

Technologies for the production of protein isolates from legumes

Tecnologías para la obtención de aislados proteicos a partir de leguminosas

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Abstract: The consumption of legume proteins has increased several years ago due to the high cost of animal protein, which has motivated the search for methods to obtain protein isolates, therefore, the objective of the research was to analyze the different technologies proposed in the literature to obtain protein isolates from legumes. For the development of the research, the SALSA methodology modified by Gunnarsdottir *et al.* (2020)(2020), which consists of 5 steps: search, evaluation, snowball technique, synthesis and analysis. The most widely used technology according to the literature for obtaining protein isolates from legumes is alkaline extraction where, proteins are solubilized at alkaline pH ranging between 8 and 11 to separate them from the rest of non-soluble compounds, followed by isoelectric precipitation by changing pH to ranges ranging between 3 and 5, However, for the application of this technology it is important to consider the composition of the raw material, since if it is rich in lipids, the sample must be degreased in order to increase the yield of protein extraction. Another technology used to obtain protein isolates consists of extracting proteins by solubilization at alkaline pH followed by ultrafiltration using membranes of different molecular weight exclusion limits (10, 50 kDa). Obtaining protein isolates from legumes by the above technologies depends on the process conditions (ratio of the raw material-water solution, pH of protein precipitation or solubilization, extraction temperature, membrane exclusion limits) and composition of the raw material.

Keywords: Isoelectric point, precipitation, solubilization, protein, ultrafiltration, ultrafiltration

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Resumen: El consumo de proteínas de leguminosas se ha incrementado hace varios años debido al elevado costo de la proteína animal, lo que ha motivado la búsqueda de métodos para la obtención aislados proteicos, por ende, el objetivo de la investigación fue analizar las diferentes tecnologías propuestas en la literatura para la obtención de aislados proteicos a partir de leguminosas. Para el desarrollo de la investigación se utilizó la metodología SALSA modificada por Gunnarsdottir *et al.* (2020), misma que consta de 5 pasos que son: búsqueda, evaluación, técnica de la bola de nieve, síntesis y análisis. La tecnología más utilizada según la literatura para la obtención de aislados proteicos a partir de leguminosas es la extracción alcalina donde, las proteínas son solubilizadas a pH alcalino que oscila entre 8 y 11 para separarlas del resto de compuestos no solubles, seguida de la precipitación isoelectrica mediante el cambio de pH a rangos que oscilan entre 3 y 5, siendo el pH 4,5 más utilizado para la precipitación de las proteínas, sin embargo, para la aplicación de esta tecnología es importante considerar la composición de la materia prima, ya que si es rica en lípidos se debe desengrasar la muestra con la finalidad de incrementar el rendimiento de la extracción de proteínas. Otra tecnología utilizada para la obtención de aislados proteicos consiste en extraer las proteínas mediante la solubilización a pH alcalino seguida de la ultrafiltración que utiliza membranas de diferentes límites de exclusión de peso molecular (10, 50 kDa). La obtención de aislados proteicos de leguminosas mediante las tecnologías mencionadas depende de las condiciones de proceso (relación de la solución materia prima-agua, pH de precipitación o solubilización de las proteínas, temperatura de extracción, límites de exclusión de la membrana) y composición de la materia prima.

Palabras clave: Punto isoelectrico, precipitación, solubilización, proteínas, ultrafiltración

Introduction

Legumes are considered one of the main sources of nutrients, especially for low-income populations in developing countries. Among the most consumed by humans are soybean (*Glycinemax*), bean (*Phaseolus vulgaris*), lentil (*Lens culinaris*), broad bean (*Vicia faba*), chickpea (*Cicerarietinum*), pea (*Pisumsativum*) and chocho (*Lupinus mutabilis*), which stand out for their high protein content ranging from 17 to 40 % depending on the spice (Caro, 2015; Jaimes *et al.*, 2012; Tello, 2018). Olmedilla Alonso *et al.* (2010)Caro (2015) and Tello (2018) mention that legumes have a higher protein content than cereals, easily assimilated carbohydrates and, compared to meats, a low lipid content. In addition, they are also characterized by having other nutrients such as dietary fiber, minerals (iron, zinc, calcium), B complex vitamins and in smaller amounts some bioactive compounds such as polyphenols, alpha-galactosides, isoflavones.

However, legumes have negative characteristics since the biological value and bioavailability of nutrients is not as high as in that of animal origin, due to the presence of toxic and anti-nutritional substances such as phytates, trypsin inhibitors, hemagglutinins or lectins, saponins, -alkaloids and cyanogenic glycosides. (Caro, 2015; Gallegos & Polo, 2013; González-Pérez & Arellano, 2009; Olmedilla Alonso *et al.*, 2010).

The most common ways to increase the nutritional value of legume components, especially proteins, are cooking, fermentation and germination, since these treatments reduce or eliminate anti-nutritional substances, thermolabile toxins and oligosaccharides while maintaining the protein and fiber content. Another way to take advantage of the nutrients and especially the nutritional potential of legume proteins is to isolate them from the rest of the antinutritional components found in legumes, eliminating protease inhibitors and increasing protein digestibility (Cantonal *et al.*, 1995). (Cantonal *et al.*, 1995; Olmedilla Alonso *et al.*, 2010).. The consumption of soy-based beverages has increased due to the development of processing technologies that improve their organoleptic properties based on their protein content, which is essential for the human diet (Enriquez Estrella *et al.*, 2022).

The consumption of legume proteins has increased several years ago due to the high cost of animal protein, which has motivated the search for methods to obtain protein concentrates and isolates by separating the rest of the legume components (Cantonal *et al.*, 1995). (Cantonal *et al.*, 1995).. Furthermore, considering that protein provides energy and taking into account the origin of the term protein (from the Greek "proteios" which means "primordial" or "first place")(Gonzalez-Torres *et al.*, 2007) means that it is the nutrient that provides the body with the amino acids necessary for the creation, repair, development and maintenance of cells and tissues during all stages of life, and many proteins perform metabolic functions (act as enzymes, hormones, antibodies). (González-Torres *et al.*, 2007). In order to obtain protein isolates as a source of this nutrient, it is necessary to obtain protein isolates.

Obtaining protein isolates from legumes has advantages compared to obtaining animal protein, since the production of animal protein uses a large amount of water and feed, which increases deforestation and greenhouse gas emissions, while obtaining protein from legumes enriches the soil through nitrogen fixation, is economical, has a water

footprint, has a low greenhouse gas level and constitutes a sustainable source of protein (González-Pérez & Arellano, 2009; Semba *et al.* (González-Pérez & Arellano, 2009; Semba *et al.*, 2021).

The importance of the consumption of protein isolates lies in the nutritional contribution they provide, which is why they are used as food for athletes, as ingredients in the supplementation of raw materials low in protein, in the preparation of different types of foods such as infant formulas, meat products, confectionery, desserts, emulsions and beverages, since proteins provide improvements in the characteristics of products due to the functional properties they possess. (Cantonal *et al.*, 1995; Fonseca, 2019; González-Pérez & Arellano, 2009; Jaimes *et al.*, 2012; Mercado *et al.*, 2015; Ulloa *et al.*, 2012)..

By saying that proteins provide improvements in the characteristics of products due to the functional properties they possess, we mean that when using protein isolates in food production it is feasible to determine the behavior of these during processing, storage or consumption, i.e., these properties and the way in which proteins act with other components directly or indirectly affect their applications, quality and acceptance of foods (Ulloa *et al.*, 2012).. Among the functional properties of proteins are solubility, water-holding capacity, oil absorption capacity, emulsifying capacity, foaming capacity and gelling capacity (Cantonal *et al.*, 1995; Fonseca, 2019; González-Pérez & Arellano, 2009; Jaimes *et al.*, 2012; Mercado *et al.*, 2015; Ulloa *et al.*, 2012)..

The importance of the properties mentioned above vary with the type of product in which the protein is intended to be used, for example, protein isolates with proteins with high water or oil retention capacities are used in meat and bakery or confectionery products, while proteins with high emulsification capacities are suitable in the preparation of salad dressings, sausages, bologna, confectionery and pastries (Ulloa *et al.*, 2012).. Additionally, obtaining protein isolates is the first step in obtaining bioactive peptides that help human beings in the reduction of diseases (Diaz, 2016).

A product may be considered as a protein isolate if it complies with the Codex Alimentarius (2007) This is achieved by reducing or eliminating the main non-protein constituents (water, lipids, carbohydrates, vitamins, minerals, anti-nutritional and toxic substances).

For the elimination of non-protein compounds and in order to obtain protein isolates, there are different technologies such as aqueous extraction, saline extraction and ultrafiltration, the most widely used being alkaline extraction/isoelectric precipitation. However, emerging methods have also been developed with the aim of increasing extraction

yields, increasing their nutritional properties and increasing their technological applications. Among the emerging methods, enzyme-assisted protein extraction, electrostatic protein separation, protein extraction assisted by cell disruption techniques are mentioned (Fonseca, 2019)

In obtaining protein isolates from legumes, the most commonly used method is alkaline extraction and isoelectric precipitation of proteins, where, proteins are solubilized at alkaline pH to separate them from the rest of the non-soluble compounds, for subsequent precipitation by changing pH (Fonseca, 2019; Mercado *et al.*, 2015; Vioque *et al.*, 2001).. Examples of this are: a) the protein isolate with a protein content of 92 % protein on a dry basis obtained by Thompson (1977) by solubilizing bean (*Phaseolus aureus*) proteins at pH 9 and 25 °C for 20 minutes followed by precipitation at pH 4; b) protein isolate from pea (*Pisum sativum*) with 90% protein obtained by (Sumner *et al.*, 1981) by solubilizing the proteins at pH 9 for 20 minutes followed by precipitation at pH 4.5.

Another technology used in obtaining protein isolates from legumes consists of alkaline extraction of proteins and subsequent filtration of the protein solution through membranes with different molecular weight exclusion limits, e.g. Des Marchais *et al.* (2011) used a 50kDa membrane to obtain a protein isolate with 96.1 % protein from peas.

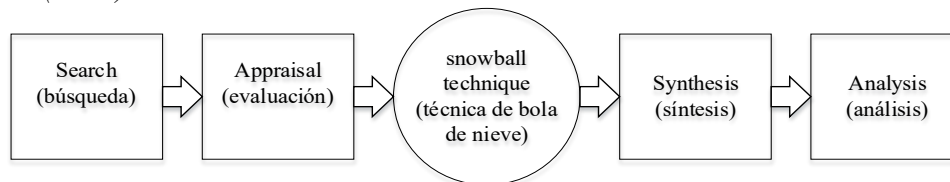
Taking into account the above mentioned, the variations in the parameters for obtaining protein isolates, the importance and usefulness of protein isolates, the objective of this research was to analyze the different technologies proposed in the literature for obtaining protein isolates from leguminous plants.

Materials and methods

The research carried out was specifically a literature review and the SALSA (Search, Appraisal, Synthesis, Analysis) methodology modified by Gunnarsdottir *et al.* (2020). The traditional SALSA method for systematic reviews involves four steps: search, appraisal, synthesis and analysis; however, Gunnarsdottir *et al.* added an additional step known as the snowball technique as shown in Figure 1 and 2.

Figure 1.

General diagram of the SALSA method as modified by Gunnarsdottir et al. (2020)

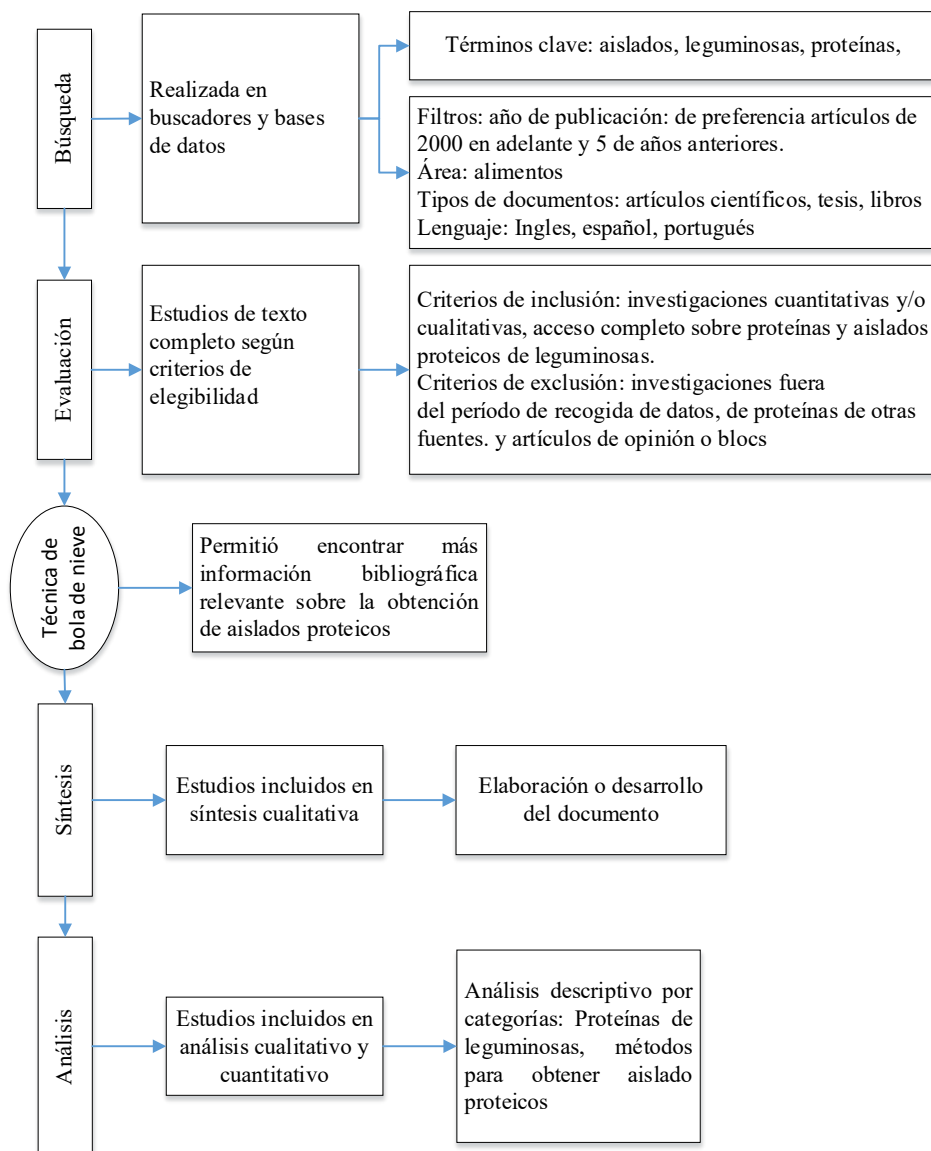


The SALSA method consists of a comprehensive search process and critical review that allows papers to be produced using the best of the available information while minimizing the potential for bias (Gunnarsdottir *et al.*, 2020; Luengo *et al.*, 2016).. The modified SALSA method used for the elaboration of this research is described below and can be visualized schematically in Figure 2.

The first step of the SALSA method consisted of searching for relevant information on legume proteins and protein isolates in undergraduate and graduate theses, scientific articles and books found in search engines and databases such as Google Scholar, Web of Science, Science, Direct, Scopus, Pubmed, Scielo. The information searched was found in English, Spanish and Portuguese, and corresponds to the years 2000 onwards, except for five documents from earlier dates.

The second step allowed further evaluation of whether the search results met the criteria for inclusion (quantitative and/or qualitative research, complete access to protein information, methods of obtaining protein isolates from legumes) and exclusion (research outside the data collection period, methods of obtaining protein from sources other than legumes, opinion articles or blogs). This point allowed the evaluation and classification of the literature used, and also served as a basis for continuing with step three.

Figure 2.
Flowchart of the modified SALSA method used for the elaboration of the article.



Source: Gunnarsdottir *et al.* (2020) with modifications for the elaboration of the work

The third step, the snowballing technique consists of using references and citations of articles to identify more relevant literature, i.e., the

review literatures found through the initial search served as the basis for snowballing to find 15 additional bibliographic investigations.

The fourth stage consisted of the synthesis or elaboration of the document based on the relevant information from the literature selected under the criteria mentioned above. For this purpose, the publications identified and evaluated in the previous stages were carefully read in order to relate the relevant information in the written document.

Finally, the synthesized information was analyzed to fulfill the objective of the research. For this purpose, unit operations, processes and raw materials involved in obtaining protein isolates from legumes were analyzed for the development of the results and discussion of the current work.

3. Result

Legumes have a higher protein content than cereals (Olmedilla Alonso et al., 2010). (Olmedilla Alonso *et al.*, 2010).which makes them the ideal raw material for obtaining protein isolates. Among the technologies for obtaining protein isolates, two types of technology stand out: a) obtaining protein isolates by alkaline extraction and subsequent isoelectric precipitation and b) obtaining protein isolates by alkaline extraction and subsequent filtration of the protein solution.

This technology consists of solubilizing the proteins at alkaline or basic pH followed by isoelectric precipitation under acid pH conditions. Tables 1, 2, 3, 4 and 5 show the aforementioned technology for obtaining protein isolates.

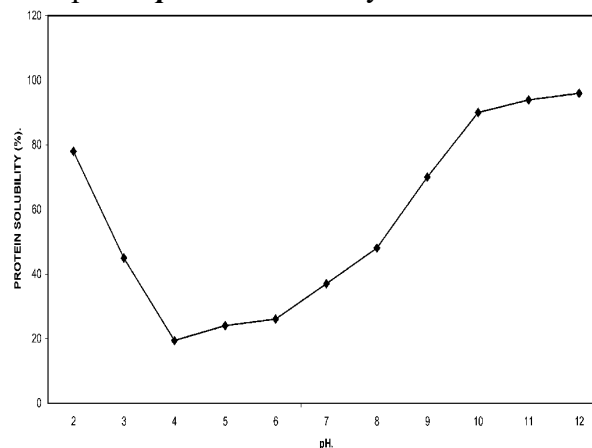
The first step, prior to protein solubilization, is to reduce the particle size of the grain (González-Pérez & Arellano, 2009). (Gonzalez-Perez & Arellano, 2009)in order to increase the contact area or surface area of the particles with the extracting medium or solution (solution at alkaline pH), which would improve the yield of the extraction process of the protein present in the food matrix. According to the research, tables 1, 2, 3, 4 and 5 show that in order to obtain protein isolates, the researchers start from flour, or in turn, the grains are reduced to flour.

Another point to consider before protein extraction is the lipid content of the legumes, since high lipid contents interfere during the process. Furthermore, this component can cause changes in the final product because it can be associated with the proteins of the isolates, giving rise to rancidity problems during processing and storage (Vioque et al.,

2001). (Vioque *et al.*, 2001) Therefore, it is advisable to defat the raw materials or to start protein extraction from defatted flour. This is especially ratified in Table 2, due to the high lipid content of soybean, which is 19.94% (Delgado-Andrade *et al.*, 2001). (Delgado-Andrade *et al.*, 2016) additionally in table 3, two authors perform defatting of beans while one does not, due to the fact that the fat content of beans is between 1.2 to 1.5 %. However, in Table 4, three out of four authors defat the chocho flour because it has a lipid content of 9.74%, a content that according to the results of the research affects the amount of protein in the final product (55.58%) since it is lower than the other treatments.

In the technologies reviewed in tables 1 to 5, the pH for protein extraction or solubilization ranges from 8 to 11, while the most commonly used pH for protein precipitation is 4.5. This is justified by the research carried out by Adebawale & Lawal (2003) and Sánchez-Vioque *et al.*, (1999)(1999), who mention that the solubility profile of the protein present in the protein concentrate or isolate is pH dependent (Figure 3), i.e., the lowest protein solubility is found in the pH range of 4 to 5, however, while protein solubility increases as the pH increases, which is why a pH close to 9 is used for the extraction of proteins from the food matrix. (Vioque *et al.*, 2001) some proteins, such as glutelins, require pH equal to or higher than 11 to be extracted.

Figure 3. Effect of pH on *protein* solubility.



Source: (Adebawale & Lawal, 2003)

Table 1 presents the steps for obtaining protein isolates from the field bean (*Phaseolus lunatus L*), for this, Tello (2018) and Tamayo (2018) work at pH 8, Chel-Guerrero *et al.* (2002) at pH 11 to solubilize or extract the proteins from the food dye, while to precipitate the proteins Tello (2018) and Tamayo (2018) work at pH 3, 4, 5 and Chel-Guerrero *et al.* (2002) at pH 4.5. From these investigations Tamayo and Tello obtain the highest amount of protein in the final product when working at an isoelectric precipitation pH of 5, agreeing that the optimum pH for protein precipitation is between 4 and 5.

Table 1. Technologies for the production of protein isolates from field beans (*Phaseolus lunatus L*) by alkaline extraction and isoelectric precipitation.

Operations	Author		
	Tello (2018)	Tamayo (2018)	Chel-Guerrero <i>et al.</i> (2002)
Suspension	5g of fava bean flour in 50 ml of distilled water	The flour was suspended in water in a 1:10 (w/v) ratio.	Flour/water was mixed in a 1:6 w/v ratio.
Protein solubilization	Adjust pH to 8 with NaOH 1 M with agitation for maintaining the pH.	Adjusting pH 8 with NaOH 2 M for one hour and constant agitation between 800-900 rpm.	Adjusted to pH 11 with NaOH 1 N and soaked for 1 h.
Separation of soluble proteins (liquid or supernatant phase)	By centrifugation for 30 minutes at 4400 rpm and room temperature. The precipitate is discarded and the supernatant is continued with the supernatant.	By centrifugation at 4400 rpm for 30 min. The precipitate is discarded and the supernatant is continued with the supernatant.	The suspension is ground and filtered to separate the solid fraction from the liquid fraction containing protein and starch. The residual solids are washed 5 times with distilled water and passed

			through a 150 mesh sieve; the wash water is then mixed with the initial supernatants. It is allowed to settle for 30 min to recover the starch and separate the solubilized protein.
Precipitation of proteins from the supernatant	pH adjustment to 3, 4, 5 and 6 with 1M HCl	Adjustment of pH to 3, 4, 5 and 6 with 2M HCl. Shake for 5 minutes	The pH was adjusted with 1 N HCl to the isoelectric point 4.5.
Rest	For 24 h	At 4° C for 24 h	
Protein separation	Remove supernatant and use the precipitate.	Remove supernatant and use the precipitate.	By centrifugation at 1317 g for 12 min. Remove supernatant and use the precipitate.
Drying/ Freeze-drying	At a pressure of 0.2 Pa and a temperature of -57°C	At -50 °C and 0.2 Pa pressure	At -47 °C and 13×10^{-3} mbar.
Storage	In sterile flasks at -80 °C	In sterile flasks at -80 °C	
Extraction yield	pH 3 8.64a ± 0.10%. pH 4 18.56 ± 1.14 %	pH 3 19.55a ± 1.55 %. pH 4 10.56b ± 0.83% pH 4 10.56b ± 0.83% pH 4	

	18.56 ± 1.14 %.	pH 5 19.56a ± 1.55%.	
	pH 5 12.86b ± 0.16%.	pH 6 27.59c ± 0.24%.	
	pH 6 32.58d ± 0.30%.		
Quantification of protein content	Biuret's method	Biuret's method	71.13 ± 0.92%
	pH 3 69.96 c ± 0.78% c ± 0.78%	pH 3 52.96a ± 0.031% pH 3 52.96a ± 0.031%	
	pH 3 69.96 c ± 0.78% pH 3	52.96a ± 0.031	
	pH 4 62.39 b ± 0.35% pH 4	pH 4 57.85b ± 0.013% ± 0.013%.	
	62.39 b ± 0.35% pH 4	pH 5 62.53c ± 0.018% ± 0.018%.	
	pH 5 71.33 c ± 0.92%.	pH 6 56.74b ± 0.026% ± 0.026%.	
	pH 6 56.80 at ± 1.77%.		
	Kjeldahl method.		
	pH 3 41.19 c ± 1.10% pH 3		
	41.19 c ± 1.10% pH 3		
	41.19 c ± 1.10% pH 3		
	pH 4 38.92 b ± 0.14% pH 4		
	38.92 b ± 0.14% pH 4		
	38.92 b ± 0.14% pH 4		
	pH 5 42.31 c ± 0.58% c ± 0.58%		
	42.31 c ± 0.58% pH 5		

pH 6.34.36 at
± 0.44%.

a, b, c, d. Indicates that there are significant differences between treatments.

Table 2. *Technologies for obtaining protein isolates from soybean (Glycine max)*

Operations	Author		
	Avila (2011)	L'HOCINE et al. (2006)	Puppo et al. (1995)
Flour production	Defatted soybean meal is milled (particle size 45 µm).	Flour defatting with hexane	Yes Part defatted soybean meal
Suspension	Flour/water mixture in a 1:6 w/v ratio with 10 min agitation.	Flour/water mixture in a 1:15 w/v ratio at 55°C.	Flour/water mixture in a 1:10 w/v ratio with 10 min agitation.
Protein solubilization	At pH 9.5 adjusted with NaOH 6N and stirring for 30 min.	The pH was adjusted and maintained at 9.0 with NaOH 2 N with stirring for 45 min at 55°C. °C	At pH 8 adjusted with NaOH 2N and stirring for 2 h at room temperature
Separation of soluble proteins (liquid or supernatant phase)	By centrifugation at 12000 rpm. The precipitate is discarded and the supernatant is continued with the supernatant.	The suspension rests for 15 min at room temperature, then centrifuged at 4 °C for 30 min at 14300g. The supernatant is continued with the supernatant	By centrifugation at 13300g for 20 min at 15°C. Work with the supernatant

Precipitation of proteins from the supernatant Rest	pH adjustment to 4.5 with HCl 6N	Adjust pH to 4.5 with 2 N HCl and stirring for 45 min at 25 °C.	At pH 4.5 adjusted with HCl 2 N
Protein separation	By centrifugation at 12000 rpm. Remove supernatant and use the precipitate.	By centrifugation at 2830 × g (4 °C) for 15 min.	By centrifugation at 3300 for 20 min.
Washing		The precipitate is washed twice with water and centrifuged each time at 2830 × g for 10 min.	
Neutralization		With NaOH 2N up to pH 7	
Storage	Freezing		
Drying/ Freeze-drying	At a pressure of 0.2 Pa and a temperature of -57°C	Freeze-dried	Lyophilized
Storage		AT 4°C	
Extraction yield	24.55%	Recovered protein: 51%.	
Quantification of protein content	85.45%	92.8 ± 0.08% on dry weight basis	82.4 ± 0.4

Table 3. Technologies for obtaining protein isolates from *Phaseolus vulgaris* (bean)

Operations	Author		
		Rui <i>et al.</i> (2011)	Ahmed <i>et al.</i> (2015)

Soaking		1 kg of sample in 4 L of water and kept at 4 °C for 12 h. Subsequently, the shells are removed.	
Particle size reduction	By milling	With blender	3 kg of fresh grain is mixed with 30 L of water and ground.
Degreasing	With hexane (1:3, w/v)		
Suspension	Ratio of flour to distilled water (1:15)	The suspension obtained was diluted ten times (v/v)	
Protein solubilization	At pH: 9. With agitation for 1 h at 55°C	With pH to 10 using NaOH 0.5 M. Mixing for 1 h.	Adjustment of pH to 8.0 with 0.2 M NaOH. Extraction is carried out at 35 °C by stirring.
Separation of the soluble phase or supernatant (soluble proteins)	Centrifugation at 11 000 × g at 20 °C for 30 min. Discard the precipitate and continue with the supernatant.	Filtered through a 75 µm mesh sieve and centrifuged at 3000 ×g for 30 min at 10 °C.	By centrifugation at 5000 × g for 15 min at 25 °C. The supernatant is continued with the supernatant once filtered.
Rest	At 4 °C for about 15 h to sediment the starch and then		

	centrifuged as above.		
Precipitation of proteins from the supernatant	Adjusting pH 4.5 with HCl 1M	Adjusted to pH 4.5 with 0.1 N HCl.	The pH is adjusted to 4.3 with dilute HCl.
Protein separation	By centrifugation at 11 000 × g at 20 °C for 30 min.	By centrifugation at 8000 ×g for 10 min at 5 °C	By centrifugation at 10000 × g for 20 min (repeat protein solubilization).
Washing	Twice more with Millipore water at 1:5 (w/w).	Wash with distilled water	
Neutralization	With 10% NaOH solution (pH 6.5-7). Then centrifuged at 4000 rpm for 5 min	Adjusting to pH 7	
Drying	Lyophilized	Freeze-drying	Freeze-drying
Extraction yield			36.15% hard bean 45.37% fresh beans
Quantification of protein content	89,25 % 83,96 %	Range of 76.96-83.96%.	71.9% hard bean 75.6% fresh beans

Table 4. *Technologies for obtaining protein isolates from chocho (Lupinus mutabilis)*

	Author			
Operations	(Guerra & Pozo, 2018)	(Aguinda, 2019)	(Acuña & Caiza Jimena, 2010)	(Urrutia Gutiérrez, 2010).

Flour preparation	The hydrated grain is peeled, dried and ground.	Grain milling	The chocho beans are ground	The grains are soaked (1 day), cooked (1h), washed (5 days), dried and ground.
Degreasing	By means of an extraction system continuous (Soxhlet) with hexane	With hexane with isolation for 18 h at TA		With 95% ethanol in a flour: solution ratio of 1:3. Three extractions during 5 hours each
Suspension		Dissolves in water in a 1:10 (w/v) ratio.	Flour to distilled water ratio (1:7, 1:9,1:11)	At 43°C with a flour: solvent ratio (1:20).
Protein solubilization	At pH 6.8-10. Adjusted with 10 % NaOH	At pH 8 adjusted with NaOH 2 M. It is left in agitation for 1 h	pH (8.5, 9.5, 10.5) adjusted with NaOH 1N, stirred for 30 min.	At pH 9.3, at 43°C
Separation of soluble proteins (liquid or supernatant phase)	By centrifugation at 12000 rpm. The precipitate is discarded and the supernatant is continued with the supernatant.	By centrifugation for 45 min and 4400 rpm. The precipitate is discarded and the supernatant is continued with the supernatant.	By centrifugation at 8000 rpm. The precipitate is discarded and the supernatant is continued with the supernatant.	By centrifugation at 4000 rpm for 20 min.
Precipitation of proteins from the supernatant	Adjusting pH 4.5 with 10 % HCl solution	Adjust pH 3, 4, 5, 6 with 2M HCl, shake for 5 minutes,	pH to 4.5 with H Cl 2N, stirred for 15min.	At pH 4.5 and stirring for 15 min.

		then stir for 5 minutes.		
Protein separation	By centrifugation at 4000 rpm for 5 min.	In refrigeration (4 °C) for 24 hours. Then remove the supernatant and obtain the protein precipitate.	By centrifugation at 12000rpm	Centrifugation 4000 rpm for 20 min.
Washing				2 times at a rate of of 1:5 (protein/water). Elimination of wash water by centrifugation at 4000 rpm for 15 min.

Table 4 (continued). Technologies for obtaining protein isolates from chocho (*Lupinus mutabilis*)

Neutralization	With 10% NaOH solution (pH 6.5-7). Then centrifuged at 4000 rpm for 5 min			
Drying/ Freeze-drying	Drying for 24h in an oven at 85 °C.	Lyophilized at pressure: < 0.2 atm; temperature < -55 °C) and stored at 4 °C	Freeze-dried	Drying
Extraction yield		pH 3.18.5± 0.62%.	At pH 10.5	tit

			pH4 28.6± 0.69%.	records 72.2%	
			pH5 31.4± 0.32%.	protein recover	
			pH6 24.7± 0.66%.	y and 42.6%	
				yield by weight.	
Quantificatio n of protein content	67,25 5,75%	±	pH3 76.8± 1.44%.	55,58%	92,83±0,18 %
			pH4 95.0± 3.17%.		
			pH5 96.9± 0.51%.		
			pH6 95.6± 2.82%.		

In addition to considering the solubilization pH of proteins, it is important to take into account that the use of extreme pH (higher than 9 and lower than 4) negatively affects the characteristics of the protein and can even cause its hydrolysis or denaturation, racemization of amino acids and therefore loss of essential amino acids such as cysteine and lysine, which would cause a reduction in protein digestibility, in addition to producing the precipitation of non-protein components that can affect the purity of the isolate (Callisaya & Alvarado, 2009; González-Pérez & Arellano, 2009). (Callisaya & Alvarado, 2009; González-Pérez & Arellano, 2009).. Therefore, for most vegetable sources, the pH values for isolating proteins should be between 4 and 9 as this should not be harmful to the proteins (González-Pérez & Arellano, 2009).

Additionally, according to what is observed in tables 1, 2, 3, 4, and 5, the process to isolate proteins from legumes is based on the same principle which is alkaline extraction and isoelectric precipitation, however, there are small variations proposed by the authors, among which is the use of centrifugation to separate the soluble part from the solid part, while others do this process by natural precipitation, that is, they wait for a time of 12 to 14 hours. Therefore, centrifugation is used to make the process faster and more efficient.

Table 5. *Technologies for obtaining protein isolates from other leguminous plants*

Operations	Author/legume		
	(Villafuerte et al., 2019)/ chachafruit (Erythrina edulis Triana)	(Sanchez-Vioque et al., 1999)/ chickpea (<i>Cicer arietinum</i>)	(Atiencia Pazmiño, 2021)/ Pea (<i>Pisum Sativum</i>)
Flour production	The grains were cut and dehydrated at 45 °C for 24 h, and then grind them		
Suspension	The flour was dispersed in distilled water (1:10 w/v).	20 g of flour was suspended in 200 ml of (a) 0.2% NaOH solution pH 12 b) 0.25% Na ₂ SO ₃ solution at pH 10.5	10 g of flour in 100 mL of distilled water (ratio 1:10)
Protein solubilization	At pH adjusted to 11.0 with 0.1 M NaOH and kept under stirring for 1 hour.	At pH 12 or 10.5 with stirring for 1h	At 8.0 with Na(OH) 1N, and stirred for 60 minutes by means of a magnetic stirrer.
Separation of the soluble phase or supernatant (soluble proteins)	By centrifugation at 2,500 rpm for 20 minutes. The supernatant is used	By centrifugation at 8000 g. Two additional extractions are made	By centrifugation for 20 minutes at 8000 rpm. Insoluble residues are suspended with distilled water at a 1:5 ratio, then pH is adjusted

			with 1N sodium hydroxide to 10.0 by mechanical shaking for 10 minutes and centrifuged.
Precipitation of proteins from the supernatant	Adjusting to pH 4 and stirring for 1 hour	At pH 4.3	At pH 4 with agitation for 20 minutes
Protein separation	By centrifugation at 2,500 rpm for 30 minutes	By centrifugation at 8000 g	By centrifugation at 12000 rpm
Washing		a) With distilled water adjusted to pH 4.3 b) with 100 ml of distilled water adjusted to pH 4.3, ethanol and acetone.	
Drying	Freeze-drying	a) Lyophilization b) Ambient temperature	
Extraction yield		Final protein recovered from chickpea flour was 65.9 and 62.1%.	11%
Quantification of protein content	96.01 % on dry basis	80.9% at pH 12 87.1% at pH 10.5	79,14%

Most researchers use freeze-drying instead of conventional drying as the operation to remove water from proteins. This is because freeze-drying works at low temperatures, which causes sensory alterations (color, shape, size, flavor, texture) and nutritional losses of the dehydrated products to be significantly reduced (García-Mora et al., 2019; Peña & Parra, 2015). The yield in the processes of obtaining proteins from raw material is less than 50% due to the fact that legumes have a series of components such as lipids, carbohydrates, among others, however, when talking about the percentage of protein recovery, this does not exceed 80%. This is due to the fact that in a matrix there are different types of proteins that have different pH solubilization (close to neutrality for example) or precipitation, but it is preferred to extract proteins at alkaline pH to favor the solubilization of proteins denatured during the preparation of the concentrates. (Vioque et al., 2001).

The technologies mentioned in Tables 1, 2, 3, 4 and 5 are applied to obtain protein isolates from various types of legumes, e.g., Li *et al.* (2010) applied the technology of alkaline extraction and isoelectric precipitation to obtain protein isolates from 16 varieties of mung bean (*Vigna radiata*). For this, they prepared five percent (w/v) solutions of the mung bean flour suspension, adjusted to pH 9 at room temperature. The solutions were mixed for 1 h and subsequently centrifuged for 15 min at 2000 - g. To obtain higher yields, the extraction and centrifugation procedures were repeated once on the residue. The extracts were combined and the pH adjusted to 4.5 to precipitate the protein. Proteins were recovered by centrifugation at 2000 - g for 15 min followed by removal of the supernatant. This procedure allowed them to obtain products with protein contents ranging from 69.22 to 74.84%.

Kaur & Singh, (2007) obtained protein isolates from chickpea (*Cicer arietinum L.*) cultures by working under the same pH conditions as Li *et al.* (2010). The protein content of the product obtained ranged from 89.9% to 94.4%. Many researches mention obtaining protein isolates, however, we must clarify that according to Codex Alimentarius (2007) products can be classified according to protein content as follows:

- a) Protein content greater than 50 and less than 65 percent: protein meal
- b) Protein content greater than 65 and less than 90 percent: protein concentrate

c) Protein content greater than 90 percent: protein isolate

That is, according to the Codex Alimentarius, the use of the alkaline extraction method and isoelectric precipitation of proteins allows obtaining not only protein isolates but also concentrates, according to the protein content reported in tables 1, 2, 3, 4 and 5.

Obtaining protein isolates by alkaline extraction and subsequent filtration.

This technology consists of extracting the proteins by solubilization at alkaline pH followed by filtration of the solution containing the proteins using filters or membranes that allow non-protein substances to pass through. The following are examples of the technology used to obtain isolates:

Des Marchais *et al*, (2011) to obtain a protein isolate proceeded as follows: they first mixed pea flour with water at room temperature in a 1:15 w/w ratio, then adjusted to pH 7.5 which allowed the proteins to be extracted from the legume matrix. Following this, they discarded the precipitate and proceeded with dissolution to ultrafiltration/diafiltration (UF/DF) operation using 50 kDa hollow fiber membranes. The product is lyophilized and after analysis they determined a protein content of $96.1 \pm 0.2\%$ on a dry basis.

Boye *et al*, (2010) conducted a comparative investigation of two methods for obtaining protein isolates, these methods are ultrafiltration (UF) and isoelectric precipitation (IEP). The first stage for the two technologies is the same, i.e. protein solubilization was performed under the following extraction conditions: pH 9.5 with solid/liquid ratio 1/15 at 35 °C for yellow pea, desi chickpea and kabuli and pH 9.0 with solid/liquid ratio 1/10 at 25 °C for red and green lentils. Subsequently, to obtain protein isolates by IEP the pH was adjusted to 4.5, while to obtain isolates by UF/DF they used a 50 kDa membrane with diafiltration (4X) at pH 6.0. The results obtained showed that the technology using UF/DF allows obtaining products with higher protein content as shown in Table 6.

Protein content of isoelectrically precipitated (IEP) and ultrafiltrated (UF) legume protein extracts.

Sample	Protein	
	IEP	UF
yellow pea	81.7 ± 0.3	83.9 ± 0.15
desi chickpea	73.6 ± 0.1	76.5 ± 0.05
kabuli chickpea	63.9 ± 1.3	68.5 ± 0.15
red lentils	78.2 ± 0.2	82.7 ± 0.20
green lentils	79.1 ± 0.3	88.6 ± 0.05

Source: Boye *et al.*, (2010)

The results by Boye *et al.*, (2010) confirm the studies carried out by Fuhrmeister & Meuser (2003) as they found that rough pea concentrates prepared by ultrafiltration had a higher protein content (70-80 %) than concentrates obtained by isoelectric precipitation (68%). Chew *et al.*,(2003) also obtained similar results when comparing the two technologies since by IEP (working at solubilization pH 8-9 and precipitation pH 4.5) they obtained a lupin protein concentrate with a protein concentration of 671 g/kg, while by UF using a 10kD membrane the protein content of the final product was higher (751 g/kg).

The bibliographic research carried out shows that the technology used to obtain the highest protein content in the final product is UF/DF; however, it also shows that the most widely used method for obtaining protein isolate is isoelectric precipitation, information that is confirmed by González-Pérez & Arellano, (2009)

4. Conclusions

According to bibliographic research, legumes have a higher protein content than cereals, and therefore represent an important source for obtaining protein isolates. However, in order to obtain protein isolates from legumes, it is important to take into account the composition of the raw material used, especially if it has a high lipid content, such as soybean or chocho. This considering that it must be defatted to obtain better results in the protein content of the final product,

The most commonly used technology for obtaining protein isolates from legumes is alkaline extraction and isoelectric precipitation of

proteins; however, to achieve the best protein extraction yield, the particle size and lipids must first be reduced, and process conditions such as raw material-water solution ratio, pH of precipitation or solubilization of proteins and extraction temperature must be taken into account.

The solubilization of the proteins should not be carried out at very high pH, while the pH of the precipitation should not be very acid because it can produce the denaturation of the proteins, therefore for the solubilization it is advisable to use a pH of maximum 10 while for the precipitation the recommended pH is 4.5.

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