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Enhancing protein extraction from soybean expeller: Exploring the impact of precipitating agents and flour-to-water ratios on functional properties

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ABSTRACT

This study investigates sustainable methods for producing protein from soybean expeller via pH-shifting processes, aiming to reduce water usage in alkaline extraction by adjusting solid-to-liquid ratios per cycle and employing isoelectric precipitants like lactic acid and lactic acid bacteria (*Lactiplantibacillus plantarum and Lactococcus Lactis*) to enhance functional and antioxidant properties over a wide pH range. Results indicate that the most efficient approach involves three 1:10 (w/v) extraction cycles with lactic acid bacteria as precipitants, demonstrating high productivity and low specific water consumption. Protein content and

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recovery yield showed no significant differences compared to alternatives with higher water consumption or less eco-friendly precipitants. Despite lower solubility, protein products precipitated with lactic acid bacteria formed stable emulsions, exhibiting superior free radical scavenging activity.

KEYWORDS

soybean expeller, pH-shifting process, protein extraction yield, water consumption, precipitating agents, lactic acid bacteria

INTRODUCTION

Soybean (*Glycine max*) is a profitable and suitable option to animal-derived protein sources (Ghumman et al., 2016). Particularly, by-products generated during the solvent oil extraction process, referred to as defatted soybean meals, constitute essential raw materials to produce highly soluble protein components, including soybean protein concentrates (SPC), soybean protein isolates (SPI), and textured soybean proteins (TSP) (Accoroni et al., 2019). On the other hand, the mechanical extraction of soybean oil yields another by-product, namely soybean expeller (EE). This partially defatted by-product, obtained through the extruding-expelling process, exhibits a fat content of 4.5–9%, protein content of 30–42%, and notably enhanced digestibility attributed to alterations in protein structures facilitated by extrusion temperatures (Ghumman et al., 2016).

In Argentina, the production of soybean expeller has exhibited almost uninterrupted growth since 2004, according to data provided by the Ministry of Agriculture, Livestock, and Fisheries from Argentina (Calzada and Ferrari, 2021). After experiencing a high average annual growth rate, expeller production reached 936,000 tons processed in 2020. In light of these trends, interest has surged in enhancing the value of Argentinean agricultural supply chains, with a specific focus on advancing the social and economic aspects within the agricultural sector.

Numerous studies have reported significant advancements in the protein extraction process from soybean expeller using alkaline extraction and isoelectric precipitation of solubilized proteins, a technique referred to as pH-shifting (Brasil et al., 2016; Das et al., 2022; Jiang et al., 2009; Zhao et al., 2023), which is a well-established, relatively inexpensive, and efficient method for attaining a high yield of protein. Other approaches have also been explored to improve protein functionality or to achieve more water-efficient processes, such as membrane separation and alternative precipitation methods using salts or selective solvents at the isoelectric point (Preece et al., 2017; Zhao et al., 2023). Nevertheless, these techniques required larger investment costs (Kim et al., 2015). The pH-shifting method implies extracting and solubilizing proteins in a pH interval from 8 to 11, and acidifying to reach the isoelectric pH, causing around 90% of globular proteins to become insoluble (Nishinari et al., 2018). Different alkalinization pH values in the extraction stage have been tested, since high pH values may improve the protein recovery performance (Vioque et al., 2001). However, a notable drawback of the pH-shifting process is its substantial water consumption during the extraction phase, leading to the generation of substantial volumes of wastewater. This aspect requires careful consideration due to environmental and economic concerns (Cheng et al., 2018; Hadnadjev et al., 2017).

Moreover, incorporating a precipitant agent generally recognized as safe (GRAS) for food applications (GRAS) during the precipitation phase requires thorough testing to ensure its seamless integration into industrial processes. Then, lactic acid could serve as a viable option, given its GRAS classification, and considering that it is a biotechnologically significant compound extensively employed in the food industry for its roles as an acidulant, pH regulator, and preservative (Ojo et al., 2023). The usage of fermentation has also been reported as a means of regulating the pH during the protein recovery process, particularly using GRAS lactic acid bacteria (LAB). This type of microorganisms has the potential to enhance the nutritional quality, prolong shelf life, and optimize gel product manufacturing in soybean processing (Cheng et al., 2018; Hadnadjev et al., 2017). Lactic acid bacteria have been assessed for their potential in reducing soybean allergens attributed to its protein composition (Liu et al., 2021; Meinlschmidt et al., 2016). Aguirre et al. (2008) explored the utilization of LAB suspensions in producing soybean hydrolysates from defatted soybean meal, noting shifts in HPLC profiles with certain peaks diminishing in intensity while new peaks emerged, although total protein content was not reported. Conversely, the precipitation step in the protein recovery from peas was also investigated by Emkani et al. (2021), where the pH was reduced solely through lactic fermentation with *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium lactis* at 37 °C. This approach increased the albumin fraction by 20–30%, thereby enhancing the pea protein solubility, possibly linked to the proteolytic activity of the bacteria. Therefore, these precipitants can constitute suitable substitutes for hydrochloric acid, are considered to be more environmentally friendly, are derived from natural sources, and do not produce hazardous by-products (Alhamad et al., 2020). Lactic acid fermentation might additionally be employed to enhance the organoleptic properties of legume proteins (i.e., product taste and texture), by reducing off-flavors and improving the solubility of proteins (Liu et al., 2023; Yang et al., 2020).

Within this framework, the aim of this investigation is to evaluate different approaches to produce a protein product from soybean expeller. The primary focus involves decreasing the water usage during the extraction phase of the pH-shifting process by adjusting the solid-to-liquid ratio per cycle, and incorporating a GRAS precipitant agent (such as lactic acid or lactic acid bacteria) in the precipitation stage to also enhance the techno functional attributes of the obtained protein products. For this purpose, a design of experiments was adopted which included 12 experimental runs in duplicate, given by the adoption of four different combinations of solid-to-liquid ratio (1:10 or 1:20 w/v) in each of the 3 cycles of the alkaline extraction stage, where the pH value was adjusted to 8.5, and the usage of three precipitant agents, hydrochloric acid (HCl), lactic acid (LA), or a mixture of two strains of lactic acid bacteria (LAB) in the isoelectric precipitation stage. In addition, 3 experimental runs in duplicate were conducted to evaluate the impact of adjusting the pH value of the alkaline extraction stage to 10, for each of the precipitant agents. The performance of the protein recovery process was evaluated by means of the protein recovery yield, productivity, and specific water consumption. Lastly, the functional and antioxidant properties of the obtained spray dried protein product were determined and analyzed.

MATERIALS AND METHODS

Materials

Soybean expeller was provided by small scale processing plants from Santa Fe province, Argentina. These plants use the expeller pressing process for producing soybean oil and discard

the expeller as a byproduct with little economic value. Samples were kept in airtight bags and maintained at −18 °C until additional processing. Chemicals here used were of analytical quality (Ciccarelli, Argentina). Commercial freeze-dried lactic acid bacteria starters (a mixture of *Lactiplantibacillus plantarum* CH6072, and *Lactococcus Lactis* SR3.54) (CHR Hansen, Denmark) were used.

Methods

Each experimental run followed the processing steps shown in Fig. 1.

Soybean expeller processing and characterization. Expeller pellets were ground at room temperature using a laboratory mill (Bühler, Germany), and sieved through ASTM-standard sieves to achieve a particle size that passed through a 25-mesh (710 µm) sieve and was retained by a 100-mesh (150 µm) sieve.

The nitrogen content of the expeller was assessed through AOAC method 2001.11 (AOAC, 2005), and subsequently reported as % db (i.e. on a dry basis or a moisture-free basis). Solubility of protein in KOH was evaluated according to the methodology described by Araba and Dale (1990). Moisture content was assessed with AOAC method 925.10 (AOAC, 2005), and expressed as % wb (i.e. on a wet basis).

Alkaline extraction. The alkaline extraction process was carried out using a batch-type extractor equipped with a 6-blade impeller (Precylec, Argentina), and involved three 15-min extraction cycles using water as the solvent. The pH was set to either 8.5 or 10 using 1 N NaOH, and the temperature was kept constant at 60 °C. Various solid-to-liquid ratios (w/v), denoted as E1, E2, E3, and E4, and outlined in Fig. 2, were used in each extraction cycle to explore the potential for minimizing water usage in the protein recovery process. After each cycle, the expeller was separated from the protein solution, and fresh water at 60 °C was added at the beginning of the second and third cycles to adjust the solid-to-liquid ratio to the required value. The three protein solutions obtained from each extraction cycle were combined into a liquid pool and transferred to a beaker for subsequent precipitation.

Isoelectric precipitation. In the first two alternatives for the isoelectric precipitation, coded as HCL and LA, the liquid pool was acidified at a temperature of 20 °C until the pH reached 4.5, using 0.1 N hydrochloric acid (as it is a strong acid commonly used in practice), coded as HCL and lactic acid (85%, food grade), coded as LA. In both cases, the resulting mixture was allowed to settle inside a refrigerator until it reached a temperature of 4 °C, which facilitated the decantation of the protein product.

The third alternative, coded as LAB, implied an acidification with a mixture of two freeze-dried lactic acid bacteria, *Lactiplantibacillus plantarum* and *Lactococcus Lactis*. These bacteria were added in a proportion of 0.003 g/L in the liquid pool at a temperature of 35–37 °C for 18 h in a thermostatic bath under aerobic conditions (Tecno Dalvo, Argentina). If necessary, lactic acid (85%, food grade) was used for final adjustments of the pH value to reach the isoelectric point. Subsequently, the mixture was left to settle in a refrigerator until it reached 4 °C, which facilitated the decantation of the protein product.

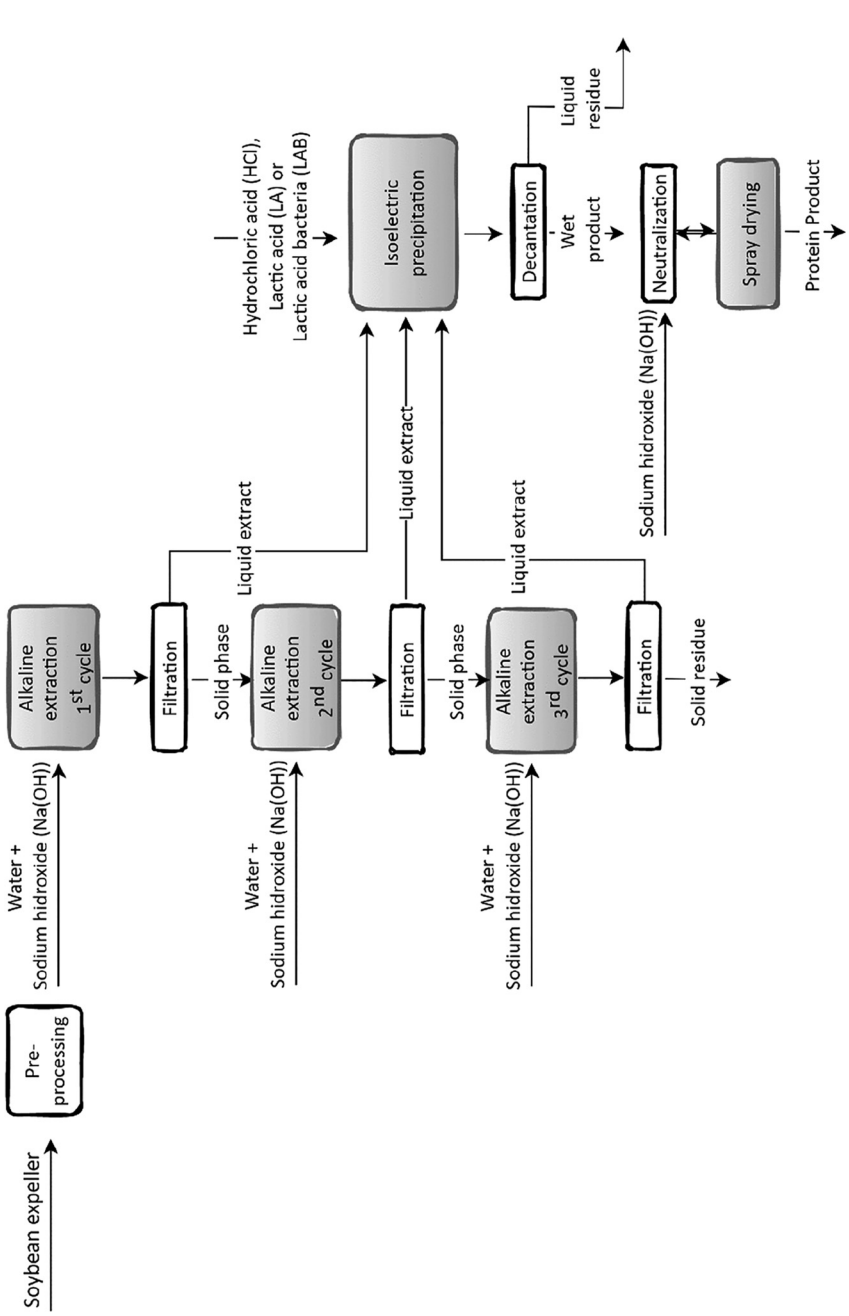

Fig. 1. Experimental methodology for the recovery of proteins from soybean expeller, using different extraction conditions and precipitant agents

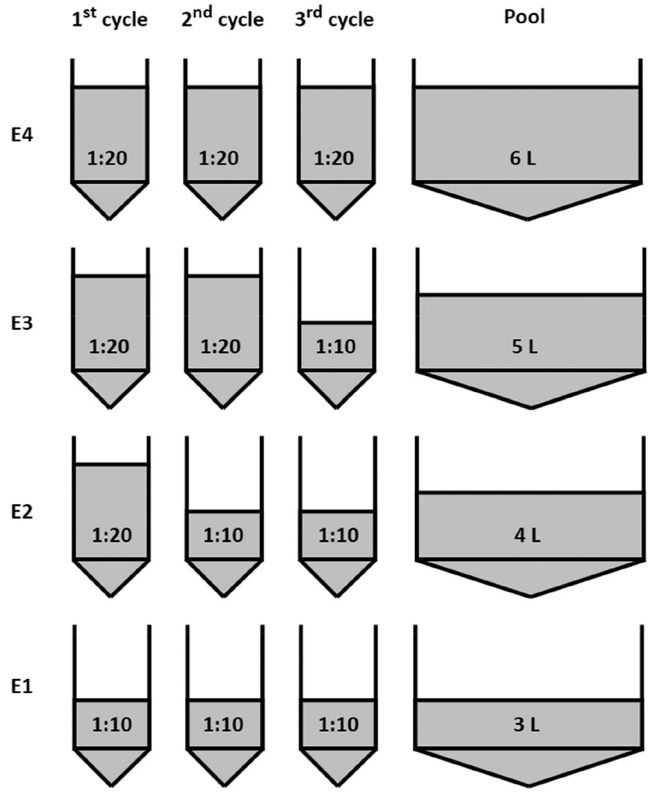

Fig. 2. Representation of the flour-to-water ratio (w/v) used in each extraction cycle at the alkaline extraction stage, coded as E1, E2, E3, and E4

Decantation, neutralization and spray drying. The liquid supernatant of the mixture obtained at the isoelectric precipitation stage was separated by decantation. Next, 5 N NaOH was added to the remaining mixture until reaching a pH of 7, with stirring for 1 h at room temperature.

Drying was done in a co-current spray dryer (TP-S15, XI'An Toption Instrument Co., Ltd, China) using a nozzle of 0.5 mm. The peristaltic pump used to feed the suspension was set at 15% of the maximum flow (2 L/h). The inlet air temperature was fixed at 180 °C, and the resulting outlet air temperature was measured at an average value of 54–60 °C. The dried powder was gathered from both the cyclone and the cylindrical components of the dryer chamber and stored in sterilized flasks.

Bacterial cell counts. The concentration of lactic acid bacteria was evaluated in experiments where they were used as the precipitant agent. Representative samples were cultured on MSR agar plates and incubated at 37 °C for 72 h under microaerophilic conditions. Viable cell counts were determined through visual inspection and expressed as colony-forming units per gram of sample (CFU/g).

Performance of the protein recovery process. The evaluation of the protein recovery was conducted by assessing the nitrogen content, yield of recovery, productivity, and specific water usage. Additionally, an analysis of the functional characteristics and antioxidant capabilities of the resulting protein products was carried out to obtain a comprehensive insight of the extraction and precipitation process.

The nitrogen content of the protein products was assessed through AOAC method 2001.11 (AOAC, 2005), and subsequently reported as % db (i.e. on a dry basis or a moisture-free basis). Moisture content was assessed with AOAC method 925.10 (AOAC, 2005), and expressed as % wb (i.e. on a wet basis).

The protein recovery yield Y_T (% db) was computed as the quantity of protein in the product relative to the initial protein content in the flour, as defined in Eq. (1).

$$Y_T = \frac{mass\ of\ protein\ in\ the\ final\ product\ (kg\ db)}{mass\ of\ protein\ in\ the\ initial\ flour\ (kg\ db)} \tag{1}$$

The productivity of each protein recovery process P_T (kg product db/kg flour db) was computed as the amount of product yielded per kilogram of flour, as defined in Eq. (2).

$$P_T = \frac{mass\ of\ final\ product\ (kg\ product\ db)}{mass\ of\ initial\ flour\ (kg\ flour\ db)} \tag{2}$$

The specific water consumption G_W (kg water/kg final product db) was computed as the water consumed for obtaining each kilogram of the final protein product, as defined in Eq. (3).

$$G_W = \frac{mass\ of\ consumed\ water\ (kg\ water)}{mass\ of\ final\ product\ (kg\ product\ db)} \tag{3}$$

Functional and antioxidant properties

Water holding capacity and oil holding capacity. Water and oil holding capacities of the soybean expeller protein products were determined according to Boye et al. (2010), Garcia-Vaquero et al. (2017) and Stone et al. (2015) with modifications. A sample (0.5 g) of the protein product was mixed in 15 mL centrifuge tubes with distilled water (3 mL) or high oleic sunflower oil (3 mL) in a vortex mixer (Precytec, Argentina). The pH was fixed to 2, 4.5, 7, 9.5, or 12, using 1 N HCl or 1 N NaOH, and mixed with a vortex mixer for 30 s. The pH-adjusted sample was centrifuged at 2,200×g for 30 min in a laboratory centrifuge (Rolco, Argentina). The liquid portion was discarded, and the centrifuge tube holding the remaining solid was weighed. The water holding capacity WHC (%) and oil holding capacity OHC (%) were computed as the quantity of water or sunflower oil held per gram of protein product, as defined in Eq. (4-5).

$$WHC = \frac{mass\ of\ water\ retained\ (g)}{mass\ of\ protein\ product\ (g)} \tag{4}$$

$$OHC = \frac{mass\ of\ sunflower\ oil\ retained\ (g)}{mass\ of\ protein\ product\ (g)} \tag{5}$$

Emulsifying capacity and emulsion stability. Emulsifying capacity and emulsion stability of the soybean expeller protein products were determined according to Garcia-Vaquero et al. (2017)

with modifications. A protein product sample (containing 0.5 g of protein db according to Kjeldahl analysis) was mixed in 50 mL laboratory tubes with distilled water (10 mL) in a vortex mixer (Precytec, Argentina). The pH was fixed to 2, 4.5, 7, 9.5, or 12, using 1 N HCl or 1 N NaOH, while mixed in the vortex mixer for 30 s. To create an emulsion, high oleic sunflower oil (7.5 mL) was incorporated and homogenized for 30 s at 14,000 rpm in a laboratory homogenizer (Labortechnik, Germany). An equal volume of high oleic sunflower oil (7.5 mL) was again added and homogenized for 90 s at 14,000 rpm in the laboratory homogenizer. Then, the homogenized sample was centrifuged at 1,100×g for 5 min in a laboratory centrifuge (Rolco, Argentina). The volume of the emulsion layer was determined. Finally, the emulsifying capacity EC (%) was calculated as the volume of the emulsion layer relative to the total volume, as defined in Eq. (6).

$$EC = \frac{volume\ of\ the\ emulsion\ layer\ (mL)}{total\ volumen\ (mL)} \tag{6}$$

Afterwards, the previously prepared emulsion was heated at 85 °C in a thermostatic bath (Tecno Dalvo, Argentina) for 15 min, allowed to cool at room temperature for 10 min, and then subjected to centrifugation at 1,100×g for 5 min in the laboratory centrifuge. The emulsion stability ES (%) was computed as the volume of the emulsion layer after heating relative to the original volume of the emulsion layer, as defined in Eq. (7).

$$ES = \frac{volume\ of\ the\ emulsion\ layer\ after\ heating\ (mL)}{volume\ of\ the\ emulsion\ layer\ (mL)} \tag{7}$$

Foaming capacity and foam stability. Foaming capacity and foam stability of the soybean expeller protein products were determined according to Garcia-Vaquero et al. (2017) and Stone et al. (2015) with slight modifications. A protein product sample (containing 0.75 g of protein db according to Kjeldahl analysis) was mixed in 50 mL laboratory tubes with distilled water (10 mL) in a vortex mixer (Precytec, Argentina). The pH was fixed to 2, 4.5, 7, 9.5, or 12, using 1 N HCl or 1 N NaOH, while mixed in the vortex mixer for 30 s. The mixture was homogenized for 60 s at 9,000 rpm in a laboratory homogenizer (Labortechnik, Germany). The foam layer volume was determined. Then, the foaming capacity FC (%) was computed as the volume of the foam layer relative to the total volume, as defined in Eq. (8).

$$FC = \frac{volume\ of\ the\ foam\ layer\ (mL)}{total\ volumen\ (mL)} \tag{8}$$

Afterwards, the previously prepared mixture was left undisturbed at ambient temperature. The remaining volume of the foam layer was determined at specified intervals (30 and 60 min). The foam stability FS (%) was computed as the foam layer volume after a given time relative to the original foam layer volume, as defined in Eq. (9).

$$FS = \frac{volume\ of\ the\ foam\ layer\ after\ specified\ time\ interval\ (mL)}{volume\ of\ the\ foam\ layer\ (mL)} \tag{9}$$

Solubility. Solubility of the soybean expeller protein products was determined according to Garcia-Vaquero et al. (2017) and Stone et al. (2015) with modifications. A product sample (containing 0.1 g of protein db according to Kjeldahl analysis) was mixed in 50 mL centrifuge

tube with distilled water (10 mL) in a vortex mixer (Precytec, Argentina). The pH was fixed to 7, using 1 N HCl or 1 N NaOH, while mixed in the vortex mixer for 30 s. The suspension underwent centrifugation at 4,000×g for 30 min in a laboratory centrifuge (Rolco, Argentina). A 1 g sample of the supernatant was separated, and its nitrogen content was assessed through AOAC method 2001.11 (AOAC, 2005), and subsequently reported as % db (i.e. on a dry basis or a moisture-free basis). The solubility S (%) was computed as the protein content post-centrifugation of the solution compared to the protein content of the dispersion, as defined in Eq. (10).

$$S = \frac{protein\ content\ after\ centrifugation\ of\ the\ supernatant\ of\ the\ pH\ adjusted\ solution\ (\%\ db)}{protein\ content\ of\ the\ full\ dispersion\ (\%\ db)} \tag{10}$$

Protein profiles by SDS-PAGE. The protein powders obtained with HCL, LA, and LAB were analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). A suspension (containing 1 mg of protein db according to Kjeldahl analysis) was solubilized in 0.125 M Tris–HCl buffer with a pH of 7.5 and dyed with Coommasie blue R-250. The sample was left to incubate at 85 °C for 15 min, and then centrifuged at 8,000 g for 5 min at ambient temperature. A 20 µg sample was loaded into 12% polyacrylamide gel slabs. The peptide electrophoretic pattern determination was conducted employing a stable current of 20 mA per gel. All Blue Marker (Biorad, US) and Protein Marker II (Serva, Germany) were used as molecular weight protein markers.

Statistical analysis

Results were assessed utilizing one-way and/or two-way ANOVA assuming normal distribution with confidence level of 95%. Each experimental measurement was done at least in duplicate. Results were presented as the mean value and standard deviation. Statistically significant differences ($P < 0.05$) were detected among the experimental data when different letters are shown next to them, according to post-hoc Tukey tests. Statistical analyses were done in R-3.6.0 software.

RESULTS & DISCUSSION

Performance evaluation of the protein recovery process from soybean expeller

The soybean expeller used as raw material was characterized as having a protein content of 43.76 ± 0.23% db, a protein solubility in KOH of 67.11 ± 0.35%, and a moisture content of 5.80 ± 0.16% wb.

Table 1 shows the protein recovery performance from soybean expeller for experimental runs using pH values of 8.5 or 10 in the alkaline extraction stage, and using hydrochloric acid, lactic acid (food grade), or a combination of lactic acid bacteria along with lactic acid as precipitating agent in the isoelectric precipitation stage. A pH value of 8.5 allowed to obtain a product with higher protein content ($P < 0.05$, one-way ANOVA of protein content with respect to pH) and expectedly better market value. Therefore, a pH value of 8.5 was selected to be used in the rest of the experimental runs, since the protein recovery yield did not significantly differ

Table 1. Performance of the protein recovery process from soybean expeller for different pH values in the alkaline extraction stage

	Protein content (% db)			Protein recovery yield, Y_T (%db)			Productivity, P_T (kg product db/kg flour db)			Specific water consumption, G_W (kg water/kg final product db)		
	HCL	LA	LAB	HCL	LA	LAB	HCL	LA	LAB	HCL	LA	LAB
E1- pH 8.5	66.78 ± 0.19 a	68.38 ± 4.50 a	59.05 ± 5.25 a	31.77 ± 6.99 a	34.14 ± 7.84 a	24.13 ± 4.58 a	0.25 ± 0.02 a	0.26 ± 0.02 a	0.21 ± 0.02 a	143.80 ± 15.70 a	140.08 ± 12.39 a	174.89 ± 11.34 a
E1- pH 10	57.94 ± 6.80 a	57.06 ± 5.20 a	54.45 ± 2.64 a	33.05 ± 7.69 a	33.74 ± 0.46 a	35.47 ± 2.21 a	0.25 ± 0.08 a	0.25 ± 0.02 a	0.28 ± 0.00 a	132.90 ± 45.90 a	123.01 ± 9.56 a	111.76 ± 1.57 a

Two-way ANOVA for each variable, where different letters represent significant differences between experimental results. The flour-to-water ratio used in the alkaline extraction stage is indicated as: E1, E2, E3, E4. The precipitant agent used in the isolectric precipitation stage is indicated as: HCL, hydrochloric acid; LA, lactic acid; LAB, lactic acid bacteria.

from the experiences where a pH value of 10 was used ($P > 0.05$, one-way ANOVA of recovery yield with respect to pH), although the productivity was lower ($P < 0.05$, one-way ANOVA of productivity with respect to pH) and the specific water consumption was higher ($P < 0.05$, one-way ANOVA of specific water consumption with respect to pH). Mardiah et al. (2014) also found no effect of alkaline extraction pH values from 8 to 10 on the final protein content of protein products obtained from soybean. Accoroni et al. (2020) reported protein concentrations of 60–65% for the alkali extraction of proteins from soybean expeller flour at a pH value of 8.5, with recovery yields of 46–48%. For two extruded expelled soy meals, Wang et al. (2004) obtained isolates with a protein content of 79.61–80.82% through an alkaline extraction at a pH value of 8.5, with values of the protein yield of 40.46–60.89%.

Table 2 shows the protein content, protein recovery yield, productivity and specific water consumption for the different alternatives in the protein recovery process from soybean expeller, including four different combinations of solid-to-liquid ratios in each of the 3 cycles of the alkaline extraction stage, and three precipitant agents in the isoelectric precipitation stage. No significant differences ($P > 0.05$, two-way ANOVA for each response with respect to solid-to-liquid ratio and precipitant agent) were found for the protein content, yield, productivity, and specific water consumption of the protein recovery process when different flour-to-water ratios or different precipitants were used. The implemented extraction strategy with the addition of fresh water in each cycle intends to simulate a continuous counter-current extraction effect, which implies improvements of the protein recovery yield with respect to the standard process with 1 or 2 extraction cycles, Accoroni et al. (2020) as the addition of fresh solvent enhances the driving force to further extract soluble proteins that are still tightly bound (Sunley, 1995). However, significant differences ($P < 0.05$, one-way ANOVA for specific water consumption respect to solid-to-liquid ratio) were found for the specific water consumption of the protein recovery process. As a general trend, the process alternatives with three 1:10 (w/v) extraction cycles used the lowest amount of water per kilogram of obtained protein product.

The efficiency of recovery process from different matrices was previously discussed in the literature. For one extraction cycle, Preece et al. (2017) proposed a model in which the results indicate that larger volumes of water used at higher solid-to-liquid ratios lead to a significant loss of proteins in the waste stream, along with its water content, thereby reducing the extraction yield. However, Sari et al. (2015) reported higher protein yields for higher solid-to-liquid ratios, reaching a recovery maximum value for ratios higher than 1:40 (with no significant differences). Results here obtained for the recovery of proteins from soybean expeller show that larger volumes of water are not required for achieving a better protein recovery performance. Contrary to the high solid-to-liquid ratio extractions usually reported in the literature, which may be suitable for protein recovery from valuable matrices at laboratory scale, the methodology here proposed could be more easily implemented at medium size scale processing plant for obtaining a food grade protein product.

For the protein recovery, experiment labeled E1, LAB cell counts were assessed and recorded as follows: the original commercial freeze-dried LAB starter had 6×10^{13} CFU/g, the isoelectric precipitation stage yielded a wet product with 3×10^{12} CFU/g, and the spray drying stage produced a protein product with 1.5×10^{12} CFU/g. Notably, a marked increase in viable LAB mass was observed during the isoelectric precipitation phase. Consistent with this observation, Rezvani et al. (2017) reported that various Lactobacilli species exhibited exponential growth after 10 h of fermentation, a duration comparable to our study, despite a decline in lactic

Table 2. Performance of the protein recovery process from soybean expeller

	Protein content (% db)			Protein recovery yield, Y_T (%db)			Productivity, P_T (kg product db/kg flour db)			Specific water consumption, G_W (kg water/kg final product db)		
	HCL	LA	LAB	HCL	LA	LAB	HCL	LA	LAB	HCL	LA	LAB
E1	66.78 ± 0.19 a	68.38 ± 4.50 a	59.05 ± 5.25 a	31.77 ± 6.99 a	34.11 ± 7.84 a	24.13 ± 4.58 a	0.20 ± 0.04 a	0.21 ± 0.03 a	0.17 ± 0.01 a	156.80 ± 34.00 a	148.70 ± 24.58 a	179.80 ± 18.30. a
E2	68.30 ± 0.50 a	53.01 ± 0.48 a	62.38 ± 2.04 a	40.21 ± 4.00 a	30.80 ± 5.64 a	30.49 ± 2.60 a	0.25 ± 0.02 a	0.25 ± 0.04 a	0.21 ± 0.02 a	165.60 ± 15.20 a	169.80 ± 29.60 a	199.50 ± 23.40 a
E3	66.47 ± 9.93 a	58.97 ± 2.38 a	56.65 ± 6.79 a	34.89 ± 6.04 a	41.09 ± 2.81 a	32.17 ± 5.56 a	0.23 ± 0.07 a	0.30 ± 0.00 a	0.24 ± 0.01 a	237.60 ± 5.70 a	174.29 ± 4.91 a	214.65 ± 11.50 a
E4	61.55 ± 4.52 a	57.23 ± 2.24 a	61.56 ± 9.17 a	31.89 ± 4.49 a	39.69 ± 4.95 a	35.59 ± 6.65 a	0.22 ± 0.04 a	0.30 ± 0.05 a	0.25 ± 0.00 a	285.20 ± 0.70 a	211.70 ± 35.20 a	252.62 ± 9.72 a

Two-way ANOVA for each variable, where different letters represent significant differences between experimental results. The flour-to-water ratio used in the alkaline extraction stage is indicated as: E1, E2, E3, E4. The precipitant agent used in the isolectric precipitation stage is indicated as: HCL, hydrochloric acid; LA, lactic acid; LAB, lactic acid bacteria.

acid production. The final pH, influenced by the bacterial strain and fermentation specifics, as noted by Engels et al. (2022), hovered around 4.5 optimal for isoelectric precipitation in the pH-shifting method. In contrast, the spray drying process led to a decrease in viable LAB mass in the protein product, albeit to a lesser extent due to the laboratory spray dryer's limited efficiency, as Moreira et al. (2021) indicated. Mora-Villalobos et al. (2020) have suggested that the residual bacterial cells could affect the functional and probiotic qualities of the final protein products.

Evaluation of functional properties of soybean expeller protein products

The advantages of incorporating soybean expeller proteins to food products like emulsions, foams, or gels could potentially be enhanced by gaining a deeper understanding of how pH affects their properties (Benelhadj et al., 2016). Given that target food products will vary in their pH, it becomes essential to assess how pH influences the functional properties of the resulting protein products.

Table 3 shows the experimental values for the functional attributes of the protein products derived from soybean expeller. For evaluating these properties, three 15-min extraction cycles using water as solvent, 1:10 (w/v) solid-to-liquid ratio, and a pH value of 8.5, were adopted in the alkaline extraction stage (previously coded as E1). The isoelectric precipitation stage was performed using hydrochloric acid (HCL), lactic acid (LA), or a combination of lactic acid bacteria starters and lactic acid (LAB). The other processing parameters were kept at the values previously stated at section 2.2. Here, water and oil holding capacities, emulsifying capacity and its stability, and foaming capacity and its stability were determined at five pH values: 2, 4.5, 7, 9.5, and 12, while the solubility was evaluated at a pH value of 7.

Water holding capacity (WHC) and oil holding capacity (OHC). The water and oil holding capacities of the protein products showed significant differences ($P < 0.05$, two-way ANOVA for each response with respect to precipitant agent and pH) with respect to some combinations of the precipitant agent for all five tested pH values, as shown in Table 3. Both water and oil holding capacities of plant proteins play a crucial role determining the textural qualities, such as juiciness and tenderness, of health-focused food products, making them a viable alternative to meat proteins (Ashaolu, 2020; Ma et al., 2022).

As general trend, the protein products showed significantly larger water holding capacity ($P < 0.05$, two-way ANOVA for water holding capacity with respect to precipitant agent and pH) when LAB was used as the precipitating agent, and specifically when the pH of the solution was alkaline (fixed to 9.5 and 12). Yang et al. (2021) discussed that lactic acid bacteria fermentation led to the denaturation of soybean protein isolates, which induced structural alterations and prompted the formation of aggregates. These aggregates retain water, forming the fundamental basis for enhancing the water holding capacity compared to non-fermented samples.

The oil holding capacity had a comparable magnitude to the values previously reported by Ma et al. (2022) for faba bean, pea, lentil and soybean protein isolates. The experimental values of this functional property are determined by the arrangement of amino acids, whether charged and polar or nonpolar (i.e., surface hydrophilicity vs. hydrophobicity), as well as surface chemistry and porosity of the protein powders, among other factors.

Emulsifying capacity (EC) and emulsion stability (ES). The emulsifying capacity and the corresponding emulsion stability of the products presented significant differences ($P < 0.05$,

Table 3. Functional properties of protein products obtained from soybean expeller

pH	Water holding capacity, *WHC* (g/g)			Oil holding capacity, *OHC* (g/g)			Emulsifying capacity, *EC* (%)			Emulsion stability, *ES* (%)		
	HCL	LA	LAB	HCL	LA	LAB	HCL	LA	LAB	HCL	LA	LAB
2	1.20 ± 0.12 fg	1.59 ± 0.08 cdef	2.08 ± 0.17 bcd	1.92 ± 0.10 bc	2.09 ± 0.12 ab	1.91 ± 0.14 bc	47.70 ± 1.97 ab	44.69 ± 3.65 abcd	46.49 ± 1.24 abc	86.74 ± 7.44 a	21.95 ± 1.59 c	96.22 ± 0.10 a
4.5	2.07 ± 0.23 bcd	1.03 ± 0.12 fg	2.02 ± 0.17 bcde	1.32 ± 0.05 def	1.70 ± 0.07 bcd	1.43 ± 0.09 de	ND	ND	39.47 ± 1.23 bcd	ND	ND	17.69 ± 5.73 c
7	1.24 ± 0.10 fg	1.03 ± 0.07 fg	2.23 ± 0.10 bc	1.92 ± 0.17 bc	1.36 ± 0.08 def	1.43 ± 0.00 de	46.36 ± 1.28 abc	46.43 ± 0.00 abc	49.04 ± 2.64 a	100.00 ± 0.00 a	46.16 ± 5.44 b	98.92 ± 2.52 a
9.5	0.22 ± 0.01 h	1.38 ± 0.17 ef	2.61 ± 0.23 ab	2.43 ± 0.12 a	1.16 ± 0.02 ef	0.99 ± 0.09 f	48.18 ± 1.28 a	45.04 ± 0.57 abc	48.67 ± 3.17 a	94.37 ± 7.96 a	100.00 ± 0.00 a	98.22 ± 2.52 a
12	1.52 ± 0.12 def	0.73 ± 0.09 gh	2.92 ± 0.39 a	1.48 ± 0.06 de	1.66 ± 0.02 cd	1.43 ± 0.19 de	36.45 ± 1.48 d	38.45 ± 0.74 cd	41.02 ± 4.02 abcd	24.29 ± 6.06 c	42.19 ± 1.82 b	100.00 ± 0.00 a

pH	Foaming capacity, *FC* (%)			Foam stability at 30 min, *FS* (%)			Foam stability at 60 min, *FS* (%)			Solubility, *S* (%)		
	HCL	LA	LAB	HCL	LA	LAB	HCL	LA	LAB	HCL	LA	LAB
2	50.00 ± 4.16 c	27.94 ± 2.08 d	70.59 ± 0.00 ab	3.80 ± 0.42 ef	2.65 ± 0.35 fg	5.30 ± 0.00 bc	2.75 ± 0.21 d	1.80 ± 0.00 e	3.50 ± 0.00 c	–	–	–
4.5	23.53 ± 0.00 d	25.00 ± 2.08 d	67.65 ± 4.16 ab	1.80 ± 0.00 g	ND	4.40 ± 0.42 cde	0.60 ± 0.00 f	ND	3.50 ± 0.00 c	–	–	–
7	29.41 ± 0.00 d	50.00 ± 4.16 c	73.53 ± 4.16 a	2.65 ± 0.35 fg	4.40 ± 0.42 cde	4.10 ± 0.00 de	1.80 ± 0.00 e	3.50 ± 0.00 c	3.50 ± 0.00 c	45.86 ± 7.21 a	21.92 ± 0.46 b	42.08 ± 0.00 a
9.5	73.53 ± 4.16 a	79.41 ± 4.16 a	76.47 ± 0.00 a	5.90 ± 0.00 b	5.30 ± 0.00 bc	5.60 ± 0.42 b	4.10 ± 0.00 b	3.50 ± 0.00 c	4.70 ± 0.00 a	–	–	–
12	55.88 ± 4.16 bc	82.35 ± 8.32 a	79.41 ± 4.16 a	5.00 ± 0.42 bcd	7.10 ± 0.00 a	5.60 ± 0.42 b	4.40 ± 0.42 ab	4.70 ± 0.00 a	3.95 ± 0.21 bc	–	–	–

Two-way ANOVA for each variable, where different letters represent significant differences between experimental results. The precipitant agent used in the isolectric precipitation stage is indicated as: HCL, hydrochloric acid; LA, lactic acid; LAB, lactic acid bacteria. ND, i.e. no detected activity, is used to indicate that the functional property could not be determined.

two-way ANOVA for each response with respect to precipitant agent and pH) with respect to some combinations of the precipitant agent for all five tested pH values, as shown in Table 3. The emulsifying capacity indicates a sample's ability to swiftly adhere to the interfaces between oil and water during the emulsification process, preventing flocculation and coalescence. This property is relevant to the elaboration of many food products by improving texture, preventing oil/water phases separation, and retaining flavors. Stable emulsions aid in distributing flavors, thus making food products more palatable (Naurzbayeva et al., 2023).

It is noted that no emulsion was formed at a pH value of 4.5 when hydrochloric acid or lactic acid was used as the precipitant agent during the protein recovery process. Under these experimental conditions, the solubilized protein product rapidly coalesced and precipitated when the pH was adjusted to this value, which is the protein's isoelectric point, thus no emulsion could be formed. Similarly, Wang et al. (2010) observed that an emulsion stabilized with soybean protein concentrate at pH 4.5 using HCl is potentially less stable, due to the proximity to the isoelectric point, where the Zeta potential tends to approach zero, indicating reduced electrostatic repulsion among the colloidal particles in the emulsion, thus increasing the influence of hydrophobic interactions and Van der Waals forces (McClements, 2004). A link between emulsifying capacity and zeta potential was evidenced by Wang et al. (2010), suggesting that emulsions exhibited enhanced stability against droplet aggregation or coalescence at elevated pH levels, because of heightened electrostatic repulsion.

On the other hand, the LAB-precipitated products exhibited some emulsifying capacity even at the isoelectric pH value. According to Aluko et al. (2009), pea protein products from lactic acid bacteria precipitation presented higher sugars contents, which may potentially enhance the protein solubility, thus improving the emulsifying capacity. In addition, the LAB-precipitated products presented similar values of the emulsion stability than the HCL-precipitated ones, and larger than the LA-precipitated ones ($P < 0.05$, one-way ANOVA for emulsion stability with respect to precipitant agent). From the previous literature, contradictory accounts were found regarding the influence of the production method of legumes protein products on their emulsion capacity and stability, where some authors found an improvement of these properties when using salt extraction, ultrafiltration, micellar precipitation, or lactic acid fermentation, while other works did not report such differences (Adebowale et al., 2011; Boye et al., 2010; Karaca et al., 2011; Stone et al., 2015).

Foaming capacity (FC) and foam stability (FS). Table 3 shows that the foaming capacity and the corresponding foam stability presented significant differences ($P < 0.05$, two-way ANOVA for each response with respect to precipitant agent and pH) with respect to some combinations of the precipitant agent for all five tested pH values, as shown in Table 3. The ability of flours to form foams could be deemed essential for their application in the production of non-dairy foods, as foam formation is essential in the manufacturing of various food products, including ice cream, cakes, fruit snacks, and foams, which underscores the significance of this functional property in the food industry (Jarpa-Parra and Chen, 2021).

As a general trend, it is observed that the foam is more stable ($P < 0.05$, one-way ANOVA for foam stability with respect to precipitant agent) when formed with protein products obtained using lactic acid bacteria rather than hydrochloric acid or lactic acid as precipitant agent during the protein recovery process. Foaming properties rely on protein features (migration to the interface, surface tension, and alignment of hydrophobic and hydrophilic components),

endogenous factors (temperature, pH, protein content, and interactions with other components), and parameters influencing foam creation (Emkani et al., 2022). Fermentation induces alterations in the electrostatic properties of macromolecules like proteins, allowing them to create dense films around each air bubble, consequently lowering the surface tension and enhancing the foam capacity and stability. Consequently, the synergy between heightened electrostatic charges and water-holding capacity contributes to an augmented foam stability (Awuchi et al., 2019).

The foaming capacity values here obtained are in agreement to the ones reported by Foh et al. (2012) for soybean protein products from a pH-shifting process, which ranged from about 4% at a pH value of 4 to about 102% at a pH value of 10. However, Foh et al. (2012) presented better foam stability values of 45% at 30 min and 25% at 60 min. The increase in foam capacity at higher pH is probably a consequence of the heightened net charges on the protein, leading to improved protein flexibility and a reduction in hydrophobic interactions. This facilitates quicker protein diffusion to the interface between air and water, resulting in improved encapsulation of air particles and enhanced foam formation, as described by Wierenga and Gruppen (2010).

Protein solubility (S). The protein solubility was significantly higher ($P < 0.05$, one-way ANOVA for protein solubility with respect to precipitant agent) for protein products obtained using hydrochloric acid and lactic acid bacteria as a precipitant agent during the protein recovery process, with respect to the solubility of protein products obtained using lactic acid, as seen in Table 3.

The state of the art regarding legume fermentation with lactic acid was reviewed by Emkani et al. (2022), as a strategy to enhance sensory and functional properties. The authors found that protein solubility depends upon factors such as production of acid by certain types of microorganisms, changes in the proteins surface, occurrence of proteolysis, diminution in the protein size, and others. Peng et al. (2020) reported a solubility index of around 90% at a pH value of 7.5 for protein products obtained from soybean cultivars with high initial protein content of 80%, using hydrochloric acid as precipitant agent. Even so, they concluded that the solubility for different pH values is case-dependent as a function of the specific protein conformation, protein surface charge, and ionic strengths during protein extraction. For the nitrogen solubility of soybean protein meal samples fermented with *Lactobacillus plantarum*, Amadou et al. (2010) reported values of 23–25% at pH 7, and an increase to 99% at pH 12. Soybean protein powders commonly exhibit limited solubility in water, especially when approaching neutral pH levels, posing a challenge for their integration into complex nutritional formulations. O'Flynn et al. (2021) obtained solubility values of unheated soybean protein isolate solutions at pH 9.0 of 28.8%, which were notably greater compared to dispersions at pH 6.9 with a solubility of 17.8%. In contrast, Meinlschmidt et al. (2016) reported solubility values of about 44, 16 and 18% for a soybean protein isolate without fermentation and fermented with *Lactobacillus helveticus* for 24 and 48 h, respectively.

As many authors reported for soybean products, such as Das et al. (2022); Peng et al. (2020); Song et al. (2023), the solubility profile of the alkaline-soluble proteins (i.e., the ones recovered through the pH-shifting process) exhibit a bell-shaped curve, with a minimum solubility index around the isoelectric point (4.5–5) and maximum values under alkaline (pH 8) and very acidic (pH 2) conditions. The solubility of proteins is primarily determined by the equilibrium between interactions among proteins and interactions with the solvent. As reported by Chang et al. (2015),

a Pearson correlation analysis revealed a strong association between solubility and protein charge, indicating that proteins with higher positive or negative charges tend to be more soluble. Conversely, hydrophobicity was not found to be significantly correlated with solubility. However, it is important to note that hydrophobic interactions are likely to aid in the stabilization of aggregates among proteins, particularly under neutral conditions.

Protein profiles by SDS-PAGE. To determine the impact of the precipitant agent on polypeptide composition of the recovered protein products, SDS-PAGE electrophoresis was used for the protein powders obtained with HCL, LA and LAB, as shown in Fig. 3. It is observed that the electrophoretic profile of the protein product obtained using LAB (Lane 3) exhibits a higher intensity band of the β-conglycinin subunit β (~50 kDa) and similar intensity bands of the β-conglycinin subunits α' (~71 kDa) and α (~67 kDa) compared to the product obtained using HCL (Lane 1) and LA (Lane 2). Moreover, the intensity of the band for glycinin, primarily composed of the acidic subunit A (29–33 kDa), is higher for LAB products.

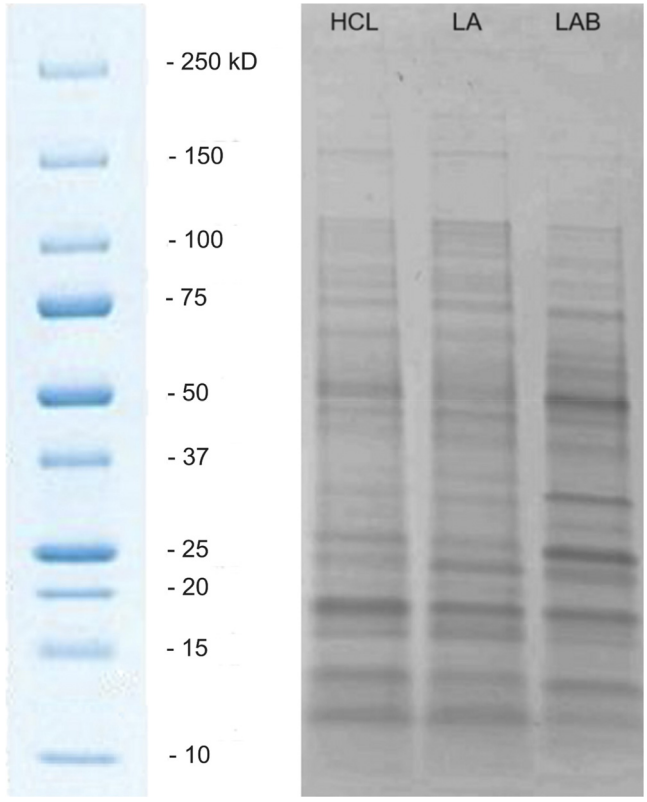

Fig. 3. Protein profiles by SDS-PAGE for protein products precipitated with hydrochloric acid (HCl), lactic acid (LA), and lactic acid bacteria (LAB)

Meanwhile, the basic subunit B (18–22 kDa) and the 2S fraction for conglycin (lower than 18 kDa) do not visually show differences among the three protein products. In general terms, the choice of precipitant agent does not appear to affect the subunits of peptides. These observations are analogous to those reported by Meinlschmidt et al. (2016), where glycinin remained unaffected by LAB fermentation, possibly attributed to the proteins structure and the presence of disulfide bonds in glycinin, which impede enzymatic protein hydrolysis. Aguirre et al. (2008) examined the proteolytic potential of 12 LAB strains, through a 6-h incubation at 37 °C of a soybean protein extract, where no discernible enzymatic activity from *Lactobacillus fermentum*, *Lactobacillus plantarum*, or *Pediococcus pentosaceus* was observed on the resultant peptide composition of the protein products.

CONCLUSIONS

The alternative pH-shifting processes for the recovery of soybean expeller proteins resulted in products with comparable protein contents and similar protein recovery yields, when different flour-water ratios were employed in the alkaline extraction or when different precipitants were used for the isoelectric precipitation. No significant differences ($P > 0.05$) were found in process indicators including protein content, productivity and specific water consumption of the protein recovery process, which implies that a lower water volume and alternative precipitant agents can be effectively employed in the protein recovery process without negatively impacting its performance.

Furthermore, protein products obtained with lactic acid bacteria exhibited improved functional properties compared to those obtained with hydrochloric acid as the precipitant agent, particularly in water holding capacity and foaming capacity. Similar values were observed for emulsion capacity, stability, and protein solubility across the analyzed pH range. These enhanced functional attributes position lactic acid bacteria as a sustainable alternative to inorganic acids as precipitant agents, which would enable the utilization of the obtained protein products in diverse food technology applications. Moreover, the resultant pH-shifting process could be used by small and medium-sized enterprises for onsite value adding to soybean expeller through a sustainable and technically feasible protein recovery alternative.

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REFERENCES

Accoroni, C., Godoy, E., and Reinheimer, M.A. (2019). Experimental data acquisition and mathematical model for soluble protein extraction from Argentinian extruded expeller soybean meal. *Journal of Food Science and Technology*, 56(7): 3492–3501, https://doi.org/10.1007/s13197-019-03838-y.

Accoroni, C., Godoy, E., and Reinheimer, M.A. (2020). Performance evaluation of protein recovery from Argentinian soybean extruded-expelled meals under different operating conditions. *Journal of Food Engineering*, 274: 109849, https://doi.org/10.1016/j.jfoodeng.2019.109849.

Adebowale, Y.A., Schwarzenbolz, U., and Henle, T. (2011). Protein isolates from Bambara groundnut (Voandzeia Subterranean L.): chemical characterization and functional properties. *International Journal of Food Properties*, 14(4): 758–775, https://doi.org/10.1080/10942910903420743.

Aguirre, L., Garro, M.S., and Savoy de Giori, G. (2008). Enzymatic hydrolysis of soybean protein using lactic acid bacteria. *Food Chemistry*, 111(4): 976–982, https://doi.org/10.1016/j.foodchem.2008.05.018.

Alhamad, L., Alrashed, A., Al Munif, E., and Miskimins, J. (2020). Organic acids for stimulation purposes: a review. *SPE Production & Operations*, 35(04): 952–978, https://doi.org/10.2118/199291-PA.

Aluko, R.E., Mofolasayo, O.A., and Watts, B.M. (2009). Emulsifying and foaming properties of commercial yellow pea (*Pisum sativum* L.) seed flours. *Journal of Agricultural and Food Chemistry*, 57(20): 9793–9800, https://doi.org/10.1021/jf902199x.

Amadou, I., Amza, T., K Foh, M.B., Kamara, M.T., and Le, G.-W. (2010). Influence of Lactobacillus plantarum Lp6 fermentation on the functional properties of soybean protein meal. *Emir. J. Food Agric.*, 22(6): 456–465, http://ffa.uaeu.ac.ae/ejfa.shtml.

AOAC (2005). *Official methods of analysis of AOAC INTERNATIONAL*. AOAC International.

Araba, M. and Dale, N.M. (1990). Evaluation of protein solubility as an indicator of underprocessing of soybean meal. *Poultry Science*, 69(10): 1749–1752, https://doi.org/10.3382/ps.0691749.

Ashaolu, T.J. (2020). Applications of soy protein hydrolysates in the emerging functional foods: a review. *International Journal of Food Science & Technology*, 55(2): 421–428, https://doi.org/10.1111/ijfs.14380.

Awuchi, G.C., Kate Echeta, C., Godswill, C., Somtochukwu, V., and Kate, C. (2019). The functional properties of foods and flours. *International Journal of Advanced Academic Research | Sciences*, 5(11), https://www.researchgate.net/publication/337403804.

Benelhadj, S., Gharsallaoui, A., Degraeve, P., Attia, H., and Ghorbel, D. (2016). Effect of pH on the functional properties of Arthrospira (Spirulina) platensis protein isolate. *Food Chemistry*, 194: 1056–1063, https://doi.org/10.1016/j.foodchem.2015.08.133.

Boye, J., Zare, F., and Pletch, A. (2010). Pulse proteins: processing, characterization, functional properties and applications in food and feed. *Food Research International*, 43(2): 414–431, https://doi.org/10.1016/j.foodres.2009.09.003.

Brasil, M., Meleiro, L.A.da C., Benincá, C., and Zanoelo, E.F. (2016). Yield of soybean protein isolate from defatted soybean flakes treated in an industrial plant and in laboratory: experiments and modeling. *Journal of Food Process Engineering*, 39(6): 636–644, https://doi.org/10.1111/jfpe.12256.

Calzada, J. and Ferrari, B. (2021). *La industria de expeller de soja no cede terreno y se aproxima al millón de toneladas procesadas*. Bolsa de Comercio Rosario.

Chang, C., Tu, S., Ghosh, S., and Nickerson, M.T. (2015). Effect of pH on the inter-relationships between the physicochemical, interfacial and emulsifying properties for pea, soy, lentil and canola protein isolates. *Food Research International*, 77: 360–367, https://doi.org/10.1016/j.foodres.2015.08.012.

Cheng, M.-H., Sekhon, J.J.K., Rosentrater, K.A., Wang, T., Jung, S., and Johnson, L.A. (2018). Environmental impact assessment of soybean oil production: extruding-expelling process, hexane extraction and aqueous extraction. *Food and Bioproducts Processing*, 108: 58–68, https://doi.org/10.1016/j.fbp.2018.01.001.

Das, D., Panesar, P.S., and Saini, C.S. (2022). pH shifting treatment of ultrasonically extracted soybean meal protein isolate: effect on functional, structural, morphological and thermal properties. *Process Biochemistry*, 120: 227–238, https://doi.org/10.1016/j.procbio.2022.06.015.

Emkani, M., Oliete, B., and Saurel, R. (2021). Pea protein extraction assisted by lactic fermentation: impact on protein profile and thermal properties. *Foods*, 10(3): 549, https://doi.org/10.3390/foods10030549.

Emkani, M., Oliete, B., and Saurel, R. (2022). Effect of lactic acid fermentation on legume protein properties, a review. *Fermentation*, 8(6): 244, https://doi.org/10.3390/fermentation8060244.

Engels, W., Siu, J., van Schalkwijk, S., Wesselink, W., Jacobs, S., and Bachmann, H. (2022). Metabolic conversions by lactic acid bacteria during plant protein fermentations. *Foods*, 11(7): 1005, https://doi.org/10.3390/foods11071005.

Foh, M.B.K., Wenshui, X., Amadou, I., and Jiang, Q. (2012). Influence of pH shift on functional properties of protein isolated of Tilapia (Oreochromis niloticus) muscles and of soy protein isolate. *Food and Bioprocess Technology*, 5(6): 2192–2200, https://doi.org/10.1007/s11947-010-0496-0.

Garcia-Vaquero, M., Lopez-Alonso, M., and Hayes, M. (2017). Assessment of the functional properties of protein extracted from the brown seaweed Himanthalia elongata (Linnaeus) S. F. Gray. *Food Research International*, 99: 971–978, https://doi.org/10.1016/j.foodres.2016.06.023.

Ghumman, A., Kaur, A., Singh, N., and Singh, B. (2016). Effect of feed moisture and extrusion temperature on protein digestibility and extrusion behaviour of lentil and horsegram. *LWT*, 70: 349–357, https://doi.org/10.1016/j.lwt.2016.02.032.

Hadnadjev, M., Dapcevic-Hadnadjev, T., Pojic, M., Saric, B., Misan, A., Jovanov, P., and Sakac, M. (2017). Progress in vegetable proteins isolation techniques: a review. *Food and Feed Research*, 44(1): 11–21, https://doi.org/10.5937/FFR1701011H.

Jarpa-Parra, M. and Chen, L. (2021). Applications of plant polymer-based solid foams: current trends in the food industry. *Applied Sciences*, 11(20): 9605, https://doi.org/10.3390/app11209605.

Jiang, J., Chen, J., and Xiong, Y.L. (2009). Structural and emulsifying properties of soy protein isolate subjected to acid and alkaline pH-shifting processes. *Journal of Agricultural and Food Chemistry*, 57(16): 7576–7583, https://doi.org/10.1021/jf901585n.

Karaca, A.C., Low, N., and Nickerson, M. (2011). Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Research International*, 44(9): 2742–2750, https://doi.org/10.1016/j.foodres.2011.06.012.

Kim, H.J. and Kim, B.K. (2015). Comparison of soy protein concentrates produced using membrane ultrafiltration and acid precipitation. *Food Science and Biotechnology*, 24(1): 67–73, https://doi.org/10.1007/s10068-015-0011-5.

Liu, Z., Fu, Y., Liu, Y., Chen, X., Jiang, M., and Rui, X. (2023). Lactic acid bacteria fermented soy β-conglycinin: assessment of structural conformational feature and immunoglobulin E reactivity. *LWT*, 173: 114246, https://doi.org/10.1016/j.lwt.2022.114246.

Liu, Z., Wang, Y., Liu, Y., Zhang, Q., Li, W., Dong, M., and Rui, X. (2021). The conformational structural change of soy glycinin via lactic acid bacteria fermentation reduced immunoglobulin E reactivity. *Foods*, 10(12): 2969, https://doi.org/10.3390/foods10122969.

Ma, K.K., Grossmann, L., Nolden, A.A., McClements, D.J., and Kinchla, A.J. (2022). Functional and physical properties of commercial pulse proteins compared to soy derived protein. *Future Foods*, 6: 100155, https://doi.org/10.1016/j.fufo.2022.100155.

Mardiah, Z., Rahardja, A.C.W., and Rovalino, A.M. (2014). Effect of different ph extraction on the yield, purity, solubility, and protein composition of lupin and soybean protein isolates. *Indonesian Student Scientific Conference*, 92.

McClements, D.J. (2004). *Food emulsions*. CRC Press, https://doi.org/10.1201/9781420039436.

Meinlschmidt, P., Ueberham, E., Lehmann, J., Schweiggert-Weisz, U., and Eisner, P. (2016). Immunoreactivity, sensory and physicochemical properties of fermented soy protein isolate. *Food Chemistry*, 205: 229–238, https://doi.org/10.1016/j.foodchem.2016.03.016.

Mora-Villalobos, J.A., Montero-Zamora, J., Barboza, N., Rojas-Garbanzo, C., Usaga, J., Redondo-Solano, M., Schroedter, L., Olszewska-Widdrat, A., and López-Gómez, J.P. (2020). Multi-product lactic acid bacteria fermentations: a review. *Fermentation*, 6(1): 23, https://doi.org/10.3390/fermentation6010023.

Moreira, M.T.C., Martins, E., Perrone, Í. T., de Freitas, R., Queiroz, L.S., and de Carvalho, A.F. (2021). Challenges associated with spray drying of lactic acid bacteria: understanding cell viability loss. *Comprehensive Reviews in Food Science and Food Safety*, 20(4): 3267–3283, https://doi.org/10.1111/1541-4337.12774.

Naurzbayeva, G., Smolnikova, F., Baytukenova, S., Kapshakbayeva, Z., Mustafayeva, A., Baytukenova, S., Smailova, Z., Dikhanbayeva, F., Temerbayeva, M., and Baybalinova, G. (2023). Incorporating carrot pomace-based emulsion to enhance the nutritional value and shelf life of butter. *International Journal of Food Properties*, 26(1): 2455–2475, https://doi.org/10.1080/10942912.2023.2251718.

Nishinari, K., Fang, Y., Nagano, T., Guo, S., and Wang, R. (2018). Soy as a food ingredient. In: *Proteins in food processing*. Elsevier, pp. 149–186, https://doi.org/10.1016/B978-0-08-100722-8.00007-3.

O′Flynn, T.D., Hogan, S.A., Daly, D.F.M., O′Mahony, J.A., and McCarthy, N.A. (2021). Rheological and solubility properties of soy protein isolate. *Molecules*, 26(10): 3015, https://doi.org/10.3390/molecules26103015.

Ojo, A.O. and de Smidt, O. (2023). Lactic acid: a comprehensive review of production to purification. *Processes*, 11(3): 688, https://doi.org/10.3390/pr11030688.

Peng, Y., Kersten, N., Kyriakopoulou, K., and van der Goot, A.J. (2020). Functional properties of mildly fractionated soy protein as influenced by the processing pH. *Journal of Food Engineering*, 275: 109875, https://doi.org/10.1016/j.jfoodeng.2019.109875.

Preece, K.E., Hooshyar, N., and Zuidam, N.J. (2017). Whole soybean protein extraction processes: a review. *Innovative Food Science & Emerging Technologies*, 43: 163–172, https://doi.org/10.1016/j.ifset.2017.07.024.

Rezvani, F., Ardestani, F., and Najafpour, G. (2017). Growth kinetic models of five species of Lactobacilli and lactose consumption in batch submerged culture. *Brazilian Journal of Microbiology*, 48(2): 251–258, https://doi.org/10.1016/j.bjm.2016.12.007.

Sari, Y.W., Mulder, W.J., Sanders, J.P.M., and Bruins, M.E. (2015). Towards plant protein refinery: review on protein extraction using alkali and potential enzymatic assistance. *Biotechnology Journal*, 10(8): 1138–1157, https://doi.org/10.1002/biot.201400569.

Song, H., Zhong, M., Sun, Y., Li, Y., and Qi, B. (2023). Recovery of proteins from soybean oil-body wastewater at various pH levels and their structural and functional characterization. *LWT*, 174: 114455, https://doi.org/10.1016/j.lwt.2023.114455.

Stone, A.K., Karalash, A., Tyler, R.T., Warkentin, T.D., and Nickerson, M.T. (2015). Functional attributes of pea protein isolates prepared using different extraction methods and cultivars. *Food Research International*, 76: 31–38, https://doi.org/10.1016/j.foodres.2014.11.017.

Sunley, N.C. (1995). Soya protein isolate production by various methods, http://researchspace.ukzn.ac.za/handle/10413/5055.

Vioque, J., Sánchez-Vioque, R., Pedroche, J., Yust, M.D.M., and Millán, F. (2001). Production and uses of protein concentrates and isolates. *Grasas y Aceites*, 52(2), https://doi.org/10.3989/gya.2001.v52.i2.384.

Wang, H., Johnson, L.A., and Wang, T. (2004). Preparation of soy protein concentrate and isolate from extruded-expelled soybean meals. *Journal of the American Oil Chemists' Society*, 81(7): 713–717, https://doi.org/10.1007/s11746-004-966-8.

Wang, B., Li, D., Wang, L.-J., Adhikari, B., and Shi, J. (2010). Ability of flaxseed and soybean protein concentrates to stabilize oil-in-water emulsions. *Journal of Food Engineering*, 100(3): 417–426, https://doi.org/10.1016/j.jfoodeng.2010.04.026.

Wierenga, P.A. and Gruppen, H. (2010). New views on foams from protein solutions. *Current Opinion in Colloid & Interface Science*, 15(5): 365–373, https://doi.org/10.1016/j.cocis.2010.05.017.

Yang, X., Ke, C., and Li, L. (2021). Physicochemical, rheological and digestive characteristics of soy protein isolate gel induced by lactic acid bacteria. *Journal of Food Engineering*, 292: 110243, https://doi.org/10.1016/j.jfoodeng.2020.110243.

Yang, X., Su, Y., and Li, L. (2020). Study of soybean gel induced by Lactobacillus plantarum: protein structure and intermolecular interaction. *LWT*, 119: 108794, https://doi.org/10.1016/j.lwt.2019.108794.

Zhao, Y., Tian, R., Xu, Z., Jiang, L., and Sui, X. (2023). Recent advances in soy protein extraction technology. *Journal of the American Oil Chemists' Society*, 100(3): 187–195, https://doi.org/10.1002/aocs.12676.