Response surface methodology guided approach for optimization of protein isolate from Faba bean. Part 1/2

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Response surface methodology guided approach for optimization of protein isolate from Faba bean. Part 1/2

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ABSTRACT

Ultrasound-assisted extraction (UAE) was evaluated as a green procedure to produce faba beans protein isolates from faba beans. Magnetic stirring was performed as conventional extraction. A three-level five-factor Box-Behnken Design (BBD) was applied to obtain the optimal UAE conditions to concurrently maximize extraction yield and protein content. The response surface methodology (RSM) showed a quadratic curvature for extraction yield and protein. The optimal extraction conditions were determined as: Power of 123 W, solute/solvent ratio of 0.06 (1:15 g/mL), sonication time of 41 min, and total volume of 623 mL with a desirability value of 0.82. Under these conditions, the extraction yield of 19. 75 ± 0.87 % (Protein yield of 67.84%) and protein content of 92.87 ± 0.53 % were obtained for optimum ultrasound extraction**.** Control samples using magnetic stirring under similar conditions without ultrasound treatment showed an extraction yield of 16.41 ± 0.02 % (Protein yield of 54.65 %) and a protein content of 89. 88 ± 0.40 %. This shows that BBD can effectively be

used to optimize the extraction of proteins from faba beans using optimal extraction conditions, resulting in a higher extraction yield and protein purity.

Keywords: Faba beans, Ultrasound extraction, response surface methodology, Box-Behnken Design, Process optimization, Protein isolate

INTRODUCTION

Expected demand for conventional proteins from animals, seafood and dairy sources is projected to increase by 2050 globally mostly for animal proteins (Hayes, 2023). Additional animal farming is linked to higher emissions of greenhouse gases (Manzano et al., 2023), increasing land and water use, along with growing concerns about risk of health issues related to red meat intake, as well as ethical and religious disagreements tied to the slaughter of animals by certain sectors of the population (Pam Ismail et al., 2020). These growing concerns and issues have driven researchers within the food industry to explore alternative environmentally friendly and renewable sources of proteins to curb these problems (Surya Ulhas et al., 2023). Thus, there has been a transition towards the search for alternatives, which generally includes proteins from aquatic sources (duckweed, microalgae, and macroalgae), bacterial and fungal sources, and plants-based sources (pulse, legume, oilseed, cereal, and food- byproducts) (Badjona et al., 2023c; Fasolin et al., 2019). In comparison to conventional sources, these alternative protein sources have several benefits, such as lower greenhouse gas emissions and carbon footprint during production, low production costs, efficient resource utilisation, and increased acceptance by consumer as the nutritional trends of individuals such as flexitarianism is on the rise (Badjona et al., 2023a; Takefuji, 2021).

Faba beans are a cool seasonal legume that is widely cultivated in Australia, Egypt, Ethiopia, Germany, Canada, and the United Kingdom. While this legume has a high protein content, ease of cultivation, and superior nitrogen-fixing capabilities; large amounts of faba bean ingredients are not employed in food systems (Khazaei et al., 2021). Whole faba beans contain 20–35% protein, 1–2% fat, 55–65% carbohydrate, 10–15% fiber, and vitamins and minerals such as iron, zinc, calcium, potassium, and magnesium. The presence of phytochemicals in faba beans has been suggested to provide numerous health benefits (Badjona et al., 2023b). According to their sedimentation coefficient, globulins, which make up 70–80 % of the storage protein in faba beans, may be divided into two classes: the 7S vicilin-type globulins and the 11S legumin-type globulins (Fiel et al., 2002). Extraction of proteins from this sustainable and renewable legume is worth considering for specialized applications in food systems such as emulsions (Dubey et al., 2016; Paximada et al., 2021).

Extraction of proteins from plant materials by alkaline-isoelectric precipitation generally involves solubilisation of the aqueous systems in alkaline condition followed by precipitation of the proteins at their isoelectric point for food applications. Unfortunately, this approach only extracts roughly half of the proteins, with the remaining lost to discarded solids and liquids (Chandran et al., 2023; Hewage et al., 2022). Lower extractability may be attributed to inherent protein-carbohydrate complexes present in certain locations of the raw material (Eze et al., 2022a). Hence, to improve the extraction yield of proteins, advanced and novel technologies such as ultrasound-assisted extraction, ohmic heating, microwave extraction supercritical fluid extraction and pulsed electrical field application have been promoted (Eze et al., 2022b). Ultrasound processing is regarded as an eco-friendly, non-toxic, relatively cheaper and time-efficient technique that can be employed to improve extraction yield (Suchintita Das et al., 2022a). The effect of ultrasound can be ascribed to cavitational effects which aid in the disruption and disintegration of cellular matrices and the subsequent release of proteins.

Thus, this present study aims to examine the efficiency of ultrasound-assisted protein extraction from faba beans by varying key processing factors such as sonication power, treatment time, solute-to-solvent ratio, and total extraction volume through the application of response surface methodology (RSM). RSM studies may also differ in the response variable. In this study, the response variable was optimized for extraction yield and protein content.

MATERIALS AND METHOD

Raw Materials and Chemicals

Faba bean seeds was obtained from Whole Foods Earth (Kent, United). NaOH, ((≥ 99.9 % pure), and HCI was also obtained from Sigma-Aldrich (United Kingdom). The seeds were milled using a cyclone mill.

Ultrasound-assisted alkaline extraction (UAE) of protein isolates from faba beans

Different dispersions of faba bean flour in water (1:5 – 1:20 w/v) with variable total volumes (500 – 1000 mL) were agitated at 25 °C for 20 minutes at 500 rpm prior to ultrasonic-assisted extraction. The dispersion was then adjusted to pH 11 using 1M NaOH, then subjected to ultrasonic treatment at varying ultrasonic power (50 – 180 W) and varying sonication duration (10 - 60 min) based on a previous study (Badjona et al., 2024a) using a S24d22D titanium ultrasonic horn (Teltow, Germany). Temperature was maintained at 20 - 25 ˚C using an ice bath. The resultant mixture was centrifuged for 20 minutes at 25 °C at 6,000 rpm (accuSpin™ 400, United Kingdom). After gathering the supernatant, 1 N HCI was used to bring the pH to 4.0 while stirring continuously for 20 minutes. Protein isolate pellets were then obtained after centrifuging at 6,000 rpm for 20 min at 25°C. After 48 hrs of lyophilization of the protein pellet, samples were stored at -20 °C for further analysis. Protein content was determined by the Dumas method using a nitrogen conversion factor of 6.25. Control protein isolate was generated using optimized conditions without ultrasound treatment.

The weight of the protein isolate obtained was divided by the initial weight of the measured faba bean flour to calculate the extraction yield, as given in Equation (1).

Extraction yield (%) = $\frac{m_p}{m_i} \times 100$ (Eq.1)

The mass of the initial flour and final protein isolate is represented by *mi* and *mp*, respectively.

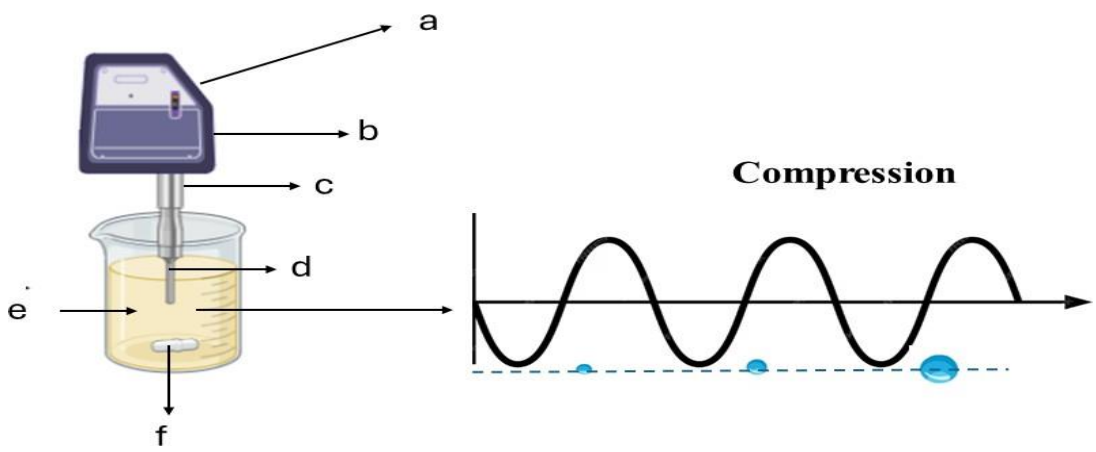

Fig.1. Schematic diagram of ultrasound-assisted extraction of faba bean protein isolate (NB: (a) screen values (b) ultrasound control system (c) Converter (d) probe horn (e) flour suspension (f) magnetic stirrer.

Experimental design and optimization

The Box-Behnken design was implemented to establish the optimal conditions for ultrasound-assisted extraction of proteins from faba beans. The response surface-based optimization method was carried out using Design Expert software to obtain the maximum extraction yield and protein content from faba bean flour. The extraction variables consisted of three distinct levels for each of the four variables. The solid/solvent ratio (g/mL) (X_1), total volume (mL) (X_2), ultrasound power (W) (X_3), and extraction time (min) (X_4) were the independent variables for the ultrasonic-assisted alkaline extraction of fava bean protein isolates that were investigated at three different levels of low (1), medium (0) and high (+1). Both the extraction yield and protein content of the freeze-dried faba bean protein isolate were used as the response variables. The coded factors for each variable are displayed in **Table 1**.

Table 1. Actual and coded variables were used in the ultrasound-assisted extraction design of the experiment.

Independent Variables	**Unit**	**Levels**		
		Low	**optimal**	**High**
Power	W	50	115	180
Solute/water ratio	*w/v*	0.06	0.15	0.25
Extraction time	min	10	35	60
Total volume	ml	500	750	1000

The experimental data were evaluated with the goal of identifying the optimal set of parameters that would produce the highest extraction yield and protein content values to identify the major influencing factors. The results of our earlier research (Badjona et al., 2024a) and those of other authors who obtained protein isolate from plant sources were used to determine the minimum and maximum amounts assigned to each factor (Alvarez-Ossorio et al., 2022; Fatima et al., 2023)**.** Actual and coded variables employed in the UAE experimental design are shown were used. The second-order polynomial model was obtained by data analysis of the response and independent variables.

$$\mathbf{EY\ (\%) = \beta_0 + \beta_1X_1 + \beta_2X_1^2 + \beta_3X_2 + \beta_4X_2^2 + \beta_5X_3 + \beta_6X_3^2 + \beta_7X^4 + \beta_8X_4^2}$$

$$\mathbf{+ \beta_9X_1X_2 + \beta_{10}X_1X^3 + \beta_{11}X_1X_4 + \beta_{12}X_2X_3 + \beta_{13}X_2X_4 + \beta_{14}X_3X_4}\ \text{(Eq.2)}$$

where X_i and X_j are independent variables; β_o is the intercept; β_i, β_{ii}, and β_{ij} are the coefficients of the linear, quadratic, and interaction term, respectively; and EY is the response variable, which includes the protein content and extraction yield.

RESULTS AND DISCUSSION

Fitting response surface models

The process of extraction has a significant impact on the functional attribute of any given protein. As a result, choosing and verifying the best extraction technique requires a thorough examination. Since the current conventional procedures have numerous drawbacks, novel enhanced extraction techniques have been suggested as an alternative (Suchintita Das et al., 2022b)**.** To achieve maximal response in terms of extraction yield and protein content simultaneously in UAE, variables such as Power (A), Solute-to-solute ratio (B), Sonication time (C), and Total volume (D) optimization were carried out using a statistical response surface model. A total of 29 runs were carried out utilizing the BBD to evaluate and optimize the combined influence of the four process parameters on both response variables. The methodology for fitting models is a significant advancement over earlier approaches because it makes explicit assumptions that might otherwise remain hidden, makes the most use of the information contained in a set of data, and provides a "goodness-of-fit test" to determine whether a model is significant prior to analysis (Boateng et al., 2023)**.** As observed in **Table 2**, the extraction yield ranged from 15.23 to 19.13 %. The highest yield value (19.13 % was achieved at a solute-to-solvent ratio of 0.06 (1:15 g/mL), sonication power of 180 W, total extraction volume of 750 mL, and 35 min of ultrasound treatment.

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Table 2. Predicted and experimental values from the Box-Behnken design matrix

	Factor 1	Factor 2	Factor 3	Factor 4	Response 1		Response 2	
Run	A: Power	B: Solute/ water	C: Sonication time	D: Total volume	Extraction yield (g/100g) Experimental	Predicted	Protein content % Experimental	Predicted
	W	S/W	min	ml	%			
6	115	0.155	35	750	17.85	17.56	92.86	91.20
15	115	0.155	35	750	17.89	17.56	91.94	91.20
24	115	0.06	35	1000	16.61	16.69	91.69	89.04
17	50	0.06	35	750	16.59	16.06	91.19	92.52
14	180	0.155	10	750	16.96	17.58	90.56	86.39
25	115	0.155	35	750	17.36	17.56	90.50	91.20
28	115	0.155	35	750	17.36	17.56	90.38	91.20
27	115	0.155	35	750	17.35	17.56	90.30	91.20
2	115	0.25	35	1000	17.01	17.45	90.06	84.74
29	115	0.06	35	500	18.52	18.66	90.00	89.11
12	50	0.155	35	1000	15.23	15.23	89.94	89.91
26	50	0.155	10	750	15.27	15.72	89.50	87.16

9	50	0.155	60	750	16.39	16.35	89.31	87.28
4	180	0.155	60	750	18.04	18.04	88.75	84.89
13	115	0.06	60	750	18.11	18.63	87.88	88.18
23	115	0.06	10	750	16.82	16.99	87.00	87.62
16	115	0.155	10	500	17.94	17.68	86.81	87.33
5	115	0.25	35	500	17.32	17.32	86.5	82.94
3	50	0.155	35	500	16.54	16.54	86.13	86.89
22	180	0.155	35	500	18.52	18.24	84.81	87.46
1	180	0.06	35	750	19.13	18.74	84.63	85.91
7	115	0.155	60	1000	17.15	17.12	84.44	87.51
10	180	0.155	35	1000	17.56	17.24	84.31	86.17
8	115	0.155	60	500	18.08	17.95	83.88	84.39
20	180	0.25	35	750	17.64	17.87	83.45	85.70
18	115	0.155	10	1000	16.34	16.17	82.88	85.94
19	115	0.25	10	750	18.79	17.98	81.31	83.63
11	50	0.25	35	750	16.77	16.86	79.94	82.25
21	115	0.25	60	750	18.01	17.56	79.69	81.69

As shown in **Table 3**, analysis of variance (ANOVA) was used to evaluate the proposed model equation. A lower p-value ($p < 0.0001$) for extraction yield demonstrated that the fitted models were significant. The F-values and p-values of lack-of-fit models implied that it was not significantly relative to the pure error indicating the suitability of the model for optimization (Sahu et al., 2020). For the quadratic regression models, the calculated correlation coefficient (R^2) was 0.83 indicating that 83 % of the variances could be explained by the fitted model (**Fig 2**). In this experiment, A, C, D, BC, and A^2 were significant model items while the other terms were insignificant ($p > 0.05$). With regards to protein content, the developed model showed a p-value of 0.20 indicating that the model was not significant.

The computed correlation coefficients (R^2) for protein content in the quadratic regression model were 0.61, meaning that 61% of the variations could be accounted for by the fitted model. In this case, B, B^2, and C^2 were the only significant model terms with regard to protein content. The reason for the insignificance in protein content could be due to the use of constant solubilization pH and precipitation pH. In this study, there was no need to optimize the pH as the precipitation pH of proteins from legumes is well documented (Jeganathan et al., 2023; Langton et al., 2020). Herein, the experimental dataset was subjected to a regression analysis to fit in the established second-order quadratic model. Regression analysis was performed on this experimental dataset to attempt to fit it into the established second-order quadratic model. The following polynomial equation expresses the predicted extraction yield and protein content.

Extraction yield (%) = $17.562 + 0.92A - 0.02B + 0.305C - 0.585D - 0.4175AB - 0.01AC + 0.0875AD - 0.5175BC + 0.4BD + 0.1675CD - 0.51A^2 + 0.33B^2 - 0.099C^2 + 0.234D^2$

Protein content (%) = $91.1975 - 0.790625A - 2.619166667B - 0.343541667C + 0.4325D + 2.5175AB - 0.40625AC - 1.078125AD - 0.625BC + 0.46875BD + 1.124375CD - 1.726979167A^2 - 2.875416667B^2 - 3.042604167C^2 - 1.862916667D^2$

where A, B, C, and D are the independent variables for Power (A), Solute-to-solvent ratio (B), Sonication time (C), and Total volume (D), respectively.

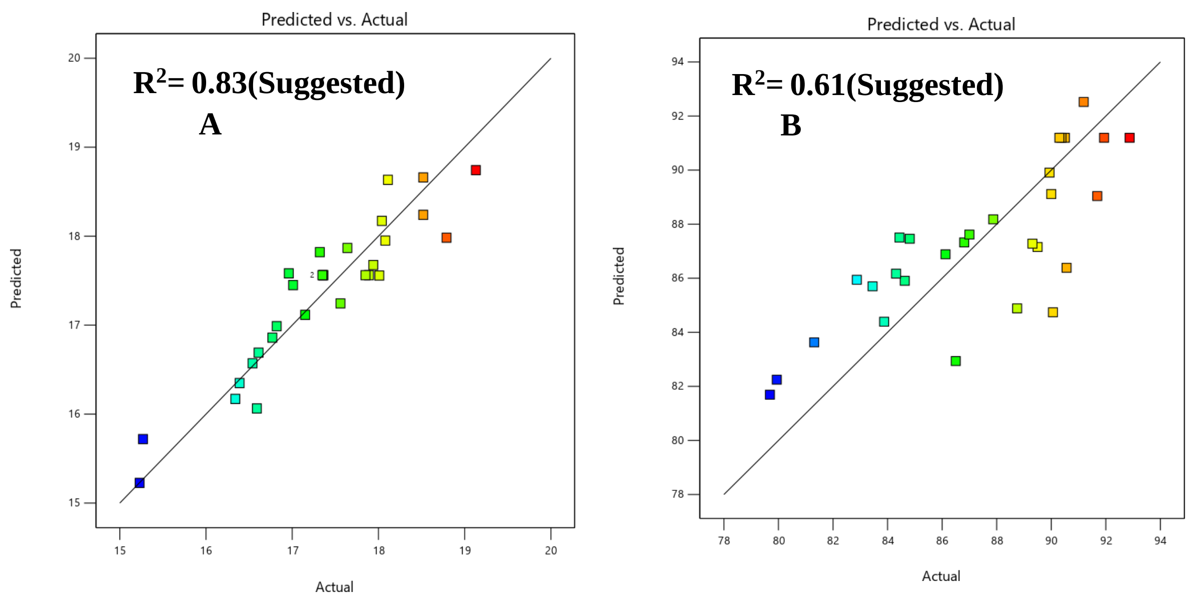

Fig 2. Regression coefficient of quadratic model for extraction yield (%) and protein content (%).

Table 3. Variance analysis for the protein content and extraction yield (%) regression model.

Source	**Extraction yield (%)**			**Protein content (%)**		
	Sum of Squares	**F-value**	**p-value**	**Sum of Squares**	**F-value**	**p-value**
Model	21.16	6.34	0.0007	236.19	1.57	0.20
A-Power (W)	10.19	42.79	<0.0001	7.50	0.70	0.42
B-Solute/water(g/ml)	0.01	0.02	0.88	82.32	7.67	0.02
C-Sonication time (min)	1.12	4.69	0.04	1.42	0.13	0.72
D-Total volume (ml)	4.11	17.24	0.0009	2.25	0.21	0.65
AB	0.69	2.93	0.11	25.35	2.36	0.15
AC	0.00	0.00	0.96	0.66	0.06	0.81
AD	0.03	0.13	0.72	4.65	0.43	0.52
BC	1.07	4.50	0.05	1.56	0.15	0.71
BD	0.64	2.68	0.12	0.89	0.08	0.78
CD	0.11	0.47	0.50	5.06	0.47	0.50
A^2	1.67	6.99	0.02	19.35	1.80	0.20
B^2	0.70	2.93	0.11	53.63	4.99	0.04
C^2	0.06	0.27	0.61	60.05	5.60	0.03
D^2	0.36	1.49	0.24	22.51	2.10	0.17
Residual	3.34			150.18		
Lack of Fit	3.02	3.81	0.11	144.85	10.87	0.02

Pure Error	0.32			5.33		
Cor Total	24.48			386.36		

significant at a 5 % level of significance.

Perturbation plot

As the focal point of the experimental design, **Fig. 3** illustrates the combined influence of factors on the yield and protein content of faba bean protein extraction. By changing one variable while keeping the other variables constant, the extraction yield perturbation plot was generated. With the exception of factor B (solute/solvent ratio), it was shown that power, sonication duration, and total volume significantly impacted extraction yield. This was indicated by the relatively flat line of factor B in **Fig 3.A** indicating lower influence on extraction yield. Power, or Factor A, has the steepest curve, indicating its exceptional significance in the extraction process. Followed by total volume D, with also a positive effect on extraction yield. In contrast to factors A and D, factor C (sonication duration) showed a comparatively flat trend, yet it significantly affected the extraction yield. Perturbation results showed increasing total volume was not suitable for maximizing extraction yield. In the case of protein content (%), the one factor that was observed to be significant was the ratio of solute to solvent ratio. Both **Fig. 3.A and B**, show that the solid-to-solvent ratio had a significant impact on the protein content and extraction yield and protein content. This behavior may be attributed to an enhanced driving force for the mass transfer of proteins, which promotes the diffusion of the solvent into cell compartments and facilitates protein release from the solute (Bedin et al., 2020).

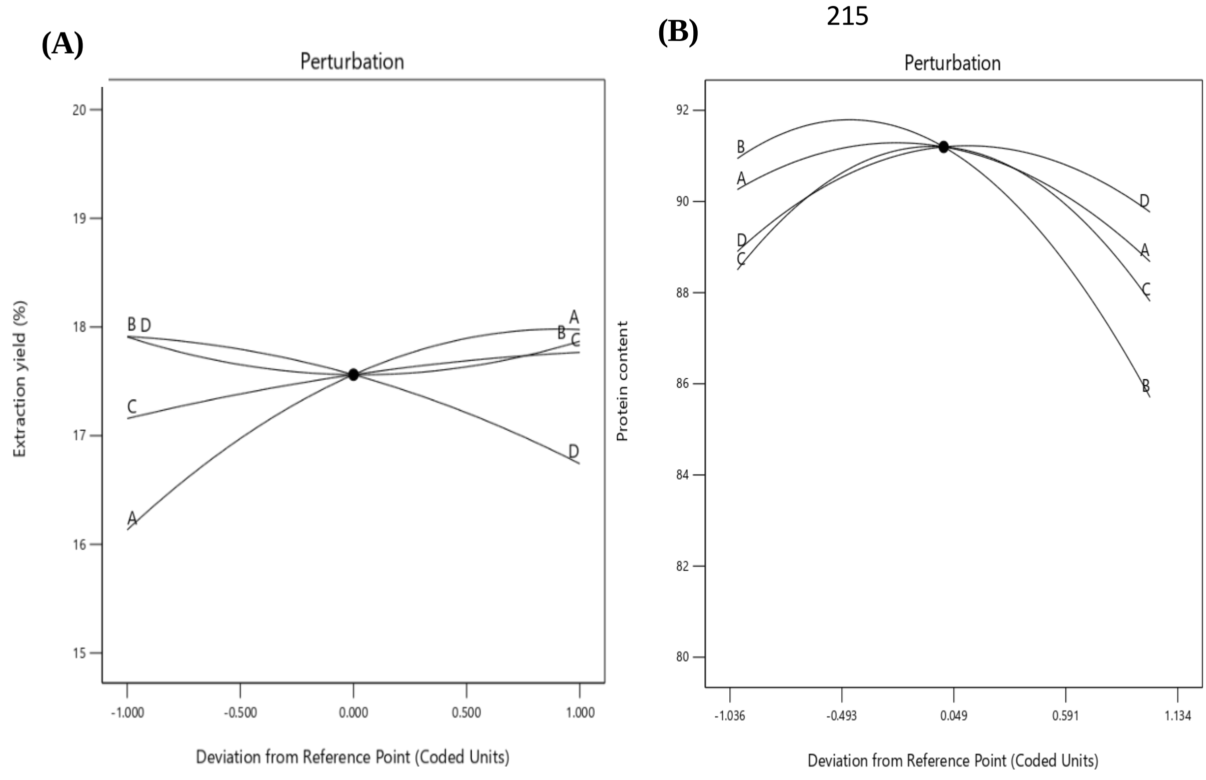

Fig 3. Perturbation plot for faba bean protein (A) extraction yield and (B) Protein content (A: Power, B: Solid/solvent ratio, C: Sonication time, D: Total volume).

Effect of independent variables on extraction yield (%) and protein content (%)

UAE was more effective in the current investigation at extracting proteins from faba beans. Given its excellent scalability, Suchintita Das et al., (2022b) claimed that the UAE represent a very promising approach in this regard. Through the combined effects of cavitation, agitation, and thermology, UAE demonstrates greater extraction efficiency from plant sources (Navaf et al., 2023)**.** Numerous research studies support UAE's effective deployment to extract proteins from plant sources such as those from mustard meal (Jahan et al., 2022)**,** alfafa flour (Hadidi et al., 2020)**,** and moringa oleifera seeds (Fatima et al., 2023)**.**

Using Design-Expert software, the three-dimensional (3D) response surface plot was constructed. The 3D plots allow the possibility to visualize the interactions between the experimental factors and the response between two test factors (Guo et al., 2018). Every response surface displays the function of two variables, while the third variable remains constant. In the event where the response surface graph was curved, the quadratic term was significant on the plot (Fatima et al., 2023). Extraction of proteins was done using a constant Alkaline solubilization of pH 11 and isoelectric precipitation of pH 4 based on previous studies (Badjona et al., 2024b; Jeganathan et al., 2023). Fig.**4. A-F** illustrates the 3-D plots interactions for extraction yield. The values of extraction yield by solute to solvent ratio and power while maintaining total volume and sonication time constant are represented in **Fig.4. A.** Increasing the solute and solvent ratio and higher ultrasonic power showed an increasing extraction yield. High solute/solvent ratio enhances the contact between faba bean flour and the solvent, resulting in an increase of protein in the dispersion.

At high solvent to solute ratios, there was a greater rate of extraction, which may indicate improved interaction with the sample environment through increased sonication power, allowing mass transfer and cell wall penetration. Further increasing sonication power results in a decrease in extraction yield due to protein gradient reduction (Rashid et al., 2022). This can also be observed in the quadratic effects where both solute-to-solvent ratio and power had a significant impact on the extraction yield. Therefore, 0.06 (1:15 g/mL) was selected as the best flour-to-water ratio. As shown in **Fig.4.B**, the relationship between sonication time and sonication power showed that increasing sonication time increased the yield of protein extraction (not significant) with minimal effect compared to ultrasonic power. High ultrasound power and relatively longer sonication time resulted in ultrasonic cavitation which was conducive to the diffusion of protein from the cell to the solvent (Liu et al., 2019a). The results of the current investigation supported the claims made by Brahmi et al., (2022), which indicated that the extraction rate of biological compounds increase in 30 minutes before subsequent reduction in yield**.**

Fig.4.C showed that increasing sonication time and solute-to-solvent ratio led to an increase in extraction yield with a significant effect observed for solute/solvent ratio. In general, maximal extraction yield was found higher between 30 – 60 min. Similar research has shown that extending the extraction period beyond 60 min did not increase the protein extraction yield (Eromosele et al., 2008; Qiu et al., 2023)**.** On the other hand, **Fig.4.D** shows the effect of total solution volume and ultrasonic power on extraction yield. A higher total extraction volume was found to be less desirable while a higher power was suitable to increase extraction yield. Total extraction volume had negative effect, meaning that the extraction yield of faba bean protein was more suitable at low extraction volume.

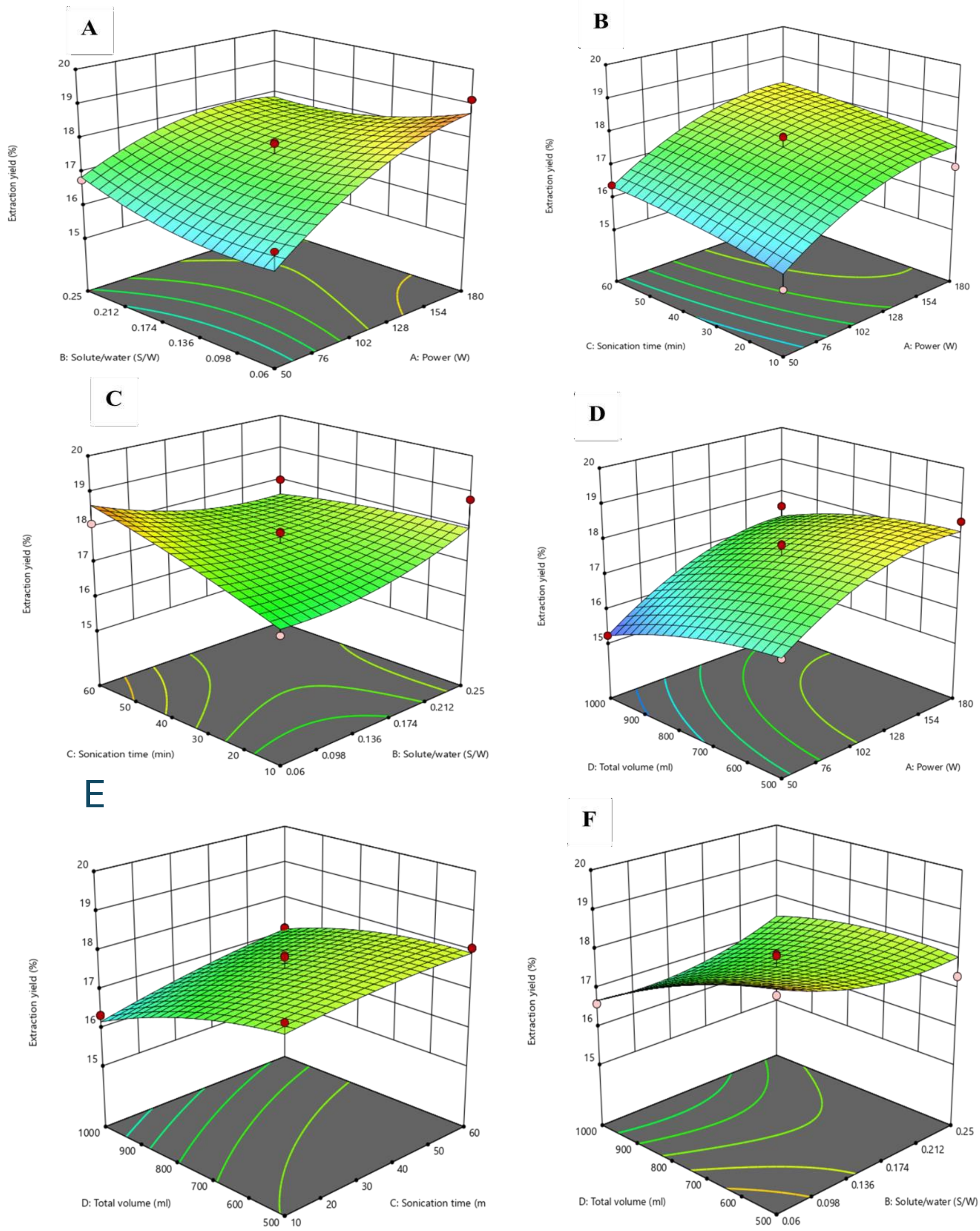

Fig.4. Response surface plots for the interaction between sonication power, sonication time, solute-to-solvent ratio, and extraction volume on extraction yield (%). (A) shows the interaction between solute/solvent ratio and Power; (B) sonication time vs Power; (C) sonication time vs solute/water ratio; (D) Total volume vs Power; (E) Total volume vs sonication time (F) Total volume VS solute/solvent ratio on extraction yield.

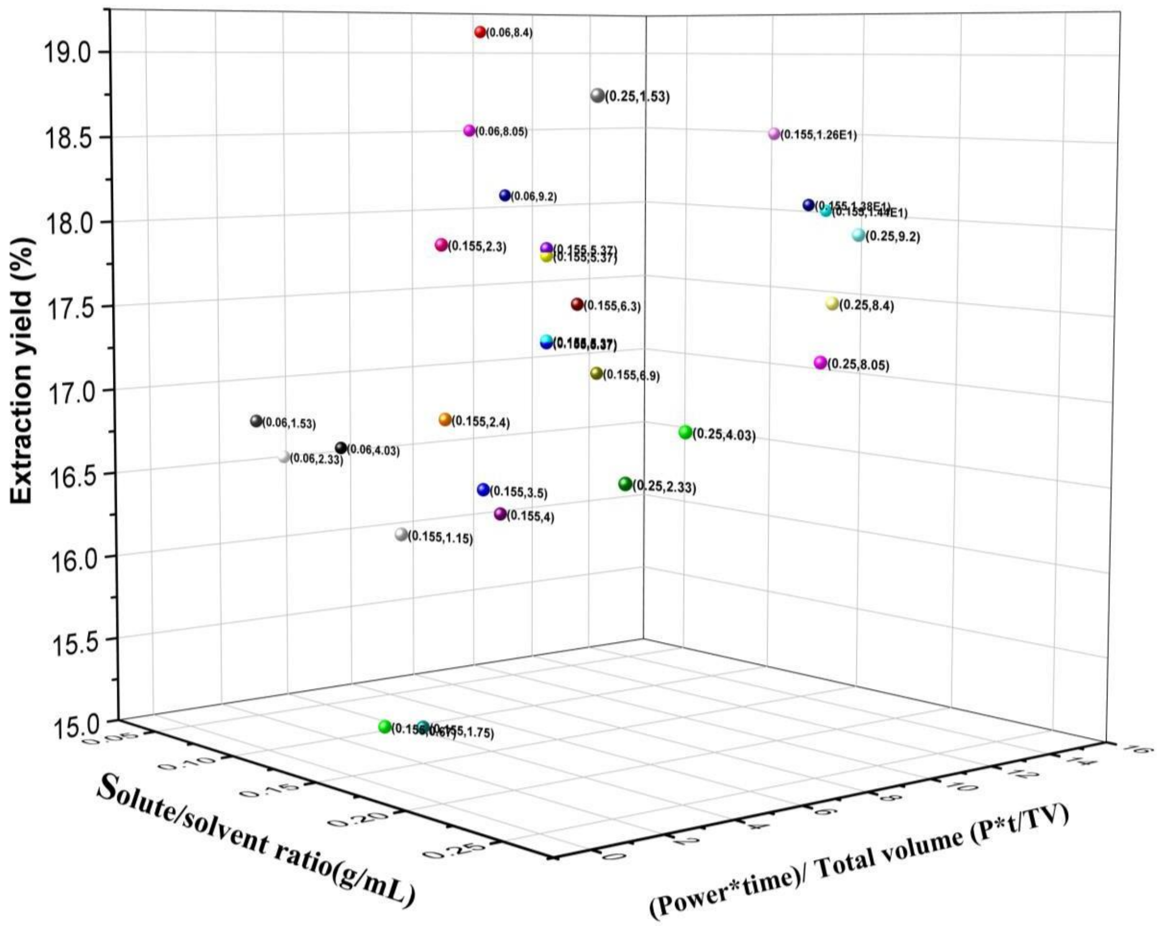

Fig.5. 3D master plot of combined effect of solute to solvent ratio(g/mL) against Wmin/mL (Power*time/Total volume). Labels (x,y) represent x(solute/solvent ratio: g/mL); y (power*Total volume : P*t/TV).

Aside from extraction yield that is mostly used to characterize extraction efficiency, protein content also represents a major variable for quantifying effectiveness of an optimization process. Depending on the process conditions, the protein content in the current study ranged from 79 to 92%. In the case of protein content, somewhat similar observations were observed as shown in **Fig.6. A-F**. Generally, higher protein content is obtained with a moderate volume of sonicating solution, sonication power and sonication time, and a higher solute-to-solvent ratio of 0.06 (1:15 g/mL). As shown in **Fig.6. A**, there was no significant increase in protein content with longer sonoprocessing times; nonetheless, the maximum protein content was reached at ~30 min as opposed to 60 min**.** The prolonged treatment may have caused a temperature rise, which in turn reduced surface tension and viscosity and increased vapour pressure, hence minimizing sonication impact (Suchintita Das et al., 2022b)**.** In contrast, the protein content increased with a high solid/solvent ratio as shown in **Fig.6. B**. A high solute-to-solvent ratio creates a high gradient in protein concentration in and out the cell matrices, thereby improving protein content (Fatima et al., 2023). Thus, an optimum value of 0.06 (1:15 g/mL) was found to be the best. Protein matrix, extraction process, source of material and other factors affects the choice of solute/solvent ratio (Chemat et al., 2017)**.** Other studies have shown an improvement in protein content after sonication, for instance soybean protein (Ding et al., 2021), yam bean protein (Eromosele et al., 2008) and wampee protein (Liu et al., 2019b)**.**

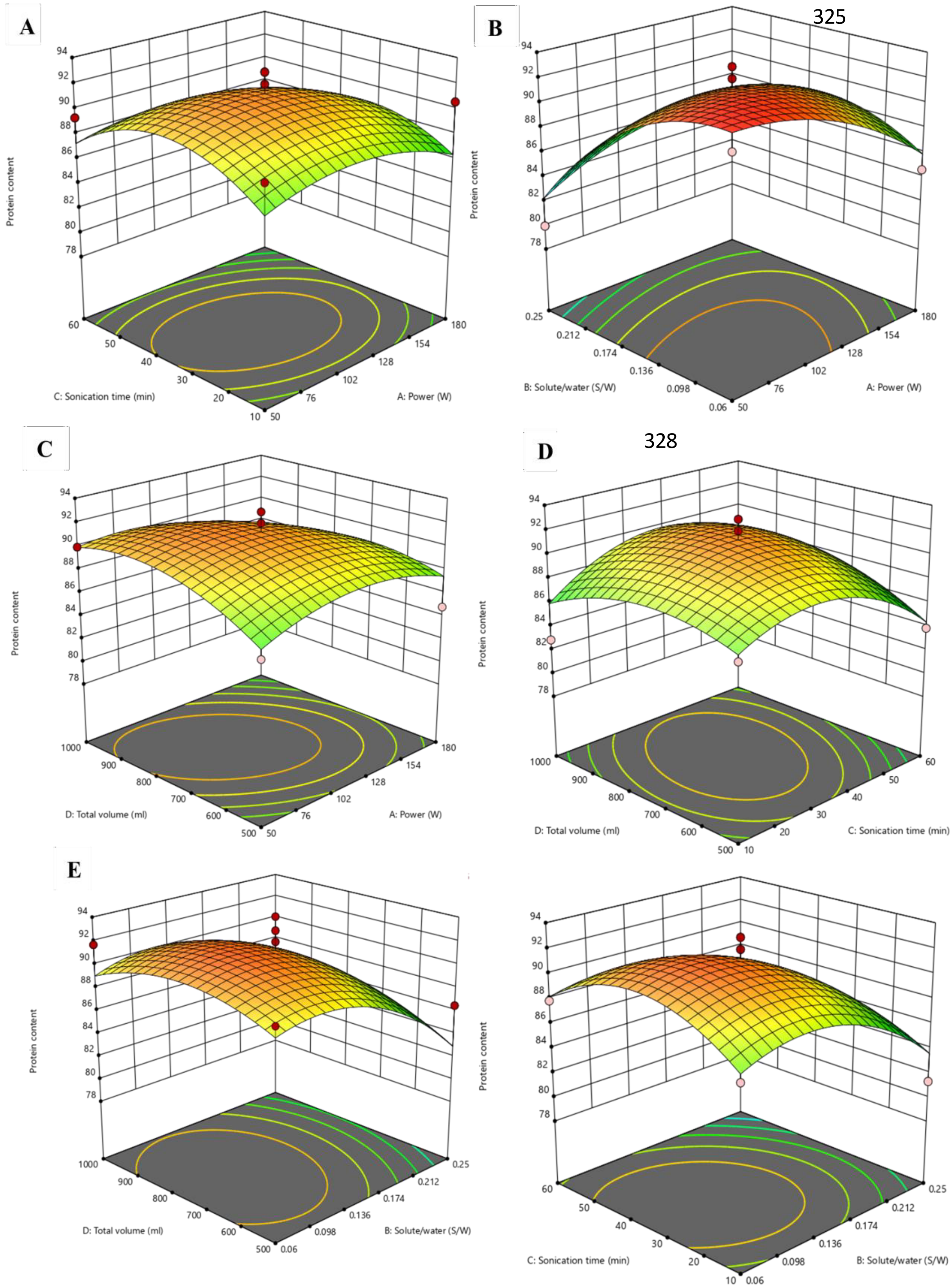
A
B
C
D
E
Protein content
C: Sonication time (min)
A: Power (W)
B: Solute/water (S/W)
D: Total volume (ml)

Fig 6. Response surface plots for the interaction between sonication power, sonication time, solute-to-solvent ratio, and extraction volume on protein content (%). (A) shows the interaction between sonication time vs Power; (B) Solute/water ratio vs Power; (C) Total volume vs Power; (D) Total volume vs sonication time; (E) Total volume vs solute/water ratio; (F) sonication time vs solute/water ratio on protein content %.

The plot in **Fig.7.** shows values of extraction yield (%) and protein content (%) variables to solute/solvent ratio and Power(W) variables. These contour diagrams were used to analyze the relationship between the three variables. One dependent variable is displayed on the z-axis, while two independent variables are displayed on the x and y axes. Contour plots are a useful tool for determining which combinations yield favorable results. With the desirability technique, responses are assigned a numerical value between 0 and 1, and variable settings are selected to increase the score for the optimisation of aggregate responses (Ares, 2014). A composite desirability of 0.6 - 0.8 is considered a satisfactory value according to Jarpa-Parra et al., 2014), hence the result of 0.83 in this present study is suitable. Verification tests were carried out in these conditions in order to assess and validate the reliability of the results.

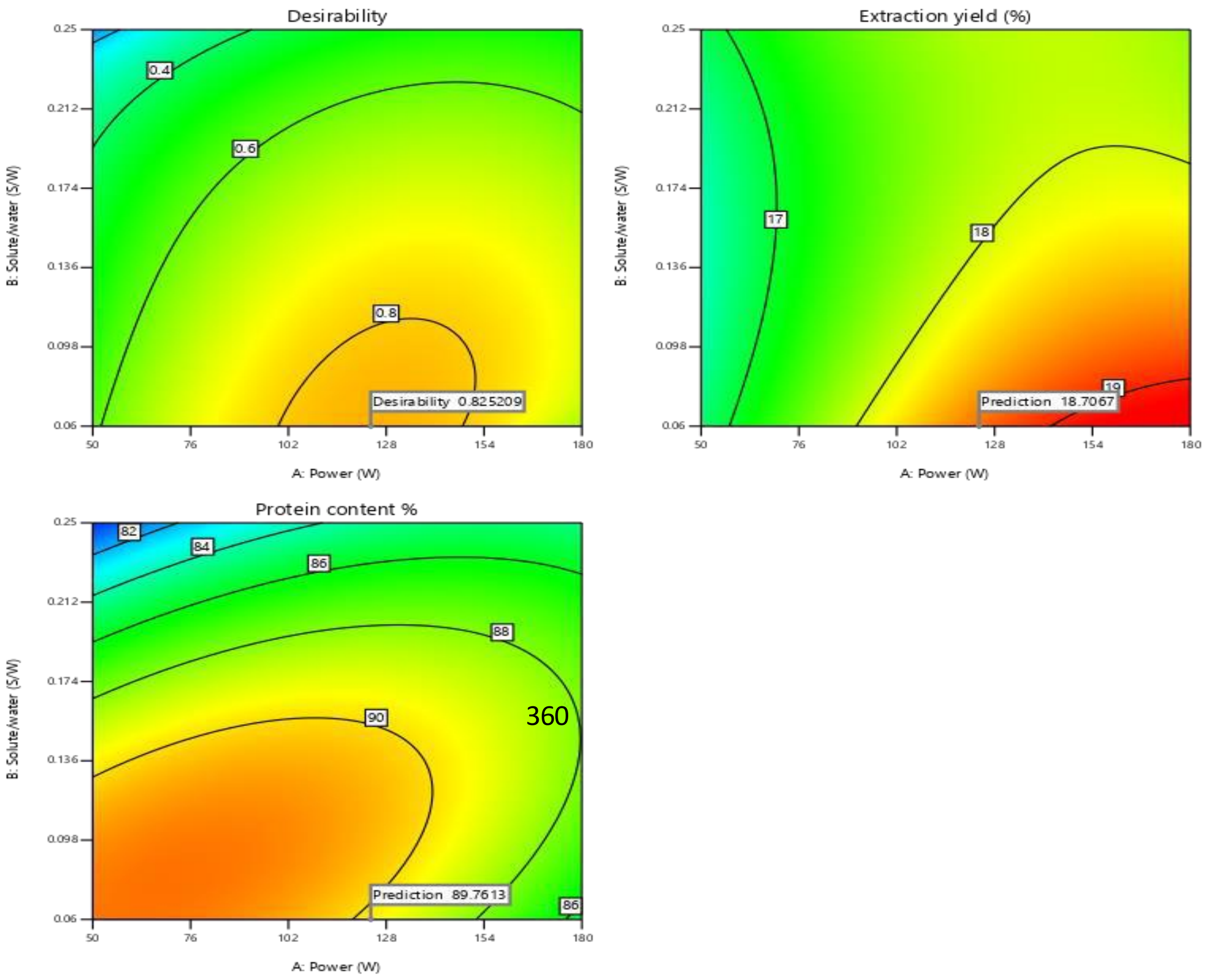

Fig 7. Contour plot on Desirability (Solute/solvent ratio vs Power).

Simultaneous validation and optimization of the isolation process

Simultaneous optimization of extraction yield and protein content of the ultrasound-assisted alkaline isoelectric precipitation on faba bean protein isolate was carried out experimentally to compare to predicted results. The suitability of the generated model was validated and tested based on the optimal conditions recommended to give maximal responses.

The following conditions: Power 123 W, solute to solvent ratio 0.06 (1:15 g/mL), sonication time 41 min, and total volume 623 mL were predicted to give a maximal yield of 18. 71 ± 1 %

and protein content of 89.76 ± 1%. In most ultrasound assisted extraction of proteins from plant materials, the crucial limit range between 20 and 60 min (Suchintita Das et al., 2022b). This duration may vary based on ultrasound equipment and other extraction conditions applied. These fixed conditions: Power 123 W, solute to solvent ratio 0.06 (1:15 g/mL), sonication time 41 min, and total volume 623 mL after experimental confirmation showed an extraction yield of 19. 75 ± 0.87 % and protein content of 92.87 ± 0.53 % (n=3). Thus, the quadratic model used in this study was useful to obtain optimal conditions necessary to produce protein isolate from faba beans flour. A control sample under similar conditions without ultrasound showed an extraction yield of 16.41± 0.02 % and protein content of 89. 88 ± 0.40 % (n=3). Using alkaline extraction of faba bean isolates, a protein purity of roughly 80 – 90 % has been attained by Krause et al., (2023). Optimised ultrasound-assisted alkaline extraction in the present study resulted in an improvement in protein purity which could be attributed to ultrasound effects (Kingwascharapong et al., 2021) as well as the optimised process conditions.

Conclusion

The market for faba bean protein is predicted to rise sharply as a result of consumers' rising interest in eco-friendly and sustainable products. For the food and other industries, faba beans can provide a reliable source of alternative protein. This work investigated the production of faba bean protein isolates using ultrasound-assisted alkaline isoelectric precipitation. A Box-Behnken RSM was used to optimize extraction yield and protein content simultaneously. The obtained findings indicated that the solid-to-solvent ratio, sonication time, Power (W), and total extraction volume, affected the measured responses. The maximum extraction yield (19.75 %) and protein content (92.87 %) were reached following optimized conditions: Power of 123 W, solute/solvent ratio of 0.06 (1:15 g/mL), sonication time of 41 min, and total volume of 623 mL. Additional control protein isolates without ultrasound application generated an extraction yield and protein content of 16.41 % and 89.99 % respectively. This work demonstrates the excellent potential of utilizing the DoE-based approach for the optimization of protein extraction from faba beans, and a BBD model with specified parameters was found to be the most effective for a quicker and more efficient protein recovery with a superior extraction yield and protein purity. The green protein extraction process presented in this study might be further explored for possible industrial scale-up to understand its limitations and cost implications.

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.

Authorship contribution statement

Abraham Badjona: Investigation, Writing – review & editing, Data curation, Formal analysis, Methodology, Writing – original draft. **Robert Bradshaw:** Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Caroline Millman:** Supervision, Writing – review & editing. **Martin Howarth:** Conceptualization, Supervision, Writing – review & editing. **Bipro Dubey:** Conceptualization, Data curation, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Data Availability Statement

The data generated during the current study are available upon reasonable request.

Rights Retention Statement

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