CHAPTER 5

Ultrasonic Processing Technology for Postharvest Disinfection

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1. INTRODUCTION

The consumption of fresh fruits and vegetables (FFVs) has increased in recent years, because they are an essential part of a healthy diet, and their intake is continually recommended by many health organizations around the globe (FAO/WHO, 2008). Increased attention has been paid to the quality and safety of FFVs and other fresh foods, in synergy between individual health-conscious consumers and government agencies (Alexandre et al., 2012).

The disinfection methods used on fresh foods during the production chain (harvesting, postharvest handling, processing, and transportation) are mainly washing with water and/or chemical sanitizing solutions (Sapers, 2001). Chlorinated solutions are widely used due to their simple handling, low cost, water solubility, and stability over prolonged storage periods. Unfortunately, chlorine is linked to the formation of potentially mutagenic or carcinogenic reaction by-products (USDA, 2011), which imposes limitations on its indiscriminate use. It has been demonstrated that certain microorganisms are more tolerant to chlorinated compounds than others (Ramos et al., 2013), which has lead several European countries to ban its use on fresh products (FAO/WHO, 2008). But the complete removal or inactivation of microorganisms from FFVs continues to be a challenge, because an effective disinfection process that can be safely and efficiently used on these kinds of products has been difficult to perfect. Some bacteria are attached or entrapped on the surfaces of FFVs and are not readily accessible to the sanitizers (Seymour et al., 2002), which further complicates the problem. Hence, an effective and safe water-based disinfection method that can be used on FFVs is still required.

Some emerging technologies are an alternative to the use of chlorine-based solutions (USDA, 2011), and in particular, ultrasound (US). It has been demonstrated that US is an excellent option to clean surfaces in the electronics industry, and its use has been recommended on FFVs (Chemat et al., 2011; São José et al., 2014b). The first study that used

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US as a disinfection agent on FFVs was conducted in 2002 by Seymour et al. Since then, many experiments have been conducted that focused on different methods to evaluate the influence of US in combination with chemical solutions on FFVs.

2. PHYSICAL PRINCIPLES OF ULTRASOUND IN AN AQUEOUS MEDIUM

The US is a form of energy that is transmitted by sound waves at frequencies of up to 20 kHz. Ultrasonic power is easily transferred through the treated medium (Mason et al., 1996), and can be measured in terms of power (W), intensity (W/cm^2), or acoustic energy density (W/mL) (O'Donnell et al., 2010). High-intensity US, with frequencies of 20–100 kHz, generates an intense pressure, shear force, and a temperature gradient into the material, which physically disrupts its structure, or promotes different chemical reactions (Earnshaw et al., 1995). When acoustic energy passes through a medium, particularly an aqueous one, a continuous undulation motion is produced by the mechanical vibrations and three types of waves are generated: (1) longitudinal waves that move in the direction of the displacement, (2) shear waves that are perpendicular to the movement to the original waves, and (3) *Rayleigh* waves, which travel very close to the surface of the medium (Mulet et al., 1999).

At the time of wave propagation, alternating compression/expansion cycles are generated by different types of waves. During these cycles, millions of small bubbles are formed, which grow by absorbing energy from the medium, and when they cannot absorb more energy, they become unstable and violently implode. This releases high amounts of energy, in a process known as cavitation, which is the most significant way that transmits ultrasonic power with in a liquid medium (Mason et al., 1996).

The collapse of a cavitation bubble can occur on the surface of a cell wall or in close proximity to it. When it happens on the surface of a cell, it can potentially punch holes through the cell wall, which further exposes new surfaces to an increasing mass and energy transfer, and culminates in the disruption of the structure and function of the cell wall, as demonstrated by Li et al. (2017).

There are discontinuous and continuous US systems, and because of the conformation of discontinuous systems (Fig. 1A and B), they are the most widely used for disinfection of FFVs (Table 1). In contrast, continuous systems (Fig. 1C) are only used in liquid products. A major advantage of US over other techniques in the food industry is that sound waves are generally considered safe, nontoxic, and they do not generate unpleasant odors and are environmentally friendly (Chemat et al., 2011).

The physical and chemical effects of an US treatment are closely related to the operational parameters such as amplitude, frequency, treatment time, temperature, volume processed among others (Ramos et al., 2013; Millan-Sango et al., 2017).

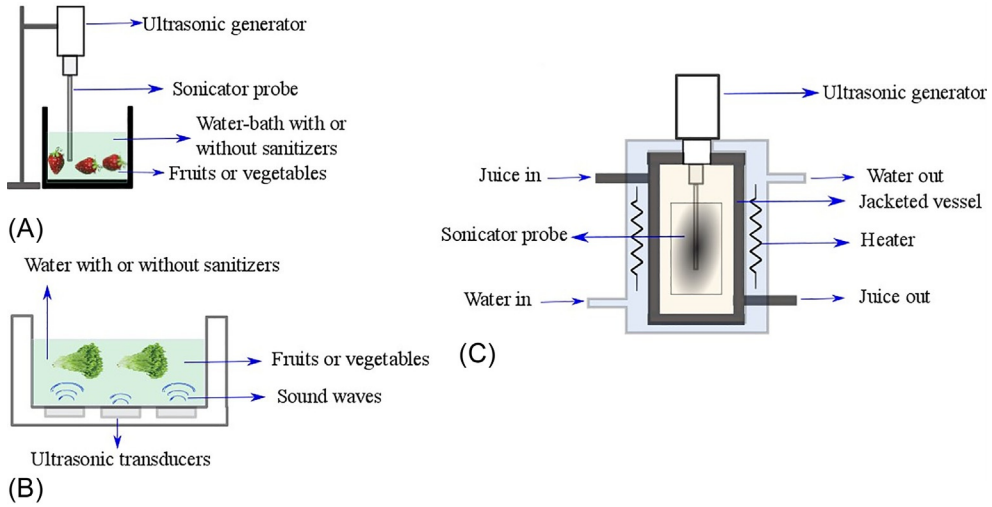

Fig. 1 (A) and (B) Immersion batch and (C) continuous flow through ultrasonic systems. *(Adapted from Anaya-Esparza, L.M., Velázquez-Estrada, R.M., Roig, A.X., García-Galindo, H.S., Sayago-Ayerdí, S.G., Montalvo-González, E., 2017. Thermosonication: an alternative processing for fruit and vegetable juices. Trends Food Sci. Technol. 61, 26–37.)*

3. EFFECTS OF ULTRASOUND ON SPOILAGE AND PATHOGENIC MICROORGANISMS

Under natural conditions, the outer layer of FFVs provides a natural barrier for microorganisms, but this natural barrier is lost during postharvest handling. The surfaces of FFVs can be smooth, rough, porous or irregularly shaped, thus, the adherence of microorganisms to them varies, as does the effectiveness of the sanitizing method. The removal of microorganisms from the surfaces of FFVs is a challenge that the industry has yet to perfect (São José et al., 2014b). High-intensity US alone, or in combination with some sanitizers, has been used to facilitate the disinfection of many FFVs (Table 1), and the results vary according to the experimental conditions and the type of microorganism.

Wang et al. (2009) demonstrated a positive correlation between roughness ($R^2 = .96$) and adhesion of *Escherichia coli* O157:H7 on the surfaces of FFVs with different surface roughness. Nonetheless, an increased elimination rate (3 log CFU/cm^2) of *Salmonella enterica* and *E. coli* from the surfaces of green pepper and melon was obtained, when US and organic acids (such as 1% lactic and acetic acid) were simultaneously applied to them (São José et al., 2014b). The authors mentioned that surface roughness of the FFVs had a direct influence on the effectiveness of the US treatment. These results are in agreement with those reported by other authors who used US (5–10 min) to disinfect the surfaces of lettuce, alfalfa, spinach, radish sprout, apples with normal surfaces and apples, pears, and truffles with cut surfaces, with or without organic/chemical

Table 1 Use of ultrasound, alone or in combination with chemicals to eliminate spoilage and pathogenic microorganisms from different fruits and vegetables

Fruit or vegetable	Microorganism	Form of the microorganism	Treatment	Ultrasonic equipment	[a]Experimental conditions	Microbial reduction (log cycles)	Reference
Strawberry	*Salmonella enterica*	Vegetative cells	US + peracetic acid (40 mg/L)	Ultrasonic water bath at 500 W	40 kHz; 5 min	2.0	Alves do Rosário et al. (2017)
Cucumber	*Cronobacter sakazakii*	Vegetative cells	US + peroxyacetic acid (150 ppm)	Ultrasonic water bath at 380 W	37 kHz; 60 min	3.1	Bang et al. (2017)
Alfalfa	1) *Salmonella enterica* 2) *Escherichia coli*	Vegetative cells	US alone	[b]Probe of 31 mm diameter	26 kHz; 90 μm; 5 min	(1) 1.4 (2) 1.9	Millan-Sango et al. (2017)
Strawberry	1) Total aerobic bacteria 2) Yeast and molds	Vegetative cells	US alone	Ultrasonic water bath at 60 W	33 kHz; 60 min	(1) 1.6 (2) 1.5	Gani et al. (2016)
Lettuce	*Salmonella enterica*	Vegetative cells	US + EOO (0.01%)	Ultrasonic water bath at 200 W	26 kHz; 90 μm; 5 min	3	Millan-Sango et al. (2016)
Lettuce	*Cronobacter sakazakii*	Vegetative cells	US + NaOCl (200 ppm)	Ultrasonic water bath at 200 W	37 kHz; 100 min	4.44	Park et al. (2016)
Tomato	1) Total aerobic bacteria 2) Yeast and molds	Vegetative cells	US alone	Ultrasonic water bath	45 kHz; 100%; 19 min	1) 2.95 2) <1	Pinheiro et al. (2015)
Lettuce	*Escherichia coli*	Vegetative cells	US + EOO (0.01%)	Ultrasonic water bath at 200 W	26 kHz; 90 μm; 5 min	4.6	Millan-Sango et al. (2015)

[a)] Watercress, [b)] Parsley [c)] Strawberry	[1)] Total aerobic bacteria [2)] Yeast and molds	Vegetative cells	US + peracetic acid (40 mg/L)	Ultrasonic water bath at 200 W	45 kHz; 10 min; 25°C	[1a)] 6.5 [2a)] 3.3 [1b)] 6 [2b)] 5 [1c)] 4.1 [c)] 4	São José and Vanetti (2015)
[a)] Cherry tomatoes [b)] Strawberries	[1)] Total aerobic bacteria [2)] Yeast and molds	Vegetative cells	US + SAEW	Ultrasonic water bath at 240 W	40 kHz; 10 min; sample-SAEW (1:10)	[1a)] 1.8 [2a)] 1.3 [1b)] 1.5 [2b)] 1.3	Ding et al. (2015)
Pear	[1)] *Salmonella enterica* Enteritidis [2)] *Escherichia coli*	Vegetative cells	US + organic acids	Ultrasonic water bath at 200 W	40 kHz; + 1% [x)]lactic/[y)]acetic acid	[1x)] 1.9 [1y)] 1.9 [2x)] 1.6 [2y)] 1.3	São José et al. (2015)
Iceberg lettuce	*Listeria monocytogenes*	Vegetative cells	US + NaOCl (200 ppm)	Ultrasonic water bath at 1200 W	37 kHz; 100 min	1.66	Lee et al. (2014)
[a)] Green peppers [b)] Melons	[1)] *Salmonella enterica* Enteritidis [2)] *Escherichia coli*	Vegetative cells	US + organic acids	Ultrasonic water bath at 200 W	40 kHz + 1% lactic/acetic acid	[1a)]2.9 [2a)]2.8 [1b)]2.5 [2b)]3.1	São José et al. (2014a, b)
[a)] Lettuce [b)] Carrots	*Bacillus cereus* ATCC 10876	Spores	US + surfactants (0.1%)	Ultrasonic water bath	40 kHz; 30 W/L; 40 μm; 5 min	a) 2.49 b) 2.22	Sagong et al. (2013)
[a)] Lettuce [b)] Radish sprout [c)] Apple (normal surface) [d)] Apple (cut surface)	[1)] *Escherichia coli* [2)] *Listeria monocytogenes* [3)] *Salmonella typhimurium*	Vegetative cells	US + CaO (2%)	Ultrasonic water bath at 130 W	20 kHz; 10 min at room temperature	[1a)] 3.6 [1b)] 3 [1c)] 2.3 [1d)] 4.4 [2a)] 3.7 [2b)] 3.7 [2c)] 1.5 [2d)] 4.7 [3a)] 2.5 [3b)] 2.8 [3c)] 1.9 [4d)] 4.1	Yoon et al. (2013)
Lettuce	[1)] Total aerobic bacteria	Vegetative cells	US + SAEW + temperature	Ultrasonic water bath	40 kHz; 400 W/L: 40°C; 3 min	7	Forghani et al. (2013)
Red bell pepper	*Listeria innocua*	Vegetative cells	US alone	Ultrasonic water bath at 120 W	35 kHz; 15°C; 2 min Sample-water (1:25)	1.98	Alexandre et al. (2013)

Continued

Table 1 Use of ultrasound, alone or in combination with chemicals to eliminate spoilage and pathogenic microorganisms from different fruits and vegetables—cont'd

Fruit or vegetable	Microorganism	Form of the microorganism	Treatment	Ultrasonic equipment	Experimental conditions	Microbial reduction (log cycles)	Reference
Strawberry	[1)] Total aerobic bacteria [2)] Yeast and molds	Vegetative cells	US alone	Ultrasonic water bath at 120 W	35 kHz; 15°C, 2 min	<1 log in both cases	Alexandre et al. (2012)
Cherry tomatoes	*Salmonella enterica* Typhimurium ATCC 14028	Vegetative cells	US + peracetic acid (40 mg/L)	Ultrasonic water bath at 200 W	45 kHz; 10 min	3.88	São José and Vanetti (2012)
Lettuce	[1)] *Salmonella typhimurium* [2)] *Escherichia coli* O157:H7 [3)] *Listeria monocytogenes*	Vegetative cells	US + organic acids	Ultrasonic water bath	40 kHz; 30 W/L; 5 min; 2% [x] lactic/ [y] citric/ [z] malic acid	[1x)] 3.2 [1y)] 2.7 [1z)] 2.7 [2x)] 2.4 [2y)] 2.7 [2z)] 2.5 [3x)] 2.9 [3y)] 2.3 [3z)] 2.5	Sagong et al. (2011)
Truffles	*Pseudomonas spp.*	Vegetative cells	US + ethanol (70%)	Ultrasonic water bath	35 kHz; 10 min	4	Rivera et al. (2011)
Plum fruit	[1)] Total aerobic bacteria [2)] Yeast and molds	Vegetative cells	US alone	Ultrasonic water bath at 100 W	40 kHz; 10 min; Sample-water (1:5)	[1)] 3 [2)] 2	Chen and Zhu (2011)
Strawberry	[1)] Total aerobic bacteria [2)] Yeast and molds	Vegetative cells	US alone	Ultrasonic water bath at 350 W	40 kHz; 10 min; 20°C	[1)] 0.6 [2)] 0.5	Cao et al. (2010)
Spinach	*E. coli*	vegetative cells	US + acidified sodium chlorine (200 mg/L)	[b]Probe of 31 mm diameter	21 kHz; 200 W/L; 2 min	4	Zhou et al. (2009)

Shredded carrot	[1)] Total aerobic bacteria [2)] Yeast and molds	Vegetative cells	US + chlorine water at 25 ppm of free chlorine	Ultrasonic water bath	45 kHz; 1 min	[1)] 1.3 [2)] 0.9	Alegria et al. (2009)
Lettuce	Total aerobic bacteria	Vegetative cells	US + $Ca(ClO)_2$ at 100 mg/L	Ultrasonic water bath	20 kHz; 50°C	2.5	Ajlouni et al. (2006)
[a)] Apple [b)] Lettuce	[1)] *Salmonella enterica* Enteritidis [2)] *Escherichia coli*	Vegetative cells	US + ClO_2 (5 ppm)	Ultrasonic water bath	170 kHz; 10 min	[1a)] 4 [2a)] 3.5 [1b)] 2 [2b)] 2	Huang et al. (2006)
Alfalfa seeds	[1)] *Salmonella* spp. [2)] *Escherichia coli* O157:H7	Vegetative cells	US + chemicals (1%) + Temperature	Ultrasonic water bath	40 kHz; 55°C; $Ca(OH)_2$	[1)] 3.3 [2)] 3.9	Scouten and Beuchat (2002)
Iceberg lettuce	*Escherichia coli*	Vegetative cells	US alone	Ultrasonic water bath at 200 W	40 kHz; 10 W/L; 10 min	1.5	Seymour et al. (2002)

[a]Optimal experimental condition: frequency (kHz); amplitude (μm or %); processing time (min); final temperature (°C); ultrasonic intensity (W); acoustic energy density (W/mL); *SAEW*, slightly acidic electrolyzed water; *ClO_2*, chlorine dioxide; *EOO*, essential oregano oil; *CaO*, calcium oxide; *NaOCl*, sodium hypochlorite; *$Ca(ClO)_2$*, calcium hypochlorite.
[b]Ultrasonic equipment coupled on circulating water bath.

sanitizers (lactic, citric, malic, and acetic acids, essential oregano oil, sodium and calcium hypochlorite, chlorine dioxide, chlorine solution, calcium oxide, and ethanol at 70%) using *S. enterica*, *Listeria monocytogenes*, *Pseudomonas* sp., and *E. coli* as indicators (Millan-Sango et al., 2016, 2017; São José et al., 2015; Lee et al., 2014; Rivera et al., 2011; Sagong et al., 2011; Zhou et al., 2009; Ajlouni et al., 2006; Huang et al., 2006).

The effect of US-generated cavitation enhances the removal of attached or entrapped microbial cells on the surfaces of FFVs, exposing the bacteria to the antimicrobial solutions (Seymour et al., 2002). Ramos et al. (2013) mentioned that the potential of US relies on improving the effectiveness of aqueous sanitizers, by increasing the penetration of these solutions to inaccessible sites, such as hydrophobic pockets and folds in leaf surfaces of FFVs.

Gani et al. (2016), during US decontamination of strawberry, reported a higher reduction in spoilage microorganisms (TAB = total aerobic bacteria, YM = yeast and molds) when the treatment time was increased. This data coincided with the report of Pinheiro et al. (2015), who treated tomatoes with US. On the other hand, São José and Vanetti (2015) showed that the highest reduction (4–6.5 log) on natural contaminant population of watercress, parsley, and strawberry was obtained when US (45 kHz, 10 min) and peracetic acid (40 mg/L) were simultaneously applied. Ding et al. (2015) used US (40 kHz, 10 min) in combination with slightly acidic electrolyzed water (SAEW) to decontaminate cherry tomatoes and strawberries, and reported a good reduction of TAB (1.8 and 1.3 log, respectively) and YM (1.5 and 1.3 log, respectively). Likewise, a high TAB reduction (sixfold log) was obtained by Forghani et al. (2013), when they treated lettuce with SAEW and US at 40°C. In the same way, a synergistic effect between US-heat chemicals (40 kHz, 55°C and $Ca(OH)_2$) was reported when *Salmonella* spp. and *E. coli* O157:H7 were inactivated on alfalfa seeds (Scouten and Beuchat, 2002).

Bang et al. (2017) evaluated the impact of US (37 kHz) in combination with peracetic acid (150 ppm) on *Cronobacter sakazakii* biofilms on fresh cucumber. After 60 min of treatment, the biofilm-producing ability of *C. sakazakii* decreased by 40%, and a cell reduction of 3.1 log was observed. Although the authors concluded that an US treatment (45 kHz) for short time (10 min) and at low concentration (40 mg/L) of the same sanitizer can effectively inhibit biofilm formation and achieved a reduction (3.8 log) of *Salmonella typhimurium* on the surface of cucumbers (São José and Vanetti, 2012).

There are many theories about the microbial detachment mechanism and/or their inactivation during US treatment, but many authors agree that cavitation is largely responsible. During stable cavitation, the microscopic bubbles on every surface/crevice of the submerged products remove the attached or entrapped cells present on the surfaces of FFVs (Sagong et al., 2011; Sapers, 2001). And during transient cavitation, free radicals are produced due to water sonolysis, which have important bactericidal properties, particularly because of DNA damage, as mentioned by Earnshaw et al. (1995). There are a number of lethal injuries that US can inflict on microbial cells, which involve the cell

wall, cytoplasmic membrane, and inner structure (Li et al., 2017). These are potentiated by low concentrations of some acids such as lactic, citric, malic, and peracetic acid, surfactants or calcium oxide among others (Sagong et al., 2011; Bang et al., 2017; São José and Vanetti 2015).

Ananta et al. (2005) reported that *E. coli* (Gram-negative) was more sensitive than *Lactobacillus rhamnosus* (Gram-positive) in response to US, concluding that the peptidoglycan layer in the cell wall of Gram-positive bacteria might provide better resistance than Gram-negative bacteria to US (Scherba et al., 1991). Li et al. (2017), using transmission electron microscopy and scanning electron microscopy, observed morphological changes in *Staphylococcus aureus* that were induced by US and SAEW. They reported both external and internal damage on microbial cells, which resulted in an outflow of some cellular contents. The authors mentioned that the microbial cells usually adhere to surfaces in a conglomerate form, in this context, US promoted colony disruption, and dispersed the aggregate into single cells. This phenomenon increases the probability of interacting with the chemicals, fragmenting cell walls and membranes, and ultimately lysing the cells. Similar to the report of Li et al. (2017), Tan et al. (2017) reported a high US-mediated damage to the flagella of *Salmonella typhimurium* cells. This reduces the possibility of attachment to the surface of FFVs. They also mentioned that cavitation facilitated the disintegration of microorganisms and increased the efficiency of the chemical sanitizers. Sagong et al. (2013) reported a significant reduction in *Bacillus cereus* spores in lettuce (2.49 log CFU/g) and carrots (2.22 log CFU/g) after an US treatment (40 kHz, 5 min) with added Tween 20 (0.1%) as surfactant.

In general, bacterial spores are more difficult to inactivate than vegetative cells, but US in combination with chemical compounds at low concentrations is a viable option for the preservation of FFVs. Unfortunately, the methods and parameters used have not been standardized (Evelyn and Silva, 2015).

4. EFFECT OF ULTRASOUND ON ENDOGENOUS ENZYMES

Most studies are regularly conducted in liquid systems, in particular in fruits and vegetables juices (Anaya-Esparza et al., 2017), but US can also be used to inhibit enzyme activity that leads to decreased quality of FFVs (Mason et al., 1996). Endogenous enzymes like pectinmethylesterase (PME), polyphenoloxidase (PPO), peroxidase (POD) and lipoxygenase (LOX) from FFVs may reduce their quality. For example, they can change their texture and develop off-flavors and browning pigments, especially in fresh-cut produce. Handling, postharvest processing, and food preparation can damage the integrity of vegetal tissues, promoting the interaction of the previously mentioned enzymes and their substrates (O'Donnell et al., 2010).

Ercan and Soysal (2011) report complete inactivation of tomato POD after US treatment (2.5 min). Chen and Zhu (2011) mentioned that a combination of US and aqueous

ClO_2 effectively inactivates the enzymes responsible for cell wall depolymerization, and inhibits the softening of plum fruit, however, no direct evidence has been reported to prove this hypothesis. The authors suggested that a faster tissue softening rate results from an increased ethylene production, promoting physiological changes in the fresh produce.

Yu et al. (2016) investigated the influence of US as an abiotic elicitor on Romaine lettuce. The mechanism that results in the production and accumulation of secondary metabolites was investigated, by examining the responses of a defense-related enzyme in US-treated lettuces. They reported that US increased phenylalanine ammonia-lyase (PAL) enzyme, resulting in an increased synthesis of phenolic compounds and antioxidant activity.

Wang et al. (2015) reported that US-treated (106 W/L) cherry tomatoes show lower ethylene production ($P < .05$), as compared to the control. The authors mentioned that the reduced and delayed ethylene production was probably related to the inactivation of enzymes responsible for its synthesis. This in turn decreased the respiration rate, and might be one of the mechanisms for decreased enzyme activities. In the same study, cherry tomatoes also showed an increase in catalase (CAT), superoxide dismutase (SOD), and POD enzyme activities in response to US. These results, obtained after a postharvest US treatment, promote a desired effect on the tomatoes, because the enzymes affected play important roles in protecting plants from oxidative stress, while also promoting their ripening. However, it is not known if the effects of US are permanent or temporary (Mason et al., 1996).

In the fresh-cut fruit and vegetable industry, thermal blanching is an important process that is applied with the aim of reducing enzyme activity. In this context, US is an alternative to blanching that leads to enzyme inactivation in FFVs (Cruz et al., 2008). Cruz et al. (2008) reported a minor impact on the nutritional composition and sensory attributes of US-treated FFVs, as compared to those that were heat blanched, suggesting that US may be a feasible alternative to heat blanching.

The theories that explain enzyme inactivation due to US propose that a synergy happens between chemical and physical effects during cavitation. This phenomenon induces protein denaturation by depolymerization and changes the protein conformation, which is related to the reduction in its specific activity (Islam et al., 2014). Micro-streaming occurs when US disrupts van der Waals interactions and hydrogen bonds in the polypeptide chains (Feng et al., 2008). Also, free radicals produced during water sonolysis attack specific sites, such as disulfide bonds, that destabilize the structural integrity of the enzyme. The free radicals can also oxidize amino acid residues, such as tryptophan, tyrosine, histidine, and cysteine, which are involved in the catalytic activity and stability of several enzymes (Islam et al., 2014). Further studies on the physiological, biochemical, and molecular responses of FFVs to US are still required (Yuting et al., 2013).

5. EFFECTS OF ULTRASOUND ON NUTRITIONAL AND QUALITY PARAMETERS OF FRUITS AND VEGETABLES

The impact of US on nutritional and quality parameters of FFV is discussed in this section. The main goal of sanitization is not only to reduce the number of pathogenic and spoilage microorganisms present in FFVs, but it is also important to preserve their physicochemical and nutritional properties (Alves do Rosário et al., 2017). Total soluble solids (TSS), titratable acidity (TA), pH, firmness, color, concentration of vitamin C, and other bioactive compounds are critical indicators of FFV quality. For example, texture is an indicator of their edible quality and shelf life, and is related to respiration rate and other physiological activities (Yuting et al., 2013).

5.1 Physicochemical and Physiological Properties

Changes caused by US treatment (positive or negative) allow us to infer if the treatment is suitable or not for the product in question. The effects of US with or without sanitizers are listed in Table 2. Ding et al. (2015) reported that an US treatment (40 kHz) in combination with SAEW (10%) had no significant effects ($P < .05$) on TSS, TA, and vitamin C content in strawberries or cherry tomatoes. Nevertheless, tomatoes, but not strawberries, presented a nearly 10% decrease in firmness, as compared to the control. These results may be attributed to the differences in surface morphology between each product. Cao et al. (2010) reported congruent results after a similar US treatment (40 kHz, 20°C, 10 min),where no changes were found in firmness, TSS, TA, and vitamin C content of strawberry. Comparable results were also documented in tomatoes (Pinheiro et al., 2015) and strawberries (Alexandre et al., 2012) that were treated with US at 45 kHz for 19 min, 35 kHz for 2 min, respectively.

The same behavior was reported in strawberries that were treated with US (20 kHz, 5 min) combined with ozone (0.075 mg/L), where no changes in color, TA, and TSS were found. These results are attributed to a slow respiration rate induced by the combined treatment (Aday and Caner, 2014). Similar responses were reported by Chen and Zhu (2011), in plums that were treated with US (40 kHz) combined with ClO_2 (40 mg/L), and by Wang et al. (2015) in cherry tomatoes treated with US (20 kHz). Wang et al. (2015) also reported that during 8–12 days of storage of cherry tomatoes, ethylene production and respiration rate were slower than the control, extending their shelf life up to 16 days, without significant changes in pH, TA, TSS, firmness, and greater retention of vitamin C and total phenolic compounds. Similar results were reported by Pinheiro et al. (2015) during a 15-day storage period of US-treated tomatoes, where the microbial counts decreased in response to the US treatment. Plum fruits treated with US (40 kHz, 10 min) combined with ClO_2 (40 mg/L), had an extended shelf life of up to 60 days, as compared to 35 days for untreated fruits (Chen and Zhu, 2011). Furthermore, no chemical residues were detected in treated samples. Bang et al. (2017) reported that a

Table 2 Influence of ultrasound treatments, with or without sanitizers, on physicochemical attributes and physiological parameters in fresh fruits and vegetables

Type of fresh produce	Ultrasonic equipment	[a]Experimental conditions	Results	Reference
Cucumber	Ultrasonic water bath at 380 W	37 kHz; 60 min; peroxyacetic acid (150 ppm)	Color, moisture content, and firmness (hardness and chewiness) did not change	Bang et al. (2017)
Strawberry	Ultrasonic water bath	40 kHz; 5 min; peracetic acid (40 mg/L)	TA, TSS, color (L, a, b) and vitamin C content did not change	Alves do Rosário et al. (2017)
Lettuce	Ultrasonic water bath at 200 W	37 kHz; 100 min; NaOCl (200 ppm)	TSS and pH did not change	Park et al. (2016)
Romaine lettuce	Ultrasonic water bath 2000 W	25 kHz; 26 W/L; 100%; 1 min	Increased TPC, firmness and color, as compared to the control	Yu et al. (2016)
Lettuce	Ultrasonic water bath at 200 W	26 kHz; 90 μm; 5 min; EOO (0.01%)	No change in electrolyte leakage rate, this parameter is related to the integrity of the surface	Millan-Sango et al. (2016)
Strawberry	Ultrasonic water bath at 60 W	33 kHz; 60 min	pH and firmness did not change, slight decrease in vitamin C and TA (<2%) and TPC (8%), increased TSS (6%) and antioxidant activity, color (L, a, b) were slightly affected	Gani et al. (2016)
Tomato	Ultrasonic water bath	45 kHz; 100%; 19 min	Preserved texture, color and increased TPC	Pinheiro et al. (2015)

Table 2 Influence of ultrasound treatments, with or without sanitizers, on physicochemical attributes and physiological parameters in fresh fruits and vegetables—cont'd

Type of fresh produce	Ultrasonic equipment	Experimental conditions	Results	Reference
Cherry tomato	[b]Probe of 25 mm diameter	20 kHz; 106 W/L; 8 min; 25°C; 35 μm	Preserved firmness, delayed ethylene production and respiration rate, TSS and TA did not change	Wang et al. (2015)
Watercress, parsley Strawberry	Ultrasonic water bath at 200 W	45 kHz; 10 min; 25°C; US + peracetic acid (40 mg/L)	Color, pH, and firmness were affected, particularly in watercress and parsley	São José and Vanetti (2015)
Lettuce	Ultrasonic water bath at 200 W	26 kHz; 90 μm; 5 min; EOO (0.01%)	No damage to the leaves was observed	Millan-Sango et al. (2015)
Cherry tomato Strawberry	Ultrasonic water bath at 240 W	40 kHz; 10 min; SAEW (1:10)	TA, TSS, and Vitamin C did not change in both cases; slight decrease in firmness (10%) as compared to the control (only in tomato); anthocyanin content did not change in strawberry	Ding et al. (2015)
Iceberg lettuce Romaine lettuce	Ultrasonic water bath at 2000 W	25 kHz; 1 min; 10°C; chlorine (100 mg/L)	Color and firmness did not change in either lettuce	Palma-Salgado et al. (2014)
Iceberg lettuce	Ultrasonic water bath at 1200 W	37 kHz; 100 min; NaOCl (200 ppm)	No changes in texture	Lee et al. (2014)
Strawberry	[b]Probe of 19 mm diameter at 30 W	20 kHz; 5 min; ozone (0.075 mg/L)	TA, TSS, color (L, a, b), electrical conductivity, and firmness did not change after treatment (1 week)	Aday and Caner (2014)

Continued

Table 2 Influence of ultrasound treatments, with or without sanitizers, on physicochemical attributes and physiological parameters in fresh fruits and vegetables—cont'd

Type of fresh produce	Ultrasonic equipment	Experimental conditions	Results	Reference
Lettuce Carrots	Ultrasonic water bath	40 kHz; 30 W/L; 40 μm; 5 min; Tween 20 (0.1%) as surfactant	No damage to lettuce leaves and carrots was observed	Sagong et al. (2013)
Red bell pepper	Ultrasonic water bath	45 kHz; 1 min; chlorine water at 25 ppm of free chlorine	Firmness and pH did not change, higher retention of vitamin C and color compared with water-washed control	Alexandre et al. (2013)
Strawberry	Ultrasonic water bath at 120 W	35 kHz; 15°C; 2 min	Increased firmness (16%), losses of color, Vitamin C, and anthocyanins were lower than those obtained in samples treated with sanitizers	Alexandre et al. (2012)
Plum fruit	Ultrasonic water bath at 100 W	40 kHz; 10 min; ClO_2 (40 mg/L)	Decreased respiration response, preservation of firmness, flavonoids, ascorbic acid, TA, and reducing sugars	Chen and Zhu (2011)
Grape berry	[b]Probe of 13 mm diameter	20 kHz; 5 min; 30°C; 92.5 μm; 80%	Damage to the structure of cuticular membrane, increased color	Fava et al. (2011)
Truffles	Ultrasonic water bath	35 kHz; 10 min; ethanol (70%)	Minimized lost weight	Rivera et al. (2011)

Table 2 Influence of ultrasound treatments, with or without sanitizers, on physicochemical attributes and physiological parameters in fresh fruits and vegetables—cont'd

Type of fresh produce	Ultrasonic equipment	Experimental conditions	Results	Reference
Lettuce	Ultrasonic water bath	40 kHz; 30 W/L; 5 min; 2% lactic/citric/malic acid	Color and texture did not change	Sagong et al. (2011)
Strawberry	Ultrasonic water bath at 350 W	40 kHz; 10 min; 20°C	TA, TSS, Vitamin C, and firmness did not change	Cao et al. (2010)
Lettuce	Ultrasonic water bath	20 kHz; 50°C; $Ca(ClO)_2$ 100 mg/L	Significant decrease in color and crispiness	Ajlouni et al. (2006)

[a]Optimal experimental condition: frequency (kHz); amplitude (μm or %); processing time (min); final temperature (°C); ultrasonic intensity (W); acoustic energy density (W/mL); *PME*, pectinmethylesterase; *POD*, peroxidase; *PPO*, polyphenoloxidase; *PG*, polygalacturonase; *LOX*, lipoxygenase; *SAEW*, slightly acidic electrolyzed water; ClO_2, chlorine dioxide; *EOO*, essential oil oregano; *CaO*, calcium oxide; *NaOCl*, sodium hypochlorite; $Ca(ClO)_2$, calcium hypochlorite; *TA*, titratable acidity; *TSS*, total soluble solids; *TPC*, total phenolic compounds.
[b]Ultrasonic equipment coupled to a circulating water bath.

combination of US (37 kHz, 60 min) and peroxyacetic acid (40 mg/L) applied to cucumber did not promote changes in color or firmness.

Long-time (10–20 min) US treatments cause significant ($P < .05$) changes in the quality of Cos lettuce tissues and fresh-cut potatoes, mainly by altering their color and crispiness (Ajlouni et al., 2006). Also, a decrease in vitamin C, phenolic compounds and changes in color can be induced by exposing strawberries to long US treatments (Gani et al., 2016). São José and Vanetti (2015) mentioned that color loss is linked to molecular changes to different colored compounds. Sanitization caused watercress and parsley to darken, particularly in response to US in combination with peracetic acid. In contrast, US alone or combined with essential oregano oil at 0.01%, or with Tween 20 at 0.1% (surfactant) had no impact on lettuce leaves after a 5-min treatment period (Millan-Sango et al., 2016; Millan-Sango et al., 2015; Sagong et al., 2013).

Similarly, US (40 kHz,5 min) in combination with organic acids (malic, lactic, and citric acid) or NaOCl (200 ppm) did not promote color or texture changes to lettuce leaves (Sagong et al., 2011; Lee et al., 2014; Palma-Salgado et al., 2014). These results are in disagreement with Fava et al. (2011), who reported damage to the structure (micro, ultra, and nano) of grape berries treated with US (20 kHz), however, a high color was observed in the treated samples. These authors mentioned that during US treatment, anthocyanins may have been released in response to cell membrane or cell wall alterations. The negative effects are apparently related to the experimental conditions of the treatments, and depend on the specific product. In some cases, negative effects have been reported regarding color or vitamin C content in response to US, but these losses are less than those obtained in the water-washed control (Alexandre et al., 2013).

5.2 Changes in Sensorial Properties and Shelf Life

The US in combination with organic sanitizers can decrease the microbiological count during FFV handling, resulting in a long shelf life, and minimal changes to the nutritional and sensorial quality (São José et al., 2014a), but studies that analyze the effect of US on FFV shelf life are scarce. Forghani et al. (2013) extended the shelf life of lettuce by 6 days at 10°C, when it was treated with a combination of US and SAEW. The US combined with $Ca(ClO)_2$ (100 mg/L) exerted similar results when applied to lettuce (Ajlouni et al., 2006). The combination of US (40 kHz, 5 min) and organic acids (2%) preserved the quality and sensorial attributes of lettuce for up to 7 days (Sagong et al., 2011). The US combined with chlorine solution (100 mg/L) extended the shelf life of lettuce for up to 14 days, without significant changes to its sensorial attributes (Palma-Salgado et al., 2014). It should also be mentioned that US-chlorine-treated samples had a significantly higher overall sensorial score, than the others treatments. Yu et al. (2016) reported that judges gave higher sensorial scores to Romaine lettuce that was treated with US (25 kHz, 1 min), as compared to an untreated sample. Rivera et al. (2011) reports that US (35 kHz, 10 min) in combination with 70% ethanol was effective in extending the shelf life of truffles for up to 28 days during refrigerated storage, without changes to their sensorial attributes.

The combination of US (40 kHz, 5 min) and peracetic acid (40 mg/L) maintained a good quality of strawberries that were stored for 9 days at 8°C (Alves do Rosário et al., 2017). Similar sensorial quality was reported in strawberries that were treated with US (35 kHz, 2 min) after 13 days of storage at 4°C (Alexandre et al., 2013), and strawberries treated with US (40 kHz, 20°C, 10 min) after 8 days of storage at 5°C (Cao et al., 2010). Gani et al. (2016) reported that an US treatment (33 kHz, 60 min) did not promote changes to the physicochemical or nutritional properties of strawberries that were stored for 15 days at 4°C. Aday and Caner (2014) reported that US (20 kHz, 5 min) combined with ozone (0.075 mg/L) can effectively prolong the shelf life of strawberries for up to 4 weeks, when stored at 4°C.

6. CONCLUSIONS

Ultrasonic processing technology can replace the use of chlorinated solutions that are commonly used to sanitize FFVs. It can be used in combination with organic sanitizers, such as lactic, citric, malic, peracetic acids among others. This increases the efficiency of US and promotes a greater reduction in the population of spoilage microorganisms, without significant changes to the sensorial attributes of FFVs. This information is of special importance into the food industry, because the development of novel sanitizing treatments can extend the shelf life of FFVs in a safe and reliable manner. This suggests that continued research is necessary to standardize and further develop the technologies.

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