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Modifications of physicochemical, functional, structural, and nutritional properties of a field bean protein isolate obtained using batch and continuous ultrasound systems

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This study aimed to investigate the effect of ultrasound (US) treatment on improving the yield and the physicochemical, functional, structural, and nutritional properties of a protein isolate from germinated field bean flour at different conditions such as 100 and 200 W at 5, 15, 25, and 35 min. Field bean is an underutilized crop with more protein content and an alternative to animal protein, thus ensuring global food security, responsible consumption, and the well-being of consumers, which is in line with the sustainable development goals (SDGs) 2, 3, 7, 12, and 13. US treatment at 25 min and 200 W gave the best result for all the properties. Upon 25 min of sonication at 200 W, there was an increase in the protein yield, foaming ability, foam stability, emulsion activity index, emulsion stability, solubility, ζ potential, and *in vitro* protein digestibility from 34.33% to 59.15%, 73.11% to 110.12%, 81.44% to 90.43%, 6.92 to 13.47 m^2 g^{-1}, 59.97 to 104.74 min, 56.29% to 73.82%, −9.92 mV to −17.5 mV, and 94.47% to 96.37%, respectively. Moreover, a decrease in the size of particles from 1766 nm to 294.1 nm in comparison to untreated samples using green technology helps in achieving clean energy and decreases extraction time. Water holding capacity and oil holding capacity increased by 52.3% and 51.8%, respectively, after 15 min of US at 200 W. The change in the microstructure of proteins because of the US treatment was analysed using SEM. FTIR analysis confirmed the changes in the secondary structures of proteins. The physical changes caused by acoustic cavitation resulted in the partial denaturation of proteins, which was shown by an increase in their surface hydrophobicity and, thus, functionalities. Outcomes of this work demonstrated that US-assisted protein extraction increased yield and adjusted characteristics to meet the needs of the food sector, indicating a possibility for industrial use and contributed to the accomplishment of SDGs 2, 3, 7, 12, and 13.

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Sustainability spotlight

This research compares the advantages of ultrasonic-assisted protein extraction to those of traditional techniques and discusses the significance of plant protein. Field beans are underutilized crops that are used as a source of protein.According to this study, ultrasound improved the functionalities, yield, extraction time and other characteristics of proteins. Germinated field bean flour was ultrasonically treated at 100 and 200 W for 5, 15, 25, and 35 minutes. All the characteristics and yield of protein increased after 25 minutes with an ultrasonic power of 200 W. Owing to their high protein content, beans can help achieve SDG 3. Additionally, they may flourish in areas that are prone to drought, which will help to achieve SDG 2. Impact on environment supporting SDGs 7 and 13 is reduced by ultrasound.

1. Introduction

The demand for proteins is expected to double by the year 2050 to meet the needs of the increasing population.[1] Dietary proteins are important for human health, survival, and reproduction.[2] A vast array of plant- and animal-based proteins are used in the food and pharmaceutical industries due to their capacity to improve viscosity, foam, emulsify, gel, and encapsulate qualities.[3] Consumers as well as researchers are showing interest in plant protein because of the dietary and religious restrictions on animal protein.[4] Plant proteins have several advantages over animal proteins, such as being more affordable, adaptable, productive, nutritious, environmentally sustainable, and highly stress tolerant as well as having a low carbon footprint,[5] which helps them meet several sustainable development goals (SDGs). Field bean crops can fix nitrogen content in soil, thus mitigating climate change and helping in

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achieving SDG 13. All these factors are crucial in achieving SDG 2 to enhance nutritional aspects and support sustainable farming. There are 17 SDGs set by the United Nations (UN) members to eradicate poverty and build a society that is equitable, prosperous, and secure for people and the planet. When compared with the proteins obtained from animal and dairy sources, legume proteins have more technical and biofunctional applications.

Field bean, also known as Dolichos bean, hyacinth bean, or Sem or Indian bean, with the scientific name *Dolichos lablab*, is an important leguminous vegetable. It is cultivated in the tropical regions of Africa, Asia, and, America. The field bean (FB) plant is believed to have its origin in India.[6] There are three varieties available in India based on the seed coat, namely, brown, cream, and white with Indian names as Vaal, Pawta, and Rangoon vaal, respectively.[7] FB has the potential to grow in saline soil, adapt to acidic conditions, and thrive in drought circumstances. It is a prominent forage and food crop in tropical lowland regions due to its tolerance to warm weather and drought resilience, which supports SDG 3 and achieves SDG 2, allowing it to reach SDG 12, 13. Being a cheap and rich source of protein, it is known as 'poor man's protein'.[8] The National Academy of Science (NAS), India classified lablab beans as a potential source of protein.[6] Aside from being a good source of protein, it also offers a lot of complex carbs, minerals, and fibre as well as a low-fat level, which helps to meet SDG 2.[9] Also, FB is a promising dietary supplement alternative to prevent infection with COVID-19.[10] Despite having these many qualities, it is an underutilized crop.[11] Thus, FB poses a good research opportunity, especially for proteins. Very few studies[12,13] are available on the yield of proteins and process modifications to the proteins of FB by conventional and few modern methods.[14] However, research was absent in the following categories, including a study on Indian seed variations and specific information such as bean type. There is a lack of studies on pretreatment procedures for protein extraction from FBs, including soaking, heating, germination roasting, and process-induced changes.[15] During extraction, processing tools can modify the proteins both in positive and negative ways. The process can be tuned to enhance the functional properties, leading to an increase in its application in the development of novel foods. Pretreatments enhance the protein digestibility, nutritional profile, and organoleptic properties of the cereals, *e.g.*, germination.[16] Pretreatments applied before the extraction of protein may also alter the constituents along with an improvement in physicochemical parameters of proteins such as an increase in protein yield from rice by soaking in dilute sulphuric acid.[17] In a study, various legumes such as mung bean, chickpea, and lentils were germinated under a controlled environment and proteins were isolated; it was found that there was an improvement in the protein yield after gemination.[18] In another study, it was mentioned that using pretreatment methods on pulses helps improve protein digestibility and decrease antinutrients.[19] The extraction of proteins was enhanced by the pretreatment of hydrothermal cooking accompanied with the action of amylase on rice bran.[20] In a study, desi chickpeas were used for the extraction of protein using soaked, germinated, boiled, and raw chickpeas. Among all these trials, germinated chickpea flour showed greater yield for the isolation of protein.[21] In a study related to field bean protein isolate, germination was selected as the pre-treatment and the germinated field bean flour was used to extract protein, which improved the protein yield. However, no significant change in the color of the isolate compared to the raw sample was observed, and along with protein increase, there is a decrease in the fat and moisture percentage, which makes it suitable for storage for a longer period.[22]

Conventional extraction methods for protein include chemical extraction methods, namely, alkali-based protein extraction and organic solvent-based protein extraction. These methods have some constraints like energy-intensive, time-consuming, and are not environment friendly as there is the involvement of chemical solvents, which is against SDG 7.[23] have reported the possibilities of undesirable reactions like a decrease in digestibility, lysinoalanine formation, loss of amino acids, and racemization of amino acids. The proteins extracted using conventional extraction technique have inferior quality with low yield.[24] Nowadays, the trend is moving towards using environment-friendly, novel non-thermal technologies like microwaves, ultrasound (US), and pulsed electric fields for the extraction of proteins. Researchers are also showing more interest in it because of its advantages over conventional extraction techniques. These techniques not only improve the protein extraction yield but also help in maintaining the protein quality by less degradation. Among other techniques, the US technique remains promising as it has the following advantages over others such as better retention of nutrients in food, providing high purity levels of the final product, low cost, energy, and time.[25] Though FB has been studied by,[12–14] the ultrasound-assisted extraction (UAE) of germinated FB proteins and the study on its process-induced modification to physicochemical, functional, structural, and quality of protein is not found elsewhere in the literature.

Cavitation is responsible for the generation and implosion of bubbles and produces high shear forces and temperature in the medium.[26] Inertial cavitation is formed, which leads to the production of abundant energy because of sonication. The basic principle behind US is 'Acoustic cavitation'.[27] Physical forces like shear forces, microjets, mechanical agitation, hot spots, shockwaves, and microstreaming are found to be efficacious in extraction and emulsification.[28] Low-frequency power US has a disruptive effect on the physical, chemical, and biological properties of food. It has applications like activation and inactivation of enzymes, crystallization, defoaming, degassing, dewatering, emulsification, extraction, extrusion, low-temperature pasteurization, particle size reduction, and viscosity alteration, which can be used commercially at a large scale. It is also used in the protein modification and extraction of bioactive compounds.[29,30] The cell walls are ruptured by ultrasonic implosion and cavitation, which improves mass transfer from the solid to liquid phase. Microchannels are also produced within the tissues using ultrasonic treatment, which improves solvent penetration into the solid matrix and hence boosts mass transfer. UAE is a viable alternative that improves yields while overcoming the shortcomings of conventional

extraction methods and so boosting sustainable energy, hence favoring SDG 7.[25] In a study, ultrasound was used for the isolation of protein from germinated chickpea flour, which resulted in increased yield, digestibility, functional, characteristics properties, and a decrease in particle size over conventional methods.[21] In another study, the extraction of protein isolates from rice dreg flour was done by ultrasound, which showed an increase in the yield of protein from 44 to 88%. After the extraction process under controlled conditions, it also improved the hydrophobic amino acids content and porosity, and a change in the structure was observed *via* SEM and FTIR analysis, which indicates that ultrasound can change or alter the microstructure of the protein to improve its quality.[31] The UAE of proteins has been studied for different varieties of pulses and legumes such as black bean,[32] pea,[33] chickpea,[34] faba bean,[35] kidney bean, and soybean.[36] These studies have reported an increase in protein yield as well as an improvement in protein quality. Plant proteins generally have low nutritional value, and poor digestibility; with the help of pre-treatments and ultrasound techniques, these limitations can be overcome.[37] These changes help improve the quality of proteins that are suitable in the food to incorporate in sports drinks, ice creams, yogurt, beverages, *etc.* To prove that proteins plays a major role in improving the quality of food, a study done by (ref. 38) shows that proteins present in the ultrasound-treated chickpea milk helps in attaining the stability of milk with lower sedimentation value. Additionally, in a related study, the mayonnaise prepared from chickpeas and green gram extract had higher viscosity; this is because the proteins in the legumes had stronger emulsifying qualities that improved the quality of the product and also had better sensory characteristics.[39,40] However, the UAE of proteins from germinated FB and also FB of Indian variety (pale brown) has not been explored yet. Therefore, this work aims to study the effect of germination on the protein yield of FB and the effect of UAE on the structural and functional properties of germinated field bean protein (FBP).

2. Materials and methods

2.1. Raw materials

Dried FBs were bought from Thanjavur, Tamil Nadu, from a local market. All of the chemicals and reagents utilized were of the analytical grade and purchased from HiMedia Laboratories Pvt. Ltd. and Thermo Fisher Scientific India Pvt. Ltd.

2.2. Preparation of field bean flour

FBs were cleaned with water and soaked for 12 h. The soaked beans were kept for germination for 24 h at room temperature and dried in a tray drier at 45–50 °C till a constant moisture content was achieved. The flour was obtained by grinding the beans using a pulverizer and was stored at room temperature. A comparison study was done for the protein isolates obtained from flour of dried beans of raw, soaked, germinated, and boiled FBs. It was observed that the protein isolate from dried germinated FB flour had higher protein content than all others and hence was chosen for further analysis in this whole study.[22]

2.3. UAE of field bean protein isolate (FBPI)

The flour was defatted before using it for protein extraction purposes. Hexane was used as a solvent for defatting by the Soxhlet method. To minimize resource use, adopting a more pragmatic way to extract protein, and promote the usage of water, or SDG 6, we used a 1 : 10 ratio of flour to water. Before protein extraction, 10.0 g of defatted FBPI was dissolved in 100 mL of distilled water and kept in a refrigerator for lowering its temperature to 4 °C. The US probe sonicator with (14 mm probe diameter, 26 kHz frequency 200 W, lab man sonicator) at 200 W input power at 50% and 100% amplitude was used to assist protein extraction. US was applied in pulsation mode with 5 s ON and 5 s OFF. The US treatment times given were 5, 15, 25, and 35 min. The entire US extraction process was performed in a temperature-controlled water bath at 3–4 °C, and the volume of all the samples was kept constant. Distilled water was used as a liquid medium for improving the extraction of protein into the medium, and the solid-solvent ratio used was 1 : 10. After giving US treatment at different amplitudes and process times, the pH value for the mixture of FB flour and deionized water was adjusted to 11 using 1 N NaOH. This mixture was then stirred using a magnetic stirrer for 2 h and then centrifuged in a refrigerated centrifuge at 4 °C for 20 min at 5000*g*. For the precipitation of proteins, the pH of the supernatant was adjusted to 4.5 after extraction. Precipitated protein was collected using a refrigerated centrifuge at 5000*g* for 20 min and washed using distilled water. Finally, FBPs were freeze-dried and stored at 4 °C for further analysis.[27,33] FBPI extracted by traditional alkaline extraction process without the usage of US was used as a control. The US-assisted extracted field bean protein isolate is denoted as UFBPI while the untreated one (0 min of sonication time) is the control sample (C).

Similarly, the sample is processed in continuous US with 26 kHz frequency, 200 W input power, 7 mm probe diameter, pulsation mode with 5 s ON and 5 s OFF, and process temperature is maintained at 4 °C.

2.4. Extraction yield

The FBPI yield extraction was calculated using the Kjeldahl technique employing the equation below (1)[42]

$$\text{Protein } (\%) = \frac{W_1 \times C_1}{W_2 \times C_2} \times 100 \quad (1)$$

where W_1 = weight of field bean protein; W_2 = weight of field bean flour; C_1 = protein content of field bean; C_2 = field bean flour protein content.

2.5. Physicochemical analysis

2.5.1. Color. The method given by (ref. 43) was used to analyze the color of FBPI with the help of the CIE L^* a^* b^* color system and values were taken in triplicates.

2.5.2. Bulk density. One gram of protein sample was taken in a 5 mL measuring cylinder and the sample was uniformly

placed. The volume was noted down and the bulk density was expressed as the weight of the sample per volume of the protein sample.[43]

2.5.3. Tapped density. After obtaining the volume for bulk density, the cylinder was tapped 50 times and the corresponding volume was noted. The tapped density was expressed as the weight of the sample per volume of the protein sample.[40,44]

2.6. Functional properties

2.6.1. Water holding capacity. The water holding capacity (WHC) of FBPI was estimated with the method described by (ref. 34 and 43) with some modifications. Protein isolate (0.1 g) was dispersed in distilled water (3 mL) and stirred for 1 min. After stirring, the sample was allowed to stand for 20 min and then centrifuged at 5000*g* for 15 min. The supernatant was removed carefully and the WHC was expressed as the weight of water absorbed per weight of the protein sample taken ($g_{water}/g_{protein}$).

2.6.2. Oil holding capacity. The oil holding capacity (OHC) of FBPI was measured using the same method as that applied for estimating WHC except for using sunflower oil instead of deionized water. The OHC was expressed as the weight of oil absorbed per weight of the protein sample taken ($g_{oil}/g_{protein}$).[43]

2.6.3. Foaming properties. The method of (ref. 42) was used to estimate the foaming properties, *i.e.*, foaming capacity (FC) and foaming stability (FS) of FBPI with some modifications. The protein solution (15 mL) having a concentration of 10 g L^{-1}, pH 7 was homogenized (IKA T18 digital) at 6500 rpm for 2 min. The foam volume was noted immediately to measure the FC, while it was also measured after 30 min to check the FS, and the calculations were as follows.

$$\text{FC}\ (\%) = \frac{\text{foam volume}}{\text{volume of solution}} \times 100 \quad (2)$$

$$\text{FS}\ (\%) = \frac{\text{foam volume after 30 min}}{\text{initial foam volume}} \times 100 \quad (3)$$

2.6.4. Emulsifying properties. The emulsifying activity index (EAI) and emulsion stability index (ESI) of FBPI were estimated using the method employed by (ref. 42 and 45) with some modifications. A 10 g L^{-1} protein solution (15 mL) was thoroughly mixed with 5 mL sunflower oil and homogenized at 6500 rpm for 2 min. From the emulsion, 50 μL of the sample was taken immediately and added with 0.1% 5 mL SDS solution. Absorbance was recorded for the diluted emulsion at 500 nm. After 30 min, the EAI values were again noted using the same method. The following equations were used to calculate the EAI and ESI values.

$$\text{EAI}\left(\text{m}^2\ \text{g}^{-1}\right) = \frac{2 \times 2.303 \times A_0}{\Phi \times \text{protein weight(g)}} \quad (4)$$

$$\text{ESI(min)} = \frac{A_0}{A_0 - A_{30}} \times T \quad (5)$$

where, Φ is the volumetric fraction of oil (0.25). A_0 is the absorbance of the sample at 0 min. A_{30} is the absorbance of a sample after 30 min.

2.6.5. Solubility. Protein solubility was determined using the method given by (ref. 46) with some modifications. FBPI dispersion was made with distilled water with a concentration of 1 mg mL^{-1} and its pH was adjusted to 7. The FBPI dispersion was centrifuged at 5000*g* for 10 minutes after being agitated for 20 minutes with a vortex shaker. The supernatant was collected and checked for its protein content using the Biuret method. The solubility was calculated using the following equation.

$$\text{protein solubility} = \frac{\text{protein content of the supernatant}}{\text{total protein content}} \times 100 \quad (6)$$

2.7. Structural properties

2.7.1. Particle size. The particle size of the FBPI solution was estimated using a Zetasizer Nano ZS (Malvern Instruments, U.K.) at 25 °C. The refractive index value for this protein sample was 1.33.[41]

2.7.2. Zeta potential. The zeta potential of the FBPI was determined using a Zetasizer Nano ZS (Malvern Instruments, U.K.) at 25 °C. The refractive index value for this protein sample was 1.33.[34]

2.7.3. Scanning electron microscopy. Morphological observations of the FBPI were done according to (ref. 41) with little modifications by scanning electron microscopy (SEM). The FBPI was scanned by a VEGA3 TESCAN (Czech Republic) system under an acceleration voltage of 10 kV to observe the surface morphology of proteins. The samples were mounted on a SEM specimen tube along double side tape before coating.

2.7.4. Fourier transform infrared spectroscopy (FTIR). FTIR was performed according to (ref. 47) with little modifications to the spectra in the wavelength range of 400–4000 cm^{-1} and was obtained using an FTIR spectrometer (Nicolet iS50). FBPI was mixed with dried KBr, and the pellet was formed by compression. Data analysis was performed by OMNIC 9.9.594 software.

2.7.5. Surface hydrophobicity. 8-Anilinonaphthalene-1-sulphonic acid (ANS) fluorescent probe technique was used to assess the surface hydrophobicity of FBPI.[34] In the beginning, FBPI was diluted in 0.01 mol L^{-1} of pH 7 phosphate buffer to make a dispersion with a concentration of 1 mg mL^{-1}. After that, the protein solution was centrifuged for 20 minutes at a speed of 10 000*g* to measure the solubility of the supernatant using the biuret reagent, as described in Section 2.6.5 above. Phosphate buffer was then used to dilute the supernatant to 1, 0.5, 0.1, 0.05, and 0.01 mg mL^{-1}, respectively. 8.0 mmol L^{-1} ANS solution was prepared by the addition of ANS in 0.01 mol L^{-1} pH^{-1} 7 phosphate buffer. 100 μL of ANS solution was added to 10 mL of protein solution and kept in the dark for 15 min. Later, using a fluorescence spectrophotometer with a 348 nm excitation wavelength and a 511 nm emission wavelength, the absorbance was measured. Through the use of linear regression analysis, the initial slope of the protein concentration and fluorescence intensity was used to determine the Ho index.

2.8. Nutritional aspects

2.8.1. *In vitro* protein digestibility. The method followed by (ref. 48) was used to determine the *in vitro* protein digestibility with few modifications. 250 mg of each sample's protein solution and 250 μL of distilled water (used as the blank) were suspended in 15 mL of 0.1 N HCl with 1.5 mg mL^{-1} pepsin, and the mixture was incubated for three hours at 37 °C in a water bath. The hydrolysis of pepsin ceased after neutralization with the addition of 7.5 mL of 0.5 N NaOH. Then, the pancreatic digestion initiated with the addition of 10 mL of 0.2 N phosphate buffer (pH 8) containing 10 mg of pancreatin was incubated at 37 °C overnight. After pancreatic hydrolysis, 1 mL of trichloroacetic acid at a concentration of 10 g/100 mL was added, and 20 minutes were spent centrifuging at 2100 rpm, the supernatant was collected, and the digestible protein was measured using the Kjeldahl method, as mentioned in the method by Wang *et al.*[36] The *in vitro* digestibility was expressed as the ratio of the protein content in the solution before and after digestion.

2.9. Comparison between batch and continuous process of US system

The protein isolate extracted earlier in this study was obtained using a batch process. Similarly, protein isolate was obtained using the continuous process. The optimized sample, *i.e.*, the UFBPI-25 min-200 W from the batch process, was chosen to compare against the continuous process (26 kHz frequency, 200 W input power, 7 mm probe diameter, pulsation mode with 5 s ON and 5 s OFF, process temperature maintained at 4 °C) using the same US system. The energy density for both the processes was equated, and the processing time required for the continuous process was calculated. Energy density (J mL^{-1}) is a product of power density (W mL^{-1}) and processing time (s) of the protein dispersion (eqn (7)).

$$\text{Energy density}\left(\text{J mL}^{-1}\right) = \frac{(\text{power drawn(NAP)(W)} \times \text{time(s)})}{\text{volume(mL)}} \quad (7)$$

where power is drawn NAP $= mC_p\frac{nT}{nt}$; "m" is the weight of the samples (g), C_p is the specific heat of the medium (4.18 kJ g^{-1} K^{-1}), and $\Delta T/\Delta t$ is the rate of temperature change with respect to time (°C s^{-1}).[49] The efficiency of the system in batch and continuous processes was studied based on the extraction yield and functional properties of the FBPI obtained from both the processes. The FBPI extracted using a continuous process is denoted as CUFBPI.

2.10. Statistical analysis

All experimental data were collected in triplicate. The findings were presented as mean ± standard deviation. The Minitab 18 program was used to evaluate the statistical data, which was then checked for significance at $p < 0.05$ using the analysis of variance (ANOVA).

3. Results and discussion

3.1. Extraction yield

The US treatment was carried out in duration (0, 5, 15, 25, and 35 minutes) and amplitude (100% and 50%), *i.e.*, with input powers of 200 W and 100 W, respectively (Fig. 1). With the increasing US treatment time, the extraction yield showed an increasing trend. The control sample showed an extraction yield of 33.64%. UAE at 100 W gave a maximum yield of 51.53% in 25 min, which decreased at 35 min of treatment to 50.24%. By increasing the US power from 100 to 200 W, the extraction yield was increased. Furthermore, by increasing the US power to 200 W, the maximum yield of 59.49% in 25 min was observed. The yield showed a decrease to 57.64% at a treatment time of 35 min. The US treatment improved the extraction yield by 76.84%. This trend of increase in the extraction yield, followed by a decrease with the increasing sonication time, was seen with the protein yield of the defatted pumpkin seed at 5 min.[50] It was also seen that the extraction done at 100% amplitude was higher than that obtained at 50% amplitude. A similar rise in protein yield was shown in a study of defatted rice bran due to the increase in the sonication power.[51] In the extraction process, intense cavitation is caused as a result of the high-power sonication, which is responsible for the increase in the yield.[50] The increase in the extraction yield may be attributed to the mechanical vibrations due to US, which increased the contact area between FB flour and alkali solution added during the extraction process. Additionally, US-induced cavitation could disintegrate plant cell walls, sever molecular connections, accelerate mass transfer, and enhance the effectiveness of protein extraction.[42] It decreased after a prolonged US treatment, which may be due to the denaturation of proteins that are soluble in the extract as the shear forces generated are also greater. Protein aggregates are formed because of the formation of the intermolecular disulfide bonds.[50,52] The maximum yield (59.15%) was found with the combination having a treatment time of 25 min. Based on protein isolate extract time and yield,

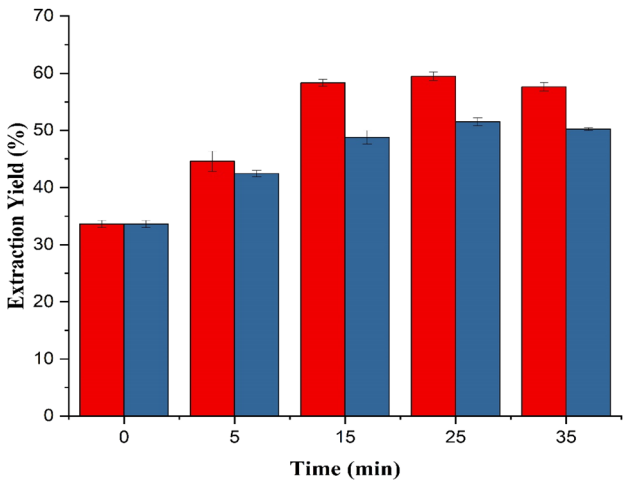

Fig. 1 Extraction yield of the FBPI at 200 W and 100 W at sonication times of 0, 5, 15, 25, and 35 min.

UAE is capable of extracting the protein effectively. Thus, using UAE instead of traditional extraction reduces the protein extraction time to 25 minutes. To promote SDG 7, the UAE will also be crucial towards the achievement of energy efficiency.

3.2. Physicochemical analysis

3.2.1. Colour. The pigments included in dietary components can either benefit from or suffer a disadvantage from sonication. It may hasten the pigments' release from proteins and obliterate the pigment-containing sites, which would absorb light. The L^* a^* b^* values of the protein are mentioned in Table 1. The value of L^* ranges from 0 to 100, indicating the white and black color, respectively. The positive values of a^* indicate redness while the negative values of a^* indicate the greenness. Similarly, the positive value of b^* indicates yellowness, and the negative value of b^* indicates the blueness.[53,54] In this study, the L^* values showed a significant decreasing trend for the 200 W-UAE samples from 5 min to 35 min of treatment in comparison to the control. It was observed that the color of the US-treated protein isolate was darker than the control sample. Also, the color became darker as the treatment time increased. This darkening of the color may be attributed to the release of pigments after US treatment. A similar darkening was found while studying the physicochemical properties of the US-treated protein isolate obtained from album seeds.[53] However, the magnitude of change is lower for the 100 W than the 200 W-UAE samples.

3.2.2. Bulk and tapped density. The values for bulk and tapped density are mentioned in Table 1. There was a significant difference ($p \leq 0.05$) between the bulk density of the US-treated and untreated samples. In addition to the moisture content and particle shape, the bulk density of the protein isolate is affected by the quantity, size, and forces of attraction between the particles.[43] In the case of the tapped density, a significant difference ($p \leq 0.05$) was observed between the control and the 25 & 35 min US-treated samples at an input power of 200 W. The bulk density affects the packaging requirement and also the degree of hydration.[55] The bulk density of canola protein isolate was reported to be 0.26 g cm^{-3}, suggesting that the obtained FBPI falls in the range.[56]

3.3. Functional properties

The most significant functional components in a food system are proteins due to their capacity for emulsification, foaming, and nutrition. Plant-based proteins offer a viable alternative to animal-based proteins for human nutritional needs because of their sustainable origin, low cost of production, accessibility, and health benefits (SDG 3).

3.3.1. Water holding capacity (WHC) and oil holding capacity (OHC). The effect of US treatment on the WHC of FBPI can be seen in Fig. 2a. The WHC of the control sample, *i.e.*, the untreated sample was 2.96 g g^{-1}, and it increased up to 4.13 g g^{-1} for the UFBPI treatment of 15 min. It was found that the US treatment increased the WHC of FBPI by 52.3% for 15 min treatment at 200 W input power. The WHC decreased after increasing the treatment time to 25 and 35 min. The US treatment decreased the particle size, thus increasing its WHC. However, prolonged US treatment decreased the WHC because of the aggregate formation and the interaction between protein molecules.[57] A similar trend was seen in the study done on ultrasonically-treated quinoa protein.[57,58] Also, proteins that are recommended for sticky and viscous foods like gravies and soups should have WHC in the range of 1.49 to 4.72 g g^{-1}. Hence, it can be concluded that proteins isolated from lablab beans have many applications in the food industry.[45,59]

The OHC also showed a similar trend to WHC (Fig. 2b). The OHC for the control sample was 3.36 g g^{-1}, and it increased after giving US treatment up to 15 min at 200 W power input. The OHC of the ultrasonically treated sample increased by 51.8% after treatment for 15 min at 100% amplitude. The OHC decreased on further increasing the treatment time. The increase in OHC may be due to the exposure of hydrophobic groups, which causes the interaction between oil and hydrophobic surface.[14,59] The exposure of hydrophobic groups may be seen by the creation of massive protein aggregates in the dry state of the US-treated freeze-dried samples.[60]

3.2.2. Foaming capacity (FC) and foam stability (FS). The FC of FBPI at 200 W and 100 W input power at 0, 5-, 15-, 25-, and 35 min sonication treatment time is given in Fig. 3a. In comparison to all of the US treatments, the control FBPI had the least FC (73.11%). There was an increasing trend in the FC and FS till 25 min treatment time, after which the values decreased.

Table 1 Physicochemical analysis of the FBPI treated at 200 W and 100 W input power at different sonication times of 0, 5, 15, 25, and 35 min[a]

Sample	Bulk density (g mL^{-1})	Tapped density (g mL^{-1})	L^*	a^*	b^*	*In vitro* protein digestibility (%)
C	0.28 ± 0.005^{c}	$0.31 \pm 0.01^{a,b}$	64.95 ± 0.23^{a}	2.87 ± 0.12^{d}	12.44 ± 0.41^{e}	94.47 ± 0.68^{b}
5A	$0.32 \pm 0.015^{a,b}$	$0.34 \pm 0.01^{a,b}$	61.19 ± 0.57^{b}	$4.50 \pm 0.07^{a,b,c}$	15.71 ± 0.03^{a}	—
5B	$0.3 \pm 0.01^{b,c}$	$0.32 \pm 0.01^{a,b}$	$62.43 \pm 0.29^{a,b}$	$4.45 \pm 0.72^{a,b,c}$	14.46 ± 0.14^{c}	—
15A	0.23 ± 0.015^{d}	$0.26 \pm 0.015^{a,b}$	56.41 ± 2.04^{c}	$4.88 \pm 0.14^{a,b}$	$14.66 \pm 0.30^{b,c}$	—
15B	0.35 ± 0.01^{a}	$0.32 \pm 0.015^{a,b}$	$62.34 \pm 0.47^{a,b}$	3.96 ± 0.05^{c}	$14.65 \pm 0.12^{b,c}$	—
25A	0.32 ± 0.015^{d}	0.40 ± 0.015^{a}	55.30 ± 1.83^{c}	5.14 ± 0.20^{a}	$15.50 \pm 0.11^{a,b}$	96.37 ± 0.38^{a}
25B	$0.31 \pm 0.01^{b,c}$	$0.34 \pm 0.020^{a,b}$	$54 \pm 1.29^{c,d}$	$4.31 \pm 0.19^{b,c}$	$14.01 \pm 0.58^{c,d}$	—
35A	0.35 ± 0.015^{a}	0.23 ± 0.15^{b}	50.72 ± 1.89^{d}	$4.88 \pm 0.20^{a,b}$	$13.28 \pm 0.36^{d,e}$	—
35B	$0.32 \pm 0.005^{a,b}$	$0.35 \pm 0.015^{a,b}$	54.32 ± 0.69^{c}	5.13 ± 0.07^{a}	15.68 ± 0.10^{a}	—

[a] C – control sample, A – 200 W input power, B – 100 W input power.

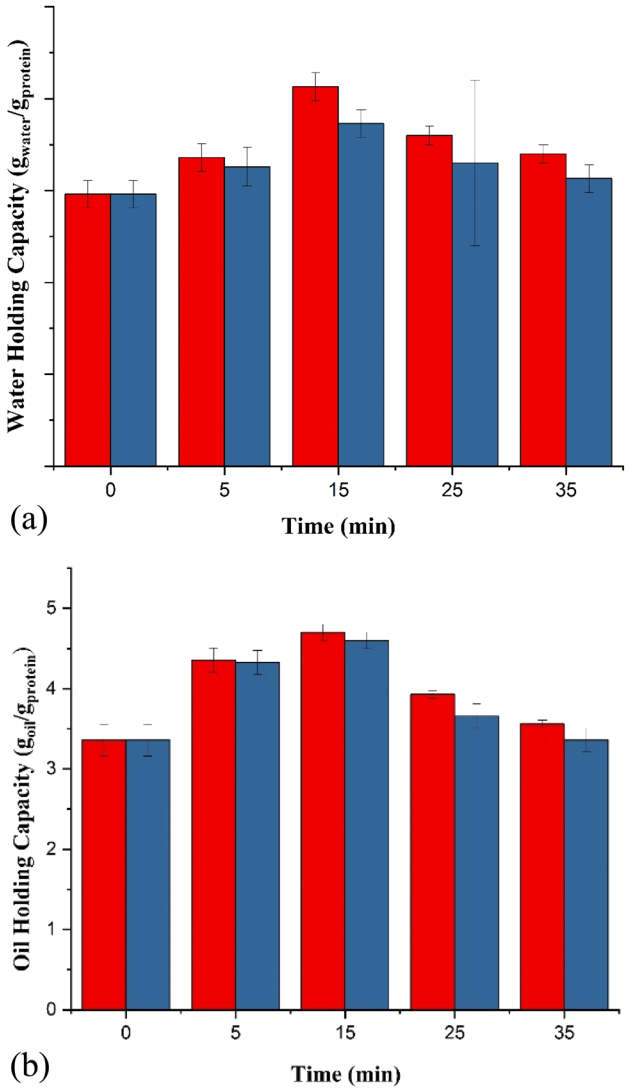

Fig. 2 (a) Water holding capacity (WHC) of the FBPI at 200 W and 100 W at 0, 5, 15, 25, and 35 min sonication treatment time. (b) Oil holding capacity (OHC) of the FBPI at 200 W and 100 W at 0, 5, 15, 25, and 35 min sonication treatment time.

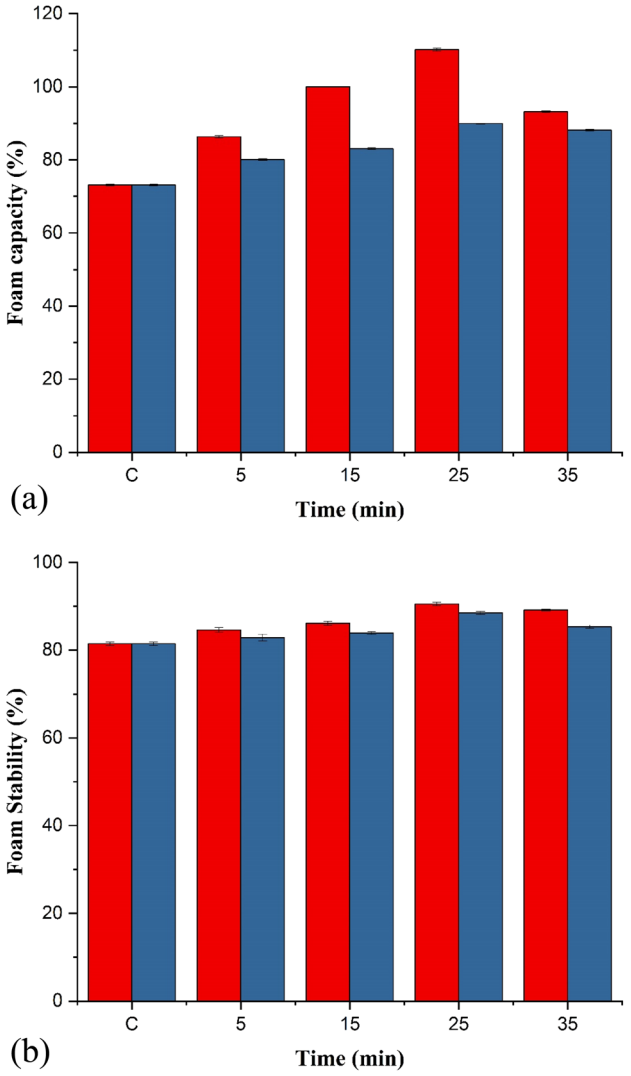

Fig. 3 (a) Foaming capacity (FC) of the FBPI at 200 W and 100 W at 0, 5, 15, 25, and 35 min sonication treatment time. (b) Foam stability (FS) of the FBPI at 200 W and 100 W at 0, 5, 15, 25, and 35 min sonication treatment time.

The FC of the UFBPI at 25 min treatment time at an input of 200 W was 110.12% and was found to be increased by 51.6% compared to the control sample. The FS was found to be maximum for the same sample as shown in Fig. 3b, *i.e.*, 90.53%. The US treatment led to the formation of more stable foam than the untreated ones. Ultrasonic treatment may cause partial changes in the protein structure, thereby enhancing the interfacial adsorption capacity at the interface of proteins, leading to increased foam formation. The enhanced foaming characteristics of UFBPI may be related to the partial unfolding of structures brought about by the US. Smaller particle sizes and improved dispersion of UFBPI might further improve the foaming capabilities.[59] The partial denaturation of protein with an increase in the sonication time for up to 25 min in a 200 W-US processed sample is supported by an increase in the surface hydrophobicity value. Protein aggregation with prolonged sonication time after 25 min, *i.e.*, the 35 min-200 W US processed sample, is supported by a decrease in the surface hydrophobicity value in the later Section 3.3.5 and Fig. 8. A similar trend was observed in the US extracted pea proteins by 19.5% and 22.7%, respectively,[59] while similar results were seen for chickpea proteins with an FC of approximately 112%.[61]

3.2.3. Emulsifying properties. The emulsion properties of FBPI are mentioned in Fig. 4a and b. Due to the structural change, UAE increased the molecular flexibility of the protein molecules that may have been adsorbed at the oil–water interface more successfully. Moreover, the FBPI particle size may decrease as a result of the mechanical impacts of the US, thus improving the molecular fluidity and emulsification potential.[42]

A favorable modification in EAI and ESI was observed in the UFBPI when compared to the control FBPI sample. The EAI and ESI rose initially as the ultrasonic duration was extended, then declined, reaching their peak at 25 minutes. A significant

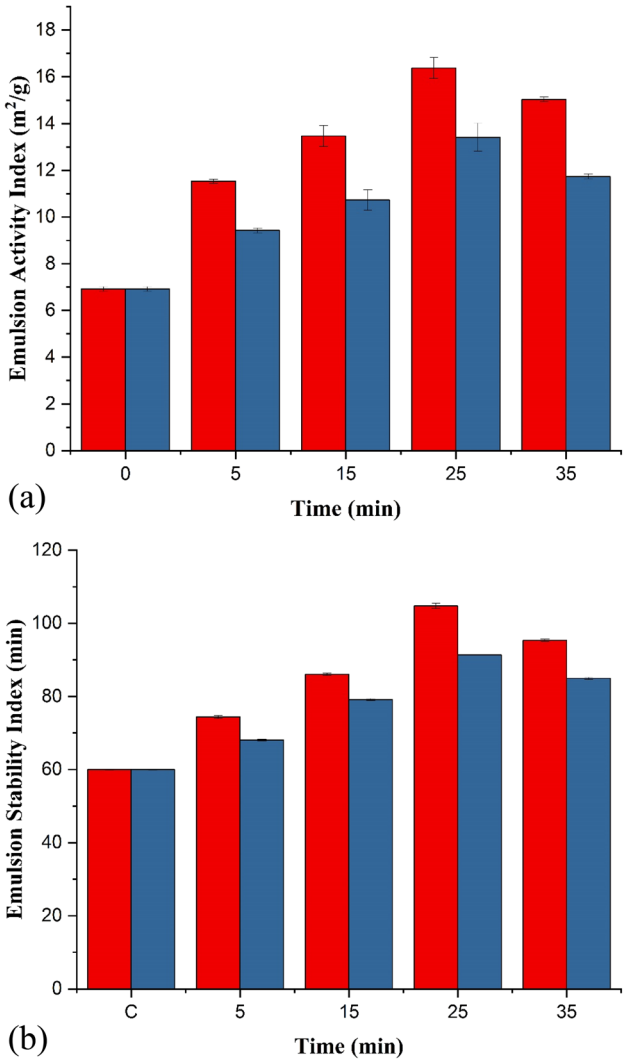

Fig. 4 (a) Emulsion activity index (EAI) of the FBPI at 200 W and 100 W at 0, 5, 15, 25, and 35 min sonication treatment time. (b) Emulsion stability index (ESI) of the FBPI at 200 W and 100 W at 0, 5, 15, 25, and 35 min sonication treatment time.

increase ($p \leq 0.05$) in EAI was recorded with a value of 16.37 m^2 g^{-1} for a US treatment of 25 minutes at 200 W in comparison to the control (6.92 m^2 g^{-1}). The control sample showed an ESI of 60 minutes while the ESI increased up to 104 minutes for UFBPI after US treatment of 25 minutes at 200 W.

It is claimed that the shear forces of US boosted the stability of the protein solution by decreasing the protein particle size and increasing the protein-specific surface area.[62] Additionally, the cavitation force brought by ultrasonic waves disrupted the non-covalent interactions that kept the protein spatial structure stable. The protein's hydrophobic group was exposed as a result, and more protein molecules moved to the water–oil interface, improving the protein's ability to emulsify. However, within 25 minutes, the threshold level of the protein's hydrophobic group exposure had been attained. Due to the proteins' molecular mobility and acceleration, hydrophobic interactions were disrupted during extended sonication, which accelerated the process of protein aggregation. Small molecules were quickly replaced by macromolecular proteins when adsorbed at the interface.[63] EAI and ESI decreased as a result of flocculation and increased emulsion interfacial tension. The results were consistent with the reported findings.[57] A similar observation is noted for UFBPI after 25 min of sonication, irrespective of the applied power. The partial denaturation of protein with an increase in the sonication time for up to 25 min in the 200 W-US processed sample is supported by the result on the increase in the surface hydrophobicity value and aggregation of the protein with prolonged sonication time after 25 min, *i.e.*, the 35 min-200 W US processed sample was supported by the decrease in the surface hydrophobicity value in the later Section 3.3.5 and Fig. 8.

3.2.4. Solubility. One of the most important characteristics of a protein is its solubility. The solubility of the proteins affects their functional properties, thus affecting their applications in the food industry. Fig. 8 depicts how the US affects the solubility of FBPI when treated with 100 W and 200 W US for different processing times. From Fig. 5, it is clear that the US treatment improved the solubility of the FBPI and also showed increased value with an increase in the processing time. The solubility of FBPI was 56.29% for the control sample, and it increased to 73.82% and 72.43% upon 25 min of US treatment at 200 W and 100 W, respectively. This may be attributed to the cavitation and the mechanical impacts of US that depolymerized larger protein clumps into smaller protein particles. The decrease in the particle size and the unfolding of protein molecules resulting from partial denaturation led to an increase in the protein and water interaction, eventually improving its solubility.[34,62,63] The solubility of FBPI was reduced after US treatment for 35 min at 200 W. The reduction in the solubility of FBPI may be because of the partial aggregation of protein molecule.[62] Protein aggregation is the result of the re-polymerization of the proteins due to excessive sonication.[34] Similar results were found in the study on soybean protein isolate.[62] The partial denaturation of protein

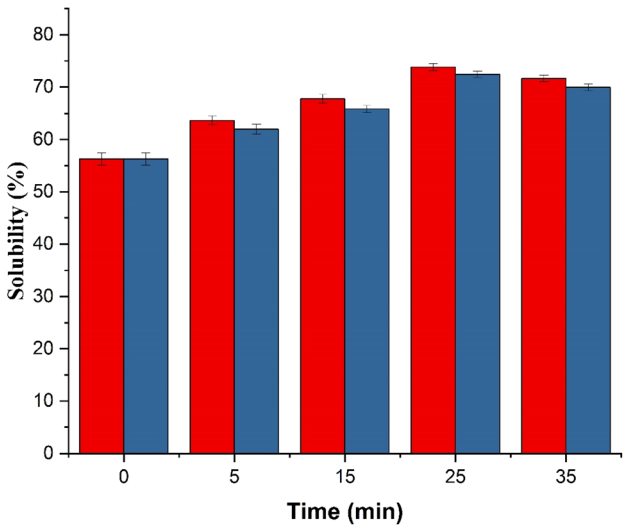

Fig. 5 Solubility of the FBPI at 200 W and 100 W at 0, 5, 15, 25, and 35 min sonication treatment time.

with an increase in the sonication time for up to 25 min in a 200 W-US processed sample is supported by the increase in the surface hydrophobicity value. Protein aggregation with prolonged sonication time after 25 min, *i.e.*, the 35 min-200 W US processed sample, is supported by the decrease in the surface hydrophobicity value in the later Section 3.3.5 and Fig. 8.

3.3. Structural properties

3.3.1. Particle size. The structural properties of proteins can occasionally change due to their functional and physical properties. Proteins undergo partial denaturation as a result of high pressure and ultrasonic vibrations, which drastically alters their physical and structural characteristics. US-induced structural alterations in proteins are linked to the partial breakdown of intermolecular hydrophobic interactions. There is an increase in the collision when shear stress is applied to the particles, which also accelerates the process of aggregation.[64] For analyzing the structural properties and nutritive properties, the 200 W-US treatment is studied henceforth as better and improved functional properties were obtained only for these samples at various sonication times in comparison to the 100 W-US treated samples at various processing times.

Table 2 depicts the particle size of FBPI proteins at 200 W US at different processing times. The particle size of the control sample was 1766 nm. The particle size decreases and the particle size distribution narrows as the ultrasonic treatment intensity rises. Up to 25 minutes of treatment time were required to see a decrease in the particle size with 294.1 nm being the lowest value. After prolonging the treatment to 35 minutes, an increase in the particle size was found. The free surface of the material rises when the particle size is decreased. The forces of cavitation in this instance lead the particles to decrease. Aggregates and agglomerates are also destroyed in this process. Aggregates, agglomerates, and even smaller particles may all be broken up by ultrasonic cavitation, which violates the van der Waals forces. Because it creates a strong enough negative pressure to produce bubble implosion and cavitation, sufficiently high-intensity US is responsible for creating friction and turbulence. As a result, it affects the treated material's surface.[65] When the ultrasonic power was constant, as the ultrasonic duration increased, protein molecules interacted with one another due to intermolecular interactions, which led to the formation of new aggregates, as noted in the size of the US 200 W 35 min US processed sample.

Table 2 Particle size and zeta potential of the FBPI treated at 200 W input power at different sonication times of 0, 5, 15, 25, and 35 min

Sample at different sonication time at 200 W	Test parameters	Result
0	Particle size	1766 nm
	Zeta potential	−9.92 mV
5	Particle size	593.5 nm
	Zeta potential	−14.5 mV
15	Particle size	471.9 nm
	Zeta potential	−15.4 mV
25	Particle size	294.1 nm
	Zeta potential	−17.5 mV
35	Particle size	369.0 nm
	Zeta potential	−15.9 mV

3.3.2. Zeta potential. The stability of a solution system can be found by determining the zeta potential. The negative value of zeta potential implies that more amino groups have having negative charge than those that have a positive charge.[66] The higher dispersion stability and greater electrostatic contact between protein molecules in the solution are both indicated by the larger absolute values of zeta potential. Table 2 depicts the zeta potential of FBPI at 200 W US at different processing times. The control sample had a zeta potential of −9.92 mV and increased up to −17.5 mV for the 25 minutes 200 W-US sample and then decreased, further increasing the treatment time to 35 minutes.

The absolute value of the ultrasonic time tends to initially increase and then decrease with increasing ultrasonic time (Table 2). These events demonstrated that the FBPI's degree of ionization was raised by ultrasonic treatment. This was most likely caused by the fact that following ultrasonic treatment, more negatively charged protein groups were exposed. However, as the sonication period and/or ultrasonic power were extended, its absolute value began to trend downward. The protein may reaggregate as a result of the cavitation effect of US, which would result in a drop in the effective charge on the protein surface.[61] The expansion of the protein secondary structure occurred during prolonged sonication, exposing the non-polar hydrophobic residues, and the interactions between them to form aggregates may be the cause of the decline in the zeta potential for 35 min 200 W-US for UFBPI, as seen in the study.[14] The partial denaturation of protein with an increase in sonication time for up to 25 min in the 200 W-US processed sample is supported by the increase in the surface hydrophobicity value and aggregation of the protein with prolonged sonication time after 25 min, *i.e.*, the 35 min-200 W US processed sample is supported by the decrease in the surface hydrophobicity value in the later Section 3.3.5 and Fig. 8.

3.3.3. SEM. To see how the cell walls are being broken or sheared, scanning electron microscopy (SEM) is utilized.[67] The surface morphology and structural characteristics of untreated field bean protein isolate powder (FBPIP) (control) and US-assisted extracted field bean protein isolate powder (UFBPIP) of US 25 min 200 W were observed at different magnification levels at 5×, 10×, 15×, and 200× (Fig. 6). The UFBPI has a rough surface when compared to the protein obtained by a conventional method. Similar results were found by (ref. 68) who studied the modification of rapeseed proteins using US-assisted alkaline pH shift method. It said that the roughness of the surface could be due to the intensified surface damage because of the US treatment. The microstructure of a protein is directly linked to its functional properties. In a liquid, ultrasonics had an impact on FB protein clumps, forcing them to depolymerize and disperse into smaller protein molecules as a result of the mechanical action of the US. On the other side, the protein aggregate's structure loosened due to the cavitation of ultrasonication. The FBPI aggregate's macromolecular structure progressively broke down and changed into smaller particles

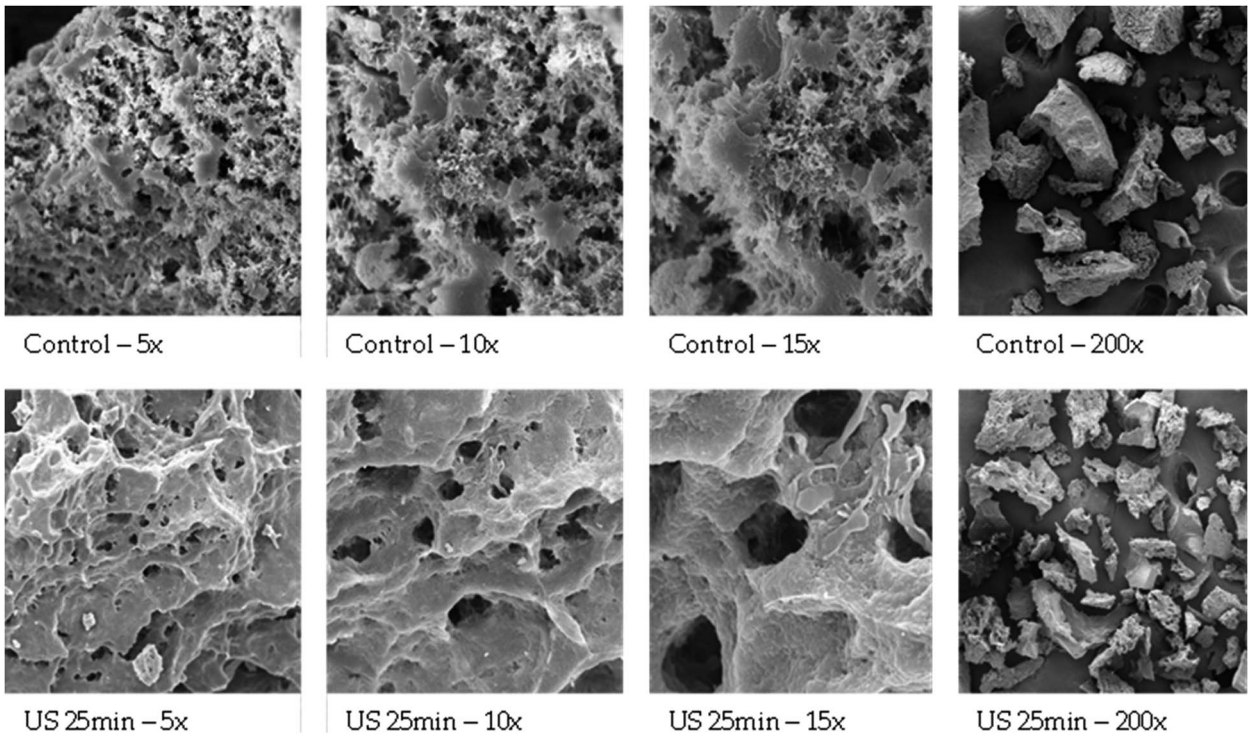

Fig. 6 SEM images of the untreated FBPI and UFBPI powder at 200 W input for 25 min at a magnification level of 5×, 10×, 15×, and 200×.

with the help of US. With the use of US, the macromolecular structure of the FBPI aggregates gradually disintegrated and transformed into smaller particles. These findings suggested that ultrasonic treatment can alter protein aggregation, which in turn alters the protein microstructure.[21] FB protein molecules were expelled from the aggregates due to the medium's vibration, which also resulted in a reduction in the aggregate's molecular weight and particle size.[57]

3.3.4. Fourier transform infrared (FT-IR). Local folded structures make up the protein's secondary structure, which often depends on interactions between backbone atoms and the amino acid sequence.[67] The effects of sonication treatments on the structure and functional groups of the untreated FBPI (control), and the 200 W US – 5-, 15-, 25-, and 35 min samples have been studied using FT-IR and are shown in Fig. 7.

The amide I band, amide II band, and amide III band are the three groupings of distinctive absorption bands that make up the protein's FTIR spectrum, and their respective wavenumbers are 1600–1700 cm^{-1}, 1530–1550 cm^{-1}, and 1260–1300 cm^{-1}, respectively. The C=O stretching vibration, which is mostly centered at 1650 cm^{-1}, is the primary cause of the amide I. The hydrogen bond interaction between molecules or within molecules can be reflected in the amide II. When the hydrogen connection between molecules or between molecules is broken, the spectrum band shifts to a high wavenumber.[68] Among all amide bands, amide I is sensitive to the type and amount of secondary structures and is not strongly influenced by side chains.[69] The amide I zone (1700–1600 cm^{-1}) is mainly used for understanding the secondary structure of proteins because it consists of overlapping component bands that characterize the structures such as α-helix, β-sheet, and β-turn.[70]

In the FT-IR analysis of FBPI, two sharp peaks were obtained for the control sample for the wavenumbers in the range of 1500–1650 cm^{-1}. One peak was found at 1530 cm^{-1}, which states the presence of N–O stretching. Another peak was found at 1633 cm^{-1}, indicating the presence of C=C stretching and corresponding to the β-strand and β-sheet structures in the native pulse protein.[70] FFBPI showed similar peaks, as seen in

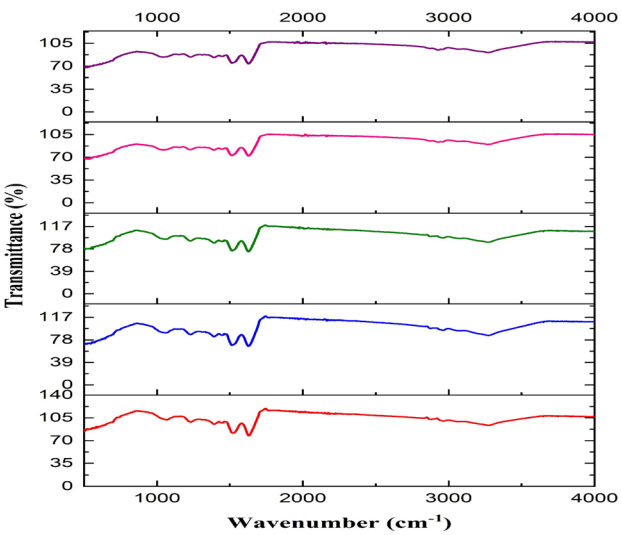

Fig. 7 FT-IR spectra of the FBPI powder at 200 W input power at 0, 5, 15, 25, and 35 min.

the control sample. The predominance of β-structures in the secondary structure also contributes to lowering the digestibility of pulse proteins. The β-sheet content of the control sample was higher than the US-treated samples. The β-sheet content decreased with increasing treatment time. The UFBPI at 25 min had the least β-sheet content, while a rise in the β-sheet content was seen when the treatment time was increased to 35 min.

Two small peaks were found in the wavenumber ranging from 1250–950 cm^{-1}, one at 1232 cm^{-1} and the other at 1065 cm^{-1} in the control and UFBPI samples. The peak at wavenumber 1232 cm^{-1} depicts C–N stretching, indicating the presence of an amine group. A broad peak was found at 3280 cm^{-1} that corresponds to O–H stretching.

3.4. Nutritional aspects

3.4.1. *In vitro* protein digestibility. Based on the results obtained from the above analysis, it was proved that the 25 minute 200 W US gave the best results and hence was chosen for this analysis. The value of *in vitro* protein digestibility of the 25 min US FBPI and the control are mentioned in Table 1. The digestibility of FBPI was altered after the US treatment. There was a significant increase ($p \leq 0.05$) in the protein digestibility value for the US-treated sample. In this study, the *in vitro* protein digestibility of the control sample was 94.47% upon US treatment of 25 min at an input power of 200 W at 100% amplitude, which raised the protein digestibility of FBPI to 96.37%. This was likely caused by the fact that under the right ultrasonic treatment conditions, the protein structure was altered, making the protein's cleavage sites accessible to digesting enzymes. The outcomes showed that the ultrasonic alteration helped to increase FBPI's digestion. A high content of β-sheets was reported to limit the access of proteolytic enzymes, leading to lower protein digestibility.[71] The presence of more content of β-sheet, which contributes towards the low digestibility in the control sample, as seen in the FT-IR analysis results given in the later section, also justifies the results. Similar results were found with chickpea.[61] The process of sonication was used to modify proteins, which improved their *in vitro* digestibility. This improved the digestibility of FPBI, helping to achieve human health and well-being and can achieve SDG 3.

3.5. Comparison between batch and continuous process of US system

Using a batch-type sonicator for 25 minutes at 200 W of 26 kHz input power, the optimal field bean protein isolate was generated based on previous findings. A sample that was jacketed with ice or water at a temperature of 3–4 °C was also taken into consideration for the continuous type. One circulation or a single pass takes 3 min for a volume of 600 mL sample in the continuous system operated at 200 W-26 kHz input US power and processed under controlled temperature using refrigerated circulation at 3–4 °C. Thus, the two systems were compared by equating their energy densities, and the processing time required for the continuous process was mathematically calculated for 150 min or 50 number of passes. The functional properties of field bean protein isolate were analyzed for a continuous system and compared against the optimized batch system for FBPI. Thus, in a continuous system, the extraction yield of the CUFBPI was 43.99%, which was lower than the extraction yield of UFBPI at 25 min-200 W (59.49%). The functional properties of the FBPI were compared to check the efficiency of the systems, *i.e.*, batch and continuous processes. The obtained results are mentioned in Table 3. The WHC, OHC, FC, FS, EAI, ESI, and solubility values obtained for CUFBPI are 3.133 ($g_{water}/g_{protein}$), 3.367 ($g_{oil}/g_{protein}$), 81.52%, 84%, 10.44 ($m^2\ g^{-1}$), 79.68 min, and 60.04%, respectively. It was observed that lower values were obtained compared to the batch-type isolation of UFBPI at 25 min-200 W for all the functional properties analyzed. The WHC and OHC values for CUFBPI were comparable with the values of UFBPI 35 min-100 W (Fig. 2a, b, 3a and b) in magnitude. Also, the result for FC, FS, EAI, and ESI was comparable with the result obtained for UFBPI 15 min-100 W for the same properties (Fig. 4–7). The surface hydrophobicity of CUFBPI was found to be 263.35, which is less than that for UFBPI-25 min (689.32) at the equivalent energy density. This explains the reason for the lower values of functional properties for CUFBPI than UFBPI-25 min, *i.e.*, the less exposure of hydrophobic groups. Hence, it can be concluded that the continuous system at the same power and energy density is less efficient than the batch system. A continuous system with higher input power and intensity operated at the same frequency may provide an equivalent or higher effect in terms of functionality and yield of protein.[72]

One of the most significant indices to assess a protein's conformational shift is its surface hydrophobicity. The surface hydrophobicity of all FBPI is mentioned in Fig. 8. The control sample has a surface hydrophobicity of 183.45. There is not a significant difference ($p > 0.05$) between the surface hydrophobicity of the control sample and the UFBPI with 5 min treatment time (183.56), indicating that the treatment time is not sufficient enough to make significant changes in the proteins. It can be noted that the surface hydrophobicity of FBPI increased with the expansion of ultrasonication duration. The highest value was seen for the UFBPI at 25 min (689.32). The majority of the hydrophobic groups in the control sample were hidden inside protein molecules. These protein groups or areas were exposed after ultrasonic treatment, which led to an increase in the surface hydrophobicity.[73] After 25 min, there is a decrease in the surface hydrophobicity of UFBPI at 35 min

Table 3 Comparison of the functional properties of the UFBPI at 25 min and CUFBPI

Functional properties	US-25 min-200 W	CUFBPI
WHC ($g_{water}/g_{protein}$)	3.6 ± 0.1	3.133 ± 0.15
OHC ($g_{oil}/g_{protein}$)	3.93 ± 0.05	3.367 ± 0.15
Foam capacity (%)	110.12 ± 0.38	81.52 ± 1.68
Foam stability (%)	90.53 ± 0.38	84.00 ± 0.61
Emulsion activity index ($m^2\ g^{-1}$)	16.37 ± 0.45	10.44 ± 0.49
Emulsion stability index (min)	104.74 ± 0.69	79.68 ± 0.56
Solubility (%)	73.82 ± 0.64	60.04 ± 0.75

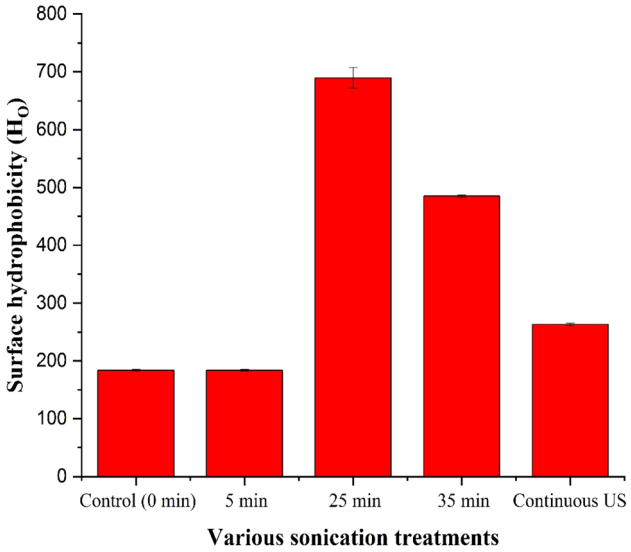

Fig. 8 Surface hydrophobicity (H_o) of FBPI at different sonication treatments at 0, 5, 25, and 35 min of 200 W and in a continuous US system.

(485.08). Along with this, CUFBPI also showed a decrease (263.55) in the protein surface hydrophobicity, which is less than 35 min of UFPBI. These results can justify the changes in the functional properties for both batch and continuous processes, as mentioned in Section 3.2, Fig. 3–5, and Table 3 for CUFBPI. Ultrasonic treatment may induce more hydrophobic groups to re-polymerize into aggregates through hydrophobic interactions, leading to a decrease in the protein's hydrophobicity.[74] Thus, from Fig. 8, it is apparent that the protein was partially denatured until the time such as 25 min at 200 W treatment and re-polymerized by hydrophobic binding and other effects as the US time is further increased to 35 min, which might have happened in the continuous ultrasound process, leading to a reduction in the values.[62,75]

4. Conclusions

Plant proteins are gaining more attention in recent times. This study has improved the yield and the physicochemical-functional, structural, and nutritional properties of protein isolate from germinated field bean flour at conditions of 200 W for 25 min. At this level, there was an increase in protein yield, foaming capacity, foam stability, emulsion activity index, emulsion stability, solubility, and zeta potential *in vitro* protein digestibility. Along with these, there is a decrease in the particle size. The change in the microstructure of the proteins due to US treatment was seen in SEM. FTIR analysis confirmed the changes in the secondary structures of proteins. The physical changes caused by acoustic cavitation resulted in the partial denaturation of proteins shown by an increase in the surface hydrophobicity and thus the functionalities. On prolonged US treatment, *i.e.*, 35 min, the decrease in the values of functional properties was seen due to the protein aggregation, suggesting that 25 min is the optimum treatment time for improving the yield, resulting in the best functional modifications of proteins that help in achieving the SDGs 2, 3, 6, 7, 12, 13. These enhanced qualities demonstrate that US-extracted germinated field bean protein has superior qualities over conventional methods and may be substituted for other proteins in food applications. This study also showed the importance of the pale brown field bean variety with its unique properties that have not received enough attention from researchers as a sustainable alternative protein to use in the food sectors. This study leads to a path to consider the US-treated field bean protein in the development of shakes, yogurt, and other products.

Author contributions

Bhakti Anand Narale: data collection, review and research work, analysis, writing – original draft and editing. Addanki Mounika: writing – original draft and editing, reviewing. Shanmugam Akalya: conceptualization, designing of experiments, reviewing, editing and supervision.

Conflicts of interest

The authors declare no conflict of interest.

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