Ultrasonics Sonochemistry 129 (2026) 107848

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

journal homepage: www.elsevier.com/locate/ultson

Extraction and characterization of protein fraction from whole chickpea (*Cicer arietinum* L.) flour through ultrasound assisted alkaline method

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ARTICLE INFO

Keywords:
Box-Behnken
Technological properties
Pulse protein
Sonoprocessing

ABSTRACT

Ultrasound-assisted alkaline extraction was applied to whole chickpea flour to optimize processing conditions and enhance the technological properties of plant protein. A Box-Behnken experimental design systematically evaluated the effects of solid-to-liquid ratio (1:05–1:15 g mL^{-1}), pH (7.0–12.0), and ultrasonication time (10–40 min), consisting of 15 runs. The most desirable assay, based on protein yield and solubility, was a ratio of 1:12.5 (g mL^{-1}), pH 9.5, and a 40-minute ultrasonication time. The extracts from the control assay (EC), the desirable one (ED), and three other experimental conditions (E6, E10, and E11) were characterized by their technological, chemical, physicochemical, and morphological properties. Among the five extracts, EC showed the highest protein content (65.13 ± 0.97%), and the ultrasound-assisted extraction method did not increase the yield but reduced the extraction time and improved the functional properties. Solubility increased from 77.33 ± 2.15% to 89.07 ± 3.06% under the optimized condition (ED); water absorption capacity from 1.76 ± 0.05% to 2.55 ± 0.02%; oil absorption from 1.42 ± 0.02% to 1.96 ± 0.06%; and foam stability from 58.04 ± 1.55% to 65.87 ± 2.75%, highlighting ED and E6. The enhanced functionality is attributed to ultrasound-induced structural modifications associated with acoustic cavitation. The optimized process provides advantages for industrial food systems, including improved efficiency, and potential sustainability benefits. Chickpea proteins show strong potential for application in baked products, pasta, meat analogues, dairy alternatives, and emerging technologies such as 3D-printed foods. Future studies should focus on process scale-up and techno-economic evaluation to support industrial implementation.

1. Introduction

The nutritional profile of legumes has significant importance in the human diet, particularly regarding the quality and bioavailability of proteins and minerals. Protein-energy malnutrition remains widespread worldwide, especially in developing countries [1]. Legumes are rich in macronutrients, particularly proteins (14.4 to 34.1%), while presenting relatively low energy density (301 to 359 kcal 100 g^{-1}), making them suitable for the development of products targeted at vulnerable populations [2]. Over the last decade, consumer demand for legume-based products with high protein and fiber content, low glycemic index, gluten-free and antioxidants potential has increased significantly [1].

Chickpea is the third most harvested pulse worldwide, with approximately 16.5 million tons produced annually, representing a crop of global relevance. Production is concentrated in South and Southeast Asian, accounting for 83.6% of global output, with India standing as the largest importer and second-largest exporter [3]. Although production is

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https://doi.org/10.1016/j.ultsonch.2026.107848
Received 12 January 2026; Received in revised form 11 March 2026; Accepted 5 April 2026
Available online 6 April 2026

dominated by these regions, chickpea cultivation has recently expanded to other areas. In Brazil, for example, commercial production is relatively recent, with virtually no cultivated area until 2010. Advances in agronomic research and suitable production systems have enabled its expansion since 2015, increasing its importance for both domestic consumption and international trade [4].

Chickpeas play a fundamental role in diet of populations in Indian and Sub-Sahara Africa due to their affordability and nutritional quality [5]. Furthermore, interest in plant proteins as food ingredients has grown because of their high production volume, low cost, balanced essential amino acid composition, high bioavailability and low allergenicity [6]. Chickpea protein has been incorporated into enriched foods such as pasta, breads, ready-to-eat snacks, and protein beverages [2,7]. These proteins are technologically attractive due to their functional properties, mild flavor, and light color [8]. The emulsifying properties of chickpea protein isolates have been reported as comparable to or superior to pea or soy protein [9], and their incorporation into rice-based gluten-free pasta enhances nutritional and cooking performance [10].

Alkaline extraction followed by isoelectric precipitation is commonly used to recover plant proteins, based on their high solubility at alkaline pH and reduced solubility near the isoelectric point (pH 4.0—5.0) [11]. Using this approach, yields of up to 73% [12] and 85.4% [8] have been reported for chickpea protein. However, the alkalization step typically requires approximately 1 h in conventional processes, prolonging extraction time and increasing energy demand.

The overcome these limitations, alternative strategies have been explored to improve extraction efficiency and functional quality. Ultrasound acts through cavitation-induced shear forces, and shockwaves generated by bubble collapse, which can disrupt plant cell walls, enhance solvent penetration, and facilitate mass transfer [13]. Ultrasonication has also been reported to improve solubility and emulsifying properties of plant proteins [14]. According to [15], ultrasound processing is an emerging green technology with broad application potential in the food industry. Studies using ultrasound as a pre-treatment for chickpea flour have reported enhanced extraction of non-protein components (starch and dietary fiber), as well as improved water- and oil-holding capacity and foaming properties [16]. Comparisons between ultrasound-assisted and conventional alkaline extraction have suggested that ultrasound may provide a more efficient, environmentally friendly, and time-saving alternative without negatively affecting technological properties.

Although previous studies have investigated protein extraction from defatted chickpea flour [17–19] and the use of ultrasound as pre- or post-treatment, there is still a lack of systematic investigation into ultrasound-assisted alkaline extractions directly applied to whole chickpea flour, combined with process optimization and comprehensive characterization of the resulting protein fraction. The use of whole flour is particularly relevant from an industrial perspective, as defatting step increases solvent consumption, processing time, equipment requirements, and environmental impact.

Therefore, this study aims to optimize ultrasound-assisted alkaline extraction followed by isoelectric precipitation for protein recovery from whole chickpea flour using a Box-Behnken experimental design. In addition, the study evaluates the effects of ultrasonic irradiation during the alkalization step on extraction efficiency and on the technological, chemical, physicochemical, and morphological properties of the selected protein extracts. By eliminating the defatting step and intensifying the extraction process, this work proposes a more sustainable and economically viable strategy for industrial plant protein production.

2. Materials and methods

2.1. Plant material

Whole chickpea flour was provided by Milhão Ingredients company (Goianira, Goiás, Brazil). The product is part of the company's portfolio, as an ingredient processed and industrially packaged, suitable for applications in snacks, soups, pasta, and baking formulations.

2.2. Chickpea protein extraction

The protein extracts from whole chickpea flour precipitated were obtained by the alkaline extraction/isoelectric precipitation method described by [20] with adaptations, as shown in the diagram in Fig. 1. The flour was dispersed in distilled water at a ratio of 1:10 (w v^{-1}) and with a solution adjusted to pH of 9.0 using 1.0 M sodium hydroxide (NaOH, analytical grade, ACS standard, Neon, São Paulo, Brazil), considering the high solubility of legume proteins in alkaline medium. The mixture was stirred for 1 h using a magnetic stirrer (TE-0851, Tecnal, Brazil) at room temperature.

After this period, the mixture was centrifuged at 7008 × *g* for 10 min, and the supernatant was collected and adjusted to pH 4.5 with 1.0 N hydrochloric acid (HCl, 37%, analytical grade, ACS standard, Neon, São Paulo, Brazil), as the isoelectric point of chickpea proteins lies between pH 4.0 and 5.0. The acidic solution was centrifuged again at 7008 × *g* for 10 min to obtain the protein precipitate. The precipitate was washed with distilled water, centrifuged again, stored at −30 °C, and freeze-dried.

Total protein content was determined by the micro Kjeldahl method [21]. Protein (%) was calculated according to equation:

$$Protein\ (\%) = Nitrogen\ (\%)\ x\ 6.25 \quad (1)$$

where 6.25 is the nitrogen-to-protein conversion factor.

2.3. Experimental design for protein extraction with ultrasound

The ultrasound-assisted protein extraction process from whole chickpea flour was evaluated using response surface methodology based on a Box–Behnken design [22]. The independent variables were solid/liquid ratio (X_1), pH (X_2), and extraction time under cavitation (X_3), evaluated at three coded levels. The actual values were selected based on preliminary experiments and [18], totaling 15 experimental runs with three repetitions at the central point (Table 1).

The procedure followed the conventional extraction described in Section 2.2 (Fig. 1), with the addition of ultrasound during the alkalization step. Whole chickpea flour dispersions were prepared at solid/liquid ratios of 1:05, 1:10, and 1:15 (w v^{-1}). The pH was adjusted to 7.0, 9.5, or 12.0 using 1.0 M NaOH.

Ultrasonic treatment was performed using an ultrasonic bath (USC-1850, Unique, Indaiatuba, Brazil) operating at a frequency of 25 kHz and nominal ultrasonic power of 154 W. Sonication was conducted for 10, 25, or 40 min. To control the temperature increase generated during sonication, ice packs were placed inside the ultrasonic bath and replaced whenever necessary to maintain the extraction temperature at 10 ± 2 °C, minimizing thermal effects during cavitation. After sonication, the dispersions were centrifuged, and the remaining steps followed the conventional alkaline extraction procedure.

The response variables, or dependent variables, evaluated were protein yield (%) and protein solubility (%). The yield was determined by Eq. (2), following the methodology of [23].

$$Protein\ yield\ (\%) = \left(\left(\frac{M_1}{M_2}\right) x\ 100\right) x \left(\frac{P_1}{P_2}\right) \quad (2)$$

where: M_1 is the mass (g) of protein on a dry basis, collected at the end of the extraction; M_2 is the initial mass (g) of whole chickpea flour used in the extraction; P_1 is the extracted protein content (%); P_2 is the whole chickpea flour protein content (%).

For solubility, protein dispersions were prepared with 0.2 g of each extracted sample (based on the protein content of the extract) in 20 g (1%, w w^{-1}) of distilled water, and pH was adjusted to 7.0 with 0.1 M NaOH or 0.1 M HCl. The dispersions were stirred on magnetic stirrers for

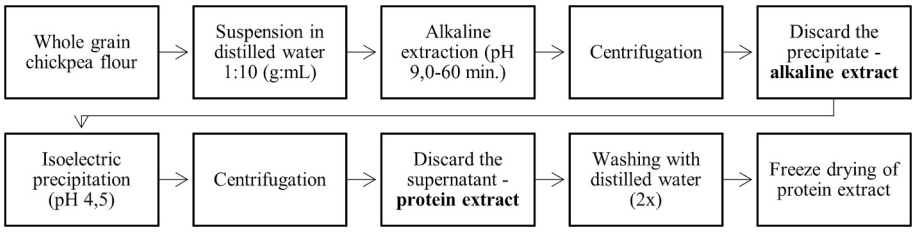

Fig. 1. Diagram of the chickpea protein extraction process using the alkaline method followed by isoelectric precipitation.

Table 1
The Box-Behnken design was utilized in the assay for protein extraction from whole chickpea flour by the alkaline/isoelectric precipitation method, assisted by ultrasound, as a function of the solid/liquid ratio, pH, and extraction/ultrasound exposure time.

Experiment	Coded value			Real value			Responses	
	X_1	X_2	X_3	Ratio [a]	pH [b]	Time [c]	Protein yield [d] (y_1)	Solubility [d] (y_2)
1	−1	−1	0	1:05	7.0	25	42.3	79.2
2	1	−1	0	1:15	7.0	25	51.9	77.6
3	−1	1	0	1:05	12.0	25	48.3	60.7
4	1	1	0	1:15	12.0	25	52.7	63.5
5	−1	0	−1	1:05	9.5	10	45.6	80.6
6	1	0	−1	1:15	9.5	10	52.8	79.6
7	−1	0	1	1:05	9.5	40	48.7	81.0
8	1	0	1	1:15	9.5	40	52.8	81.8
9	0	−1	−1	1:10	7.0	10	45.0	83.7
10	0	1	−1	1:10	12.0	10	55.0	79.9
11	0	−1	1	1:10	7.0	40	48.6	81.7
12	0	1	1	1:10	12.0	40	49.5	73.4
13	0	0	0	1:10	9.5	25	50.9	81.0
14	0	0	0	1:10	9.5	25	51.0	78.1
15	0	0	0	1:10	9.5	25	52.7	81.5

[a] g mL^{-1}; [b] dimensionless; [c] min; [d] %.

1 h at room temperature to solubilize the proteins in the medium. Then, the mixtures were centrifuged at 4180 × g for 10 min at room temperature, and 5 g aliquots were collected for nitrogen content determination by the micro Kjeldahl method, with a conversion factor of 6.25 [21].

The percentage of protein solubility was quantified using Eq. (3) [24].

$$\textit{Protein solubility}\ (\%) = \frac{\textit{Protein content of the supernatant}\ (\%)}{\textit{Total protein conten t}(\%)} \quad (3)$$

Through desirability testing, conducted using Statistica software (Statsoft Inc., version 7.10, Tulsa, OK, USA), the most desirable conditions were determined, which combined the highest protein yield in extraction with the highest protein solubility. The most desirable experiment (ED) and three other experiments were selected to determine the technological, chemical, physicochemical, and morphological properties of the protein extracts and compare the results with the control experiment extract (EC), which involved traditional alkaline extraction without ultrasound. These aspects are important for the food industry, and the information gathered can expand the applications of protein extracts obtained from whole chickpea flour.

The experiment 6 (E6) was chosen because the levels of solid/liquid ratio and pH were close to those of the most desirable experiment (ED), but with a shorter extraction time; experiment 10 (E10) was selected due to its maximum protein yield considered in isolation; and experiment 11 (E11) was chosen because it presented results close to the protein yield and solubility of E10, but with different pH and cavitation time, aiming to observe how pH and cavitation time can affect the properties of the protein fraction. For the comparison of properties, a completely randomized design was used, with 5 treatments (Table 2), each with 3 repetitions, totaling 15 experimental units. All the analytical determinations described below were performed in triplicate.

Table 2
Selected treatments for characterization of the technological, chemical, physicochemical and morphological properties of the protein extracts.

Tratament s	Extraction levels
EC (Control experiment)	1:10 g mL^{-1}; 9.5; 60 min.
ED (Desirable experiment)	1:12,5 g mL^{-1}; 9.5; 40 min.
E6 (Experiment 6)	1:15 g mL^{-1}; 9.5; 10 min.
E10 (Experiment 10)	1:10 g mL^{-1}; 12.0; 10 min.
E11 (Experiment 11)	1:10 g mL^{-1}; 7.0; 40 min.

2.4. Technological, chemical, physicochemical and morphological properties of protein extracts

2.4.1. Water and oil absorption capacity

Water absorption capacity (WAC) and oil absorption capacity (OAC) were determined following [24] methodology, with adaptations. Protein extracts (0.5 g of protein on dry basis) were dispersed in 4 g of soybean oil or distilled water, in 50 mL centrifuge tubes and vortexed (VWR Vortex Mixer, VWR International, Radnor, PA, USA) for 10 s every 5 min during 30 min at maximum speed. Then, every sample was centrifuged (TG16-WS, Xiangyi Centrifuge, Changsha, Hunan, China) at 1000 × g for 15 min, followed by supernatant decantation. WAC and OAC were calculated using Eq. (4):

$$\textit{WAC or OAC}\ (\%) = \frac{(\textit{wet sample weight}\ (g) - \textit{dry sample weight}\ (g))}{\textit{dry sample weight}\ (g)} \quad (4)$$

2.4.2. Foam capacity and stability

According to [25] methodology with adaptations; a sample of 65 ml solution with 1% protein (g g^{-1}) (based on dry extract protein content) was prepared with pH adjusted to 7.0 with 0.1 M NaOH or 0.1 M HCl.

The solution was agitated for 1 h at room temperature. Therefore, an aliquot of 15 ml of this solution was transferred to a beaker and then homogenized for 2 min at 3870 × g using homogenizer (3000 N/10, Dremel, Mexico City, Mexico) with the tip of the equipment at air–water interface. The foaming solution was immediately transferred to a graduated cylinder, using an additional 10 ml of distilled water to rinse the remaining solution in the beaker, and the initial foam volume and volume after 30 min were recorded. Foam capacity (FC) was calculated using Eq. (5), and foam stability (FS) was calculated using Eq. (6).

$$FC\ (\%) = \left(\frac{foam\ volume\ (mL)}{initial\ sample\ volume\ (mL)} \right) x\ 100 \quad (5)$$

$$FS\ (\%) = \left(\frac{foam\ volume\ after\ 30\ min\ (mL)}{initial\ sample\ volume\ (mL)} \right) x\ 100 \quad (6)$$

2.4.3. Emulsifying activity and stability indices

Emulsifying activity (EAI) and emulsifying stability (ESI) indices were determined with adaptations from [17] methodology. A sample of 25 g of protein solution with a concentration of 0.25% (g g^{-1}) was prepared and neutralized as described earlier. Therefore, a 5 g aliquot of the solution was homogenized with 4 g of soybean oil using a homogenizer (3000 N/10, Dremel, Mexico City, Mexico) for 2 min at 2688 × g. From this emulsion, an aliquot of 50 µL was immediately taken from the bottom of the tube and diluted in 7.5 mL of 0.1% sodium dodecyl sulfate (SDS). The solution was vortexed for 10 s and transferred to a glass cuvette for absorbance measurement, at 500 nm, using spectrophotometer (S-2000, Bel Spectro, Piracicaba, Brazil). After 10 min, another 50 µL sample was taken and the same procedures were followed. EAI and ESI were calculated using Eqs. (7) and (8).

$$EAI\ (m^2\ g^{-1}) = \frac{2\ x\ 2\ x\ 303\ x\ A_0\ x\ N}{C\ x\ \varphi\ x\ 1000} \quad (7)$$

$$ESI\ (min) = \left(\frac{A_0}{\Delta A} \right) x\ t \quad (8)$$

where: A_0 is the diluted emulsion absorbance after homogenization; N is the dilution factor (×150); C is the protein mass per volume (g mL^{-1}); φ is the emulsion oil volume fraction; ΔA is the absorbance variance between 0 and 10 min ($A_0 - A_{10}$); and t is the time interval (10 min).

2.4.4. Chemical composition

The proximate composition of the raw material (whole chickpea flour) and the experimental protein fractions were determined according to [21]. moisture content by method 925.45b, dried in oven at 105 °C; lipid content by Soxhlet extraction following method 920.39; total nitrogen content by micro Kjeldahl method, with conversion factor of 6.25 (method 960.52) and ash content by incineration in muffle furnace at 550 °C (method 923.03). Total carbohydrate content was obtained estimating the difference between 100 and the sum of the other components, following [26] methodology. For the raw material only, the total dietary fiber content was also determined using the enzymatic–gravimetric method (method number 985.29) [21].

2.4.5. Differential scanning calorimetry (DSC)

Thermal analysis was determined by Differential Scanning Calorimetry (DSC) (Q20, TA Instruments, New Castle, UK) following methodology described [27], with modifications. 2 mg (db) of protein extract was weighed into an aluminum sample pan and mixed with distilled water (6 µL). The sample pan was hermetically sealed under specific press (Tzero press, TA Instruments, New Castle, UK) and kept at room temperature for 2 h, allowing uniform water distribution. Therefore, analysis was performed by heating the sample from 35 to 150 °C, to obtain enthalpy change (ΔH), and denaturation temperature peak (Tp). An empty pan was used as reference during measurement. To register data from the analysis, a TA Universal Analysis software (TA Instruments, New Castle, UK) was used.

2.4.6. Fourier transform infrared spectroscopy (FTIR)

The analysis was conducted using FTIR spectrometer (Spectrum 400, PerkinElmer, USA), operating from 400 to 4000 cm^{-1}, performing 12 scans with resolution of 4 cm^{-1}. A sample of 1 mg was mixed with 100 mg of potassium bromide (KBr), compressed under high pressure (~8 tons) until a translucent pellet, without deformations, were formed [28].

2.4.7. Granule morphology

Protein granules morphology was evaluated using scanning electron microscopy (SEM) analysis (JSM-6610, Jeol, Tokyo, Japan). The protein extract was diluted in ethyl alcohol (1%) and coated with gold. Then images were taken with 104 applications at 100x and 1500x magnification.

2.5. Statistical analysis of data

The statistic application (Statsoft, version 7.10, Tulsa, USA) was employed for multiple regression analysis, construction of 3D graphs, and desirability testing to determine the most desirable combination of variables regarding both the highest protein yield and solubility simultaneously. The models were validated using analysis of variance and Tukey's test ($p < 0.05$) to compare the means of the technological, chemical, physicochemical, and morphological characteristics of the selected protein extracts in the response surface assay.

3. Results and discussion

3.1. Characterization of the raw material

The whole chickpea flour used as a raw material for protein extraction presented proximate composition data (Table 3) within the expected range, compared to the literature. The data are consistent with the research by [29], who evaluated the composition of Kabuli chickpeas produced in Pelotas-RS, where they observed 5.8% lipids, 18.4% protein, and 63.4% total carbohydrates. The values of moisture, ash, and lipids also agree with those of [30], despite the different variety. However, the protein content reported by these authors (22.34%) was higher than that determined in the present study (18.79%).

The total carbohydrate content determined in whole chickpea flour was 62.13%, with 20.55% corresponding to the total dietary fiber content and the remainder (41.58%) to other carbohydrates (Table 3), which may mainly include starch and, to a lesser extent, sugars. The total dietary fiber content is within the range indicated in the literature, which varies from 18 to 22% in whole chickpeas, highlighting the potential use of this pulse in the food industry [31]. The soluble sugars that are most abundant in Kabuli chickpeas, used in this experiment, are sucrose, glucose, and fructose [32]. In addition to providing sweetness to foods, these sugars also play an important role in the texture, color, and preservation of many food products.

According to [32], chickpeas have the highest carbohydrate content among legumes, with starch content ranging from 41 to 50% of the total

Table 3
Proximate composition of whole chickpea flour used as raw material for protein extraction.

Component[a]	Mean ± standard deviation
Moisture	9.18 ± 0.04
Ash	3.34 ± 0.05
Lipid	6.56 ± 0.16
Protein	18.79 ± 0.52
Total Carbohydrates	62.13
Total Dietary Fiber	20.55 ± 0.03
Other Carbohydrates	41.58

[a] %.

carbohydrates, which is consistent with the estimated content of other carbohydrates in this study. The authors also stated that, of the total starch present in chickpeas, 35% is identified as resistant starch and 65% as available starch. The resistant starch content has a physiological function similar to dietary fiber and combined with the high amylose content in this starch, it promotes the low glycemic index of chickpea flour, making it suitable for incorporation into diets that control blood sugar levels. The high amylose content also indicates a higher rate of starch retrogradation, an important characteristic in applications such as custards and puddings [33].

There is also a close relationship between starch retrogradation and resistant starch, and therefore, starches with a higher rate of retrogradation may contain a significant amount of resistant starch in foods. This occurs because during the retrogradation process, starch molecules are realigned into more crystalline structures, making them less accessible to digestive enzymes in the small intestine. This results in a larger portion of starch passing through the small intestine undigested and therefore classified as resistant starch [34].

The relatively high starch and fiber content of whole chickpea flour may influence protein extraction efficiency, particularly under alkaline and ultrasonic conditions, due to possible interactions between proteins and polysaccharides affecting solubilization behavior [35].

3.2. Optimization of the protein extraction method from whole chickpea flour

The adjusted mathematical models for the optimization of protein extraction, as shown in Table 4, were validated by variance analysis, and the data are provided in Appendix A. Additionally, the effect of different interactions of factors in ultrasound-assisted alkaline extraction on the response variables is depicted in Fig. 2.

The protein extraction yield from whole chickpea flour, using the ultrasound-assisted alkaline method, ranged from 42.3 to 55.0% (Table 1, Fig. 2). This result was significantly influenced by two of the three extraction factors evaluated, the solid/liquid ratio and pH (Table 4). The adjusted multiple regression model for protein yield was significant ($p = 0.018$), explaining 74% of the variation in the responses with a non-significant lack of fit (LOF = 0.20), demonstrating a reasonable predictive capability of the model [36].

The solubility of chickpea whole flour protein ranged from 60.7 to 83.7% (Table 1, Fig. 2). The adjusted model was significant ($p = 0.12$), with a coefficient of determination of 0.88 and non-significant lack of fit (LOF = 0.31), indicating good predictive capability (Table 4). Protein solubility was significantly influenced by the linear effect of pH and extraction time under sonication, as well as by the quadratic effects of chickpea flour/water ratio (solid/liquid) and pH.

Protein yield was higher as pH and solid/liquid ratio increased, with the maximum value obtained between pH 9.5 and 12.0 and a ratio of 1:12 and 1:15 g mL^{-1} (Fig. 2). In contrast to yield, solubility showed maximum values at pH below 10.5, with a ratio between 1:7.5 and 1:12.5, and extraction/sonication time both near the lowest level (10 min) and the highest (40 min) (Fig. 2B). Similar findings were observed by [18], who tested ultrasound-assisted protein extraction from peas, obtaining higher extraction yield (83.6%) at a solid/liquid ratio of 1:11.5 g mL^{-1}, pH 9.6, and sonication time of 13.5 min. On the other hand, [37] used ultrasound as a pretreatment on peeled chickpea flour and reported a reduction of about 20 percentage points (from 54.17% without ultrasound to 34.17% and 35.44% with sonication at different powers) in protein extraction yield after sonication, explaining this by the possible protein-lipid interaction inhibiting protein dissolution and limiting their isolation.

Although sonication time did not show significant differences in extraction yield, for solubility, an important property of plant proteins, maximum values were observed at both ends studied (10 and 40 min of sonication). This indicates that using shorter time may be important for the industry, as yield and solubility values were similar, contributing to cost reduction such as energy consumption and working time.

[38] observed higher solubility in samples treated with 30 min of high-intensity ultrasound compared to 15 min of treatment, but unlike this research, the authors subjected the extracted protein to ultrasound treatment afterward. [19] also performed ultrasound treatment on chickpea protein extracts, combining different pH values (2.0 and 12.0) and sonication time (2 and 30 min), and observed an increase in protein solubility after sonication (up to 1.2 mg mL^{-1}) as in this study. On the other hand, the authors achieved higher solubility in protein extracts with pH 12.0, differing from this study, where solubility was lower at pH above 9.5. However, the methodology with ultrasound assisting the protein extraction from whole chickpea flour contributed to time reduction, expenditure on reagents and energy, and could be adapted for possible industrial-scale production.

3.2.1. Model validation and desirability test

The most desirable treatment, the solid/liquid ratio, pH, and sonication time combination that presented the most desirable values in both response variables, as shown in Fig. 3, was determined by the Statistica software. In graphs A, B, and C, the values predicted by the mathematical model considering the combination of factors are depicted, while graphs D and E show the maximum, mean, and minimum values of the response variables. Graphs F, G, and H demonstrate the coded levels of the independent variables corresponding to the most desirable responses (ED), which are +0.5 for solid/liquid ratio, 0.0 for pH, and +1.0 for time. In real values, these levels were 1:12.5 g mL-1 for solid/liquid ratio, pH 9.5, and 40 min, achieving 89.79% desirability.

The validation of the models was conducted by extracting chickpea protein at the desirable levels to obtain the best responses, in triplicate. The adjusted model yielded results of 52.5% for yield and 83.8% for solubility (Fig. 3), while the measured values were 54.7% for protein yield and 89.1% for protein solubility, resulting in deviations of 4.19% and 6.32%, respectively. Thus, considering that the deviations were below 5% and 10%, respectively, the models can be considered validated and can be applied in optimizing protein yield in chickpea whole flour extraction by ultrasound-assisted alkaline method.

Although the desirability analysis (ED) indicated that 40 min of ultrasonication provided the optimal condition, treatment E6 (10 min) also showed promising results, presenting comparable protein yield with slightly lower solubility. From an industrial perspective, shorter processing times may represent an important advantage due to reduced energy consumption and improved process efficiency. However, the higher solubility obtained under the optimized condition may be technologically relevant, since protein solubility strongly influences the functional performance of plant proteins in food systems. Therefore, depending on the intended application, both conditions may be considered viable alternatives.

3.3. Technological and chemical characteristics of selected protein extracts

Only the means of foam stability index (FS) and ash content did not differ significantly ($p > 0.05$) among the selected protein extracts from whole-grain chickpea flour obtained by the alkaline-assisted ultrasound

Table 4
Adjusted mathematical models, significance level (p-value), coefficient of determination (R^2), and lack of fit (LOF) for yield and solubility of chickpea protein extracted by ultrasound-assisted alkaline method as a function of solid/liquid ratio, pH, and extraction/exposure time.

Parameter	Adjusted model	p	R^2	LOF
Yield[a]	$y_1 = 50.631 + 3.152x_1 + 2.203x_2 - 1.465x_2^2$	0.018	0.74	0.20
Solubility[a]	$y_2 = 80.215 - 4.507x_1^2 - 5.785x_2 - 5.422x_2^2 + 5.307x_3$	0.012	0.88	0.31

[a] %.

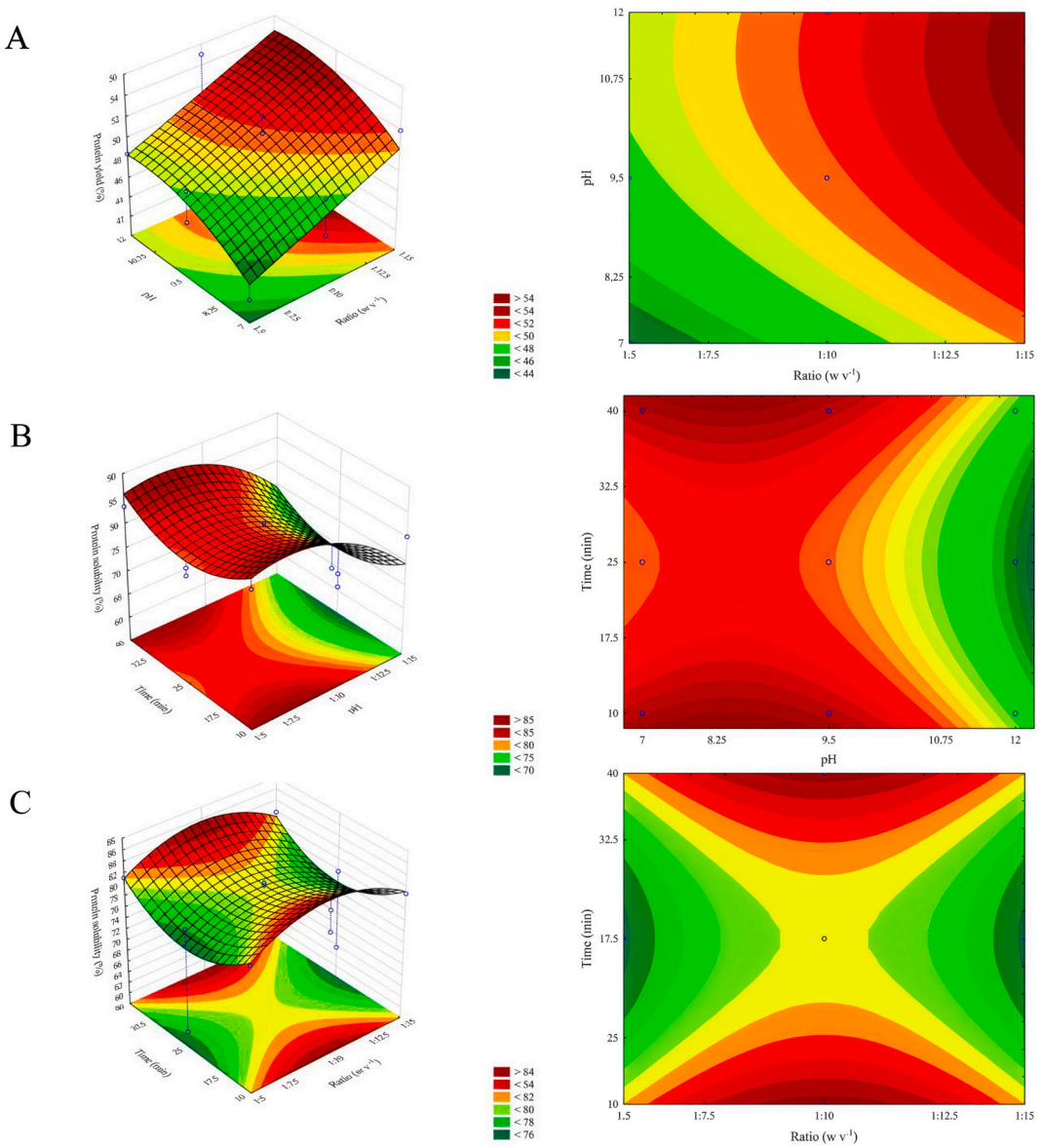

Fig. 2. Yield (A) and Solubility (B and C) of chickpea flour protein extracted by ultrasound-assisted alkaline method as a function of solid/liquid ratio, pH, and sonication time.

method (Table 5). Moisture, lipids, and protein were higher, while the total carbohydrate content was lower in the control experiment, indicating that the use of ultrasound was not efficient in increasing the protein content of the extracts. However, the control showed a 55.5% protein yield (PY) after the extraction process, and the other experiments showed 56.3%, 56.6%, 53.0%, and 49.2% for ED, E6, E10, and E11, respectively. Therefore, despite the lower protein content of the extracts compared to the control, the protein yield after extraction for ED and E6 was statistically equal to the control. This probably occurred due to the different flour-to-water ratios among the experiments, confirming that a smaller amount of chickpea flour relative to water (1:12.5 for ED and 1:15 for E6) can be used and still ensure equal or higher protein yield, since E10 and E11 had higher amounts of chickpea flour relative to water (1 g:10 mL).

Although ultrasound-assisted extraction did not substantially increase protein yield or protein content, a marked increase in carbohydrate content was observed in the ultrasound-treated extracts. This may be related to the co-extraction of non-protein components such as starch and dietary fiber. When comparing the control extract (4.04%) with the ultrasound-assisted extracts (27.74–34.59%), carbohydrate content increased considerably. In pulses, starch granules are embedded within a protein matrix through hydrogen bonds and electrostatic interactions and are surrounded by a cellulose-rich cell wall. Ultrasonic cavitation can disrupt these structures by rupturing cell walls and breaking protein–polysaccharide interactions, which enhances mass transfer and facilitates the release of intracellular components such as starch and dietary fibers into the extraction medium [39].

[12] also evaluated the chemical composition of chickpea protein isolates extracted by the traditional alkaline method, but with defatted flour, which has a higher protein content compared to whole-grain flour, and obtained up to 73% protein, 5% moisture, 2% ash, 2% oil, and about 18% carbohydrates, where only the ash value showed similar results to this research. However, the combination of methods to defatted the flour, soaking in ethanol solution, followed by extraction in the Soxhlet, used by these authors, contributed to the determination of higher protein content, in addition to using pH 4.7 in the isoelectric precipitation step. This may explain the difference with the results of this research, since the flour was not defatted initially, and pH 4.5 was used for isoelectric precipitation.

The lipid content significantly decreased following ultrasound

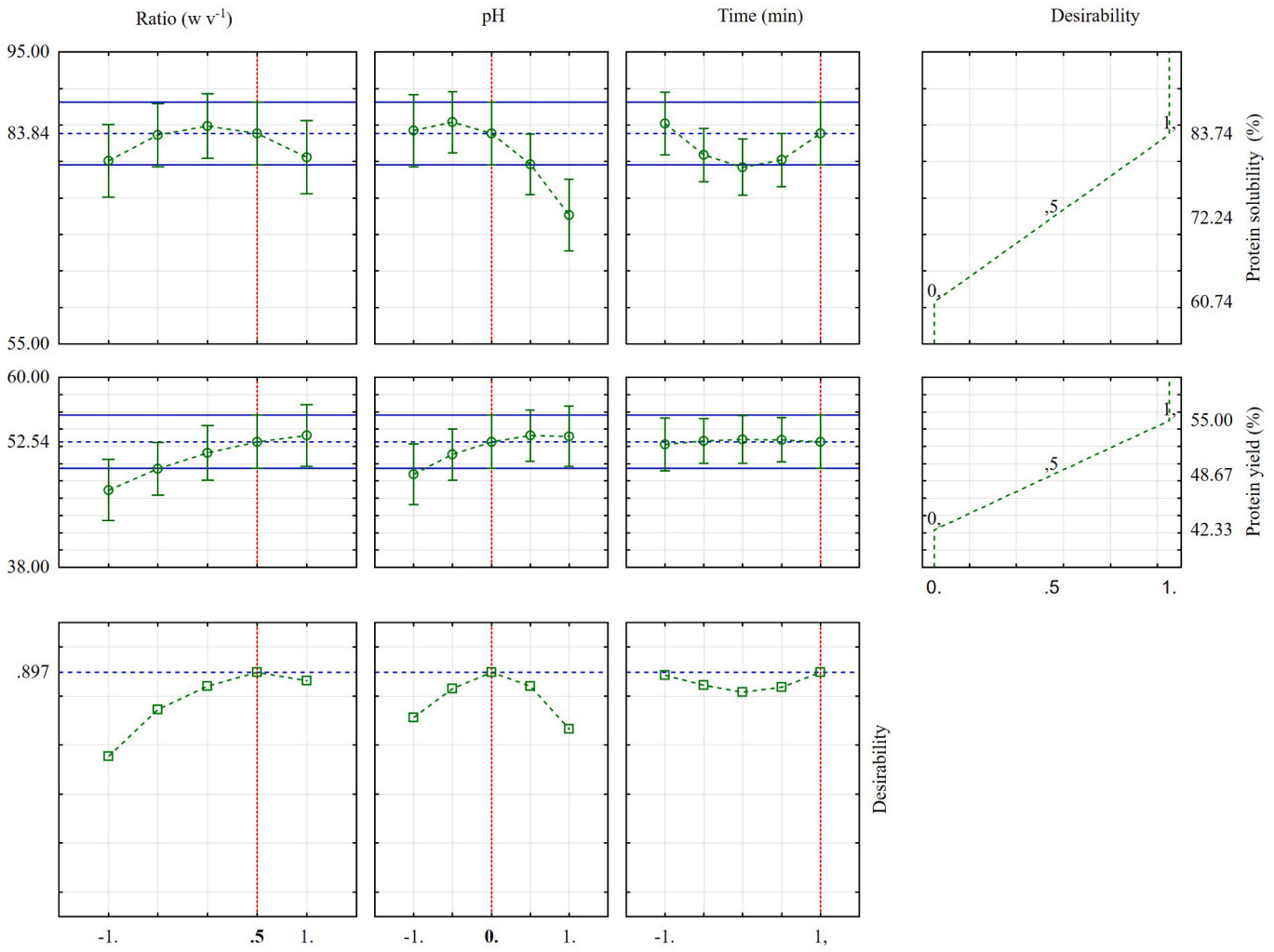

Fig. 3. Desirability test diagram using Yield (%) and Solubility (%) parameters of chickpea protein extracted by ultrasound-assisted alkaline method as a function of solid/liquid ratio, pH, and sonication time.

treatment, reflecting an estimated decrease of 65–81% compared with the control extract. This suggests that ultrasonic cavitation enhanced lipid separation during the extraction process. The reduction in fat content offers several advantages, particularly in the context of food formulation and the development of health-oriented products, such as improved oxidative stability, extended shelf life, and better digestibility [40]. Other authors have conducted protein extraction using defatted flour [17,18,41], arguing that oil can negatively affect the separation of fractions, hindering flour dispersion. However, the use of ultrasound was effective in removing fat from the sample (Table 5), ensuring extracts with low lipid content and eliminating the need for an additional extraction step that incurs costs and requires the use of solvents that may be harmful to health. The substantial lipid reduction observed in ultrasound-assisted treatments suggests that ultrasonication may act as a physical degreasing mechanism, enabling protein extraction from whole chickpea flour without the conventional solvent-based defatting step. Furthermore, the low oil content in the extracts favors their application due to greater flexibility in formulation, enabling better control over the final product's quality, both in terms of total fat content and in preventing deterioration such as oxidation and rancidity in foods, as well as contributing to the development of healthier foods with lower total cholesterol and LDL-C levels [42].

[43] explains that the effect of ultrasonic cavitation accelerates lipid diffusion after cell disruption, while the collapse of cavitation bubbles generates microjets that promote the release of intracellular lipids. In addition, the aqueous medium facilitates the separation of lipids into the upper layer during centrifugation, allowing their efficient removal during the process. On the other hand, sonication may have facilitated the increase in carbohydrate content in the extracts, possibly by increasing the solubility of some of these compounds, which are also soluble in an alkaline medium, justifying the increase in this component after ultrasound-assisted extraction [44].

The protein solubility (PS), water absorption capacity (WAC), and oil absorption capacity (OAC) values increased with the use of ultrasound or remained statistically unchanged, except for E11 for WAC. Meanwhile, the protein yield (PY), foaming capacity (FC), and emulsifying activity index (EAI) of the extracts obtained with ultrasound remained statistically similar to the control or decreased. Foam stability (FS) showed variable behavior. These properties are important in food processing and can influence the appearance, texture, and other sensory characteristics of foods [25].

PS increased with ultrasound only for ED, which used longer time, pH, and intermediate solid/liquid ratio compared to the control extract, while the other extracts showed no statistical differences from the control. This property influences the degree of cooking, protein denaturation, and protein digestibility in foods [45], especially in beverage incorporation, as higher PS results in a more uniform mixture without lumps or altered consistency, leading to more homogeneous final products [46].

More soluble proteins, as seen in SP, are advantageous for food

Table 5
Protein yield (PY), protein solubility (PS), water absorption capacity (WAC), oil absorption capacity (OAC), foam capacity (FC), foam stability (FS), emulsifying activity index (EAI), emulsifying stability index (ESI), and proximate composition (mean ± standard deviation) of chickpea protein extracts (E) obtained under different experimental conditions.

Parâmetro[a]	EC	ED	E6	E10	E11
PY[2]	55.45 ± 0.92^{A}	56.28 ± 0.37^{A}	56.64 ± 1.36^{A}	53.00 ± 0.49^{AB}	49.20 ± 2.28^{B}
PS[2]	77.33 ± 2.15^{B}	89.07 ± 3.06^{A}	79.61 ± 3.44^{B}	79.95 ± 1.93^{B}	81.74 ± 2.19^{B}
WAC[2]	1.76 ± 0.05^{D}	2.06 ± 0.04^{B}	1.89 ± 0.01^{C}	2.55 ± 0.02^{A}	1.59 ± 0.02^{E}
OAC[2]	1.42 ± 0.02^{C}	1.75 ± 0.05^{B}	1.96 ± 0.06^{A}	1.95 ± 0.07^{A}	1.75 ± 0.05^{B}
FC[2]	45.56 ± 1.92^{AB}	46.66 ± 0.00^{AB}	48.89 ± 1.92^{A}	44.44 ± 1.92^{B}	27.22 ± 0.96^{C}
FS[2]	58.04 ± 1.55^{B}	51.59 ± 2.25^{C}	59.62 ± 2.39^{B}	65.87 ± 2.75^{A}	56.58 ± 1.22^{BC}
EAI[3]	41.18 ± 0.99^{A}	42.41 ± 0.52^{A}	41.10 ± 1.66^{A}	38.17 ± 0.83^{B}	26.01 ± 1.17^{C}
ESI[4] n.s.	11.56 ± 0.41	11.20 ± 0.36	11.29 ± 0.28	11.14 ± 0.06	10.91 ± 0.11
Moisture[2]	2.24 ± 0.06^{A}	1.04 ± 0.04^{BC}	1.43 ± 0.03^{B}	0.73 ± 0.01^{CD}	0.53 ± 0.00^{D}
Ashes[2] n.s.	2.28 ± 0.10	2.14 ± 0.07	2.07 ± 0.06	2.32 ± 0.03	2.11 ± 0.10
Lipids[2]	26.32 ± 0.03^{A}	5.57 ± 0.16^{D}	4.99 ± 0.05^{D}	6.57 ± 0.29^{C}	9.25 ± 0.32^{B}
Protein[2]	65.13 ± 0.97^{A}	61.21 ± 2.21^{B}	57.00 ± 0.76^{C}	57.22 ± 0.59^{C}	60.37 ± 1.47^{B}
Carbohydrate[2]	4.04 ± 1.03^{D}	30.04 ± 1.81^{BC}	34.59 ± 0.83^{A}	33.17 ± 0.50^{AB}	27.74 ± 1.72^{C}

[a]Different letters on the same line indicate significant differences between treatments according to the Tukey test ($p < 0.05$); [2]%; [3] $m^2\ g^{-1}$; [4] min; [n.s.] no significant differences.
EC (control – ratio 1:10 g ml^{-1}; pH 9.5; 60 min), ED (desirable – ratio 1:12.5 g ml^{-1}; pH 9.5; 40 min), E6 (ratio 1:15 g ml^{-1}; pH 9.5; 10 min), E10 (ratio 1:10 g ml^{-1}; pH 12.0; 10 min) e E11 (ratio 1:10 g ml^{-1}; pH 7.0; 40 min).

production due to several factors, such as: they are easier to handle and process during food production, contributing to the formation of soft and smooth structures in products like ice creams, yogurts, creams, and mousses; they can help prevent phase separation, sedimentation, and the formation of unwanted crystals in some foods, making them more palatable and acceptable to consumers; moreover, they can provide a source of high-quality protein that is readily available to the body, helping to promote health and nutrition. The combination of pH with time was ideal for improving this property, as the treatment that came closest had the same ultrasound time (40 min), but at a neutral pH. [18] also observed that ultrasonic treatment improved the SP of chickpea protein isolates. This occurs due to the reduction in the size of the proteins, which are partially depolymerized, forming peptides due to cavitation and its mechanical effects, and with the reduced size, the interaction between protein and water was enhanced, resulting in increased solubility in water [47].

Regarding WAC and OAC, E10 showed higher results, with E6 also standing out in OAC, where there were no statistical differences between them (Table 5). [12] observed 1.65 mL $H_2O\ g^{-1}$ for CAA and 1.72 mL oil g^{-1} for OAC in chickpea protein isolates produced in Monywa, Myanmar, values similar to those reported in this study. The higher results obtained for WAC may be due to the ultrasound process, which tends to reduce the size of protein particles, making them disperse more evenly in water and oil [13]. This can facilitate the incorporation of proteins into a variety of food products, including beverages, sauces, and baked goods, thereby improving their functionality and processability.

WAC indicates the protein-water interaction that occurs in the food system, while OAC is related to the protein-oil interaction and can affect the taste and texture, altering the volume, viscosity, and consistency of foods [13]. Proteins with higher WAC retain water in the food, which is important in frozen products, processed meats, and sausages, contributing to the texture of the final product. Similarly, higher OAC retains more oil, providing greater tenderness and juiciness in processed products [48].

Moreover, higher WAC tends to positively affect the availability of nutrients in foods, as it retains more water-soluble nutrients, in addition to greater consumer acceptance by maintaining the moisture and juiciness of the food, and greater stability by preventing phase separation and particle sedimentation, a very important property in products such as sauces, dairy beverages, and soups. Sonication for 10 min combined with high pH (12.0) and a 1:10 g mL^{-1} ratio was sufficient to improve WAC compared to traditional extraction. Similarly, OAC and WAC values were statistically higher in ultrasound-assisted extractions, but there is a tendency for reduction as the time in the equipment increases (E6 and E10, 10 min; E11 and ED, 40 min; EC, 60 min) (Table 5).

This fact corroborates with the work of [18], who determined increases of up to 40% in WAC and 6.9% in OAC in pea proteins extracted by ultrasound-assisted alkaline method, compared to the conventional (alkaline) method. [27] also found that OAC increased after the use of ultrasound in the extraction of *Dolichos lablab* L. seeds and reported that this phenomenon could be due to the exposure of the hydrophobic surface to a solvent, caused by sonication. In both cited studies, the authors reported an increase in the hydrophobicity of the protein surface with the use of ultrasound, which led to changes in its structure exposing hydrophobic groups that interact with lipids, providing an increase in OAC.

The foaming and emulsifying characteristics showed a similar trend. [33] assert that chickpea protein stands out in thermal expansion, foaming capacity, and stability when compared to other legumes, important traits in gluten-free bread development as they confer a high specific volume to the bread, besides enhancing its nutritional characteristics. [49] also suggest canned chickpea water (aquafaba) as an emulsifier in cake and mayonnaise production, demonstrating potential for egg replacement.

For the analyses of FC and EAI, experiments ED and E6 did not differ statistically from the control, and E11 showed lower values for these properties (Table 5). It was also observed that experiments that stood out the most used a pH of 9.5 to alkalize the solution, while the more basic pH (12.0) presented median values, and lower values were obtained with neutral pH (7.0). Regarding FS, E10 showed better results, followed by E6, FC, and E11, which did not show statistical differences, and with lower stability, ED, which was statistically equal to E11. [50] also observed lower values of foaming and emulsifying properties in alfalfa protein extracted by alkaline extraction followed by ultrasound-assisted isoelectric precipitation and ultrafiltration, when compared to other extraction methods like heat coagulation and alkaline isoelectric precipitation. However, similar to this research, the authors reported higher solubility, water, and oil retention for the method that used ultrasound.

Ultrasonication is capable of reducing particle size and increasing protein absorption, as the energy released by the equipment promotes protein structure unfolding and disorder, enhancing absorption at the water–oil interface. Additionally, FS also depends on the turbulence generated by the sonication treatment and the binding of oil bubbles in an emulsion [13]. Although ultrasonication benefited some functional properties such as PS, WAC and OAC, the characteristics of the equipment used in this study were probably not efficient enough to improve foam and emulsion properties statistically compared to conventional treatment (Table 5).

[18,27,41,51] confirmed through their research that the use of ultrasound, either as a post-extraction treatment or as an instrument to assist the extraction process, contributed to improving extraction yield and, primarily, the functional properties of protein extracts, supporting the findings of this study. Although the extraction yield and protein content in this work were not statistically superior (Table 5), most functional properties benefited from the use of ultrasound or remained

statistically similar to the traditional method. Furthermore, considering that the agitation time in the alkaline solution in the conventional method is 60 min, and with 10 min, a superior or similar result is achieved with sonication, the combined method could be viable, aiming to optimize the process time.

It is important to highlight that protein extracts, whether concentrated or isolated, are manufactured as singular ingredients, but are often mixed with other ingredients in formulation. These ingredients can directly influence the structure and techno-functionality of the protein and, thus, guide the industry in optimizing the formulation for a wide range of food applications [48]. Additionally, the ability to use ultrasound to enhance the functionality of proteins has shown new possibilities for innovation and the development of new food products with improved texture, stability, flavor, and nutritional value.

From an application perspective, the improved solubility and absorption capacities suggest potential use of these protein extracts in beverage formulations, meat analogues, and bakery systems. Future studies should investigate their performance in model food systems and assess scalability under industrial ultrasonic reactors.

3.4. Physical-chemical and morphological characteristics of selected protein extracts

The assessment by differential scanning calorimetry (DSC) demonstrated the thermal stability of the protein extracts from whole grain chickpea flour (Fig. 4). The denaturation kinetics were generally comparable among the treatments, with peak temperatures occurring within a relatively narrow range. The initial temperature (Ti), the maximum denaturation temperature or peak (Tp), and the enthalpy (ΔH) were 76.87 °C, 93.08 °C, and 2.33 J g^{-1} for EC, and 74.46 °C, 91.33 °C, and 3.07 J g^{-1} for E11 (Fig. 4A); 75.55 °C, 92.20 °C, and 1.94 J g^{-1} for ED (Fig. 4B); 78.15 °C, 93.05 °C, and 1.58 J g^{-1} for E6 (Fig. 4C); and 82.50 °C, 96.24 °C, and 1.11 J g^{-1} for E10 (Fig. 4D).

The differences in enthalpy values suggest that extraction conditions such as pH and ultrasonication influenced the structural organization of the proteins. The control sample presented an intermediate ΔH value (2.33 J g^{-1}), while the lowest value was observed for E10 (1.11 J g^{-1}), obtained strongly alkaline conditions (pH 12). In contrast, the highest enthalpy value was observed for E11 (3.07 J g^{-1}), extracted at neutral pH (7). Lower ΔH values generally indicate that the protein structure undergone partial unfolding prior to thermal analysis, requiring less energy for denaturation [52]. Under alkaline conditions, protein molecules may experience structural rearrangements due to the disruption of intermolecular interactions and possible cleavage of disulfide bonds, resulting in partial unfolding [53]. In addition, ultrasonic cavitation can promote structural modifications through shear forces, turbulence, and microstreaming effects, which weaken internal hydrophobic interactions. These structural rearrangements may expose hydrophilic groups and enhance protein–water interactions, which may partially explain the improved solubility observed in ultrasound-assisted extracts [54].

Among the treatments, E10 showed slightly higher Ti and Tp values compared to the other samples, suggesting greater resistance to the onset of thermal denaturation. However, this treatment also exhibited the lowest ΔH value, indicating that structural modifications induced during extraction may have altered protein organization without substantially compromising thermal stability.

[25] reported denaturation temperatures for chickpea protein concentrates above 100 °C, ranging from 127 to 135 °C, while [55] observed lower values between 98.1 and 99.8 °C. These authors explained that globulins, the major protein fraction in legumes, typically present Tp values in the range of 83.8–107.8 °C, which is consistent with the results obtained in this study. Although the ultrasound-assisted treatments produced variations in ΔH values, the denaturation temperatures remained within similar ranges among treatments, suggesting that the overall thermal stability of the protein fractions was not substantially altered. However, ultrasound-assisted extraction combined with alkaline conditions may induce moderate structural rearrangements that influence protein functionality without significantly compromising thermal stability. Further studies evaluating different ultrasound intensities and extraction parameters may help optimize plant protein functionality [56].

Functional properties directly influence the application and processing of protein extracts. Therefore, understanding their structural characteristics allows the food industry to monitor protein stability during storage and predict their thermal behavior in food systems, including extruded products, bakery and confectionery products enriched with chickpea protein [25]. Fourier transform infrared

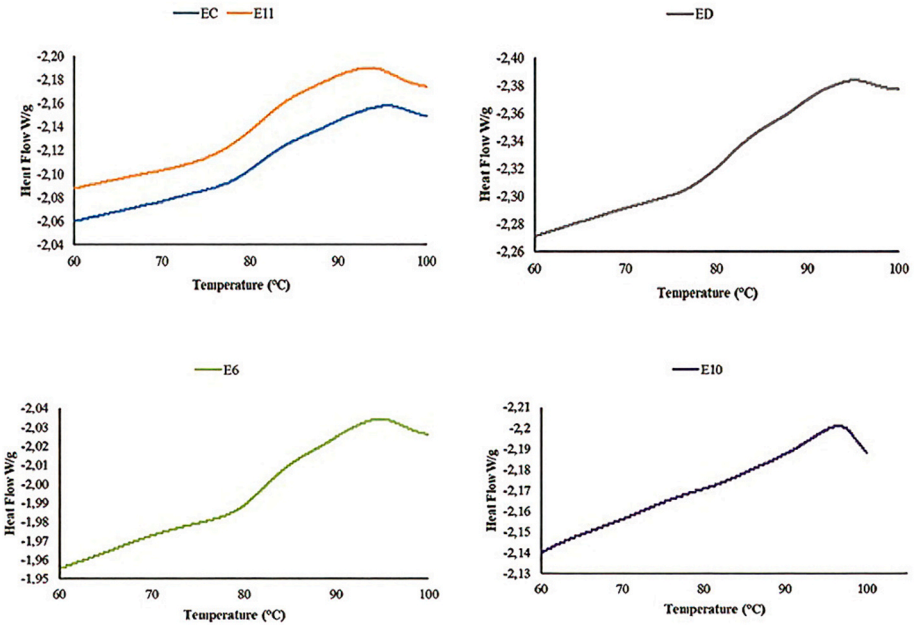

Fig. 4. Thermograms of selected chickpea whole flour protein extracts: EC (no ultrasound), ED (ratio 1:12.5 g mL^{-1}; pH 9.5; 40 min), E6 (ratio 1:15 g mL^{-1}; pH 9.5; 10 min), E10 (ratio 1:10 g mL^{-1}; pH 12.0; 10 min), and E11 (ratio 1:10 g mL^{-1}; pH 7.0; 40 min).

spectroscopy (FTIR) is a vibrational spectroscopic technique widely used to determine the secondary structure of proteins (α-helix, β-sheets, β-turns, and irregular structures) by identifying characteristic vibrational bands of specific functional groups [57]. The spectra obtained are presented in Fig. 5, indicating the percentage of transmittance of the samples across wavelength, ranging from 400 to 4000 cm^{-1}.

Peaks around 1650 cm^{-1} were identified in all experiments. According to [58], peaks at this wavelength represent proteins conformed in α-helix in the amide I band, a region corresponding to wavelengths from 1600 to 1700 cm^{-1}, where information about protein secondary structures is found. These authors also identified bands around 1654 cm^{-1} attributed to α-helix in chickpea protein isolates obtained from enzyme-assisted extractions. Moreover, they identified strong bands in the range of 1612 to 1640 cm^{-1} and 1680 cm^{-1} attributed to β-sheets, at 1645 cm^{-1} random coils, and at 1670 cm^{-1} representing β-turns. All ultrasound-assisted treatments showed similar results, suggesting that the extracted proteins, in all mentioned experiments, have a significant amount of α-helix secondary structure, indicating that the extraction process, including the ultrasound method, did not compromise the secondary structure of the proteins, maintaining their native conformation during the process. Proteins with preserved secondary structures, such as α-helix, tend to have better functional properties, such as water binding capacity, gel formation, thermal stability, and emulsification capacity.

[59] observed, through absorbance data, a higher portion of α-helices in chickpea protein isolates via enzyme-assisted alkaline extraction than in isolates obtained only from alkaline extraction, which showed a higher quantity of β-sheets. They indicate that the higher solubility of the isolate is usually associated with a higher content of α-helix, which are more flexible structures, unlike β-sheets which are more rigid. Considering that absorbance is inversely proportional to transmittance [57], the ED, for presenting lower transmittance, may have a higher content of α-helix than the other treatments, confirming its higher solubility. This information is relevant to the food industry, as it highlights the importance of selecting the extraction method to obtain proteins with desirable characteristics for specific applications.

Other peaks can be identified in the FTIR analysis, such as those between 2800 and 3000 cm^{-1} (Fig. 5). This range represents CH bonds, which occur in aliphatic compounds, and the peaks in the region of 2850 and 2920 cm^{-1} are associated with the stretching of methyl bonds (CH_3), with values closely matching those determined by [60] in roasted and raw chickpeas. The authors also identified peaks between 1740 and 1750 cm^{-1}, as observed for all treatments in the region of 1745 cm^{-1}, indicating the presence of the carbonyl function (C=O) of lipid ester groups, indicating the significant presence of lipids associated with the extracted proteins.

The increase in solubility observed for ED may be associated with the higher α-helix content suggested by FTIR analysis, since more flexible secondary structures facilitate protein–water interactions.

The protein structures were altered by sonication (Fig. 6). Scanning electron microscopy (SEM) images showed that the control sample (EC) presented larger particles with a more compact and lamellar surface and relatively smooth morphology (Fig. 6A), suggesting minimal structural disruption in the absence of ultrasonic treatment. With the use of ultrasound, structural modifications became evident, including the presence of smaller particles and increased surface irregularity, particularly in treatment E6 (Fig. 6C). These observations confirm the reduction in particle size caused by the mechanical forces and cavitation effects generated during ultrasonication.

Previous studies, such as [47], also reported variations in particle size after sonication of walnut protein isolates, observing that shorter ultrasound times decreased particle size, while longer sonication periods

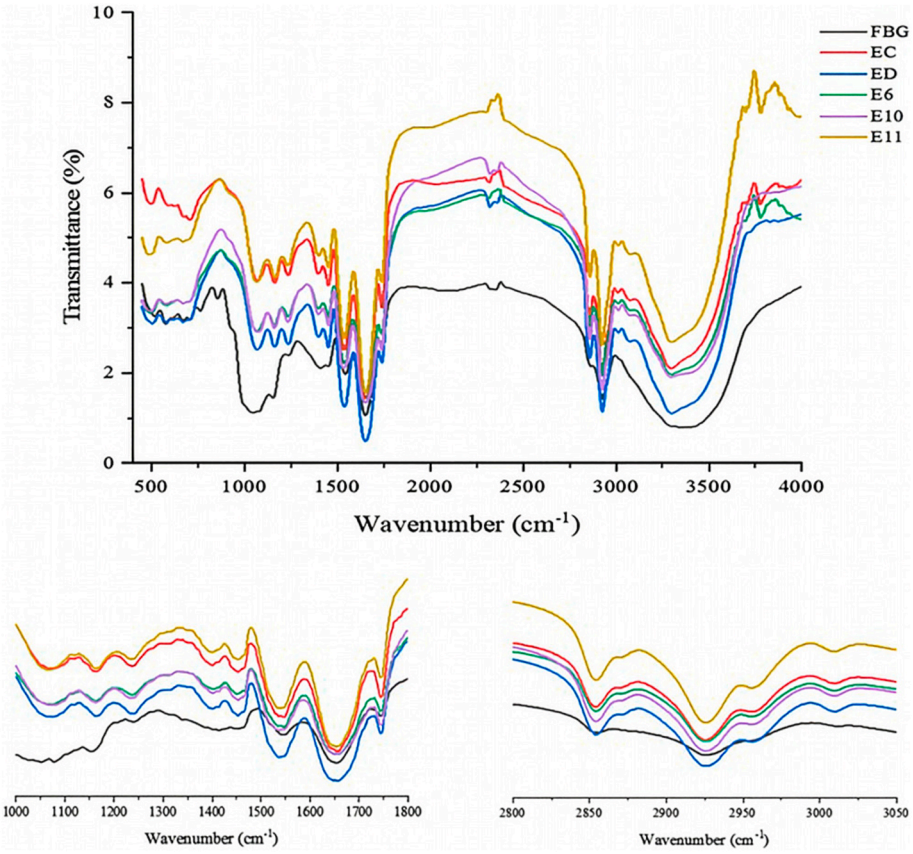

Fig. 5. FTIR of chickpea flour (CF) and protein extracts: EC (without ultrasound), ED (ratio 1:12.5 g mL^{-1}; pH 9.5; 40 min), E6 (ratio 1:15 g mL^{-1}; pH 9.5; 10 min), E10 (ratio 1:10 g mL^{-1}; pH 12.0; 10 min), and E11 (ratio 1:10 g mL^{-1}; pH 7.0; 40 min).

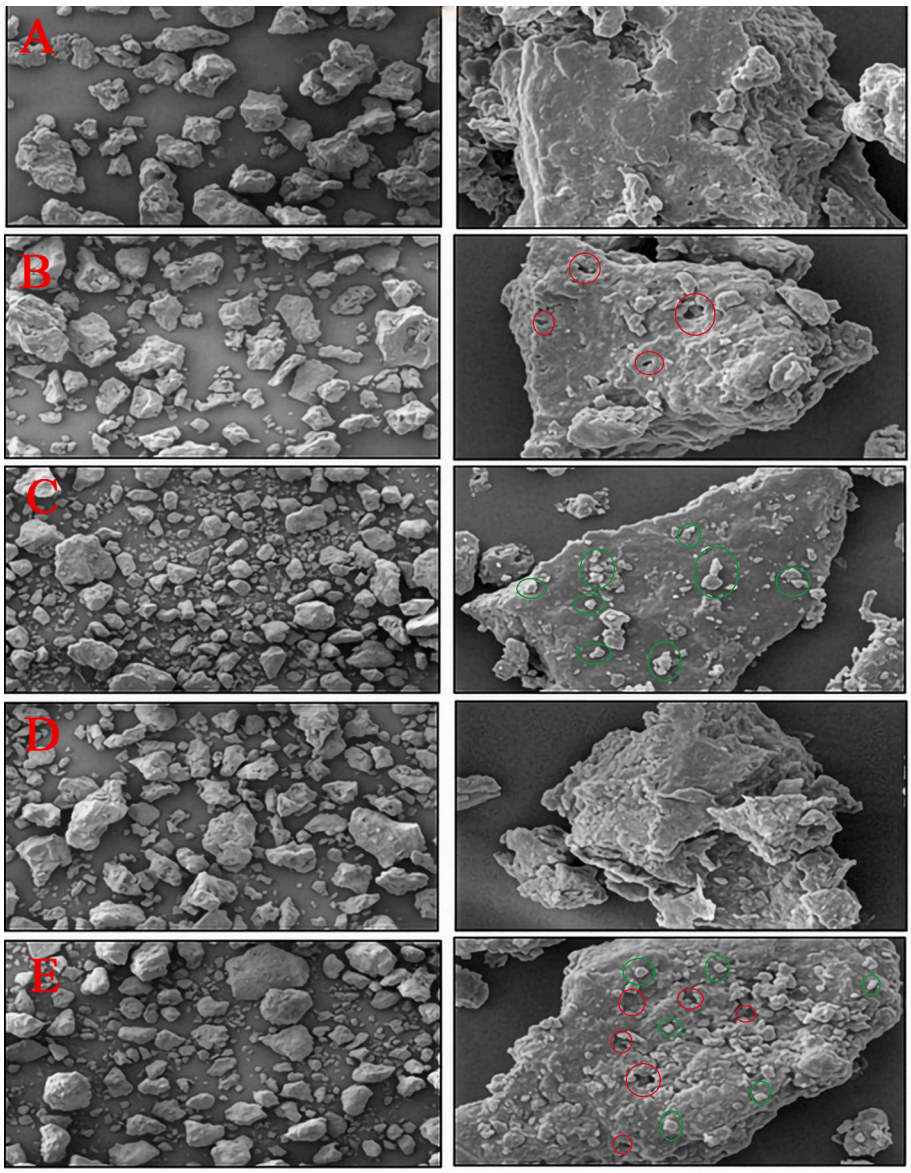

Fig. 6. Scanning electron microscopy of chickpea whole flour protein, with magnifications of 100x and 1500x, from treatments (A) EC – without ultrasound; (B) ED – ratio 1:12.5 g mL^{-1}; pH 9.5; 40 min.; (C) E6 – ratio 1:15 g mL^{-1}; pH 9.5; 10 min.; (D) E10 – ratio 1:10 g mL^{-1}; pH 12.0; 10 min.; (E) E11 – ratio 1:10 g mL^{-1}; pH 7.0; 40 min.

could promote particle aggregation. In the present study, E6 presented particles with smaller dimensions under shorter sonication time (10 min), while ED (Fig. 6B) exhibited larger particles under longer ultrasound exposure (40 min) when comparing treatments subjected to ultrasound.

Morphological differences among the treatments were also observed. The surface in Fig. 6B became more irregular with the appearance of micro-cavities and structural disruption, suggesting the onset of ultrasonic cavitation effects, where pores are highlighted by the red circles. In Fig. 6C, the surface still appeared relatively smooth, but small particle fragments adhered to the surface, indicating mechanical fragmentation of the protein matrix. In Fig. 6D, the structure appeared more disrupted and layered, suggesting mechanical stress and particle breakage caused by sonication. Finally, Fig. 6E showed the most porous morphology, with numerous micro-pores and fragmented particles distributed across the surface.

As observed in Fig. 6B and E, the most porous structures also showed a greater number of adhered particles (highlighted by green circles). This may be associated with the longer ultrasonication time (40 min), which likely intensified cavitation effects. In contrast, although Fig. 6D corresponded to a shorter extraction time (10 min), the extraction was performed at pH 12, which may have also contributed to structural modifications in the protein matrix. The structural differences observed are consistent with the DSC and FTIR results discussed previously.

The reduction in particle size observed by SEM likely contributed to the increased water absorption capacity (WAC), since smaller particles provide a larger surface area for water interaction. Another important aspect of protein structures is porosity, which, together with particle size, directly influences technological properties such as water retention capacity and consequently affects textural properties important for food applications, including restructured meats, vegetarian burgers, and sausages.

In addition, the desirable treatment showed a more porous surface compared to the others, which helps explain the higher solubility and water absorption capacity observed in this extract. Similar findings were reported by [18], who observed larger pores and increased pore formation in chickpea protein isolates subjected to ultrasound treatment. These morphological changes are characteristic of acoustic cavitation,

where the collapse of microbubbles generates localized shear forces and microjets capable of disrupting protein matrices and modifying their surface structure.

4. Conclusions

The findings indicate that the alkaline extraction method assisted by ultrasonication did not increase protein yield or content (maximum 65.13 ± 0.97% in the control extract) but significantly improved the technological properties of the protein extracts. Protein solubility increased from 77.33 ± 2.15% to 89.07 ± 3.06%, water absorption capacity from 1.76 ± 0.05 to 2.55 ± 0.02 g g^{-1}, oil absorption from 1.42 ± 0.02 to 1.96 ± 0.06 g g^{-1}, and foam stability from 58.04 ± 1.55% to 65.87 ± 2.75%. These results highlight the potential of ultrasound-assisted extraction to enhance the functional performance of chickpea protein extracts. Such improvements may support their application in food systems such as beverages, bakery products, and meat analogues. Future studies should focus on process scale-up, evaluation in model food matrices, and techno-economic assessment to determine the feasibility of industrial implementation. Overall, the results demonstrate how emerging technologies such as ultrasound can contribute to the development of functional ingredients and more sustainable food processing strategies.

CRediT authorship contribution statement

Priscylla Martins Carrijo Prado: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gabriela Silva Mendes Coutinho:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Aryane Ribeiro Oliveira:** Writing – review & editing, Supervision, Project administration, Data curation. **Vitor Hugo Pacheco Jardim:** Supervision, Formal analysis, Data curation. **Gabriella L. Magalhães:** Writing – review & editing, Methodology, Formal analysis, Conceptualization, Data curation, Investigation. **Larissa Silva Couto:** Investigation, Formal analysis, Data curation. **Nathale Jordana Clemente Santos:** Methodology, Formal analysis, Investigation. **Tâmila Gouveia Raimundo:** Methodology, Formal analysis, Investigation. **Alline Emannuele Chaves Ribeiro:** Validation, Supervision, Project administration, Methodology. **Ivan Torres Nicolau de Campos:** Validation, Supervision, Methodology, Formal analysis. **Luciano Morais Liao:** Validation, Supervision, Methodology, Data curation. **Márcio Caliari:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Manoel Soares Soares Júnior:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (88887.647679/2021-00) and Universidade Federal de Goiás (UFG). Milhão Ingredients for providing the whole chickpea flour sample. The Multiuser High-Resolution Microscopy Laboratory (LabMic/UFG), and the Multiuser Analysis Center (CAM) of the Institute of Chemistry at UFG for access to their research facilities.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2026.107848.

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