Ultrasound processing alone or in combination with other chemical or physical treatments as a safety and quality preservation strategy of fresh and processed fruits and vegetables: A review

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Abbreviations:

AA, ascorbic acid; CFU, colony forming units; EO, essential oil; FV, fruits and vegetables; GRAS, generally recognized as safe; POD, phenol peroxidase; PPO, polyphenol oxidase; SAEW, slightly acidic electrolyzed water; TPC, total phenolic compound; US, ultrasound.

Abstract

Ultrasound (US) processing has emerged as a novel food preservation technology. This strategy has proved antimicrobial effects due to cavitation, which is the formation, growth, and collapse of bubbles that generate a localized mechanical and chemical energy. This technology can be applied by water so introducing it in the washing step to obtain safe fresh or fresh-cut products could be promising. The current review provides an overview of the current knowledge and recent findings on the use of US, alone or in combination with other mild physical technologies or chemical agents, to reduce microbial loads, and to better retain their quality attributes including color and texture, as well as the content of bioactive compounds such as antioxidant, phenolic compounds, or vitamins of minimally processed fruits and vegetables. As the effects of US depends on several factors related with treatment parameters, target microorganism, and matrix characteristics, further research efforts should be directed on optimizing US processes in accordance with their further application.

Keywords: sonication, microorganisms, fresh-cut, non-thermal technologies, antimicrobial.

1. Introduction

Minimally processed or fresh-cut produce has been defined by the International Fresh-cut Produce Association as “any fruit, vegetable or their combination subjected to a physical alteration from its original form, remaining in a fresh state” (Grau-Rojas, 2010). These products are completely edible, packaged, and should be stored under refrigerated conditions thus providing convenience to consumers (Grau-Rojas, 2010). The fruit and vegetable processing industry is experiencing an expanding period as the global demand for healthy, fresh, and sustainable products is increasing (Qadri, 2015). However, consumption of minimally processed fruits and vegetables (FV) has been associated to concerns on their safety due to the emergence of several outbreaks of foodborne pathogens linked to their consumption (Pinela, 2015).

Main causes of foodborne diseases are due to bacteria 53.0%, viruses 42.5%, and parasites 4.5% (Ramos, 2013). *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes* were the main contaminants involved in outbreaks related to FV over the past years (Birmpa, 2013; Park, 2012; Silva, 2017; Tango, 2017). According to the European Food Safety Authority (EFSA), the top ranking food/pathogen combinations are *Salmonella* spp. and leafy greens, bulb, stem vegetables, tomatoes, or melons, and *E. coli* and fresh pods, legumes, or grains (Andreoletti, 2013). Another major problem of the FV processing industry is related to alterative microbiota, which do not suppose any health risk to humans, but can lead to quality deteriorations, shortening the products’ shelf-life, and causing significant economic losses (Rico, 2007). For example, strawberry spoilage losses can be as high as 40 % (Luksiene, 2013) leading to strawberry producers to look for strategies to extend their shelf life. In addition, fresh-cut operations such as peeling or cutting result in increased nutrient availability and senescence rates as a consequence of the natural epidermal breach leading to the growth of microorganisms (Qadri, 2015; Ramos, 2013). For these reasons, disinfection procedures are crucial to maintain safety and quality of fresh-cut FV.

Among available sanitizers, chlorine is the most widely used due to its low cost, ease of use, and effectiveness against vegetative bacteria and some enteric viruses (Luo, 2018). However, chlorine has been associated with negative health outcomes and it has already been banned in some European countries including Belgium, Denmark, Germany and the Netherlands (Meireles, 2016). Therefore, the need to find effective alternatives to chlorine led to a number of novel chemical and non-thermal strategies. Proposed methods include the use of electrolyzed water, high pressure processing, ozone, Generally Recognized As Safe (GRAS) substances such as organic acids or essential oils, pulsed electric fields, ultraviolet irradiation, ultrasounds (US), or combinations of chemical and physical strategies (Barba, 2017; Cebrián, 2016).

US has been reported as a green food processing technology, as it implies a saving of energy and water use, and it is environmentally friendly, with a reduced carbon and water footprint when compared with traditional techniques (Chemat, 2017). It offers an advantage in terms of productivity and yield, with improved processing times and enhanced quality, and it has been reported to improve processes such as freezing and crystallization, drying, degassing, emulsification and demolding (Chemat, 2011).

US consists on the use of ultrasonic waves at a frequency beyond 18 kHz with a specific intensity and amplitude (Bevilacqua, 2018). It has been acknowledged and reviewed that microorganism lethality caused by sonication is due to a phenomenon known as transient cavitation (Pérez-Andrés, 2018). Generated bubbles collapse and, consequently, molecules collide, spots of extremely high temperature and pressure occur (5000 ºC and 50 MPa), and cellular envelopes and other microbial components are destroyed (Leong, 2017), thus reducing the viable microorganisms (Van Impe, 2018). Moreover, US can induce other chemical and structural changes, affecting quality and nutritional values of the processed products (Leong, 2017).

Most of the review papers published to date discussed the potential utilisation of sonication in liquid matrices such as milk or juices(Anaya-Esparza, 2017; Ortega-Rivas, 2014; Potoroko, 2018; Van Impe, 2018). The current manuscript summarises the most recent findings on the effect of sonication on the physicochemical and nutritional attributes as well on the safety of fresh and minimally processed fruit and vegetables. To the best of the authors' knowledge, this is the first paper that reviews the effect of sonication of fresh and minimally processed vegetables. Furthermore, this paper also highlights the possible use of sonication combined with other physical or chemical aids and discusses the potentinal large-scale utilisation of this technology if a correct optimisation and scaling-up is conducted.

2. Antimicrobial effects of US processing

2.1. Effect of sonication on natural-occurring and inoculated microorganisms

Antimicrobial effects of US have been attributed to two main causes: cavitation and free radical formation. The former can shear and break cell wall and membrane structures, thus increasing permeability and losing selectivity (Bilek, 2013). The micro-mechanical shocks of the collapsing bubbles, can cause disruption of cell components and DNA injuries, breakages, and fragmentation (Birmpa, 2013). The latter is caused by the high pressure and temperature reached within the bubbles, which promotes the generation of primary hydroxyl radicals and the acceleration of single electron transfer. This originates a series of reactions that form, among others, hydrogen peroxide with bactericidal properties (Bilek, 2013). Hydroxyl radicals are also able to react with the sugar-phosphate backbone of DNA, causing the withdrawal of phosphate-ester bonds and breaking the double strand microbial DNA, leading to cell unviability, dysfunction, and further death of microorganisms (Mañas, 2005).

Fresh and minimally processed FV must be microbiologically safe as they are generally consumed raw. As US processing has been reported to be a potential alternative to chlorine in disinfection steps, studies have been and must be carried out in order to better understand the outcomes of US processing of FV. The most commonly used conditions when applying US to FV are shown in Table 1 and Table 2. Briefly, the most commonly used frequencies ranged between 20 and 40 kHz, obtained by applying sonication powers between 10 and 200 W and temperatures ranging from 20 to 40 °C. Treatment times ranged from 1 to 60 min. In addition, several food matrices have been evaluated, and these included vegetables such as lettuce, kale, or carrots, and fruits such as strawberries, plums, or kiwis. So far, most studies reported the effects of US processing on either alterative microbiota, typically mesophilic bacteria, yeasts and molds (see Table 1) or pathogenic microorganisms, namely *E. coli*, *Salmonella* spp., and *L. monocytogenes* (see Table 2).

Antimicrobial effects of US depend on treatment parameters. Indeed, Mansur (2016) recently suggested that sonication power is a factor which has a key impact on the antimicrobial efficacy of US. Moreover, the higher was the intensity used when treating kale (ranging between 100 to 400 W/L), the higher were the reductions observed for all the microorganisms studied (ranging between 3.2 to 3.9 log cycles). Overall, it seems that US application mode is not a factor that has a significant effect on microorganisms, as there seems to be no differences between continuous – constant sonication – or pulsed – intermittent sonication – modes (Hashemi, 2018a). Still, the use of continuous or pulsed US affected differently the content of certain compounds in fruits and vegetables previously. For example, Pan (2012), reported different values of total phenolics content and antioxidant activity on pomegranate after application of continuous US when compared to pulsed US. We would like to highlight that this does not mean that US processing can alter the content of polyphenols in fruits. It is likely that continuous or pulsed ultrasonic waves can lead to different extraction yields and therefore higher phenolic contents and antioxidant activities of the water and organic extracts. Another parameter that affects cavitation activity is frequency, as bubble size is inversely proportional to it. Application of lower frequencies results in larger bubbles, liberating higher energy (São José, 2014a).

Effects of US on microorganisms also depend on the target specie and on matrix properties. In this sense, São José (2014b) obtained different antimicrobial effects depending on the studied matrix. They reported a reduction of *E. coli* populations of 2.3 or 1.6 log cycles when processing green pepper or melon at 40 kHz for 2 min, respectively. When using these same conditions, reductions of *S. enterica* Enteritidis on green pepper or melon were lower, 1.8 and 1.9 log cycles, respectively. These differences were attributed to the behavior of each microorganism on different surfaces. According to Tan (2017), sonication alone significantly affects the flagella and fimbriae of bacteria, decreasing the cell adhesion of artificially inoculated *S. enterica* Typhimurium by 0.5 to 1.0 log cycles, a relevant reduction if taken into account that *Salmonella* contamination in real production lines typically contains <1 log CFU/mL of this bacteria. US capacity to remove bacterial cells from the surface is recognized, as it influences the attachment ability of microorganisms before and after biofilm formation. Biofilms, or aggregates of microorganisms whose cells are frequently embedded within a self-produced matrix of extracellular polymeric substances, may be another source of resistance to sanitizers and surfactants (Brilhante de São José, 2012). In fact, several studies have evidenced its effects on *L. monocytogenes* biofilms alone (Hamman, 2018) or combined with surfactants (Torlak & Sert, 2004). US are widely used on machinery surfaces and food pipelines, as a physical method to eliminate biofilms, since there is no residue left over in the removal process (Zhao, 2017).

Yeasts and molds can also be inhibited by US. Some authors have reported 0.5 log cycles reductions in strawberry processed at 33 kHz and 60 W for 10 min (Gani, 2016). Other studies observed reductions of 2.3 log cycles in kiwi, when processed at 30 kHz and 368 W/cm^2 during 8 min (Vivek, 2016). Overall, in the majority of the studies published to date, decay incidence, or percentage of fruits with visible mold growth, was significantly reduced when comparing US processed with a non-treated control (Muzaffar, 2016; Vivek, 2016). Even though reported reductions of pathogenic bacteria seem to be higher than those of epiphytic microbiota, pathogenic microorganisms are normally artificially inoculated in the food matrix before the assay, so internalization and attachment are typically lower than what occurs regarding natural microbiota. Moreover, total bacteria count includes a wide range of microorganisms within which some strains could be more resistant to specific US conditions. In this regard, more assays should be carried out in order to elucidate whether US could be capable of reducing microbial loads that occur in the stomata, vasculature, cut edges or intercellular tissues, where other strategies have proven to be ineffective (Meireles, 2016).

Another factor that affects the effectiveness of US is processing or dipping time. It seems that longer processing times result in higher microbial inactivation (Birmpa, 2013). It is important to highlight that for each target microorganism, matrix, and US conditions, a minimum application time is necessary to report significant changes on the microbiota (Hashemi, 2018a). Temperature of the matrix and the media can increase by the application of US for a period of time, due to acoustic energy produced (Marques Silva, 2017). The temperature achieved could affect the results, leading to a possible increase in microorganism inactivation but also to an alteration or degradation of biochemical and nutritional compounds. In order to implement this technology at large scale production of minimally processed fruits or vegetables, processing times should be minimized and should not exceed a few minutes. Although US processing alone can exert antimicrobial effects (do Rosário, 2017), to reduce treatment time and to achieve a sufficient microbial inactivation, US can be combined with other chemical or physical strategies, because synergistic or additive effects may take place when it is combined (Barba, 2017; Park, 2018).

2.2. US combined with mild temperatures

So far, there are no publications that use a combination of mild temperatures and US (thermosonication) to disinfect FV for fresh-cut produce. It has been widely studied in FV juices, with good results on pathogenic microorganism reductions (Sánchez-Rubio, 2018), alterative microbiota growth and bioactive compounds maintenance (Lafarga, 2018; Hashemi, 2018b), and enzyme inactivation (Illera, 2018). Nonetheless, application of thermosonication on FV for fresh-cut industry could lead to changes in texture that may not be a shortcoming in juices but could have detrimental effects on fresh-cut FV. As previously mentioned, long processing times are not feasible in industry, as they can have detrimental effect on firmness (Terefe, 2011). However, further studies are needed in order to assess the real potential of this technology in the fresh and minimally processed fruit and vegetable industry.

2.3. US processing combined with chemical agents

Because of the limitations of US processing alone and the limited applications of the combinations of US with mild temperature for fresh produce, chemical agents used as sanitizers may become effective alternatives to chlorine. Among others, organic acids and ozone have proved to be able to reduce microbial load in FV (Meireles, 2016). Many of these compounds have GRAS status, and have already demonstrated to exert antimicrobial activity. For example, carvacrol, vainillin, or peracetic acid were used against *E. coli* O157:H7, *Listeria* spp., and *Salmonella* spp. and reductions between 1.0 and 3.0 log cycles were observed (Abadias, 2011). This, together with the possibility of combining them with US, makes them good choices for the fresh-cut industry.

2.3.1. Organic acids

Organic acids seem to have two distinct antimicrobial action modes. The first involves pH depression, as a release of protons to the surrounding media creates unfavorable conditions for bacterial growth. The second is based on the diffusion of the non-dissociated form of the organic acid across the semi-permeable membrane of the microorganisms. Once within the cell, the acid may undergo a dissociation process, as the pH of the cytoplasm, which is approximately 7, may be different to the pH outside the cell. Once the organic acid is dissociated, the pH drop can suppress cell enzymes and nutrient transport systems, causing the death of the pathogen (Calmont, 2010).

The most widely studied organic acids are lactic, citric, acetic, and peracetic acid, at concentrations ranging between 0.04 and 2%. Reductions of 3.2 or 3.0 log cycles have been achieved against *Salmonella* Typhimurium when combining US with citric (2%) or peracetic acid (5%) respectively (Sagong, 2011; Silveira, 2018), which were higher than those of non-treated product. Lower reductions were observed when using lactic acid 1% against *Salmonella* Enteritidis, which reduced by 1.9 to 2.8 log units (Sâo José, 2014a; São José, 2015). *L. monocytogenes* and *E. coli* have also been studied, and reductions of approximately 2.5 log cycles have been reported when processing lettuce leaves at 40 kHz and 90 W for 5 min combined with lactic, citric, or malic acid at 2% (Sagong, 2011).

Except for Silveira (2018), who reported no significant differences between US alone (40 kHz, 500 W, 5 min) or in combination with peracetic acid 50 mg/L, studies published to date show a significant synergistic or additive effect on the combination of both mechanisms (Table 1 and Table 2). The intense pressure gradients caused by US seem to enhance the penetration of the organic acids through the cell membrane of the microorganisms, and along with cavitation, it assists the disaggregation of the microorganisms, leading to an increased efficiency of the sanitization treatment (Sâo José, 2015).

2.3.2. Essential oils

Sonication can also be combined with essential oils (EOs). EOs are effective antimicrobials (Ribeiro-Santos 2018). Their action mechanism includes membrane rupture, ATP-ase inhibition, leakage of essential biomolecules, proton motive force disruption, and enzyme inactivation (Pisoschi, 2018). According to Salvia-Trujillo (2015), the key features for the effectiveness of EOs are their composition, concentration, and droplet size, that promotes faster inactivation of microorganisms. Millan-Sango (2016) suggested that EOs' droplet size is not as important. However, when EOs and US processing are combined, US frequency and processing time are directly related with antimicrobial effects.

In fact, cinnamon, oregano, and thyme EOs have been studied against several pathogens. When using cinnamon EO (2%), reductions of *L. monocytogenes* ranging from 0.8 to 1.6 log cycles have been reported. Cinnamon EO in combination with 140W, 5 min US processing, also stopped the growth of the microorganism during 9 days of storage (Park, 2018). Oregano (10-18 mg/L) and thyme (14-18 mg/L) EOs in combination with US processing at 26 kHz and 200W for 5 min, were used against *Salmonella* spp. increasing the effect achieved when using US alone (Millan-Sango, 2015). In addition, a 4- to 5-fold higher decrease in the *E. coli* O157:H7 populations was observed when compared with disinfection with EOs only (Millan-Sango, 2016).

This synergism, or the greater effect observed when combining US and EOs compared to the sum of their individual effects, and the ease to apply both methods together, make the tandem a promising alternative for disinfection processes in FV industry.

2.3.3. Ozone

Briefly, the antimicrobial action mode of ozone consists of two mechanisms. On the one hand, the oxidation of sulfhydryl groups and amino acids of enzymes and proteins generating small peptides. On the other hand, oxidation of polyunsaturated fatty acids to acid peroxides by ozone induces cell envelope damage or disintegration, leakage of cell content, and lysis (Brodowska, 2017; Horvitz, 2014). Ozone is one of the most potent oxidizing agents, and it is more soluble in water than it is in air, making it suitable to be combined with US (Aguayo, 2014). Moreover, as ozone is unstable in aqueous phases, it decomposes to form oxygen and therefore, food products treated with ozone are free from chemical residues (Souza, 2018).

To the best of the authors' knowledge, there is only one study published so far evaluating the combined effect of ozone and US. Aday (2014) combined US (20 kHz, 30 W, 5 min) and ozonation (0.075 mg/L) on strawberries. The authors reported a 21 and 35% incidence of *Botrytis cinerea* in non-treated fruits at the 3rd and 4th weeks respectively, whereas a complete inhibition of mold growth was observed after the treatment with ultrasound and ozone during the whole storage (See Table 2). In order to determine whether the combination of ozonation and sonication could be an effective option for FV disinfection, more studies should be carried out using both methodologies and applying them to a range of matrices, target microorganisms and at different conditions.

2.3.4. Slightly acidic electrolyzed water

Slightly acidic electrolyzed water (SAEW) is produced by means of an electrolytic cell without a separating membrane, producing the electrolysis of dilute sodium chloride and hydrochloric acid solutions. Its bactericidal effect is attributed to the available chlorine compounds including ClO^-, HClO, and Cl_2 (Ye, 2017). SAEW is commonly used at pH values ranging from 5.0 to 5.5 and oxide-reduction potential values of 930-980 mW.

Despite the potential of SAEW for disinfecting fresh foods, it seems that this technique alone might not be able to completely disinfect all FV, especially those that might have hidden places where adherent microorganisms are difficult to remove by aqueous sanitizers (Luo, 2016b). Indeed, Koide (2009) found that SAEW was effective to remove bacteria from the surface of fresh-cut cabbage but residual contamination could be caused by microorganisms embedded inside the cellular tissues, namely stomata. The combined effect of SAEW and US has been proved to be more efficient in reducing microbial loads when compared to their individual application. For instance, SAEW has been applied in lettuce or tomato in combination with US at 20 kHz, 130/210 W, for 5 to 15 min against *L. monocytogenes* and *E. coli*, achieving reductions of 4.0 log cycles (Afari, 2016). The combination did not only reduce the population but also allowed the control of the remaining microorganisms in FV. Indeed, for *Bacillus cereus* in potato processed with US at 40 ºC 40W/L for 3 min, the lag time increased by 0.2-10.5 h, and the specific growth rate decreased 0.01-0.23 log cfu/h in comparison to the 0.46 log cfu/h of the non-treated control. The authors indicated that the cells stressed by the treatment had lower metabolic activity compared to those untreated (Luo, 2016a). In addition, SAEW and US combination was also effective reduce spoilage microbiota, as it was reported by Wu (2018), who applied pH 5.5 and ORP 514 mV water combined with 40 kHz, 200 W, 3 min US treatment to mushrooms and found significant differences in spoilage microbiota in comparison to the water-treated control. The combination is worthy as well to inactivate the pathogens that could remain in water (Afari, 2016).

2.4. High pressure CO_2

Supercritical CO_2 is being increasingly studied as an antimicrobial agent, due to its advantageous characteristics. These include being a GRAS substance, and that its critical temperature (31.1 ºC) and pressure (7.3 MPa) are compatible with the thermal stability of most food matrices, facilitating its application in industrial processes (Hossain, 2013; Hossain, 2016; Tamburini, 2014).

So far, most commonly used pressures and temperatures were 6 to 12 MPa and 22 to 35 °C, respectively (Table 1 and Table 2). Studies published to date have reported an 8.0 log cycle reduction of *E. coli* when combining supercritical CO_2 10 MPa, 22ºC with US at 40 kHz, 10 W, after processing for 5 min, while 15 min were needed to achieve the same levels using CO_2 alone (Ferrentino, 2015b). Ferrentino (2015a) detailed that mesophilic microorganisms, coliforms, yeasts and molds were also reduced by 3.0 log cycles when combining CO_2 at 12 MPa, with US at 40 kHz, 10 W for 30 min, at a mean temperature of 39.7ºC. Also, a 7.0 log cycle reduction of *S. typhimurium* was achieved with the same treatment. Effect on *S. tyhphimurium* was not observed when applying US alone.

Combination of supercritical CO_2 with US demonstrated to have an improved effect than it had when US was applied alone (Ferrentino, 2015a; Ferrentino, 2015b). As one of the main drawbacks of US is that the transmitting media seems to partially absorb the acoustic energy, preventing its transfer to the solids to be treated, the use of CO_2 could potentially overcome this issue as it is a dense fluid, and acoustic waves would not be reflected but absorbed by the solid (García-Pérez, 2006). Moreover, with an increase of temperature from 22 to 40ºC, higher diffusivity of CO_2 and increased fluidity of cell membrane allows a faster penetration of CO_2 into it. US enhances this effect, as it induces a better contact between CO_2 and the membrane, accelerating the diffusion through the membrane, thus causing a drastic drop in intracellular pH and extraction of vital constituents (Ferrentino, 2015).

3. Nutritional changes

The effect of US processing on FV nutritional components has been widely studied. Results listed in Table 3 suggest a higher content of phytochemicals in extracts obtained from sonicated fruits and vegetables. US is commonly used to promote the extraction of compounds from food sources including phenols (Soquetta, 2018), carbohydrates (Vilkhu, 2008), or proteins (Lafarga, 2018). This does not mean that US processing promotes the generation of these valuable compounds. Higher yields reported in the literature could be attributed to the enhanced extraction efficacy when US have been applied. US causes cell disruption, allowing permeation of intracellular compounds and therefore, a higher liberation of molecules to the extracting media (Hidalgo, 2017). In order to obtain improved extraction yields, processing times in the range 20-60 min are generally required (Annegowda, 2012; Lafarga, 2019). However, it has been suggested that sonication can increase the degradation of natural products (Pingret, 2013). Two chemical reactions have been proposed as probable mechanisms responsible for the degradation connected with sonication. One is related with pyrolysis within cavitation bubbles or gas pockets trapped in the crevices of the solid boundaries, which cause the degradation of polar compounds, and the other is the generation hydrogen ions ($H+$), free radicals ($O-$, $OH-$,) and hydrogen peroxide (H_2O_2) that are produced by cavitation effect (Rawson, 2011). For instance, isomerization of carotenoids can also occur, as there are extreme physical conditions of temperature and pressure during processing (Kumcuoglu, 2014). Also, antioxidant capacity of cyaniding 3-glucoside was evaluated after US treatment (20 kHz) and showed a 20% of its original antioxidant capacity. The authors suggested that hydroxylation occurred during sonication, causing such decrease (Ashokkumar, 2008). Degradation or oxidation of biochemical compounds has been related with increased treatment times (Gani et al., 2016; Jahouach-Rabai, 2008)

There are scarce studies focusing on the effects of US on the macromolecules of FV. In fact, from the recent past years, there is only one paper reporting values of fat content, and the results showed that there was no statistical effect on this parameter when combining US and high pressure CO_2 on coconut (Ferrentino, 2015a). Regarding proteins, US could induce changes in native form: conformational changes, damage to secondary structure, re-structuration of disulfide bond or generation of other intra/ inter molecular interactions (Chizoba-Ekezie, 2018). Studies on proteins in FV after US have focused mostly on its extraction yield and allergenicity (Nayak, 2017). Only one study carried on by Li (2017) evaluated the effect of US (40 kHz, 350 W) on total soluble proteins of straw mushrooms. They reported that this parameter – an indicative of tissue destruction – was negatively affected by over-time treatments (30 min), but 1 to 10 min served to prevent soluble protein utilization, allowing metabolic activity prolongation.

The effect of US processing on the total phenolic content (TPC), of FV has been largely studied. However, only few studies evaluated whole pieces and most of them focused on processed products such as juices or purees. Bal (2017) processed grapes with US (32 kHz, 60 W/L, 10 min) and observed an increase on the yield TPC of the sonicated product at the end of a 60-day storage period when comparing to the untreated control. Related to flavonoids, Bal (2017) suggested that even though there were no statistical differences between samples, total anthocyanin content of grapes processed with US tended to increase during storage. Other authors observed an increment of 7.9% in TPC values on strawberries processed with US (33 kHz, 60 W, 10-40 min, US bath maintained at 25ºC) from day 1 (Gani, 2016). Increase of TPC was partially explained by a better extraction of polyphenols attributed to an increase in temperature that occurs in US treatment as a consequence of cavitation phenomena, and it was also attributed to hydroxylation of flavanols, which has a positive effect on antioxidant activity (Soria, 2010). Increases in yield of TPC were reported by Yu (2016), who found that the TPC values of US-treated romaine lettuce (25 kHz, 26 W/L, 1-3 min) were up to 22% higher than those quantified in the untreated product. As an abiotic stress, US could enhance the biosynthesis of secondary metabolites in plant cells, through stimulating their physiological activities. That could partially explain why TPC increases during storage when compared with the non-sonicated products (Wang, 2015). In addition, US could promote the liberation of phenols, as these compounds can be bound to other compounds present in cell walls (polysaccharides, proteins, etc.) and be disrupted by US cavitation (Khan, 2018). On the contrary, Ferrentino (2015b) found that applying US (30 kHz, 40 W, 30 min) and high pressure CO_2 (12 MPa, 35ºC), the TPC decreased when compared with the untreated product. Still, these results could not be attributed directly to the ultrasonic effect, because no control of both individual treatments was used in that study.

Ascorbic acid (AA) forms part of Vitamin C, and its content can be affected by US processing. Alexandre (2013) reported that sonication reduced the loss of AA during the freezing of red bell pepper when processing at 35 kHz, 120 W and 15 ºC compared to water-washed ones. In terms of the US mode application, Hashemi (2018a) did not observe significant differences between the use of pulsed or continuous mode in the AA content of plums. Treatment time was a significant factor to take into account in US processing. The same authors reported an increase in the AA content when longer US treatment times (1, 15, 30, 45 and 60 min) were applied to plums at 30 kHz and 100 W. The increase of this compound was attributed to the elimination of entrapped oxygen due to cavitation, which is essential for AA degradation (Bhat, 2011; Cheng, 2007).

As summarized above, effects of US on nutritional values of FV may differ between studies, conditions, and matrices, and they can partially be attributed to different extraction yields when applying US (Chemat, 2017). These differences may also occur when scaling up from lab or pilot plant scales to industry. With this purpose, several papers reviewing the potential of US in food industry have been published to date (Bilek, 2013; Kentish, 2014; Prakash, 2003).

4. Effect of US processing on FV quality

As highlighted in previous sections, US processing combined with chemical sanitizers shows potential for being used for the large scale disinfection of fresh and minimally processed fruit and vegetables. However, US processing can result not only in antimicrobial or increased extraction yields but also in a detriment in quality attributes. The quality of FV is based on several properties: physical parameters, such as texture or color, organoleptic attributes like aroma or flavor, and nutritional and bioactive properties including TPC or antioxidant capacity. Therefore, in order to obtain high-quality products, it is important to assess the effects of processing on these key parameters.

4.1. Overall quality changes

Physical properties of FV processed with US generally remain unchanged after treatment. As it is shown in Table 3, pH and titratable acidity tend to maintain the values of the control samples after the US treatment. In some cases FV processed with US have higher total soluble sugars values than those from the control. This has been attributed to the fact that US might accelerate the depolymerization process of the starch gel (Amaral, 2015; Bal, 2017) in the outer parts (< 1 mm) and at deeper tissues, changes are attributed to water removal (Schössler, 2012). These structure alterations can be related with the increment of exposure time to US, increasing the temperature and the further destruction of cellular structure (Jurek, 2012).

4.2. Color

Color is an important sensory attribute of a fruit or vegetable that provides an indication of freshness and flavor quality. It could affect the consumer buying decision to acquire a certain product or to prefer one to another. Not appropriate color will suggest loss of freshness or lack of ripeness that will repel the potential buyer (Barrett, 2010), thus the importance of monitoring the effect of US on this attribute.

Several studies, including those listed in Table 4, evaluated the effect of US processing on the color parameters of FV. Overall, no changes in color were observed, processing with US alone or in combination with chlorine or high pressure CO_2 (Ferrentino, 2015b; Salgado, 2014). However, some studies reported significant differences between US-processed and untreated samples in the a* and b* values, either once treated or after storage (Ferrentino, 2015a) or in L* values in different matrices such as coconut, mango, or strawberries (Aday, 2014; Amaral, 2015; Santos, 2015).

The observed changes in color can be attributed to the possible inactivation of enzymes such as poly-phenol oxidase (PPO) and phenol peroxidase (POD). These enzymes are proposed to cause off-colors in raw and frozen vegetables and browning reactions (São José, 2014a; Toivonen, 2008). US treatments have demonstrated to be able to inactivate such enzymes in certain conditions, occurring at higher rates when combining US technology with heat (40-60℃) (Illera, 2018). Enzyme inactivation also depends on treatment time (Cao, 2018; Zhu, 2017) and US intensity (Liu, 2017). Causes of enzyme inactivation involve shear stress and pressure, which cause oligomeric enzymes dissociation, free radicals affecting the structure, and creation of a large interfacial area by US that disturbs the hydrophobic interaction and hydrogen bonding, thus destabilizing proteins (Terefe, 2015). For instance, Li (2017) observed an inhibition in PPO activity when processing straw mushrooms with US (40 kHz, 350 W, 1-30 min). A decrease in POD and PPO activities of fresh-cut pineapple was reported by Yeoh (2017) after processing pineapple slices with 25-29 W, 37 kHz, for 10 to 15 min US . These enzymes had significantly lower activity in sonicated fruit than they had in water-washed fruit. Besides, after a 5-day storage period, POD and PPO activities were 3.8 and 4.5-fold lower than they were in the water-dipped control. Moreover, US was suggested to facilitate the penetration of ascorbic acid to vegetable cells, as cell wall disruption occurred, thus enhancing antioxidant processes. On the contrary, Wang (2015) found an increase on POD activity of cherry tomatoes and Ferrentino (2015a) of coconut. It has been proposed that low US power level could promote enzyme production, whereas high power US could induce the contrary effect, but it could affect the quality parameters of the product. Also, the effectiveness of US depends on the differences in the resistance of each enzyme to the treatment (Kentish, 2014).

In red fruit, changes in color may be attributed to the degradation of anthocyanins when cavitation occurs for long period times (Gani, 2016). For mushrooms, it has been suggested that US exerts a protective effect on surface color changes as hydrogen peroxide is formed in distilled water when cavitation occurs, and this compound helps to maintain their whiteness (Lagnika, 2013). Factors affecting other FV are pigments, such as carotenoids and chlorophyll, or other compounds like ascorbic acid (Bermúdez-Aguirre, 2013), that may be altered by US treatments. Carotenoids, lycopene, and other liposoluble pigments undergo isomerization processes that can lead to color alterations (Adekunte, 2010). Indeed, Sun (2010) observed that the appliance of US at 21-25 kHz, 950W, for 10 min to β-carotene resulted in several carotene degradation products, including 15-*cis*-β-carotene and di-*cis*-β-carotene. Eh (2012) also found that processing tomatoes with US with 37 kHz, at 140 W for 45 min resulted into changes in lycopene forms *cis* and *trans*.

For what has been reviewed, changes in FV color after US treatment can occur as a consequence of a number of reasons, namely activity reduction or inactivation of browning enzymes, penetration of antioxidant agents to vegetable cells, and alteration or degradation of pigments. As so, more studies should be carried out in order that US conditions be optimized for each purpose in order to maintain overall color quality of FV.

4.3. Texture

There are two main factors influencing the consumer's mouth feel of a fruit or vegetable: firmness and juiciness. Firmness is determined by the physical anatomy of the plant tissue, cell size and shape, wall thickness and strength, and cell-to-cell adhesion. In turn, juiciness is related to the cell sap content and the ease to be split (Toivonen, 2008). Consumers have clear expectations for the texture of fresh-cut FV, and panel testing indicates that they are more sensitive to small differences in texture than in flavor, being textural defects and the interaction of flavor and texture the features that cause most reject (Barrett, 2010).

A review of the data found to date is summarized on Table 4. Some studies report no significant differences in textural parameters after US processing. However, most of the accounts suggest that US processing can affect the firmness of fresh FV depending on the intensity of the treatment. The effect of US processing on texture also depends on several parameters, which include food matrix, variety, maturity stage, intensity, or processing duration of US treatment. Results obtained so far seem to be contradictory and matrix-dependant, texture changes should be assessed independently for each fruit or vegetable. Softening of fruits has been attributed to inner changes of cell wall constituents, mostly pectin, which can be de-esterified by the activity of pectin methyl esterase, followed by a depolymerization of methoxy pectin or pectic acids due to endo-polygalacturonase activity (Wang, 2018). For instance, Saeeduddin (2015) found that 20 kHz, 0.30 W/mL US applied for 10 min at 45 or 25 ºC could inactivate pear pectin methyl esterase by 60 or 7%, respectively. Thus, US, combined or not with mild temperatures or high pressures, can cause partial or total inactivation of enzyme activity, thus leading to changes on the textural quality of the FV (Marques-Silva, 2017).

From above, one can gather that texture and color change or maintenance depends on a number of factors including matrix, treatment conditions, enzymes and plant components. Therefore, the effect of US processing on FV quality parameters should be assessed for each product independently.

4.4. Antioxidant capacity

Fruit and, to a less extent, vegetables, are along with beverages the main sources of the daily intake of phenolic antioxidants (Shahidi, 2015). Apart from preventing browning and deterioration of different constituents of FV, antioxidant compounds are now on the focus for health reasons, as they are presumed to prevent the deleterious effects of free radicals in the human body (Pisoschi, 2012).

According to recent studies, antioxidant activity values obtained by *in vitro* methods of FV processed with US increases in comparison with the control samples. As an example, Wang (2015) reported that applying US (22kHz, 100 W) to tomatoes led to an increase DPPH· inhibition by 8.22 to 17.56%, depending on the power intensity used (66.64 and 106 W/L respectively) and an increase in FRAP values from 6.03 to 13.18% respectively. Yu (2016) observed similar results in romaine lettuce treated with US (25 kHz, 26 W, 1 or 3 min). Gani (2016) also stated that antioxidant activity of US treated samples increased with processing and was higher proportionally to the treatment time. However, a slight decrease was observed at 60 min, attributed to the excessive damage to cell structure which could lead to greater chances of oxidation as well as degradation of polyphenolic compounds. It has been suggested that due to the generation of hydroxyl radicals, hydroxylation of food materials could be increased during US, leading to an increased antioxidant activity (Ashokkumar, 2008). Increased antioxidant capacity can also be attributed to an increased phenolic content in FV, as this two values are positive correlated (Gani, 2016). Nonetheless, and as it has been previously described, antioxidant compounds may be maintained in amount in FV but better extracted due to tissue disruption, leading to higher antioxidant capacity values regarding sonication time does not exceed. More studies should be done concerning antioxidant capacity of sonicated fruits, in order to find a relationship between the higher yields observed and a higher bioavailability once ingested.

4.5. Flavor

In relation to flavor, data that can be found in the literature is not extensive. Feng (2018) reported no differences in astringency, aftertaste, bitterness, umami, richness, and saltiness between US processed cucumber (20 kHz, 200 W, 10 min) and the non-sonicated control. The concentration of the main volatile compounds of cucumber increased with treatment. Yu (2016) found that 1 min sonicated romaine lettuce had a good punctuation on overall sensory evaluation, and it was higher than it was for the control and samples processed for 2 or 3 min.

Effects of US on flavor has not been thoroughly studied, so more investigation in this line could be done in order to elucidate the effects of US on aromatic and sapid molecules.

Conclusion

Ultrasound is a mild technology that has been studied with the aim to reduce microbial load of food, and its application in fresh and fresh-cut fruits and vegetables has potential interest for manufacturers, as it is versatile and reasonably easy to use. It has been reported to be relatively effective as an antimicrobial agent, and its effects can be improved if it is combined with other physical technologies, such as mild temperatures or supercritical CO_2, or with chemical agents, including organic acids and essential oils, ozone, or slightly acidic electrolyzed water. A part from being able to reduce pathogenic and alterative microbiota in FV, US may have a consequence on other key features, such as color or texture, or components, namely phenols or vitamins. Overall, it seems that results of US processing on FV do not follow general trend, as they depend on several parameters related with treatment conditions and matrix. Targeted microorganisms may not respond equally in the same conditions, and reductions may also vary depending on parameters stated before. Accounting on this review's information and knowing are capable to achieve the desired outcomes, each case should be studied and scaled-up individually in order to preserve safety, quality and nutrition values of fresh and fresh-cut FV.

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Conflict of interests

The authors declare no conflict of interest

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Table 1. Effect of US processing alone or in combination with other strategies on FV natural microbiota.

Fruit / vegetable	US conditions	Target microorganisms	Effect	Source
Kale	40 kHz, 100 – 400 W/L, 1 min, 40°C	TBC, yeasts and molds, and Enterobacteriaceae	Reductions: 1.0, 0.9 and 1.0 log cfu/g (100 W/L) and 1.8, 1.5 and 1.7 log cfu/g (400 W/L). Reductions at 20°C were lower than they were at 40°C.	(Mansur, 2015)
Cherry tomatoes	20 kHz, 100 W, 8 min	TBC, and yeasts and molds	Reductions: 0.8 and 0.7 log cfu/g. Microorganism populations were reduced by US treatments compared with the control group. The higher the power density was, the lower the counts.	(Wang, 2015)
Kiwis	30 kHz, 368 W/cm^2, 8 min	TBC and yeasts and molds	Reductions: 2.3 and 3.5 log cfu/cm^2. Not better compared with treatment using NaOCl, that achieved 5.83 and 3.68 log cfu/cm^2 respectively.	(Vivek, 2016)
Strawberries	33 kHz, 60 W, 10 – 60 min	TBC and yeasts and molds	Reductions: 3.6 ± 0.1 and 2.0 log cfu/mL. After 15 days storage, best conditions to preserve were 40 min, and reduced 3.9 and 3.3 log cfu/mL respectively.	(Gani, 2016)

Grapes	32 kHz, 60 W/L, 10 min	Decay incidence	Decay incidence was lower when compared with the control.	(Bal, 2017)
Mirabelle plums	30 kHz, 100 W, 0 – 60 min, pulsed/continuous	TBC and decay incidence	Reductions: 0.4 – 1.5 log cfu/g. Decay incidence was reduced when compared with the control. No differences between pulsed and continuous mode. Highest decrease was observed at 60 min.	(Hashemi, 2018a)
Strawberries	20 kHz, 30 W, 5 min combined with 0.075 mg/L ozone or 6 mg/L chlorine dioxide	Decay incidence	US combined with ozone or chlorine dioxide prevented mold growth, while in control group, mold presence was of 21 and 35% at the 3rd and 4th week.	(Aday, 2014)
Carrots	40 kHz, 10 W, 30 min combined with high pressure CO_2 (12 MPa, 22°C)	Mesophyll microorganisms, acid lactic bacteria, total coliforms and yeasts and molds	Reductions: 3.7, 2.5, >6, and 3 log cfu/g for mesophyll microorganisms, acid lactic bacteria, total coliforms, and yeasts and molds.	(Ferrentino, 2015b)
Strawberries	40 kHz, 100 W, 5 min combined with acetic acid (800 mg/L), SDS (1,200 mg/L) or PAA (40 mg/L)	TBC and yeasts and molds	Reductions: 1.0 ± 0.2 log cfu/g and 1.2 ± 0.2 log cfu/g higher when compared with the control. The most effective treatment was US combined with PAA, which achieved 2.0 ± 0.8 log cfu/g reductions more than the control.	(do Rosário, 2017)

Calçot (*Allium cepa* L.)	40 kHz, 250 W, 1 to 45 min	TBC	Reductions: 1.0 log cfu/g after 45 min of ultrasonication. Populations did not exceed 10^6 cfu/g in any case.	(Zudaire, 2018)
Melons	40 kHz, 500 W, 5 min, combined or not with NaOCl (100 mg/L)	TBC	Reductions: 0.4 log cfu/g after combination US+NaOCl. Statistically different from the application of NaOCl or US individually, where reductions were of 0.1 and 0.2 log cfu/g, respectively.	(do Rosário, 2018)

CFU, colony forming units; ORP, oxide-reduction potential; PAA, peracetic acid; SDS, sodium dodecylbenzenesulfonate; TBC, total bacteria counts; US, ultrasounds. Decay incidence, % of fruits with visible mold growth

868 Table 2. Effect of US processing alone or in combination with other strategies on pathogenic microorganisms in FV.

Fruit / vegetable	**US conditions**	**Combined with**	**Target microorganisms**	**Reductions (log cfu/g)**	**Source**
Lettuce leaves	37 kHz, 90 W, 10 - 60 min	-	*E. coli*	2.3 ± 0.3	(Birmpa, 2013)
			S. aureus	1.7 ± 0.2	
			Salmonella Enteritidis	5.7 ± 0.1	
			L. innocua	1.9 ± 0.6	
Strawberries	37 kHz, 90 W, 10 - 60 min	-	*E. coli*	3.0 ± 0.7	(Birmpa, 2013)
			S. aureus	2.1 ± 0.6	
			Salmonella Enteritidis	5.5 ± 0.1	
			L. innocua	6.1 ± 0.0	
Kale	40 kHz, 100 W/L, 1 min	-	*E. coli* O157:H7	2.5 ± 0.2	(Mansur, 2015)
			L. monocytogenes	2.6 ± 0.1	
Lettuce leaves	40 kHz, 90 W, 5 min	Malic acid (2%)	*S.* Typhimurium	2.7 ± 0.5	(Sagong, 2011)
			L. monocytogenes	2.8 ± 0.3	
			E. coli O157:H7	2.5 ± 0.6	
		Lactic acid (2%)	*S.* Typhimurium	2.7 ± 0.4	
			L. monocytogenes	2.5 ± 0.8	
			E. coli O157:H7	2.8 ± 0.7	
		Citric acid (2%)	*S.* Typhimurium	3.2 ± 0.2	
			L. monocytogenes	2.3 ± 0.3	
			E. coli O157:H7	2.4 ± 0.1	

Lettuce leaves	40 kHz, 500 W, 5 min	PAA (50 mg/L)	*S.* Typhimurium	3.0	(Silveira 2018)
Pears	40 kHz, N/A	-	*S.* Enteritidis	0.9 ± 0.6 [1]	(Brilhante de Sâo José, 2015)
			E. coli	1.5 ± 0.4 [1]	
		Lactic acid (1%)	*S.* Enteritidis	1.9 ± 0.4	
			E. coli	1.9 ± 0.4	
		Acetid acid (1%)	*S.* Enteritidis	1.6 ± 0.3	
			E. coli	1.4 ± 0.6	
Strawberries	40 kHz, 500 W, 5 min	-	*S.* Enterica	1.2 ± 0.3	(do Rosário, 2017)
		Acetic acid (800 mg/L)	*S.* Enterica	1.0 ± 0.3	
		SDS (1200 mg/L)	*S.* Enterica	1.0 ± 0.4	
		PAA (40 mg/L)	*S.* Enterica	2.0 ± 0.4	
Green Peppers	40 kHz, 2 min	-	*S.* Enteritidis ATCC 13076	1.8 ± 0.2	(Brilhante de Sâo José, 2015)(São José, 2014b)
			E. coli ATCC 11229	2.3 ± 0.3	
		Lactic acid (1%)	*S.* Enteritidis ATCC 13076	2.8 ± 0.6	
			E. coli ATCC 11229	2.9 ± 0.5	
		Acetic acid (1%)	*S.* Enteritidis ATCC 13076	2.4 ± 0.3	
			E. coli ATCC 11229	2.6 ± 0.3	
Melons	40 kHz, 2 min	-	*S.* Enteritidis ATCC 13076	1.9 ± 0.3	

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[1] Log cfu/cm^2

			E. coli ATCC 11229	1.6 ± 0.5	(Brilhante de São José, 2015)(São José, 2014b)
		Lactic acid (1%)	*S.* Enteritidis ATCC 13076	3.1 ± 0.7	
			E. coli ATCC 11229	2.5 ± 0.3	
		Acetic acid (1%)	*S.* Enteritidis ATCC 13076	2.4 ± 0.2	
			E. coli ATCC 11229	2.1 ± 0.2	
Carrots	40 kHz, 10 W, 30 min	-	*E. coli* ATCC 25922	No effect	(Ferrentino, 2015b)
		High pressure CO_2 6-12 MPa, 22/35°C	*E. coli* ATCC 25922	8.0	
Coconuts	30 kHz, 40 W, 30 min	-	*S.* Typhimurium	No effect	(Ferrentino, 2015a)
		High pressure CO_2 12 MPa, 35°C	*S.* Typhimurium	7.0	
Endives	N/A, 140 W, 5 min, 20ºC	-	*L. monocytogenes* (KCTC 13064, ATCC 15313)	0.4 0.5	(Park, 2018)
			E. coli O157:H7 (ATCC 43889, NCTC 12079)		
		Cinnamon leaf oil + surfactants CPC or BC	*L. monocytogenes* (KCTC 13064, ATCC 15313)	1.6 (CPC), 1.5 (BC)	
			E. coli O157:H7 ATCC 43889, NCTC 12079)	1.6 (CPC), 1.5 (BC)	
Lettuce leaves	26 kHz, 200 W, 5 - 25 min	Oregano EO (10 mg/L)	*E. coli* 0157:H7 NCTC 12900	4.0 ± 0.1 [2]	

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[2] Log cfu/mL

		Oregano EO (14 mg/L)	*E. coli* 0157:H7 NCTC 12900	> 5.0 *[2]	(Millan-Sango, 2015)
Lettuce leaves	26 kHz, 200 W, 6 min	Oregano EO (18 mg/L)	*Salmonella* spp.	3.1 ± 0.3 [1]	(Millan-Sango, 2016)
		Thyme EO (18 mg/L)	*Salmonella* spp.	2.9 ± 0.3[1]	
Parsley, lettuce and dill mix	20 kHz, 500 W, 5 min	Cinnamon EO	*L. monocytogenes*	0.8 ± 0.1	(Özcan, 2016)
Tomatoes	-	Calcium oxide, fumaric acid, SAEW	*L. monocytogenes* (ATCC 19111, 19118, Scott A)	4.5 ± 0.1	(Tango, 2017)
			E. coli O157:H7 (ATCC 23150, 43894, 43895)	4.3 ± 0.6	
	40 kHz, 400 W, 3 min	Calcium oxide, fumaric acid, SAEW	*L. monocytogenes* (ATCC 19111, 19118, Scott A)	> 5	
			E. coli O157:H7 (ATCC 23150, 43894, 43895)	> 5	
Potatoes	40 kHz, 400 W/L, 40°C, 3 min	-	*B. cereus*	2.9 ± 0.2	(Luo, 2016a)
		SAEW (pH 5.3-5.5, ORP 958-981 mV)	*B. cereus*	3.0 ± 0.2	
Lettuce leaves	20 kHz, 130 - 210 W, 5 – 10 - 15 min	Near neutral electrolyzed water (pH 6.5)	*E. coli* O157:H7	4.7 ± 0.5	(Afari, 2016)
			S. enterica Typhimurium	4.3 ± 0.5	
Tomatoes			*E. coli* O157:H7	8.4 ± 0.5	
			S. enterica Typhimurium	8.5 ± 0.5	

Bell peppers	40 kHz, 400 W/L, 10 min, 60 °C	SAEW (pH 5.0-5.2, ORP 930-950 mV)	*L. monocytogenes*	3.0 ± 0.1	(Luo, 2015)
			S. enterica Typhimurium	3.0 ± 0.1	

CFU, colony forming units; EO, essential oil; ORP, oxide-reduction potential; PAA, peracetic acid; SAEW, slightly acidic electrolyzed water; SDS, sodium dodecylbenzenesulfonate; US, ultrasounds.

Table 3. Changes in quality parameters of FV after US processing.

Fruit / vegetable	US conditions	Parameter	Obtained results	Source
Strawberries	20 kHz, 30 W, 5 min, combined with 0.075 mg/L ozone or 6 mg/L chlorine dioxide	pH	The greatest increase in pH during the storage was observed in untreated samples in comparison to the individual or combined treatments.	(Aday, 2014)
		TSS	Untreated samples had lower TSS content than other treatments. No significant difference between the treatments.	
		Respiration rate	Samples treated with US + ClO_2 and US + O_3 had a lower respiration rate than the individual treatments.	
Potatoes	24 kHz, 400 W, 1/5/10 min	pH	pH of sonicated potato was reduced after 5 and 10 min of treatment. Longer time the sonication, the greatest decrease in pH	(Amaral, 2015)
		TSS	TSS was higher on samples treated for 10 min.	
		Dry matter	No significant differences (p>0.05).	
		Cell structure	Differences in microstructure of potato after 10 min US. Disruption of the vacuole and the polygonal cell wall.	
Coconut	40 kHz, 10 W, 30 min, combined with high pressure CO_2 12 MPa, 35ºC	pH, TA	pH and TA of processed samples remained unchanged during storage. Contrarily, in control samples, pH values decreased and TA increased after 21 d storage	(Ferrentino, 2015b)
		POD	Treatment was not able to induce POD inactivation. Its activity slightly increased by the end of storage period.	
		Fat content	No significant differences (p>0.05).	

		TPC	Processed samples showed lower TPC values than controls did.	
		Antioxidant activity	A slight decrease was observed after the combined treatment compared with the untreated samples.	
Strawberries	33 kHz, 60 W, 10 / 20 / 30 / 40 / 60 min	TPC	TPC increased when strawberries were processed with US. The longer the time was, the higher the TPC.	(Gani, 2016)
		Antioxidant activity	Antioxidant activity of US treated samples increased with the increase in treatment time.	
Mirabelle plums	30 kHz, 100 W, 0 /15 / 30 / 45 /60 min, pulsed/continuous	TA	No significant differences (p>0.05) between the control and 15 min US processed samples. 30, 45 and 60 min sonication significantly inhibited the decrease of TA content.	(Hashemi, 2018a)
		TSS	Only 60 min treatment showed significant differences in TSS compared with the control. Higher amounts were observed.	
		AA	Significant increase in all sonicated samples when compared with the control	
Cucumber	20 kHz, 100 / 200 W, 10 min	TSS	100 and 200 W better retained SSC in samples. 300 W had a negative effect on TSS value	(Feng, 2018)
		Flavor	No significant difference in astringency, umami, richness or saltiness between processed samples and fresh ones.	
		Volatile compounds	Characteristic aromatic compounds, although decreased with time, were better retained if samples had been sonicated.	

Straw mushroom	40 kHz, 300 W, 3, 10, 30 min	Respiration rate	US significantly inhibited the respiration od straw mushroom. 10 min US treatment resulted in the minimum CO_2 production rate.	(Li, 2017)
		Weight loss	Us treatment delayed the weight loss. 10 min treatment had the greatest effect.	
		TSS	In all tested groups, TSS increased after the first 12 h period	
		Total soluble proteins	Over-time US treatment (30 min) had a negative effect on total soluble proteins, indicating tissue destruction.	
		PPO	US processing inhibited PPO.	
Romaine lettuce	25 kHz, 70 W, 1 / 2 / 3 min	TPC	Samples processed with US had higher TPC than control had. Only 1 min treatment was statistically significant ($p<0.05$)	(Yu, 2016)
		Antioxidant activity	During the first 30 h of storage, DPPH· inhibition was higher on sonicated samples, and they were followed by a significant increase	
		PAL	Samples processed during 2 and 3 min expressed higher PAL activity than the control did.	
		Sensory evaluation	Samples treated with US 1 min were rated higher than the control and maintained an acceptable score after 150 h. No significant differences ($p>0.05$) between samples treated with US 2 and 3 min and the control.	
Kiwi	400 W, 8 min	pH, TSS, TA	No significant differences ($p>0.05$).	(Vivek, 2016)
Cherry tomatoes	20 kHz, 100 W	Ethylene production	Ethylene production of treated samples was lower than it was for the control after 12 days storage. Climacteric peak was delayed by 4 d.	(Wang, 2015)

		TSS, TA	No significant differences (p>0.05).	
		POD	US processed fruits had higher POD activity than control group after 0 to 8 days.	
		TPC	At the end of the 16 d storage, US processed fruits showed higher TPC than the control did.	
		AA	At the end of the 16 d storage, US processed fruits had higher ascorbic acid content than the control had.	
		Antioxidant activity	At the end of the 16 d storage, US processed fruits had DPPH·, FRAP and ORAC values than the control had.	
Red bell pepper	35 kHz, 120 W, 15ºC	pH	No significant differences (p>0.05)	(Alexandre, 2013)
		AA	US treated samples retained more ascorbic acid than water washed ones did.	
Grapes	32 kHz, 600 W, 10 min	TSS	No significant differences (p>0.05) immediately after the treatment. US processed samples had the highest TSS compared with the control.	(Bal, 2017)
		TA	No significant differences (p>0.05)	
		Anthocyanin content	No significant differences (p>0.05)	
		TPC	US processed samples had the highest TPC values, and control samples had the lowest TPC values	

Pear	42 kHz, 200 W, 5-15 min	AA	No changes were observed in ascorbic acid content after US treatment.	(Plaza, 2015)
		TPC	Total phenolic content was significantly higher in US treated pears for 5 min than it was in non-treated samples. No differences in TPC were observed at 10 or 15 min treatments.	
Melon	40 kHz, 500 W, 5 min	pH	No significant differences (p>0.05)	(do Rosário, 2018)
		TA	No significant differences (p>0.05)	

AA, ascorbic acid; DPPH·, 2,2-Diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; ORAC, ozygen radical absorbance capacity; POD, phenol peroxidase; PPO, polyphenol oxidase; TA, titratable acidity; TPC, total phenolic content; TSS, total soluble solids; US, ultrasound.

Table 4. Changes in color and texture of FV after US processing

Fruit / vegetable	US conditions	Color	Texture	Source
Lettuce leaves	40 kHz, 90 W, 5 min combined with organic acids (malic, citric, and lactic) 0.3, 0.5, 0.7, 1.0 and 2.0%	Processing did not affect color parameters immediately after the treatment nor at 7 days of storage	No significant differences immediately after processing or after 7 days of storage.	(Sagong, 2011)
Lettuce leaves	37 kHz, 90 W, 10 / 20 / 30 / 45 / 60 min	Decrease in L* when treated with US. TCD was higher and positively correlated with treatment time (significantly different after 30 min)	Not significantly affected	(Birmpa, 2013)
Strawberries		Significant differences in L*, a*, and b* values when treatment time was higher than 30 min	Not significantly affected	
Strawberries	20 kHz, 30 W, 5 min, combined with 0.075 mg/L ozone or 6 mg/L chlorine dioxide	Ozone caused an increase in L* due to its bleaching effect. a* values of untreated strawberries were lower than treated ones. Strawberries treated with ultrasound plus ClO_2 preserved their a* values significantly better than other treatments.	All treated strawberries had higher firmness values than the controls. No difference was noticed between strawberries treated with ultrasound or ozone	(Aday, 2014)
Romain and iceberg lettuce leaves	25 kHz, 2 000 W, 1 min, combined with chlorine, surfactantants and Sodium dodecylbenzenesulfonate (1200 mg/L)	No significant effect on color. TCD between samples not significant. TCD<4 Chlorine helped to retain color.	No difference between samples immediately after processing or after storage for 14 days. Firmness evolved equally for all treatments.	(Salgado, 2014)
Coconuts	40 kHz, 10 W, 30 min, combined with high pressure CO_2 12 MPa, 35ºC	L* values were not statistically different after the treatment or during 4 weeks of storage. a* and b* parameters decreased. TCD of treated samples was higher than 4 after 3 weeks of storage.	No differences in hardness were observed between treated and non-treated samples. Hardness significantly increased after 2 weeks of storage in treated samples.	(Ferrentino, 2015a)

Mangoes	25 kHz, 50 W, 30 min	TCD was higher for US processed samples. º Hue was the most affected by US. Significant differences were observed immediately after the process, and a greater decrease occurred after 7 days of storage.	Firmness decreased when products were US processed. Firmness had more decay after 7 days of storage in treated samples.	(Santos, 2015)
Potatoes	24 kHz, 400 W, 1/5/10 min	L* was affected by US for all treatment times. After frying, color was correct (L* > 60) for all the treatments. L* and chroma decreased with time when US for 1 min. Hue values were not affected.	Losses of texture were observed but there were no statistical differences with the control.	(Amaral, 2015)
Carrots	40 kHz, 10 W, 30 min, combined with high pressure CO_2 12 MPa, 22ºC	Color did not show significant modifications. Thermally processed did affect L*, a*, b* parameters, decreasing their values.	Combined treatment induced a significant reduction of firmness about 92%, compared with fresh-cut carrot. Similar results than when thermally processed.	(Ferrentino, 2015b)
Cherries	33 kHz, 60 W, 10 / 20 / 30 / 40 / 60 min	TCD increased when > 30 min. 20 min treatment was the most effective to maintain color red brightness for 15 days.	Significant decrease in firmness after when samples treated for more than 20 min.	(Muzaffar, 2016)
Strawberries	33 kHz, 60 W, 10 / 20 / 30 / 40 / 60 min	Loss of brightness L* when exceeded 30 min of treatment.	Fruit firmness was better retained throughout all refrigerated storage if samples had been previously sonicated.	(Gani, 2016)
Apple slices	40 kHz, 1 / 2 min, combined with ascorbic acid, citric acid, NaCl or Ca-ascorbate	US alone did not help to prevent browning. When used with antibrowning solutions, especially with Ca-ascorbate, US enhanced this effect on some apple varieties.	N/A	(Putnik, 2017)

Straw mushroom	40 kHz, 300 W, 3, 10, 30 min	No significant reduction of browning was observed when samples were treated by US for 3 or 30 min. 10-min US treatment significantly improved the storage life to 72 h keeping straw mushrooms with stable color without spoilage.	US retained the straw mushrooms firmness. 3-min US treatment at 95% RH led to the maximum firmness retention of 1.90 N.	(Li, 2017)
Romaine lettuce	25 kHz, 26 W, 1 / 2 / 3 min	Hue angle decreased in all samples, indicating that enzymatic browning was not affected by US.	Samples processed by US exhibited higher firmness (maximum force, N) than the control (water washed) did right after treatment and during storage.	(Yu, 2016)
Mirabelle plums	30 kHz, 100 W, 0 /15 / 30 / 45 / 60 min, pulsed/continuous	Highest changes in control. US preserved color better.	US helped maintaining firmness. Pulsed gives higher firmness than continuous.	(Hashemi, 2018a)
Cucumber	20 kHz, 200 W, 10 min	Combined with controlled atmosphere, US substantially improved the appearance of the cucumber samples up to 25 days and preserved the original green color.	Ultrasound treatment significantly retained the firmness. A decrease of 35.60% when applying US was observed compared with 56.78% of the control.	(Feng, 2018)
Melon	40 kHz, 500 W, 5 min	N/A	Firmness, adhesiveness, cohesiveness, guminess and chewiness increased after US processing.	(do Rosário, 2018)

TCD, total color difference (TCD value of 4 is considered a clearly distinguishable color difference to the average person); US, ultrasounds.