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

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14 Abbreviations:

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

18 **Abstract**

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

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

31 **1. Introduction**

32 Minimally processed or fresh-cut produce has been defined by the International Fresh-cut Produce  
33 Association as “any fruit, vegetable or their combination subjected to a physical alteration from its original  
34 form, remaining in a fresh state” (Grau-Rojas, 2010). These products are completely edible, packaged, and  
35 should be stored under refrigerated conditions thus providing convenience to consumers (Grau-Rojas,  
36 2010). The fruit and vegetable processing industry is experiencing an expanding period as the global  
37 demand for healthy, fresh, and sustainable products is increasing (Qadri, 2015). However, consumption of  
38 minimally processed fruits and vegetables (FV) has been associated to concerns on their safety due to the  
39 emergence of several outbreaks of foodborne pathogens linked to their consumption (Pinela, 2015).

40 Main causes of foodborne diseases are due to bacteria 53.0%, viruses 42.5%, and parasites 4.5% (Ramos,  
41 2013). *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes* were the main contaminants involved  
42 in outbreaks related to FV over the past years (Birmipa, 2013; Park, 2012; Silva, 2017; Tango, 2017).  
43 According to the European Food Safety Authority (EFSA), the top ranking food/pathogen combinations  
44 are *Salmonella* spp. and leafy greens, bulb, stem vegetables, tomatoes, or melons, and *E. coli* and fresh  
45 pods, legumes, or grains (Andreoletti, 2013). Another major problem of the FV processing industry is  
46 related to alterative microbiota, which do not suppose any health risk to humans, but can lead to quality  
47 deteriorations, shortening the products’ shelf-life, and causing significant economic losses (Rico, 2007).  
48 For example, strawberry spoilage losses can be as high as 40 % (Luksiene, 2013) leading to strawberry  
49 producers to look for strategies to extend their shelf life. In addition, fresh-cut operations such as peeling  
50 or cutting result in increased nutrient availability and senescence rates as a consequence of the natural  
51 epidermal breach leading to the growth of microorganisms (Qadri, 2015; Ramos, 2013). For these reasons,  
52 disinfection procedures are crucial to maintain safety and quality of fresh-cut FV.

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

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

66 US consists on the use of ultrasonic waves at a frequency beyond 18 kHz with a specific intensity and  
67 amplitude (Bevilacqua, 2018). It has been acknowledged and reviewed that microorganism lethality caused  
68 by sonication is due to a phenomenon known as transient cavitation (Pérez-Andrés, 2018). Generated  
69 bubbles collapse and, consequently, molecules collide, spots of extremely high temperature and pressure  
70 occur (5000 °C and 50 MPa), and cellular envelopes and other microbial components are destroyed (Leong,  
71 2017), thus reducing the viable microorganisms (Van Impe, 2018). Moreover, US can induce other  
72 chemical and structural changes, affecting quality and nutritional values of the processed products (Leong,  
73 2017).

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

## 82 2. Antimicrobial effects of US processing

### 83 2.1. Effect of sonication on natural-occurring and inoculated microorganisms

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

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

104 Antimicrobial effects of US depend on treatment parameters. Indeed, Mansur (2016) recently suggested  
105 that sonication power is a factor which has a key impact on the antimicrobial efficacy of US. Moreover, the  
106 higher was the intensity used when treating kale (ranging between 100 to 400 W/L), the higher were the  
107 reductions observed for all the microorganisms studied (ranging between 3.2 to 3.9 log cycles). Overall, it  
108 seems that US application mode is not a factor that has a significant effect on microorganisms, as there  
109 seems to be no differences between continuous – constant sonication – or pulsed – intermittent sonication  
110 – modes (Hashemi, 2018a). Still, the use of continuous or pulsed US affected differently the content of

111 certain compounds in fruits and vegetables previously. For example, Pan (2012), reported different values  
112 of total phenolics content and antioxidant activity on pomegranate after application of continuous US when  
113 compared to pulsed US. We would like to highlight that this does not mean that US processing can alter  
114 the content of polyphenols in fruits. It is likely that continuous or pulsed ultrasonic waves can lead to  
115 different extraction yields and therefore higher phenolic contents and antioxidant activities of the water and  
116 organic extracts. Another parameter that affects cavitation activity is frequency, as bubble size is inversely  
117 proportional to it. Application of lower frequencies results in larger bubbles, liberating higher energy (São  
118 José, 2014a).

119 Effects of US on microorganisms also depend on the target specie and on matrix properties. In this sense,  
120 São José (2014b) obtained different antimicrobial effects depending on the studied matrix. They reported a  
121 reduction of *E. coli* populations of 2.3 or 1.6 log cycles when processing green pepper or melon at 40 kHz  
122 for 2 min, respectively. When using these same conditions, reductions of *S. enterica* Enteritidis on green  
123 pepper or melon were lower, 1.8 and 1.9 log cycles, respectively. These differences were attributed to the  
124 behavior of each microorganism on different surfaces. According to Tan (2017), sonication alone  
125 significantly affects the flagella and fimbriae of bacteria, decreasing the cell adhesion of artificially  
126 inoculated *S. enterica* Typhimurium by 0.5 to 1.0 log cycles, a relevant reduction if taken into account that  
127 *Salmonella* contamination in real production lines typically contains <1 log CFU/mL of this bacteria. US  
128 capacity to remove bacterial cells from the surface is recognized, as it influences the attachment ability of  
129 microorganisms before and after biofilm formation. Biofilms, or aggregates of microorganisms whose cells  
130 are frequently embedded within a self-produced matrix of extracellular polymeric substances, may be  
131 another source of resistance to sanitizers and surfactants (Brilhante de São José, 2012). In fact, several  
132 studies have evidenced its effects on *L. monocytogenes* biofilms alone (Hamman, 2018) or combined with  
133 surfactants (Torlak & Sert, 2004). US are widely used on machinery surfaces and food pipelines, as a  
134 physical method to eliminate biofilms, since there is no residue left over in the removal process (Zhao,  
135 2017).

136 Yeasts and molds can also be inhibited by US. Some authors have reported 0.5 log cycles reductions in  
137 strawberry processed at 33 kHz and 60 W for 10 min (Gani, 2016). Other studies observed reductions of  
138 2.3 log cycles in kiwi, when processed at 30 kHz and 368 W/cm<sup>2</sup> during 8 min (Vivek, 2016). Overall, in  
139 the majority of the studies published to date, decay incidence, or percentage of fruits with visible mold  
140 growth, was significantly reduced when comparing US processed with a non-treated control (Muzaffar,

141 2016; Vivek, 2016). Even though reported reductions of pathogenic bacteria seem to be higher than those  
142 of epiphytic microbiota, pathogenic microorganisms are normally artificially inoculated in the food matrix  
143 before the assay, so internalization and attachment are typically lower than what occurs regarding natural  
144 microbiota. Moreover, total bacteria count includes a wide range of microorganisms within which some  
145 strains could be more resistant to specific US conditions. In this regard, more assays should be carried out  
146 in order to elucidate whether US could be capable of reducing microbial loads that occur in the stomata,  
147 vasculature, cut edges or intercellular tissues, where other strategies have proven to be ineffective  
148 (Meireles, 2016).

149 Another factor that affects the effectiveness of US is processing or dipping time. It seems that longer  
150 processing times result in higher microbial inactivation (Birmpa, 2013). It is important to highlight that for  
151 each target microorganism, matrix, and US conditions, a minimum application time is necessary to report  
152 significant changes on the microbiota (Hashemi, 2018a). Temperature of the matrix and the media can  
153 increase by the application of US for a period of time, due to acoustic energy produced (Marques Silva,  
154 2017). The temperature achieved could affect the results, leading to a possible increase in microorganism  
155 inactivation but also to an alteration or degradation of biochemical and nutritional compounds. In order to  
156 implement this technology at large scale production of minimally processed fruits or vegetables, processing  
157 times should be minimized and should not exceed a few minutes. Although US processing alone can exert  
158 antimicrobial effects (do Rosário, 2017), to reduce treatment time and to achieve a sufficient microbial  
159 inactivation, US can be combined with other chemical or physical strategies, because synergistic or additive  
160 effects may take place when it is combined (Barba, 2017; Park, 2018).

## 161 **2.2. US combined with mild temperatures**

162 So far, there are no publications that use a combination of mild temperatures and US (thermosonication) to  
163 disinfect FV for fresh-cut produce. It has been widely studied in FV juices, with good results on pathogenic  
164 microorganism reductions (Sánchez-Rubio, 2018), alternative microbiota growth and bioactive compounds  
165 maintenance (Lafarga, 2018; Hashemi, 2018b), and enzyme inactivation (Illera, 2018). Nonetheless,  
166 application of thermosonication on FV for fresh-cut industry could lead to changes in texture that may not  
167 be a shortcoming in juices but could have detrimental effects on fresh-cut FV. As previously mentioned,  
168 long processing times are not feasible in industry, as they can have detrimental effect on firmness (Terefe,  
169 2011). However, further studies are needed in order to assess the real potential of this technology in the  
170 fresh and minimally processed fruit and vegetable industry.

171 **2.3. US processing combined with chemical agents**

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

180 **2.3.1. Organic acids**

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

188 The most widely studied organic acids are lactic, citric, acetic, and peracetic acid, at concentrations ranging  
189 between 0.04 and 2%. Reductions of 3.2 or 3.0 log cycles have been achieved against *Salmonella*  
190 Typhimurium when combining US with citric (2%) or peracetic acid (5%) respectively (Sagong, 2011;  
191 Silveira, 2018), which were higher than those of non-treated product. Lower reductions were observed  
192 when using lactic acid 1% against *Salmonella* Enteritidis, which reduced by 1.9 to 2.8 log units (São José,  
193 2014a; São José, 2015). *L. monocytogenes* and *E. coli* have also been studied, and reductions of  
194 approximately 2.5 log cycles have been reported when processing lettuce leaves at 40 kHz and 90 W for 5  
195 min combined with lactic, citric, or malic acid at 2% (Sagong, 2011).

196 Except for Silveira (2018), who reported no significant differences between US alone (40 kHz, 500 W, 5  
197 min) or in combination with peracetic acid 50 mg/L, studies published to date show a significant synergistic  
198 or additive effect on the combination of both mechanisms (Table 1 and Table 2). The intense pressure  
199 gradients caused by US seem to enhance the penetration of the organic acids through the cell membrane of

200 the microorganisms, and along with cavitation, it assists the disaggregation of the microorganisms, leading  
201 to an increased efficiency of the sanitization treatment (São José, 2015).

### 202 **2.3.2. Essential oils**

203 Sonication can also be combined with essential oils (EOs). EOs are effective antimicrobials (Ribeiro-Santos  
204 2018). Their action mechanism includes membrane rupture, ATP-ase inhibition, leakage of essential  
205 biomolecules, proton motive force disruption, and enzyme inactivation (Pisoschi, 2018). According to  
206 Salvia-Trujillo (2015), the key features for the effectiveness of EOs are their composition, concentration,  
207 and droplet size, that promotes faster inactivation of microorganisms. Millan-Sango (2016) suggested that  
208 EOs' droplet size is not as important. However, when EOs and US processing are combined, US frequency  
209 and processing time are directly related with antimicrobial effects.

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

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

### 221 **2.3.3. Ozone**

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

229 To the best of the authors' knowledge, there is only one study published so far evaluating the combined  
230 effect of ozone and US. Aday (2014) combined US (20 kHz, 30 W, 5 min) and ozonation (0.075 mg/L) on  
231 strawberries. The authors reported a 21 and 35% incidence of *Botrytis cinerea* in non-treated fruits at the  
232 3<sup>rd</sup> and 4<sup>th</sup> weeks respectively, whereas a complete inhibition of mold growth was observed after the  
233 treatment with ultrasound and ozone during the whole storage (See Table 2). In order to determine whether  
234 the combination of ozonation and sonication could be an effective option for FV disinfection, more studies  
235 should be carried out using both methodologies and applying them to a range of matrices, target  
236 microorganisms and at different conditions.

#### 237 **2.3.4. Slightly acidic electrolyzed water**

238 Slightly acidic electrolyzed water (SAEW) is produced by means of an electrolytic cell without a separating  
239 membrane, producing the electrolysis of dilute sodium chloride and hydrochloric acid solutions. Its  
240 bactericidal effect is attributed to the available chlorine compounds including  $\text{ClO}^-$ ,  $\text{HClO}$ , and  $\text{Cl}_2$  (Ye,  
241 2017). SAEW is commonly used at pH values ranging from 5.0 to 5.5 and oxide-reduction potential values  
242 of 930-980 mW.

243 Despite the potential of SAEW for disinfecting fresh foods, it seems that this technique alone might not be  
244 able to completely disinfect all FV, especially those that might have hidden places where adherent  
245 microorganisms are difficult to remove by aqueous sanitizers (Luo, 2016b). Indeed, Koide (2009) found  
246 that SAEW was effective to remove bacteria from the surface of fresh-cut cabbage but residual  
247 contamination could be caused by microorganisms embedded inside the cellular tissues, namely stomata.  
248 The combined effect of SAEW and US has been proved to be more efficient in reducing microbial loads  
249 when compared to their individual application. For instance, SAEW has been applied in lettuce or tomato  
250 in combination with US at 20 kHz, 130/210 W, for 5 to 15 min against *L. monocytogenes* and *E. coli*,  
251 achieving reductions of 4.0 log cycles (Afari, 2016). The combination did not only reduce the population  
252 but also allowed the control of the remaining microorganisms in FV. Indeed, for *Bacillus cereus* in potato  
253 processed with US at 40 °C 40W/L for 3 min, the lag time increased by 0.2-10.5 h, and the specific growth  
254 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  
255 indicated that the cells stressed by the treatment had lower metabolic activity compared to those untreated  
256 (Luo, 2016a). In addition, SAEW and US combination was also effective reduce spoilage microbiota, as it  
257 was reported by Wu (2018), who applied pH 5.5 and ORP 514 mV water combined with 40 kHz, 200 W,  
258 3 min US treatment to mushrooms and found significant differences in spoilage microbiota in comparison

259 to the water-treated control. The combination is worthy as well to inactivate the pathogens that could remain  
260 in water (Afari, 2016).

#### 261 **2.4. High pressure CO<sub>2</sub>**

262 Supercritical CO<sub>2</sub> is being increasingly studied as an antimicrobial agent, due to its advantageous  
263 characteristics. These include being a GRAS substance, and that its critical temperature (31.1 °C) and  
264 pressure (7.3 MPa) are compatible with the thermal stability of most food matrices, facilitating its  
265 application in industrial processes (Hossain, 2013; Hossain, 2016; Tamburini, 2014).

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

274 Combination of supercritical CO<sub>2</sub> with US demonstrated to have an improved effect than it had when US  
275 was applied alone (Ferrentino, 2015a; Ferrentino, 2015b). As one of the main drawbacks of US is that the  
276 transmitting media seems to partially absorb the acoustic energy, preventing its transfer to the solids to be  
277 treated, the use of CO<sub>2</sub> could potentially overcome this issue as it is a dense fluid, and acoustic waves would  
278 not be reflected but absorbed by the solid (García-Pérez, 2006). Moreover, with an increase of temperature  
279 from 22 to 40°C, higher diffusivity of CO<sub>2</sub> and increased fluidity of cell membrane allows a faster  
280 penetration of CO<sub>2</sub> into it. US enhances this effect, as it induces a better contact between CO<sub>2</sub> and the  
281 membrane, accelerating the diffusion through the membrane, thus causing a drastic drop in intracellular pH  
282 and extraction of vital constituents (Ferrentino, 2015).

### 283 3. Nutritional changes

284 The effect of US processing on FV nutritional components has been widely studied. Results listed in Table  
285 3 suggest a higher content of phytochemicals in extracts obtained from sonicated fruits and vegetables. US  
286 is commonly used to promote the extraction of compounds from food sources including phenols (Soquetta,  
287 2018), carbohydrates (Vilkhu, 2008), or proteins (Lafarga, 2018). This does not mean that US processing  
288 promotes the generation of these valuable compounds. Higher yields reported in the literature could be  
289 attributed to the enhanced extraction efficacy when US have been applied. US causes cell disruption,  
290 allowing permeation of intracellular compounds and therefore, a higher liberation of molecules to the  
291 extracting media (Hidalgo, 2017). In order to obtain improved extraction yields, processing times in the  
292 range 20-60 min are generally required (Annegowda, 2012; Lafarga, 2019). However, it has been suggested  
293 that sonication can increase the degradation of natural products (Pingret, 2013). Two chemical reactions  
294 have been proposed as probable mechanisms responsible for the degradation connected with sonication.  
295 One is related with pyrolysis within cavitation bubbles or gas pockets trapped in the crevices of the solid  
296 boundaries, which cause the degradation of polar compounds, and the other is the generation hydrogen ions  
297 ( $H^+$ ), free radicals ( $O^-$ ,  $OH^-$ ), and hydrogen peroxide ( $H_2O_2$ ) that are produced by cavitation effect  
298 (Rawson, 2011). For instance, isomerization of carotenoids can also occur, as there are extreme physical  
299 conditions of temperature and pressure during processing (Kumcuoglu, 2014). Also, antioxidant capacity  
300 of cyaniding 3-glucoside was evaluated after US treatment (20 kHz) and showed a 20% of its original  
301 antioxidant capacity. The authors suggested that hydroxylation occurred during sonication, causing such  
302 decrease (Ashokkumar, 2008). Degradation or oxidation of biochemical compounds has been related with  
303 increased treatment times (Gani et al., 2016; Jahouach-Rabai, 2008)

304 There are scarce studies focusing on the effects of US on the macromolecules of FV. In fact, from the recent  
305 past years, there is only one paper reporting values of fat content, and the results showed that there was no  
306 statistical effect on this parameter when combining US and high pressure  $CO_2$  on coconut (Ferrentino,  
307 2015a). Regarding proteins, US could induce changes in native form: conformational changes, damage to  
308 secondary structure, re-structuration of disulfide bond or generation of other intra/ inter molecular  
309 interactions (Chizoba-Ekezie, 2018). Studies on proteins in FV after US have focused mostly on its  
310 extraction yield and allergenicity (Nayak, 2017). Only one study carried on by Li (2017) evaluated the  
311 effect of US (40 kHz, 350 W) on total soluble proteins of straw mushrooms. They reported that this

312 parameter – an indicative of tissue destruction – was negatively affected by over-time treatments (30 min),  
313 but 1 to 10 min served to prevent soluble protein utilization, allowing metabolic activity prolongation.

314 The effect of US processing on the total phenolic content (TPC), of FV has been largely studied. However,  
315 only few studies evaluated whole pieces and most of them focused on processed products such as juices or  
316 purees. Bal (2017) processed grapes with US (32 kHz, 60 W/L, 10 min) and observed an increase on the  
317 yield TPC of the sonicated product at the end of a 60-day storage period when comparing to the untreated  
318 control. Related to flavonoids, Bal (2017) suggested that even though there were no statistical differences  
319 between samples, total anthocyanin content of grapes processed with US tended to increase during storage.  
320 Other authors observed an increment of 7.9% in TPC values on strawberries processed with US (33 kHz,  
321 60 W, 10-40 min, US bath maintained at 25°C) from day 1 (Gani, 2016). Increase of TPC was partially  
322 explained by a better extraction of polyphenols attributed to an increase in temperature that occurs in US  
323 treatment as a consequence of cavitation phenomena, and it was also attributed to hydroxylation of  
324 flavanols, which has a positive effect on antioxidant activity (Soria, 2010). Increases in yield of TPC were  
325 reported by Yu (2016), who found that the TPC values of US-treated romaine lettuce (25 kHz, 26 W/L, 1-  
326 3 min) were up to 22% higher than those quantified in the untreated product. As an abiotic stress, US could  
327 enhance the biosynthesis of secondary metabolites in plant cells, through stimulating their physiological  
328 activities. That could partially explain why TPC increases during storage when compared with the non-  
329 sonicated products (Wang, 2015). In addition, US could promote the liberation of phenols, as these  
330 compounds can be bound to other compounds present in cell walls (polysaccharides, proteins, etc.) and be  
331 disrupted by US cavitation (Khan, 2018). On the contrary, Ferrentino (2015b) found that applying US (30  
332 kHz, 40 W, 30 min) and high pressure CO<sub>2</sub> (12 MPa, 35°C), the TPC decreased when compared with the  
333 untreated product. Still, these results could not be attributed directly to the ultrasonic effect, because no  
334 control of both individual treatments was used in that study.

335 Ascorbic acid (AA) forms part of Vitamin C, and its content can be affected by US processing. Alexandre  
336 (2013) reported that sonication reduced the loss of AA during the freezing of red bell pepper when  
337 processing at 35 kHz, 120 W and 15 °C compared to water-washed ones. In terms of the US mode  
338 application, Hashemi (2018a) did not observe significant differences between the use of pulsed or  
339 continuous mode in the AA content of plums. Treatment time was a significant factor to take into account  
340 in US processing. The same authors reported an increase in the AA content when longer US treatment times  
341 (1, 15, 30, 45 and 60 min) were applied to plums at 30 kHz and 100 W. The increase of this compound was

342 attributed to the elimination of entrapped oxygen due to cavitation, which is essential for AA degradation  
343 (Bhat, 2011; Cheng, 2007).

344 As summarized above, effects of US on nutritional values of FV may differ between studies, conditions,  
345 and matrices, and they can partially be attributed to different extraction yields when applying US (Chemat,  
346 2017). These differences may also occur when scaling up from lab or pilot plant scales to industry. With  
347 this purpose, several papers reviewing the potential of US in food industry have been published to date  
348 (Bilek, 2013; Kentish, 2014; Prakash, 2003).

#### 349 **4. Effect of US processing on FV quality**

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

##### 357 **4.1. Overall quality changes**

358 Physical properties of FV processed with US generally remain unchanged after treatment. As it is shown  
359 in Table 3, pH and titratable acidity tend to maintain the values of the control samples after the US  
360 treatment. In some cases FV processed with US have higher total soluble sugars values than those from the  
361 control. This has been attributed to the fact that US might accelerate the depolymerization process of the  
362 starch gel (Amaral, 2015; Bal, 2017) in the outer parts (< 1 mm) and at deeper tissues, changes are attributed  
363 to water removal (Schössler, 2012). These structure alterations can be related with the increment of  
364 exposure time to US, increasing the temperature and the further destruction of cellular structure (Jurek,  
365 2012).

##### 366 **4.2. Color**

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

371 Several studies, including those listed in Table 4, evaluated the effect of US processing on the color  
372 parameters of FV. Overall, no changes in color were observed, processing with US alone or in combination  
373 with chlorine or high pressure CO<sub>2</sub> (Ferrentino, 2015b; Salgado, 2014). However, some studies reported  
374 significant differences between US-processed and untreated samples in the a\* and b\* values, either once  
375 treated or after storage (Ferrentino, 2015a) or in L\* values in different matrices such as coconut, mango, or  
376 strawberries (Aday, 2014; Amaral, 2015; Santos, 2015).

377 The observed changes in color can be attributed to the possible inactivation of enzymes such as poly-phenol  
378 oxidase (PPO) and phenol peroxidase (POD). These enzymes are proposed to cause off-colors in raw and  
379 frozen vegetables and browning reactions (São José, 2014a; Toivonen, 2008). US treatments have  
380 demonstrated to be able to inactivate such enzymes in certain conditions, occurring at higher rates when  
381 combining US technology with heat (40-60°C) (Illera, 2018). Enzyme inactivation also depends on  
382 treatment time (Cao, 2018; Zhu, 2017) and US intensity (Liu, 2017). Causes of enzyme inactivation involve  
383 shear stress and pressure, which cause oligomeric enzymes dissociation, free radicals affecting the structure,  
384 and creation of a large interfacial area by US that disturbs the hydrophobic interaction and hydrogen  
385 bonding, thus destabilizing proteins (Terefe, 2015). For instance, Li (2017) observed an inhibition in PPO  
386 activity when processing straw mushrooms with US (40 kHz, 350 W, 1-30 min). A decrease in POD and  
387 PPO activities of fresh-cut pineapple was reported by Yeoh (2017) after processing pineapple slices with  
388 25-29 W, 37 kHz, for 10 to 15 min US . These enzymes had significantly lower activity in sonicated fruit  
389 than they had in water-washed fruit. Besides, after a 5-day storage period, POD and PPO activities were  
390 3.8 and 4.5-fold lower than they were in the water-dipped control. Moreover, US was suggested to facilitate  
391 the penetration of ascorbic acid to vegetable cells, as cell wall disruption occurred, thus enhancing  
392 antioxidant processes. On the contrary, Wang (2015) found an increase on POD activity of cherry tomatoes  
393 and Ferrentino (2015a) of coconut. It has been proposed that low US power level could promote enzyme  
394 production, whereas high power US could induce the contrary effect, but it could affect the quality  
395 parameters of the product. Also, the effectiveness of US depends on the differences in the resistance of each  
396 enzyme to the treatment (Kentish, 2014).

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

406 processing tomatoes with US with 37 kHz, at 140 W for 45 min resulted into changes in lycopene forms  
407 *cis* and *trans*.

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

### 413 **4.3. Texture**

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

420 A review of the data found to date is summarized on Table 4. Some studies report no significant differences  
421 in textural parameters after US processing. However, most of the accounts suggest that US processing can  
422 affect the firmness of fresh FV depending on the intensity of the treatment. The effect of US processing on  
423 texture also depends on several parameters, which include food matrix, variety, maturity stage, intensity,  
424 or processing duration of US treatment. Results obtained so far seem to be contradictory and matrix-  
425 dependant, texture changes should be assessed independently for each fruit or vegetable. Softening of fruits  
426 has been attributed to inner changes of cell wall constituents, mostly pectin, which can be de-esterified by  
427 the activity of pectin methyl esterase, followed by a depolymerization of methoxy pectin or pectic acids  
428 due to endo-polygalacturonase activity (Wang, 2018). For instance, Saeeduddin (2015) found that 20 kHz,  
429 0.30 W/mL US applied for 10 min at 45 or 25 °C could inactivate pear pectin methyl esterase by 60 or 7%,  
430 respectively. Thus, US, combined or not with mild temperatures or high pressures, can cause partial or total  
431 inactivation of enzyme activity, thus leading to changes on the textural quality of the FV (Marques-Silva,  
432 2017).

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

#### 436 **4.4. Antioxidant capacity**

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

441 According to recent studies, antioxidant activity values obtained by *in vitro* methods of FV processed with  
442 US increases in comparison with the control samples. As an example, Wang (2015) reported that applying  
443 US (22kHz, 100 W) to tomatoes led to an increase DPPH· inhibition by 8.22 to 17.56%, depending on the  
444 power intensity used (66.64 and 106 W/L respectively) and an increase in FRAP values from 6.03 to  
445 13.18% respectively. Yu (2016) observed similar results in romaine lettuce treated with US (25 kHz, 26 W,  
446 1 or 3 min). Gani (2016) also stated that antioxidant activity of US treated samples increased with  
447 processing and was higher proportionally to the treatment time. However, a slight decrease was observed  
448 at 60 min, attributed to the excessive damage to cell structure which could lead to greater chances of  
449 oxidation as well as degradation of polyphenolic compounds. It has been suggested that due to the  
450 generation of hydroxyl radicals, hydroxylation of food materials could be increased during US, leading to  
451 an increased antioxidant activity (Ashokkumar, 2008). Increased antioxidant capacity can also be attributed  
452 to an increased phenolic content in FV, as this two values are positive correlated (Gani, 2016). Nonetheless,  
453 and as it has been previously described, antioxidant compounds may be maintained in amount in FV but  
454 better extracted due to tissue disruption, leading to higher antioxidant capacity values regarding sonication  
455 time does not exceed. More studies should be done concerning antioxidant capacity of sonicated fruits, in  
456 order to find a relationship between the higher yields observed and a higher bioavailability once ingested.

#### 457 **4.5. Flavor**

458 In relation to flavor, data that can be found in the literature is not extensive. Feng (2018) reported no  
459 differences in astringency, aftertaste, bitterness, umami, richness, and saltiness between US processed  
460 cucumber (20 kHz, 200 W, 10 min) and the non-sonicated control. The concentration of the main volatile  
461 compounds of cucumber increased with treatment. Yu (2016) found that 1 min sonicated romaine lettuce

462 had a good punctuation on overall sensory evaluation, and it was higher than it was for the control and  
463 samples processed for 2 or 3 min.

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

466 **Conclusion**

467 Ultrasound is a mild technology that has been studied with the aim to reduce microbial load of food, and  
468 its application in fresh and fresh-cut fruits and vegetables has potential interest for manufacturers, as it is  
469 versatile and reasonably easy to use. It has been reported to be relatively effective as an antimicrobial agent,  
470 and its effects can be improved if it is combined with other physical technologies, such as mild temperatures  
471 or supercritical CO<sub>2</sub>, or with chemical agents, including organic acids and essential oils, ozone, or slightly  
472 acidic electrolyzed water. A part from being able to reduce pathogenic and alterative microbiota in FV, US  
473 may have a consequence on other key features, such as color or texture, or components, namely phenols or  
474 vitamins. Overall, it seems that results of US processing on FV do not follow general trend, as they depend  
475 on several parameters related with treatment conditions and matrix. Targeted microorganisms may not  
476 respond equally in the same conditions, and reductions may also vary depending on parameters stated  
477 before. Accounting on this review's information and knowing are capable to achieve the desired outcomes,  
478 each case should be studied and scaled-up individually in order to preserve safety, quality and nutrition  
479 values of fresh and fresh-cut FV.

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487 **Conflict of interests**

488 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.

<b>Fruit / vegetable</b>	<b>US conditions</b>	<b>Target microorganisms</b>	<b>Effect</b>	<b>Source</b>
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 <sup>2</sup> , 8 min	TBC and yeasts and molds	Reductions: 2.3 and 3.5 log cfu/cm <sup>2</sup> . Not better compared with treatment using NaOCl, that achieved 5.83 and 3.68 log cfu/cm <sup>2</sup> 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 3 <sup>rd</sup> and 4 <sup>th</sup> week.	(Aday, 2014)
Carrots	40 kHz, 10 W, 30 min combined with high pressure CO <sub>2</sub> (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)

<i>Calçot (Allium cepa L.)</i>	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 <sup>6</sup> 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)

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

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

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

<sup>1</sup> Log cfu/cm<sup>2</sup>

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

<sup>2</sup> Log cfu/mL

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

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

<b>Fruit / vegetable</b>	<b>US conditions</b>	<b>Parameter</b>	<b>Obtained results</b>	<b>Source</b>
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 <sub>2</sub> and US + O <sub>3</sub> 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 <sub>2</sub> 12 MPa, 35°C	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 of straw mushroom. 10 min US treatment resulted in the minimum CO <sub>2</sub> 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, 15°C	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)	

873 AA, ascorbic acid; DPPH·, 2,2-Diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; ORAC, oxygen radical absorbance capacity; POD, phenol peroxidase; PPO, polyphenol  
874 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

<b>Fruit / vegetable</b>	<b>US conditions</b>	<b>Color</b>	<b>Texture</b>	<b>Source</b>
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 <sub>2</sub> 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, surfactants 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 <sub>2</sub> 12 MPa, 35°C	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. ° 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 <sub>2</sub> 12 MPa, 22°C	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.