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Ultrasound-assisted innovations in protein processing: review

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ABSTRACT

The contemporary landscape of protein processing is witnessing a paradigm shift propelled by innovative technologies. This review unveils innovations in protein processing through the lens of an ultrasound-assisted approach. The focus was on the interplay between ultrasound waves and proteins during ultrasound extraction technology. The realm of protein extraction, where traditional methods face challenges and ultrasound emerges as a transformative force, was highlighted, as well as ultrasound's role in enhancing protein yield and quality in relationship to protein structure and function. Comparative analyses have showcased the remarkable advancements ushered in by ultrasound-assisted techniques, and this review also extends to enzymatic hydrolysis, where ultrasound catalyses reactions, unlocking new dimensions in the production of bioactive peptides and nutritionally enriched proteins. In the bio-industrial sectors, ultrasound facilitates protein refolding and revolutionises recombinant protein production, stability and bioavailability. Ultrasound has emerged as a catalyst for efficiency and bioactivity enhancement, defeating conventional limitations to the intricate optimisation strategies of refolding. This review envisages the advantages of ultrasound technology and its applications in the bio-industrial sector. The prospects of ultrasound-assisted protein processing are outlined, and roadmaps and processing techniques are offered.

Keywords: protein processing, ultrasound-assisted innovations, protein extraction, enzymatic hydrolysis, biopharmaceutical industry.

INTRODUCTION

Ultrasound-assisted protein processing advances have arisen as a focus of study, motivated by their potential to improve substrate efficiency and biological activity. This technological paradigm has been used in various fields, including extraction, modification, and mitigating freezing/thawing-induced oxidation. Key breakthroughs and applications demonstrate the adaptability and effectiveness of ultrasound-assisted protein processing. In enzymatic protein hydrolysis, ultrasound is a non-thermal processing method that is both environmentally friendly and efficient. Notably, ultrasonic pretreatment-assisted enzymatic hydrolysis stands out for its ability to considerably improve the efficiency of enzymatic processes while also increasing the biological activity of substrates. This method is mostly helpful for extracting bioactive compounds and breaking down biological macromolecules **[1]**. Another remarkable use is the extraction of proteins from watermelon seeds, which are considered food processing waste. Although watermelon seeds are a byproduct, they contain high-quality proteins. The inquiry into ultrasound-assisted extraction techniques has shown encouraging results. The optimal conditions, which included a pH of 11, a sonication temperature of 45 °C, and a sonication period of 10 minutes, resulted in maximal protein recovery at an astounding rate of 85.81% **[2]**. Additionally, ultrasound-assisted extraction techniques have been explored to obtain proteins from faba beans, complementing conventional methods. This research extends beyond ultrasound to encompass high-pressure processing and hydrodynamic cavitation. The overarching goal is to pioneer efficient and innovative protein extraction methods, focusing on plant-based sources. This approach represents a concerted effort to advance extraction technologies, offering sustainable solutions for protein procurement from diverse agricultural resources **[3]**.

Protein processing techniques have evolved due to advances in biotechnology and molecular biology and the continual development of protein purification, characterisation, and analysis methods. Notable trends in protein processing include a variety of transformational advances. Artificial intelligence and machine learning have played critical roles in transforming the environment. Advances in AI and machine learning have resulted in unique methods for processing and evaluating protein data. Inspired by biological systems, these strategies seek to improve our knowledge of protein interactions and their consequences in illness. Biophysical methods have advanced significantly, allowing researchers to analyse protein interactions **[3]**. X-ray crystallography, nuclear magnetic resonance (NMR), and atomic force microscopy (AFM) have all evolved to help researchers investigate protein structures, domains, and sequence patterns involved in protein-protein interactions. These advances allow for the quick examination of numerous samples with tiny quantities of material, making them ideal for genome- or proteome-wide research to uncover new therapeutic targets **[4]**. The field of Protein Extraction Technologies has seen significant advances. New approaches have arisen, such as ultrasound-assisted extraction, high-pressure processing, and hydrodynamic cavitation, which improve the efficiency and efficacy of protein extraction from a variety of sources. These approaches have practical uses in sectors such as food processing and pharmaceuticals, helping to acquire high-quality proteins **[5]**. Genomic Fishing and Data Processing tools have also emerged, allowing for extracting and analysing particular gene sequences from taxonomically varied genomic data. This technique contributes to molecular evolution research by detecting adaptive/purifying selection and reconstructing ancestral proteins **[6]**.

Challenges in Traditional Protein Processing

Traditional protein processing technologies face problems, prompting the investigation of novel options. Traditional heat-based processes like salting, smoking, and frying are notoriously energy-intensive and time-consuming. These approaches result to higher carbon footprints and greenhouse gas emissions, creating sustainability issues. Second, classic technologies such as ultrasonography have limits in thick and complicated food matrices, notably those high in protein. Penetrating deeply into such matrices is difficult, preventing consistent processing and extraction inside these complex structures. Third, standard approaches for limiting microbial growth and eliminating pathogens may not always provide optimal food safety and quality. This variation raises issues regarding shelf life and potential food loss **[7]**.

Ultrasound-assisted technology emerges as a key actor in protein processing, delivering significant benefits while addressing unique obstacles. Recent advances highlight its promise in extracting, modifying, and mitigating freezing/thawing-induced protein oxidation. The role of ultrasonography in protein processing is diverse. For starters, it is significantly more efficient and sustainable in food processing, making it a possible alternative to existing techniques. Ultrasound technology can provide identical outcomes with less energy input, resulting in lower energy consumption, a smaller carbon footprint, and fewer greenhouse gas emissions **[8]**. Second, ultrasonic processing technology improves food safety and quality. This application demonstrates the potential of ultrasound technology to bring about novel advancements in protein modification within food manufacturing. Despite these promising attributes, ultrasound technology faces challenges, particularly in processing dense and complex food matrices like proteins. Ongoing research and development efforts are poised to address these limitations, with the expectation of expanding the scope and applications of ultrasound **[9]**. Ultrasound technology is a potential force in protein processing, providing various benefits such as increased energy efficiency, higher food safety standards, and the capacity to extract proteins from unorthodox sources. Recent findings from comprehensive research highlight the comparative benefits of ultrasound in food processing, positioning it as a more energy-efficient and ecologically friendly alternative to existing techniques. This difference substantially contributes to the overall aim of reducing energy usage and greenhouse gas emissions. One prominent characteristic of ultrasonic technology is its ability to improve food safety by successfully suppressing microbial development and eradicating infections. This feature results in a longer shelf life for processed foods and a corresponding reduction in food waste, which aligns with larger sustainability goals. Furthermore, ultrasound-assisted extraction techniques have been examined for their usefulness in extracting significant protein yields from alternate sources, as demonstrated by experiments on watermelon seeds. This novel technique helps to repurpose food processing waste and promotes extracting high-quality proteins from non-traditional sources. However, it is critical to recognise the limitations of ultrasonic efficacy, especially in thick and complicated food matrices, including proteins. This intrinsic problem needs continual research and development efforts to address and overcome these limitations, ensuring the technology performs optimally in varied processing contexts **[7]**. Figure 1 depicts how the laboratory-scale ultrasonic technique works.

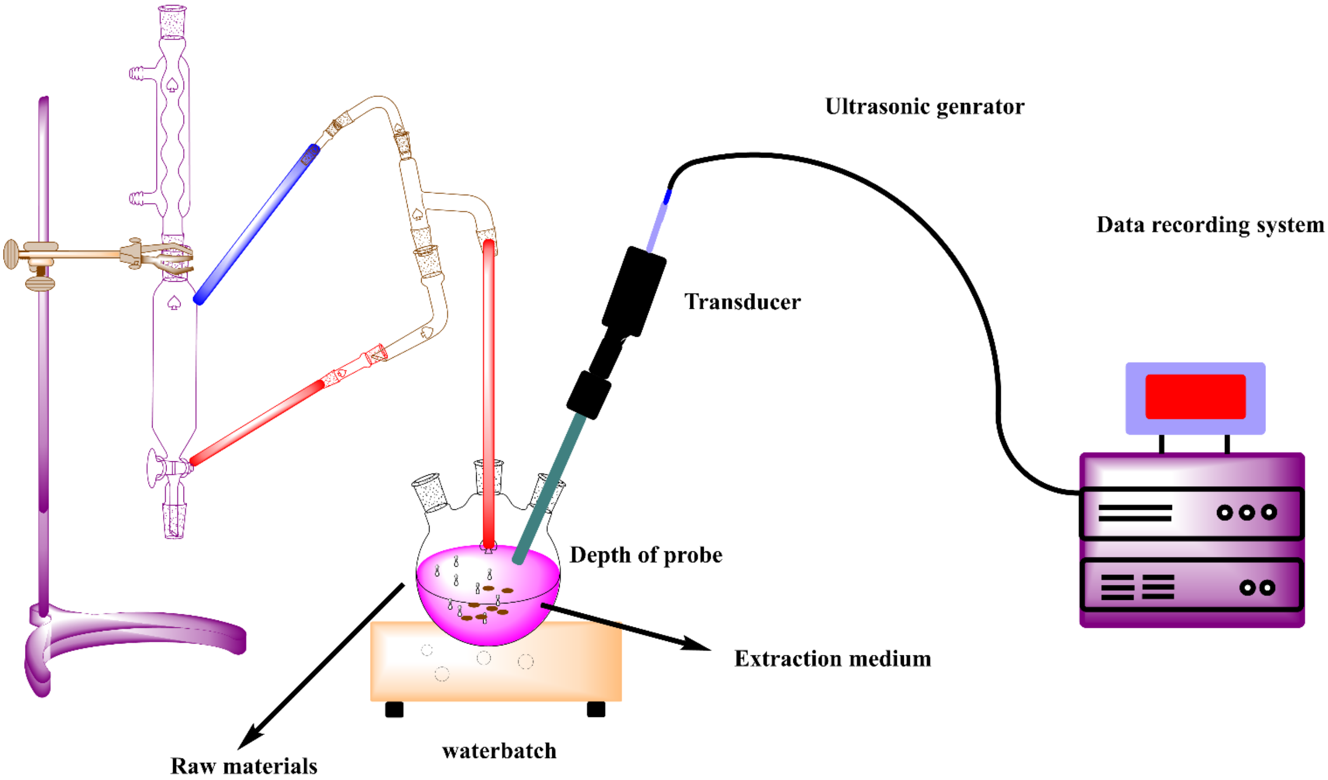

Figure 1. Lab scale of ultrasonic-assisted extraction technique.

Advantages of Ultrasound Technology

Ultrasound technology has emerged as a key tool in protein processing, with several benefits supported by study findings. It is known for its superior energy efficiency and environmental effect compared to standard processing methods. This technical technique can provide equivalent effects with less energy input, considerably contributing to decreasing energy consumption, lowering the carbon footprint, and reducing greenhouse gas emissions **[7]**. Second, ultrasonic processing technology is essential in improving food safety and quality. Its capacity to prevent microbial development and remove pathogens results in longer shelf life for processed goods, reducing food waste. This quality is consistent with the food industry's broader sustainability and resource optimisation goals. Furthermore, ultrasonic technology aids in the extraction of valuable biochemicals from biomass. This feature makes extraction more accessible and allows their use in waste valorisation and biorefinery processes. The extraction of essential biochemicals aids in biomass's more sustainable and resource-efficient use. Enzymatic protein hydrolysis is one example of ultrasonic technology's use in protein processing. Ultrasound, a green and efficient non-thermal processing technology, aids enzymatic hydrolysis, considerably boosting efficiency and increasing substrate biological activity. This use is especially noteworthy for the extraction of bioactive compounds and the breakdown of biological macromolecules **[1]**.

Ultrasound technology has the potential to significantly affect the food business, as highlighted in several significant areas. To begin with, ultrasonic technology is considered superior to standard processing methods regarding energy efficiency and environmental effects. This distinction helps to reduce energy use, carbon footprint, and GHG emissions. The potential cost reductions and enhanced sustainability performance provide significant opportunities for food processing enterprises **[10]**. Second, ultrasonic processing technology is a game changer for improving food safety and quality. It increases the shelf life of processed foods by successfully preventing microbial growth and removing pathogens, resulting in less food waste. This protects the quality and safety of food items and promotes consumer happiness and confidence, which are critical in the food sector **[11]**. Furthermore, ultrasonic technology is essential for extracting valuable biochemicals from biomass. This skill offers opportunities for their use in waste valorisation and biorefinery processes, promoting the development of new sustainable food products and processing procedures. Ultrasound has found valuable applications in meat preparation, replacing or supplementing existing procedures. Its functions in cutting, degassing, and meat tenderisation provide prospects for enhanced meat processing and the development of innovative products. However, it is critical to recognise the current limits, most notably the efficiency of ultrasound in dense and complicated dietary matrices, particularly those high in protein. Addressing these problems necessitates continual research and development activities, which have the potential to broaden the uses of ultrasound technology in the food business. Successfully overcoming these limits may signal a transformational age in which ultrasonic technology contributes significantly to the food business's sustainability, innovation, and development **[12]**.

Ultrasound emerges as an effective tool for illuminating the complexities of structures, including characteristics such as forms, sizes, and textures, as well as determining spatial linkages and placements. It functions as a type of energy that travels through media such as air or water in the form of waves **[13]**. The fundamental characteristics of ultrasonography include numerous crucial components. To begin, ultrasound is created by a transducer, which converts electrical energy into mechanical vibrations that eventually produce sound waves. The transducer, made up of piezoelectric crystals, vibrates when an electrical current is applied, causing sound waves to travel through the surrounding material. Understanding the physics and apparatus of ultrasonography is critical for enhancing diagnostic pictures and assuring safety. This understanding entails comprehending the relationship between frequency, penetration, and resolution. It involves knowing how the size and shape of a transducer's footprint assist beam access to target structures and recognising the medium's effect on ultrasonic energy **[14]**. ultrasonic-assisted extraction is a prominent application that uses ultrasonic waves to facilitate the extraction of bioactive substances from various sources, including plants and animal tissue. These waves damage cell walls by forming cavitation bubbles, resulting in better yields and shorter extraction times. Recent developments in ultrasound technology have accelerated its use in clinical practice, particularly at the point of care. Ultrasound investigations are conducted and interpreted by trained doctors from many healthcare areas, who contribute to patient evaluation and management. The real-time nature of point-of-care ultrasonography allows doctors to integrate generated information smoothly into the current assessment and management procedures **[15]**.

Basic principles of ultrasound

The fundamental concepts of ultrasonography include production, propagation, and applications. Ultrasound is a type of mechanical energy with a frequency that exceeds the human hearing range, usually exceeding 20 kHz. It is created by a transducer, which transforms electrical energy into mechanical vibrations that result in sound waves. These sound waves propagate across a medium, such as air or water, and may be employed for various applications, including medical imaging, industrial testing, and therapeutic treatments **[16]**. The fundamental concepts of ultrasonic sources include the employment of piezoelectric crystals within the transducer to produce sound waves. When an electrical current is supplied to these crystals, they vibrate, generating ultrasonic waves. This procedure creates sound waves with the required frequency and strength for specific purposes **[17]**. In environmental research and engineering, ultrasonography has been investigated for its potential to destroy low quantities of estrogen hormones in aqueous solutions. According to research, ultrasound can influence the breakdown of estrogen molecules, with parameters such as solution temperature and fluid pressure impacting the reaction's efficiency. These principles underpin ultrasound's many uses, from medical diagnostics to environmental cleanup, making it a flexible and essential technology in various sectors **[18]**.

Acoustic Waves: Their Properties

Acoustic waves, such as surface acoustic waves and shear-horizontal (SH) acoustic waves, have been investigated for their sensitivity to changes in the parameters of their travel medium **[19]**. The properties of these waves, such as their velocity and existence range, have been studied theoretically in various materials, including layered media, lithium and potassium niobate plates, and graphene. These investigations help to better understand the behaviour of acoustic waves in specific materials and under varied situations **[20]**.

A transducer generates ultrasonic waves by converting electrical energy into mechanical vibrations that produce sound waves. The transducer consists of piezoelectric crystals that vibrate when an electrical current is supplied to them, resulting in sound waves that flow through the medium. The frequency of the sound waves produced by the transducer is governed by the electrical signal given to the crystals. The sound waves produced by the transducer can be focussed or defocused by changing the form of the transducer or employing lenses **[21]**. Ultrasound waves may be employed in various applications, including medical imaging, industrial testing, and therapeutic therapies. The creation of ultrasonic waves is an essential feature of ultrasound technology, enabling its many uses **[14]**.

Ultrasound waves are employed in various applications, each having unique features. Pulse waves are used in medical imaging and Doppler ultrasonography **[22],** whereas chirp waves assess bone quality and quantity **[23]**. Tone-burst waves are utilised in industrial testing and non-invasive pain management, whereas continuous waves are used in ultrasound imaging to provide high-resolution pictures. These waves find applications in various domains, from unravelling the complexities of the human body to analysing materials in industrial settings **[24]**.

The differences between low-frequency and high-frequency ultrasonic waves include different qualities and uses. Low-frequency ultrasonic waves, less than 1 MHz, are used in deep tissue imaging and treatments such as physical therapy and pain management **[25]**. In contrast, high-frequency ultrasonic waves with frequencies more than 10 MHz are used in superficial tissue imaging, particularly in dermatology and ophthalmology. The ultrasonic frequency is determined by the individual application requirements and the depth of the tissue to be scanned or treated. Recognising the characteristics and uses of low-frequency and high-frequency ultrasonic waves is critical for achieving the best diagnostic results and safe operations in various settings. This information is helpful in multiple applications, including medical imaging, industrial testing, and therapeutic therapies. Understanding the subtle features of these ultrasonic waves aids educated decision-making, contributing to improved efficacy and safety in medical, industrial, and therapeutic applications **[26]**.

Continuous wave and pulsed ultrasound are separate forms of ultrasonic waves, each with distinctive qualities and uses in various domains, including medical imaging and treatments. Sinusoidal waves distinguish continuous wave ultrasound with a consistent frequency. Its principal application is in ultrasonic imaging, where it excels at producing high-resolution pictures of inside body components **[27]**. Continuous wave ultrasound is also helpful for deep tissue imaging and treatments such as physical therapy and pain management. Notably, research comparing the effects of constant and short-pulsed focused ultrasound on muscle tissue found that continuous wave ultrasound produced a considerably greater diffusion coefficient. Pulsed ultrasound, conversely, consists of brief, constant-frequency sound pulses **[28]**.

The search results highlight the promising applications of ultrasound technology in protein extraction and processing. Ultrasound-assisted extraction (UAE) has emerged as an effective technique for enhancing the extraction of bioactive compounds, including proteins, from various sources. The key mechanisms behind the effectiveness of UAE include cell wall disruption and improved mass transfer. Cell wall disruption is facilitated by the cavitation bubbles generated by ultrasound waves, which effectively disrupt cell walls, thereby facilitating the release of intracellular compounds, including proteins. This leads to higher extraction yields compared to conventional extraction methods. Additionally, ultrasound enhances the mass transfer of solutes from the solid matrix into the solvent, accelerating the extraction process and reducing extraction times **[29]**. Several successful applications of UAE for protein extraction have been reported. For instance, in extracting proteins from Sacha inchi meal biomass, UAE combined with deep eutectic solvents resulted in higher protein yields than conventional extraction methods. Similarly, optimising UAE parameters, such as pH, temperature, and time, led to a maximum protein recovery of 85.81% in extracting proteins from watermelon seeds **[2]**. Moreover, UAE has been utilised to extract proteins from excess sludge, where low-intensity ultrasound-assisted enzymatic hydrolysis enhanced enzyme activity, improved the protein extraction rate, and reduced overall processing costs **[30]**. Furthermore, UAE has been explored as a potential technology for protein extraction from faba bean, along with other novel techniques like high-pressure processing and hydrodynamic cavitation **[3]**. Figure 2. depicts the mechanism of how ultrasonic waves work.

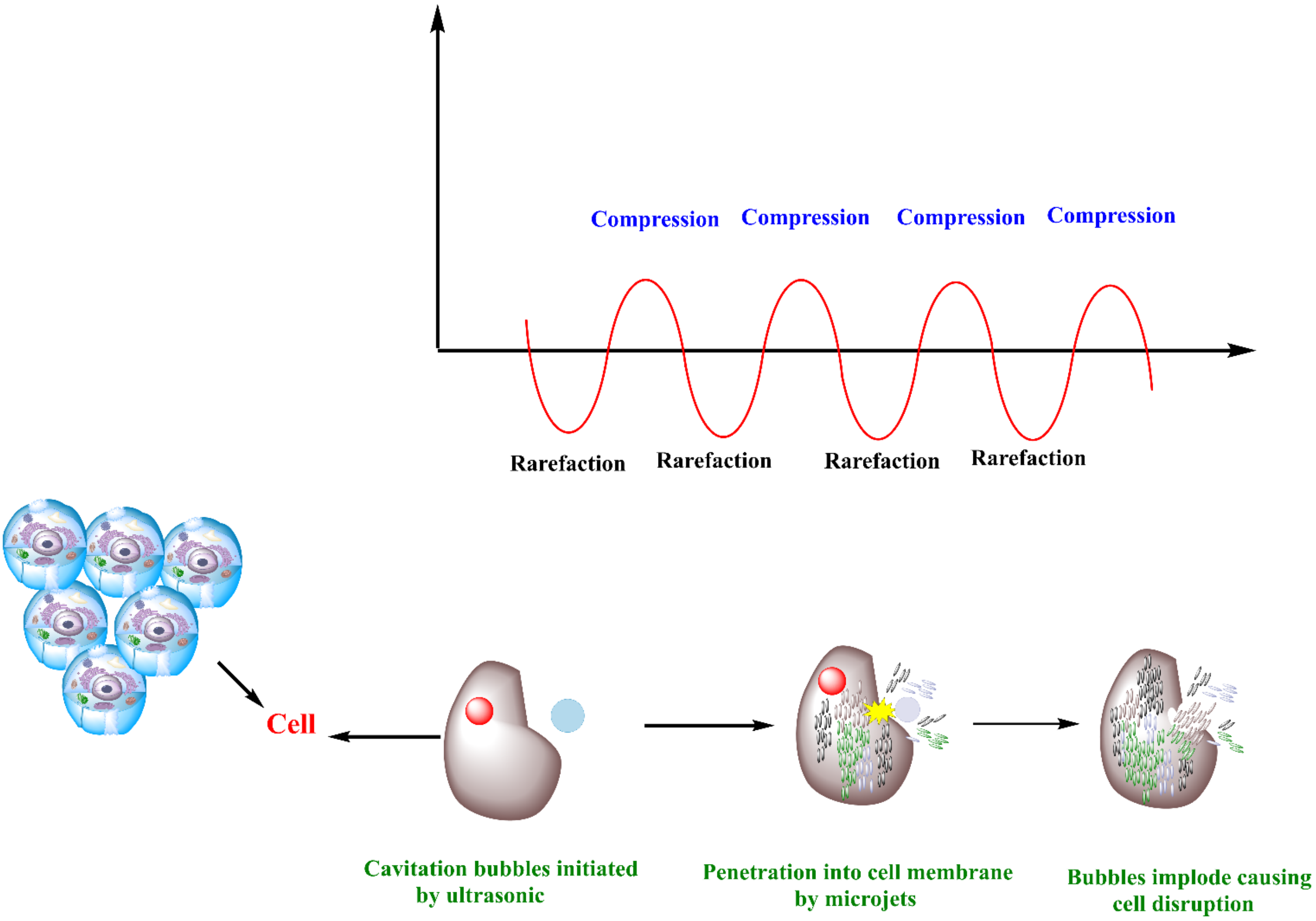

Figure 2. Ultrasonic wave's destructive mechanism.

Enzymatic protein hydrolysis has emerged as a new focus in investigating ultrasound-protein interactions. Ultrasound is an important non-thermal processing technology for helping enzymatic hydrolysis since it is environmentally friendly and effective **[31]**. Compared to ordinary enzymatic hydrolysis, ultrasonic-pretreatment-assisted enzymatic hydrolysis has a much higher efficiency. This enhancement goes beyond simply increasing efficiency and dramatically increasing substrate biological activity. This breakthrough can potentially improve enzymatic hydrolysis processes and hence increase total protein processing efficiency **[32]**.

Furthermore, the study of Ultrasound's interaction with proteins includes Protein Modification. The production of a myofibrillar protein-gallic acid combination exemplifies ultrasound's potential for generating new protein modifications, notably in the food manufacturing industry. These applications highlight ultrasound's flexibility in modifying proteins to desired specifications, thereby widening the vista of possibilities within the field of food preparation **[33]**.

The processes governing the interaction of ultrasound with proteins are complex and not yet fully understood. Among the postulated processes, cavitation stands out because ultrasonic waves cause cavitation bubbles to develop inside the medium. These bubbles, in turn, generate mechanical stress and shear forces that damage protein structure and facilitate the release of bioactive chemicals. This sophisticated procedure improves the overall efficiency of protein processing technologies, paving the way for new approaches in the food business **[34]**. Another discovered process is acoustic streaming, which occurs when ultrasound waves cause fluid to flow. This movement, mediated by the propagation of sound waves, improves the mass transfer of bioactive chemicals from the protein matrix to the solvent. Acoustic streaming significantly impacts the extraction process, increasing the intricacy of ultrasound-protein interactions **[35]**. Thermal impacts are yet another aspect of this relationship. Ultrasound waves can create heat by absorbing energy from the medium, which causes protein denaturation and the consequent release of bioactive chemicals. This thermal element adds a temperature dimension to the interaction, contributing to more nuanced results in protein processing processes **[36]**. Enzymatic activation is a method in which ultrasonic waves activate enzymes. This activation increases enzymatic activity, which improves the efficiency of enzymatic hydrolysis. The synergy between ultrasonic and enzymatic processes increases the capacities of protein processing technologies, creating opportunities for better outcomes **[37]**.

Effects on Protein Structure

The effects of ultrasound on protein structure are complicated and dependent on several parameters, including the frequency, intensity, and duration of the ultrasound exposure. According to specific research, ultrasonography can produce changes in protein structure, such as denaturation, aggregation, and fragmentation. Other research has found that ultrasonic can improve protein biological activity by increasing solubility and accessibility to enzymes. The kind of protein and the medium in which it is suspended also have an impact on ultrasound's effects on protein structure **[38]**.

Ultrasound-Assisted Protein Extraction

Ultrasound-assisted protein extraction has emerged as a transformational field of study, offering several benefits over existing approaches. One notable advantage is its potential to obtain better extraction yields due to the cavitation effect caused by ultrasonic vibrations, which shatter cell walls and facilitate the release of target chemicals. This increased efficiency is combined with decreased solvent use, which aligns with sustainable standards and contributes to an environmentally benign extraction process **[31]**. Furthermore, the simplicity of ultrasound-assisted extraction equipment reduces maintenance costs compared to previous methods, highlighting its cost-effectiveness **[39]**. Beyond extraction efficiency, research has shown that it can increase proteins' physical, structural, and functional characteristics, including solubility, stability, and biological activity. Multi-mode ultrasound technology highlights continuous attempts to improve protein extraction rates. Real-time process monitoring, including Fourier Transform Infrared (FT-IR) analysis, sheds light on the structural features of proteins during extraction, enhancing knowledge of the relationship between protein structure and extraction levels **[40]**. Furthermore, ultrasonic is used in enzymatic protein hydrolysis, exhibiting its capabilities as a green and practical non-thermal processing approach, considerably boosting enzymatic hydrolysis efficiency and increasing substrate biological activity **[37]**.

Ultrasound-Assisted Protein Extraction

Mechanical Methods

Ultrasound-assisted protein extraction has gained popularity recently, offering various benefits over standard extraction techniques. One significant advantage is its capacity to provide high extraction yields. Ultrasound waves provide a cavitation effect, disrupting cell walls and enabling the release of target substances, resulting in enhanced extraction efficiency **[41]**. Furthermore, the use of ultrasound promotes environmental sustainability by minimizing solvent usage. This strategy correlates with a more environmentally friendly extraction procedure, making it a better option than traditional approaches, which frequently use more solvents **[42]**. Another advantage of ultrasound-assisted extraction is that it has fewer maintenance expenses. This approach does not require sophisticated equipment or intricate procedures, simplifying the extraction process and reducing maintenance costs **[43]**. According to studies, ultrasound-assisted protein extraction improves proteins' physical, structural, and functional characteristics and efficiency. Improvements in solubility, stability, and biological activity have been found, demonstrating the adaptability and usefulness of this extraction method **[44]**. Multi-mode ultrasound technology has emerged as a significant advancement in enhancing protein extraction levels. According to research, various ultrasonic working modes have a considerable impact on the overall success of the extraction process, and multi-mode ultrasound devices have been built to take advantage of these differences. Fourier Transform Infrared (FT-IR) analysis allows real-time process monitoring during ultrasound-assisted extraction. This in situ monitoring enables researchers to investigate proteins' structural features during extraction, providing insights into the relationship between protein structure and extraction levels **[42]**. In addition to protein extraction, ultrasound is helpful for enzymatic protein hydrolysis. Ultrasound is a green and efficient non-thermal processing technology that aids in enzymatic hydrolysis, considerably boosting efficiency and increasing the biological activity of substrates as compared to typical enzymatic hydrolysis procedures **[45]**.

Challenges in Conventional Methods

Sonication, high-intensity focused ultrasound (HIFU), and an ultrasonic bath are all methods for extracting proteins using ultrasound. Sonication uses high-frequency sound waves to shatter cell walls and release proteins. HIFU employs high-intensity ultrasound waves to cause localized heating and pressure changes, resulting in protein extraction **[46]**. The ultrasonic bath immerses the sample in ultrasound waves to aid protein extraction. Studies have demonstrated that ultrasound-assisted protein extraction can result in greater extraction yields, decreased solvent consumption, and enhanced physical, structural, and functional characteristics of proteins compared to traditional approaches. Multi-mode ultrasound equipment has been created to increase protein extraction levels. In situ, real-time process monitoring may investigate protein structural features during ultrasound-assisted extraction **[29]**. Ultrasound-assisted enzymatic hydrolysis has also been widely employed as a green and practical non-thermal processing approach to aid enzymatic hydrolysis, considerably boosting its efficiency and increasing substrate biological activity. A comparative investigation of multiple ultrasound-assisted extraction procedures for pectin from tomato processing waste revealed that ultrasonic bath and sonication were the most successful methods for pectin extraction **[47]**.

Ultrasound-assisted protein extraction has been proven to increase protein output and quality compared to traditional approaches. Ultrasound-assisted extraction has been shown in studies to increase protein yields, enhance techno-functional attributes, and modify proteins' physical, structural, and functional properties **[48]**. For example, a pumpkin seed protein extraction survey discovered that ultrasonic treatment increased protein output and enhanced the extracted protein's techno-functional qualities **[49]**. Another study on walnut dregs protein extraction using multi-mode ultrasound found that ultrasonic-assisted protein extraction effectively increases yield and changes proteins' physical, structural, and functional aspects. Ultrasound-assisted enzymatic hydrolysis has also been found to improve the efficiency and biological activity of substrates dramatically **[40]**.

Mechanisms of Ultrasound-Enhanced Enzymatic Reactions

The processes of ultrasound-enhanced enzymatic reactions have been investigated in various disciplines, including food processing, analytical chemistry, and nanomaterial-enhanced biosensors. Ultrasound has been proven to aid in beneficial dietary processes such as enzymatic crosslinking, protein hydrolysis, and fermentation **[50]**, **[51]**. In analytical chemistry, ultrasound has been used to accelerate the enzymatic hydrolysis of substances, resulting in a significant decrease in the time necessary for this step. Furthermore, ultrasound has been shown to improve the performance of first-generation amperometric biosensing schemes in nanomaterial-enhanced biosensors, notably in the context of enzymatic processes **[52]**. Furthermore, ultrasound has been studied for its ability to enhance the activity of enzymes in cascade-catalytic tumor treatment. In metal extraction, ultrasound has been investigated for its capacity to improve sulfuric acid leaching for zinc extraction, with results indicating a higher leaching rate than traditional approaches. These studies highlight the many uses and processes of ultrasound-enhanced enzymatic reactions in a variety of domains **[53]**.

Ultrasound-assisted hydrolysis has several noticeable benefits in a variety of disciplines. For starters, it dramatically improves the efficiency of enzymatic hydrolysis operations, resulting in much shorter reaction times. For example, studies have shown that ultrasound may reduce reaction times from hours to minutes, making it a time-saving tool, particularly in applications involving the enzymatic hydrolysis of chemicals detected in urine samples **[54]**. Furthermore, ultrasound promotes favourable processes in food processing, such as enzymatic cross-linking, protein hydrolysis, fermentation, and marination. These procedures increase yield and alter physical and functional features, improving enzymatic reaction efficiency. Furthermore, ultrasound-assisted hydrolysis has several uses in food processing, analytical chemistry, and the production of nanomaterial-enhanced biosensors **[55]**. However, this strategy is not without its drawbacks. One key difficulty is to optimize the settings for ultrasound-assisted hydrolysis. Parameters such as sonication strength, duration, enzyme activity, and water-substrate ratio must be carefully adjusted to produce the desired results. Another source of worry is the possibility of hydrolysis artefacts, as shown in situations where acidification during extraction resulted in unwanted chemical changes. Addressing these problems necessitates careful control of experimental settings to avoid unwanted reactions or changes in target molecules **[56]**.

Bioactive Peptide Production

Enzymatic hydrolysis is the primary method for producing bioactive peptides, with ultrasonography, microbial fermentation, or recombinant DNA technologies typically used as auxiliary methods. Enzymatic hydrolysis, significantly when helped by ultrasound, improves efficiency and yield, as proven by experiments on diverse protein sources such as chicken feathers and tilapia fish skin waste. These peptides have many health benefits, including antibacterial, antioxidant, anticancer, and immunomodulatory capabilities, making them potential constituents for highly nutritious and functional food items **[57]**, **[58]**. In the biomedical arena, continuous research strives to enhance peptide synthesis technologies to realise their broad prospective uses. However, problems remain, necessitating careful optimization of manufacturing variables such as temperature, pH, and enzyme-to-substrate ratio to achieve hydrolysis degree and bioactivity. Developing more effective ultrasound-assisted hydrolysis devices might boost peptide output even more **[59]**. Furthermore, constant research and innovation are required in peptide synthesis, separation, identification, and functionality evaluation to realize their potential across several industries fully. Overall, the search results illustrate the bright outlook for bioactive peptides, emphasizing the critical role of ultrasound-assisted enzymatic hydrolysis in their creation while also identifying areas for future refinement and research **[60]**.

Ultrasound in Protein Refolding

Low-amplitude ultrasound can induce specific structural changes in protein monomers, forming hydrogen-bonded β-sheet-rich structures, which serve as primary nucleation sites for protein refolding. These changes are initiated by pressure perturbations and accelerated by temperature factors **[61]**. Additionally, prolonged exposure to low-amplitude ultrasound enables the controlled elongation of amyloid protein nanofibrils directly from monomeric lysozyme proteins. Remarkably, the nanofibrillar assemblies formed under ultrasound exhibit identical structural characteristics to those formed by native fibrillation, as determined by solution X-ray scattering. The study indicates that ultrasound can effectively induce structural changes in proteins and facilitate the formation of amyloid protein nanofibrils with properties akin to native fibrillation **[62]**.

Protein Denaturation and Refolding

The role of ultrasound in aiding protein refolding is becoming increasingly evident, with studies showing its ability to induce refolding of specific motifs in protein monomers, leading to primary nucleation characterized by adopting a hydrogen-bonded β-sheet-rich structure **[63]**, **[64]**. Additionally, ultrasound has been observed to displace small heat shock proteins from protein aggregates, thereby initiating the refolding process. These findings suggest that ultrasound could play a crucial role in protein refolding, with promising applications in the biopharmaceutical industry and the refolding of recombinant proteins. However, ultrasound in protein refolding is still an area of ongoing research, and further studies are required to fully comprehend its mechanisms and explore its practical applications. In summary, while ultrasound shows promise in aiding protein refolding by inducing specific motifs and displacing chaperones from aggregates, further research is necessary to unlock its full potential in this domain **[65]**, **[66]**.

Challenges in Protein Denaturation

Protein refolding presents numerous substantial issues, such as protein aggregation, refolding condition optimization, protein structure and function preservation, process scalability, and impurity elimination. Protein aggregation can hamper refolding efficiency by generating insoluble complexes but optimizing parameters such as temperature and pH is critical for successful refolding. Preserving the protein's original structure and function is critical to ensuring the quality of the finished product, and scaling up the process offers logistical hurdles as demand increases **[67]**. Furthermore, contaminants such as tiny heat shock proteins might impede refolding and degrade product quality, demanding effective removal techniques. Ultrasound has emerged as a promising method for addressing some of the issues of protein refolding **[68]**.

Challenges in Protein Denaturation

Protein denaturation and refolding present several issues, including protein aggregation, optimizing refolding conditions, preserving protein structure and function, process scalability, and impurity removal. Protein aggregation reduces refolding efficiency, necessitating mitigating techniques **[69]**. To get the best results, refolding conditions, governed by parameters such as temperature and pH, must be precisely adjusted for each protein type. Preserving proteins' original structure and function is critical to the quality of the final product, demanding careful refolding technique selection. Scaling up the refolding process to meet increasing demand creates logistical challenges that must be overcome **[70]**. Impurities like tiny heat shock proteins might impair refolding efficiency and need effective removal procedures. Additionally, ultrasound-assisted refolding is a potential path for investigation, with additional studies required to elucidate its mechanisms and possible applications **[71]**, **[72]**.

Importance of Proper Refolding

The search results give helpful information on the variables influencing protein refolding, notably cysteine-rich proteins that tend to aggregate when overexpressed in E. coli. The major processes in the refolding process are the purification of inclusion bodies comprising insoluble, aggregated proteins, solubilization using denaturants such as guanidinium chloride, and refolding with a redox system, co-solvents and additives to encourage correct folding **[73]**. The dilution approach and using detergents with cosolvents are valuable methods for refolding cysteine-rich proteins **[74]**. Several factors influence the efficiency of the refolding process, including protein concentration, disulfide bond formation, chaperone proteins (such as small heat shock proteins and Hsp70 chaperones), micelle size and composition, and salt concentration. Increasing salt concentration up to 1 M NaCl can enhance the refolding process **[75]**. Additionally, synthetic nano chaperones with hydrophobic microdomains can stabilize denatured proteins and facilitate their refolding with high efficiency **[76]**.

Ultrasound as a Refolding Aid

Ultrasound has emerged as a viable method for aiding protein refolding in various ways. Firstly, it causes structural changes in protein monomers, leading to adopting a hydrogen-bonded β-sheet-rich structure, which is essential for commencing protein refolding **[77]**. Furthermore, ultrasonic removes tiny heat shock proteins from protein aggregates, a necessary step in solubilizing and refolding aggregated proteins with the help of chaperones such as Hsp70 **[78]**, **[79]**. Furthermore, extended exposure to low-amplitude ultrasound enables the controlled elongation of amyloid protein nanofibrils directly from monomeric proteins, indicating possible uses in material manufacturing. These findings highlight ultrasound's critical function in protein refolding, notably in biopharmaceuticals and recombinant protein manufacturing. Despite its potential, the use of ultrasound in protein refolding remains a subject of the current study, demanding further studies to thoroughly understand its mechanisms and explore its potential applications **[80]**.

Comparison of structural characteristics between ultrasound-influenced and natively fibrillated proteins

The search results do not include precise information regarding the structural differences between ultrasound-influenced and naturally fibrillated proteins. Low-amplitude ultrasound may refold certain motifs in protein monomers, resulting in initial nucleation with a hydrogen-bonded β-sheet-rich structure. These structural changes are triggered by pressure disturbances and enhanced by temperature variations **[81]**. Furthermore, the prolonged action of low-amplitude ultrasound allows for the controlled elongation of amyloid protein nanofibrils directly from monomeric proteins until they reach a critical length—nanofibrillar assemblies formed under ultrasound share identical structural characteristics with natively fibrillated proteins **[81], [82]**.

CONCLUSION

In conclusion, studying ultrasound-assisted protein processing has yielded numerous significant discoveries. Ultrasound in protein extraction has been shown to increase yields, minimize solvent consumption, and improve protein physical, structural, and functional characteristics. Furthermore, the use of ultrasound in enzymatic hydrolysis has demonstrated encouraging results in terms of efficiency and biological activity. The interaction of ultrasound with proteins has enabled novel alterations, giving new opportunities in food processing. The implications of ultrasound-assisted protein processing for the food and biopharmaceutical industries are substantial. The technique offers a more sustainable and environmentally friendly approach in the food sector, aligning with the growing demand for greener practices. The improved quality of proteins and efficient extraction methods hold promise for developing novel food products with enhanced nutritional profiles. In the biopharmaceutical realm, ultrasound's role in protein refolding and enzymatic reactions signifies potential advancements in producing recombinant proteins and pharmaceutical formulations. These implications underscore the transformative impact ultrasound can have on various industrial applications. The impact of ultrasound-assisted protein processing on the food and pharmaceutical sectors is significant. In the food industry, the technology provides a more sustainable and ecologically friendly approach, meeting the rising need for greener practices. Protein quality improvements, along with effective extraction technologies, show promise for the development of innovative food items with increased nutritional profiles. In the biopharmaceutical field, ultrasound's function in protein refolding and enzymatic processes represents possible advances in creating recombinant proteins and medicinal formulations. These consequences highlight the transformational effect ultrasonography may have on various industrial applications. Prospects in ultrasound-assisted protein processing research provide exciting opportunities. Further research is needed to dive into the subtle mechanics of ultrasound-protein interactions, offering a better grasp of the technology's full potential. Refining and refining ultrasonic settings for specific applications, such as enzymatic hydrolysis and protein refolding, is critical for optimum efficiency. Furthermore, studying alternative industrial uses outside food and biopharmaceuticals, such as cosmetics and nutraceuticals, provides opportunities to widen the scope of ultrasound technology. Continued research and innovation in ultrasound-assisted protein processing are critical for realizing its full potential advantages and uses.

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