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ORIGINAL ARTICLE

Common bean processing to obtain a protein ingredient for the plant-based market

Processamento de feijão carioca para obtenção de ingrediente proteico para o mercado de produtos vegetais

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Abstract

Common beans are cultivated worldwide and constitute a raw material for obtaining protein ingredients for novel product development, especially for the plant-based food market. They are naturally rich sources of proteins, ranging from 19% to 24%. This study aimed to define conditions for obtaining protein concentrate from common beans using alkaline extraction with subsequent acid precipitation. Parameters for alkaline extraction (pH, solid-to-water ratio, and stirring time) and acid precipitation (pH and stirring time) of proteins from common beans were defined. Four scaling-up tests were performed to validate the selected parameters (n=4). Results showed that the process should follow a protein water extraction step (water and bean flour ratio of 1:8, pH of 9.0, and 30 minutes stirring) followed by an acid precipitation step (pH of 5.5 and 10 minutes stirring) and spray drying. The protein concentrate obtained is a powder with 76.5% (± 3.76) protein on a dry basis, and the process presented a mass yield of 11.0% (± 0.91) and a protein yield of 39.7% (± 2.50). Techno-functional properties of water holding capacity (1.77 ± 0.05 g water/g), oil holding capacity (1.22 ± 0.05 g oil/g), emulsifying activity index (18.87 ± 0.35 m^2/g), foaming capacity (73.33 ± 6.67%), and least gelling concentration (20.00 ± 0.00 g/100g water) of the concentrate were also determined. The process for obtaining common bean protein concentrate, including the steps of alkaline extraction and acid precipitation, was defined along with its yield and the concentrate performed similarly to that of others obtained from different types of beans in terms of techno-functional properties.

Keywords: Food ingredient; *Phaseolus vulgaris* cv Carioca; Protein extraction; Protein processing; Pulses; Vegetable ingredient.

Resumo

O feijão carioca é cultivado em todo o mundo e pode ser considerado uma matéria-prima para obtenção de ingredientes proteicos para o desenvolvimento de novos produtos, principalmente para o mercado de alimentos vegetais. Ele é uma fonte naturalmente rica em proteínas, variando de 19% a 24%. O objetivo deste trabalho foi definir as condições para obtenção de concentrado proteico de feijão carioca utilizando extração alcalina com posterior precipitação ácida. Foram definidos parâmetros para extração alcalina (pH, relação sólido/água e tempo de agitação) e precipitação ácida (pH e tempo de agitação) das proteínas de feijão carioca. Quatro testes de escalonamento foram realizados para validar os parâmetros selecionados (n=4). Os resultados mostraram que o processo deve seguir uma etapa de extração de proteína com água (proporção de água e farinha de feijão de 1:8, pH de 9,0 e 30 minutos de agitação), seguida de uma etapa de precipitação ácida (pH de 5,5 e 10 minutos de agitação) e de secagem por *spray drying*. O concentrado proteico obtido se trata de um pó contendo 76,5% (± 3,76) de proteína em base seca e o processo apresentou rendimento em massa de 11,0% (± 0,91) e rendimento de proteína de 39,7% (± 2,50). As propriedades tecnofuncionais de capacidade de absorção de água (1,77 ± 0,05 g água/g), capacidade de absorção de óleo (1,22 ± 0,05 g óleo/g), atividade emulsificante (18,87 ± 0,35 m^2/g), capacidade espumante (73,33 ± 6,67%) e de formação de gel (20,00 ± 0,00 g/100g água) do concentrado também foram determinadas. O processo de obtenção do concentrado proteico de feijão carioca, incluindo as etapas de extração alcalina e precipitação ácida, foi definido juntamente com seu rendimento, e o concentrado teve desempenho semelhante ao de outros obtidos de diferentes tipos de feijão em termos de propriedades técnico-funcionais.

Palavras-chave: Ingrediente alimentício; *Phaseolus vulgaris* cv Carioca; Extração de proteína; Processamento de proteínas; Pulses; Ingrediente vegetal.

Highlights

- Common beans were used to obtain the protein ingredient
- Concentrate obtained from common beans contained 76.5% proteins
- Yield for common beans protein concentrate reached 11.0%

1 Introduction

Plant-based proteins are used as ingredients in many food formulations due to their nutritional and techno-functional properties (Gundogan & Karaca, 2020), as well as to enhance the sensory properties of the formulated foods (Lima et al., 2021; Shevkani et al., 2015).

The conventional alkaline method is the basis for the industrial production of protein concentrates and isolates (Hojilla-Evangelista et al., 2018). The unit operations involved are quite simple, in which proteins are solubilized by increasing the pH, usually at pH 8 to 11, followed by centrifugation to remove the insoluble materials. Then, the solubilized proteins are precipitated by acidification, usually at pH 4 to 5 (Hewage et al., 2022). However, process parameters must be adapted for each material since protein purity and yield are affected by processing conditions (Boye et al., 2010).

Currently, plant-based protein concentrates and isolates are mainly derived from soy and pea (Shrestha et al., 2023; Vogelsang-O'Dwyer et al., 2020); however, due to dietary restrictions and consumer preferences, food manufacturers have sought alternative protein sources (Shevkani et al., 2015).

Common beans (*Phaseolus vulgaris* L.) are cultivated and consumed worldwide (Boye et al., 2010; Karaman et al., 2022), being used for preparing protein ingredients based on their high protein content and low cost (Shrestha et al., 2023). The cultivar Carioca is the most popular type in Brazil and represents nearly 70% of the domestic market (Los et al., 2020), with a protein content ranging from 19% to 24% (Donadel & Prudencio-Ferreira,1999). In addition, these beans present minor amounts of oil (< 2%) (Donadel & Prudencio-Ferreira,1999), implying that a defatting step is not required, unlike the most used materials for obtaining protein ingredients (Lima & Mellinger, 2022).

This study aimed to define conditions for obtaining protein concentrate from a common bean (*Phaseolus vulgaris* cv Carioca) using alkaline extraction with subsequent acid precipitation. A first scaling-up effort was carried out to validate the bench process, which was associated with spray drying. The whole seed was used as an innovation in the process, thus avoiding dehulling and defatting steps, which implies fewer unit operations for the potential commercial production of the protein concentrate.

2 Materials and methods

2.1 Material

Common beans (*Phaseolus vulgaris* cv Carioca) were purchased in the local market (Rio de Janeiro, Brazil) and ground in a Perten LM3100 hammer mill with a 0.8 mm sieve opening (Perten Instruments AB, Huddinge, Sweden). The obtained flour was analyzed for moisture and protein content (N x 6.25) (AOAC, 2010) and stored under refrigeration at 6 to 8°C for further use.

2.2 Alkaline extraction

Protein extraction was performed by suspending the flour in water, adjusting the pH, stirring, and performing centrifugation at 5600 *xg* for 15 min (Thermo Scientific Heraeus Multifinge-R, Osterode, Germany). Tests were carried out in three different stages to assess the effect of pH (8.0, 8.5, 9.0, 9.5, and 10.0) using fixed parameters for flour to water ratio (1:10, 3 g/30 mL) and stirring time (60 min). Next, the stirring time (10, 15, 30, 60, and 120 min) was determined using the pH determined in the previous test and a flour-to-water ratio of 1:10 (3 g/30 mL). Subsequently, the proportion of flour and water (1:6, 1:8, 1:10, 1:12, 1:14, and 1:16) was established based on the pH and stirring time previously determined. The soluble protein content (Bradford, 1976) was determined in the extracts as a parameter for choosing the best extraction conditions, and the results were submitted to analysis of variance and Tukey's test (α=0.05).

2.3 Acid precipitation

The extract obtained according to the best conditions defined in the alkaline extraction step was subjected to acid precipitation and the effect of pH (4.0, 4.5, 5.0, 5.5, and 6.0), and time after pH adjustment (10min, and overnight storage under refrigeration) in the protein content of the obtained common bean protein concentrate (CBPC) was assessed. The precipitate was separated by centrifugation (5600 *xg* for 15 min) (Thermo Scientific Heraeus Multifinge-R, Osterode, Germany), dried in an oven with forced air at 60ºC overnight and analyzed for moisture and protein content (N x 6.25) (Association of Official Analytical Chemists, 2010). Protein yield (g protein in the concentrate/100 g protein in the flour) was also determined. The results are expressed on a dry basis and were submitted to an analysis of variance and Tukey's test when applicable (α=0.05).

2.4 Process scaling-up

Scale-up experiments were performed (10 times increase, 1 kg flour/batch) based on the best conditions determined in the alkaline extraction and acid precipitation steps. A washing step was added to reduce the acidity of the product; therefore, the precipitate was re-suspended in water (1:1 in weight), stirred for 10 min, and centrifuged (5600 *xg* for 15 min) (Thermo Scientific Heraeus Multifinge-R, Osterode, Germany). The precipitate protein was dried in a pilot-scale spray dryer (NIRO Atomizer, Soborg, Dinamarca) with an inlet air temperature of 160 ºC, outlet air temperature of 85 ºC, air flow of 460 m/s, and process flow of 10 L/h. The operations were repeated in four batches. Moisture, protein content (N x 6.25) (Association of Official Analytical Chemists, 2010), mass yield (g concentrate/100g of flour), and protein yield (g protein in the concentrate/100g protein in the flour) were determined. The results were submitted to an analysis of variance and Tukey's test (α=0.05).

2.5 Techno-functional properties

The CBPC obtained was characterized based on water and oil holding capacities, emulsifying activity index, foaming capacity, and least gelling concentration (Silva et al., 2022).

3 Results and discussion

3.1 Alkaline extraction

Soluble protein values were higher in pH, ranging from 9.0 to 9.5 (Figure 1A). pH of 9.0 was chosen for the extraction process, considering a milder extraction condition and less chemical addition. As for the time, after 30 minutes, no increase in the amount of extracted protein was observed (Figure 1B), hence, the shortest time was chosen for the process. For the flour-to-water ratio, although statistical differences occurred for protein recovery (Figure 1C) among the tested values, the beneficial effect of using less water in an industrial process was also considered, which influences the effluent volume. Thus, a 1:8 ratio was used to minimize this effect, since 1:6 provided a too viscous material that was difficult to deal with.

Working with four different types of beans (*P. vulgaris*), Gundogan & Karaca (2020) and Hojilla-Evangelista et al. (2018) observed maximum protein solubility at pH 9.0 and pH 10.0, respectively. However, both extractions were performed with defatted beans at a higher water ratio (1:10) and for longer periods (60 to 180 minutes) compared with the conditions used in our study.

The processing parameters determined for the alkaline extraction in this research are also less harsh than those reported by Karaman et al. (2022), who worked with five genotypes of common bean and reached maximum protein content for extraction at pH 11.0 and 0.4% salt solution for producing protein concentrate.

The usefulness of the milder conditions obtained in our experiment is apparent, as increasing extraction pH may harm the functionality of the concentrate (Vogelsang-O'Dwyer et al., 2020). In addition, a shorter time in the extraction step and less water in the process can account for a better solution, considering the environment and production expenses.

3.2 Acid precipitation

Protein content in the concentrate increased with higher precipitation pH; however, protein yield was higher in pH ranging from 4.5 to 5.5 (Figure 2). Thus, pH 5.5 was chosen for acid precipitation due to less chemical use.

The isoelectric point of most plant proteins ranges from pH 4 to 5 (Hewage et al., 2022) and several researchers used pH 4.5 for protein precipitation from different types of beans (Gundogan & Karaca, 2020; Los et al., 2020; Ribeiro et al., 2009; Shevkani et al., 2015). The pH value of 5.5 observed in our research confirmed the importance of adapting process parameters for each material since a milder condition was defined.

The precipitation process showed that flocculation occurs as soon as pH 5.5 is reached. Thus, an experiment was performed to confirm the effect of time after pH adjustment on the protein content of the dry concentrate obtained. There was no significant difference between treatments (Table 1), which indicates that agitation for 10 minutes after pH adjustment is sufficient for the precipitation process.

Table 1. Effect of time after pH adjustment on protein content of CBPC.

Time (after pH adjustment and stirring)	Protein (%, dry basis)
10 min	75.38 ± 0.95 ns
Overnight storage under refrigeration (~10ºC)	75.70 ± 0.03 ns

ns= not significant (t student, $p > 0.05$).

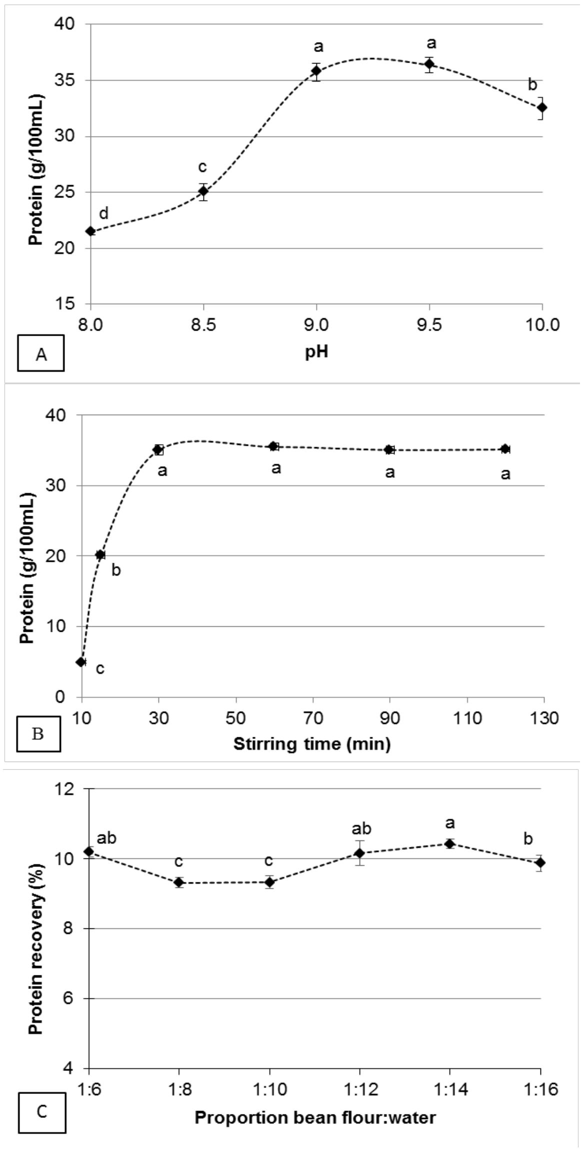

Figure 1. (A) Effect of pH on common bean protein extraction; (B) Effect of time on common bean protein extraction; (C) Effect of bean flour:water proportion on common bean protein extraction. Different letters in the same line mean significant differences between samples (Tukey test, α=0.05).

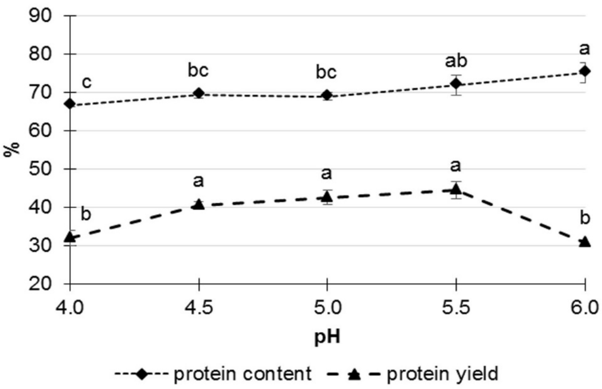

Figure 2. Effect of precipitation pH on protein content of CBPC and on protein yield. Different letters in the same line mean significant differences between samples (Tukey test, α=0.05).

3.3 Process scaling-up

The CBPC obtaining process (Figure 3) showed a mass yield of 11.0%, a protein yield of 39.7%, and a CBPC protein content of 76.5% (Table 2). Considering an initial protein content of 20.0% in the common bean, the process increased its value by 3.8 times.

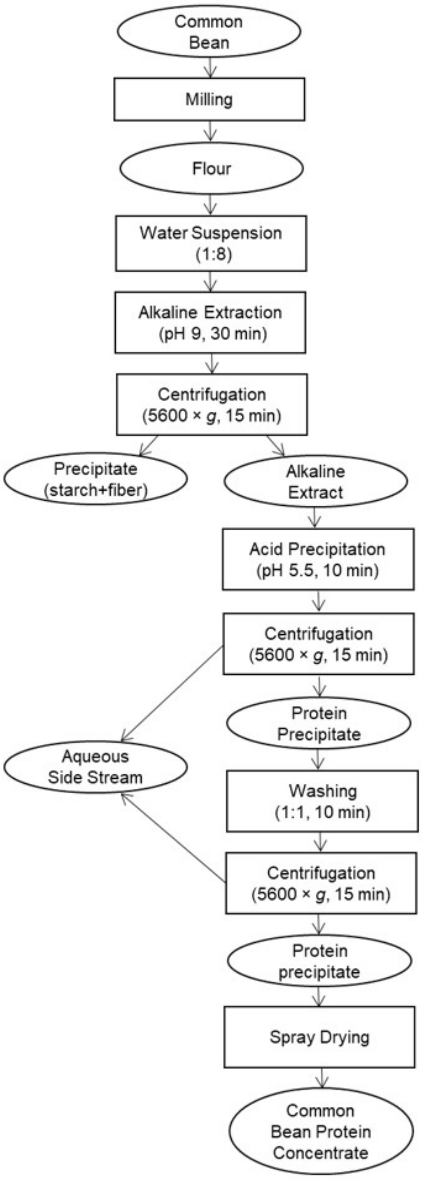

Figure 3. Process flowchart for common bean protein concentrate (CBPC) obtainment. Squares represent the processing steps; circles are the main products and by-products.

Table 2. Protein content, yield, and recovery of common bean protein concentrate production process.

Batch number*	1	2	3	4	mean ± sd
Protein (% dry basis, N x 6.25)	78.68	80.14	75.36	71.69	76.47 ± 3.76
Mass yield (%)	10.48	8.80	10.84	11.41	11.02 ± 0.91
Protein recovery (%)	40.97	35.70	40.54	37.62	39.71 ± 2.50

*Each batch corresponds to 1 kg of flour processing.

Reported results on protein content of concentrates obtained from different types of beans (*P. vulgaris*) range from 56 to 89% with protein yield ranging from 9.7% to 77.0% (Gundogan & Karaca, 2020; Hojilla-Evangelista et al., 2018; Karaman et al., 2022; Los et al., 2020; Quintero-Quiroz et al., 2022; Ribeiro et al., 2009; Shevkani et al., 2015). However, the comparison with those results is difficult due to differences in processing parameters, such as the steps of dehulling and defatting, as well as the use of temperature, ultrasound, dialysis, and enzymes. Protein content and yield are easily affected by processing conditions, which could explain the variation in results (Boye et al., 2010).

Although the conventional method of alkaline extraction of the proteins used in the process described, the lack of need for the defatting step of the material for common beans is a great technological advantage. Organic solvents, particularly n-hexane used for defatting, contribute to air pollution and are reported to be toxic (Hewage et al. 2022).

3.4 Techno-functional properties

Even though CBPC presented a lower oil-holding capacity and less gelling concentration (Table 3) than the protein concentrates obtained from five different types of beans by Gundogan & Karaca (2020), the water-holding capacity, emulsifying, and foaming activities were similar. Those differences may have occurred due to the process conditions to obtain the concentrates that affect the techno-functional properties of the materials. The results followed our previous work comparing the properties of eight protein ingredients from different legumes, including common bean, which showed a water holding capacity ranging from 1.34 to 4.52 g water/g, oil holding capacity from 1.22 to 2.84 g oil/g, emulsifying activity index from 14.03 m^2/g to 19.39 m^2/g, foaming capacity from 73.33% to 93.33%, and least gelling concentration from 12% to 20% (Gouvêa et al., 2023). These are key properties of the ingredients for influencing food production in general, especially in meat analog products (Lima et al., 2021).

Table 3. Techno-functional properties of common bean protein concentrate.

Property	Value
Water holding capacity (g water/g)	1.77 ± 0.05
Oil holding capacity (g oil/g)	1.22 ± 0.05
Emulsifying activity index (m^2/g)	18.87 ± 0.35
Foaming capacity (%)	73.33 ± 6.67
Least gelling concentration (g/100g water)	20.00 ± 0.00

4 Conclusions

Conventional methods employed for protein obtainment from different food materials involve some drawbacks, including the production of wastewater causing environmental hazards, consumption of chemicals, and low extraction yield. The process reported in this paper helps diminish these bottlenecks for food industries since 76.5% protein was achieved in the common bean ingredient using milder pH conditions for the extraction and precipitation steps, as well as less water and shorter time for the protein extraction. In addition, using the whole grain in the process, without the need for dehulling and defatting steps, has an impact on production costs. Consumers have sought a greater variety of foods in their diet; therefore, common beans are available and affordable for manufacturers as a raw material for obtaining ingredients for novel product development.

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