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Review

Decontamination efficiency of high power ultrasound in the fruit and vegetable industry, a review

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ABSTRACT

Decontamination of fresh fruits and vegetables is an important unsolved technological problem. The main focus of this review is to summarize and synthesize the results of studies and articles about ultrasonic processing which can be adapted to the wash water decontamination process for fruits and vegetables. This review will also provide an overview about the importance of an effective wash water decontamination process in fruits and vegetables, the increase of foodborne outbreaks caused by fresh fruits and vegetables, microbial inactivation using ultrasound, and an interpretation of the high power ultrasound results in the fruits and vegetable industry. In addition, the limitations of ultrasonic processing in commercial applications have also been introduced.

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1. Introduction

Today, food chains are becoming more complicated in the handling, processing, and transportation of food; hence obtaining safe food is becoming more difficult day by day. Most of the antimicrobial substances and sanitizers used in the food industry for preservation and sanitation are dangerous for human health and harmful to the environment. In recent years, there has been an increasing demand for safe antimicrobial substances and sanitizers for the food industry (Lopez-Gomez et al., 2009). Similar trends are also valid for fresh fruits, vegetables, and organic foods.

Thus, novel and complementary food preservation technologies are continuously being investigated. Among the alternative food preservation technologies, particular attention has been paid to the physical methods and biopreservation to extend the shelf-life and inhibit undesirable microorganisms, minimizing the impact on the nutritional and organoleptic properties of food products.

No method of treatment or sanitation that is currently used in the food industry has been proven capable of inactivating microorganisms attached to fruit or vegetable tissues. Therefore, this review will summarize the basic knowledge and current applications of ultrasound technology as an alternative washing method for avoiding attachment of microorganisms to fruit and vegetable tissues. Ultrasound technology is mostly combined with other sanitizing agents

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for decontamination purposes in fruit and vegetable washing. In this review, simulation of the existing literature data was also accomplished for an estimation of single ultrasonic application in wash water.

2. The necessity for an effective wash-water decontamination process in the fruit and vegetable industry

Decontamination of fresh fruits and vegetables is an important unsolved technological problem. Over the past two decades, fruits and vegetables have repeatedly become a source of foodborne illnesses. The different pathogens most frequently linked to fruit and vegetable produce-related outbreaks generally include bacteria such as *Escherichia coli* O157: H7, *Salmonella* spp. and *Listeria* spp. which are a public health concern (Buck et al., 2003; Sivapalasingam et al., 2004; Nguyen-The, 2012; Olaimat and Holley, 2012; Batz et al., 2012). In fact, the foodborne outbreaks caused by *E. coli* and *Salmonella* isolated from fruits and vegetables resulted with 727 cases/6 deaths and 2288 cases/3 deaths, respectively, between the years 2006 and 2010 in the USA (CDC, 2012). In recent years, food borne outbreaks caused by fruits and vegetables have shown an increasing trend.

Many bacteria including *Bacillus*, *Salmonella*, *Listeria*, *Staphylococcus*, and *Escherichia* are capable of adhering to and forming a biofilm on different surfaces (Sinde and Carballo, 2000; Ryu and Beuchat, 2005); however, there are limited investigations that are interested in the adhering and forming of biofilm on the surface of fresh vegetables (Elhariry, 2011). When spoilage and pathogenic microorganisms come in contact with produce in the fruit and vegetable production environment, they can rapidly attach and strongly adhere themselves (Liao and Sapers, 2000; Ukuku and Fett, 2006; Sapers and Doyle, 2009). Some pathogens can also form biofilms on fruit and vegetable surfaces (Annous et al., 2005; Sapers and Doyle, 2009; Solomon and Sharma, 2009; Elhariry, 2011).

The necessity for an effective wash water decontamination process in the raw material department of the fruit and vegetable industry is undeniable as well as being a very critical step. In fruit and vegetable cultivation, the possible contamination sources are seed, soil, irrigation water, animals, manure, and the use of sewage sludge (Sivapalasingam et al., 2004). The washing methods can reduce the microbial load of the product. On the other hand if the washing treatment has not been applied properly, this step can cause cross-contamination (Buck et al., 2003; Olaimat and Holley, 2012). There is only one study that determined the microbial count in wash water after ultrasonic treatment. In this study, ultrasound treatment provided a 4.4 log reduction of *E. coli* O157:H7 count in the wash water (0.28 W/L, 20 kHz, 53 min, 10^6 CFU/mL inoculation) (Elizaquivel et al., 2011). Future studies about the total microbial quality of wash water are needed to determine important and valuable information concerning the antimicrobial effect of ultrasound to avoid cross-contamination in wash water.

Vegetables are washed typically with water that generally contains free chlorine from approx. 0 to 30 ppm. The chlorine and chlorinated compounds have already been used for several decades and these compounds are still the most widely used sanitizers in the food industry (Behrsing et al., 2000; Sapers, 2001; Beuchat et al., 2004; Hua and Reckhow, 2007; Al-Zenki et al., 2012). Despite not having very clear scientific data, many researchers mentioned that excessive use of chlorine can be harmful due to the formation of carcinogenic disinfection by-products such as trihalomethanes, chloramines, haloketones, chloropicrins, and haloacetic acids caused by the reaction of residual chlorine with organic matter (Akbaş and Ölmez, 2007; Ukuku and Fett, 2006; Gil et al., 2009; Ölmez and Kretzschmar, 2009; Cao et al., 2010; Cho et al., 2010; Hernandez et al., 2010). Due to the risks posed by the use of chlorine in the food industry, the use of these compounds is forbidden in European countries such as the Netherlands, Sweden, Germany, and Belgium (Rico et al., 2007; Ölmez and Kretzschmar, 2009; Issa-Zacharia et al., 2010). Actually, there is a trend in eliminating chlorine based compounds from the decontamination and disinfection process and applying innovative and emerging technologies in the food industry (Ölmez and Akbaş, 2009; Cao et al., 2010; Hernandez et al., 2010).

3. Ultrasound

The application of ultrasound is a non-thermal technology which contributes to the increase of microbial safety and prolongs shelf-life, especially in food with heat-sensitive, nutritional, sensory, and functional characteristics (Alegria et al., 2009; Cao et al., 2010; O'Donnell et al., 2010; Wang et al., 2011; Bhat et al., 2011). Ultrasound refers to pressure waves with a frequency of 20 kHz or more and generally, ultrasound equipment uses frequencies from 20 kHz to 10 MHz. Higher-power ultrasound at lower frequencies (20 to 100 kHz), is referred to as “power ultrasound” and has the ability to cause cavitation, which has uses in food processing to inactivate microorganisms (Piyasena et al., 2003). A major advantage of ultrasound over other techniques in the food industry is that sound waves are generally considered safe, non-toxic, and environmentally friendly (Kentish and Ashokkumar, 2011).

The combination of ultrasound with some non-thermal and/or physical–biological methods constitutes an attractive approach to enhance microbial inactivation and elimination (Guerrero et al., 2001; Kuldiloke, 2002; Vercet et al., 2002). Additionally, from the stand point of consumer demand, ultrasound and physical–biological combined processes show a potential for further investigation and application in a plant scale and dependent on this, ultrasound technology could have a wide range of current and future applications in the food industry (Earnshaw, 1998; Zenker et al., 2003; D'Amico et al., 2006; Valero et al., 2007; Chen et al., 2007; Zhao et al., 2007; Alegria et al., 2009; Cao et al., 2010; O'Donnell et al., 2010; Wang et al., 2011; Bhat et al., 2011).

Most published data indicates that the antimicrobial efficiency of ultrasound is relatively low in some conditions and only under special situations could ultrasound become an actual and effective alternative to the decontamination process (Arce-Garcia et al., 2002; Guerrero et al., 2005; López-Malo et al., 2005).

The multiple hurdles concept is a widely accepted approach in food preservation and the hurdle technology is generally defined as using the simultaneous or the sequential application of factors and/or treatments affecting microbial growth. The principle of this concept can be explained as; two or more inhibition and inactivation methods at suboptimal levels are more effective than one. In this manner, ultrasound technology can be adapted in the washing tank for decontamination of fruit and vegetables where the ultrasonic waves can be generated from the surface of the tank. In the hurdle concept, the application of combining different factors with ultrasound has important synergistic effects on the microorganisms (McClements, 1995; Leistner, 2000). The combination of ultrasound with some methods, constitutes an attractive approach to enhance microbial inactivation as previous works have demonstrated about the hurdle effect in different fruits and vegetables such as plum fruit (Chen and Zhu, 2011), strawberries (Cao et al., 2010; Alexandre et al., 2012), alfalfa seeds (Scouten and Beuchat, 2002), fruit and vegetable juices (Kuldiloke, 2002), apples and lettuce (Huang et al., 2006) and red bell pepper (Alexandre et al., 2013).

3.1. Physical and chemical effects of ultrasound and microbial inactivation

The primary antimicrobial effects and the driving force of the processing of ultrasonication are attributed to intracellular acoustic cavitations which cause an increase in the permeability of membranes and lost selectivity, thinning of cell membranes (Sams and Feria, 1991), localized heating (Suslick, 1998), and production of free radicals (Fellows, 2000; Butz and Tauscher, 2002). The cavitation bubbles are generated by the ultrasound waves. These bubbles pass through the solution and create a series of compression/rarefaction (expansion/collapse) cycles creating a negative pressure affecting the molecules of the liquid. When the distance between the molecules exceeds the minimum molecular distance the liquid breaks down and a void is formed. In successive cycles, voids or cavities continuously grow with a small amount of vapor from the liquid. During ultrasound applications, many thousands of such bubbles, which are categorized by two different structures, are formed; the first groups of bubbles, defined as stable cavitation bubbles are non-linear, have some equilibrium size during pressure cycles, and form large bubble clouds. The second, internal (transient) cavitation bubbles are nonstable and collapse quickly in a very short time period and then disintegrate into a mass of smaller bubbles. These bubbles are often small and they also collapse rapidly. Small bubbles will simply dissolve; however, the mass transfer boundary layer is thinner, and the interfacial area is greater during bubble expansion than during bubble collapse. This means that more air transfers into the bubble during the expansion phase than leaks out during the collapse (Lauternborn and Ohl, 1997; Lee et al., 2005; Kentish and Ashokkumar, 2011; Tiwari and Mason, 2012).

Unstable internal cavitations are generally observed at low frequencies (20–100 kHz) and undergo collapse to generate temperature and pressures in the medium. The gas and vapor within the bubble may be heated to a high temperature and the hot spots of high temperature (up to 5500 °C) and pressure (up to 50,000 kPa) occur in very short-time periods (on the order of microseconds). Shock waves radiated by collapsing bubbles could be strong enough to shear and break the cell wall and membrane structures. Finally it can be said that the components of the microbial cells disrupt by means of the micro-mechanical shocks of ultrasound technology (Fellows, 2000; Butz and Tauscher, 2002).

The second antimicrobial effect comes from the chemical effect of ultrasonication. In fact, literature mentions that sonolysis using a 20 kHz ultrasonic unit was found to enhance the inactivation of microorganisms due to the antimicrobial mechanisms of hydroxyl radicals (Suslick, 1998; Phull and Mason, 1999; Butz and Tauscher, 2002; Kadkhodaee and Povey, 2008). Previous studies have shown that ultrasound generates a temperature increase at a localized level inside a collapsing bubble which generates primary hydroxyl radicals (Makino et al., 1983; Suslick, 1989; Ashokkumar and Mason, 2007; Kentish and Ashokkumar, 2011). In addition, it was reported that reactions that involve single electron transfer are accelerated in ultrasonic applications (Weiss et al., 2011). All the chemical effects of cavitation include free radical generation and involve single electron transfer during the cooling phase and hydrogen atoms and hydroxyl radicals recombine to form hydrogen peroxide (H_2O_2) which have important bactericidal properties (Lee and Feng, 2011). If other compounds are added to water irradiated with ultrasound, a wide range of secondary reactions can occur and organic compounds can be oxidized and reduced (Suslick, 1989). At the end of these successive reactions, normally the amount of free radicals increases. Moreover, the hydroxyl radical (OH^-) is able to react with the sugar-phosphate backbone of the DNA chain and causes the secession of the phosphate-ester bonds and breaks in the double strand microbial DNA (Manas and Pagan, 2005).

3.2. Commercial applications of high power ultrasound in food industry

There have been numerous studies about various applications of high power (low frequency) ultrasound in food science and technology. All of these applications and principles were reviewed by Awad et al. (2012), Carcel et al. (2012) and Chandrapala et al. (2012). Researchers, in the past decades, were able to optimize many ultrasound applications either for testing or processing of food products. In addition, the commercial ultrasonic applications existed for defoaming, emulsification, extraction and decontamination, extrusion, waste water treatment, and tenderization of meat (Cardoni and Lucas, 2005; Clark, 2008; Patist and Bates, 2008; Awad, 2011; Chemat et al., 2011; Quan, 2011; Anon., 2012). For antimicrobial purposes, ultrasound was mostly used for the cleaning and disinfecting of factory surfaces in the food industry. Commercially, there are no plant scale applications of ultrasound in the decontamination and inhibition of microorganisms in foods. Although, in an industrial water system, high frequency ultrasound treatment, patented as Sonoxide, has shown excellent results in controlling bacteria and algae and has over 600 applications worldwide (Broekman et al., 2010).

Recently, it has been observed that intensive research concerning the appropriate ultrasound sensing or processing system in terms of probe design, geometry, and characteristics (e.g., frequency) as well as operating conditions, that meet the demands of specific applications in different food materials or provide optimum results for each individual application, are being carried out. As a result, it can be said that the effectiveness of ultrasound technology is a very important issue for ensuring the robustness of this technology in possible areas of industrial applications (Patist and Bates, 2010; Soria and Villamiel, 2010; Knorr et al., 2011; Awad et al., 2012). An important factor causing difficulties that is effecting the adaptation of ultrasound to existing food production lines is the commitment of food producers, to traditional methods.

From the stand point of the tremendous trend for the use of new technologies, it can be said that ultrasound is one of the most important green technologies used in processing and preservation (Chemat et al., 2011; Awad et al., 2012). More research efforts are still needed to develop efficient systems for various problems related to specific foods and production lines.

3.3. High-power ultrasound processing in the fruit and vegetable industry

Fruits and vegetables become microbiologically safe by using inhibition or elimination processes. Washing is the main step for removing microorganisms or reducing microbial load. It is widely acknowledged in the food industry that the washing step, which aims to remove the dirt and cell exudes from damaged surfaces, along with immersion of the product in a washing tank with a sanitizing agent, and an optional rinsing step, reduces the microbial load. According to the type and the concentration of sanitizing agents, the total count of the microbiological populations on different kinds of fruits and vegetables after washing generally varies between 1.0 and 3.0 log CFU/g (Sapers, 2001; Gil et al., 2009).

Currently, for decreasing the microbial load of fresh fruits and vegetables, decontamination techniques of one or a combination of methods and antimicrobials take place due to the washing of products with chlorine, chlorine dioxide, acidified sodium chloride, organic acid formulations, alkaline-based sanitizers, hydrogen peroxide, ozonated water, electrolyzed water, peroxyacetic acid, and mild heat treatments, as well as other physical methods including ultrasound, ultraviolet radiation, pulsed electric field, oscillating magnetic fields, and high pressure (Gil et al., 2011).

New decontamination methods are needed to contact and kill microorganisms without any negative effects. The application of ultrasound in fruit and vegetable washing is one of the alternative methods and is recommended for the food industry (Sapers, 2001; Seymour et al., 2002; Huang et al., 2006; Knorr et al., 2004; Alegria et al., 2009; Cao et al., 2010; Zhou et al., 2009; Elizaquivel et al., 2011; Rivera et al., 2011; Sagong et al., 2011; São José and Vanetti, 2012; Alexandre et al., 2012, 2013). The limited research, carried out until today, regarding ultrasound applications in the washing step of fruits and vegetables is summarized in Table 1.

Despite there being no knowledge of the commercial application of ultrasound in the wash-water decontamination processes, nowadays most studies are concentrated on studying the physical cleaning and decontamination effect of ultrasound on fruit and vegetable surfaces. Moreover, researchers were trying to evaluate the effectiveness of

Table 1
High power ultrasound applications (single and combined) and microbial reductions in the wash-water decontamination process of some fruits and vegetables.

Product	Ultrasound (US) parameters	Treatments	Microbial reductions[a] (log_{10} CFU/g sample)	References
Strawberry	350 W/L, 40 kHz, 20 °C, 10 min	US alone	TVC: 0.6 YMC: 0.5	Cao et al. (2010)
Lettuce	280 W/L, 20 kHz, 53 min	US alone	*E. coli* O157:H7: 4.4 in wash water	Elizaquivel et al. (2012)
Strawberry	120 W, 35 kHz, 15 °C Sample/water: 1/25	US alone	TVC: 0.6 YMC: 1.4	Alexandre et al. (2012)
Red bell pepper	120 W, 35 kHz, 15 °C Sample/water: 1/25	US alone	*L. innocua*: 1.98	Alexandre et al. (2013)
Iceberg lettuce	10 W/L, 32–40 kHz, 10 min Sample/water:1/20	US alone	*S. typhimurium*: 1.5	Seymour et al. (2002)
		US + Chlorinated water (25 ppm free chlorine)	*S. typhimurium*: 2.7	
Shredded carrot	45 kHz, 1 min	US alone US + chlorinated water (200 ppm free chlorine)	TVC: 1.3 YMC: 0.9 TVC: 1.0 YMC: 0.9	Alegria et al. (2009)
Cherry tomatoes	45 kHz, 10 min, 25 °C	US alone US + peracetic acid (40 mg/L)	*S. enterica typhimurium*: 0.8 *S. enterica typhimurium*: 3.9	São José and Vanetti (2012)
Lettuce	170 kHz, 6–10 min	US + ClO_2 (5 and 10 ppm)	*Salmonella* spp.: 2.2–2.9 *E. coli* O157:H7: 1.3–2.2	Huang et al. (2006)
Apple			*Salmonella* spp.: 3.1–4.2 *E. coli* O157:H7: 2.2–3.8	
Spinach leaves	200 W/L, 21.2 kHz, 2 min	US + acidified sodium chloride (200 mg/L)	*E. coli* O157:H7: 4	Zhou et al. (2009)
Lettuce	30 W/L, 40 kHz, 5 min	US + lactic/citric/malic acid (2%)	*E. coli* O157:H7: 2.7 *S. typhimurium*: 3.2 *L. monocytogenes*: 2.9	Sagong et al. (2011)
Truffles	35 kHz, 4 °C, 10 min	US + ethanol (70%)	TVC: 4 Mold count: <1.7 Yeast count: <0.5 *Pseudomonas* spp.: >4 Enterobacteriaceae: 3.6 Lactic acid bacteria: 3.5	Rivera et al. (2011)
Plum fruit	100 W, 40 kHz, 20 °C, 10 min Sample/water: 1/5	US + ClO_2 (40 mg/L) US + peracetic acid (40 mg/L)	TVC (mesophilic):3 TVC (psychrotrophic):2.9 YMC: 2	Chen and Zhu (2011)

TVC: total viable counts (mesophilic).
YMC: yeast and mold count.
[a]Microbial reductions in given range changed depending on chemical concentrations in combined applications.

ultrasound in washing procedures. As seen in Table 1, most of the research was carried out and published in the years between 2002 and 2012 and are directly related to the decontamination treatments of fruits and vegetables. In studies using ultrasound for decontamination purposes, mostly lettuce, spinach, shredded carrot, truffles, cherry tomatoes, and strawberries were used as food materials. The high power ultrasound with low frequencies and treatment times between 20–45 kHz and 1–10 min were generally used in the applications. In different applications in combination with the parameters such as power, frequency, temperature, and time, the microbial reduction with ultrasound varies between 0.5 and 1.98 log CFU/g (Huang et al., 2006; Zhou et al., 2009; Alegria et al., 2009; Cao et al., 2010; Sagong et al., 2011; Chen and Zhu, 2011; Rivera et al., 2011; Elizaquivel et al., 2012; São José and Vanetti, 2012; Alexandre et al., 2012, 2013). Seymour et al. (2002), studied the effect of tap water, chlorinated water (25 ppm free chlorine), ultrasound in water (10 W/L, 32–40 kHz, 10 min), and ultrasound in chlorinated water in four different treatments and tried to determine the decontamination efficiency of these treatments on ampicillin resistant strains of *Salmonella typhimurium*, *E. coli* and *Listeria monocytogenes* in iceberg lettuce, cucumber, carrot, pepper, white cabbage, onion, parsley, strawberry, mint, and other herbs. Table 1, shows the results of *S. typhimurium* and iceberg lettuce regarding the researchers' conclusion. Literature reports that all experiments were also repeated for *E. coli* and *L. monocytogenes* but no significant differences in attachment efficiency were found and for this reason, these results are not given in Table 1. For the frequency effect between the given range for high power ultrasound, it was suggested that the different frequencies of ultrasound treatment had no significant effect on the decontamination efficiency of *S. typhimurium* ($P > 0.05$) in the washing of iceberg lettuce, the average reductions for 25, 32–40, and 62–70 kHz treatments were 1.4, 1.3, and 1.3 log_{10} CFU/g respectively (not shown in Table 1). The ultrasound application in water significantly reduced the numbers of *S. typhimurium* (approx. 1.5 log_{10} CFU/g reduction, 97.9% reduction). These reductions were significantly different ($P < 0.05$) from the water control in the decontamination of fresh produce.

Simple water washings allowed microbial log reductions of 1.43 ± 0.04 CFU/g red bell peppers. Among the technologies applied ozone in aqueous solution, ultrasounds and ultraviolet C radiation, ultrasound was found one of the most effective process. On average, 1.98 ± 0.21 log CFU/g reductions on *Listeria innocua* occurred when red bell pepper samples had been washed with aqueous ultrasounds (Alexandre et al., 2013).

There are some studies designed to investigate the single and combined effects of ultrasound with some chemicals such as organic acids, acidified sodium chloride, ethanol, chlorine dioxide, and peracetic acid on the microbial inactivation of some fruits and vegetables (Huang et al., 2006; Zhou et al., 2009; Sagong et al., 2011; Rivera et al., 2011; São José and Vanetti, 2012). Sagong et al. (2011) compared the effectiveness of combining treatments of ultrasound (30 W/L, 40 kHz, 5–10 min) with different organic acid (malic, citric and lactic acids) concentrations (0, 0.3, 0.5, 0.7, 1, and 2), and treatment times (5, 10, 20, 30, and 60 min) with mild agitation at 20 °C against *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes*. The maximum reductions of *E. coli* O157:H7, *S. typhimurium* and *L. monocytogenes* were determined as 2.7 (lactic acid), 3.2 (citric acid), and 2.9 (malic acid) log_{10} CFU/g after a combined treatment with ultrasound and 2% organic acid for 5 min., respectively ($P < 0.05$). The reduction effect of ultrasound on *S. typhimurium*, *E. coli* O157:H7, and *L. monocytogenes* counts between the 5 and 10 min treatments were not significantly ($P > 0.05$) different on fresh lettuce in an ultrasound treatment with organic acid applications. The similar data obtained from different studies suggest that the reduction effect of ultrasound occurred primarily during the first 5 min and did not significantly increased even after a 10 min treatment in different samples such as parsley, lettuce, cabbage, carrot, cucumber, strawberry, onion, and pepper (mentioned in Seymour et al., 2002; Sagong et al., 2011).

Alegria et al. (2009) evaluated the alternative decontamination processes of shredded carrots, applied the following processes: chlorination (50 or 200 ppm free chlorine/1 min at 5 °C), ozonization (1 ppm/5 min, 5 °C), hot water (100 °C/45 s), and ultrasonication (45 kHz/1 min). The reduction of the initial microbial load of the shredded carrots after singular and combined decontamination treatments are given in Table 2. As shown in Tables 1 and 2, it was observed that the logarithmic reductions of 1.3 and 0.9 in precut treatments were determined for a single ultrasound treatment for TVC and YMC, respectively. In some decontamination outcome studies, the chlorine combined ultrasound treatments did not exceed the efficacy of the single ultrasound application, which is a very important result from the stand point of the antimicrobial effect of ultrasound. In both treatments with and without chlorine the number of microorganisms was reduced by approx. 1 logarithmic unit in these experimental conditions which was applied for decontamination purposes.

Huang et al. (2006) used the combination of chlorine dioxide and ultrasound to kill the nalidixic acid resistant *Salmonella enterica*, serotypes *Enteritidis*, *Typhimurium*, and *Mission* and nalidixic-novobiocin resistant *E. coli* O157:H7 on apples and lettuce. The studies regarding the microbial reduction in these samples by chlorine dioxide at 0, 5, 10, 20, and 40 ppm with and without 170 kHz ultrasonic treatment for 10 min are shown in Table 3. The results of Huang et al. (2006), demonstrate that chlorine dioxide can effectively reduce the numbers of test organisms from samples, and ultrasound application can promote the antimicrobial effect of chlorine dioxide on *Salmonella* and *E. coli* O157:H7 inoculated apples and lettuce samples and a single treatment of ultrasound caused an additional 1.2–1.9 $\log_{10}$ CFU/g reduction in the samples. The decontamination efficiency of chlorine dioxide when combined with ultrasonication and applied to both test organisms showed that the inoculated apple samples were higher than the inoculated lettuce. This result could be that the structural differences and irregular surfaces of lettuce may provide some protection for the microbial cells. As shown in Table 4, a 1.52 $\log_{10}$ CFU/g additional reduction was obtained with an ultrasound application on *E. coli* O157:H7 inoculated apples, in experiments which applied ultrasound with the chlorine dioxide, the reduction values were additionally increased in the range of 0.6–2.4 $\log_{10}$ CFU/g depending on the chlorine dioxide concentrations (5–40 ppm). In the lettuce experiments, it was determined that an additional reduction in *Salmonella* spp. was obtained between 0.3 and 0.65 $\log_{10}$ CFU/g using the ultrasound treatment.

Table 2
The effects of singular and combined decontamination treatments applied in pre-cut and post-cut shredded carrots on the reduction[1] ($\log_{10}$ CFU/g) of mesophilic total viable counts (TVC) and yeast and mold counts (YMC) (summarized from Alegria et al., 2009).

Treatments	Pre-cut		Post-cut	
	TVC	YMC	TVC	YMC
Ultrasound – US (45 kHz, 1 min)	1.3[b]	0.9[e]	0.5[a]	0.5[c]
Combined applications				
Chlorinated water (200 ppm free chlorine/5 min, 5 °C) + US (45 kHz, 1 min)	1.0[b]	0.9[e]	0.9[b]	0.8[de]
Ozonated water (1 ppm/5 min, 5 °C), +US (45 kHz, 1 min)	0.2[a]	0.5[c]	0.4[a]	0.6[cd]

[1]Data are given as means ± SD with different letters in the same column.
[a,b]Different small letters represent significant differences ($P < 0.05$) for TVC.
[c,d,e]Different capital letters represent significant differences ($P < 0.05$) for YMC.

Table 3
The reduction values of different concentrations of chlorine dioxide single and combined with ultrasound on *Salmonella* spp. and *E. coli* O157:H7 inoculated apples and lettuce samples (summarized from Huang et al., 2006).

Salmonella strains reduction ($\log_{10}$ CFU/g sample)				
Concentrations (ClO_2-ppm)	Apples		Lettuce	
	ClO_2 alone	ClO_2 + US	ClO_2 alone	ClO_2 + US
5	2.5[a]	3.7[b]	1.7[a]	1.7[a]
10	2.5[a]	3.9[b]	2.1[a]	2.2[b]
20	2.5[a]	3.7[b]	2.1[a]	3.0[c]
40	2.5[a]	4.2[b]	2.2[a]	3.6[d]
E. coli O157:H7 reduction ($\log_{10}$ CFU/g sample)				
Concentrations (ClO_2-ppm)	Apples		Lettuce	
	ClO_2 alone	ClO_2 + US	ClO_2 alone	ClO_2 + US
5	1.7[a]	3.2[b]	1.5[a]	1.7[a]
10	1.8[a]	3.1[b]	1.7[a]	1.7[a]
20	1.8[a]	3.7[b]	2.3[a]	1.8[a]
40	2.2[a]	3.8[b]	2.4[a]	1.9[a]

[a,b,c,d]Data are given as means ± SD with different letters in the same column are significantly different ($P < 0.05$).
US: ultrasound application (170 kHz, 10 min).

São José and Vanetti (2012) studied the effect of ultrasound (45 kHz, 10 min, 25 °C) in the presence of 5% hydrogen peroxide and 40 mg/L peracetic acid on cherry tomatoes. The reduction of the total viable count, yeast and mold count, and inoculated *S. enterica typhimurium* that adhered to the surface of the tomatoes was evaluated (Table 5). Treatments with ultrasound alone, 5% hydrogen peroxide and 40 mg/L peracetic acid individually lead to reductions of 1.2, 2.1, and 2.6 $\log_{10}$ CFU/g (TVC) and 0.7, 2.3, and 3.3 $\log_{10}$ CFU/g (YMC), respectively. In combined applications with hydrogen peroxide and peracetic acid with ultrasound, the additional reduction values caused by ultrasound increased to 0.5–0.8 $\log_{10}$ CFU/g (TVC), and 0.2–1.1 $\log_{10}$ CFU/g (YMC) (Table 5).

Similarly, Rivera et al. (2011), studied the antimicrobial effect of sodium hypochlorite (500 ppm), hydrogen peroxide (500 ppm), and 70% ethanol combined with ultrasound (35 kHz, 10 min, 4 °C) on truffle samples. Ultrasound applied alone eliminated 1 $\log_{10}$ CFU/g TVC (mesophilic), 1.6 $\log_{10}$ CFU/g *Pseudomonas* spp., 1.6 $\log_{10}$ CFU/g Enterobacteriaceae count, and 0.9 $\log_{10}$ CFU/g lactic acid bacteria, and 0.9 $\log_{10}$ CFU/g YMC. When ultrasound was combined with sodium hypochlorite and hydrogen peroxide, an additional effect (approx. 1 $\log_{10}$ CFU/g) was found.

Zhou et al. (2009) searched the microbial load of spinach leaves and reported that acidified sodium chloride reduced the *E. coli* O157:H7 population by 2.1 $\log_{10}$ CFU/g over that of water wash, while the reduction from other sanitizers such as chlorine, peroxyacetic acid, and acidic electrolyzed water was about 1–1.2 $\log_{10}$ CFU/g (Table 6).

Table 4
The reduction values of chlorine dioxide applications single and combined with ultrasound (US) on *Salmonella* spp. and *E. coli* O157:H7 inoculated apples and lettuce samples (summarized from Huang et al., 2006).

Applications	Sample	Test organisms	Reduction* ($\log_{10}$ CFU/gsample)
Washing with water (10 min)	Apples	*E. coli* O157:H7	0.97[a]
US (170 kHz-10 min)	Apples	*E. coli* O157:H7	1.52[a]
ClO_2 (5–40 ppm)	Lettuce	*Salmonella* spp.	~1.97–2.35[b1]
ClO_2 (5–40 ppm) + US (170 kHz, 10 min)	Lettuce	*Salmonella* spp.	~2.26–3.00[b1]
ClO_2(5–40 ppm) + US (170 kHz, 10 min)	Apples	*E. coli* O157:H7	~2.14–3.90[c1]

*The reduction values are shown as means of log reduction ± SD.
[1]The values in given range changed depending on ClO_2 concentrations (5–40 ppm).
[a,b,c]The letters in the same column are significantly different ($P < 0.05$).

Table 5
Effect of ultrasound (US), hydrogen peroxide (HP) and peracetic acid (PAA) alone and combined applications in reducing the total viable count (TVC), yeast and mold count (YMC) and *Salmonella Typhimurium* in cherry tomatoes (summarized from São José and Vanetti, 2012).

Treatments	Reduction (log_{10} CFU/g sample)		
	TVC (mesophilic)	YMC	*Salmonella*
US	1.2^a	0.7^a	0.8^a
HP (5%)	2.1^b	2.3^b	nd
HP (5%) + US (45 kHz, 25 °C, 10 min)	2.6^b	2.5^b	nd
PAA (40 mg/L)	2.6^b	3.3^b	nd
PAA (40 mg/L) + US (45 kHz, 25 °C,10 min)	3.4^c	4.4^c	3.9^c

All data are means of determination with standard deviation (±).
a,b,c Treatments indicated with same letter did not differ (P > 0.05) between themselves.
nd: not determined.

Ultrasonication (21.2 kHz, 200 W/L, 2 min) significantly enhanced the reduction of *E. coli* O157:H7 on spinach for all treatments by 0.7 to 1.1 log_{10} CFU/g over that of washes with sanitizers alone (P < 0.05).

To prove the effects of ultrasound on plum fruit, the combined effects of this technique with chlorine dioxide were also reported by Chen and Zhu (2011). Microbial counts decreased in the three different treatments given below:

- Washing with tap water without US (Control),
- ClO_2 (40 mg/L) + US in ClO_2 solution (40 kHz, 20 °C, 10 min, 100 W) (Treatment I)
- ClO_2 (40 mg/L) + US in tap water (40 kHz, 20 °C, 10 min, 100 W) (Treatment II).

The ultrasound and chlorine dioxide treatments (I and II) significantly reduced the number of the total viable counts and yeast and mold counts in plum fruit by 2.3–3.0 and 1.4–2.0 log_{10} CFU/g respectively (P < 0.05 — Table 7) when compared to the control. When the ultrasound was applied in water, it gave a higher microbial reduction (approx. 0.7 log CFU/g for TVC and YMC) than ultrasound in chlorine dioxide.

As a result of this study, combined applications of chemicals and ultrasound on fruits and vegetables are suggested and that simultaneous ultrasonic waves and cavitation synergistically improved the antimicrobial effects of the chemical treatment compared with using them sequentially. The data in literature showed that there is a synergistic effect enhanced by approximately 0.7–1.7 logarithmic unit in the reduction of TVC, YMC, *E. coli* O157:H7, and *Salmonella* when ultrasound combined with some antimicrobial chemical agents, depending on the concentrations used, the ultrasound experimental conditions, the strains of microorganisms, and the type of vegetable. There are limited researches which determined totally the antimicrobial effect of ultrasound alone, ultrasound application reduce approx. 0.6–1.5 log_{10} CFU/g mL in general experimental conditions.

Table 6
The reduction of *E. coli* O157:H7 inoculated on the surface of spinach with ultrasound (US, 21.2 kHz, 200 W/L, 2 min) in combination with selected sanitizers (summarized from Zhou et al., 2009).

Sanitizer	Reduction (log_{10} CFU/g sample)	
	Alone sanitizer	Sanitizer + US (21.2 kHz, 200 W/L, 2 min)
Water	1.0^a	2.1^b
Chlorinated water (200 mg/L)	2.0^b	3.1^c
Acidic electrolysed water (80 mg/L)	2.2^b	3.1^c
Peroxyacetic acid (80 mg/L)	2.2^b	2.9^c
Acidified sodium chlorite (200 mg/L)	3.1^c	4.0^d

a,b,c,d Data are given as means ± SD with different letters in the same column are significantly different (P < 0.05).

Table 7
The microbial counts of (log_{10} CFU/g) plum fruit treated with combined ClO_2 and ultrasound (summarized from Chen and Zhu, 2011).

Treatments	Microbial counts (log_{10} CFU/g sample)		
	TVC (mesophilic)	TVC (psychrotrophic)	YMC
Control (tap water washing, without US)	3.9^a	3.7^a	2.7^a
ClO_2 (40 mg/L) + US in ClO_2 solution (40 kHz, 20 °C, 10 min, 100 W)	1.6^b	1.5^b	1.3^b
ClO_2 (40 mg/L) + US in tap water (40 kHz, 20 °C, 10 min, 100 W)	0.9^c	0.8^c	0.7^c

a,b,c Data are given as means ± SD with different letters in the same column are significantly different (P < 0.05).
TVC: total viable count.
YMC: yeast and mold count.

Microbial reduction by ultrasound is very important from the stand point of green decontamination and the hurdle concept of inhibition and elimination methods for food preservation technologies in fruits and vegetables. Additionally, from existing literature we concluded that these results could be helpful for estimating the decontamination effect of ultrasound and the possible use of ultrasound technology in different processes instead of antimicrobial chemical agents in fruits and vegetable washing processes.

4. Conclusion

Until today, the results obtained from different studies carried out using decontamination washing treatments combined with ultrasound applications are variable. Findings from different studies are also difficult to compare because they use different parameters such as ultrasound frequency, efficiency, acoustic energy density, time of treatment, temperature, water/sample ratios, agitation-washing protocol, species and strains of test organisms such as *E. coli* O157:H7, *S. typhimurium*, *L. monocytogenes*, and type of fruits and vegetables. There are a lot of parameters and factors which are not interpreted the same in all experimental conditions. Because of these differences, the harmonization of the results of the ultrasound applications may be very difficult.

As a result, finding the best conditions, doses, and combination of treatments for different hurdle decontamination technologies is a further challenge for the commercial adaptation of ultrasound. Future studies are needed to use ultrasound technology for decontamination purposes in the commercial food industry in place, for the purpose of scale up and optimization. These realistic studies are the only way to determine the best operating conditions.

It was also shown that, ultrasound applied by itself and with the chemical agents chlorine, peroxyacetic acid, and acidic electrolyzed water showed no significant microbial reduction (approx. 1 log CFU/g) between the two processes. In light of this knowledge, future research is necessary to determine the antimicrobial effects using ultrasound or chemicals in order to compare the results for decontamination washing processes in the fruit and vegetable industries.

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