Performance evaluation of protein recovery from Argentinian soybean extruded-expelled meals under different operating conditions

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Abstract

The soybean extruded-expelled (EE) meals are the byproduct of the process commonly used by small or medium-sized Argentinian companies for obtaining soy oil. In this work, the performance of the pH-shifting process for obtaining a protein product from the EE meals was evaluated as a strategy for on-site value-adding.

The EE meals were subjected to the proposed pH-shifting process under different operating parameters at the alkaline extraction stage (2 and 3 cycles at 55, 60 and 65 ºC with and without sodium sulfite) and isoelectric precipitation stage (0 ºC and 20 ºC with hydrochloric and phosphoric acids), which constitute the controlling steps in an industrial scaling of the process. The pH-shifting process consisting of 3 alkaline extraction cycles at 60 ºC followed by isoelectric precipitation at low temperature using hydrochloric acid was found to be well suited for obtaining a final product with a protein content upwards of 75 %.

Keywords: soybean extruded-expelled meals; pH-shifting process; value-adding strategies

1. Introduction

Finding food sources for the rapidly growing human population is one of the most important challenges facing mankind. The demand for protein is particularly heightened by the changing dietary habits in developing countries, owing to the improving economy and rising per capita income (Preece et al., 2017). In this context, the use of soybean as a protein source has seen a significant scientific and technological interest in the last decades, as a viable alternative to proteins from animal origin, such as milk, meat, and egg (Endres, 2001).

During the industrial processing of soybean, the oil is extracted by chemical (solvent extraction) or physical (expelled-pressed extraction) technologies (Johnson, 2008). The remaining meal can be used to obtain a protein by-product through an extraction and concentration process. Such strategy would increase its added-value (Sunley, 1995), offering advantages such as providing a more concentrated source of protein according to market requirements, improving the functional properties of proteins, and reducing its undesirable properties (e.g., anti-nutritional factors).

Traditional processing technologies for soybean meal (i.e. resulting from solvent extraction) have been extensively developed for producing highly soluble protein ingredients such as concentrates (SPC), isolates (SPI) and texturized products (TSP) (Endres, 2001; Preece et al., 2017; Wang, et al., 2004). These alternatives include pH-shifting, salting-in extraction, aqueous alcohol extraction and heat processes (steam injection or jet cooking) (Endress, 2001; Johnson, 2008).

1.1 pH-shifting technology

The pH-shifting process, based on the manipulation of the protein solubility as a function of pH, is a method satisfactorily applied worldwide for protein recovery from different food matrices, such as soybean (Wang et al., 2004), sunflower (Raphael, 1997), rice (Ju et al., 2001),

among others. Moreover, this technology has been commonly adopted by small and medium-sized companies because it is simpler to scale for their production capacity, and the associated operating and capital costs are usually smaller (Endress, 2001; Johnson, 2008).

The pH-shifting process consists of the extraction and solubilization of proteins at a pH range of 8-11, followed by the acidification at a pH value of 4.5-4.8 which causes the insolubilization of approximately 90% of the globular proteins (Nishinari et al., 2014). In addition, the manipulation of temperature during the precipitation step can assist in separating proteins from other solubilized components (Barać et al., 2004).

The most commonly implemented prior art method for isolating vegetable protein from soybean meal involves a general step of protein solubilization by addition of alkali during the extraction stage (Heywood et al., 2002; Sunley, 1995). Several authors have used NaOH for the alkaline extraction with enhanced results for the refunctionalization of EE meals, since it usually leads to better results than calcium hydroxide or sodium bicarbonate (Heywood et al., 2002; Lawhon et al., 1981; Li et al., 2016; Sunley, 1995; Wang et al., 2004). Additionally, the usage of sodium sulphite during the extraction stage was previously reported as a strategy for lowering the levels of polyphenols oxidation (Govindaraju, 2003) and reducing disulfide bonds (Raphael, 1997; Yust et al., 2003) during oilseed protein extraction.

The operating temperature has been reported in the literature as a relevant condition that influences the process yield (Preece et al., 2017), within a commonly established range for soybean protein extraction (Sunley, 1995). Likewise, the usage of two consecutive cycles for the alkaline extraction of proteins from soybeans is usually recommended in the literature (Sunley, 1995); although previous work by Accoroni (2019) implied the presence of a large quantity of remnant proteins in the solid at the end of the 2nd processing cycle, thus suggesting the implementation of a 3rd extraction cycle could improve the overall proteins recovery efficiency.

The precipitation stage has the objective of converting soluble proteins into insoluble ones. The isoelectric point of soy protein fractions (2S, 7S and 11S) varies from pH values of 4.2 to 4.8 (Deak et al., 2008; Endres, 2001), where previous experimental results identified satisfactory precipitation yields at a pH value of 4.5 (Sunley, 1995). Additionally, decreasing the operating temperatures at the isoelectric precipitation stage entail an improvement in the amount of recovered proteins (Raphael, 1997). On the other hand, the usage of hydrochloric acid may not be advised in the food processing industry due to potential health risks, even if a posterior neutralization stage is carried out (Heywood et al., 2002); so, usage of phosphoric acid as alternative precipitation media could be also analyzed by quantifying the variation in the amount of recovered proteins.

1.2 Soybean processing in small and medium-sized companies in Argentina

Extrusion-expelling processing is gaining importance in Argentina, since more than 500 small and medium-sized companies have adopted this technology as it requires a low initial capital investment. Argentinian processing plants have an average processing capacity of 36 ton/day of soybeans, and represent 10% of the Argentinian soybean production (Juan et al., 2015). In spite of this relevant fact, limited technological advancements have been accomplished as to value- adding to the supply chain of the soybean EE meals (Heywood et al., 2002; Wang et al., 2004). Nevertheless, due to the increased interest in the expansion of the social economy, understood as the development of small or medium-sized companies and cooperatives, as well as the increasing importance of soybean protein as a food source, an optimal processing strategy for obtaining protein products from soybean EE meals has become a relevant challenge in order to add value to this byproduct.

Soybean EE meals present higher digestible energy and amino-acid availability than solvent meals (Endres, 2001). On the other hand, the disadvantages of soybean EE meals

processing, compared with defatted soybean flakes, can be attributed to the remnant anti-nutritional factors related with the shorter exposure to heat treatment, where the levels of trypsin inhibitors and nutrient digestibility could be inappropriate for usage as animal feeding or food ingredient (Endres, 2001). Another potential disadvantage is related to the higher content of remnant oil that persists in the EE meals due to the lower extraction efficiency for the pressing process compared with solvent extraction (Li et al., 2016). Additional work should include microbiological and functional studies for assessing the safety of usage of the protein products obtained from EE meals as a food ingredient (Heywood, 2001).

The soybean EE meals samples were obtained by the extrusion-expelling method at four processing plants (N1-N4) located in the Argentinian central region. The conditioned soybeans (dehulled and crushed) were processed in single screw extruders, and exited the die at temperatures between 125 and 140 °C, before entering the screw presses where the oil is extracted. Even though an early survey of these producers did not reveal large differences in the processing conditions, further analysis of the influence of extrusion parameters on the EE meals quality should be carried out through a rigorous long-term monitoring at each processing location, since it was previously reported that the EE meals composition varies because of the implemented technologies during drying, pressing and extrusion (Juan et al., 2015), and that a more severe thermal treatment usually results in lower value of protein solubility (Campbell, 2010).

For using the soybean EE meals as raw material for the pH-shifting process, a previous grinding step is required, as Rosenthal et al. (1998) found that lower particle sizes resulted in higher protein and oil extraction yields. The fraction of interest for the subsequent extraction is comprised of particle sizes between 25-mesh through and 100-mesh retained (Rosenthal et al., 1998), where D'Emanuele Ares et al. (2017) obtained an 85% recovery of the milled product with a roller miller at pilot plant scale.

1.3 Aim and objective

The objective of this work is to implement the pH shifting method, widely applied in the literature for soy protein extraction from defatted flakes (Deak, et al., 2008; Sunley, 1995; Wang et al., 2004), to obtain a soy protein product from soybean extruded-expelled meals produced at different Argentinian establishments. Specifically, different operating conditions are tested at the alkaline extraction stage (number of cycles, temperature, and addition of an auxiliary reagent) and at the isoelectric precipitation stage (temperature and precipitating acid), evaluating their impact on the performance of the pH shifting method, and assessing the overall feasibility of the proposed strategy.

2. Materials and methods

2.1 Raw materials characterization

The soybean EE meals samples were obtained by the extrusion-expelling method at various processing plants (N1-N4) located in the Argentinian central region. Samples were stored using sealed bags at freezer temperatures (-18 °C) until processing. Analytical methods by AOAC (2019) were used to determine protein content by Kjeldahl (method 954.01), moisture content (method 925.10), and crude oil content (method 920.39). The protein solubility index in potassium hydroxide was determined according to Araba and Dale (1990). The characteristics of the samples are presented in Table 1 (in dry base), where differences ($p<0.05$) between samples are noted in the protein and lipid contents, while no differences ($p>0.05$) were found for the moisture content and the KOH protein solubility (see section 2.4 for further details on statistical analysis). Note that Juan et al. (2015) reported some additional variability in the characteristics of Argentinian soybean EE meals related for example to crops seasonality and specific processing parameters in the expelled-pressed process.

Urease activity and aflatoxin level were evaluated as anti-nutritional factors. The urease activity was measured as the pH difference of ammonia released from urea by residual urease enzyme (method Ba 9-58, AOCS (2017)). The aflatoxin level was determined by the Elisa method (Leszczyńska et al., 2001). According to Argentinian food quality standards, the allowed upper level for urease activity is 0.3 ΔpH, while the allowed upper limit for aflatoxins is 0.03 µg/g. Then, it is here observed that both parameters are within acceptable levels for all samples.

2.2 Experimental methodology

The proposed experimental methodology was designed for evaluating the impact of variations in the main design and operating variables over the pH-shifting process of soybean protein extraction from EE meals provided by different processing plants located in Argentina, as presented in Figure 1. The variables here analyzed are the ones considered inherent to the processing equipment, which were also previously reported in the literature to have a large impact on the performance of pH-shifting process (Barać et al., 2004; Nishinari et al., 2014; Sunley, 1995; Wang et al., 2004). Other variables were set at values recommended in the literature, including agitation speed, flour/solvent ratio, particle size distribution and extraction pH.

2.2.1 Samples pre-processing

Soybean EE meals were ground into flour using a Blade mill (Sojamet, Argentina). For sieving, a sieve shaker (Ro-Tap, US) and sieves (Macotest, Argentina) corresponding to the ASTM series No. 4, 8, 12, 25, 40, and 50, 100 and blind were used. The fraction of interest for the subsequent extraction was comprised of particle sizes between 25-mesh through and 100-mesh retained (D'Emanuele Ares et al., 2017), and amounted to 65 % of the milled product at laboratory scale.

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186 *2.2.2 Alkaline Extraction*

187 The EE meals flour was mixed with distilled water as solvent, in a solid to liquid ratio of
188 1:20 g/ml, within a batch extractor with continuous stirring at 140 rpm, where the temperature
189 was set and maintained at 55, 60 or 65 °C by means of a thermostatic bath (Lauda, Germany).
190 Immediately, the pH of the mixture was adjusted to 8.5 using a 0.1 N sodium hydroxide solution,
191 as measured with a pH meter (Hanna, Spain). These adopted operating conditions agree with
192 those proposed by Sunley (1995) and Wang et al. (2004).

193 The alkaline extraction stage consisted of 2 or 3 cycles spanning 15 minutes each. At
194 2.5, 5, 10 and 15 minutes of extraction, aliquots of the liquid at the extractor were taken and
195 filtered, and the soluble protein content was determined by the Bradford technique (Bradford,
196 1976), measuring the absorbance at 595 nm in a spectrophotometer (UV-1800, Shimadzu,
197 Japan). Additional experimental runs with 2 or 3 cycles at 60 °C were carried out were 0.25 %
198 Na_2SO_3 was added in the first cycle, for testing the impact of sodium sulphite in the extraction
199 performance.

200 At the end of each extraction cycle, the solid and liquid phases were separated by vacuum
201 filtration. The solid fraction was used at the subsequent extraction stage, maintaining the same
202 operating parameters than at the 1st extraction cycle. The moisture content of the filtered residual
203 EE meal was considered as a reduction in the necessary volume of freshwater in the solid to
204 liquid ratio calculation of the subsequent cycle. Finally, the liquid extracts obtained from every
205 cycle were grouped together, while the remnant solid fraction was discarded.

206 In order to determine the soluble proteins profiles, several aliquots of the pooled liquid
207 extract obtained at different processing temperatures were analyzed by SDS–PAGE. Samples
208 were solubilized in 0.125 M Tris–HCl buffer and dyed with Coommasie blue R-250. The
209 homogenate was incubated at 90 °C for 5 min, followed by centrifugation at 8000g for 5 min at

room temperature. Then, 20 µg samples were loaded into 12 % polyacrylamide gel slabs. The electrophoretic pattern of proteins was determined using a constant current of 20 mA per gel.

2.2.3 Isoelectric precipitation

The obtained pooled liquid extract was cooled down prior to the isoelectric precipitation stage up to two different levels identified as low and high precipitation temperatures, corresponding to average values of 0 ºC and 20 ºC, respectively. Then, the pH of the pooled liquid extract was lowered to a value of 4.5 under constant agitation at 140 rpm, using hydrochloric or phosphoric acid, where proteins precipitated and were selectively separated from the remaining soluble components. Note that the isoelectric precipitation is a fast phenomenon and almost all proteins with the characteristics of being precipitated came out of the solution within the first minute (Raphael, 1997).

The precipitate was separated by centrifugation as a wet protein product and the residual liquid fraction was discarded. Then, the obtained product was kept at -18 ºC for usage at the freeze-drying stage.

2.2.4 Freeze-drying stage

Freeze-drying of the wet protein product was carried out with a laboratory lyophilization equipment (L-I-E300-CRT, Rificor, Argentina) operated at -35 ºC shelf temperature and -40 ºC condenser temperature during 36 hours. Finally, the obtained dry protein product was weighed and stored for later analysis, using the analytic techniques previously detailed in Section 2.1.

2.3 Process yield

Different instances of the process yield are hereafter defined in order to evaluate the performance of the protein recovery process from the soybean EE meals.

The alkaline extraction yield $Y_{E,c}$ (%) defined by Eq. (1), measures the amount of protein solubilized during each alkaline extraction step $c = c1, c2, c3$(by Bradford, 1976), with respect to the initial total protein content of the EE meal (by Kjeldahl; AOAC, 2019).

$$Y_{E,c} = \frac{volume\ of\ extract\ [l] \cdot soluble\ protein\ concentration\ at\ cycle\ c\ [\frac{g\ protein}{l}]}{mass\ of\ EE\ meal\ [g\ EE\ meal] \cdot initial\ protein\ concentration\ [\frac{g\ protein}{g\ EE\ meal}]} \cdot 100 \qquad (1)$$

The total alkaline extraction yield $Y_{E,T}$ (%) quantifies the total amount of protein solubilized at the two or three extraction cycles, defined by Eq. (2).

$$Y_{E,T} = \sum_c Y_{E,c} \qquad (2)$$

The isoelectric precipitation yield Y_P (%) defined by Eq. (3), computes the amount of protein precipitated at the isoelectric precipitation step (by Kjeldahl; AOAC, 2019), with respect to the total protein previously solubilized at the alkaline extraction step (by Bradford, 1976).

$$Y_P = \frac{mass\ of\ final\ product\ [g\ product] \cdot final\ protein\ concentration\ [\frac{g\ protein}{g\ product}]}{\sum_c (volume\ of\ extract\ at\ cycle\ c\ [l] \cdot soluble\ protein\ concentration\ at\ cycle\ c [\frac{g\ protein}{l}])} \cdot 100 \qquad (3)$$

The protein recovery yield Y_T (%) defined by Eq. (4), quantifies the total recovered protein throughout the pH-shifting process, with respect to the initial total protein content of the EE meal (both by Kjeldahl; AOAC, 2019).

$$Y_T = \frac{mass\ of\ final\ product\ [g\ product] \cdot final\ protein\ concentration\ [\frac{g\ protein}{g\ product}]}{mass\ of\ EE\ meal\ [g\ EE\ meal] \cdot initial\ protein\ concentration\ [\frac{g\ protein}{g\ EE\ meal}]} \cdot 100 \qquad (4)$$

The productivity of the pH-shifting process P_T (kg product/kg EE meal) is defined by Eq. (5), as the quantity of protein product obtained per kilogram of processed EE meal.

$$P_T = \frac{mass\ of\ final\ product\ [kg\ product]}{mass\ of\ EE\ meal\ [kg\ EE\ meal]} \qquad (5)$$

2.4 Statistical analysis

Comprising samples obtained from the four processing plants, 28 experimental runs were carried out for testing the previously described different operating conditions at the pH-shifting

process. The obtained data were processed in order to compute the yields and productivity, and were subjected to analysis of variance (one-way ANOVA), assuming normal distribution with a two-sided confidence level of 95 % (α=0.05). Experimental measurements were performed at least by duplicate, and the obtained results are here presented as the mean value with its standard deviation. Note that different letters next to the experimental values indicate that significant differences were found among them.

3. Results and discussion

3.1 Performance evaluation of the alkaline extraction stage

Figure 2 presents the evolution of the soluble protein concentration (g protein/l) at each extraction stage, exemplified for an operating temperature of 60 °C, noting that similar results were obtained for 55 and 65 °C. A final average protein concentration of 17.0±5.6 g/l is obtained at the 1st cycle, , while this value decreases to 10.3±4.1 g/l for the 2nd cycle and 5.7±3.1 g/l for the 3rd cycle, since the rate of mass transfer decreases with the decrease in protein content in the meal (note that freshwater was used as solvent in each extraction cycle).

Another consequence of the decreasing driving force for mass transfer is that the soluble proteins concentration remains almost constant from 10 minutes up to the end of the extraction cycle. Other authors previously concluded that prolonged extraction times may lead to protein denaturalization when using alkaline solutions (Raphael, 1997; Wang et al., 2004). Therefore, savings on operating costs, including decreased processing time and usage of heating agent, may be attained if the extraction process is ended at the 10 minutes mark.

Figure 3 portrays the alkaline extraction yields $Y_{E,c}$ (%) for 3 extraction cycles operating at temperatures of 55, 60 and 65 °C. Significant differences ($p<0.05$) are found for the final soluble protein concentration and the corresponding total alkaline extraction yields $Y_{E,T}$ among the three different processing temperatures, as noted by the different letters above the respective

columns. The observed increment in protein concentration is a consequence of increased protein solubility at higher temperatures, considering that the process of thermal-denaturation for soy proteins begins at around 72 ºC for β–conglycinin (Endres, 2001), which imposes an upper limit for the operating temperature at the extraction process. In addition, heating temperatures over 70 ºC also cause dissociation of the quaternary structures of proteins, with the consequent unstable structure being susceptible to protein aggregates via different interchange mechanisms (Barać et al., 2004).

A weaker dependence of the soluble proteins concentration on temperature was observed during the 2nd extraction cycle with respect to the 1st cycle, since the rate of mass transfer decreases with the decrease in protein content in the meal. For the 1st cycle, the variation in the extraction temperature from 55 °C to 60 °C and from 60 °C to 65 °C increases the extraction yield on average 10.6±1.6 % and 15.5±0.7 %, respectively. Meanwhile, for the 2nd cycle, the extraction yield remains almost invariable with the extraction temperature, where an average value of 23.7±0.4 % was achieved.

When using only two extraction cycles, a considerable amount of soluble proteins still remained non-extracted in the EE meal sample. It was then observed that the additional 3rd cycle recovers an extra average 12.8±0.2 %, 12.1±2.3 % and 8.2±0.1 % of the soluble proteins from the EE meals when operating at 55, 60 and 65 ºC, respectively. Then, the effectiveness of this strategy decreases with temperature as a consequence of the lower driving force for mass transfer, where the gain in per-cycle-yield $Y_{E,c}$ is incrementally lower, but otherwise allows reaching higher values of the total protein recovery $Y_{E,T}$. Therefore, the cost-benefit ratio of the introduction of a 3rd extraction cycle in the process should be thoroughly analyzed as this strategy was previously successfully implemented for example for protein recovery from fish (Reinheimer et al., 2013).

306 The impact of sodium sulphite in the extraction performance was tested by additional
307 experimental runs with 2 or 3 cycles at 60 °C were 0.25 % Na2SO3 was added in the first cycle,
308 as suggested in the literature (Govindaraju, 2003; Raphael, 1997; Yust et al., 2003). For the
309 protein recovery from soybean EE meals, no significant differences ($p>0.05$) in the extraction
310 yield $Y_{E,c}$ were observed between the experimental runs with and without the addition of sodium
311 sulfite, thus rendering this strategy unadvisable as it incurs in additional material costs.
312 Additionally, a strong sulfur odor remained in the liquid extract, which would likely cause
313 unacceptability issues if used in a product for human consumption.
314 Figure 4 presents two randomly selected samples where the recovered protein profiles
315 were analyzed by means of the SDS-PAGE method (note that similar results were obtained
316 throughout the rest of the analyzed aliquots, see section 2.2.2). Here, no noticeable differences
317 are observed at the distribution of the protein molecular weights nor between extraction cycles
318 neither between operating temperatures. Soybean proteins can be classified according to their
319 sedimentation coefficients, and three main groups can be appreciated. The 7S fraction consists of
320 the globulin subunits α (67 kDa), α' (71 kDa) and β (50 kDa); while the 11S fraction comprises
321 two main subunits with molecular weights of 35 and 20 kDa. The 7S (β-conglycinin) and 11S
322 (glycinin) fractions represent approximately 80 % of the total soybean proteins (Nishinari et al.,
323 2014). The molecular weight of proteins in the 2S fraction (conglycin) is the range of 8-20 kDa.
324 These proteins confer to the product foaming, emulsification and water holding capacity (Tay et
325 al. 2006), and contain essential amino-acids as a nutrition source (Hidayat et al. 2011).
326
327 *3.2 Overall performance evaluation of the pH-shifting process*
328 The overall performance of the ph-shifting process was analyzed as reported in Table 2
329 by computing the isoelectric precipitation yield Y_P (%), the protein recovery yield Y_T (%), the
330 productivity P_T (kg product/kg EE meal), and the protein concentration in the final product C_T

(%, in dry base). No significant differences were found between the yields and productivity when comparing the process performance for high and low precipitation temperatures. Then, savings on cooling services may be achieved if the operative temperature of the precipitation stage was only lowered to around 20 ºC (instead of the usually proposed value of 0 ºC).

Phosphoric acid was used in additional experimental runs to test the feasibility of replacing the hydrochloric acid for lowering the pH of the solution during the precipitation stage. No significant differences were found for the yields and productivity between experimental runs with phosphoric and hydrochloric acids. Therefore, in order to avoid potential health risks associated to the usage of hydrochloric acid in food products for human consumption, precipitating with phosphoric acid may constitute a technically feasible alternative.

The overall performance of the pH-shifting process was furthermore evaluated with respect to the operating conditions during the alkaline extraction stage, as reported in Table 3, by means of the protein recovery yield Y_T (%), the productivity P_T (kg product/kg EE meal), and the protein concentration in the final product C_T (%, in dry base). It was observed that the extraction temperature does not significantly impact the overall process performance, as similar values of the recovery yield and productivity are obtained. During soy protein isolate production, Rickert et al. (2004) revealed that soy isoflavone proteins content decreased at the final protein product when the extraction temperature increased at the same pH value. Therefore, it may seem preferable to operate at a lower temperature at the extraction stage, since fewer resources would be consumed in heating the solvent. On the other hand, using three extraction cycles instead of two significantly increased the protein recovery yield and productivity, as a larger amount of soluble proteins are recovered in the additional 3rd extraction cycle.

Regarding the protein concentration in the final product, no significant differences were observed as a function of the tested operating conditions of the extraction and precipitation steps. Even though, the final protein concentration was larger than 60 % in most experimental runs, and

values upwards of 75 % were obtained for individual experimental runs with 3 extraction cycles at 60 ºC and precipitation at low temperature with HCl, which translates in a final product with high value-added.

Results obtained at individual experimental runs with 3 extraction cycles at 60 ºC and precipitation at low temperature with HCl are comparable to the values reported by Wang et al. (2004) for EE meals with low protein dispersibility index with one alkaline extraction cycle under similar operating conditions. Similarly, Sunley (1995) obtained higher yields and final protein contents when using hexane-defatted soy white flakes as raw material, aided by the lower residual oil content of the flakes. As analyzed samples of Argentinian soybean EE meals presented lower initial protein contents, additional extraction cycles were here required to secure similar values of the overall process yields.

Even though lipid reduction was not an explicit target of the ph-shifting process, similar percentage values of the lipid content were found between the EE meals and the protein products, according to the values reported by Godoy et al. (2019). The mass of the EE meal used as raw material was around double that of the final freeze-dried protein product, and consequently, the residual EE meal. Therefore, through a mass balance, it is found that the lipid mass was almost halved between both the residual EE meal and the protein product. This split of the lipid mass occurred as the hot water emulsified part of the oil during the alkaline leaching step, that then precipitated because of the pH-shift. The rest of the lipid mass remained in the residual EE meal forming lipid-protein interactions, which cause an undesirable reduction of the foaming capacity of the protein product (Lamsal et al., 2006). Consequently, the methodology here implemented allowed overcoming the disadvantage of using EE meals in the pH-shifting process associated with its larger lipid content (with respect to other commonly used raw materials), by halving the lipid mass of the final protein product.

4. Conclusions

In this work, the performance evaluation of the pH-shifting process for obtaining protein products from soybean extruded-expelled meals was addressed. The methodology here proposed intends to provide a novel alternative for further value-adding at a large number of small to medium-sized processing plants located in the Argentinian central region, for which the EE meals are a low-value byproduct of the extrusion-expelling process for producing soy oil.

Different operating conditions at the alkaline extraction stage were comprehensively tested, including 2 and 3 cycles at 55, 60 and 65 ºC with and without sodium sulfite. At the isoelectric precipitation stage, the impact of two operating temperatures of 0 ºC and 20 ºC was evaluated, while using hydrochloric and phosphoric acids for lowering the pH. The results of several experimental runs were processed in order to determine which ones significantly impact different indicators of the process performance, including the extraction and overall yield, productivity and protein concentration in the final product.

Higher temperatures and the addition of a 3rd cycle increased the alkaline extraction yield. However, the isoelectric precipitation yield, protein recovery yield and productivity did not present significant differences with respect to the extraction temperature, being then preferred operating at a lower extraction temperature in order to decrease the heating requirement of this stage. At the isoelectric precipitation, the operating temperature did not significantly affect the performance indicators when using hydrochloric acid. On the other hand, the use of phosphoric acid as precipitation media may be further evaluated as a feasible alternative since similar yields and productivity were observed, while being its usage safer in food products for human consumption.

A compromise alternative from the technical viewpoint comprises extracting with 3 cycles at 60 ºC, followed by a precipitation at low temperature using hydrochloric acid. These operating conditions enable achieving an overall protein recovery yield of 45-50 % and a

productivity of 0.25-0.28 kg of protein product per kg of soybean EE meal, with a final protein concentration mostly larger than 60 % and the possibility of getting values upwards of 75 %. In this context, the industrial scaling of the protein recovery from soybean EE meals by means of the pH-shifting process should take into consideration the economics of the project as well as the environmental impact of the implemented solution.

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Tables

Table 1 Characteristics of the soybean EE meals obtained from four Argentinian processing plants (different letters in a row indicate significant differences between samples)

Composition	N1	N2	N3	N4
Protein (%)	43.06 ± 0.45 (b)	44.80 ± 1.52 (a,b)	43.75 ± 0.21 (a,b)	47.40 ± 0.81 (a)
Moisture (%)	7.55 ± 0.78 (a)	5.95 ± 1.49 (a)	7.69 ± 0.33 (a)	6.97 ± 1.25 (a)
Crude oil content (%)	6.74 ± 0.49 (b)	7.83 ± 0.89 (a)	7.53 ± 0.66 (a)	6.26 ± 0.68 (b)
KOH protein solubility (%)	87.80 ± 0.63 (a)	87.91 ± 0.17 (a)	89.31 ± 1.45 (a)	88.46 ± 0.63 (a)
Aflatoxins (µg/g)	0.0069 ± 0.001	<0.0017	0.0046 ± 0.001	<0.0017
Urease activity (ΔpH)	0.071 ± 0.0073	0.093 ± 0.0045	0.046 ± 0.0049	0.026 ± 0.0031

507

Table 2 Performance of the pH shifting process for different operating conditions at the isoelectric precipitation stage (different letters in a column indicate significant differences for different operating conditions)

Conditions at the isoelectric precipitation stage	Isoelectric precipitation yield - Y_P(%)	Protein recovery yield - Y_T (%)	Productivity of the pH-shifting process - P_T(kg/kg)	Protein concentration in the final product - C_T (%)
Precipitation with HCl at low temperature	58.40 ± 11.82 (a)	48.21 ± 8.08 (a)	0.2701 ± 0.0582 (a)	63.92 ± 10.0 (a)
Precipitation with HCl at high temperature	54.29 ± 9.50 (a)	41.33 ± 9.20 (a)	0.2295 ± 0.0437 (a)	60.93 ± 4.45 (a)
Precipitation with H_3PO_4 at low temperature	54.40 ± 10.93 (a)	47.11 ± 8.01 (a)	0.2715 ± 0.0496 (a)	54.48 ± 8.01 (a)
p-value	0.673	0.243	0.289	0.574

508

509

Table 3 Performance of the pH shifting process for different operating conditions at the alkaline extraction stage (different letters in a column indicate significant differences for different operating conditions)

Conditions at the alkaline extraction stage	Isoelectric precipitation yield - Y_P(%)	Protein recovery yield - Y_T (%)	Productivity of the pH-shifting process - P_T(kg/kg)	Protein concentration in the final product - C_T (%)
Extraction at 55 °C	72.30 ± 2.19 (a)	52.53 ± 1.59 (a)	0.3062 ± 0.0034 (a)	54.03 ± 1.59 (a)
Extraction at 60 °C	58.79 ± 9.87 (a,b)	46.22 ± 9.30 (a)	0.2585 ± 0.0605 (a)	62.83 ± 8.73 (a)
Extraction at 65 °C	49.06 ± 8.62 (b)	45.71 ± 7.77 (a)	0.2545 ± 0.0476 (a)	63.78 ± 10.0 (a)
p-value	0.008	0.580	0.490	0.629
Extraction with 2 cycles	55.81 ± 8.14 (a)	41.80 ± 7.48 (b)	0.2316 ± 0.0412 (b)	60.66 ± 6.45 (a)
Extraction with 3 cycles	57.38 ± 12.41 (a)	48.92 ± 8.14 (a)	0.2764 ± 0.0588 (a)	63.72 ± 10.2 (a)
p-value	0.737	0.039	0.045	0.528

510

511 **List of Figures**

512