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Techno-Functionalities of White Bean Protein Concentrate: A Comparative Study with Soy and Pea Proteins

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Abstract: The study of the techno-functional properties of novel plant-based proteins has gained importance due to their as alternatives to conventional proteins in food systems. This work evaluated the techno-functional and structural properties of white bean protein concentrate (WBPC) in comparison with commercial soy and pea proteins. The WBPC exhibited a higher foaming capacity (FC) at neutral pH and excellent foam stability (FS) at both tested pH levels, outperforming the commercial proteins. Although the WBPC's gelation occurred only at concentrations above 16% and its water-holding capacity (WHC) was lower than that of the soy and pea proteins, the WBPC showed a high binding capacity for nonpolar molecules, excelling in its oil-holding capacity (OHC) and forming stable emulsions, which are relevant for stabilization in food products. Additionally, WBPC can form more rigid gel networks, suitable for systems requiring greater mechanical strength. These techno-functional properties indicate that WBPC is a promising alternative source for the plant-based food industry, helping to meet the demand for innovative, sustainable products and contributing to the diversification of protein sources.

Keywords: alternative proteins; *Phaseolus vulgaris* L.; foam capacity; emulsion stability; vegetable proteins



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1. Introduction

The accelerated growth of the plant-based product market has driven the need for further studies on new protein sources and the impacts of both traditional industrial processes and emerging technologies on these sources [1–7]. Therefore, research on these proteins is crucial to elucidate the potential of their properties, as plant proteins represent a sustainable and economically viable option compared to animal proteins [8,9].

Legume proteins, such as those found in beans, lentils, soybeans, and peas, play an essential role not only as nutritional sources but also due to their techno-functional properties, which make them valuable for various industrial applications [10]. These proteins exhibit attractive technological properties, including good water-holding capacity, foam formation

ability, and emulsifying activity, characteristics that position them as promising ingredients for widespread use in the food industry [10–13]. The techno-functional properties of these proteins underscore their potential as innovative alternatives for the development of food formulations, while also contributing to sustainability within the food sector. Understanding these properties is crucial to fully exploring the potential of legumes in various contexts, highlighting their importance in the realm of techno-functional applications.

Among the most extensively studied plant proteins, soy protein is the standard for both the isolated and concentrated forms due to its high availability, high protein content, and favorable functional properties, such as its water-holding capacity, solubility, and hydrophobicity [4,13]. These proteins are widely used in processed meats, plant-based beverages, and meat analogs [14,15]. Recent research efforts have expanded the horizon for new alternative plant proteins [6,7,16–19] due to concerns about allergenicity, the presence of antinutritional factors, and considerations regarding the environmental impact and sustainability associated with soy use [20,21].

Beans are an accessible and economical source of proteins, carbohydrates, dietary fibers, vitamins, minerals, and phenolic compounds. They are an essential component of vegetarian and vegan diets, as the only plant-based food providing a significant amount of the essential amino acid lysine. Moreover, beans are gluten-free, making them an excellent fortifying ingredient in various food products [22]. Bean proteins are characterized by their balanced amino acid content, with high amounts of proline, glutamic acid, arginine, leucine, tryptophan, tyrosine, lysine, and phenylalanine [17,23–25]. Despite the numerous health benefits of beans, such as reducing cardiovascular disease risk factors and promoting gut microbiota health [22], beans remain underexplored legumes regarding their techno-functional properties [26].

Characterizing, modifying, and exploring the use of white common bean protein concentrate represents an important research area in the food industry, as it may serve as a useful protein source for flexitarian, vegetarian, and vegan consumers. Thus, the purpose of this research is to investigate the techno-functional characteristics of white bean protein concentrate (*Phaseolus vulgaris* L.), comparing it with commercial proteins (soy and pea), to broaden the potential for developing food products with technologically modulated ingredients.

2. Materials and Methods

2.1. Extraction and Preparation of Protein Concentrate

The method used to extract the protein concentrate involved alkaline extraction with 1 M NaOH followed by isoelectric precipitation using concentrated HCl. The process of extraction and preparation of white bean protein concentrate is shown schematically in Figure 1. Initially, 100 g of raw white beans was submerged in 1 L of distilled water (ratio 1:10 w.w^{−1}) and soaked for 12 h at room temperature. Subsequently, the beans were blended and filtered, and the fibrous portion was discarded. The aqueous portion was adjusted to an alkaline medium (pH 10) for enhanced protein solubilization, followed by agitation for 12 h. The sample was then centrifuged at 10,000 rpm at 4 °C for 20 min. The sediment was discarded, and the supernatant was collected and adjusted to pH 3 for isoelectric precipitation. This supernatant was centrifuged again at 10,000 rpm at 4 °C for an additional 20 min. After this centrifugation, the supernatant was discarded and the precipitate was washed with distilled water (pH 7), then centrifuged again at 10,000 rpm for 20 min at 4 °C. After washing, the white bean protein concentrate was stored in an ultra-freezer for subsequent lyophilization.

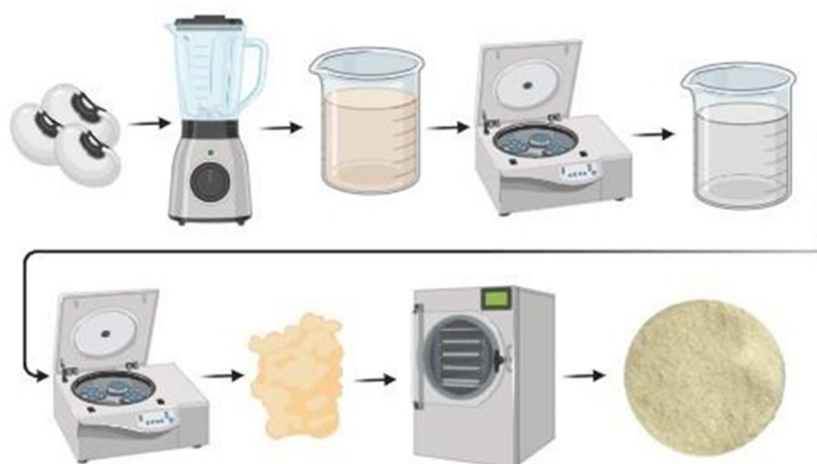


Figure 1. Flowchart of the white bean protein concentrate extraction process. The steps include grinding the beans in a blender (initially on the left), adjusting the pH to 10, centrifugation for fraction separation, subsequent pH adjustment (to 3), a second centrifugation to obtain the precipitated protein, and finally drying the protein concentrate, resulting in a powder.

2.2. Protein Content

The total nitrogen content of the WBPC was determined using a nitrogen-to-protein conversion factor of 6.25 [27].

2.3. Fourier Transform Infrared Spectroscopy

The extracts were analyzed via Fourier transform infrared spectroscopy (FTIR) using an Agilent Cary 630 spectrometer. The FTIR spectrum was collected in the range of 400–4000 cm^{-1} with a spectral resolution of 1 cm^{-1} .

To obtain the values of the secondary structure of the proteins, the FTIR spectra were deconvoluted using the “Peak Deconvolution” application in OriginPRO 2022 through the second derivative method [28].

2.4. Hydrophobicity

The hydrophobicity of the nonsolubilized proteins was determined according to the method described by Chelch, Gatellier, and Santé-Lhoutellier (2006) [29], with minor modifications, using bromophenol blue sodium salt for electrophoresis (from Sigma, St. Louis, MO, USA). In 1 mL of protein suspension, 200 μL of a 1 $\text{mg}\cdot\text{mL}^{-1}$ BPB solution (in distilled water) was added, and the mixture was homogenized thoroughly. The control, free of proteins, consisted of adding 200 μL of the same BPB solution (in distilled water) to 1 mL of 20 mM phosphate buffer at pH 7. The samples and control were subjected to continuous agitation in a vortex mixer (Vortex Multi 0-3000—Craltech, Barcelona, Spain) at room temperature for 10 min, followed by centrifugation (Microcentrifuge for microtubes, Daiki—Model DT-10DK, Osaka, Japan) at 10,000 rpm for 10 min. The absorbance of the supernatant (diluted 1.10^{-1}) was measured at 595 nm, using phosphate buffer as the blank. The amount of BPB bound to the proteins was calculated using Equation (1):

$$BPB \text{ bound} = \frac{200 \mu\text{g} \times (A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \quad (1)$$

With A = absorbance at 595 nm.

2.5. Solubility as a Function of pH, NaCl, and Glucose

The solubility percentage of the WBPC was evaluated based on variations in pH (2.0–10.0), NaCl concentration (0–1 M), and glucose concentration (0–1 M). For the exper-

iment, 3 g of protein was dissolved in 60 mL of distilled water. The pH adjustment was conducted using 1 M HCl and NaOH solutions. For each experimental condition, three 2 mL aliquots were transferred into Eppendorf tubes. Then, 6 mL of distilled water was added to each aliquot to adjust the final pH. In the assays involving varying NaCl concentrations, 1.5 μ L of NaCl solution was added to the samples, resulting in concentrations ranging from 0 to 1 M, followed by the addition of 0.5 μ L of the protein solution. In the glucose assays, 1.5 μ L of glucose solution was added to reach concentrations ranging from 0 to 1 M, with 0.5 μ L of protein solution. The samples were stirred for 1 h at room temperature, followed by centrifugation at 4 °C at 8000 $\times$ g for 10 min. Then, 2 μ L of the supernatant from each tube was pipetted into microplates, and protein quantification was performed using the Bradford method.

2.6. Water-Holding Capacity (WHC) and Oil-Holding Capacity (OHC)

The WHC and OHC of the WBPC were determined following the methodology described by Tan, Ying-Yuan, and Gan (2014) [25], with modifications. The WBPC (0.1 g) was mixed with 1.5 mL of distilled water and with 1.5 mL of soybean oil in a pre-weighed microcentrifuge tube, then vortexed (Vortex Multi 0-3000—Craltech) for 1 min. The sample was incubated at room temperature for 30 min and then centrifuged (Microcentrifuge for microtubes, Daiki—Model DT-10DK) at 10,000 rpm for 30 min. The resulting supernatant was carefully decanted and the sample was weighed. The WHC and OHC were expressed in grams of water or oil bound per gram of sample using Equation (2).

$$WHC/OHC(g \text{ de } \acute{a}gua \text{ ou } \acute{o}leo/g \text{ de } prote\acute{i}na) = \frac{W_2 - W_1}{W_0} \times 100 \quad (2)$$

where W_0 is the weight of the dry sample (g), W_1 is the weight of the tube plus the dry sample (g), and W_2 is the weight of the tube plus the sediment (g).

2.7. Emulsion Analysis

2.7.1. Emulsion Production

The emulsions were prepared with a fixed concentration of 1 g of protein in two ratios of commercial soybean oil to protein solution (1:1 and 2:1 oil/water) at pH 4 and 7, for a total volume of 30 mL. The mixture was homogenized using a high-speed disperser (Ultra-Turrax IKA T25 digital; Model T 25 D S32, DAIKURE, Tokyo, Japan) at 25,000 rpm for 90 s, and the emulsion stability was assessed using a Turbiscan Lab.

2.7.2. Turbiscan Analysis

The emulsion stability was measured according to the adapted methodology of Zhu et al. (2020) [30] using the Turbiscan Lab analyzer (Serial Number: 2302-TL-2395; SMLS 880 nm; Formulacion, France). This equipment enables separate measurements of the transmission and backscattering of the sample. A 20 mL volume of the emulsion was transferred into a specific glass test vial for the instrument and subjected to a scan analysis along the height of the tube, performed by two detectors at a temperature of 25 °C. Measurements were taken at 1 min intervals from 0 to 10 min, 2 min intervals from 10 to 20 min, and 5 min intervals from 20 to 60 min, totaling a 1 h continuous analysis. The emulsion stability was determined using the Turbiscan Stability Index (TSI) via the Turbiscan software (TurbiSoft-Lab-3.0.2.0).

2.8. Least Gelation Concentration (LGC)

The least gelation concentration (LGC) of the WBPC was determined as described by the conventional methodology [13]. Protein suspensions with concentrations ranging from

2% to 22% were prepared in 20 mL of distilled water and transferred to sealed glass test tubes. The tubes were subjected to a water bath (Dubnoff Microprocessed Bath—Q226M) at 100 °C for 1 h, followed by immediate cooling in an ice bath and storage at 4 °C overnight. The samples were then inverted and categorized into three gelation categories: (1) absence of gel, indicated by a liquid solution that flowed without resistance; (2) weak gel, characterized by a solution that flowed with some resistance; (3) gel formation, evidenced by a solution that did not flow when the tube was inverted. The LGC was defined as the lowest concentration that formed a firm gel.

2.9. Rheological Analysis

A Haake Mars IQ Air stress- and strain-controlled oscillatory and rotational rheometer (Thermo Scientific Inc., Remote, Germany) equipped with a steel parallel plate (35 mm diameter/1 mm GAP) and with a Peltier heating system was applied the dynamic rheological properties of the samples. After the sample was placed on the plate, the temperature was allowed to stabilize for 5 min. All rheological measurements were conducted in triplicate and the average values are reported here.

Dynamic Shear Properties

The amplitude sweep test was performed in the strain range of 0.01–100% at 1 Hz to determine the linear viscoelastic region (LVR). A frequency sweep test was performed at 1.0% strain (within LVR) over a frequency (ω) range of 0.1–10 Hz at 25 °C and the temperature sweep test at 25–95–25 °C at the same heating and cooling rate of 5 K/min at 1 Hz of frequency to record the storage (G'), loss modulus (G''), tangent phase ($\tan \delta$), and complex viscosity (η^*) values.

The dependence of G' , G'' , and η^* of the samples on the angular frequency (ω) was modeled by a power-type relationship (Equations (3)–(5)) and on the temperature by an Arrhenius model (XX):

$$G' = K'(\omega)^{n'} \quad (3)$$

$$G'' = K''(\omega)^{n''} \quad (4)$$

$$\eta^* = K^*(\omega)^{n^*} \quad (5)$$

where K' , K'' , K^* , n' , n'' , and n^* are empirical constants [31].

2.10. Foam Capacity (FC) and Foam Stability (FS)

The foaming properties of WBPC were determined according to the method of Ge et al. (2024) [23], with minor modifications. Approximately 0.3 g of WBPC was dispersed in 10 mL of deionized water adjusted to pH 4 and 7, using 0.1 M HCl or NaOH. The dispersion was whipped at 25,000 rpm for 90 s using a high-speed disperser (Ultra-Turrax IKA T25 digital; Model T 25 D S32). The foam volume (mL) was recorded from 0 to 60 min. The FC and FS were calculated using Equations (6) and (7), respectively:

$$FC(\%) = \frac{V_1}{V_0} \times 100 \quad (6)$$

$$FS(\%) = \frac{V_2}{V_1} \times 100 \quad (7)$$

where V_1 is the volume after agitation, V_0 is the initial volume before agitation, and V_2 is the volume after resting.

2.11. Statistical Data Analysis

All analyses were performed in quadruplicate. The results are expressed as the mean $\pm$ standard deviation. The mean comparisons were conducted using a one-way analysis of variance (ANOVA), followed by Tukey's test ($p < 0.05$). The statistical analyses were performed using JAMOV software (version 2.4.14).

3. Results and Discussion

3.1. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR results provide detailed insights into the secondary structure of the analyzed proteins [32]. Table 1 presents the percentage values of the different secondary structures (α -helix, β -sheet, β -turn, and random coil) for the white bean protein concentrate (WBPC), soy protein isolate (SPI), and pea protein concentrate (PPC). It is noteworthy that β -structures were predominant in the secondary structures of all analyzed proteins, with β -sheet being the major component across all proteins, showing no significant differences between them (p -value > 0.05). Other isolates and concentrates from various bean varieties also exhibited β -sheet as the main structural component [10,33–35].

Table 1. Secondary structure of white bean and pea protein concentrates and soy protein isolate.

Sample	α -Helix	β -Sheet	β -Turn	Random Coil
White beans	11.12 $\pm$ 0.2 c	56.13 $\pm$ 1.5 a	17.39 $\pm$ 0.1 b	15.34 $\pm$ 0.1 a
Pea	12.73 $\pm$ 0.3 b	55.14 $\pm$ 3.2 a	19.63 $\pm$ 0.2 a	12.48 $\pm$ 0.1 b
Soy	15.03 $\pm$ 0.5 a	57.22 $\pm$ 2.2 a	19.63 $\pm$ 0.2 a	8.10 $\pm$ 0.3 c

The letters a, b, and c indicate significant differences between the data in the same column (p -value < 0.05).

The β -sheet content may be associated with the hydrophobic interactions of the protein, as hydrophobic sites present in the molecule can be exposed, thereby increasing the surface hydrophobicity [36]. WBPC exhibits the lowest percentage of α -helices and the highest percentage of random coils compared to the other proteins in this study. The higher random coil percentage in the WBPC may be related to a more disordered protein structure [37,38], with a significant difference (p -value < 0.05) when compared to conventional proteins. The lower α -helix content could also indicate a less ordered protein structure [39] compared to soy and pea proteins, which show higher percentages (p -value < 0.05) of α -helices, suggesting a more compact and stable conformation for these proteins. This difference may explain the better functional properties observed in their native forms, which will be discussed in later sections.

From the results of the secondary structure composition of the proteins, we can begin to understand the stability and potential functionalities of WBPC. Although the structure of soy protein isolate and pea protein concentrate appears more organized, white bean protein exhibits a less defined conformation, which may indicate greater flexibility to changes compared to conventional proteins. These disordered regions of WBPC may facilitate binding with different molecules more easily, and the flexibility could enable structural changes that improve functional properties.

3.2. Surface Hydrophobicity

The surface hydrophobicity of a protein can be understood as a property that relates to the extent of hydrophobic groups exposed on the protein surface [40,41]. Figure 2 compares the surface hydrophobicity of white bean protein concentrate (WBPC) with commercial soy protein isolate (SPI) and pea protein concentrate (PPC). The results indicate that PPC exhibits greater hydrophobicity compared to the other proteins, likely due to a higher amount of nonpolar regions exposed on the surface of the pea protein.

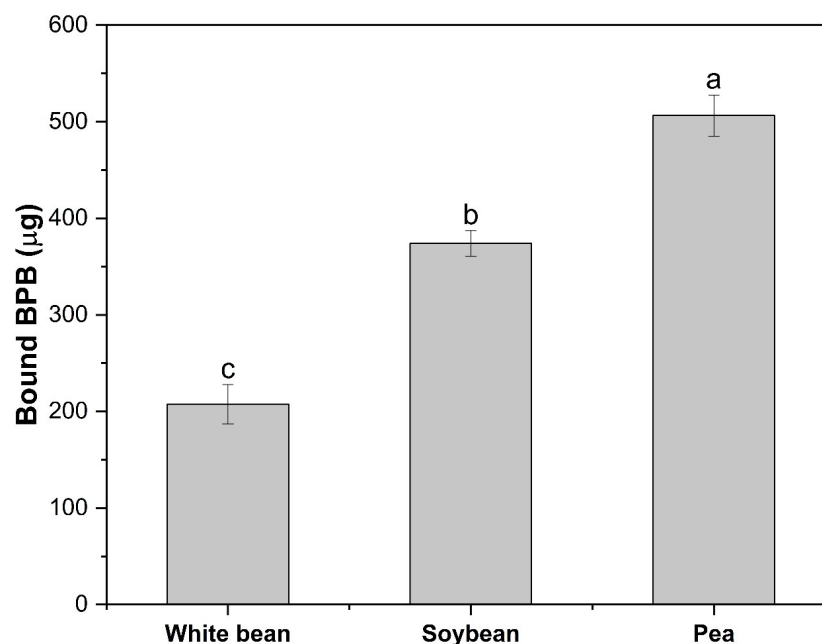


Figure 2. Surface hydrophobicity of white bean protein concentrate compared with commercial soy protein isolate and pea protein concentrate. The letters a, b, and c indicate significant differences between the data in the same column (p -value < 0.05). Bound BPB refers to the amount of Bromophenol Blue bound to proteins, with the unit of measurement expressed in micrograms (μg), indicating the quantity of this substance or the complexes formed.

In contrast, WBPC shows significantly lower hydrophobicity (p -value < 0.05) when compared to the commercial proteins. More hydrophobic proteins tend to exhibit better emulsifying properties due to their ability to adsorb at the oil–water interface, as well as a high capacity for foam formation, since hydrophobic regions aggregate at air–water surfaces [42]. Conversely, proteins that are more hydrophilic typically bind more readily to water molecules, which imparts resistance to precipitation and aggregation.

Although hydrophobicity is generally associated with improved interaction of the protein with nonpolar molecules [33,40,43], oil retention may be favored by less hydrophobic proteins, which tend to be more flexible and present a larger contact surface, facilitating interaction with oil, depending on its polarity [44]. Another relevant factor is that the flexibility of less hydrophobic proteins may enhance the interaction with water, resulting in better solubility, which favors air retention. Consequently, these proteins can exhibit a good capacity for foam formation [12] by reducing the coalescence rate of bubbles.

While WBPC has lower hydrophobicity compared to commercial plant proteins, this does not necessarily imply reduced effectiveness in functional properties within food systems that rely on protein–oil and protein–water interactions.

3.3. Solubility

3.3.1. Solubility as a Function of pH

Understanding protein solubility is essential, as it is linked to the performance and properties of proteins in high-moisture food systems [7,23,40,43,45]. Figure 3 presents the solubility results for WBPC as a function of pH variations. WBPC's solubility is shown to be strongly pH-dependent, with minimum solubility observed at pH 3, which can be attributed to its isoelectric point (pI). At this pH, the lowest solubility is due to the minimal net charge of the protein molecules, resulting in reduced electrostatic repulsion and leading to protein aggregation. Conversely, maximum solubility occurs in highly acidic (pH 2), neutral (pH 7), and alkaline (pH 8 and 10) environments, where surface charges

promote electrostatic repulsion between protein molecules, preventing aggregation and precipitation. These conditions enhance protein–solvent interactions, thereby increasing solubility [7,11]. When applied to food systems, higher protein solubility enhances water interactions, improving properties such as the water-holding capacity, foam formation, and stability. It may also increase the oil droplet coverage, favoring better stabilization [40].

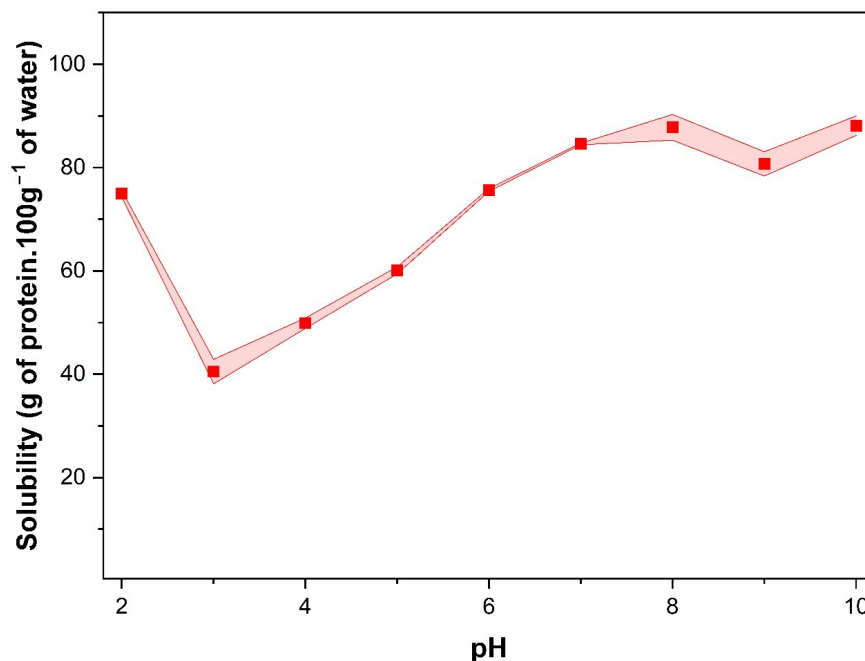


Figure 3. Solubility of white bean protein concentrate as a function of pH variation. Solubility is expressed as the amount of protein (g) dissolved in 100 g of water, showing variation with pH. The points represent the experimental measurements.

3.3.2. Solubility as a Function of Salt (NaCl) Concentration

Figure 4 shows the WBPC's solubility as a function of the NaCl concentration, highlighting an initial increase with salt addition, reaching peak solubility at approximately 0.8 M. This pattern suggests that salt enhances protein solubilization, likely due to the salting-in effect. In this phenomenon, low salt concentrations can increase the protein solubility by reducing electrostatic interactions among protein molecules, facilitating protein–water interactions and preventing protein aggregation [46–49].

However, as the NaCl concentration surpasses > 0.8 M, the solubility declines significantly due to the salting-out effect. At high salt concentrations, water preferentially solvates NaCl ions, reducing its availability to interact with proteins, leading to protein aggregation and precipitation [46–50]. With the addition of 1 M NaCl, the WBPC's solubility reaches a minimum, indicating that further salt additions have no additional effect on the protein solubility.

Studies such as that by Mu et al. (2009) [51] on the influence of salts on sweet potato protein's emulsifying properties demonstrated that adding 0.2 mol.L⁻¹ of CaCl₂ enhanced the emulsifying activity and stability, reducing the dependency of the emulsifying activity index on the pH. Such correlations between salt and protein solubility could reduce the need for synthetic additives in formulations [52].

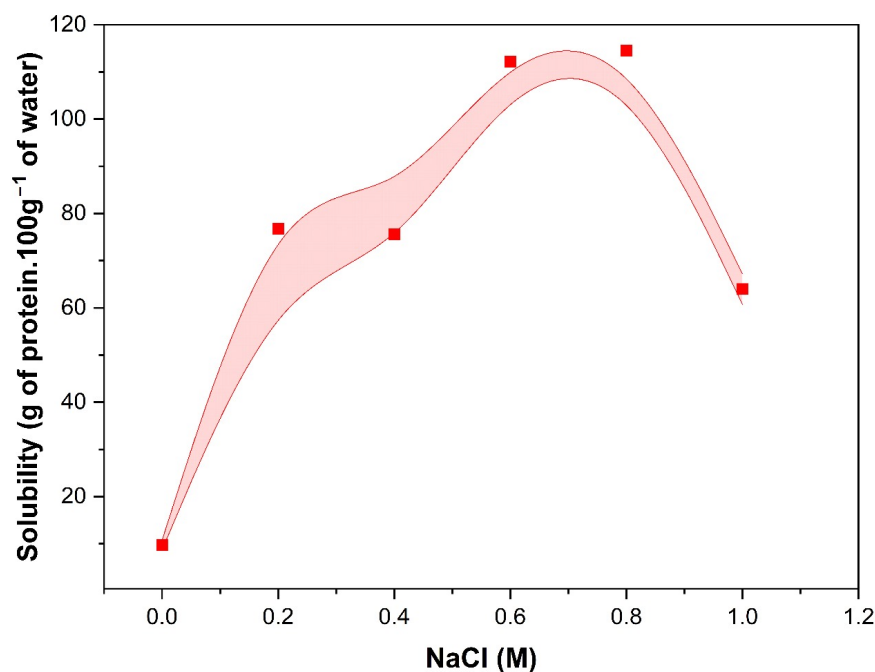


Figure 4. Solubility of white bean protein concentrate as a function of NaCl concentration. Solubility is expressed as the amount of protein (g) dissolved in 100 g of water. The points represent the experimental measurements.

Schuldt et al. (2014) [53] reported that the soy protein isolate solubility increased, contributing to the formation of softer gels by promoting electrostatic interactions that enable molecular rearrangements. Additional repulsive forces seemed to promote protein rearrangements, enabling optimal gel network configuration. Furthermore, Yuliana et al. (2014) [48] observed that NaCl concentrations of up to 0.5 mol.dm^{-3} improved the solubility, foam stability, and emulsions of cashew nut shell protein isolate, due to weakened hydrophobic interactions and protein adhesion, forming interfacial layers with improved rheological properties that enhance the foam and emulsion integrity.

Understanding the effect of the NaCl concentration on protein solubility is relevant for the application of WBPC in food products containing salt. Optimizing the solubility at low salt concentrations can enhance important functional properties [54,55], such as the emulsification and foaming capacity [48], while allowing control and mitigation of the negative effects associated with high ionic strength [54,56]. This understanding also provides a foundation for developing formulations with reduced reliance on synthetic additives, thereby expanding the techno-functional potential of WBPC. The evaluation of this property is common in the analysis of new vegetable proteins [54,56,57], and it is important to understand the behavior of the protein at different NaCl concentrations, as many of these proteins can be applied in the production of meat analogs and hybrid meat products.

These results are significant for the application of WBPC in food products containing salt, providing insights into its behavior in various formulations. The limited solubility of plant proteins, such as WBPC, can be optimized in low-salt solutions, enhancing functional properties such as emulsification and gel formation.

3.3.3. Solubility as a Function of Carbohydrate (Glucose)

Figure 5 presents WBPC's solubility as a function of the glucose concentration, showing fluctuations throughout the curve. Initially, the addition of glucose results in increased solubility, reaching a peak between 0.1 and 0.3 M glucose. This increase can be attributed

to glucose's stabilizing effect, as it may form a hydration layer around protein molecules and through hydrophilic covalent bonding, enhancing the interactions between protein molecules and water, thereby promoting solubility [58].

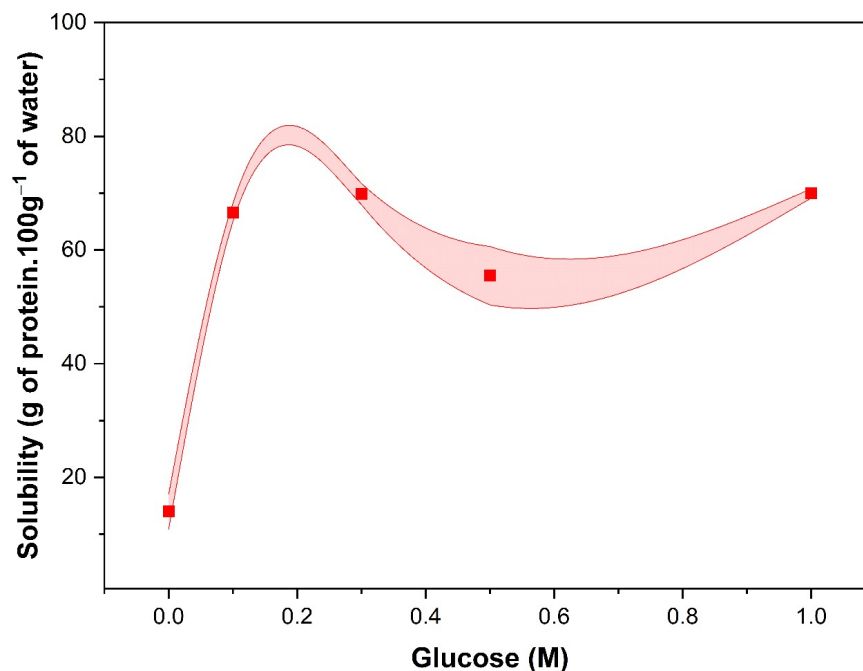


Figure 5. Correlation between glucose concentration (M) and protein solubility (g of protein per 100 g of water): the curve represents the variation in protein solubility as a function of glucose concentration.

Beyond 0.2 M of glucose, the WBPC's solubility decreases, suggesting protein destabilization at intermediate glucose concentrations. This behavior may be explained by the glucose forming a network that competes with the protein for available water, due to glucose's high hydrophilicity. This may enhance the hydrophobic interactions among protein molecules, resulting in decreased protein solubility [59].

At higher glucose concentrations (~1 M), the protein solubility increases again. The presence of hydrophilic or charged carbohydrate fractions can enhance the protein surface hydrophilicity or charge, leading to increased hydration and electrostatic repulsion among protein molecules, which promotes solubility. Additionally, carbohydrates may increase the steric repulsion between proteins, preventing aggregation [60]. Studies indicate that different plant proteins, such as rice protein [61] and pea protein [58], when conjugated with sugars, can show improvements in properties such as solubility, activity, and emulsification stability.

Glucose exhibits a unique effect on the WBPC's solubility, reflecting the complex interaction that can exist between carbohydrates and proteins in aqueous solutions. These findings are particularly relevant for food systems applications, especially given the lack of prior studies on glucose and white bean protein interactions.

3.4. Foam Capacity (FC) and Foam Stability (FS)

The FC values of the WBPC, PPC, and SPI at pH 4 and 7 are shown in Figure 6A. Our analysis of the pH conditions applied to the proteins reveals that the pH acts as a critical factor in foam formation, influenced by parameters such as solubility and the protein's behavior in acidic and basic environments. Under acidic conditions, PPC and SPI exhibit greater foam capacity compared to WBPC. This suggests that these proteins are structurally more flexible and capable of forming foam in this environment. The lower foam capacity

of WBPC under these conditions may be associated with its low solubility at this pH, likely due to its isoelectric point (pI) near pH 4, where the electrical charges are reduced and solubility is minimal. Proteins solubilized and precipitated near their isoelectric point tend to show reduced functional properties, such as the FC, at pH values close to the pI, as evidenced by previous studies [62,63].

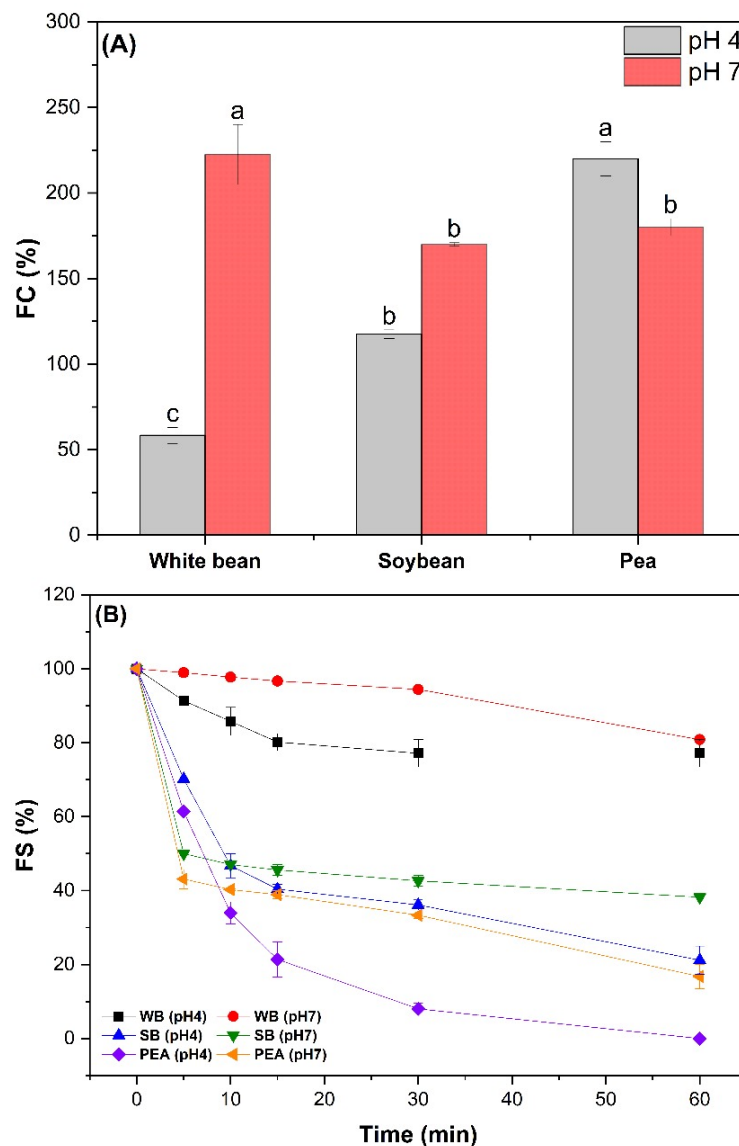


Figure 6. Comparison of foam functional properties at different pH levels and protein sources. (A) Foam capacity (FC%) of proteins from white bean, soybean, and pea at pH 4 and pH 7. Bars represent mean values $\pm$ standard error and different letters indicate statistically significant differences ($p < 0.05$) within the same pH range. (B) Foam stability (FS%) over time (0–60 min) for the same protein sources at pH 4 and pH 7. Stability is represented by the remaining foam fraction over time, with distinct symbols used for each condition.

Regarding the FS under acidic conditions, Figure 6A shows that the WBPC demonstrated greater foam stability at pH 4, despite the PPC and SPI exhibiting higher FC in this condition. This behavior may be attributed to the protein's specific interaction with the medium. Although pea protein shows good FC, its stability is considerably lower, with only around 13% stability after 15 min, rapidly decreasing over time and reaching 0% after 60 min, which limits its application in products requiring stable foams. Conversely, WBPC's

high stability in acidic environments indicates its potential for application in products that demand high foam stability.

At neutral pH, WBPC exhibits high FC, outperforming the commercial proteins analyzed. This performance may be related to the increased solubility and structural conformation of white bean protein concentrate, favoring foam formation in this environment. While SPI and PPC also demonstrate good foam-forming abilities, both are inferior to WBPC. Regarding the FS, Figure 6B shows that WBPC retains approximately 94% and 80% of its foam after 30 and 60 min, respectively, indicating good performance at neutral pH. Although SPI maintains good stability over prolonged periods and is more stable than PPC, its stability is inferior to that of WBPC, which may limit its efficacy in foods requiring stability at neutral pH. The initial foam formation alone is not a definitive indicator of the food application potential. PPC, for example, experiences a rapid decline in stability at neutral pH even after forming foam initially, with only about 17% of its foam being retained after 60 min, indicating that WBPC may serve as a suitable substitute. Globular proteins such as WBPC can form an interfacial film alongside carbohydrates, allowing air bubbles to be suspended, reducing their coalescence rate, and improving the foam stability [64]. The higher FC at neutral pH may be explained by the increase in the net surface charges of the proteins, related to weakened hydrophobic interactions and increased protein–protein repulsions, resulting in greater flexibility. This flexibility allows proteins to diffuse more rapidly at the air–water interface, effectively encapsulating air particles and enhancing the FC under neutral pH conditions [35,48,62].

Gundogan and Karaca (2020) [35] investigated the FC and FS in bean proteins, which also exhibited good foaming properties. Gouvêa et al. (2023) [13] demonstrated in their study that common bean protein concentrate maintained approximately 95% FS after 60 min, outperforming SPI, which showed significant loss, with less than 65% foam retention. Similar to WBPC, lupin protein fractions studied by Burgos-Díaz et al. (2016) [63] showed favorable FS and FC under different pH conditions. Additionally, Tang, Roos, and Miao (2023) [65] studied chickpea and lentil protein isolates, revealing these proteins' potential in food foam formulations, independent of the pH, making them promising alternatives to animal-derived proteins.

These results suggest that WBPC could serve as a valuable ingredient for applications in food products. In addition to being an alternative to commercial plant proteins with lower foam stability, WBPC stands out for its efficiency in maintaining the foam structure and volume over prolonged periods, which is beneficial for applications in products such as toppings and confections [13] and beverages such as beer and coffee [62].

3.5. Least Gelation Concentration (LGC)

The least gelation concentration (LGC) values of the white bean protein concentrate (WBPC) at different protein concentrations (2% to 22%) are presented in Figure 7. No gel formation was observed at concentrations between 2% and 10%. Gel formation occurred only at concentrations above 16%, with firm gel formation observed at higher concentrations of WBPC ($\geq 20\%$). Results similar to those of this study were reported by Gouvêa et al. (2023) [13], who studied the LGC in common beans and found firm gel formation only at the highest concentration (20%). Tan, Ying-Yuan, and Gan (2014) [25] found that when analyzing the LGC in pinto bean protein isolate, a weak gel began forming at 16% and a firm gel at 12% for soybean protein isolate.

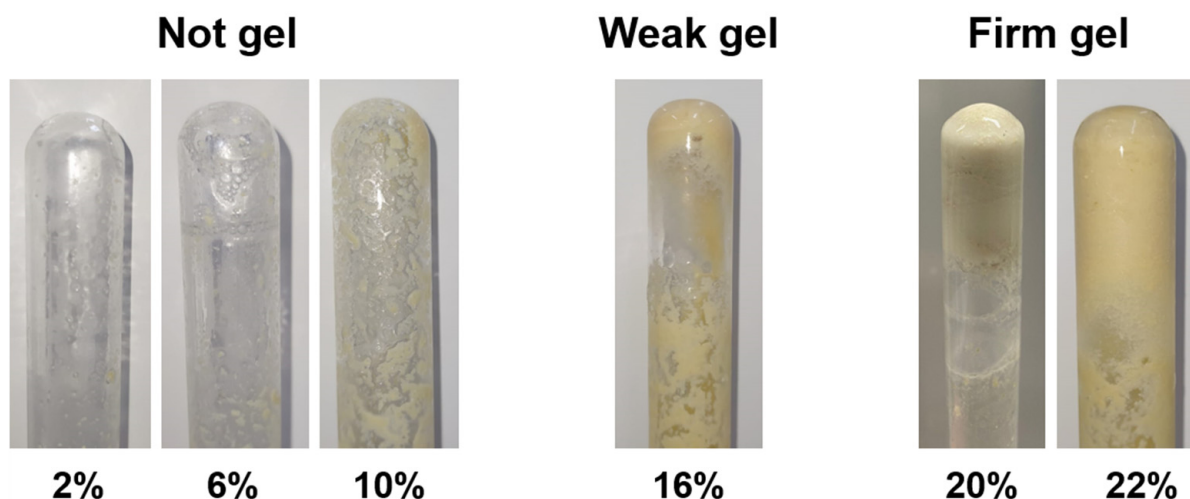


Figure 7. Minimum gelation capacity of white bean protein concentrate. The images represent the behavior of proteins at increasing concentrations (2% to 22%) and the corresponding gel formation. The samples are classified into three categories, absence of gel (2%, 6%, 10%), weak gel formation (16%), and firm gel formation (20%, 22%), indicating the dependence of the protein concentration on gel structuring.

However, the LGC of WBPC appears to be higher than that reported by Peyrano, Speroni, and Avana (2016) [66], who investigated cowpea protein isolate with an LGC of 12% without heat treatment, as well as Ogunwolu et al. (2009) [67], who studied cashew protein concentrate ($10.0 \pm 1.81\%$) and cashew protein isolate ($13.5 \pm 0.01\%$) at their natural pH. This suggests that proteins from other bean varieties and plant protein sources may have lower LGCs compared to WBPC. According to Ma et al. (2022) [43] and Ogunwolu et al. (2009) [67], these LGC variations may be due to the fact that smaller globular proteins generally require higher concentrations to form a gel compared to larger, more extended proteins. Thus, different protein fractions can significantly influence the LGC.

3.6. Gel Rheology

3.6.1. Gel Rheology as a Function of Frequency

The rheological behavior of the WBPC, SPI, and PPC was studied with respect to their oscillation frequency, as shown in Figure 8. In rheology, G' , the storage modulus, indicates the material's elasticity, while G'' , the loss modulus, indicates the material's viscosity [23,45]. Figure 8 illustrates the variations in G' and G'' for WBPC as a function of frequency and G' and G'' for SPI and PPC. Overall, both moduli increase to different extents at higher frequencies across all protein gels. However, when comparing the WBPC with the conventional proteins analyzed, differences in gel rigidity and structural stability are evident. With an elastic modulus reaching 10 kPa, the WBPC gel has a more rigid structure compared to the weaker, less rigid gels of the commercial proteins.

Rigid gels are advantageous for applications requiring higher resistance to external forces and a more stable structure, such as gelatin and certain desserts. In contrast, less rigid gels are ideal for applications where a firm structure is unnecessary but viscosity and fluidity are desirable.

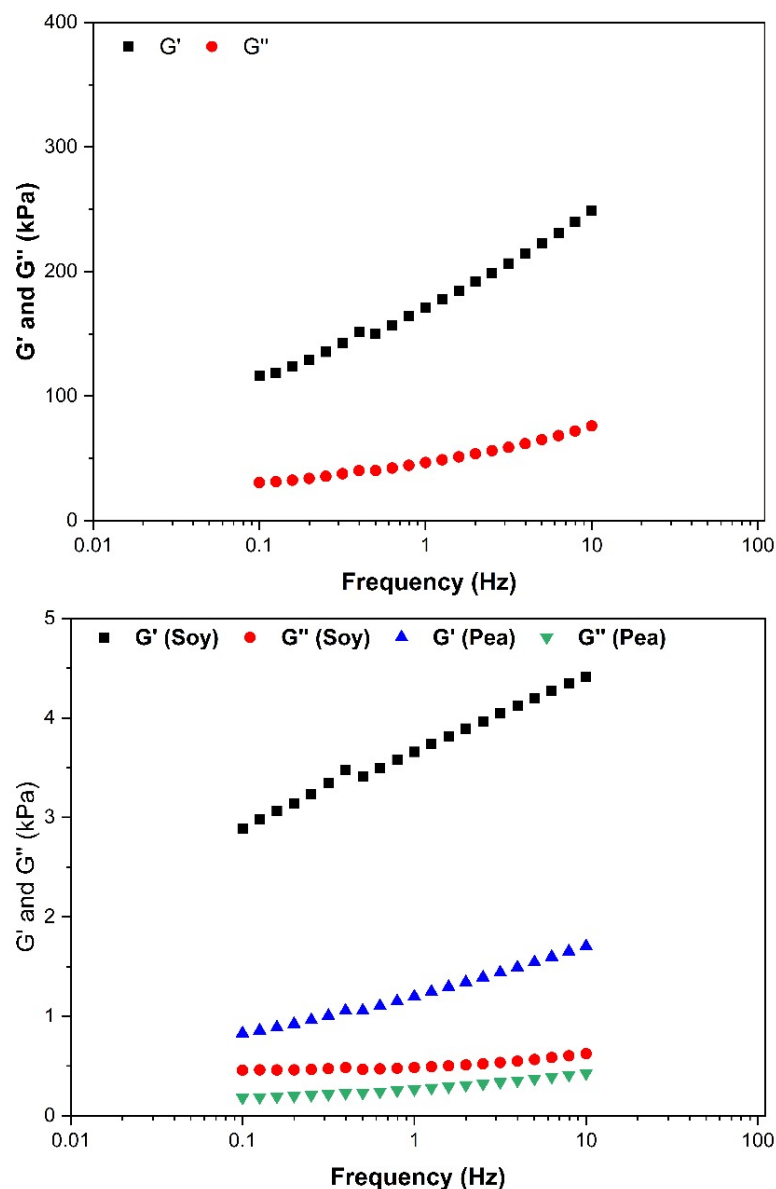


Figure 8. Rheological behavior of white bean, soy, and pea protein gels as a function of frequency. The figures present the elastic (G') and viscous (G'') moduli as a function of frequency (0.01–10 Hz) for white bean protein (**upper** part) and soy and pea proteins (**lower** part). The results show the rheological dependence of the protein systems, highlighting the formation of distinct structural networks.

3.6.2. Rheological Analysis as a Function of Shear Strain

Figure 9 shows the rheological analysis of the WBPC, SPI, and PPC as a function of shear strain. In Figure 9, which corresponds to the white bean protein concentrate (WBPC), $G' > G''$ across nearly the entire range of shear deformation, indicating predominantly elastic behavior. This profile suggests a rigid network structure (with G' and G'' in kPa), characterizing it as a strong gel suitable for applications requiring high mechanical strength and elasticity. However, near 10% deformation, the G' and G'' values decline, indicating that at higher deformation levels the gel structure begins to break down—an expected behavior for viscoelastic materials [45,68,69]. Despite the white bean protein appearing to form a well-defined gel structure, the inflection point between G' and G'' can be considered a stability threshold, where increased shear deformation leads to a loss of the network's mechanical integrity.

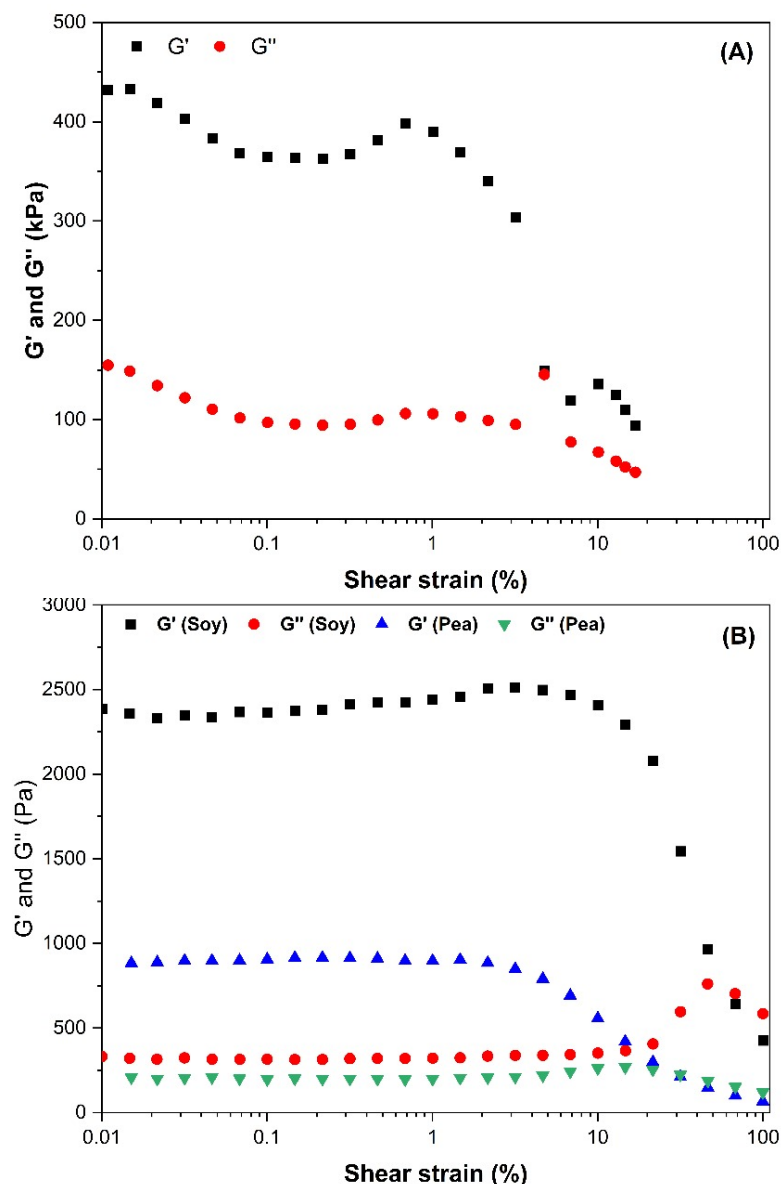


Figure 9. Deformation resistance of white bean, soy, and pea protein gels. The figures present the elastic (G') and viscous (G'') moduli as a function of shear strain (%): (A) white bean protein gels; (B) soy and pea protein gels. The analysis demonstrates the structural stability of the gels under increasing deformation, indicating the structural rupture point at different protein concentrations.

In contrast, Figure 9, which displays the shear strain curves for the PPC and SPI, shows that both exhibit elastic behavior. The soy protein has higher G' values, remaining relatively constant up to a greater strain level compared to pea protein, which shows lower G' and G'' values, suggesting a less cohesive and weaker network. This indicates that soy protein forms a more rigid and structured network than pea protein, which likely has lower long-term stability.

These data suggest that soy and pea proteins may offer sufficient structural stability for food applications requiring gels with lower rigidity and greater flexibility. By comparison, white bean protein, with its higher G' and G'' values (in kPa), demonstrates a greater potential to form rigid networks depending on the level of shear deformation, making it more suitable for systems that demand high mechanical resistance.

3.7. Water-Holding Capacity (WHC) and Oil-Holding Capacity (OHC)

The WHC and OHC of the analyzed proteins are presented in Figure 10. The WBPC exhibited lower WHC values compared to the SPI and PPC. These variations in WHC may be due to differences in protein structure, the availability of polar amino acids on protein surfaces that interact with water [10,11,70,71], and the presence of hydrophilic carbohydrates that contribute to water absorption [10].

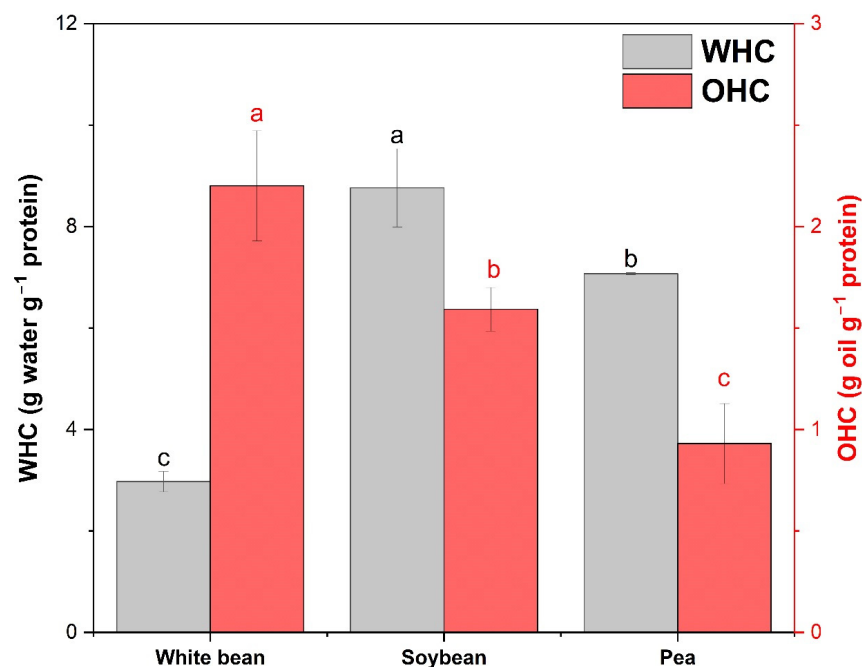


Figure 10. Water-holding capacity (WHC) and oil-holding capacity (OHC) of white bean protein concentrate, pea protein concentrate, and soy protein isolate. The water-holding capacity (WHC) is presented on the left axis (gray bars) and the oil-holding capacity (OHC) on the right axis (red bars). The bars represent the mean $\pm$ standard deviation. Different letters indicate statistically significant differences ($p < 0.05$) between groups for each evaluated parameter. WHC is expressed in g of water per g of protein, while OHC is expressed in g of oil per g of protein.

In comparison to recent studies on the functional properties of other protein fractions [10,70,72], the WBPC demonstrated a relatively high WHC, highlighting its potential compared to alternative proteins, potentially due to protein unfolding and increased molecular spacing. Enhancing the interaction of these proteins with water can be achieved by applying specific concentrations of salts [46] and sugars [60], which improve the WBPC's solubilization, as observed in this experiment. Furthermore, the use of structural modification through emerging technologies is a promising approach for this purpose [36,73]. However, additional studies are needed to fully explore these possibilities for white bean protein concentrate.

On the other hand, the WBPC showed a significantly higher OHC (p -value < 0.05) than the other proteins (Figure 10). This result is consistent with Lafarga et al. (2018) [74], who reported a high OHC for Ganxet bean protein concentrate, and Tabatabaei et al. (2019) [71], who found that navy bean exhibited a higher OHC than commercial soybean protein concentrate and other protein-rich fractions.

The high OHC may be attributed to denaturation, alterations in surface properties, and exposure of protein hydrophobic groups [23,71,75]. Elevated hydrophobicity enhances oil retention as lipid chains interact with the nonpolar side chains of amino acids. This hydrophobic interaction provides a strong driving force for oil retention, a key component in effective oil binding [23]. Hydrophobicity is considered one of the most significant

intrinsic factors affecting the OHC and emulsifying properties in legume proteins [35]. The WBPC's good diffusion at the oil–water interface may be linked to these hydrophobic groups on its protein surface due to the higher presence of β -sheet in its secondary structure.

There is a strong positive correlation between the OHC and the emulsifying capacity and emulsion stability [72]. In the food industry, a protein with good OHC is valuable for use in products and emulsion-stabilizing applications, such as sausages, fat-rich gels, and beverages.

Gouvêa et al. (2023) [13], who also studied a protein concentrate from another bean variety, confirmed that bean proteins have promising potential for the plant-based market due to their better emulsifying activity index than soybean protein concentrate and excellent emulsion stability, surpassing other isolates and concentrates such as soybean, pea, and fava bean. Tan, Ying-Yuan, and Gan (2014) [25], as well as Tabatabaei et al. (2019) [71], also reported high emulsifying capacity for pinto beans and good emulsifying activity for navy beans, respectively.

3.8. Emulsion Stability

The Turbiscan Stability Index (TSI) is a parameter used to evaluate the stability of emulsions and suspensions, measured using the Turbiscan instrument. This device analyzes the transmission and scattering of light along the height of the sample, detecting changes in optical properties that indicate variations in particle concentration, droplet size, and structural alterations in the emulsion or suspension over time. Generally, low TSI values indicate more stable emulsions, while higher values suggest instability associated with phenomena such as flocculation, sedimentation, cream formation, and coalescence [76–78].

Figure 11 presents the TSI values for emulsions of WBPC, PPC, and SPI under different pH conditions (4 and 7) and oil-to-water ratios (1:1 and 2:1). Overall, emulsions at pH 7 exhibit greater stability, characterized by lower TSI values, which can be attributed to the higher net charge of the proteins in neutral pH, reducing aggregation due to electrostatic repulsion and being further from the isoelectric point. Conversely, at pH 4, near the isoelectric point of the proteins, the emulsions show a tendency toward aggregation and phase separation, resulting in higher TSI values and indicating lower stability.

Among the proteins, the emulsions containing pea protein (represented by blue stars in the 2:1 water/oil ratio at pH 7) and white bean protein (represented by black squares and red circles) display the lowest TSI values, indicating these emulsions are more stable compared to those with soy protein and pea protein, showing less variation over time. The emulsions of soy and pea proteins at pH 4 in both oil/water ratios exhibit an increase in TSI over time, with higher values indicating instability, possibly due to sedimentation or phase separation. This suggests that these proteins exhibit lower stabilization capacity in emulsions under these conditions compared to white bean protein.

The greater stability observed for emulsions with white bean protein can be explained by its higher oil retention capacity (HOC) and predominant β -sheet secondary structure, which appears to possess a more flexible structure, allowing for more efficient interaction at the oil–water interface. This characteristic makes white bean protein a promising alternative for food applications requiring stable emulsions. Additionally, another factor potentially contributing to the lower TSI values for white bean protein emulsions is their superior viscosity, which may enhance their physical stability by reducing the particle mobility and consequently the frequency of collisions between them, thereby promoting stability [15].

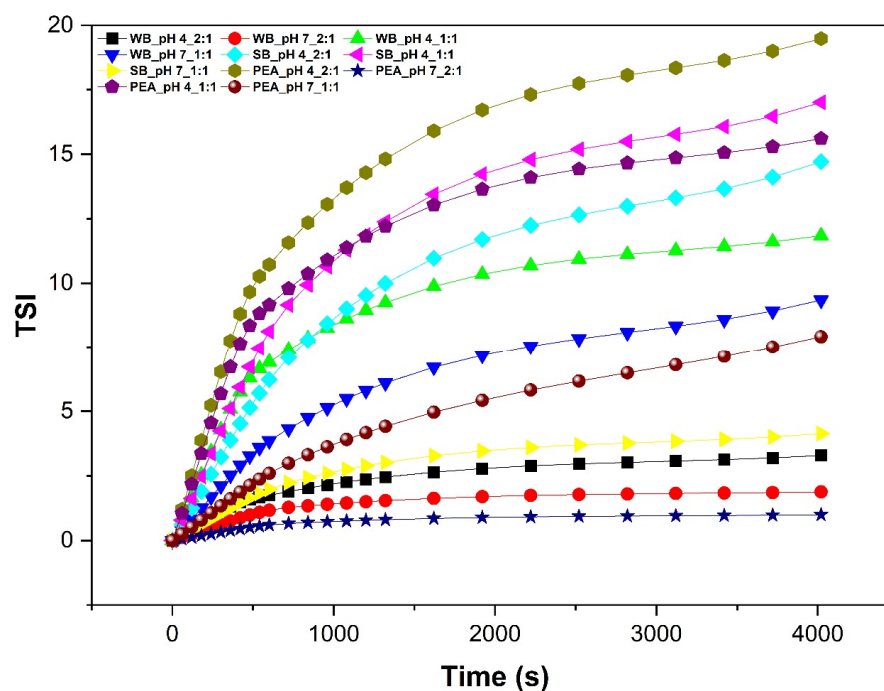


Figure 11. Turbiscan Stability Index (TSI) as a function of time (seconds) for emulsions formulated with white bean (WB), soy (SB), and pea (PEA) proteins at different pH values and oil/water ratios. The samples were evaluated at pH 4 and pH 7, with oil/water ratios of 2:1 and 1:1. The symbols represent the different treatments. The evolution of the TSI values reflects the stability of the emulsions, with lower values indicating greater stability.

4. Conclusions

The results of this study suggest that white bean protein concentrate (WBPC) exhibits unique and promising functional properties for application in the food industry. Compared to soy protein isolate (SPI) and pea protein concentrate (PPC), the WBPC demonstrated distinct characteristics in its secondary structure, including a higher proportion of β -sheets and a more flexible conformation, which may facilitate interactions with different molecules. The rheology analysis of the WBPC indicated a rigid gel structure at high protein concentrations (above 20%), showcasing its potential for use in formulations requiring high mechanical strength and elasticity.

In the emulsion stability analysis, the WBPC showed a competitive stability index (TSI), especially at neutral pH, where it was able to form more stable emulsions compared to the pea and soy concentrates. This indicates good oil retention capacity (OHC) and resistance to phase separation. These results may be attributed to WBPC's hydrophobic structure and high oil-holding capacity, factors that contribute to effective diffusion at the oil–water interface, making it a valuable option for emulsified systems, such as meat products and protein-based beverages.

Although the WBPC exhibited lower surface hydrophobicity and water-holding capacity (WHC) compared to soy and pea proteins, its high OHC suggests it is suitable for fat-rich products that require emulsification and long-term stability. The lower hydrophobicity may further confer WBPC greater flexibility for structural changes, favoring solubility and interaction with polar compounds, which broadens its application potential across various food systems.

Overall, this study highlights WBPC as a competitive alternative to traditional plant proteins, with characteristics that could be leveraged to develop innovative and stable food products in the industry. However, future research is required to optimize its solubility

and explore structural modification techniques that could further enhance its functional properties and applications.

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