#### REVIEW



# Impact of Thermosonication Processing on Food Quality and Safety: a Review

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## Abstract

The thermosonication processing has been evaluated as a smart alternative to overcoming the heat treatment limitations associated with longer holding times at high temperatures. In this regard, this innovative technique combines acoustic energy with moderate heat treatments to inactivate pathogenic and spoilage microorganisms and endogenous enzymes in foods and beverages. Thermosonication treatment employs short holding times, which can promote the maintenance of phytochemical compounds and sensory characteristics of food products. The challenges and advantages of this emerging technology were reviewed in contrast to conventional heat treatments. Additionally, the process design of ultrasound combined with mild thermal treatments was discussed. Thermosonication is presented as a promising technique to inactivate microorganisms and enzymes. Also, it has not affected the nutritional and sensory quality of different food products. However, thermosonication treatments must be standardized. Some studies used only acoustic cavitation as a source of heat. Furthermore, many studies did not calculate or present the actual thermal energy provided by thermosonication processes. Therefore, temperature monitoring throughout thermosonication processing is crucial to ensure the suitable process design, avoiding the negative impacts observed in thermally treated products, such as degradation of bioactive compounds and off-flavors formation.

Keywords Emerging technology · High-intensity ultrasound · Microbial inactivation · Thermal history

# Introduction

The current demand for safer, healthier, and more nutritious food products has boosted the search for new manufacturing strategies. Conventional thermal treatments, such as pasteurization (60 to 100 °C) and sterilization (up to 140 °C), have been applied to produce safe food products. Heat treatments ensure consumer safety through the inactivation of pathogenic and spoilage microorganisms and endogenous enzymes of foods and beverages (Ağçam et al., 2018; Lima Gomes et al., 2020; Muñoz et al., 2018). However, severe thermal treatments can cause undesirable changes in sensory attributes of food products. Likewise, nutrients also are affected after heat processing. Degradation of vitamins and thermolabile bioactive compounds and protein denaturation can be verified in thermally treated products. Therefore, there is a growing demand for non-thermal technologies or those that use a lower process temperature to inactivate microorganisms and enzymes in foods and beverages. These innovative technologies are also evaluated to minimize product degradation, maintaining the aspects and characteristics of the fresh or unprocessed product (Chen et al., 2010; Monteiro et al., 2018).

New processes based on low-frequency (16 to 100 kHz) and high-power (> 1 W/cm<sup>2</sup>) ultrasound technology have been proposed as a potential alternative to conventional heat treatments. In this way, limitations and drawbacks concerning the application of severe heat processing in food products have been overcome using acoustic energy. Additionally, this technology has been recognized for enabling clean or green processes. Ultrasound-assisted processes for phytochemical extraction (Sukor et al., 2021; Tekin et al., 2015), food stabilization (Saeeduddin et al., 2015; Yildiz et al., 2020), emulsification (Huerta et al., 2020; Silva et al., 2015), and chemical modification of

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macromolecules (Chen et al., 2019; Zhong & Xiong, 2020) promote less impact on natural resources than conventional processes. Ultrasound technology enables the reduction or even elimination of the use of toxic solvents, besides shorter processing times, and consequently less energy expenditure and operational cost. Therefore, ultrasound technology is promising for developing new sustainable food production systems (Guimarães et al., 2019; Oladunjoye et al., 2021; Scudino et al., 2020; Tappi et al., 2020).

Innovative food stabilization processes based only on the application of acoustic energy have been assessed for different products (Ansari et al., 2017; Inmanee et al., 2020; Križanović et al., 2020; Režek Jambrak et al., 2017). Despite that, studies concerning the synergistic effect of acoustic cavitation combined with mild thermal treatments (~50 to 70 °C) have increased in the last years. Combined treatments can enhance the inactivation of microorganisms and enzymes in foods without compromising their sensory and nutritional quality. The combination of acoustic energy and mild heat treatment is known as thermosonication (Bansal et al., 2018). The beneficial effects of thermosonication processing have been observed on vegetables (Cruz et al., 2007; Mansur & Oh, 2015), fruit and vegetable juices (Anaya-Esparza et al., 2017a, b; Jabbar et al., 2015), meat products (Inmanee et al., 2020; Pennisi et al., 2020), dairy products (Gursoy et al., 2016; Ragab et al., 2019), fermented beverages (Deng et al., 2018; Milani & Silva, 2017), and others (Al-Juboori et al., 2012; Zhong & Xiong, 2020).

In this context, this review evaluate the impact of thermosonication processing on the safety and quality aspects of different foods and beverages. The challenges and advantages of this emerging technology were discussed in contrast to conventional heat treatments. Also, the process design of ultrasound combined with mild thermal treatments was assessed.

## Fundamentals of Ultrasound Technology

Sound is a mechanical wave that propagates longitudinally, promoting compression and rarefaction of atmospheric air throughout its propagation due to instantaneous pressure variations. The sound can be classified as infrasound ( $\leq 20$  Hz) and ultrasound ( $\geq 20$  kHz) (Alves et al., 2013; Cárcel et al., 2012; Yu et al., 2020). The frequency range of the ultrasound also defines its technological application since ultrasound technology is divided into processes based on low frequency (20 a 100 kHz) and high frequency (> 100 kHz) (Kentish & Feng, 2014; Knorr et al., 2004).

Figure 1 presents the applicability of ultrasound technology according to the intensity and frequency of energy provided by the ultrasonic system. Low-energy and highfrequency ultrasound technology is applied for imaging diagnostic analysis (Correia et al., 2008; Elvira et al., 2005). In contrast, high-energy and low-frequency ultrasound waves are applied in liquid systems to promote mechanical, physical, and chemical changes (Monteiro et al., 2020; Strieder et al., 2020).

Acoustic energy has been characterized in the literature in different ways (Knorr et al., 2004):

- Acoustic power: refers to the amount of energy delivered by the ultrasonic system to each unit of time (energy/ time)
- Acoustic power intensity: the ratio between acoustic power emitted by the ultrasonic system and the emission area (power/area)
- Acoustic power density: the ratio between acoustic power emitted by the ultrasonic system and the volume of the sonicated medium (power/volume)

Since most sonication processes were carried out on a laboratory scale, the magnitudes of intensity and density



are commonly presented in the units of W/cm<sup>2</sup> and W/cm<sup>3</sup>, respectively. The acoustic energy provided by the ultrasonic system also can be expressed considering the processing time. Equation 1 presents the energy density that expresses the energy applied per volume. Equation 2 shows the specific energy that expresses the energy delivered per mass (Strieder et al., 2020).

$$Energy \ density \left[ \frac{Energy}{Volume} \right] = \frac{Ultrasound \ power \times Processing \ time}{Volume}$$
(1)

$$Specific \ energy\left[\frac{Energy}{Mass}\right] = \frac{Ultrasound \ power \times Processing \ time}{Mass}$$
(2)

Acoustic cavitation is a physical phenomenon that occurs due to the application of low-frequency and high power ultrasound in liquid media. Ultrasound waves promote alternative cycles of compression and rarefaction (expansion) of the molecules of a liquid medium (Swamy et al., 2020). This leads to pressure fluctuations in the liquid medium that favor the formation of bubbles throughout the expansion cycle. The bubble formation starts when a high negative pressure exceeds the tensile strength of the liquid. The produced bubbles absorb small amounts of vapor from the liquid and continue to grow. The bubbles explode, releasing energy when they reach a critical size (Yu et al., 2020). This energy is transformed or released in various forms (microjet, heat, or chemical reactions) throughout the bubble collapse stage (Wu et al., 2020). Thus, acoustic cavitation this phenomenon performs the main effect of the ultrasound application in food matrices (Marques Silva & Sulaiman, 2017).

The cavitation intensity depends on the energy converted to acoustic cavitation during sonication (Soltani-Firouz et al., 2019). Frequency influences the bubble size and, consequently, also impacts the cavitation intensity. Low-frequency ultrasound generates transient cavitation, characterized by the formation of large unstable bubbles that can collapse violently. The violent collapse generates points of high temperature and pressure in the sonicated medium (Soltani-Firouz et al., 2019; Welti-Chanes et al., 2017). Different ultrasonic frequencies from the same acoustic intensity provide different acoustic pressures and wave interactions in the liquid medium. The higher the ultrasonic frequencies (>80 kHz), the shorter the period and, consequently, the shorter the wavelength. Thus, bubbles produced will not have enough time to execute the collapse mechanism (Rashwan et al., 2020).

In addition to frequency, the physical properties of the sonicated medium, such as viscosity, density, surface tension, and vapor pressure, also affect acoustic cavitation. Tzanakis et al. (2017) demonstrated that the impact of vapor pressure, surface tension, and viscosity, plays a decisive role in the development and activity of cavitation in sonicated liquids. A high viscosity contributed to the cushioning of bubble oscillations in glycerin. Thereby less violent collapses were observed with energy dissipation into the medium. High energy amplitudes are required for greater penetration of acoustic radiation into high viscosity liquids due to their greater resistance to acoustic energy (O'Sullivan et al., 2018). On the other hand, liquids with low vapor pressure generate fewer cavitation bubbles and, consequently, less bubble collapse. Thus, there is less conversion of acoustic energy into heat in the sonicated medium (Merouani et al., 2018).

Acoustic energy can also cause different effects on the sonicated matrices depending on the frequency, power, processing time, amplitude, and temperature conditions. The explosion of the cavitation bubbles explosion generates microjets that promote shear forces on products, specifically on the external surface of cellular tissues (plant or animal), the cell wall of microorganisms, and on particles, such as starch granules and sugar crystals. The formation of cracks and pores in the physical structures induced by lowfrequency and high-power ultrasound is known as sonoporation (Knorr et al., 2004; Yu et al., 2020). The sonoporation is responsible for microbial inactivation and extraction of phytochemicals because it promotes cell lysis of microorganisms and disruption of plant cell structures (Guo et al., 2020; Tabib et al., 2020). In addition to sonoporation, acoustic cavitation may favor the production of reactive species and free radicals. The decomposition of the water molecule under sonication can generate free radicals, such as H<sup>+</sup> and OH<sup>-</sup>. These can also recombine to form hydrogen peroxide  $(H_2O_2)$ . The chemical changes in the liquid medium attributed to acoustic cavitation contribute to microbial inactivation. Free radicals, together with localized heating, can lead to the thinning of the microbial cell membrane. Microbicidal properties of hydrogen peroxide also act synergistically for microbial inactivation (Dolas et al., 2019; Swamy et al., 2020). Free radicals can also react with the amino acids of enzymes affecting their activity and catalytic function (Marques Silva & Sulaiman, 2017).

The characteristics and properties of the matrices, such as their physical properties and composition, also impact the sonoporation results. Recent reviews have demonstrated new proposals for the application of ultrasound technology, such as for the growth of beneficial microorganisms in probiotic cultures (Guimarães et al., 2019), improvements in the functional properties of polymers (Wang et al., 2020), and a promising alternative for producing food flavors obtained from the Maillard reaction (Yu et al., 2020).

#### Ultrasound System

Figure 2 presents ultrasonic systems. They are composed of an electric power generator, a transducer, and an emitter,



Fig. 2 Ultrasound equipment and types of ultrasonic systems

which produce ultrasonic waves in the liquid medium. The two configurations most used are probe-type ultrasound, in which a probe is used as an emitter, and bath-type ultrasound (Fig. 2) (Bermúdez-Aguirre et al., 2011; Welti-Chanes et al., 2017). The application of acoustic energy in food products can be carried out directly or indirectly (Miano et al., 2017; Rashwan et al., 2020). However, energy is usually applied directly to the sample using probe-type ultrasound (Miano et al., 2017). In this application, food matrices are placed in a container, and the ultrasonic probe is subsequently immersed inside it (Fig. 2). Figure 2 presents an example using the bath system. The matrices are placed inside a container that is inside the bath. The bath stays filled with liquid that can be water. Thus, acoustic energy is supplied to matrices at the bottom of the ultrasonic bath, where the transducers are located. Probe ultrasonic systems provide high acoustic intensity (W/cm<sup>2</sup>) into a liquid system confined in the vessel or chamber. In contrast, ultrasonic bath systems allow a greater liquid volume in the chamber, and their transducers have a large surface area. In this way, they can provide a low acoustic intensity and low acoustic power density compared to ultrasonic probes (Kentish & Feng, 2014). Therefore, the energy intensity required in the processing defines the condition and type of ultrasonic system to be used (Kentish & Feng, 2014).

The probe diameter is an important characteristic of the ultrasound system (Bermúdez-Aguirre et al., 2011). O'Sullivan et al. (2018) observed that a 3-mm diameter probe generated higher acoustic intensity in a liquid medium than a 12-mm probe. A smaller probe diameter provided greater penetration and, thus, a better distribution of acoustic cavitation. The probe shape also affects the acoustic energy transmission and, consequently, the cavitation efficiency. Conical probes generate a larger cavitation zone by increasing the amplitude of the waves and thus improving the process efficiency (Fang et al., 2018). The acoustic cavitation performance also depends on the geometry and ultrasound reactor size because they impact acoustic pressure. For the application of acoustic energy directly on scales larger than the laboratory, higher ultrasonic amplitudes would be needed to achieve a larger area and greater cavitation intensity (Rashwan et al., 2020).

# Thermosonication

Thermosonication is a technique that combines the application of acoustic energy with mild moderate heat treatments. Therefore, the acoustic energy and the heat act simultaneously on the cell structures, promoting the inactivation of pathogenic and spoilage microorganisms and endogenous enzymes (Amador-Espejo et al., 2020). Thermosonication treatments are energetically more efficient because they promote microbial and enzymatic inactivation in shorter holding times (Sango et al., 2014; Welti-Chanes et al., 2017). According to the temperature range employed, the process can be classified as sub-lethal (<45 °C) and lethal (>45 °C) (Anaya-Esparza et al., 2017a, b).

The temperature control during thermosonication processing is critical to ensure its effectiveness. Since high temperatures can affect nutritional and sensory properties altering food quality. Furthermore, high temperatures can attenuate the action of acoustic cavitation in liquid media. The temperature rise promotes an increase in the vapor of pressure of liquids decreasing their viscosity. In its turn, lower viscosities favor the cavitation bubbles formation. However, the bubbles formed in this low-viscosity liquid medium contain more steam, which reduces their collapse intensity. Thus, the energy released throughout bubble eclosion is reduced. This phenomenon is known as the damping effect (Sango et al., 2014; Ugarte-Romero et al., 2007). Therefore, there is an optimum temperature to be reached for each ultrasound process for obtaining the best acoustic cavitation performance and the desired effects (Schössler et al., 2014).

In this regard, the heat provided by an external source during the thermosonication treatment is an additional energy since sonication treatment alone promotes a temperature rise in the liquid medium. On the other hand, in some processes, the increase temperature provided by sonication is undesirable. Thus, cooling circulating baths are widely employed to reduce the temperature increase. Another alternative is setting up the ultrasound equipment to apply pulses of energy. For example. using 5 s on/10 s off or 5 s on/5 s off, as opposed to a continuous power supply (Marques Silva & Sulaiman, 2017). In thermosonication processing, heating circulating baths are used to provide thermal energy and maintain the working temperature throughout the treatment. However, in most cases, the working temperature conditioned by the external heat source is not the actual temperature of the thermosonication treatment because sonication also provides thermal energy to the sonicated medium due to acoustic cavitation. For example, thermosonication processes carried out at mild temperatures between 40 and 70 °C can reach high process temperatures, such as 100 °C, depending on applied acoustic energy conditions, such as ultrasound intensity and power density Therefore, temperature monitoring throughout thermosonication processing is fundamental to ensure the maintenance of nutritional and sensory quality aspects of thermosonicated food products.

#### Thermosonication on Food Processing

# **Plant Products**

Plant products, such as fruit and vegetable juices and fresh fruits and vegetables, were grouped in this section. Table 1 presents the main remarks regarding thermosonication processing on this plant-based products. Thermosonication treatments have been evidenced as a promising technique for the inactivation of microorganisms and enzymes of plant products. Fruit and vegetable juices have been subjected to a shorter exposure time to high temperatures during thermosonication treaetment. Thus, this emerging technology has allowed the maintenance or even increased availability and retention of bioactive compounds in products, including throughout their storage.

After the thermosonication treatment (45 kHz, 40 °C, 30 min) of a whole tomato sample, Pinheiro et al. (2019) observed an 8% reduction in the total phenolic compounds content. However, they verified an increase of 26% after 15 days of storage. This effect was attributed to the sonoporation of cell structures, which potentiated the bioaccessibility of phenolic compounds. A reduction in the total phenolic content was also observed in thermosonicated red pitaya juice samples. The working temperature had a significant impact on the degradation of phenolic compounds. The higher working temperature of 50 °C (475 W, 20 min) also reduced the retention of bioactive compounds (Liao et al., 2020). Similar behavior was observed for thermosonicated carrot juice samples (20 kHz, 48 W/cm<sup>2</sup>, 10 min) according to Jabbar et al. (2015). A significant reduction in the total phenolic compounds, flavonoids, tannins, and ascorbic acid content was associated with the working temperature rise from 40 to 60 °C. Despite this, the retention levels of bioactive compounds in thermosonicated juices were higher than in thermally treated juices at 80 °C for 1 min. On the other hand, all thermosonicated juice samples presented higher total carotenoid content than fresh juice and heat-treated. The disruption of cell membranes and protein-carotenoid complex contributed to the carotenoids extraction throughout thermosonication treatment (Jabbar et al., 2015). Different results were observed in thermosonicated hazelnut-based non-dairy alternative milk (Atalar et al., 2019). Thermosonication treatments (20 kHz, 750 W, 40-80% amplitude) promoted a significant increase in the phenolic compound contents of the samples. The treatment applying 60% amplitude for 20 min increased the phenolic content from 162.78 to 178.82 µg GAE/g, which increased the antioxidant activity. Atalar et al. (2019) attributed these results to the sonoporation of the cell walls. The sonoporation contributed to the disruption of cell structures, favoring the release of bioactive phytochemical compounds.

Thermosonication did not promote significant changes in pH, acidity index, and total solids content of hazelnut-based non-dairy alternative milk (Atalar et al., 2019), carrot juice (Jabbar et al., 2015), fruit smoothie (Amador-Espejo et al., 2020), soursop puree (Martínez-Moreno et al., 2020), and pumpkin juice (Demir & Kılınç, 2019). On the other hand, different effects on the color attributes were observed after thermosonication treatments. The reconstituted orange juice

Table 1         Thermosonication effects on t	the safety and quality aspects of plant pro	oducts		
Matrix	Ultrasonic system	Process conditions	Main remarks	Reference
Orange juice	Ultrasonic bath	300 W; 20 kHz; 10 s/3 s; 47 °C; 30 min; 100 mL inoculated with A. <i>acidoterrestris</i> . Storage: 4 and 25 °C for 24 days	The juice storage at 4 °C increased its shelf life, preventing microbiological degradation. The bioactive compounds (antioxidants, phenolic and ascorbic acid) were preserved	Wahia et al. (2020)
Orange juice	Ultrasonic processor with 22-mm diameter probe	400 W; 24 kHz; 100 $\mu$ m; 85 W/cm <sup>2</sup> ; $TS_{J'}$ 1d4 mL/min, 40 °C, 2.8 min. $TS_{2'}$ 8 mL/min, 50 °C, 5 min. <i>High-</i> <i>intensity pulse of light (360 µs, 3</i> $Hz_{J}$ : PL <sub>1</sub> : 4.03 $J$ /cm <sup>2</sup> and PL <sub>H</sub> : 5.1 $J$ / cm <sup>2</sup> ; 38 mL inoculated with <i>E. coli</i>	<i>Individual application</i> : Maximum reduction was 1.60 and 2.42 logs CFU/mL for TS <sub>1</sub> and PL <sub>H</sub> , respectively <i>Combined applications</i> : Inactivation of 2.5 to 3.93 log CFU/mL for the combined treatments, regardless of the sequence	Muñoz et al. (2011)
Orange juice	Ultrasonic bath	30 kHz; 55 °C; 5, 10, 20 min; 200 mL inoculated with <i>S. aureus. PEF:</i> 1 µs and 15 Hz pulse width	TS promoted a reduction of 3.3 logs cycles in 20 min, retention of 98% ascorbic acid, and a 96% reduction in residual activity of pectin methylesterase (PME). <i>TS for 10 min</i> + <i>PEF at 40 kV/cm</i> , <i>150 µs</i> : maximized the reduction in 6.8 log cycles and decreased the activity of the PME (12%).	Walkling-Ribeiro et al. (2009)
Red grape, sour cherry, and pomegranate juice	Ultrasonic processor with 12-mm diameter probe	20 kHz; 42.7 µm; 60 °C; 8 min; 25 mm; 200 mL	The antioxidant activity and the total phenolic content of the samples increased. Minimal anthocyanin degradation (10%, 4%, and 1% for grapes, pomegranates, and cherry respectively). Significant reduction in the population of aerobic bacteria, coliforms, yeasts, and fungi	Hooshyar et al. (2020)
Fruit smoothie (Mango Manguifera indica L., cv Ataulfo, jaekfruit Artocarpus heterophylius L., and rice)	Ultrasonic processor with 25-mm diameter probe	1500 W; 20 kHz; 70, 77.5, and 85%; 2 s/4 s; 15, 20, 25 min; $T_{fi}$ 40, 47.5, and 55 °C; 400 mL	Loads of Mesophiles and <i>Enterobacteriaceae</i> and the PME activity were reduced by increasing process amplitude and temperature. The inactivation of the polyphenol oxidase activity was influenced by the processing time and amplitude. Optimal process condition: 77.5% of amplitude, 20 min, and 47.5 °C	Amador-Espejo et al. (2020)
Red pitaya juice	Ultrasonic processor with 10-mm diameter probe	190–570 W; 20–25 kHz; 3 s/3 s; 5–40 min; 10 to 70 °C; 60 mL	Accelerated decrease in betacyanins at temperatures > 50 °C. The TS at 380 W, 50 °C for 40 min retained > 87.2% of beta-cyaninase and for 20 min retained > 92.97% of polyphenols	Liao et al. (2020)

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Table 1 (continued)				
Matrix	Ultrasonic system	Process conditions	Main remarks	Reference
Soursop puree	Ultrasonic processor with 22-mm diameter probe coupled to a vacuum reactor	85 W; 20 kHz; 40, 45, and 50 °C; 10 min; 8.46, 11, and 16.93 kPa. PIVs-5 s: 150, 200, and 300 s; 250 g inoculated with <i>E. coli</i> and <i>S. aureus</i>	Optimal conditions: 16.5 kPa, 3 PIVs of 150 s, 50 °C for 10 min. Residual enzyme activity of 0.20% and a reduction of almost 7 logs CFU were achieved	Martínez-Moreno et al. (2020)
Soursop nectar	Ultrasonic processor with 22-mm diameter probe	400 W; 24 kHz; 1.1, 1.2, and 1.4 W/ mL; 34, 44, and 54 °C; 2, 6, and 10 min; 200 mL inoculated with <i>E.</i> <i>coli</i> and <i>S. aureus</i> ; storage: 4 °C to 45 days	Optimal conditions: 1.4 W/mL, 51 °C for 10 min arrived a reduction> 5 logs CFU/mL for <i>E. coli</i> and <i>S. aureus</i> The TS decreased 99% of antioxidant activity, retained 85% of vitamin C and 93% of total polyphenols for 30 days	Anaya-Esparza et al. (2017a, b)
Pumpkin (Cucurbita moschata) juice	Ultrasonic bath without and with continuous circulation	Discontinuous TS: 150 W; 37 kHz; 40, 50, and 60 °C; 30 min; 10 mL inoculated with <i>E. coli</i> <i>Continuous TS</i> : Vessel 250 mL; 0.029 L/min; 60 °C. Three cycles with holding time of 2.87 min	Discontinuous TS: maximum reduction: 6.62 log CFU/mL at 60 °C <i>Continuous TS:</i> microbial inactivation was achieved after 8.6 min	Demir and Kılınç (2019)
Apple juice	Ultrasonic processor with 19-mm diameter probe	20 kHz; 5 min TS <sub>j</sub> : 17.5 W/cm <sup>2</sup> ; T <sub>j</sub> : 42.5 °C TS <sub>j</sub> : 25 W/cm <sup>2</sup> ; T <sub>j</sub> : 48.5 °C; 200 mL. Storage: 4 and 20 °C for 9 days	TS did not modify the chemical properties of the juice but reduced the anthocyamin content (92 and 82%). A TS <sub>2</sub> extended the shelf life of the juice stored under refrigeration up to 7 days	Nadulski et al. (2019)
Aloe vera ( <i>Aloe barbadensis</i> Miller) juice	Ultrasonic processor with 13-mm diameter probe	750 W; 20 kHz; 50%; 5 s/5 s; 25, 50 °C; 3, 6, and 9 min; 10 mm; 150 mL inoculated with <i>Lactobacillus</i> <i>plantarum</i>	Best condition: 50 °C for 6 min. This promoted an improvement in the swelling properties and fat adsorption capacity	Alvarado-Morales et al. (2019)
Tangerine juice	Probe ultrasonic processor	100 W; 30 kHz; 25, 50, and 75%; 15 s/5 s; 60, 70, and 80 °C; 20 mL inoculated with <i>B. fulva, L.</i> <i>monocytogenes</i> , <i>S. sonnei</i> , and <i>S.</i> <i>cerevisiae</i>	The temperature presented a greater influence on microbial inactivation. On the other hand, the increase in ultrasound amplitude promoted the degradation of $\beta$ -carotene and ascorbic acid in the juice. Ideal condition: 80 °C and 75% amplitude	Hashemi et al. (2019)
Strawberry (Fragaria × ananassa Duch.) nectar	Ultrasonic processor with standard probe	150 W; 3.6, 82.1, 2.71, 460.9, and 539.4 J/g; 25 to 75 °C; 0.1 and 15 min; 10 mm from the bottom; 250 g	TS conditions of 59 °C and 455 J/g maximized the content of polyphenols, monomeric anthocyanin, and ascorbic acid in the sample. These also allowed the inactivation in $75\%$ of PPO activity	Dündar et al. (2019)

Table 1 (continued)				
Matrix	Ultrasonic system	Process conditions	Main remarks	Reference
Strawberry puree	Ultrasonic processor with 3-mm diameter probe	Pre-treatment: $80 ^{\circ}$ C for 15 min; 33 W; 24 kHz; 100%; 0.33 W/mL; 65, 70, and 75 °C; 0 to 60 min; 10 mm from the bottom; 100 mL inoculated with <i>B. nivea</i>	The temperature increase favored the inactivation of ascospores up to 1.8 logs after 15 min at 75 °C. Increasing inactivation was observed in the first 5 min, followed by linear inactivation	Evelyn and Silva (2015a)
Watermelon peel juice with honey	7.5 L ultrasonic bath	40 kHz; 25, 45, and 65 °C; 10, 35, and 60 min. Storage: 4 and 25 °C for 8 days	TS temperature provided changes in the kinetic stability, color, vitamin C content, and microbial load of the juice. Ideal condition: 65 °C for 60 min. The juice storage at 4 °C extended its shelf life	Hussain et al. (2019)
Watermelon ( <i>Citrullus lanatus cv.</i> ) juice	Ultrasonic processor with 19-mm diameter probe	1500 W; 20 kHz; 24.4 to 61 µm; 5 s/5 s; 25 to 45 °C; 2 to 10 min; 25 mm; 80 mL	Higher retention of ascorbic acid (AA) and lycopene (LP) were observed between amplitude levels of 24.4 and 42.7 μm	Rawson et al. (2011)
Tomato juice enriched with anthocyanin	16.8 L ultrasonic bath	250 W; 35, 130 kHz; 20, 40, and 60 °C; 5 and 10 min;100 mL. Storage: 4 °C for 7 days	Ideal condition: 60 °C and 35/130 kHz for 5 min. This promoted greater retention of bioactive compounds and a decrease in 5.1 logs CFU/g of microbial load. During storage, there was an 81.6% reduction in anthocyanin content, and enzyme activity increased	Lafarga et al. (2019)
Barberry juice with added copigments	Ultrasonic processor with 13-mm diameter probe	200 W; 20 kHz; 70 and 100%; 18.65 and 37.97 W/cm <sup>2</sup> ; 45 °C; 15 s/5 s; 5 min; 20 mm; 60 mL. Storage: 4 °C for 60 days	Amplitude 70%: Did not promote changes in the phytochemical properties of the samples Amplitude 100%: Complete reduction of the microbial load. The addition of copigments increased the color stability and the content of anthocyanins in the juice	Farhadi Chitgar et al. (2018)
Apple (Golden delicious) juice	Ultrasonic processor with 22-mm diameter probe	24 kHz; 50 and 100 µm. Pulse durations: 50% /100%; 40, 50, and 60 °C; 5 and 10 min; 25 mm; 200 mL. Storage: 25 °C for 4 months	TS increased the cloudiness level by 16.9 times and the cloud stability by 9.8 times. After treatment, up to 58% of the solid particles were suspended. There was total inactivation of yeasts and molds. The best conditions for TS were 60 °C, 10 min, and 100 µm	Ertugay and Başlar (2014)

Matrix	Ultrasonic system	Process conditions	Main remarks	Reference
Apple (Malus domestica) juice	Ultrasonic bath/ultrasonic processor with 12.7-mm diameter probe	20, 40, and 60 °C; 80 mL Ultrasonic bath: 500 W; 25 kHz; 0.06 W/cm <sup>3</sup> ; 30 min Ultrasonic processor probe: 750 W; 20 kHz; 5 and 10 min; 0.30 W/cm <sup>3</sup> ; 5 s/5 s; 25 mm	TS treatment using the probe system at 60 °C promoted greater enzymatic inactivation (>91%) and avoided the degradation of ascorbic acid (6%), phenolics, flavonoids, and flavonoids. However, it maintained microbial cells	Abid et al. (2014)
Apple (Golden delicious) juice	Ultrasonic processor with 13-mm diameter probe	750 W; 20 kHz; 25 to 100% (19 to 76 µm); 1.57, 1.36, 1.21, 1.09 W/mL; T <sub>i</sub> : 44 to 67 °C; 15 min; 20 from the bottom; 60, 80, 100, and 120 mL	The increase in amplitude, power density, and temperature (100%, > 1.15 W/mL, 67 °C) promoted greater enzymatic inactivation. They acquired residual activities of 3% of polyphenol oxidase (PPO) and 50% of pectin methylesterase (PME)	Illera et al. (2018)
Apple juice	Ultrasonic processor with 22-mm diameter probe	400 W; 24 kHz; 100 µm; 85 W/cm <sup>2</sup> ; $TS_{f'}$ 14 mL/min, 40 °C, 2.9 min; $TS_{2}$ ; 8 mL/min, 50 °C, 5 min High-intensity pulse of light (360 µs, $3 H_{2}$ ): PL <sub>L</sub> : 4.03 J/cm <sup>2</sup> and PL <sub>H</sub> : 5.1 J/cm <sup>2</sup> 40 mL inoculated with <i>E. coli</i>	Individual application: Maximum inactivation of 2.7 and 4.9 logs CFU/mL by TS <sub>2</sub> and PL <sub>H</sub> , respectively <i>Combined</i> : Inactivation of 4.8 to 5.9 logs CFU/mL, regardless of the treatment sequence. The apple juice color was significantly affected by the PL+TS sequence	Muñoz et al. (2012)
Apple juice	Ultrasonic processor with 6-mm diameter probe	950 W; 20–25 kHz; 20% (6.48 W); 37, 42, 47, and 52 °C; 3 s/3 s; 40 min; 60 mL. Nisina: 100 ppm. Storage: 8 °C for 15 days	TS and TS-Nisina at 52 °C for 30 min retained 89% of vitamin C and extended the sample shelf life to 15 days. The temperature of 52 °C inactivated 2.79 logs of aerobic bacteria and 3.12 logs of mold veasts	Liao et al. (2020)
Carrot ( <i>Daucus carota L.</i> ) juice with added orange peel and pulp extracts ( <i>Citrus sinensis</i> )	Ultrasonic bath	110 W; 40 kHz; 40, 50, and 60 °C; 5, 7.5, and 10 min; 100, 125, 150, and 200 mL	The polyphenol content and antioxidant activity of the sample were affected by temperature and processing time. Ideal conditions: juice with pulp extract (125 mL, 60 °C, 5 min) and juice with peel extract (125 ml, 52 °C, 6.5 min)	Adiamo et al. (2018)
Carrot juice with added orange peel and pulp extracts	Ultrasonic bath	110 W; 40 kHz. Juice with peel extract: 52 °C; 6 min Juice with pulp extract: 60 °C; 5 min Concentration: 0.5, 2, 4 mg GAE/g of extract; 125 mL. Storage: 4 °C for 21 days	The process at 60 °C employing 4 mg of GAE/g of orange peel and pulp extract promoted higher microbial inactivation and better retained bioactive compounds (phenols and antioxidant activity) in the samples	Adiamo et al. (2017)

Table 1 (continued)

Table 1 (continued)				
Matrix	Ultrasonic system	Process conditions	Main remarks	Reference
Carrot (Daucus carota cv. Heitian-5) juice	Ultrasonic processor with 13-mm diameter probe	750 W; 20 kHz; 70%; 48 W/cm <sup>2</sup> ; 20, 40, and 60 °C; 5 s/5 s; 5 and 10 min; 20 mm; 250 mL	The temperature of 60 °C promoted higher inactivation of enzymes (PME, PPO, POD, LOX) and microbial cells maintaining the pigments, ascorbic acid, total phenolics, total flavonoids, and tannins of the sample	Jabbar et al. (2015)
Blueberry nectar	Ultrasonic processor with 12.7-mm diameter probe	600 W; 20 kHz; 50, 75, and 100% (60, 90, and 120 $\mu$ m); T <sub>f</sub> : 39 to 70 °C; 3, 6, and 9 min; 100 mL	The TS process at 60 °C allowed complete inactivation of yeasts and molds The TS at 50% for 3 and 6 min promoted less oxidative deterioration of the nectar	Režek Jambrak et al. (2017)
Fresh <i>Ke lulut</i> honey	Ultrasonic bath	2.5 kW; 25 kHz; 45, 55, 67.5, 80, and 90 °C; 30, 50, 75, 100, and 120 min; 20 g	The increase in temperature and processing time promoted positive effects on the quality of the honey. The process at 90 °C for 111 min allowed the best results	Chong et al. (2017)
Coconut water	Ultrasonic processor with 10-mm diameter probe	20 kHz; 50, 90%; 6.5, 13.5 min; 5 mm; 70 mL	TS using 500 and 550 mW/mL allowed greater inactivation of polyphenol oxidase and peroxidase. The enzymes complete inactivation required the specific acoustic energy of 655.80 mW/mL	Ribeiro et al. (2017)
Tomato ( <i>Solanum lycopersicum</i> , cv. Zinac)	45 L ultrasonic bath	80%; 45 kHz; 32, 35, 40 °C; 13, 20, 30, 40, and 47 min; 10 tomato units. Storage: 10 °C up to 15 days	TS delayed tomato color modifications during ripening and promoted an increase in its total phenolic content. The treatment also maintained the general quality of the fruits. Ideal conditions: 40 °C for 30 min	Pinheiro et al. (2019)
Tomato (Solanum lycopersicum 'Zinac')	45 L ultrasonic bath	80%; 45 kHz; 40 °C; 30 min; 15 kg. Storage: 10 °C and 90% relative humidity for 30 days	TS minimized changes in fruit color, increased the content of its antioxidants by 16%, and decreased the activities of peroxidase (25%) and PME (49%). The reduction in microbial load was observed up to 26 days after processing	Pinheiro et al. (2016)
Tomato (Variedade Heinz 3402) juice	Ultrasonic processor with 10-mm diameter probe	40 W, 80%; 20 kHz; 65 µm; 50 to 75 °C; 40 mm; 20 mL	TS at 75 °C for 4 min promoted almost complete inactivation of PME and 72% of polygalacturonase (PG)	Terefe et al. (2009)

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Table 1 (continued)				
Matrix	Ultrasonic system	Process conditions	Main remarks	Reference
Tomato and pomegranate juice	Ultrasonic processor with 22-mm diameter probe	400 W; 24 kHz; 25, 50, and 75 µm (60, 90, and 120 W); <i>TS<sub>1</sub></i> ; 60 °C for 20 min; <i>TS</i> <sub>2</sub> : 65 °C for 10 min; <i>TS<sub>3</sub></i> : 70 °C for 5 min; bath temperature: 59, 63, and 68 °C; 200 mL	The TS at 60 and 65 °C allowed higher PME inactivation ( $90\%$ ). The treatments also reduced the average particle size < 30 µm and increased the viscosity of the juice (2 to 4 times)	Wu et al. (2008)
Sliced cabbage (Brassica oleracea L. var. acephala)	Ultrasonic bath	40 kHz; 100, 200, and 400 W/L; 25, 40, and 50 °C; 1, 3, and 5 min; disinfectant solution: deionized water, electrolyzed water (5, 15, and 30 mg/L), NaOCI and NaCIO <sub>2</sub> (100 mg/L); 10 g inoculated with <i>E. coli</i> , <i>L. monocytogenes</i> and immersed in 1.5 L of each sanitizing solution	TS (40 °C, 3 min, 400 W/L) combined with electrolyzed water (5 mg/L) was more effective on the inactivation of pathogens and deteriorating microorganisms (> 3 logs CFU/g)	Mansur and Oh (2015a)
Sliced cabbage (B. oleracea L. var. acephala)	Ultrasonic bath	40 kHz; 400 W/L; 40 °C; 3 min; 10 g inoculadas com <i>L monocytogenes</i> e imersas em 200 ml de soluções desinfetantes. Armazenamento: 4 and 7 °C	A TS em combinação com água eletrolisada levemente ácida (5 mg/L) prolongou vida útil microbiana e sensorial (≥14 dias) da couve	Mansur and Oh (2015b)
Artemísia argy leaves	Ultrasonic processor with 20-mm diameter probe	20 kHz; 7.96, 11.94, and 15.92 W/cm <sup>2</sup> (40, 60, and 80% of amplitude); 70, 75, 80, 85, and 90 °C; 0.5 to 3 min; 25 mm; 3 g introduced in 90 mL of distilled water	TS temperature increase (70 to 85 °C) promoted a higher rate of inactivation of peroxidase Best condition: 85 °C for 1 min (11.94 W/cm <sup>2</sup> ). This condition allowed the reduction in 92.7% of the enzyme activity and maintained 96.7% of the chlorophyll content	Xin et al. (2015)
Grapefruit juice	Ultrasonic bath	70%, 420 W; 28 kHz; 20, 30, 40, 50, and 60 °C; 30 and 60 min; 250 mL	TS at 60 °C for 60 min promoted a reduction of PME (91%), PPO (90%), and POD (89%) activities and increased the content of bioactive compounds	Aadil et al. (2015)
Watercress (N. <i>officinale</i> ) leaves	Ultrasonic processor with 13-mm diameter probe	50%, 125 W; 20 kHz; 82.5 to 92.5 °C; 0.5 to 2 min; 3 g submerged in 100 mL of water	The increase in TS temperature promoted the degradation of vitamin C by 40 to 60%. TS promoted in a shorter time the same effects as heat treatment under the same conditions	Cruz et al. (2008)

Table 1 (continued)				
Matrix	Ultrasonic system	Process conditions	Main remarks	Reference
Watercress (N. officinale) leaves	Ultrasonic processor with 13-mm diameter probe	50%, 125 W; 20 kHz; $TS_j$ : 30 seg at 86 °C. $TS_2$ : 2 s at 92 °C; 3 g immersed in 100 mL of water	$TS_1$ maximized the retention of vitamin C (95%) and improved the green color (5%) of the leaves. $TS_2$ presented the best characteristics in terms of industrial viability. This treatment allowed the retention of 75% of the vitamin C content and improved the green color by 8%	Cruz et al. (2011)
Watercress (N. officinale) leaves	Ultrasonic processor with 13-mm diameter probe	125 W; 20 kHz; 82.5 to 92.5 °C; 3 g immersed in 100 mL of water	TS promoted a slight increase in chlorophyll content, which consequently influenced the green color of the leaves	Cruz et al. (2007)
Red pepper ( <i>Capsicum annuum</i> L.)/ watercress ( <i>Nusturtium officinale</i> R.Br.)/strawberries ( <i>Fragaria</i> <i>ananassa</i> D.)	5.6 L ultrasonic bath	120 W; 35 kHz; 2 min; 50, 60, and 65 °C (pepper); 50, 55, and 65 °C (watercress); 50, 55, 60, and 65 °C (strawberries) Sample ratio with bath water volume: 40 g/L	The increase in the TS temperature promoted a reduction in the microbiological load of the samples. The highest inactivation was 8.24 log cycles The TS at 50 °C promoted the retention of 89% of the anthocyanins in the strawberry and maintained the color of the pepper and the watercress	Alexandre et al. (2011)

Power (W); frequency (kHz); ultrasound intensity (W/cm<sup>2</sup>); acoustic energy density (W/mL or W/cm<sup>3</sup>) specific energy (J/g); amplitude (µm or %); temperature (°C); final sample temperature (T<sub>P</sub>); pulsation on and off time (s/s or %/%); processing time (min or s); probe immersion (mm); sample (mL, g or kg); flow (mL/min); colony forming units (CFU); thermosonication (TS); low-energy pulsed light (PLL); high-energy pulsed light (PLL); high-energy pulsed light (PLL); pulsed electric field (PEF); vacuum (kPa); intermittent vacuum pulses (PIVs); flow (mL/min); ascorbic acid (AA); lycopene (LP); methylesterase pectin (PME); polyphenol oxidase (POO); lipoxygenase (LOX); polygalacturonase (PG); sodium hypochlorite (NaOCI); sodium chlorite (NaCIO<sub>2</sub>) samples presented smaller changes ( $\Delta E = 1.8$ ) in their color instrumental parameters after thermosonication processing (30 kHz, 500 W, 55 °C, 10 min) compared to heat treatment at 94 °C for 26 s ( $\Delta E = 2.7$ ) (Walkling-Ribeiro et al., 2009). However, fresh orange juice samples thermosonicated at 20 kHz and 47 °C applying a nominal power of 300 W for 30 min presented changes in their color parameters  $\Delta E > 3.0$ (Wahia et al., 2020). Longer processing times (> 20 min) and temperatures above 50 °C also led to a visual color change  $(\Delta E > 3.0)$  in red pitaya juice. Despite that, no difference concerning visual color appearance was observed between thermosonicated and untreated pitaya juice samples. Also, UV-visible absorbance spectra demonstrated that thermosonication processes were more efficient in preserving the color of the juice than heat treatment at 83 °C for 1.5 min (Liao et al., 2020). Thermosonication treatment also delayed the development and ripening of the color of tomatoes. The ultrasound processing combined with mild heat treatment possibly inhibited the ethylene production in tomatoes and delayed other ripening processes. Color changes in fruit and aroma development were associated with acoustic cavitation, which may have promoted an adverse effect on the tomato's pigment stability (Pinheiro et al., 2016, 2019). Therefore, thermosonication processing can cause different impacts on the plant products' color depending on process conditions and the characteristics of the product.

## **Meat Products**

Studies related to thermosonication of meat products, such as fish, sausage, chicken, and beef, were grouped in this section. Thermosonication treatments have been less efficient in microbial inactivation of these products, mainly of meat. The chemical and physicochemical nature of the meat is the primary reason for this low efficiency. Meat and meat products present nutrients and a pH range that can benefit the development, adhesion, or even the protection of microorganisms. Besides that, studies have been observed that aerobic bacteria are more resistant to thermosonication treatments. The formed set of gram-negative and gram-positive bacteria on the skin of the chicken difficult their inactivation. Gram-positive bacteria have thicker cell walls that physically protect other microorganisms making them more resistant to thermosonication. In this way, another practical and promising strategy is the combination of thermosonication with other treatments for industrial applications. These other treatments have been employed in chemical products or thermal shocks to enhance the microbial inactivation of meat products. Table 2 presents some examples of thermosonication effects on meat products.

Inmanee et al. (2020) evaluated the thermosonication treatment of pork sausage at 80 °C and 40 kHz applying a nominal power of 150 W. They observed that the innovative treatment did not affect the sensory characteristics and the

chemical properties of the product. However, longer processing times (> 20 min) affected more the texture characteristics, such as the hardness, elasticity, cohesiveness, and chewability, of the sausages than conventional pasteurization at 80 °C for 15 min. The best thermosonication condition for sausages processing was 80 °C for 20 min. This process condition inactivated total bacteria, molds, and yeast in the sausages. After 30 days of cold storage at 4 °C, the sausages presented a small microbiological load of  $5.0 \times 10^1$  CFU/g and less than 1 log CFU/g for total bacteria and molds and yeasts, respectively. Additionally, the thermosonication treatment promoted the extension of the self life of the sausages, maintaining their quality attributes.

Kassem et al. (2018) observed that the increase in working temperature and processing time caused a higher decrease in the microbial load of the thighs of raw chickens after thermosonication processing. The thermosonication treatment at 54 °C for 3 min applying 80 W/L as an individual treatment promoted a low inactivation of Campylobacter jejuni and total enterobacteriaceae. Also, this process condition did not affect total viable count (TVC). However, the combination of thermosonication processing with chemical treatments enhanced the microbial load reduction. The combination of sodium decanoate (3%) + thermosonication (54 °C, 3 min, 80 W/L) enhanced the inactivation of the microbial load. Similar results were reported by Haughton et al. (2012). They observed higher resistance of TVCs to thermosonication treatments using energy densities of 20 kW/L and 20 W/L at 53 °C for 16 min. However, C. jejuni and enterobacteriaceae groups were more sensitive to thermosonication treatments. The count of these microorganisms was reduced to undetectable values using an energy density of 20 kW/L. The resistance of the aerobic bacteria was associated with the variety of microorganisms present in the chicken's skin. Gram-positive bacteria and sporeforming bacteria may be more resistant to sonication than gram-negative bacteria.

Evelyn and Silva (2015b) observed a low inactivation of two strains of *Clostridium perfringens* spores (NZRM 2621 and NZRM 898) in a beef paste. A slight logarithmic reduction of 1 and 1.5 was observed after 60 min of thermosonication at 75 °C and 24 kHz, applying 33 W with a specific energy of 0.33 W/g. An association of treatments was proposed to enhance the inactivation microorganism. The authors combined a thermal shock treatment at 80 °C for 10 min with thermosonication at 24 kHz applying 162 W and energy density of 16.2 W/mL using different temperatures (75 to 105 °C). The combination of thermal shock + thermosonication treatment doubled spore inactivation. However, temperatures higher than 85 °C were required to ensure *C. perfringens* spores inactivation.

Pennisi et al. (2020) evaluated the effect of thermosonication treatments on the inactivation *Listeria* 

Matrix	Ultrasonic system	Process conditions	Main remarks	Reference
Smoked salmon	30 L ultrasonic bath	0.8 kW; 20 kHz; 100%; 20, 25, 30, 40, and 50 °C; 5, 10, and 15 min; 25 g inoculated with <i>L. monocytogenes</i>	The processes that applied 30 °C for 15 min, 40 °C for 15 min, and 50 °C for 5 min promoted microbial inactivation of 2.02, 2.12, and 2.44 log CFU/g, respectively. The TS treatments preserved the sensory characteristics of the salmon	Pennisi et al. (2020)
Pork sausage	Ultrasonic bath	<ul> <li>150 W; 40 kHz; 80 °C; 20, 30, and 40 min;</li> <li>5 units (2.2 cm diameter, 10 cm length)</li> <li>vacuum-packed. Storage: 4 °C for 30 days</li> </ul>	Ideal TS conditions: 80 °C for 20 min. This promoted the inhibition of microbial growth during storage. Temperatures > 80 °C affected the sausage's hardness, elasticity, cohesion, and chew properties	Inmanee et al. (2020)
Raw chicken thighs	5 L ultrasonic bath	120 W; 40 kHz; 4, 25, and 54 °C; 1, 2, and 3 min; chicken inoculated with <i>C. jejuni</i>	The TS at 54 °C for 3 min + 3% sodium decanoate promoted the highest bacterial inactivation of 2.8, 2.0, and 2.2 log <sub>10</sub> CFU/g for <i>C. jejuni</i> , TVC, and total count <i>Enterobacteriaceae</i> , respectively	Kassem et al. (2018)
Raw chicken skin and thighs	54 L ultrasonic bath/Ultrasonic processor with 3-mm diameter probe	<i>TS/LI</i> : 20 W/L; 40 kHz; 53 °C; 16 min; 5 chicken thighs <i>TS/HI</i> : 20 kW/L; 24 kHz; 53 °C; 16 min; 2 g of chicken skin	The TS/LI decreased in 2.74 and 1.60 logs CFU/g de Enterobacteriaceae and TVC, respectively The TS/HI reduced to an undetectable amount of <i>Campylobacter</i> or viable <i>Enterobacteriaceae</i> and decreased 2.49 log CFU/g of the TVC	Haughton et al. (2012)
Beef paste	Ultrasonic processor with 3-mm diameter probe	33 W; 24 kHz; 0.33 W/g; 75 °C; 5 to 60 min; 10 mm from the bottom; 99 g l inoculated with C. <i>perfringens</i>	TS promoted low spore inactivation of 1 to 1.5 log after 60 min of the process. Thermal shock (80 °C, 10 min) followed by US treatment (1.62 W/mL for 1 min) inactivated half the number of spores in the suspension	Evelyn and Silva (2015b)
Power (W); frequency (kHz) sion (mm); sample (mL, g c (TVC)	; ultrasound intensity (W/cm <sup>2</sup> ); acoustic energ or kg); colony forming units (CFU); thermos	y density (W/mL or W/cm <sup>3</sup> or J/g); amplitude onication (TS); low-intensity thermosonicatio	(µm ou %); temperature (°C); processing time (TS/LJ); high-intensity thermosonication (	e (min or s); probe immer- TS/HI); total viable count

 Table 2
 Thermosonication effects on the safety and quality aspects of meat products

*monocytogenes* inactivation in smoked salmon. Thermosonication treatments (800 W, 20 kHz) at 30 and 40 °C for 15 min and 50 °C for 5 min promoted the same microbial load reduction of 2.19 log CFU/g. The processes carried out at 40 and 50 °C for 10 min also promote low inactivation of *L. monocytogenes*. The resistance of *L. monocytogenes* to thermosonication may be associated with the damping effect of the matrix. Also, thermosonication treatments did not modify the sensory characteristics of the salmon samples, such as flavor and color.

#### **Dairy Products**

Thermosonication treatments have been applied to milk and dairy products to inactivate their microbial load. Additionally, products prepared with thermosonicated milk have shown different characteristics of water retention, viscosity, consistency, syneresis, and sedimentation. Thermosonication processing has promoted the kinetic stability of the dairy productss by the reduction in the droplet size distribution of milk emulsions. The self size dairy products also has been extended by the inactivation of pathogenic microorganisms naturally found in milk. Table 3 presents the main remarks of these thermosonication effects on milk and dairy products.

Erkaya et al. (2015) applied thermosonication treatments on the milk to improve the technological properties of Ayran, an acidified milk beverage. The sample produced with thermosonicated milk presented an increase in water retention capacity and viscosity. The thermosonicated sample at 70 °C for 3 min presented 31% less of the syneresis/ phase separation than the control sample. This effect was still observed in the samples for up to 30 days after their processing. The increase of ultrasonic power (100 to 150 W) also reduced the phase separation (21 to 0%) of the acidified milk. minThe yogurt produced with thermosonicated milk (70 °C for 15 min) also presented a higher apparent viscosity than the control sample (Gursoy et al., 2016). These effects were observed up to 10 days after processing. According to the authors, thermosonication treatment promoted the dissociation of the casein micelles in subunits, which favored the formation of strong networks by aggregating the subunits between them and/or partially denatured whey proteins throughout the fermentation step. These strong networks improved the gel structure formation in the yogurt samples produced with thermosonicated milk. Another study carried out by Riener et al. (2010) demonstrated that the thermosonicated milk samples produced stronger yogurt gels, even with a low-fat content (0.1%). However, the firmness of the yogurt gels produced with thermosonicated milk (10 min at 400 W) was not associated with a whey protein denaturation. The percentage of whey protein denaturation was 26.0, 26.9, and 28.1%, for skimmed (0.1%), semi-skimmed (1.5%), and whole milk (3.5%), respectively. In contrast, the milk samples treated by a conventional thermal process at 90 °C for 10 min presented 49.1, 49.3, and 52.2% of whey protein denaturation for the skimmed, semi-skimmed, and whole milk, respectively (Riener et al., 2009).

Almanza-Rubio et al. (2016) also evaluated the use of thermosonicated milk to manufacture cream cheese. They attributed the cheese water retention (from 55 to 60%) and production yield (from 10.9 to 19.5%) to the synergistic effect between acoustic energy and heat treatment. The denatured whey proteins showed greater binding capacity with the casein micelles. Thermosonication processing also promoted the formation of large aggregates with casein micelles and reduced the milk fat globules diameter from 7 µm to values below 2 µm. This size reduction improved cheese fat retention. The authors also observed an increase in the interactions between fat globules and fat globules-proteins, possibly due to an alteration in the fat globule membrane. The cream cheese produced with thermosonicated milk also presented better texture and rheological properties, besides thermal stability (Almanza-Rubio et al., 2016).

#### **Fermented Beverages**

Few studies have evaluated the thermosonication effects on wine and beer production. Additionally, most of these studies examined the impacts of thermosonication processing on the inactivation of microorganisms, such as Brettanomyce bruxellensis, Saccharomyces cerevisiae, and Lactobacillus acetotolerans, responsible for the deterioration of these drinks. Thermosonication treatments have induced the transformation of these microorganisms to a viable putative non-culturable (VPNC) state. The beverages' alcohol content and pH of the beverages have not influenced the thermosonication effects on microbial inactivation. However, the sugar content of the beverage can help in the adaptation of microorganisms to adverse conditions promoted by thermosonication treatment. Furthermore, thermosonication did not affect the sensory attributes and physicochemical properties of fermented drinks. Table 4 presents the studies concerning the impact of thermosonication processing on the safety and quality aspects of beer and wine.

Deng et al. (2018) evaluated thermosonication treatments (538.7 W and 24 kHz) at 50 and 60 °C for 2 min on microbial inactivation of lager beer. These treatments inactivated yeast, lactic acid bacteria, and mesophilic microorganisms similarly to the conventional heat pasteurization at 60 °C for 15 min. Beer quality parameters, such as pH, ethanol content, and bitterness, were not affected after thermosonication processing. Furthermore, the foam stability of the thermosonicated beer samples was enhanced. Likewise, beer samples subjected to thermosonication processing at 50 °C had long-term stability concerning their color, flavor,

Matrix	Ultrasonic system	Process conditions	Main remarks	Reference
Goat milk	Ultrasonic processor with 13-mm diameter probe	150, 200, 300, and 400 W; 20 kHz; 10 min; 30 mm; 200 mL previously pasteurized at 72 °C for 15 s	Reduction of the total bacterial count and the size of the casein particles with the increase in power. Best results: Powers of 300 and 400 W. The samples also showed lower viscosity, turbidity, and size of the fat micelles	Ragab et al. (2019)
Whey	Ultrasonic processor with 10-mm diameter probe	100 to 250 W; 20 kHz; 60 °C; 5, 10, and 15 min; 10 mm; 100 mL	The increase in TS power and processing time promoted higher permeate recovery (94.8%) and the recovery after the crystallization of lactose (94.6%). TS decreased the whey particle size	Khaire and Gogate (2018)
Cream cheese produced with thermosonicated milk	Ultrasonic processor with 12.7-mm diameter probe	450 W; 20 kHz; P <sub>diss</sub> : 20, 50, 80, and 100 W; 7 to 30 min; 4 to 63 °C; 30 mm; 500 g of milk (3% fat)	TS treatment decreased the size of fat globules (7 to <2 µm) and increased the fat content and cheese yield (10.9 to 19.5%). TS also improved the texture and the rheological properties of cream cheese. Best condition: $P_{diss} < 25$ W, $\leq 30$ min, 35 to 50 °C	Almanza-Rubio et al. (2016)
Yogurt beverage produced with thermosonicated milk	Ultrasonic processor with 13-mm diameter probe	100, 125, and 150 W; 24 kHz; 70 °C; 15 min; 800 mL. Storage: 4 °C for 10 days	The increase in TS power reduced the serum separation and increased the viscosity of the yogurt beverage. The 150 W power did not provide serum separation during storage. TS did not change the proximal composition and color of the beverage	Gursoy et al. (2016)
Yogurt produced with thermosonicated milk	Ultrasonic processor with 22-mm diameter probe	400 W; 24 kHz; T <sub>i</sub> : 45 °C; T <sub>i</sub> :72 °C; 10 min; 30 mm; 200 mL of milk different fat content 0.1, 1.5, 3.5%	Yogurts produced with thermosonicated milk (1.5% or 3.5% fat percentage) showed greater water retention capacity, greater firmness, and viscosity than those produced with non-thermosonicated milk. The average particle size of these yogurts was also reduced to <1 $\mu$ m	Riener et al. (2009)
Yogurt produced with thermosonicated milk	Ultrasonic processor with 22-mm diameter probe	400 W; 24 kHz; 45 °C; 10 min; 30 mm; 200 mL of milk different fat content 0.1, 1.5, 3.5%	Thermosonicated milk samples with higher fat content provided yogurts with fewer syneresis effects than milk treated by a conventional thermal process. The yogurts produced with thermosonicated milk also present stronger gel structures	Riener et al. (2010)

Table 3 (continued)				
Matrix	Ultrasonic system	Process conditions	Main remarks	Reference
Ayran (acidified milk bevearge)	Ultrasonic bath	140 W; 35 kHz; 60, 70, and 80 °C; 1, 3, and 5 min; 300 mL. Storage: 4 °C for 30 days	TS increased the viscosity and consistency and decreased the phase separation of the serum (higher reduction at TS conditions of 31% and 70 °C for 3 min) of the beverage. TS at 60 °C for 1 min was sufficient to protect the bacteria from lactic acid and avoid the growth of yeasts and molds (<1 log CFU/mL) during storage	Erkaya et al. (2015)
Rice porridge/whole milk/water	Ultrasonic processor with 13-mm diameter probe	750 W; 20 kHz; 100% (114 $\mu$ m); 1.1 W/mL; T <sub>1</sub> ; 20 °C; T <sub>1</sub> ; 75 °C., 5 min; 100 ml inoculated with <i>B. subtilis</i> (~ 10 <sup>6-7</sup> spores/mL). After US, the samples were heat treated at 100 °C for 5 min	The TS allowed the production of porridges with whole milk with a lower D value (35 times lower) than those produced by thermal processing (100 °C). On the other hand, the effects of TS on porridge produced with water were not as pronounced. TS and heat treatments reduced the D values of porridge produced with water by 18% and 4%, respectively	Ansari et al. (2017)
Power (W); dissipated power ( $P_{diss}$ ); f (°C); initial sample temperature ( $T_1$ ); (TS)	requency (kHz); ultrasound intensity (W/ final sample temperature $(T_F)$ ; processin	$cm^2$ ); acoustic energy density (W/mL or V g time (min or s); probe immersion (mm);	//cm <sup>3</sup> or J/g); specific energy (W/g); amp sample (mL, g or kg); colony forming u	litude (µm or %); temperature units (CFU); thermosonication

Matrix	Ultrasonic system	Process conditions	Main remarks	Reference
Cabernet Sauvignon young red wine	Ultrasonic processor with 12.7-mm diameter probe	Pre-treatment: 43 °C for 2 min; 600 W; 20 kHz; 120 µm; 1, 2, and 3 min; 25 mm; 200 mL inoculated with <i>Brettanomyces bruxellensis</i> . Storage: 30, 60, and 90 days	TS for 3 min inactivated yeast: $100\%$ in dry red wines and 92.3% in wines with added sugar. Decreased cell viability: 7.5 to $20.5\%$ TS reduced the population of <i>B. bruxellensis</i> only in the initial stages of wine contamination	Križanović et al. (2020)
Lager beer	Ultrasonic processor with 22-mm diameter probe	538.7 W; 24 kHz; 2 min; 4 s/2 s; 40, 50, and 60 °C; 200 mL. Storage: 20 °C for 12 months	TS improved the foam stability and maintained the ethanol content, pH value, and bitterness of the beer. The processes carried out at 50 °C favored the quality, oxidative stability, and flavor of the beer	Deng et al. (2018)
Lager beer	Ultrasonic processor with 14-mm diameter probe	24 kHz; 50 °C; 22 mm; 0.6 W/mL (5, 10, 15, 20, and 25 min); 1.2 W/mL (3, 6, 9, 12, and 15 min); 1.8 W/mL (2, 4, 6, 8, 10, and 12 min); 2.4 W/mL (2, 4, 6, 8, 10, and 12 min); 60 mL inoculated with <i>L acetotolerans</i>	The application of lower densities of acoustic energy (0.6, 1.2, and 1.8 W/mL) induced the growth of VPNC bacterial cells in beer. VPNC cells can remain in beer after TS and promote its deterioration	Piao et al. (2019)
Lager beer	Ultrasonic processor with 14-mm diameter probe	2.7, 5.8, and 8.9 W/mL; 24 kHz; 40, 50, and 60 °C; 2, 4, and 6 min; 22 mm; 60 mL inoculated with <i>L. acetotolerans</i> in normal state and VPNC	Optimal conditions: 8.9 W/mL, 40 °C, and 4 min for 6 log inactivation of normal cells. 8.9 W/mL, 60 °C, and processing time > 4 min for reduction of VPNC cells	Yin et al. (2018)
Beer	Ultrasonic processor with 14-mm diameter probe	161.6 W; 24 kHz; 125 µm; 43 to 70 °C; 40 mm. <i>Continuous mode</i> : 10.8 W/mL; 0.5 min; 15 mL. <i>Discontinuous mode</i> : 16.2 W/mL; 0.5 to 60 min; 10 mL inoculated with S. <i>cerevisiae</i>	TS treatments at 50 °C employed 3.0, 1.9, and 4.5 min for "pasteurizing" beers with 0, 4.8, and 7.0% v/v ethanol, respectively The beers were "pasteurized" at range temperatures of 50 and 55 °C, regardless of the beer level of alcohol content	Milani and Silva (2017)
Chinese rice wine	Ultrasonic processor with 12-mm diameter probe	600, 750 W; 20 kHz; 30, 35, and 40 °C; 1 s/1 s; 20 to 120 min; 15 mm; 150 mL inoculated with S. <i>cerevisiae</i> <i>PEF</i> : 3 μs and 300 Hz in monopolar mode	TS at 750 W promoted higher bacterial inactivation (<1.2 logs CFU/mL) by increasing the TS temperature and after 60 min of processing time The treatment combination of TS (750 W, 120 min, 35 °C) followed by PFE (12 kV/cm, 120 µs, 35° C) promoted an additive effect on the inactivation of <i>S. cerevisiae</i> (3.72 logs CFU/ mL)	Lyu et al. (2016)
Power (W); freque tion on and off tim (PEF)	sucy (kHz); ultrasound intensity (W/cm <sup>2</sup> ); specifitie (s/s); probe immersion (mm); sample (mL); flow	c energy (W/g); acoustic energy density (W/mL); w (mL/min); colony forming units (CFU); viable b	amplitude ( $\mu$ m ou %); temperature (°C); processin ut not cultivable state (VPNC); termosonication (1	ig time (min or s); pulsa- [S); pulsed electric fields

 Table 4
 Thermosonication effects on the safety and quality aspects of fermented beverages

and oxidative stability. Other studies have also evaluated the inactivation of L. acetotolerans CGMCC 7.150 in larger beers through thermosonication processing (Piao et al., 2019; Yin et al., 2018). Piao et al. (2019) observed that low power densities (0.6, 1.2, or 1.8 W/mL, 24 kHz) induced the transformation of the L. acetotolerans cells to VPNC state. However, the application of 2.4 W/mL for 12 min at 24 kHz resulted in a beer with a lower viable cell count (almost zero). In this way, thermosonication can inactivate exponentially growing and VPNC cells. Yin et al. (2018) also observed that the cells of L. acetotolerans in the VPNC state are more resistant to thermosonication treatments than cells in the normal state. They applied 8.9 W/mL at 24 kHz and 40 °C for 4 min and observed a reduction of normal cells in 6 log cycles. An energy density of 8.9 W/mL at 60 °C and 24 kHz was required to inactivate the same amount of VPNC cells.

Different alcohol contents (0.0, 4.8, and 7.0% v/v ethanol) promoted the same effects on the Saccharomyces cerevisiae ascospores inactivation in beer samples subjected to thermosonication processing. However, process parameters, such as holding time and temperature, affected the ascospores inactivation. A logarithmic reduction of 3.6 log cycles was achieved by applying 16.2 W/mL at 24 kHz for 20 min. Additionally, the batch processing resulted in a higher inactivation of the microbial load compared to the continuous process. The differences between the results achieved in each type of process were related to specific energies applied. In the continuous process, lower energy density (10.8 W/ mL) was applied to the beer samples compared to the batch process (16.2 W/mL) for the same holding time (Milani & Silva, 2017). Therefore, higher specific densities favored the inactivation of S. cerevisiae ascospores.

Otherwise, Križanović et al. (2020) reported that the sugar content of the red wine and thermosonication holding time significantly influenced the reduction in the cultivability of Brettanomyces bruxellensis CBS 2499. Higher sugar content ensured yeast adaptation to unfavorable conditions promoting a protective effect. In contrast, longer holding times were associated with a total reduction in the yeast cultivability. Thermosonication processing also induced the yeast to pass for a VPNC state in dry wines (without sugar) after 3 min of treatment applying 600 W at 43 °C and 20 kHz, which affected 20.5% of yeast viability. The yeast's metabolic activity also was affected by thermosonication after 90 days of storage. Lower production of deteriorating compounds was observed in wines with a higher ethanol content (14%) and lower pH values (3.5 and 3.7) after storage time. However, the sugar content promoted the growth and proliferation of yeast in the wine, increasing the production of deteriorating compounds (4-ethylphenol and 4-ethylguaiacol). Lyu et al. (2016) studied the impacts of thermosonication on Chinese rice wine. The higher S.

*cerevisiae* inactivation (< 1.2 log CFU/mL) was achieved by applying higher nominal power and a low working temperature (750 W at 40 °C and 20 kHz) for 60 min. The low *S. cerevisiae* inactivation may be due to the high carbohydrate content of rice. As explained earlier, carbohydrates can protect the yeast against thermosonication treatment. Additionally, a lower power density of 5 W/mL was applied in this study compared to the power density of 16.2 W/mL used to inactivate ascospores in beers, as mentioned earlier.

# Impact of the Food Matrix on Thermosonication Efficiency

Some intrinsic characteristics of each food group hinder the microbial or enzyme inactivation through thermosonication. On the other hand, the combined effect of acoustic energy and mild heat treatments can modify food the physicochemical and technological properties of the food products. In this topic, the characteristics of each food product (plant, meat, dairy, and fermented beverage) were grouped to emphasize those most affected the thermosonication efficiency.

**Plant Products** The main objective of the application of thermosonication on plant products, such as fruit and vegetables and their juices and beverages, has been the inactivation of microorganisms and enzymes. Thus, many studies aimed to reduce the microbial load and enzyme activity, avoiding bioactive compounds degradation. Several studies reported losses in these compounds using high thermosonication temperatures and holding times. Thus, the thermal resistance of bioactive phytochemical compounds is the most relevant characteristic of plant products. Thermosonication treatments must be performed to inactivate microorganisms and enzymes at temperatures that preserve these phytochemical.

**Meat Products** Thermosonication treatments have been applied to meat products for microbial inactivation. However, some strains of microorganisms, such as gram-positive bacteria, are resistent to these treatments. Thus, the identification of the microorganisms present in each meat product is a crucial factor to be considered in the processing of this food group. Thereby, thermosonication can be applied to inactivate these resistant microorganisms combined with natural chemical agents.

**Dairy Products** Thermosonication treatments have been applied to milk to inactivate microorganisms and improve the technological properties of their derivatives. The microbial inactivation of dairy products through ultrasound processing is describe in the literature (Guimarães et al., 2018; Scudino et al., 2020). High acoustic energy is required for

the inactivation of microorganisms in milk. On the other hand, the impact of thermosonication on the technological properties of dairy products has been discussed in the literature recently. The application of thermosonication in these products has mainly promoted the breakdown of fat globules and structural changes in dairy proteins. In this way, technological characteristics associated with texture and rheological properties, such as water retention, viscosity, consistency, spreadability, viscoelastic, and kinetic stability, have been affected. Therefore, different thermosonication process conditions can affect the fats and proteins of dairy products, promoting several technological changes.

**Fermented Beverage** Few studies were carried out, but they reported that the wine sugar content difficult the thermosonication action on the inactivation of enzymes. Sugar promotes a protective effect on wine yeast. Therefore, the sugar content must be considered in thermosonication treatments focused in microbial and enzymatic inactivation.

# Impact of Thermosonication Processing on the Microbial Inactivation

Most thermosonication treatments have been performed for the microbial inactivation of food products. Ramteke et al. (2020) and Anaya-Esparza et al. (2017a, b) reviewed more specifically the effects of thermosonication on the microbial inactivation of dairy and plant products, respectively. They explain some theories about the possible mechanisms for the inactivation of microorganisms during thermosonication. Most agree that the acoustic cavitation is the main effect responsible for microbial inactivation. In addition to thermal effects, acoustic cavitation weakens the cell membrane of microorganisms due to shear stress (Bermudez-Aguirre et al., 2011). This cell membrane weakening and/or rupture promote the leakage of the internal content of the cellular organelles. Thus, a lethal or sub-lethal effect on microbial cells and spores is observed (Anaya-Esparza et al., 2017a, b; Bermudez-Aguirre et al., 2011; Wordon et al., 2012). In this section, we discussed the impact of thermosonication on the activation of microbial suspensions. Table 5 presents some examples of these impacts on the inactivation of microbial suspensions usually associated with technological issues of food products.

Fan et al. (2019a, b) demonstrated that thermosonication causes damage to the internal membrane of *Bacillus subtilis* spores favoring their inactivation. Despite thermosonication ability to promote microbial inactivation, the effectiveness of this treatment is strongly affected by the morphology (shape or size) and the intrinsic characteristics of microorganisms. Some of these are more resistant to adverse conditions. Deshpande and Walsh (2020) obtained smaller reductions in the spore count of *B. subtilis* compared to vegetative cells of *Geobacillus* stearothermophilus and Anoxybacillus flavithermus applying the same holding times. In another study, only 1.8 log cycles of *Byssochlamys nivea* spores were inactivated at 75 °C and 24 kHz applying 0.33 W/mL for 15 min. However, thermosonication processing carried out for 30 min demonstrated the same or higher viable spore inactivation compared to high-pressure processing associated with moderate heat treatment (600 MPa, 75 °C for 30 min) and to a conventional heat treatment performed at 75 °C for 30 min (Evelyn & Silva, 2015a).

On the other hand, thermosonication processing of soursop nectar did not promote differences in reducing gram-positive and gram-negative microorganisms. The highest lethality values were observed for Escherichia coli (5.16 log CFU/mL) and Staphylococcus aureus (5.18 log CFU/mL) using the same thermosonication treatment at 54 °C applying 1.4 W/mL for 10 min (Anaya-Esparza et al., 2017a, b). Logarithmic reductions in the E. coli count of 6.62 log CFU/mL were also observed in a pumpkin juice after thermosonication at 60 °C applying 150 W for 30 min (Demir & Kılınc, 2019). Yin et al. (2018) also reported reductions of 6 log cycles in the count of the microorganism responsible for the beer quality deterioration (L. acetotolerans). This study demonstrated that an energy density of 8.9 W/mL (24 kHz,  $\geq$  4 min, 40/60 °C) was needed to inactivate the exponential growth and cells in a viable but non-culturable (VPNC) state. Bacteria in VPNC state are unable to grow and develop in colonies in environments where they usually would. However, they are alive and capable of metabolic activity (Deng et al., 2015).

Other studies have reported lower reductions in the count of microorganisms. They have even assessed the additive effect of two emerging technologies on their inactivation. Thermosonication processing of apple juice reduced 2.7 log cycles in the *E. coli* count (400 W, 50 °C, 5 min, 8 mL/min). However, the combined heat treatment with pulsed light achieved decreases of up to 5.9 log cycles (Muñoz et al., 2012). Martínez-Moreno et al. (2020) observed that a thermosonication treatment applying pulsed ultrasound on soursop puree in the vacuum (16.5 kPa, 3 IVPs, 50 °C, 10 min) reached the highest values of inactivation of *E. coli* (7.58 log CFU) and *S. aureus* (7.35 log CFU).

# Impact of Thermosonication Processing on the Enzyme Inactivation

Thermosonication has also promoted the inactivation of enzymes in food products, mainly in vegetables and beverages. Acoustic energy-based treatments can promote the denaturation of proteins by depolymerizing and changing

Table 5         Impact of the thermosonication	I treatment on the inactivation of microbi	al suspensions		
Matrix	Ultrasonic system	Process conditions	Main remarks	Reference
Supension of G. stearothermophilus, A. flavithermus, B. subtilis in triptych soy broth/milk	Ultrasonic processor with 3-mm diameter probe	500 W; 20 kHz; 30%; 72 and 73 °C; 0 to 120 s; 40 mm; 5 mL <i>B. subtilis</i> (only spores)	The temperature of 73 °C provided the reduction of vegetative cells after 120 s of treatment. The highest reduction was 1.94 log for G. <i>stearothermophilus</i> . The highest reduction of spores ( $\leq$ 0.2 logs) was on A. flavithermus at 73 °C for 120 s	Deshpande and Walsh (2020)
Suspension of <i>B. subtilis</i> in distilled water	Ultrasonic processor with 10-mm diameter probe	20 kHz; 6.67, 13.3, and 20 W/mL; 80 °C; 40 min; 15 mm; 30 mL (10 <sup>7</sup> spores/mL)	The spore load was reduced in 1.8, 2.09, and 2.43 log CFU/mL using the AEDs of 6.67, 13.3, and 20 W/ mL, respectively. TS damaged the proteins of the inner membrane (IM) or the IM itself leading to leakage of intracellular substances and cell death	Fan et al. (2019a, b)
Suspension of <i>B. subtilis</i> in distilled water	Ultrasonic processor with 10-mm diameter probe	620 kHz; 67, 13.3, and 20 W/mL; 60, 70, and 80 °C; 10 to 40 min; 15 mm; 30 mL (10 <sup>7</sup> spores/ml, pH 7.0)	The spore inactivation was recorded at higher temperatures (2.43 log CFU/ mL at 80 °C, 600 W). The spore inactivation occurred due to lesions promoted in key enzymes of the intermediate metabolism	Fan et al. (2019a, b)
Suspension of <i>B. subtilis</i> in distilled water	Ultrasonic processor with 10-mm diameter probe	20 kHz; 6.67, 13.3, and 20 W/mL; 80 °C; 40 min; 30 mL (10 <sup>7</sup> spores/mL)	The TS damaged the spores after 40 min of processing time using the AED of 6.67 W/mL and 80 °C. TS mainly affected proteins associated with metabolism	Fan et al. (2020)
Suspension of <i>B. cereus</i> in sterile distilled water	Ultrasonic processor with 13-mm diameter probe	800 W; 20 kHz; 100%; 80 °C; 5 to 30 min; 9 mm of background; 40 mL (10 <sup>7</sup> spores/mL)	Spore reduction in 0.41 and 0.51 log after 15 and 30 min, respectively. Inactivation occurred through damage to the membrane structure and the release of 33.99% dipicolinic acid	Lv et al. (2019)
Suspension of <i>E. coli</i> and <i>P. fluorescens</i> in Ringer's solution	Two continuous ultrasonic processors with 40-mm diameter probe	1000 W; 20 kHz; 18.6 $\mu$ m (TS-L) and 27.9 $\mu$ m (TS-H); 55 °C; 2.1 min; 160 mL/min (8.6 × 10 <sup>8</sup> and 6.07 × 10 <sup>8</sup> CFU/mL for <i>P. fluorescens</i> and <i>E.</i> <i>coli</i> , respectively)	<i>P. fluorescens:</i> Inactivation was 9.2% (TS-L) and 6.4% (TS-H), without sub-lethal cell damage <i>E. Coli:</i> Inactivation was 1.1% (TS-L) and 6.3% (TS-H). 1.5% sub-lethal lesion was observed after TS-L	Halpin et al. (2014)
Suspension of <i>E. coli</i> in distilled water	Ultrasonic processor with 22-mm diameter probe	55 kHz; 45, 50, 55, and 60 °C; 1 to 5 min; 17.56, 21.49, and 24.17 W/cm <sup>2</sup> ; 2 and 4 mm; 50 mL (10 <sup>6</sup> CFU/mL)	Ideal condition: 21.49 W/cm <sup>2</sup> and 45 °C for 4 min (5 log reduction) TS required less specific energy (583.6 kJ/kg) to achieve the maximum microbial reduction (5 logs) compared to manosonication and heat treatment	Al-Juboori and Yusaf (2010)

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Matrix	Ultrasonic system	Process conditions	Main remarks	Reference
Suspension of <i>E. coli</i> in phosphate buffer (0.01 M)	Ultrasonic processor with 13-mm diameter probe	750 W; 20 kHz; 124 µm; 40, 47, 54, and 61 °C; 0.25 to 4 min; 14 mm from the bottom; 50 mL (10 <sup>8</sup> CFU/ mL, pH: 7.5)	TS significantly reduced the time required for a 5 log reduction of <i>E.</i> <i>coli</i> (61 °C, 0.75 min) Extensive cellular damage caused by physical forces was observed	Lee et al. (2009)
Suspension of <i>S. aureus</i> in trypticasein soy broth	Ultrasonic processor with 25.4-mm diameter probe	400 W; 20 kHz; 60, 75, and 90 µm; 40, 50, and 60 °C; 5 to 40 min; 40 mm from the bottom. Antimicrobial: vanillin at 200, 350, and 500 mg/kg; 50 mL ( $10^4$ CFU/mL), $a_w = 0.96$ , and pH = 3.5	At temperatures > 55 °C, the dominant factor in microbial inactivation was the temperature	Avila-Sosa et al. (2010)
Power (W); frequency (kHz); ultrasounc	d intensity (W/cm <sup>2</sup> ); acoustic energy den	nsity (W/mL); amplitude (µm ou %); temp	erature (°C); processing time (min or s)	; probe immersion (mm); sam-

ple (mL); flow (mL/min); colony forming units (CFU); thermosonication (TS); low-amplitude thermosonication (TS-L); high-amplitude thermosonication (TS-H)

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the conformation of their tertiary structure. They also can promote obstructions in Van der Waals interactions and hydrogen bonds (Amador-Espejo et al., 2020). These obstructions are caused by the formation of free radicals in the sonicated medium, as related in the "Fundamentals of Ultrasound Technology" section. However, some enzymes may exhibit resistance to thermosonication processing, which may be associated with an intrinsic characteristic of the thermal resistance of the enzyme. Because of this, the most thermally resistant enzymes found in the food matrix are used for perform the thermosonication process design (Marques Silva & Sulaiman, 2017).

Amador-Espejo et al. (2020) evaluated the effects of thermosonication treatment in a fruit smoothie. The increase of ultrasound amplitude decreased the activity of pectin methylesterase (PME). On the other hand, the polyphenol oxidase (PPO) activity decreased using longer treatments. The thermosonication treatment carried out at 77.5% of amplitude, and 47.5 °C for 20 min resulted in PPO and PME activities of 4.82 and 0.12 UEA/mL, respectively.

The inactivation of PPO was also observed in pitaya juice. A PPO residual activity of 2.17% was observed after the treatment performed at 50 °C applying 380 W for 20 min. The enzyme was inactivated by extending the holding time to 40 min (Liao et al., 2020). Baltacioğlu et al. (2017) also studied PPO inactivation in mushroom suspensions (pH 6.5). They used a 3-mm diameter probe at 24 kHz with 400 W in the amplitudes of 60, 80, and 100%. The inactivation kinetics was evaluated from 5 to 30 min using the working temperatures from 20 to 60 °C. The results showed a significant decrease in the PPO residual activity with increased ultrasonic power, temperature, and holding time. A PPO inactivation higher than 99% was observed after the treatment carried out at 60 °C with an amplitude of 100% for 10 min. The PPO inactivation by thermosonication occurred due to the irreversible alteration in the secondary structure of the enzyme.

Raviyan et al. (2005) studied the inactivation of PMEs in tomato suspensions (pH 7.5). They used a 13-mm diameter probe operating at 20 kHz by applying 750 W in the amplitude of 20 µm. The working temperatures were 61 °C and 72 °C. The temperature rise favored the PME inactivation. The highest PME inactivation regarding D value was obtained after treatments at 61 °C for 0.8 min and at 72 °C for 0.3 min. PME activity was also reduced by 90% in tomato juice (24 kHz, 25–75 µm). The exposure time to heat significantly affected the inactivation of this enzyme. Otherwise, the use of different amplitude levels did not promote a significant effect. Additionally, the working temperatures of 60 °C and 65 °C resulted in a higher PME inactivation. These temperatures reduced the enzyme activity by about twice as much as 70 °C. The authors explained that this higher temperature might have

increased the vapor pressure cavitation bubbles. In this way, the working temperature of 70 °C may have favored the damping effect, reducing the acoustic cavitation intensity (Wu et al., 2008). Terefe et al. (2009) also observed a decrease in the PME inactivation rate by increasing the working temperature from 60 to 75 °C. They studied the thermosonication processing at 20 kHz and 75 µm of tomato juice. The PME inactivation rate was 6 and 1.5 times for treatments performed at 60 and 75 °C, respectively. Although the PME inactivation was low, applying working temperatures of 70 and 75 °C, these were 3.9 and 1.4 times higher than the inactivation of thermal treatments performed at the same temperatures. The discrepancy in the results obtained in the two studies presented may be associated with the variety of tomatoes, the process conditions, such as the differences in the sonotrode geometry and energy density applied. The energy density applied in the first study was 0.48 W/mL, while in the second was 1.6 W/mL. The PME and peroxidase (POD) activities were also evaluated by Pinheiro et al. (2016) in whole tomatoes. A thermosonication treatment at 40 °C and 45 kHz for 30 min resulted in a PME inactivation of only 25%. Furthermore, a gradual increase in PME activity occurred throughout the cold storage at 10 °C. The thermosonication promoted a different effect on the POD activity. The thermosonication increased POD activity by 26%. After that, its activity was reduced throughout storage. On the other hand, a thermosonication treatment at 51 °C applying 1.4 W/mL for 10 min reduced the PPO activity by 99% in soursop nectar. Besides that, no PPO activity was detected during the storage (Anaya-Esparza et al., 2017a, b). Thus, thermosonication treatments using higher temperatures may favor the PPO inactivation. Additionally, the product's intrinsic characteristics of the product on which the treatment is applied can affect enzyme inactivation.

# **Critical Observations**

The process standardization applied to food products is crucial to ensure the safety and quality of foods and beverages, mainly when innovative technologies are introduced to the manufacturing process. Furthermore, the scaling up of the thermosonication process for industrial lines depends on its standardization. However, some inconsistencies were verified in the thermosonication methodologies. The application of low-frequency ultrasound in a liquid medium provides heat due to the phenomenon of acoustic cavitation, as previously discussed. The cavitation bubble explosion releases high energy promoting the homogenization of the liquid medium and temperature rise. In this way, the energy provided to sonication modulates the acoustic cavitation intensity and, consequently, the temperature increase. Since thermosonication treatment is based on the combined action of acoustic energy with a mild heat treatment, monitoring the total thermal energy supplied for the system is fundamental for understanding the actual heat impact of the heat on thermosonicated foods products. Thus, the monitoring of thermal histories of thermosonicated samples is recommended, or at least the temperature differentials before and after thermosonication processing. Many studies did not report the temperature changes throughout thermosonication treatments. They studies have reported only working temperatures. In other words, they are only reporting the heat supplied by the external heat source. Thus, the actual heat treatment to which the thermosonicated samples were subjected was omitted (Almanza-Rubio et al., 2016; Avila-Sosa et al., 2010; Erkaya et al., 2015; Evelyn & Silva, 2015b; Fan et al., 2019a, b; Gursoy et al., 2016; Illera et al., 2018; Inmanee et al., 2020; Jabbar et al., 2015; Kassem et al., 2018; Khaire & Gogate, 2018; Liao et al., 2020; Lv et al., 2019; Pennisi et al., 2020; Pinheiro et al., 2016; Pinheiro et al., 2019).

Some studies have monitored the temperature during thermosonication treatment. Riener et al. (2009, 2010) observed that samples 75 °C after 6 min in a thermosonication process which set a working temperature of 45°C. Samples processed in bath-type ultrasound presented a lower temperature rise. The greater heat exchange area of bath-type ultrasound equipment contributes to energy dissipation in heat form, reducing the system temperature. In this type of ultrasound equipment are observed increases from 1 to 4 °C in the working temperature (Martínez-Moreno et al., 2020; Milani & Silva, 2017; Yin et al., 2018). Other studies also stated the need to set 2 or 3 °C below the working temperature to avoid overheating throughout thermosonication treatments at 50 to 75 °C (Terefe et al., 2009; Wu et al., 2008). In contrast, other studies have ensured that the process temperature was similar to working temperature (40, 50, 60, 55, 75, and 85 °C) throughout the treatment (Demir & Kılınç, 2019; Deng et al., 2018; Fan et al., 2020; Haughton et al., 2012; Hooshyar et al., 2020; Piao et al., 2019; Walkling-Ribeiro et al., 2009).

The keyword "thermosonication" has also been associated with processes that did not use an external heat source to maintain constant working temperature. These studies used only the heat provided by sonication. Goat's milk samples, for example, were previously pasteurized at 72 °C for 15 s and then sonicated in an ice-coated beaker (Ragab et al., 2019). Amador-Espejo et al. (2020) also heated samples of fruit smoothies to 40 or 55 °C previously to thermosonication. However, the working temperature was not maintained by an external heat source throughout the treatment. Other studies established working temperatures of 40 and 50 °C but used water recirculation baths at 10 or 23 °C (Muñoz et al., 2011, 2012). Cold-water recirculation was also used in a thermosonication treatment (Halpin et al., 2014; Križanović et al., 2020). Ribeiro et al. (2017) sonicated samples of coconut water at room temperature without the aid of an external heat source. The thermal histories of this study demonstrated an increase in the sample temperature from 23 to 80 °C throughout sonication time. Blueberry juice samples, treated in the same way, presented an increase in their temperature from 17 to 70 °C due to increased processing time (Režek Jambrak et al., 2017). From the point of view of most studies on thermosonication processing examined in this review, the thermosonication treatment must be performed by combining acoustic energy with an external heat source to maintain the working temperature. However, inconsistencies in the process methodology may be solved with the own name of this emerging technology, which defines the application of ultrasound and heat simultaneously.

## **Conclusion and Perspectives**

Thermosonication has shown promise for the inactivation of microorganisms and enzymes in foods and beverages, providing them lower impact on their nutritional and sensory quality compared to severe thermal treatments. Furthermore, the undesirable effects of this emerging technology on the quality aspects of food products can be minimized by modulating thermosonication process conditions. On the other hand, many treatments even increased the availability and retention of bioactive compounds in foods and beverages, including throughout their storage.

Thermosonication has been widely studied in the processing of fruit and vegetable juices. However, few studies have been performed to evaluate thermosonication effects on meat and dairy products and fermented beverages. In this way, there is a demand for more studies about these food groups.

Depending on the thermosonication process design, high thermal energy may be delivered to food products due to the coupled treatment between sonication and heat by an external source. Therefore, the monitoring of thermal history throughout the processes is a crucial requirement to achieve the aims set for the process. Monitoring thermal history ensures the maintenance of food quality and safety.

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## Declarations

Conflict of Interest The authors declare no competing interests.

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