

# Technological-functional features of chia, almond, pumpkin seed, soybean and pea proteins

## Caracterização tecnológica e funcional das proteínas de chia, amêndoas, semente de abóbora, soja e ervilha

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### Highlights

Pea protein showed better functional properties than the other proteins.

Soy protein showed no gel formation for the analyzed concentration.

The five plant proteins showed more soluble at pH > 6.

### Abstract

Plant proteins are gaining prominence in plant-based product development. Plant protein isolates and concentrates work as stabilizing, gelling and dispersing agents. Pea (86% protein), pumpkin seed (60% protein), almond (57.5% protein), chia (42% protein) and soybean (43% protein) protein technological-functional properties were determined. Samples were assessed based on the methodology proposed by the Brazilian Agricultural and Research Corporation to find emulsifying activity (EAI) and emulsion stability indices (ESI), foaming capacity (FC) and stability (FS), water solubility, gelling capacity, water holding capacity (WHC) and oil holding capacity (OHC). Peas recorded the highest FC (94.07 ± 6.87 %) and EAI (312.96 ± 14.32  $\frac{m^2}{g}$ ) ( $p < 0.05$ ) values. Soybean and chia accounted for the highest ESI (291.02 ± 15.68 min; 269.58 ± 19.84 min) and pumpkin seed recorded the lowest FS (82 ± 0.51 %) ( $p > 0.05$ ) values. Chia and pea proteins showed higher WHC (4.60 ± 0.26 g/g sample; 4.56 ± 0.01 g/g sample) value, whereas soybean and pea proteins presented higher OHC (2.58 ± 0.25 g/g sample; 2.43 ± 0.26 g/g sample) ( $p < 0.05$ ) value. Almond protein recorded better gel formation at 0.06 g/mL, whereas soybean protein did not form gel at any of the tested concentrations. The five proteins were less soluble at pH ranging from 4 to 6 and they were more soluble at pH > 6. Pea protein showed the highest technological potential to develop new products among the assessed proteins, and this finding is likely related to its protein content, origin, extraction method and globulins found in it.

**Key words:** Functional properties. Plant based. Plant protein solubility. Plant protein isolates and concentrates.

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## Resumo

As proteínas vegetais ganham destaque no desenvolvimento de produtos *plant based*. Os isolados e concentrados proteicos vegetais atuam como agentes estabilizantes, gelificantes e dispersantes. Realizou-se a caracterização tecnológica-funcional das proteínas de ervilha (86%), semente de abóbora (60%), amêndoas (57,5%), chia (42%) e soja (43%). As amostras foram avaliadas usando a metodologia proposta pela Empresa Brasileira de Pesquisa e Agropecuária obtendo-se a capacidade emulsificante (CE) e estabilidade de emulsão (EE), capacidade de formação de espuma (CFE) e estabilidade de espuma (CEE), solubilidade em água, formação de gel e capacidade de absorção de água (CAA) e de óleo (CAO). A ervilha apresentou maior CFE ( $94,07 \pm 6,87\%$ ) e CE ( $312,96 \pm 14,32 \frac{m^2}{g}$ ) ( $p<0,05$ ). A soja e a chia apresentaram maiores EE ( $291,02 \pm 15,68$  min;  $269,58 \pm 19,84$  min) e a semente de abóbora menor CFE ( $82 \pm 0,51\%$ ) ( $p>0,05$ ). A proteína de chia e de ervilha apresentaram maiores CAA ( $4,60 \pm 0,26$  g/g amostra;  $4,56 \pm 0,01$  g/g amostra) enquanto a de soja e de ervilha maiores CAO ( $2,58 \pm 0,25$  g/g amostra;  $2,43 \pm 0,26$  g/g amostra) ( $p < 0,05$ ). A proteína de amêndoas apresentou melhor formação de gel a partir de  $0,06$  g/mL, enquanto que não houve formação nas concentrações testadas para proteína de soja. Para a solubilidade, as cinco proteínas apresentaram menores valores na faixa de pH 4 a 6 e melhor solubilidade em pH > 6. Dentre as proteínas estudadas, a ervilha apresentou maior potencial tecnológico para desenvolvimento de novos produtos, possivelmente está relacionado ao teor proteico, a origem e o método de extração e as globulinas presentes nesta proteína.

**Palavras-chave:** Propriedades funcionais. *Plant based*. Solubilidade de proteínas vegetais. Isolados e concentrados proteicos vegetais.

## Introduction

Plant-based products are gaining prominence in the food industry, which is developing new products by partially or completely replacing animal protein by the vegetable ones in order to target vegan, vegetarian and flexitarian consumers. A number of benefits are associated with a moderate diet focused on plant-based products, among them, weight loss, prevention of cardiovascular diseases, lower cholesterol and reduced risk of stroke (Satija & Hu, 2018). Incorporating plant proteins into animal products is an alternative given current concerns with environmental impacts caused by animal proteins, such as greenhouse gas emissions caused by farming, the use of larger areas than the necessary to grow

plant-based food, and high energy and water consumption to produce animal-based food. Accordingly, there is a growing demand for fully plant-based or alternative products, and it opens room for innovation aimed at meeting the demand for reducing farming negative impacts and, at the same time, offering healthy and tasty products (Boeck et al., 2021; Guyomarc'h et al., 2021).

Pumpkin species *Cucurbita* belongs to family *Cucurbitaceae*. Pumpkin seeds have been used in the healthcare field and in the food industry due to their rich nutritional composition (polyunsaturated fatty acids, fiber and bioactive compounds) and anti-cancer, anti-inflammatory, anthelmintic, antiviral and antioxidant properties (Vale et al., 2019).

Almond is the seed of almond tree *Prunus dulcis*, which belongs to family Rosaceae. Almond seeds are used to develop new health and food products. Their nutritional properties have stressed them as a rich source of lipids, proteins, vitamin E, besides being a good source of fiber, manganese and copper (Chen et al., 2006). Studies have shown that eating almonds on a regular basis helps reduce the risk of diseases such as hypertension, obesity and diabetes mellitus (Kalita et al., 2018).

Pea species *Pisum sativum* L. is a legume used in the food industry to develop new products given its high nutritional value, low allergenic effects and good availability (Lam et al., 2018; Daba & Morris, 2021). It is rich in phosphorus, potassium, magnesium, calcium, B-complex vitamins like B3 and B6, vitamin E, protein and carbohydrates.

Chia species *Salvia hispanica* L. is used to develop food and pharmaceutical products because of its rich nutritional composition ( $\alpha$ -linolenic acid (omega-3) and linoleic acid (omega-6), dietary fiber, antioxidants and protein, which accounts for health benefits and for helping to prevent obesity, cardiovascular diseases and type 2 diabetes (Marcinek & Krejpcio, 2017; Kotecka-Majchrzak et al., 2020).

Soybean species *Glycine max* is widely used in food preparation given its rich nutritional composition (protein, dietary fiber, bioactive compounds and vitamins), which helps improving and preventing osteoporosis, hyperlipidemia, cancers, hypertension, atherosclerosis, type 2 diabetes mellitus and obesity (Tan et al., 2023). Its use is already well established; furthermore, it has excellent emulsion stability and improves products' oil absorption.

It is known that plant protein isolates and concentrates have technological-functional properties that justify their use as stabilizing, emulsifying, foaming, gelling and dispersing agents in several products. Therefore, it is necessary to investigate these proteins' properties in order to target their possible applications. Thus, the aim of the present study was to assess the technological-functional properties of pea, pumpkin seed, almond, chia and soybean proteins in order to properly characterize them (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2022) and to provide information on their likely different applications in the development of planted-based products.

## Materials and Methods

The methodology proposed by the Brazilian Agricultural Research Corporation was used (EMBRAPA, 2022) to technologically and functionally feature pea, pumpkin seed, almond, chia (RS Blumos, Cotia, Brazil) and soybean (Kinino, Mirrasol, Brazil) proteins at concentrations of 86%, 60%, 57%, 42% and 43%, respectively. Emulsifying activity (EAI) and emulsion stability indices (ESI), foaming capacity (FC) and foaming stability (FS), water solubility, gelling capacity, water holding capacity (WHC) and oil holding capacity (OHC) were assessed.

### Emulsifying capacity and emulsion stability

These were assessed by adjusting protein and distilled water samples' pH to 7.0 (NaOH or HCl 0.1 mol/L) through the addition of 20 mL commercial soybean oil

to homogenize the solution in Ultra-Turrax homogenizer at 9500 rpm for one minute. An aliquot of 50  $\mu$ L was removed from the middle and the bottom of the beaker, added to a test tube filled with 5 mL of 0.1% (w/v) SDS (sodium dodecyl sulfate) and vortexed for 30 seconds to measure absorbance in spectrophotometer. Two absorbance measurements were taken at 500 nm: time zero and after 10 minutes. Emulsifying capacity and emulsion stability were determined through equations (1) and (2).

$$EAI \left( \frac{m^2}{g} \right) = \left( \frac{2x2.303x100xA0}{cx0.25x10000} \right) \quad \dots (1)$$

$$ESI (min) = \left( \frac{A0}{A0-A10} \right) x10 \quad \dots (2)$$

Wherein:

- EAI = emulsifying activity index;
- ESI = emulsion stability index;
- A0 = emulsion absorbance at time zero, i.e., right after homogenization is over;
- c = protein sample concentration (g/mL), i.e., sample weight divided by 60 mL;
- A10 = emulsion absorbance after 10 minutes.

### Foaming capacity and stability

These were assessed by adjusting the sample's pH to 7.0 (NaOH or HCl 0.1 mol/L); 45 mL solution was removed and homogenized in Ultra-Turrax for 2 minutes at the following speed/time: 6500 rpm/30 s, 9500 rpm/30 s and 13500 rpm/60 s. The sample was transferred to 100 mL beaker and foam volume was measured and recorded at 0, 10, 30 and 60 minutes. Foaming capacity and stability were determined through equations (3) and (4).

$$FC (\%) = \left( \frac{V1-V0}{V0} \right) x 100 \quad \dots (3)$$

$$FS (\%) = \left( \frac{V2}{V1} \right) x 100 \quad \dots (4)$$

Wherein:

- FC is foaming capacity;
- FS is foaming stability;
- V0 is the initial volume of the protein-sample solution;
- V1 is volume after homogenization (solution + foam);
- V2 is the remaining volume (solution + foam) after 10-minute, 30-minute or 60-minute resting at room temperature.

### Water and oil holding capacity

The total of 100 mg protein ingredient was weighed and 1 mL distilled water (water absorption capacity), or 1 mL soybean oil (oil absorption capacity), were added. The homogenized samples were shaken in vortex mixer for 1 minute, left to rest for 30 minutes at room temperature and centrifuged for 30 minutes (15 minutes at 5000 rpm and 15 minutes at 12000 rpm). Water or oil holding capacity was determined through equation (5).

$$WHC \text{ or } OHC \left( \frac{g}{g} \text{ sample} \right) = \left( \frac{M1-Mt-M0}{M0} \right) \quad \dots (5)$$

Wherein:

- WHC is water holding capacity;
- OHC is oil holding capacity;
- M1 is the mass (g) of the tube filled with the wet sample after residual water or oil supernatant were discarded;

- $M_0$  is the initial sample mass (g);
- $M_t$  is the mass (g) of the Eppendorf microtube.

### Water solubility

It was assessed by adjusting the pH from 3.0 to 9.0 by using HCl (0.1 M or 1M) or NaOH (0.1 M or 1M) and control pH (protein plus NaOH 0.1 M). The samples were shaken for 30 minutes in orbital shaker. Subsequently, an aliquot of 2 mL was taken from each analyzed pH. These samples were centrifuged at 5000 rpm for 16 minutes and assessed for protein concentration through the Lowry method (Lowry et al., 1951). Solubility was determined through equation (6).

$$\text{Solubility (\%)} = \left( \frac{C}{C_t} \right) \times 100 \quad \dots (6)$$

Wherein:

- $C$  is protein concentration in the supernatant at each tested pH;
- $C_t$  is the total protein concentration in the control sample.

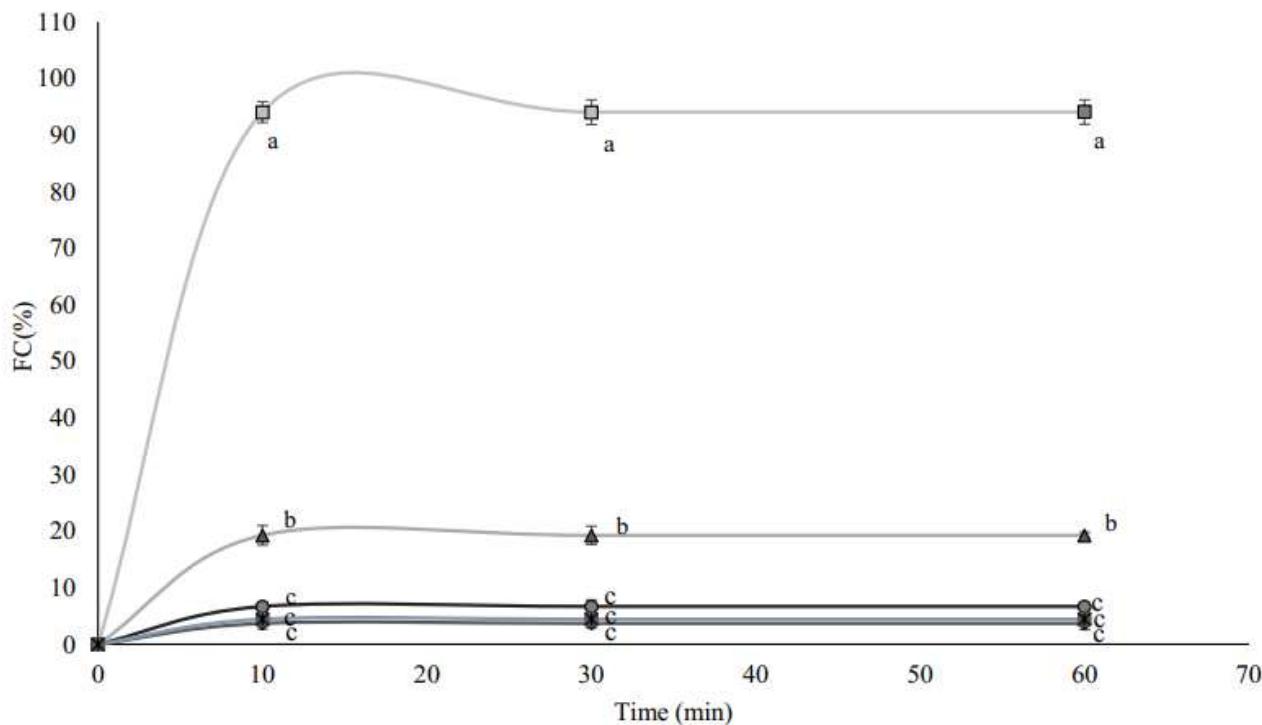
### Gelling capacity

It was determined by using 0.1 g to 1 g sample. The aliquot of 5 mL distilled water was added to each tube, homogenized in vortex for 1 minute and heated in water bath at 100 °C for 60 minutes. They were cooled in an ice bath and kept refrigerated for 2 hours. The tubes were poured in increasing concentration order to trigger gel formation. The answer to the test followed the legend below:

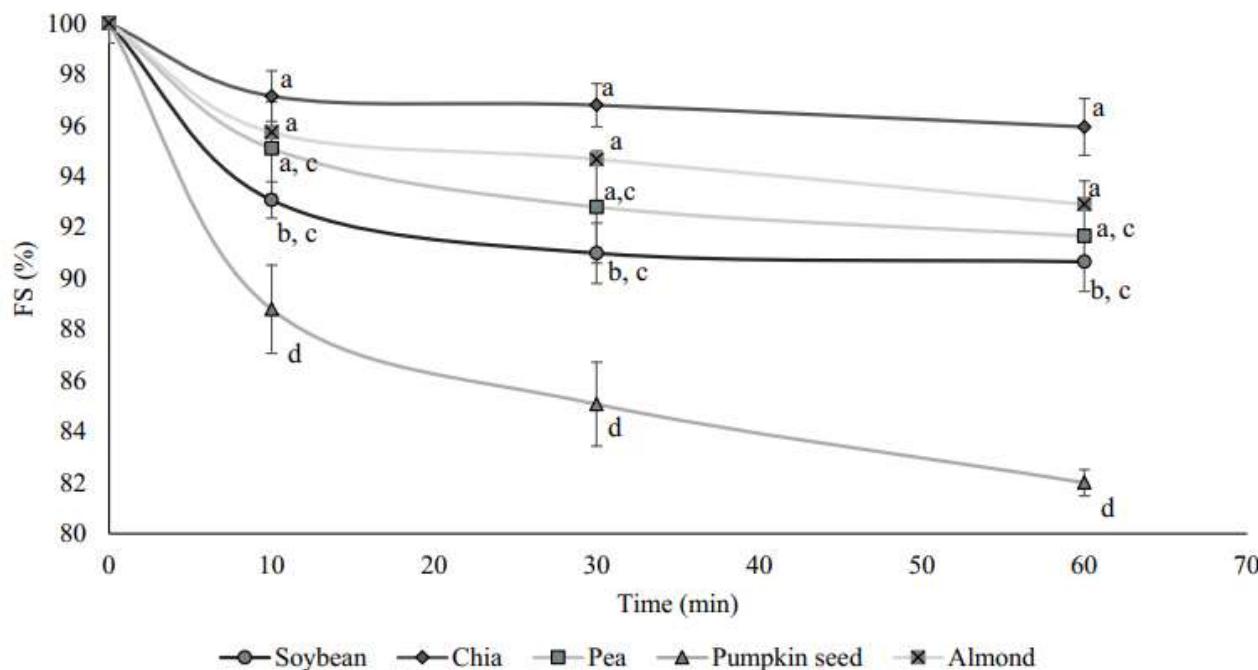
- (-) no gel, in case of liquid solution;
- (±) weak gel, when the solution flows, but it is more viscous;
- (+) gel, when the tube is turned up-side down, but the solution does not flow.

### Results and Discussion

Figures 1 and 2 show the foaming capacity (FC) and stability (FS) of vegetable proteins. The pea protein recorded the best FC value (94.07%  $\pm$  6.87 %), in comparison to the other proteins ( $p < 0.05$ ), and it was followed by pumpkin seed (19.26%  $\pm$  2.77 %). Pumpkin seed showed the lowest FS value (82.00 %  $\pm$  0.51 %;  $p < 0.05$ ), and the other samples showed no significant difference from each other. These properties are necessary for their application in food development, such as in mousses, toppings and confectionery products.



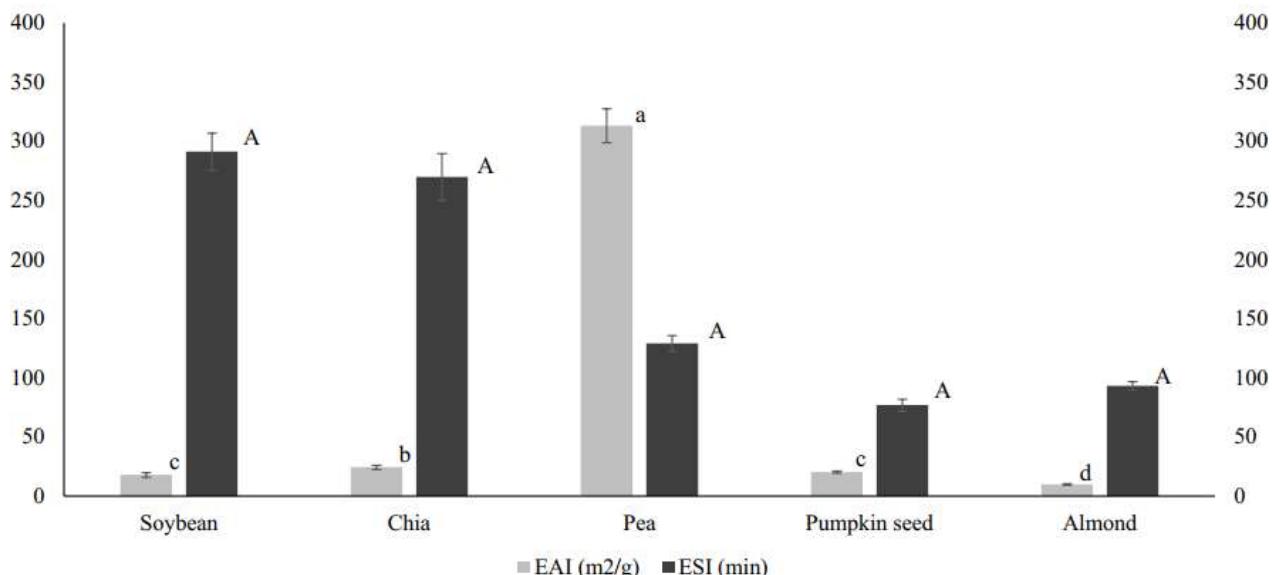
**Figure 1.** Foaming capacity of pea (86%), pumpkin seed (60%), almonds (57.5%), chia (42%) and soybean (43%) proteins. <sup>a-c</sup> Average values with different letters are significantly different ( $p<0.05$ ).



**Figure 2.** Foaming stability of pea (86%), pumpkin seed (60%), almonds (57.5%), chia (42%) and soybean (43%) proteins. <sup>a-d</sup> Average values with different letters are significantly different ( $p<0.05$ ).

Figure 3 shows the emulsifying activity (EAI) and emulsion stability indices (ESI). The pea protein recorded the best EAI result ( $312.96 \pm 14.32 \text{ m}^2/\text{g}$ ) in comparison to the other proteins ( $p<0.05$ ). There was no

significant ESI ( $p>0.05$ ) difference between vegetable proteins, which are essential for making sausages, sauces, desserts and burgers.



**Figure 3.** Emulsifying activity (EAI) and Emulsion stability indices (ESI) recorded for pea (86%), pumpkin seed (60%), almonds (57.5%), chia (42%) and soybean (43%) proteins. <sup>a-d</sup>: Average values with different letters are significantly different ( $p<0.05$ ). Equal capital letters do not point out significant differences between samples in the Tukey's test ( $p>0.05$ ).

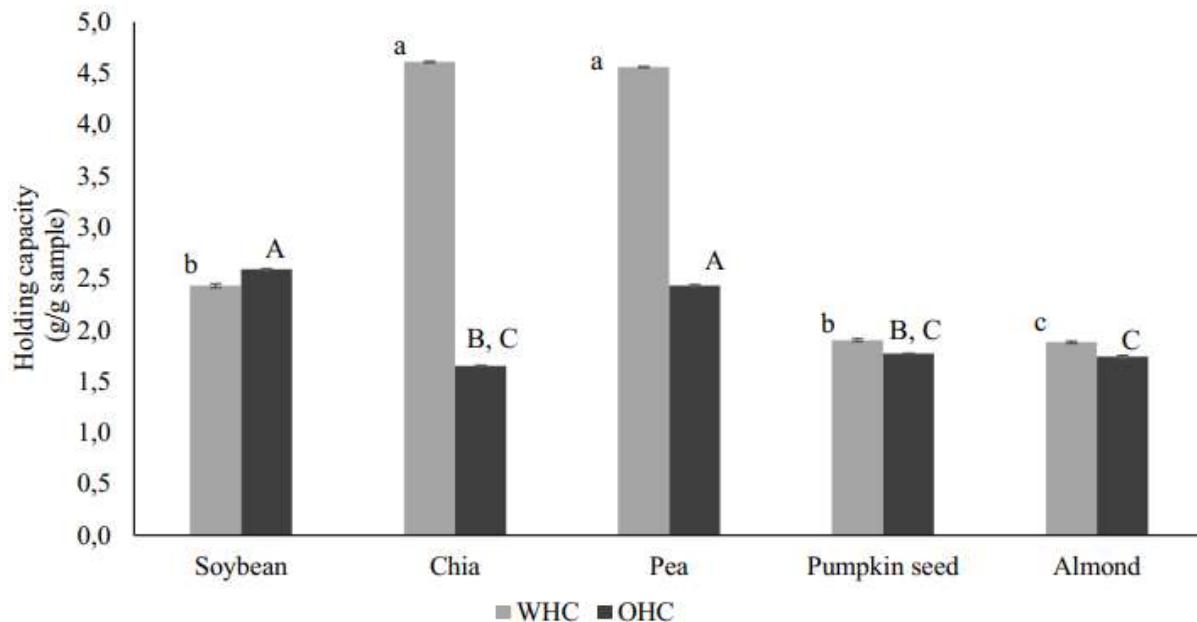
In addition to proteins, plant protein ingredients have polysaccharides, lipids and polyphenols in their composition. According to Stephen and Phillips (2016); Le et al. (2017); Albano et al. (2018), polysaccharides help structure and stabilize thickening, emulsifying and gelling properties.

Wouters et al. (2016) and Kumar et al. (2022) found that both FC and FS depend on interfacial stability. Increased stability is related to hydrophobic proteins and molecular mass, besides leading

to increased retention of air bubbles in suspension. Carbohydrates found in flours also affect this property. According to Kumar et al. (2022) and Shevkani et al. (2015), the main differences in EAI and ESI results are associated with soluble and insoluble protein constituents, lipids and starches. This property is determined by interactions between water and polar amino acids of protein molecules. These factors may have had direct effect on results recorded for the herein analyzed proteins.

Figure 4 shows the water holding (WHC) and oil holding capacity (OHC) of vegetable proteins. Chia and pea proteins recorded the highest WHC values ( $4.60 \pm 0.26$  g/g sample;  $4.56 \pm 0.01$  g/g sample),

whereas soybean ( $2.58 \pm 0.25$  g/g sample) and pea ( $2.43 \pm 0.26$  g/g sample) proteins presented the highest OHC values ( $p < 0.05$ ). These properties are essential to develop viscous food, bakery dough and sausages.



**Figure 4.** Water and oil holding capacities recorded for pea (86%), pumpkin seed (60%), almonds (57.5%), chia (42%) and soybean (43%) proteins. <sup>a-b; A-C</sup> Average values with different letters are significantly different ( $p < 0.05$ ).

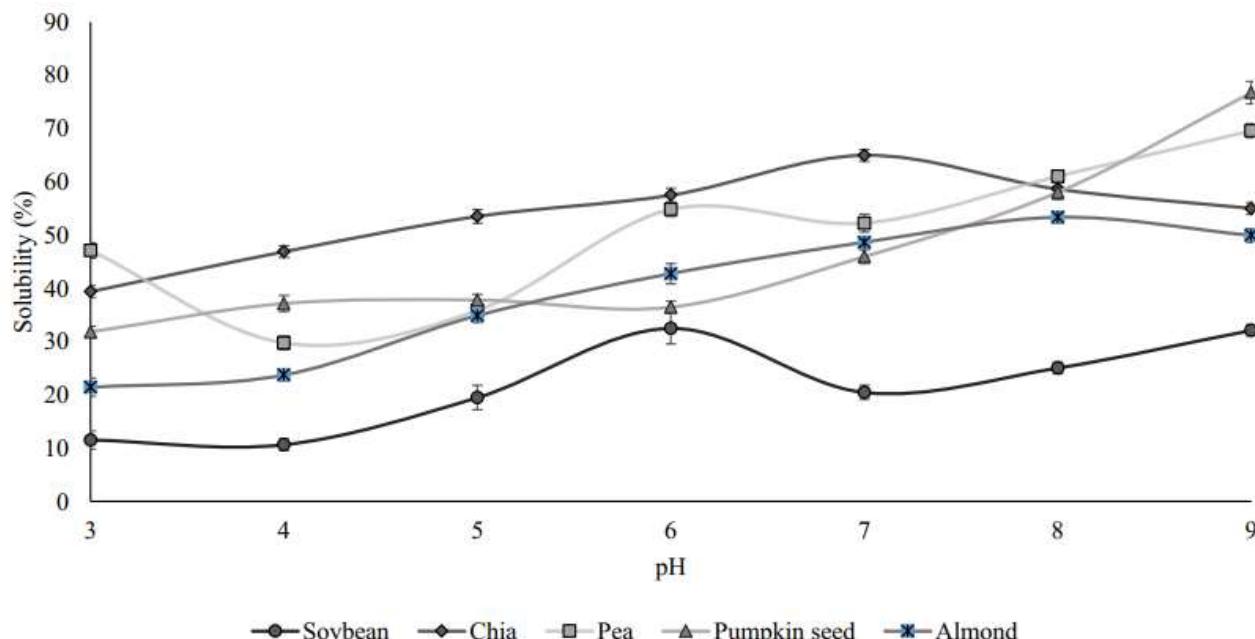
According to Day et al. (2022), proteins' intrinsic hydrophobicity also affects OHC; hydrophobic amino acid proteins can interact with apolar lipids due to van der Waals forces. The tertiary structure determining surface hydrophobicity relates the inner distribution of hydrophobic amino acids to the exterior of the folded peptide chain. Kaur and Singh (2005) observed that WHC increases in the presence of hydrophilic compounds such as polysaccharides. These factors likely acted directly in protein results. Soybean and pea proteins presented a more hydrophobic

profile, and chia protein hydrophilic profile was more remarkable than that of the other proteins.

Figure 5 shows plant-proteins' water solubility. Five proteins recorded lower pH values (ranging from 4 to 6) and better solubility at  $\text{pH} > 6$ . This finding points towards low solubility - close to these proteins' isoelectric point. Data in the literature corroborated these results (Ma et al., 2022; Malomo & He, 2014; Miranda et al., 2022; Chang et al., 2022; Q. Tang et al., 2023). Extraction method also affects this property.

Boye et al. (2010) observed that lentil protein concentrated by precipitation has lower

solubility than lentil protein concentrated by ultrafiltration.



**Figure 5.** Water solubility recorded for pea (86%), pumpkin seed (60%), almonds (57.5%), chia (42%) and soybean (43%) proteins.

Figure 6 shows proteins' gel formation. The almond protein recorded the best gel formation at concentration of 0.06 g/mL, whereas pea and chia proteins formed at 0.14 g/mL, and pumpkin seed protein formed at 0.16 g/mL. No gel formation was observed for soybean protein at these concentrations. This is an essential feature to develop puddings, flans and jellies. With respect to this property, Ma et al. (2022) observed that most vegetable proteins gelled at the range of 10% to 18%, although it is also possible to find lower values, such as in chickpea protein (approximately 5%

to 7%) (Aydemir & Yemencioglu, 2013). However, X. Tang et al. (2021) found 16% gel formation for pea protein (89.2% protein content), and this outcome was similar to that recorded in the current study. However, Fernández-Quintela et al. (1997); Aydemir and Yemencioglu (2013); X. Tang et al. (2021) recorded soybean gelation at 14%, 16% and 10%, at 70%, 82.2% and 92.4% protein content, respectively. Assumingly, the soybean sample in the present study did not gel because its protein content (43%) was lower than that of the other studies.

Replicate	Plant proteins									
	Concentration (g/mL)									
0,02	0,04	0,06	0,08	0,10	0,12	0,14	0,16	0,18	0,20	
Soybean	-	-	-	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	-	
Chia	-	-	-	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	-	
Pea	-	-	-	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	-	
Pumpkin seed	-	-	-	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	-	
Almond	-	-	-	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	-	

**Figure 6.** Gelling capacity recorded for pea (86%), pumpkin seed (60%), almonds (57.5%), chia (42%) and soybean (43%) proteins.

Globular proteins (albumins, globulins, prolamins and glutelins) comprise polypeptide chains and they are the main plant-protein components. Based on Sim et al. (1967), these proteins work as good emulsifying and foaming agents due to the apolar regions on their surfaces, which allow their absorption at the oil-water or air-water interfaces. According to Day et al. (2022), globulin represents 50%-70% of total globular proteins in plant proteins.

According to Nicolai and Chassenieux (2019), globulins form gels when they are heated in aqueous solution. The difference between plant origin source and extraction method determines globulins role in the gel-network formation. Assumingly, globulins played key role in determining the gel-forming properties of almonds, as well as the

foaming and emulsifying properties of the herein assessed pea protein.

Results suggest that each protein can be used to replace animal proteins in plant-based product development. If the recorded values are taken into consideration, the almond protein stands out as the most suited to develop jellies and puddings given its excellent gel formation. Pumpkin seed showed good foam formation for the production of toppings and mousses. The pea protein was most suited to confectionery, sausages and sauces production, since it is an excellent emulsifying and foaming agent. Chia can be used to help improving emulsification and foam stability. Soybean can be used to improve emulsion stability and oil absorption by products.

## Conclusion

The analysis highlighted the possibility of obtaining functional plant protein properties (almond, pumpkin seed, pea, soybean and chia) to assist the development of plant-based products. The pea protein showed the highest potential for food application in comparison to the other analyzed proteins, given its excellent technological properties, among them, excellent oil and water holding, emulsification and foaming capacities.

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