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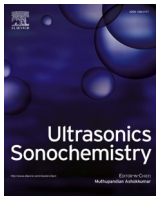

Review

Applications of ultrasonication on food enzyme inactivation- recent review report (2017–2022)

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ABSTRACT

Ultrasound processing has been widely applied in food sector for various applications such as decontamination and structural and functional components modifications in food. Enzymes are proteinaceous in nature and are widely used due to its catalytic activity. To mitigate the undesirable effects caused by the enzymes various technologies have been utilized to inactive the enzymes and improve the enzyme efficiency. Ultrasound is an emerging technology that produces acoustic waves which causes rapid formation and collapse of bubbles. It has the capacity to break the hydrogen bonds and interact with the polypeptide chains due to Vander Waals forces leading to the alteration of the secondary and tertiary structure of the enzymes thereby leading to loss in their biological activity. US effectively inactivates various dairy-related enzymes, including alkaline phosphatase (ALP), lactoperoxidase (LPO), and γ-glutamyl transpeptidase (GGTP) with increased US intensity and time without affecting the natural dairy flavors. The review also demonstrates that inactivation of enzymes presents in fruit and vegetables such as polyphenol oxidase (PPO), polygalacturonase (PG), Pectin methyl esterase (PME), and peroxidase. The presence of the enzymes causes detrimental effects causes off-flavors, off-colors, cloudiness, reduction in viscosity of juices, therefore the formation of high-energy free molecules during sonication affects the catalytic function of enzymes and thereby causing inactivation. Therefore this manuscript elucidates the recent advances made in the inactivation of common, enzymes infruits, vegetables and dairy products by the application of ultrasound and also explains the enzyme inactivation kinetics associated. Further this manuscript also discusses the ultrasound with other combined technologies, mechanisms, and its effects on the enzyme inactivation.

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1. Introduction

Enzymes are proteinaceous molecules or compounds present in almost all classes of food systems for accelerating specific chemical reactions incurring remarkable changes in the biochemical and organoleptic properties of the commodity. Based on the application requirements, the enzymatic reactions are either favorable or detrimental. Besides using certain enzymes intentionally for the particular role of action in food processing explicitly, utilization of amylases for starch hydrolysis in baking and brewing, catalases for the hydrolysis of hydrogen peroxide residues in cheese production, different proteases for meat tenderization, and so on, number of enzymes are required to be inactivated for retaining or preserving the quality of the food products. To elucidate, major enzymes in fruits and vegetables namely polyphenol oxidase (PPO), peroxidase (POD), and ascorbic acid oxidase (AAO) lead to the off-flavor, off-odor, and off-color productions via oxidative degradations while pectinases, polygalacturonase (PG) and pectin methyl esterase (PME) soften the tissues thereby affect the texture of the products. Similarly, thermostable enzymes in raw milk including alkaline phosphatase and lactose peroxidase might produce milk flavor changes and thus are considered the indicators of the effectiveness of pasteurization. The uncontrollable reaction of proteases would also affect the texture of meats by bringing in soft exudate tissues. Also, lipolytic enzymes lipases and lipoxygenase would cause degradation of the fatty acid chains affecting the flavor as well as stability of the fat-containing products. Thus, it becomes essential in distinct scenarios to inactivate the enzymes responsible for the aforementioned adverse reactions.

As an alternative to conventional heat and chemicals involving processes, enzyme inactivation by several non-thermal technologies is gaining attention because of their target-specific reactions which tend to retain the freshness attributes of the products [1]. Ultrasound (U/S) is one of the non-thermal processing technologies involving the application of sound waves with a frequency (20–100 kHz) not at the human hearing level [2]. Exposing the food commodity to strong mechanical sound waves produces different physicochemical effects in the product which aids in ensuring the safety of the product via microbial decontamination [3], pesticide metabolization [4], and allergen diminishing [5] as well as in enhancing the process efficiency via increasing the rate of mass transfer in extraction[6], drying [7], and filtration [8]. In addition, the ultrasonic inactivation of enzymes is a significant application as it not only ensures microbial safety but also provides qualitative stability to the ultrasonicated products [9,10].

In the past two decades, the studies on the intervention of ultrasound for enzyme inactivation were substantial, however, only a few works of literature [2,11,12] reviewed the investigations on enzyme inactivation by ultrasound (US). Also, there are nearly 25 reviews since 2017 on ultrasound technologies in food processing, yet there exists no distinct review analyzing the effectiveness of US for enzyme inactivation in both dairy and fruit and vegetable products. Thus, the present review exclusively discusses the application of ultrasonic inactivation of enzymes based on the recently reported investigations from 2017 to 2022 expounding the principle of action; kinetics of inactivation; US as a standalone technique for enzyme inactivation in dairy, fruits, and vegetable products; US as a synergistic technique in combination with other thermal and non-thermal technologies for enzyme inactivation.

Through a methodical search for research articles from 2017 to 2022 in different databases such as the web of science, Scopus, and google

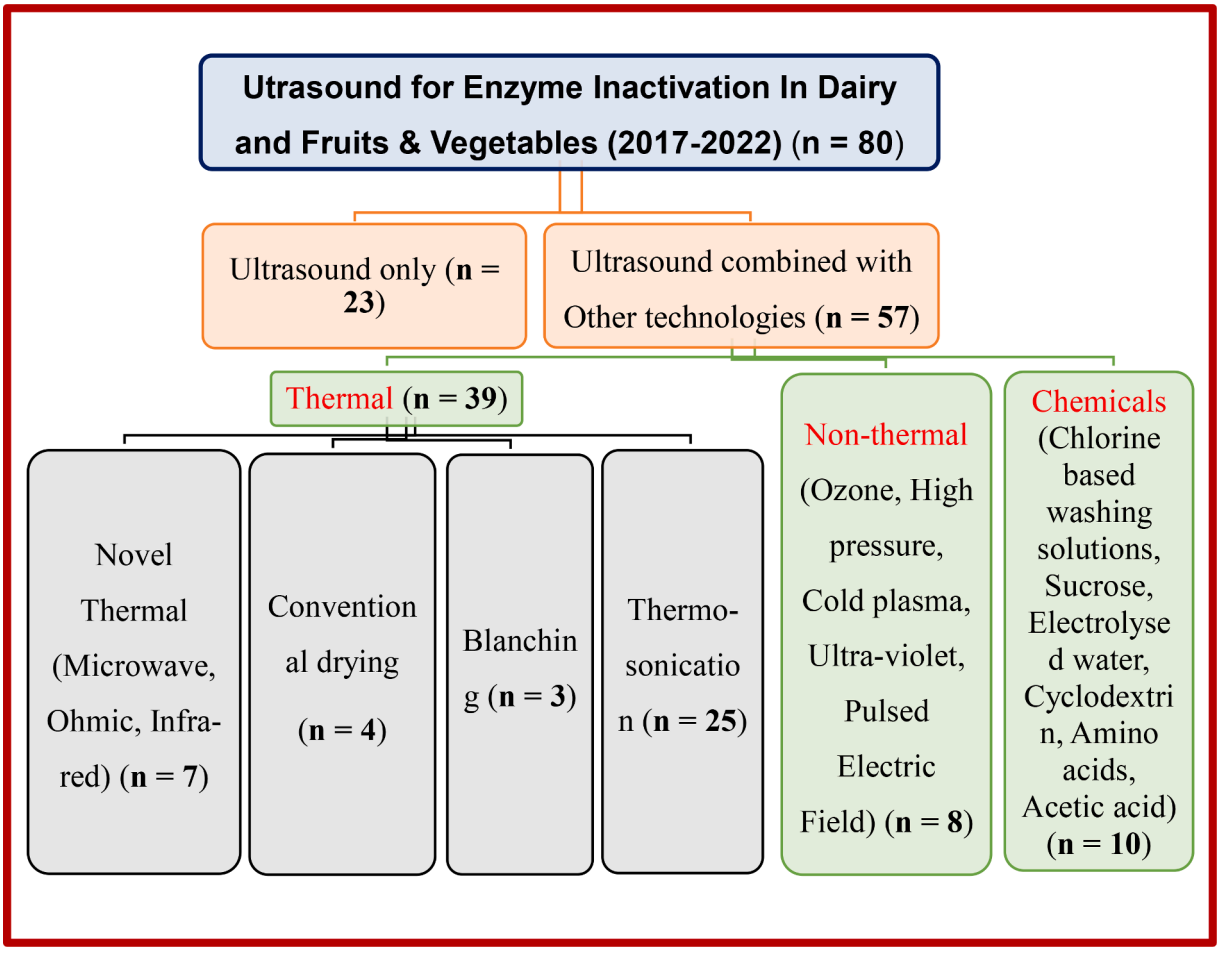

Fig. 1. Quantitative screening of studies for the review.

scholar using the keywords 'ultrasound' 'enzyme'; 'inactivation' almost all the investigations (nearer to n = 80) were screened out. The quantitative details of the articles taken into consideration under different sub-categories are given in Fig. 1. The review is restricted to articles studying the enzymes in dairy, fruits, and vegetables, and the studies focussing on absolute enzyme solutions are excluded. Also, a few studies on enzymes in edible flowers were excepted from the review.

2. Ultrasound – Principles, concepts and types

Ultrasound is defined as sound waves of higher frequencies beyond the audible range of mankind i.e., ranging from 20 kHz to 10 MHz [13] which tends to cause subsequent increases and decreases in the pressure of the vapor-air interface of the product subjected to it. This change in the pressure tends to form vapor bubbles in the medium. When the pressure inside the bubble cavity exceeds the intermolecular forces, a bubble explosion occurs which is widely known as the phenomenon of 'cavitation' [14]. Physical, chemical, and thermal outcomes of ultrasonic cavitation have been utilized as the principle of action for many applications corresponding to the need [15]. The 'sono-physical' effect represents the intense shear forces of ultrasound resulting in surface erosion and poration in the product. Particularly when the ultrasound waves are propagated in a liquid medium, a profusion of microjets arises after the bubble collapse which engenders the surface changes in the product immersed in it. When these bubbles undergo vicious bursts, the vapor molecules trapped inside the cavity will be dissociated to produce $OH^{\cdot}$radicals. Such radicals produced on sonolysis of water tend to produce incessant chemical reactions with the compounds of the product treated until they become stable. This event of occurrence is widely known as a 'sono-chemical' reaction. In addition, the temperature and pressure of the medium hit the extreme (5000 K and 1000 atm) at the spot of the bubble burst which provides localized thermal shocks to the product and devotes to the 'sono-thermal' effect [16]. To produce these intense sound waves of varying frequencies causing the physical, chemical, and thermal impacts, the reverse piezoelectricity phenomenon is used [17] by employing three essential components of an ultrasonic system i.e. the generator, converter (transducer), and dissipator (emitter). Electrical energy imparted by the generator will be converted into mechanical sound waves by the transducer which will further emit ultrasonic waves to the product exposed.

The ultrasonic systems are classified into different categories based on the frequency of the sound waves, based on the mode of application, and also based on the geometry of the emitter. Based on the sonic frequency, there exists high and low-frequency US. The frequency (f) of the US waves has an inverse relation with the radius (r) of the bubbles formed on cavitation as given in Eq. (1).[18].

$$f.r = 3 \tag{1}$$

The higher the frequency (>1000 Hz), the smaller will be the bubble radius which on collapse would provide less intense after-effects whereas at a lower frequency (20–1000 Hz), because of the violent collapse of larger bubbles, the intensity will be higher to produce localized temperature and pressure hotspots along with the vigorous shear forces [19]. Thus, the high-frequency or low-power (<1 W/cm^2) US is utilized for on-destructive diagnostic purposes as it would not incur acute modifications in the structure and characteristics of the product subjected to it rather than getting absorbed, reflected, or refracted while the low-frequency or high-power (>1 W/cm^2) US [20] amends the product attributes in a conducive way and is employed in processes for improving the quality and safety of the food products as mentioned in the fore section. Based on the mode of application, US systems are classified as contact and non-contact types. In the contact-type US system, the medium of sonic propagation is liquid inside which the emitter is immersed and in direct contact with the product submerged in the liquid. Whereas in non-contact type US systems air is the medium of propagation and thus this type of US system is termed as 'air-borne US'. Though the air is a worse propagator of sonic waves compared to the liquid medium due to the high acoustic impedance incompatibility [21], airborne US waves bring in pressure fluctuations in the solid product being treated and produce interior microchannels. This is called a 'sponge effect' which facilitates enhancing the mass transfer processes such as extraction and drying [22]. Based on the geometry of the ultrasound emitter, there are horn/probe US and ultrasonic baths. Comparing these two systems, horn US is considered to be more efficient than the US bath as the sonic intensity is lesser and heterogeneous in the latter. However, US bath systems are relatively cost-efficient and are widely used for surface cleaning, solubilization, and degassing purpose [23].

The effect of US on the product depends on the characteristics of the sound wave which will be defined through its frequency, amplitude, power, and intensity. The frequency of the sound wave is described as the number of wave displacements or oscillations per second while the amplitude of the sound wave is the degree of maximum displacement of the wave from its equatorial position [24]. Among these two, the size of the cavitation bubbles is determined by the frequency of the ultrasound along with the power [25]. The acoustic power is estimated by measuring the adiabatic change in the temperature with the sonication time ($\frac{dT}{dt}$) calorimetrically as given in Eq. (2).[26]

$$Acousticpower(AP) = mCp\frac{dT}{dt} \tag{2}$$

where m is the mass and C_p is the specific heat capacity. The intensity of the ultrasound is the acoustic power exposed per area (A) as in Eq. (3). while the acoustic power propagated per volume (V) is known as the acoustic power density (APD) mentioned in Eq. (4) [27]

$$AcousticIntensity(AI) = \frac{P}{A} \tag{3}$$

$$Acousticpowerdensity(APD) = \frac{P}{V} \tag{4}$$

Besides the above factors, the sonication time and the duty cycle of the process will also influence the efficiency and impact of ultrasonication. Additionally, the product or the intrinsic parameters including the pH, temperature, pressure, and structural attributes also affect the implications of US. Thus, these factors have also been taken into consideration and employed in combination with ultrasonication as a hurdle approach to obtain desirable and maximized outcomes [28].

2.1. Enzyme inactivation mechanism and kinetics

Enzymes are proteins with an active site to bind the substrate and activate specific biochemical reactions. Thus, the chemical nature of enzymes contains the primary, secondary, tertiary, and quaternary structures as in the case of proteins. The primary structure of enzymes is composed of amino acids linked with peptide bonds. These polypeptide chains tend to form secondary structures including α-helices, β-sheets, β-turns, and random coils via hydrogen bonding. Such secondary structures tuck over three-dimensionally to become the subunit establishing the tertiary structures through hydrophobic interactions while the quaternary structure comprises those subunits associated via van der Waals attractive forces [29]. Modification in the structure of an enzyme will alter its functionality, stability, and residual activity, thus inactivating the enzyme requires the alteration in its structure by affecting the molecular interactions among the amino acids [30]. The sono-physical, sono-chemical, and sono-thermal effects of ultrasound would invoke structural changes in the enzymes subjected to it as illustrated in Fig. 2.

Subsequent cycles of compression and rarefaction occur as ultrasonic waves travel through the liquid medium. When the rarefaction surpasses the intramolecular attractive forces of the molecules, the formation of bubbles takes place which is widely known as cavitation. By moving around, these bubbles create strong eddy currents in the medium, which

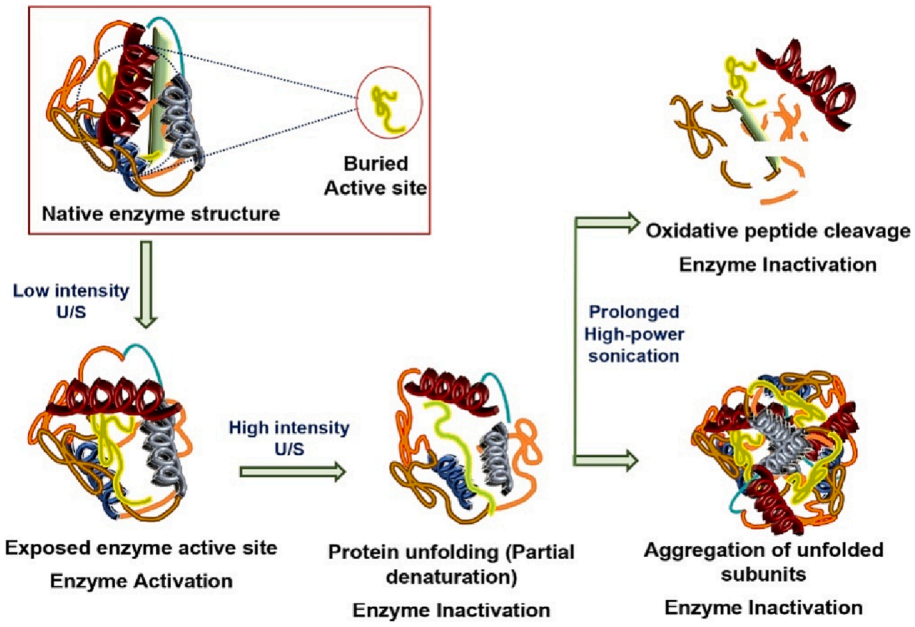

Fig. 2. Schematic diagram of enzyme inactivation mechanism of ultrasound.

would convey constant shear forces. Thus, every bubble formed will be majorly influenced by the impact created by the adjacent bubble, making them unstable and leading to its collapse. The effect of ultrasound produced by these physical forces is termed the sono-physical effect. The eddy currents and the energy released on bubble collapse incur localized hotspots (>5000 K) and high-pressure zones which is termed the sono-thermal effect. The sono-physical effect distributes incessant microjets in the medium. These microjets diffuse intensified shear forces amidst the molecules of protein and thus affect the catalytic activity of the enzymatic proteins. Sonic shearing causes the unfolding of the protein secondary structures and aid in the exposure of internally buried hydrophobic amino acids to the exterior [31]. Due to the enhanced surface hydrophobicity of the ultrasonicated enzymatic proteins, the hydrophilic affinity to the liquid medium reduces and the inter-molecular protein–protein interaction increases via the disulfide linkages and hydrophobic interactions. The protein–protein interactions facilitate protein aggregations which would enshroud the substrate binding site of the enzyme thereby inactivating the enzyme. Also, the aggregate formation initiates partial denaturation of the enzyme. Structural characterization of ultrasonicated mushroom PPO also exhibited a corresponding observation signifying partially denatured enzyme protein [32]. However, the denaturation of enzyme protein structure requires substantially longer sonication time with higher ultrasonic intensity [2]. Thus, less intense sonication just causes unfolding in the enzyme structure without affecting the integrity. This would enhance the exposure of the active site thereby improving the activation kinetics of enzymes [33] which is outside the scope of the present review.

The localized hotspot (>5000 K) at the site of bubble collapse would cause denaturation of the enzymatic protein through the intense energy transfer. The denatured enzyme exhibit loss in its activity and is thus considered to be inactivated. Elucidating the sono-chemical effect, the production of hydroxy radicals on sonolysis of water proceeds to form hydrogen peroxide radicals as mentioned in Eq.5 and Eq.6.

$$H_2O \rightarrow OH^- + H^+ \tag{5}$$

$$H_2O + OH^- + H^+ \rightarrow H_2O_2 + H_2 \tag{6}$$

The rate at which hydrogen peroxide radicals are formed is measured as the cavitational intensity [34]. As these H_2O_2 radicals are of higher oxidative potential, the subjected enzymatic proteins would also undergo oxidative reactions. Oxidation of amino acid in the enzymatic polypeptide chains generates peptide alkoxy radicals' reactions which would be terminated with the production of diamide derivative, isocyanate derivative, amide derivative, and keto acyl derivatives on oxidative peptide cleavage via two different pathways [35], although no specific evidence of such oxidative product was reported in any ultrasound inactivated enzymes. However, the oxidization of amino acids in the enzyme after ultrasound treatment was efficiently studied by evaluating the fluorescence intensity of the enzymes. It was reported that the fluorescence intensity contributed by the aromatic amino acids (tyrosine, tryptophan) reduced after the ultrasonication indicating the possible oxidation of the amino acids [36]. Besides, the phenomenon of amino acid oxidation, the produced radicals also cause remarkable changes in the conformation and aggregation. The energetic free radicals strike the disulfide linkages in the protein and convert it into a thiol moiety at the terminal which would alter the enzymatic protein conformation. Since the catalytic activity, substrate binding, and stability of the enzyme majorly depend on its conformation, alteration in the enzyme conformation inactivates the enzyme. Additionally, the secondary structure of the protein would also be affected by the ultrasonic cleavage of hydrogen bonding [37]. Thus, the mechanism of ultrasonic enzyme inactivation by the sono-physical, sono-thermal, and sono-chemical effects depends on the amino-acid composition and the structural conformation of the enzymatic protein.

Typically, the kinetics of enzyme inactivation is fitted and modeled using the first-order rate kinetics as given in Eq.7.

$$ln\frac{A_t}{A_o} = -Kt \tag{7}$$

where A_t and A_o is the residual enzyme activity at time t and 0 min and K is the rate constant (min^{-1}). Time of sonication required to cause a 90% reduction in the residual activity is calculated through the obtained rate constant of a specific ultrasonic power or intensity which is equivalent to the D-value of microbial decontamination (Eq. (8)).

$$D - value = \frac{ln(10)}{K} \tag{8}$$

Similarly, the acoustic power (AP) or intensity (AI) required for the 90% reduction in the D value can be estimated by taking the slope of the

plot $\log_{10}$D values vs acoustic power/intensity [38] and is termed Z-value ($Z_{AP/AI}$) as mentioned in Eq.9.

$$Z-value(Z_P)=\frac{log_{10}(D_2-D_1)}{P_1-P_2} \quad (9)$$

where D_1 and D_2 are the D values corresponding to the different acoustic powers P_1 and P_2. In certain instances, to explain the non-linear inactivation behavior of the enzyme, the biphasic model (Eq.10) has also been employed using the residual activities of both heat stable and labile enzyme fractions (A_S and A_L) with the corresponding rate constants (k_S and k_L)[39].

$$A=A_S e^{(-k_S t)}+A_L e^{(-k_L t)} \quad (10)$$

Similarly, a fractional conversion model (Eq. (11)) is used to describe the enzyme resistance behavior at longer sonication times with higher acoustic energy densities.

$$A=A_\infty+(A_0-A_\infty)e^{(-kt)} \quad (11)$$

where A_∞ is the residual enzyme activity of the stable enzyme fraction. Thus, the existing models would facilitate understanding the effect of sonication time and intensity on the stable as well as labile enzyme fractions. Besides being an efficient technology for enzyme inactivation, ultrasound technology is one of the most feasible non-thermal technologies because of which exploring the applications of US technology is widening as a standalone technology as well as in combination with other thermal, chemical, and non-thermal technologies which will be discussed in the following sections.

3. Dairy enzymes

Thermal processing has been considered the most common and widely applied technique for pasteurizing food and dairy products for microbial and enzyme inactivation. However, these techniques can harm the natural dairy flavors and their nutritive value. Therefore, researchers have investigated novel techniques to meet consumer demand for higher-quality products with extended shelf life [40].

US treatment is one of the emerging technologies, which is frequently marked as non-thermal. In the dairy industry, ultrasound-based techniques can be used for diverse applications such as fat separation, pasteurization, controlling lipid oxidation, homogenization, emulsification, disinfection, protein denaturation, enzyme activation or inactivation, etc. [41]. Several studies have demonstrated the significant potential of US with heat and/or pressure than implemented alone [42]. The efficacy of the US for enzyme inactivation in dairy products has been outlined in this section.

3.1. Native milk enzymes

The effect of US alone or in combination with heat has been presented below. Various researchers have reported that US could be effective against various dairy-related food enzymes, including alkaline phosphatase (ALP), lactoperoxidase (LPO), and γ-glutamyl transpeptidase (GGTP) [43] and with varying effectiveness against the respective enzyme. Typically, the enzyme activity decreases as the enzyme concentration increases, but higher solid content (i.e., high protein and fat) can enhance enzyme inactivation (Ahmad et al., 2019).

In dairy systems, ultrasound can homogenize protein aggregates and cause whey protein denaturation. Enzyme inhibition occurs when pressure and heat are applied, such as 3.5 kg/cm^2, 126.5 °C, while mild sonication conditions can increase enzyme activity [44]. Pegu and Arya [45] also depicted that shorter duration and intensity (at 200 W for 4 and 6 min) resulted in a 1–2% increase in ALP activity; however, as time and intensity were increased, ALP activity decreased. Enzyme activity can rise due to increased mass transfer, impart substrate availability, making enzymes more readily available for reaction. In contrast, it can fall as a result of protein denaturation caused by the disruption of Van der Waals interactions and hydrogen bonds in the polypeptide.

Enzymes produced by microbial contaminants are also present in milk. Extracellular compounds delivered by psychrotrophic microorganisms are among milk's main microbial catalysts. These proteolytic and lipolytic enzymes resist heat and remain active even after UHT treatment and pasteurization. The bitter and rancid off-flavors caused by protein and fat degradation threaten the dairy products' shelf life after pasteurization [12,42,46]. No studies have been reported regarding the inactivation of heat resistance extracellular enzymes from psychrotrophic bacteria in milk via US alone [42]. However, the non-thermal inactivation of enzymes in milk does not guarantee the killing of pathogens [40]. The ultrasound treatment not limited to inactivated or elimination of the bacteria it also helped in maintaining the nutritional parameters like protein, amino acids during the processing, which could beadvantageous for dairy products processing.

US as a solitary non-thermal process or combined with heat and/ or pressure can achieve the desired enzyme inactivation. However, US has been observed to be more beneficial when used in combination. Integration of US-treatment with pasteurization or the HTST process can be helpful in the deactivation of enzymes that compromise the quality of the product. US has yet to gain practical application in the dairy industry, probably because of a need for high capacity, continuous processing units with sufficiently short processing time, a combination that cannot be met by US equipment currently available. Also, the effect on physicochemical properties should be extensively studied to evaluate the advantages of US-treatment over other treatments.

4. Enzymes from fruit and vegetable processing

The endogenous enzymes released from fruits and vegetables affect the quality and shelf life of the final products. The undesirable change caused by endogenous enzymes varies depending on the type of enzyme and its three-dimensional structure [47]. The commonly observed changes in the food products are the development of browning pigments, release of off-flavors, softening of texture, and shifts in the rheological properties. For example, in fruit juice processing, endogenous enzymes such as polyphenol oxidase, peroxidase, and pectin methylesterase present in the fruits are released into the final products. The presence of these enzymes reduces the viscosity of juice, causes cloudiness, and develops off-flavors.

US is a promising method that can inactivate the endogenous enzymes released from fruits and vegetables [48]. In recent years, several studies have utilized ultrasonication to inactivate these endogenous enzymes (Table 1). Depending on the processing parameters involved in US, it could either partially or completely inactivate the enzymes. The inactivation occurs as the US alters the protein's three-dimensional structure and changes it from the native to a denatured state. During US, bubble formation and collapsing occur, which changes the environment of enzymes, including the pH, shear stress, temperature, and pressure. The change in environmental conditions breaks the hydrogen bonds in the polypeptide chain of the endogenous enzyme and results in inactivation. Further, the formation of high-energy free molecules during sonication affects the catalytic function of enzymes [49].

The major negative impacts caused by these enzymes on food products are summarized in Fig. 3.In addition, the enzyme inactivation is affected by the medium in which the enzyme is held. The following section discusses the effect of ultrasonication on the inactivation of these endogenous enzymes.

4.1. Polyphenol oxidase

Browning occurs in fruits and vegetables due to non-enzymatic and enzymatic reactions [39]. The maillard reaction and caramelization are major non-enzymatic browning reactions that are commonly observed in thermal processing techniques such as extrusion. Enzymatic browning

Table 1
Application of US in the inactivation of fruits and vegetables.

Source	Enzyme inactivated	Optimum condition for enzyme inactivation	Enzyme inactivation efficiency	Reference
Fruit and vegetable smoothie	Peroxidase	20 kHz, 750 W, 70 % amplitude, 4 min	US inactivated 71.6% of peroxidase activity	[69]
Carrot juice	Pectin methylesterase and polyphenol oxidase	20 kHz, 3.80 W, 10 min	The activity of pectin methylesterase and polyphenol oxidase was reduced by 37.95% and 43.90%, respectively.	[70]
Carrots	Peroxidase	22 kHz, 300 W, 12 min	Reduced peroxidase activity by 18%.	[71]
Sugarcane juice	Peroxidase	20 kHz, 75% power intensity, 25 min	US inactivated 77.3% of peroxidase activity	[63]
Green asparagus	Phenylalanine ammonia lyase (PAL)	40 kHz, 360 W, 10 min	Reduced the residual activity of PAL by 75%	[72]
Green coconut water	Peroxidase	20 kHz, 286 W, 30 min	Inactivated peroxidase activity by 27%	[64]
Spinach juice	Peroxidase and polyphenol oxidase	40 kHz, 180 W, 21 min	US reduced the enzyme activity of peroxidase and polyphenol oxidase by 31.76 and 36%, respectively.	[73]
Dried ginger slices	Polyphenol oxidase, peroxidase	33 kHz, 600 W, 30 min	Inactivated 75% of polyphenol oxidase and peroxidase activity	[74]
Fresh-cut pineapple	Peroxidase	37 kHz, 25 W, 10 min	Inactivated 53.2% of peroxidase activity	[68]
Plum nectar	Polyphenol oxidase	20 kHz, 400 W, 10 min	Polyphenol oxidase activity was reduced to 46%.	[75]
Pineapple juice	Pectin methylesterase	20 kHz, 500 W, 15 min	Enzyme activity was reduced by 70–80% during 60 days of storage.	[61]

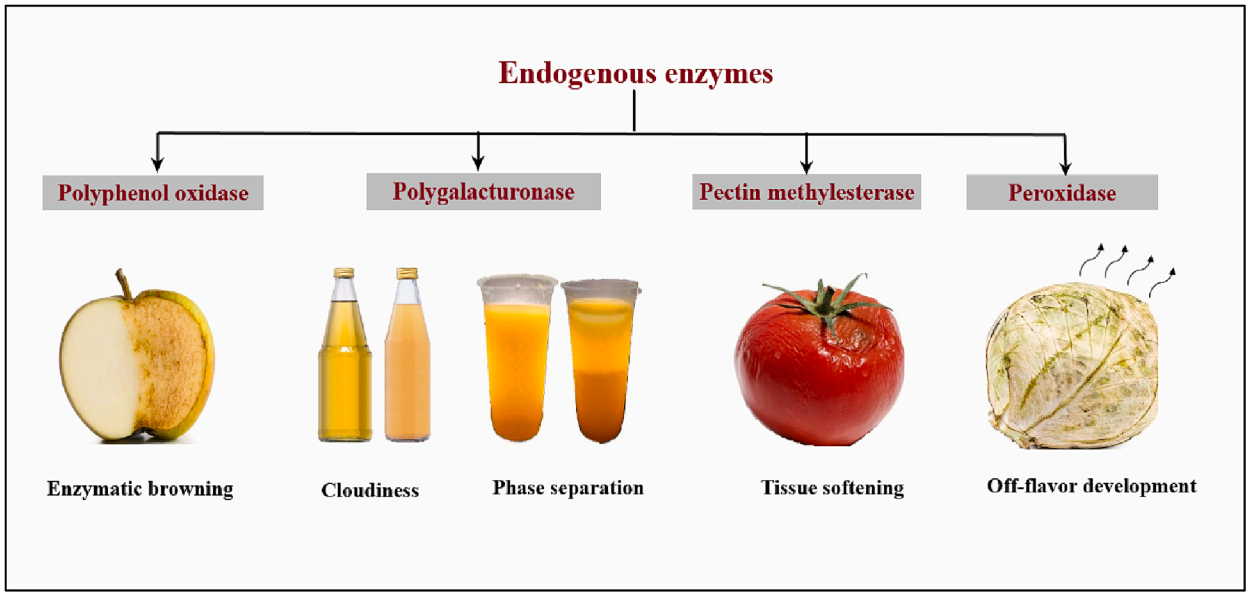

Fig. 3. Major detrimental effects caused by endogenous enzymes released from fruits and vegetables.

occurs due to the exposure of fruits and vegetables to air during processing, handling, and/or storage. Polyphenol oxidase (PPO) is the key enzyme in fruits and vegetables that causes browning by catalyzing the formation of o-quinones. The polymerization of o-quinones leads to the development of undesirable brown pigments, which affect the flavor, color, and nutritional quality of food products [50].

US is an energy-efficient technology that can inactivate the PPO by altering its secondary and tertiary structure. Studies have shown that USexhibits higher enzyme inactivation efficiency compared to thermal and other non-thermal techniques. For instance [38] compared the efficiency of ultrasonication, thermal process (55–75 °C), and US combined with cooling (USC) for inactivating PPO in the bayberry juice. The results illustrated that the PPO inactivation efficiency was higher for US than the thermal process and USC. The residual activity of PPO decreased as the sonication intensity increased from 90 to 452 W/cm^2, indicating that high-intensity US is an efficient treatment to inactivate PPO compared to thermal techniques. Similar results were observed during PPO inactivation in the sweet melon as they were subjected to varying US intensities (100–500 W for 20 min) and thermal treatments (25–85 °C for 25 min) [51]. The PPO activity was reduced with the increase in US intensity, and thermal treatment was found to be less efficient than US. The authors concluded that high-intensity US could inactivate the PPO by generating less heat compared to thermal treatment. Studies have revealed that high-intensity US causes structural modification of PPO by initially dissociating and later aggregating the protein structure. Specifically, the α-helix conformation is affected, and it leads to the reorganization of the secondary structure. The fluorescence analysis has revealed that the tertiary structures are also disrupted at higher intensities. These studies indicate the potential of US in inactivating the PPO [52].

Apart from the enzyme inactivation efficiency, it is crucial to consider factors such as energy consumption and energy density for scaling up to an industrial scale [53]. Compared the potential of US, US with temperature control, and high-pressure homogenization (0–150 MPa) for inactivating the PPO at an industrial scale. US was found to be more economical due to less processing time (6 min), low energy density (444 MJ/m^3), and less energy consumption compared to the other treatments. The high-pressure homogenization required ten passes at 150 MPa to achieve 50% enzyme inactivation, and the US with temperature control inactivated 90% PPO in 45 min. While the highest efficiency was exhibited by US as it inactivated the total PPO content in 6 min, indicating its suitability for industrial scale-up.

In recent years, the combined effect of US with other treatments (e.g., high-pressure, temperature) for enzyme inactivation has been continuously explored, and it has been found to be effective. Silva et al [39]utilized thermosonication(TS) (71 °C—1.3 W/g), thermal treatment (71 °C), and high-pressure processing combined with thermal treatment (600 MPa—71 °C) to inactivate PPO present in apples, pears, and strawberries. The TS was highly efficient for PPO inactivation, followed by thermal treatment, and high-pressure processing—thermal treatment. In another research, mushrooms were TS at the following conditions: temperature (20–60 °C), sonication power (60, 80, or 100%), and treatment time (0–30 min) to inactivate the PPO. The combined effect of temperature and ultrasonication (100% power and 60 °C for 10 min) was more efficient compared to sonication alone. Fourier transform infrared spectroscopy (FTIR) revealed that the TS decreased the α-helix and β-sheets contents and aggregated the random coils, β-sheet, and turns, thus causing denaturation of enzymes. These studies illustrate the possibilities of utilizing the US process together with other processing conditions to inactivate PPO. More information related to these hurdle technologies is discussed below.

4.2. Pectin methylesterase

Pectin is an important cell wall polysaccharide present in plants. The esterification of pectin softens the texture of fruits and vegetables and consequently leads to food spoilage and wastage [54]. Pectin methylesterase (PME) is a major enzyme in plants that causes pectin de-esterification, hydrolyzation, and degradation. The PME activity causes cell wall loosening, fruit tissue softening, decreases the viscosity of fruit and vegetable products, and increases the turbidity, phase separation, and cloudiness in juice products [55]. Studies have shown that ultrasound can inactivate the PME to a certain extent and promote the formation of networks within the treated products, which positively influences their texture, rheological properties, and stability.

In a recent study, the post-harvested apricot fruit was subjected to an US intensity of 400 W for 20 min to inactivate the PME activity [51]. After the US treatment, the residual activity of PME was reduced by 56.71%. Subsequently, it increased the firmness of apricots by 3.83% during the 16 days storage period. The decreasing trend of PME activity due to US and subsequent texture enhancement was also reported in quince fruit during the 14 days storage period [56].

In addition to the texture enhancement by inactivating PME, several studies have also focused on improving the rheological properties and stability of the juice products. [57] used US to inactivate the residual activity of PME in the strawberry pulp. The sonication intensity of 605 W/cm^2 for 16 min decreased the PME activity by 22.6%. The US treatment increased the apparent viscosity, loss modulus, and storage modulus of the strawberry pulp by reducing the esterification of pectin and promoting the formation of complexes between the free calcium ions and low methylated pectin. Likewise, the rheological properties and storage stability were enhanced in the kiwifruit juice US at 400 W for 16 min due to PME inactivation. In another study, carrot juice was subjected to US intensities ranging between 0.95 and 3.80 W/ml [58]. The increase in sonication intensity reduced the activity of PME, and the highest reduction of 37.95% was observed after treating the juices at 3.80 W/ml for 10 min. Similar to the previously discussed studies, US increased the apparent viscosity. Furthermore, it maintained the stability of juice and preserved its nutritional value.

Despite studies indicating the potential of ultrasound in inactivating PME, few contradicting results indicate that US is ineffective. For instance, the author Gomes et al. [55] compared the efficiency of US and thermal processing for inactivating PME in fresh orange juices. The juices were subjected to US intensity of 17 W for 15–150 s and thermal processing at 90 °C for 1 min. The US was not capable of inactivating the PME, while the heat treatment was found to be efficient as it inactivated 96.3% of PME. The study concluded that ultrasound is not suitable for PME inactivation and stated it is necessary to combine US with other technologies. Adding to it, [59]elsewhere it was pointed out that TS (40 kHz—60 °C) is efficient for inactivating the PME present in the orange juices compared to US. Similar results were also observed in custard apple juices treated by TS (20 kHz, 68 W/cm^2). Due to the contradicting results, it is important to conduct more studies to test the efficiency of US and combined technologies for inactivating PME.

4.3. Polygalacturonase

Polygalacturonase (PG) is a pectic enzyme that causes pectin degradation by hydrolyzing the homogalacturonan regions [60]. During juice production, the PG could cause both desirable and undesirable effects. The desirable effects include increased juice extract and providing better clarification. At the same time, it is also capable of increasing cloudiness and causing phase separation, which is considered an undesirable outcome [61]. Additionally, the synergistic interaction between the PG and PME decreases the viscosity of the juice products.

Until now, thermal processing, pulsed electric field, and high-pressure processing have been widely used for inactivating PG, and only one study has utilizedUS. [62] inactivated PG in apple juice using US treatment. The study maintained a constant frequency of 28 kHz and 90% amplitude for 0–10 min. The residual activity of PG decreased with the treatment time, and at the end of 10 min, the residual activity was 51%. The inactivation was mainly attributed to the breakdown of van der Waals force between the molecules due to the forces resulting from cavitation. In addition to the PG inactivation, US improved the sugar value, color, and antioxidant activity of the juices. The authors also compared the stability of PG and peroxidase to ultrasonic treatment and revealed that PG is more sensitive than peroxidase.

4.4. Peroxidase

Peroxidase (POD) is a ubiquitous heme-containing enzyme that plays a critical role in the physiological functions of plants [63]. It is mainly involved in plant development, lignin biosynthesis and degradation, and response to abiotic stress conditions. The POD is known as an indicator enzyme of quality deterioration due to its high thermal stability. It is generally assumed that if the POD is inactivated, then the remaining quality deteriorating enzymes are inactivated [64]. The presence of POD in food products leads to the development of browning pigments and off-flavors. Hence, inactivating the POD is crucial to maintain the original flavor and color of the food products.

Due to the high thermal stability of POD, thermal processing is considered a suitable method for inactivation. However, most thermal treatments used for inactivating POD degraded the functional compounds in the food product [38]. Due to this reason, an increasing trend toward the utilization of US for POD inactivation is widely seen, and several studies have revealed that US is more efficient than thermal processing. For example, the effect of ultrasonication and thermal processing for POD inactivation in custard apples was examined [65]. US intensity ranging from 40 to 100 W for 5 min and the thermal treatment at 91 °C for 22 min was adapted for the study. The thermal treatment completely inactivated the POD after 22 min, while the US operated at 85 W was able to achieve 100% inactivation in 5 min. During the inactivation, the thermal treatment caused a loss of 52.7% of vitamins C, and relatively less loss (21.6%) was observed in the US process. In another study, bayberry juices were subjected to US (90–452 W/ cm^2 for 12 min) and thermal processing (55–75 °C for 1–30 min [38]. The increase in sonication intensity from 90 to 452 W/cm^2 decreased the D value by 55.23 to 6.77 min. After US at 452 W/cm^2 for 12 min, the POD was completely inactivated. While the thermal treatment performed at 75 °C required 17 min for inactivation. Similarly, the study performed on the goldenberry puree indicated that USis capable of both inactivating POD and preserving the carotenoids compared to thermal treatment [66]. These studies point out the efficiency and suitability of US in the inactivation of POD and preserving bioactive compared to thermal

processes.

US treated fresh pumpkin juices at 0–600 W for 10 min to inactivate POD. The increase in US intensity decreased the POD residual activity in the juice. The sonication intensity of 600 W was identified to be an optimal condition as it decreased the POD activity by 63.75%. Despite applying high-intensity ultrasound, the carotenoid content in juices remained unaffected. On top of this, US enhanced the rheological properties, sensory attributes, and nutritional value of pumpkin juice [67]. The US treatment (29 W for 15 min) was also found to be efficient in inactivating POD in fresh-cut pineapples [68]. Overall, the current literature indicates that US is suitable for POD inactivation.

The whole manuscript focusses on the inactivation of enzymes, the ultrasound treatment is widely used in various food commodities and has an influence on the nutritional parameters. US has positive effect on nutritional status of various fruit juices and vegetables by enhancing the total phenolic, flavonoid, reducing power, antioxidant properties, Vitamin, protein and including minerals. The bioactive properties significantly increased and were maintained during the US process, and this greatly played a role in storage and shelf extension of the products. Consequently, the US application showed benefits on both the nutritional and physical properties. Therefore, US applicability in industry is suggested as an alternative emerging technique to successfully replace the pasteurization for improving the quality.

5. Hurdle approach- US with other combined technologies

5.1. Synergistic effect of US with thermal technologies

5.1.1. Thermosonication (TS)

Modifying the temperature of the liquid medium of ultrasound propagation along with the sonication process is termed 'thermo-sonication' (TS). As the enzymatic proteins are thermally sensitive and alter their structure and conformation with temperature intervention, combining US processing with thermal processing would effectively inactivate the enzymes. Also, the compounding effect of sonication facilitates better enzyme inactivation with relatively lesser and intermediate temperature involvement compared to conventional thermal processing. Thereby the organoleptic properties of the product were expected to be unaffected. As a result of understanding this fact, several studies from 2017 to 2022 evaluated the TS effect for microbial and enzyme inactivation rather than just employing the ultrasonic treatment. Significant studies on TS for enzyme inactivation are briefly given in Table 2.

[39] studied the effect of TS on PPO in three different fruits (strawberry, pear, apple) and compared its effect with thermal processing as well as high-pressure processing and found that TS is outperforming the other technologies in all the fruits for the inactivation of PPO. At the extreme condition of the TS (71℃; 460 W/cm^2; 24 kHz) for 20 min, the residual activity is reduced to 0.7% while exclusive thermal treatment of 71 °C for 20 min was observed with the residual activity of 89% in pear. This is a definite indication of the superiority of the synergistic effect of thermal and US technology over conventional thermal processing. Although, the authors did not study the effect of US alone to draw an explicit conclusion about the contributive effect of US on enzyme inactivation. Additionally, the inactivation of PPO in the strawberry was observed at a relatively lower temperature of 57 °C which must be because of the softer texture of the strawberry compared to that of pear and apple for the sonic waves to penetrate and act on the enzymes. Thus, the tissue matrix in whole fruit and vegetable commodities would also influence the inactivation efficiency. However, in liquid products such as clear juice [76], the pulp [77], paste [78], and nectars [79,80] disregarding the consistency of the product, enzyme inactivation was efficient and predominantly depended on the process and product parameters.

The difference in the enzyme structure will also influence the percentage reduction in the residual enzyme activity. The TS inactivation of

Table 2
Thermosonication for enzyme inactivation.

S. No	Product	Enzyme	Process Parameters	Findings	Reference
	Pear Apple Strawberry	PPO	Probe US; 24 kHz; 460 W/cm^2; Temperature 57 °C & 71 °C; 10–20 min.	Highest and quicker inactivation at 71 °C TS	[113]
	Strawberry clear juice	PPO	Probe US; Flat sweep (15 min) & dual frequency (15 min) 20/40 kHz; 60 °C; Storage till 28 days at 4, 25, 37 °C.	Maximum of 90.7 % reduction in the residual enzyme activity after TS with dual frequency mode; No reversible change in the enzyme activity till 28 days of storage irrespective of the storage temperature.	[114]
	Tomato paste	PME, PG	Probe US; 20–25 kHz; 87.52 W/cm^2; 22 °C & 65 °C for 10 min.	PME- 9.78% residual activity at TS of 65 °C for 10 min.PG - 20% residual activity at TS of 65 °C for 10 min.	[53]
	Spinach juice	POD, PPO	Probe US; 30 kHz, 200–600 W; 60 °C for 20 min.	Highest reduction at TS with 600 W, 60 °C for 20 min	[115]
	Vacuum packed soursop pulp	POD, PPO	Ultrasonic bath; 40 kHz; 100 W; 20–60 °C.	Maximum inactivation observed at 60 °C. Vitamin C retention reduced to 71% at the highest temperature.	[116]
	Orange juice	PME	Ultrasonic bath; 40 kHz; 25–60 °C for 10 min.	Minimum enzyme activity of 16% observed at 60 °C of TS. 40% reduction in ascorbic acid content.	[117]
	Jackfruit nectar	PME	Probe US; 80% amplitude, 40–50 °C for 15–25 min.	Maximized enzyme reduction observed at 50 °C for 25 min.	[117]
	Carrot slices	POD	US bath; Mono, dual, tri frequency mode (22/33/40 kHz); 300 W; 60 °C for 2–12 min.	Minimized residual activity of 22.44% at dual frequency mode TS (22/40 kHz) at 12 min. Hydrogen radicals' intensity reduced at TS.	[39]
	Cloudy apple juice	PPO, PME	Probe US; 750 W; 100% amplitude; 62 °C for 20	Reduction in the enzyme activity of PPO after TS to	[76]

(continued on next page)

Table 2 (*continued*)

S. No	Product	Enzyme	Process Parameters	Findings	Reference
			min; Storage till 28 days at 4 °C.	12.7% while PME was reduced minimally about 93.1%.	
	Fruits (Mango and jackfruit) and rice smoothie	PPO, PME	Probe US; 1500 W; 20 kHz; 70–85% amplitude; 40–55 °C for 15–25 min.	Highest reduction at 85% amplitude, 55 °C for 25 min.	[78]
	Grape juice	PPO, POD	Probe US; 1000 W; 20–25 kHz; 55 °C; Nisin concentration of 200 ppm.	Highest reduction was with TS and Nisin incorporation.	[82]
	Sugarcane juice	PPO, POD	Probe US; 20 kHz; 50 W/cm^2; 50–80 °C for 2.5–25 min; storage till 32 days at 4 °C.	Enzyme inactivation observed above 60 °C.	[83]
	Orange juice	PME	Ultrasonic bath; 300 W;20–60 kHz; 45–75 °C for 20–40 min.	20 kHz, 75 °C for 30 min incurred the highest inactivation of PME.	[84]
	Guava slices	PPO, POD	Ultrasonic bath; 240 W; 37 kHz; 65 °C for 5 and 10 min followed by freeze drying.	Maximum inactivation after 10 min; TS guava slices required lesser time of freeze drying.	[85]
	Cloudy strawberry nectar	PPO	Probe US; 150 W; 25–75 °C for 0.1 and 15 min.	Maximum inactivation at 75 °C at 15 min.	[71]
	Camu-camu nectars	POD, PPO	Ultrasonic bath; 40–60 °C for 30 and 60 min.	Maximum inactivation attained at 60 °C for 30 min.	[81]
	Cloudy apple juice	PME, PPO	Probe US; 20 kHz; 100% amplitude; 20–50 °C for 15 min.	Complete inactivation at the maximum temperature.	[77]
	Blood fruit juice	PME, PPO, POD	Probe US; 1500 W; 20 kHz; 80% amplitude; 1.2, 1.4 W/cm$^{2;}$ 44 and 55 °C for 5, 10 min.	Maximum reduction nearer to that of pasteurized sample at 55 °C for 10 min at 1.4 W/cm^2.	[87]
	Black berry juice	PME, PPO	Probe US; 1500 W; 20 kHz; 40 and 50 °C for 25 min.	Highest inactivation at 50 °C for 25 min. with the increase in ascorbic acid, total phenolics and antioxidant activity.	[88]
	Custard apple juice	POD, PME	Probe US; 400 W; 20 kHz; 30 °C.	PME complete inactivation after 50 s and POD complete inactivation after 40 min.	[89]

PME and PG in tomato paste showed an equivalent trend in the reduction of residual activity, however, the values of residual activity of both enzymes vary at the same processing time [78] which would be because of their difference in the resistance towards degradation. Increasing the TS temperature from 22 °C to 65 °C had a significant rise in the reduction. Additionally, TS at 22 °C was observed to show effects commensurate to that of thermal processing at 65 °C. This distinctly manifests the ability of U/S to inactivate the enzymes at low-temperature conditions.

Similarly, [76] investigated the effect of multimode TS on the microbial and biological quality of the strawberry clear juice with the inactivation of PPO during the storage period of 28 days at different temperatures (4 °C, 25 °C, and 37 °C). The authors exhibited the efficiency of the TS process at (60 °C; 15 min) to yield irreversible change in the enzyme activity i.e., a reduction in the residual activity of 90.7% was retained till the 28th day of storage at all the temperatures. Such an irreversible change was also seen in cloudy apple juice for PPO inactivation at 62 °C for 20 min with 750 W probe US [81]. [76] have also parallelly studied the thermal pre-treatment effects (90 °C for 1 min) and showed that though the enzyme inactivation caused is two times higher than that of TS, other bioactive properties including total phenolics, total flavonoids, total anthocyanins, and total ascorbic acid contents are retained in the thermo-sonicated samples than in the thermally processed samples. Therefore, TS can be an efficient enzyme inactivation technology with all the biochemical constituents unaffected. Likewise [82] also reported a retainment in total phenolics, total flavanols, chlorophylls, carotenoids, and anthocyanins in TS (600 W, 60 °C, 20 min) spinach juice along with the efficient enzyme PPO and POD inactivation closest to that of pasteurized samples (60 °C for 30 min).

On a contrary [83], reported a decrease in vitamin C retention in TS (100 W, 60 °C) vacuum-packed sour soup pulp beside the PPO and POD enzyme inactivation. The high thermolabile nature of vitamin C would be the reason for such a reduction. Such a thermally affected ascorbic acid content was reported in the case of thermo-sonicated orange juice as well (40 kHz, 60 °C, 10 min) [84] besides the reduction in the residual activity of PME. Furthermore, conversely, ascorbic acid content was observed to be increasing after TS (80% amplitude; 50 °C, 25 min) of jackfruit nectar [85]. The authors stated that the shear forces in cavitation aid in the release of ascorbic acid from the cell tissues leading to its increase. It was also observed that at the highest treatment condition PME activity was significantly reduced. A comparable rise in the ascorbic acid content was also observed in thermo-sonicated, PPO and PME-inactivated blackberry juice [86]. The reported reduction in the bioactive contents in the TS commodities was processed mostly with US bath systems. Thus, it might also be predicted that the geometry of the propagator would also influence the efficiency of processing in terms of the retention of bioactive constituents.

Since the effect of temperature combined with US was well established at temperatures above 40 °C, the effect of other parameters was also explored. [71] reported that the frequency mode of sonication would also influence the enzyme inactivation efficiency. The author found that the highest inactivation of POD in carrot slices was observed with the dual frequency mode of 22/40 kHz due to the interaction of two different wavelength US waves producing a sequential microstreaming effect at a fixed temperature of 60 °C. Surprisingly, the free radicals (hydrogen, hydroxyl, and alkyl radicals) intensity was found to be lesser in the thermo-sonicated medium than that in the ultrasonicated medium which represents the thermal instability of radicals produced on sonication. From this, it can be conferred that the sono-thermal effect is a predominant mechanism of inactivation in thermo-sonication rather than the sono-chemical effect. Additionally, the impact of changing the sonic wave amplitude on enzyme inactivation was shown by [77]. The study exhibited that increasing the amplitude of US increases enzyme inactivation. Thus, it is decisive that process parameters have a significant role in the inactivation even though the thermal effect is distinct.

In addition to the TS, the incorporation of nisin in a concentration of

200 ppm in grape juice has also inactivated the PPO and PME enzymes effectively. However, the principle of action of nisin on the enzyme inactivation is yet to be investigated [87]. Many other different studies have exhibited the efficacy of TS in varied products like sugarcane juice [88], orange juice [89], cloudy strawberry nectar [79], Camu-Camu nectar [80], cloudy apple juice [90], blood fruit juice [91], and blueberry juice. Furthermore, thermo-sonicated guava slices have shown better enzyme inactivation along with a faster drying process after the sonication. The surface modification of the product after US would be the cause of this enhancement in the mass transfer during drying resulting in lesser energy consumption [92]. Also, the guava slices were processed in the ultrasonic bath which would explain the possibility of scaling up the technology for commercial processing.

Enzyme inactivation efficiency is influenced by several factors including the temperature, ultrasound frequency, amplitude, sonication time, and the enzyme's structure and conformation. In many of the TS studies, the maximum percentage of inactivation was attained at temperatures $< 60\ ^{\circ}C$ particularly, [93] observed a complete inactivation of PME and POD after 50 s and 40 min of TS respectively at 30 °C. Though lower temperature usage may require prolonged sonication for complete inactivation, the bioactive profile of the product will be preserved at low-temperature processing technologies. Since most of the TS studies focussed on the microbial safety of the products, combining US with thermal processing would be a promising hurdle technology for microbial as well as enzyme inactivation with the better nutritional quality of the product.

5.1.2. US combined with conventional drying techniques

The intervention of thermal processing during or after the sonication imparts the difference between thermo-sonication and other US + thermal processes. Specific studies employed ultrasonication as a pre-treatment for dehydration to obtain better mass transfer during the drying process. As discussed in the US-principles section, the sponge effect and the surface modification of the sonicated product by cavitational shear forces aid in the movement of moisture from the product through the microchannels when subjected to an environment of temperature or solute gradients. Some of the studies on US-assisted dehydration exhibited the effect of the combination technology on enzyme inactivation and are briefed in Table 3.

[94] explored the influence of US combined with a vacuum (V) and osmotic dehydration (OD) on the inactivation of the PPO enzyme in garlic slices. About 30% of calcium chloride was used as the osmotic solution and the effect of US, OD, and vacuum pressure was evaluated individually. The authors reported that the PPO residual activity was reduced to the least level when US was used alone as well as when combined with V + OD. The cavitational effect in synergy with the V and osmotic pressure facilitated the highest enzyme inactivation along with the increase in the total phenolic, total flavonoid, and antioxidant activities. As anticipated, the sonication process enhanced the rate of drying and thereby reduced the specific energy consumption for drying besides the improved rehydration properties of the sonic pre-treated dried products. Similar results were observed with PPO and POD inactivation in ginger slices using a 20% sucrose solution as the osmotic solution [92]. Even with the convective hot air drying, the POD, PPO, and AAO enzymes in banana slices were inactivated by US (20 kHz; 300 W; 30 min). Additionally, the kinetics of enzymatic and non-enzymatic browning of dried slices with and without US pre-treatment was investigated and it was found that the rate of enzymatic browning of sonic-pretreated slices was relatively lesser which would be the correlative outcome of the inactivated enzyme by sonication [95].

Though the principal objective of US -assisted drying is to enhance the kinetics of drying through increased mass transfer, it has also exhibited an enzyme inactivation ability to yield desirable dehydrated products. Though drying and dehydration are the primeval processing technologies, the loss of nutrients on drying is considered to be the major limitation. However, the reported studies with ultrasonic assistance quoted the increase in the bio-actives in US pre-treated dried products which would be a parallel response of the inactivated enzymes as those bio-actives act as substrates for these oxidative enzymes.

5.1.3. US combined with Novel-thermal techniques

To utilize the efficiency of thermal processing besides subduing the limitations of conventional thermal processing such as heterogeneous heating, substandard end products, and loss of nutrients, certain novel thermal technologies have emerged which include microwave, infrared, radio-frequency, and ohmic heating. Ultrasonic processing combined with these novel thermal technologies has also been investigated for drying, microbial decontamination, and enzyme inactivation. Some of the studies discussed the enzyme inactivation effect have been given in Table 4.

US combined with microwave blanching of the garlic bulbs incurred a maximum of 88% reduction in the PPO and POD enzyme activity along with the degradation in the predominant black mold *Aspergillus niger* [96]. The US -induced pores in the structure of garlic bulbs promote homogeneous heating with microwave processing which thereby resulted in the microbial and enzyme inactivation of the product. US pre-treated pineapple slices have also shown higher enzyme inactivation of three different enzymes (PPO, POD, and bromelain) after infra-red drying [97]. The authors have investigated the influence of multi-frequency mode US and reported that the tri-frequency (20/40/60 kHz) mode of sonication combined with ethanol immersion and infrared drying engendered the highest inactivation. Furthermore, the microstructure, rehydration, appearance, and flavor profile characteristics were also preserved in the tri-frequency sonicated dried slices. Similar results were also reported with PPO and POD inactivation in sweet potato slices by US combined with infrared drying and also in the case of POD inactivation by infrared drying of carrot slices.

Apart from the drying processes, microbial safety of the liquid products is also ensured through these novel thermal methods, one of which is the ohmic heating technology where the electrical resistance of

Table 3
Enzyme inactivation by US combined drying technologies.

S. No	Product	Combined drying technology	Enzyme	Process Parameters	Findings	Reference
1.	Garlic slices	Vacuum osmotic dehydration and convective drying	PPO	Ultrasonic bath; 40 kHz; 600 W; 30 °C 40 min; 30 % $CaCl^2$ osmotic solution; 100 mbar pressure; Drying at 60 °C	Enzyme activity reduced to 30% with just U/S; 20% with U/S and osmotic drying (OD); 10% with Vacuum + U/S + OD. Specific energy consumption is reduced Antioxidant and bioactive properties increased with sonication and drying	[93]
2.	Ginger slices	Osmotic dehydration and convective drying	PPO, POD	Ultrasonic bath; 33 kHz; 600 W; 30 °C 30 min; 20% sucrose solution;	Lowest enzyme residual activity obtained with U/S combined with osmotic dehydration	[79]
3.	Banana slices	Convective hot air drying	PPO, POD, AAO	Ultrasonic bath; 20 kHz; 300 W/l; 10–30 min; Drying at 70 °C 45 min;	Longer sonication before drying reduced the enzyme activity. Rate of enzymatic browning decreased in longer sonicated samples. Increased drying rate and reduced drying time.	[80]

Table 4
Enzyme inactivation by US combined with novel thermal technology.

S. No	Product	Combined novel thermal technology	Enzyme	Process Parameters	Findings	Reference
1.	Garlic bulbs	Microwave blanching	POD, PPO	Ultrasonic bath; 40 kHz; 100 W; 40–60 °C for 20–40 min; Microwave of 1–4 W/g for 40–180 s;	Maximum percentage of reduction in POD and PPO was 85% and 88%.	[90]
2.	Pineapple slices	Infra-red drying	PPO, POD, Bromelain	Ultrasonic bath; Multifrequency mode (20 kHz; 20/40 kHz; 20/40/60 kHz); 50 W/l; 30 min; Infrared power 675 W; temperature 60 °C;	Higher enzyme inactivation observed with tri-frequency ultrasound treated dried slices.	[91]
3.	Sweet potato slices	Infra-red drying	PPO, POD	Ultrasonic bath; 20–60 kHz; 300 W/cm^2; 30 °C; 30 min. Temperature 60,70,80 °C.	Enzyme activity reduced to 10% with sonication and drying at 80 °C.	
4.	Carrot slices	Infra-red drying	POD	Ultrasonic bath; tri-frequency mode 20, 28, 40 kHz; 100 W; 60,70, 80 °C for 1–5 min. Infrared drying temperature 70 °C.	POD activity reduced to 18.57% after sonication at 80 °C for 5 min.	
5.	Orange juice	Ohmic-sonication	PME	Probe ultrasound; 550 W; 20 kHz; 100% amplitude; 60 −70C for 2–8 min. Ohmic heating at 40 V; 68 °C; 60 s	Highest percentage inhibition after 5 min sonication at 70 °C along with ohmic heating.	[86]

the product is utilized to generate heat and further impart the benefits of thermal processing. [92] reported that combining US with ohmic heating potentially inhibits microbial growth with the effective inactivation of PME in the orange juice. Moreover, carotenoids, flavonoids, phenolics, and vitamin C retention was found to be higher in the ohmic + US orange juice compared to that of conventionally heat-processed juice and fresh juice. Though there are very limited studies on the combination of US with novel thermal technologies focussing on enzyme inactivation, the existing reports drew an explicit prospect of combining these technologies.

5.2. Synergistic effect of US with chemical agents

US is a proven technique for the inactivation of enzymes in various food products. The activity of the enzymes is highly dependent on the structural conformation of the enzymes. In recent studies, the combined effect of ultrasound and chemical agents has been shown to improve the microbial quality of various fruits and vegetables through a synergistic effect (given in Table 5) and also for reducing the enzymatic browning induced during the processing of fruits, vegetables, and juices (See Table 6).

[98] explored the combined effect of US and chlorine dioxide (ClO_2) on the enzyme activities of minimally processed bok choy and it was

Table 5
Enzyme inactivation by US combined with chemical agents.

S. No	Product	Combined non-thermal technology	Enzyme	Process Parameters	Findings	Reference
1.	Button Mushroom slices	Low-Concentration Acidic Electrolyzed Water	PPO, POD	Ultrasonic bath; 40-kHz; 200 W; 3 min. Low-Concentration Electrolyzed Water (LcEW) (1% HCl) at pH 5.50	The PPO and POD activities of LcEW + US-treated mushrooms were remarkably lower than those of the mushrooms treated with LcEW alone and control	[94]
2.	Bok Choy	Aqueous chlorine dioxide	PAL, PPO, POD	Ultrasonic bath; 80 kHz; 120–210 W; 10 min. 50 ppm aqueous ClO_2 solution	A significant reduction in POD and PPO enzyme activity was observed between 6 and 9 days of storage. Whereas PAL activity has increased after treatment.	[95]
3.	Apple Juice	β-cyclodextrin	POD	Probe US 1000 W; 20 kHz; 100–400 W. 5–30 min. β-Cyclodextrin concentration 0.002–0.008 g mL^{-1}	At 300 W with 0.006 g mL-1 β-CD had a maximum of 17.82% reduction in PPO activity.	[96]
4	Cauliflower	Zinc Acetate (ZA), Tea Saponin (TS), Ethanol (ET)	PAL, PPO, POD	US tank; Dual frequencies (20 + 28 kHz, 28 + 40 kHz, 20 + 40 kHz); 100 W/L; 15 min.Washing solution concentration of ZA (0.1–0.7%), TS (0.02–0.08%), ET (1–7%)	The enzyme activity of PPO, POD, PAL, reduced effectively with US + (ZA, TS, ET) treatment as compared to US alone. The ZA and TS showed higher inactivation efficiency in all the three enzymes.	[97]
5	Chinese cabbage	Sodium hypochlorite	PPO	Ultrasonic bath; 40 kHz, for 10 min. 100 mg/L sodium hypochlorite solution,	US + NaOCl on PPO activity became predominant especially after day 4 of storage, leading to a 2.31–fold reduction at the end of storage.	[98]
6	Lettuce	ε-polylysine	POD, PPO	Probe US; 20 kHz, 17, 23, 29 W/L, for 10 min at 20 °C.ε-polylysine solution (0.1–0.6 g/L)	At 12 days storage period, the least POD and PPO activity was observed for US - ε-polylysine treatment.	[99]
7	Potato	L-Cysteine	PPO	Ultrasonic bath; 28 kHz; 100–500 W; time 2–10 min; 20 °C. L-cys concentration 0.5 – 2.5 g L^{-1}.	The highest enzyme activity inactivation of 83.10% was observed with 2.5 g/L L-cysteine, powder 300 W and 6 min treatment.	[92]
8	Green asparagus	Acetic acid and Gibberellic acid	POD, PAL	Ultrasonic bath; 40 kHz; 360 W; 10 min. Concentration 2% acetic acid and 50 mg/kg gibberellic acid	US + AG treatment decreased POD activity from the day 8 during the storage period.	[92]
9	Tender Coconut water	Nisin	PPO, POD, PAL	Probe US; Amplitude 60%; (1–10 min); 20 kHz; 20 °C. Nisin addition – 25 IU/ml.	Enzyme activity reduction by ultrasound with nisin treatment was 50% for PPO, 30% for POD, and 35% for PAL,	[100]
10	Ginger slices	Sucrose solution	PPO	Ultrasonic bath; 33, 50, and 68 kHz; 10–30 min; 600 W; 30 °C. Sucrose solution 20–60%, Sample to solution ratio 1:10	At UF (50 kHz), SC (35% w/v), and PT (30 min), PPO activity was reduced to 13.21 ± 0.05%	[104]

Table 6
Enzyme inactivation by US combined non-thermal technologies.

S. No	Product	Combined non-thermal technology	Enzyme	Process Parameters	Findings	Reference
1.	Blueberry Juice	Mano-sonication	PPO	Ultrasonic bath; 40 kHz; 280–700 W; 20 °C; 5 min. 350 MPa; 20 °C; length of exposure to pressure: 5–20 min	PPO residual activities reduced to 24.03% for 5 min treatment, which further decreased to 4.56% after 10 min.	[101]
2.	Blueberry Juice	Thermo-mano-sonication	PPO	Ultrasonic bath; 40 kHz; 280–700 W; 40 °C; 5 min. 350 MPa; 40 °C; length of exposure to pressure: 5–20 min	PPO residual activities reduced to 10.91% for 5 min treatment, which further decreased to 0.12% after 10 min.	[118]
3.	Apple juice	High pressure homogenisation	PPO	ProbeUS; 24 kHz; 100 μm amplitude; 7–45 min, Temperature – controlled (42 °C) and uncontrolled. 0–150 Mpa, 10 successive passes, flow rate 2.5 cm^3/s.	The residual activity of PPO was reduced to 59 % after 15 min sonication for controlled temperature, whereas 2 % after 4 min sonication for uncontrolled process.	[106]
4	Acai juice	ozone	POD, PPO	Probe US; 19 kHz; 350 &700 W; 5 min; 32 °C. Ozone concentration 1.50 ppm, exposure time 5 and 10 min	The reduction in PPO and POD enzyme activity observed in higher ozone exposure time with 700 W US treatment.	[102]
5	Mango Juice	Ultraviolet	PPO, POD, PME	Probe US; 40 kHz; 0–40 min;0–600 W2 UV lamp (8 W and 254 nm), distance from sample 5 cm.	US-UV treatment at 10 min, 600 W, all enzymes injuicewere inactivated	[105]
6	Spinach Juice	Pulsed electric field	POD, PPO	Ultrasonic bath; 40 kHz; 200 W; 30 °C for 21 min.PEF treatment (pulse frequency: 1 kHz, pulse width 80 μs; flow rate: 60 ml/min, temperature: 30 °C, time: 335 μs, and electric field strength 9 kV/cm).	The enzyme activity reduction of 78.82% and56% was observed in POD and PPO.	[107]

observed that different enzymes were affected differently by the combined US and ClO_2 treatment. To illustrate, the activity of PPO was observed to be reduced after 6–9 days of storage, before and after when the enzyme activity was increased whereas the POD activity showed a significant reduction throughout the storage period when compared to the control. The reduction in enzyme activity was expected to be due to the combined effect of US cavitation which breaks the peptide bond and chlorine dioxide-induced oxidation of amino acid and disulfide bond in the enzyme structure. Contradictorily, the Phenylalanine ammonia-lyase (PAL) activity increased with the treatment showing that the impact of US on enzyme inactivation is majorly dependent on enzyme structure and its resistance to the treatment conditions [99]. In another study, sodium hypochlorite (NaOCl) solution combined with US was applied to the washing of Chinese cabbage particularly for microbial reduction and quality improvement [92]. However, the PPO and POD activities were distinguishably reduced after the combined treatment of NaOCl and US. The PPO activity was reduced to 2.31-fold after day 4 storage in combined treatment whereas the individual treatment of NaOCl and US showed only 1.46- and 1.57-fold reduction (at day 7). Similarly, the synergistic effect reduced the POD activity by 1.17-fold whereas the individual treatment showed a lower reduction at the end of the storage [92].

Though chlorine-based solutions are effective against microbes and browning enzymes, they are found to be harmful and carcinogenic since they produce by-products like trihalomethane [92]. This led to the exploration of another eco-friendly chemical agent for effective microbial and enzyme activity reduction. Button mushrooms washed with low-concentration electrolyzed water (LcEW) combined with US technology were investigated for improving the storage quality. The study reported that the combined LcEW with US reduced the PPO and POD activity significantly by 1.83 and 1.57-fold respectively, which is due to the combined effect of microbubble cavitation and reduced pH of the LcEW (5–6.5) solution causing the effective enzyme inactivation [100]. Likewise [101], studied the combined effect of US with ε-polysine, which is a non-toxic, natural antimicrobial component for its efficiency in microbial load reduction as well as on enzyme activity. Both US and US + ε-polysine showed reduced enzyme activity of PPO and POD when compared to the control. The reduction in enzyme activity is attributed to the mechanical and chemical effects of the US treatment, however, the significance of ε-polysine addition on enzyme inactivation was not provided. Similarly, in US treatment of tender coconut water combined with a natural bacteriocin nisin, the enzyme activity of PPO, POD, and PAL was reduced by 50%, 30%, and 35% as compared to the control [101]. Some of the other natural and chemical surfactants such as tea saponin, zinc acetate, and ethanol were also examined for their efficacy in microbial reduction of cabbage in combination with the US. The study showed the effective reduction in enzyme activity of PPO, POD, and PAL with combined treatment rather than U/S treatment alone. The PPO activity was found to be least with US + 0.5% ZA treatment, whereas the POD and PAL activity was least at US + 0.06% TS treatment after 8 days of storage [102]. Though these combined agents helped in microbial and enzyme activity reduction, the mechanism of action was not known, which opens up the scope for future studies to explore the enzyme and its interaction with combined US hurdle technologies, particularly for methods employing chemical agents.

The enzyme inactivation is also important to inhibit certain biosynthesis pathways which lead to the quality deterioration of the fresh produce. Lignification of green asparagus is one such process affecting the quality parameters, where POD and PAL are playing an important role in the lignin biosynthesis pathway. So [102], studied the effect of acetic acid (A) and gibberellic acid (G) in combination with the US for the inactivation of POD and PAL enzymes in green asparagus. The US + AG treatment showed a higher reduction in POD and PAL activity as compared to the control and other individual treatments. The control activity for POD was 105 ± 4.83 U/g was reduced to 69.16 ± 4.90 U/g after the combined treatment. In the case of PAL, the activity was reduced by 2.2 fold after the combined treatment during storage, which might be due to increased acidity induced by the combination of acetic acid and gibberellic acid along with free radical-induced acidity in the washing solution [103].

The synergistic effect of high-intensity US in combination with the complexing carbohydrate β-cyclodextrin was studied for the inactivation of browning enzyme in apple juice. The result showed that the inhibition of PPO activity with combined treatment of β-cyclodextrin with US was more, while there was no significant inhibition observed with varying concentrations of β-cyclodextrin. The US treatment was observed to increase the solubility of β-cyclodextrin, which could help β-cyclodextrin to trap more enzymes in its hydrophobic cavity leading to a reduction in enzyme activity. A similar study of browning enzyme inactivation was also done in fresh-cut potatoes with the combined

effect of ultrasound and L-cysteine (U-L-cys). Having the thiol group in its structure, L-cysteine acts as an effective antioxidant and binds with the PPO enzymes and thus inhibiting the enzymatic browning by reducing the availability of enzymes for substrate reaction. The result showed that the U-L-cys caused a reduction of 41.10% in the enzyme activity as compared to the control, whereas the only U/S treatment reduced the enzyme activity by 23.64% [104].

Apart from these chemical agents, class I preservatives such as sugar solution are also used in enzyme inactivation in the osmo-sonication process due to the antioxidant properties of sucrose solution [105]. In a study by [50] it was observed that the sucrose concentration of 44% and U/S pre-treatment of 20 min reduced the PPO enzyme activity in ginger slices whereas the lower sucrose concentration of around 20% and shorter sonication for 10 min increased the enzyme activity. This depicts the importance of sucrose concentration and the osmotic pressure gradient to promote or inactivate the enzyme activity. Even though specific chemical agents investigated along with US showed a prospective enzyme inactivation, the lack of actual principles governing the inactivation is significant and needs to be addressed in further detail in future aspects.

5.3. Synergistic effect of US with other non-thermal technologies

The synergistic effect of US with other thermal, novel thermal, and chemical treatments have demonstrated to be more effective in enzyme inactivation than the ultrasound treatment alone in various food products. However, these combinations also have their disadvantages such as reducing bioactive and nutritional components in thermal-based treatment and producing toxic chemical residue and human health issues in case of chemical treatments. Various non-thermal technologies such as high-pressure processing [106], ozone [72,92], ultraviolet [11] have already proven to be effective in increasing the shelf life and storage quality of food products by effective microbial decontamination and enzyme inactivation. However, ultrasound in combination with other non-thermal treatments can be efficacious in processing enzymatically stable and better-quality products. Though the studies on nonthermal technologies combined with the US principally focusing on enzyme inactivation are limited (given in Table 6), the demand for exploration has to be increased among the research community to produce nutritionally better products.

[107] have reported the combined effect of US, heat, and pressure-based nonthermal technologies on the inactivation of E. coli and enzyme inactivation of blueberry juice. The residual activity of PPO after ultrasound treatment (560 W) was found to be reduced only up to 35%, whereas it reduced to 24.03% when combined with a high-pressure treatment of 350 MPa for 5 min at 20 °C, and the activity further reduced to 4.56% on extending the pressure treatment to 10 min. Thus, the pressure treatment in combination with the US impacted the enzyme activity due to the denaturation of enzyme protein and structural damage by the breakdown of hydrogen bonding and hydrophobic interactions. They have also studied the effect of thermo-mano-sonication where the high-pressure treatment was done at 40 °C to add the impact of heat along with mano-sonication keeping the remaining parameters the same. The residual enzyme activity was further reduced to 10.91% and 0.12% after 5 min and 10 min of the thermo-mano-sonication process. This depicts the importance of the combined effect of pressure and temperature along with US on the effective inactivation of enzymes.

The US was also combined with high-pressure homogenization (HPH) for the treatment of apple juice. High-pressure homogenization also works on a similar principle as that of ultrasonication such as the denaturation of the protein structure of the enzyme through mechanical stress and cavitation [108]. These synergistic effects of HPH along with the US were observed to be more effective in enzyme inactivation. The impact of HPH-US was studied along with the influence of temperature through controlled (USct) and uncontrolled US setups during the process. In HPH-USct combined process, the minimum residual activity of PPO was observed as 59% after 15 min treatment with the temperature 46 °C. However, the uncontrolled temperature (73.9 ± 5.8 °C) HPH-US process resulted in 2% residual activity obtained within 4 min as the temperature raise remarkably influenced the enzyme inactivation [74]. In another study, the US was combined with ozone treatment for the treatment of acai juice. The ozone is proven to be an effective oxidative antimicrobial technique in various studies due to its high permeability into the tissue supporting efficient enzyme inactivation. In theUS and ozone combined treatment of acai juice, the enzyme inactivation by ozone alone was higher than in the ultrasound treatment, however, the combined ozone + US treatment showed better PPO and POD inactivation. The increase in US power with 5 min ozone treatment has not significantly reduced the enzyme activity, however, the increase in US power during longer ozone treatment of 10 min synergistically created higher inactivation of up to 20 % residual enzyme activity. Thus, the US combined with ozone treatment improved the enzyme inactivation due to cavitation on US and oxidation of protein structure by oxygen radicals in ozone combined treatment [109].

Similarly, other non-thermal techniques like ultraviolet (UV) and pulsed electric field (PEF) were also fused with the US in the treatment of mango juice and spinach juice respectively [110,111]. The US-UV treatment at ultrasound powder 600 W and 10 min treatment completely inactivated the browning enzymes (PPO and POD) and pectin methylesterase (PME) in mango juice. The UV-PEF treatment of spinach juice exhibited enzymatic inactivation of 78.82 % in POD and 56% in PPO. While the individual non-thermal treatments showed 31.76% (POD) and 36% (PPO) activity reduction with the US and 43.52% (POD) and 44% (PPO) activity reduction with PEF which shows the higher potential of combined treatment on enzyme inactivation [112]. Hence, the non-thermal combined US treatment is proven to be a better and more efficient option to inactivate the harmful and browning enzymes besides their potential for microbial inactivation.

5.4. Ultrasound technology: non-thermal blanching method

Blanching is the conventional processing or pre-processing method focussed to inactivate the enzymes in the fruits and vegetables with the employment of hot water (98 °C for 1 min) or steam (2 min). Despite the effective inactivation of enzymes by thermal-blanching, the high thermal energy applied to destruct the thermo-labile constituents of the product treated especially the bioactive such as phenolics, flavonoids, carotenoids, and vitamins. Also, the sensitive water-soluble elements leach out vigorously with the application of very high temperatures. Though the enzymes affecting the texture of fruits and vegetables are inactivated by thermal blanching, the intense thermal energy would depolymerize the polymers (pectin) responsible for the structure causing the loss of turgor pressure. Thus, the fresh-like attributes of the blanched products are matters of concern. To attain the enzyme inactivation function of blanching without mild thermal interference, several studies were carried out with non-thermal technologies such as high-pressure processing, pulsed light, and ultrasound. Since the focus of the present review is ultrasound technology for enzyme inactivation, the other non-thermal blanching studies were not reviewed systematically.

However commenting on non-thermal blanching methods, the high-pressure processing technology involves the homogenous pressurization of the product through a liquid medium. Such a high pressure (200–800 Mpa) affects the covalent bonds in the macromolecules without affecting the small biological elements (vitamins and phenolics). Considering the enzymatic protein structure, covalent bonds in the peptide and disulfide linkages were affected and denaturation of the protein occurs. High-pressure denaturation of enzymatic protein can be augmented along with the mild heating of the pressurizing medium. Similarly, in pulsed light processing, exposing the product to high-fluence intensity pulses of UV-C incurs primary as well as secondary radical-induced protein oxidation causing enzyme inactivation. This pulsed light blanching

method can also be employed as a pre-treatment to ultrasonication for accelerating enzyme inactivation. Terming the processing method used for enzyme inactivation irrespective of the temperature used as 'blanching', ultrasonic blanching was thoroughly investigated in the present review. Regarding, enzyme inactivation efficiency and the bioactive retention or enhancement with the preservation of products' freshness ultrasonic blanching is considered to be superior to thermal blanching. Furthermore, the possibility of combining the ultrasonic process with mild heating (thermo-sonication) and high-pressure involvement (mano-sonication) facilitates supreme enzyme inactivation over any other thermal processing.

6. Conclusions and future outlook

Ultrasound technology is one of the most feasible technologies among the non-thermal technologies with multiple applications in both liquid and solid food products which imparts the incessant investigations emerging in the field. The most common applications in the food industry include cell destruction and extraction of intracellular material. Depending on its intensity, ultrasound is used for the activation or deactivation of enzymes, mixing and homogenization, emulsification, dispersion, preservation, stabilization, dissolution and crystallization, hydrogenation, tenderization of meat, ripening, ageing and oxidation, and as an adjuvant for solid–liquid extraction for maceration to accelerate and to improve the extraction of active ingredients from different matrices and atomization for food preparations. The present review elaborated on the mechanism of ultrasound on enzyme inactivation and evaluated the recent reports (2017–2022) in dairy, fruit, and vegetable systems. As an evolution, many of the studies directed towards the hurdle approach of integrating and improving the benefits of existing chemical, thermal, and other non-thermal technologies with the sonication effects. However, investigations exclusively focussing the influence of sonication on enzyme activity are limited as compared to that on microbial safety which necessitates and widens the future scope. Also, the mechanism of action of ultrasound on the primary, secondary and tertiary structure of enzymes extracted from the treated product matrix has to be assessed to distinctly comprehend the interaction which would be useful for the standardization of process parameters to obtain desired outcomes either activation or inactivation of the enzymes. High-intensity US has become an effective tool for large-scale commercial applications and the researchers are keenly working on the equipment design optimization and the performance of continuous-flow systems improvement. As with all other more innovative processing technologies, high-power US is not a standard technology and therefore must be studied and developed for each type of application in the near furture.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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