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Optimization of ultrasound-assisted extraction of faba bean protein isolate: structural, functional, and thermal properties. Part 2/2

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ABSTRACT

Environmental concerns linked to animal-based protein production have intensified interest in sustainable alternatives, with a focus on underutilized plant proteins. Faba beans, primarily used for animal feed, offer a high-quality protein source with promising bioactive compounds for food applications. This study explores the efficacy of ultrasound-assisted extraction under optimal conditions (123 W power, 1:15 g/mL solute/solvent ratio, 41 minutes sonication, 623 mL total volume) to isolate faba bean protein (U-FBPI). The ultrasound-assisted method achieved a protein extraction yield of 19.75 % and a protein content of 92.87 %, outperforming the control method's yield of 16.41% and protein content of 89.88%. Electrophoretic analysis confirmed no significant changes in the primary structure of U-FBPI compared to the control. However, Fourier-transform infrared spectroscopy revealed modifications in the secondary structure due to ultrasound treatment. The U-FBPI demonstrated superior water and oil holding capacities compared to the control protein isolate, although its foaming capacity was reduced by ultrasound. Thermal analysis indicated minimal impact on the protein's thermal properties

under the applied ultrasound conditions. This research highlights the potential of ultrasound-assisted extraction for improving the functional properties of faba bean protein isolates, presenting a viable approach for advancing plant-based food production and contributing to sustainable protein consumption.

Keywords: Ultrasound, faba bean, Protein isolate, Structural properties, functional properties, thermal analysis

INTRODUCTION

Economic growth heightened cultural and social awareness, and an improved standard of living over the past decade has driven consumer preference for nutritious and flavourful foods. This shift has resulted in over $30 billion in revenue for the dietary supplement industry (1). To meet the rising nutritional demands of the world's growing population, plant protein has become a crucial part of diets. One of its key benefits is providing sufficient amounts of essential amino acids (2). Moreover, the unique physicochemical properties of plant proteins affect food processing, storage, and consumption, thereby impacting food quality and sensory attributes. This shift is often referred to as the "protein transition" (3).

Compared to other common protein crops, faba bean seeds (V. fabacaeae) have a higher protein content, approximately 30 % of dry matter, making them an appealing raw material for producing protein-rich foods (4). Additionally, faba beans are increasingly favoured as a plant-based protein source due to their effective nitrogen-fixing abilities and ease of cultivation (5). Moreover, faba bean protein offers a quality of amino acid profile and digestibility comparable to animal proteins, distinguishing it from many other plant-based proteins (6–8). A whole faba bean contains approximately 27 % protein, 2 % fat, 60 % carbohydrates, and 15 % fibre, along with various vitamins and minerals (9,10). Globulins, making up 70 – 80 % of the storage protein in faba bean seeds, are classified into two types based on their sedimentation coefficient: 7S vicilin-type globulins and 11S legumin-type globulins. Additionally, the albumin fraction in faba beans is notable for its high content of sulphur-containing amino acids compared to other seed proteins (11). The physicochemical and functional properties of globulin and other protein fractions vary due to differences in their structures. These structural variations lead to differences in attributes such as denaturation temperature, solubility profile, and functional characteristics (12,13). Protein-rich products from faba beans are typically classified into two categories: isolates, which contain approximately 90 % protein, and

concentrates, which have around 65 % protein (9). Protein isolates from pulses are generally obtained using various methods, including alkaline solubilization with subsequent precipitation at the isoelectric point, or salt extraction followed by micellization (9,14). Traditional extraction methods are often limited by their lower extraction yields and reduced protein purity. Additionally, many commercially available proteins exhibit poor solubility and are prone to significant denaturation, which can adversely affect their structural integrity and functional performance (15).

Ultrasound, a novel non-thermal technology for processing and chemical applications, is classified as a method for process intensification (16). Enhanced extraction with ultrasound primarily results from the benefits of acoustic cavitation, which is induced by ultrasonic waves traveling through the suspension (17). Ultrasound enhances extraction by mechanically increasing the contact area between liquid and solid phases, which improves solvent penetration into the sample and accelerates the diffusion of solutes into the solvent (18,19). However, optimizing the process for ultrasound-assisted extraction (UAE) remains limited. Effective UAE can lead to improved extraction yields and modified functional properties of proteins (20). Successful protein extraction relies on understanding how UAE parameters such as time, power, frequency, solvent-to-sample ratio, and temperature affect the extraction of faba bean proteins. Using response surface methodology to optimize these variables helps in selecting the best conditions to maximize extraction yield and purity.

Although ultrasound-assisted extraction (UAE) is a promising technique, there is a lack of in-depth studies on optimizing protein extraction from faba bean seeds. This research aims to fill this gap by introducing UAE for faba bean protein isolation. It involves: (i) optimizing UAE parameters using Box-Behnken Design (BD) to enhance extraction efficiency; and (ii) evaluating and comparing the structural, thermal, and functional properties of proteins isolated

through this method. The study offers a detailed analysis of the protein physicochemical and thermal characteristics obtained through optimized UAE.

MATERIALS AND METHOD

Raw Materials and Chemicals

Faba bean seeds were purchased from Whole Foods Earth (Kent, United). NaOH, ((≥ 99.9 % pure), HCI, phosphate-buffered saline (PBS) was also obtained from Sigma-Aldrich (United Kingdom). β-mercaptoethanol was obtained from Thermo Fisher scientific. Rapeseed oil was obtained from a local shop in Sheffield (United Kingdom). All the chemicals and reagents used in this study were of analytical grade. The seeds were finely powdered using a cyclone mill and kept at -20 °C until needed.

Optimization of ultrasonic-assisted extraction of faba bean protein

The optimization process aimed to determine the optimal set of parameters for achieving the highest extraction yield and protein content, thereby identifying the key influencing factors. This was informed by previous research findings (21) and other studies on protein isolation from plant sources, which guided the selection of minimum and maximum values for each factor (22,23). 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 min at 500 rpm before ultrasonic-assisted extraction. PH of the dispersion was then adjusted to pH 11, then subjected to ultrasonic treatment at varying ultrasonic power (50 – 180 W) and varying sonication duration (10 - 60 min) using a S24d22D titanium ultrasonic horn with a sonotrode diameter of 22 mm and radiating surface of 3.8 cm^2 (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 using a centrifuge (accuSpin^{TM} 400, United Kingdom). After gathering the supernatant, 1 N

121 HCI was used to bring the pH to 4.0 while stirring continuously for 20 min. Protein isolate
122 pellets were then obtained after centrifuging at 6,000 rpm for 20 min at 25 °C. After 48 hrs of
123 lyophilization of the protein pellet, samples were stored at -20 °C for further analysis. Protein
124 content was determined by the Dumas method. Control protein isolate was generated using
125 optimized conditions without ultrasound treatment.

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

128 **Extraction yield** (%) = $\frac{m_i}{m_s}\ x\ 100$ (Eq.1)

129 The mass of the initial flour and final protein isolate are represented by m_s and m_i, respectively.

130 **Box-Behnken analysis experiment**

131 The independent variables in the study were ultrasonic power, sonication time, solid/solvent
132 ratio, and total extraction volume. To maximize extraction yield and protein content from faba
133 bean flour, a response surface-based optimization method was employed using Design Expert
134 software. Each of the four variables was tested at three distinct levels: low (1), medium (0),
135 and high (+1). These variables were explored for their effects on the ultrasonic-assisted alkaline
136 extraction of faba bean protein isolates. Both the extraction yield and protein content of the
137 freeze-dried faba bean protein isolate served as the response variables. The comprehensive
138 results of the optimization process are documented (24). The coded factors for each variable
139 are displayed in Table 1.

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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

Functional properties

Protein oil and water holding capacity (OHC and WHC).

For WHC and OHC, 1.0 g of faba bean protein isolate was dispersed in 40 mL of distilled water and rapeseed oil, respectively. The mixtures were vortexed at maximum speed for one minute, and then allowed to stand at room temperature (20 – 23 ºC) for six hours. After that, samples were centrifuged for 30 minutes at 20 ºC at 3000 x g.

$$\text{WHC/OHC} = \frac{W_0 - W_1}{W_3}$$

Where W_0 is the mass of the tube and protein isolate and absorbed water or oil; W_1 is the mass of the tube and protein isolate while W_3 is the mass of faba bean protein.

Foaming properties

To study the foam stability and capacity, the method described by Loushigam et al., (25) was employed. Faba bean protein isolate solutions (1 % weight protein basis, pH 7) were dissolved in distilled water and stirred at room temperature for one hour. 15 mL of the protein mixture were homogenized for three minutes at room temperature using a homogenizer (IKA T18 ultra-turrax basic, United Kingdom). Using a graduated cylinder, the total volumes (mL) were measured before and after whipping. The following formula was used to compare the volume of the foam layer at 15 and 30 minutes to the initial foam volume of the samples to determine the foaming capacity (FC) and stability (FS):

$$\text{FC (\%)} = \frac{foam\ volume}{15\ mL}\ \text{X}\ 100\ \%$$

$$\text{FS (\%)} = \frac{foam\ volume\ after\ 15\ and\ 30\ min}{Inital\ foam\ volume}\text{X}\ 100\ \%$$

Qualitative analysis of proteins using electrophoresis (SDS-PAGE)

Electrophoresis was carried out on SDS-PAGE in a reducing solution of β-mercaptoethanol (26). 50 mg protein powders were dissolved in 10 mL of PBS buffer (0.01 M, pH 7) and stirred at 200 rpm for 2 hr at room temperature. 10 µL protein solution was dissolved, and vortexed with 10 µL loading buffer (reducing solution containing 10% 2-mercaptoethanol). The samples were heated for 4 min at 95 ˚C, followed by cooling, and centrifugation at 13300 x g for 3 min. An aliquot was injected into the pocket of the Bio-Rad 4 % acrylamide stacking gel. Separation at a current of 25 mA was performed for one hour at a voltage of 200 V for 35 mins. SDS-PAGE pre-stained ladder ranging from 260 – 8 kDa was used as standard marker. The gel was rinsed with water and stained sequentially with commasie blue and imperial stain. Destained gel was scanned using Gel analysis instrument (Nugenius, United Kingdom).

Fourier-transform infrared spectroscopy analysis

For the FTIR investigation, an Attenuated Total Reflectance (ATR)-FTIR spectrophotometer (Spectrum 100 FT-IR, PerkinElmer, USA) was used. A total of 16 scans were conducted within the wavenumber range 4000 – 650 cm^{-1} at a resolution of 4 cm^{-1}.

Thermal properties

Thermogravimetric analysis (TGA)

A thermogravimetric analyzer type Q50 was used to study the thermal properties of the protein isolates. About 2 mg of the material was heated from 30 to 900 °C under ambient nitrogen (200 mL/min).

Differential scanning calorimetry

The thermal profile of the protein isolates was measured using differential scanning calorimetry. Aluminum pans with a hermetically sealed interior held 10 – 20 mg of protein isolates. In an inert nitrogen environment, samples were heated at a rate of 10 °$Cmin^{-1}$ using a heat ramp from 25 to 180 °C.

X-ray diffraction (XRD)

For XRD studies, an X'pert X-ray diffractometer was employed. The anti-scatter slits were set at 0.04 mm along with 1 mm diverging and receiving slits. The diffractogram was measured between 5 and 70 º (2θ) with a step size of 0.05 º.

Statistical analysis

All statistical analyses were performed by Origin 2019. All the values were expressed as means ± standard deviation (SD).

RESULTS AND DISCUSSION

Optimal extraction conditions by RSM

The experimental results indicated that the conditions of 123 W power, a solute to solvent ratio of 0.06 (1:15 g/mL), a sonication time of 41 minutes, and a total volume of 623 mL were predicted to yield a maximum extraction yield of 18.71 ± 1 % and a protein content of 89.76 ± 1 % after optimization. Upon experimental validation, these conditions achieved an extraction yield of 19.75 ± 0.87 % and a protein content of 92.87 ± 0.53%. Therefore, the quadratic model used in this study proved effective in determining the optimal conditions for producing protein isolate from faba bean flour. In contrast, a control sample processed under similar conditions but without ultrasound treatment yielded an extraction rate of 16.41 ± 0.02 % and a protein content of 89.88 ± 0.40 % (24). Thus, the ultrasound treatment enhanced both the extraction efficiency and the protein content of the extracts, consistent with findings reported in the literature for other plant materials (27). The effectiveness of ultrasonication could be linked to cell disruption during the extraction process. Plant proteins are often covalently bound to other macromolecules like carbohydrates, cellulose, hemicellulose, and pectin. These bonds hinder protein release from plant matrices and reduce their availability in the extraction medium, necessitating an extraction method capable of effectively disrupting the cell matrix (28). In alkaline medium, ultrasonic waves produce cavitation, turbulence, and shear forces near the plant matrix, disrupting plant tissue and enhancing protein extraction (29). This is attributed to cavitation and the mechanical vibrations of ultrasound waves, which break cell walls and molecular bonds, increasing the contact surface area between the solid and liquid matrices (30).

Functional properties

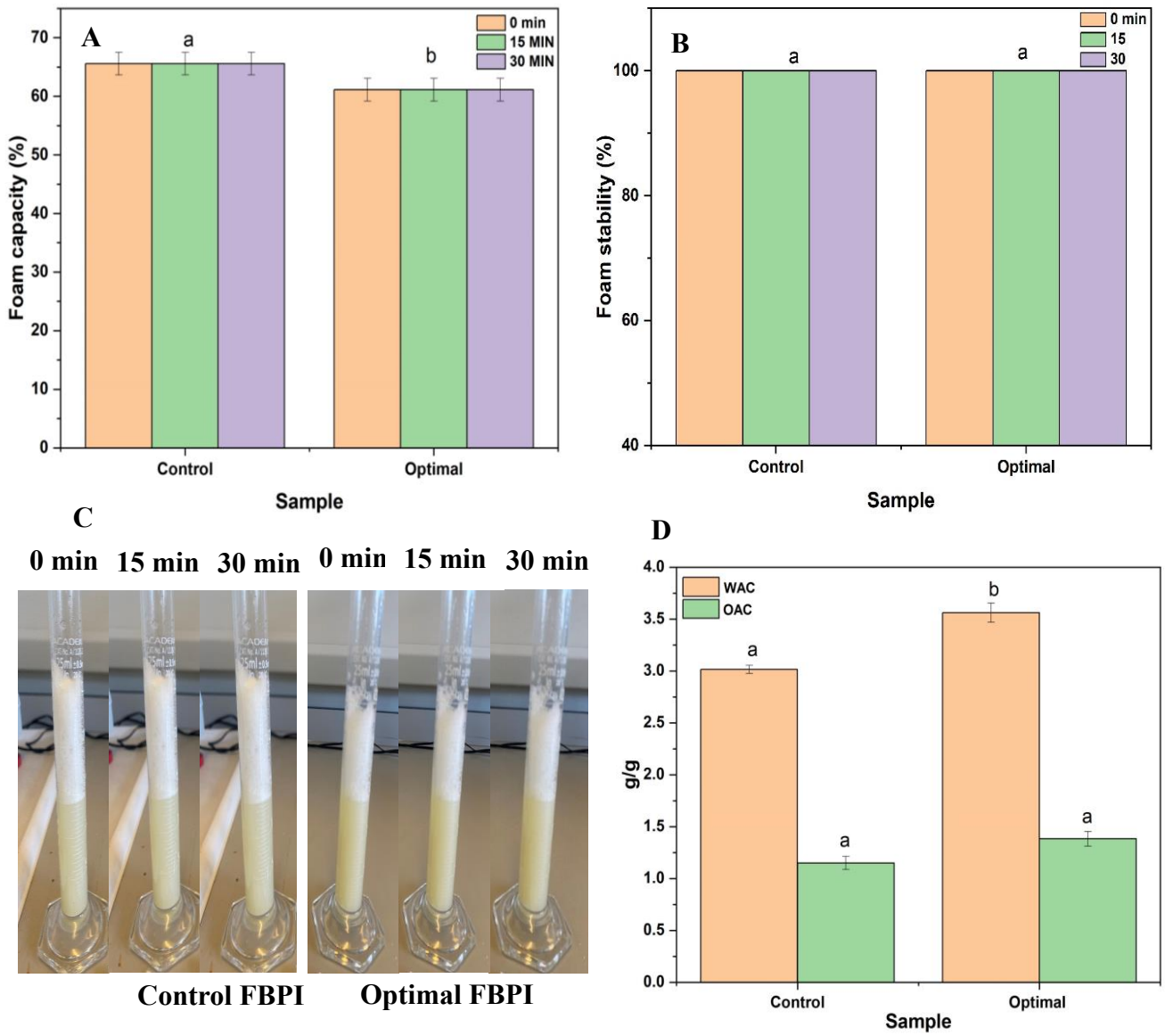
Foaming capacity (FC) and foaming stability (FS)

The foaming capacity and foaming stability of control and ultrasound FBPI are presented in **Fig 6.A& B**. While foaming stability (FS) refers to a protein's ability to produce stable foams by constructing a continuous intermolecular polymer network that encloses air cells, foaming capacity (FC) describes a protein's ability to unfold quickly forming a cohesive layer that surrounds gas bubbles (31). In theory, the main phases for protein to form foams are transportation, penetration, and reorganization of protein molecules at the air/water interface. The Foaming capacity of the control FBPI was 65.56 % which was higher than the optimal U-FBPI (61.11 %). Several studies have reported an improvement in foaming capacity after ultrasound treatment (32,33) which are contrary to those observed in these studies. However, a study by Gao et al. (34) on soluble pea proteins showed that ultrasound treatment can reduce foaming capacity through major modifications in protein structure and network which may affect FC. Apart from variations in the foaming method used, final power input per unit volume and other ultrasonic parameters may influence foaming capacity. In terms of foam stability, both samples showed 100 % stability as shown in **Fig.6.B & C** after 10, 15, and 30 min. This indicates that both protein isolates had sufficient mechanical strength, and flexibility to keep the foams intact. Chittapalo et al. (35) found that the alkaline extraction method resulted in a higher foaming capacity for rice bran protein compared to the ultrasonic extraction method. This reduction may be due to ultrasonic treatment altering the protein structure and changing the ratio of hydrophilic to hydrophobic groups. This increases surface tension and decreases surface activity, affecting the protein's adsorption capacity and migration speed at the air-water interface (36)**.**

252 **Water holding capacity and oil holding capacity (WHC)**

253 Information on the water and oil absorption capacity of proteins is useful in predicting protein
254 behaviour in food systems such as meat analogs, yogurt analogs, and bakery products. This is
255 necessary to prevent liquid loss (water or oil) during processing and avoiding undesirable
256 textural and sensorial properties (37,38). The water and oil absorption capacity of the optimal
257 ultrasound-assisted faba bean protein isolate and the control is shown in **Fig.6. D**. The water
258 holding capacity was significantly higher in the optimal ultrasound protein isolate (3.56 g/g)
259 compared to the control sample (3.01 g/g). The WHC of proteins is influenced by numerous
260 factors such as conformational structure, particle size, surface hydrophobicity as well as the
261 amino acid sequence (39). Similarly, a higher OHC was observed in the optimal ultrasound
262 protein isolate (1.38 g/g) than in the control sample (1.15 g/g). Lipid-protein interactions are
263 attributed to the binding of non-polar amino acid side chains to aliphatic chains of lipids, thus
264 proteins with high surface hydrophobicity tend to have a high OHC (40).

265 The relatively high fluid binding properties of the faba bean protein after ultrasound treatment
266 may have been due to the formation of a more porous structure (41). An increase in OBC values
267 may also be because ultrasonic cavitation caused partial denaturation of the faba bean's
268 proteins, which exposed hydrophobic groups at the surfaces of the protein powders, thereby
269 leading to greater oil retention (42). Proteins with higher hydrophobicity typically exhibit better
270 fat absorption, as the non-polar amino acid chains interact easily with lipid aliphatic chains
271 (43). The application of protein in specific foods often depends on the WHC to OHC ratio.
272 Non-protein substances such as starch and lipids can interact with proteins, altering the WHC
273 and OHC, highlighting the importance of efficient protein isolation from the plant matrix.



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275 **Fig 6.** Functional properties of optimal ultrasound-assisted and control extracted faba bean
276 protein isolate (A) foaming capacity (B) foaming stability (C) representative photographs of
277 the foam prepared from (1 % wt., protein basis, pH 7) at 0, 15 and, 30 (D) water and oil
278 absorption capacity.

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SDS-PAGE analysis

SDS electrophoresis was performed to analyze the protein profiles of the two protein isolates: optimized ultrasound-assisted extraction and conventional faba bean protein isolates. **Fig 7**. shows the protein profile of the two isolation conditions under reducing conditions. Several bands were observed from 8 - 90 kDa. An additional smearing band was observed from 260-90 kDa. Similar subunit bands were observed between optimized and controlled protein isolates. These results are in agreement with different studies which observed no changes on primary structure after ultrasound application in soy protein isolate, moringa protein and pea protein isolate (44,45). Two strong protein bands detected ~90 kDa could correspond to seed lipoxygenase (46). A strong protein band was detected at ~50 – 55 kDa in both samples. This band could be the globulin convicilin (46). The trimeric protein is one of the primary storage proteins in Vicia faba L. The dense protein bands detected at from ~38kDa may represent vicilin subunits while protein subunits at 15 kDa and below may be associated with albumins (46).

The results indicate that ultrasound treatment did not alter the primary structure of macromolecules, as the applied sonication conditions were insufficient to disrupt this structure. Polypeptides in the 20–29 kDa and 29–44 kDa ranges are likely related to the acidic and basic subunits of glycinin (47,48). Subunits around 50 kDa and 70 kDa are probably vicilin and convicilin, respectively (47). Singh & Kaur, (2019) identified polypeptides with molecular weights of 12–120 kDa and 11.5–122 kDa as corresponding to albumin and globulin fractions, respectively. The SDS-PAGE results support the findings from Zou et al. (50) and O'sullivan et al. (51), which demonstrated that ultrasound does not significantly alter the molecular structure of protein isolates.

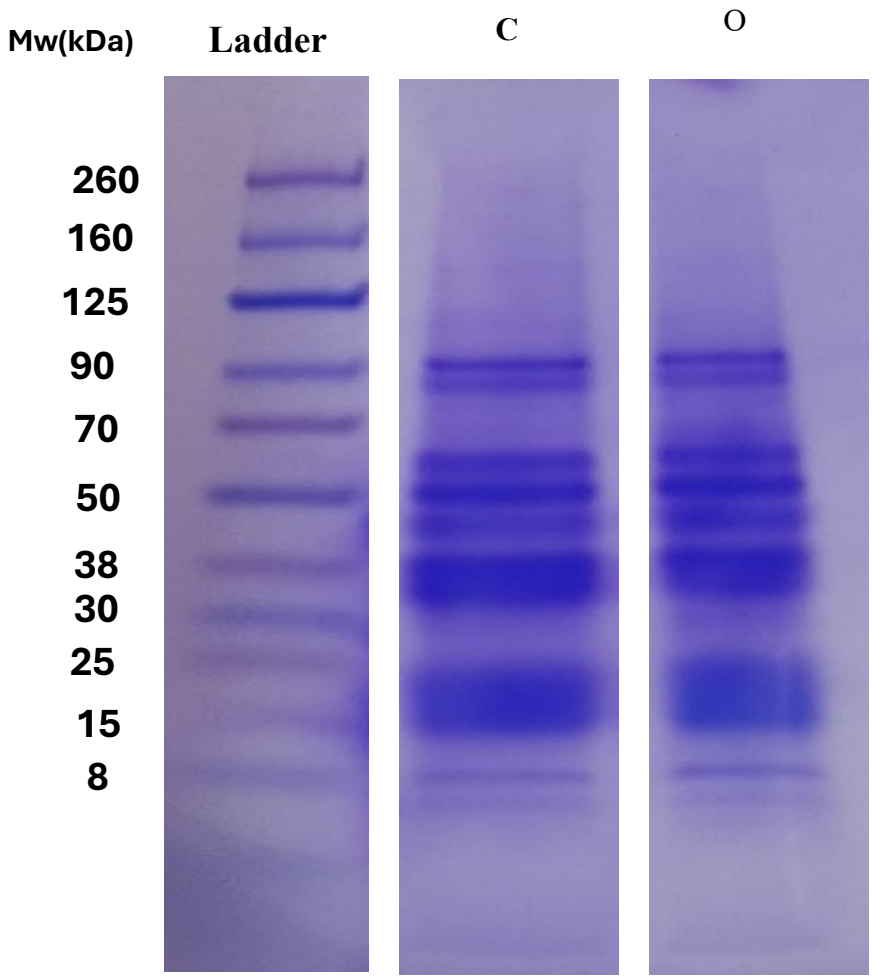

Fig. 7.SDS-PAGE protein profile of faba bean isolates under reducing conditions. C represents conventional protein extraction while O represent ultrasound-assisted extraction.

FTIR

FTIR spectra of optimised protein isolate and conventionally extracted protein were both in accordance with those found from other protein isolates such as pea, quinoa, and album proteins (52,53) (**Fig. 8**). Dominant regions attributed to protein functional groups Amide I, Amide II and Amide II (**Fig 8.B**) were found in both spectra. Additionally, other regions such as Amide A and B (region 3500 – 2500 cm^{-1}) were also observed in both samples. Bands ranging from 1200–1000 cm^{-1} mostly ascribed to carbohydrate regions showed major

differences between the two samples (54). Major differences were observed in the spectra in all amide regions especially in amide A and B regions however slight differences in the Amide I, II, and III regions. Optimized ultrasound-extracted proteins showed lower absorbance in amide A and B regions in comparison with the control protein isolate. The lower peak intensity of ultrasound-produced protein isolates could be attributed to protein-protein interaction occurrence via hydrogen bonds in a higher degree. Amide I band which is the most sensitive protein region was also confirmed to result from C = O stretching vibrations and N-H bending vibrations (52,55). The amide I band of both optimized and control samples showed similar wavenumber and intensities, however, the Amide II band of optimized protein isolate showed a higher intensity compared to control isolates. Additionally, the carbohydrate region (1200 – 1000 cm^{-1}), showed lower intensity in ultrasound extracted proteins compared to the control, indicating high protein purity in ultrasound-assisted protein extraction**.** Modification of the secondary structure after ultrasonic-assisted treatment can be attributed to the breaking of β-sheet secondary bonds resulting in the rearrangement into α-helices and β-turns (36). Additionally, the reduction in absorption intensity in the Amide A and B regions may be attributed to partial swelling of the protein structure and disruption of hydrogen bonding interactions. This results in the cleavage of β-sheet and β-turn bonds and their reorganization into α-helices, altering the protein structure (36).

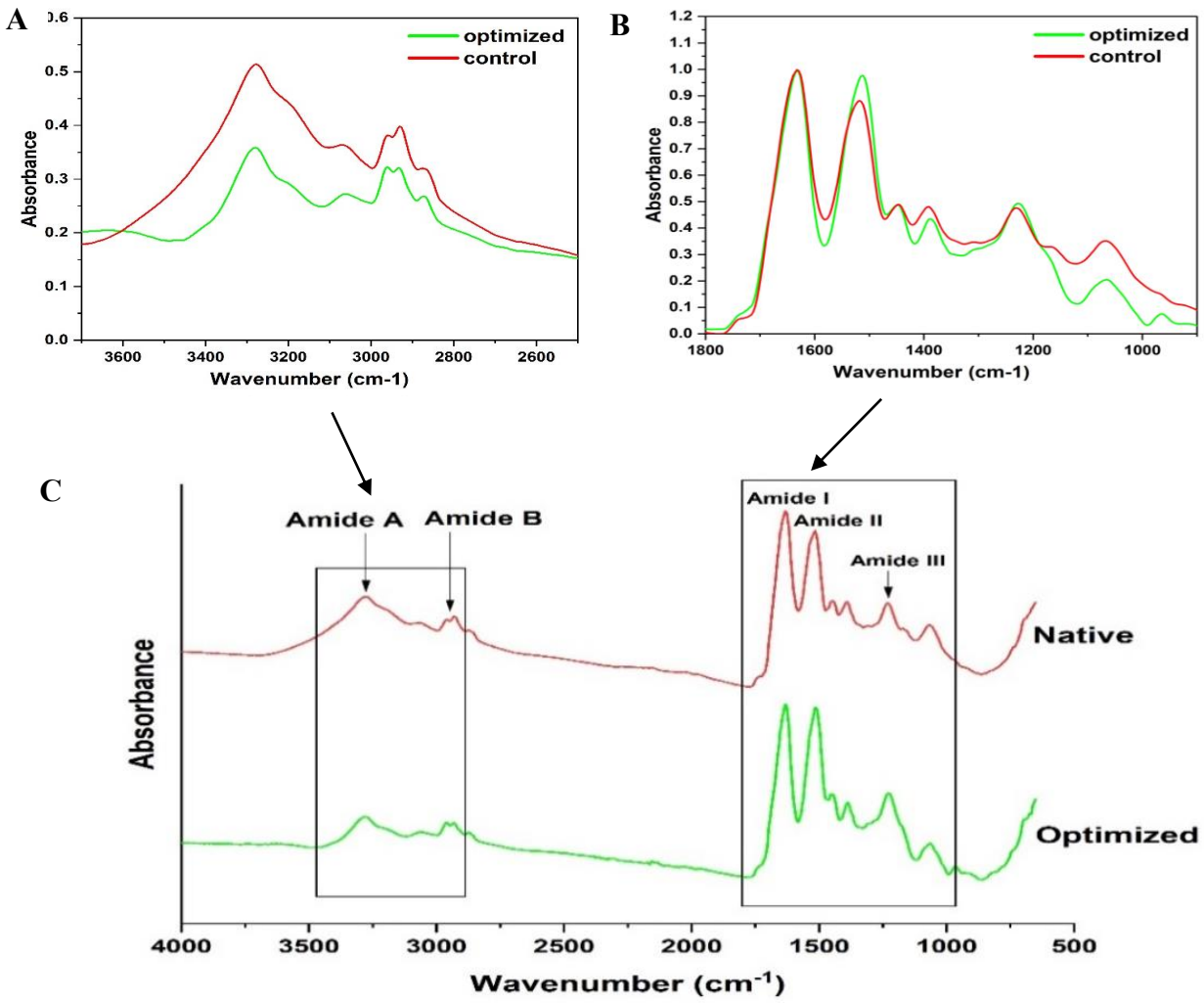

Fig 8. FTIR spectra of freeze-dried protein isolate from faba beans flour by optimized ultrasound-assisted and conventional extraction process.

X-ray diffraction

X-ray diffraction is used to investigate the phase composition and crystal structural properties (56). XRD patterns of protein obtained from faba bean flour by ultrasound and conventional extraction process were studied to provide further structural information. The diffractogram of ultrasonic-assisted and conventional faba bean isolates is shown in **Fig. 9**. Slight differences between both samples showed that extraction conditions influenced diffractogram patterns. The

first diffraction peak was observed between 5° and 10° (low intensity), which is related to a relatively sharp diffraction peak. The second peak was observed around 20° (high intensity). A very small peak was observed between 40° – 45° for untreated extracted faba bean protein isolate. The crystalline region I and II is attributed to 2θ=10° and 2θ=20° respectively. Information on crystalline size can be obtained from diffraction intensity and area. A small crystal size usually shows a low diffraction intensity and vice versa (57). The results are similar to those of 7S and 11S proteins obtained from soy proteins (58) as well as results obtained from mung bean proteins (59). Whey protein isolates were also reported to have comparable peaks at 2θ = 8° and 19.5° (60).

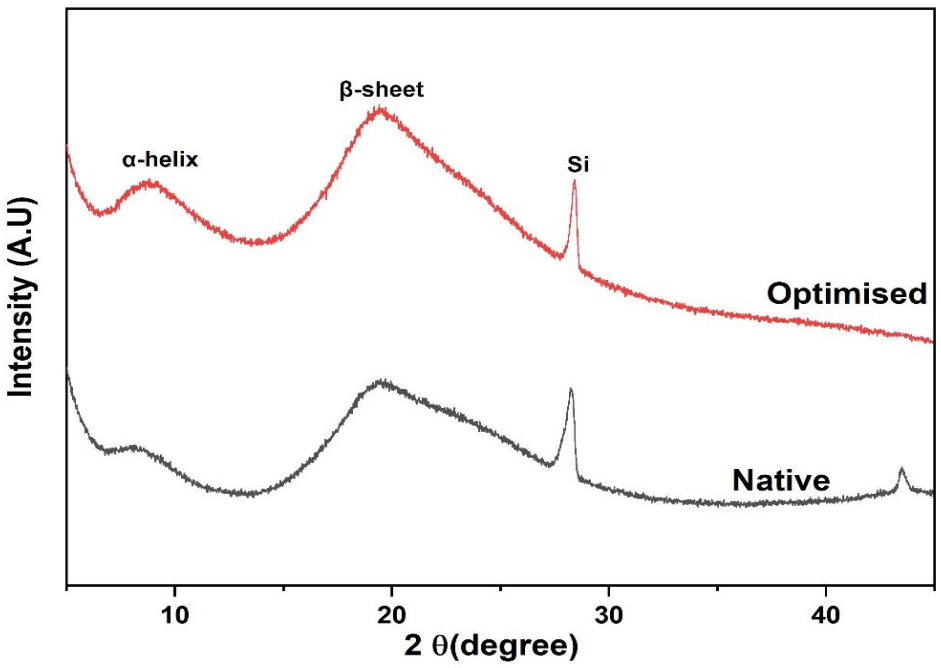

Fig 9. XRD diffraction pattern of optimised and control faba bean protein isolate.

Differential scanning calorimetry (DSC)

The thermal stability of proteins plays a key role in the functionality and hence their applicability in food systems. The thermal properties are useful in different food application as

most processes involve some heating steps. DSC can serve as a means of examining protein properties during processing. The $T_{denaturation}$ is the temperature of protein degradation and is useful observed as a peak and reflects thermal stability of proteins. DSC thermogram of the optimized and control faba bean protein isolate is shown in **Fig 10**. Control FBPI displayed an endothermic peak at 109.5 °C while the peak of ultrasound-assisted optimized FPPI had a similar endothermic peak but slightly lower. Slightly lower denaturation temperature may be due to physical structural modification resulting from ultrasound treatment. Ultrasonicated protein did not denature after extraction, instead, proteins were stable. This indicates that native and optimized ultrasound treatments are both thermally stable and could serve several benefits in the food industry. Previous researchers have reported different denaturation temperatures for commercially and laboratory-prepared faba bean protein isolates. Kimura et al. (12) observed that the 11S fraction of faba bean protein showed T_d value of 95.4 °C while the 7S fraction showed a T_d value of 83.8 °C. Variations in the denaturation temperature of protein isolates from faba beans could be attributed to extraction conditions, varietal differences, and technique used.

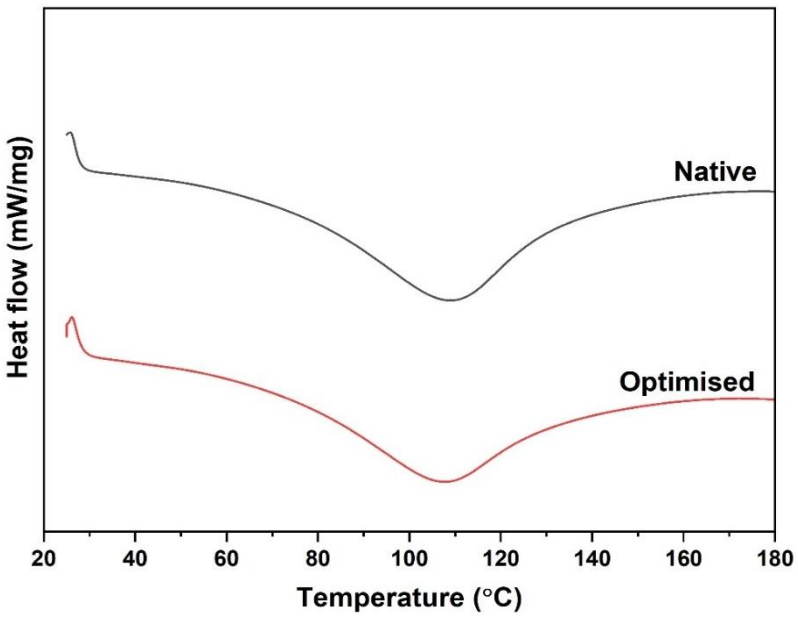

Fig 10. DSC spectrum of optimized and control Faba bean protein isolates**.**

Thermogravimetric analysis (TGA)

Thermal investigation of proteins is useful for determining the temperature-dependent behaviour of proteins before significant thermal decomposition occurs, which is critical for various food applications. This is of particular interest during high-temperature processes such as cooking, pasteurization, and sterilization (61)**.** TGA curves of ultrasound-treated and conventional faba bean protein isolates are shown in **Fig. 11**. Both samples showed similar degradation curves up to 950 °C. Weight loss in the temperature range 0 to 200 °C is often associated with water loss and some volatile compounds. Control samples showed a slightly rapid water loss compared to optimal ultrasound-assisted protein isolate. At temperatures between 200 to 400 °C, there was a drastic weight loss in both samples mostly attributed to the presence of polymeric substances which in this case is proteins. Control protein isolate however showed slightly lower degradation compared to optimal ultrasound-aided extraction from 400 to 900 °C. This observation is related to the structural modifications induced by ultrasound, such as the modification in secondary structure. This finding agrees with the results reported by Mir et al. (62) for album seed protein isolates treated with high-intensity ultrasound. Finally, a slight constant weight loss was observed from 400 to 900 °C, which may be attributed to residual materials such as oxidation products. Comparable results have been reported by Yılmaz & Gultekin Subasi (63) for laurel and Olive protein isolates, and He et al (64) noted a similar pattern for quinoa protein isolates.

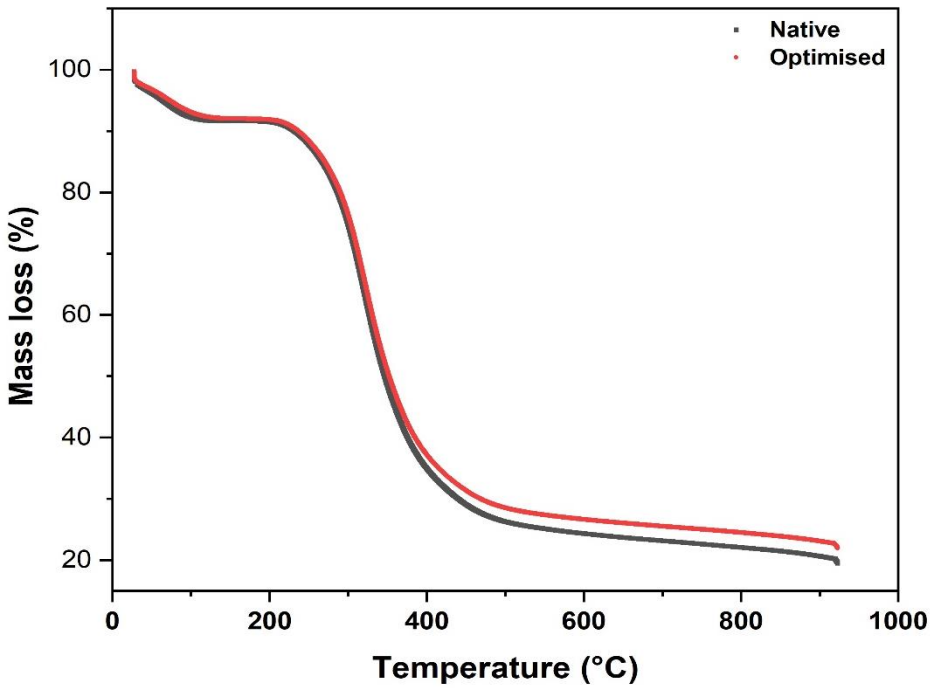

Fig. 11. TGA profile of optimal ultrasound-aid and control faba beans protein isolates

Conclusion

In this study ultrasound-assisted process parameters for the production of faba bean protein isolate were compared to the conventional alkaline extraction process. Following BBD optimization of the ultrasound process, a higher extraction yield (19. 75 vs 16.41 %) and protein content (92.87 vs 89.88 %) were obtained under optimum conditions (Power (123 W), solute/solvent ratio (0.06) (1:15 g/mL), sonication time (41 min), and total volume (623 mL)) compared to the conventional approach. When comparing the ultrasound-treated FBPI to the conventional protein isolate, the ultrasound FBPI demonstrated enhanced water holding and oil absorption capacities. However, it exhibited a decreased foaming capacity. Both protein isolates showed similar foaming stability. FTIR analysis indicated modifications in the secondary structure and fingerprint regions of the ultrasound FBPI, while electrophoresis studies showed no changes in the primary structure. Thermal analysis using DSC and TGA revealed changes in the thermal characteristic profile of ultrasound-treated FBPI. This provides

460 the opportunity to use the recommended ultrasound optimum parameters in food and industrial
461 settings to produce functional faba bean protein for different food applications.

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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

Conceptualization: Abraham Badjona, Bipro Dubey, Robert Bradshaw, and Martin Howarth; methodology: Abraham Badjona, Bipro Dubey, Robert Bradshaw and Martin Howarth; Investigation: Abraham Badjona, Bipro Dubey, and Robert Bradshaw; Writing—original draft preparation: Abraham Badjona, Robert Bradshaw, Bipro Dubey, Martin Howarth.; Project administration: Bipro Dubey, Robert Bradshaw, Caroline Millman and Martin Howarth. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

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

Rights Retention Statement

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