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Priti Sharad Mali

1950132@sliet.ac.in

Sant Longowal Institute of Engineering and Technology

Pradyuman Kumar

Sant Longowal Institute of Engineering and Technology

Research Article

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Posted Date: September 29th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7318492/v1>

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Additional Declarations: No competing interests reported.

Modification of black bean (*Phaseolus vulgaris* L.) protein isolate by high-intensity ultrasound: Effect on the rheological structure, amino acid composition, and nutritional profiling

Priti Sharad Mali* and Pradyuman Kumar

Department of Food Engineering and Technology

Sant Longowal Institute of Engineering and Technology

Longowal 148106 (Punjab) INDIA

primali1111@gmail.com and pradyuman2002@hotmail.com

+91-8380846035, +91-9417321580

Corresponding Author*

Highlights

- CD spectra confirmed the significant impact of High-Intensity ultrasound on the structure and conformation of BBPs.
- HIU with 40% amplitude for 30 min significantly improved the rheological properties of BBPs.
- The effect of high-intensity ultrasound on the *in vitro* digestibility of BBPs was evaluated.
- HIU enhances the amino acid composition and nutritional attributes of BBPs

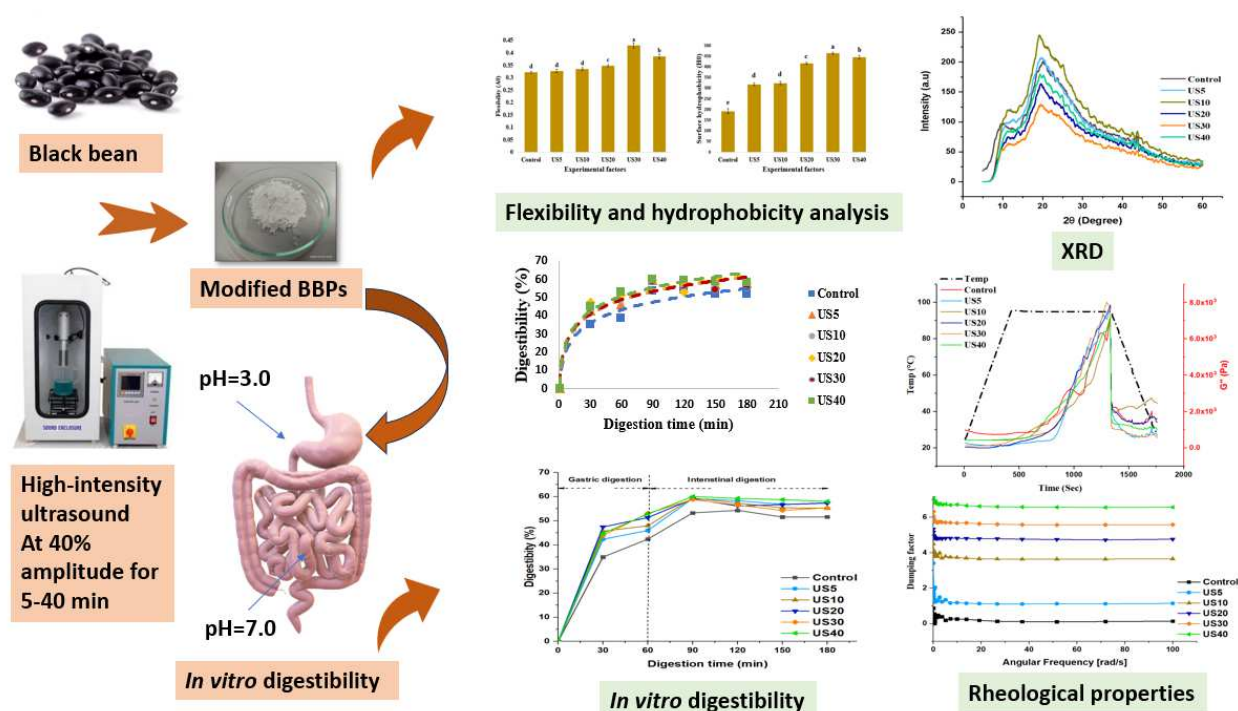
Abstract

High-intensity ultrasound (HIU), a promising ‘green’ technology, can potentially improve the functionality of biomolecules, particularly proteins and peptides. The current research aimed to evaluate the influence of HIU (40% amplitude for 5, 10, 20, 30, and 40 min) on black bean protein isolate (BBPs), which was assessed with nutritional profile, structural and rheological properties, and digestion behaviour. The results showed that HIU treatment improved the molecular flexibility of proteins from 0.31 to 0.39 by disrupting non-covalent interactions and

promoting partial unfolding. The *in vitro* digestibility of HIU-treated BBPs increased to 44.76%, compared to 34.95% in the control sample, indicating improved protein structure for better enzymatic hydrolysis and bioavailability. HIU treatment alters the pattern of BBPs by modifying their crystalline structure, increasing in amorphous regions. The circular dichroism spectroscopy showed that the β -sheet of HIU-treated BBPs increased, whereas the random coil and α -helix decreased. During the temperature sweep evaluation, HIU-treated BBPs exhibited a higher storage modulus (G') throughout the temperature profile while improving their viscosity properties in the frequency sweep analysis. HIU treatment (30 min) enhanced hydrophobic amino acid content, resulting in a higher total amino acid content than the control sample. Therefore, HIU treatment significantly improved black bean protein isolate properties with promising applications in food supplements.

Keywords: Black bean protein isolate, high-intensity ultrasound, CD, amino acid profile, rheology.

Graphical abstract



1. Introduction

Proteins are vital nutrients that support the growth and conservation of the human body, playing a significant role in increasing bone density, enhancing lean body mass, and promoting overall well-being. Sufficient protein intake is crucial for physiological functions such as muscle development, tissue repair, and metabolic regulation (Chai et al., 2023). FAO/WHO (2007) states that adult females (excluding lactating and pregnant women) and males of all ages require a daily intake of approximately 0.85 g/Kg of body weight. The rising global demand for plant-based protein is driven by consumers seeking sustainable, nutritious, and allergen-free alternatives. As concerns about the environmental impacts of animal farming and health issues associated with animal proteins become more prominent, plant proteins are receiving considerable attention in the food and nutrition sectors.

Among the various plant sources, black beans (*Phaseolus vulgaris* L.) stand out as a promising alternative due to their high protein content (16-33%), impressive nutritional profile, and versatile functional properties, which enable their use in various food formulations (Rodríguez et al., 2022). Black bean protein isolate (BBPs) offers a superior essential amino acid profile and composition ratio to soybean protein. It is especially rich in lysine, a limiting amino acid in cereals like corn and wheat. As a result of their wide existence, distribution, and absorption, processes in humans have drawn increasing attention. Additionally, it has been reported that black bean bioactive peptides possess antioxidant, anticancer, anti-inflammatory, and antihypertensive properties (Mali and Kumar, 2025). Several *in vivo* and *in vitro* clinical trials have discovered that common bean consumption showed promising results by reducing the risk and/or prevalence of non-transmissible diseases. In particular, common beans incorporated in the diet can reduce obesity levels and modulate cardiovascular processes. It has also been recognized as a chemopreventive agent, primarily against colorectal cancer *in vivo* and *in vitro* (Luna-vital and Diego, 2015). Extracting protein from black beans presents a valuable

66 opportunity to satisfy the rising demand for plant-derived proteins using an affordable and
67 widely accessible crop.

68 Various physical, enzymatic, and chemical pretreatments can modify protein functionality in
69 food processing. However, chemical and enzymatic methods, such as cross-linking and
70 deamination, are often expensive. Additionally, chemical modifications may increase toxicity,
71 allergenicity, and nutritional impairment, posing potential health risks (Sengar et al., 2022).
72 Thus, an urgent need to figure out how to enhance the functional characteristics of black bean
73 protein by modifying the molecular structure, thereby widening its field of implementation in
74 the pharmaceutical and food sectors. Furthermore, the physical modification of plant proteins
75 may help to reduce toxicity, minimize food allergies, and improve nutritional quality, thereby
76 preventing potential adverse health effects. High-intensity ultrasound is considered one of the
77 advanced non-thermal, eco-friendly food processing technique with a minimal environmental
78 impact and preserves high product quality. It is widely used for reconstruction, as it generates
79 high shear force and turbulence, disrupting non-covalent interactions such as hydrogen bonding
80 and hydrophobic interactions (Zhang et al., 2020). Therefore, identifying and implementing
81 suitable strategies that can effectively enhance the nutritional profile and rheological
82 characteristics of BBPs. It is crucial for optimizing its functionality and widening its
83 applications in food and industrial processes. Extensive research has been conducted on the
84 application of ultrasound in protein modifications, including alterations to the molecular
85 structure of soy protein isolates (Wang et al., 2024), pumpkin seed protein (Habib et al., 2025),
86 myofibrillar protein (Ni et al., 2024), pea protein (Ozkan et al., 2024), rapeseed protein (Li et
87 al., 2020), and faba bean protein isolate (Martínez-Velasco et al., 2018).

88 Based on these backgrounds, it can be inferred that high-intensity ultrasound (HIU)
89 pretreatment would help to modify the structure and aggregation state of protein molecules,
90 which could profoundly influence protein nutritional composition. No studies were reported on

the nutritional profile, amino acid composition, and digestion behaviour of black bean protein isolates. The present article estimated the effect of HIU (40% amplitude for 5, 10, 20, 30, and 40 min) on the rheological properties, nutritional profiling, amino acid composition, and digestion behavior of black bean protein isolate.

2. Materials and methods

2.1 Raw materials and reagents

Black beans (*Phaseolus vulgaris* L.) were obtained from a local producer from the Neelam Foodland, Mumbai, Maharashtra. All the reagents and chemicals employed in this research were of analytical grade. The enzymes pancreatin (obtained from porcine pancreas) and trypsin (produced from bovine pancreas), gallic acid, ascorbic acid, DPPH, ABTS, and FRAP reagents were sourced from Sigma-Aldrich.

2.2 Sample preparation

Black bean (BB) seeds were disinfected using 2% NaOCl (sodium hypochlorite) solution for 2 min and then thoroughly washed to remove any residues. Afterward, the samples were soaked in distilled water for 8 h, dehulled manually, and oven-dried at 40°C for 24 h. The soaked seeds were pulverized using a laboratory grinder (SAMSUNG) and then sieved using a 60-μm mesh to achieve a consistent particle size. By employing petroleum ether in a Soxhlet assembly, the flour was entirely defatted for 7 h. Then, the flour was stored at 4°C until use.

2.3 Black bean protein extraction

The black bean flour (BBFs) was immersed in distilled water (10% w/v), and the pH was set to 9.5 using 2 M NaOH. The samples were centrifuged at 9000×g for 20 min, after which 2 M HCl was added to adjust the pH of the supernatant to 4.5. The protein precipitate was isolated following a 20 min incubation by centrifuging at 6000×g for 20 min. The pH of the protein

mixture was regulated to 7.0 using 2 M NaOH. The protein solution was lyophilized, producing a powder according to the methods of Malik and Saini (2018).

2.4 High-intensity ultrasound (HIU)

Black bean protein isolates (BBPs) were modified using the method of Mir et al. (2019) with slight adjustments. A probe-type ultrasonicator (Nextgen Lab 500 Ultrasonic Platform sonicator, India) was inserted at the 2/3rd level of the solution for the HIU treatment. HIU was performed at 40% of the maximum amplitude, with a pulse cycle of 10 seconds on and 5 seconds off, for varying durations of 5 min (US5), 10 min (US10), 20 min (US20), 30 min (US30), and 40 min (US40). All the samples were placed in an ice bath to ensure the heat remained under 10°C, while the untreated sample served as a control. Afterward, centrifugation was done at 4293×g for 30 min, and the protein precipitate was collected. Subsequently, the protein mixture was neutralized, freeze-dried, and stored at 4°C. The protein content of the mixture resulting from the Kjeldahl method was 92.15 g per 100 g (w/w).

2.5 Surface hydrophobicity (H₀)

The H₀ of BBPs was assessed using 1-anilinoaphthalene-8-sulfonic acid (ANS) using the method ascribed by Zhu et al. (2020). Briefly, 1 mg/mL sample of the extraction mixture was transferred to 10 mmol/L PBS buffer (pH 7.0) solution with a concentration of 0.06, 0.12, 0.18, 0.24, and 0.3 mg/mL. The fully dissolved BBPs solution (8 mL) was extracted and added to 40 µL of 8.0 mmol/L ANS solution (pH 7.0), employing ANS in phosphate buffer as the blank. After 2 h of incubation, fluorescence was detected using an RF-6000 spectrophotometer (Shimadzu Co.). The fluorescence settings were as follows: The excitation and emission wavelengths of 390 nm and 484 nm, slit width set to 5 nm, and scanning speed of 600 nm/min. The fluorescence intensity showed a linear correlation with protein concentration, and the slope of this relationship represented the protein surface hydrophobicity.

2.6 Molecular flexibility (MF)

MF of the BBPs was assessed with some modifications in the method described by Kato (1985). Briefly, 250 μ L of trypsin solution (0.05 mol/L, pH 7.5 Tris-HCl buffer, potency $\geq$ 2500 units/mg) was combined with 4 mL of a 1 mg/mL sample solution and kept in a water bath at 37°C for 10 min. Subsequently, all the BBPs samples were centrifuged at 6000 $\times$ g for 15 min. The absorption of protein solution was read at 280 nm (V-670, JASCO, Tokyo, Japan). The recorded absorbance value was applied to analyze the molecular flexibility of BBPs.

2.7 Circular dichroism (CD)

CD spectra measurements were recorded with a CD spectropolarimeter (Model J-1500, JASCO Corporation, Japan) equipped with a quartz cell with a 1-mm optical path at 26 $\pm$ 1°C. CD spectra covering a wavelength range of 190-260 nm with a bandwidth of 1 nm. The scanning speed of 100 nm/min and an average of 3 repetitions were set to generate the data. About 2.5 mg/mL protein concentration was used to obtain the spectrum acquisition.

2.8 X-Ray diffraction

XRD analysis of BBPs was carried out on a X'pert PRO X-ray diffractometer with CuK (A°) =1.54056. The diffractograms were scanned at a rate of 2 θ per min from 5° to 70° (2 θ) with a step size of 0.05°. A 1 mm divergence and receiving slits and 0.04 mm anti-scatter slit were used to control beam scattering. Diffractograms from the control and BBPs were used to determine variations in crystalline size.

2.9 Antioxidant properties

2.9.1 Total phenolic content (TPC)

The TPC assay was estimated based on the assay reported by El-Hadary et al. (2023). Samples (0.5 mg/mL) were dissolved in PBS solution (pH 7.0), then agitated at 25°C for 30 min and

centrifuged at 4538g for 10 min. Briefly, 0.8 mL samples were mixed with 4 mL of a 10.0% FCR reagent, then 2.5 mL sodium carbonate (7.5%). The mixture was kept in the dark at (26±1°C) for 30 min. The absorbance was recorded at 765 nm using a UV-visible spectrophotometer (V-670, JASCO, Tokyo, Japan). The TPC was quantified as mg GAE/g of the sample.

2.9.2 DPPH, ABTS, and FRAP analysis

The assay developed by Al-Ruwaih et al. (2019) and Yu et al. (2023) was used to determine the radical scavenging activity of BBPs against the DPPH and ABTS radicals. The antioxidant potency of the BBPs sample was evaluated using the FRAP assay, following the method established by Sanwal et al. (2023).

2.10 Rheological properties

2.10.1 Temperature sweep

The rheometer (Anton Paar, MCR 300, Austria) was used to determine the temperature sweep test of the BBPs suspension. In a typical experiment, G' (storage modulus) and G'' (loss modulus) were recorded at a 1% strain and frequency ranges from 0.05 to 10 Hz. During the heating stage, the temperature of the protein dispersion was raised from 25 to 95°C with a 1°C/min heating rate.

2.10.2 Frequency sweep

The frequency sweep test was performed to identify the linear viscoelastic region using a rheometer (Anton Paar, MCR 300, Austria) with a constant strain of 0.1% and frequency increasing from 0.1-100 rad/s. The temperature was maintained at 25°C, and the steel parallel plate geometry was set at a 1 mm gap and 30 mm diameter. BBPs (20% w/v) suspension was used to perform the frequency sweep test.

2.11 *In vitro* digestibility

2.11.1 Digestibility

The digestion of BBPs was evaluated as described by Zhang et al. (2023). The BBPs dispersion was diluted to 0.5 g/mL in PBS at pH 7.0. A 1 mL suspension aliquot was mixed with simulated gastrointestinal fluid (SGF) at a 1:1 volume ratio. The SGF contained pepsin (4800 U/mL, pH 3.0), 0.5 M KCl, 0.3 M CaCl₂, 0.5 M (NH₄)₂ CO₃, 1 M NaHCO₃, 2 M NaCl, 0.5 M KH₂PO₄, and 0.15 M MgCl₂ (H₂O)₆. The samples were incubated at 37°C for 2 h. For simulated intestinal conditions, the pH was adjusted to 7.0, followed by 20 mL of each gastric digest combined with 20 mL of simulated intestinal fluid (SIF). The SIF contained trypsin (19740 U/mL, pH 7.0), 0.15M MgCl₂(H₂O)₆, 0.3M CaCl₂, 1M NaHCO₃, 2M NaCl, 0.5M KH₂PO₄, and 0.5M KCl. The dispersion was then incubated for 2 h at 37°C and halted in a water bath (95°C for 5 min). The digestion mixture and undigested protein from both the gastric and intestinal digestion phases were precipitated by combining 15% trichloroacetic acid (1:1 v/v). Afterward, centrifugation was employed at 4293×g for 15 min, and the digestibility was assessed using the following Eq. (1).

$$\text{Digestibility (\%)} = \frac{W_2 - W_0}{W_1} \quad \dots (1)$$

Here, W_0 represents the blank group protein content (mg), W_1 and W_2 represent protein content (mg) before and after digestion of the supernatant.

2.11.2 Digestion kinetics

The digestion kinetics of BBPs were studied using simulated gastrointestinal digestion. A first-order fractional conversion model was applied for the evaluation of digestibility, as represented by Eq. (2).

$$D (\%) = C_k (1 - e^{-kt}) \quad \dots (2)$$

Here, D represents the digestibility (%) of BBPs at a given digestion time (t), C_k denotes the estimated plateau of BBPs at prolonged digestion times, and k is the estimated reaction rate constant per min. The goodness of fit was assessed using the coefficient of determination (R^2).

2.12 Amino acid composition

Black bean protein extracted by high-intensity ultrasound at five different durations, i.e., 5 (US5), 10 (US10), 20 (US20), 30 (US30), and 40 (US40) min, with the highest protein content and overall properties, was used for the amino acid profile valuation. The assessment of the overall amino acid composition of 30 min (US30) was obtained after acid hydrolysis through HR-LCMS utilizing a Q-Exactive Plus Biopharma-High Resolution Orbitrap Liquid Chromatography Mass Spectroscopy (Thermo Fischer Scientific Pvt. Ltd., USA). The samples were hydrolyzed in a sealed tube for 24 h at 110°C with 6 M HCl. Afterward, the mixtures were filtered, and the final volume was made up to 50 mL. Approximately 1 mL of the sample was collected, passed through a 0.22 µm membrane filter, and analyzed for amino acid composition (Ayim et al., 2018).

2.13 Nutritional profiling

2.13.1 Protein efficiency ratio (PER)

The nutritional profile of the BBPs sample was determined using the PER, which is the proportion of body weight gained relative to the amount of protein consumed over a specific period.

PER for all BBPs samples was determined using five modified equations (Rawat et al., 2020).

$$PER_1 = -0.684 + 0.456 \times \text{Leu} - 0.047 \times \text{Pro} \quad \dots (3)$$

$$PER_2 = -0.468 + 0.454 \times \text{Leu} - 0.105 \times \text{Tyr} \quad \dots (4)$$

$$PER_3 = -1.186 + 0.435 \times \text{Meth} + 0.78 \times \text{Leu} + 0.211 \times \text{His} - 0.944 \times \text{Tyr} \quad \dots (5)$$

$$PER_4 = 0.08084 (\text{Val} + \text{Thr} + \text{Ile} + \text{Meth} + \text{Leu} + \text{Lys} + \text{Phe}) - 0.1094 \quad \dots (6)$$

$$PER_5 = 0.06320 (\text{Val} + \text{Thr} + \text{Ile} + \text{Meth} + \text{Leu} + \text{Lys} + \text{Phe} + \text{His} + \text{Arg} + \text{Tyr}) - 0.1539 \quad \dots (7)$$

2.13.2 Essential amino acid index (EAAI)

The % EAAI of BBPs was determined using the method described by Oser (1959).

$$EAAI = \sqrt{\frac{\text{Lys}_a \times \text{Tyr}_a \times \dots \times \text{His}_a}{\text{Lys}_s \times \text{Tyr}_s \times \dots \times \text{His}_s}} \quad \dots (8)$$

Where, a represents amino acid content in BBPs, s represents the amino acid content in reference protein

2.13.3 Biological value (BV)

The BV of BBPs samples was performed by the method described previous study of Oser, (1959).

$$BV \text{ of BBPs} = 1.09 \times EAAI - 11.7 \quad \dots (9)$$

2.13.4 Nutritional index (NI)

The NI of BBPs was determined based on the EAAI and total protein, following the method outlined by Sharma and Saini (2022).

$$NI = EAAI \times \text{Protein (\%)} / 100 \quad \dots (10)$$

2.13.5 Amino acid scoring (AAS)

AAS of black bean protein isolates was performed using the method described in a previous study by Rawat and Saini (2020).

2.13.6 Digestible indispensable amino acid score

DIAAS for black bean protein isolates was performed using the method described in a previous study by Sharma and Saini (2022). The calculation of DIAAS was done using Eq. (11):

$$\text{DIAAS (\%)} = 100 \times (\text{mg of DDIAA in 1 g of dietary protein} / \text{mg IDIAA in 1 g of reference protein}) \dots (11)$$

Where, IDIAA represents the identical dietary indispensable amino acid and DDIAA represents the digestible dietary indispensable amino acid.

The True IIAAD (True Ileal Indispensable Amino Acid Digestibility) coefficient values were used to assess TIDIAA (True Ileal Digestible Indispensable Amino Acid) in BBPs samples. The coefficients, derived from reference figures, were established at 0.95 for Lysine, 0.94 for Sulfur Amino Acids, and 0.90 for Threonine. For comparison purposes, reference scoring patterns from the FAO manual for infants and adults were employed.

2.14 Statistical analysis

Each experiment was performed in triplicate, and the findings are shown as mean $\pm$ SD. The statistical product and service solution (SPSS, version 12.0 software) was used to make statistical differences using the Duncan test ($p < 0.05$) level. The Origin Pro 8.0 software was used to generate graphs, and Microsoft Office Excel 2007 was used for calculations.

3. Results and discussion

3.1 Surface hydrophobicity

Hydrophobicity is determined to elucidate changes in the protein conformation after HIU treatment. Markedly, the distribution of the hydrophobic residues on the protein surfaces is usually characterised by H_0 . As illustrated in Fig. 1, with an increase in ultrasonic duration from 5-40 min, the protein surface hydrophobicity increases from 163.75 ± 7.32 to 297.04 ± 4.25 . This may be attributed to the cavitation and shear force generated by ultrasound, which causes

protein unfolding and subsequently enhances the hydrophobicity of protein isolates (Cao et al., 2021), as evidenced by the increased flexibility (Fig. 1), along with enhanced β -sheet content (Fig. 2) and amino acid composition (Table 1). The ultrasonic treatment time increases as the protein structure unfolds, leading to insoluble aggregates from soluble forms. This unfolding causes the protein molecules to expand, exposing hydrophobic groups and increasing surface hydrophobicity. Similarly, Cao et al. (2021) stated that the increase in the HIU duration led to an increase in the H_0 of quinoa protein isolates. Also, previous studies are consistent with the findings of whey protein isolate (Meng et al., 2021), peanut protein (Wang et al., 2023), and soy protein (Wang et al., 2022). However, when ultrasonic treatment exceeded 30 min, significant conformational changes occurred in the protein. Hydrophilic amino acid residues became more exposed to the aqueous environment, promoting re-aggregation and decreasing surface hydrophobicity. This might be due to the gradually unfolded structure aggregated and the binding degree between the hydrophobic groups and the fluorescence probe ANS reduced, subsequently decreasing surface hydrophobicity.

3.2 Molecular flexibility

Protease susceptibility is a key indicator of protein molecular flexibility, with more flexible proteins exhibiting greater susceptibility to proteolytic degradation than their more rigid counterparts. Fig. 1 displays the molecular flexibility of control and HIU-treated black bean protein isolates. Upon exposure to ultrasound, BBPs exhibit the highest flexibility, aligning with alterations observed in their secondary structure, including α -helix and β -sheet (Table 1). The molecular flexibility of HIU-treated BBPs was higher than that of untreated (control) samples. This indicates that high-intensity ultrasound can directly influence the protein molecules and disrupt rigid structures to form a smaller molecular structure (Yan et al., 2021). The molecular flexibility of proteins is influenced by non-covalent bonds such as hydrophobic interactions, van der Waals forces, electrostatic attraction, and hydrogen bonds. The results

align with Tang (2017), who reported that high-flexibility denatured proteins can enhance their surface properties. The molecular flexibility of protein peptides alters, resulting in corresponding changes in the functional properties (Li et al., 2019). Increased molecular flexibility of the protein structure was positively correlated with enhanced protein digestibility, indicating that more flexible conformations allow better accessibility for digestive enzymes. Moreover, in the study conducted by Yan et al. (2021), it was found that proteins exhibited greater molecular flexibility when the α -helix content was reduced.

3.3 Circular dichroism

The changes in the secondary structure of the control, US5, US10, US20, US30, and US40 black bean protein isolates have been outlined in Fig. 2. The proportion of α -helix, β -sheet, β -turn, and random coil shows a significant difference after HIU treatment. The α -helix structure is characterized by a positive spectral band near 193 nm and negative spectral bands at 208 and 222 nm. In contrast, β -sheet structure typically exhibits positive spectral bands around 195 nm and negative spectral bands near 218 nm (Kurli et al., 2025). The data showed that the β -sheet of HIU-treated BBPs increased, while the α -helix and random coil content decreased. The secondary structure of proteins depends on hydrogen bonds and electrostatic interactions; sonication may disrupt these forces, altering the protein secondary structure (Rahman et al., 2020). This may be attributed to the breakdown of interactions among certain protein molecules and local amino acid sequences. This could be due to structural differences in hydrogen bonding, which were significantly lower than in the control BBPs. Coincidentally, Hu and Li (2021) also found a decrease in α -helix content and an increase in β -sheet content for soy protein isolates. In contrast, Yang et al. (2024) observed reduced β -sheet content and increasing α -helix content in soy protein isolates after ultrasound treatment at 400 W.

3.4 XRD

X-ray diffraction is the main technique used to analyze the crystal structure of the BBPs. The diffraction angle (2θ) and intensity are related to the crystal size. As the crystal size decreases, the diffraction intensity diminishes, while the diffraction angle increases as depicted in Fig. 3. The diffractogram of BBPs displayed two characteristic peaks at (low intensity) $2\theta=10^\circ$ and (high intensity) $2\theta=19.5^\circ$, and the smallest peak was shown at $2\theta=44^\circ$. The peak observed at $2\theta=10^\circ$ is designated as crystalline region I, while the peak at $2\theta=19.5^\circ$ is referred to as crystalline II. The crystalline intensity increased after HIU treatment, suggesting that high-intensity ultrasound pretreatment promoted the transformation of protein from an amorphous structure to a crystalline structure. Malik and Saini (2018) reported a slightly different arrangement of two diffraction peaks at approximately $2\theta=10^\circ$ and $2\theta=20^\circ$ for sunflower protein. Ma et al. (2018) also identified two similar peaks at $2\theta=8.5^\circ$ and $2\theta=19.5^\circ$ in proteins extracted from cottonseed meal. Malik and Saini (2017) observed that decrease in crystallinity after HIU treatment, indicating the formation of new chemical bonds and alterations in the orderly arrangement of protein fragments. This emphasizes the beneficial impact of HIU treatment on the protein particle size.

3.5 Effect of HIU on TPC and antioxidant activity of BBPs

The influence of HIU treatment on the total phenolic content and antioxidant activity of BBPs is associated with several key factors. These processes include the enhanced release of amino acids, breakdown of insoluble aggregates, and the subsequent liberation of reactants containing free radicals, which consist of various hydrophobic groups and antioxidants.

3.5.1 Total phenolic content

The effect of HIU on the total phenolic content (TPC) of control and HIU-treated BBPs is displayed in Table 1. The TPC content varied from 2.69 ± 0.24 mg GAE/g (control) to 6.31 ± 0.34 mg GAE/g (30 min; US30), representing a 57% increase over the control BBPs. The results

indicate that HIU significantly ($p<0.05$) enhanced the TPC of BBPs. Moreover, the TPC of all samples treated by HIU was found to be significantly higher than that of control BBPs. High-intensity ultrasound may enhance the release of total phenolic compounds and therefore increase the amount of TPC in HIU-treated BBPs. According to a prior study, Yeasmin et al. (2024) found a trend of continuous increase in TPC values of kidney bean protein isolate extract up to 20 min of ultrasound treatment. The increased release of TPC at higher HIU power levels and extended treatment durations is primarily driven by cavitation. This may collapse cavitation bubbles near colloidal particles, enhancing the efficient extraction of phenolic constituents (Liao et al. 2022). Furthermore, the TPC contents results align with the findings of DPPH, ABTS, and FRAP radical scavenging activity (as presented in Table 1). RSA is strongly correlated with the TPC content in the sample, with higher TPC value leading to a greater antioxidant potential and vice versa.

3.5.2 Antioxidant properties (DPPH, ABTS, and FRAP analysis)

The DPPH, ABTS radical scavenging assay, and FRAP content of the BBPs extract are shown in Table 1. Control BBPs exhibited a weak DPPH radical scavenging activity of $23.36\pm1.00\%$, significantly improving to $43.06\pm0.91\%$ (US4, 30 min) following HIU treatment. As shown in Table 1, HIU-treated BBPs exhibited a 10% higher radical scavenging activity, which may be due to HIU-induced exposure and led to its inherent hydrophobic peptides on the protein surface, thereby resulting in an augmentation of the ferric iron-reducing capability and electron donor capacity (Hu and Li, 2022). (Zheng et al., 2021) demonstrated that higher levels of sulphur-containing amino acids, such as methionine and cysteine, enhance the antioxidant radical scavenging activity of BBPs. Yeasmin et al. (2024) reported that applying ultrasound pretreatment effectively increased the DPPH radical scavenging activity of kidney bean protein extract than the control sample. The antioxidant activity of ultrasound-treated soy protein

isolate protein nanofibrils was superior to that of untreated samples (approximately 35.98 to 39.82 %), as reported by Hu and Li (2022).

Black bean protein isolates exhibited effective ABTS radical scavenging activity (RSA) as the HIU duration increased from 5 to 40 min (Table 1). The ABTS radical scavenging activity of BBPs (Control, US5, US10, US20, US30, and US40) was 22.49%, 29.65%, 34.91%, 35.55%, 53.05%, and 34.30%, respectively. These results showed that the ABTS activity of all HIU-treated BBPs, except 40 min (US5), had a higher ABTS RSA activity (29.65-53.05%). BBPs demonstrate a strong activity effect, largely attributed to their high antioxidant amino acids, such as phenylalanine, asparagine, tryptophan, and glutamine (Liu et al., 2022). The current findings are consistent with those reported by Liu et al. (2022), who observe mung bean protein isolates ABTS activity values ranging from 19 to 99.10%. The shear and turbulent force generated by HIU treatment made the particle smaller and exposed more hydrophobic ends to improve the antioxidant capacity. Furthermore, aromatic amino acids can enhance radical scavenging activity by stabilizing free radicals through electron donors (Xie et al., 2019).

The FRAP assay is a commonly used method to evaluate the scavenging potential by assessing the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), facilitated by peptides capable of donating hydrogen atoms (Ikram et al., 2020). As shown in Table 1, HIU treated with 5-30 min durations significantly ($p<0.05$) enhanced the FRAP of the BBPs. This enhancement in FRAP values is attributed to a reduction in the protein granule size, and the increase in the surface area caused by high-intensity ultrasound might also have resulted in a higher FRAP activity. The results of this study are consistent with those reported in a previous study of Kingwascharapong and Karnjanapratum (2021) for Bombay locusts protein. However, extending the HIU duration from 30 to 40 min resulted in a decline in FRAP activity, as observed in the US5 sample, due to a decrease in the free sulfhydryl (SH) groups.

3.6 Rheological property

3.6.1 Temperature sweep

The rheological properties of proteins are closely linked to their functional characteristics, including their ability to form gels. Fig. 4 illustrates the oscillation temperature sweep (25-95°C) conducted for all BBPs samples using a rheometer. The phase angle ($\tan \delta$), storage modulus (G'), and loss modulus (G''), are commonly used to describe the viscoelastic and structural characteristics of the proteins. Compared to control BBPs samples, the HIU-treated sample exhibits a G' value greater than G'' during the temperature changing process, suggesting the development of a continuous network structure in the BBPs gel. The protein treated in the HIU showed a notably higher storage modulus (G') throughout the entire temperature profile compared to control BBPs. This increase is mainly due to enhanced protein aggregation during heating treatment, revealing a more elastic and stronger gel. Additionally, this may be attributed to the pressure generated and the movement of molecules and chemical bonds (Chen et al., 2018). Similar findings were described by Wang et al., (2021), HIU unfolds the myofibrillar proteins chain, facilitating the formation of the junction zone. The G' values increase rapidly from 30 to 95°C, reaching a peak at 95°C, indicating a transition from a viscous sol to an elastic gel. This could be attributed to forming a hydrophobic group and a disulfide bond within the black bean protein isolates, resulting in an irreversible reaction. At around 30°C, the $\tan \delta$ curve begins to rise, reaching its peak at approximately 64°C before gradually decreasing from 64°C to 50°C and declining steadily until 95°C, which was the final heating temperature. This drop in $\tan \delta$ indicates that the development of heat-induced, stable gel is linked to the unfolding and rearrangement of myosin structures (Jiang et al., 2019). Coincidentally, Huang et al. (2018) suggested that a lower phase angle ($\tan \delta$) reflects a more robust protein gel network during the final heating stage.

3.6.2 Frequency sweep

Rheological properties are strongly linked to the functional characteristics of proteins, including their gelling capacity. After the cooling period at 25°C, a frequency sweep test was conducted to examine the effect of frequency on gel strength. A gel is considered strong when G' stays constant over various frequencies, whereas a weak gel exhibits a G' that varies depending on frequency. Fig. 5 shows the influence of high-intensity ultrasound on the variations of the phase angle ($\tan \delta$), storage modulus (G'), and loss modulus (G'') of the BBPs. The G' , G'' , and $\tan \delta$ are usually used to represent the structural and viscoelastic characteristics of proteins. An increase in both G' and G'' with rising frequency was observed for all samples, indicating strong frequency dependency and suggesting the existence of a refined gel structure. The control BBPs exhibited greater frequency dependence than the HIU-treated BBPs, suggesting that high-intensity ultrasound treatment led to the formation of well well-structured and stronger gel. This trend was reported earlier for soy protein isolate dispersion (Zhao et al., 2021). As depicted in Fig. 5, High-intensity ultrasound resulted in a reduction in both G' and G'' of black bean protein isolate dispersions, which aligns with the results observed for wheat gluten (Zhang et al., 2011). This phenomenon could be due to the ultrasonic treatment reducing the granule size of BBPI suspension (Jambrak et al., 2009) and the distribution of particle size influences flow properties (Arzeni et al., 2011).

3.7 *In vitro* protein digestibility

The modified protein molecules should have improved digestibility to ensure effective nutrient absorption and utilization in the body. It is used as an enrichment source in many food products, enhancing overall nutrition and health benefits. Fig. 6 illustrates the *in vitro* digestibility of control and HIU-treated BBPs. After 5-10 min of gastric digestion, the absorption rate increased rapidly until the end of intestinal digestion (180 min) during sonication, suggesting

the breakdown of a significant portion of the fibrous structure. This could result from pepsin hydrolyzing peptides, the building blocks of nanofibrils, into smaller peptides (Wei et al., 2020). All BBPs that were sonicated at 40% amplitude of the acoustic wave for 5 to 40 min showed significantly higher *in vitro* protein digestibility (IVPD) compared to the control BBPs ($p<0.05$). The ultrasonication caused the unfolding of BBPs by disrupting various interactions. These conformational changes in the BBPs structure resulted in more exposed sites, making them available for enzymatic action. As a result, this mechanism enhanced the *in vitro* protein digestibility of the treated BBPs. According to previous findings, Sullivan et al. (2018) investigated the changes in sorghum flour digestibility by altering the overall secondary structure of kafirin protein. In contrast, Martinez-Velasco et al. (2018) observed a decrease of approximately 3.6% in faba bean protein *in vitro* digestibility, due to structural changes induced by HIU. During intestinal *in vitro* digestion, digestibility initially increased to 120 min, then slightly decreased as the duration extended to 180 min, while all HIU-treated samples had higher digestibility compared to the control sample. These findings suggest that the HIU technique can facilitate BBPs hydrolysis during the digestive process in the intestine by increasing enzyme accessibility, including gastric and pancreatic enzymes, and promoting substrate degradation, also indicating that BBPs were mainly digested in the intestine. Dai et al. (2024) reported similar results for the *Sipunculus nudus* protein.

3.8 Digestion kinetics

The HIU treatment time plays an essential role in influencing the digestion kinetics of proteins. As shown in Fig. 7, the kinetic behavior of the high-intensity ultrasound of BBPs was analyzed using the first-order fractional conversion model at various durations (0 to 40 min), amplitudes, and ultrasonic powers (60% and 450W). The coefficient of determination (R^2) measures the goodness of fit, indicating how well a model explains the variability of the dependent variable. A high R^2 value (e.g., 0.90 or above) suggests a strong correlation between HIU duration and

digestion efficiency. During the overall digestion process, all samples exhibited a gradual increase in digestibility due to the enzymatic activity of proteases. The HIU-treated BBPs samples exhibited greater proteolysis than the control BBPs samples, resulting in an enhanced digestibility trend. According to Table 2, C_k values increase as HIU duration increases, while k values decrease with increasing HIU duration. High-intensity ultrasound enhanced the solubility of black bean protein isolates by promoting the unfolding of protein isolate structures. In contrast, for extended HIU duration (120-180 min), the digestibility of BBPs decreased due to protein aggregate formation. Additionally, in the control (untreated) sample, digestibility appeared to plateau toward the end of the digestion curve (Fig. 7), aligning with the relatively higher rate constants showed in this group, which suggests a faster attainment of the final plateau. Overall, the findings indicate that the fundamental characteristics of the protein play a significant role in modifying its stability in a dynamic gastric condition.

3.9 Amino acid composition

The control and HIU-treated black bean protein isolates were used to analyze the amino acid profile. The findings obtained are systematically presented in Table 3. Among the twenty-two analyzed amino acids, seventeen were found in both control and HIU-treated BBPs, thereby confirming the ideal essential amino acid model proposed by FAO/WHO (2013), and showed no obvious difference. The overall essential amino acid revealed a total content of 25.37 g/100 g of protein isolate in the control sample, while in the HIU-treated BBPs, it increased to 29.29 g/100 g of protein isolate. HIU disrupts protein aggregates and promotes unfolding, which exposes previously buried amino acid groups (Kang et al., 2022). This increases the solubility and accessibility of essential amino acids, resulting in enhanced detection and quantification. A previous study by Ding et al. (2018) found that applying sonication at 20–50 kHz to grape seed protein notably enhanced the release of total amino acids, which was attributing this effect to the greater exposure of hydrophobic groups. Aspartic acid and glutamic acid are the most

widely released amino acids in common beans, with significantly higher levels observed in HIU-treated samples, measuring 16.17 g and 14.18 g/100g of BBPs, respectively. The high content of essential amino acids, including leucine, lysine, phenylalanine, threonine, tyrosine, valine, and isoleucine, is believed to contribute to its significant nutritional value (Yang et al., 2021). Black bean protein can be regarded as having an adequate level of sulfur-containing amino acids, primarily methionine (1.61 g/100 of protein), which is rarely found in plant-based proteins. Comparable results were obtained when buckwheat protein isolates underwent ultrasound treatment for a frequency of 20 kHz, a time of 10 min, and an amplitude of 60% (Jin et al., 2021). In contrast, Li et al. (2016) described a reduction in total amino acid content and total hydrophobic amino acid content after sonication (15 min, 58 W/L) as compared to untreated rice protein.

3.10 Nutritional profiling of BBPs

Amino acid composition analysis of BBPs was used to evaluate their nutritional properties. The HIU-treated BBPs exhibited a higher total amino acid content (TAA) than the control sample (refer to Table 4). The higher TTA content in the US4 sample (30 min) indicates an improvement in nutritional value after high-intensity ultrasonication. Protein efficiency ratio (PER) is considered an important indicator of protein quality, reflecting the ability of a protein source to support growth and meet essential nutritional requirements.

Biological value (BV) measures protein quality, indicating how efficiently the body can utilize and absorb protein for growth, maintenance, and repair. A biological value between 70% and 100% indicates a protein of high nutritional quality. The calculated BV of HIU-treated BBPs was found to be 92.55%, which exceeded the BV of 78.23% of control BBPs. Rawat et al. (2023) reported similar results for HIU-treated sunnhemp protein isolate (91.96% for untreated and 95.22% for HIU-treated). Biological value is determined by the essential amino acid index,

with a higher EAAI indicating a greater BV of food protein. Therefore, the US30 sample exhibited a higher BV than the control sample. In the present study, the nutritional index of black bean protein isolates was found to be 67.69% in the control sample and 85.21% in the US30 sample, which is consistent with the findings of flaxseed protein isolates reported by Sharma and Saini (2022).

The protein efficiency ratio (PER), calculated theoretically, is a useful indicator for evaluating the nutritional value of a protein source. The observed PER₁, PER₂, PER₃, PER₄, and PER₅ values of 2.07, 2.01, 1.80, 1.93, and 1.97 for HIU-treated BBPs suggest that HIU treatment influenced the protein quality to varying degrees. These dissimilarities in PER can be ascribed to variations in structural and functional properties of the protein induced by ultrasound. Limiting amino acids are defined as the essential amino acids present in the lowest proportion compared to human nutritional requirements, thereby restricting the overall utilization of dietary protein. In the case of BBPs, leucine was recognized as the first limiting amino acid for both infant and preschool children (up to 1-2 years) as well as for adults, according to the amino acid scoring pattern established by FAO/WHO (Sharma & Saini, 2022). This indicates that leucine availability is the primary factor constraining the protein quality of BBPs. In addition to leucine, isoleucine and valine emerged as the second and third limiting amino acids found in both control and HIU-treated (US30) BBPs samples.

The Digestible Indispensable Amino Acid Score (DIAAS) provides a more accurate evaluation of nutritional quality by considering both the physiological importance and digestibility of individual amino acids. For BBPs, the DIAAS analysis revealed that threonine had the lowest score among indispensable amino acids for infants in control and (US30) samples. In case of adults lowest scores were observed for threonine in the (US30) sample, as indicated in Table 6. Despite these limitations, the overall DIAAS values suggest that HIU treatment positively influences the nutritional profile of BBPs. The enhancement is likely due to improved protein

digestibility (Fig. 6) and structural modifications that increase amino acid bioavailability, although it may not significantly alter the amino acid composition.

4. Conclusions

In this study, we emphatically surveyed the influence of HIU treatment on the black bean protein isolates (BBPs) across varying treatment durations, revealing significant structural and conformational modifications. The findings showed that the HIU improved the antioxidant activity (ABTS, DPPH, FRAP) and molecular flexibility of BBPs. HIU treatment at 30 min (US30) increased surface hydrophobicity compared to untreated BBPs, due to the partial unfolding of protein molecules. The result of circular dichroism (CD) indicates that high-intensity ultrasound caused conformational changes in BBPs. *In vitro* digestibility findings demonstrated that ultrasound processing effectively enhances protein digestibility. Nutritional index (NI), essential amino acid index (EAAI), and biological value (BV) of HIU-treated (US30) were enhanced compared to untreated BBPs. High-intensity ultrasound enhances the amino acid content in BBPs, as revealed by amino acid composition analysis. The excellent nutritional value and favorable functional properties make it valuable for manufacturing various specialty food products with potential health benefits.

Authorship contribution statement

Priti Sharad Mali: Conceptualization, Investigation, Methodology, Formal analysis, Data curation, Writing- original draft preparation.

Pradyuman Kumar: Conceptualization, Resources, Supervision, Writing- review and editing

Declaration of competing interest

The authors report no declarations of interest.

Acknowledgments

The author acknowledges the Department of Science and Technology, Government of India, for providing an INSPIRE fellowship.

Funding declaration

The research received no external funding.

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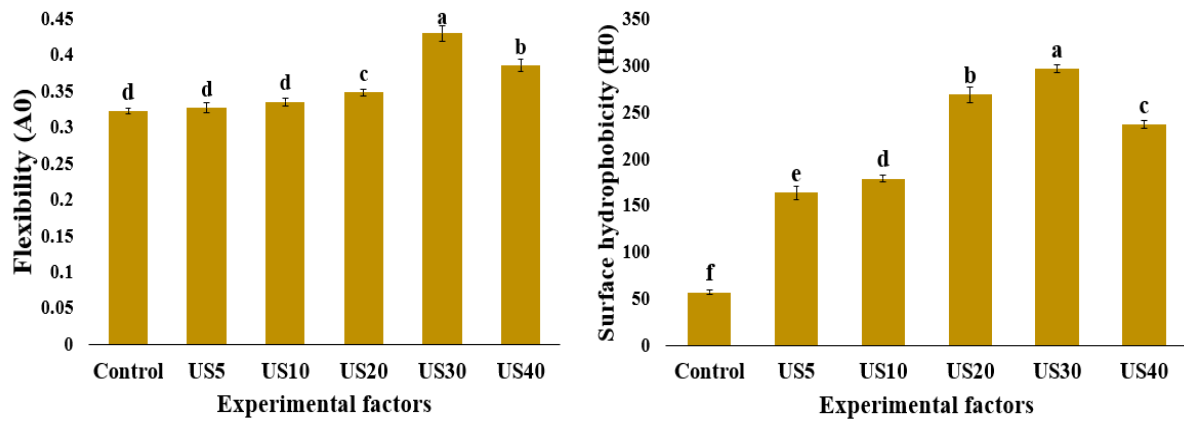


Fig. 1. Flexibility and hydrophobicity of control and HIU-treated BBPs at 5 min, 10 min, 20 min, 30 min, and 40 min.

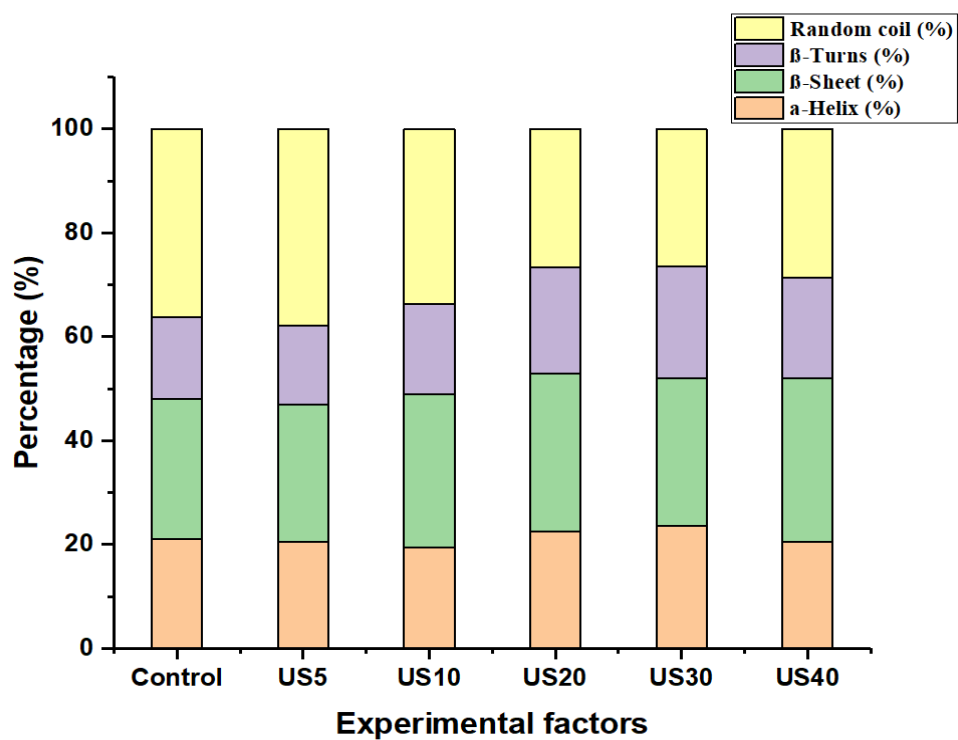
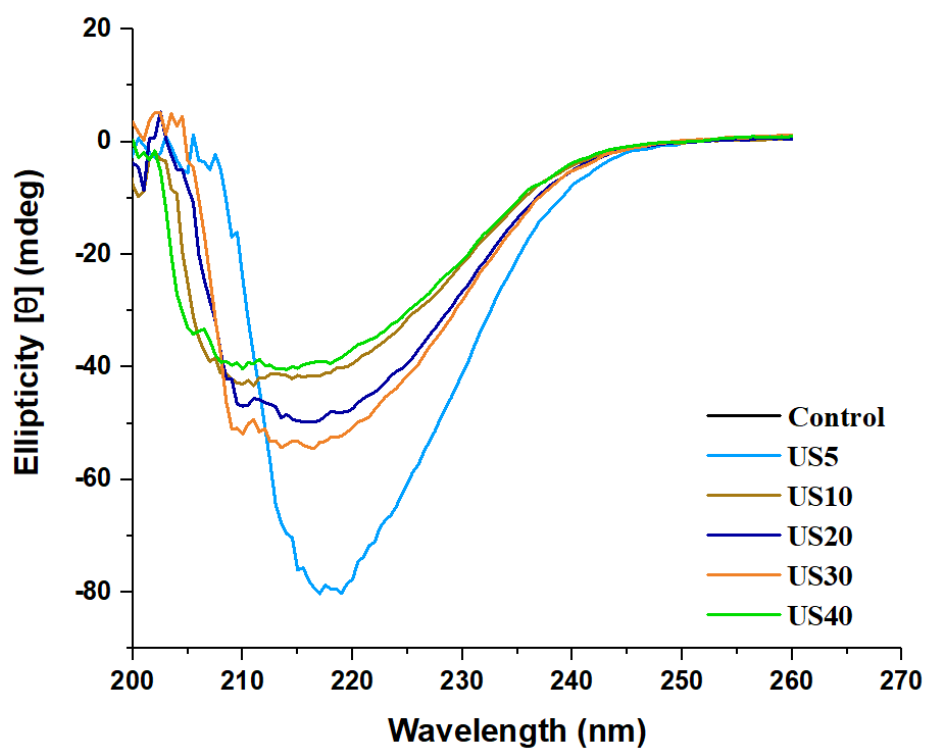


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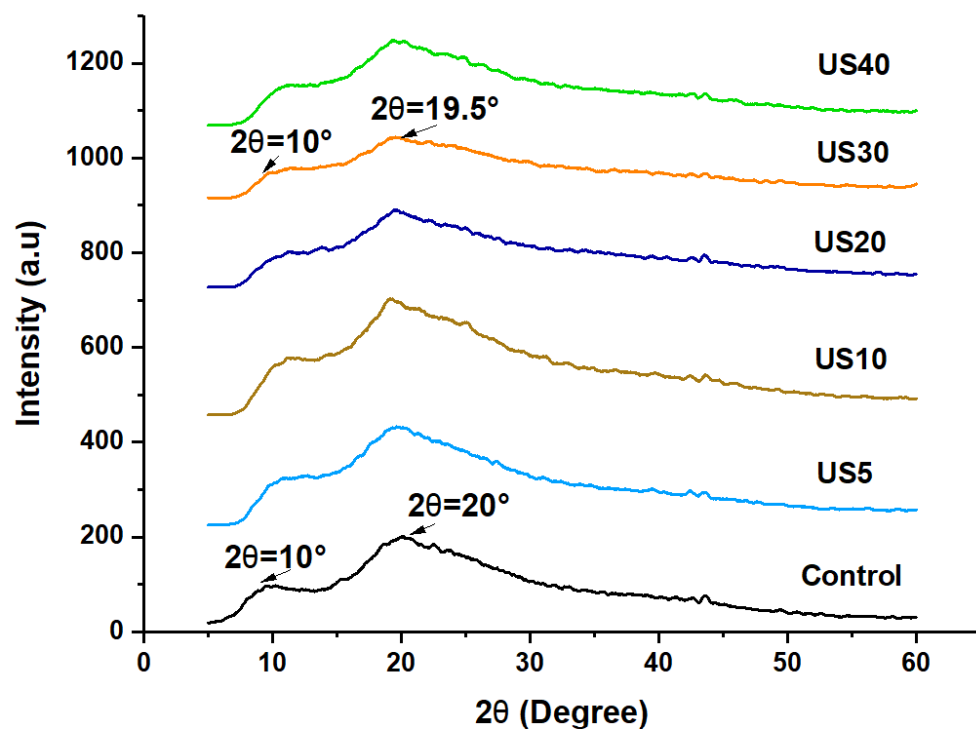
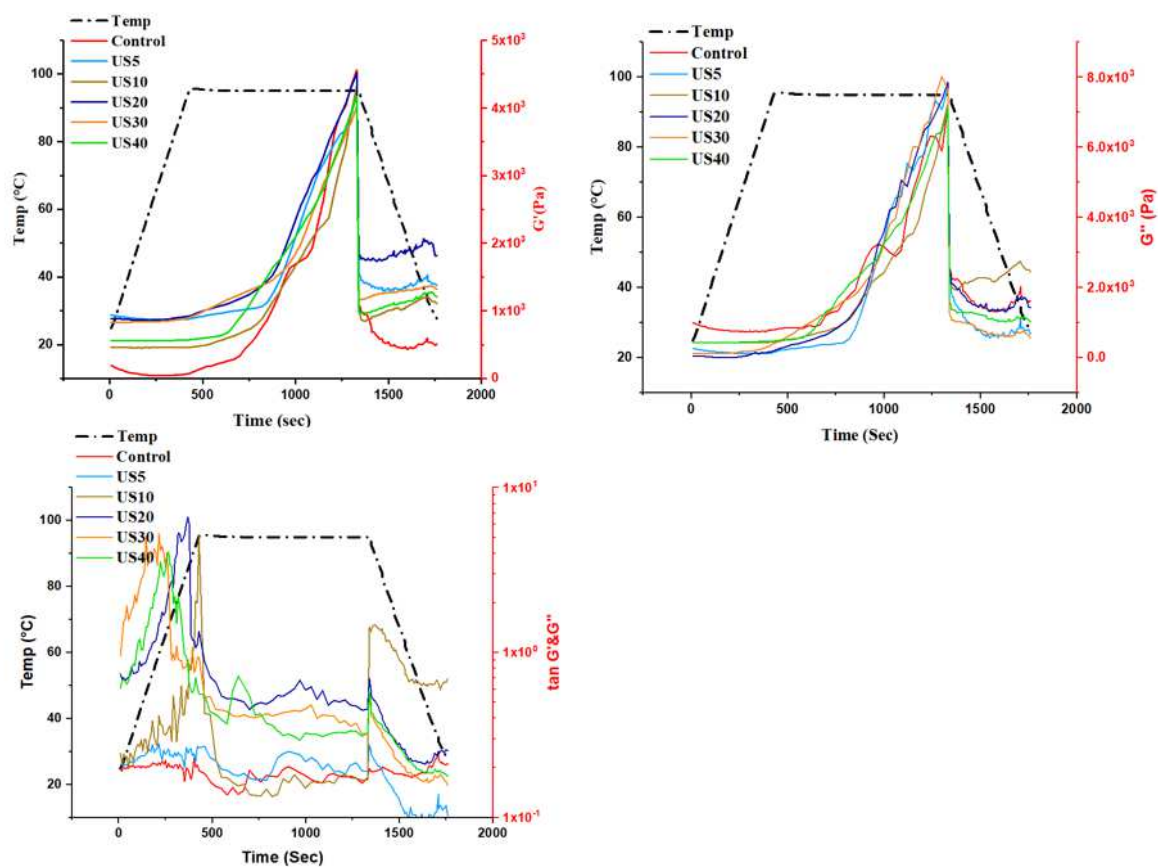


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834 Fig. 4. Temperature sweep analysis of control and HIU-treated BBPs at 5 min, 10 min, 20 min,

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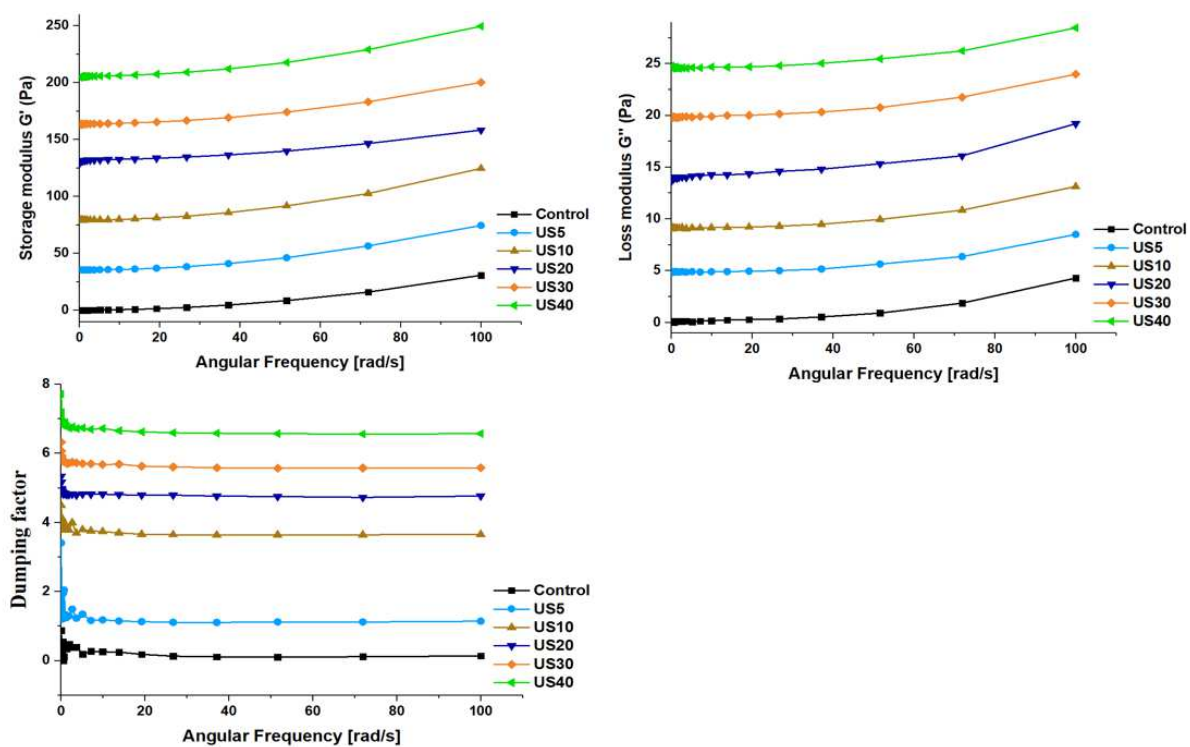


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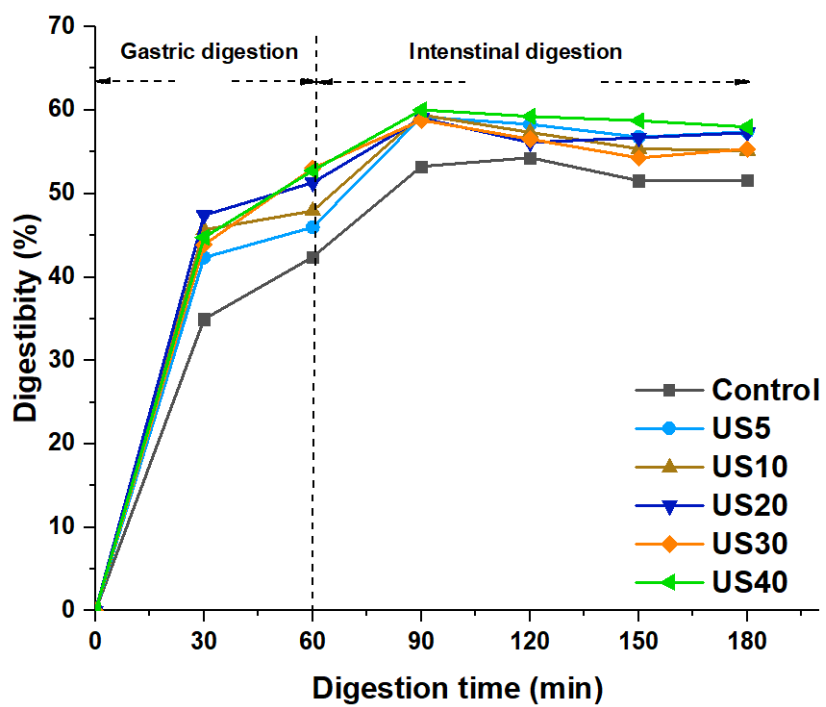


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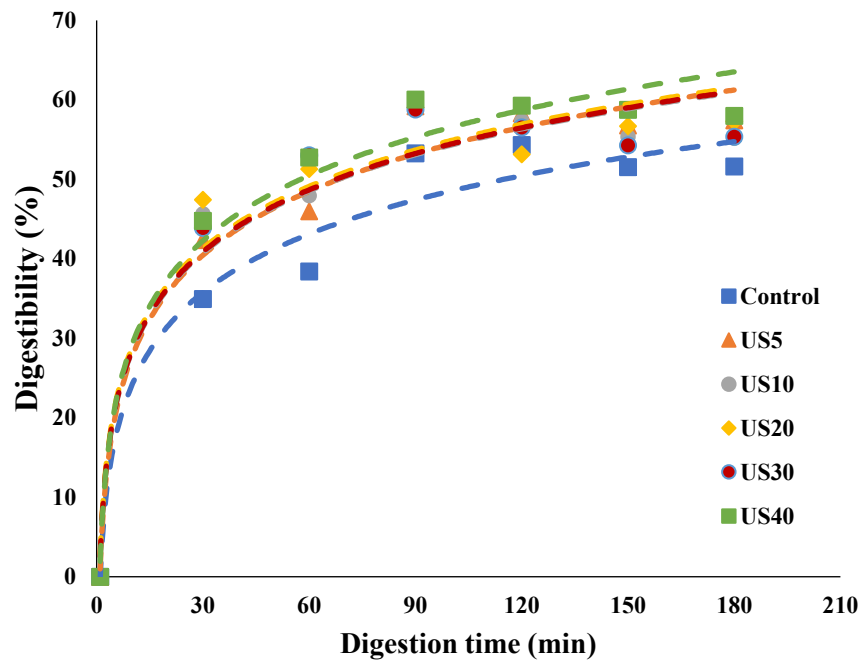


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Table 1. Effect of different HIU durations on total phenolic content and antioxidant properties of BBPs.

Sample	TPC (mg GAE/g)	FRAP (mg AAS/g)	DPPH (%)	ABTS (%)
Control	2.69±0.24 ^d	6.52±0.39 ^d	23.36±1.00 ^d	22.49±0.63 ^d
US5	4.33±0.24 ^c	9.18±0.29 ^c	34.41±0.82 ^c	29.65±1.76 ^c
US10	5.28±0.12 ^b	9.73±0.33 ^c	35.22±0.49 ^c	34.91±0.49 ^b
US20	5.29±0.18 ^b	10.71±0.5 ^b	38.07±0.59 ^b	35.55±0.01 ^b
US30	6.31±0.34 ^a	12.89±0.64 ^a	43.06±0.91 ^a	53.05±0.71 ^a
US40	4.30±0.38 ^c	7.19±0.61 ^d	36.34±0.54 ^c	34.30±0.66 ^b

Mean ± SD (n = 3). Within the column, means having different superscript letters are significantly different ($p < 0.05$).

Table 2. Effect of different HIU durations on kinetic parameters of in vitro digestion of BBPs.

Treatment	C _k (%)	k (min ⁻¹)	R ²
Control	45.52 ± 11.15	0.81 ± 0.02	0.8407
US5	53.34 ± 7.24	0.72 ± 0.05	0.9028
US10	53.49 ± 5.45	0.58 ± 0.04	0.9429
US30	54.15 ± 4.35	0.98 ± 0.05	0.9637
US40	53.65 ± 5.17	0.95 ± 0.01	0.9484
US50	55.60 ± 5.90	0.91 ± 0.05	0.9383

C_k represents the final protein digestibility after 3 h of digestion, k represents the digestion rate constant, and values are the estimated kinetic values ± SD of the model's kinetic parameters.

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915 Table 3. Amino acid composition of the control and optimized (30 min) HIU-treated black bean
916 protein isolate.

Amino acid	Essential amino acids (g amino acid/100 g protein isolate)		
	Control	US30	FAO/WHO (2013)
Histidine	0.73±0.06 ^b	1.16±0.05 ^a	1.5
Isoleucine	1.68±0.04 ^b	1.9±0.05 ^a	3.0
Leucine	5.38±0.01 ^b	6.14±0.02 ^a	5.9
Lysine	4.33±0.10 ^b	5.05±0.07 ^a	4.5
Methionine	1.6±0.09 ^a	1.61±0.01 ^a	1.6
Cysteine	ND	ND	0.6
Phenylalanine	4.11±0.02 ^b	4.64±0.10 ^a	-

Threonine	2.95±0.01 ^b	3.59±0.02 ^a	2.3
Valine	1.99±0.01 ^b	2.3±0.05 ^a	3.9
Tyrosine	2.6±0.02 ^b	2.9±0.06 ^a	3.8
Tryptophan	ND	ND	0.6
Total	25.37±0.05 ^b	29.29±0.02 ^a	27.7

Non-essential amino acids (g amino acid/100 g protein isolate)

Arginine	3.69±0.05 ^b	4.39±0.08 ^a
Aspartic acid	16.17±0.06 ^b	17.48±0.05 ^a
Glutamic acid	14.18±0.12 ^b	12.37±0.05 ^a
Glycine	3.72±0.05 ^b	4.35±0.02 ^a
Hydroxyproline	6.37±0.02 ^b	7.63±0.10 ^a
Serine	8.87±0.04 ^b	9.94±0.07 ^a
Asparagine	ND	ND
Glutamine	ND	ND
Alanine	3.68±0.04 ^b	4.38±0.01 ^a
Norvaline	ND	ND
Total	56.68±0.04 ^b	60.54±0.03 ^a

*ND: Not detected.

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921 Table 4. Nutritional profiling of the control and optimized (30 min) HIU-treated black bean
 922 protein isolate.

Attributes	Control	US30
Total amino acids	82.05±3.49 ^b	89.82±1.85 ^a
Total essential amino acids with histidine	25.37±0.48 ^b	29.28±0.49 ^a
Total essential amino acids without histidine	24.64±0.96 ^b	28.12±0.38 ^a
Total non-essential amino acids	56.68±0.02 ^b	60.54±0.05 ^a
Total non-polar amino acids	22.16±0.02 ^b	25.31±0.03 ^a
Total polar amino acids	53.52±0.01 ^b	56.88±0.04 ^a
Total amino acids with aliphatic side chains	12.73±0.04 ^b	19.07±0.06 ^a
Total neutral amino acids	34.59±0.03 ^b	41.74±0.03 ^a
Total amino acids with aromatic groups	6.71±0.06 ^b	7.54±0.03 ^a
Total amino acids with acidic group	30.35±0.05 ^b	29.85±0.03 ^a
Total amino acids with basic group	8.75±0.06 ^b	10.6±0.04 ^a
Total amino acids with hydroxyl group	14.42±0.04 ^b	13.53±0.08 ^a
Total sulfur-containing amino acids	1.6±0.05 ^a	1.61±0.04 ^a

%EAA	25.37±0.08 ^b	29.28±0.04 ^a
%NEAA	56.68±0.08 ^b	60.54±0.07 ^a
Leucine/isoleucine ratio	3.20±0.10 ^b	3.23±0.05 ^a
Lysine/arginine ratio	1.17±0.05 ^b	1.15±0.06 ^a
PER ₁	1.72±0.06 ^b	2.07±0.08 ^a
PER ₂	1.7±0.20 ^b	2.01±0.04 ^a
PER ₃	1.18±0.10 ^b	1.8±0.04 ^a
PER ₄	1.68±0.05 ^b	1.93±0.07 ^a
PER ₅	1.67±0.05 ^b	1.97±0.05 ^a
BV	94.99±0.04 ^b	114.14±0.0 ^{a8}
EAAI	91.84±0.04 ^b	112.72±0.08 ^a
NI	77.94±0.12 ^b	102.52±0.20 ^a

Mean ± SD (n = 3). Within the column, means that having different superscript letters are significantly different ($p < 0.05$). *NEAA*: Non-essential amino acids; *EAA*: Essential amino acids; PER: protein efficiency ratio; BV: Biological value; EAAI: Essential amino acid index; NI: Nutritional index.

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932 Table 5. Digestible indispensable amino acid score (DIAAS) of the control and optimized (30
933 min) HIU-treated black bean protein isolates.

Sample	BBPIs (mg/g)			TIDIAA content in IAA reference pattern					
				BBPI (mg/g)					
	Lys	SAA	Thr	Lys	SAA	Thr	Amino acid	Infants	Adults
Control	43.3	16.0	29.5	41.14	15.04	26.60	Lys	69	48
US30	50.5	16.1	35.9	47.80	15.04	32.31	SAA	33	23
							Thr	44	25
DIAA reference ratio				DIAAS for BBPIs (%)					
Amino acids	Infants		Adults		Age group		Control	US30	
	Control	US30	Control	US30					
Lys	0.60	0.70	0.86	0.10	Infants (Thr)		60.34	73.43	
SAA	0.46	0.46	0.65	0.65	Adults (SAA)		65.39	65.39	
Thr	0.60	0.73	1.06	1.029	Adults (Thr)		106.2	129.24	

934 Control: Untreated; US30: HIU for 30 min; SHPICD: TIDIAA: True ileal digestible
935 indispensable amino acid; IAA: Indispensable amino acid; Lys: Lysine; SAA: Sulphur
936 containing amino acids; Thr: Threonine; DIAA Reference ratio: Digestible indispensable
937 amino acid in 1 g BBPs, observed as % for each.

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Table 6. Amino acid scoring pattern for the infants and adults (FAO,2013) of the control and optimized (30 min) HIU-treated black bean protein isolates.

Amino acid	Amino acid score (infants/preschool children (1-2 yrs))		Amino acid score (adults)	
	Control	US30	Control	US30
Histidine	34.29	55.23	36.00	58.00
Isoleucine	30.55	34.54	52.50	59.38
Leucine	56.04	63.96	81.52	93.03
Lysine	62.61	73.19	75.79	88.60
Meth +Cyst	48.48	48.48	59.26	59.26
Phe +Tyr	71.38	80.32	129.04	145.19
Threonine	67.04	81.59	95.16	115.80
Valine	36.00	41.82	46.05	53.49

Control: Untreated; US30: HIU for 30 min; Meth + Cyst: Methionine + Cysteine; Phe + Tyr: Phenylalanine + Tryptophan; amino acid scoring pattern was observed in mg/g protein requirement as recommended by FAO.