Structure–Function Relationships of Ultrasound-Extracted Faba Bean Protein in Stabilizing Oil-in-Water Emulsions

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RESEARCH

Structure–Function Relationships of Ultrasound-Extracted Faba Bean Protein in Stabilizing Oil-in-Water Emulsions

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Abstract

Faba bean protein is emerging as a valuable ingredient in fortified beverages due to its high nutritional quality; however, its functional attributes remain insufficiently understood. This study examined the structure–function relationships of ultrasound-extracted faba bean protein isolate in stabilizing oil-in-water emulsions under different high-pressure homogenization conditions (500 and 1500 bar). Structural analyses showed that ultrasound extracted faba protein retained its secondary structure and exhibited strong thermal stability. Functionally, the extracted faba protein demonstrated high oil-holding capacity and reduced surface tension, supporting enhanced interfacial activity. Emulsion characteristics, including droplet size distribution, rheology, creaming, and gravitational stability, were monitored over 20 days at 4 °C. At 1500 bar, ultrasound-extracted faba protein generated fine emulsions with D_v 50 droplet sizes below 0.4 µm at 1% protein, maintaining long-term stability. Collectively, these results highlight ultrasound extracted faba protein as a promising clean-label emulsifier for next-generation plant-based food formulations.

Keywords Plant protein · High-pressure homogenization · Emulsifying properties · Rheology · stability

Introduction

The growth of the plant-based foods market has surged due to increasing concerns about the environmental, animal welfare, and health impacts associated with animal-based foods [1]. Livestock agriculture significantly contributes to greenhouse gas emissions, water depletion, pollution, and biodiversity loss [2, 3], while also raising ethical concerns about animal treatment [4]. Factory farming practices pose risks such as zoonotic disease transmission and antibiotic resistance [5, 6], underscoring the need for sustainable protein alternatives [7, 8]. The cultivation of pulses not only provides nutritional and functional proteins (e.g. emulsifiers) but is also beneficial to the environment [9]. Recognized as a viable protein source in the protein transition, pulses provide functional benefits like emulsification [10, 11]. Pulse proteins are complex mixtures, primarily composed of globulins (70–80%) and albumins (10–20%) [12]. Globulins include legumins (hexamers, 300–400 kDa, stabilized by disulfide bonds), vicilins (trimers, 145–190 kDa, cysteine-free), and convicilins (trimers, 220–290 kDa) [13, 14]. Albumins comprise diverse low-molecular-weight proteins, including enzymes, storage proteins, and cytoplasmic proteins, with broad size variability [15, 16]. The functional behaviour of these proteins is influenced by their structure, amino acid composition, surface properties which is altered by extraction and processing conditions such as pH, ionic strength, solvent type, heat and mechanical treatment [17, 18]. Due to structural and molecular weight differences, plant proteins like soy, pea, and faba bean exhibit distinct interfacial and emulsifying behaviours [19].

Oil-in-water emulsion systems often employed in cosmetic, pharmaceutical, food industries require effective emulsifiers. Replacing synthetic and animal-based emulsifiers with natural, plant-derived alternatives remains a major challenge [20–22]. Many proteins can function as natural emulsifiers due to their amphiphilic nature and 3D

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conformations that expose hydrophobic groups at interfaces. Upon adsorption to oil droplets during homogenization, proteins reduce interfacial tension and facilitate droplet breakup. Conformational rearrangements at the interface may enhance hydrophobic interactions and promote interfacial cross-linking, while the resulting protein layer provides electrostatic and steric stabilization against aggregation [23, 24]. However, the emulsification mechanisms of plant proteins remain less understood compared to those of animal proteins [25, 26].

The functional properties of plant proteins can exhibit substantial variability across different suppliers and production batches [27, 28], posing challenges for achieving consistent quality in commercial food formulations. This study explores how modulating high-pressure homogenization (HPH) intensity unlocks the emulsifying and rheological potential of ultrasound-extracted faba bean protein isolate, revealing its promise as a next-generation plant-based emulsifier. Protein dispersions at different concentrations were subjected to HPH treatments at 500 and 1500 bar, followed by assessment of their rheological, stability and emulsifying characteristics. Furthermore, the structural, thermal, and functional properties of the protein isolates were analysed to better understand the observed emulsification behaviours.

Material and Method

Raw Materials and Chemicals

Faba bean seeds were sourced from Whole Foods Earth (Kent, UK), while analytical-grade sodium hydroxide (NaOH, ≥99.9%) and hydrochloric acid (HCl, 37%) were obtained from Sigma-Aldrich (UK). The seeds were milled using a cyclone mill (Retsch Twister, Germany) operating at 12,000 rpm with a 0.5 mm sieve, and the resulting flour was stored until further use. Sunflower oil was purchased from a local retailer. All experimental preparations were carried out using distilled water. All other reagents used in the study were of analytical grade.

Extraction of Ultrasound-assisted Faba Bean Protein Isolate (US-FBP)

Ultrasound-assisted protein extraction was performed based on previously optimized conditions [29, 30], using 123 W power, a 1:15 g/mL solute-to-solvent ratio, and 41 min sonication. Faba bean flour was dispersed in water (623mL), stirred at 25 °C for 20 min (500 rpm), and the pH was adjusted to 11 before sonication using a S24d22D titanium horn (Teltow, Germany), with temperature maintained between 20 and 25 °C using an ice bath. The extract was centrifuged at 6000 rpm for 20 min at 25 °C (accuSpin™ 400, UK). The supernatant pH was lowered to 4.0 with 1 N HCl to precipitate proteins, which were recovered by centrifugation, freeze-dried for 48 h, and stored at −20 °C for further use. The ultrasound extracted faba bean protein isolate had a protein content of 92% [29].

Surface Tension

The variation of the surface tension of ultrasound extracted faba protein solution at different concentrations (0.01, 0.5 and 1%) was measured using pendant drop tensiometer (OCA 15EC Dataphysics, Germany) [15].

Water and Oil Holding Capacity

Water holding capacity (WHC) and oil holding capacity (OHC) were measured following the method of Badjona et al., (2024) [31]. A 1.0 g sample of faba bean protein isolate was mixed with varying volumes (2,5,10,12,15,20 and 25mL) of distilled water or sunflower oil, vortexed for 1 min, and equilibrated at room temperature (20–23 °C) for 2 h. Samples were then centrifuged at 3000×g for 15 min at 20 °C. WHC and OHC were calculated based on the weight of absorbed water or oil as follows:

$$\mathrm{WHC} = \frac{\mathrm{w}_0 - \mathrm{w}_1}{\mathrm{w}_3} \times 100 \quad (1)$$

$$\mathrm{OHC} = \frac{\mathrm{w}_0 - \mathrm{w}_1}{\mathrm{w}_3} \times 100 \quad (2)$$

Where w_0 is the mass of the tube and protein isolate and absorbed water or oil after centrifuge; w_1 is the mass of the tube and protein isolate, while w_3 is the mass of faba bean protein.

Fourier-transform Infrared Spectroscopy Analysis

Fourier-transform infrared (FTIR) analysis was conducted using an Attenuated Total Reflectance (ATR)-FTIR spectrophotometer (Spectrum 100 FT-IR, PerkinElmer, USA) on freeze dried protein powder. Spectra were recorded with 16 scans at a resolution of 4 cm^{-1}, covering the wavenumber range of 4000 to 650 cm^{-1}.Spectras was subjected to baseline correction, Gaussian smoothing, and normalization [15].

X-Ray Diffraction

X-ray diffraction (XRD) analysis was performed using an X'pert PRO X-ray diffractometer on freeze dried protein powder. An anti-scatter slit of 0.04 mm and divergence and receiving slits of 1 mm were used. The diffractogram was recorded over the 2θ range of 5–70° at a scan rate of 2°/min with a step size of 0.05°.

Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) was conducted using a Q50 Thermogravimetric Analyzer (TA Instruments, New Castle, Germany). Approximately 2 mg of the protein powder was heated from 30 to 900 °C under an atmospheric nitrogen flow of 200mL/min. The degradation temperature was identified at the second step of weight loss, while the initial weight loss was attributed to water evaporation.

Preparation of Emulsions Sby US-FBP and Their Characterization

Ultrasound-extracted faba bean protein (US-FBP) was dissolved in deionized water to prepare stock solutions with protein concentrations of 0.01, 0.5, 1, and 5% (w/v). The dispersions were gently stirred overnight at 4 °C using a magnetic stirrer to ensure complete hydration. During the first 2 h of stirring, the pH was adjusted to 7.0 using 1 M NaOH or HCl. All samples were prepared based on protein mass fraction.

Emulsions were initially prepared through pre-emulsification using an L5M-A high-shear mixer (Silverson, UK). An oil-to-water phase ratio of 1:9(w/w) was selected, as higher oil content may lead to clogging of the homogenizer due to high viscosity or emulsion gel formation, and this ratio closely aligns with optimal conditions for food emulsions. Following the preparation of both phases, the pre-emulsification process was conducted by setting the mixer speed to 10,000 rpm. The aqueous phase was introduced into the homogeniser probe, after which the equipment base was secured, and agitation was initiated. Sunflower oil (dispersed phase) was then gradually introduced into the system under continuous mixing, and the emulsification process was maintained for 2 min to ensure proper dispersion of the phases.

The pre-emulsion was further processed using an EmulsiFlex-C50 high-pressure homogenizer (Ontario, Canada) to produce a fine emulsion. The pre-emulsion was passed through the homogenizer once at two different pressure settings (500 and 1500 bar) to evaluate the effect of homogenization intensity. The outlet temperature following homogenization was maintained at approximately 32 °C or below. The resulting fine emulsions were collected into 50mL centrifuge tubes and stored under refrigerated conditions at 4 ± 2 °C until further analysis (Fig. 1).

Emulsion Rheological Properties

Rheological measurements were conducted on freshly prepared emulsion samples using a rotational rheometer (MRC 302, Anton Paar) equipped with a 17 mm by 43 mm concentric cylinder with a gap of 1 mm (CC17/T200/SS) and an attached vane geometry (SR15-2 V/2 V-32/100). The unit was temperature-controlled at 20 °C ± 0.2 using an integrated water bath. The viscosity of the samples was measured through a shear sweep ranging from 0 to 100s^{-1}. Each sample was analysed in replicates (n = 3). Apparent viscosity and shear stress data were generated and plotted.

Emulsion Particle Size Analysis

The droplet size distribution of the emulsions was measured using laser diffraction. To minimize multiple scattering effects, emulsion samples were diluted 100-fold with

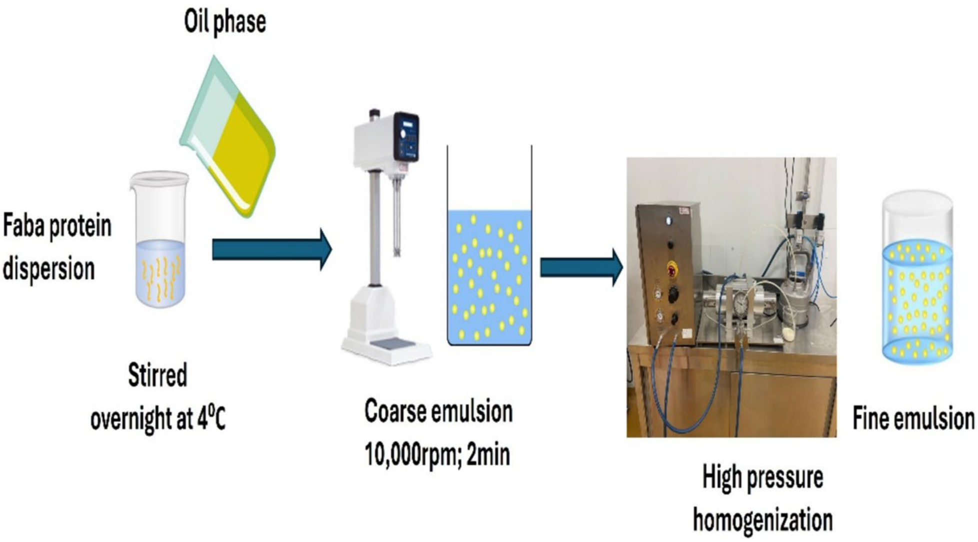

Fig. 1 Schematic representation of emulsion preparation using ultrasound-extracted faba bean protein isolate (US-FBP) and high-pressure homogenization (HPH)

deionized water prior to analysis, and obscuration was set between 8 and 9%. Measurements were performed using a Malvern Mastersizer 3000 (Malvern Instruments Co., Ltd.), and each sample was analysed in triplicate to ensure reproducibility. Data were processed using Malvern analytical software, and particle size distributions were characterized using multiple parameters:

- $d_{3,2}$ - surface weighted mean or surface area mean diameter,
- particle diameters - d(50), d(10) and d(90) which represent diameters at 10, 50, and 90% cumulative volume, respectively,
- Span - the width of a droplet size distribution. Its value is calculated.

$$\mathrm{Span} = \frac{\mathrm{d}\,(90) - \mathrm{d}\,(10)}{\mathrm{d}\,(50)} \tag{3}$$

Emulsifying Creaming Index (CI)

The creaming index (*CI*) was used as a key parameter to assess emulsion stability over time. The height of the cream layer (V_1) and the total height of the emulsion (V) were measured at regular intervals over a 20-day storage period. *CI* was calculated as the percentage ratio of the cream layer height to the total emulsion height as described by [32]

$$CI\,(\%) = \frac{V_1}{V} \times 100 \tag{4}$$

Emulsion Destabilization Under Accelerated Gravitation

Emulsifying stability (*ES*) under centrifugal force was also evaluated. The total volume of the freshly prepared emulsion was recorded as Wv. The emulsions were then centrifuged at 3000×g for 15 min, after which the volume of the remaining emulsified layer was measured and denoted as *Elv*. Emulsifying stability was calculated using the following equation, as described by [32]:

$$ES\,(\%) = \frac{Elv}{W_v} \times 100 \tag{5}$$

Statistical Analysis

Statistical analyses were conducted using Origin 2019 and Excel 2024 (v2406). Data are presented as mean ± standard deviation (SD) of experiments performed in triplicate. All analysis was carried out in triplicate except XRD. A one-way ANOVA test with 95% confidence to identify possible significant differences between the protein types used. A $p \leq 0.05$ represented a significant difference.

Results and Discussion

Physicochemical Characterisation of US-FBP

Surface Tension

Figure 2A shows the surface tension values as a function of time of protein solution at pH 7. The results show that surface tension values decrease with increasing protein concentration for US-FBP protein isolates. Similar behavior has been reported by previous studies. At this 0.01, 0.5 and 1% protein concentration (Fig. 2A), the difference in surface tension was most pronounced. At 0.01% protein rapidly reduced surface tension from approximately 73mN/m to below 65mN/m within the first few hundred seconds, demonstrating strong and fast adsorption at the air–water interface. For 0.5% protein suspension (Fig. 2A), the initial surface tension of the proteins was ~ 55mN/m, however after 1000 s, a drastic reduction in surface tension was observed. However, ultrasound-extracted faba protein at 1% (Fig. 2A) showed a drop after 1000 s, likely due to slower adsorption kinetics or conformational resistance. Increased interfacial adsorption leads to reduced surface tension. Namely, Increasing the protein concentration caused a sharp reduction in surface tension, which continued until a plateau was attained at a specific critical protein concentration. Similar trends have been observed in whey protein isolates, where protein unfolding and secondary structure changes contribute to decreased surface tension [33].

The surface activity and consequently the emulsifying behaviour of low-molecular-weight surfactants can be readily estimated from their hydrophilic–lipophilic balance (HLB). In contrast, predicting the surface behaviour of proteins is considerably more complex because their diverse amino acid compositions govern a wide range of physicochemical properties that ultimately influence surface activity [34]. Even proteins with comparable proportions of hydrophobic and hydrophilic residues may display markedly different physicochemical behaviours. Based on the measured surface tension values, however, the proteins examined in this study demonstrated different surface activity despite their structural similarity.

Water and Oil-holding Capacity

The water and oil holding capacity of protein isolates are functional indices that reflect their ability to bind water

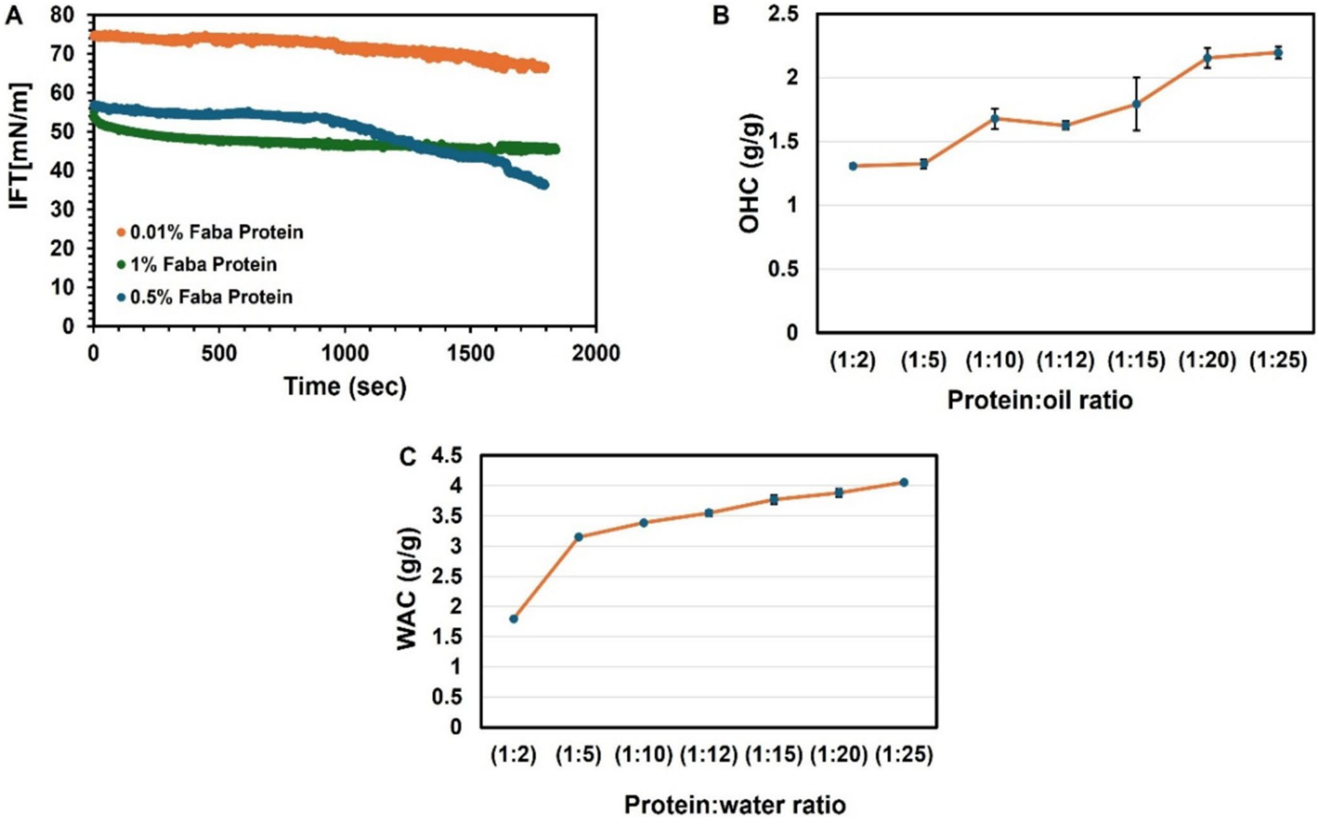

Fig. 2 **A** Surface tension at the air–water interface as a function of time for protein solutions at different concentrations (0.01, 0.5 and 1% w/v) at pH 7; **B** oil holding capacity and **(C)** Water-holding capacity (WHC) of US-FBP determined at different protein-to-oil or protein-to-water ratios. Values represent means ± SD (*n* = 3)

and lipids in a matrix. Water and oil holding capacities are governed by several intrinsic factors, including protein molecular size, amino acid composition, conformational arrangement, and the presence of residual carbohydrates or lipids. Proteins enriched in hydrophilic functional groups typically exhibit greater WHC because they can form more extensive hydrogen bonding interactions with water. Conversely, OHC is largely determined by the exposure of hydrophobic and non-polar side chains on the particle surface, which facilitates interactions with oil molecules [26]. Major differences in WHC and OHC were observed for ultrasound-extracted faba protein at different protein to water/oil ratio Fig. 2A & B. The interaction of proteins with water is influenced by protein structure, and the level of exposure of hydrophilic groups as well as charge amino acids [35]. There was an observed increase in WHC with increasing protein to water ratio. The average WHC of faba protein ranged from 1.80 to 4.06 g/g, with the higher WHC observed at protein to water ratio of 1:25 g/mL. Additionally, the oil holding capacity (OHC) of ultrasound-extracted faba bean protein also increased with increasing protein to oil ratio (Fig. 2B). A high OHC may suggest the presence of more hydrophobic regions that can bind and entrap oil.

In plant proteins, high oil-holding capacity (OHC) is often associated with improved emulsifying performance, as greater oil-binding ability enables the formation of a thicker and more cohesive interfacial film around oil droplets. This reinforced interface helps prevent droplet coalescence and phase separation, thereby enhancing emulsion stability [18, 36]. Nonetheless, the extent of this effect is also strongly influenced by the protein's molecular structure and the processing conditions applied. A protein with excellent amphiphilicity such as US-FBP may indicate its ability to achieve equilibrium at the oi-water interfaces.

TGA, FTIR and XRD analysis

Structural and thermal analysis, Fig. 3 revealed key structural properties of ultrasound-extracted faba bean protein isolate that can help elucidate their emulsifying behavior. FTIR spectroscopy is a reliable tool for analyzing differences in structural conformation, offering valuable insights into secondary structure of proteins [37]. Infrared analysis of proteins can reveal both the strength and relative abundance of hydrogen bonds associated with different amino acid conformations [34]. Approximately 70–78% of the proteins

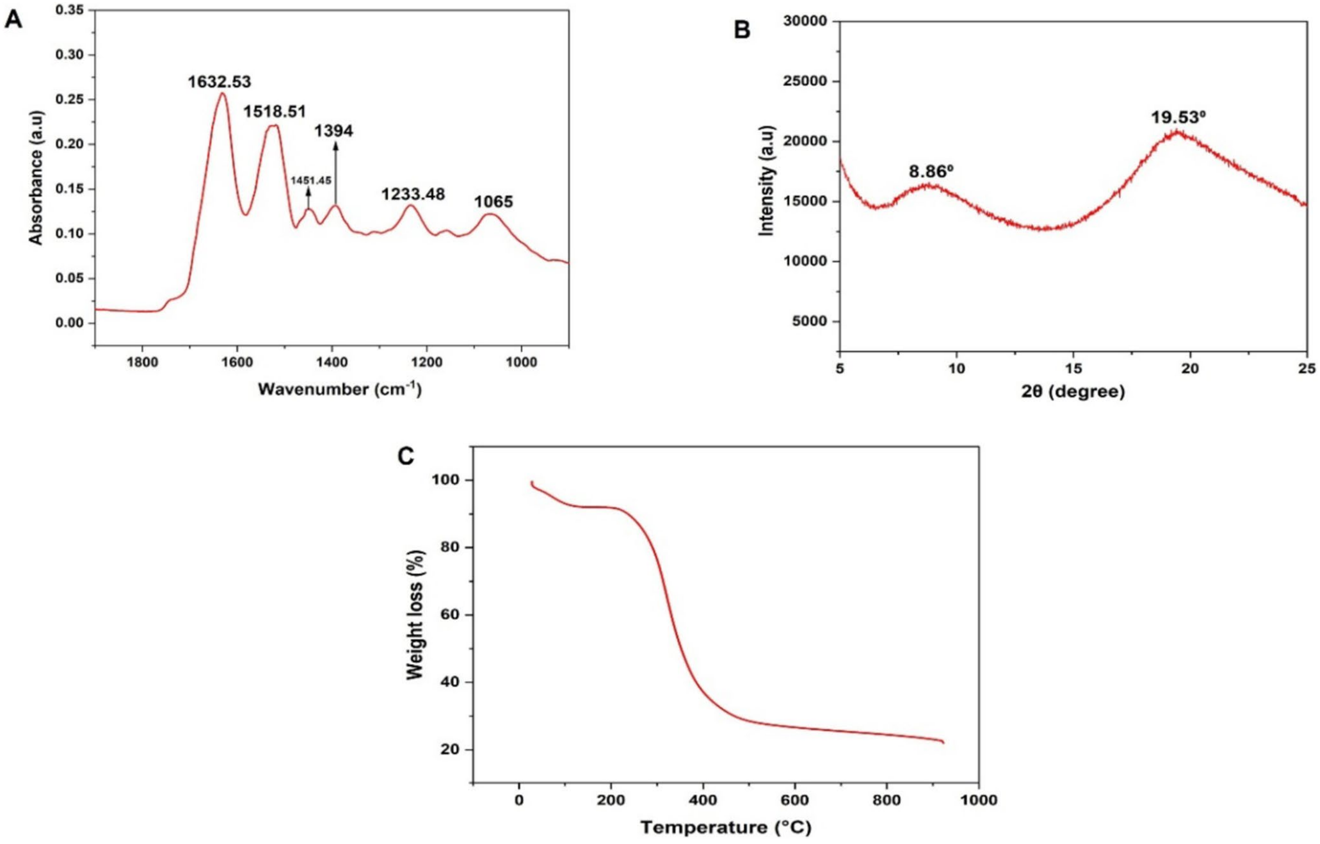

Fig. 3 ATR-FTIR spectra highlighting amide I, II and III regions (**A**); XRD diffractogram (**B**) and (**C**)Thermogravimetric analysis (TGA) curve showing mass loss as a function of temperature of ultrasound-extracted faba bean protein

in pulses are globulins, followed by 10–20% of albumins [38]. From Fig. 3A, there were notable peaks in the spectra of US-FBP. US-FBP showed distinct peaks in amide I, II and III regions. The amide I band (1600–1700 cm^{-1}), corresponding to C=O stretching vibrations exhibits sensitivity to alterations in the secondary of proteins. The peak at 1632.53 cm^{-1} is the characteristic absorption peak of the amide I band, indicating the presence of high-intensity hydrogen bonds in the β-sheet structure, which directly reflects the secondary structure of protein and has a positive effect on its stability [39].

The XRD pattern of US-FBP protein is represented in Fig. 3B. Sharp peak shapes are commonly associated with crystalline compound while broad and diffuse backgrounds are associated with crystalline structure [40]. Faba bean protein showed a weak peak at ~8.86⁰ and 19.53⁰. These two distinct peaks are attributed to the presence of a-helix and B sheets respectively in proteins [41]. The XED experiments showed that US-FBP had an intact structure, and the proteins did not have obvious structural changes during extraction. These findings align with observations for 7 S and 11 S soy proteins [42], mung bean protein [43], and whey protein isolates, all of which display comparable diffraction peaks at 2θ≈8° and 19.5° [44], indicative of proteins retaining their native structural state.

Protein functionality and thus their suitability for food applications is strongly affected by thermal stability. This is particularly important because most food processing operations involve heat treatments that can modify both functional and nutritional properties [29]. To better understand the structure of US-FBP and the properties of functional groups, TGA was used to analyse the thermodynamic behaviours of US-FBP. Thermogravimetric analysis showed that faba bean protein possesses high thermal stability (Fig. 3C). The high thermal stability may indicate that faba protein still possess high functionality under processing conditions. Faba protein showed a delayed mass loss with higher onset temperature. Although high thermal stability may seem beneficial, in emulsion preparations, it may reduce protein flexibility. TGA thermograms revealed a typical two step weight loss for US-FBP. An initial ~ 5–8% mass loss below 150 °C (due to moisture evaporation and volatile compounds) and a major degradation step starting around 280–300 °C attributed to protein decomposition.

O/W emulsions using US-FBP and their characterization

Emulsion rheological properties

Proteins are macromolecules which increase the viscosity of the surrounding aqueous medium. This bulk-phase viscosity enhancement, alongside their inherent surface activity, is a key factor contributing to their emulsifying and stabilizing performance [36, 45]. The steady-state rheological behaviour is an essential parameter reflecting the change in the speed of dispersive motion and external resistance of the liquid [45]. The rheological properties of ultrasound-extracted faba bean protein emulsions were measured at different shear rates from 1 to $100s^{-1}$ at 20 ± 1 °C, as shown in Fig. 4. Faba bean emulsions showed weak shear-thinning (viscosity decreases with increasing shear rate) or Newtonian behaviour. At a given concentration (especially 5%), faba bean emulsions exhibit slightly higher viscosity compared to lower protein concentrations. Shear-thinning behaviour is characteristic of protein-stabilized emulsions and can be attributed to the alignment and deformation of emulsion droplets and protein aggregates under shear, leading to reduced resistance to flow [46, 47]. The extent of shear-thinning reflects droplet interaction and network structure. The lower viscosity of faba emulsions at lower protein concentration may suggest weaker protein–protein or droplet–droplet interactions, smaller or less structured flocs in faba emulsions due to lower dispersed phase (10%) and emulsifier concentration (≤1%). For instance, at a shear rate of $100s^{-1}$, 5% protein concentration, US-faba bean protein emulsion showed apparent viscosity of 4.8 ± 0.00mPa.s (significantly high) (Fig. 4B), while at 0.5% concentration emulsion viscosity showed a lower viscosity of 2.01 ± 0.01mPa.s. Proteins typically behave as polyelectrolytes macromolecules containing numerous ionizable functional groups. Upon dissociation in aqueous solution, these groups generate a charged macromolecular ion along with associated counterions. Because the charged sites are covalently bound along the polymer chain, their behaviour is interdependent rather than independent [34]. Hence, in this study higher protein concentrations led to increased viscosity, attributable to more extensive interfacial protein film, reduced droplet size, intensified droplet–droplet interactions or flocculation, and elevated continuous phase viscosity arising from unadsorbed proteins (Fig. 5).

Emulsion Particle Size

High-pressure homogenization (HPH) significantly influenced the droplet size distribution of US-FBP-stabilized emulsions, with finer emulsions produced at 1500 bar compared to 500bar (Fig. 6A–B). Generally, freshly prepared emulsions prepared at 1500 bar displayed smaller droplets (Fig. 6.A) compared to treatment at 500bar (Fig. 6.B). The difference in particle size distribution between freshly prepared emulsion at 1500 bar (0.1–5 mm) and 500 bar (0.8–100 mm) was clear in Fig. 6A&B.

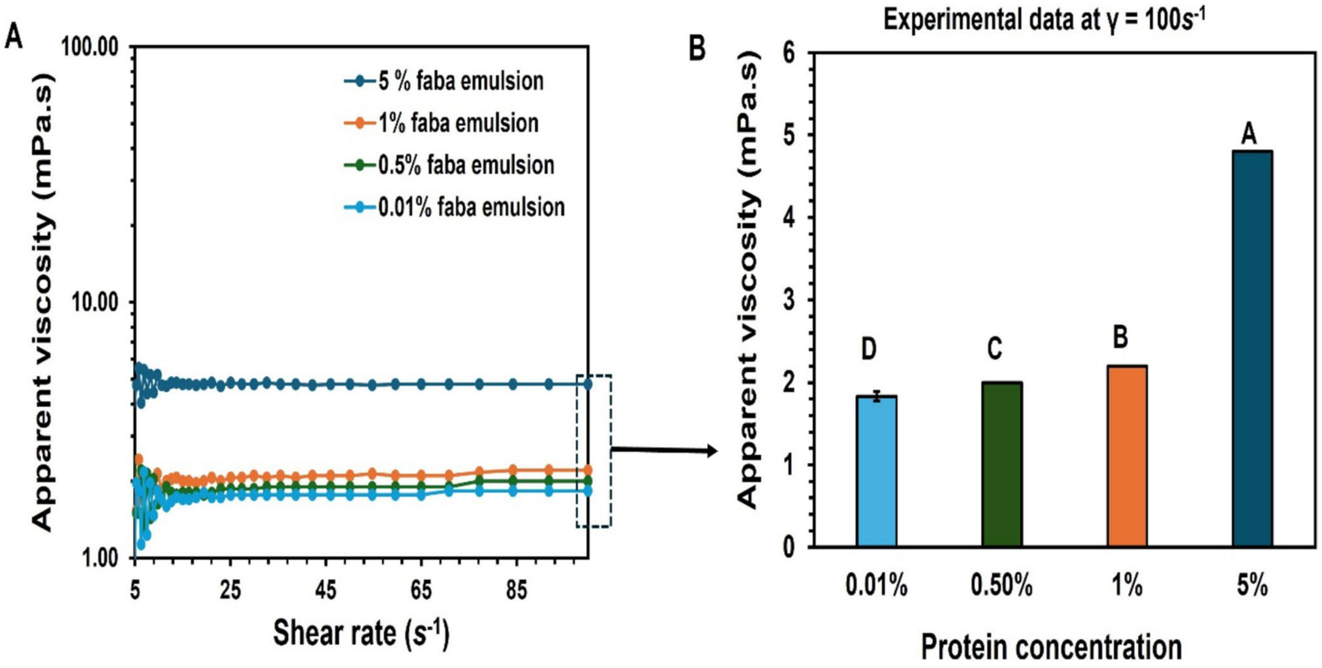

Fig. 4 Rheological properties of ultrasound-extracted faba protein emulsions prepared at a homogenisation pressure of 1500 bar at different protein concentrations (0.01-5%). **A** represent apparent viscosity as a function of shear rate of protein emulsions, measured at 20 °C and (**B**) indicates values of the apparent viscosity at $100s^{-1}$. The different letters denote significant differences ($P < 0.05$) between samples

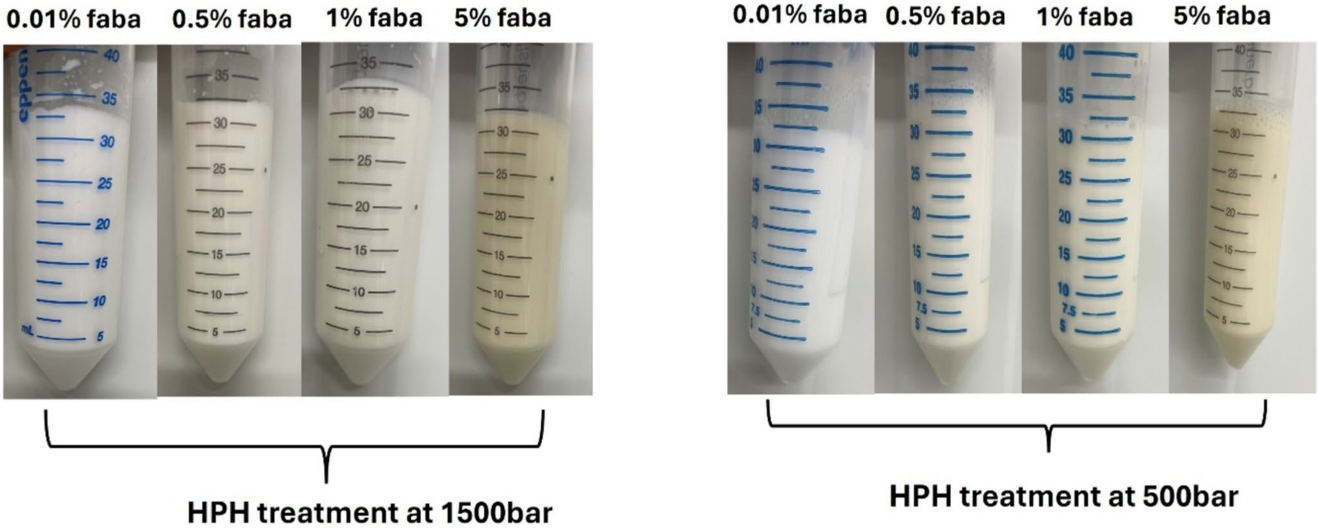

Fig. 5 Digital photos of freshly prepared oil-in-water emulsions stabilized by ultrasound extracted faba bean protein: **A** HPH processing at 1500 bar and (**B**) HPH processing at 500 bar

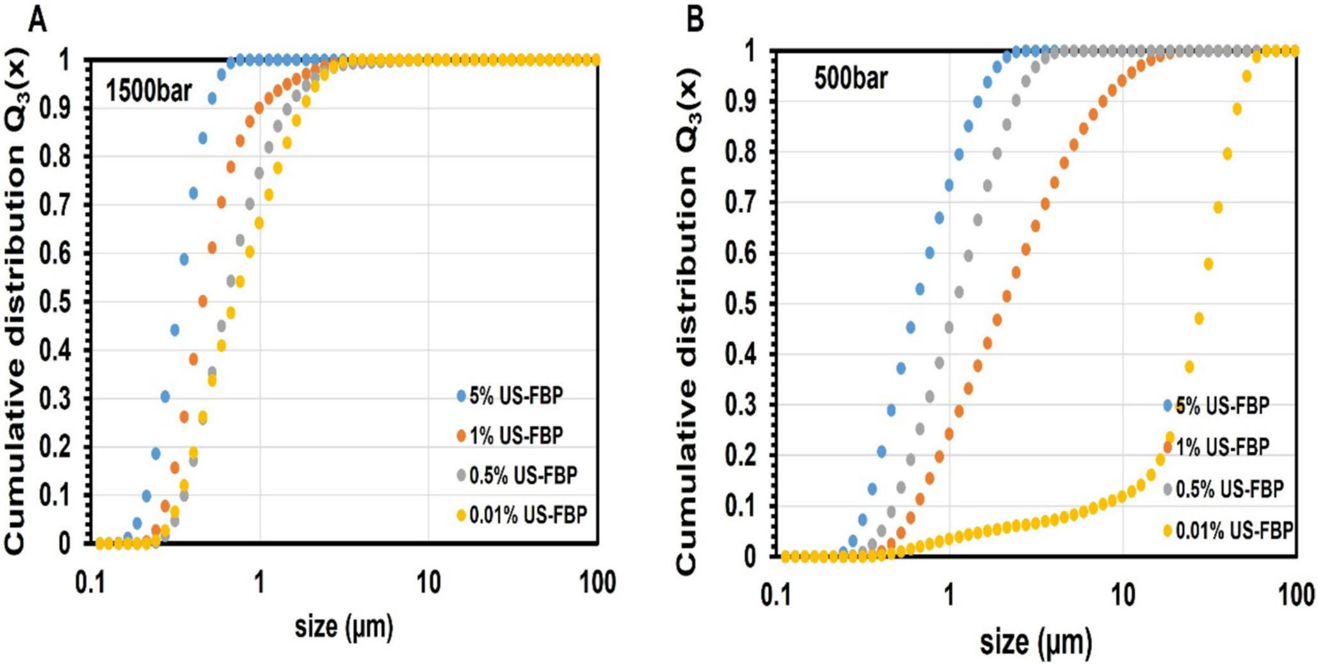

Fig. 6 Cumulative mass distribution Q3 as a function of particle size of fresh emulsions stabilized by ultrasound-extracted faba bean protein isolate at emulsification pressure of (**A**) 1500 bar and (**B**) 500 bar at different protein concentrations (0.01–5% w/v)

The volume-average particle sizes represent a combined measurement of individual and aggregated oil droplets and protein particles, as the particle size analyser cannot differentiate between these components. The D_v 50 values of emulsions prepared at lower processing pressure were significantly higher compared to higher pressure as shown in Table 1. At 1% protein concentrations, the emulsion produced at 1500 bar (0.52 mm) was smaller than at 500 bar (2.33 mm). As shown in the Table 1 D_v 10 and D_v 90 values for US-FBP emulsion increased with decreasing protein concentration. Interestingly, at low protein concentrations (≤0.5%), droplet size distributions broadened, and span values increased, indicating less effective interfacial coverage and greater susceptibility to droplet aggregation or flocculation. For example, emulsions prepared with 0.01% US-FBP at 500 bar exhibited a D_v 90 of 53.17 µm, reflecting unstable structures and partial phase separation. In contrast, emulsions with 1% protein at 1500 bar formed tightly distributed droplets (D_v 90 = 1.13 µm), highlighting the critical role of both protein concentration and processing intensity

Table 1 Comparative effect of homogenization pressure effect on particle size for freshly prepared ultrasound extracted faba protein emulsions at 1500 bar and500 bar

Protein concentration	Pressure (bar)	D_v 10(μm)	D_v 50(μm)	D_v 90(μm)
5% faba	1500	0.24 ± 0.00	0.38 ± 0.01	0.58 ± 0.00
5% faba	500	0.38 ± 0.00	0.73 ± 0.00	1.66 ± 0.04
1% faba	1500	0.33 ± 0.33	0.52 ± 0.00	1.13 ± 0.05
1% faba	500	0.733 ± 0.01	2.33 ± 0.13	8.72 ± 0.92
0.5% faba	1500	0.41 ± 0.00	0.2 ± 0.01	1.67 ± 0.09
0.5% faba	500	0.54 ± 0.00	1.23 ± 0.02	2.74 ± 0.04
0.01% faba	1500	0.39 ± 0.39	0.8 ± 0.00	2.03 ± 0.01
0.01% faba	500	5.70 ± 4.47	32.2 ± 0.83	53.17 ± 1.82

in achieving stable emulsions. This phenomenon could be because at lower protein concentration, there was less protein available to form an effective network, leading to larger droplets. The improved emulsification ability of faba protein can be attributed to various reasons. Ultrasound in particular likely produced faba bean protein isolates with higher solubility and more flexible conformation enabling it to adsorb rapidly and reduce interfacial tension effectively during homogenization [48]. Similar observation has been reported with other proteins; for instance, high pressure micro-fluidization and sonication was observed to disrupt proteins, increase exposure of hydrophobic groups and ultimately yield smaller droplets [49]. A similar result had been reported by Tong et al., (2024) [50] in which pea and rice protein emulsion displayed lower particle size with increasing pressure. However, the authors claimed a low average particle size doesn't result is superior emulsion stability.

Effect of Storage Time

The resistance of fat droplets to aggregation and coalescence is an important quality index for O/W emulsions, which is influenced by several factors. As a thermodynamically unstable food system, the free energy of the oil-water interface induces reversible and irreversible separation during storage, i.e. creaming, Ostwald ripening, coalescence and flocculation [36]. As shown in Table 2; Fig. 7, emulsion destabilization, indicated by the aggregation of fusion of oil droplets, leads to particle size increase in all protein samples. The particle size of the different proteins at varying concentrations exhibited a progressing ascendency trend during 20 days of storage at 4 °C (Fig. 7B).

Table 2 provides the value-weighed mean droplet sizes (Dv10, Dv50 and Dv90) and span values for emulsion stabilized by US-FBP. With increasing storage time (5 and 20 days), the droplet size increased at all protein concentrations; however, the rate of increase differed significantly. In the case of faba emulsion dramatic increase in droplet size at 5% protein was observed. The D_v 90 increased from 0.58 µm (fresh) to 47.2 µm during 20days, demonstrating a higher susceptibility for instability at high protein concentration and longer storage time. This instability can be explained by several mechanisms. Excess proteins in the continuous phase can promote bridging flocculation between oil droplets. Over time, this enhances aggregation and coalescence, leading to larger droplet clusters. Additionally, at high protein concentration, multilayer adsorption or rearrangement at the oil–water interface may reduce the net charge density and steric barrier. This weakens electrostatic stabilization, accelerating droplet–droplet collisions and coalescence during storage. In contrast at 1 and 0.5% concentration, faba emulsion showed superior stability over time with lower particle size evolution. A similar observation was observed by [51] after 7 and 30 days of storing pea protein emulsion showing increasing particle size distribution. The span values (a measure of the distribution width) also increased with storage, especially for lower protein concentration, indicating broader droplet size distribution and reduced emulsion uniformity with time. In contrast, at 0.01% protein concentration, the initially unstable emulsion experiences rapid creaming and partial phase separation during early storage. This removes large droplets from the sampled dispersion phase. We hypothesise that particle size measurements at later storage times reflect predominantly the remaining fine

Table 2 Effect of storage and protein concentration on particle size distribution of fresh and 20-day storage emulsions stabilized by ultrasound-extracted faba bean protein isolate at emulsification pressure of 1500 bar. The different letters denote significant differences ($P<0.05$) between samples

Protein concentration	Days	D_v 10(μm)	D_v 50(μm)	D_v 90(μm)	span
5% faba	0	0.24 ± 0.00^{k}	0.38 ± 0.00^{d}	0.58 ± 0.00^{d}	0.89 ± 0.00^{c}
	5	0.23 ± 0.00^{k}	0.35 ± 0.00^{d}	0.54 ± 0.00^{d}	0.90 ± 0.00^{c}
	20	7.02 ± 0.12^{a}	$18.10\pm0.36a$	47.20 ± 2.55^{c}	2.21 ± 0.10^{c}
1% faba	0	0.33 ± 0.33^{hij}	0.52 ± 0.00^{d}	1.13 ± 0.05^{d}	1.54 ± 0.10^{c}
	5	0.33 ± 0.00^{ghij}	0.53 ± 0.00^{d}	1.12 ± 0.03^{d}	1.51 ± 0.04^{c}
	20	0.33 ± 0.00^{ghij}	0.53 ± 0.00^{d}	$1.30\pm0.05_{d}$	1.83 ± 0.09^{c}
0.5% faba	0	0.41 ± 0.00^{efgh}	0.2 ± 0.01^{d}	1.67 ± 0.09^{d}	$1.76\pm0.10c$
	5	0.41 ± 0.00^{efgh}	0.72 ± 0.01^{d}	1.55 ± 0.07^{d}	1.60 ± 0.09^{c}
	20	0.42 ± 0.00^{ef}	0.74 ± 0.01^{d}	1.67 ± 0.09^{d}	1.71 ± 0.11^{c}
0.01% faba	0	0.39 ± 0.39^{efgh}	0.80 ± 0.00^{d}	2.03 ± 0.01^{d}	2.05 ± 0.00^{c}
	5	0.39 ± 0.00^{efgh}	0.80 ± 0.00^{d}	2.03 ± 0.01^{d}	2.05 ± 0.00^{c}
	20	0.35 ± 0.00^{fghi}	0.57 ± 0.00^{d}	1.11 ± 0.00^{d}	1.36 ± 0.00^{c}

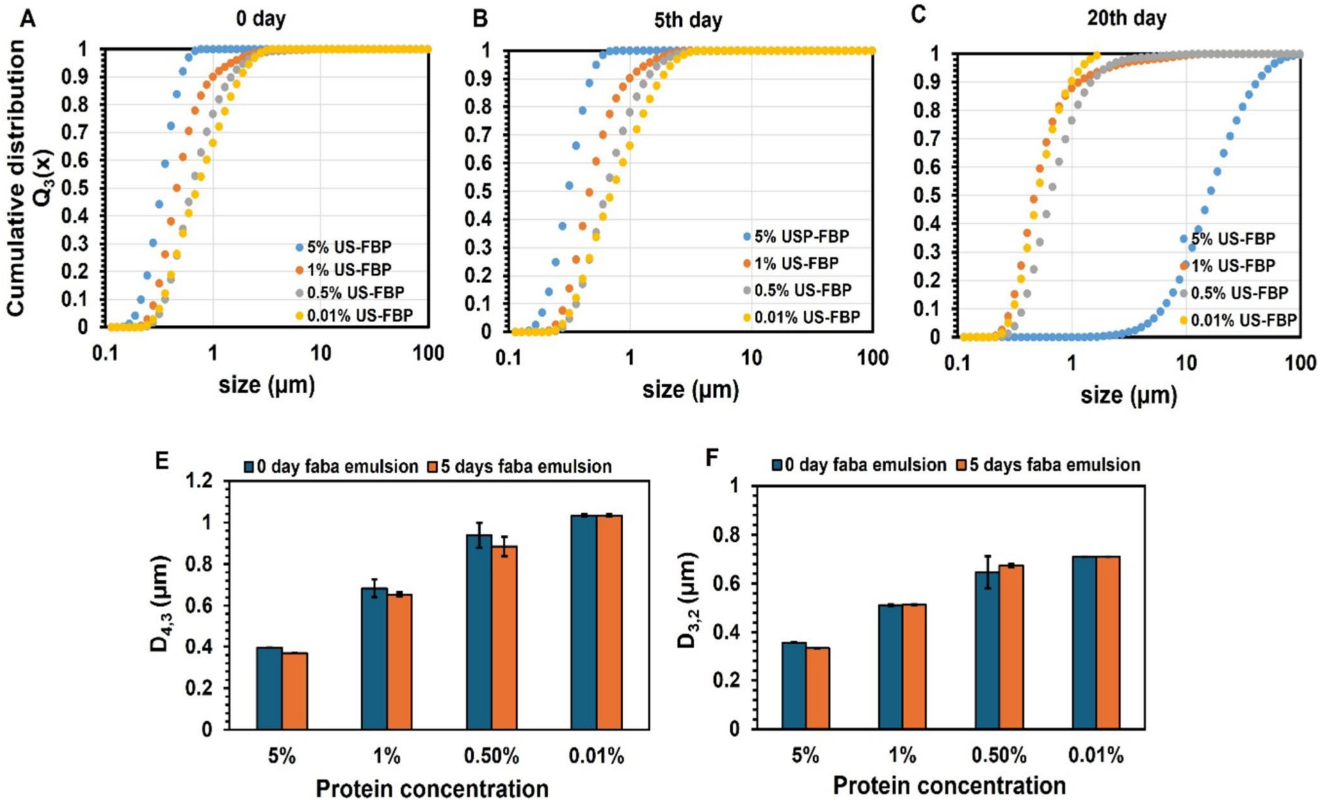

Fig. 7 Effect of storage on Cumulative mass distribution Q_3 as a function of particle size for US-FBP emulsions for (**A**) 0days; **B** 5days and 20days (**C**) of emulsions produced at 1500 bar at different protein concentrations (0.01–5% w/v). The change of volume-weighted mean droplet size $D_{4,3}$ µm (**E**) and volume-surface mean diameter $D_{3,2}$ µm (**F**) of fresh and 5 days stored emulsions at emulsification pressure of 1500 bar

droplet population, leading to an apparent decrease in measured particle size. This phenomenon may be attributed to dilute protein-stabilized emulsions and represents selective loss of large droplets, rather than true stabilization.

Emulsion creaming index

Due to particle aggregation leading to phase separation, the creaming index was used to evaluate and describe the stability of the developed plant-based emulsions. Creaming index was monitored over 20days at 4 °C. In all cases, higher HPH at 1500 bar (Fig. 8A) improved stability relative to 500 bar (Fig. 8C), as smaller droplets cream slowly under gravity (Stoke's law) and are more effectively Brownian-stabilized. At lower HPH processing, there was an observed creaming during 5 to 20days of storage at 0.5 and 0.01% protein concentration for faba protein emulsions. At 500 bar faba bean protein at 0.5% was more susceptible to creaming. The low concentration of protein could not meet the requirement of a stable emulsion for prolonged storage and thus resulting in bridging and flocculation between oil phases. Liu & Tang, (2014) [52] found that the interaction force between oil phases of soybean protein emulsion was enhanced after high-pressure homogenization, and serious emulsion flocculation occurred at low protein concentrations. However, when the protein concentration was increased to 1%, the degree of flocculation of the emulsion was improved, indicating that the flocculation state of the emulsion was closely related to the protein concentration. This indicates that there are critical concentration and pressure to achieve a stable emulsion for prolonged storage. According to Stoke's law, the terminal velocity (rate of phase separation) in O/W systems is directly proportional to the square of the oil droplet diameter. As previously discussed, the particle size at lower HPH showed higher droplet size compared to higher HPH, since higher HPH pressures provide greater disruptive forces, producing finer emulsions with smaller and more uniform droplet sizes due to more efficient droplet breakup, and thus there was a reduced propensity of phase separation at 1500 bar. In general, the creaming index of the protein emulsions was positively correlated with the emulsifier concentration. This was because a higher concentration of the emulsifier generally leads to lower interfacial tension and smaller droplet sizes, reduced coalescence, and improved long-term stability of emulsions, up to a certain optimal concentration beyond which no further stability benefit may

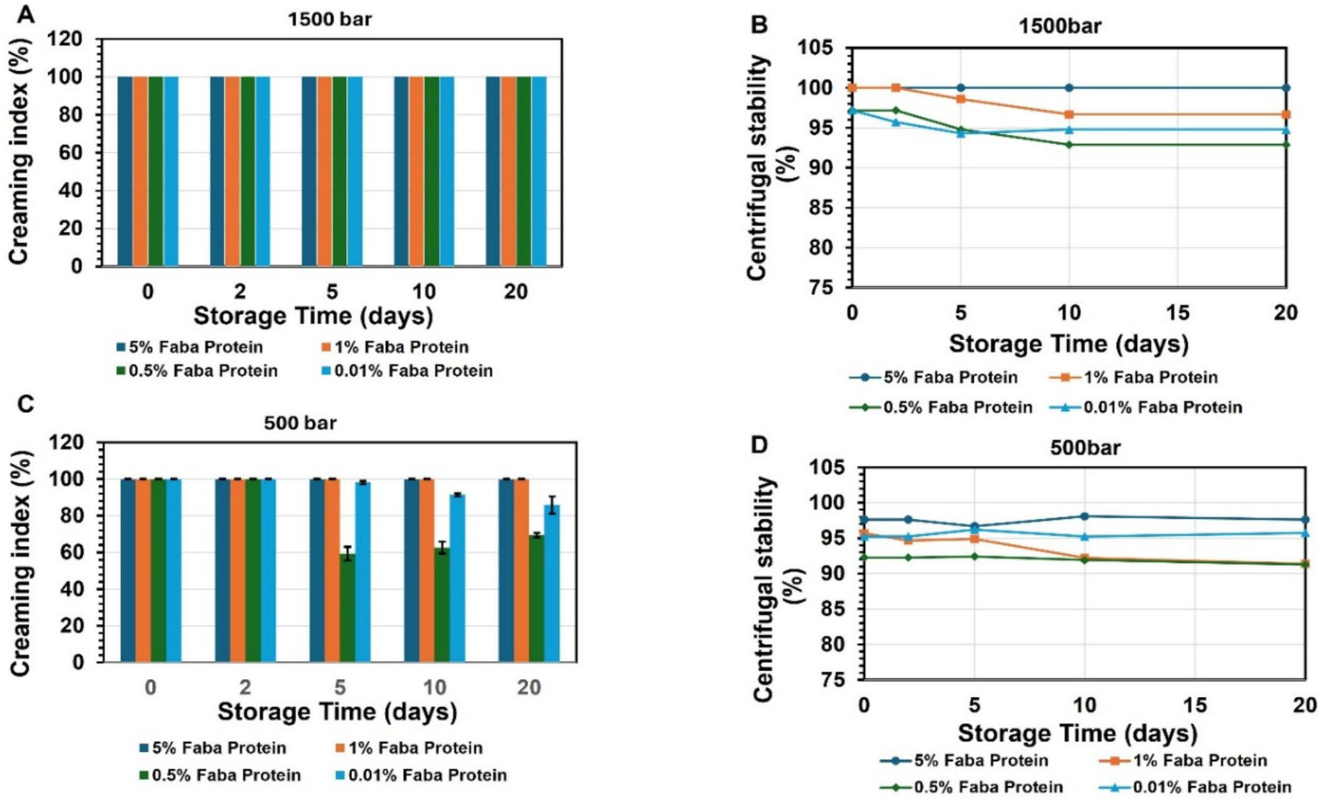

Fig. 8 Creaming index evolution of emulsions stabilized by ultrasound extracted faba bean protein at HPH treatment at 1500bar (**A**); **C** HPH treatment at 500 bar; **B** Centrifugal stability evolution at 1500 bar and (**D**) HPH treatment at 500 bar at different protein concentrations (0.01–5% w/v)

occur. It was basically in line with the rule that the greater the emulsifier concentration, the higher the emulsification index [32]. More emulsifiers not only stabilised the oil droplets by adsorbing on the interface but also restricted the continuous phase and slowed down the relative movement of the oil droplets.

Emulsion destabilization under accelerated gravitation

Generally, centrifugation is considered an effective method to destabilize emulsions, which will lead to the separation of the oil and water phases, and the agglomeration of emulsified oil droplets serves as an indicator for predicting the long-term stability of emulsions against gravitational separation and coalescence. A centrifugal test was therefore performed (3000 g for 15 min) to further compare emulsion stability under accelerated gravity. Immediately after preparation (fresh emulsion), US-FBP emulsions exhibited high emulsifying stability indices after centrifugation at 1500 bar using 1 and 5% protein concentration (Fig. 8B). At higher concentrations of protein, the proteins created enough interfacial networks to resist coalescence and phase separation under stress. However, after storage (5, 10 and 20 days) at 4 °C, differences in stability emerge for all emulsions. The faba bean emulsion retained slightly higher fractions of emulsified oil upon centrifugation at higher homogenization pressure (Fig. 8B) than at lower processing pressure. Ultrasound-extracted faba protein emulsions stored for several days showed a drop in centrifugal stability, indicating slight coarsening of the droplet size under stress. These results are consistent with the creaming data. It is noteworthy that the faba protein emulsions still exhibited promising stability which can serve as a plant-based emulsifier.

Conclusion

This study investigated the potential of ultrasound-extracted faba bean protein isolate (US-FBP) as a plant-based emulsifier by systematically linking extraction, structure, processing, and emulsion performance. Ultrasound-assisted extraction was employed to obtain a high-purity protein isolate, and its structural, thermal, and functional properties were characterized to understand their relevance to oil-in-water emulsion stabilization under different high-pressure homogenization (HPH) conditions.

At 1500 bar high-pressure homogenization and 1% protein concentration, US-FBP stabilized emulsions (1% O/W) with *Dv50* droplet sizes as small as 0.38 µm. Over 20 days of storage at 4 °C, emulsions with 0.5–1% US-FBP retained significantly smaller droplet sizes (*Dv90* = 1.67 µm at 0.5% and 1.30 µm at 1%). The rheological properties of US-FBP emulsions at 5% protein concentration exhibited an apparent viscosity of 4.8 ± 0.00mPa·s at 100s^{-1}, indicating weaker droplet–droplet interactions and a more fluid consistency desirable for applications like protein-fortified beverages. Emulsion stability further supported US-FBP's performance with improved creaming index at 1% protein over 20 days at 1500 bar. The centrifugal stability revealed that US-FBP emulsions retained over 80% of their emulsified volume after centrifugation, particularly at 1–5% concentrations, highlighting their resistance to coalescence.

A key outcome of this work is the identification of an optimal processing–formulation in which ultrasound-extracted faba bean protein delivers maximum emulsifying performance without compromising stability. These findings highlight the importance of balancing protein concentration and homogenization intensity rather than relying solely on droplet size reduction. Despite these promising results, the study is limited to neutral pH and a single oil phase, and molecular-level changes in protein aggregation were not directly probed. Overall, this study supports the use of ultrasound-extracted faba bean protein as a clean-label, plant-based emulsifier and provides mechanistic insight into how processing conditions govern its structure–function relationships in emulsion systems.

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

Declarations

Competing interests The authors declare no competing interests.

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