

## Review Article

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## Ultrasound - Assisted Treatment in Protein and Bioactive Ingredients Processing

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### ABSTRACT

Derive of Protein by processing is now shifting to advanced, modest and innovative technology these days. This review shows the possibility of deriving proteins via processing through the use of ultrasound approaches. The target is between ultrasound waves and proteins behavior during ultrasound extraction technology. The extent of protein extraction by traditional methods faces structural, biostability and economic challenges, but ultrasounds emerge as an alternative to these challenges. In this work, highlights on ultrasound's role in enhancing protein yield and bio-quality in relationship to bioavailability and functional role were discussed. Analyses have shown some cases where remarkable advancement by ultrasound techniques has also extended to enzymatic hydrolysis, using ultrasound catalyzed activities to unlocked dimensions in the production of bioactive - peptides and enriched proteins. Bio industrial sector that uses ultrasound approach to extract bioactive and proteins not only do they facilitates protein reshapes but also changes protein production, biostability and bioavailability. Ultrasound has emerged as a catalyst for effective bioactive enhancement, overthrowing conventional methods to optimization strategies of protein reshaping and rebouncing. The review envisages the advantages of ultrasound technology and applications in bio - industrial sectors, the future prospects of ultrasound assisted protein extraction with offered roadmaps and processing advantages.

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### Introduction

Ultrasound-assisted innovations bioactive processing emerged as a focal research interest, driven by potential to enhance the efficiency and biological activity of protein substrates. This technological revolution has found applications across diverse domains, such as extraction, modification, freezing/thawing-induced oxidations [1, 2].

The technology in ultrasound-assisted protein has become versatile. Enzymatic protein hydrolysis, ultrasound extracting is a green and non-thermal processing tool. The pretreatment-assisted enzymatic hydrolysis stands out for capacity to enhance enzymatic reactions while concurrently amplifying the biological stability of substrates. Thus, this technology finds primary utility in the extraction of bioactive substances and the degradation of biological macromolecules [3].

The study by on protein extraction from watermelon (*Citrullus lanatus*) seeds using ultrasound assisted processing was able yield desired outcome following optimal pH of 11, sonication temperature of 45 °C and sonication time of 10 minutes. The processing conditions have demonstrated its ability for good protein recovery at a rate of 86% from water melon [4]. Additionally, UAET (ultrasound-assisted extraction techniques)

have been explored to obtain proteins from faba bean (*Vicia faba*) and pumpkin seed and this approach have been able to complementing conventional method of protein extraction [5]. When ultrasound processing (UP) is encompassed with high-pressure and hydrodynamic cavitation, but in an advanced extraction technology, the process had offered sustainable solutions for protein production from diverse agricultural resources [1,3,4,6]. These new techniques of UAET with high-pressure and hydrodynamic cavitations have, enhanced the efficiency and effectiveness of protein extraction from various sources [7]. These methods have found practical applications in industries like food processing and pharmaceuticals, thus contributed to obtain quality proteins.

Another sphere called fish genomics and data processing techniques have evolved, facilitating the extraction and analysis of specific genetic sequence from taxonomically diverse genomic data. The approach supports molecular surg, contributing to the detection of adaptive peptides, purification, selection and the reconstruction of ancestral proteins [3,7,8,9].

The evolution of protein processing techniques has fueled a lot of advancements geared towards protein purification, characterization and analysis [3,7]. The wave in protein processing encompasses a range of transformative developments. Such as in artificial intelligence and machine learning that had given rise to innovative algorithms dedicated to processing and

analyzing protein data. These innovation technology of UAET have enabled the rapid analysis of multiple samples using small amounts of material, making them particularly suitable for genomic and proteomic-wide studies that are aimed at identifying new therapeutic functional food ingredient (FFI) that could be however geared toward in-vivo bio-formatic technology and study but limited by cost however aimed at simple protein extraction to enhance food enrichment like ultra sound processing technology. Recognizing the properties and applications of frequencies of ultrasound waves, these had been assumed to be very importance for ensuring optimal diagnostic outcomes and safe operations across diverse domains of functional food sciences (FFS). This knowledge proves indispensable from industrial application and therapeutic treatments. The importance and characteristics of ultrasound waves, could help in an informed decision-making, and enhanced efficiency and safety UAET across food processing, medical, bio-industry and therapeutic realm [10-12].

### The basic Principles of Ultra Sound Wave

The basic principle of ultrasound production (UP) encompasses generation, division and uses. Ultrasounds are forms of energy with a frequency above the human ear range, usual above 20 kHz. It is produced by a transducer, that converts electrical energy into mechanical sound waves. These sound waves could travel through medium, such as air, water and can be used for various purposes, including industrial extraction and therapeutic treatments.

The fundamental principles in (UP) involves piezoelectric crystals put within the transducer to generate sound waves [13]. When current is applied to these piezoelectric crystals, they vibrate, thereby producing the ultrasound energy-related waves. The rate -frequency of the sound waves generated by the transducer is determined by the frequency of the electrical signal applied to the crystals from source. Thus, energy of the sound waves produced by the transducer can be focused or defocused using shape adjuster or by using lense. This process enables the creation of energy from sound wave within the desired frequency and intensity for specific applications [13,11]. Research have shown the ability of ultrasound energy (UE) to impact on the degradation of estrogen compounds, when the following factors such as solution temperature and fluid pressure which can influence the efficiency of the reaction are in place [14]. The ultrasound waves can be used for various purposes in food and functional food processing sectors. The generation of ultrasound waves are fundamental to ultrasound technology, enabling its diverse applications in various fields [11,14] such as protein and bioactive extractions. Ultrasound waves are generated using piezoelectric crystals transducer, which converts electrical energy into sound vibrations that produce sound waves energy [13].

### Application of Ultrasound Assisted Waves

Ultra-sound waves are used in various food and industrial applications, each with distinct properties. Pulse waves, Doppler ultrasounds and chirp waves are used in medica imaging, estimate bone quality and quantity [10]. While tone-burst waves are used in industrial testing and non-invasive pain relief, and waves are prevalent in ultrasound imaging for high-resolution imaging [11]. These waves find applications in diverse fields, from unraveling the intricacy of the human body to assessing material in industrial and food settings [10,11,15].

Assisted ultrasound extraction (AUE) use generated wave energy and solvent to extract target compounds from various plant or animal matrices at given Ultrasound frequency say (>20 kHz) [16]. These wave energy consist of series of compression and rarefaction cycles that are propagated through solid, liquid or gas medium to induce displacement and dislodgement of the molecules from their original positions thereby creating cavitations The negative pressure during rarefaction waves ,when it exceeds the attractive force joining the molecules together, this forces pull the pressure apart and create cavitation bubbles. The temperature created by this bubble may move up to 5000 K and pressure increase may be up to 1000 atm [17,18].

The recoveries of bioactive compounds through solvent extraction, mechanical expelling, supercritical extraction, and microwave extraction methods have limitations like use of extra solvent in solvent extraction, low yield in mechanical expelling, large capital in supercritical fluid extraction, and requirement of aqueous phase in microwave assisted extraction [19]. when Compared to the methods, of UAE with the advantages such as less time and energy requirement, low temperature extraction and retention of the quality of the extract. UAE extract bioactive compounds from fruit and vegetable waste and by-products using high intensity sound waves have been reported to cause disruption in the plant tissue through physical forces developed and during acoustic cavitation and helps in release of extractable components in the solvent in very less time by enhancing the mass transport [20,7].

### Challenges in Protein Processing

Traditional protein processing methods encounter challenges that prompt the exploration of innovative alternatives. Firstly, conventional heat-based techniques such as salting, smoking, and frying are known for both energy-intensive and time-consuming. These methods contribute to increased carbon footprint and greenhouse gas emissions, raising sustainability concerns. Similarly, the dense and complex food matrices, including those rich in proteins, traditional methods face limitations. Penetrating deeply into such matrices poses challenges, hindering the achievement of uniform processing and extraction within these intricate structures. The traditional methods designed to curtail microbial growth and eliminate pathogens may not consistently ensure optimal food safety and quality. This inconsistency can lead to concerns about shelf life and potential food waste. The efficiency in extracting beneficial bio-chemicals from biomass remains a challenge in traditional protein processing. This inefficiency presents obstacles in waste valorization and the implementation of bio-refinery processes, that can impact overall sustainability [21].

### Importance of Ultrasound-Assisted Protein Extraction

Ultrasound-assisted protein extraction has garnered considerable attention in recent years, presenting several advantages over traditional extraction methods.

### High Yield and Energy Efficiency

One notable benefit is its ability to achieve high extraction yields. Ultrasound wave energy create cavitations that disrupts cell walls, facilitating the release of target compounds and leading to increased extraction efficiency. Has been opined and that there is comparative advantages of ultrasound wave energy in food processing, presented as a more energy-efficient and environmentally sustainable alternative to traditional methods [1]. This distinction contributes significantly to the goals of diminished energy consumption and reduced greenhouse gas

emissions. Ultrasound-assisted extraction techniques (UAET) have been investigated for their efficacy in obtaining substantial protein yields from alternative sources, exemplified by study that focused on watermelon seeds. This innovative approach not only contributes to repositioning food processing waste but also facilitates the extraction of high-quality proteins from non-traditional sources

### **Economy of Solvent and Material use**

The use of ultrasound contributes to environmental sustainability by reducing solvent consumption. This approach aligns with eco-friendly extraction process, making it favorable choice in comparison to conventional method that often involve higher solvent use. Other advantage lies in low maintenance costs associated with ultrasound-assisted extraction process. This method does not necessitate complex equipment or involve multiple intricate steps, that stream from extraction process and minimizing maintenance requirements. Ultrasound-assisted protein extraction goes beyond mere efficiency, positively impacting the physical, structural and functional properties of proteins. The enhancements in solubility, stability and biological activity have been observed, further establishing the versatility and efficacy of this extraction technique.

### **Monitoring Effects**

Real-time process monitoring during ultrasound-assisted extraction is facilitated by techniques like Fourier Transform Infrared (FT-IR) analysis. This in situ monitoring allow researchers to study the structural properties of proteins throughout the extraction process, offering insights into the correlation between protein structure and extraction levels.

### **Green House Co-extraction Friendly Method**

Ultrasound has found utility in enzymatic protein hydrolysis. Serving as a green and efficient non-thermal processing technique, ultrasound assists in enzymatic hydrolysis, significantly improving efficiency and enhancing the biological activity of substrates compared to traditional enzymatic hydrolysis methods [6,22,16,23,24].

### **Enhance Food Safety**

One notable aspect of ultrasound technology lies in its capacity to enhance food safety by effectively inhibiting microbial growth and eliminating pathogens. This attribute translates into extended shelf life for processed foods and a consequential reduction in food waste, aligning with broader sustainability and objectives.

### **Ultra-sound-Assisted Protein Extraction Mechanism**

Assisted ultrasound protein extraction (AUPE) has emerged as a transformative area of research, that has presented myriads of advantages over traditional methods. One significant benefit lie in the ability to achieve higher extraction yields, attributed to the cavitations effect induced ultrasound waves that disrupt cellular wall facilitate and e release of target bioactive compounds. This enhanced efficiency coupled with a reduction in solvent consumption, aligned sustainable practices and contribution to eco-friendly extraction process. Furthermore, the simplicity of ultrasound-assisted extraction equipment leads to lower maintenance costs compared to traditional methods, emphasizing cost-effectiveness. Beyond mere extract efficiency, the capacity to improve the physical, structural, and functional properties of proteins, such as solubility, stability, and bio-ceutical activities are improved. The utilization of multi-mode ultrasound equipment underscores ongoing efforts to optimize bioactive extraction and levels. Real-time process monitoring, including

fourier transform infrared (FT-IR) analysis, provides valuable insights into structural properties during extraction, enhancing the understanding of the correlation between protein structure and extraction levels.

The UAPEST (Ultrasound-assisted protein extraction sonication technique), high-intensity focused ultrasound (HIFU) and ultrasonic bath (UB) are invoked nowadays. Sonication involves the use of high-frequency sound waves to disrupt cell walls and release proteins. HIFU uses high-intensity ultrasound waves to create localized heating and pressure changes, resulting in protein extraction. Ultrasonic bath involves immersing the sample in a bath of ultrasound waves to facilitate protein extraction. Studies have shown that ultrasound-assisted protein extraction can result in higher extraction yields, less solvent consumption, and improved physical, structural and functional properties of proteins compared to conventional methods.

Ultrasound-assisted enzymatic hydrolysis (UAEH) has been widely used as a green and efficient non-thermal processing technique to assist with enzymatic hydrolysis, significantly improving the efficiency of enzymatic hydrolysis and enhancing the biological activity of substrates [26]. A comparative analysis of different ultrasound-assisted extraction techniques for pectin extraction from tomato processing waste, it was found out about ultrasonic bath and sonication to be the most effective methods for pectin extraction [6,22,25].

Ultrasound-assisted protein extraction has been shown to improve protein yield and quality compared to conventional methods [3,4]. Study have demonstrated that ultrasound-assisted extraction can result in higher protein yields, improved bifunctional properties, and modified physical, structural, and functional properties of proteins [22,16,23]. For example, a study on pumpkin seed protein extraction found that ultrasonic treatment during extraction resulted in a significant increase in protein yield and improved techno-functional properties of the isolated protein [4]. Study on walnut dregs protein extract using multi-mode ultrasound reported a potent attribute of ultrasound-assisted protein extraction for improving yield and modifying the physical, structural, and functional properties of proteins [1].

Wave approach of ultrasound-assisted enzymatic hydrolysis (UAEH) has been significant improver and aid in the efficiency of enzymatic hydrolysis with enhance biological activity in substrates [6,22,23]. Ultrasound's wave application extends to enzymatic protein hydrolysis which demonstrate its prowess as a green and efficient non-thermal processing technique, significantly improving enzymatic hydrolysis effectiveness and substrate enhancement [26].

### **Ultra-sound Wave and Enzyme Reactions**

Mechanisms of ultrasound-enhanced enzymatic reactions have been studied in various fields, including food processing, analytical chemistry, and nonmaterial, biosensors. Ultrasound has been shown to facilitate beneficial food reactions such as enzymatic cross-linking, protein hydroxylation and fermentation. In analytical chemistry, ultrasound has been used to enhance the enzymatic hydrolysis of compounds, leading to a drastic reduction in the time required for these unit steps. Additionally, in the field of nonmaterial-enhanced biosensors, ultrasound has been found to play roles in enhancing the performance of first-generation biosensing schemes, particularly the context of enzymatic reactions. Much more, ultrasound has been investigated for its role in enhancing the activity of nanozymes in catalytic tumor therapy.

In the context of metal extraction, ultrasound has been linked, for its ability to enhance sulfuric acid leaching for zinc extraction [24], revealing an increased leaching rate compared to conventional methods. These studies collectively demonstrated the diverse applications and mechanisms of ultrasound-enhanced enzymatic reactions in various fields such as food, pharmacy and biotechnology [25,27,28,29].

### Ultra-Sound Wave in Enzymatic Hydrolysis

Enzymatic Protein hydrolysis emerges as another focal point in the study of protein interactions. Serving as a green and efficient non-thermal processing technique, ultrasound wave could play pivotal role in assisting enzymatic hydrolysis. In compare to traditional enzymatic hydrolysis, ultrasonic-pretreatment-assisted enzymatic hydrolysis has demonstrated substantial enhancement in bio efficiency. This improvement extends above mere bio efficiency, as it significantly elevates the biological activity and stability of substrates. This advancement holds promise for optimizing enzymatic hydrolysis processes, thereby contributing to the overall efficiency of protein processing.

Exploration of ultrasound's interaction with proteins extends to Protein modification as well. This is exemplified by the synthesis of a myo fibrillar protein and gallic acid mix complex, showcasing ultrasound's potential for driving innovative protein modifications, particularly in the domain of food manufacturing [2]. The application underscores the versatility of ultrasound in tailoring proteins to desire specifics, thereby expanding the horizon of possibilities within the realm of food and functional food processing [1, 2].

### Mechanism of Ultrasound in Bio-active extraction

Extensive research into interaction of ultrasound with proteins has uncovered promising applications within the realm of protein processing and extraction. One noteworthy area is UAE (ultrasound-assisted extraction), where ultrasound technology proves instrumental in augmenting extraction of bioactive compounds from plant to animal tissues. The utilization of ultrasound waves generates cavitations bubbles, effectively disrupting cellular tissues and facilitate the release of bioactive compounds. This innovative approach not only leads to higher yields but also significantly reduces extraction times, presenting an efficient and time-saving solution in the process.

The mechanisms underlining interaction between ultrasound and proteins are intricate and un-comprehending. Among the proposed mechanisms, cavitations as ultrasound wave induce the formation of cavitation bubble within medium. These bubbles, in turn, create mechanical stress and shear forces, disrupting the protein structure and facilitating the release of bioactive compounds [30]. This intricate process contributes to the overall efficiency of protein processing method called (UAEPP) offering a pathway for innovative approaches in the food industry. Another identified mechanism is acoustic streaming, a phenomenon where ultrasound waves induce the motion of fluid [11,12,13,15,31-34,35]. The motion, caused by the propagation of sound waves, enhances the mass transfer of bioactive compounds from the protein matrix to the solvent. Acoustic streaming play crucial role in influencing the extraction process, adding near complexity of the dynamics of ultrasound-protein interactions [36].

Heat effects represent yet another facet of this interaction. Ultrasound waves can generate heat through the absorption of energy by the medium, leading to the denaturation of proteins

and the subsequent release of bioactive compounds. This therma or heat l aspect introduces a temperature dimension to the interaction, contributing to the simple outcomes in protein processing methods. Enzymatic activation, as a mechanism, involves ultrasound wave energy activating enzymes. This activation results in increased enzymatic activity, enhancing efficiency of enzymatic hydrolysis. The synergy between ultrasound and enzymatic processes further advances the capabilities of protein processing methods, providing avenues for improved outcomes [14,36].

### Effects of Ultrasound wave on Protein Structure

The effects of ultrasound on protein structure are complex and depend on various factors, such as the frequency, intensity, and duration of the ultrasound exposure. Some studies have shown that ultrasound can cause changes in protein structure, including denaturation, aggregation and fragmentation [1,2]. However, other studies have shown that ultrasound can enhance the biological activity of proteins by improving their solubility and accessibility to enzymes [36]. The effects of ultrasound on protein structure are also influenced by the type of protein and the medium in which it is suspended [2, 36].

### Bioactive Peptide Production

Bioactive peptides are specific protein fragments that have health-promoting potential for humans, including antimicrobial, antioxidant, anticancer, and immunomodulatory activities. The production technology and processes of bioactive peptides have attracted excessive attention, especially concerning peptides' synthesis, separation, identification, and functionality. Among the production technology of bioactive peptides, enzymatic hydrolysis, microbial fermentation, and recombinant DNA technology are commonly used. Ultrasound technology has been shown to enhance the enzymatic hydrolysis of compounds, leading to an increase in hydrolysis levels and bioactivity. The UAEH (Ultrasound-assisted enzymatic hydrolysis) has been used to prepare high-calcium and high-amino acid fish frame hydrolysates, which have potential as a foundation for creating highly nutritious food additives [30]. The potential applications of bioactive peptides in biomedicine are vast, however projects are needed to further optimize and improve efficiency of peptide production technology [37].

### Effects of Ultrasound on Protein Stability, Folding Patterns and Behavior

The effects of ultrasound on protein stability, folding patterns, and self-assembling behavior have been explored in various research studies, revealing intriguing findings across different protein systems.

In the context of amyloid fibrils, low-amplitude ultrasound has been identified as a potential inducer of refolding in specific motifs within protein monomers. This induction leads to Io nucleation characterized by the adoption of a hydrogen-bonded  $\beta$ -sheet-rich structure [38]. long exposure to low-amplitude ultrasound facilitates controlled elongation of amyloid protein nanofibrils directly from monomeric proteins. The resulting nanofibrillar assemblies formed under ultrasound exhibit structural characteristics identical to naturally fibrillated proteins, suggesting that ultrasound can play certain role in sharpening self-assembly behavior of amyloid proteins [39].

In the case with bovine serum albumin (BSA), ultrasound measurements are employed. The study reveals changes in volume and adiabatic compressibility of BSA. Interestingly,

sorbitol has been identified as a restorative agent, recovering BSA's volume and compressibility values and restoring original alpha-helix content [40]. This implies that the observed compressibility variation reflects conformational changes during the transition of BSA, highlighting ultrasound's potential in studying protein conformational dynamics.

Concerning protein refolding, the interplay between small heat shock proteins (sHsps) and Hsp70 under the influence of ultrasound has been explored. sHsps form assemblies with misfolded proteins, and Hsp70 displaces surface-bound sHsps from these assemblies. This displacement initiates the protein refolding process and has significant implications for protein homeostasis. The cooperation between Hsp70 and sHsps, influenced by ultrasound, ensures efficient refolding of damaged proteins under favorable folding conditions, thus emphasizing the potential applications of ultrasound in protein refolding processes [40, 41].

### Comparison of Structural Characteristics Between Ultrasound-Influenced and Natively Fibrillated Proteins

The contrasting quality of structural characteristics ultrasound-influenced and natively fibrillated proteins revealed that when ultrasonic energy is sufficiently low, it can induce refolding of specific motifs in protein monomers, which is sufficient for primary nucleation, characterized by adopting a hydrogen-bonded  $\beta$ -sheet-rich structure initiated by pressure perturbations and are accelerated by a temperature factor [2]. Furthermore, the prolonged action of low-amplitude ultrasound enables the elongation of amyloid protein nanofibrils directly from monomeric lysozyme proteins, in a controlled manner, until they reach a critical length [39,42]. Using solution X-ray scattering, it was determined that nanofibrillar assemblies formed under the influence of ultrasound energy and natively fibrillated lysozyme share identical structural characteristics [40].

### pH Effect During Ultrasound Assisted Extraction

Acid base balance is an important factor that does affect the extraction, yield and properties of bioactive compound during UAE. The extraction of polysaccharides (pectin) from the fruit and vegetable by-products has been studied in the pH range of 1–5. The recovery of pectin is high when the pH maintained is low. The high acidic medium improves the cell wall fragmentation, hydrolyses the insoluble pectin into soluble form and reduces the molecular weight of pectin increasing their dissolution into the surrounding medium and thus enhancing their recovery. The optimum pH for the extraction of pectin from pomegranate peel is 1.2, from orange peel, eggplant peel, jackfruit peel is ~1.5, from, and from banana peel and sunflower head is 3.2 [43-47].

pH has also been reported to exhibit maximum effect among the studied extraction variables for example in extraction of pectin from grape pomace and orange peel [48-50]. Has given linear effect of pH on the molecular weight of extracted pectin [51]. pH also affects the degree of esterification of extracted pectin. At low pH, degree of extraction is low which is due to de-esterification of pectin in the high acidic condition. However, it is interesting to note that effect of pH on degree of extraction was reported differently on UAE of pectin from grape pomace and orange peel. Grape pomace degree of extraction increased on decreasing the pH and opposite trend was found for orange peel. This may be due to the interactive effect of other variables such as temperature, power and time with pH.

The effect of pH on extraction of phenolic compounds has not been reported vividly. Gave effect of pH range 4.5–6.5 of ethanol solvent adjusted by HCl on the extraction yield of phenolics from coconut shell powder and observed positive linear effect with maximum yield at pH 6.5 [20]. Gave significant quadratic and negative effect of pH on the extraction of monomeric anthocyanin from jaboticaba peels which meant that increase in pH favoured the monomeric anthocyanin extraction upto certain value and thereafter discovered that increase in pH decreased the monomeric anthocyanin yield. It could be concluded that pH for the extraction of anthocyanin and phenolics lies in the range of pH 1–3 [43].

### pH-Induced Conformational Transitions and Ultrasound Measurements

The search results provided information related to ultrasound on the conformational changes of proteins at acid pH [41]. Specifically, a study on bovine serum albumin (BSA) demonstrated that ultrasound measurements were used to investigate the structural transition of BSA at acid pH, showing changes in volume and adiabatic compressibility. The study also found that sorbitol could restore of BSA volume and compressibility values, as well as a substantial recovery of its original alpha-helix content, implying that the compressibility variation observed reflects the structural changes during the transition [10,14]. Additionally, another study investigated ultrasound waves on the structural characteristics and oxidative stability of walnut oil oleogel coated with soy protein isolate-phosphatidylserine [8,7]. The study found that ultrasound produced cavitation activity (UPCA) and high local shear force, causes formation and rupture of bubbles or cavities, increasing the number of nucleation sites in the entire system, and reducing the size of crystals in the internal structure, thus showing a more even and finer distribution. The drift in the internal and external structure had been reported to have a positive effect on the oxidative stability of the oleogel. These findings demonstrate the potential of ultrasound wave (UW) in influencing the conformational changes and structural characteristics of proteins, as well as their self-assembly, with potential applications in protein processing and bio separation techniques [31-33,52].

### Benefits and Challenges of Ultrasound-Assisted Hydrolysis

Ultrasound-assisted hydrolysis (UAH) has several benefits, including increased efficiency and reduced reaction time of biological active ingredient or extracts. Ultrasound can also enhance the enzymatic hydrolysis of compounds which could lead to drastic reduction in the time required during unit steps. Ultrasound-assisted hydrolysis (UAH) can facilitate beneficial food reactions such as enzyme cross-linking, protein hydrolyzation, fermentation, and marination effects [53]. Furthermore, the optimal conditions for UAH, such as sonication power, sonication time, enzyme activity, and water-substrate ratio, need to be carefully optimized to achieve the desired results. [54,30,46]

### Conclusion

The exploration of ultrasound-assisted protein processing has unveiled several key findings. The application of ultrasound in protein and bioactive extraction has demonstrated enhanced yields, manage chemicals and their consumption, as well improved physical, structural, and functional properties of proteins. Additionally, application of ultrasound energy in enzymatic hydrolysis has shown promising results in increasing efficiency and enhancing biological activity. The

interaction of ultrasound with proteins has paved the way for innovative modifications, presenting new possibilities in food manufacturing and for functional food ingredient making. The use of UAEPP (ultrasound-assisted protein processing) for the food and biopharmaceutical industries are very substantial. In the food sector, the technique offers a more robust, sustainable and environmentally friendly approaches, aligning with the growing demand for greener practices and making functional food ingredient and bioactives stable. The quality of proteins upsurged with efficient extraction methods, holds promise for developing novel food products with enhanced nutritional profiles. In the biopharmaceutical realms, ultrasound's role in protein refolding and enzyme reactions signify and potentiate advancements in the production of recombinant proteins, functional ingredients and pharmaceutical formulations. These implications underscore the transformative impact ultrasound can have on various industrial applications [55-82].

### Recommendation

Future directions in UAPP (ultrasound-assisted protein processing) hold exciting possibilities. Investigations are warranted to delve into the intricate mechanisms of ultrasound-protein interactions, providing a deeper understanding of the technology in full with its potentials. Refining and optimizing ultrasound parameters for specific applications, such as enzymatic hydrolysis and protein refolding, will be crucial for maximum efficiency. Moreover, exploring additional industrial applications beyond food and biopharmaceuticals, such as in nutraceuticals, presents avenues for broadening the reach of ultrasound technology. Continued research and innovation in ultrasound-assisted protein processing are essential for unlocking full range of benefits and application in making potent bioactive and functional ingredient for the growing functional food industries

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