UNIVERSITAT POLITÈCNICA DE VALÈNCIA

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

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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/cm^2. 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 (5000ºC 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 CO_2 (SC-CO_2) 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/cm^2). 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.

Keywords (connected by AND)			Date	Results	Publications used in this work	Choosing criteria
Ultrasound	Pathogenic bacteria	Juice	All years	9	4 (Gabriel et al. (2012), Gabriel et al. (2014), Patil et al. (2009), Anaya-Esparza et al. (2017), Tamarin et al. (2019))	*E. coli, Salmonella, Listeria* and *A. acidoterrestris* inactivation
Sonication	*Listeria*	Juice	All years	22	1 (Guzel et al. (2014))	*Listeria* and *E. coli* inactivation
Sonication	*Salmonella*	Juice	All years	14	1 (Kiang et al. (2012)	*Salmonella* and *E. coli* inactivation was investigated
Sonication	*Escherichia coli*	Juice	All years	59	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), Dinçer and Topuz (2015), Guzel (2014), Aligourchi et al. (2014), Lee et al. (2009), Muñoz et al. (2011 and 2012), Salleh-Mack and Roberts (2007)	*E. coli* inactivation was studied; Influence of sonication in different quality parameters
Sonication	*acidoterrestris*	Juice	All years	6	3 (Tremarin et al. (2019), Roobab et al. (2018), Evelyn and Silva (2016))	*A. acidoterrestris* and spores inactivation
Sonication	*cerevisiae*	Juice	All years	23	2 (Aligourchi et al. (2014), Bermúdez-Aguirre and Barbosa-Cánovas (2012))	*S. cerevisiae* inactivation
Ultrasound	Enzymatic inactivation	Juice	All years	30	2 (Cervantes-Elizarrarás et al. (2017), Saeeduddin et al. (2015))	PPO inactivation
Nonthermal technologies	Juices	Document types: Reviews	2016-2018	7	2 (Bevilacqua et al (2018), Roobab et al. (2017))	Microbial inactivation with HPU and combined with other nonthermal technologies
Ultrasound	Supercritical carbon dioxide	Authors: Ortuño	All years	7	1 (Ortuño et al, (2014))	*S. cerevisiae, E. coli* and PME inactivation

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. (Abbreviatures: TPC (Total Plate Count), Y&M (Yeasts and Molds), f (frecuency), T (Temperature), a/p (after process)).

Fruit juice	f (kHz)	Power (W)	Other power conditions	T (ºC)	Time (min)	Sonication mode	Microorganism	log CFU/ml reduction	Quality changes	Reference
Apple	20	130	60%	40 (a/p)	6	6s pulse	*S. cerevisiae*	2.40	Similar sensory attributes to untreated sample	Bevilacqua et al., 2014
					4	2s pulse		2.16		
					6	6s pulse	*Z. bailii*	1.75		
					4	2s pulse		1.75		
					6	6s pulse	*Z. rouxii*	1.95		
					4	2s pulse		0.4		
					6	6s pulse	*P. membranifaciens*	2.00		
					4	2s pulse		1.80		
					6	6s pulse	*W. anomalus*	2.20		
					4	2s pulse		0.65		
					6	6s pulse	*C. norvegica*	2.25		
					4	2s pulse		1.50		
red fruit	20	130	60%	40 (a/p)	6	6s pulse	*S. cerevisiae*	2.00	Similar attributes to untreated sample	Bevilacqua et al., 2014
					4	2s pulse		0.50		
strawberry	20	130	60%	40 (a/p)	6	6s pulse	*S. cerevisiae*	2.40	Similar attributes to untreated sample	Bevilacqua et al., 2014
					4	2s pulse		1.00		
orange	20	130	60%	40 a/p	6	6s pulse	*S. cerevisiae*	2.00	Similar attributes to untreated sample	Bevilacqua et al., 2014
					4	2s pulse		0.20		
pineapple	20	130	60%	40 (a/p)	6	6s pulse	*S. cerevisiae*	0.20	Similar attributes to untreated sample	Bevilacqua et al., 2014
					4	2s pulse		0.70		
Barberry juice	20	200	70%	25	10	15s pulse on and 5s pulse off	Aerobic	1.99	No effects on anthocyanins, pH and ºBrix, increased total phenolic content, color parameters affected	Farhadi Chitgar et al., 2017
							Y&M	2.04		
	20	200	100%		10		Aerobic	3.68		
							Y&M	3.34		
	20	200	70%		15		Aerobic	3.68		
							Y&M	3.34		
Apple juice (natural)	20	600	80% 95.2 µm	30	30	-	*S. cerevisiae*	2.50	-	Ferrario et al., 2015
				44				2.80		
	20	600	80% 95.2 µm	30	30	-	*A. acidoterrestris*	0.00		
				44				0.00		
Apple juice (commercial)	20	600	80% 95.2 µm	30	30	-	*S. cerevisiae*	2.50		
				44				2.50		
	20	600	80% 95.2 µm	30	30	-	*A. acidoterrestris*	0.00		
				44				0.00		
apple	20	600	80% 95.2 µm	44	10	-	*S. cerevisiae*	1.20	-	Ferrario and Guerrero, 2017
				44	30			2.60		
Black mulberry	20	750	60%	25	15	5s pulses	*E. coli*	3.93	Increased color values and turbidity. No changes in pH, antioxidant activity	Dinçer and Topuz, 2015
			80%					4.49		
			100%					5.14		
Orange	20	-	0.4µm	30	15	0.5s pulses	*E. coli*	1.10	-	Patil et al., 2009
			7,5µm					2.50		
			37,5µm					2.70		
Apple	20	-	0.4µm	30	15	0.5s pulses	*E. coli*	3.00	-	
Pome-granate	20	-	50% 24,4µm	25	15	-	*E. coli*	1.58	-	Alighourchi et al., 2014
							S. cerevisiae	0.89		
			75%		15	-	*E. coli*	1.85		
							S. cerevisiae	1.06		
			100% 60µm		15	-	*E. coli*	3.37		
							S. cerevisiae	1.84		

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. anomalus* 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.

Juice	f (kHz)	Power (W)	Other power conditions	T (ºC)	Time (min)	Microorganism	log CFU/ml reduction	Quality changes	Reference
Strawberry	25	Not provided	70%	20	30	TPC	0.15	Same pH, a_{w}, ºBrix, color, titratable acidity and turbidity. Lower viscosity, increased cloud assessment and anthocyanine	Bhat and Goh, 2017
						Y&M	0.12		
Pear	25	500	70%	25	15	TPC	0.5	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.	Saeeduddin et al., 2016
					30		0.7		
					45		1.3		
					60		2		
	25	500	70%	25	15	Y&M	0.1		
					30		0.3		
					45		0.7		
					60		1.1		
Grapefruit	28	600	-	20	30	TPC	0.5	Increase in lycopene, anthocyanin and carotenoids	Aadil et al., 2017
						Y&M	0.5		
Blueberry	40	280	-	20	10	*E. coli*	0.13	Not-significant Anthocyanin reduction (3% from untreated juice)	Zhu et al., 2017
		420					0.2		
		560					0.23		
		700					0.25		

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

Fruit juice	f (kHz)	Power (W)	Other power conditions	Time (min)	T (ºC)	Microorganism	log CFU/ml reduction	Quality changes	Reference
Pear	20kHz	750W		10min	45˚C	TPC	1.4	light decrease of ascorbic acid and phenolic compounds. No significant changes in ºBrix, pH and titratable acidity	Saeeduddin et al., 2015
						Y&M	2.5		
				10min	65˚C	TPC	4		
						Y&M	3.4		
Sugarcane	20kHz	750W	100%	10min	50˚C	*E. coli*	5.5	-	Garud et al., 2017
						B. cereus	2.5		
Black mulberry	20kHz	750W	100%	10min	40˚C	*E. coli*	4	Increased color values and turbidity. Decreased anthocyanin content. Same pH and antioxidant activity	Dinçer and Topuz, 2015
					50˚C	*E. coli*	4.8		
Apple	24kHz	400W	120µm	20 min	50˚C	*E. coli*	7.3	Continuous mode: reddish and brighter; pulsed mode: greenish	Moody et al., 2014
				5min	60˚C		6.5		
Orange	24 kHz		33,31W/ml, 105µm	10min	43 ˚C	TPC	0.8	Increase in total phenolic content, flavonoids, antioxidant compounds, carotenoids, ascorbic acid. Decrease in pH. No significant changes in total soluble solids. Decrease aroma and taste after 20 and 30 min treatment.	Guerrouj et al., 2016
						Y&M	0.7		
				20min	43 ˚C	TPC	1.3		
						Y&M	0.9		
				30min	46°C	TPC	1.5		
						Y&M	-		
Soursop nectar	24 kHz	400W	1.4W/ml	10min	34 ˚C	*E. coli*	3.8	Similar content in ascorbic acid to untreated juice, decrease when temperature increase. No significant changes in color, pH, titratable acidity and total soluble solids.	Anaya-Esparza et al., 2017
						S. aureus	3.4		
					44 ˚C	*E. coli*	4.02		
						S. aureus	4.03		
					54 °C	*E. coli*	5.1		
						S. aureus	5.1		
Orange	24 kHz	400W	Pulsed and continuos mode	10min	60˚C	*S. cerevisiae*	5.2	Decrease in pH. Changes in color	Bermúdez-Aguirre and Barbosa-Cánovas 2012
							6.4		
Cranberry	24 kHz	400W		20min		*S. cerevisiae*	5.1	Increase in pH. Changes in color	
							5.7		
Grape	24 kHz	400W		30min		*S. cerevisiae*	5.9		
							6.2		
Apple	20kHz	600W	60µm	3min	40˚C	*S. cerevisiae*	0	-	Režek Jambrak et al., 2018
			90µm	3 min			0		
			90µm	6min			0		
			90µm	9min			0		
			60µm	6min	60˚C		4.98		
			60µm	9min			4.99		
			120µm	3min			4.8		
			120µm	9min			5.15		
	20kHz	600W	60µm	6min	60˚C	*A. acidoterrestris*	0.029	-	
			60µm	9min	60˚C		0		
			120µm	3min	60˚C		0		
			120µm	9min	60˚C		0		
	20kHz	600W	60µm	6min	60˚C	*A. ochraceus*	3.5	-	
			60µm	9min	60˚C		3.8		
			120µm	3min	60˚C		3.7		
			120µm	9min	60˚C		3.9		
	20kHz	600W	60µm	6min	60˚C	*P. expansum*	4.26	-	
			60µm	9min	60˚C		4.09		
			120µm	3min	60˚C		4.15		
			120µm	9min	60˚C		3.77		
	20kHz	600W	60µm	6min	60˚C	*Rhodotorula sp*	5.194	-	
			60µm	9min	60˚C		4.93		
			120µm	3min	60˚C		4.86		
			120µm	9min	60˚C		4.99		
Orange	24kHz		100%, 460 W/cm2, 0.33 W/mL	40min	75˚C	*N. fischeri* ascospores	3.6	-	Evelyn et al., 2016

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 10min, 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 65ºC (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 5.5log reduction in sugarcane juice treated at 50˚C, 20kHz and 100% of 750W for 10min. 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 7.3log reduction of *E. coli* after 20min. Increasing the temperature to 60ºC, 6.5log reduction was achieved after only 5 min. For another bacteria, *B. cereus*, treated at the same conditions, Garud et al. (2017) obtained only 2.5log 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 33.31W/mL and 43ºC in orange juice. In the same way, Saeeduddin et al. (2015) observed that for bacteria and Y&M inactivation, TS treatment (750W during 10min) at temperatures lower than 50ºC were not able to achieve a high reducing rate of microorganisms (until 2.5log reduction). However, total inactivation of these microorganisms (4 and 3.4 log reduction) was obtained when temperature was increased 15ºC. Anaya-Esparza et al. (2017) also found that it was not until 54ºC when 5log reduction of *E. coli* and *S. aureus* were achieved after 10 min of TS at 400W in soursop nectar. The influence of temperature was also highlighted by Režek Jambrak et al., (2018). These authors found that at 40ºC the log reduction of *S. cerevisiae* in apple juice was minimum (0-0.12), while the inactivation rate rised until 5.15log at 60ºC applying the same ultrasound conditions. Similar results were observed by Bermúdez-Aguirre and Barbosa-Cánovas (2012), reporting more than 5log reductions of *S. cerevisiae* after 10 min of TS treatment at 400W and 60ºC. This authors also observed that continuous sonication mode was more effective than pulsed mode. Thus, in orange juice TS treated for 10 min at 60ºC (400W), 5.2log reduction was obtained when using pulsed mode, while 6.4log 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, 600W, 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/cm^2) 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

Juice	HPU system	f (kHz)	Power (W)	Time (min)	T (ºC)	Microorganism	log CFU/ml reduction	Quality changes	Reference
Blueberry	bath-type sonicator	40	280-700	10	30-40	*E. coli*	0.2-0.6	No differences in anthocyanin	Zhu et al., 2017
			280-700		50		2.2-2.4		
			280-420		60		3.87-4.1	Anthocyanin reduction (8.4%)	
			560-700		60		4.8-5.1		
Mango	Bath-type sonicator	25	200	10	50	*Salmonella enteritidis*	9	-	Kiang et al., 2012
						E.coli	3		
				7	60	*Salmonella enteritidis*	9		
						E.coli	5		
Apple	Bath-type sonicator	28, 45 and 100	600	30min of 1ms pulses	44.03 a/p	*E. coli*	1	-	Gabriel, 2012
						Salmonella spp	0.9		
						L. monocytogenes	1.6		
Orange	Bath-type sonicator	28, 45 and 100	600	40min of 1ms pulses	55.43 a/p	*E. coli*	4.5	-	Gabriel, 2014
						Salmonella spp	4.6		
						L. monocytogenes	4.2		
Apple	Bath-type sonicator	35	480	90	85	*A. acidoterrestris*	4.8	-	Tremarin et al. 2019
				60	90		4.7		
				20	95		5.5		

Zhu et al. (2017) studied *E. coli* inactivation in 50mL of blueberry juice submitted to TS at different temperatures (30-60ºC) 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 60ºC 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 60ºC 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 55.43ºC 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 20min of TS (480W) at the temperature of 95ºC 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 60ºC for 9min, 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 50ºC, 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

Juice	Sonication system	f (kHz)	Power (W)	Pressure	Time	T (˚C)	Microorganism	log CFU/ml reduction	Quality changes	Reference
Blueberry	bath-type sonicator	40	560	350 MPa	10min	20	*E. coli*	5.2	Anthocyanin reduction (0.6%)	Zhu et al., 2017
						40		5.85		
Apple	Horn system	20	450	200 kPa	7.2min	35	*L. monocytogenes*	4	-	Guzel et al., 2014
					3.6 min		*E. coli*	4		
Apple	Horn system	20	450	200 kPa	0.92min	60	*L. monocytogenes*	4	-	
					1.08 min		*E. coli*	4		
Apple-carrot	Horn system	20	750	300 kPa	15s	60	*E. coli*	4.89	-	Ozan et al. 2017
				100 kPa	30s	60		5		
				100 kPa	60s	50		5		

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 20ºC, and 5.85log reductions at 40ºC. 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 35ºC in apple juice. When temperature increased to 60ºC, 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 (60-50ºC). 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 7. Combined HPU and PL for microbial inactivation: processing conditions

Juice	TS						PULSED LIGHT				Microorganism	log CFU/ml reduction	Reference
Natural apple	horn system	20 kHz	600 W	80 %	44 ˚C	30 min	3 pulses/s	0.1 m distance	71.6 J/cm^2	60 s	*S. cerevisiae*	5.9	Ferrario et al., 2015
											A. acidoterrestris	1.8	
Commercial apple	horn system	20 kHz	600 W	80 %	44 ˚C	30 min	3 pulses/s	0.1 m distance	71.6 J/cm^2	60 s	*S. cerevisiae*	5.9	
											A. acidoterrestris	3.03	
Orange	horn system	24 kHz	400 W	100 %	40 ˚C	2.9 min	360us; 3Hz	0.019 m distance	5J/cm^2	2.81s	*E. coli*	3.9	Muñoz et al., 2011
					50 ˚C	5 min						2.9	
Apple	horn system	24 kHz	400 W	100 %	50 ˚C	5 min	360us; 3Hz	0.019 m distance	4J/cm^2	2.22s	*E. coli*	5.9	Muñoz et al., 2012
									5J/cm^2	2.81s		5.9	

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 50ºC combined with 2.81s of PL at 5J /cm^2). 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. acidoterrestris* 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. acidoterrestris* 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 applicated, the higher inactivation is obtained.

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

Juice	HHP			TS					Microorganism	log CFU/ml reduction	Reference
Orange	200MPa	15min	39ºC	Horn system	24kHz	100%	60 min	78°C	*A. acidoterrestris* spores	2.7	Evelyn and Silva, 2016
	600MPa								*A. acidoterrestris* spores	4.4	

The application of HPU during a treatment with supercritical carbon dioxide (SC-CO_2) 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-CO_2 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

Product	SC-CO2		Sonication			Time (min)	Microorganism	log CFU/ml reduction	Reference
Orange juice	100 bar	36 ºC	horn system	30kHz	40W	1	*E. coli*	2.5	Ortuño et al., 2014
						7		5.8	
	225 bar	36 ºC	horn system	30kHz	40W	1		4.6	
						7		7.2	
	350 bar	36 ºC	horn system	30kHz	40W	1		5.4	
						7		7.9	
	225 bar	31 ºC	horn system	30kHz	40W	7		7.0	
		36 ºC				7		8.0	
		41 ºC				3		8.0	
Orange juice	100 bar	36 ºC	horn system	30kHz	40W	4	*S. cerevisiae*	7.0	Ortuño et al., 2014
	225 bar					1.5		7.0	
	350 bar					2		7.0	
	225 bar	31 ºC	horn system	30kHz	40W	6		4.0	
		36 ºC				3		7.0	
		41 ºC				3		7.0	
Orange juice	100 bar	31 ºC	horn system	-	40W	3.06	*E. coli*	3.4	Paniagua-Martinez et al., 2018
		36 ºC				3.06		3.8	
		41 ºC				3.06		3.8	
	100 bar	31 ºC	horn system	-	40W	3.06	*S. crevisiae*	2.6	
		36 ºC				3.06		2.24	
		41 ºC				3.06		2.19	

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. acidoterrestris* 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 50ºC of 1500W of 26μm amplitude along 17min (Cervantes-Elizarrarás et al., 2017), 1.97% after TS at 50ºC of 580W treatment (Zhu et al., 2017) or 1.91% after TS at 65ºC 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).

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

Juice	HPU system	f (kHz)	Power (W)	T (ºC)	Time (min)	Enzyme	Residual Activity	Quality changes	Reference
Blueberry	bath type sonicator	40	280	20	10	PPO	64%	No significant anthocyanin reduction	Zhu et al., 2017
			420				48.30%		
			560				40.70%		
			700				40.10%		
			560	40	10	PPO	7.23%		
				50	10	PPO	1.97%		
				60	10	PPO	0.03%	Anthocyanin reduction until 8.6%	
			700	60	10	PPO	0.00%		
Blackberry	Horn system	20	1500 28μm	50	17	PPO	1.72%	No changes in pH and total soluble solids. Cloud index increase. Enhanced antioxidant activity, ascorbic acid and total polyphenol content	Cervantes-Elizarrarás et al., 2017
						PME	35.35%		
Pear	Horn system	20	750	25	10	PPO	89.3%	Higher retention of ascorbic acid and phenolic compounds	Saeeduddin et al., 2015
						POD	94.7%		
						PME	92.5%		
		20	750	45	10	PPO	37.83%	Decrease of antioxidant capacity. No significant changes in ºBrix, pH and titratable acidity	
						POD	43.2%		
						PME	40.22%		
		20	750	65	10	PPO	1.91%		
						POD	4.3%		
						PME	3.25%		
Orange	Horn system		80	63	10	PME	9%	-	Koshani et al., 2014
Orange - sweet potato	Horn system	26	200 90%	39	8	PPO	71.62%	Preservation of b-carotene, phenolic compounds, antioxidant activity.	Ríos-Romero et al., 2018
						POD	53.72%		

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 40ºC (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

Juice	T (ºC)	Time	Enzyme	Residual Activity	Reference
Blueberry	50	10min	PPO	59.00%	Zhu et al., 2017
	60			14.20%	
	70			2.67%	
	80			0.07%	
Blackberry	90	15s	PPO	10.25%	Cervantes-Elizarrarás et al., 2017
			PME	85.33%	
Pear	65	10 min	POD	66%	Saeeduddin et al., 2015
			PPO	59%	
			PME	63%	
	95	2 min	POD	0%	
			PPO	0%	
			PME	0%	
Orange	75	22 min	PME	0.14%	Koshani et al., 2014

The comparison between Tables 10 and 11, shows that TS permits a decrease of 20ºC to obtain the same rates of enzyme inactivation (near 2% of residual activity) after 10 min of application of TS at 50ºC at 560W than conventional thermal treatment (10 min at 70ºC) (Zhu et al., 2017). A thermal treatment for 10min at 60ºC 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), 20% of decrease in temperature and 50% in processing time of a thermal treatment at 75ºC 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

Product	Sonication						HHP			Enzyme	Residual Activity	Reference
Apple juice	bath-type sonicator	25kHz	500W	70%	60min	20 ºC	250MPa	10 min	25 ºC	PME	66%	Abid et al., 2014
										PPO	63%	
										POD	71%	
		25kHz	500W	70%	60min	20 ºC	350MPa	10 min	25 ºC	PME	47%	
										PPO	43%	
										POD	52%	
		25kHz	500W	70%	60min	20 ºC	450MPa	10 min	25 ºC	PME	24%	
										PPO	21%	
										POD	32%	

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-CO_2 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

Product	SC-CO2		Sonication			Time	Enzyme	Residual Activity	Reference
Orange juice	100 bar	36 ºC	horn system	30kHz	40W	10min	PME	54.20%	Ortuño et al., 2014
	225 bar							54.20%	
	350 bar					8min		32.38%	
						10min		15.90%	
	225 bar	31 ºC	horn system	30kHz	40W	10min		47.50%	
		36 ºC						47.50%	
		41 ºC						10.65%	

In enzymatic inactivation, the effectiveness of residual activity reduction is linked with the temperature. However, SC-CO_2 assisted by HPU is capable to reduce the most resistant enzyme to the acceptable rate of 10% of its activity, thus, SC-CO_2 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), ºBrix (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 ºBrix, 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.

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