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REVIEW ARTICLE

Assisted ultrasound applications for the production of safe foods

D. M. Sango[1], D. Abela[1,2], A. McElhatton[1] and V.P. Valdramidis[1]

1 Department of Food Studies and Environmental Health, Faculty of Health Sciences, University of Malta, Msida, Malta
2 Institute of Applied Science, MCAST, Triq Kordin, Paola, Malta

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Correspondence
Vasilis P. Valdramidis, Department of Food Studies and Environmental Health, Faculty of Health Sciences, University of Malta, Msida, MSD 2080, Malta.
E-mail: vasilis.valdramidis@um.edu.mt

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Summary

Ultrasound requires high power and longer treatment times to inactivate micro-organisms when compared to ultrasound combined with other technologies. Previous reports have shown that the effectiveness of ultrasound as a decontamination technology can be increased by combining it with another treatment such as pressure, heat and antimicrobial solutions. Assisted ultrasound, the combination of ultrasound with another technology, is more energy efficient, and it has less impact on the food properties. In this review paper, the power ultrasound antimicrobial mechanisms of action, the antimicrobial effects of ultrasound in combination with other physical processes and antimicrobial solutions are comprehensively discussed. Furthermore, the present interest on using these technologies as alternative processing and decontamination methods is presented. Research outputs on the application of ultrasound combined with physical processes are showcased including applications of thermosonication, manosonication, manothermosonication and osmosonication. Antimicrobial efficacy, energy requirements and optimal operation conditions of the different assisted ultrasound technologies are critically discussed, and their impact on the food industry for future applications is presented. Overall, this review paper highlights the importance and recent developments of assisted ultrasound for enhancing food safety.

Introduction

Food-borne illnesses are still a huge concern both for the food authorities and the food industry. The Centre for Disease Control and Prevention (CDC) reported 48 million food-borne illnesses caused by food-borne pathogens in the United States in 2011 that resulted in 128 000 hospitalizations and 3000 deaths (CDC 2011). In Europe, the Rapid Alert System for Food and Feed (RASFF) (RASFF 2011) stated that the number of notifications regarding microbial pathogens had increased in recent years and reached 600 reports during 2011. It is therefore evident that the efficient application of decontamination and processing technologies by the fresh produce and food processing industry is a prerequisite to achieve the production of safe products.

Thermal technologies such as pasteurization and sterilization have commonly been used by the food industry for food processing applications and production of safe and added value food products. However, thermal technologies cause significant changes in food properties such as the alteration of colour [e.g. darkening of pineapple juice in pasteurization process when the temperature is increased from 55 to 95°C (Rattanathanalerk *et al.* 2005)], development of undesirable flavour [e.g. pasteurization over 79°C can decrease milk acceptability by consumers due to the appearance of cooked flavours (Gandy *et al.* 2008)] and the decrease in the nutritional value [e.g. loss of carotenoid compounds in orange juice is observed after pasteurization process (Lee and Coates 2003)]. Thus, the food industry is focusing on developing nonthermal processing technologies, able to inactivate undesired micro-organisms while being capable of preserving food nutritional values and also physicochemical properties. Moreover, these technologies are intended to

be eco-friendly and resulting in considerable energy and water saving (Chemat and Khan 2011). Some of these innovative and emerging technologies are the pulse electromagnetic field, high pressure, microwave processing and (assisted) ultrasound.

In the case of the fresh produce industry, the main industry practices rely heavily on pre- or postharvest interventions, for example surface decontamination, to ensure microbiological safety and quality. Washing with different antimicrobial solutions is the most commonly applied practice to decontaminate minimally processed produce (MPP), in which chlorine (between 50 and 200 mg l^{-1}) as aqueous sanitizer is the most common to ensure the safety of fresh produce. Nonetheless, its efficacy on produce has been questioned in some cases (Zhang and Farber 1996; Park and Beuchat 1999; Gil *et al.* 2009). In addition, treatments with chlorine have adverse effects, such as the formation of chlorinated compounds when chlorine reacts with organic material in water (producing chloramines and trihalomethanes) (Wei *et al.* 1995). Nevertheless, it appears that chlorine dioxide does not create carcinogenic products on contact with organic matter (White 1992). Hence, it has been used as a substitute of chlorine, and studies were performed using concentration levels of 15–20 ppm. This could result in achieving 3·95 log reduction of the target microorganisms in fresh produce (Singh *et al.* 2002; Wu and Kim 2007). However, such levels of aqueous chlorine dioxide are in excess of legal limits defined by FDA (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=173.300: accessed date: 17/06/13), which requires that the maximum concentration of aqueous chlorine dioxide must not exceed 3 ppm. Therefore, other antimicrobial solutions such as peroxyacetic acid, hydrogen peroxide, trisodium phosphate and solutions with citric acid, ascorbic acid and calcium-based solutions (e.g. calcium lactate) have been considered for replacing chlorine decontamination. Nevertheless, the use of aqueous sanitizers alone is not always successful in controlling food-borne pathogens (Annous *et al.* 2001; Ölmez and Kretzschmar 2009).

Moreover, the food industry is seeking technologies able to reduce the water use and wastewater discharge rates. According to UNESCO's Intergovernmental Scientific Cooperative Programme in Hydrology and Water Resources, the industry sector in Europe will withdraw around 250 km^3 of water in 2025, placing it ahead of the agriculture sector with water needs of 200 km^3 (http://webworld.unesco.org/water/ihp/db/shiklomanov/part%273/HTML/Fi_17%271.html, accessed date 09/08/2013). In addition, the food industry is in third position regarding water use, ranking behind the chemical and refinery industries (Ölmez and Kretzschmar 2009). The additional impetus of reducing water usage together with chemical emissions changes the focus of processing and decontamination technologies towards reduced water usage or non-water based processes.

Ultrasound

Ultrasound processing can be classified among the non-water based or reduced water usage technologies. Ultrasound generates sonic waves of specific intensity and amplitude depending on the operational frequency. Ultrasound can be classified into two main categories, low-power ultrasound (from 100 kHz and above) and high-power ultrasound (from 20 to 100 kHz), both of which have been used in the food industry. The food industry has been using low-power ultrasound to assess the physicochemical properties of food products such as the composition and structure, and other quality control assessments (Demirdöven and Baysal 2008; Awad *et al.* 2012), while high-power ultrasound has been applied to a wide spectrum of applications that include sonocrystallization, emulsification, drying and freezing processes, inactivation of enzymes such as pectin methylesterase, polyphenoloxidases and peroxidases responsible for deterioration of juices; modification of functional properties such as gelation, viscosity and solubility in proteins used at industrial levels (e.g. whey protein concentrate, soya protein isolate and egg white protein) (Arzeni *et al.* 2012); and inactivation of microbes during decontamination and processing treatments. The application of power ultrasound requires the presence of a liquid phase.

Mechanism of action

The ability of ultrasound to be applied for microbial inactivation and consequently food decontamination is linked with two phenomena called acoustic cavitation (Fig. 1) and acoustic streaming. During acoustic cavitation, longitudinal waves are created which cause areas of alternating compression and expansion. In the expansion cycle, small bubbles in the liquid medium grow due to reduction in local pressure below that of the vapour pressure of the liquid resulting in changing intensities. In the compression phase, bubble surface area is reduced. When the bubbles grow in size and have a bigger area, the liquid medium is unable to absorb all the gas contained in the bubbles and the bubbles continue growing. When the energy supplied by the ultrasound matches with the fluctuation of the bubble wall, these bubbles become unstable and implode, generating the areas of high temperature (up to 5000°C) and high pressure (up to 1000 atmospheres) (Patist and Bates 2008; Mukhopadhyay and Ramaswamy 2012).

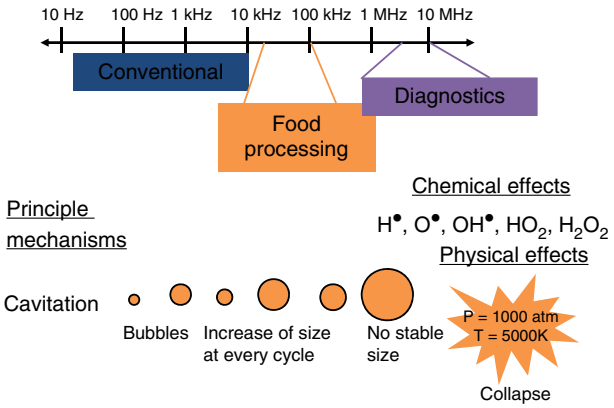

Figure 1 Ultrasound antimicrobial mechanism of action.

Acoustic streaming generates a dissipation of the acoustic energy leading the gradients in momentum, thereby the fluid currents (Lighthill 1978). The speed acquired by the fluid permits a better convection heat transfer coefficient near the solid boundaries, sometimes leading to turbulence and promoting heat transfer rate. This effect allows the better heating of the medium due to dissipation of the mechanical energy (Legay *et al.* 2011). All these phenomena contribute to bacterial disruption.

Apart from high temperatures and pressures, high shear energy waves and turbulence are created in the cavitation zones (Soria and Villamiel 2010). Additionally, the gases located in the bubbles in the presence of high temperatures and pressures, like those generated during the implosion, are transformed in reactive species and free radicals (Gogate and Kabadi 2009). Chemical (free radicals) and physical (pressure and heat gradients) phenomena created during ultrasound, act on the cell envelope, disrupting the cell walls, and consequently, the intracellular content is released leading to cellular death.

Regarding the effect of ultrasound on micro-organisms, spore-forming bacteria have been reported to be more resistant than vegetative forms. Fungi are more resistant than bacteria, aerobes more resistant than anaerobes and cocci typically more resistant than bacilli, due to the relationship of cell surface and cell size (Chandrapala *et al.* 2012). The effectiveness of ultrasonic waves against Gram-positive and Gram-negative bacteria has generated some controversial reports. According to some researchers, Gram-positive bacteria are more resistant than Gram-negative bacteria (Alliger 1975; Hülsen 1999; Villamiel and de Jong 2000). However, other studies reported no significant differences between these groups of bacteria (Scherba *et al.* 1991). The resistance of the different micro-organisms depends on many factors such as the properties of the medium and the processing conditions, that is, time, intensity of the treatment, temperature, pH (Chandrapala *et al.* 2012), which require further focused research studies to draw specific conclusions.

Applications of high-power ultrasound

High-power ultrasound (HPU) technology could be used for the pasteurization of a number of fruit juices (fresh or concentrated). It could then replace the heating steps of fruit juice or the microbial filtration step of concentrated juices. The main advantages of its application in food processing and preservation include the reduced energy consumption, the minimal flavour loss in liquid foods (e.g. juices), the increase in homogeneity and the breakdown of agglomerates of bacteria (Mason *et al.* 1996; Vercet *et al.* 2001; Piyasena *et al.* 2003; Awad *et al.* 2012). Ultrasound treatment of fruit juices was found to have a minimal effect on vitamin contents during processing and resulted in improved cloud stability during storage when compared to thermal treatment. This positive effect of ultrasound compared with heating is assumed to be due to the effective removal of occluded oxygen from the juice (Knorr *et al.* 2004), which is a critical parameter influencing the stability of ascorbic acid (Solomon *et al.* 1995). Tiwari *et al.* (2009) reported a maximum degradation of only 5% in the ascorbic acid content of orange juice when sonicated at high acoustic energy densities (0·81 W ml^{-1}) and treatment times (10 min).

Ultrasound has also been applied for the decontamination of abiotic surfaces, dental and medical equipment (Detwiler 1989; Huang *et al.* 2006b). More recently, ultrasound has been used to decontaminate fresh produce (Kwak *et al.* 2011; Sagong *et al.* 2011; Zhou *et al.* 2012) as a substitute of the traditional washing with aqueous sanitizers. Brilhante Sao Jose and Dantas Vanetti (2012) reported 1 log CFU g^{-1} reduction in aerobic mesophiles on cherry tomatoes using ultrasound at 45 kHz for 10 min. A study carried out by Seymour *et al.* (2002) has shown that *Salmonella* Typhimurium is reduced by 1·70 log in iceberg lettuce when ultrasound is applied at a frequency of 32–40 kHz for 10 min, while Rivera *et al.* (2011) reported a reduction of 1 log CFU g^{-1} of mesophilic organisms and 1·50 log CFU g^{-1} of *Enterobacteriaceae* in truffles at 35 kHz for 10 min at 4°C. From the previous reports and the achieved microbial reductions, it is evident that ultrasound alone is not very effective for inactivating micro-organisms on produce (Piyasena *et al.* 2003).

Assisted ultrasound

Power ultrasound alone requires high power and longer treatment times to inactivate micro-organisms (Chan-

drapala *et al.* 2012), leading to high costs for the food industry. The effectiveness of ultrasound as a decontamination technique can be increased by combining it with another treatment such as pressure, heat and antimicrobial solutions (McClements 1995; Ulusoy *et al.* 2007; Chemat and Khan 2011; Awad *et al.* 2012). Moreover, assisted ultrasound is more energy efficient and because lower intensities for shorter times can be used, it can have a positive impact on some food properties such as appearance and consistency in milk (Bermúdez-Aguirre *et al.* 2009). Additionally, ultrasound combined with another technology reduces processing times and increases efficiency at the industrial level (Demirdöven and Baysal 2008). An overview of these combined treatments is given hereunder and is summarized in Table 1.

Thermosonication

The combination of ultrasound with temperature treatments is able to reduce the operating requirements (e.g. temperature levels and process times) while achieving a microbial inactivation similar to conventional heat treatments. Food properties such as shelf life and surface colour stability (lightness) in orange juice (Zenker *et al.* 2003) could be better enhanced, while organoleptic parameters could also be retained (López-Malo *et al.* 2005). Bermúdez-Aguirre and Barbosa-Cánovas (2012) applied continuous (24 kHz, 400 W, 120 μm) and pulsing ultrasound treatments (ultrasound conditions were same as described previously with a cycle of 5 s on and 5 s off) at 60°C during 10 min in different juices (i.e. pineapple, cranberry and grape). All the continuous modes were more effective than pulsed modes in bacterial inactivation with the exception of cranberry juice, although the difference was not more than 1 log.

Bermúdez-Aguirre and Barbosa-Cánovas (2012) also described the microbial inactivation kinetics during thermosonication. All microbial kinetics of *Saccharomyces cerevisiae* for the tested juices showed tailing effects, indicating the presence of a resistant subpopulation, with an exception of grape juice treated by pulsed mode that showed a shoulder (i.e. initial resistance to the applied treatment) effect. Tailing effects were also observed in other thermosonication treatments of *Listeria innocua* and *Escherichia coli* (Ugarte-Romero *et al.* 2006; Bermúdez-Aguirre *et al.* 2009).

Researchers have also reported that heat during thermosonication contributes to the mechanical disruption of cells, making them more susceptible to cavitation (Chandrapala *et al.* 2012). However, some studies (Raso *et al.* 1998a; López-Malo *et al.* 1999) have shown that the effectiveness of the cavitation phenomena can decrease by increasing temperature. At high temperatures, vapour pressure is higher and the viscosity is lower, producing a reduction in the energy release during the bubble implosion (Guerrero *et al.* 2001). Nonetheless, Herceg *et al.* (2012) applied a combination of ultrasound at different temperatures (20 and 60°C) and reported that the maximum inactivation of *Staphylococcus aureus* was achieved when milk was treated in a thermosonication process at 20 kHz, 600 W, 120 μm and 60°C for 12 min. Herceg *et al.* (2012) also reported that the antagonistic effect of temperature was not detected due to the presence of solid elements in the milk suspension which increased the cavitation phenomena. Wordon *et al.* (2012) argued that the synergetic effect between ultrasound and heat can also be linked to the ability of ultrasound to produce nonlethal intracellular injuries, resulting in more vulnerable cells to heat treatments increasing their disruption rate.

Overall, thermosonication is a valuable method to reduce the microbial load, saving cost and energy and being less aggressive against food properties than heat treatments alone. Nevertheless, high temperatures can produce an opposite effect as they can reduce the cavitation phenomena. Additionally, the enhancement of the sonication effects when treating suspensions containing solid particles indicates that liquid food products such as milk and juices when having solid particles are the most suitable for thermosonication.

Manosonication

Pressure can also be combined with ultrasound to enhance microbial inactivation. This can be assigned to different reasons, such as an increase in free radical production (Vercet *et al.* 1998) and higher bubble implosion (Whillock and Harvey 1997). The microbial responses presented in the literature were described by first-order reaction kinetics. These kinetics were reported using *D*-values, that is, decimal reduction time. A research study carried out by Mañas *et al.* (2000) reported that the *D*-value of *Listeria* in citrate phosphate buffer decreased from 5·70 to 2·50 min when the pressure was raised from 0 to 200 kPa in combination with ultrasound at 20 kHz and 90 μm. Raso *et al.* (1998a) have shown a reduction in *D*-values from 1·52 to 0·28 min in *Yersinia enterocolitica* in citrate phosphate buffer when there was an increase in pressure from 0 to 300 kPa with ultrasound at 150 μm and 20 kHz. However, the same study has shown that an increase in pressure from 300 to 600 kPa at the same ultrasound operating conditions reduced the *D*-value from 0·28 to 0·20 min, which was not statistically different. This phenomenon can be attributed to the hydrostatic pressures that enhance some effects such as sonoluminescence (i.e. emission of short

Table 1 Overview of assisted ultrasound applications for microbial decontamination

Ultrasound assisted by	Operating conditions		Food (model) system	Micro-organisms studied	Microbial reduction	References
Temperature (thermosonication)	24 kHz, 400 W, 120 W cm^{-2}, 10 min, 60°C	Continuous	Pineapple juice	*Saccharomyces cerevisiae*	6·40 log CFU ml^{-1}	Bermúdez-Aguirre and Barbosa-Cánovas (2012)
		Pulsed (5 on 5 off)			5·20 log CFU ml^{-1}	
		Continuous	Cranberry juice	*Saccharomyces cerevisiae*	5·10 log CFU ml^{-1}	
		Pulsed (5 on 5 off)			5·70 log CFU ml^{-1}	
		Continuous	Grape Juice	*Saccharomyces cerevisiae*	7 log CFU ml^{-1}	
		Pulsed (5 on 5 off)			5·90 log CFU ml^{-1}	
	20 kHz, 600 W, 120 μm, 12 min	20°C	Milk	*Staphylococcus aureus*	1·49 log CFU ml^{-1}	Herceg *et al.* (2012)
				Escherichia coli	3·07 log CFU ml^{-1}	
		60°C		*Staphylococcus aureus*	0·94 log CFU ml^{-1}	
				Escherichia coli	2·49 log CFU ml^{-1}	
Pressure (manosonication)	20 kHz, 150 μm	0 kPa	Citrate phosphate buffer	*Yersinia enterocolitica*	*D*-value 1·52 min	Raso *et al.* (1998a)
		300 kPa		*Yersinia enterocolitica*	*D*-value 0·28 min	
		600 kPa		*Yersinia enterocolitica*	*D*-value 0·20 min	
Pressure (manosonication)	20 kHz,90 μm	0 kPa	Citrate phosphate buffer	*Listeria monocytogenes*	*D*-value 5·70 min	Mañas *et al.* (>2000)
		200 kPa		*Listeria monocytogenes*	*D*-value 2·50 min	
		300 kPa		*Listeria monocytogenes*	*D*-value 2·30 min	
Pressure and heat (manothermosonication)	20 kHz, 117 μm, 175 kPa	35°C	McIlvaine's citrate–phosphate buffer	*Salmonella* Senftenberg	*D*-value 1·71 min	Alvarez *et al.* (2006)
		67°C		*Salmonella* Senftenberg	*D*-value 0·02 min	
	20 kHz, 100 kPa, 4 min	40°C	Buffer system	*Escherichia coli*	4 log CFU ml^{-1}¶	Lee *et al.* (2009)
		47°C		*Escherichia coli*	4·50 log CFU ml^{-1}¶	
		54°C		*Escherichia coli*	4·50 log CFU ml^{-1}¶	
		61°C		*Escherichia coli*	7 log CFU ml^{-1}+	
	20 kHz, 117 μm 12 min	500 kPa, 70°C	Liquid medium	*Bacillus subtilis* spores	99% of the population	Raso *et al.* (1998b)
	20 kHz, 117 μm	Room temperature Low-pressure	Broth media	*Listeria monocytogenes*	*D*-value 4·30 min	Pagán *et al.* (1999)
		Room temperature 200 kPa		*Listeria monocytogenes*	*D*-value 1·50 min	
		55°C, 200 kPa		*Listeria monocytogenes*	*D*-value 1 min	
Osmotic pressure (osmosonication)	20 kHz, 50W, 48 μm, 25°C, 13 min	10·90 MPa, 650 g kg^{-1} TSS, 72 h	Orange juice	*Salmonella* spp.	>5 log CFU ml^{-1}	Wong *et al.* (2012)
Ozone (sonozonation)	Horn 22 kHz, 240 W Water bath 20·50 kHz, 120 W	0·5 mg l^{-1} ozone, 15 min	Water	Total coliforms, faecal coliforms and faecal streptococci	Between 95–99·9%	Jyoti and Pandit (2004)

(Continued)

Table 1 (*Continued*)

Ultrasound assisted by	Operating conditions		Food (model) system	Micro-organisms studied	Microbial reduction	References
Pulsed electric fields (PEF)	20 kHz, 31 μm 40 W cm^{-2}, 200 kPa, 52°C	34 KV cm^{-1}, 32 μs	Smoothies	*Listeria innocua*	5·60 log CFU ml^{-1}	Palgan *et al.* (2012)
	34 KV cm^{-1}, 32 μs	20 kHz, 31 μm, 40 W cm^{-2}, 200 kPa, 52°C		*Listeria innocua*	4·20 log CFU ml^{-1}	
	20 kHz, 40 W, 55°C, 5 min	30 pulses 5·67 KV mm^{-1}	Liquid whole eggs	*Salmonella* Enteritidis	2·25 log of reduction	Huang *et al.* (2006a)
	30 pulses, 5·67 KV mm^{-1}	20 kHz 40 W, 55°C, 5 min		*Salmonella* Enteritidis	2·30 log	
UV radiation	1400 W, 15 min	2 UV-C lamps of 150 W	Wastewater	*Escherichia coli*	Below the limits of the legislation	Naddeo *et al.* (2009)
UV radiation	30 s of UV radiations (14 W of which 3 W are emitted at 254 nm)	20 kHz, 5 s, 50 W l^{-1} 20 kHz, 5 s, 310 W l^{-1}	Wastewater	Faecal coliforms Faecal coliforms	3·30 log CFU ml^{-1} 3·70 log CFU ml^{-1}	Blume and Neis (2004)
	20 kHz, 95 μm, 20 min		Orange juice	*Escherichia coli*	1·86 log CFU ml^{-1}¶	Char *et al.* (2010)
	20 kHz, 95 μm, 10 min	UV at 100W at 257·70 nm 10 min		*Escherichia coli*	1·94 log CFU ml^{-1}¶	
	20 kHz, 95 μm, simultaneously with UV at 100W at 257·70 nm, 20 min			*Escherichia coli*	3·56 log CFU ml^{-1}¶	
Plasma	47 kHz, 140 W, 30 min	13 kV/60 Hz	Liquid medium	*Escherichia coli* *S. cerevisiae*	6 log CFU ml^{-1}	Chen *et al.* (2009)
Antimicrobial solutions (antimicrobials substances)	20 kHz 120 μm, 60°C	No antimicrobial 500 ppm vanillin 500 ppm potassium sorbate	Sterile broth	*Aspergillus flavus* *Aspergillus flavus* *Aspergillus flavus*	*D*-value 1·20 min *D*-value <0·50 min *D*-value <050 min	López-Malo *et al.* (2005)
	70 kHz, 20 mW cm^{-2}, 2 h	24 μg ml^{-1} of gentamicin	Biopolymeric surfaces	*Escherichia coli*	97%	Johnson *et al.* (1998)
Antimicrobial solutions (antimicrobials substances)	20 kHz, 150 μm, 40W cm^{-2},43°C 126 s	2·5 mg l^{-1} of nisin	Buffer system	*Listeria innocua*	2·80 log CFU ml^{-1}	Muñoz *et al.* (2012)
	20 kHz, 600 W, 95·2 μm, 45°C, 8 min	Combination of 1500 ppm of vanillin and 100 ppm of citral	Orange juice	*Listeria monocytogenes*	6 log CFU ml^{-1}¶	Ferrante *et al.* (2007)

(Continued)

Table 1 (*Continued*)

Ultrasound assisted by	Operating conditions		Food (model) system	Micro-organisms studied	Microbial reduction	References
Antimicrobial solutions (organic Acids)	24 kHz, 9 min	Citric acid (pH=2·5)	Orange juice	*Escherichia coli*	5·42 log CFU g^{-1}	Salleh-Mack and Roberts (2007)
		Malic acid (pH = 2·5)		*Escherichia coli*	5·37 log CFU g^{-1}	
	40 kHz 30 W l^{-1} 5 min	Malic acid at 2% (v/v)	Lettuce	*Escherichia coli* O157:H7	2·52 log CFU g^{-1}	Sagong *et al.* (2011)
				Salmonella Typhimurium	2·73 log CFU g^{-1}	
		Lactic acid at 2% (v/v)		*Escherichia coli* O157:H7	2·75 log CFU g^{-1}	
				Salmonella Typhimurium	2·71 log CFU g^{-1}	
		Citric acid at 2%(v/v)		*Escherichia coli* O157:H7	2·43 log CFU g^{-1}	
				Salmonella Typhimurium	3·18 log CFU g^{-1}	
	23·3 kHz, 150 W, 3 h	Chlorhexidine gluconate 5 w/v	Liquid suspension	*Bacillus subtilis*	52·30%	Gorman *et al.* (1990)
		Chlorhexidine gluconate 10 w/v		*Bacillus subtilis*	95·20%	
Antimicrobial solutions (sanitizers)	170 kHz, 10 min	40 ppm of ClO_2	Apples	*Salmonella* Typhimurium	4·25 log CFU g^{-1}¶	Huang *et al.* (2006b)
				Escherichia coli O157:H7	3·86 log CFU g^{-1}¶	
	32–40 kHz, 10 W l^{-1}, 10 min	25 ppm of Chlorine	Cut iceberg lettuce	*Salmonella* Typhimurium	2·70 log CFU g^{-1}	Seymour *et al.* (2002)
	45 kHz, 10 min	40 mg l^{-1} of peracetic acid	Cherry tomatoes	*S.* Typhimurium	3·88 log CFU g^{-1}	Brilhante Sao Jose and Dantas Vanetti (2012)
	2 mg l^{-1} chlorine dioxide, 10 min	No US treatment*	Waste water	*Escherichia coli*	0·80 log CFU g^{-1}	Ayyildiz *et al.* (2011)
		20 kHz, 500W, 150W l^{-1}		*Escherichia coli*	3·20 log CFU g^{-1}	
		20 kHz, 500W, 300W l^{-1}		*Escherichia coli*	3·50 log CFU g^{-1}	
	21·20 kHz, 200W l^{-1}, 2 min	Chlorinated water (200 mg l^{-1})	Spinach leaves	*Escherichia coli* O157:H7	3·10 log CFU g^{-1}	Zhou *et al.* (2009)
		AEW (80 mg l^{-1})†		*Escherichia coli* O157:H7	3·10 log CFU g^{-1}	
		POAA (80 mg l^{-1})‡		*Escherichia coli* O157:H7	2·90 log CFU g^{-1}	
		ASC (200 mg l^{-1})§		*Escherichia coli* O157:H7	4 log CFU g^{-1}	
Antimicrobial solutions (sanitizers)	40 kHz, 100 W, 10 min	40 mg l^{-1} ClO_2	Plums		Extend shelf life up to 60 days	Chen and Zhu (2011)
	37 kHz, 380 W, 5–20 min, 600–1000 ppm of NaClO	0·10 kGy	Raw rice	*Bacillus cereus* spores	Complete inactivation	Ha *et al.* (2012)
		0·20 kGy				
		0·30 kGy				

(Continued)

Table 1 (*Continued*)

Ultrasound assisted by	Operating conditions		Food (model) system	Micro-organisms studied	Microbial reduction	References
Antimicrobial solutions (enzymatic solutions)	40 kHz, 10 s	Protease (36 U ml^{-1})	Milk biofilms on stainless steel sheets	*Escherichia coli*	61%	Oulahal-Lagsir *et al.* (2003)
		Trypsin (7600 U ml^{-1})		*Escherichia coli*	76%	
		Amyloglucosidase (50 U ml^{-1})		*Escherichia coli*	96%	
		Papain (3 U ml^{-1})		*Escherichia coli*	76%	
		Lysozyme (6000 U ml^{-1})		*Escherichia coli*	70%	
	40 kHz, 10 s EDTA (0·025 mol l^{-1})	Trypsin (7600 U ml^{-1})	Meat biofilms on stainless steel sheets	*Escherichia coli*	50%	Oulahal *et al.* (2007)
		Protease (3 U ml^{-1})		*Staphylococcus aureus*	79%	
		Papain (3 U ml^{-1})		*Escherichia coli*	75%	
		Protease (36 U ml^{-1})		*Staphylococcus aureus*	70%	
Antimicrobial solutions (enzymatic solutions)	40 kHz, 10 s EDTA (0·025 mol l^{-1})	Trypsin (7600 U ml^{-1})	Meat biofilms on stainless steel sheets	*Escherichia coli*	27%	Oulahal *et al.* (2007)
		Lysozyme (6000 U ml^{-1})		*Staphylococcus aureus*	100%	
		Protease (36 U ml^{-1})		*Escherichia coli*	56%	
		Lysozyme (6000 U ml^{-1})		*Staphylococcus aureus*	74%	
		Lysozyme (6000 U ml^{-1})		*Escherichia coli*	54%	
		Papain (3 U ml^{-1})		*Staphylococcus aureus*	30%	
		Amyloglucosidase (50 U ml^{-1})		*Escherichia coli*	42%	
				Staphylococcus aureus	100%	

*Ultrasound.
†Acidic electrolysed water.
‡Peroxyacetic water.
§Acidified sodium chlorite.
¶As extracted from graph of the published work.

bursts of light from imploding bubbles in a liquid when excited by ultrasound) and free radical production until a certain maximum of pressure (Mañas *et al.* 2000). Overall, it is important to determine the critical pressure level for achieving the maximum synergetic effect. Above this pressure, there is a decrease in effectiveness, associated with a decrease of cavitation phenomena, because ultrasound waves are unable to overcome the combined cohesive forces of overpressure and the cohesive force of the liquid molecules (Condon *et al.* 2005).

Manothermosonication

Combination of heat, pressure and ultrasound can be applied to achieve a higher microbial inactivation. The effectiveness of this treatment can be related to two mechanisms acting separately, that is, heat treatment and manosonication (Raso *et al.* 1998a). Pagán *et al.* (1999) reported that the application of ultrasound waves at 117 μm and 20 kHz at ambient temperature and low pressure produced a *D*-value for *L. monocytogenes* of 4·30 min. When pressure was applied (200 kPa) (manosonication), the *D*-value was reduced to 1·50 min, while when heat was applied in combination with pressure (manothermosonication), no effect was observed in the inactivation rate up to 50°C. Nevertheless, *D*-values decreased significantly when higher temperatures were applied compared with cases that temperature and pressure were applied alone (e.g. the *D*-value for manothermosonication was 1 min, and the *D* value without the combined use of ultrasound was 2·37 min at 55°C and 200 kPa—data were extracted based on a graph illustration from the referred manuscript). Similar results were obtained by Alvarez *et al.* (2006), where ultrasound at 117 μm and 20 kHz in combination with pressure at 175 kPa and 35°C reduced the *D*-value of *Salmonella* Senftenberg in McIlvaine's citrate–phosphate buffer until 1·71 min. When the temperature was raised to 67°C, the *D*-value decreased to 0·02 min. Same synergetic effect of temperature was also described by Lee *et al.* (2009). In addition, manothermosonication is also able to inactivate sporulating bacteria. Raso *et al.* (1998b) reported that the application of manothermosonication (20 kHz, 117 μm, 70°C, 500 kPa) after 12 min reduced the *Bacillus subtilis* spore population by *c.* 99% in liquid medium.

According to the available literature, the separate effect of the two mechanisms is evident and the efficacy of manothermosonication is dependent on the applied temperature. At lower temperatures, <50°C, the predominant effect of inactivation is due to manosonication, and the effect of the heat is negligible. At higher temperatures, the microbial reduction rate is a result of the synergistic effect of heat and manosonication. Therefore, the identification of the synergistic effect of temperature on the microbial inactivation is a key point for optimizing this technology.

Osmosonication

Combination of ultrasound and high osmotic pressure has been named osmosonication. This technology can be used in products where heat treatments can damage nutritional compounds such as vitamins. A predictive modelling study carried by Wong *et al.* (2012) reported that osmosonication (ultrasound waves at 20 kHz, 50 W, 48 μm, for 13 min, treatment temperature of 25°C) followed by storage for 72 h at an osmotic pressure of 10·90 MPa with the use of sucrose [i.e. solution of 650 g kg^{-1} total suspended solids (TSS)] can be used in orange juice to reduce *Salmonella* spp. by more than 5 logs without affecting the properties of the juice. In addition, a response surface model was developed to describe the microbial reduction in relation to the sonication time and storage of the treated product. This statistical approach could give an indication on the required combinations of treatment and storage time to achieve at least a 5 log reduction.

Osmosonication may be an alternative to thermal processing in order to produce high-quality produce. However, more studies will be required to further assess the efficacy of this technology for a number of different food products.

Sonozonation

Sonozonation, the combination of ultrasound and ozone, has also been used for water decontamination. Jyoti and Pandit (2004) have shown that the application of ultrasound either by horn at 22 kHz and 240 W or water bath at 20·50 kHz and 120 W in combination with ozone (0·50 mg l^{-1}) for 15 min was able to decrease the total number of coliforms, faecal coliforms and faecal streptococci between 95 and 99·9% while reducing the dosage of chemical agent in water decontamination. This effectiveness can be related to the ability of ultrasound to increase the ozone gas–liquid transfer and decompose the dissolved ozone (Dahl 1976). Similarly, further studies will be required especially regarding the mechanism of action of this technology and its efficacy under different processing conditions.

Ultrasound and pulsed electric fields

Pulsed electric fields (PEF) is another nonthermal technology that can be combined with ultrasound to inactivate micro-organisms. The bactericidal mechanism of

PEF is based on the electroporation of microbial cell membranes due to repetitive application of short pulses of high-intensity electric fields (Jaeger *et al.* 2009). Palgan *et al.* (2012) reported 5·60 log CFU ml^{-1} reduction of *L. innocua* when Manothermosonication (MTS) was applied in smoothies at 20 kHz, 31 μm, 40 W cm^{-2} at 200 kPa and 52°C followed by PEF at 34 KV cm^{-1} and 32 μs. Nevertheless, when PEF was followed by MTS, by applying the same treatments as above, *L. innocua* reduction was only 4·20 log CFU ml^{-1}. In a similar approach, Huang *et al.* (2006a) carried out two variations in the procedure; ultrasound (20 kHz, 40 W, at 55°C during 5 min) followed by PEF (30 pulses at 5·67 kV mm^{-1}) and a similar PEF followed by similar ultrasound treatment to inactivate *Salmonella* enteritidis in liquid whole eggs, achieving 2·25 and 2·30 log reductions, respectively.

Overall, the sequence of the treatments plays a key role to achieve the highest inactivation level. It is evident that when ultrasound is applied firstly, it could affect the cell membrane properties and can have a synergistic effect with PEF. Gram-positive bacteria are less sensitive to PEF than Gram-negative bacteria (Hülsheger *et al.* 1983); therefore, the efficacy of ultrasound and PEF is dependent on the type of the target bacteria.

Ultrasound and UV radiation

Water contamination has become a serious issue for consumers' health. Recent outbreaks associated with fresh produce have been associated with the use of contaminated water for irrigation. This has led to the recognition of the need to establish new decontamination protocols that allow a higher and more effective microbial inactivation. According to Naddeo *et al.* (2009), wastewater treated with two low-pressure UV-C lamps of 150 W for 15 min was ineffective to reduce *E. coli* below the limit required by the waterwaste reuse according to the Italian legislation (10 CFU per 100 ml). However, UV radiations in combination with ultrasound (1400 W) at the same conditions were able to decrease the microbial load down to 2 CFU per 100 ml. In the same way, a research study carried out by Blume and Neis (2004) has shown that 30 s of UV radiation treatment (14 W of which 3 W are emitted at 254 nm) reduced faecal coliforms by up to 2·50 log units in water. Additionally, when the same process was followed by 5 s of ultrasound at 50 or 310 W l^{-1}, the microbial reduction achieved was 3·30 and 3·70 log units, respectively. Similarly, Char *et al.* (2010) reported that application of ultrasound at 20 kHz and 95 μm for 20 min resulted in 1·86 log reduction of *E. coli* in orange juice. Similar reduction was shown when ultrasound (20 kHz, 95 μm, 10 min) was followed by UV treatment (100 W at 257·70 nm and 10 min). However, when ultrasound was combined simultaneously with UV irradiation at the same conditions during 20 min, it led to an additional 1·60 log reduction. This proved that there was a synergistic effect when compared with treatments performed separately or in a sequence. However, the exact mechanism of action of the synergistic effects of UV and ultrasound still remains unknown, and further studies will be required to elucidate it.

Ultrasound and plasma

Ultrasound can be combined with other emerging technologies such as plasma. Preliminary studies carried out by Chen *et al.* (2009) reported that ultrasound at 140 W and 47 kHz in combination with plasma at 13 kV and 60 Hz for 30 min was able to reduce 6 log CFU ml^{-1} *E. coli* and *Saccharomyces cerevisiae* in liquid phase. This could be an interesting process for the decontamination of wastewater, and future studies will be needed.

Ultrasound and antimicrobial solutions

Assisted ultrasonic treatment processes could drastically enhance decontamination capabilities by increasing the diffusion of antimicrobial solutions in products, thereby addressing microbiological safety and quality issues. The phenomenon of acceleration of the mass transfer by the application of ultrasound in food systems has been reported and explained by several mechanisms (Knorr *et al.* 2004). These include the asymmetric cavitation near the solid surface that generates microjets in the direction of the surface (Lamminen *et al.* 2004). These microjets affect the mass transfer by disturbing the boundary layer of the ultrasound-treated surfaces. So far the phenomenon of microjet generation has been used for the injection of brine into meat samples, which produces an increase in both moisture and salt solutions (Cárcel *et al.* 2007; Siró *et al.* 2009). The development of assisted ultrasonic processes could enhance the efficacy of bioactive compounds, where much lower concentrations are required by comparison with water-assisted surface-washing treatments.

Ultrasound with natural antimicrobials

In recent years, food industry focused on using natural antimicrobial substances in food control. Plant extracts are widely used due to their antioxidant and antimicrobial properties. These properties can be related to the presence of chemical compounds (e.g. polyphenols, terpenes). These compounds can interact with the microbial membrane producing an increase in membrane permeability and leakage of intracellular compounds (Lambert *et al.* 2001; Schoenbach *et al.* 2002). Ferrante *et al.*

(2007) reported that thermosonication treatment at 45°C, frequency of 20 kHz, amplitude of 95·2 μm and energy of 600 W in combination with vanillin (1500 ppm) and citral (100 ppm) achieved a 6 log CFU ml^{-1} reduction of *L. monocytogenes* in orange juice. The reduction curves followed a Weibull model. According to several studies (Guerrero *et al.* 2001; Ferrante *et al.* 2007; Char *et al.* 2010), when ultrasound is applied on its own, first-order microbial kinetics were reported. However, when another stress factor is combined, the reduction curves are not linear (Guerrero *et al.* 2005).

According to the hurdle theory, similar decontamination approaches can be combined to improve microbial inactivation. For example, a study carried out by López-Malo *et al.* (2005) combined heat, ultrasound and antimicrobials to inactive *Aspergillus flavus*. Temperatures around 60°C and ultrasound at 20 kHz and 120 μm resulted in a *D*-value of 1·20 min for *A. flavus* spores in sterile broth. When the same treatment was performed in combination with either vanillin (500 ppm) or potassium sorbate (500 ppm), the *D*-value of *A. flavus* spores decreased to <0·5 min.

Micro-organisms can be a source of other natural compounds with antimicrobial properties. As an example, nisin, synthesized by *Lactococus lactis*, has generally been used as an antimicrobial agent, especially against Gram-positive bacteria. The capacity of nisin to kill Gram-positive bacteria is due to the presence of the uncommon amino acid of lanthionine and its interaction with microbial membranes. A study by Muñoz *et al.* (2012) has shown that the application of ultrasonic waves at 20 kHz of frequency, 150 μm of amplitude and 40 W cm^{-2} of acoustic power energy without nisin produced a 0·30 log CFU ml^{-1} reduction in *L. innocua*. However, the addition of nisin at 2·50 mg l^{-1} resulted in pronounced antimicrobial effect of the ultrasound against *L. innocua* resulting in reduction of 2·80 log CFU ml^{-1}. Moreover, the combination of ultrasound with nisin and either high-intensity pulse light or pulsed electric fields raised the microbial inactivation to 5·60 and 4·80 logs, respectively. Gentamicin is another natural antimicrobial, in this case synthesized by *Micromonospora*. Its effectiveness has been reported against Gram-negative bacteria, by interrupting protein synthesis. Johnson *et al.* (1998) reported that a combination of ultrasound treatment of 70 kHz and of 20 mW cm^{-2} for 2 h combined with the use of gentamicin sulphate at 24 μg ml^{-1} on a biofilm reduced the populations of *E. coli* by 97%, due to the ability of ultrasound to destabilize the cell membrane and therefore allowing the diffusion of the antibiotic through it.

Consumers demand food products with low levels or absence of chemical preservatives. To reduce the dosage of these natural and synthetic antimicrobials, the application of assisted ultrasound is a viable alternative. However, natural antimicrobials can have an effect on the sensory attributes of the products. Therefore, studies based on laboratory media should be further validated in real foods to identify a range of tolerable taste thresholds (Alzamora *et al.* 2003).

Ultrasound with sanitizers

Ultrasound can also be combined with chlorine to improve its efficacy. Research carried out by Seymour *et al.* (2002) has shown that high-power ultrasound (32–40 kHz) increased the antimicrobial activity of chlorine because through ultrasound cells of *S.* Typhimurium were removed from the surface of the fresh produce. Huang *et al.* (2006b) reported that ultrasound at 170 kHz in combination with 40 ppm of chlorine dioxide during treatment of 10 min was able to reduce *S.* Typhimurium by 4·25 log CFU g^{-1} and *E. coli* O157:H7 by 3·56 log CFU g^{-1} on previously inoculated apples. A study carried out by Chen and Zhu reported that the shelf life of plums becomes unacceptable after 40 days of storage (4°C). However, a combination of aqueous solution of chlorine dioxide at 40 mg l^{-1} and ultrasound at 40 kHz of frequency and 100 W of energy during 10 min can extend the shell life of plums by 60 days. This technology can also be applied to decontaminate wastewater. Ayyildiz *et al.* (2011) have shown that the application of chlorine dioxide at 2 mg l^{-1} with ultrasound at 150 and 300 W l^{-1} of acoustic energy density produced a synergetic effect on the decontamination of *E. coli* in wastewater and led to 3·20 and 3·50 log of reduction, respectively. Chlorine dioxide treatment alone only reduced *E. coli* 0·80 logs. The explanation of this synergetic effect can be related not only to the ability of ultrasound to kill microbial cells by cavitation but also to its capacity of detaching microbes from the sample surfaces, enhancing the sanitizers' decontamination efficacy (Ayyildiz *et al.* 2011; Chen and Zhu 2011).

The ability of ultrasound to be combined with other decontamination technologies can contribute to the disruption of sporulating bacteria, which are more resistant than vegetative forms. Thus, ultrasound with sanitizers can be combined with ionizing radiations to enhance its effectiveness and inactivate spores. Ha *et al.* (2012) reported that ultrasound at 37 kHz, 380 W for 5–20 min with sodium hypochlorite (600–1000 ppm) in combination with ionizing radiations (0·10, 0·20, 0·30 kGy) was able to inactivate completely an initial population of 2·90 CFU g^{-1}of *Bacillus cereus* spores in raw rice.

Concluding, traditional washing methods using sanitizers may not work effectively for microbial inactivation in cases that bacteria are attached on rough surfaces or form

biofilms. Therefore, the ability of ultrasound to disengage the bacteria can contribute to enhancing the microbial decontamination by the combined use of disinfectants. This assisted ultrasound technology could be considered for the effective decontamination of fruits and vegetables.

Ultrasound with organic acids

Organic acids can replace the use of chlorine in fresh produce sanitation with the advantage of not exhibiting any risk to consumer health. These acid compounds have also been commonly used by the food industry as additives in produce conservation. The ability of organic acids to reduce environmental pH and penetrate through the microbial membrane dissociating inside the bacterial cytoplasm has been described as the main mechanism for microbial inactivation. According to Sagong *et al.* (2011), a combination of several organic acids [malic acid, lactic acid, citric acid at 2%(w/v)] and ultrasound (40 kHz of frequency and 30 W l^{-1} of Acoustic Energy Density) produced an increase in reduction of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* on lettuce by about 0·80–1 log CFU g^{-1} compared with the individual treatments. Salleh-Mack and Roberts (2007) have shown that ultrasound at frequency of 24 kHz in combination with citric acid and malic acid at pH 2·5 resulted in 5·42 and 5·37 log reduction of *E. coli* in orange juice after 9 min of treatment.

Moreover, other traditional decontamination solutions are being used. A study carried out by Brilhante Sao Jose and Dantas Vanetti (2012) reported that an ultrasound treatment at 45 kHz for 10 min in the presence of 40 mg l^{-1} of peracetic acid led to reduction of 3·88 log CFU g^{-1} of *S.* Typhimurium in whole cherry tomatoes. In the same way, Zhou *et al.* (2009) applied ultrasound at 21·20 kHz and 200 W l^{-1} in a solution of peroxyacetic (POAA) acid at 80 mg l^{-1} to improve the reduction in *E. coli* O157:H7 on spinach leaves (by up to 2·90 log CFU g^{-1}). In the same study, application of ultrasound at the same conditions but in this case in combination with acid electrolysed water (AEW) (80 mg l^{-1}), chlorinated water (200 mg l^{-1}) or Acidified Sodium Chlorite (ASC) (200 mg l^{-1}) reduced the population of *E. coli* O157:H7 by 3·10, 3·10 and 4 log CFU g^{-1}, respectively.

Additionally, organic acids can be used in combination with ultrasound to inactivate bacterial spores. One of the first reports is that of Gorman *et al.* (1990) who showed that a treatment with ultrasound at 150 W and 23·3 kHz in combination with chlorhexidine gluconate at 5% and 10% w/v reduced the viability of the initial *B. subtilis* population (10^6 CFU ml^{-1}) to levels of 47·70 and 4·80%, respectively. Nevertheless, the process took 3 h, making it unattractive for industry applications.

Ultrasound in combination with organic acids can be widely applied to a range of solid and liquid food products such as lettuce leaves, spinach leaves, tomatoes and juices. However, the effectiveness of the treatment is dependent on the uniformity of the ultrasound propagation in the ultrasonic baths. This is because of the formation of standing waves and the ultrasound propagation blockage by the solid food materials (Zhou *et al.* 2009). In addition, the type of organic acid, the sonication time and pH achieved in the medium are critical factors in increasing the inactivation rate of the target bacteria (Utsunomiya and Kosaka 1979; Hsiao and Siebert 1999).

Ultrasound with enzymatic solutions

Ultrasound can also be combined with enzymatic solutions. Oulahal-Lagsir *et al.* (2003) reported that ultrasound at 40 kHz for 10 s alone was able to remove only 30% of an *E. coli* model biofilm that was constructed using milk on stainless steel sheets. By contrast, when ultrasound was combined with different enzymatic treatments such as protease (36 U ml^{-1}), trypsin (7600 U ml^{-1}), amyloglucosidase (50 U ml^{-1}), papain (3 U ml^{-1}) and lysozyme (6000 U ml^{-1}), the percentage of biofilm removed was in the range of 61, 76, 96, 76 and 70%, respectively.

Further work carried out by Oulahal *et al.* (2007) was performed on meat biofilms of *E. coli* and *Staph. aureus* on stainless surfaces. Ultrasound alone, at the same conditions as those described previously, was able to detach 49% for *E. coli* and 39% of *Staph. aureus.* However, when ultrasound was combined with EDTA (0·025 mol l^{-1}) and a cocktail of enzymes, these values were increased. A cocktail of papain (3 U ml^{-1}) and protease (36 U ml^{-1}) resulted in the highest value (75%) of *E. coli* detachment. Regarding *Staph. aureus* detachment, amyloglucosidase (50 U ml^{-1}) and a mix of trypsin (7600 U ml^{-1}) and lysozyme (6000 U ml^{-1}) achieved 100%.

Biofilms created on food processing pipes contribute to microbial regrowth (Chandy and Angles 2001). The ability of ultrasound to remove biofilms from surfaces as well as its ability to be combined with enzymatic treatments provides an effective technology to decontaminate food contact surfaces (Brisou 1995).

Industrial applications and energy needs

One of the main benefits of installing ultrasonic technology in industrial applications is the absence of moving parts, that is, lack of rotors, seals, grease, which also contributes to resource efficient manufacturing. Herceg *et al.* (2012) assessed the energy requirements for milk treatment at amplitude levels as low as 90 μm, temperature of 60°C and treatment time of 9 min. They found that the

amount of energy required is about 0·19 J ml^{-1}. This treatment reduced *Staph. aureus* populations from 2·10 log CFU ml^{-1} to below the maximum acceptable limits for this hazard (<1 log CFU ml^{-1} after pasteurization process). In addition, previous reports have shown that *c.* 85% of the power sent to the transducer is transferred into the medium. Jyoti and Pandit (2004) reported that the energy consumption to inactivate coliforms in drinking water using an ultrasonic horn at 240 W and 24 kHz had a cost as low as 0·035 US $ l^{-1}.

The energy for conventional thermal pasteurization of juice with heat regeneration or recovery is about 35 J ml^{-1} (Kozempel *et al.* 1998). Additionally, the energy for nonthermal pasteurization of juice by electromagnetic processes, pulsed electric fields and radiofrequency electric fields, is estimated to be in the range of 100–400 J ml^{-1} (Barsotti *et al.* 2001; Schoenbach *et al.* 2002; Geveke and Brunkhorst 2008), which is much higher than that reported for ultrasound treatments of other fluid foods (see the reference above for 0·19 J ml^{-1} energy requirements for milk treatment). Moreover, the application of ultrasound does not require the use of water; it is implemented directly to the product. It is therefore evident that this technology will have a huge impact on reducing water usage. Nevertheless, to the knowledge of the authors, there are no reported studies on the energy requirements of assisted ultrasound and their comparison with conventional technologies.

Impact

Assisted ultrasound is expected to increase the safety of fresh produce and processed foods and extend their shelf life, while maintaining or improving organoleptic characteristics. This will provide the industry with a powerful marketing advantage and is expected to amplify consumer confidence and instil a positive perception of fresh produce and processed products. In particular, the market of 'fresh convenient foods' could be further supported considering its significant growth in the coming years as a result of the lifestyle trends and the increased consumer demand for products that are not only healthy, but also convenient. This will promote the replacement of chlorine by some of the discussed technologies, and consequently, it will reduce or even eliminate the potential health and environmental risks associated with the formation of carcinogenic halogenated decontamination by-products. Consequently, small-medium enterprises (SMEs) will produce competitive products free from harmful chemicals and safer for human health and the environment with potential for accessing wider markets in a cost-effective manner. Environmental and economic benefits will also be derived from reduced water usage. Overall, assisted ultrasound technologies will help with decreasing the environmental impact associated with the whole supply chain as they are expected to increase the shelf life of food products and reduce the associated food waste.

Future prospects

Ultrasound has widely been applied in combination with other decontamination technologies to decrease the number of micro-organisms on different food products. However, further research should be focused on studying the cavitation phenomena when ultrasound is 'assisted' by other technologies. This would allow understanding of the different microbial inactivation mechanisms and the synergistic effects from the application of the different technologies.

Studies with knockout mutants (like those proposed by Patil *et al.* 2011) having different susceptibility to thermal, oxidative and other stresses will permit the identification of specific regulons playing a key role in microbial stress responses generated during assisted ultrasound treatments. Additionally, transcriptome analysis using, for example, next-generation sequencing technology will revolutionize the identification of critical genes in individual microbial species during the application of these process technologies. This could facilitate the quantification of (a) genomic biomarker(s) related to the microbial-assisted ultrasound stress adaptation of selected food-borne pathogenic bacteria.

More studies on Gram-positive- and Gram-negative-resistant vegetative micro-organisms must be performed to assess and optimize assisted ultrasound technology in order to produce safe food products or produce. Furthermore, few studies have shown the effect of assisted ultrasound technologies on spores (e.g. Gorman *et al.* 1990; Raso *et al.* 1998b; Ha *et al.* 2012; Sagong *et al.* 2012).

Assisted ultrasound technologies have the potential to be adapted to any kind of food production line and any kind of food product application. Optimization of the operating parameters, allowing energy and cost savings, is appearing as a major objective for the further application of these technologies. Similarly, Bilek and Turantaş (2013) in a recent review (which appeared during the preparation of this manuscript) of HPU in fruit and vegetable industry pinpointed the need of identifying best conditions, doses and combination of treatments for the further commercial adaptation of ultrasound.

Conclusion

Recent concerns about food-borne illness have highlighted the need of producing safe food products for consumers. Although traditional decontamination technologies may be appropriate for producing safe food

products, new consumer habits demand food produce with the maximum amount of health-promoting ingredients, preserving all the nutritional compounds and being free of chemical residues. Therefore, this has highlighted the need of developing new decontamination technologies. Ultrasound has been considered a decontamination technology with high potential due to its eco-friendly and nonthermal properties, also allowing energy and cost savings. Nonetheless, ultrasound alone is not sufficient to decrease microbial loads in order to meet existing food legislation limits. Thus, ultrasound can be combined with other decontamination technologies (e.g. heat, pressure, antimicrobial solutions and other emerging technologies) to enhance its efficacy. Assisted ultrasound technology can be applied to a wide set of food produce and food products. However, further research must be performed to understand the microbial inactivation kinetics, the different inactivation mechanisms and synergetic effects of assisted ultrasound technology in order to achieve a feasible decontamination technology.

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Conflict of interest

The authors declare that they have no conflict of interest.

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