HIGH POWER ULTRASOUND AS A NONTHERMAL TECHNOLOGY FOR MICROBIAL AND ENZYMATIC INACTIVATION OF JUICES

TRABAJO FIN DE MÁSTER UNIVERSITARIO EN GESTIÓN DE LA SEGURIDAD Y CALIDAD ALIMENTARIA

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HIGH POWER ULTRASOUND AS A NONTHERMAL TECHNOLOGY FOR MICROBIAL AND ENZYMATIC INACTIVATION OF JUICES

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RESUMEN

Las tecnologías térmicas son las más utilizadas para garantizar la seguridad alimentaria de los zumos de frutas. No obstante, el uso de temperaturas elevadas tiene efectos negativos, como la disminución del valor nutricional o la variación de la calidad organoléptica. Es importante destacar que los consumidores demandan cada vez más productos de alta calidad y elevado valor nutricional, sin olvidar que estos productos deben cumplir con los requisitos de seguridad alimentaria. El objetivo del presente trabajo es realizar una revisión bibliográfica sobre el uso de ultrasonidos de potencia (HPU) como tecnología de inactivación no térmica para la conservación de zumos de frutas. Así, se describen y discuten los objetivos de seguridad alimentaria, las condiciones de proceso, la inactivación microbiana y enzimática, la calidad de los alimentos y los objetivos de rendimiento y así como la relación entre todos estos factores.

PALABRAS CLAVE: zumos, ultrasonidos, inactivación microbiana, inactivación enzimática, efectos organolépticos, tecnologías no térmicas

RESUM

Les tecnologies tèrmiques són les més utilitzades per a garantir la seguretat alimentària dels sucs de fruites. No obstant això, l'ús de temperatures elevades té efectes negatius, com la disminució del valor nutricional o la variació de la qualitat organolèptica. És important destacar que els consumidors demanden cada vegada més productes d'alta qualitat i elevat valor nutricional, sense oblidar que aquests productes han de complir amb els requisits de seguretat alimentària. L'objectiu del present treball és realitzar una revisió bibliogràfica sobre l'ús d'ultrasons de potència (HPU) com a tecnologia d'inactivació no-tèrmica per a la conservació de sucs de fruites. Així, es descriuen i discuteixen els objectius de seguretat alimentària, les condicions de procés, la inactivació microbiana i enzimàtica, la qualitat dels aliments i els objectius de rendiment i així com la relació entre tots aquests factors.

PARAULES CLAU: sucs, ultrasons, inactivació microbiana, inactivació enzimàtica, efectes organolèptics, tecnologies no tèrmiques

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ABSTRACT

Thermal techniques are the most widely used to ensure food safety of fruit juices. Nevertheless, the use of high temperature has negative effects, such as nutritional value decrease or the changes in organoleptic quality. It is important to highlight that consumers are increasingly demanding products with high quality and high nutritional value together with the food safety requirements. The aim of this work is, through a literature research, to review the state of the art of the use of High Power Ultrasound (HPU) as a non-thermal technology for the preservation of fruit juices. Thus, food safety objectives, HPU processing conditions, microbial and enzymatic inactivation, food quality and performance objectives, and the relationship between all of them are described and discussed.

KEYWORDS: Juices, sonication, microbial inactivation, enzymatic inactivation, quality, nonthermal processing
1- INTRODUCTION

The juice industry has an important role in the Spanish economy. According to the MERCASA (2018) report, the consumption of juices and nectars in Spain during 2017 reached 808.15 million of liters. Moreover, Spain stood at the head of the European Union in exporting fruit juices (775.672 tonnes exported), being citrus (42%) and grape juices (25%) the most ones exported. Orange juice is the favorite for the Spanish consumers, with a market share of around 34% of the total. Far behind, pineapple (19.2%) and peach juices (17.8%) are localized, followed by multifruit (12.6%) and apple juices (3.9%).

Fruit juices are mainly characterized by their high water activity, with varying sugar content according to the type and ripening state of fruit. Their low pH values, between 1.6 - 4.0, can partially prevent the growing of pathogenic microorganisms. Nevertheless, in fresh juices, it is possible the short-term survival of pathogenic bacteria, such us Escherichia coli, Salmonella and Listeria (Asozumos, 2013). The high content of fermentable sugars in juices (5 to 80 Brix) can lead to recontamination after treatment by spoilage microorganisms (A. acidoterrestris, S. cerevisiae) that can damage the organoleptic characteristics of juices or lead to the formation of mycotoxins because of their ability of adaption to low pH in presence of oxygen (AINIA, 2012 and Asozumos, 2013).

On the other hand, some fruits also contain enzymes responsible for the oxidation of certain juices’ compounds, which could change their appearance or color (peroxidase, POD; polyphenol oxidase, PPO) or their rheological characteristics (pectin methylesterase, PME).

In Spain, the minimum quality parameters of fruit juices and methods of analysis are established in RD 1518/2007, November, the 6th, involving the seven most consumed juices in this country (orange, pineapple, peach, pear, apricot, tangerine and apple juices), and RD 1044/1987, July, the 31st, which regulates the production of grape juices in accordance with Community regulations. Also, safety limits of E. coli, Listeria and Salmonella for fruit juices are established in the Commission Regulation (EC) No 1441/2007 of 5th December 2007 amending Regulation (EC) No 2073/2005.

To prevent quality degradation and ensure food safety, thermal technologies are the most currently used techniques by the food industry. They consist of the treatment of packed or unpacked juice to temperatures in the range of 70-95°C for 15-30s. The treatment can be more aggressive (99-120°C) in the case of juices with higher pH. Nevertheless, the thermal treatments can produce another negative effects such as nutritional values decrease (e. g. vitamin loss) and changes in organoleptic quality, affecting parameters such as texture (water loss, hardening/softening), flavor, taste and smell (cooked, rancid, strange) or color changes (darkening, whitening) (Fellows, 2017). It is important to highlight that consumers are increasingly looking for products of high quality and nutritional value together with the food safety requirements.

High power ultrasound (HPU) has attracted considerable interest in food preservation because it could constitute a non-thermal technology capable to
ensure food safety while minimize quality decrease due to the decrease of temperature of treatment. It consists of mechanical waves usually applied in a range of frequency of 20-40kHz and intensities above 1 W/cm². In liquid media, ultrasound can induce transient cavitation, which is the growth and collapse of bubbles inside the liquid. When each cavitation bubble implodes, it acts as a hotspot, leading to reach for an instant in localized points extremely high pressure and temperature (5000°C y 100 MPa) and producing an intense shear stress. These extreme conditions are able to cause the rupture of OH bonds in aqueous systems, leading to produce free radicals, like hydrogen peroxide, or small quantities of oxygen gas (Cebrián et al., 2016, Mason et al., 2005). The enzyme activity and its catalytic function are altered by HPU due to the change of enzyme’s environment, by breaking of hydrogen bonds and van der Waals bonding in the enzyme structure, and the reaction of the free radicals with the amino acids of the enzyme structure (Sulaiman et al., 2015). On the other hand, bacterial cells suffer oxidative damage, cytological disruption of organelles, perforation of the cell wall, wall fragmentation, disorganization of internal content or breakage of the plasma membrane (Guerrero et al., 2017).

Thus, effectiveness of HPU application on microbial or enzymatic inactivation depends on ultrasonic parameters (amplitude and frequency of ultrasonic waves), and also on external factors (pressure, temperature, surface tension of the media), physicochemical properties of food and resistance of microbial cells to this technology (Alzamora et al., 2011). HPU combined with another technology, for example temperature (thermosonication, TS), pressure (manosonication, MS), or both (manothermosonication, MTS), high hydrostatic pressure (HHP), supercritical CO₂ (SC-CO₂) or pulsed light (IPL) can increase the inactivation by varying the external factors, allowing to soften processing conditions.

This diversity of conditions, both intrinsic and extrinsic, and the possibility to operate with different modes (pulsed or continuous) or types of ultrasonic equipment (bath or horn system), translates into the handling of several process variables which expression vary from one study to another, what adds an additional difficulty when comparing different works. For example, the main variable of HPU, the applied power, can be found referenced as the electrical power of the equipment (W), or even as a percentage of this power; as amplitude of vibration of the tip of the probe in horn type system (μm), as power per unit of volume treated (W/l), or, as power applied by each probe surface (W/cm²). In addition, when some variables are not described, such as temperature, power, or if temperature changes during the process is not controlled, the results obtained could deviate from the general trend of the studies reviewed.

The aim of this work is, through a literature research, to review the state of the art of the use of HPU as a non-thermal technology for the preservation of juices. Thus, food safety objectives, HPU processing conditions, microbial and enzymatic inactivation, food quality, and the relationship between them are described and discussed.
2- MATERIALS AND METHODS

To this end, the microorganisms and enzymes mentioned above, were submitted to literature research using the scientific database Web of Science with keywords and choosing criteria shown in Table 1.

TABLE 1. Keywords and choosing criteria used in literature data base search.

<table>
<thead>
<tr>
<th>Keywords (connected by AND)</th>
<th>Date</th>
<th>Results</th>
<th>Publications used in this work</th>
<th>Choosing criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound</td>
<td>Pathogenic bacteria</td>
<td>Juice</td>
<td>All years</td>
<td>9</td>
</tr>
<tr>
<td>Sonication Listeria</td>
<td>Juice</td>
<td>All years</td>
<td>22</td>
<td>1 (Guzel et al. (2014))</td>
</tr>
<tr>
<td>Sonication Salmonella</td>
<td>Juice</td>
<td>All years</td>
<td>14</td>
<td>1 (Kiang et al. (2012))</td>
</tr>
<tr>
<td>Sonication E. coli</td>
<td>Juice</td>
<td>All years</td>
<td>59</td>
<td>12 (Gabriel et al. (2012), Gabriel et al. (2014), Patil et al. (2009), Anaya-Esparza et al. (2017), Ozan et al. (2017), Roobab et al. (2018), Dişer and Topuz (2015), Guzel (2014), Aligourchi et al. (2014), Lee et al. (2009), Muñoz et al. (2011 and 2012), Salleh-Mack and Roberts (2007))</td>
</tr>
<tr>
<td>Sonication A. acidoterrestris</td>
<td>Juice</td>
<td>All years</td>
<td>6</td>
<td>3 (Tremarin et al. (2019), Roobab et al. (2018), Evelyn and Silva (2016))</td>
</tr>
<tr>
<td>Sonication S. cerevisiae</td>
<td>Juice</td>
<td>All years</td>
<td>23</td>
<td>2 (Aligourchi et al. (2014), Bermúdez-Aguirre and Barroso Canovas (2012))</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>Enzymatic inactivation</td>
<td>Juice</td>
<td>All years</td>
<td>30</td>
</tr>
<tr>
<td>Nonthermal technologies</td>
<td>Juices</td>
<td>Document types: Reviews</td>
<td>2016-2016</td>
<td>7</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>Supercritical carbon dioxide</td>
<td>Authors: Ortuño</td>
<td>All years</td>
<td>7</td>
</tr>
</tbody>
</table>

Also, references cited in these publications were consulted to amplify information about A. acidoterrestris, yeasts and molds (Y&M), and PME and POD enzymes. Thus, a total of 52 publications and 3 laws were used in this review to analyze food safety objectives, HPU processing conditions, microbial and enzymatic inactivation, food quality and performance objectives, and the relationship between all of them.

3- RESULTS AND DISCUSSION

3.1 Microbial inactivation

The microbial inactivation is measured in terms of log reductions. FDA (2004) recommendation for fruit juices was used in this work as reference to determine the effectiveness of the treatment, which is established in 5 log cycles reduction of most resistant pathogenic microorganism population. However, the European legislation does not indicate this decimal reduction as reference to the effectiveness of a treatment but only the maximum concentration allowed of the microorganisms at the end of the period of its shelf life (Commission Regulation (EC) No 1441/2007, of 5th December 2007, on microbiological criteria for foodstuffs). For no pasteurized juices, the maximum tolerance for E. coli (for 5 samples tested (n = 5)) is 100 - 1.000 ufc/g; and for Salmonella (n=5): the absence in 25g. For all ready-to-eat foods
unable to support the growth of *L. monocytogenes* the level allowed (n=5) is 100 ufc/g. Table 2 collect the treatment conditions for microbial inactivation using HPU.

**Table 2.** Microbial inactivation by HPU with a horn system: processing conditions and quality changes. (Abbreviations: TPC (Total Plate Count), Y&M (Yeasts and Molds), f (frequency), T (Temperature), a/p (after process)).

<table>
<thead>
<tr>
<th>Fruit juice</th>
<th>f (kHz)</th>
<th>Power (W)</th>
<th>Other power conditions</th>
<th>T (°C)</th>
<th>Time (min)</th>
<th>Sonication mode</th>
<th>Microorganism</th>
<th>log CFU/ml reduction</th>
<th>Quality changes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple</td>
<td>20</td>
<td>130</td>
<td>60%</td>
<td>40</td>
<td>6</td>
<td>6s pulse</td>
<td>S. cerevisiae</td>
<td>2.40</td>
<td>Similar sensory attributes to untreated sample</td>
<td>Bevilacqua et al., 2014</td>
</tr>
<tr>
<td>red fruit</td>
<td>20</td>
<td>130</td>
<td>60%</td>
<td>40</td>
<td>6</td>
<td>6s pulse</td>
<td>S. cerevisiae</td>
<td>2.00</td>
<td>Similar attributes to untreated sample</td>
<td>Bevilacqua et al., 2014</td>
</tr>
<tr>
<td>strawberry</td>
<td>20</td>
<td>130</td>
<td>60%</td>
<td>40</td>
<td>6</td>
<td>6s pulse</td>
<td>S. cerevisiae</td>
<td>2.40</td>
<td>Similar attributes to untreated sample</td>
<td>Bevilacqua et al., 2014</td>
</tr>
<tr>
<td>orange</td>
<td>20</td>
<td>130</td>
<td>60%</td>
<td>40</td>
<td>6</td>
<td>6s pulse</td>
<td>S. cerevisiae</td>
<td>2.00</td>
<td>Similar attributes to untreated sample</td>
<td>Bevilacqua et al., 2014</td>
</tr>
<tr>
<td>pineapple</td>
<td>20</td>
<td>130</td>
<td>60%</td>
<td>40</td>
<td>6</td>
<td>6s pulse</td>
<td>S. cerevisiae</td>
<td>0.20</td>
<td>Similar attributes to untreated sample</td>
<td>Bevilacqua et al., 2014</td>
</tr>
<tr>
<td>Barberry juice</td>
<td>20</td>
<td>200</td>
<td>70%</td>
<td>25</td>
<td>10</td>
<td>15s pulse on and 5s pulse off</td>
<td>Aerobic</td>
<td>1.99</td>
<td>No effects on anthocyanins, pH and °Brix, increased total phenolic content, color parameters affected</td>
<td>Farhadi Chitgar et al., 2017</td>
</tr>
<tr>
<td>Apple juice (natural)</td>
<td>20</td>
<td>600</td>
<td>80%</td>
<td>95.2 µm</td>
<td>30</td>
<td>10</td>
<td>6s pulse</td>
<td>S. cerevisiae</td>
<td>2.50</td>
<td>Similar attributes to untreated sample</td>
</tr>
<tr>
<td>Black mulberry</td>
<td>20</td>
<td>750</td>
<td>60%</td>
<td>80%</td>
<td>25</td>
<td>15</td>
<td>5s pulses</td>
<td>E. coli</td>
<td>3.93</td>
<td>Increased color values and turbidity, No changes in pH, antioxidant activity</td>
</tr>
<tr>
<td>Orange</td>
<td>20</td>
<td>600</td>
<td>100%</td>
<td>44</td>
<td>30</td>
<td>0.5s pulse</td>
<td>E. coli</td>
<td>1.10</td>
<td>Similar attributes to untreated sample</td>
<td>Patil et al., 2009</td>
</tr>
<tr>
<td>Pomegranate</td>
<td>20</td>
<td>600</td>
<td>100%</td>
<td>25</td>
<td>15</td>
<td>0.5s pulse</td>
<td>E. coli</td>
<td>1.58</td>
<td>Similar attributes to untreated sample</td>
<td>Alighourchi et al., 2014</td>
</tr>
</tbody>
</table>

Bevilacqua et al. (2014), studied microorganisms’ inactivation in different juices applicating HPU in pulsed mode. In apple juice, *S. cerevisiae* suffer the highest inactivation (2.4 log reductions) after 6 min of pulsed HPU treatment (6s pulses at 60% of 130W). Similar values of inactivation were found in *P. membranifaciens, W. anomalus* and *C. norvegica*, with 2, 2.2, and 2.25 log reductions, respectively. However, *Z. bailii and Z. rouxii* suffered a significant lower inactivation (1.75-1.95 log). This could indicate that microbial characteristics determine their resistance to the treatment. Decreasing HPU...
exposition time to 4 min with 2s pulses produce a decrease of the inactivation rates as it is shown in Table 2. The different microorganisms had different rates of reduction, being, in this case, W. anomalous and Z. rouxii the most resistant ones. This could indicate that the treatment time applied, and the mode of operation differently affects the microorganisms inactivation, but, these results also maybe were caused because temperature was not stable during the treatment, and only was measured at the end of the process, reaching 40ºC. Thus, if the temperature is not controlled there is not possible to differentiate ultrasound effects from temperature effects.

A similar difference in microbial resistance was observed by Alighourchi et al. (2014), studying E. coli and S. cerevisiae inactivation in pomegranate juice at different amplitude of energy applied (50-100% of power of the sonicator used, not mentioned in the study). They found that E. coli was more labile (1.58-3.37log reduction) than S. cerevisiae, (0.89-1.84log reduction). Ferrario et al. (2015) observed that the behavior of S. cerevisiae was completely different compared to A. acidoterrestris. When treated in same condition of HPU (80% of 600W, 30min) in apple juice, A. acidoterrestris did not suffer any inactivation, and S. cerevisiae reduced 2.5log CFU/ml.

Thus, the type of microorganisms (pathogenic, probiotic or spoilage bacteria), their characteristics (shape, size, softness, Gram-positive or Gram-negative, growth stage) and their ability of recovering from stressing agents, are some of limiting factors which HPU effectivity will depend on (Roobab et al., 2018). Greater levels of inactivation are produced in Gram negative bacteria, as it is E. coli, than in Gram positive bacteria or yeasts, such S. cerevisiae, because of the greater rigidity of their layer. Cavitation causes more damage to cells provided with a thin wall, while rigid cells are difficult to rupture. So, their inactivation is assumed to be mainly due to formation of hydrogen peroxide free radicals and its release of intracellular protein (Mohideen et al., 2015).

On the other hand, the effectiveness of inactivation could depend on the type of juice. For example, S. cerevisiae suffered near 2 log reductions in all juices studied (Bevilacqua et al., 2014), except for pineapple juice inactivation, where only 0.2 log reduction was reached after 6 min of treatment at 20kHz and 60% of 130W. Patil et al. (2009) also have seen than at the same treatment conditions (0.4µm, 30ºC, 15min) there was differences in E. coli reduction depending on the juice treated.

Finally, HPU intensity and processing time also plays an important role in inactivation. Farhadi Chitgar et al. (2017) obtained the total inactivation of Y&M and aerobic microorganisms, after 10min of treatment at 20kHz and 100% of 200W. When only 70% of 200W was applied, treatment time had to be increased to 15 min for obtaining the same result. Dinçer and Topuz (2015) submitted a suspension of E. coli in black mulberry juice to 20kHz, 750W, varying the power applied in the range 60-100%, for 15min pulsed HPU treatment, and obtained 3.93-5.14log reduction depending of the power (the higher the amplitude, the higher the inactivation).

Similar tendency for treatments in a bath type sonicator is shown in Table 3. Zhu et al. (2017) reduced E. coli in blueberry juice at different power (280W,
420W, 560W and 700W), and shown a clear tendency of higher inactivation with more intense treatments.

### TABLE 3. Microbial inactivation by HPU with a bath-type sonicator: processing conditions and quality changes.

<table>
<thead>
<tr>
<th>Juice</th>
<th>f (kHz)</th>
<th>Power (W)</th>
<th>Other power conditions</th>
<th>T (ºC)</th>
<th>Time (min)</th>
<th>Microorganism</th>
<th>log CFU/ml reduction</th>
<th>Quality changes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strawberry</td>
<td>25</td>
<td>Not provided</td>
<td>70%</td>
<td>20</td>
<td>30</td>
<td>TPC</td>
<td>0.15</td>
<td>Same pH, aº, °Brix, color, titratable acidity and turbidity, Lower viscosity, increased cloud assessment and anthocyanine</td>
<td>Bhat and Goh, 2017</td>
</tr>
<tr>
<td>Pear</td>
<td>25</td>
<td>500</td>
<td>70%</td>
<td>25</td>
<td>15</td>
<td>TPC</td>
<td>0.5</td>
<td>No color, pH, titratable acidity and total soluble solids changes; Reduction of particle size, Improved cloud values; better consistency and homogeneity, Increased content of ascorbic acid</td>
<td>Saeeduddin et al., 2016</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>500</td>
<td>70%</td>
<td>25</td>
<td>30</td>
<td>Y&amp;M</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>500</td>
<td>70%</td>
<td>25</td>
<td>45</td>
<td>Y&amp;M</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>500</td>
<td>70%</td>
<td>25</td>
<td>60</td>
<td>Y&amp;M</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grapefruit</td>
<td>28</td>
<td>600</td>
<td>-</td>
<td>20</td>
<td>30</td>
<td>TPC</td>
<td>0.5</td>
<td>Increase in lycopene, anthocyanin and carotenoids</td>
<td>Aadil et al., 2017</td>
</tr>
<tr>
<td>Blueberry</td>
<td>40</td>
<td>280</td>
<td>-</td>
<td>20</td>
<td>10</td>
<td>E. coli</td>
<td>0.13</td>
<td></td>
<td>Zhu et al., 2017</td>
</tr>
<tr>
<td></td>
<td>420</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.2</td>
<td>Not significant Anthocyanin in reduction (3% from untreated juice)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>560</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>700</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

On the other hand, when comparing the results of Zhu et al. (2017) with the obtained by Dincer and Topuz (2015), it is observed a clear difference in *E. coli* inactivation rates, higher when using a horn sonicator system. Similar results can be observed comparing Farhadi Chitgar et al. (2017) with the studies of Y&M inactivation showed in Table 3. When Farhadi Chitgar et al. (2017) obtained 3.34log reduction in berberry juice after 15min treatment at 20kHz and 70% of 200W at 25ºC, Saeeduddin et al. (2016), for same time, temperature and amplitude, but at higher power (500W), obtained only 0.1 log reductions of Y&M (the volume of pear juice treated is not mentioned in the study). Similar small rates of Y&M reduction was described by Aadil et al., (2017) (0.5log reduction after 60min of 600W treatment of a unknown volume of grapefruit juice) and Bhat and Goh (2017) (0.12log reductions after 30min of 70% of power not mentioned in the study). Besides some important variables as power and volume treated were not included in these studies, horn sonicators seem being more effective than bath system cleaners, allowing better inactivation rates. This fact could be because of the distribution and transmission of the ultrasound waves when horn system is used, giving higher power in a smaller volume.

In resume, effectiveness and efficiency of ultrasound in microorganism inactivation depend on the characteristics of the microorganism, duration of the ultrasound treatment and the power applied. The influence of the juice composition also can be an interfering factor in microbial reduction, but variables as pH, viscosity or density must be compared to affirm this hypothesis. Despite the trend found, HPU alone is not enough to reach the 5log reduction recommended by FDA (2004) for most of the microorganisms cited in previous tables. Thus, the effect of HPU combined with other physical treatments or non-thermal technologies must be reviewed.
3.1.1. COMBINATION OF HPU WITH TEMPERATURE AND PRESSURE

The role of temperature in microbial inactivation is well known, thus, its combination with HPU could be interesting to accelerate the rate of microbial inactivation, reduce process time and, therefore, decrease nutritional losses. In Tables 4 and 5, some results for the combination of temperature with HPU applied with horn and bath systems respectively are shown.

**TABLE 4.** Microbial inactivation by HPU horn system combined with heat: processing conditions and quality changes

<table>
<thead>
<tr>
<th>Fruit juice</th>
<th>Power (W)</th>
<th>Other power conditions</th>
<th>Time (min)</th>
<th>T (°C)</th>
<th>Microorganism</th>
<th>log CFU/ml reduction</th>
<th>Quality changes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pear</td>
<td>20kHz</td>
<td></td>
<td>10min</td>
<td>45°C</td>
<td>TPC</td>
<td>1.4</td>
<td>light decrease of ascorbic acid and phenolic compounds. No significant changes in Brix, pH and titratable acidity</td>
<td>Saeeduddin et al., 2015</td>
</tr>
<tr>
<td></td>
<td>20kHz</td>
<td></td>
<td>10min</td>
<td>65°C</td>
<td>Y&amp;M</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20kHz</td>
<td></td>
<td>10min</td>
<td>50°C</td>
<td>E. coli</td>
<td>4</td>
<td></td>
<td>Granul et al., 2017</td>
</tr>
<tr>
<td>Sugarcane</td>
<td>20kHz</td>
<td>100%</td>
<td>10min</td>
<td>40°C</td>
<td>E. coli</td>
<td>4</td>
<td></td>
<td>Dincer and Topuz, 2015</td>
</tr>
<tr>
<td>Black mulberry</td>
<td>20kHz</td>
<td></td>
<td>20min</td>
<td>50°C</td>
<td>E. coli</td>
<td>4.8</td>
<td>Increased color values and turbidity. Decreased anthocyanin content. Same pH and antioxidant activity</td>
<td>Saeeduddin et al., 2015</td>
</tr>
<tr>
<td>Apple</td>
<td>24kHz</td>
<td>120µm</td>
<td>30min</td>
<td>50°C</td>
<td>E. coli</td>
<td>7.3</td>
<td>Continuous mode: reddish and brighter; pulsed mode: greenish</td>
<td>Moody et al., 2014</td>
</tr>
<tr>
<td>Orange</td>
<td>24 kHz</td>
<td>33.31 W/ml, 105µm</td>
<td>10min</td>
<td>43°C</td>
<td>TPC</td>
<td>0.8</td>
<td></td>
<td>Guerrouj et al., 2016</td>
</tr>
<tr>
<td>Soursop nectar</td>
<td>24 kHz</td>
<td></td>
<td>30 min</td>
<td>46°C</td>
<td>TPC</td>
<td>1.5</td>
<td></td>
<td>Anaya-Esparrza et al., 2017</td>
</tr>
<tr>
<td>Orange</td>
<td>24 kHz</td>
<td></td>
<td>10min</td>
<td>60°C</td>
<td>S. aureus</td>
<td>3.8</td>
<td>Similar content in ascorbic acid to untreated juice, decrease when temperature increase. No significant changes in color, pH, titratable acidity and total soluble solids.</td>
<td>Bermúdez-Aquiar-Barrabosa-Cánovas 2012</td>
</tr>
<tr>
<td>Cranberry</td>
<td>24 kHz</td>
<td></td>
<td>20 min</td>
<td>60°C</td>
<td>S. cerevisiae</td>
<td>5.2</td>
<td>Decrease in pH. Changes in color</td>
<td>Bermúdez-Aquiar-Barrabosa-Cánovas 2012</td>
</tr>
<tr>
<td>Grape</td>
<td>24 kHz</td>
<td></td>
<td>30 min</td>
<td>60°C</td>
<td>S. cerevisiae</td>
<td>5.9</td>
<td>InCREASE IN pH. Changes in color</td>
<td>Bermúdez-Aquiar-Barrabosa-Cánovas 2012</td>
</tr>
<tr>
<td>Apple</td>
<td>20kHz</td>
<td></td>
<td>60 min</td>
<td>40°C</td>
<td>S. cerevisiae</td>
<td>5.2</td>
<td></td>
<td>Režek Jambrak et al., 2018</td>
</tr>
<tr>
<td>Apple</td>
<td>20kHz</td>
<td></td>
<td>60 min</td>
<td>60°C</td>
<td>A. acidothermstis</td>
<td>0</td>
<td></td>
<td>Režek Jambrak et al., 2018</td>
</tr>
<tr>
<td>Apple</td>
<td>20kHz</td>
<td></td>
<td>60 min</td>
<td>60°C</td>
<td>A. ochraceus</td>
<td>0</td>
<td></td>
<td>Režek Jambrak et al., 2018</td>
</tr>
<tr>
<td>Apple</td>
<td>20kHz</td>
<td></td>
<td>60 min</td>
<td>60°C</td>
<td>P. expansum</td>
<td>0</td>
<td></td>
<td>Režek Jambrak et al., 2018</td>
</tr>
<tr>
<td>Orange</td>
<td>24kHz</td>
<td>100µl, 460 W/cm², 0.33 W/ml</td>
<td>40 min</td>
<td>75°C</td>
<td>N. fischeri ascospores</td>
<td>3.6</td>
<td></td>
<td>Evelyn et al., 2016</td>
</tr>
</tbody>
</table>

Saeeduddin et al. (2015) compared the TS and commercial thermal treatments of pear juice and their effects on bacteria and Y&M inactivation.
They observed that TS at 45°C (20kHz, 750W) for 10 min had a similar effect on the inactivation of total plate count (TPC) of bacteria (1 log reduction) and Y&M (2.1 log reduction) than thermal process at 65°C for the same treatment time. Similarly, TS at 65°C (20kHz, 750W) for 10 min achieved total inactivation of microorganisms studied, the same result if the pear juice was submitted to 92°C for 2 min. Thus, HPU coupled with thermal treatment could reduce in 20–27°C the treatment temperature, which may be decisive in the improvement of the sensorial quality of the product.

Regarding *E. coli* inactivation with TS, Garud et al. (2017) obtained 5.5 log reduction in sugarcane juice treated at 50°C, 20kHz and 75% of 750W for 10 min. At the same conditions, Dinçer and Topuz (2015) obtained lower reduction (4 log) in black mulberry juice. The same temperature, 24kHz and 400W treatment were applied by Moody et al. (2014). They obtained 7.3 log reduction of *E. coli* after 20 min. Increasing the temperature to 60°C, 6.5 log reduction was achieved after only 5 min. For another bacteria, *B. cereus*, treated at the same conditions, Garud et al. (2017) obtained only 2.5 log reduction. This result shows the importance of the microorganism characteristics.

The results found by Guerrouj et al., (2016) showed near 1 log reduction of bacteria TPC and Y&M after 20 min of TS at 33.31 W/mL and 43°C in orange juice. In the same way, Saeeduddin et al. (2015) observed that for bacteria and Y&M inactivation, TS treatment (750W during 10 min) at temperatures lower than 50°C were not able to achieve a high reducing rate of microorganisms (until 2.5 log reduction). However, total inactivation of these microorganisms (3 and 3.4 log reduction) was obtained when temperature was increased 15°C. Anaya-Esparza et al. (2017) also found that it was not until 54°C when 5 log reduction of *E. coli* and *S. aureus* were achieved after 10 min of TS at 400 W in soursop nectar. The influence of temperature was also highlighted by Režek Jambrak et al., (2018). These authors found that at 40°C the log reduction of *S. cerevisiae* in apple juice was minimum (0-0.12), while the inactivation rate rised until 5.15 log at 60°C applying the same ultrasound conditions. Similar results were observed by Bermúdez-Aguirre and Barbosa-Cánovas (2012), reporting more than 5 log reductions of *S. cerevisiae* after 10 min of TS treatment at 400 W and 60°C. This authors also observed that continuous sonication mode was more effective than pulsed mode. Thus, in orange juice TS treated for 10 min at 60°C (400 W), 5.2 log reduction was obtained when using pulsed mode, while 6.4 log reduction was reached in continuous mode.

On the other hand, Režek Jambrak et al., (2018), described the inactivation of *A. ochraceus*, *P. expansum*, *Rhodotorula sp* and *S. cerevisiae* in fruit juices and nectars using TS treatments at 20kHz, 600 W, 60°C, for 3-9 min. Thus, log reductions ranging from 4.2 to 5.9 were reached in every microorganism. On the contrary, in the case of *A. acidoterrestris*, only 0.24-0.05 log reduction was obtained. This spore-forming bacterium have a thermoacidophilic character. In fact, *A. acidoterrestris* was suggested as reference microorganism for thermal treatment processes in high-acid fruit products (Ferrario et al., 2015), and
reaffirm that microbial characteristics has an important role in the resistance to the treatment.

Spore inactivation is also an interesting point to be reviewed. Evelyn et al. (2016) reported that long TS at 75°C (24kHz, 460 W/cm²) treatments were needed (40 min) to obtain 3.6 log reductions of Neosartorya fischeri ascospores in orange juice. The authors stated that during the first 10 min of treatment, the US application induce the activation of the spores. After that, a longer time was needed to obtain the required reduction. This fact makes TS not really attractive for spore inactivation, because a commercial application requires shorter times of treatment for better industrial productivity.

When HPU was applied in an ultrasonic cleaner bath, the rates of inactivation lightly decrease (Table 5).

**TABLE 5. Microbial inactivation by HPU bath system combined with heat: processing conditions and quality changes**

<table>
<thead>
<tr>
<th>Juice</th>
<th>HPU system</th>
<th>f (kHz)</th>
<th>Power (W)</th>
<th>Time (min)</th>
<th>T (°C)</th>
<th>Microorganism</th>
<th>log CFU/ml reduction</th>
<th>Quality changes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blueberry bath-type sonicator</td>
<td>40</td>
<td>280-700</td>
<td>10</td>
<td>30-40</td>
<td>E. coli</td>
<td>0.2-5.5</td>
<td>No differences in anthocyanin</td>
<td>Zhu et al., 2017</td>
<td></td>
</tr>
<tr>
<td>Mango Bath-type sonicator</td>
<td>25</td>
<td>200</td>
<td>10</td>
<td>50</td>
<td>Salmonella enteritidis</td>
<td>9</td>
<td>-</td>
<td>Kiang et al., 2012</td>
<td></td>
</tr>
<tr>
<td>Apple Bath-type sonicator</td>
<td>28, 45,</td>
<td>600</td>
<td>60</td>
<td>55.43 a/p</td>
<td>E. coli</td>
<td>4</td>
<td>Anthocyanin reduction (8.4%)</td>
<td>Gabriel, 2012</td>
<td></td>
</tr>
<tr>
<td>Orange Bath-type sonicator</td>
<td>28, 45, 100</td>
<td>600</td>
<td>40min at 1ms pulses</td>
<td>Salmonella spp</td>
<td>4.6</td>
<td>-</td>
<td>Gabriel, 2014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple Bath-type sonicator</td>
<td>35</td>
<td>480</td>
<td>60</td>
<td>80</td>
<td>A. acidoterrestris</td>
<td>4.7</td>
<td>-</td>
<td>Tremarin et al., 2019</td>
<td></td>
</tr>
</tbody>
</table>

Zhu et al. (2017) studied E. coli inactivation in 50mL of blueberry juice submitted to TS at different temperatures (30-60°C) using different ultrasonic power applied (200-700W). Results shown that 5 log reduction was only reached at the more intense tested conditions (10 min at 60°C and 700W). On the contrary, Kiang et al. (2012) obtained 5log and 9log of E. coli and Salmonella reductions respectively in mango juice, after 7 min of TS at 60°C applying only an ultrasonic power of 200W. In this study, the volume of juice treated was not mentioned, thus it is difficult to determine if the inactivation was conditioned by the relationship between power applied and volume treated, or the physicochemical characteristics of the juice.

Gabriel (2012 and 2014) used Dynashock ultrasound waves for the microorganism’s inactivation in orange juice. The particularity of this system is the combination of three frequencies (28, 45, and 100 kHz) that are alternately generated at a speed of 1ms to maximize microbubble generation and cavitation. Thus, 1L of apple juice (Gabriel, 2012) and 1L of orange juice (Gabriel, 2014) was processed at 600W, along 30 and 40 min respectively. At the end of the treatment, the temperature had reached until 44.03 and 55.43°C respectively. In both experiments, inactivation of E. coli, Salmonella spp and L. monocytogenes did not reach 5log reduction, and inactivation rates completely differed from each other (Table 5). Thus, when the treated medium
was not forced to maintain a constant temperature, the effect of ultrasound increased the temperature of the medium, therefore, inactivation was greater due to temperature but not to the ultrasonic treatment itself.

The inactivation of the thermoresistant *A. acidoterrestris* was studied by Tremarin et al. (2019) in a bath cleaner. The 5 log reductions were achieved after 20 min of TS (480W) at the temperature of 95°C in apple juice. Since the inactivation of this microorganism, mentioned previously in the study of Režek Jambrak et al. (2018), was minimal (0.02-0.05 log reductions) after horn system sonication at 600W at 60°C for 9 min, the role of the high temperature used in Tremarin et al. (2019) was highlighted. Anyway, even high temperature influence, sonication also fulfills its effect, reducing approximately half of the treatment time to attain the same inactivation than a conventional thermal process.

In short, it can be stated that TS efficiency depends on the microorganism’s characteristics, the temperature, the type of sonicator used and the application mode (pulsed/continuous). Moreover, the microbial inactivation with TS is more effective when temperature is higher than 50°C, facing the 5log reduction recommended by FDA (2014) to microorganisms as *E. coli*, *S. aureus* and *S. cerevisiae*. This process is effective to reduce thermal treatment time, obtaining the same inactivation because of its synergetic effect. Therefore, TS could be a viable technology for increasing the shelf life of juices, reducing the impact of temperature on thermal-sensitive attributes. On the other hand, spores and thermoresistant microorganisms such as *A. acidoterrestris* are not viable to being reduced by TS, so another way to damage their cells must to be investigated.

The pressure increase in a media induce changes in the structure of microbial membrane, altering nuclei and intracellular organelles, or releasing intracellular constituents outside the cell. Thus, the effect of combination of HPU with pressure (MS) or with pressure added to a mild thermal treatment (MTS) is shown in Table 6.

**TABLE 6.** Microbial inactivation by MTS and MS

<table>
<thead>
<tr>
<th>Juice</th>
<th>Sonication system</th>
<th>f (kHz)</th>
<th>Power (W)</th>
<th>Pressure</th>
<th>Time</th>
<th>T (°C)</th>
<th>Microorganism</th>
<th>log CFU/ml reduction</th>
<th>Quality changes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blueberry</td>
<td>bath-type sonicator</td>
<td>40</td>
<td>560</td>
<td>350 MPa</td>
<td>10 min</td>
<td>20</td>
<td>E. coli</td>
<td>5.2</td>
<td>Anthocyanin reduction (0.6%)</td>
<td>Zhu et al., 2017</td>
</tr>
<tr>
<td>Apple</td>
<td>Horn system</td>
<td>20</td>
<td>450</td>
<td>200 kPa</td>
<td>7.5 min</td>
<td>35</td>
<td>L. monocytogenes</td>
<td>4</td>
<td>-</td>
<td>Guzel et al., 2014</td>
</tr>
<tr>
<td>Apple</td>
<td>Horn system</td>
<td>20</td>
<td>450</td>
<td>200 kPa</td>
<td>0.92 min</td>
<td>60</td>
<td>E. coli</td>
<td>4</td>
<td>-</td>
<td>Ozan et al. 2017</td>
</tr>
<tr>
<td>Apple-carrot</td>
<td>Horn system</td>
<td>20</td>
<td>750</td>
<td>300 kPa</td>
<td>15 s</td>
<td>60</td>
<td>E. coli</td>
<td>4.89</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Zhu et al. (2017) studied *E. coli* inactivation by MS. After the treatment under pressure (350MPa) combined with sonication (560W) during 10 min, 5.2 log reduction was reached at only 20°C, and 5.85 log reductions at 40°C. In this sense, high inactivation rates can be reached without thermal treatments, avoiding the nutritional degradation and undesirable quality changes due to high temperatures.
On the other hand, when the MS is carried out at a moderate temperature (MTS), it produces an added stress to microorganisms leading to a higher inactivation rates. Thus, Guzel et al. (2014) found 4 log reduction of *L. monocytogenes* and *E. coli* after 7.2 and 3.6 min respectively of MS at 450W and 200kPa at 35°C in apple juice. When temperature increased to 60°C, maintaining the power and pressure conditions, the time to reach the same inactivation rates decrease to 0.92 and 1.08 min, respectively to *L. monocytogenes* and *E. coli*. These results show that MTS allows to decrease the treatment time. Ozan et al. (2017) obtained high inactivation rates of *E. coli* (5log reduction) in apple-carrot juice after only 30-60s of MTS at 750W and 100kPa using mild temperatures (60-50°C). Thus, MTS could be a promising technology to ensure microbial inactivation in short time treatments, and MS can ensure high inactivation without increasing the temperature.

### 3.1.2. MICROBIAL INACTIVATION BY COMBINING ULTRASOUND WITH OTHER NONTHERMAL TECHNOLOGIES

Another way to induce damages to microbial cells is the combination of HPU with other nonthermal technology, like Pulsed Light (PL), (Table 7).

<table>
<thead>
<tr>
<th>Juice</th>
<th>MTS</th>
<th>PULSED LIGHT</th>
<th>Microorganism</th>
<th>log CFU/ml reduction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural apple</td>
<td>hom system</td>
<td>600 kHz</td>
<td>30 min</td>
<td>0.1 m pulse/s 1.0 J/cm²</td>
<td>60 s</td>
</tr>
<tr>
<td>Commercial apple</td>
<td>hom system</td>
<td>600 kHz</td>
<td>30 min</td>
<td>0.1 m pulse/s 1.0 J/cm²</td>
<td>60 s</td>
</tr>
<tr>
<td>Orange</td>
<td>hom system</td>
<td>400 kHz</td>
<td>5 min</td>
<td>3600us 3Hz 0.019 J/cm²</td>
<td>2.81s</td>
</tr>
<tr>
<td>Apple</td>
<td>hom system</td>
<td>400 kHz</td>
<td>5 min</td>
<td>3600us 3Hz 0.019 J/cm²</td>
<td>2.81s</td>
</tr>
</tbody>
</table>

Ferrario et al. (2015) studied the inactivation of *S. cerevisiae* and *A. acidoterrestris* spores in apple juice with HPU coupled to PL. They found a 5.9 log reduction in *S. cerevisiae* at conditions shown in Table 7. As expected, spores had less level of inactivation than cells, being the *A. acidoterrestris* spores reduced only 1.8-3.03 log.

Muñoz et al. (2011 and 2012) studied the *E. coli* inactivation in orange and apple juice. Different rates of inactivation were obtained depending on the juice, being 5.9 log reduction in apple juice, while only 2.9 log reductions in orange juice in same treatment conditions (5 min of TS at 400W and 50°C combined with 2.81s of PL at 5J /cm²). This could be due to juices physicochemical properties, which can affect the sound propagation because of the density, viscosity, compressibility or elasticity of the fluid. Thus, more viscous and concentrated juices will present higher resistance to HPU treatments, reducing the degree to which cavitation occurs. In this case, lower frequency and higher power of ultrasound will be more effective than higher frequency ultrasound, which is easily dispersed within the medium. Salleh-Mack and Roberts (2007) observed that the presence of solids significantly
affects the inactivation. The pH also influenced microbial resistance to the treatment being the resistance lower as lower the pH. On the other hand, Lee et al. (2009) studied E. coli behavior at different pH (7, 5, 4 and 3) and no significant differences in inactivation were observed.

Back to combination of TS and PL, these combined technologies meet the FDA requirements for fruit juice processing and are able to inactivate S. cerevisiae and E. coli. However, A. acidotherrestris inactivation still have to be studied with another technologies. Anyway, the combination of PL with TS duplicates the rate of inactivation compared with TS alone (Ferrario et al., 2015).

Evelyn and Silva (2016) studied the inactivation of A. acidotherrestris spores in orange juice combining a pre-treatment of High Hydrostatic Pressures (HHP) with a treatment of HPU at the conditions shown in Table 8. They found a 4.4 log reduction when a TS treatment for 60min was applied after a 600MPa of HHP pretreatment for 15min. This is one of the highest reductions obtained of this spore among every treatment analyzed in this work. However, the total time of treatment is high. Thus, it is important to investigate the impact of this long processing time on the juice quality attributes, and it still not facing FDA requirement of 5log reduction. Anyway, the higher HHP pressure applied, the higher inactivation is obtained.

### TABLE 8. Combined HHP and HPU for microbial inactivation: processing conditions

<table>
<thead>
<tr>
<th>Juice</th>
<th>HHP 200MPa</th>
<th>TS 600MPa</th>
<th>Microorganism</th>
<th>log CFU/ml reduction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange</td>
<td>15min 39ºC</td>
<td>Horn system</td>
<td>24kHz 100%</td>
<td>60 min 78 C</td>
<td>Ferriano et al., 2015</td>
</tr>
</tbody>
</table>

The application of HPU during a treatment with supercritical carbon dioxide (SC-CO2) was studied by Ortuño et al. (2014). Specifically, they investigated the S. cerevisiae and E. coli inactivation in orange juice. The treatment conditions are shown in Table 9. The log reduction rates obtained were very promising, reaching total inactivation of E. coli and S. cerevisiae (7-8 log reduction) in only 3min after a treatment with SC-CO2 at 225 bar, 36 ºC and applying HPU at 30kHz and 40 W.

### TABLE 9. Combined HPU and SC-CO2 for microbial inactivation: processing conditions

<table>
<thead>
<tr>
<th>Product</th>
<th>SC-CO2</th>
<th>Sonication</th>
<th>Time (min)</th>
<th>Microorganism</th>
<th>log CFU/ml reduction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange juice</td>
<td>100 bar</td>
<td>36 ºC</td>
<td>horn system</td>
<td>30kHz 40W</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Orange juice</td>
<td>225 bar</td>
<td>36 ºC</td>
<td>horn system</td>
<td>30kHz 40W</td>
<td>1</td>
<td>3.00</td>
</tr>
<tr>
<td>Orange juice</td>
<td>350 bar</td>
<td>36 ºC</td>
<td>horn system</td>
<td>30kHz 40W</td>
<td>1</td>
<td>3.00</td>
</tr>
<tr>
<td>Orange juice</td>
<td>225 bar</td>
<td>31 ºC</td>
<td>horn system</td>
<td>30kHz 40W</td>
<td>1</td>
<td>3.00</td>
</tr>
<tr>
<td>Orange juice</td>
<td>350 bar</td>
<td>36 ºC</td>
<td>horn system</td>
<td>30kHz 40W</td>
<td>1</td>
<td>3.00</td>
</tr>
<tr>
<td>Orange juice</td>
<td>225 bar</td>
<td>31 ºC</td>
<td>horn system</td>
<td>30kHz 40W</td>
<td>1</td>
<td>3.00</td>
</tr>
<tr>
<td>Orange juice</td>
<td>350 bar</td>
<td>36 ºC</td>
<td>horn system</td>
<td>30kHz 40W</td>
<td>1</td>
<td>3.00</td>
</tr>
<tr>
<td>Orange juice</td>
<td>225 bar</td>
<td>31 ºC</td>
<td>horn system</td>
<td>30kHz 40W</td>
<td>1</td>
<td>3.00</td>
</tr>
<tr>
<td>Orange juice</td>
<td>350 bar</td>
<td>36 ºC</td>
<td>horn system</td>
<td>30kHz 40W</td>
<td>1</td>
<td>3.00</td>
</tr>
</tbody>
</table>
Similar results were obtained by Paniagua-Martinez et al. (2018). They achieved the complete inactivation for *E. coli* and the 99.7% inactivation for *S. cerevisiae* (2.1-2.6 log reduction) in continuous mode (3.06 min residence time) in orange juice (conditions described in Table 9).

The high inactivation rates obtained in these studies state SC-CO$_2$ assisted by HPU treatment as one of the most effective treatments reviewed in this work. This treatment could ensure 5log inactivation required by FDA (2014) at mild temperature conditions. This is due to the SC-CO$_2$ high diffusivity properties, in addition to cavitation which induces a better contact of CO$_2$ with cell’s surface, allowing the faster penetration into the cell membrane and the extraction of the cell material from the cytoplasm. A temperature increment could accelerate the diffusivity increasing the inactivation in short time rates (Spilimbergo et al., 2014).

In short, HPU combined with nonthermal technologies allowed to obtain great inactivation rates of microorganisms that faces FDA requirements of 5log reduction, being SC-CO$_2$ assisted by HPU the one with the best inactivation for the more common microorganisms. The inactivation of *A. acidothermophilus* and spores with combined technologies has not been widely studied in literature.

### 3.2. Enzymatic inactivation

The inactivation of undesirable enzymes is one of the main goals pursued for food industry. Browning is an important problem during the juice production. Fruit and vegetables darkening is produced when PPO and POD react with the phenolic compounds from the food matrix, bringing to the product an undesirable appearance. PME is the enzyme that breaks ester bonds in pectin, leading to cloud stability reduction in juices. This phenomenon is responsible of a no homogeneous appearance of the product (separation between the juice and its pulp). Traditional thermal techniques are applied to inactivate these enzymes, but the high temperatures produce loses of some valuable compounds, like antioxidants, ascorbic acid, total phenols and flavonoids or color (Koshani et al., 2014).

Different studies referred to PPO, PME and POD inactivation in juices are collected in Table 10. In general, HPU assisted enzymatic inactivation process depends on the same parameters than the microbial one. Thus, the effectiveness increases at higher ultrasonic power applied, temperature and processing time. According to the results of PPO inactivation (Table 10), it can be observed that temperature conditions below 50°C are not effective to reduce the activity of the enzymes (Zhu et al., 2017, Cervantes-Elizarrarás et al., 2017, Saeeduddin et al., 2015, Ríos-Romero et al., 2018). Above this temperature, PPO activity highly decreases, until 1.72% after TS at 50°C of 1500W of 26µm amplitude along 17min (Cervantes-Elizarrarás et al., 2017), 1.97% after TS at 50°C of 580W treatment (Zhu et al., 2017) or 1.91% after TS at 65°C at 750W (Saeeduddin et al., 2015). PME and POD seems being more resistant to HPU than PPO (Cervantes-Elizarrarás et al., 2017, Saeeduddin et al., 2015).
In the same way, according to Koshani et al. (2014), the comparison between Tables 10 and 11, shows that TS permits a decrease of 20°C to obtain the same rates of enzyme inactivation (near 2% of residual activity) after 10 min of application of TS at 50°C at 560W than conventional thermal treatment (10 min at 70°C) (Zhu et al., 2017). A thermal treatment for 10 min at 60°C remained a POD, PPO and PME residual activity of 66%, 59% and 63% respectively. However, when this thermal treatment was complemented with ultrasound application at 20kHz and 750W, the POD, PPO and PME activity decreased until 4.3%, 1.91% and 3.25% respectively (Saeeduddin et al., 2015). In the same way, according to Koshani et al. (2014),

### TABLE 10. Enzymatic inactivation by HPU/TS: processing conditions and quality changes

<table>
<thead>
<tr>
<th>Juice</th>
<th>HPU system</th>
<th>T (kHz)</th>
<th>Power (W)</th>
<th>T (ºC)</th>
<th>Time (min)</th>
<th>Enzyme</th>
<th>Residual Activity</th>
<th>Quality changes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Blueberry</em></td>
<td>bath type sonicator</td>
<td>40</td>
<td>280</td>
<td>20</td>
<td>10</td>
<td>PPO</td>
<td>64%</td>
<td>No significant anthocyanin reduction</td>
<td>Zhu et al., 2017</td>
</tr>
<tr>
<td></td>
<td>420</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>560</td>
<td>700</td>
<td>40</td>
<td>10</td>
<td>10</td>
<td>PPO</td>
<td>40.10%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>560</td>
<td>700</td>
<td>50</td>
<td>10</td>
<td>10</td>
<td>PPO</td>
<td>37.63%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>65</td>
<td>50</td>
<td>10</td>
<td>10</td>
<td>PPO</td>
<td>35.35%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Blackberry</em></td>
<td>Horn system</td>
<td>20</td>
<td>1500</td>
<td>28µm</td>
<td>50</td>
<td>PME</td>
<td>99%</td>
<td>No changes in pH and total soluble solids. Cloud index increase. Enhanced antioxidant activity, ascorbic acid and total polyphenol content</td>
<td>Carnavantes-Elizarraras et al., 2017</td>
</tr>
<tr>
<td><em>Pear</em></td>
<td>Horn system</td>
<td>20</td>
<td>750</td>
<td>25</td>
<td>10</td>
<td>POD</td>
<td>93.3%</td>
<td>Higher retention of ascorbic acid and phenolic compounds</td>
<td>Saeeduddin et al., 2015</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>750</td>
<td>45</td>
<td>10</td>
<td>10</td>
<td>POD</td>
<td>43.2%</td>
<td>Decrease of antioxidant capacity. No significant changes in °Brix, pH and titratable acidity</td>
<td></td>
</tr>
<tr>
<td><em>Orange</em></td>
<td>Horn system</td>
<td>80</td>
<td>63</td>
<td>10</td>
<td>10</td>
<td>PME</td>
<td>3.25%</td>
<td></td>
<td>Koshani et al., 2014</td>
</tr>
<tr>
<td><em>Orange-sweet potato</em></td>
<td>Horn system</td>
<td>26</td>
<td>200</td>
<td>90%</td>
<td>8</td>
<td>POD</td>
<td>53.72%</td>
<td>Preservation of β-carotene, phenolic compounds, antioxidant activity.</td>
<td>Ríos-Romeros et al., 2018</td>
</tr>
</tbody>
</table>

Zhu et al. (2017) obtained that the increase of HPU power (from 280 to 700W) do not produce a decrease of PPO activity (from 64% to 40.1%) greater than the obtained by increasing the treatment temperature from 20 to 40°C (PPO activity decrease from 64% to 7.2%). The system selected to apply ultrasound, bath type sonicator or horn sonicator, didn’t show important influence in the effectiveness of the process. This indicate that the ultrasonic power applied have less influence in the process than temperature. In Table 11 some literature about the effect of temperature on thermal treatment of enzymes are shown.

### TABLE 11. Effect of temperature of thermal treatment in the enzyme inactivation

<table>
<thead>
<tr>
<th>Juice</th>
<th>T (ºC)</th>
<th>Time</th>
<th>Enzyme</th>
<th>Residual Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Blueberry</em></td>
<td>50</td>
<td>10min</td>
<td>PPO</td>
<td>59.00%</td>
<td>Zhu et al., 2017</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td></td>
<td>PPO</td>
<td>52.00%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>70</td>
<td></td>
<td>PPO</td>
<td>42.67%</td>
<td></td>
</tr>
<tr>
<td><em>Blackberry</em></td>
<td>90</td>
<td>15s</td>
<td>PPO</td>
<td>10.25%</td>
<td>Cervantes-Elizarraras et al., 2017</td>
</tr>
<tr>
<td><em>Pear</em></td>
<td>65</td>
<td>10min</td>
<td>PPO</td>
<td>66%</td>
<td>Saeeduddin et al., 2015</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>2min</td>
<td>PME</td>
<td>63%</td>
<td></td>
</tr>
<tr>
<td><em>Orange</em></td>
<td>75</td>
<td>22min</td>
<td>PME</td>
<td>0.14%</td>
<td>Koshani et al., 2014</td>
</tr>
</tbody>
</table>
20% of decrease in temperature and 50% in processing time of a thermal treatment at 75°C for 22min might be achieved for inactivation of PME by applying 10min of HPU (80W) at 63ºC, to obtain less of 10% of PME residual activity in sour orange juice.

Enzyme inactivation is caused by implosion of cavitation bubbles which induces structural damages in the enzyme. These damages increase the surface able to be affected by environmental conditions, resulting with a denaturation phenomenon (Aadil, et al., 2015). PPO resulted to be the most sensitive enzyme to the increase of temperature, followed by POD, and PME as the most resistant one. At moderate temperatures (25-36°C), ultrasound is not effective to inactivate the main enzymes responsible of degradation of juice quality. However, inactivation rates comparable to industrial heat treatment can be obtained when temperature conditions are mildly increased to 50-65ºC. Nevertheless, it is still temperature the main factor that determines enzymatic inactivation.

To study enzyme inactivation at lower temperatures, a list of papers dealing with other nonthermal treatments are shown in Tables 12 and 13.

TABLE 12. Combined US and other nonthermal technologies for enzymatic inactivation: processing conditions

<table>
<thead>
<tr>
<th>Product</th>
<th>Sonication</th>
<th>HHP</th>
<th>Enzyme</th>
<th>Residual Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple juice</td>
<td>25kHz</td>
<td>500W</td>
<td>70%</td>
<td>60min 20 ºC</td>
<td>PME 66%</td>
</tr>
<tr>
<td>bath-type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pod 63%</td>
</tr>
<tr>
<td>sonicator</td>
<td>25kHz</td>
<td>500W</td>
<td>70%</td>
<td>60min 20 ºC</td>
<td>PME 71%</td>
</tr>
<tr>
<td></td>
<td>25kHz</td>
<td>500W</td>
<td>70%</td>
<td>60min 20 ºC</td>
<td>Pod 63%</td>
</tr>
<tr>
<td></td>
<td>25kHz</td>
<td>500W</td>
<td>70%</td>
<td>60min 20 ºC</td>
<td>Pod 52%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Abid et al., 2014</td>
</tr>
</tbody>
</table>

Abid et al. (2014) investigated PME, PPO and POD inactivation by HHP at 250-450MPa with sonication at 70% of 500W pre-treatment, but inactivation rates at higher treatment conditions did not permit a residual inactivation lower than 24%, 21% and 32% to PME, PPO and POD respectively.

The use of SC-CO₂ assisted by HPU to inactivate the most resistant enzyme has been also studied (Ortuño et al., 2014). In this case, PME, only reduced the residual activity under 10% at most intense conditions tested (225 bar, 41°C) after 10 min at 30kHz and 40W.

TABLE 13. Combined US and other nonthermal technologies for enzymatic inactivation: processing conditions

<table>
<thead>
<tr>
<th>Product</th>
<th>SC-CO₂</th>
<th>Sonication</th>
<th>Time</th>
<th>Enzyme</th>
<th>Residual Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange juice</td>
<td>100 bar</td>
<td>horn system</td>
<td>36 ºC</td>
<td>30KHz</td>
<td>10min 40W</td>
<td>PME 54%</td>
</tr>
<tr>
<td></td>
<td>225 bar</td>
<td>horn system</td>
<td></td>
<td></td>
<td></td>
<td>Pod 44%</td>
</tr>
<tr>
<td></td>
<td>350 bar</td>
<td>36 ºC</td>
<td>10min</td>
<td></td>
<td></td>
<td>Pod 47%</td>
</tr>
<tr>
<td></td>
<td>225 bar</td>
<td>36 ºC</td>
<td>10min</td>
<td></td>
<td></td>
<td>Pod 47%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>41 ºC</td>
<td></td>
<td></td>
<td></td>
<td>Pod 18%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>horn system</td>
<td>30KHz</td>
<td>10min</td>
<td></td>
<td>Ortuno et al., 2014</td>
</tr>
</tbody>
</table>

In enzymatic inactivation, the effectiveness of residual activity reduction is linked with the temperature. However, SC-CO₂ assisted by HPU is capable to reduce the most resistant enzyme to the acceptable rate of 10% of its activity,
thus, SC-CO₂ assisted by HPU could be an interesting alternative to thermal treatments.

3.3. Influence on quality

The intense effects produced by HPU cavitation can affect not only microorganism or enzymes but also quality attributes of the treated product. For example, free radicals generated during cavitation can interact with organic compounds leading to oxidation problems.

In literature reviewed, few papers describe quality changes in the juices treated. Most of them show similar quality attributes to the untreated sample (Bevilacqua et al., 2014 Dinçer and Topuz, 2015), where color (Zhu et al., 2017 Farhadi Chitgar et al., 2017, Bhat and Goh, 2017, Saeeduddin et al., 2016 Anaya-Esparza et al., 2017), pH (Farhadi Chitgar et al., 2017, Bhat and Goh, 2017, Saeeduddin et al., 2016, Saeeduddin et al., 2015, Dinçer and Topuz, 2015, Anaya-Esparza et al., 2017), antioxidant content (Zhu et al., 2017 Farhadi Chitgar et al., 2017, Dinçer and Topuz, 2015, Anaya-Esparza et al., 2017), total soluble solids (Anaya-Esparza et al., 2017, Guerrouj et al., 2016, Saeeduddin et al., 2016), °Brix (Farhadi Chitgar et al., 2017, Bhat and Goh, 2017, Saeeduddin et al., 2015) and turbidity (Bhat and Goh, 2017) had no changed after the different treatments described in Tables 2, 3, 4, 5, 6 and 10.

Moreover, some authors described enhanced effects of HPU in fruit juices. Some of them are the increase of phenolic content (Farhadi Chitgar et al., 2017, Guerrouj et al., 2016), the increase of carotenoids (Aadil et al., 2017, Guerrouj et al., 2016), anthocyanins (Dinçer and Topuz, 2015, Bhat and Goh, 2017, Aadil et al., 2017), ascorbic acid (Saeeduddin et al., 2016, Guerrouj et al., 2016), lycopene (Aadil et al., 2017), and the reduction of particle size improving cloud values and turbidity, giving to the juice a better consistency and homogeneity (Dinçer and Topuz, 2015, Bhat and Goh, 2017, Saeeduddin et al., 2016). The enhancement of the total phenolic content (carotenoids, anthocyanins and lycopene) could be due to addition of hydroxyl group, produced by cavitation, to the aromatic ring of phenolic compounds (Farhadi Chirgar et al. 2017). It might be also attributed to the changes in the surface structure of plant cells making them easier to break, thus, the disruption of cell walls facilitate the release of bound phenolic contents (Guerrouj et al., 2016).

Referring to the enhancement of the ascorbic acid, the main problem of its degradation by temperature and oxygen exposition. Thus, during sonication the increment of ascorbic acid content could be attributed to the elimination of dissolved oxygen in the juice, preserving it from oxidation (Guerrouj et al., 2016, Saeeduddin et al., 2016).

The increase in turbidity could be due to the effect of high pressure gradient exerted by cavitation which breaks the larger particles into smaller ones, incrementing the number of suspended molecules and reducing the distance between them, which translates in a turbidity increase (Dinçer and Topuz, 2015).

Some not desirable changes has also been observed in literature, like color changes (Farhadi Chitgar et al., 2017, Moody et al., 2014, Dinçer and Topuz, 2015), anthocyanin reduction (Zhu et al., 2017 50ºC Dinçer and Topuz, 2015),
viscosity decrease (Bhat and Goh, 2017), decrease in ascorbic acid (Anaya-Esparza et al., 2017, Saeeduddin et al., 2015), pH (Guerrouj et al., 2016, Bermúdez-Aguirre and Barbosa-Cánovas, 2012) and aroma and taste after long processing treatment (Guerrouj et al., 2016). Most of these changes are produced while combining HPU with different temperatures during TS, so these degradations of color and antioxidant compounds might mostly be linked to the temperature effect.

The reduction in viscosity can be assigned to pressure, shear and temperature changes on the media during cavitation, which induce the fragmentation of polymeric structures (usually pectin in strawberry juice) (Bhat and Goh, 2017). The change of pH after TS in juices could be because of the formation of some chemical products in the medium such as nitrite, hydrogen peroxide and nitrate, depending on the medium treated (Bermúdez-Aguirre and Barbosa-Cánovas 2012). Moreover, it can appear the sonotrode erosion during cavitation, promoted by high temperature and pressure generated during the process. This provokes the deposition of metal particles in the food, giving it a metallic odor (Bermudez-Aguirre, 2018).

Compared with quality effects induced by thermal treatments, the color changes and antioxidant compounds degradation resulted be less harmful during HPU treatments (Zhu et al., 2017, Saeeduddin et al., 2015), positioning HPU as an alternative nonthermal technology capable of preserving these characteristics of juices. Also, the preservation of °Brix, acidity, and other characteristics of juices treated by HPU faces the RD 1518/2007, November, the 16th, in relation of the minimum quality parameters for fruit juices. Nevertheless, analysis of important organoleptic parameters for the consumers such as the taste and the smell of juices have not been found in most of the literature consulted, so it cannot be concluded that HPU is an alternative nonthermal technology that avoids non desirable quality changes, despite the nutritional improvements it can offer. More research is needed in that sense.

4- CONCLUSION

Microbial and enzymatic inactivation was studied in different juices according to review HPU efficacy for the legislated juices in Spanish legislation. Orange, pineapple, pear, and apple juices have been an extended object of study, but only few studies was about grape juice, and there was not found any study about peach, apricot and tangerine juice. Despite the difficulty of comparing different processing conditions compiled in literature, it can be stated that HPU alone was not effective in bacterial and enzymatic inactivation whereas synergetic effects of nonthermal technologies combination or combining with pressure or temperature noticed very promising results for more resistant microorganisms.

Microbial and enzymatic inactivation depends on their own characteristics, and on the composition of the juice treated. E. coli, Salmonella, Listeria, S. cerevisiae and S. aureus were 5log reduced with TS, MTS, and combined HPU
with nonthermal technologies, facing FDA recommendation. The inactivation of *A. acidoterrestris* and its spores must be studied yet. TS at middle temperatures is able to inactivate POD, PPO and PME enzymes. SC-CO$_2$ assisted by HPU resulted be interesting for both microbial and enzymatic inactivation.

The characteristics of juice that influence the effectiveness of the treatment are not totally clear, but it was observed that the pH does not affect the efficacy, and viscosity and juice composition could interfere in the acoustic wave propagation.

The evaluation of HPU influence on quality shows that HPU and HPU combined with other thermal or nonthermal technologies are able to obtain better nutritional and sensory quality than conventional thermal treatments, allowing in some cases an enhancement in antioxidant content or texture.

In conclusion, further studies are still needed to determine the better processing conditions of HPU combined with the technologies reviewed, to obtain the formula for juice treatment that provides the processing conditions needed for validation of HPU at industrial scale, taking into account the composition of juices and the organoleptic impact they may suffer during their treatment.

**REFERENCES**


Asozumos, Guía de aplicación del sistema APPCC en la industria de zumos de frutas, [en línea]. Centro Nacional de Tecnología y Seguridad Alimentaria 2013 URL link:<http://www.aecosan.msssi.gob.es/AECOSAN/docs/docum


España, Real Decreto 1044/1987, de 31 de julio, por el que se regula la elaboración de zumos de uva en armonización con la normativa comunitaria [on line]. URL link: https://www.boe.es/eli/es/rd/1987/07/31/1044 [Consulted: 20th May 2019]

España, Real Decreto 1518/2007, de 16 de noviembre, por el que se establecen parámetros mínimos de calidad en zumos de frutas y los métodos de análisis aplicables [on line]. URL link: <https://www.boe.es/eli/es/rd/2007/11/16/1518> [Consulted: 20th May 2019]


Farhadi Chitgar, M., Aalami, M., Maghsoudlou, Y., Milani, E. 2017. Comparative study on the effect of heat treatment and sonication on the quality of barberry (Berberis vulgaris) juice. Journal of Food Processing and Preservation, 41:3


Režek Jambrak, A., Šimunek, M., Evačić, S., Markov, K., Smoljanić, G., Frece, J. 2018. Influence of high-power ultrasound on selected moulds, yeasts and *Alicyclobacillus acidoterrestris* in apple, cranberry and blueberry juice and nectar, *Ultrasonics*, 83:3-17


