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Extraction and Characterization of Protein Isolates from two Varieties of Broad Bean *(Vicia faba)*

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***Abstract* — The objective of this research was the obtaining and characterization of protein isolates from two varieties of *Vicia faba*, with the purpose of evaluating whether of this legume grain can take advantage in the production of high-quality protein foods. The importance of this study lies in the need to identify new sources of plant protein that diversify the supply of functional ingredients and respond to the growing demand for sustainable alternatives to animal protein. They were rehearsed different extraction and precipitation conditions were tested, with the best treatment being alkaline extraction (pH 11) of the flour of Peruvian variety, followed by isoelectric precipitation at pH 5.5. Under these conditions, a yield of 21.41 g/100 g dw, a protein content of 84.17 g/100 g, a digestibility of 83.68 %, and a PDCAAS of 19.25 % were obtained, reflecting good nutritional quality. Regarding its functional properties, the following stood out: oil retention capacity of 5.25 mL/g, water solubility index of 30.60 %, water absorption capacity of 2.74 g water/g protein, and foaming capacity of 30 %. The results indicate that the protein isolate from the Peruvian variety of *Vicia faba* has significant nutritional and functional potential, making it a promising ingredient for the development of supplements, functional foods, and innovative formulations in the food industry.**

***Keywords:* isolated protean; functional properties; PDCAAS; amino acids; protein digestibility.**

***Resumen* — El objetivo de esta investigación fue la obtención y caracterización de aislados proteicos de dos variedades de Vicia faba, con el propósito de evaluar si este grano de leguminosa puede aprovecharse en la producción de alimentos proteicos de alta calidad. La importancia de este estudio radica en la necesidad de identificar nuevas fuentes de proteína vegetal que diversifiquen la oferta de ingredientes funcionales y responder a la creciente demanda de alternativas sostenibles frente a la proteína animal. Se ensayaron diferentes condiciones de extracción y precipitación, siendo el mejor tratamiento la extracción alcalina (pH 11) de la harina de variedad peruana, seguida de precipitación isoeléctrica a pH 5.5. Bajo estas condiciones, se obtuvo un rendimiento de 21.41 g/100 g ps, un contenido proteico de 84.17 g/100 g, una digestibilidad de 83.68 % y 19.25 % de PDCASS, lo que refleja una buena calidad nutricional. En cuanto a sus propiedades funcionales, se destacaron: la capacidad de retención de aceite de 5.25 mL.g^{-1}, índice de solubilidad en agua de 30.60 %, capacidad de absorción de agua de 2.74 g agua/g proteína y capacidad de formación de espuma de 30 %. Los resultados indican que el aislado proteico de la variedad peruana de Vicia faba posee un potencial nutricional y funcional significativo, convirtiéndolo en un ingrediente prometedor para el desarrollo de suplementos, alimentos funcionales y formulaciones innovadoras en la industria alimentaria.**

***Palabras Clave:* proteína aislada; propiedades funcionales; PDCAAS; aminoácidos; digestibilidad de proteína.**

I. INTRODUCTION

THE broad bean *(Vicia faba)* is a legume with a cultivation history that dates back over 8000 years, being domesticated and valued in various cultures as an essential food. Its importance lies not only in its nutritional value for human consumption but also in its widespread use in animal feed, reflecting its versatility and relevance in agriculture [1]. This legume is distinguished by its nutritional composition, which includes carbohydrates, fiber, essential vitamins and minerals, as well as a notable protein content ranging from 20-35 g/100 g dw, according to the variety and the growing conditions [2].

This nutritional profile makes it an exceptional source of plant proteins and a valuable resource to combat issues such as malnutrition [3]. Additionally, fava beans are especially beneficial for those following vegetarian or vegan diets, providing a rich protein alternative. Their potential extends to the formulation of enriched foods, contributing to food security and reducing reliance on animal protein sources. Fava bean flour is recognized for its high protein content; however, obtaining a protean isolate allows for maximizing this nutritional value by concentrating its beneficial components [4].

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The extraction and purification of proteins beginning with these legumes offer great potential not only for obtain protein isolates with direct applications in the food and nutritional industry [5], but also for innovating in the development of functional food products. These isolates can be used as ingredients in a wide range of applications, including nutraceuticals, sports supplements, and alternative meat products. In addition to their extraordinary nutritional qualities, the use of protein isolates derived from fava beans presents environmental benefits. Since fava beans are sustainable crops that improve soil health and contribute to the reduction of greenhouse gas emissions, their integration into food systems can be a key step toward more responsible and sustainable agricultural practices, [3]. The objective of this research was to obtain and characterize the proteins isolates of two broad bean varieties, in order to project their use in the fortification of food products.

II. MATERIALS AND METHODS

A. Plant material

The peruvian broad bean was obtained in the free market in the city of Latacunga. The sultana broad bean was provided by the legume program of INIAP and was cultivated in the Santa Catalina Station, whose geographic coordinates are: 00°, 22 ‘ S, 78° 33 ‘ W and 2050 m.a.s.l. The average rainfall of this station is 1342.6 mm per year and the average annual temperature is 11.5 °C. This variety comes from the crossing between the parents ECU-8395 x ECU-2522, its cultivation cycle ranges from 140 to 175 days, and it shows an average yield of 21 t/ha [6]. (Fig. 1).

B. Methods

1) Flour preparation

To obtain the flour, the dry grains (13 % moisture) were subjected to selection and cleaning operations, subsequently, they were ground in an industrial equipment (FAIRUZ EQUIPOS SDLM S.A.S, Ecuador) and sifted, selecting for the research the flour fraction with a particle size of 50 µm [5]. (Fig. 1). The flours were packaged in airtight polyethylene bags and stored at 4 °C until analysis. The analyses were made after one month of storage.

2) Preparation of protean isolate

The extraction of protein from the flours, was carried out following the methodology described by [5], which is detailed below. (Fig. 1).

3) Alkaline solubilization

A flour: distilled water suspension was prepared in a 1:10 (w/v) ratio, and the pH was adjusted with a 24 % NaOH solution until to reach pHs of 9 and 11 (Table I). Each suspension was stirred for 1 hour in an electric stirrer (MICROMAT CO. Mahwah. New Jersey, USA), subsequently the samples were centrifuged in an equipment (WIFUG STOCKHOLM, USA) at 18 °C, 10.000 rpm for 20 minutes. Was rescued the supernatant for protein isolation.

4) Acid precipitation

With the obtained supernatant, the pH was adjusted with a 30 % citric acid solution to reach pHs of 4.5 and 5.5. The samples were centrifuged in an equipment (WIFUG STOCKHOLM, USA) at 10,000 rpm for 20 minutes; the precipitate was recovered and freeze-dried in an equipment (LABCONCO, USA). The freeze-dried samples were packaged in polyethylene bags and stored at 4 °C until analysis.

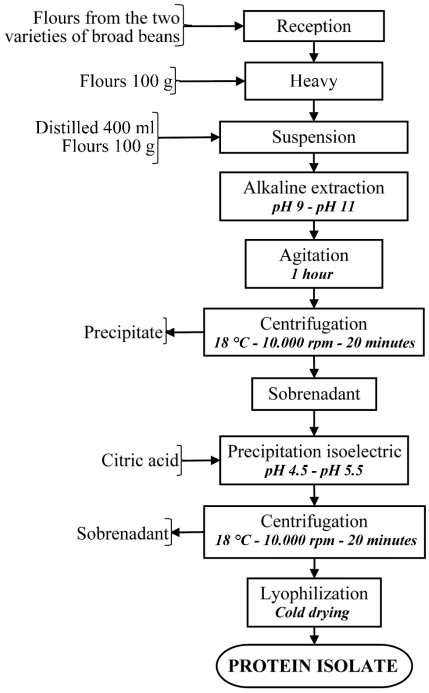

Fig. 1. Flowchart for the extraction of protean isolates.
Note: The parameters in italics indicate operating conditions.

TABLE I
TREATMENTS FOR PROTEIN ISOLATION

Treatments	Variety of broad bean	pH of alkaline extraction	pH of isoelectric precipitation
T1	Sultana	pH 9	pH 4.5
T2	Sultana	pH 9	pH 5.5
T3	Sultana	pH 11	pH 4.5
T4	Sultana	pH 11	pH 5.5
T5	Peruvian	pH 9	pH 4.5
T6	Peruvian	pH 9	pH 5.5
T7	Peruvian	pH 11	pH 4.5
T8	Peruvian	pH 11	pH 5.5

C. Proximal analysis of the flours

Methodologies of the AOAC with some modifications were used to evaluate: moisture (930.15), crude protein (total N x 6.25) (955.39), crude fat (920.39), crude fiber (978.10), ash (942.05), and the determination of carbohydrates was obtained (by difference) according to the equation described by [7], with all components expressed as a percentage.

D. Obtaining and characterization of the protean isolates

1) Yield

It was evaluated by gravimetry, from the weight of the isolate with relation to the flour of each variety of faba bean.

2) Nutritional properties

Crude protein content, CPC. It was evaluated by the method Kjeldahl, according the AOAC (2005).

Soluble protein. 0.2 g of the isolate was mixed with 20 mL of distilled water and stirred in a shaker (Cole Parmer, Cole-Parmer instrument, company, Vernon Hills, Illinois, USA) for 30 minutes. It was centrifuged in an equipment (International Equipment. CO. USA) for 25 minutes. In the supernatant, 30 % trichloroacetic acid was added to determine the soluble protein using the Kjeldahl method, described by AOAC (2005).

E. Functional properties

1) Water absorption capacity (WAC) and Water solubility index (WSI)

Was mixed 0.5 gram of sample in a centrifuge tube with 20 mL of distilled water at 30 °C and stirred for 30 minutes using a equipment (Thermo scientific variomag telesystem, Spain), then, they were centrifuged in a centrifuge (International Equipment. CO. USA) at 3000 rpm for 15 minutes, and the volume of the supernatant was recorded in a graduated cylinder [8], (Eq. 1). To determine WSI, the empty Petri dishes were weighed, and 3 mL of the previously separated supernatant was added. The Petri dishes were then placed in the stove for 24 hours at 0 °C [9]. The results were calculated using (Eq. 2).

$$\mathrm{WAC} = \frac{W_p - T_m}{n} \times 100 \quad (1)$$

$$\mathrm{WSI}(\%) = \frac{\mathrm{CDE} - \mathrm{CV}}{m} \times 100 \quad (2)$$

Where:
Wp = weight of the precipitate
Tm = empty sample tube
n = sample
CDE = weight of the Petri dishes after the oven
CV = weight of the empty Petri dishes
m = represents the sample

2) Oil retention capacity

The oil retention capacity was determined according to the method described by [8]. One gram of sample was mixed with 10 mL of oil (edible maize oil, La favorita, Ecuador) for 30 seconds. The samples were allowed to rest at room temperature (25 ± 2 °C) for 30 minutes, after centrifuged at 3000 rpm for 30 minutes. The volume of the supernatant was recorded in a graduated cylinder of 10 mL. The oil absorption ($mL.g^{-1}$) was calculated with the difference between the initial volume of oil added to the sample and the volume of the supernatant.

3) Turbidity

The turbidity of protean isolate from faba bean flours (sultana-peruana) was evaluated following the procedure described by [10]. The protein solution was stirred for 30 minutes at 25 °C. The turbidity was recorded at 600 nm using a (Photometry Macherey Nagel, PF-11, France). Ultrapure water was used as a reference for the measurements.

4) Foaming capacity (FC), Foaming stability (FS)

FC and FS were determined according to a published report, with some modifications [8]. 2.5 g of the isolate was shaken with 20 mL of water in a shaker (Cole Parmer, Cole-Parmer instrument, company, Vernon Hills, Illinois, United States) for 2 minutes, and the protein solution was liquefied for 1 minute (Eq. 3). The volume of the dispersion was measured at the beginning (0 min) and 30 min after foaming to determine FS (Eq. 4) in a 50 mL graduated cylinder. The (FC) and (FS) values were calculated as follows:

$$\mathrm{FC}\left(\%\right) = \frac{V_1 - V_0}{V_0} \times 100 \quad (3)$$

$$\mathrm{FS}\left(\%\right) = \frac{V_2}{V_1} \times 100 \quad (4)$$

Where:
V_o = volume of the protein solution
V_1 = volume of the foam after mixing
V_2 = volume of the foam after 30 minutes of resting

5) Emulsifying capacity (EC), Emulsion stability (ES)

The emulsifying activity and foam stability were evaluated according to the method of [8], with some modifications. It was liquefied with 250 milligrams of the isolate in 25 mL of distilled water plus 5 mL of oil for 1 minute. Subsequently, the emulsions were centrifuged at 1.100 rpm for 5 minutes. To determine the emulsion stability (ES), the samples were subjected to water bath at 80 °C for 30 minutes before being centrifuged again at 1.100 rpm for 5 minutes. The results of EC and ES were calculated using the Eq. 5, Eq. 6.

$$\mathrm{EC}(\%) = \frac{\mathrm{ELTH}}{\mathrm{TC}} \times 100 \quad (5)$$

$$\mathrm{ES}(\%) = \frac{\mathrm{ELTH}}{\mathrm{TCA}} \times 100 \quad (6)$$

Where:
ELTH = height of the emulsified layer after heating (cm)
TC = height of the total content of the tube
TCA = height of the total content before heating (cm)

F. Characterization of the selected treatment

1) In vitro protein digestibility

The digestibility of proteins was determined using the method of [11], with some modifications. The samples (6.25 mg of crude protein (N x 6.25)) were dispersed in double-distilled water and heated to 37 °C for 30 minutes. Then, the sample solution was adjusted to pH 8 using 0.2 N NaH. A 2 mL solution containing chymotrypsin (11.2 mg) and trypsin (10 mg) was added to the protein suspension at a ratio of 1:10 (v/v). After 10 minutes of reaction at 25 °C, the pH of the mixture was measured, and in vitro digestibility was calculated as the percentage of digestible protein (Y) using Eq. 7.

$$Y = 210.46 - x(18.10) \quad (7)$$

Where:
Y = Digestibility
x = final pH of the protein after 10 minutes of digestion.

2) Tryptophan content

For the determination of tryptophan, 85 mg of protein isolate was added, it suspended in 3 mL of papain solution (4 $mg.mL^{-1}$). The sample was incubated in an oven (Memmert, Germany) at 63 ± 2 °C for 16 hours, then cooled to 25 °C and centrifuged in a centrifuge (International Equipment CO. USA) at 2,500 rpm for 5 minutes. The quantification of tryptophan was performed using a photo colorimeter (Thermo Scientific, Evolution 201, Waltham, Massachusetts, USA) at 560 nm fluorescence in a standard curve with a concentration range of 0 to 35 µg.mL.

3) PDCAAS determination

The percentage of the limiting amino acid of the selected protein isolate was calculated using Eq. 8.

$$\mathrm{AAS}\left(\%\right) = \frac{\mathrm{Cia(mg/g\ of\ protein)}}{\mathrm{Crp(mg/g\ of\ protein)}} \quad (8)$$

Where:
AAS = Scoring-limiting amino acids
Cla = Content of the limiting amino acid (mg/ g of protein)
Crp = Content of the limiting amino acid in the reference profile (mg/ of protein)

The limiting amino acid in the selected protein isolate was compared to the standard values established by the [9], for children > 1 year and adults, with 25 mg-g-¹ of (methionine + cysteine) and 7.0 mg-g-¹ of tryptophan. The PDCAAS was calculated based on the limiting amino acid (Score) with the lowest value from the broad bean isolate, using the following equation: (Eq. 9).

$$\mathrm{PDCAAS}(\%) = \mathrm{AAS(Score)} \times \mathrm{Digestibility} \quad (9)$$

Where:
PDCAAS is the Protein Digestibility Corrected Amino Acid Score.

G. Statistical analysis

All analyses were performed in duplicate, and the results are presented as mean ± standard deviation. The data were analyzed using factorial analysis of variance, ANOVA of AxBxC, using the statistical package INFOSTAT. The honest significant test of Tukey was applied to determine significant differences at 5 %.

III. RESULTS AND DISCUSSION

A. Proximal analysis of the flours

The proximal analysis of the flours from two varieties of faba bean shows a significant difference ($p \leq 0.05$) in the content of protein, ash, fat, fiber, and moisture with a higher value in the sultana variety (Table II), while carbohydrates exhibited a lower content (68.05 g/100 g dw) with relation to the peruvian variety. These results are similar to the lentil flour, which contains 25.2 g/100 g dw of protein, 5.2 g/100 g dw of fiber, 0.7 g/100 g dw of fat, 2.8 g/100 g dw of ash, 53.8 g/100 g dw of carbohydrates, and 12.2 g/100 g dw of moisture [12]. However, the experimental values varied compared to chickpea flour and dried green pea flour in terms of ash, fat, and carbohydrates, [13]. The variation in the chemical composition of the flours can be attributed to the genetic of the specie, cultivation conditions (geographical location and growing season), and agronomic management, [14].

TABLE II
PROXIMAL ANALYSIS OF THE TWO VARIETIES OF BROAD BEAN FLOURS (G/100G DW)

Analysis	Peruvian	Sultana
Protein	24.06 ± 1.03[b]	25.38 ± 1.24[a]
Ash	3,75 ± 0.10[b]	3.83 ±0.14[a]
Fat	0,8 ± 0.28[b]	1 ± 0.2[8]a
Crude Fiber	1,2 ± 0.28[b]	1.75 ± 0.4[9]a
Moisture	8,1 ± 0.42[b]	9.7 ± 0.0[7]a
Carbohydrates	70,19 ± 0.93[a]	68.05 ± 1.3[1]b

Note: Mean value ± Standard deviation with n=2. The different letters indicate a significant difference ($p < 0.05$).

TABLE III
PHYSICAL CHARACTERISTIC, CHEMICAL COMPOSITION, NUTRITIONAL AND FUNCTIONAL PROPERTIES OF THE PROTEIN ISOLATES

	Unit	TREATMENTS							
		Sultana				Peruvian			
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8*
Yield	g/ 100 g dw	20.38±0.02^{ab}	16.35±0.01^{c}	21.01±0.02^{ab}	21.18±0.01^{ab}	19.68±0.01^{abc}	17.76±1.04^{bc}	20.69±0.66^{ab}	21.41±2.02^{a}
CPC	g/ 100 g dw	73.94±3.26^{ab}	68.78±2.23^{b}	71.96±4.42^{ab}	73.13±3.17^{ab}	76.22±4.08^{ab}	72.42±2.60^{ab}	74.15±2.81^{ab}	84.17±4.29^{a}
SP	g/ 100 g dw	0.72±0.03^{b}	0.68±0.03^{b}	0.68±0.03^{b}	0.72±0.03^{b}	0.77±0.04^{ab}	0.68±0.0^{3}b	0.68 ± 0.03^{b}	0.86 ± 0.0^{4}a
WAC	g H_2O/g protein	1.57±0.03^{e}	1.82±0.05^{d}	1.37±0.02^{e}	1.51±0.03^{b}	2.53±0.08^{b}	1.53±0.01^{e}	2.13±0.01^{c}	2.74±0.10^{a}
WSI	%	24.80±0.85^{bc}	24.1±1.27^{bc}	22.8±0.85^{c}	25.10±0.14^{bc}	23.25±2.62^{bc}	28.20±0.28^{ab}	23.70±0.99^{bc}	30.60±1.70^{a}
ORC	mL.$^{g-1}$	2.75±0,35^{b}	2.8±0.28^{b}	2.7±0.14^{b}	3.30±0.14^{b}	3.00±0.42^{b}	2.95±0.07^{b}	2.55±0.64^{b}	5.25±0.35^{a}
Turbidity	FAU	1890 ±1.55^{c}	1540±1.41^{bc}	1060±1.97^{ab}	1935±1.92^{c}	940±1.71^{a}	2075±1.50^{c}	2015±1.48^{c}	820±1.71^{a}
FC	3 %	11.15±0.45^{bc}	10.21±0.3^{0}c	13.96±0.88^{ab}	10.94±0.74^{bc}	13.02±0.74^{abc}	11.05±0.88^{bc}	11.98±0.74^{abc}	14.59±1.4^{8}a
	10 %	17.00±1.41^{bc}	18.00±2.83^{bc}	10.00±2.83^{c}	15.00±2.24^{bc}	18±1.83^{bc}	18±2.83^{bc}	22.00±2.83^{ab}	30.00±2.83^{a}
FS	3 %	5.73±0.74^{b}	6.77±0.74^{b}	6.77±0.74^{b}	7.29±1.47^{b}	7.29±1.47^{b}	7.29±1.47^{b}	7.81±0.74^{b}	13.54±1.4^{7}a
	10 %	11.00±1.41^{bc}	14.8±1.96^{bc}	9.00±1.41^{c}	11.60±2.26^{bc}	18.00±1.83^{ab}	14±1.83^{bc}	17.00±1.41^{abc}	22.00±1.4^{1}a
EC	%	13.51±1.10^{bc}	15.95±0.23^{bc}	16.52±0.62^{bc}	15.23±2.04^{bc}	12.02±1.85^{c}	16.96±0.40^{b}	12.21±0.10^{bc}	23.36±0.40^{a}
ES	%	12.62±0.16^{cd}	14.57±0.75^{bc}	14.62±0.86^{bc}	14.4±0.86^{bc}	1.19±0.68^{d}	15.26±0,37^{b}	10.62±0.13^{d}	21.49±0.47^{a}

Note: Mean value ± Standard deviation with n=2. The different letters indicate a significant difference ($p < 0.05$). T_1**:** (Sultana, pH 9 of alkaline extraction, pH 4.5 of isoelectric precipitation), T_2**:** (Sultana, pH 9 of alkaline extraction, pH 5.5 of isoelectric precipitation), T_3**:** (Sultana, pH 11 of alkaline extraction, pH 4.5 of isoelectric precipitation), T_4**:** (Sultana, pH 11 of alkaline extraction, pH 5.5 of isoelectric precipitation), T_5**:** (Peruvian, pH 9 of alkaline extraction, pH 4.5 of isoelectric precipitation), T_6**:** (Peruvian, pH 9 of alkaline extraction, pH 5.5 of isoelectric precipitation), T_7**:** (Peruvian, pH 11 of alkaline extraction, pH 4.5 of isoelectric precipitation), T_8**:** (Peruvian, pH 11 of alkaline extraction, pH 5.5 of isoelectric precipitation).
CPC: Crude protein content, **SP:** Soluble protein **WAC:** Water absorption capacity, **WSI:** Water solubility index, **ORC:** Oil retention capacity, **FC:** Foaming capacity, **FS:** Foaming stability, **EC:** Emulsifying capacity, **ES:** Emulsion stability, **FAU:** Formazine attenuation units

B. Obtaining and characterizing protein isolates

1) Yield

A significant difference ($p \leq 0.05$) was observed in the yield, with the highest value (21.41 g/100 g dw) in treatment T_8 (Peruvian variety, solubilization pH 11, precipitation pH 5.5), which could be attributed to the lower content of fiber and fat in this variety (Table III). Another predominant factor is pH, which significantly influences protein solubilization; very basic or acidic pHs prevent protein aggregation. This factor also affects the interactions between protein functional groups (such as amino and carboxyl groups) and water. These interactions are key for the protein to remain in solution. In contrast, at the isoelectric point, the electrostatic interactions that normally keep the protein dissolved in solution are reduced, which favors protein aggregation and precipitation.

The extraction yields of the following treatments did not vary significantly from treatment T_8: T_6 and T_2. According to [15], variety, alkaline extraction pH, and isoelectric precipitation pH are key factors that significantly influence the yield of protein isolates, as reported in the study on amaranth protein extraction [15]. The results obtained suggest that plant proteins are more soluble in alkaline media (pH 8-12), which helps separate the proteins from the grain matrix [16]. Then, these proteins precipitate by lowering the pH to reach the isoelectric point (pH 4-5) using citric acid or hydrochloric acid solutions, [17].

2) Nutritional properties

Crude protein content, CPC. The contents of the crudes proteins varied significantly ($p \leq 0.05$) among the different treatments (Table III), with the highest value (84.17 g/100 g dw) for the peruvian variety, a result that correlated with the highest extraction yield from this variety. These results are different from those mentioned by [18], who reported a protein content of 69.31 % at pH 10 and isoelectric precipitation at pH 4.5, while at pH 8 and isoelectric precipitation at pH 3, they obtained 42.11 % protein in the isolate from the lentil seed. In another investigation, a protein content of 70.10 ± 0.77 % was reported for the protein isolate of quinoa variety INIAP-Tunkahuan at alkaline pH (11) and acid precipitation at pH 5 [19]. Factors such as variety, analysis method, sample conditions, solubilization parameters, and precipitation can influence protein content [20]. The results obtained highlight the

importance of faba bean as a raw material for obtaining isolates with high protein content, which are essential compounds for growth, tissue maintenance, and cell repair; even the people following vegetarian or vegan diets may rely on these grains to meet their protein needs, [4]. Protein also plays a critical role in the agri-food chain, from the food industry to feed and the production of vegetable drinks, gluten-free food production, and improving food texture, with opportunities for new markets, especially for consumers with special diets or preferences for vegan products, [19]. Therefore, protein isolates could be an important part of a balanced diet, providing benefits for health, sustainability, and overall nutrition.

Soluble protein, SP. A significant difference in soluble protein content was observed among the treatments (ANOVA, $p \leq 0.05$). Treatment T_8 showed the highest value (0.86 ± 0.03 g/100 g dw), significantly different from T_2, T_3, T_6, and T_7, which ranged from 0.68 ± 0.03 to 0.77 ± 0.04 g/100 g dw (Table III). These differences could be attributed to the chemical and structural characteristics of faba bean proteins, which are mainly globular and susceptible to conformational changes depending on the extraction conditions [20]. Factors such as the applied thermal treatment, the pH used during the isolation process, and protein–matrix interactions likely influenced solubility [17]. Compared to other protein isolates, the soluble protein content of the faba bean treatments was lower than that reported for quinoa varieties (10.5 g/100 g dw) and for cañihua protein isolates obtained through alkaline solubilization and acid precipitation at pH 4.5 [20]. Similarly, olive seed protein isolates showed a higher solubility (11 g/100 g dw) at pH 4.0 [21], suggesting that faba bean proteins may possess a more compact structure or exhibit stronger aggregation tendencies under the tested conditions.

C. Functional properties

1) Water absorption capacity, WAC

A significant difference in WAC ($p \leq 0.05$) was observed among the analyzed treatments. Treatment T_8 showed a higher WAC of 2.74 (g water/g protein) (Table III). This result is different from quinoa protein, which reached a capacity of 3.94 g/mL, and soy isolate that presented 4.05 g water/g protein, [22]. The lower WAC of the faba bean isolate may be influenced by the protein structure, amino acid composition, pH, temperature, and other environmental factors, matrix [16]. The three-dimensional structure of the protein, including its primary conformation (the amino acid sequence) and secondary structure (alpha helices, beta sheets, etc.), influences how the protein interacts with water [22]. It is likely that faba bean proteins have more compact and less hydrophilic structures. Another influencing factor is pH, which can alter the charge of functional groups in amino acids, affecting solubility and the interaction of the protein with water. In environments with very acidic or basic pH, proteins may undergo conformational changes that affect their ability to attract water, [23].

2) Water solubility index, WSI

WSI of protein isolates provides important information about their possible technological applications [24]. In the analysis of the solubility of faba bean isolates, significant differences ($p \leq 0.05$) were observed among the treatments applied for protein isolation. Treatment T_8 showed a higher solubility (30.60 %), while treatment T_3 showed the lowest value (22.8 %) (Table III). The pH of solubilization and precipitation of the protein influenced these results. The latter value is similar to that reported by other authors for lentil, faba bean, and chickpea isolates produced at a pilot level, which reached an average of 24.02 % with pH 10 in solubilization and pH 2 in protein precipitation, [25]. These authors indicate that the low solubility of legume proteins at a pH between 4 and 5 is attributed to the formation of aggregates, which occurs near the isoelectric point of the proteins. In another study related to protein isolation from wild turnip seeds *(Brassica rapa L)*, a WSI of 23 % was reported, which is similar to our experimental values obtained in treatments T_1, T_2, T_4, T_5, and T_7 and is relevant for the formulation of products such as instant cereals, soups, and whole grain cookies, [23].

3) Oil retention capacity, ORC

A significant difference in ORC was observed among the different treatments ($p \leq 0.05$). Treatment T_8 presented the highest ORC (5.25 $mL.g^{-1}$ protein) (Table III). This result could be directly related to the amino acid composition of the protein isolate, its chemical structure, the interaction with oil, and the conditions of protein extraction (pH, temperature, presence of other compounds), [22]. They mention that proteins with a higher proportion of hydrophobic amino acids, more open or denatured structures, have a greater ORC. Treatments T_1-T_7 showed lower ORC values, similar to those reported for soy and quinoa isolates with 2.10 and 1.88 mL/g, [22]. These results highlight the importance of faba bean protein isolates' ability to retain oil; this property is useful in the food industry for making bakery products, dressings, and sausages, [26]. The isolate's ability to retain oil not only improves the functional properties of baked goods but also opens the door to applications in creating vegetarian and vegan alternatives, where there is a desire to replicate the texture and flavor of meat, [27]. The inclusion of these isolates could contribute to producing products with better texture and flavor, benefiting to producers and consumers. Their versatility and unique properties make them an attractive option for innovation in multiple food categories.

4) Turbidity

A significant difference in turbidity was observed among the different treatments ($p \leq 0.05$). Treatment T_8 presented the lowest turbidity (820 FAU), indicating greater purity and quality of the isolate (Table III). This characteristic could be related to smaller molecule size, the nature of the suspended

proteins, temperature, pH, solid concentration, and the preparation of the protein isolate. Meanwhile, treatment T_6 showed the highest turbidity (2075 FAU), possibly due to the presence of protein aggregates or other undesirable components, [10]. These factors also influence the digestibility and bioavailability of proteins, as well as their behavior in food matrices, [10].

5) Foaming capacity (EC) and Foam stability (FS)

A significant difference in EC was observed among the different treatments ($p \leq 0.05$) (Table III). Treatment T_8 presented an EC of 14.59 % at a protein concentration of 3 %, which increased to 30.00 % at a concentration of 10 %, demonstrating the effect of concentration on FS. At higher protein concentrations, the EC improved, likely because there are more molecules available to stabilize the air bubbles. In the remaining treatments, EC varied between 10.21 and 13.96 % at a protein concentration of 3 %, and between 10.2 and 22.00 % at a concentration of 10 %. Foam is a dispersion of gas (air) in a liquid or a mixture of liquids, and its stability is determined by the ability of the bubbles to remain intact without collapsing or disintegrating, [28] Treatment T8 exhibited an FS of 13.54 % at a concentration of 3 and 22 % at a concentration of 10 %, after resting for 30 minutes (Table III). These results show that, at higher protein concentrations, the foam is more stable since there are more proteins available to stabilize the bubbles, [29]. However, the experimental FS values are lower compared to animal proteins such as egg albumin, which exhibited stability of 33-54 %, due to its different chemical structure that affects its ability to stabilize bubbles, [28].

6) Emulsifying capacity (EC) and Emulsion stability (ES)

Treatment T_8 showed a significant difference ($p \leq 0.05$) with a value of 23.36 % in EC. Meanwhile, the other treatments exhibited values between 12.02 and 16.96 % (Table III). This result was lower than that reported for vitabosa flour at 48.7 % and sorghum flour at 49 %, [26]. In this regard, [27] indicate that the EC of plant proteins depends on key factors such as their solubility, structure, charge, the presence of electrolytes, the type of protein, concentration, emulsion droplet size, and processing conditions (such as temperature and agitation method). The ES of the protein isolates from broad beans varied with the treatments applied for protein extraction and precipitation; treatment T_8 presented the highest value in ES with 21.49 %. This result correlated with the highest ORC, while the other treatments T_1-T_7 showed values between 1.19 and 15.26 % (Table III). This behavior seems to be influenced by the conditions of protein extraction and its ability to reduce surface tension and stabilize the oil-water interface. The results of our studies surpassed those reported by [26], in the vitabosa and sorghum flours at different times with increasing flour concentrations starting from 6 % p/v onwards. The unfolding of proteins at the water/oil interface plays an important role in emulsifying capacity and stability due to the increase in hydrophobicity, [30].

D. Characterization of the selected treatment

Based on the obtained results, it was determined that treatment T8 allows for the production of a protein with good physical characteristics, chemical composition, and nutritional and functional properties. Therefore, this treatment was selected for the digestibility and protein quality assay.

TABLE IV
LIMITING AMINO ACIDS AND PROTEIN QUALITY

Amino acids	Raw broad bean (mg-g^{-1})	Protein isolate (mg-g^{-1})	Children's requirements (mg-g^{-1})	Score Dimensionless	Protein Digestibility %	PDCAAS %
Methionine + cysteine	5.30	5.66	25	0.23	83.68	19.25
Tryptophan	10.2	10.5	7	1.50		

* Standard amino acid values for children > 1 year and adults.

1) Digestibility

The digestion of proteins begins in the stomach, where they come into contact with the main proteolytic enzyme known as pepsin. This enzyme operates in an acidic environment, generating a pH of 2, characteristic of healthy individuals. Subsequently, the digestive process moves to the small intestine, where various pancreatic enzymes intervene, such as trypsin, chymotrypsin, aminopeptidases, and carboxypeptidases, [2]. The selected protein isolate (T_8) achieved 83.68 % digestibility (Table IV). This result is similar to that reported by [29], for broad bean protein (77.73 %). This property makes it a favorable option for good nutrition, ranging from sports supplementation, where rapid availability of amino acids is prioritized to promote muscle recovery, to balanced diets aimed at satisfy the protein needs of different population groups.

2) Protein quality -PDCAAS

The quality of the protein was determined based on the content of limiting amino acids (methionine + cysteine and tryptophan) and the digestibility of the protein. The selected treatment T_8 presented 5.66 and 10.5 mg-g^{-1} of limiting amino acids (methionine + cysteine and tryptophan), from which, in relation to the requirements for children > 1 year and adults [9], the score for each amino acid was obtained (Table IV). When multiplied by the digestibility of the protein, this resulted in a PDCAAS of 19.25 % (Table IV), suggesting that the broad bean isolate not would fully meet the nutritional needs necessary for the proper development of children > 1 year and adults that would need to be complemented with other plant proteins (*lupin, sacha inchi*), [3].

IV. CONCLUSION

According to the proximate analysis, the two evaluated varieties of Vicia faba had a moderate contribution of proteins and carbohydrates, which motivated the isolation of the protein in order to identify a new source of nutrients for the food industry. For this purpose, two pH levels (9 and 11) of solubilization

and precipitation pH (4.5 and 5.5) were tested, resulting in a yield of 21.41 % in the Peruvian variety at a solubilization pH of 11 and precipitation pH of 5.5, with a crude protein content of 84.17 %, while the Sultana variety showed 73.94 % protein. As for functional properties, the Peruvian isolate showed better functional properties than the protein isolate of the Sultana variety, expressed in a high-water absorption capacity (2.74 g of water/g of protein), water solubility index (30.60 %), correlating these characteristics with a higher oil retention capacity (5.25 %), lower turbidity (820 FAU), foaming capacity at 10 % concentration (22.00 %), and foam stability at 30 %. In relation to emulsifying capacity (EC) and foam stability (FS), the values were 23.36 % and 20.17 %. These results show the nutritional and functional potential of the protein isolate of the Peruvian variety for its application in various food formulations with nutritional and functional improvement.

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