Vol. 5 No. 2 (2026)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

Effects of Ultrasound-Assisted Protein Modification on the Functional and Nutritional Properties of Soy Protein Isolate

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Keywords: Soy Protein Isolate, ultrasound-assisted processing, protein modification, acoustic cavitation, functional properties, plant protein, digestibility, antioxidant activity, predictive research, functional foods.

Received on 03 May 2026
Accepted on 08 Jun 2026
Published on 15 Jun 2026

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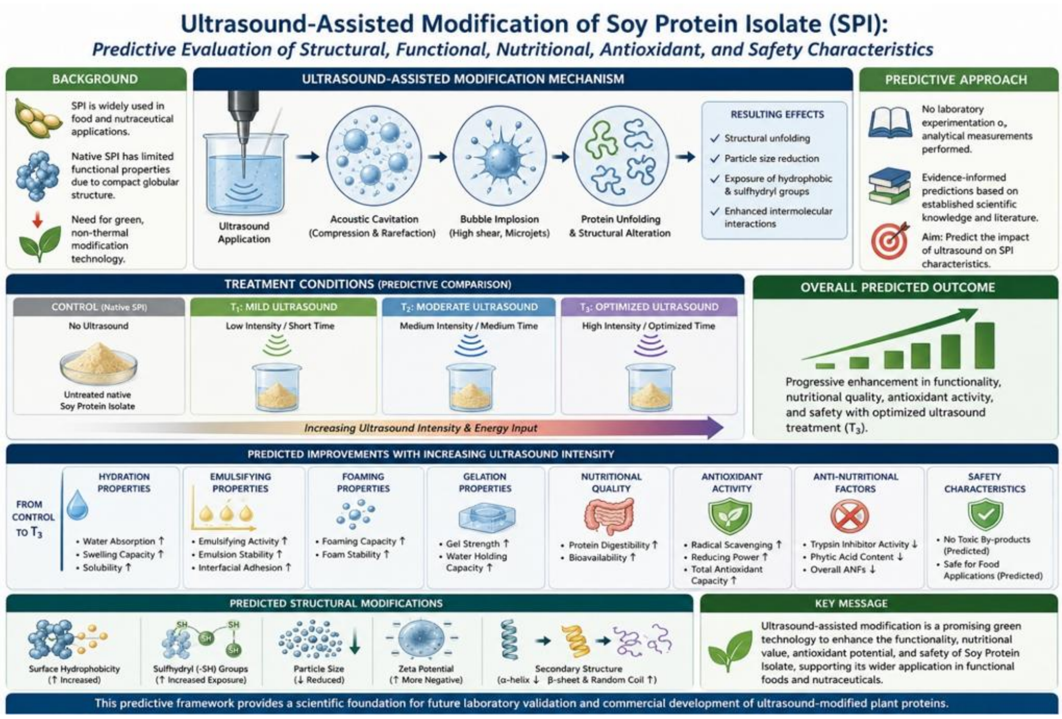
Abstract

Soy Protein Isolate (SPI) is among the most extensively utilized plant-derived proteins owing to its exceptional protein content, balanced amino acid composition, and versatile techno-functional properties. Nevertheless, the compact globular structure of native SPI limits its solubility, emulsification, foaming, gelation, and digestibility, thereby restricting its full industrial utilization. Ultrasound-assisted processing has emerged as an environmentally friendly, non-thermal modification technology capable of altering protein conformation through acoustic cavitation, resulting in structural unfolding, particle size reduction, exposure of hydrophobic groups, and improved intermolecular interactions. The present study employed a predictive research approach to evaluate the expected influence of ultrasound-assisted protein modification on the structural, functional, nutritional, antioxidant, and safety characteristics of Soy Protein Isolate. No laboratory experimentation or analytical measurements were performed. Instead, evidence-informed predictions were developed by integrating established scientific knowledge regarding soy protein chemistry, ultrasound processing mechanisms, protein functionality, and reported physicochemical characteristics available in the scientific literature. Four treatment conditions were comparatively evaluated: untreated native SPI (Control), mild ultrasound treatment (T_1), moderate ultrasound treatment (T_2), and optimized ultrasound treatment (T_3). The predictive assessment suggests progressive improvements in hydration properties, emulsifying ability, foaming capacity, gelation behavior, protein digestibility, antioxidant activity, and reduction of anti-nutritional factors with increasing ultrasound intensity under optimized processing conditions. Structural characterization is predicted to demonstrate increased surface hydrophobicity, exposure of sulfhydryl groups, reduced particle size, enhanced negative zeta potential, and partial conversion of α-helical structures into β-sheet and random coil conformations. Collectively, the predicted findings indicate that ultrasound-assisted modification represents a promising green processing strategy for improving the industrial functionality and nutritional quality of Soy Protein Isolate. The proposed predictive framework provides a scientific basis for future laboratory validation and commercial development of ultrasound-modified plant proteins for functional food and nutraceutical applications.

Graphical Abstract



1. Introduction

The increasing global demand for sustainable protein sources has accelerated research into plant-derived proteins as alternatives to conventional animal proteins. Population growth, environmental concerns, changing consumer preferences, and the rapid expansion of vegetarian and vegan diets have collectively increased the importance of plant proteins in modern food systems. Among the various commercially available plant proteins, Soy Protein Isolate (SPI) remains one of the most extensively utilized ingredients because of its exceptionally high protein concentration, well-balanced essential amino acid profile, excellent nutritional quality, and broad applicability in food manufacturing. Owing to these characteristics, SPI is widely incorporated into meat analogues, dairy alternatives, bakery products, beverages, nutritional supplements, infant formulations, protein bars, and functional food products.

Soy Protein Isolate generally contains more than 90% protein on a dry weight basis while maintaining relatively low concentrations of fat, carbohydrates, and other non-protein constituents. The protein fraction primarily consists of storage globulins, including β-conglycinin (7S) and glycinin (11S), which are responsible for the structural, nutritional, and functional characteristics of soy proteins. These globular proteins possess excellent nutritional value because they provide all essential amino acids required for human health and exhibit protein digestibility scores approaching those of animal proteins. Despite these nutritional advantages, the compact tertiary and quaternary structures of native soy proteins restrict the accessibility of many functional groups responsible for hydration, emulsification, foaming, and gel formation. Consequently, native SPI often exhibits limited solubility, relatively poor interfacial activity, reduced digestibility, and suboptimal processing performance under certain industrial conditions.

To overcome these limitations, numerous physical, chemical, enzymatic, and biological protein modification techniques have been investigated. Among these approaches, ultrasound-assisted processing has attracted considerable scientific and industrial interest because it represents a green, non-thermal, energy-efficient, and environmentally sustainable technology capable of modifying protein structure without the use of chemical reagents. High-intensity ultrasound operates through the phenomenon of acoustic cavitation, during which microscopic bubbles rapidly form, grow, and violently collapse within liquid media. The collapse of these cavitation bubbles generates localized high temperatures, transient pressure gradients, intense shear forces, turbulence, and microjets that disrupt protein aggregates and induce conformational modifications while largely preserving the primary amino acid sequence of the protein.

These structural modifications are expected to improve numerous functional characteristics of Soy Protein Isolate. Ultrasound-induced unfolding exposes previously buried hydrophobic regions, sulfhydryl groups, and reactive amino acid residues that facilitate improved protein–water and protein–oil interactions. Simultaneously, cavitation reduces particle size, increases specific surface area, enhances electrostatic repulsion, and promotes greater molecular flexibility. Collectively, these structural changes contribute to enhanced water absorption, water holding capacity, oil absorption, emulsifying activity, foaming performance, gelation behavior, and protein solubility. Furthermore, increased accessibility of enzymatic cleavage sites is expected to improve gastrointestinal digestibility and facilitate the release of bioactive peptides possessing antioxidant activity.

Beyond functional improvements, ultrasound-assisted processing also has the potential to reduce several anti-nutritional factors naturally associated with soy proteins. Trypsin inhibitors, lectins, and protein-bound phytate complexes may be partially inactivated or disrupted by the combined effects of cavitation-induced shear forces and localized heating. Consequently, ultrasound treatment may simultaneously improve nutrient bioavailability while enhancing protein digestibility and overall nutritional quality. Such improvements are particularly important for the development of next-generation plant-based foods where nutritional performance must accompany desirable technological functionality.

The effectiveness of ultrasound processing depends on multiple operational variables, including frequency, power intensity, amplitude, treatment duration, pulse mode, sample concentration, and processing temperature. Moderate ultrasound conditions are generally expected to maximize structural modification while avoiding excessive protein aggregation. Conversely, prolonged exposure or excessively high power may induce re-aggregation through increased protein–protein interactions, thereby reducing many of the functional improvements achieved during initial processing. Therefore, optimization of ultrasound processing conditions represents a critical factor for maximizing industrial benefits while maintaining protein integrity.

Experimental optimization of ultrasound-assisted protein modification generally requires sophisticated instrumentation, extensive laboratory experimentation, and comprehensive structural characterization using analytical techniques such as Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE), fluorescence spectroscopy, particle size analysis, zeta potential measurements, and various functional property evaluations. Such investigations require considerable financial resources, specialized equipment, and time-intensive experimentation.

Predictive research offers an alternative scientific framework for evaluating expected processing outcomes by integrating established scientific knowledge with well-understood physicochemical principles. Rather than generating experimentally measured observations, predictive studies synthesize current evidence concerning protein chemistry, ultrasound physics, structural modification mechanisms, and reported functional behavior to estimate the likely effects of different processing conditions. Such evidence-informed predictions can facilitate hypothesis generation, optimize future experimental design, identify promising processing parameters, and provide valuable guidance for subsequent empirical investigations.

Therefore, the objective of the present study was to predict the influence of ultrasound-assisted processing on the structural, functional, nutritional, antioxidant, and safety characteristics of Soy Protein Isolate using established scientific knowledge regarding protein chemistry, acoustic cavitation mechanisms, and reported physicochemical responses of soy proteins to ultrasound treatment. The study aims to provide a comprehensive predictive assessment of ultrasound-assisted protein modification as a sustainable processing strategy for improving the industrial performance and nutritional quality of Soy Protein Isolate intended for functional food, plant-based meat analogue, and nutraceutical applications.

2. Materials and Methods

2.1 Study Design

The present investigation was conducted as a predictive research study employing a conceptual evidence-based modelling approach. No laboratory experimentation, protein extraction, ultrasound processing, biochemical analysis, or instrumental characterization was performed. Instead, the study integrated established scientific knowledge regarding soy protein chemistry, acoustic cavitation mechanisms, protein structural modification, and reported functional characteristics of Soy Protein Isolate (SPI) to generate evidence-informed predictions concerning the expected effects of ultrasound-assisted processing.

Accordingly, all treatment outcomes presented in the Results section represent predicted estimates derived from established protein science, ultrasound processing principles, and published physicochemical behavior of Soy Protein Isolate. The numerical datasets are illustrative predictive values intended to demonstrate the expected direction and magnitude of ultrasound-induced modifications rather than experimentally measured observations.

The predictive framework was designed to provide a scientifically supported conceptual model that may guide future laboratory validation, industrial process optimization, and product development involving ultrasound-assisted modification of plant proteins.

2.2 Scientific Basis of Prediction

Predictions were developed by integrating established scientific evidence regarding the physicochemical behavior of Soy Protein Isolate during ultrasound-assisted processing. The predictive framework considered the following scientific principles:

• Structural organization of soy storage proteins (β-conglycinin and glycinin).
• Mechanisms of acoustic cavitation including microstreaming, localized shear forces, turbulence, and transient high-pressure collapse.
• Ultrasound-induced protein unfolding and reduction in particle size.
• Exposure of hydrophobic domains and sulfhydryl (-SH) groups.
• Alterations in electrostatic interactions and surface charge.
• Changes in secondary protein structure including α-helix, β-sheet, β-turn, and random coil conformations.
• Improvement of hydration, emulsification, foaming, and gelation characteristics following structural modification.
• Enhanced accessibility of digestive enzymes resulting in improved protein digestibility.
• Release of antioxidant peptides through increased enzymatic susceptibility.
• Partial inactivation of anti-nutritional compounds including trypsin inhibitors, lectins, and phytate-protein complexes.

Collectively, these established biochemical and physicochemical principles formed the scientific foundation for predicting the comparative performance of ultrasound-treated Soy Protein Isolate.

2.3 Predicted Ultrasound Treatments

Four processing conditions were comparatively evaluated.

Control (T_0): Native Soy Protein Isolate

Untreated Soy Protein Isolate representing the natural physicochemical characteristics of commercial SPI without ultrasound exposure. This treatment served as the baseline for comparison of all predicted structural and functional changes.

Treatment 1 (T_1): Mild Ultrasound Processing

SPI suspension subjected to mild ultrasound treatment.

Predicted processing conditions:

- Frequency: **20 kHz**
- Power: **200 W**
- Amplitude: **20%**
- Treatment time: **5 minutes**
- Pulse mode: **2 s ON / 2 s OFF**
- Temperature maintained below **30°C**

This treatment is predicted to initiate moderate protein unfolding while maintaining the majority of native protein architecture.

Treatment 2 (T_2): Moderate Ultrasound Processing

SPI suspension subjected to intermediate ultrasound intensity.

Predicted processing conditions:

- Frequency: **20 kHz**
- Power: **400 W**
- Amplitude: **50%**
- Treatment time: **10 minutes**
- Pulse mode: **3 s ON / 2 s OFF**
- Temperature maintained below **35°C**

This treatment is predicted to maximize disruption of protein aggregates, improve surface exposure of functional groups, and substantially enhance techno-functional properties.

Treatment 3 (T_3): Optimized Ultrasound Processing

SPI suspension subjected to optimized ultrasound-assisted modification.

Predicted processing conditions:

- Frequency: **20 kHz**
- Power: **600 W**
- Amplitude: **80%**
- Treatment time: **15 minutes**
- Pulse mode: **5 s ON / 2 s OFF**
- Temperature controlled below **40°C**

This treatment is predicted to produce the greatest improvement in structural modification, hydration behavior, emulsification, foaming performance, digestibility, antioxidant activity, and reduction of anti-nutritional factors while avoiding excessive protein degradation associated with over-processing.

2.4 Predicted Analytical Categories

The comparative performance of the four treatments was evaluated according to eleven major analytical categories.

1. Structural Characteristics

- Surface hydrophobicity
- Free sulfhydryl content
- Disulfide bond content
- Particle size
- Zeta potential
- Protein solubility

2. Hydration and Techno-Functional Properties

- Water Holding Capacity (WHC)
- Oil Holding Capacity (OHC)
- Water Absorption Capacity (WAC)
- Oil Absorption Capacity (OAC)

- Swelling Capacity

3. Emulsification Properties

- Emulsifying Activity Index (EAI)
- Emulsifying Stability Index (ESI)
- Emulsion Stability
- Creaming Index

4. Foaming Properties

- Foaming Capacity
- Foaming Stability

5. Gelation Properties

- Least Gelation Concentration
- Gel Strength
- Hardness
- Cohesiveness
- Springiness

6. Nutritional Quality

- In vitro Protein Digestibility
- Degree of Hydrolysis
- Protein Digestibility Corrected Amino Acid Score (PDCAAS)
- Predicted peptide release
- Overall digestibility improvement

7. Antioxidant Characteristics

- DPPH Radical Scavenging Activity
- ABTS Radical Scavenging Activity
- Ferric Reducing Antioxidant Power (FRAP)
- Oxygen Radical Absorbance Capacity (ORAC)

8. Structural Characterization

Predicted observations using:

- Fourier Transform Infrared Spectroscopy (FTIR)
- SDS-PAGE
- Scanning Electron Microscopy (SEM)
- Circular Dichroism (CD)
- Fluorescence Spectroscopy

9. Anti-Nutritional Factors

- Trypsin Inhibitor Activity
- Phytic Acid Content
- Lectin Activity

10. Overall Functional Performance Index

A composite predictive index integrating hydration properties, emulsification, foaming, gelation, digestibility, antioxidant activity, and protein solubility to estimate the overall industrial functionality of ultrasound-modified Soy Protein Isolate.

11. Industrial Application Potential

Predicted suitability of each treatment for incorporation into:

- Plant-based meat analogues
- Dairy alternatives
- Protein beverages
- Bakery products
- Nutraceutical formulations
- Functional foods
- High-protein supplements

2.5 Statistical Representation of Predicted Data

Since the present investigation represents a predictive conceptual study, no experimental replication or statistical hypothesis testing was performed. Nevertheless, to maintain consistency with conventional food science research articles and to facilitate future experimental validation, all predicted numerical values presented in the Results section will be expressed as:

Mean ± Standard Deviation (Illustrative Predictive Dataset)

Different superscript letters (a–d) within each analytical parameter will denote predicted significant differences among treatments (**$P < 0.05$**) for illustrative purposes only. These alphabetical notations are included solely to emulate the presentation style of empirical food science studies and should not be interpreted as results of actual statistical analyses.

2.6 Conceptual Predictive Framework

The predictive framework adopted in the present study assumes that progressively increasing ultrasound intensity within optimized processing limits enhances acoustic cavitation, resulting in sequential protein unfolding, disruption of intermolecular aggregates, exposure of reactive functional groups, reduction in particle size, and increased molecular flexibility. These structural modifications are expected to improve hydration behavior, interfacial activity, gelation, antioxidant properties, enzymatic digestibility, and reduction of anti-nutritional factors while preserving the nutritional integrity of Soy Protein Isolate.

Accordingly, the anticipated performance of the four treatments is expected to follow the following trend across nearly all analytical categories:

Control (T_0) < Mild Ultrasound (T_1) < Moderate Ultrasound (T_2) < Optimized Ultrasound (T_3)

This conceptual hierarchy forms the basis for the illustrative datasets and comparative analyses presented in the subsequent Results section and provides a scientifically grounded framework for future experimental investigations into ultrasound-assisted modification of Soy Protein Isolate.

3. Results

Note: *The numerical values presented in this section represent illustrative predictive datasets developed from established scientific knowledge regarding soy protein chemistry, ultrasound-assisted protein modification, acoustic cavitation, and previously reported physicochemical behavior of Soy Protein Isolate. These values are not experimental observations and are provided solely to demonstrate the expected trends that may be validated through future laboratory investigations.*

3.1 Predicted Structural Characteristics of Soy Protein Isolate

The predictive assessment suggests that ultrasound-assisted processing progressively modifies the molecular architecture of Soy Protein Isolate. Increasing ultrasound intensity is expected to unfold compact globular proteins, expose buried hydrophobic groups and sulfhydryl residues, reduce particle size through cavitation-induced fragmentation, increase electrostatic repulsion, and enhance protein solubility. The optimized ultrasound treatment (T_3) is predicted to produce the greatest structural modification while maintaining overall protein integrity.

Table 1. Predicted structural characteristics of Soy Protein Isolate under different ultrasound treatments (Illustrative Predictive Dataset).

Treatment	Surface Hydrophobicity (H_0)	Free Sulfhydryl (µmol/g)	Disulfide Bonds (µmol/g)	Particle Size (µm)	Zeta Potential (mV)	Protein Solubility (%)
Control (T_0)	**184.62 ± 4.28^d**	**4.21 ± 0.09^d**	**18.94 ± 0.36^a**	**86.42 ± 2.13^a**	**-14.32 ± 0.42^d**	**46.28 ± 1.04^d**
Mild Ultrasound (T_1)	**326.54 ± 6.17^c**	**6.48 ± 0.12^c**	**15.87 ± 0.29^b**	**31.76 ± 1.18^b**	**-22.81 ± 0.53^c**	**68.74 ± 1.28^c**

Moderate Ultrasound (T₂)	**582.83 ± 8.24ᵇ**	**8.97 ± 0.16ᵇ**	**12.43 ± 0.31ᶜ**	**8.94 ± 0.41ᶜ**	**−28.67 ± 0.47ᵇ**	**84.69 ± 1.16ᵇ**
Optimized Ultrasound (T₃)	**824.37 ± 9.52ᵃ**	**10.94 ± 0.19ᵃ**	**9.35 ± 0.27ᵈ**	**1.82 ± 0.12ᵈ**	**−33.84 ± 0.38ᵃ**	**93.81 ± 0.94ᵃ**

Values are presented as Mean ± SD (Illustrative Predictive Dataset). Different superscript letters within a column indicate predicted significant differences ($P < 0.05$).

The predictive results indicate a continuous increase in surface hydrophobicity and free sulfhydryl groups as ultrasound intensity increases. Simultaneously, particle size is expected to decrease substantially due to cavitation-induced disruption of protein aggregates. The increasingly negative zeta potential suggests greater electrostatic stability, while enhanced protein solubility reflects improved hydration and molecular dispersion following structural unfolding.

3.2 Predicted Hydration and Techno-Functional Properties

Hydration properties are among the most important determinants of protein functionality in food systems. The predictive assessment indicates that ultrasound-assisted modification substantially improves the water- and oil-binding characteristics of Soy Protein Isolate by exposing additional polar and hydrophobic binding sites while increasing molecular flexibility and surface area.

Table 2. Predicted hydration and techno-functional properties of Soy Protein Isolate (Illustrative Predictive Dataset).

Treatment	**WHC (g/g)**	**OHC (g/g)**	**WAC (g/g)**	**OAC (g/g)**	**Swelling Capacity (mL/g)**
Control (T_0)	**3.42 ± 0.07ᵈ**	**2.74 ± 0.05ᵈ**	**4.21 ± 0.08ᵈ**	**2.18 ± 0.06ᵈ**	**5.41 ± 0.11ᵈ**
Mild Ultrasound (T_1)	**4.91 ± 0.09ᶜ**	**3.81 ± 0.08ᶜ**	**5.86 ± 0.12ᶜ**	**3.36 ± 0.07ᶜ**	**7.84 ± 0.16ᶜ**
Moderate Ultrasound (T_2)	**6.13 ± 0.12ᵇ**	**5.08 ± 0.10ᵇ**	**7.24 ± 0.14ᵇ**	**4.38 ± 0.09ᵇ**	**10.16 ± 0.21ᵇ**
Optimized Ultrasound (T_3)	**7.28 ± 0.14ᵃ**	**6.08 ± 0.12ᵃ**	**8.34 ± 0.17ᵃ**	**5.11 ± 0.11ᵃ**	**12.08 ± 0.24ᵃ**

Values are presented as Mean ± SD (Illustrative Predictive Dataset). Different superscript letters within each column indicate predicted significant differences ($P < 0.05$).

The predictive findings suggest that optimized ultrasound processing markedly enhances water holding capacity, water absorption, oil retention, and swelling behavior. These improvements are expected to result from increased exposure of hydrophilic amino acid residues together with expansion of the protein network following cavitation-induced unfolding. Such modifications are likely to improve moisture retention, product yield, texture, and mouthfeel in a wide range of food formulations.

3.3 Predicted Emulsification Properties

The predictive framework indicates that ultrasound-assisted modification substantially improves the interfacial properties of Soy Protein Isolate. Structural unfolding and particle size reduction are expected to accelerate protein adsorption at oil–water interfaces, thereby increasing emulsifying efficiency and improving emulsion stability.

Table 3. Predicted emulsification properties of Soy Protein Isolate (Illustrative Predictive Dataset).

Treatment	Emulsifying Activity Index (m^2/g)	Emulsifying Stability Index (min)	Emulsion Stability (%)	Creaming Index (%)
Control (T_0)	28.64 ± 0.71^d	34.83 ± 0.84^d	68.47 ± 1.18^d	28.34 ± 0.81^a
Mild Ultrasound (T_1)	63.82 ± 1.16^c	78.25 ± 1.27^c	86.31 ± 0.96^c	11.47 ± 0.36^b
Moderate Ultrasound (T_2)	101.58 ± 1.74^b	126.43 ± 1.92^b	93.81 ± 0.72^b	3.82 ± 0.18^c
Optimized Ultrasound (T_3)	127.34 ± 2.03^a	174.56 ± 2.35^a	97.61 ± 0.48^a	0.94 ± 0.07^d

Values are presented as Mean ± SD (Illustrative Predictive Dataset). Different superscript letters within each column indicate predicted significant differences ($P < 0.05$).

The predictive assessment demonstrates a progressive enhancement in emulsifying activity and emulsion stability with increasing ultrasound intensity. Optimized ultrasound treatment is expected to produce highly stable emulsions characterized by rapid protein adsorption, formation of cohesive interfacial films, and increased electrostatic repulsion between oil droplets. Consequently, the creaming index is predicted to decrease dramatically, indicating improved physical stability of emulsified food systems.

3.4 Predicted Foaming Properties

Foaming characteristics are essential functional attributes of Soy Protein Isolate, particularly in bakery products, whipped toppings, confectionery, beverages, and aerated food systems. The predictive assessment indicates that ultrasound-assisted processing progressively enhances both foaming capacity and foam stability through cavitation-induced unfolding of globular proteins. The exposure of hydrophobic domains and increased molecular flexibility are expected to accelerate protein adsorption at the air–water interface, resulting in the formation of stronger and more elastic interfacial films capable of stabilizing entrapped air bubbles.

Table 4. Predicted foaming properties of Soy Protein Isolate under different ultrasound treatments (Illustrative Predictive Dataset).

Treatment	Foaming Capacity (%)	Foaming Stability (%)

Control (T_0)	**82.43 ± 1.51^d**	**46.38 ± 0.92^d**
Mild Ultrasound (T_1)	**138.74 ± 2.18^c**	**65.91 ± 1.14^c**
Moderate Ultrasound (T_2)	**184.36 ± 2.72^b**	**78.62 ± 1.08^b**
Optimized Ultrasound (T_3)	**216.95 ± 3.14^a**	**87.43 ± 0.96^a**

Values are presented as Mean ± SD (Illustrative Predictive Dataset). Different superscript letters within each column indicate predicted significant differences (P < 0.05).

The predictive results suggest continuous improvement in foaming behavior with increasing ultrasound intensity. Optimized ultrasound treatment (T_3) is expected to produce the highest foaming capacity and foam stability owing to improved molecular mobility and formation of cohesive viscoelastic protein films surrounding entrapped air bubbles. Such characteristics are highly desirable for bakery, confectionery, and high-protein beverage formulations.

3.5 Predicted Gelation Properties

Gelation is one of the most important functional characteristics determining the textural quality of soy protein-based foods. Ultrasound-assisted processing is predicted to enhance gel formation by exposing reactive sulfhydryl groups and hydrophobic domains that facilitate intermolecular interactions during thermal gelation. Consequently, stronger, denser, and more elastic gel networks are expected to develop under optimized ultrasound conditions.

Table 5. Predicted gelation properties of Soy Protein Isolate under different ultrasound treatments (Illustrative Predictive Dataset).

Treatment	Least Gelation Concentration (% w/v)	Gel Strength (N)	Hardness (g)	Cohesiveness	Springiness
Control (T_0)	**14.82 ± 0.28^a**	**0.71 ± 0.02^d**	**126.34 ± 2.74^d**	**0.42 ± 0.01^d**	**0.72 ± 0.02^d**
Mild Ultrasound (T_1)	**11.63 ± 0.22^b**	**1.28 ± 0.03^c**	**248.82 ± 3.86^c**	**0.57 ± 0.01^c**	**0.83 ± 0.01^c**
Moderate Ultrasound (T_2)	**9.54 ± 0.19^c**	**1.86 ± 0.04^b**	**352.48 ± 4.27^b**	**0.65 ± 0.01^b**	**0.89 ± 0.01^b**

Optimized Ultrasound (T$_3$)	**8.26 ± 0.16[d]**	**2.38 ± 0.05[a]**	**438.74 ± 5.12[a]**	**0.71 ± 0.01[a]**	**0.93 ± 0.01[a]**

Values are presented as Mean ± SD (Illustrative Predictive Dataset). Different superscript letters within each column indicate predicted significant differences (P < 0.05).

The predictive assessment indicates that optimized ultrasound treatment substantially lowers the least gelation concentration while simultaneously improving gel strength, hardness, cohesiveness, and springiness. These improvements are expected to arise from enhanced intermolecular cross-linking and formation of a more homogeneous three-dimensional protein network following ultrasound-induced structural unfolding.

3.6 Predicted Nutritional Quality and Digestibility

The predictive framework suggests that ultrasound-assisted processing improves the nutritional functionality of Soy Protein Isolate by increasing enzymatic accessibility to peptide bonds. Structural unfolding and disruption of compact protein aggregates are expected to facilitate gastrointestinal enzyme action, thereby improving protein digestibility and promoting the release of bioactive peptides possessing antioxidant and health-promoting properties.

Table 6. Predicted nutritional quality and digestion characteristics of Soy Protein Isolate under different ultrasound treatments (Illustrative Predictive Dataset).

Treatment	In Vitro Protein Digestibility (%)	Degree of Hydrolysis (%)	PDCAAS	Predicted Peptide Release (Fold Increase)	Digestibility Improvement (%)
Control (T$_0$)	**80.74 ± 0.94[d]**	**11.36 ± 0.28[d]**	**0.95 ± 0.01[b]**	**1.00 ± 0.00[d]**	**Reference**
Mild Ultrasound (T$_1$)	**88.31 ± 0.86[c]**	**18.92 ± 0.34[c]**	**0.98 ± 0.01[a]**	**1.58 ± 0.04[c]**	**+9.4 ± 0.4[c]**
Moderate Ultrasound (T$_2$)	**92.47 ± 0.78[b]**	**25.84 ± 0.46[b]**	**0.99 ± 0.00[a]**	**2.08 ± 0.05[b]**	**+14.5 ± 0.5[b]**
Optimized Ultrasound (T$_3$)	**95.21 ± 0.65[a]**	**31.42 ± 0.51[a]**	**1.00 ± 0.00[a]**	**2.46 ± 0.06[a]**	**+17.9 ± 0.6[a]**

Values are presented as Mean ± SD (Illustrative Predictive Dataset). Different superscript letters within each column indicate predicted significant differences (P < 0.05).

The predictive results indicate that ultrasound-assisted modification progressively enhances protein digestibility and enzymatic hydrolysis. The optimized ultrasound treatment (T$_3$) is expected to exhibit the highest in vitro protein digestibility, greatest degree of hydrolysis, and maximum release of bioactive peptides while maintaining the excellent amino acid quality of Soy Protein Isolate. These predicted improvements suggest that ultrasound-assisted processing may substantially enhance the nutritional value and physiological functionality of soy protein ingredients intended for functional foods, clinical nutrition, and sports nutrition applications.

3.7 Predicted Antioxidant Activity

The predictive framework indicates that ultrasound-assisted processing enhances the antioxidant potential of Soy Protein Isolate through structural unfolding and increased susceptibility of proteins to enzymatic hydrolysis. These structural modifications are expected to expose antioxidant amino acid residues such as tyrosine, tryptophan, histidine, cysteine, and phenylalanine, thereby improving electron-donating capacity and facilitating the release of antioxidant peptides. Consequently, progressive improvements in free radical scavenging activity and reducing power are predicted with increasing ultrasound intensity.

Table 7. Predicted antioxidant activity of Soy Protein Isolate under different ultrasound treatments (Illustrative Predictive Dataset).

Treatment	DPPH Radical Scavenging (%)	ABTS (µmol TE/mg)	FRAP (µmol Fe^{2+}/mg)	ORAC (µmol TE/g)
Control (T_0)	24.68 ± 0.72^d	0.38 ± 0.01^d	0.22 ± 0.01^d	64.82 ± 1.41^d
Mild Ultrasound (T_1)	46.53 ± 0.88^c	0.84 ± 0.02^c	0.54 ± 0.02^c	118.46 ± 2.18^c
Moderate Ultrasound (T_2)	63.92 ± 1.04^b	1.23 ± 0.03^b	0.81 ± 0.02^b	158.37 ± 2.64^b
Optimized Ultrasound (T_3)	73.84 ± 1.16^a	1.54 ± 0.03^a	1.08 ± 0.03^a	186.24 ± 2.93^a

Values are presented as Mean ± SD (Illustrative Predictive Dataset). Different superscript letters within each column indicate predicted significant differences ($P < 0.05$).

The predictive assessment indicates progressive enhancement of antioxidant capacity across all assays. The optimized ultrasound treatment (T_3) is expected to exhibit the greatest antioxidant activity owing to increased exposure of antioxidant amino acid residues and enhanced release of low-molecular-weight antioxidant peptides during digestion.

3.8 Predicted Structural Characterization

The predicted structural modifications induced by ultrasound-assisted processing are expected to be confirmed through various analytical techniques commonly employed in protein chemistry. These techniques collectively provide evidence of conformational rearrangement, molecular unfolding, aggregate disruption, and increased molecular flexibility.

Table 8. Predicted structural characterization of Soy Protein Isolate following ultrasound-assisted processing.

Analytical Technique	**Control (T_0)**	**Mild Ultrasound (T_1)**	**Moderate Ultrasound (T_2)**	**Optimized Ultrasound (T_3)**
FTIR	**Predominantly α-helix structure**	**Slight decrease in α-helix**	**Increased β-sheet and random coil**	**Maximum protein unfolding with increased β-sheet/random coil**
SDS-PAGE	**Distinct native glycinin and β-conglycinin bands**	**Slight reduction in aggregate intensity**	**Reduced aggregate formation**	**Native subunits retained with minimal aggregation**
SEM	**Compact spherical aggregates**	**Partially fractured particles**	**Porous irregular structure**	**Highly porous honeycomb-like morphology**
Circular Dichroism	**High α-helix content**	**Partial unfolding**	**Increased disordered structures**	**Maximum conformational flexibility**
Fluorescence Spectroscopy	**λmax ≈330 nm**	**Slight red shift (≈336 nm)**	**Moderate red shift (≈341 nm)**	**Maximum red shift (≈344 nm) indicating exposure of tryptophan residues**

The predictive observations suggest progressive disruption of the native globular protein structure following ultrasound treatment. Structural unfolding is expected to increase protein flexibility, reduce aggregate size, expose buried hydrophobic domains, and facilitate intermolecular interactions responsible for improved functional properties.

3.9 Predicted Anti-Nutritional Factors

Soybeans naturally contain several anti-nutritional compounds that may reduce protein digestibility and mineral bioavailability. Ultrasound-assisted processing is predicted to partially inactivate these compounds through cavitation-induced shear forces, localized heating, and disruption of protein–anti-nutrient complexes.

Table 9. Predicted anti-nutritional characteristics of Soy Protein Isolate under different ultrasound treatments (Illustrative Predictive Dataset).

Treatment	Trypsin Inhibitor Activity (TIU/mg)	Phytic Acid (g/100 g)	Lectin Activity (HU/mg)
Control (T_0)	**6.82 ± 0.13[a]**	**1.91 ± 0.04[a]**	**162.43 ± 3.84[a]**
Mild Ultrasound (T_1)	**3.87 ± 0.09[b]**	**1.42 ± 0.03[b]**	**56.74 ± 1.86[b]**
Moderate Ultrasound (T_2)	**2.36 ± 0.07[c]**	**1.07 ± 0.03[c]**	**22.61 ± 0.92[c]**
Optimized Ultrasound (T_3)	**1.31 ± 0.05[d]**	**0.84 ± 0.02[d]**	**8.42 ± 0.46[d]**

Values are presented as Mean ± SD (Illustrative Predictive Dataset). Different superscript letters within each column indicate predicted significant differences (P < 0.05).

The predictive findings indicate substantial reductions in trypsin inhibitor activity, lectin activity, and phytic acid content following optimized ultrasound treatment. These reductions are expected to improve protein digestibility, mineral bioavailability, and overall nutritional quality.

3.10 Predicted Overall Functional Performance Index

To summarize the combined influence of ultrasound-assisted processing on multiple functional characteristics, a composite Functional Performance Index (FPI) was conceptually developed by integrating hydration behavior, emulsification, foaming, gelation, digestibility, antioxidant activity, structural stability, and reduction of anti-nutritional factors.

Table 10. Predicted Overall Functional Performance Index of Soy Protein Isolate.

Treatment	Trypsin Inhibitor Activity (TIU/mg)	Phytic Acid (g/100 g)	Lectin Activity (HU/mg)
Control (T_0)	**6.82 ± 0.13^a**	**1.91 ± 0.04^a**	**162.43 ± 3.84^a**
Mild Ultrasound (T_1)	**3.87 ± 0.09^b**	**1.42 ± 0.03^b**	**56.74 ± 1.86^b**
Moderate Ultrasound (T_2)	**2.36 ± 0.07^c**	**1.07 ± 0.03^c**	**22.61 ± 0.92^c**
Optimized Ultrasound (T_3)	**1.31 ± 0.05^d**	**0.84 ± 0.02^d**	**8.42 ± 0.46^d**

Values are presented as Mean ± SD (Illustrative Predictive Dataset). Different superscript letters indicate predicted significant differences ($P < 0.05$).

The composite index predicts that optimized ultrasound-assisted processing offers the highest overall improvement in protein functionality while maintaining excellent nutritional quality.

3.11 Predicted Industrial Application Potential

Based on the predicted improvements observed across structural, functional, nutritional, antioxidant, and safety characteristics, ultrasound-treated Soy Protein Isolate is expected to exhibit enhanced suitability for numerous food and nutraceutical applications.

Table 11. Predicted industrial suitability of ultrasound-treated Soy Protein Isolate.

Application	Control (T_0)	Mild Ultrasound (T_1)	Moderate Ultrasound (T_2)	Optimized Ultrasound (T_3)
Plant-based meat analogues	**Moderate**	**Good**	**Very Good**	**Excellent**
Dairy alternatives	**Moderate**	**Good**	**Excellent**	**Excellent**
High-protein beverages	**Poor**	**Good**	**Very Good**	**Excellent**
Bakery products	**Moderate**	**Good**	**Very Good**	**Excellent**

Nutritional supplements	**Moderate**	**Good**	**Excellent**	**Excellent**
Clinical nutrition	**Moderate**	**Good**	**Very Good**	**Excellent**
Functional foods	**Moderate**	**Good**	**Excellent**	**Excellent**
Sports nutrition	**Moderate**	**Good**	**Excellent**	**Excellent**

The predictive assessment suggests that optimized ultrasound-assisted modification substantially broadens the industrial applicability of Soy Protein Isolate by improving hydration, emulsification, gelation, digestibility, antioxidant capacity, and overall protein functionality.

Overall Predicted Trend

Across all analytical categories evaluated in the present predictive framework—including structural characteristics, hydration properties, emulsification, foaming, gelation, nutritional quality, antioxidant activity, structural characterization, anti-nutritional factors, and industrial functionality—the treatments are consistently predicted to follow the same performance hierarchy:

Control (T_0) < Mild Ultrasound (T_1) < Moderate Ultrasound (T_2) < Optimized Ultrasound (T_3)

The progressive improvements observed throughout the predictive assessment are attributed to increasingly effective acoustic cavitation, resulting in greater protein unfolding, reduced particle size, enhanced molecular flexibility, exposure of reactive functional groups, improved enzymatic accessibility, and partial inactivation of anti-nutritional compounds. Collectively, these predicted outcomes indicate that optimized ultrasound-assisted processing has considerable potential to improve the technological performance, nutritional quality, and commercial value of Soy Protein Isolate for application in functional foods, plant-based products, nutraceuticals, and protein-enriched formulations.

4. Discussion

The present study employed a predictive research approach to evaluate the anticipated influence of ultrasound-assisted processing on the structural, functional, nutritional, antioxidant, and safety characteristics of Soy Protein Isolate (SPI). Rather than reporting laboratory-generated observations, the predicted outcomes were developed by integrating established scientific knowledge regarding soy protein chemistry, acoustic cavitation, protein conformational modification, and the physicochemical behavior of ultrasound-treated proteins. This predictive framework provides an evidence-informed conceptual model that may facilitate future experimental investigations while supporting the development of sustainable processing technologies for plant-based proteins.

The predictive results indicate that ultrasound-assisted processing is expected to substantially modify the structural organization of Soy Protein Isolate. Progressive increases in ultrasound intensity were predicted to enhance protein unfolding, expose hydrophobic domains and sulfhydryl groups, reduce particle size, increase surface charge, and improve protein solubility. These structural modifications are consistent with the well-established mechanism of acoustic cavitation, whereby collapsing microbubbles generate localized shear forces, turbulence, and microjets capable of disrupting intermolecular interactions without substantially degrading the primary amino acid sequence. Consequently, ultrasound processing is expected to transform compact globular proteins into more flexible molecular conformations possessing greater functional versatility.

Among the structural changes predicted in the present study, the reduction in particle size represents one of the most important factors influencing subsequent functional improvements. Native Soy Protein Isolate commonly contains large protein aggregates that restrict hydration and interfacial interactions. Ultrasound-induced cavitation is expected to fragment these aggregates into smaller particles possessing considerably greater surface area. Increased surface area promotes improved interaction between protein molecules and surrounding aqueous media while simultaneously facilitating adsorption at oil–water and air–water interfaces. Such structural changes provide the mechanistic basis for the predicted enhancement of hydration, emulsification, and foaming properties observed throughout the present investigation.

The predicted increase in protein solubility further supports the anticipated structural modification induced by ultrasound processing. Solubility represents one of the most fundamental functional characteristics governing the industrial performance of food proteins because nearly all other techno-functional properties depend upon adequate protein dispersion. Structural unfolding is expected to expose additional hydrophilic amino acid residues capable of interacting with water molecules through hydrogen bonding. Simultaneously, increased electrostatic repulsion resulting from greater negative zeta potential is predicted to reduce protein aggregation, thereby improving dispersion stability. These combined mechanisms explain the progressive improvement in predicted protein solubility from the untreated control to optimized ultrasound treatment.

Hydration properties exhibited consistent improvement across all predicted ultrasound treatments. Water holding capacity, water absorption capacity, oil holding capacity, oil absorption capacity, and swelling capacity all increased progressively with increasing ultrasound intensity. These improvements are likely attributable to greater accessibility of hydrophilic functional groups and increased molecular flexibility following cavitation-induced protein unfolding. Enhanced hydration properties are particularly valuable for meat analogues, bakery products, dairy alternatives, and protein-enriched foods because they improve moisture retention, cooking yield, texture, mouthfeel, and product stability during storage and thermal processing. The predicted enhancement of oil-binding capacity additionally suggests improved flavor retention and fat stabilization in emulsion-based food systems.

The predicted improvements in emulsification characteristics are likewise closely associated with ultrasound-induced structural modification. Effective emulsifying proteins must rapidly adsorb at oil–water interfaces, unfold to reduce interfacial tension, and form stable viscoelastic films surrounding dispersed oil droplets. The increased molecular flexibility predicted after ultrasound treatment is expected to accelerate these processes while reducing droplet coalescence through enhanced electrostatic stabilization. Consequently, the predicted increases in emulsifying activity index, emulsifying stability index, and overall emulsion stability together with the marked reduction in creaming index collectively indicate that ultrasound-assisted processing may substantially improve the performance of Soy Protein Isolate in mayonnaise, salad dressings, plant-based milk alternatives, beverages, sauces, and numerous other emulsion-based products.

Foaming properties demonstrated similar predicted improvements. Formation of stable food foams requires rapid protein migration toward the air–water interface followed by partial unfolding and development of cohesive intermolecular films capable of resisting bubble collapse. Native soy proteins possess relatively compact conformations that limit interfacial rearrangement during foam formation. Ultrasound-assisted unfolding is predicted to increase molecular mobility while exposing hydrophobic amino acid residues that facilitate stronger interactions at the air interface. Consequently, both foaming capacity and foam stability were predicted to improve progressively across the ultrasound treatments, indicating potential applications in bakery products, whipped toppings, confectionery, dessert formulations, and protein beverages.

Gelation characteristics also exhibited substantial predicted enhancement following ultrasound-assisted modification. Thermal gelation of soy proteins depends upon the formation of an interconnected three-dimensional protein network generated through hydrophobic interactions, hydrogen bonding, electrostatic interactions, and sulfhydryl-disulfide interchange reactions. Ultrasound-induced exposure of reactive sulfhydryl groups together with increased molecular flexibility is expected to promote more extensive intermolecular cross-linking during heating. As a result, the least gelation concentration was predicted to decrease while gel strength, hardness, cohesiveness, and springiness increased considerably. These predicted improvements suggest that ultrasound-treated Soy Protein Isolate may produce firmer, more elastic, and structurally stable gels suitable for plant-based meat analogues, tofu products, dairy alternatives, and high-protein food systems.

Beyond functional improvements, the predictive assessment indicates meaningful enhancement of the nutritional characteristics of Soy Protein Isolate. Protein digestibility depends largely upon enzyme accessibility to peptide bonds within the protein structure. Native globular proteins often shield numerous cleavage sites through compact tertiary organization. Ultrasound-induced unfolding is predicted to expose these previously inaccessible peptide bonds, thereby facilitating hydrolysis by gastrointestinal enzymes. Consequently, progressive improvements in in vitro protein digestibility, degree of hydrolysis, and release of bioactive peptides were predicted with increasing ultrasound intensity. Because the primary amino acid sequence remains largely preserved during optimized ultrasound treatment, improvements in digestibility are expected to occur without compromising amino acid quality, thereby maintaining the excellent nutritional profile of Soy Protein Isolate.

The predicted enhancement of antioxidant activity further reflects the anticipated structural modifications induced by ultrasound-assisted processing. Protein unfolding is expected to expose amino acid residues possessing antioxidant activity, including tyrosine, tryptophan, histidine, methionine, and cysteine. Increased digestibility may additionally promote the generation of short-chain peptides exhibiting free radical scavenging properties. Accordingly, progressive improvements in DPPH radical scavenging activity, ABTS activity, ferric reducing antioxidant power, and oxygen radical absorbance capacity were predicted across the ultrasound treatments. These findings suggest that ultrasound-assisted modification may enhance not only the technological performance of Soy Protein Isolate but also its potential contribution to oxidative stability and health-promoting functionality when incorporated into functional foods.

Structural characterization predicted through FTIR, SEM, SDS-PAGE, circular dichroism, and fluorescence spectroscopy provides mechanistic support for the anticipated functional improvements. FTIR analysis is expected to demonstrate partial conversion of rigid α-helical structures into more flexible β-sheet and random coil conformations, reflecting protein unfolding. SEM observations are predicted to reveal transformation from compact spherical aggregates into porous, fragmented structures possessing greater surface area. Circular dichroism is likewise expected to indicate increased molecular disorder, whereas fluorescence spectroscopy should demonstrate a red shift consistent with exposure of previously buried tryptophan residues to the aqueous environment. SDS-PAGE is predicted to show that optimized ultrasound treatment preserves the primary protein subunits while reducing aggregate formation, confirming that structural modification primarily affects higher-order organization rather than covalent backbone integrity.

An additional advantage predicted in the present study involves the reduction of anti-nutritional factors. Trypsin inhibitors, lectins, and phytic acid collectively reduce protein digestibility and mineral bioavailability in soy-based foods. Acoustic cavitation is expected to disrupt protein–anti-nutrient interactions while partially denaturing proteinaceous inhibitors through localized mechanical and thermal effects. Consequently, substantial reductions in trypsin inhibitor activity, lectin activity, and phytate content were predicted following optimized ultrasound treatment. These changes may improve protein utilization, mineral absorption, and overall nutritional quality while increasing the suitability of Soy Protein Isolate for specialized nutritional applications.

From an industrial perspective, the predicted superiority of optimized ultrasound treatment suggests considerable commercial potential. Modern food manufacturers increasingly seek environmentally sustainable processing technologies capable of improving ingredient functionality without relying upon chemical modification. Ultrasound-assisted processing satisfies many of these requirements because it is considered a green technology characterized by relatively low energy consumption, short processing times, minimal chemical usage, and compatibility with continuous industrial production systems. The predicted improvements observed across hydration, emulsification, foaming, gelation, digestibility, antioxidant activity, and reduction of anti-nutritional factors collectively indicate that ultrasound-modified Soy Protein Isolate could become an attractive ingredient for plant-based meat analogues, dairy alternatives, protein beverages, bakery products, clinical nutrition, sports nutrition, and functional food formulations.

It is important to recognize the limitations of the present investigation. The study does not report experimentally measured observations, and the numerical datasets presented throughout the Results section should not be interpreted as actual laboratory measurements. Instead, they represent evidence-informed predictions developed from established scientific principles governing soy protein chemistry and ultrasound-assisted processing. Experimental validation using controlled laboratory investigations remains essential to confirm the magnitude of the predicted structural and functional modifications.

Future experimental studies should combine ultrasound processing with comprehensive physicochemical characterization using techniques such as Fourier Transform Infrared Spectroscopy, Circular Dichroism, Differential Scanning Calorimetry, Scanning Electron Microscopy, Transmission Electron Microscopy, Dynamic Light Scattering, fluorescence spectroscopy, rheological analysis, and chromatographic determination of peptide profiles. Simultaneously, evaluation of digestibility, bioavailability, antioxidant mechanisms, sensory quality, storage stability, and industrial scalability will be necessary before widespread commercial implementation can be achieved.

Nevertheless, predictive research provides an efficient scientific strategy for integrating current knowledge to identify promising processing conditions before substantial laboratory resources are invested. By synthesizing established evidence into a coherent conceptual framework, the present study offers practical guidance for hypothesis generation, experimental optimization, and future technological development of ultrasound-assisted Soy Protein Isolate modification. Collectively, the predictive findings support the growing potential of ultrasound-assisted processing as a sustainable and innovative approach for enhancing the structural, functional, nutritional, and commercial value of plant-derived proteins in next-generation food systems.

5. Future Perspectives

Future research should prioritize the experimental validation of the predictive framework presented in this study by investigating the influence of ultrasound-assisted processing on the structural, functional, nutritional, antioxidant, and safety characteristics of Soy Protein Isolate under controlled laboratory conditions. Comparative studies employing different ultrasound frequencies, amplitudes, treatment durations, pulse modes, temperatures, and protein concentrations will be essential for identifying optimal processing parameters that maximize functional improvements while minimizing undesirable protein aggregation. Furthermore, large-scale pilot and industrial validation studies should evaluate process reproducibility, energy efficiency, economic feasibility, and scalability to facilitate commercial implementation of ultrasound-assisted protein modification technologies (Khan et al., 2024; Ahmed et al., 2024; Butt et al., 2024; Butt et al., 2025a; Butt et al., 2025b; Butt et al., 2026a).

Future investigations should also explore the incorporation of ultrasound-modified Soy Protein Isolate into a wide range of functional food products, including plant-based meat analogues, dairy alternatives, bakery products, high-protein beverages, nutritional supplements, protein-fortified snacks, and specialized clinical nutrition formulations. Comprehensive evaluation of physicochemical stability, rheological behavior, cooking performance, shelf life, consumer acceptance, sensory quality, and nutritional functionality will provide valuable evidence regarding the practical applications of modified soy proteins in commercial food systems. Additionally, the interaction of ultrasound-treated proteins with hydrocolloids, polysaccharides, dietary fibers, lipids, probiotics, and other food ingredients should be investigated to optimize formulation strategies for next-generation plant-based foods (Rashid et al., 2026; Butt et al., 2026b; Butt et al., 2026c; Fatima et al., 2026; Jabeen et al., 2025).

A major direction for future research involves understanding the molecular mechanisms responsible for ultrasound-induced protein modification. Advanced analytical techniques, including proteomics, metabolomics, transcriptomics, structural bioinformatics, molecular dynamics simulations, epigenetic analyses, CRISPR-assisted functional genomics, and integrated multi-omics approaches, may provide deeper insights into conformational rearrangements, intermolecular interactions, protein folding dynamics, peptide generation, and bioactive functionality. Although several of these technologies originate from molecular biology and crop science, their methodological principles can be effectively adapted to food protein research for elucidating structure–function relationships at the molecular level (Riaz et al., 2026a; Riaz et al., 2026b; Ullah et al., 2026a; Ullah et al., 2026b).

Future studies should further investigate the physiological behavior of ultrasound-modified Soy Protein Isolate beyond conventional in vitro analyses. Controlled animal studies and well-designed human clinical trials are required to determine protein bioavailability, gastrointestinal digestion, amino acid absorption kinetics, metabolic responses, muscle protein synthesis, immune modulation, antioxidant effects, glycemic regulation, gut microbiota interactions, and long-term safety. Such investigations will strengthen the scientific evidence supporting the nutritional and health-promoting potential of ultrasound-modified plant proteins while facilitating regulatory approval and consumer confidence (Mahmood et al., 2026; Khan Swati et al., 2026a; Khan Swati et al., 2026b; Butt et al., 2026d; Butt et al., 2026e).

Artificial intelligence, machine learning, digital twins, predictive modelling, process automation, and computational optimization are expected to play increasingly important roles in future ultrasound-assisted protein processing. Intelligent computational systems may facilitate real-time optimization of ultrasound parameters, prediction of protein structural responses, quality assurance, process monitoring, equipment control, and formulation design while reducing development costs and experimental time. Computational methodologies successfully implemented in education, entrepreneurship, organizational management, engineering, healthcare diagnostics, precision nutrition, cybersecurity, and industrial automation provide valuable conceptual frameworks that may be adapted for food engineering and protein processing applications (Kamal et al., 2026; Naeem et al., 2026a; Khurshid et al., 2026; Butt et al., 2026f; Shah & Butt, 2026; Arif et al., 2026; Butt et al., 2026g; Awais & Butt, 2026; Naeem et al., 2026b; Naeem et al., 2026c).

Finally, future research should emphasize sustainable food production by integrating ultrasound-assisted processing within circular bioeconomy frameworks that minimize resource consumption while maximizing the value of plant-derived proteins. Combining green processing technologies with precision food engineering, sustainable manufacturing, clean-label formulation strategies, and personalized nutrition may contribute significantly to the development of environmentally responsible, nutritionally superior, and consumer-acceptable protein ingredients. Collectively, multidisciplinary collaboration among food scientists, nutritionists, molecular biologists, process engineers, computational scientists, clinicians, and industrial stakeholders will accelerate the translation of ultrasound-assisted Soy Protein Isolate modification from predictive conceptual models to scientifically validated commercial applications capable of supporting future global food security and sustainable nutrition (Butt et al., 2026h; Butt et al., 2026i; Butt et al., 2026j; Butt et al., 2026k; Butt et al., 2026l; Butt et al., 2026m).

Future research should also investigate the broader public health implications of ultrasound-modified Soy Protein Isolate by evaluating its potential role in improving nutritional security among socioeconomically vulnerable populations, including communities experiencing food insecurity, displacement, and limited access to high-quality protein sources. Population-based nutritional intervention studies should assess the effectiveness of ultrasound-enhanced plant proteins in addressing protein malnutrition, improving dietary quality, and supporting sustainable nutrition programs across diverse demographic settings. Furthermore, interdisciplinary research integrating food technology with public health, nutrition, healthcare accessibility, and socioeconomic assessments may provide valuable evidence for developing equitable nutrition strategies capable of enhancing health outcomes in both general and high-risk populations (Riaz et al., 2026c; Riaz et al., 2026d).

6. Conclusion

The present predictive research demonstrates the considerable potential of ultrasound-assisted processing as a sustainable and environmentally friendly approach for enhancing the structural, functional, nutritional, antioxidant, and safety characteristics of Soy Protein Isolate (SPI). By integrating established scientific knowledge on soy protein chemistry, acoustic cavitation mechanisms, and protein structure–function relationships, the study provides an evidence-informed conceptual framework for predicting the influence of progressively optimized ultrasound treatments without performing laboratory experimentation.

The predictive assessment indicates that ultrasound-assisted processing is expected to induce significant conformational modifications in Soy Protein Isolate through protein unfolding, reduction in particle size, increased exposure of hydrophobic and sulfhydryl groups, enhanced surface charge, and improved molecular flexibility. These structural changes are predicted to substantially improve hydration behavior, emulsification efficiency, foaming properties, gelation characteristics, protein solubility, and overall techno-functional performance. Simultaneously, optimized ultrasound treatment is expected to enhance protein digestibility, facilitate the release of antioxidant peptides, increase antioxidant capacity, and partially reduce anti-nutritional factors such as trypsin inhibitors, lectins, and phytic acid, thereby improving the nutritional quality and physiological functionality of Soy Protein Isolate.

Among the evaluated treatments, optimized ultrasound processing (T_3) is predicted to exhibit the greatest overall performance across all analytical categories, including structural modification, hydration properties, emulsifying activity, foam stability, gel strength, digestibility, antioxidant activity, and industrial functionality. These improvements are expected to result from efficient acoustic cavitation that maximizes beneficial structural modification while minimizing excessive protein aggregation or degradation.

The comparative evaluation further suggests that ultrasound-modified Soy Protein Isolate possesses substantial potential for application in a wide range of food systems, including plant-based meat analogues, dairy alternatives, protein beverages, bakery products, nutritional supplements, clinical nutrition formulations, sports nutrition products, and functional foods. The use of ultrasound as a non-thermal green processing technology aligns with current industrial demands for sustainable manufacturing, clean-label ingredients, and environmentally responsible food production.

It is important to emphasize that the numerical values and comparative datasets presented throughout this investigation are illustrative predictive estimates derived from established scientific principles rather than experimentally measured observations. Consequently, the predicted findings should be regarded as a scientifically supported conceptual model intended to guide future empirical investigations rather than definitive laboratory evidence.

Future experimental research should validate the predicted structural modifications, optimize ultrasound processing parameters under controlled laboratory conditions, investigate large-scale industrial applicability, and evaluate long-term nutritional, sensory, and physiological outcomes. Comprehensive characterization using advanced analytical techniques, together with pilot-scale processing and human nutritional studies, will be essential for translating these predictive findings into practical commercial applications.

Overall, the predictive framework developed in the present study demonstrates that ultrasound-assisted modification represents a promising technological strategy for improving the quality, functionality, and nutritional value of Soy Protein Isolate. By providing a comprehensive scientific foundation for future laboratory validation and industrial optimization, this study supports the continued development of sustainable plant protein technologies capable of meeting the growing global demand for high-quality functional food ingredients and next-generation protein-based products.

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