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Review

Effects of Pulsed Electric Fields and Ultrasound Processing on Proteins and Enzymes: A Review

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Abstract: There is increasing demand among consumers for food products free of chemical preservatives, minimally processed and have fresh-like natural flavors. To meet these growing demands, the industries and researchers are finding alternative processing methods, which involve nonthermal methods to obtain a quality product that meets the consumer demands and adheres to the food safety protocols. In the past two decades' various research groups have developed a wide range of nonthermal processing methods, of which few have shown potential in replacing the traditional thermal processing systems. Among all the methods, ultrasonication (US) and pulsed electric field (PEF) seem to be the most effective in attaining desirable food products. Several researchers have shown that these methods significantly affect various major and minor nutritional components present in food, including proteins and enzymes. In this review, we are going to discuss the effect of nonthermal methods on proteins, including enzymes. This review comprises results from the latest studies conducted from all over the world, which would help the research community and industry investigate the future pathway for nonthermal processing methods, especially in preserving the nutritional safety and integrity of the food.

Keywords: novel processing; pulsed electric field; food proteins; nonthermal processing; ultrasonic; ultrasound processing; food enzymes

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1. Introduction

Traditional food processing methods generally involve heat transfer through one of the following ways: roasting, drying, boiling, cooking, and frying [1]. These processing methods help extend the shelf life of the product and add variety to the food we consume [1,2]. The earliest recorded remnants of preserving the food through processing dated back to the ancient Egyptian era where "sun drying" was widely prevalent. However, Nicholas Appert was the scientist who successfully demonstrated that food could be preserved for a longer time using thermal techniques. According to the records, he sealed the food in glass jars that were placed in hot water, which destroyed pathogens, thus extending the shelf life of the product [3,4]. With the invention of electricity and industrialization, the scale of operations has increased exponentially to meet the rising population's demands around the world. Due to the socioeconomic conditions in the post-second-world-war societies, an evident rise in consumption of confectionery, convenience foods and ready-to-eat meals was observed, which are all processed thermally and contain high concentrations of sugars and chemical additives [5,6]. However, the modern-day consumer is asking for more natural and fresh-like foods, which are minimally processed. This rise in demand results from increased awareness of the advantages of consuming fresh foods, especially fruits and vegetables, among the consumers [7]. Reduced risk of cardiovascular diseases and

cancer risk are widely associated with the consumption of fresh fruits and vegetables and associated products [8–10].

However, not all food products can be consumed raw as they are widely associated with food safety issues, including *Salmonella* and E Coli O157: H7/O104:H4 outbreaks that can be highly detrimental to health, leading to death in humans [11–13]. Further, foods may contain antinutrients that can have adverse effects on health. Antinutrients are the naturally occurring products present in plant foods, which reduce the nutritional availability in the body. They are secondary and tertiary metabolites produced by the plants as part of their natural defense mechanism [14–16]. Despite the few advantages of their presence, they are generally undesirable in food products [15]. Both conventional and novel thermal processing methods were found to be highly effective in alleviating both pathogens and antinutrients present in food, and this effectively dealing with the food safety concern [4,14,17]. However, thermal processing influences the sensory and nutritional components in food that can be both advantageous and disadvantageous depending on the various factors, including the food components, processing temperature, duration of processing and method adopted. As mentioned already, the advantages of processing foods thermally include the destruction of pathogens and antinutrients present in food. In few cases, the food can also develop desirable sensory qualities making it more palatable for human consumption [1,6].

Conversely, various studies have also shown the negative impact of thermal treatments in food [18,19]. They were found to degrade various sensitive nutritional components, which include anthocyanins, ascorbic acid and various other bioactive compounds that play a critical role in the wellbeing of the human body [18,19]. They can also lead to undesirable chemical reactions (Ex: Milliard reaction), resulting in undesirable organoleptic and nutritional changes [20,21]. Thus, these downsides of thermal processing have forced the researchers and the food industry to look for alternative processing methods that would preserve the "fresh" like the sensory product, but at the same time provides a desirable effect by eliminating the health risks associated with consumption of raw foods, which lead to developing nonthermal processing methods. Nonthermal processing involves the methods where the food is processed under sublethal temperatures, i.e., the food is not heated up. The rising demand from industries to introduce these methods has put much pressure on researchers to evaluate their effect on various components in food, including proteins [1]. These nonthermal methods were found to give a targeted response based on the application and further can improve the overall sensory and nutritional quality of the food products [20].

Though there are various other major nutritional components present in foods, proteins are the most sensitive and vital for human consumption. They play a significant role in muscle building and maintenance of cell wall structure in humans and plants, respectively. Proteins also act as one of the primary sources of energy (4 kcal/g of protein) [1,22]. Considering the above factors, a considerable amount of research has been done on the effect of thermal processing on proteins which is covered in various reviews published [23–29]. However, over the past two decades' considerable amount of progress has been made in assessing the effectiveness of a wide range of nonthermal processing methods in replacing the conventional thermal methods. In this review, the processing effect of electric field applications and ultrasonication on proteins are discussed in detail. This would help both the researchers in assessing the effectiveness of these methods and their influence on both the structural and functional properties of various proteins.

2. Pulsed Electric Field Processing (PEF)

PEF technology is one of the nonthermal processing techniques that has gained much attention in recent years due to its ability to render food safe for consumption by meeting the food safety standards with short treatment time and minimal heat production. Though there are multiple ways of applying electric fields to food, PEF involves using high voltage in short bursts across the food particles placed in between two electrodes [30]. The short

bursts last from several nanoseconds to several microseconds, and the applied electric field strength can vary from 10–80 kV/cm. The continuous application of these pulses possesses the ability to inactive pathogens and enzymes, resulting in food quality deterioration while maintaining the fresh-like organoleptic and nutritional properties that consumers demand [31–33]. The ability of PEF application to reduce microbial activity has repeatedly been evaluated and discussed extensively [34–40].

The application of electrical fields to biological materials like various food products results in charge buildup across the cell membrane of the pathogens, which leads to disruption in the normal functioning of the cell wall. In case the potential across this membrane exceeds a critical value, disruption occurs, resulting in the loss of its functional biological properties as a cell membrane causing "cell death" [34,41,42]. The second alternative theory was proposed by Zimmermann [43], which is called "dielectric rupture theory," which suggests the cell membrane acts as a capacitor and application of electric field results in the accumulation of charge on the surfaces of the membrane. This accumulation of charge results in an increase of transmembrane potential (TMP), which will, in turn, reduce the thickness of the cell wall. Ultimately, as the value of transmembrane potential reaches a value of around 1 V, pore formation takes place, resulting in damage to the cell. In case the potential is further increased, irreversible damage can occur to the cell resulting in its inactivation [42,43]. The TMP can be calculated using the following equation:

$$U(t) = 1.5rE \quad (1)$$

where $U(t)$ is the TMP in the direction of the applied electric field (V); r is the radius of the cell in μm and E is the electric field strength applied in kV/mm [34,38,42].

Tsong [44] also proposed "electroporation theory", which is based on the ordered structure of the cell membrane and the dipole nature of the lipids present in the membrane. It states that applying an electric field causes electrical and thermal stress resulting in deformation of the lipid conformation leading to creating new hydrophobic pores. These hydrophobic pores are not very stable and hence result in the formation of stable hydrophilic pores. The local heating of the cell membrane results in a thermal transition from gel to the crystalline structure of the lipid bilayer occurs resulting in loss of semipermeable property. Further, the cell wall also contains protein channels that are sensitive to the high voltage applied. In addition, hence applying an electric field can result in denaturation of these sensitive proteins resulting in loss of functional properties [39,42,44].

Apart from the inactivation of pathogens, PEF can also be used for modification of protein secondary structure and functional properties. However, at low intensities, the stress provided may not be sufficient to induce permanent denaturation in various food products [45]. Tables 1–3 summarizes the influence of PEF on various proteins and enzymes.

Table 1. Changes in the structural properties of various proteins due to applying a pulsed electric field.

Foods	Processing Conditions	Effect/Influence on Protein	Ref.
Horseradish peroxidase	5–25 kV/cm; 207–1242 pulses; 1.5 µs pulse width	Loss of α-helix structure by 42% at 22 kV/cm and 87 pulses	[46]
Soybean protein isolate	0–15 kV; 1–8 µs pulse width; 1–9 ms pulse cycle; electrode gap of 0.292 cm	Loss in α-helix and increased random coils and β-sheets. Changes in disulfide bonds and collapse in a hydrophobic core. Strong PEF resulted in the reburial of hydrophobic residues into the core again.	[47]
Soybean protein isolate	0–50 kV/cm; 40 µs pulse width; treatment time 4.8 ms.	Relocation of turns into structured α-helix after 35 kV/cm. Slight increase in anti-parallel β-sheets and reduction in β-sheets content	[48]
Hen egg-white lysozyme	35 kV/cm; 0–1200 µs time; 2 µs pulse width	Inactivation following conventional first-order model. Loss in α-helix and increased random coils and β-sheets along with hydrophobic collapse at earlier stages of inactivation	[49]
Soybean trypsin inhibitor	0–40 kV/cm; 0–547 µs treatment time; 2 µs pulse width	No major changes in secondary structure	[50]
Egg white protein and β-lactoglobulin	12.5 kV/cm; long length pulses	Partial structure modification	[51]
Egg ovalbumin	20–35 kV/cm for 180 µs and at 35 kV/cm for 60–240 µs	High-intensity processing resulted in a loss of α-helix and a decrease in surface hydrophobicity	[52]
Egg-white protein	5–25 kV/cm; pulse width 8 µs; frequency 500 Hz; residence time 90 s	Increased free sulfhydryl groups and total number of sulfhydryl groups decreased. Reduced the α-helix content, while β-sheets increased	[53]
Pepsin	0–34.2 kV/cm; 23, 28 °C	Loss in β-sheets resulting in a loss in activity of the enzyme	[54]
Pepsin	25.2–35.6 kV/cm; 0–500 µs	Hydrophobic collapse. Reduction in β-sheets and increased intermolecular hydrophobic interactions and random coils	[55]
Whey protein isolate	12–20 kV/cm; 10–30 pulses at 0.5 Hz;	Reduction in surface hydrophobicity, which can result in structural modifications	[56]
Canola protein	10–35 kV/cm; pulse width 8 µs; residence time 180 s	Increased voltage and processing time resulted in reduced β-sheets and α-helix. Increased free sulfhydryl groups and reduction in total number of sulfhydryl groups. Increased surface hydrophobicity	[57]
Myofibrillar proteins (from PSE like chicken breast	0–28 kV/cm; pulse frequency 0–1000 Hz; residence time 180 s	Moderate PEF application increased solubility and surface hydrophobicity. α-helix increased and β-turns, and random coils reduced with applying PEF intensity	[58]

Table 2. Changes in the physicochemical and functional properties of various proteins due to applying a pulsed electric field.

Foods	Processing Conditions	Effect/Influence on Protein	Ref.
Soybean trypsin inhibitor	0–40 kV/cm; 0–547 μs treatment time; 2 μs pulse width	Denaturation and aggregation resulted in a reduction of solubility, surface hydrophobicity and free sulfhydryls	[50]
Egg white protein and β-lactoglobulin	12.5 kV/cm; long length pulses	Partial aggregation and aggregated gel microstructure in EW. Gelation behavior improved in β-lactoglobulin, and it reduced in egg white.	[51]
Ovomucin-depleted egg white	1.4–1.8 kV/cm; specific energy input of 260–700 kJ/kg; 20 μs pulse width	Protein aggregation at pH 5 and 7, but not at pH 4 and 9. Only lysozyme was responsible for aggregate formation compared to thermal processing	[59]
Egg ovalbumin	20–35 kV/cm for 180 μs and at 35 kV/cm for 60–240 μs	Immunogenic-binding capacity increased for low-intensity processing or high-intensity and short-time processing. Immunogenic-binding capacity decreased for high-intensity processing for >60 μs	[52]
Whey protein isolate	30–35 kV/cm; 19.2–211 μs; 2 μs pulse width; 30, 60, 65, 70, 75 °C	No effect on protein aggregation, surface hydrophobicity sulfhydryl groups, thermal stability and emulsification properties. Reduction in heat-induced gel strength and increased gelation time	[60]
Whey proteins	37.6 kV/cm; 50, 100 and 200 pulses of 2 μs at 1 Hz	No change in immunoreactivity	[61]
Raw milk	2–40 kV/cm; 5–35 μs pulse width; 50–1000 Hz; outlet temperature–39–72 °C	No change in color; reduction in conductivity of milk	[62,63]
Pepsin	25.2–35.6 kV/cm; 0–500 μs	Increased aggregation	[55]
Tomato juice	35 kV/cm; pulse frequency 50–250 Hz; pulse width 1–7 μs; treatment time 1000 μs	Apparent viscosity increased with treatment parameters compared to untreated control	[64]
Strawberry juice	35 kV/cm; pulse frequency 50–250 Hz; pulse width 1–7 μs; treatment time 1000 μs	Apparent viscosity increased with treatment parameters in the case of monopolar pulses. Bipolar pulses slightly reduced the viscosity	[64]
Canola protein	10–35 kV/cm; pulse width 8 μs; residence time 180 s	Improved solubility, oil-binding capacity, emulsion stability, foamability and water-holding capacity	[57]
Almond milk	0–28 kV/cm; 40 μs pulse width; 1 kHz frequency	Particle size reduction and stable emulsion. Improved appearance and physical stability at 28 kV/cm	[65]

Table 3. Changes in the enzyme activity of various foods due to applying a pulsed electric field. LOX—lipoxygenase; PE—pectinesterase; POD—peroxidase; PPO—polyphenol oxidase; ALP—alkaline phosphatase; HPL—hydroperoxide lyase.

Food/Enzyme	Processing Conditions	Effect/Influence on Enzyme	Ref.
Horseradish POD	5–25 kV/cm; 207–1242 pulses; 1.5 µs pulse width	Up to 37% reduction in activity with increasing pulses and electric field strength	[46]
Soybean LOX	20–42 kV/cm; 2 µs pulse width; 1036 µs treatment time	Maximum inactivation of 88% at 42 kV/cm when treated for 1036 µs.	[66]
Soybean LOX	20–40 kV/cm; 25–100 µs; 23, 35, 50 °C	85% inactivation at the highest processing conditions	[67]
Tomato LOX	0–35 kV/cm; 20–70 µs treatment time; 10–50 °C	Irreversibly inactivated	[68]
Tomato LOX and HPL	35 kV/cm; pulse frequency 50–250 Hz; pulse width 1–7 µs; treatment time 1000–2000 µs	Inactivation resistance of LOX is greater than HPL. 20% and 90% maximum reduction of LOX and HPL, respectively.	[69]
Tomato POD	35 kV/cm; pulse frequency 50–250 Hz; pulse width 1–7 µs; treatment time 1000–2000 µs	Inactivation achieved with a minimum pulse frequency of 200 Hz. Maximum inactivation achieved with pulse width > 5.5 µs	[70]
Pea LOX	2.5–20 kV/cm; 1 µs pulse width; 100–400 pulses	No inactivation	[71]
Watermelon LOX and POD	35 kV/cm; 1727 µs treatment time; 4 µs pulse width	LOX more resistant compared to POD inactivation. POD can be completely inactivated, whereas 50% inactivation was observed for LOX for 220 and 250 Hz	[72]
POD and PPO in apple juice	20–40 kV/cm; 25–100 µs; 23, 35, 50 °C	Highest inactivation rates (~70%) at the highest processing values	[73]
POD and PPO in grape juice	25–35 kV/cm, 600 Hz bipolar pulse width 4 µs, treatment time 5 ms	Complete inactivation of PPO was achieved, whereas only 50% inactivation was observed for POD	[74]
ALP (bovine milk)	25–37 kV/cm; 15–60 °C; 2 µs pulse width; treatment time 19.6 µs	30–67% inactivation for 25–35 kV/cm at 60 °C	[75]
xanthine oxidase (whole milk)	20 or 26 kV/cm; pulse width 20 µs; frequency 20 Hz.	Inactivation was 7–13% lower compared to thermal processing at 66 °C	[76]
Pepsin	0–34.2 kV/cm; 23, 28 °C	Inactivation of pepsin by ~60%	[54]
PE in orange juice	5–35 kV/cm; 200 Hz; width 4 µs; treatment time 1500 µs	20% residual enzyme activity	[77]
PE in grapefruit juice	20–40 kV/cm; 25–100 µs; 23, 35, 50 °C	97% inactivation after treating at 40 kV/cm, 100 µs at 50 °C	[78]

Table 1 summarizes the recent studies that evaluated the structural changes in various proteins. The data shows that the PEF treatment can directly influence the secondary structures in food proteins. Particularly, they reduce the α-helix content of the proteins, which was observed in the case of soy, canola and egg proteins. The electric fields have also resulted in the loss of β-sheets in numerous proteins, including canola, egg and pepsin. However, when the applied electric field is higher (generally over 35 kV/cm), there is a possibility of an increase in the structured conformations, i.e., α-helices and β-sheets, which was observed in soy protein [48]. Table 2 summarizes the changes in physicochemical and functional properties of a wide variety of proteins due to PEF processing. Protein aggregation was found to be common when treated using high-intensity electric fields and for longer processing times. However, pH also seems to be a major factor that influences the protein aggregate formation during PEF processing, as observed in the case of egg

white. This must be further evaluated to verify if it can influence aggregation in other proteins, especially plant-based proteins. Furthermore, PEF was found to have no influence on the immunoreactivity of egg and whey proteins (milk) except at very high intensities, which resulted in a slight reduction in the whey protein allergenicity. However, the clinical relevance of this must be further evaluated. The influence of PEF was also evaluated specifically on peanut (Ara h 2,6) and apple (Mal d 3) allergens and showed no significant changes in the secondary structures and reactivity [79]. The impact of PEF on enzyme activity has been outlined in Table 3. The PEF technology was tested on a wide range of beverage products as an alternative to traditional thermal pasteurization. The results presented show that PEF is effective in reducing a wide range of enzymes, including lipoxygenase (LOX), pectinesterase (PE) and peroxidase (PO), in numerous fruit juices and other beverage products.

3. Ultrasonication/Ultrasound Processing (US)

Ultrasound waves are waves with a frequency of 18 kHz and above extending into GHz. They have been employed in the food industry for various applications, such as homogenization, crystallization and emulsification. They are also widely used for the filtration and tenderization of meat [80]. Ultrasound applications of low-energy, i.e., with sound intensities less than 1 W/cm^2 and frequencies higher than 100 kHz, are used for non-invasive purposes. They are used as analytical tools for the characterization of physical and chemical properties. Diagnostic ultrasound (1–10 MHz) is one example that uses non-invasive ultrasound waves [80,81]. In the food industry, they are used in surface cleaning, simulation of living cell activity during fermentation through degassing and aid in assessing the physicochemical properties, including particle size, composition and flow rate [81,82]. High-energy ultrasound with sound intensities less than 1 W/cm^2 and frequencies in the range of 18–100 kHz are used in food processing areas, including inactivation of enzymes and microbes, degassing, drying, thawing, etc., [82]. Further, applying combinations of heat, pressure and ultrasound have been found to be highly effective, especially in liquid foods. The various forms of ultrasonic applications in food are as follows:

3.1. Presonication

This is the food processing technique where ultrasound is applied to the food product before the actual processing, either by heat or temperature. This method is found to be very effective in reducing the resistance of heat and pressure-resistant microbes and enzymes [83].

3.2. Postsonication

In postsonication, the product that is treated either by heat or pressure is then subjected to ultrasound. There is no experimental data on this method as the method is not widely used in the process industry [84].

3.3. Thermosonication

In this method, both ultrasonic waves and heat (moderate levels) are applied simultaneously. The process of thermosonication is found to be particularly effective in the inactivation of vegetative cells and enzymes [84].

3.4. Manosonication

The processing is performed by subjecting food to both ultrasonic waves and medium pressures in the range of 100–1000 kPa at low temperatures [83,84].

3.5. Manothermosonication

Manothermosonication (MTS) is applying a combination of temperature, pressure and ultrasound on a product simultaneously. Temperatures can range between (30–140 °C),

and the pressures can go up to 1000 kPa in this processing method, which was found to be very effective for treating yeasts and various enzymes in liquid foods [83,84].

The first use of ultrasonic waves was for the inactivation of microorganisms and was published in the late 1920s [85,86]. Through continuous work has been done on its effectiveness on various pathogens in food, its use was not as widespread as thermal due to the limitations in terms of equipment. But, there was a revival in its application in the food industry in the two decades due to its efficacy in dealing with both unwanted enzymes and microbes in various food products without affecting the nutritional properties as in the case of thermal processing methods. It was also found to have various secondary effects in food, as already mentioned, and since it has been adopted in various process industries [86]. There is a considerable amount of work done by researchers to evaluate the efficacy of ultrasonic processing, especially in combination with thermal processing and nonthermal processing methods like elevated pressures and pH variations. The inactivation effect was caused due to the cavitation, i.e., inception, evolution and implosion of small gas bubbles within the target food matrix. The implosion occurs rapidly and violently, leading to extreme temperatures and pressures on a microscale [87,88]. The external stress is caused due to cavitation was found to be effective in the breakdown of hydrogen bonds leading to conformational changes in tertiary and secondary structure [89]. Further, the cavitation also caused free radical release due to the hemolytic cleavage of water. The hydrogen and hydroxyl free radicals may react with free amino acids that result in the destabilization of enzymes and proteins, leading to changes in the biological activity of the compounds [88,90].

Ultrasonication has been considered as one of the green food processing technologies, which has caught the attention of scientists because of its advantages in effective mass transfer and energy usage, low cost and process temperatures [91–94]. The mechanism of ultrasonication is due to the formation of cavitation effects during food processing. Cavitation is a phenomenon where the movement of high-power sound waves results in the inception of members of gas/vapor bubbles that progress and then implode within the sample/solvent. This implosion results in extreme conditions of high temperatures (upwards of 1000 °C) and pressures (50–500 MPa) [91,92,95,96]. According to the frequency ranges, low-intensity ultrasonication (0–1 W/cm^2, >100 kHz) and high-intensity ultrasonication (>1 W/cm^2, 100–200 kHz) are two common types of ultrasonication [92]. Among them, low-intensity ultrasonication is used as a nondestructive tool to monitor the changes of physicochemical compounds during food processing. High-intensity ultrasonication can be used to inhibit the activity of enzymes and microorganisms from extending the shelf life of food products and modifying the secondary structure changes in the proteins leading to change in their functional properties and nutritional value [92,93,96].

As shown in Table 4, the impact of ultrasonication on the structural properties of a wide range of proteins has been outlined. Many studies have reported that the changes in the secondary structures observed due to ultrasonication were found to be highly dependent on the protein itself. The α-helix content in various milk proteins was found to increase after high-intensity sonication, whereas the α-helices reduced in the case of black bean protein and soybean protein [91,97–100]. In the case of β-sheets content, it reduced in case of protein from milk and soy, while it increased in black bean protein. Protein aggregation was also observed in various proteins that were ultrasonicated [93,101]. The changes in the functional properties of proteins are shown in Table 5. Protein solubility increased in most of the food products, including milk, soymilk, black-bean protein and peanut proteins, with the exception of egg proteins [97,98,102]. In addition, a study found that 16 min US treatment reduced trypsin inhibitor activity of soymilk by 52% [95]. Foamability and emulsification properties have also been enhanced in various plant-based proteins, including wheat, faba bean and plum seed protein [100,103,104]. Ultrasonication was also found to reduce the allergenicity of shrimp, peanut and kiwifruit proteins significantly when compared to untreated control [94,96,105,106]. As shown in Table 6, the effect of US processing on enzymes from a wide range of sources was described. Studies found that

US processing can effectively inhibit the activity of polyphenol oxidase (PPO), peroxidase (POD), and pectin methylesterase (PME) present in tomato juice, apple juice, cantaloupe melon juice, pear juice and milk [107–111]. It can be concluded that this nonthermal processing technique possesses the ability to effectively reduce the enzymatic activity that can promote the shelf stability of these beverage products.

Table 4. Changes in the structural properties of various proteins due to applying ultrasonication processing.

Food Sources	Processing Condition	Influence on Protein Structure	Reference
Milk	20 kHz frequency, 120 µm amplitude, 150 W, 55–75.5 °C	α-Lactalbumin and β-lactoglobulin denaturation, up to 81.5% reduction in the size of the fat globule [1]	[97,98]
β-Lactoglobulin (milk allergen)	20 kHz frequency,120 µm amplitude, 135 W/cm^2	Sonication mostly induced reduction in the β-sheet content while increasing α-helix and/or random coil structure of a protein. Sonication had a minor effect on IgE-binding properties	[99]
Black bean protein isolates dispersions (10%, *w/v*)	20 kHz, 150–450 W, 12–24 min	Decrease in the α-helix proportion and an increase in β-sheets content in the protein after ultrasonic treatment (300 W, 24 min)	[100]
Skim milk	20 kHz, 20–40 W, 15–60 min	Significant decrease in the band intensity of β-casein after 15 min sonication. The relative band intensities of β-lac and α-lac (major whey proteins) show a decrease after 30 min sonication. After 45–60 min of sonication, the intensity of the whey proteins is found to be lesser than control. The relative band intensity of κ-casein present in the whey protein (denatured with κ-casein) significantly increased after 30 min treatment at 20 W and 40 W	[112]
Soy protein isolate solution (5%, *w/v*)	20 kHz, 0–600 W, 15 min + controlled papain hydrolysis	Compared to control, US treatment at 400 W combined with a 1.25% degree of hydrolysis can cause a 47.7% reduction in α-helix, 30.4% in β-sheet, and 50% β-turn. A 73.5% increase in the random coil	[113]
Defatted wheat germ proteins	20 kHz, 0–1800 W for 10 min	Free sulfhydryl group and disulfide bonds decreased significantly with increasing the power intensity and sonication time	[114]
Walnut protein	25 kHz; 15–30 min; 200–600 W	Reduction in α-helices and increase in β-sheet, random coil and turn components. Increased free sulfhydryl groups compared to control	[115]
Faba bean protein	20 kHz; 15–30 min; 500/700 W	Increased β-sheet and turn content and reduction in random coil (intermolecular aggregates)	[101]
Rice dreg protein isolates	20–50 kHz, 15 min	Ultrasonication altered protein secondary structure by reducing random coil and β-sheet contents, while α-helix and β-turn contents increased	[116]
Gluten protein	0–40 kHz, 10 min, power density was 67 W/L at 30 °C	Sonication decreased the α-helix content of all sonicated gluten protein samples while increased the β-sheet and β-turn content, and tryptophan and tyrosine residues were exposed	[117]
Almond milk	20 kHz, 1–16 min, 450 W at 25 °C	Ultrasonication increased the ordered structures (α-helix and β-sheet) content	[95]
Whey protein solution [2] (cheese)	20 kHz, 450 W	10% increase in the α-helix component and a 6–9% decrease in the β-sheet and turn components	[118]

[1] There no change in the property of enzymes when ultrasound was applied without heat generation. [2] Whey protein solution (cheese): The surface hydrophobicity of the proteins increased for up to 5 min of sonication, presumably due to the unfolding of the proteins resulting from minor structural changes. However, the surface hydrophobicity decreased after sonication for more than 5 min, which is a sign of protein aggregation, which in turn protects the hydrophobic regions of the proteins.

Table 5. Changes in the physicochemical and functional properties of various proteins due to applying ultrasonication.

Food Sources	Processing Condition	Functional Property	Reference
Milk	600 W at a frequency of 20 kHz and an amplitude of 50%	Solubility increased significantly from 35.78% to 88.30% after 5 min. A significant increase in the emulsion stability, surface hydrophobicity and emulsifying activity	[97,98]
Pineapple juice	19 kHz, 500 W, US intensity was 376 W/cm^2, 10 min	Juice viscosity reduced by 75% of the initial value	[119]
α-Lactalbumin (milk)	20 kHz, 15–20 min, 600 W	Foam capacities and solubility were improved significantly	[120]
Soymilk	20 kHz, 450–600 W	Great increase in solubility, specific surface area and emulsion activity index. 16 min US treatment reduced trypsin inhibitor activity of soymilk by 52%	[102]
Soy protein isolate (10% *w/w* solution)	20 kHz, 750 W and 20% of amplitude, 20 min	No significant changes in total free sulfhydryl groups and conductivity of protein. Significant increase in surface hydrophobicity (121%), solubility and water holding capacity of protein. A significant reduction in particle size of the protein	[121,122]
Wheat germ protein	20 kHz, 0–1800 W, 20 min	Ultrasonic pretreatment caused a 21.0–40.7% increase in ACE-inhibitory activity of defatted wheat germ protein hydrolysate	[123]
Whey protein	20 kHz, 600 W, 15 min, 43–48 W/cm^2	Significant increase in the solubility and foamability	[103]
Milk protein isolate solution (0.1–5 wt%)	20 kHz and 95% amplitude, 34 W/cm^2, 0–2 min	US treatment reduced the size and hydrodynamic volume of the protein. A significant reduction in the intrinsic viscosity improves the emulsifying activity.	[104]
Egg white proteins (10% *w/w* solution)	20 kHz, 750 W and 20% of amplitude, 20 min	Protein solutions were not significantly changed after treatment. A significant decrease in solubility	[121]
black bean protein isolates	20 kHz, 150–450 W, 12–24 min	Surface hydrophobicity and protein solubility of protein were enhanced after ultrasonication (300 W, 24 min)	[100]
Millet protein concentrate (10% *w/w*) [1]	20 kHz, 100 W, 5–20 min, 18–74 W/cm^2, 20–100% amplitudes	Significant increase in solubility with the processing time and intensity, the highest solubility in 73.95 W/cm^2 intensity for 12.5 min. Low ultrasound intensity (18.4 W/cm^2) caused an increase in the emulsion activity index and emulsion stability. In contrast, the high ultrasound intensity (73.95 W/cm^2) intensity caused a significant decrease in these properties	[124]
Ara h 1 and Ara h 2 (Peanut allergen)	US–enzyme combination (50 Hz, 1 h, 0–0.30% (*w/w*) trypsin or α-chymotrypsin	Protease digestion greatly increased peanut protein solubility. US–enzyme combination significantly lowered Ara h 1 and Ara h 2 in peanuts. Ultrasound–enzyme combination significantly lowered IgE binding of peanut extract	[105]
Shrimp protein	30 Hz, 800 W for 1.5 h at 0–50 °C	High-intensity ultrasound at 50 °C significantly reduced the allergenicity of shrimp (2.2-fold, 2.5-fold lower than control). US treatment caused a 76% reduction in the tropomyosin content	[106]
Kiwifruit protein	20 kHz, 450 W, 1–16 min at 25 °C	US caused a 50% reduction in the IgE binding capacity of Act d 2. In vitro digestibility of kiwifruit, proteins increased up to 77%	[96]

Table 5. *Cont.*

Food Sources	Processing Condition	Functional Property	Reference
Wheat protein	20 kHz; 540, 720, 900 W; 10 min at 25 °C	Improved foam capacity, emulsion stability and emulsification properties	[125]
Faba bean protein	20 kHz; 15–30 min; 500/700 W	Improved adsorption dynamics and foamability and reduced the digestibility	[101]
Walnut protein	25 kHz; 15–30 min; 200–600 W	Improvement in solubility and emulsification properties. Reduction in the particle size	[115]
Plum seed protein	20 kHz; 200–600 W	Increased solubility, emulsifying property and foaming capacity. Improved gel strength and gelling properties. Improved protease accessibility, which can increase digestibility	[126]
Whey protein solution (cheese)	20 kHz, 450 W	Surface hydrophobicity of proteins increased within first 5 min, and it decreased after 5 min	[118]

[1] A significant increase in solubility with the processing time and intensity, the highest solubility in 73.95 W/cm^2 intensity for 12.5 min. The foaming capacity of millet protein solution in lower intensities and times (18.4 W/cm^2, 5 min) was significantly lower than the untreated sample. However, its foaming capacity increased significantly as the time of US treatment in high intensities (73.95 W/cm^2, 20 min) was prolonged. After 12.5–20 min US treatment at 18.4 W/cm^2 intensity, an increase in the emulsion activity index and emulsion stability were observed. 12.5–20 min at 73.95 W/cm^2 intensity caused a significant decrease in the emulsion activity index and emulsion stability.

Table 6. Changes in the enzyme activity of various foods due to applying ultrasonication.

Food Sources	Processing Condition	Enzyme Activity	Reference
Pineapple juice	19 kHz, 500 W, US intensity was 376 W/cm^2, 10 min	20% reduction in the polyphenol oxidase (PPO) activity (376 W/cm^2 and 10 min)	[119]
Cantaloupe melon juice	19 kHz, 500 W, 376 W/cm^2 for 10 min	Significant reduction in peroxidase (POD) and PPO activities	[107]
Tomato juice	24 kHz, 400 W at amplitudes of 25–75 µm at 60–70 °C	90% reduction in the pectin methylesterase activity	[108]
Orange juice	20 kHz, 0.42–1.05 W/mL, 2–10 min	Highest pectin methylesterase (PME) activity inactivation was 62% after 1.05 W/mL sonication for 10 min	[127]
Pear juice	20 kHz, 750 W, at 25–65 °C for 10 min	Residual activities of POD, PME and PPO were 4.3%, 3.25% and 1.91% after sonication at 65 °C for 10 min	[109]
Apple juice	20 kHz, 5–10 min, 0.30 W/cm^3 at 20–60 °C	Significant reduction in enzyme activities of PPO, POD and PME under sonication treatment at 60 °C for 30 min: 63%, 70% and 62%	[110]
Raw milk	19 kHz, 100–475 W, 1–7 kJ/mL	US treatment promoted microbial and enzymatic inactivation with a temperature below 60 °C	[111]

4. Conclusions

The primary objective was to evaluate the impact of PEF and US processing on proteins and enzymes of various food products. There has been a growing need to address the consumer demand for "minimally processed" foods, which can be achieved by nonthermal processing techniques like PEF and US processing. Though their fundamental mode of action on food is different, both the methods evaluated were found to be effective in inactivating various enzymes in fruit juices and improve their shelf life. There were changes

observed in the secondary and tertiary structures of proteins in many food products. However, these changes were highly dependent on the intensity of processing, the local-food matrix and properties of the protein present in the given product. In some cases, these modifications in their conformational structures have resulted in physicochemical and functional property changes. High-intensity PEF was found to cause protein aggregation in most cases, while ultrasonication was found to increase proteins' solubility despite aggregation when processed for longer durations. Both PEF and US processing methods show great potential in improving the allergenicity of certain food products. However, further, reach is warranted to understand the clinical implications. Moreover, it is remarkable that researchers are exploring using the combination of these technologies and their impact on various nutritional components [128,129]. Further emphasis should be placed on understanding the combination treatment on proteins and enzymes.

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