alternative to soy in plant-based beverages, fresh and fermented. *Lebensmittel- Wissenschaft and Technology*, 97, 570–572 (2018).
  35. Nawaz, M.A., Singh, T.K., Stockmann, R., Jegasothy, H. and Buckow, R. Quality Attributes of Ultra-High Temperature-Treated Model Beverages Prepared with Faba Bean Protein Concentrates. *Foods*, 10, 1244 (2021). <https://www.mdpi.com/journal/foods>
  36. Mao, X., and Hua, Y., Composition, structure, and functional properties of protein concentrates and isolates produced from walnut (*Juglans regia L.*). *International journal of molecular sciences*, 13(2), 1561-1581 (2012).

37. Jitngarmkusol, S., Hongsuwankul, J., and Tananuwong, K., Chemical compositions, functional properties, and microstructure of defatted macadamia flours. *Food Chemistry*, 110(1), 23-30 (2008).
38. Suliman, M.A., El Tinay, A.H., Elkhaila, A.E., Babiker, E. & Elkhailil, E.A. Solubility as influenced by pH and NaCl concentration and functional properties of lentil protein isolate. *Pakistan Journal of Nutrition*, 5, 589–593 (2006).
39. Lawal, O. S., Adebawale, K. O., and Adebawale, Y. A. Functional properties of native and chemically modified protein concentrates from Bambara groundnut. *Food Research International*, 40(8), 1003-1011 (2007).
40. Damodaran S., Protein-stabilized foams and emulsions. In: Damodaran S, Paraf A, editors. *Food proteins and their applications*. New York: Marcel Dekker. p 57–110 (1997).