INSIGHTS-JOURNAL OF
HEALTH AND REHABILITATION

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

A COMPARATIVE STUDY OF EXTRACTION AND QUANTIFICATION METHODS OF PROTEIN ISOLATED FROM BEANS

Original Research

Saliha Khalid[1]*, Drakhshaan[2], Alaa Ghaleb Alsawwa[3], Md Kamrul Islam[4], Khizra Hashmat[5], Ahmad Ibne Yousaf[6], Abu Baker Sidiq[7], Muhammad Sikandar Subhani[8]

[1]Department of Material Science and Nanotechnology, School of Engineering and Natural Sciences, Kadir Has University, Istanbul Turkey.
[2]Institute of Pharmaceutical Sciences, University of Veterinary and Animal Sciences Lahore.
[3]Faculty of Sciences and Arts, Aqaba University of Technology.
[4]Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka.
[5]School of Human Nutrition and Dietetics, Minhaj University Lahore.
[6]School of Human Nutrition and Dietetics/Assistant Registrar, Minhaj University Lahore.
[7]Department of Plant Breeding & Genetics, Faculty of Agriculture, Gomal University, Dera Ismail Khan, Pakistan.
[8]Department of Chemistry, Riphah International University Islamabad.

Corresponding Author: Saliha Khalid*, Department of Material Science and Nanotechnology, School of Engineering and Natural Sciences, Kadir Has University, Istanbul Turkey, salihamughal80@gmail.com

Conflict of Interest: None

Grant Support & Financial Support: None

Abstract

Background: Beans serve as a significant source of protein, minerals, and plant-derived micronutrients, holding social and economic importance. Their consumption is linked to numerous health benefits, including reducing the risk of cardiovascular disease, obesity, and diabetes. Plant-based proteins are also environmentally advantageous, as their production requires fewer natural resources and results in a smaller carbon footprint than animal-derived proteins. This study explores protein extraction methods from beans and assesses analytical techniques for protein quantification.

Objective: To examine various protein extraction and quantification techniques from beans, focusing on four primary analytical methods: SDS-PAGE, Bradford, Lowry, and spectrophotometry.

Methods: Proteins were extracted from bean samples using standardized cell disruption, solubilization, enrichment, and digestion techniques. Four analytical methods were then applied to quantify the proteins: SDS-PAGE (for molecular weight separation), Bradford (dye-binding assay), Lowry (colorimetric assay), and spectrophotometry (UV absorption at 280 nm). Each method's precision, sensitivity, and compatibility with bean protein extracts were evaluated.

Results: SDS-PAGE effectively separated proteins in the 5-250 kDa range with a 95% consistency in molecular weight determination. The Bradford method detected protein concentrations as low as 1 µg/mL, while the Lowry method demonstrated a 10-fold sensitivity increase over spectrophotometry, which was limited by UV interference. Both Bradford and Lowry were modified for specific protein compositions, with Bradford showing less interference.

Conclusion: The Bradford method demonstrated the greatest adaptability and minimal interference in bean protein quantification, whereas SDS-PAGE proved optimal for protein fraction analysis. These findings suggest the continued development of extraction and quantification techniques to meet the increasing demand for plant-based proteins.

Keywords: Analytical Techniques, Beans, Bradford Assay, Environmental Impact, Plant-derived Proteins, Protein Extraction, SDS-PAGE

INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

INTRODUCTION

Proteins are fundamental to biological function and serve as major structural components within cells. Distinctly more complex than fats or carbohydrates, proteins form intricate polymer chains of amino acids that exhibit significant molecular diversity and complexity. The molecular mass of these chains can vary, ranging from approximately 5000 Daltons to over a million (1). Structurally, proteins are assembled from twenty amino acids connected by peptide bonds, involving elements like hydrogen, oxygen, carbon, sulfur, and nitrogen. Nitrogen's distinct presence is a basis for protein quantification in several analytical methods, including the Kjeldahl and Dumas methods (1, 2). Proteins are categorized according to their structural conformation, amino acid composition, biological functions, and solubility properties. While simple proteins are composed solely of amino acids, complex proteins may include additional, non-amino acid components. Structural conformation and solubility of proteins can be altered by factors such as heat, detergents, organic solvents, guanidine-HCl, alkali, and urea, leading to denaturation and changes in function (1). Key protein sources span both animal and plant origins, including dairy, meat, eggs, and various grains and legumes. Beans and other legumes, known as a "poor man's meat," play a pivotal role as a primary protein source in developing countries, where protein-energy malnutrition is prevalent. Besides protein, beans provide a rich array of phytochemicals, antioxidants, and flavonoids (3-6). In Pakistan, beans and pulses are especially significant, contributing around 25% of dietary iron, with widespread consumption of mung beans (7, 8).

Food Proteins and Proteomics

Food proteins are inherently complex, presenting challenges for proteomics, a field that aims to study and characterize protein diversity. Proteomics can reveal insights into cellular functions and enhance environmental outcomes, especially through modern methods such as two-dimensional (2D) electrophoresis and mass spectrometry (MS) (9). By integrating proteomics with genomics and transcriptomics, a more holistic understanding of protein structure and function can be achieved (10). Current applications of proteomics include non-thermal food processing, genetically modified organisms (GMOs), functional foods, and food irradiation (10-12).

Health Benefits of Plant Proteins

Research underscores the health benefits of plant-based proteins, highlighting their potential to reduce the risk of cardiometabolic diseases. For instance, replacing animal proteins with plant proteins has been associated with reductions in HbA1c, blood pressure, low-density lipoprotein (LDL) cholesterol, and insulin levels, with protective effects against type 2 diabetes (13-15). Plant proteins are further linked to reduced mortality risks compared to animal proteins, especially red meat, which is correlated with higher mortality rates. Overall, emerging evidence suggests that incorporating plant proteins in the diet promotes both health and environmental sustainability (16, 17).

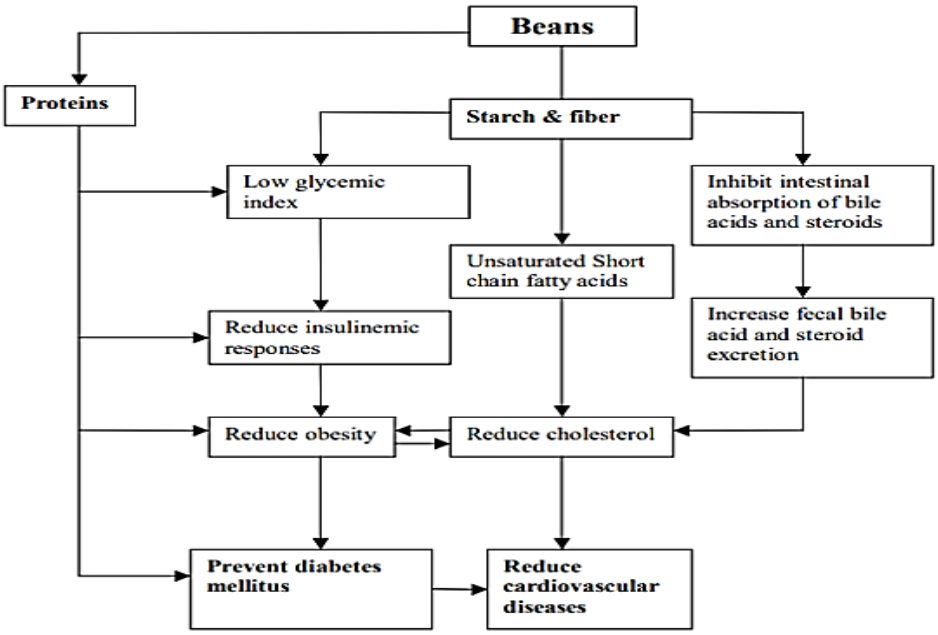

Figure 1: Mechanisms involving reduction of cardiovascular diseases, obesity, and diabetes (18). Proteins, starch and fiber present in beans are involved in reducing incidence of cardiovascular diseases and diabetes mellitus through various mechanisms.

Protein Content of Beans

Beans are high in protein, primarily comprising globulins and albumins, with lesser amounts of protamines and glutelins (18, 19). Different beans exhibit varying protein concentrations, with soybeans, mung beans, and peanuts ranking high in protein per 100 grams. Table 1 (not included here) lists the approximate protein composition in various types of beans, demonstrating their nutritional value as compared to other plant sources (20-23). Notably, beans and legumes from the Phaseolus vulgaris L. family are particularly rich in protein, although they are relatively low in sulfur-containing amino acids like methionine and cysteine (24, 25).

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

Protein Analysis: Extraction and Quantification Methods

Proteomics, which explores the protein composition of cells or organisms, relies on specific techniques to accurately assess protein quantity and quality. The typical workflow for protein analysis includes four stages: extraction, separation and quantification, enrichment, and data interpretation (26). Protein analysis methods vary according to the sample type and analytical principle, from determining total nitrogen content to detecting the presence of aromatic amino acids, peptide bonds, and UV absorptive properties (1). Figure 2 (not included here) illustrates the protein extraction and fractionation process, highlighting techniques for cell disruption, solubilization, precipitation, enrichment, cleanup, and fractionation of proteins(27).

Table 1: Approximate Protein Composition of some Beans

Beans	Proteins (g/100 g)
Kidney beans (Phaseolus vulgaris)	8.7
Bambara groundnut (Vigna subterranean)	14–24
Black bean (Phaseolus vulgaris)	8.86
Mung bean (Vigna radiate)	28.86
Peanut (Arachis hypogaea)	25
Soybeans (Glycine max)	36.49
White beans	7.20
Chickpea (Cicer arietinum)	7.82
Green lentils (Lens culinaris)	8.31
Brown lentils (Lens culinaris)	5.08
Pinto beans (Phaseolus vulgaris)	10.71
Pea (Pisum)	5.42
Flageolet	6.92

(20-23)

Methods of Protein Extraction and Fractionation

To analyze the protein content of food, proteins or peptides must first be extracted from the food source, ideally with minimal modification. Extraction methods are carefully tailored to different food types, aiming to maximize yield while preserving protein structure (28). A significant challenge in protein extraction from plant sources is the presence of compounds that may hinder the extraction process. Consequently, cell disruption is essential to release proteins enclosed within plant cells (29). Cell disruption methods vary depending on the plant type, with specific techniques used for different types of beans as outlined in Table 2 (not included here)(30).

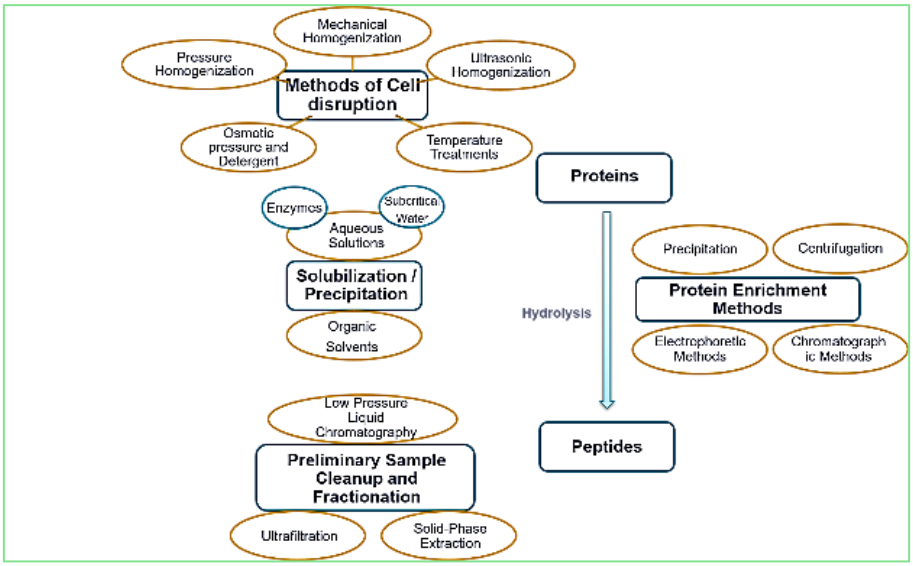

Figure 2: Extraction and Fractionation Techniques for Proteins (26). These techniques involve various steps for cell disruption, solubilization and precipitation of isolates, protein enrichment methods, cleanup and fractionation of preliminary samples.

Techniques for Protein Extraction

Several mechanical, chemical, and thermal methods facilitate cell disruption for protein extraction. Mechanical homogenization uses tools like rotor-stator homogenizers and blenders to break down plant cells, which have

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

robust cell walls (31). Wet-milling with sulfur dioxide is commonly employed for grains, utilizing chemicals like sulfur dioxide (SO_2) and sodium hydrogen sulfite (NaHSO3) to aid protein separation (32). Pressure homogenization is another widely used technique that applies high pressure to release proteins from cells, yielding twice the amount of protein as atmospheric pressure methods. While effective, high-pressure techniques may expose proteins to proteolytic enzymes, potentially causing denaturation and loss of function (33, 34). Ultrasonic homogenization, or sonication, uses an acoustic transducer to break down proteins within cellular pellets. This method has proven effective in increasing protein yield and is extensively used in commercial applications (35). Temperature treatment, particularly the freeze-thaw method, also aids in cell disruption. Liquid nitrogen is often applied to flash-freeze cells, followed by mechanical force to release proteins. This process boosts protein solubility, foaming, and emulsifying properties, though it may impede overall extraction (36, 37). High temperatures are also applied in certain extraction processes, such as wet-milling, though heating can compromise protein recovery (32, 38).

Table 2: Types of Cell Disruption methods corresponding to the type of beans and food

Type of cell disruption	Procedure	Food	Referenced from
Mechanical Homogenization	Centrifugal grinding and air dehulling	Pea, chickpea, and lentil	(39)
Mechanical Homogenization	Wet-milling with sulfur dioxide	Sorghum	(32)
Ultrasonic Homogenization	Acoustic Transducer	Soybean	(40)
Pressure Homogenization	High-pressure homogenization	Peanut	(33)
Pressure Homogenization	High-pressure homogenization	Rapeseed	(41)
Temperature treatments	Mortar and Pestle with liquid N2	Peanut	(36)
Temperature Treatments	Wet-milling with temperature	Sorghum	(32)

Protein analysis is a multi-faceted process, where selective isolation and solubilization of proteins are crucial to obtaining accurate results. This isolation step, known as protein solubilization, enables the removal of compounds that may interfere with quantification, ensuring that the protein sample is of high quality and purity (26). Effective protein solubilization is pivotal for high-quality analysis, as it directly influences the accuracy of subsequent analytical methods. Given the variability in plant tissue composition, the solubilization process must be carefully tailored to the specific food sample, especially when handling samples from complex sources like beans. These samples often contain numerous interfering substances that challenge complete protein solubilization, necessitating optimal conditions to prevent protein modifications or degradation through proteolysis (31, 42).

Table 3: Organic solvents used against the different types of beans and food source

Organic Solvents	**Food**	**Referenced from**
Aqueous Isopropanol	Soybean	(28)
Aqueous Isopropanol	Rapeseed	(41)
Glacial Acetic Acid	Sorghum	(32)
Phenol	Soybean	(28)
TCA	Bean	(45)
TCA / Acetone	Soybean	(46)
Thiourea / urea	Soybean	(28)

Organic Solvents in Protein Solubilization

A variety of organic solvents are used for protein solubilization, selected based on the food source, with some effective options listed in Table 3. Common solvents include trichloroacetic acid (TCA) and acetone, which, when used in combination, aid in extracting proteins from beans, cereals, and other plants by inhibiting proteolytic enzyme activity due to TCA's negative charge and extreme pH. Although highly effective, TCA-treated proteins often pose challenges in re-dissolution, presenting a limitation of this solvent (43). In recent years, phenol has gained attention for protein precipitation due to its superior clean-up capacity. Capable of dissolving nucleic acids and polysaccharides to some extent, phenol exerts a potent solvent effect on proteins, though it is time-consuming and relatively toxic(44).

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

Aqueous Solutions as Environmentally Friendly Alternatives

Driven by environmental concerns, aqueous solutions have become increasingly preferred over organic solvents for protein extraction. Water-based solutions are neither toxic nor flammable, unlike organic and alcoholic solvents, making them safer for both the environment and laboratory personnel. However, the effectiveness of these aqueous solutions depends on several factors, such as pH, net charge, salt concentration, electrostatic repulsions, and ionic strength. Each of these parameters can significantly impact protein yield and quality(47). A commonly used aqueous method, based on the Osborne method developed in 1924, involves the use of alkaline solutions to extract plant proteins. However, this approach requires rigorous washing to eliminate excess alkali, which can generate large amounts of wastewater (21, 48). The addition of enzymes in aqueous solutions, known as aqueous enzymatic extraction, is an emerging approach that aids the solubilization process. Enzymes such as carbohydrases target the plant cell wall, enhancing protein yield by facilitating cell wall breakdown (47). Aqueous enzymatic extraction is eco-friendly and minimizes protein damage during processing, though its high cost and lengthy processing times can limit its scalability. Immobilizing enzymes for reuse offers a potential solution to these economic challenges, helping to lower costs and reduce waste (26, 49).

Table 4: Enzymes used in Aqueous Enzymatic Extraction of proteins from various beans and seeds

Enzymes used	Food	Extracted protein	Referenced from
Alcalase 2.4 L	Rapeseed	66.7 %	(50)
Alcalase 2.4 L	Peanut	82.5 %	(29)
Alcalase 2.4 L	Peanut	80.1 %	(51)
Glucoamylase	Lentil	-	(52)
Protex 6 L	Soybean	84.6 %	(53)
Protex 6 L	Soybean	96.0 %	(54)
Protex 7 L	Sesame seed	87.1%	(55)

Subcritical Water Extraction

Subcritical water extraction, using water at temperatures above its boiling point (100–374°C) but under pressurized conditions to maintain its liquid state, has emerged as an effective and eco-friendly protein extraction method. This approach offers unique properties, such as high ion concentration and low dielectric constant, enhancing protein yield from beans, seeds, and bran (56). As an innovative and sustainable method, subcritical water extraction aligns with current priorities for minimizing environmental impact in laboratory processes(57).

Protein Enrichment Methods

Following isolation, protein enrichment becomes a critical step to improve concentration before analytical techniques such as mass spectrometry (MS) are applied. Despite advances in proteomics, no single method can measure specific protein concentrations accurately, necessitating enrichment to address low protein levels within extracts (26). Protein enrichment encompasses various techniques, including precipitation, centrifugation, electrophoresis, and chromatography, each playing a role in refining protein extracts and removing residual contaminants(58). Precipitation or "salting out" involves adding salts, commonly ammonium sulfate, to increase protein interactions, resulting in protein aggregation and subsequent precipitation. Ammonium sulfate is particularly advantageous due to its high solubility and ability to form saturated solutions, although its acidic pH requires adjustment prior to use (31, 59). Centrifugation offers a straightforward approach for protein enrichment, initially used in protein isolation to create a homogenized mixture and separate cellular components. Later stages of protein solubilization and enrichment rely on centrifugation to refine and isolate protein fractions by their density, allowing gradient centrifugation to optimize fractionation productivity (26, 60). Electrophoresis, a method of separating proteins based on shape, charge, or size, serves as both a preparative and analytical tool. Generally performed as one-dimensional separation, electrophoresis helps evaluate protein purity and molecular weight before proceeding to two-dimensional analysis, like SDS-PAGE. In cases of high protein complexity, ampholytes are often removed prior to MS analysis to prevent interference (61, 62). Chromatographic methods such as Liquid Chromatography (LC) and Ion-Exchange Chromatography (IEX) separate proteins based on characteristics like size, charge, and hydrophobicity. Techniques like size-exclusion chromatography are valuable for refining protein fractions from complex mixtures (63). Affinity chromatography may also be used to detect post-translational modifications such as glycosylation or phosphorylation, enriching the proteomic analysis by revealing functional protein characteristics.

Protein Hydrolysis and Digestion for Amino Acid Analysis

Protein hydrolysis, essential for determining amino acid composition, utilizes various methods including hydrochloric acid, sodium hydroxide, performic acid, and proteolytic enzymes. Hydrolysis with 6 M hydrochloric acid is a common method, conducted at high temperatures over extended periods to release amino acids, though amino acids like Valine and Isoleucine require longer hydrolysis times to be accurately quantified (36, 64). Alternatively, sodium hydroxide hydrolysis at 110 °C for 16 hours is effective for amino acids like Tryptophan and Leucine (36). Performic acid oxidation, followed by hydrochloric acid hydrolysis, provides accurate assays for cysteine and methionine, while proteolytic enzymes such as trypsin and chymotrypsin are frequently used for targeted digestion of specific peptide bonds. Trypsin, widely applied for its affordability and high purity, selectively cleaves peptide bonds at lysine and arginine, generating peptides with strong C-terminal residues that are well-suited for MS analysis (65, 66). This comprehensive overview highlights the significance of protein solubilization and enrichment techniques in analyzing plant-based proteins, specifically from beans. The use of advanced methods such as subcritical water extraction and enzymatic approaches underscores the shift toward eco-friendly practices in protein extraction. By evaluating various methods for protein solubilization, enrichment, and hydrolysis, this study aims to determine optimal strategies for isolating and quantifying proteins from beans, offering a robust foundation for future research in nutritional science and food proteomics(66).

METHODS

The methodology employed for protein analysis encompasses various spectrophotometric, colorimetric, dye-binding, and electrophoretic techniques, each selected based on the specific requirements of protein quantification and molecular weight determination. Each method aimed to accurately quantify or characterize proteins while considering the limitations and interferences that may arise from specific sample types. The following sections outline the procedures used, adapted to standardize the process for optimal precision and reliability.

Spectrophotometric Analysis

A spectrophotometer was utilized to determine protein content based on absorbance at specific wavelengths. Proteins containing tyrosine and tryptophan residues exhibited strong absorption at 280 nm due to these aromatic amino acids, enabling the quantification of protein concentration using Beer's Law. For this analysis, proteins were solubilized in an alkaline solution or buffer, and their absorbance was measured at 280 nm against a blank reagent. The protein concentration was calculated using the formula A=abcA = abcA=abc, where AAA represents absorbance, aaa the absorption coefficient, bbb the cuvette path length, and ccc the concentration. Additionally, peptide bonds absorbed between 190 and 220 nm; however, this lower UV range made quantification challenging for broader commercial applications (1).

Lowry Method

The Lowry method, a colorimetric technique, was employed for protein quantification by leveraging the Folin-Ciocalteu reagent's reaction with peptide bonds under alkaline conditions. The method involved two steps: initially, the protein sample formed a complex with copper ions (Cu^{2+}), followed by the reduction of the Folin reagent by the copper-protein complex to form molybdenum blue. The resulting color intensity was measured at either 750 nm or 500 nm, with 750 nm suited for low protein concentrations and 500 nm for higher concentrations. A standard curve of bovine serum albumin (BSA) was constructed to estimate protein concentration in unknown samples (1).

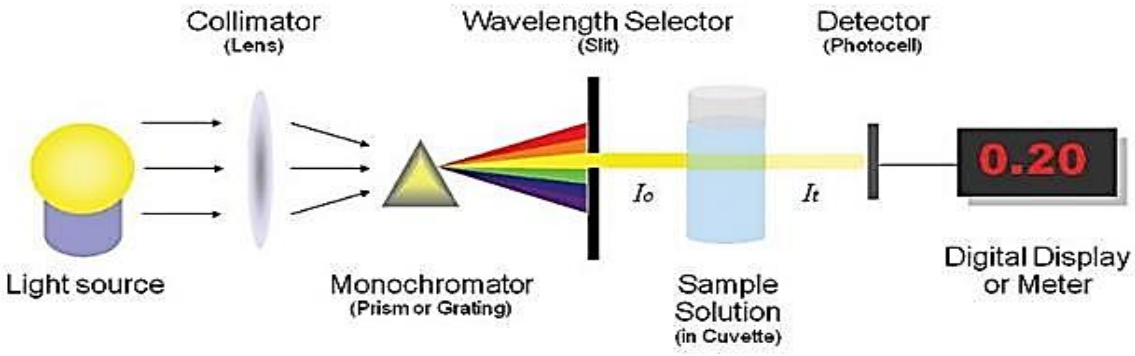

Figure 3: Basic structure of spectrophotometer (It consists of a light source, a collimator, a monochromatic, a wavelength selector, a cuvette for sample solution, a photoelectric detector, and a digital display or a meter).

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

While effective, the Lowry method had limitations due to interference from various compounds, such as thiols, free amino acids, and chelating agents, which reacted with the Folin-Ciocalteu reagent. To mitigate interference, several modifications were applied, including heating the sample pre- and post-reagent mixing, removing lipids with organic solvents, and adding SDS and chloramine-T (67). Despite alternative advancements, the traditional Lowry method remains widely used due to its high sensitivity.

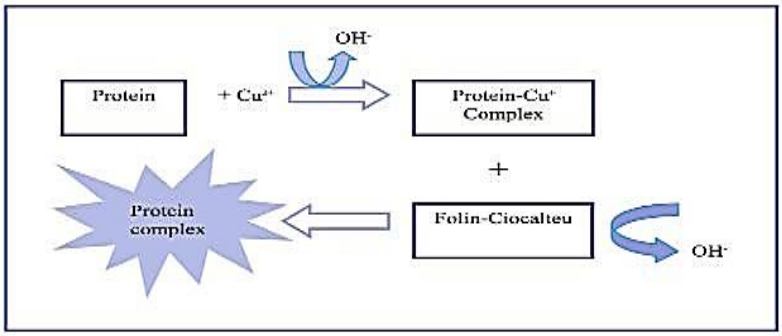

Figure 4: Principle of Lowry Method (The method relies on two different reactions. The first reaction is the formation of a copper ion complex with amide bonds, forming reduced copper in alkaline solutions. The second reaction is the reduction of Folin-Ciocalteu reagent giving an intense blue compound)

Bradford Method

The Bradford method, a dye-binding technique, relied on the color change of Coomassie Brilliant Blue G-250 upon binding with proteins, shifting its absorbance from 465 nm to 595 nm. This change was proportional to the protein concentration in the sample. To prepare the sample, Coomassie dye was mixed with ethanol and phosphoric acid, then combined with the extracted protein solution. The absorbance of the resulting mixture was read at 595 nm, and a BSA standard curve was generated to quantify protein levels (1). The Bradford method offered simplicity, speed, and minimal interference from contaminants, as commonly seen with the Lowry method. However, variations in protein composition influenced the dye's affinity, requiring specific adjustments for different protein types. Smaller peptides often went undetected due to weak interactions with the dye, while proline-rich proteins altered the dye color, demanding customized calibration (68).

SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis)

SDS-PAGE, an electrophoretic technique, was utilized to separate proteins based on molecular weight, ranging from 5 kDa to 250 kDa. Polyacrylamide gels, consisting of acrylamide and a cross-linker methylene bisacrylamide, were catalyzed by ammonium persulfate (AP) and tetramethylethylenediamine (TEMED) to create a three-dimensional network that facilitated protein migration. Sodium dodecyl sulfate (SDS), an anionic detergent, provided a uniform negative charge to proteins, ensuring that migration depended solely on molecular weight. A known molecular weight marker was included for relative comparison, and proteins were visualized via staining (69). To ensure accurate migration, SDS concentration was carefully maintained at 1.4 g per 1 g of protein. Deviations in SDS concentration resulted in rigid-rod conformations, complicating separation. Proteins with high charge densities, like histones, and low molecular weight proteins sometimes displayed inconsistencies in migration. Adjustments to gel calibration standards were applied as needed to address these anomalies. The SDS-PAGE protocols followed foundational methods outlined by Shapiro et al. (70) and Laemmli (71), which were later modified to accommodate low molecular weight proteins ranging from approximately 1,400 to 25,000 Da.

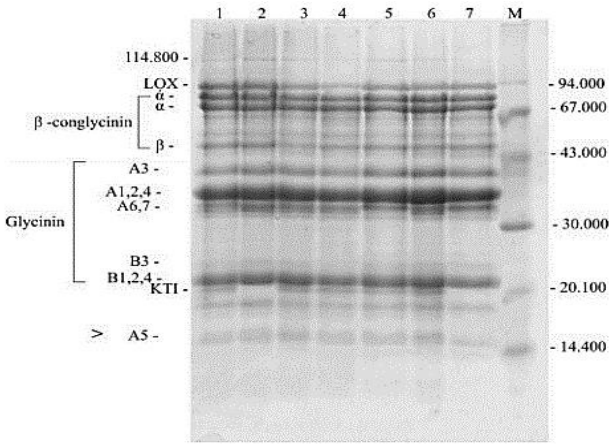

Figure 5: SDS-PAGE patterns of proteins from soybean varieties. α′, α and β indicate subunits of β-conglycinin. A and B indicate acidic and basic polypeptides of glycinin, respectively. LOX, lipoxygenase; KTI, Kunitz trypsin inhibitor; 1-7, soybean varieties; M, molecular weight standards (72).

RESULTS & DISCUSSION

The rising global demand for protein-rich foods necessitates a shift toward plant-derived proteins, not only for their health benefits but also for environmental sustainability. As awareness of the positive health impacts of plant proteins grows, an increased focus is anticipated on developing techniques that maximize protein extraction from plant sources. Beans, pulses, and legumes are ideal for such extraction due to their high protein content, ease of cultivation, and relatively efficient isolation techniques. Among beans, soybeans exhibit the highest protein concentration, followed closely by peanuts and kidney beans. The extraction and purification of proteins from beans involve steps similar to those used for proteins from other plant tissues, although analytical quantification techniques may vary based on the specific protein source(30, 73).

This study evaluated four primary analytical methods for protein quantification. The Lowry method, while historically popular, is often limited by its sensitivity to interference from non-protein compounds in the sample. Despite its high sensitivity, this method's reactions with other extract components complicate accurate quantification, reducing its suitability in complex mixtures. The Lowry method's steps and sensitivity are advantageous for some applications; however, its practical limitations suggest that alternative methods might be preferable when quantifying protein in less controlled, plant-based extracts(34, 74). The Bradford method, a dye-binding technique, emerged as an effective alternative due to its simplicity, rapidity, and reduced interference from other substances within the sample. It demonstrated versatility by measuring protein concentrations down to the microgram level, a strength further amplified by recent modifications that improve compatibility with various buffers. The Bradford method's high sensitivity and adaptability make it a preferred choice for low-concentration protein assays, including enzyme characterization and quantification. While the Lowry method remains widely used for specific applications due to its high sensitivity, the Bradford method's resilience against interference and its rapid process make it more favorable for a broader range of protein analysis tasks. Yet, the Bradford assay can be influenced by protein composition, as certain proteins with high proline content may interact differently with the dye, indicating a potential need for optimization depending on the sample(9, 38).

Spectrophotometric analysis at 280 nm, leveraging the absorbance of aromatic amino acids like tyrosine and tryptophan, offered another approach to protein quantification. This method is generally less suitable for high-throughput analysis of plant-based proteins, as the absorbance of peptide bonds in the lower UV range poses challenges in obtaining precise measurements. Due to its limitations with non-animal sources, spectrophotometric analysis is often reserved for applications in milk and meat products rather than plant-derived proteins. Although effective for these specific sources, it lacks the flexibility and broad applicability required for routine plant protein analysis(2, 8). SDS-PAGE was also examined for its utility in protein analysis, particularly for its ability to separate proteins based on molecular weight, allowing for the profiling of protein fractions within complex food extracts. SDS-PAGE provided clear advantages in terms of its straightforward operation and its effectiveness in determining protein profiles, particularly when coupled with mass spectrometry for more intricate analyses. However, SDS-PAGE's relatively slower process limits its throughput, and the proteins' susceptibility to denaturation due to the presence of SDS remains a notable drawback. This denaturation risk underscores the need for improved electrophoresis methods or alternative approaches that reduce the dependency on SDS, thereby preserving protein structure and activity(12, 17).

The Bradford method proved to be the most adaptable and interference-resistant for quantifying proteins in diverse food samples, while SDS-PAGE excelled in separating and analyzing protein fractions from complex extracts. The Lowry and spectrophotometric methods, though useful, were found to have limitations in the context of plant-derived protein analysis. These findings highlight the need for continued development of protein quantification techniques that balance sensitivity, accuracy, and resistance to interference for reliable analysis across a variety of food sources(25, 27).

CONCLUSION

The shift towards plant-derived proteins has the potential to impact both health outcomes and environmental sustainability. Embracing these proteins, particularly from beans and legumes, offers a viable alternative to animal-based sources, promoting better nutrition and reducing ecological strain. Developing functional foods and nutraceuticals from plant sources like beans could play a critical role in addressing global health challenges. Although advancing food proteomics and related technologies will require significant investment, consumers can contribute by making conscious food choices now. Current methods, such as phenol-based protein extractions, show promise but require further refinement for optimized application in plant proteomics. While 2-D electrophoresis remains useful for general protein analysis, advancements are needed to better handle the complex protein expression levels found in food systems. Altogether, the continued development of these methods could accelerate progress in nutrition, health, and environmental preservation.

REFERENCES

1. Chang SK, Zhang Y. Protein analysis. Food analysis. 2017:315-31.

2. Ladjal-Ettoumi Y, Hamadi M, Douik LH, Cherifi Z, Nazir A. Physicochemical, functional, and nutraceutical properties of Spirulina and Chlorella biomass: a comparative study. Algal Research. 2024;81:103561.

3. Choung M-G, Choi B-R, An Y-N, Chu Y-H, Cho Y-S. Anthocyanin profile of Korean cultivated kidney bean (Phaseolus vulgaris L.). Journal of Agricultural and Food Chemistry. 2003;51(24):7040-3.

4. Lin L-Z, Harnly JM, Pastor-Corrales MS, Luthria DL. The polyphenolic profiles of common bean (Phaseolus vulgaris L.). Food chemistry. 2008;107(1):399-410.

5. Granito M, Paolini M, Pérez S. Polyphenols and antioxidant capacity of Phaseolus vulgaris stored under extreme conditions and processed. LWT-Food Science and Technology. 2008;41(6):994-9.

6. Beninger CW, Hosfield GL. Antioxidant activity of extracts, condensed tannin fractions, and pure flavonoids from Phaseolus vulgaris L. seed coat color genotypes. Journal of agricultural and Food chemistry. 2003;51(27):7879-83.

7. Nair RM, Yang RY, Easdown WJ, Thavarajah D, Thavarajah P, Hughes JdA, et al. Biofortification of mungbean (Vigna radiata) as a whole food to enhance human health. Journal of the Science of Food and Agriculture. 2013;93(8):1805-13.

8. Kaur S, Singh V, Chopra HK, Panesar PS. Extraction and characterization of phenolic compounds from mandarin peels using conventional and green techniques: a comparative study. Discover Food. 2024;4(1):60.

9. Yokota H. Applications of proteomics in pharmaceutical research and development. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics. 2019;1867(1):17-21.

10. Pedreschi R, Hertog M, Lilley KS, Nicolai B. Proteomics for the food industry: opportunities and challenges. Critical reviews in food science and nutrition. 2010;50(7):680-92.

11. Jorrin-Novo JV. Plant proteomics methods and protocols: Springer; 2014.

12. Dong X, Rawiwan P, Middleditch M, Guo G, Woo MW, Quek SY. Effects of protein variations by different extraction and dehydration approaches on hempseed protein isolate: Protein pattern, amino acid profiling and label-free proteomics. Food Chemistry. 2024;460:140426.

13. Li SS, Blanco Mejia S, Lytvyn L, Stewart SE, Viguiliouk E, Ha V, et al. Effect of plant protein on blood lipids: A systematic review and meta-analysis of randomized controlled trials. Journal of the American Heart Association. 2017;6(12):e006659.

14. Song M, Fung TT, Hu FB, Willett WC, Longo VD, Chan AT, et al. Association of animal and plant protein intake with all-cause and cause-specific mortality. JAMA internal medicine. 2016;176(10):1453-63.

15. Luo C, Zhang Y, Ding Y, Shan Z, Chen S, Yu M, et al. Nut consumption and risk of type 2 diabetes, cardiovascular disease, and all-cause mortality: a systematic review and meta-analysis. The American journal of clinical nutrition. 2014;100(1):256-69.

16. Tremblay F, Lavigne C, Jacques H, Marette A. Role of dietary proteins and amino acids in the pathogenesis of insulin resistance. Annu Rev Nutr. 2007;27(1):293-310.

17. Badjona A, Bradshaw R, Millman C, Howarth M, Dubey B. Optimization of ultrasound-assisted extraction of faba bean protein isolate: Structural, functional, and thermal properties. Part 2/2. Ultrasonics sonochemistry. 2024;110:107030.

18. Hayat I, Ahmad A, Masud T, Ahmed A, Bashir S. Nutritional and health perspectives of beans (Phaseolus vulgaris L.): an overview. Critical reviews in food science and nutrition. 2014;54(5):580-92.

19. Vidigal Filho PS, Gonçalves-Vidigal MC, da Rocha AB, Hammerschmidt R, Kirk WW, Poletine JP, et al. Characterization and content of total soluble protein and amino acids of traditional common bean cultivars collected in Parana state, Brazil. J Food Agric Environ. 2011;9:143-7.

20. Margier M, Georgé S, Hafnaoui N, Remond D, Nowicki M, Du Chaffaut L, et al. Nutritional composition and bioactive content of legumes: Characterization of pulses frequently consumed in France and effect of the cooking method. Nutrients. 2018;10(11):1668.

21. Tanwar B, Goyal A. Oilseeds: health attributes and food applications: Springer; 2021.

22. Kudre TG, Benjakul S, Kishimura H. Comparative study on chemical compositions and properties of protein isolates from mung bean, black bean and bambara groundnut. Journal of the Science of Food and Agriculture. 2013;93(10):2429-36.

23. Bouchenak M, Lamri-Senhadji M. Nutritional quality of legumes, and their role in cardiometabolic risk prevention: a review. Journal of medicinal food. 2013;16(3):185-98.

24. Lafarga T, Álvarez C, Bobo G, Aguiló-Aguayo I. Characterization of functional properties of proteins from Ganxet beans (Phaseolus vulgaris L. var. Ganxet) isolated using an ultrasound-assisted methodology. Lwt. 2018;98:106-12.

25. Badjona A, Bradshaw R, Millman C, Howarth M, Dubey B. Response surface methodology guided approach for optimization of protein isolate from Faba bean. Part 1/2. Ultrasonics Sonochemistry. 2024;109:107012.

26. Martínez-Maqueda D, Hernández-Ledesma B, Amigo L, Miralles B, Gómez-Ruiz JÁ. Extraction/fractionation techniques for proteins and peptides and protein digestion. Proteomics in foods: principles and applications. 2013:21-50.

27. Nasrollahzadeh F, Roman L, Skov K, Jakobsen LM, Trinh BM, Tsochatzis ED, et al. A comparative investigation of seed storage protein fractions: The synergistic impact of molecular properties and composition on anisotropic structuring. Food Hydrocolloids. 2023;137:108400.

28. Natarajan S, Xu C, Caperna TJ, Garrett WM. Comparison of protein solubilization methods suitable for proteomic analysis of soybean seed proteins. Analytical biochemistry. 2005;342(2):214-20.

29. Wang W, Tai F, Chen S. Optimizing protein extraction from plant tissues for enhanced proteomics analysis. Journal of separation science. 2008;31(11):2032-9.

30. Stefanucci A, Scioli G, Marinaccio L, Zengin G, Locatelli M, Tartaglia A, et al. A comparative study on phytochemical fingerprint of two diverse Phaseolus vulgaris var. Tondino del Tavo and Cannellino bio extracts. Antioxidants. 2022;11(8):1474.

31. Bodzon-Kulakowska A, Bierczynska-Krzysik A, Dylag T, Drabik A, Suder P, Noga M, et al. Methods for samples preparation in proteomic research. Journal of Chromatography B. 2007;849(1-2):1-31.

32. De Mesa-Stonestreet NJ, Alavi S, Bean SR. Sorghum proteins: the concentration, isolation, modification, and food applications of kafirins. Journal of food science. 2010;75(5):R90-R104.

33. Dong X, Zhao M, Shi J, Yang B, Li J, Luo D, et al. Effects of combined high-pressure homogenization and enzymatic treatment on extraction yield, hydrolysis and function properties of peanut proteins. Innovative Food Science & Emerging Technologies. 2011;12(4):478-83.

34. Sha L, Xiong YL. Comparative structural and emulsifying properties of ultrasound-treated pea (Pisum sativum L.) protein isolate and the legumin and vicilin fractions. Food Research International. 2022;156:111179.

35. Chittapalo T, Noomhorm A. Ultrasonic assisted alkali extraction of protein from defatted rice bran and properties of the protein concentrates. International journal of food science & technology. 2009;44(9):1843-9.

36. Liang X, Luo M, Holbrook C, Guo B. Storage protein profiles in Spanish and runner market type peanuts and potential markers. BMC Plant Biology. 2006;6:1-9.

37. Méchin V, Thévenot C, Le Guilloux M, Prioul J-L, Damerval C. Developmental analysis of maize endosperm proteome suggests a pivotal role for pyruvate orthophosphate dikinase. Plant Physiology. 2007;143(3):1203-19.

38. Pawar VS, Bhande D, Pawar SD, Mudila H, Kaushik A, Kumar A. Investigating purification and activity analysis of urease enzyme extracted from jack bean source: A green chemistry approach. Analytical biochemistry. 2022;659:114925.

39. Boye J, Aksay S, Roufik S, Ribéreau S, Mondor M, Farnworth E, et al. Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. Food Research International. 2010;43(2):537-46.

40. Toorchi M, Nouri M-Z, Tsumura M, Komatsu S. Acoustic technology for high-performance disruption and extraction of plant proteins. Journal of Proteome Research. 2008;7(7):3035-41.

41. Barbin D, Natsch A, Müller K. Improvement of functional properties of rapeseed protein concentrates produced via alcoholic processes by thermal and mechanical treatments. Journal of Food Processing and Preservation. 2011;35(3):369-75.

42. Karami Z, Butkinaree C, Yingchutrakul Y, Simanon N, Duangmal K. Comparative study on structural, biological and functional activities of hydrolysates from Adzuki bean (Vigna angularis) and mung bean (Vigna radiata) protein concentrates using Alcalase and Flavourzyme. Food Research International. 2022;161:111797.

43. Nandakumar M, Shen J, Raman B, Marten MR. Solubilization of trichloroacetic acid (TCA) precipitated microbial proteins via NaOH for two-dimensional electrophoresis. Journal of proteome research. 2003;2(1):89-93.

44. Chang L, Lan Y, Bandillo N, Ohm J-B, Chen B, Rao J. Plant proteins from green pea and chickpea: Extraction, fractionation, structural characterization and functional properties. Food Hydrocolloids. 2022;123:107165.

45. Wu F-S, Wang M-Y. Extraction of proteins for sodium dodecyl sulfate-polyacrylamide gel electrophoresis from protease-rich plant tissues. Analytical biochemistry. 1984;139(1):100-3.

46. Natarajan SS, Xu C, Bae H, Caperna TJ, Garrett WM. Characterization of storage proteins in wild (Glycine soja) and cultivated (Glycine max) soybean seeds using proteomic analysis. Journal of Agricultural and Food Chemistry. 2006;54(8):3114-20.

47. Tang S, Hettiarachchy NS, Shellhammer TH. Protein extraction from heat-stabilized defatted rice bran. 1. Physical processing and enzyme treatments. Journal of Agricultural and Food Chemistry. 2002;50(25):7444-8.

48. Sereewatthanawut I, Prapintip S, Watchiraruji K, Goto M, Sasaki M, Shotipruk A. Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis. Bioresource technology. 2008;99(3):555-61.

49. Quintero-Quiroz J, Celis-Torres A, Ciro-Gómez G, Torres J, Corrales-García L, Rojas J. Physicochemical properties and functional characteristics of ultrasound-assisted legume-protein isolates: a comparative study. Journal of Food Science and Technology. 2021:1-12.

50. Zhang SB, Wang Z, Xu SY. Downstream processes for aqueous enzymatic extraction of rapeseed oil and protein hydrolysates. Journal of the American Oil Chemists' Society. 2007;84:693-700.

51. Zhang SB, Lu QY, Yang H, Li Y, Wang S. Aqueous enzymatic extraction of oil and protein hydrolysates from roasted peanut seeds. Journal of the American Oil Chemists' Society. 2011;88:727-32.

52. Bildstein M, Lohmann M, Hennigs C, Krause A, Hilz H. An enzyme-based extraction process for the purification and enrichment of vegetable proteins to be applied in bakery products. European Food Research and Technology. 2008;228(2):177-86.

53. Jung S. Aqueous extraction of oil and protein from soybean and lupin: a comparative study. Journal of food processing and preservation. 2009;33(4):547-59.

54. De Moura J, Campbell K, De Almeida N, Glatz C, Johnson L. Protein extraction and membrane recovery in enzyme-assisted aqueous extraction processing of soybeans. Journal of the American Oil Chemists' Society. 2011;88:877-89.

55. Latif S, Anwar F. Aqueous enzymatic sesame oil and protein extraction. Food chemistry. 2011;125(2):679-84.

56. Hata S, Wiboonsirikul J, Maeda A, Kimura Y, Adachi S. Extraction of defatted rice bran by subcritical water treatment. Biochemical Engineering Journal. 2008;40(1):44-53.

57. Ismail MA, Amin MA, Eid AM, Hassan SE-D, Mahgoub HA, Lashin I, et al. Comparative study between exogenously applied plant growth hormones versus metabolites of microbial endophytes as plant growth-promoting for Phaseolus vulgaris L. Cells. 2021;10(5):1059.

58. Warsame AO, Michael N, O'Sullivan DM, Tosi P. Identification and quantification of major faba bean seed proteins. Journal of agricultural and food chemistry. 2020;68(32):8535-44.

59. Vogelsang-O'Dwyer M, Petersen IL, Joehnke MS, Sørensen JC, Bez J, Detzel A, et al. Comparison of faba bean protein ingredients produced using dry fractionation and isoelectric precipitation: Techno-functional, nutritional and environmental performance. Foods. 2020;9(3):322.

60. Pedrosa MM, Varela A, Domínguez-Timón F, Tovar CA, Moreno HM, Borderías AJ, et al. Comparison of bioactive compounds content and techno-functional properties of pea and bean flours and their protein isolates. Plant Foods for Human Nutrition. 2020;75:642-50.

61. Chen B, Dong J-Q, Chen Y-J, Wang J-M, Tian J, Wang C-B, et al. Two-dimensional electrophoresis for comparative proteomic analysis of human bile. Hepatobiliary & Pancreatic Diseases International: HBPD INT. 2007;6(4):402-6.

62. Kotha RR, Finley JW, Luthria DL. Determination of soluble mono, di, and oligosaccharide content in 23 dry beans (Phaseolus vulgaris L.). Journal of agricultural and food chemistry. 2020;68(23):6412-9.

63. Ohm JB, Hareland G, Simsek S, Seabourn B. Size-exclusion HPLC of protein using a narrow-bore column for evaluation of breadmaking quality of hard spring wheat flours. Cereal chemistry. 2009;86(4):463-9.

64. Ibáñez M, De Blas C, Cámara L, Mateos G. Chemical composition, protein quality and nutritive value of commercial soybean meals produced from beans from different countries: A meta-analytical study. Animal Feed Science and Technology. 2020;267:114531.

65. Couto N, Barber J, Gaskell SJ. Matrix-assisted laser desorption/ionisation mass spectrometric response factors of peptides generated using different proteolytic enzymes. Journal of mass spectrometry. 2011;46(12):1233-40.

66. Hertzler SR, Lieblein-Boff JC, Weiler M, Allgeier C. Plant proteins: assessing their nutritional quality and effects on health and physical function. Nutrients. 2020;12(12):3704.

67. Sikorski ZE. Functional properties of proteins in food systems. Chemical and functional properties of food proteins. 2001:113-35.

68. Owusu-Apenten R. Food protein analysis: quantitative effects on processing: CRC press; 2002.

69. Nowakowski AB, Wobig WJ, Petering DH. Native SDS-PAGE: high resolution electrophoretic separation of proteins with retention of native properties including bound metal ions. Metallomics. 2014;6(5):1068-78.

70. Shapiro AL, Viñuela E, Maizel Jr JV. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. Biochemical and biophysical research communications. 1967;28(5):815-20.

71. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. nature. 1970;227(5259):680-5.

72. Žilić SM, Barać MB, Pešić MB, Mladenović Drinić SD, Ignjatović-Micić DD, Srebrić MB. Characterization of proteins from kernel of different soybean varieties. Journal of the Science of Food and Agriculture. 2011;91(1):60-7.

73. Byanju B, Rahman MM, Hojilla-Evangelista MP, Lamsal BP. Effect of high-power sonication pretreatment on extraction and some physicochemical properties of proteins from chickpea, kidney bean, and soybean. International Journal of Biological Macromolecules. 2020;145:712-21.

74. Brishti FH, Chay SY, Muhammad K, Ismail-Fitry MR, Zarei M, Karthikeyan S, et al. Effects of drying techniques on the physicochemical, functional, thermal, structural and rheological properties of mung bean (Vigna radiata) protein isolate powder. Food Research International. 2020;138:109783.