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Microbial decontamination assisted by ultrasound-based processing technologies in food and model systems: A review

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Abstract

Ultrasound (US) technology is recognized as one of the emerging technologies that arise from the current trends for improving nutritional and organoleptic properties while providing food safety. However, when applying the US alone, higher power and longer treatment times than conventional thermal treatments are needed to achieve a comparable level of microbial inactivation. This results in risks, damaging food products' composition, structure, or sensory properties, and can lead to higher processing costs. Therefore, the US has often been investigated in combination with other approaches, like heating at mild temperatures and/or treatments at elevated pressure, use of antimicrobial substances, or other emerging technologies (e.g., high-pressure processing, pulsed electric fields, nonthermal plasma, or microwaves). A combination of US with different approaches has been reported to be less energy and time consuming. This manuscript aims to provide a broad review of the microbial inactivation efficacy of US technology in different food matrices and model systems. In particular, emphasis is given to the US in combination with the two most industrially viable physical processes, that is, heating at mild temperatures and/or treatments at elevated pressure, resulting in techniques known as thermosonication, manosonication, and manothermosonication. The available literature is reviewed, and critically discussed, and potential research gaps are identified. Additionally, discussions on the US's inactivation mechanisms and lethal effects are included. Finally, mathematical modeling approaches of microbial inactivation kinetics due to US-based processing technologies are also outlined. Overall, this review focuses only on the uses of the US and its combinations with other processes relevant to microbial food decontamination.

KEYWORDS
decontamination, food, inactivation, manosonication, manothermosonication, microbiology, modeling, sonication, thermosonication, ultrasound

1 | INTRODUCTION

Ultrasound (US) technology, along with high-pressure processing, microwave, or pulsed electric fields, is recognized as one of several promising food processing technologies (Jermann et al., 2015). It is based on using acoustic waves with frequencies higher than the human hearing threshold (>14–16 kHz). These waves can travel and propagate through a material, with liquid media being the most often used and reported (Soria & Villamiel, 2010). Depending on the frequency and intensity of the US waves, two significant categories relevant to food processing can be defined: high-frequency ultrasound (HFU) and low-frequency ultrasound (LFU).

HFU is based on applying frequencies higher than 100 kHz, typically at low energy intensities (<1 W/cm^2). Main applications can be found in food quality determination and assurance as a noninvasive technique for assessing different quality parameters with the help of sound waves, for example, the assessment of emulsion stability in the production of dairy products, such as butter, chocolate, and ice cream (Awad et al., 2012). Other applications of HFU include the estimation of food composition, the detection of metal particles and other foreign materials, and the monitoring of physicochemical and structural properties during processing and storage (Awad et al., 2012; Dolas et al., 2019). On the other hand, LFU uses a lower range of frequencies from 20 to 100 kHz and higher energy intensities (>1 W/cm^2) (Iorio et al., 2019). Its application induces modifications to the food properties due to mechanical, physical, chemical, and biochemical changes in the food components. The primary effect of LFU is based on a phenomenon called cavitation, and its principles are described in the following section (McClements, 1995).

2 | TECHNICAL ASPECTS OF US TECHNOLOGY

2.1 | Cavitation as the driving force of US technology

The cavitation phenomenon is known to be originated from a sudden pressure drop due to the propagation of ultrasonic waves, which triggers the generation of vapor and gas microbubbles in a liquid medium (Zupanc et al., 2019). Subsequently, after the pressure recovers, the microbubbles quickly grow in size during the compression and expand at low-pressure cycles until there is no capacity to withstand the external pressure, and, finally, they undergo violent collapse. For instance, the pressure threshold for cavitation in a water medium is above 200 kPa (Caupin & Herbert, 2006). As a result of the pressure changes in materials and surroundings in very short bursts (in the order of microseconds, μs), continuous cycles of microbubbles creation and implosion cycles occur, resulting in the release of large amounts of energy and localized mechanical, thermal, and sonochemical effects. This situation results in extreme conditions, called "hot spots," associated with sudden pressure rises to 100 MPa and high temperatures of around 5000°K (Ferrante et al., 2007; Herceg et al., 2013; Scherba et al., 1991). Consequently, this results in the formation of highly reactive free radical species of hydroxyl radical ($OH^{\bullet}$) and hydrogen atoms ($H^{\bullet}$) due to the fragmentation of water molecules (H_2O), a phenomenon called sonolysis. In parallel, hydrogen peroxide (H_2O_2) generation from the $OH^{\bullet}$ species reaction or organic solutes occurs (Gogate & Kabadi, 2009; Hua & Thompson, 2000). In addition, other reactive species are also formed depending on the dissolved gases in the liquid medium ($O_2H^{\bullet}$, $N^{\bullet}$, $O^{\bullet}$). Likewise, the implosion bursts may be accompanied by local shock waves, liquid high-speed microjets (>100 m/s), high shear forces (turbulence and eddies), and microstreaming (Adekunte et al., 2010a; Brilhante São José & Dantas Vanetti, 2012; Condón et al., 2004; Patil et al., 2009; Piyasena et al., 2003; Valero et al., 2007; Zupanc et al., 2019). An overview of the cavitation phenomenon and its effects is given in Figure 1.

2.2 | Devices and modes of application

The US waves are usually generated by a piezoelectric transducer, which transforms the electrical energy into mechanical vibrations. These generated waves may typically be applied to a liquid product by two devices, either an ultrasonic bath or a probe system (Figure 2). The ultrasonic bath consists of a vessel containing the liquid product to be treated with the US. The acoustic waves are propagated from transducers, usually located at the bottom of the device (Chemat et al., 2017). The main advantage of this type of device is the possibility of simultaneously treating several samples of different sizes. However, many limitations in terms of microbial inactivation can be seen. For instance, ultrasonic baths have a generally low acoustic energy application, and the wave propagation is irregular because of reflection on the bath walls. This means that the implosion of the cavitation microbubbles is not heterogeneous inside the bath, not providing the treatment with the same effectiveness in all regions. In addition, only a batch mode operation can be applied in ultrasonic baths (Mason, 1998; Rodríguez et al., 2018; Santos et al., 2008).

On the other hand, the probe system consists of a transducer connected from a component known as the horn to

the sonotrode, the part immersed in the treated medium for acoustic wave propagation, as shown in Figure 2. Furthermore, an additional part, known as a booster, can be incorporated into the setup to increase or reduce the amplitude of the waves during the treatment, a parameter discussed later. The position of the probe in the ultrasonic reactor, as well as the geometry and design of the reactor, is important (Lee et al., 2009b). Among the essential features of the probe in terms of microbial inactivation efficiency are its length, geometry, design, and tip diameter (Awad et al., 2012; Zupanc et al., 2019). In fact, the acoustic energy is transmitted to the product through the probe's tip to generate the cavitation directly underneath it. This type of system is the most commonly used US device because of being more powerful and able to achieve more effective microbial inactivation than the ultrasonic bath (Chemat et al., 2017; Rodríguez et al., 2018). Nevertheless, due to the implosion of the cavitation microbubbles, the probe tip may be pitted over time, and, therefore, a metal powder may be released into the treated medium (Palma et al., 2017).

Regarding the application modes of the US, two different modes can be mentioned: continuous and pulsed. In a continuous mode, acoustic waves are emitted throughout the medium, with the transducer continuously excited with an electrical sine wave. As a result of the continuous supply of acoustic energy, a notable temperature increase in the treated medium is recorded, which can damage the food product. By contrast, better performance in terms of microbial inactivation is achieved with this application mode (Bermúdez-Aguirre & Barbosa-Cánovas, 2012). In contrast, in the pulsed mode, also called "duty-cycles," the acoustic waves are emitted intermittently for a specific time, resulting in the excitation of the transducer and the discharge of very short electrical signals. This is interrupted by pauses for a short time to repeat the electrical discharge, and thus, the increase in temperature of the treated sample during the US treatment is lower than in the continuous mode (Dolas et al., 2019; Zupanc et al., 2019). The duty-cycles are repeated throughout the treatment, but the microbial inactivation efficiency in pulsed mode is more limited than in continuous mode (Bermúdez-Aguirre & Barbosa-Cánovas, 2012).

2.3 | Factors influencing the inactivation of microorganisms by US

2.3.1 | Processing factors

In the application of US treatments, several factors directly impact the extent to which the cavitation threshold can be reached in a tested medium. Apart from the processing time, the most important processing parameters, especially when considering the inactivation of microorganisms, are:

I. *Frequency*, expressed in Hz, is known to be inversely proportional to the microbubble size: in LFU, the low frequencies applied (20–100 kHz) generate large cavitation microbubbles leading to an efficient microbial inactivation, different from the HFU (>100 kHz) where small microbubbles are formed, and the resulting cavitation is not enough to inactivate bacteria (Cao et al., 2010). There are two major frequency modes to consider during treatments: constant frequency or multi-frequency treatments, for example, dual-frequency ultrasound (DFU) (20–40 kHz) and tri-frequency ultrasound (TFU) (20–40–60 kHz). According to the review of Bermúdez-Aguirre (2017),

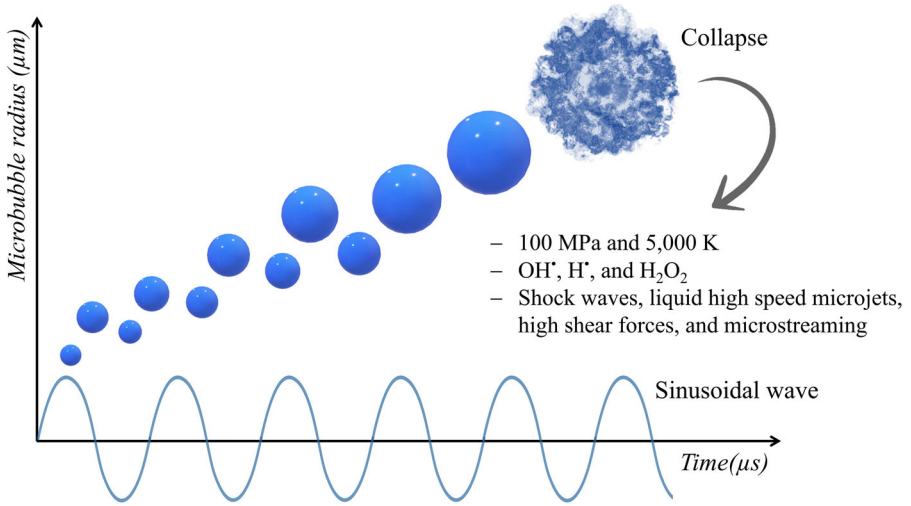

FIGURE 1 Cavitation phenomenon: growth and collapse of the cavitation microbubble according to the sinusoidal wave and mechanical, thermal, and chemical effects. Adapted from Jiang et al. (2020).

the inactivation of microorganisms is most effective within the frequency range of 20–24 kHz.

II. *US intensity* is considered a parameter whose definition differs between groups: some researchers associate the intensity of the treatment with the nominal power input, referring to US intensity as the electrical power of the device used (Cameron et al., 2008; Su et al., 2010). However, this conception can be considered inaccurate enough, as not all electrical energy is converted and emitted as acoustic waves. Hence, other authors determine the actual acoustic power delivered to the treated medium during the process by using different methods, for example, with calorimetry. However, this approach can only be used when assuming that all acoustic energy is dissipated into heat (Yamamoto et al., 2015). As a result of this ambiguity, at least four different ways of reporting US intensity can be found in the literature: (1) power capacity (W), (2) power over volume or weight (W/L, W/kg), (3) power over US emitting area (W/cm^2), and (4) total energy emitted per unit of volume (J/L) (Zupanc et al., 2019). Regarding microbial decontamination efficiency, the higher the US intensity, the greater the mechanical, thermal, and sonochemical effects, resulting in a higher inactivation of microorganisms (Marques-Silva & Sulaiman, 2017).

III. *Wave amplitude* is expressed in μm or %, according to the amplitude level selected in the US device used, and because it differs between types of equipment, this parameter has a limited value for comparison between different setups. However, the wave amplitude has a strong influence on the microbubble size. The larger the amplitude used, the larger the cavitation microbubbles, thus increasing the cavitation intensity and the microbial inactivation efficiency during the treatment (López-Malo et al., 1999; Manson, 1990). Nevertheless, it is crucial to consider that using high amplitudes over a long time may cause damage to the US equipment and possibly release eroded metal particles from the sonication probe to the treated medium (Guerrero et al., 2001).

IV. *Squeeze film* refers to the space, commonly expressed in cm or mm, separating the end of the sonication probe and the bottom of the reactor where the treatments occur, that is, the probe depth inside the reactor. This parameter's optimization is fundamental to guarantee a homogeneous distribution of the US intensity and, therefore, a homogeneous microbial inactivation efficiency throughout the treatment medium (Furuta et al., 2004).

V. *Temperature and pressure* are two main physical factors that can increase microbial inactivation when combined with the US. Thus, both parameters and their influence on decontamination will be discussed later in this manuscript.

2.3.2 | Product factors

Regarding the assurance of the effectiveness of the treatments, it is crucial to consider variables related to the composition and physical properties of the processed product. The most important product properties include viscosity, water activity (a_w), food composition (like the presence of suspended solid particles), pH value, dissolved

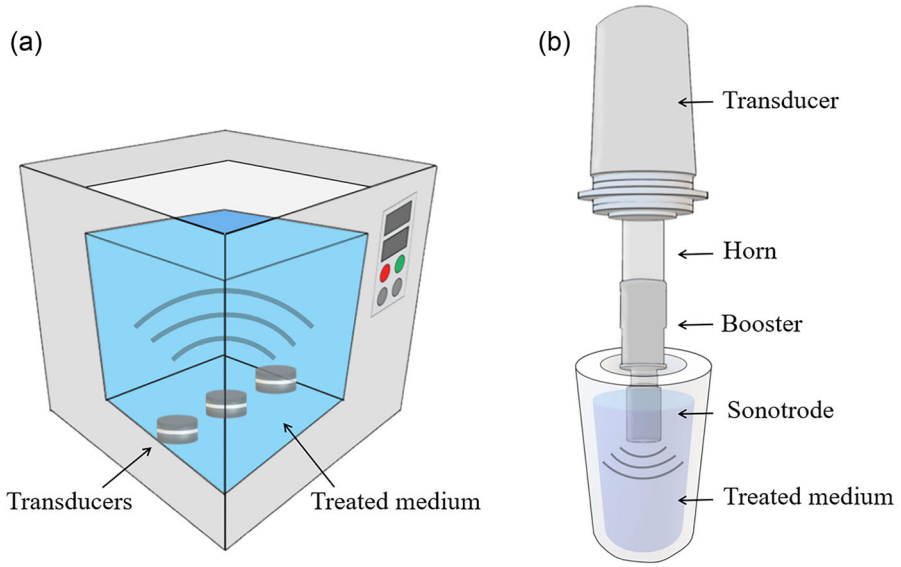

FIGURE 2 Ultrasound application systems: (A) ultrasonic bath; (B) probe system.

gas, and the volume of the product to be processed. In general, microbial tolerance against the US is reported to be higher in real food products compared with culture media and model foods due to the protective effects of specific food components, such as fat content in dairy products or sugar and acid concentration in fruit juices (Bermúdez-Aguirre et al., 2009; Chemat et al., 2011; Guerrero et al., 2001; Huang et al., 2006; Jacobs & Thornley, 1954; Raso et al., 1998; Valero et al., 2007).

2.3.3 | Microbiological factors

Besides processing and product parameters, some bacterial features may also influence the effectiveness of the inactivation by US treatment. First, the difference between the monoderm (Gram-positive) and diderm (Gram-negative) bacteria plays an important role. The latter are generally more tolerant against the US technology due to the thicker cell wall and the more tightly adherent peptidoglycan layer in this group. As a result, the outer membrane may be considered the primary target for the inactivation of diderm bacteria, whereas the cytoplasmic membrane and internal cell structure might be the target for inactivation in the case of monoderm bacteria (Iorio et al., 2019; Li et al., 2016). Furthermore, the cell size and shape might also influence the inactivation, with more significant bacteria being more sensitive to the US. Also, rod-shaped bacteria are more sensitive than coccoid forms (Jacobs & Thornley, 1954). Likewise, aerobic bacteria are more resilient to being inactivated by the US than anaerobic species, and as expected, the bacterial spore formers exhibit higher tolerance than vegetative cells (Ahmed & Russell, 1975).

Taking all of the above mentioned into account and because US technology is not a thoroughly investigated and standardized technology yet, there are still certain aspects related to the inactivation of microorganisms by the US, that is, safety and food quality properties following the US treatments.

3 | US AND COMBINATIONS WITH TEMPERATURE AND/OR PRESSURE FOR INACTIVATION OF MICROORGANISMS IN CULTURE MEDIA AND FOOD

One of the first studies suggesting the application of the US as a technology for the inactivation of microorganisms is dated 1928 when Harvey and Loomis investigated the bactericidal effects of ultrasonic waves in a test tube (Harvey & Loomis, 1929). Despite exhibiting that the treatment achieved lysis of bacteria and/or severe retardation of growth, there was no follow-up research at that time, most likely because the treatment was considered too expensive (Bermúdez-Aguirre, 2017). However, in the 1960s, this technology was investigated more intensively after discovering that the acoustic waves used in submarine warfare could kill fish (Earnshaw et al., 1995). However, only after the 1990s, US technology was widely studied as a non-thermal technology with high potential. For this reason, and apart from microbial inactivation, a broad range of application areas for the US were considered, such as filtration, freezing, crystallization, thawing, drying, foaming, degassing, mixing, tenderization, cooking, emulsification, cutting, extraction, rehydration, homogenization, separation, or microbial growth and fermentation (Guimarães et al., 2021; Singla & Sit, 2021; Soria & Villamiel, 2010). The main reason for the intensification of such research lies in developing powerful, efficient, and more durable transducers (Ugarte-Romero et al., 2006).

During the last 30 years, significant scientific progress has been made in describing the mechanisms of cell inactivation induced and/or assisted by the US. This has led to the investigation of the US with other preservation methods to overcome some drawbacks of applying the technology alone, thus improving microbial inactivation while minimizing food quality loss (Bermúdez-Aguirre, 2017). Table 1 summarizes different possibilities of US-combined strategies investigated so far.

However, food US-assisted decontamination is currently not available at a commercialized industrial level. Some of the reasons probably lie in the lack of scientific documentation and comprehensive understanding of the basics of the technology, that is, the cavitation phenomenon and its possible side effects, lack of expertise and awareness of the potential users, and the reluctance to abandon conventional treatments (Singla & Sit, 2021). In contrast, according to some surveys and market studies, small-medium enterprises are willing to introduce new technologies in their food production to increase competitiveness and efficiency, allowing them to access broader markets (Chemat et al., 2011; Sango et al., 2014).

Therefore, this review aims to gather and discuss studies performed on US and microbial inactivation, including the two most commonly combined industrially viable physical approaches, that is, mild heating temperatures (40–70°C) and/or treatments at elevated pressure. Additionally, the mathematical modeling approaches to describe microbial inactivation kinetics are also outlined. Hereafter, the technology's benefits and drawbacks, the need for further optimization, future research, and potential up-scaling strategies are presented. In this sense, the inactivation of bacterial spore formers and biofilms is not included in this manuscript because recent extensive reviews in these fields have been summarized in the work of Evelyn & Silva (2020) and Yu et al. (2020).

TABLE 1 US-based combination strategies for microbial food decontamination as appearing in the literature and representative examples

Strategy combined with ultrasound		References
Mild temperatures, thermosonication (TS)		Bermúdez-Aguirre et al., 2009
Elevated pressure, manosonication (MS)		Guzel et al., 2014
Mild temperatures + elevated pressure, manothermosonication (MTS)		Mañas et al., 2000b
High-pressure processing (HPP)		Song et al., 2021a
Pulsed electric fields (PEF)		Gomez-Gomez et al., 2021a
Nonthermal plasma (NTP)		Liao et al., 2018a
Ultraviolet light radiation (UV)		Anjaly et al., 2022
High-intensity light pulses (HILP)		Ferrario & Guerrero, 2016
Gamma irradiation (GI)		Shi et al., 2022
Ohmic heating (OH)		Abedelmaksoud et al., 2019
Microwave (MW)		Kernou et al., 2021
Supercritical carbon dioxide (SC-CO_2)		Gomez-Gomez et al., 2021b
Microfiltration (MF)		Zhang et al., 2021b
Ozone, sonozonation		Aday & Caner, 2014
Osmotic pressure, osmosonication		Wong et al., 2012
Steam, sonosteam		Musavian et al., 2014
Photosensitizers, sonophotodynamic		Bhavya & Hebbar, 2019
Antimicrobial substances	Natural antimicrobials, e.g.:	
	- ε-Polylysine	Fan et al., 2019b
	- Nisin	Song et al., 2021b
	Sanitizers, e.g.:	
	- Peroxyacetic acid	Joo et al., 2020
	- Slightly acid electrolyzed water	Guo et al., 2021
	Organic acids, e.g.:	
	- Citral nanoemulsion	Yang et al., 2023a
	- Thyme essential oil	He et al., 2021
	Enzymatic solutions, e.g.:	
	- Lysozyme	Bi et al., 2020
	Surfactants, e.g.:	
	- Tween 20	Sagong et al., 2013
Other combinations	MS + cysteamine	Raso et al., 1998
	MTS + cysteamine	Raso et al., 1998
	TS + PEF + nisin	Muñoz et al., 2012
	MTS + PEF	Palgan et al., 2012
	TS + PEF	Halpin et al., 2014
	TS + UV	Tremarin et al., 2017
	TS + gallic acid	Adiamo et al., 2017
	TS + nisin	Zhao et al., 2021
	TS + HPP	Chen et al., 2021
	TS + slightly acid electrolyzed water	Li et al., 2021
	TS + ascorbic acid	Park et al., 2021

3.1 | Inactivation of microorganisms by US

The application of US alone for microbial inactivation in food has been widely studied in the last 30 years, and an overview of the studies is presented in Table 2. As can be seen from the table, several vegetative bacterial species, yeasts, molds, and viruses have been investigated in different culture media and food products, showing them grouped into categories to facilitate comparison between matrices with similar features, for example, the acidity level in liquid food products.

Generally, when new technologies for microbial inactivation are investigated, initial studies in culture media and model foods are performed, which are later transferred to real food products and matrices. Usually, as in many other inactivation technologies, higher bacterial tolerance is seen in real food products compared with model foods and culture media. For instance, the tolerance of *Salmonella* Enteritidis against US (13 mm-diameter probe, 20 kHz, 750 W, 80%, 30 s-on and 30 s-off pulsed mode) in phosphate-buffered saline (PBS) compared with liquid whole egg was lower in culture media, achieving a microbial reduction of 3.6 Log_{10} CFU/mL and a change in cell concentration of 0.1 Log_{10} CFU/mL, respectively, after 10 min of US (≤20°C) (Techathuvanan & D'Souza, 2018). Indeed, the inactivation of bacteria and the inactivation of viruses show similar trends (Su et al., 2010). This fact can probably be related to the food matrix's possible protective effect (Bermúdez-Aguirre & Barbosa-Cánovas, 2008; Char et al., 2010; Techathuvanan & D'Souza, 2018). Therefore, inactivation levels achieved with the US below 0.5 Log_{10} CFU/mL or CFU/g cannot be considered "microbial reduction" when extrapolating from what is generally accepted in the scientific community as an increase in the microbial concentration (ISO, 2019; NACMCF, 2010).

Some authors consider microbial inactivation assisted by the US as an "all-or-nothing" phenomenon, which means that, generally, after applying an efficient treatment, the reviving of ruptured and disintegrated monoderm cells, such as *Staphylococcus aureus* (Li et al., 2016; Liao et al., 2018b) or *Listeria monocytogenes*, and diderm cells, like *Escherichia coli* (Gera & Doores, 2011; Li et al., 2016, 2018; Liao et al., 2018b; Wang et al., 2018) is impossible. This means the absence of subpopulations having any sublethal injuries which may have the chance of recovering after US treatments (Gera & Doores, 2011). However, this assumption has been challenged, for example, after confirming the presence of sublethal cell injuries following US treatment in experiments conducted in vitro, for example, in phosphate buffer (pH 7.0) with *E. coli* and *Lactobacillus rhamnosus* (Ananta et al., 2005) as well as in situ, for example, in almond milk, contaminated with *E. coli* O157:H7 and *L. monocytogenes* (Iorio et al., 2019).

Furthermore, some bacterial species have shown tolerance to the US. For example, the US's inactivation of *S. aureus* in whole raw milk, orange juice, and phosphate buffer (pH 7.0) was much lower than other microorganisms investigated. As a result, a reduction of the cell counts of *S. aureus* by 0.6 Log_{10} CFU/mL (22 mm-diameter probe, 24 kHz, 400 W, 100%, continuous mode), 0.2 Log_{10} CFU/mL (13 mm-diameter probe, 20 kHz, 700 W, 30 μm, 5 s-on and 10 s-off pulsed mode), and 0.3 Log_{10} CFU/mL (13 mm-diameter probe, 20 kHz, 800 W, 114 μm, 3 s-on and 2 s-off pulsed mode) were achieved, respectively, depending on the US setup (Bhavya & Hebbar, 2019; Chantapakul et al., 2019; Marchesini et al., 2015). Likewise, Baumann et al. (2005) compared the US tolerance (13 mm-diameter probe, 20 kHz, 750 W, 0.46 W/mL, 100%, continuous mode) of eight strains of *L. monocytogenes* of different origins, such as human, animal, food, or their mutations, in saline solution. From this study, *L. monocytogenes* 10403S, isolated from mice, was found to be the most tolerant strain against the technology.

3.1.1 | Inactivation in high-acid liquid foods

The US application can be regarded as included in a strategy to extend the shelf-life of high-acid fruit juices by delaying the growth of bacteria, yeasts, and molds throughout the storage time. The mesophilic aerobic bacteria (MAB) and yeast and molds (YM) are important in these products, and their development has been investigated after the US processing of the juice and throughout storage. For instance, Gómez-López et al. (2010) evaluated the inactivation of MAB and YM in calcium-added orange juice by the US (13 mm-diameter probe, 20 kHz, 500 W, 89.25 μm, continuous mode). After 8 min of treatment at 10°C, reductions of 1.4 and 0.6 Log_{10} CFU/mL were achieved, respectively, resulting in a shelf-life of 4°C up to 10 days, extended by 4 days compared with the untreated juice at the same temperature. Several examples of the preservation of fruit juices with the US can be found in the literature, as seen in Table 2. Recently, de Albuquerque et al. (2021) evaluated the potential preservation effect of the US in Brazilian nopal (*Opuntia ficus-indica*) beverage. Treatments (ultrasonic bath, 42 kHz, 240 W, 0.22 W/mL, continuous mode) were applied for 40 min (30°C) to assess the products' microbiological quality during 28 days of storage at 4°C. Counts of MAB, YM, coliforms, and *Salmonella* spp. were reported. As a result of US treatment, only YM were detected in the beverage and only on day 28 of storage, while the other microbial groups were under the detection limit at all times during storage.

TABLE 2 Review of microbial inactivation in culture media and food products assisted by ultrasound (US). Treatments were applied in a batch system if not otherwise specified

Food/model system	Microorganism	US operating conditions	Processing conditions	Microbial inactivation, in Log_{10} CFU/mL or Log_{10} CFU/g	References
Culture media and model foods					
Model apple juice	*Escherichia coli*	Probe (13 mm), 20 kHz, 750 W, 37.5 μm	<14 min, <30°C	3.0	Patil et al., 2009
Model orange juice				2.7	
Phosphate buffer (pH 7.0)	*Escherichia coli* K12	Probe (12.5 mm), 20 kHz, 6 W/mL, 124 μm	4 min, 40°C	4.0	Lee et al., 2009b
	Escherichia coli	Probe (13 mm), 20 kHz, 800 W, 114 μm	5 min, 30°C	2.0	Chantapakul et al., 2019
	Staphylococcus aureus			0.3	
Phosphate-buffered saline, PBS	Murine norovirus	Probe (13 mm), 20 kHz, 750 W, 80%	30 min, <21°C	6.0	Su et al., 2010
	Feline calicivirus		5 min, <21°C	6.0	
	Salmonella Enteritidis	Probe (13 mm), 20 kHz, 750 W, 80%	10 min, ≤20°C	3.6	Techathuvanan & D'Souza, 2018
	Escherichia coli O157:H7	Ultrasonic bath, 40 kHz, 700 W	9 min, room temperature	1.3	Park & Ha, 2019
	Salmonella Typhimurium			0.7	
	Listeria monocytogenes			0.6	
Saline solution	*Enterobacter aerogenes*	Probe (12.7 mm), 20 kHz, 13 W	13 min, ≤30°C	4.5	Gao et al., 2014
	Staphylococcus spp.			0.5	
Water					
Water	*Escherichia coli*	Ultrasonic bath, 42 kHz, 70 W	>60 min	>2	Dehghani, 2005
High-acid liquid foods					
Apple juice	*Alicyclobacillus acidoterrestris*	Probe (6 mm), 25 kHz, 600 W, 1.2 W/L	30 min, <50°C	4.6	Wang et al., 2010
	Alicyclobacillus acidiphilus			4.3	

(Continues)

TABLE 2 (Continued)

High-acid liquid foods					
	MAB[a]	Ultrasonic bath, 25 kHz, 2 W/cm^2	60 min, 20°C	1.0	Abid et al., 2013
	YM[b]			1.0	
	Escherichia coli O157:H7	Ultrasonic bath, 40 kHz, 700 W	5 min, room temperature	0.8	Park & Ha, 2019
	Salmonella Typhimurium			0.9	
	Listeria monocytogenes			1.1	
Barberry juice	MAB	Probe (13 mm), 20 kHz, 140 W, 70%	15 min, 25°C	3.7	Farhadi Chitgar et al., 2017
	YM			3.3	
	Escherichia coli	Probe, 30 kHz, 100 W, 100%	20 min, <25°C	6.0	Hashemi & Roohi, 2021
	Salmonella Typhi			5.0	
	Bacillus cereus			4.0	
	Enterococcus faecalis			4.9	
Blackberry juice	MAB	Probe, 20 kHz, 1500 W, 80%	25 min	2.9	Ramírez-Moreno et al., 2018
	Enterobacteriaceae			0.1	
Blueberry juice	MAB	Probe (10 mm), 20 kHz, 500 W, 100%	Continuous treatment (93.5 mL/min), 25°C	1.4	Mohideen et al., 2015
	Coliforms			1.3	
	YM			1.2	
Calcium-added orange juice	MAB	Probe (13 mm), 20 kHz, 500 W, 89.25 μm	8 min, 10°C	1.4	Gómez-López et al., 2010
	YM			0.6	
Carrot juice	MAB	Ultrasonic bath, 40 kHz, 0.5 W/cm^2	60 min	1.0	Zou & Jiang, 2016
	YM			0.9	

(Continues)

TABLE 2 (Continued)

High-acid liquid foods					
Grapefruit juice	MAB	Ultrasonic bath, 28 kHz, 600 W	30 min, 20°C	0.5	Aadil et al., 2018
	YM			0.5	
Kasturi lime fruit juice	MAB	Ultrasonic bath, 25 kHz, 392 W	60 min, 20°C	1.9	Bhat et al., 2011
	YM			0.5	
Mango juice	MAB	Ultrasonic bath, 40 kHz, 130 W	60 min, 25°C	<0.3	Santhirasegaram et al., 2013
	YM			<0.2	
Orange juice	Murine norovirus	Probe (13 mm), 20 kHz, 750 W, 80%	30 min, <21°C	1.6	Su et al., 2010
	Feline calicivirus		15 min, <21°C	4.0	
	Escherichia coli	Probe (13 mm), 20 kHz, 700 W, 30 μm	5 min, <35°C	3.0	Bhavya & Hebbar, 2019
	Staphylococcus aureus			0.2	
Orange and celery juice blend	MAB	Ultrasonic bath, 20 kHz, 5.57 kW/m^3	90 min, ≤25°C	0.4	Ruiz-De Anda et al., 2019
	YM			0.3	
Passion fruit juice	MAB	Probe (13 mm), 20 kHz, 263 W, 89.25 μm	8 min, 10°C	1.4	Gómez-López et al., 2017
	YM			0.7	
Pear juice	MAB	Ultrasonic bath, 25 kHz, 500 W, 2 W/cm^2	60 min, 25°C	2.0	Saeeduddin et al., 2016
	YM			1.1	
Pomegranate juice	*Escherichia coli*	Probe (19 mm), 20 kHz, 500 W, 100%	30 min, 35°C	>5.0	Pala et al., 2015
	Saccharomyces cerevisiae			1.4	

(Continues)

TABLE 2 (Continued)

High-acid liquid foods					
Prebiotic soursop whey beverage	MAB	Probe (13 mm), 20 kHz, 600 W	3 min, <53°C	2.0	Guimarães et al., 2018
	YM			0.2	
Strawberry juice	MAB	Ultrasonic bath, 25 kHz, 140 W	30 min, 20°C	0.2	Bhat & Goh, 2017
	YM			0.1	
	MAB	Probe (10 mm), 20 kHz, 968 W/cm^2	10 min, <25°C	2.1	Chen et al., 2018
	YM			1.1	
	Escherichia coli O157:H7	Probe (12.5 mm), 20 kHz, 750 W, 100%	5 min, room temperature	5.0	Yildiz & Aadil, 2020
Sweet lemon juice	*Salmonella* Typhi	Probe (10 mm), 30 kHz, 100 W	8 min, temperature control system	1.4	Hashemi & Jafarpour, 2020
	Shigella dysenteriae			1.9	
	Listeria monocytogenes			2.5	
	Shigella flexneri			2.6	
Tomato juice	*Pichia fermentans*	Probe (19 mm), 20 kHz, 1500 W, 61 μm	7.5 min, <45°C	5.0	Adekunte et al., 2010a
Low-acid liquid foods					
Almond milk	*Escherichia coli* O157:H7	Probe (5 mm), 20 kHz, 104 W	8 min, <40°C	1.3	Iorio et al., 2019
	Listeria monocytogenes		2 min, <40°C		
Camel milk	*Escherichia coli* O157:H7	Probe (13 mm), 20 kHz, 900 W	15 min, <23°C	6.0	Dhahir et al., 2020
	Salmonella Typhimurium			4.4	
Chocolate milk beverage	MAB	Probe (13 mm), 19 kHz, 400 W	<42°C	3.6	Monteiro et al., 2018

(Continues)

TABLE 2 (Continued)

Low-acid liquid foods					
Liquid whole egg	*Escherichia coli* K12	Probe, 20 kHz, 42 W	5 min, 5°C	2.0	Lee et al., 2003
	Salmonella Enteritidis	Probe (13 mm), 20 kHz, 750 W, 80%	10 min, ≤20°C	0.1	Techathuvanan & D'Souza, 2018
	Salmonella Typhimurium	Probe (10 mm), 968 W/cm^2	20 min, 35°C	3.3	Bi et al., 2020
Pasteurized retentate of ultrafiltered cow's milk	*Escherichia coli* O157:H7	Probe (13 mm), 60 kHz, 200 W, 80%	20 min	4.3	Jalilzadeh et al., 2018
	Staphylococcus aureus			2.0	
	Penicillium chrysogenum			1.1	
	Clostridium sporogenes			2.2	
Peanut milk	MAB	Probe (13 mm), 20 kHz, 400 W	3 min, <51°C	1.1	Salve et al., 2019
	YM			0.9	
Raw whole cow's milk	*Debaryomyces hansenii*	Probe (22 mm) 24 kHz, 400 W, 100%	5 min, ≤46°C	4.6	Marchesini et al., 2015
	Pseudomonas fluorescens			2.8	
	Escherichia coli			2.1	
	Staphylococcus aureus			0.6	
	MAB	Probe (13 mm), 19 kHz, 475 W	<35°C	3.9	Scudino et al., 2020
Rice beverage	*Salmonella enterica*	Probe (5 mm), 20 kHz, 130 W	10 min, <48°C	<DL of 1 Log_{10} CFU/mL[h]	Campaniello et al., 2018
Soybean milk	MAB	Probe (6.36 mm), 20 kHz, 400 W	9 min, 25°C	0.6	Mu et al., 2022
UHT whole cow's milk[i]	*Listeria monocytogenes*	Probe (13 mm), 20 kHz, 750 W, 124 μm	10 min, ≤26°C	2.0	Cameron et al., 2010

(Continues)

TABLE 2 (Continued)

Alcoholic beverages					
Chinese rice wine	*Saccharomyces cerevisiae*	Probe (12 mm), 20 kHz, 750 W	120 min, 35°C	0.8	Lyu et al., 2016
Wine	*Brettanomyces*	Probe (22 mm), 24 kHz, 400 W, 100 μm	Continuous treatment (1.0 L/min), 1 min (retention time), <40°C	<3.0	Gracin et al., 2016
	LAB[e]			>2.0	
Honey					
Raw honey	MAB	Probe (19 mm), 20 kHz, 750 W, 60%	8 min, <40°C	1.5	Janghu et al., 2017
	Coliforms			<DL	
	YM			<DL	
Fruits and vegetables					
Arugula	MAB	Ultrasonic bath, 40 kHz, 70 W	5 min, 25°C	0.4	Francisco et al., 2018
Blueberry fruits	*Listeria innocua*	Probe, 20 kHz, 500 W, 230.8 W/L	10 min, 20°C	2.0	Zhang et al., 2021a
Cherry tomato fruits	*Escherichia coli*	Ultrasonic bath, DFU (20-40 kHz) [f], 300 W	10 min, <25°C	>2.0	Mustapha et al., 2019
Fresh-cut cucumber	MAB	Probe (15 mm), 20 kHz, 226 W/cm^2	10 min, <22°C	1.0	Fan et al., 2019a
	YM			0.8	

(Continues)

TABLE 2 (Continued)

Fruits and vegetables					
Gulupa fruit	Yeast count	Ultrasonic bath, 40 kHz, 325 W	30 min, 5°C	2.1	Calderón-Martínez et al., 2021
Lettuce leaves	*Escherichia coli* O157:H7	Probe (14 mm), 26 kHz, 200 W, 90 μm	5 min, <45°C	2.7	Millan-Sango et al., 2015
	Salmonella enterica Abony	Probe (14 mm), 26 kHz, 200 W, 90 μm	10 min, <45°C	1.8	Millan-Sango et al., 2016
Strawberry fruits	MAB	Ultrasonic bath, 40 kHz, 350 W	10 min, 20°C	0.8	Cao et al., 2010
	YM			0.9	
Tomato fruits	BMY[c]	Ultrasonic bath, TFU (20-40-60 kHz)[g], 300 W, 120 W/L	20 min, <30°C	>1.5	Alenyorege et al., 2019
Fish and fishery products					
Cod	PMAB[d]	Ultrasonic bath, 30 kHz, 51.41 W/L	45 min, <14°C	0.5	Pedrós-Garrido et al., 2017
Hake				0.5	
Mackerel				1.1	
Salmon				1.5	

Microbial inactivation values are rounded up to the first decimal.
[a]MAB: total mesophilic aerobic bacteria.
[b]YM: yeast and molds.
[c]BMY: total bacteria, mold, and yeast.
[d]PMAB: psychrophilic and mesophilic aerobic bacteria.
[e]LAB: lactic acid bacteria.
[f]DFU: dual-frequency US.
[g]TFU: tri-frequency US.
[h]DL: detection limit.
[i]UHT: ultra-high-temperature.

However, US treatment has been considered for preserving fruit juices against spoilage and the inactivation of pathogenic bacteria. For example, the inactivation of *E. coli* O157:H7 was evaluated in strawberry juice, a commonly investigated fruit juice matrix. When US (12.5 mm-diameter probe, 20 kHz, 750 W, 100%, continuous mode) was applied for 5 min at room temperature, a reduction of 5.0 Log_{10} CFU/mL of the pathogenic microorganism was achieved (Yildiz & Aadil, 2020).

3.1.2 | Inactivation in low-acid liquid foods

The potential of US against microorganisms has also been studied in low-acid products, where the inactivation of pathogenic bacteria was more of interest when compared with high-acid products. For example, the survival of *S. enterica* in rice beverage after treatment with the US (5 mm-diameter probe, 20 kHz, 130 W, 2 s-on and 10 s-off pulsed mode) was evaluated during 13 days of storage at 4°C. After 10 min of treatment (<48°C), the cell counts of the microorganism were reduced below the detection limit (1.0 Log_{10} CFU/mL) and remained undetectable throughout the storage time (Campaniello et al., 2018). Likewise, the preservation of animal milk, like cow and camel, and vegetable beverages, for example, from rice or almond by use of US, have been investigated. As an example, Iorio et al. (2019) evaluated the survival and growth of *E. coli* O157:H7 and *L. monocytogenes* in almond milk after the US (5 mm-diameter probe, 20 kHz, 104 W, 6 s-on and 6 s-off pulsed mode) and during storage at 4°C for 14 days. After 8 and 2 min of treatment, both microorganisms achieved cell count reductions of 1.3 Log_{10} CFU/mL. Furthermore, in the case of *E. coli* O157:H7, the growth rate (μmax) was reduced from 1.2 to 0.8 Log_{10} CFU/mL/day compared with the untreated samples. On the contrary, *L. monocytogenes* remained potentially in a state of sublethally injury, exhibiting a lag phase for 4.9 days.

3.1.3 | Inactivation in fruits and vegetables

On the other hand, the efficiency of US treatments in food preservation has been evaluated in several fruits and vegetables by immersing the solid food product in a liquid medium, generally water, where the US is emitted. For instance, the inactivation of MAB and YM was assessed in strawberry fruits by Cao et al. (2010). The inactivation achieved with US (ultrasonic bath, 40 kHz, 350 W, continuous mode) after 10 min of treatment (20°C) was 0.8 and 0.9 Log_{10} CFU/g in the counts of MAB and YM, respectively, and resulted in a reduction of 43.7% of the decay index in comparison with the untreated samples after 8 days of storage at 5°C. Regarding the preservation potential in fresh-cut vegetables, Fan et al. (2019a) applied the US to extend the shelf-life of modified atmospheric fresh-cut cucumber. As a result, US (15 mm-diameter probe, 20 kHz, 226 W/cm^2, 10 s-on and 5 s-off pulsed mode) was applied to inhibit the growth of MAB and YM. As a result, treatments with a duration of 10 min (<22°C) achieved reductions up to 1.0 and 0.8 Log_{10} CFU/g in the counts of MAB and YM, respectively. This improved the microbiological quality of the treated samples compared with the untreated samples over a storage period of 15 days at 4°C.

3.1.4 | Inactivation in meat products

Moreover, the US has been studied as a technology for microbial decontamination in other solid food, such as meat products. For instance, Sams & Feria (1991) studied the inactivation of MAB in broiler drumstick skin by the US (ultrasonic bath, 47 kHz, 200 W, continuous mode) and growth over 14 days-storage times. Treatments up to 30 min (<40°C) did not lead to any significant inactivation effects, probably due to irregular skin surface, providing a physical shield to microorganisms. Indeed, the MAB cell counts in the US-treated samples on day 7 of storage were lower than the untreated samples, but the cell counts in both treated and untreated samples reached the same level at the end of the storage time (day 14). This could be explained due to increased extraction of nutrients from the food, leading to a faster rate of microbial growth in the products. In the same way, Piñon et al. (2019) evaluated the inactivation of total psychrophilic and mesophilic aerobic bacteria (PMAB) and lactic acid bacteria (LAB) in chicken meat. US treatments (probe, 20 kHz, 27.6 W/cm^2, continuous mode) applied for 5 min at 4°C led to the inhibition of the microbial growth in samples during 7 days of storage at 4°C.

3.1.5 | General statements on US

Overall, studies that evaluated the influence of US processing parameters during microbial inactivation found that higher intensity, as well as lengthier treatment times and amplitude levels, lead to higher inactivation rates of bacteria (Campaniello et al., 2018; Margean et al., 2020; Patil et al., 2009; Scherba et al., 1991; Starek et al., 2021; Türken & Erge, 2017; Wang et al., 2010). In addition, in the case of continuous treatments, faster flow rates are considered more efficient in inactivating microorganisms (Gracin et al., 2016; Valero et al., 2007; Van Hekken et al., 2019).

An essential consideration of US processing is the increased sample temperature recorded because of the

nature of the treatment. For instance, US treatments applied in fruit and vegetable juices increased temperature by around 50°C during 15 min of treatment (13 mm-diameter probe, 20 kHz, 100 W, 70%, continuous mode) (Margean et al., 2020). It is easily understood that controlling and monitoring the temperature during the treatment is crucial to evaluate the microbial inactivation effect of US and separate its impact on the nutritional, organoleptic, and sensorial food properties from that of heat. On the other hand, studies in the presence or absence of the temperature effects concluded that higher microbial lethal effects were seen when temperature increased during the US treatments (Türken & Erge, 2017).

Generally, effective microbial inactivation that will ensure microbial food safety requires long and high-intensity treatment, often associated with increased energy demands and a higher risk for more significant changes in the treated food's chemical, nutritional, and sensory properties. This is probably one of the reasons that hindered a possible introduction of the US to any industrial and commercial scale application of food decontamination (Chen et al., 2020; Lee et al., 2013; Sala et al., 1995). However, there are other food applications, such as extraction, emulsification, or homogenization, where the US became an established technology for large-scale commercial applications. Furthermore, scale-up applications on improving system designs for the higher efficiency of large-scale continuous flow systems for each application have been reported (Patist & Bates, 2008). In addition, certain pathogenic microorganisms show tolerance against US treatment alone, such as *S. aureus* species and some *L. monocytogenes* strains (Baumann et al., 2005; Bhavya & Hebbar, 2019; Chantapakul et al., 2019; Marchesini et al., 2015). For this reason, some authors recommended that the US should be combined with other hurdle strategies with greater antimicrobial potency, such as mild heating temperatures, treatments at elevated pressure, use of antimicrobial substances, or other emerging technologies to obtain better inactivation results (Valero et al., 2007; Wang et al., 2015). Indeed, the majority of the studies presented in Table 2 are concerned with the inactivation of spoilage microorganisms rather than with pathogenic microorganisms, being clear that there is a lack of studies on the inactivation of pathogenic microorganisms with the US. One of the reasons may be potential safety issues associated with the high inocula with pathogenic microorganisms needed in the setup of such experiments.

Consequently, research in microbial food decontamination with the US has focused on further increasing its efficacy through its application in combination with other decontamination methods. In this sense, available literature reported higher microbial inactivation levels with lower processing times when combining US with mild heating temperatures and/or treatments at elevated pressure. Moreover, the combination of US with antimicrobials solutions (Francisco et al., 2018; Millan-Sango et al., 2015, 2016; Zhang et al., 2021a) and with other emerging technologies, for example, pulsed electric fields or blue light treatment (Bhavya & Hebbar, 2019; Lyu et al., 2016; Saeeduddin et al., 2017) has also been studied, but these combinations are out of scope of this review.

3.2 | Inactivation of microorganisms by thermosonication

The limited potential of the US to inactivate microorganisms at low temperatures (<40°C) seemed to have motivated scientists to investigate possible combinations of US with other inactivation approaches, such as heat treatments at mild temperatures (40–70°C) and atmospheric pressure, a treatment known as thermosonication (TS).

3.2.1 | Mechanism of action and beginning of TS

During TS treatments, a reduction of surface tension and viscosity of the medium occurs due to the temperature increase (López-Malo et al., 1999). Thus, a higher vapor pressure is required in the medium to achieve the formation of microbubbles. As a result, a large number of smaller microbubbles are generated in TS treatments. Because of that, the released energy during the microbubble collapse is reduced, and the cavitation intensity decreases accordingly (Guerrero et al., 2001; Mason, 1998; Patist & Bates, 2008). However, the tolerance of microorganisms to the US seems to be also reduced due to the sum of the damaging effects of cavitation and mild heat (Raso et al., 1998). In comparison with the conventional thermal processes, temperatures applied during TS are significantly reduced (by 16–55%) while achieving the same lethality values (Das et al., 2020) and maintaining food quality (Piyasena et al., 2003).

An optimum temperature range for TS has been considered the one at which the viscosity of the medium is low enough to generate a violent enough microbubble collapse, which, in turn, allows for a reasonable microbial inactivation (Patist & Bates, 2008). Most studies have reported enhanced microbial inactivation when temperatures above 50°C are combined with the US (Bermúdez-Aguirre, 2017; Dubrović et al., 2011). However, it has also been reported that when temperature increases higher than the optimum temperature, the combined effect is reduced, resulting in no differences in the lethality between TS and the heat-based treatment alone at

the same temperature (Ugarte-Romero et al., 2006, 2007; Zenker et al., 2003). This critical temperature value is referred to as *upper temperature limit* and differs depending on the target microorganisms and the treated media (Ugarte-Romero et al., 2006). Table 3 gives an overview regarding some of the upper temperature limits found in the literature for TS treatments.

The first reports on combining US and mild heat were first released in the 1980s, when US at 20 kHz, 160 W was used in assistance to thermal treatments (≤62°C) for inactivation of *S. aureus* in acid buffer media (pH 6.6) (Ordoñez et al., 1984). The obtained results suggested that the combination of both treatments was much more effective in terms of inactivation regarding the treatment time and energy applied, compared with both treatments used individually. Since then, a significant number of studies have been done regarding microbial inactivation by TS, as summarized in Table 4. As can be seen from the studies, the inactivation of microorganisms by TS in different food products, such as milk, apple cider, or fruit juices, has been reported. In addition, TS effectiveness on microbial inactivation was frequently compared with inactivation results obtained with US and thermal treatment alone.

3.2.2 | Inactivation in high-acid liquid foods

Decontamination by TS of apple products, such as apple juice or apple cider, may be the most investigated area when treating high-acid food products with TS. Baumann et al. (2005) studied the effects of TS treatments (13 mm-diameter probe, 20 kHz, 750 W, 0.46 W/mL, 100%, continuous mode) on the inactivation of *L. monocytogenes* in apple cider and reported an improved microbial inactivation by using TS, but also attributed part of the inactivation to the low pH value of the product. An additional 5.0 Log_{10} CFU/mL reduction of *L. monocytogenes* was achieved after 6 h of the TS-treated apple cider storage at 20°C. These results indicate that TS might cause sublethal injuries, as seen in other studies (Anaya-Esparza et al., 2017). In another study with apple cider, where TS was compared with heat treatment to inactivate *E. coli* K12, only applying thermal treatment at 40°C for 20 min resulted in no microbial inactivation (Ugarte-Romero et al., 2006). However, when TS (probe, 20 kHz, 0.46 W/mL, continuous mode) was applied at the same conditions, a reduction of 5.3 Log_{10} CFU/mL of the same microorganisms was achieved. This study set the upper temperature limit at 40°C, obtaining a lower microbial inactivation (5.1 Log_{10} CFU/mL) with TS at 60°C during the same processing time (20 min). Nevertheless, in work conducted by Baumann et al. (2005), the higher inactivation of *L. monocytogenes* in apple cider

TABLE 3 Overview of upper temperature limit values found in different matrices and microorganisms when applying TS

Food/model system	Microorganism	US operating conditions	Temperature ranges	Upper temperature limit	References
Culture media					
Phosphate buffer	*Escherichia coli* K12	Probe, 19.3 kHz, 800 W, 55 μm	48–67°C	60°C	Zenker et al., 2003
Sabouroud broth (pH 3.0)	*Saccharomyces cerevisiae*	Probe (13 mm), 20 kHz, 600 W, 83.3 μm	35, 45, and 55°C	45°C	Guerrero et al., 2001
High-acid liquid foods					
Apple cider	*Escherichia coli* K12	Probe, 20 kHz, 0.46 W/mL	40–60°C	40°C	Ugarte-Romero et al., 2006
Orange juice	*Escherichia coli* K12	Probe, 19.3 kHz, 800 W, 55 μm	48–67°C	60°C	Zenker et al., 2003
Low-acid liquid foods					
UHT whole goat's milk	*Escherichia coli* O157:H7	Ultrasonic bath, 40 kHz, 54.4 W, 1.9 kJ/mL	45.9–74.1°C	70°C	Bernardo et al., 2022

TABLE 4 Review of microbial inactivation in culture media and food products assisted by thermosonication (TS). Treatments were applied in a batch system if not otherwise specified

Food/model system	Microorganism	US operating conditions	Processing conditions	Microbial inactivation, in Log_{10} CFU/mL or Log_{10} CFU/g	References
Culture media					
Bacterial suspension	*Shigella boydii*	22.3 kHz, 1.43 W/mL	10 min, 40°C	5.0	Ugarte-Romero et al., 2007
	Listeria monocytogenes		2.5 min, 65°C		
Brain heart infusion broth	*Salmonella* Typhimurium	Ultrasonic bath, 40 kHz	30 min, 40°C	3.0	Wrigley & Llorca, 1992
Peptone water	*Escherichia coli*	Probe (13 mm), 20 kHz, 600 W, 95.2 μm	Continuous treatment (0.2L/min), 20 min, 40°C	2.0	Char et al., 2010
		Probe (13 mm), 20 kHz, 45%	0.38 min, 60°C	5.0	Baboli et al., 2020a
	Staphylococcus aureus	Probe (13 mm), 20 kHz, 50%	0.5 min, 64°C		
Phosphate buffer (pH 7.0)	*Escherichia coli* K12	Probe, 19.3 kHz, 800 W, 55 μm	Continuous treatment (26 L/h), 60°C	3.8	Zenker et al., 2003
		Probe (12.5 mm), 20 kHz, 6 W/mL, 124 μm	0.5 min, 61°C	5.0	Lee et al., 2009b
	Escherichia coli	Probe (13 mm), 20 kHz, 800 W, 114 μm	5 min, 50°C	3.5	Chantapakul et al., 2019
	Staphylococcus aureus			1.3	
High-acid liquid foods					
Apple cider	*Escherichia coli* K12	Probe, 20 kHz, 0.46 W/mL	20 min, 40°C	5.3	Ugarte-Romero et al., 2006
		Probe (12.5 mm), 20 kHz, 6 W/mL, 124 μm	3.8 min, 59°C	5.0	Lee et al., 2013
	Listeria monocytogenes	Probe (13 mm), 20 kHz, 750 W, 0.46 W/mL, 100%	5 min, 60°C	5.0	Baumann et al., 2005

(Continues)

TABLE 4 (Continued)

High-acid liquid foods					
	Escherichia coli O157:H7	Probe, 20 kHz, 150 W, 118 W/cm^2	Continuous treatment (50 mL/min), 18 min, 57°C	5.1	D'Amico et al., 2006
Apple juice	*Escherichia coli*	Probe (13 mm), 20 kHz, 600 W, 95.2 μm	Continuous treatment (0.2 L/ min), 20 min, 40°C	1.0	Char et al., 2010
		Probe (13 mm), 20 kHz, 18.3 W/mL, 45%	0.6 min, 60°C	5.0	Baboli et al., 2020b
	Staphylococcus aureus	Probe (13 mm), 20 kHz, 18.3 W/mL, 50%	0.5 min, 62°C	5.0	
	MAB[a]	Probe, 20 kHz, 525 W, 0.30 W/cm^2	10 min, 40°C	1.8	Abid et al., 2014
	YM[b]			1.5	
	MAB	Probe (15 mm), 525 W	12 min, 60°C	4.0	Shen et al., 2021
	YM			3.8	
	Saccharomyces cerevisiae	Probe (12.7 mm), 20 kHz, 600 W, 120 μm	9 min, 60°C	5.2	Režek Jambrak et al., 2018
	Aspergillus ochraceus			3.9	
	Penicillium expansum	Probe (12.7 mm), 20 kHz, 600 W, 60 μm	6 min, 60°C	4.3	
	Rhodotorula spp.			5.2	
Beetroot juice	MAB	Probe (25 mm), 20 kHz, 1500 W, 85%	13 min, 50°C	3.1	Ramírez-Melo et al., 2022
	Enterobacteriaceae	Probe (25 mm), 20 kHz, 1500 W, 80%	10 min, 50°C	4.3	
Black mulberry juice	*Escherichia coli*	Probe (13 mm), 20 kHz, 750 W, 100%	10.5 min, 50°C	5.1	Dinçer & Topuz, 2015
Chinese bayberry juice	*Bacillus subtilis*	Probe (10 mm), 20 kHz, 400 W	9.6 min, 63°C	5.0	Li et al., 2019
Cranberry juice	*Saccharomyces cerevisiae*	Probe (22 mm), 24 kHz, 400 W, 120 μm	10 min, 60°C	>7.0	Bermúdez-Aguirre & Barbosa-Cánovas, 2012

(Continues)

TABLE 4 (Continued)

High-acid liquid foods					
Grape juice	*Saccharomyces cerevisiae*	Probe (22 mm), 24 kHz, 400 W, 120 μm	10 min, 60°C	>5.0	Bermúdez-Aguirre and Barbosa-Cánovas, 2012
Mango juice	*Escherichia coli* O157:H7	Ultrasonic bath, 25 kHz, 200 W	7 min, 60°C	5.0	Kiang et al., 2013
Orange juice	*Escherichia coli* K12	Probe, 19.3 kHz, 800 W, 55 μm	Continuous treatment (26 L/h), 60°C	2.5	Zenker et al., 2003
	Escherichia coli	Probe (13 mm), 20 kHz, 600 W, 95.2 μm	Continuous treatment (0.2 L/min), 20 min, 40°C	1.9	Char et al., 2010
	Staphylococcus aureus	Ultrasonic bath, 30 kHz, 100 W	20 min, 55°C	3.3	Walkling-Ribeiro et al., 2009
Orange juice whey drink	MAB	Probe (10 mm), 20 kHz, 520 W	70°C	1.7	Oliveira et al., 2022
	YM			1.8	
	LAB[c]			2.6	
Pear juice	MAB	Probe (12.7 mm), 20 kHz, 750 W, 70%	10 min, 65°C	4.0	Saeeduddin et al., 2015
	YM			3.4	
Pineapple juice	*Saccharomyces cerevisiae*	Probe (22 mm), 24 kHz, 400 W, 120 μm	10 min, 60°C	>6.0	Bermúdez-Aguirre & Barbosa-Cánovas, 2012
	MAB	Probe (13 mm), 24 kHz, 200 W, 35%	2 min, 62°C	2.0	Mala et al., 2021
	YM			2.2	
Pumpkin juice	*Escherichia coli* K12	Ultrasonic bath, 37 kHz, 150 W	Batch (30 min), 60°C	6.6	Demir & Kılınç, 2019
			Continuous treatment (0.029 L/min), 2.87 min (retention time), 60°C	6.2	
Soursop nectar	*Escherichia coli* O157:H7 *Staphylococcus aureus*	Probe, 24 kHz, 400 W, 1.4 W/mL	10 min, 51°C	4.5-5.0	Anaya-Esparza et al., 2017

(Continues)

TABLE 4 (Continued)

High-acid liquid foods					
Watermelon rind-honey beverage	MAB	Ultrasonic bath, 40 kHz, 150 W	60 min, 65°C	2.6	Hussain et al., 2019
	YM			2.4	
Low-acid liquid foods					
Fruit smoothie (mango, jackfruit, and rice milk)	MAB	Probe (25 mm), 20 kHz, 1500 W, 70%	20 min, 40°C	3.2	Amador-Espejo et al., 2020
	Enterobacteriaceae			2.9	
Liquid whole egg	*Salmonella* Enteritidis	Probe (3.2 mm), 20 kHz, 40 W	5 min, 55°C	2.3	Huang et al., 2006
	Salmonella Typhimurium	Ultrasonic bath, 40 kHz	30 min, 50°C	>2.0	Wrigley & Llorca, 1992
Powdered infant formula	*Cronobacter sakazakii*	Probe (19 mm), 20 kHz, 1500 W, 61 μm	2.5 min, 50°C	7.0	Adekunte et al., 2010b
Raw human milk	*Escherichia coli*	Ultrasonic bath, 40 kHz, 100 W, 14.69 mW/mL	4 min, 65°C	6.0	Parreiras et al., 2020
	Salmonella spp.				
	Staphylococcus aureus				
Raw whole cow's milk	*Listeria innocua*	Probe (22 mm), 24 kHz, 400 W, 2.85 W/cm^2, 120 μm	10 min, 63°C	5.0	Bermúdez-Aguirre et al., 2009, 2011
	Escherichia coli	Probe (12 mm), 20 kHz, 600 W, 54.59 W/cm^2, 120 μm	12 min, 60°C	3.1	Herceg et al., 2012
	Staphylococcus aureus			1.5	
	MAB	Probe, 20 kHz, 150 W, 118 W/cm^2	Continuous treatment (50 mL/min), 18 min, 57°C	4.8	D'Amico et al., 2006
Reconstituted skim cow's milk powder (31.5% total solids)	*Geobacillus stearothermophilus*	Probe (3.2 mm), 20 kHz, 600 W, 240 μm	0.3 min, 67.5°C	4.8	Beatty & Walsh, 2016
Sterilized human milk	*Staphylococcus aureus*	Ultrasonic bath, 40 kHz, 110 W	10 min, 60°C	4.6	Gomes et al., 2022

(Continues)

TABLE 4 (Continued)

Low-acid liquid foods					
UHT fat free cow's milk[d]	*Listeria innocua*	Probe (22 mm), 24 kHz, 400 W, 120 μm	30 min, 63°C	4.9	Bermúdez-Aguirre & Barbosa-Cánovas, 2008
UHT low fat cow's milk	*Listeria innocua*	Probe (22 mm), 24 kHz, 400 W, 85 W/cm^2, 117 μm	1.3 min, 55°C	1.2	Noci et al., 2009
UHT skim cow's milk	*Salmonella* Typhimurium	Ultrasonic bath, 40 kHz	30 min, 50°C	>2.0	Wrigley & Llorca, 1992
UHT whole cow's milk	*Bacillus subtilis*	Ultrasonic bath, 40 kHz, 240 W	25 min, 50°C	3.2	Yang et al., 2023b
	Listeria monocytogenes	Probe, 20 kHz, 150 W, 118 W/cm^2	Continuous treatment (50 mL/min), 18 min, 57°C	5.3	D'Amico et al., 2006
	Listeria innocua	Probe (22 mm), 24 kHz, 400 W, 120 μm	30 min, 63°C	2.5	Bermúdez-Aguirre & Barbosa-Cánovas, 2008
UHT whole goat's milk	*Escherichia coli* O157:H7	Ultrasonic bath, 40 kHz, 54.4 W, 1.9 kJ/mL	6 min, 70°C	6.6	Bernardo et al., 2022
Wheat plantlets juice	MAB	Ultrasonic bath, 40 kHz, 294 W	40 min, 60°C	4.1	Ahmed et al., 2019
	YM			3.5	
Fruits and vegetables					
Green olives	MAB	Probe, 30 kHz, 100 W	15 min, 55°C	5.3	Jafarpour, 2022
	TAB[e]			2.6	
	YM			3.9	
Red bell peppers	*Listeria innocua*	Ultrasonic bath, 35 kHz, 120 W, 21.4 W/L	2 min, 65°C	7.4	Alexandre et al., 2011
Strawberry fruits					
Fish and fishery products					
Smoked salmon	*Listeria monocytogenes*	Ultrasonic bath, 20 kHz, 100%	5 min, 50°C	2.4	Pennisi et al., 2020

Microbial inactivation values are rounded up to the first decimal.
[a]MAB: total mesophilic aerobic bacteria.
[b]YM: yeast and molds.
[c]LAB: lactic acid bacteria.
[d]UHT: ultra-high-temperature.
[e]TAB: total thermophilic aerobic bacteria.

was seen at the highest temperature investigated (60°C), without an upper temperature limit reported.

Microbial inactivation in other fruit juices assisted by TS has also been widely investigated, comparing the efficacy of the treatment against conventional heat processing. For instance, the inactivation of *E. coli* and *Lactobacillus acidophilus* in orange juice achieved the optimal temperature range and a synergistic lethal effect from 48 to 60°C and from 52 to 60°C, respectively (Zenker et al., 2003). Furthermore, it was reported that TS (probe, 19.3 kHz, 800 W, 55 μm, continuous mode) required 3.5-times and 1.5-times shorter processing time for the inactivation of *E. coli* and *L. acidophilus*, respectively, than heat treatments to achieve the same level of inactivation. In addition, the lethal temperature values were reduced from 68 to 56°C and from 60 to 52°C for the inactivation by TS of *E. coli* and *L. acidophilus*, respectively. Likewise, the efficacy of TS (10 mm-diameter probe, 20 kHz, 400 W, continuous mode) was also evident by the reduction of the processing time to achieve inactivation of 5.0 Log_{10} CFU/mL of vegetative cells of *Bacillus subtilis* in Chinese bayberry juice. More specifically, when thermal treatment at 63°C was used, 36.9 min was needed to achieve this reduction. In contrast, 9.6 min was required with TS treatment at the same temperature, indicating a decrease in the treatment time by more than 74% (Li et al., 2019).

A comparison between TS, US, and heat treatment was performed by Demir & Kılınç (2019) in a study focused on the inactivation of *E. coli* K12 in pumpkin juice. The least effective treatment was US (23°C), where a difference of only 0.4 Log_{10} CFU/mL in the cell concentration was achieved, followed by the thermal treatment (60°C), which resulted in the microbial reduction of 3.6 Log_{10} CFU/mL. However, TS (ultrasonic bath, 37 kHz, 150 W, continuous mode) at 60°C resulted in bacterial reductions of 6.2 and 6.6 Log_{10} CFU/mL for treatments applied in continuous and batch mode, respectively. It was also concluded that a decrease of 71% in the treatment time could be achieved by using TS instead of conventional heat treatment to reach the same level of microbial inactivation. Regarding the influence of wave amplitude during TS, most studies pointed out its relevance to microbial inactivation potential. Nevertheless, one study in apple juice and molds revealed that when applying TS treatments (12.7 mm-diameter probe, 20 kHz, 600 W, continuous mode) at 60°C during different processing times (3, 6, and 9 min), similar inactivation levels of *Penicillium expansum* and *Rhodotorula* spp. was achieved regardless the value of wave amplitude (60–120 μm) (Jambrak et al., 2017).

In addition, the inactivation of spoilage yeasts by TS has been studied. For example, the inactivation of *Brettanomyces bruxellensis* in red wine, considered the main spoilage microorganism in the wine industry, was more effective with TS (12.7 mm-diameter probe, 20 kHz, 600 W, 120 μm, continuous mode) at 43°C compared with the US (25°C) during 3 min (Gracin et al., 2017).

3.2.3 | Inactivation in low-acid liquid foods

Another widely investigated application of TS is cow's milk processing. Several types of this low-acid matrix are studied, such as different fat content (whole, fat free, or low fat), raw milk or ultra-high-temperature (UHT) treated, or even reconstituted milk powder. For instance, TS (22 mm-diameter probe, 24 kHz, 400 W, 2.85 W/cm^2, 120 μm, continuous mode) has been applied to reduce processing time in the conventional batch milk pasteurization (63°C by 30 min), that is, a low-temperature-long-time treatment, to achieve a reduction of 5.0 Log_{10} CFU/mL of nonpathogenic *L. innocua*, a bacterial surrogate for a pathogenic *L. monocytogenes*. Furthermore, the application of TS treatment in batch mode in a walled vessel resulted in 20 min treatment time reduction, that is, a 63°C/10 min treatment, compared with the conventional treatment (63°C, 30 min) (Bermúdez-Aguirre et al., 2009). Moreover, thermal treatment at the temperature of 63°C for a treatment time of 10 min resulted in a difference of only 0.5 Log_{10} CFU/mL in the concentration of *L. innocua* (Bermúdez-Aguirre et al., 2011).

3.2.4 | General statements on TS

The main process parameters that may influence the inactivation of microorganisms by TS are temperature and processing time, but the influence of other parameters, which have been mentioned in the previous section where US is described, such as amplitude and acoustic intensity, is also important (Adekunte et al., 2010a; Amador-Espejo et al., 2020; Guerrero et al., 2001; Herceg et al., 2012; Ugarte-Romero et al., 2007; Zenker et al., 2003). Overall, the microbial inactivation levels achieved in almost all cases are higher than in US treatments, resulting in highly reduced processing times to achieve an optimal level of food decontamination. Likewise, for most studies where TS has been compared with conventional heat treatments or even the US, TS is seen as an alternative method to reduce the microbial and thermal load applied to the product. Therefore, it can be considered an energy-efficient alternative to traditional thermal treatments. However, efficient process parameters should be considered, such as the optimal microbial inactivation level, without compromising the quality of the processed food.

Based on the literature data, just some apple products and different types of cow milk products have been investigated in depth. Thus a wide processing window can be identified, and further inactivation and validation studies should be performed to confirm these results, especially for new products that have not been assessed so far, or already investigated products where the upper temperature limit for different microorganisms was not stated, being required to be found in terms of optimizing the efficiency of TS in microbial food decontamination.

3.3 | Inactivation of microorganisms by manosonication

Another approach to enhance antimicrobial efficacy is using the US at elevated pressure. The elevated pressure in the treated medium is typically 200−500 kPa, and the technology is called manosonication (MS). In MS, the temperature is generally maintained at lower values, less than 40°C.

3.3.1 | Mechanism of action and beginning of MS

As a result of the combination of US and pressure, a more aggressive microbubble implosion is generated (Whillock & Harvey, 1997), increasing the free radical formation (Vercet et al., 1998) and the subsequent enhanced mechanical disruption of microbial cells and microbial inactivation (Raso et al., 1998). In this sense, due to the increased pressure in the US, more than fourfold microbial lethal effect could be achieved compared with only US, making MS a potential technology to inactivate vegetative cells at room temperature (Condón et al., 2004).

After investigating seawater disinfection with cavitation phenomenon and different pressure inlets, Badve et al. (2015) observed that the microbial inactivation rate increased up to a certain pressure level, but above a certain level, further pressure elevation indicated no further reduction. The decrease in the bactericidal effectiveness of MS is suggested to be associated with a drop in the number of collapsing cavitation microbubbles. This happens because of the incapacity of US waves to overcome the sum of the cohesive forces of the reached overpressure and the ones present in the liquid molecules (Condón et al., 2004). For this reason, similar to TS, where the upper temperature level is of major importance, in MS, it is crucial to determine the *upper pressure limit* to achieve the maximum synergistic effect for microbial inactivation.

A pioneering investigation of MS was conducted by Neppiras & Hughes (1964), who studied the possibility of working with additional relative pressure to increase yeast disintegration when applying US treatments. However, the reported effect was very slight because the treatments were performed with very weak acoustic fields. There need to be more studies focusing on MS and even fewer studies where the treated medium was real food. In Table 5, several studies in which MS has been investigated for microbial inactivation so far are listed, mostly focusing on assessments in culture media, where the preliminary knowledge of the lethal microbial potential of MS was found.

3.3.2 | Inactivation in culture media

In the study of Raso et al. (1998), an MS treatment (13 mm-diameter probe, 20 kHz, 450 W, continuous mode) was applied in McIlvaine citrate-phosphate buffer (pH 7.0) to study the inactivation of *Yersinia enterocolitica*. The upper pressure limit was found at 400 kPa because no further improvement of the inactivation was reached with a pressure higher than 600 kPa. Further in the study, an exponential reduction in the D value from 4 to 0.4 min was achieved with increased wave amplitude from 21 to 150 μm (30°C, 200 kPa). Assessments on the same medium with MS treatments (13 mm-diameter probe, 20 kHz, 450 W, 90 μm, continuous mode) at 40°C resulted in a significant decrease of the D values of *L. monocytogenes* from 5.70 to 2.5 min when pressure increased from the atmospheric level (≈100 kPa) to 200 kPa (Mañas et al., 2000a). Moreover, a linear relationship was reported between the amount of US power applied and the D value reduction.

Other microbial assessments include the work of Pagán et al. (1999b), who investigated the application of MS treatments (13 mm-diameter probe, 20 kHz, 450 W, continuous mode) at 40°C in McIlvaine citrate-phosphate buffer (pH 7.0) for inactivating *Streptococcus faecium*, *L. monocytogenes*, *S.* Enteritidis, and *Aeromonas hydrophila*. The obtained results suggested that the level of wave amplitude was more significant than the pressure applied in reducing the D values. When amplitude was increased from 62 to 150 μm, a sixfold reduction was recorded, compared with the fivefold reduction when relative pressure increased from 0 to 400 kPa. Moreover, when comparing MS with thermal treatment alone, the advantage of lower microbial recovery potential was seen in MS, independent of the treated medium used. In addition, the cellular damage caused by MS was also evaluated in the mentioned bacteria. The experimental results suggested that irreversible damages are found when applying this technology, meaning an advantage compared with reversible cellular damages caused by heat treatments. Similarly, a reduction of 3.5 Log_{10} CFU/mL of *Cronobacter sakazakii* in McIlvaine citrate-phosphate buffer (pH 7.0) after

TABLE 5 Review of microbial inactivation in culture media and food products assisted by manosonication (MS). Treatments were applied in a batch system if not otherwise specified

Food/model system	Microorganism	US operating conditions	Processing conditions	Microbial inactivation, in Log_{10} CFU/mL	References
Culture media					
McIlvaine citrate-phosphate buffer (pH 7.0)	*Cronobacter sakazakii*	Probe (13 mm), 20 kHz, 450 W, 5 W/mL, 117 μm	1 min, 35°C, 200 kPa	3.5	Arroyo et al., 2011
Phosphate buffer (pH 7.0)	*Escherichia coli* K12	Probe (12.5 mm), 20 kHz, 6 W/mL, 124 μm	2 min, <40°C, 300 kPa	5.0	Lee et al., 2009b
	Escherichia coli	Probe (13 mm), 20 kHz, 800 W, 114 μm	5 min, 30°C, 400 kPa	3.9	Chantapakul et al., 2019
	Staphylococcus aureus			1.9	
High-acid liquid foods					
Apple and orange juice	*Escherichia coli* O157:H7	Probe (13 mm), 20 kHz, 450 W, 110 μm	3.6 min, 35°C, 200 kPa	4.0	Guzel et al., 2014
	Listeria monocytogenes		7.2 min, 35°C, 200 kPa		
Apple juice	*Cronobacter sakazakii*	Probe (13 mm), 20 kHz, 450 W, 5 W/mL, 117 μm	1 min, 35°C, 200 kPa	1.1	Arroyo et al., 2012
Low-acid liquid foods					
Raw whole cow's milk	*Escherichia coli*	Two probes (40 mm), 20 kHz, 90 W/cm^2, 75%	4 min, 36°C, 225 kPa	1.6	Cregenzán-Alberti et al., 2014
	Pseudomonas fluorescens			1.6	
	Staphylococcus aureus			1.1	

Microbial inactivation values are rounded up to the first decimal.

1 min of MS treatment (13 mm-diameter probe, 20 kHz, 450 W, 117 μm, continuous mode) at 35°C and 200 kPa was achieved, without resulting to any sublethal cellular injuries (Arroyo et al., 2011). This study reported an exponential relationship between the ultrasonic power applied and the *D* value reduction.

Likewise, the tolerance of heat-shocked cells of *L. monocytogenes* against MS (13 mm-diameter probe, 20 kHz, 450 W, 117 μm, continuous mode) was evaluated by Pagán et al. (1999a). Experimental results suggested that the development of heat-shock proteins leads to a higher tolerance when applying conventional heat treatments, but the generation of this type of structure does not seem to affect their tolerance to MS.

3.3.3 | Inactivation in real foods

On the other side, few works were conducted on real food products treated by MS compared with the research on real food products carried out in US and TS. This could probably be because of the more complex configurations of MS because a system to apply and control the pressure must be incorporated. As an example, apple juice products treated by MS (13 mm-diameter probe, 20 kHz, 450 W, 110 or 117 μm, continuous mode) at 35°C and 200 kPa resulted in the inactivation of *E. coli* O157:H7, *L. monocytogenes*, and *C. sakazakii*. Among the bacteria, *C. sakazakii* was the most resilient against MS, achieving reductions of only 1.1 Log_{10} CFU/mL after 1 min or even with longer treatment times. However, a reduction of 4.0 Log_{10} CFU/mL was reached in the inactivation of *E. coli* O157:H7 and *L. monocytogenes* after 3.6 and 7.2 min of MS, respectively (Arroyo et al., 2012; Guzel et al., 2014). Another example of real food treated by MS is found in raw whole cow's milk and the inactivation of *E. coli*, *P. fluorescens*, and *S. aureus*. MS treatments (two 40 mm-diameter probes, 20 kHz, 90 W/cm^2, 75%) at 36°C and 225 kPa for 4 min resulted in the microbial reduction of 1.6 Log_{10} CFU/mL, except for *S. aureus* inactivation, due to close to inactivation of 1.0 Log_{10} CFU/mL was achieved (Cregenzán-Alberti et al., 2014).

3.3.4 | General statements on MS

Microbial inactivation by MS can be seen as a promising preservation technology of food products due to its antimicrobial efficiency compared with conventional heat treatments and other processes described in this review. Furthermore, it is crucial to determine the upper pressure limit to achieve the maximum synergetic effect of microbial inactivation. The application of elevated pressure values in MS does not necessarily result in greater lethal effects, contrary to what is observed with other processing parameters, such as wave amplitude, where higher values generally lead to higher levels of microbial inactivation.

Considering the results obtained so far, which were obtained in culture media, studies in real food products are lacking, and more studies are required to assess the impact of MS on microbial inhibition, such as the inactivation of different microbial species, including viruses, molds, and yeasts, in different food matrices.

3.4 | Inactivation of microorganisms by manothermosonication

Manothermosonication (MTS) is the inactivation strategy resulting from the combination of elevated pressure (200–500 kPa), mild temperatures (40–70°C), and the US. In the literature and review manuscripts, very often, this strategy is claimed to have the highest efficiency in terms of microbial inactivation compared with mild temperature, US, TS, and MS treatments, and it even requires lower processing times and temperatures during its effective operation (Dolas & Kaur, 2018).

3.4.1 | Mechanism of action and beginning of MTS

As mentioned, combining the US with mild heating temperatures reduces the cavitation intensity. However, one option to overcome this issue is by increasing the pressure in the treated medium, which leads to a pressure increase inside the microbubbles, and consequently results in a more rapid and violent collapse of the bubbles (Lorimer & Mason, 1987; Muthukumaran et al., 2006; Raso et al., 1999; Whillock & Harvey, 1997). A schematic representation of the increase in the size of microbubbles during MTS and its comparison with the size increases resulting in US-based technologies described in this review is shown in Figure 3.

The inactivation effectiveness of MTS is related to the antimicrobial effects of thermal and MS treatments, so when these three hurdles (sonication, mild temperatures, and elevated pressure) are combined, additive or even synergetic effects are observed, which can lead to improved microbial inactivation compared with the corresponding single treatments (Raso et al., 1998). Furthermore, as a result of this combination, a more rapid microbial inactivation can be achieved, requiring half of the treatment time at the optimum temperature compared with the necessary time of conventional heat treatments (Condón et al., 2004).

In MTS treatments, the crucial point is determining the upper limits of temperature and pressure to identify

the optimum processing window for a synergistic effect. For instance, Raso et al. (1998) found an upper temperature limit of 58°C when the pressure was set at 200 kPa for MTS treatment aiming at inactivating *Y. entercolitica* in McIlvaine citrate-phosphate buffer (pH 7.0). Another relevant correlation of these parameters regarding energy consumption was found by Raso et al. (1999), who demonstrated that if the medium temperature increases during the treatment, the pressure applied in the system must also be increased to achieve constant power output. Furthermore, it has been demonstrated that the lethal effects of MTS at the same pressure level were more pronounced when higher temperatures were applied rather than with the increase in the pressure level (Kahraman et al., 2017).

First studies involving and building special equipment to realize MTS treatments were performed by Sala et al. (1995) and Raso et al. (1998). Since then, MTS technology has been studied for microbial inactivation in buffered media, fruit and vegetable juices, and liquid egg products. The most recent and relevant data are collected and presented in Table 6.

3.4.2 | Inactivation in culture media

The preliminary studies investigated the lethal effects of MTS (13 mm-diameter probe, 20 kHz, 450 W, 117 μm, continuous mode) in different culture media and bacterial species. For instance, the inactivation of *L. monocytogenes* by MS in a McIlvaine citrate-phosphate buffer (pH 7.0) showed an additive effect of elevated pressure and US compared with the application of US only, but bacteria inactivated with MTS indicated synergistic inactivation effects in temperature ranges of 62–68°C (Pagán et al., 1999a). Similarly, in McIlvaine citrate-phosphate buffer (pH 7.0), the influence of different a_w values on the inactivation of *Salmonella* spp. strains were studied after MTS treatment (13 mm-diameter probe, 20 kHz, 450 W, 117 μm, continuous mode) (65°C, 175 kPa). When a_w dropped from >0.99 to 0.93 and heat treatments at 65°C were applied, the *D* value of *S*. Senftenberg 775 W increased up to 18-folds (Álvarez et al., 2006), while in the case of *S*. Enteritidis, tolerance increased up to 30-folds for dropping a_w from 1 to 0.96 (Álvarez et al., 2003). However, due to the application of MS and MTS, *D* values hardly increased in *S*. Senftenberg 775 W (Álvarez et al., 2006) or only twofold in *S*. Enteritidis (Álvarez et al., 2003). This indicates a potential advantage of MS and MTS for the inactivation of microorganisms in media with low a_w values. However, additive effects were seen when MTS and MS treatments were applied in media with high a_w values.

Furthermore, when evaluating the reduction of *D* values in heat-shocked *L. monocytogenes* cells (180 min, 45°C) in buffered media, the tolerance of bacteria following exposure to heat treatments of 62°C increased by sixfold (D_{62} of 1.8 min) when compared with the nonheat exposed

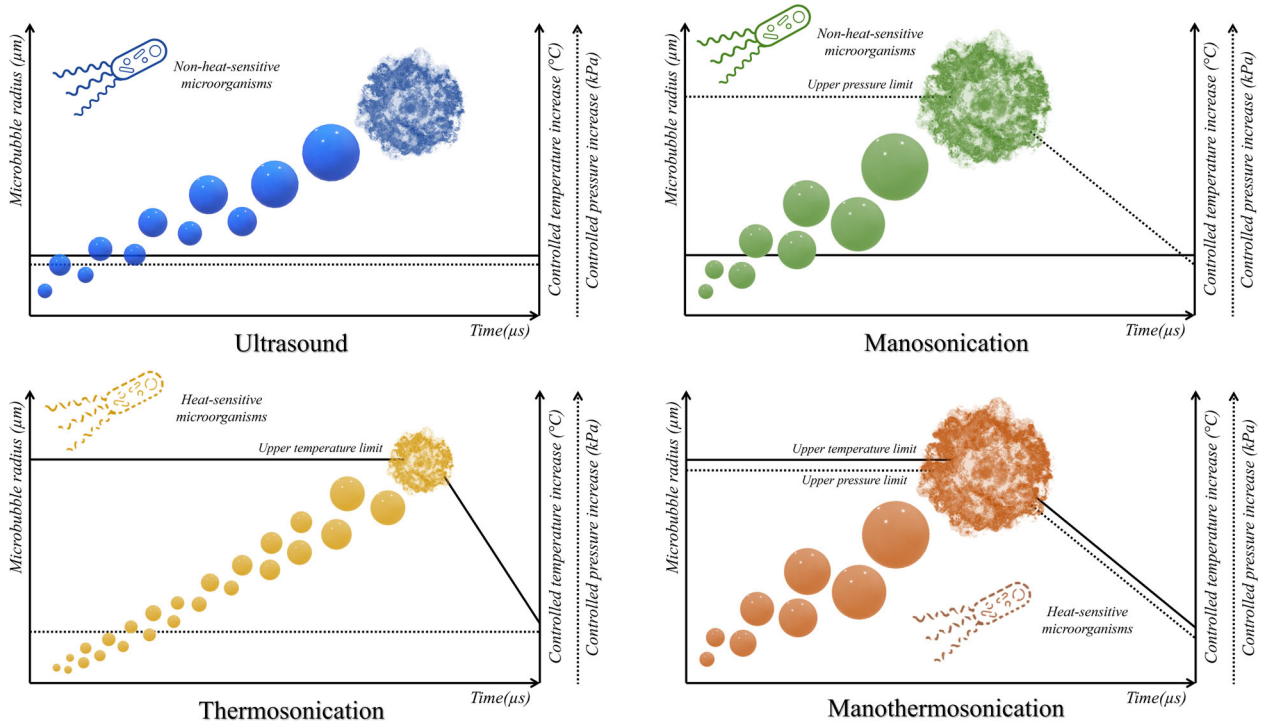

FIGURE 3 Schematic representation of the microbubble size increase during US-based processing technologies as a result of the controlled increase in temperature and/or pressure.

TABLE 6 Review of microbial inactivation in culture media and food products assisted by manothermosonication (MTS)

Food/model system	Microorganism	US operating conditions	Processing conditions	Microbial inactivation, in Log_{10} CFU/mL	References
Culture media					
Phosphate buffer (pH 7.0)	*Escherichia coli* K12	Probe (12.5 mm), 20 kHz, 6 W/mL, 124 μm	0.5 min, 60°C, 300 kPa	5.0	Lee et al., 2009b
	Escherichia coli	Probe (13 mm), 20 kHz, 800 W, 114 μm	5 min, 50°C, 400 kPa	6.3	Chantapakul et al., 2019
	Staphylococcus aureus			4.6	
Phosphate-buffered saline, PBS (pH 4.0)	*Escherichia coli* K12	Probe (12.5 mm), 20 kHz, 6 W/mL, 124 μm	0.25 min, 60°C, 400 kPa	5.0	Lee et al., 2009a
High-acid liquid foods					
Apple and carrot juice	*Escherichia coli* O157:H7	Probe (13 mm), 20 kHz, 750 W	Continuous treatment, 0.5 min, 60°C, 300 kPa	5.0	Kahraman et al., 2017
Apple and orange juice	*Escherichia coli* O157:H7	Probe (13 mm), 20 kHz, 450 W, 110 μm	1.1 min, 60°C, 200 kPa	4.0	Guzel et al., 2014
	Listeria monocytogenes		0.9 min, 60°C, 200 kPa		
Apple cider	*Escherichia coli* K12	Probe (12.5 mm), 20 kHz, 6 W/mL, 124 μm	1.4 min, 59°C, 400 kPa	5.0	Lee et al., 2013
Apple juice	*Cronobacter sakazakii*	Probe (13 mm), 20 kHz, 450 W, 5 W/mL, 117 μm	1 min, 54°C, 200 kPa	2.7	Arroyo et al., 2012
Low-acid liquid foods					
Liquid whole egg	*Salmonella* Senftenberg 775W	Probe (13 mm), 20 kHz, 450 W, 117 μm	3.5 min, 60°C, 200 kPa	>3.0	Mañas et al., 2000b

Treatments were applied in a batch system if not otherwise specified. Microbial inactivation values are rounded up to the first decimal.

cells (D_{62} of 0.24 min). However, when applying MTS (13 mm-diameter probe, 20 kHz, 450 W, 117 μm, continuous mode), synergistic effects regarding microbial inactivation were seen in the range 62–68°C at 200 kPa with the heat-shocked cells (Pagán et al., 1999a). In addition, the sensitivity of both heat-shocked and nonheat-shocked cells was the same (D_{MS} of 1.6 min) after the MS treatments (40°C, 200 kPa). Similarly, Pagán et al. (1999b) investigated the bacterial tolerance of *S. faecium*, *L. monocytogenes*, *S.* Enteritidis, and *A. hydrophila* in McIlvaine citrate-phosphate buffer (pH 7.0) after MTS (13 mm-diameter probe, 20 kHz, 450 W, 117 μm, continuous mode). Additive lethal effects of heat and MS were seen in all microbial species except *S. faecium*, where synergistic effects were recorded at 62°C and 200 kPa.

Recently, Condón-Abanto et al. (2018) suggested that the simultaneous application of MTS may provide additive or synergistic effects depending on the studied target bacterial species. For instance, after MTS treatments in McIlvaine citrate-phosphate buffer (pH 7.0), bacteria such as *A. hydrophila* and *Y. enterocolitica* showed additive effects, but synergistic effects were seen in *S. bayanus*, *L. monocytogenes*, *Salmonella* spp., and *Bacillus* spp. However, further studies suggested that microorganisms recognized for their high heat tolerance are, at the same time, the ones in which higher synergistic effects are seen when MTS is applied. This fact proves there is a reduction in the treatment time to achieve a certain level of microbial inactivation compared with heat treatments. In addition, the lowest synergistic effect is seen for bacterial vegetative cells, whereas the largest synergistic effect is reported for yeasts and bacterial spores (Raso et al., 1998).

The effectiveness of MTS microbial inactivation has often been compared with other US-based treatments described previously in this review. For example, in a study about the lethal effects of US, TS, MS, and MTS (12.5 mm-diameter probe, 20 kHz, 6 W/mL, 124 μm, continuous mode) on *E. coli* K12 in a PBS, results indicated that the inactivation rate was higher in treatments where mild temperatures were applied (TS and MTS) (Lee et al., 2009a). Moreover, after investigating the effect of the different pH values in the treatment medium, it was reported that the lethal effect of the MTS was enhanced at lower pH values (pH values of 3.0 and 4.0) compared with mild pH values (pH values of 5.0 and 7.0). In addition, this lethal effect (>1.0 Log_{10} CFU/mL) was recorded at mild temperatures (50, 55, and 60°C) but not at low temperatures (40°C). In a different study, Lee et al. (2009b) also compared microbial inactivation by TS, MS, and MTS (12.5 mm-diameter probe, 20 kHz, 6 W/mL, 124 μm, continuous mode) for inactivation of *E. coli* K12 in phosphate buffer (pH 7.0) and reported a 5.0 Log_{10} CFU/mL of microbial reduction with MTS (61°C, 300 kPa) after 0.5 min. Furthermore, the upper pressure limit was found at 300 kPa among the three investigated pressure levels (300, 400, and 500 kPa), as no further inactivation was found at pressures higher than 300 kPa. However, it is worth mentioning that the inactivation achieved with MTS treatment was comparable to that found with TS treatment of the same treatment time, temperature, and pressure (0.5 min, 61°C, 100 kPa). On the other hand, the required treatment times to reach the same microbial inactivation level with MS (40°C, 300 kPa) were found to be 2 min longer.

After application of MTS (13 mm-diameter probe, 20 kHz, 800 W, 114 μm, 3 s-on and 2 s-off pulsed mode) (50°C, 400 kPa), reductions of 6.3 Log_{10} CFU/mL of *E. coli* and 4.6 Log_{10} CFU/mL of *S. aureus* in phosphate buffer (pH 7.0) were achieved (Chantapakul et al., 2019). Comparing these results with mild a temperature treatment (50°C, 100 kPa), both microorganisms were inactivated by less than an inactivation of 1.0 Log_{10} CFU/mL. When applying only US treatment (30°C, 100 kPa), changes in microbial concentration of 2.0 and 0.3 Log_{10} CFU/mL of *E. coli* and *S. aureus* were reached, respectively. After TS treatment (50°C, 100 kPa), the achieved inactivation levels were 3.5 and 1.3 Log_{10} CFU/mL of *E. coli* and *S. aureus*, respectively. Finally, the application of MS (30°C, 400 kPa) resulted in reductions of 3.9 and 1.9 Log_{10} CFU/mL of *E. coli* and *S. aureus*, respectively. Consequently, it can be stated that MTS was the most effective treatment for microbial decontamination of the investigated treatments, followed by MS, TS, US, and mild temperature treatment, when *E. coli* was the target microorganism. In the case of the monoderm bacteria *S. aureus*, MTS was also the most effective treatment, followed by MS and TS, but heat treatment at mild temperature was more effective than the US treatment.

3.4.3 | Inactivation in real foods

Regarding applying this technology in real food products, MTS may be seen as an alternative to liquid whole egg pasteurization. Traditional pasteurization treatments in liquid whole egg (60°C, 3.5 min) should reduce >5.0 Log_{10} CFU/mL in the population of the most prevalent *S. enterica* subsp. *enterica* serotypes are *S.* Enteritidis and *S.* Typhimurium (Ceylan et al., 2021; Doyle & Mazzotta, 2000; Froning et al., 2002; Mañas et al., 2003; Shah et al., 1991). However, *S.* Senftenberg 775 W is considered the most heat-resistant serotype, resulting in no further than 1.0–4.0 Log_{10} CFU/mL reductions with the conventional pasteurization heat treatment in plain whole egg, and although this heat-resistant strain is not frequently found, low safety level for pasteurized liquid whole egg in case of contamination is expected (Davidson et al., 1966; Ng et al., 1969; Osborne et al., 1954; Anellis et al., 1954; Mañas et al., 2003). For this reason, Mañas et al. (2000b) investigated the inactivation of *S.* Senftenberg 775 W in liquid whole

egg with MTS (13 mm-diameter probe, 20 kHz, 450 W, 117 μm, continuous mode). MTS at 60°C and 200 kPa, resulted in the reduction of 3.0 Log_{10} CFU/mL of *S.* Senftenberg 775 W in 3.5 min due to the additive effect of heat and MS and achieving no more than 2.0 Log_{10} CFU/mL microbial reduction when only heat treatment was applied under the same conditions (60°C, 3.5 min). In addition, an exponential increase in the microbial inactivation rate was seen with a linear increase in the applied wave amplitude. Another example of real food products in which MTS has been studied as a microbial decontaminant technology is apple juice. Arroyo et al. (2012) investigated the inactivation of *C. sakazakii* in this product after MTS treatment (13 mm-diameter probe, 20 kHz, 450 W, 117 μm, continuous mode), finding a higher inactivation rate between 45 and 64°C, and the highest synergistic lethal effect at 54°C, leading to a reduction of 2.7 Log_{10} CFU/mL of the pathogenic microorganism.

A comparison of the US-based technologies in real food products, namely apple juice, was performed by Arroyo et al. (2012), where the lethal effect of mild temperatures (54°C), MS (35°C, 200 kPa), and MTS (54°C, 200 kPa) (13 mm-diameter probe, 20 kHz, 450 W, 117 μm, continuous mode) for inactivation of *C. sakazakii* after 1 min of processing time and subsequent storage at 4°C for 96 h were studied. Applying only mild heat treatment resulted in a change in cell concentration of 0.5 Log_{10} CFU/mL. In contrast, the MS reduced 1.1 Log_{10} CFU/mL, and the MTS resulted in the inactivation of 2.7 Log_{10} CFU/mL. Furthermore, during the storage period (96 h, 4°C), microbial counts for MTS-treated samples were further decreased to 5.3 Log_{10} CFU/mL. This indicates the presence of sublethally injured cells. On the contrary, only 1.8 Log_{10} CFU/mL of microbial reduction was achieved after applying only mild heat treatment. When the required treatment time to achieve a reduction of 5.0 Log_{10} CFU/mL of *E. coli* K12 in apple cider by using TS (59°C, 100 kPa), MS (55°C, 400 kPa), and MTS (59°C, 400 kPa) (12.5 mm-diameter probe, 20 kHz, 6 W/mL, 124 μm, continuous mode) was studied (Lee et al., 2013), treatment time for MTS was significantly reduced down to 1.4 min, compared with 3.8 min and 2.5 min for TS and MS, respectively.

Moreover, intending to compare the lethal effect of MS and MTS (13 mm-diameter probe, 20 kHz, 450 W, 110 μm, continuous mode) on both Gram-type bacteria in real food products, Guzel et al. (2014) investigated the effects of MS (35°C, 200 kPa) and MTS (60°C, 200 kPa) on inactivation of *E. coli* O157:H7 and *L. monocytogenes* in apple and orange juice. The processing times to achieve a microbial reduction of 4.0 Log_{10} CFU/mL obtained with MS showed higher tolerance for *L. monocytogenes* (7.2 min) than for *E. coli* O157:H7 (3.6 min). The same tendency was found in US and TS with higher ultrasonic tolerance in monoderm bacteria due to the thick peptidoglycan layer, which is missed in the diderm bacteria. However, the same inactivation level without significant differences in treatment times for both bacteria was achieved by MTS treatment after 1.1- and 0.9-min treatment times of *E. coli* O157:H7 and *L. monocytogenes*, respectively. Even though lower required processing times were seen in the monoderm species, this bacterial group is considered the most tolerant against conventional physical treatments. As a result, MTS treatment might be proposed as a strategy in which physiological differences between monoderm and diderm bacteria are not as crucial to consider in terms of microbial inactivation as in other conventional or novel food processing technologies.

3.4.4 | General statements on MTS

Based on the reviewed results, the MTS technology is the most promising US-based strategy of the proposed approaches in which desired microbial inactivation can be achieved with a significant reduction of the processing time. Although some research is carried out, this field is still in its early phase, and certain aspects need to be studied before a safe use of the technology can be recommended. For example, more studies must be conducted on MTS' inactivation of viruses, molds, and yeasts. On the other hand, MTS has been proven to have the potential to inactivate spore-forming bacteria, such as *Bacillus* spp. spores, as suggested in several studies gathered in the literature (Condón-Abanto et al., 2016; Raso et al., 1998; Sala et al., 1995).

Comparing MTS with heat processing, the heat provided by MTS means a more effective microbial inactivation method, with which the processing time could be reduced (Raso et al., 1998). Moreover, the use of shorter processing times makes possible the increase in the amount of product that can be processed simultaneously and shorter processing periods (Lee et al., 2013). However, despite the mentioned advantages, some limitations make this technique unsuitable for all applications. The major drawback is that MTS can only be used to treat liquid food products due to its inability to generate the cavitation phenomenon in solid systems successfully. In addition, studies carried out so far have been performed in complex and rather time-consuming laboratory-scale equipment. These new instrument designs and optimizations are needed to improve MTS performance concerning industrial upscaling. Hence, MTS is still a novel technology that has not been widely explored in real food products; for this reason, more studies that evaluate its microbial decontamination efficacy in these matrix types should be conducted (Chantapakul et al., 2019).

Nevertheless, the current microbial food decontamination efficacy of the setups of US-based approaches

investigated so far, including MTS, is only sometimes enough when reaching certain inactivation levels of pathogenic microorganisms, which are crucial to controlling the food chain. Therefore, Table 7 compares the inactivation levels of target pathogenic microorganisms with these technologies and traditional heat treatments in different food categories. The main conclusions drawn from Table 7 are as follows:

a. Generally, the achieved inactivation of the pathogenic microorganisms with the US-based processing technologies does not meet target reductions achieved with conventional heat processing, for example, target reductions for *L. monocytogenes* in fish and fishery products and *Salmonella* spp. in the plain whole egg are not met.
b. There is not enough validated data to show that reduction targets are met to propose the novel technologies as an alternative to conventional heat processing, for example, *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. in fruit juices (pH 4.0 or less).
c. There is no reported data on the inactivation of the target pathogenic microorganism in milk and milk products, that is, *Coxiella burnetii*, probably due to the difficulties and the risk associated with handling the pathogen, which is classified as biosafety level 3 according to current legislation on biological materials (Seitz, 2014). Therefore, applying these novel technologies as decontamination techniques in the dairy sector would not be possible without these investigations.

For this reason, validation data on the decontamination efficiency of these technologies are required for the target pathogenic microorganisms to be inactivated in the respective food sector. Considering all these things, US-based technologies, especially MTS, may represent promising alternatives to traditional thermal pasteurization treatments for liquid foods. However, optimization of current setups and a better understanding of the processing factors' lethal effects are still required to overcome limitations and achieve their implementation as food decontamination technologies.

4 | MATHEMATICAL MODELING OF MICROBIAL INACTIVATION KINETICS BY US-BASED PROCESSING TECHNOLOGIES

Microbial kinetic modeling is a useful tool for applying a US model-based optimization and performing predictions during food preservation. These approaches in the field of predictive microbiology lead to a quantitative description of microbial inactivation kinetic in food products for a specific range of environmental conditions (Van Impe et al., 2018). In this way, kinetic models can be employed to predict the inactivation and assess the impact of different processing parameters on different microorganisms and food products (Valdramidis et al., 2010).

Two approaches can generally describe microbial inactivation behavior (Gomez-Gomez et al., 2020). The first one assumes that the microbial population in the food subjected to the treatment has a homogeneous resistance and follows first-order kinetics. In this case, a first-order model needs to be used. This model is the most common and straightforward based on estimating one inactivation parameter, often associated with the D value (Chantapakul et al., 2019). On the other hand, another approach considers more complex microbial inactivation kinetics where nonlinear models must be employed to describe survival behavior accurately. The nonlinearity in microbial inactivation is described by different modes, for example, downward concavity or shoulder before the log-linear part of the inactivation period and/or an upward concavity or tail after the log-linear part of the inactivation period (Gomez-Gomez et al., 2020; Lee et al., 2013). In this case, models are based on more than one parameter, such as the D value and δ value, and are used to predict inactivation parameters for microbial populations with heterogeneous resistance (Chantapakul et al., 2019). In terms of modeling microbial inactivation using US-based technologies, a significant number of studies, mainly including nonlinear models to describe the inactivation, are summarized in Table 8.

As it can be seen from Table 8, several nonlinear kinetics models such as the Weibull model, modified Gompertz model, log-logistic model, biphasic linear model, log-linear and shoulder model, among others, have been used in monoderm and diderm bacteria or yeasts to predict the microbial inactivation assisted by the US-based processing technologies. Regarding the US modeling, microbial kinetics can be described by the first-order model, also known as the log-linear model, because of the presence of one single lethal factor (sonication), similar to modeling in conventional thermal inactivation processes (Lee et al., 2009b). However, the US modeling can also be adequately described by nonlinear models, such as the Weibull model, being considered a flexible nonlinear model with suitable functions in describing microbial inactivation in food products (Adekunte et al., 2010a; Pala et al., 2015). Less frequently, other nonlinear models, like the biphasic linear model and the log-linear and shoulder model, could describe the inactivation kinetics by the US (Gómez-López et al., 2017; Mustapha et al., 2019).

Alternatively, when more than one lethal factor is applied, such as the combination of sonication with mild thermal treatment and/or treatments at elevated pressure, nonlinear microbial inactivation kinetics have been

TABLE 7 Comparison of US, TS, MS, and MTS with traditional heat pasteurization treatments guidelines on the inactivation of target pathogenic microorganisms in food product categories

Food category	Target microorganism for conventional processing	Target reduction with traditional processing	Inactivation achieved with US-based processing technologies	References
Fish and fishery products	*Listeria monocytogenes*	6.0 Log_{10} CFU/g ($F_{70} = 2$ min; $z = 7.5°C$)	TS, 2.4 Log_{10} CFU/g in smoked salmon	Pennisi et al., 2020
Fruit juices (pH 4.0 or less)	*Escherichia coli* O157:H7	5.0 Log_{10} CFU/mL (71.1°C, 3 s)	US, 0.8 Log_{10} CFU/mL in apple juice	Park & Ha, 2019
			US, 5.0 Log_{10} CFU/mL in strawberry juice	Yildiz & Aadil, 2020
			TS, 5.1 Log_{10} CFU/mL in apple cider	D'Amico et al., 2006
			TS, 5.0 Log_{10} CFU/mL in mango juice	Kiang et al., 2013
			TS, 4.5-5.0 Log_{10} CFU/mL in soursop nectar	Anaya-Esparza et al., 2017
			MS, 4.0 Log_{10} CFU/mL in apple and orange juice	Guzel et al., 2014
			MTS, 5.0 Log_{10} CFU/mL in apple and carrot juice	Kahraman et al., 2017
			MTS, 4.0 Log_{10} CFU/mL in apple and orange juice	Guzel et al., 2014
Fruit juices (pH 4.0 or less)	*Listeria monocytogenes*	5.0 Log_{10} CFU/mL (71.1°C, 3 s)	US, 1.1 Log_{10} CFU/mL in apple juice	Park & Ha, 2019
			US, 2.5 Log_{10} CFU/mL in sweet lemon juice	Hashemi & Jafarpour, 2020
			TS, 5.0 Log_{10} CFU/mL in apple cider	Baumann et al., 2005
			MS, 4.0 Log_{10} CFU/mL in apple and orange juice	Guzel et al., 2014
			MTS, 4.0 Log_{10} CFU/mL in apple and orange juice	Guzel et al., 2014

(Continues)

TABLE 7 (Continued)

Food category	Target microorganism for conventional processing	Target reduction with traditional processing	Inactivation achieved with US-based processing technologies	References
Fruit juices (pH 4.0 or less)	*Salmonella* spp.	5.0 Log_{10} CFU/mL (71.1°C, 3 s)	US, 0.9 Log_{10} CFU/mL of *S.* Typhimurium in apple juice	Park & Ha, 2019
			US, 1.4 Log_{10} CFU/mL of *S.* Typhi in sweet lemon juice	Hashemi & Jafarpour, 2020
			US, 5.0 Log_{10} CFU/mL of *S.* Typhi in barberry juice	Hashemi & Roohi, 2021
Milk and milk products	*Coxiella burnetii*	5.0 Log_{10} CFU/mL (63°C, 30 min; 72°C, 15 s)	–	–
Plain whole egg	*Salmonella* spp.	5.0 Log_{10} CFU/mL (60°C, 3.5 min)	US, 0.1 Log_{10} CFU/mL of *S.* Enteritidis in liquid whole egg	Techathuvanan & D'Souza, 2018
			US, 3.3 Log_{10} CFU/mL of *S.* Typhimurium in liquid whole egg	Bi et al., 2020
			TS, >2.0 Log_{10} CFU/mL of *S.* Typhimurium in liquid whole egg	Wrigley & Llorca, 1992
			TS, 2.3 Log_{10} CFU/mL of *S.* Enteritidis in liquid whole egg	Huang et al., 2006
			MTS, >3.0 Log_{10} CFU/mL of *S.* Senftenberg 775 W in liquid whole egg[a]	Mañas et al., 2000b

Data on target microorganisms and target reductions for conventional processing are extracted from the review by Ceylan et al., 2021.

[a]*S.* Senftenberg 775 W is known to be resistant to guidelines of conventional heat pasteurization of plain whole egg, achieving no more than 1.0–4.0 Log_{10} CFU/mL.

TABLE 8 Review of mathematical models used in culture media and food products to describe microbial inactivation kinetics by US-based processing technologies

Food/model system	Microorganism	Microbial inactivation models	References
Ultrasound			
Apple juice	*Alicyclobacillus acidoterrestris* *Alicyclobacillus acidiphilus*	Models studied: Weibull model, log-logistic model, modified Gompertz model, biphasic linear model Best-fitting model: biphasic linear model for *Alicyclobacillus acidoterrestris*; Weibull model for *Alicyclobacillus acidiphilus*	Wang et al., 2010
Barberry juice	*Escherichia coli* *Salmonella* Typhi *Bacillus cereus* *Enterococcus faecalis*	Models studied: Weibull model, modified Gompertz model, polynomial model, biphasic linear model Best-fitting model: Weibull model	Hashemi & Roohi, 2021
Calcium-added orange juice	MAB[a] YM[b]	Models studied: GInaFiT model-fitting tool[c] Best-fitting model: Weibull model, log-linear and shoulder model	Gómez-López et al., 2010
Cherry tomato fruits	*Escherichia coli*	Models studied: first-order model, Weibull model, Weibull model with tail, log-logistic model, log-linear and shoulder model, log-linear and shoulder and tail model, biphasic linear model, multi-target model, single-target model Best-fitting model: Weibull model, log-linear and shoulder model, biphasic linear model	Mustapha et al., 2019

(Continues)

TABLE 8 (Continued)

Food/model system	Microorganism	Microbial inactivation models	References
Ultrasound			
Passion fruit juice	MAB YM	Models studied: GInaFiT model-fitting tool Best-fitting model: Weibull model, log-linear and shoulder model	Gómez-López et al., 2017
Phosphate buffer (pH 7.0)	*Escherichia coli* K12	Models studied: first-order model, Weibull model, modified Gompertz model, log-logistic model, biphasic linear model Best-fitting model: first-order model	Lee et al., 2009b
	Escherichia coli *Staphylococcus aureus*	Models studied: first-order model, biphasic linear model Best-fitting model: first-order model	Chantapakul et al., 2019
Phosphate-buffered saline, PBS (pH 4.0)	*Escherichia coli* K12	Models studied: first-order model, Weibull model, modified Gompertz model, log-logistic model, biphasic linear model Best-fitting model: first-order model	Lee et al., 2009a
Pomegranate juice	*Escherichia coli* *Saccharomyces cerevisiae*	Models studied: first-order model, Weibull model	Pala et al., 2015
Thermosonication			
Apple cider	*Escherichia coli* K12	Models studied: Weibull model, log-logistic model, biphasic linear model Best-fitting model: biphasic linear model	Lee et al., 2013
Chinese bayberry juice	*Bacillus subtilis*	Models studied: Weibull model	Li et al., 2019

(Continues)

TABLE 8 (Continued)

Thermosonication			
Cranberry juice	*Saccharomyces cerevisiae*	Models studied: Weibull model, four-parameter model, modified Gompertz model Best-fitting model: modified Gompertz model	Bermúdez-Aguirre & Barbosa-Cánovas, 2012
Phosphate buffer (pH 7.0)	*Escherichia coli* K12	Models studied: first-order model, Weibull model, modified Gompertz model, log-logistic model, biphasic linear model Best-fitting model: biphasic linear model	Lee et al., 2009b
	Escherichia coli *Staphylococcus aureus*	Models studied: first-order model, biphasic linear model Best-fitting model: biphasic linear model	Chantapakul et al., 2019
Phosphate-buffered saline, PBS (pH 4.0)	*Escherichia coli* K12	Models studied: first-order model, Weibull model, modified Gompertz model, log-logistic model, biphasic linear model Best-fitting model: modified Gompertz model, log-logistic model, biphasic linear model	Lee et al., 2009a
Powdered infant formula	*Cronobacter sakazakii*	Models studied: GInaFiT model-fitting tool Best-fitting model: biphasic linear with shoulder model	Adekunte et al., 2010b
Raw whole cow's milk	*Listeria innocua*	Models studied: Weibull model, four-parameter model	Bermúdez-Aguirre et al., 2009
Tomato juice	*Pichia fermentans*	Models studied: Weibull model	Adekunte et al., 2010a

(Continues)

TABLE 8 (Continued)

Manosonication			
Apple cider	*Escherichia coli* K12	Models studied: Weibull model, log-logistic model, biphasic linear model Best-fitting model: biphasic linear model	Lee et al., 2013
Phosphate buffer (pH 7.0)	*Escherichia coli* K12	Models studied: first-order model, Weibull model, modified Gompertz model, log-logistic model, biphasic linear model Best-fitting model: biphasic linear model	Lee et al., 2009b
	Escherichia coli *Staphylococcus aureus*	Models studied: first-order model, biphasic linear model Best-fitting model: biphasic linear model	Chantapakul et al., 2019
Phosphate-buffered saline, PBS (pH 4.0)	*Escherichia coli* K12	Models studied: first-order model, Weibull model, modified Gompertz model, log-logistic model, biphasic linear model Best-fitting model: modified Gompertz model, log-logistic model, biphasic linear model	Lee et al., 2009a
Manothermosonication			
Apple and carrot juice	*Escherichia coli* O157:H7	Models studied: first-order model, Weibull model, modified Gompertz model, log-logistic model, biphasic linear model, K-L-M model Best-fitting model: Weibull model, log-logistic model	Kahraman et al., 2017
Apple cider	*Escherichia coli* K12	Models studied: Weibull model, log-logistic model, biphasic linear model Best-fitting model: biphasic linear model	Lee et al., 2013

(Continues)

TABLE 8 (Continued)

Manothermosonication			
McIlvaine citrate-phosphate buffer (pH 7.0)	*Aeromonas hydrophila* *Yersinia enterocolitica* *Saccharomyces bayanus* *Listeria monocytogenes* *Salmonella* spp. *Bacillus* spp.	Models studied: GInaFiT model-fitting tool Best-fitting model: log-linear and shoulder model	Condón-Abanto et al., 2018
Phosphate buffer (pH 7.0)	*Escherichia coli* K12	Models studied: first-order model, Weibull model, modified Gompertz model, log-logistic model, biphasic linear model Best-fitting model: biphasic linear model	Lee et al., 2009b
	Escherichia coli *Staphylococcus aureus*	Models studied: first-order model, biphasic linear model Best-fitting model: biphasic linear model	Chantapakul et al., 2019
Phosphate-buffered saline, PBS (pH 4.0)	*Escherichia coli* K12	Models studied: first-order model, Weibull model, modified Gompertz model, log-logistic model, biphasic linear model Best-fitting model: modified Gompertz model, log-logistic model, biphasic linear model	Lee et al., 2009a

[a]MAB: total mesophilic aerobic bacteria.
[b]YM: yeast and molds.
[c]GInaFiT model-fitting tool: first-order model, Weibull model, fixed β-parameter Weibull model, Weibull model with tail, log-linear and shoulder model, log-linear and tail model, log-linear and shoulder and tail model, biphasic linear model, biphasic linear with shoulder model (Geeraerd et al., 2005).

reported (Lee et al., 2009b, 2013). For example, when TS, MS, and MTS were investigated, the biphasic linear model had the best fitting for the inactivation of *E. coli* K12 in apple cider (Lee et al., 2013) or *E. coli* and *S. aureus* in phosphate buffer (pH 7.0) (Chantapakul et al., 2019; Lee et al., 2009b). The biphasic linear model may be the most suitable option for fitting models by US-based processing technologies, being often reported as the best-fitting model in TS, MS, and MTS treatments. Moreover, Lee et al. (2009b) reported this model as the most suitable kinetic model to describe microbial inactivation by sonication-based treatments. The biphasic linear model assumes the fitting of two independent first-order kinetics involving the differentiation of sensitive and resistant populations (Chantapakul et al., 2019).

On the other hand, less often, other nonlinear models adequately described microbial survival in TS, MS, and MTS treatments, for example, the modified Gompertz model and the log-logistic model (Lee et al., 2009a). Contrary to microbial inactivation by the US alone, the Weibull model may not be a suitable option to describe inactivation kinetics by sonication-based treatments when more than one lethal factor is applied.

Finally, current microbial modeling approaches evaluate the inactivation kinetics assuming that processing parameters are not changing with time, for example, the temperature is considered constant, which differs from the real processing conditions at the industrial level (Cattani et al., 2016). Nevertheless, in US-based technologies the temperature profile of the treated medium will change over time if no temperature control system is used, such as a cooling bath. As a result, there will be a direct impact on the accurate and precise evaluation of the microbial inactivation parameters (Dolan et al., 2013; Valdramidis et al., 2008). Therefore, future research should focus on implementing approaches to estimate inactivation parameters under realistic dynamic environments for the actual values of the parameters to represent reliable ones, being measured accurately by changing processing factors, such as medium temperature, and used as inputs for the microbial models to perform the microbial regression analysis.

5 | CONCLUSIONS

US technology is one of the emerging food processing technologies with the potential to deliver safe food by inactivating pathogenic and spoilage bacteria. However, mostly, it can be stated that only limited microbial inactivation can be achieved when the US is applied alone. In contrast, combining US with other hurdle approaches, such as heat treatments at mild temperatures and/or treatments at elevated pressure, has been proposed to overcome US technology's drawbacks and thus improve microbial inactivation. From the combinations, MTS is reported to be the most promising for the inactivation of different microorganisms in food products, such as fruit or vegetable juices and liquid egg products. In order to describe the microbial inactivation kinetics, often nonlinear mathematical models are required, most often used in previous studies. However, despite the great potential of these technologies, there are still numerous research questions regarding their safe use, impact on the quality of different foods and their sensorial aspects, scalability, economic viability, and sustainability aspects. Additionally, current microbial modeling approaches are limited to assessing microbial resistance under dynamic conditions, assuming that all these issues need to be investigated before these technologies can reach the market-ready level.

AUTHOR CONTRIBUTIONS

E. Beitia: Conceptualization, writing—original draft, visualization. **E. Gkogka**: Writing—review and editing **P. Chanos**: Writing—review and editing. **C. Hertel**: Writing—review and editing. **V. Heinz**: Funding acquisition, writing—review and editing, resources. **V. Valdramidis**: Conceptualization, Funding acquisition, writing—review and editing, project administration, supervision. **K. Aganovic**: Conceptualization, funding acquisition, writing—review and editing, project administration, supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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