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Review

Thermosonication: An alternative processing for fruit and vegetable juices

Luis M. Anaya-Esparza [a], Rita M. Velázquez-Estrada [a], Artur X. Roig [b], Hugo S. García-Galindo [c], Sonia G. Sayago-Ayerdi [a], Efigenia Montalvo-González [a, *]

[a] Laboratorio de Integral de Investigación de Alimentos, Instituto Tecnológico de Tepic, Av. Tecnológico 2595, Lagos del Country, C.P. 63175, Nayarit, Mexico
[b] CERPTA-Animal and Food Science Department, Universitat Autònoma de Barcelona, Travessera dels Turons s/n, 08193, Bellaterra, Barcelona, Spain
[c] UNIDA, Instituto Tecnológico de Veracruz, M.A. de Quevedo 2779, Col. Formando Hogar, C.P. 91897, Veracruz, Mexico

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ABSTRACT

Background: Alternative methods of pasteurization have gained relevance in the food industry. Nowadays there are new technologies that offer options for food processing to ensure the stability and quality of products. Particularly in processed fruit and vegetable juices, consumers search for additive-free and minimally processed products.

Scope and approach: Thermosonication (TS) is a novel and viable technique that is employed to replace the conventional thermal processing. It can increase the microbial and enzymatic inactivation rates, extend product shelf life and reduce the impact on the nutritional content and overall quality of fruit and vegetable juices. This article reviews the advantages and limitations offered by the application of TS on fruit and vegetable juices. Additionally, its effects on bioactive compounds, physicochemical, microbiological, enzymatic and sensory parameters in fruit and vegetable juices are also discussed.

Key findings and conclusions: Scientific evidence shows that TS is a viable technology for processing of fruit and vegetable juices that preserve the quality of fruit and vegetable juices, compared with conventional thermal processing (60 °C for 30 min). An additive effect between ultrasound and heat has the potential to ensure product stability and also is effective for inactivation of enzymes present in juices. This technology represents a rapid, efficient and reliable alternative to retain the quality and extend the shelf life of fruit and vegetable juices.

1. Introduction

Beverages, in particular fruit and vegetable juices have been employed as vehicles to deliver high concentrations of bioactive compounds, because juices are a convenient way for their consumption (Carrillo, Fiszman, Lähteenmäki, & Varela, 2014). However, and because of the rapid deterioration by microbial growth and enzymatic activities on these products it is necessary to apply treatments that ensure their stability and sensory quality (Dias et al., 2015). Thermal pasteurization is the most common heat treatment applied for food preservation to date. It assures good shelf-life and stability of fruit and vegetable juices; however, this process may affect the juices quality in terms of nutritional and physicochemical parameters such as: vitamins (C and E), carotenoids, polyphenols, organic acids, pH and color (Dubrovic, Herceg, Rezek, Badanjak, & Dragovic-Uzelac, 2011; Giner, Hizarci, Martí, Saura, & Valero, 2013; Santhirasegaram, Razali, & Somasundram, 2013).

Novel food technologies are described as complete or partial alternatives to thermal processing, among which ultrasound applications stand out, which have a proven potential for use in the food industry, especially in the beverage industry (Knorr et al., 2011). Several researchers have studied the application of ultrasound at atmospheric pressure and controlled temperatures on different food products and culture media, and it has proved to be effective against food-borne pathogens (Adekunte et al., 2010a; Ferrante, Guerrero, & Alzamora, 2007; Sagong et al., 2011; Salleh-Mack & Roberts, 2007; Ugarte-Romero, Feng, & Martin, 2007) and spoilage flora (Adekunte, Tiwari, Cullen, Scannell, & O'Donnell, 2010b; Santhirasegaram et al., 2013; Ertugay & Baslar, 2014). However, some authors have suggested that ultrasound at a

* Corresponding author.
E-mail address: efimontalvo@gmail.com (E. Montalvo-González).

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controlled temperature (25 °C) in some cases may not be very efficient for inactivation of some types of microorganisms (Gabriel, 2012) and enzymes on fruit juices (Dias et al., 2015; Tiwari, Muthukumarappan, O'Donnell, & Cullen, 2009a). Therefore, these authors considered that ultrasound is often more effective when combined with moderate heat and is known as thermosonication (TS). This combined treatment increases enzymatic and microbial inactivation by combined heat and cavitation; which produces an effect on bacterial membrane and depolymerization of macromolecules, without effecting changes on juice quality (Feng, Yang, & Hielscher, 2008; Herceg et al., 2013a; Muñoz et al., 2012). Recently, Sánchez-Rubio, Taboada-Rodríguez, Cava-Roda, López-Gómez, and Marín-Iniesta (2016) combined TS (24 kHz; 105 μm; 33.31 W mL^{-1}, 30 min, 50 °C) and cinnamon leaf essential oil (0.02 mg mL^{-1}) to inactivate *Saccharomyces cerevisiae* in natural orange and pomegranate juices; obtaining a reduction near to 3 log cycles. In this context, TS can be applied in combination whit other barrier technologies, as was mentioned by Ferrante et al. (2007) who applied TS in combination with antimicrobials (vanillin) to control *Listeria monocytogenes* growth in orange juice.

This review describes the advantages and limitations that offer the application of TS in processing of fruit and vegetable juices. It includes an overview of the effects of this treatment on the bioactive compounds and sensory attributes of juices, as well as on the inactivation of enzymes and microorganisms responsible for spoilage and the corresponding reduction in their shelf-life.

2. Physical principles of sonication and thermosonication

Ultrasound is generated by an electric current with a frequency of 20 kHz or more, which is converted to sound energy through a transducer. The most widely used are the piezoelectric transducers (McClements & Gunasekaran, 1997). The generated sound propagates through food materials (especially in liquid media) causing a complex phenomenon known as “cavitation” (Chandrapala, Oliver, Kentish, & Ashokkumar, 2012; Lee, Zhou, Liang, Feng, & Martin, 2009). The combination of cavitation with heat produces an important effect on heat-shocked cell structure that increases the lethal range for bacterial and enzyme inactivation (Cruz, Vieira, Fonseca, & Silva, 2011; Muñoz et al., 2012). When both heat and sound waves act simultaneously, high sensitivity on microbial cell wall occurs, causing damage in the cell structure (Kiang, Bhat, Rosma, & Cheng, 2013), as a consequence of the called “additive effect” (Huang, Mittal, & Griffiths, 2006). However, to ensure the effectiveness of TS treatment, it is necessary to consider variables such as: viscosity, suspended solids content, pH in the medium, but also the acoustic energy density (frequency, amplitude) temperature and time of the treatment, among others (Guerrero, López-Malo, & Alzamora, 2001; McClements & Gunasekaran, 1997). Nonetheless, sometimes the additive effect between ultrasound and heat cannot occur as a consequence of high temperatures. Normally, it occurs when temperature exceeds 55 °C. This is probably the result of an increase of temperature, hampering the formation of large bubbles during cavitation (Guerrero et al., 2001; Mason, 1990; Sala, Burgos, Condón & Raso, 1995).

TS according to the temperature range can be classified into sublethal (<45 °C) and lethal (>45 °C) (Ugarte-Romero et al., 2007; Tiwari, O'Donnell, Patras, Brunton, & Cullen, 2009b). It is considered an excellent substitute to thermal treatment (Aadil et al., 2015) and can be applied for discontinuous (most common) or for continuous processes, according to the configuration of the ultrasound equipment (Fig. 1). Discontinuous TS (Fig. 1A and B) consists of a sonicator probe and an external circulator water bath, or by immersion into an ultrasonic bath; while the continuous TS (Fig. 1C) consists of a flow-cell which contains the sonicator probe with control temperature (Chemat, Zill-e-Huma, & Khan, 2011); that is intended to reduce the temperature and/or time during food and beverages processing (Alzamora, Guerrero, Schenk, Raffellini, & López-malo, 2011).

Temperature plays an important role in food preservation; however, an increase of temperature might affect juice quality. Some authors have reported a considerable increase in temperature of juices when ultrasound was applied at room temperature using a re-circulating water bath for temperature control. In addition, it has been reported a temperature increase up to 55 °C when ultrasound was applied to orange (Ferrante et al., 2007; Valero et al., 2007), pineapple (Costa et al., 2013), Cantaloupe melon (Fonteles et al., 2012), purple cactus pear (Zafra-Rojas et al., 2013) and soursop (Dias et al., 2015) juices. According to most authors, this temperature increase was attributable to the ultrasonic intensity and processing time used during the treatment. Heat transfer from cavitational bubbles caused gradual temperature increases in the medium and the effect of cavitation by increasing medium temperature, can be minimized (Li, Pordesimo, & Weiss, 2004). High temperature is one of the most important variables that affect bioactive compounds or enzymatic and microbial inactivation (Abid et al., 2014; Herceg, Lelas, Jambrak, Vukušić, & Levaj, 2013b; Rawson et al., 2011; Wu, Gamage, Vilkhu, Simons, & Mawson, 2008). For these reasons, it is necessary that this technology should always be applied with temperature control to preserve the cavitation effects.

3. Effects of thermosonication on spoilage and pathogenic microorganisms

According to the Food and Drug Administration (FDA, 2004) in its “Guidance for Industry: Juice, Hazard Analysis and Critical Control Points (HACCP)” the purpose of any technology is to ensure a stable product and comply with the provisions of regulatory agencies to reduce at least five log cycles of the target bacteria (e.g. *Escherichia coli* and *Listeria monocytogenes*). Because of this, some spoilage and pathogenic microorganisms have been identified as being of special concern for fruit juice producers and academia, especially in the fruit juice industry. In this context, several research groups have studied the effect of TS on inactivation rates of microorganisms in juices as is shown in Table 1. Some of the microorganisms studied include: *Saccaromyces cerevisiae* (Char, Mitilinaki, Guerrero, & Alzamora, 2010; Marx, Moody, & Bermúdez-Aguirre, 2011; Bermúdez-Aguirre & Barbosa-Cánovas, 2012; Wordon, Mortimer, & Mcmaster, 2012; Ferrario, Alzamora, & Guerrero, 2015), *Escherichia coli* O157H:7 and *Salmonella enteritidis* (Char et al., 2010; Kiang et al., 2013; Muñoz et al., 2011, 2012; Sagong et al., 2011; Ugarte-Romero, Feng, Martin, Cadwallader, & Robinson, 2006), *Cronobacter sakazakii* (Adekunte et al., 2010a; Arroyo, Cebrián, Pagán, & Condón, 2012), *Alicyclobacillus acidophilus* and *Alicyclobacillus acidoterrestris* (Evelyn & Silva, 2016; Ferrario et al., 2015; Wang, Hu, & Wang, 2010), *Staphylococcus aureus* (Walkling-Ribeiro et al., 2009b) and *Neosartorya fischeri* (Evelyn, Kim, & Silva, 2016) among others. Additionally, some of these microorganisms are able to produce spores. Spores can survive the pasteurization process and exhibit high thermal resistance compared with major spoilage microbes in vegetative cell forms (Evelyn & Silva, 2016).

There are many theories about the possible mechanisms controlled for microorganism inactivation involved during TS processing, but most agree that the cavitation phenomenon is directly responsible for this. There are two types of cavitation, transient and stable. Transient cavitation entails mechanical effects such as localized high pressure and high temperatures, while stable cavitation produces micro-streaming creating shear stress caused by

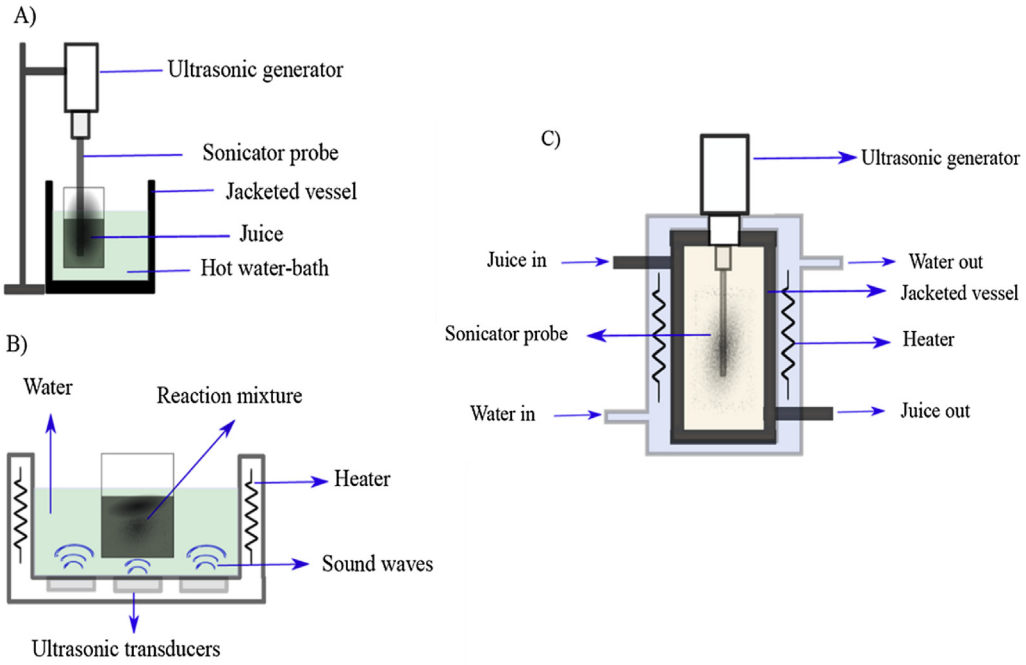

Fig. 1. Batch (A and B) and continuous (C) flow through thermosonication systems.

constant implosion and explosion of bubbles surrounding the cells (Feng et al., 2008). Some authors have called this event as a "stochastic process" (Chapman, Ferguson, Consalo, & Bliss, 2013). In addition to this, during transient cavitation free radicals are produced; these species have important bactericidal properties because the primary target site of free radicals in the cell is the DNA (Earnshaw, Appleyard, & Hurst, 1995). Kinsloe, Ackerman, and Reid (1954) reported a collapse of cells and loss of some pieces of cell wall on *Saccharomyces cerevisae* when TS was applied. Similar findings by Marx et al. (2011) were reported when they applied TS (24 kHz, 60 °C and 30 min) on *Saccharomyces cerevisiae* (ATCC 4113) and reported a lethal injury on the yeasts. According to Ciccolini, Taillandier, Wilhem, Delmas, and Strehaiano (1997) there are a number of lethal injuries for the disruptive action of TS on microbial cells. Cameron, McMaster, and Britz (2008) reported both internal and external damage on different microbial cells after TS. In a similar way, Lee et al. (2009) described damage on intracellular constituents on *Escherichia coli* by TS. Also, cytoplasmic material from the cell (*Listeria innocua*) to the medium was observed by Bermúdez-Aguirre, Mawson, and Barbosa-Cánovas (2011); these results agreed with Gao, Lewis, Ashokkumar, and Hemar (2014). Evidence shows that TS is effective to achieve microbial inactivation beyond five log cycles of vegetative microbial cells.

There is little information about spore inactivation by TS in fruit and vegetable juices. Ferrario et al. (2015) reported a significant reduction of *Saccharomyces cerevisiae* (6.4 log cycles) and *Alicyclobacillus acidoterrestris* (3.0 log cycles) after TS treatment (20 kHz; 95.2 μm; 30 min; 44 °C) in commercial apple juice. Evelyn et al. (2016) observed that temperature plays an important role in the inactivation of *Neosartorya fischeri* ascospores in apple juice during TS treatment (24 kHz, 210 μm, 0.33 W mL^{-1}; 75 °C, 30 min). According to these authors, the spores become more sensitive to TS than heat alone. Similar trends by Evelyn and Silva (2015a) were reported during inactivation of *Byssochlamys nivea* ascospores in strawberry puree by TS. In another study, Evelyn and Silva (2016) using high pressure processing (600 MPa) (HPP) as pretreatment prior to application of TS (24 kHz; 100%; 20 W mL^{-1}; 42 min; 78 °C) in orange juice, reported that combination of these technologies may be used to inactivate spores in orange juice. They observed that an increase of acoustic energy density (AED) (20 W mL^{-1}) during TS treatment, producing a 6-fold reduction at the same temperature than thermal process and decrease the time of processing. Likewise, research works have been conducted and demonstrated that the effectiveness of TS for spore inactivation can vary with different media and experimental conditions such as: Lopez-Malo, Palou, Jiménez-Fernández, Alzamora, and Guerrero (2005) on *Aspergillus flavus* ascospores in sabouraud broth (TS: 20 kHz, 120 μm, 0.5 min, 57.5 °C); Evelyn and Silva (2015b) on psychrotrophic *Bacillus cereus* spores in skim milk and beef slurry (TS: 24 kHz, 210 μm, 0.33 WmL^{-1}, 20 min, 70 °C); Milani, Ramsey, and Silva (2016) on *Saccharomyces cerevisiae* ascospores in beer (TS: 24 kHz, 125 μm, 5 min, 60 °C); Beatty and Walsh (2016) on *Geobacillus stearothermophilus* in skim milk (TS: 20 kHz, 240 μm, 30 s, 45 °C) and Campaniello, Bevilacqua, Sinigaglia, Rosaria (2016) on *Penicillium* spp., and *Mucor* spp. in distilled water (TS: 20 kHz, 130 W, 12 min, 60 °C).

According to Herceg et al. (2013a), Wordon et al. (2012) and Evelyn and Silva (2016) the bacterial cells and spores generally become more sensitive after thermosonication as a result of energy absorption by membranes and biomaterials. This causes weakening and/or disruption of the cell membrane through the combined effects of heat and ultrasound waves. The development of pores and modifications on microbial cell membranes (*Saccharomyces cerevisiae*, *Escherichia coli* and *Listeria innocua*) during TS were reported by Marx et al. (2011), Guerrero et al. (2001) and Bermúdez-Aguirre et al. (2011), caused by the combination of stress factors (temperature, pH, micro streaming and mechanical damage and its cumulative effects during TS (Kinsloe et al., 1954). The modification, erosion and perforation of cell membranes expose the intracellular content to the environment and generate a lethal effect (Cameron et al., 2008; Lee et al., 2009; Mackey, Miles, Parsons, & Seymour, 1991; Tsong, 1991). Additionally, disruption of cell membranes can favor the intake of antimicrobial agents into the cell, thereby reducing the viability of microorganisms (Lopez-Malo et al., 2005).

Table 1
Effect of thermosonication on spoilage and pathogenic microorganisms in different fruit juices.

Type of juice	Microorganism	Form of the microorganism	Ultrasonic equipment	Experimental conditions[a]	Initial concentration (log CFU mL^{-1})	Microbial reduction (log cycles)	Reference
Orange Pomegranate	*Saccharomyces cerevisiae*	Vegetative cells	Probe of 3 mm diameter at 200 W[b]	24 kHz, 105 μm, 33.31 W mL^{-1}, 30 min, 50 °C, 0.02 mg mL^{-1} CLEO	5.13	2.81 2.52	Sánchez-Rubio et al. (2016)
Orange	*Alicyclobacillus acidoterrestris*	Spores	Probe of 3 mm diameter at 200 W[b]	Pretreatment of 600 MPa, 15 min, 39 °C; 24 kHz; 100%; 20.20 WmL^{-1}; 60 min, 78 °C	7	4.4	Evelyn and Silva (2016)
Apple	*Neosartorya fischeri*	Ascospores	Probe of 3 mm diameter at 200 W[b]	24 kHz, 210 μm, 0.33 W mL^{-1}; 75 °C, 30 min	7	1.6	Evelyn et al. (2016)
Sugar cane	*E. coli* ATCC 25922 *B. cereus* F4810	Vegetative cells	Probe of 10 mm diameter at 750 W[b]	20 kHz, 120 μm, 10 min, 50 °C	6	5.0 2.5	Garud, Priyanka, Negi, and Rastogi (2016)
Commercial apple juice	*Saccharomyces cerevisiae* KE162 *Alicyclobacillus acidoterrestris* ATCC 49025	Spore Spore	Probe of 22 mm diameter at 600 W[b]	20 kHz; 95.2 μm; 30 min; 44 °C	8–9	6.4 3.0	Ferrario et al. (2015)
Natural apple juice	*Saccharomyces cerevisiae* KE162 *Alicyclobacillus acidoterrestris* ATCC 49025	Spore Spore	Probe of 22 mm diameter at 600 W[b]	20 kHz; 95.2 μm; 30 min; 44 °C	8–9	5.8 2.0	Ferrario et al. (2015)
Mango	*Escherichia coli O157H:7* *Salmonella enteritidis*	Vegetative cells	Ultrasound water bath at 200 W	25 kHz; 7 min; 60 °C	9	5 9	Kiang et al. (2013)
Apple	*Chronobacter sakazakii* ATCC 29544	Vegetative cells	Probe of 13 mm diameter at 450 W[b]	20 kHz; 117 μm; 5 W mL^{-1}; 0.28 min; 56 °C	9.7	1.01	Arroyo et al. (2012)
Pineapple, grape and cranberry	*Saccharomyces cerevisiae* ATCC 4113	Vegetative cells	Probe of 22 mm diameter at 400 W[b]	24 kHz; 117 μm; 6 min; 60 °C	8	5	Bermúdez-Aguirre and Barbosa-Cánovas (2012)
Apple	*Escherichia coli*	Vegetative cells	Probe of 22 mm diameter at 400 W[b]	24 kHz; 100 μm; 2.9 min; 50 °C	8	4.9	Muñoz et al. (2012)
Apple	*Saccharomyces cerevisiae* ATCC 4113	Vegetative cells	Probe of 22 mm diameter at 400 W[b]	24 kHz; 120 μm; 30 min; 60 °C	7	7	Marx et al. (2011)
Orange	*Escherichia coli* K12	Vegetative cells	Probe of 22 mm diameter at 400 W[b]	24 kHz; 100 μm; 5 min; 50 °C	8	3.37	Muñoz et al. (2011)
Apple	*Alicyclobacillus acidiphilus* DSM14558T *Alicyclobacillus acidoterrestris* DSM3922T	Vegetative cells	Probe of 6 mm diameter at 600 W[b]	25 kHz; 1.2 W mL^{-1}; 30 min; 50 °C	Range from 5 to 5.72	4.56	Wang et al. (2010)
Orange	*Staphylococcus aureus*	Vegetative cells	Ultrasound water bath at 100 W	30 kHz; 100 W; 30 min; 55 °C	12	5.5	Walkling-Ribeiro et al. (2009b)

[a] Optimal experimental condition: Frequency (kHz); Amplitude (μm or %); Processing time (min); Final temperature (°C); Ultrasonic intensity (W); Acoustic Energy Density (W mL^{-1}); Megapascals (MPa); Cinnamon leaf essential oil (CLEO).
[b] Ultrasonic equipment coupled on circulating water bath.

Upon membrane disruption, antimicrobials absorption may occur, together with the release of intracellular components such as proteins, lipids and other compounds (Liu, Zeng, Sun, & Han, 2013; Wu et al., 2015; Zhang, Jin, Xie, Wu, & Wu, 2014).

Some microorganisms may be more susceptible than others to TS, or that the size and shape of the microorganism can affect treatment efficiency; this is probably caused by increased surface area (Yusaf, 2014). Scherba, Weigel, and Brien (1991) proposed that these morphological features were not significantly different in the numbers of microorganisms killed by ultrasonic energy; however, spores are more difficult to inactivate than vegetative cells, which can be in several stages of growth (Bevilacqua, Sinigaglia, & Corbo, 2013). Cameron et al. (2008) mentioned that the peptidoglycan layer in the cell wall might provide more resistance to TS of gram-positive than gram-negative bacteria; making a rigid envelope of the cell, meanwhile that membranes of gram-negative bacteria are exposed to the surrounding environment (Herceg, Jambrak, Lelas, & Thagard, 2012). Also, low pH values can affect membrane permeability and reduce cell resistance to TS (Coronel, Jiménez, López-Malo, & Palou, 2011; Kiang et al., 2013; Marx et al., 2011). The effects of pH on inactivation rates were negligible at sub-lethal temperatures, but were significant at lethal temperatures in combination with ultrasound (Alzamora et al., 2011; Guerrero et al., 2001; Salleh-Mack and Roberts (2007)).

It was shown that TS is a viable option for inactivation of microorganisms and differences in cell sensitivity are evident; but there are other variables that could be crucial to consider, such as: sonotrode design, frequency and acoustic energy density,

characteristics and complexity of the medium (viscosity, food matrix composition), the type of interactions and chemical reactions that occur with other molecules (Chemat et al., 2011; Feng et al., 2008; McClements & Gunasekaran, 1997) and high temperatures may produce an attenuated cavitation effect (Ugarte-Romero et al., 2007). Another variable that can affect the efficiency of TS on microorganism inactivation is the initial bacterial numbers; with populations up to 12 log CFU, it is possible that during ultrasonic treatment not all bacterial cells may be affected (Walkling-Ribeiro et al., 2009b). In this context, Wordon et al. (2012) reported a temporary increase (14%) in total cell population of *Saccharomyces cerevisiae* after the first minute of sonication, caused by a breakage of filaments of the microbial cells, which increased the total counts. Phase stage of the inoculum and stress adaptation mechanism can also affect, because bacterial cells are more sensitive during the exponential growth phase than on the stationary phase, providing that microorganisms can manifest distinct characteristics, behaviors, and responses to environmental conditions (Gao et al., 2014; Mañas & Pagán, 2005).

In order to use TS as a potential substitute for pasteurization in fruit and vegetable juice production, it is necessary to consider the sub-lethal injury caused in microbial cells. Sub-lethal injury is related to the higher sensitivity of survivors to stress conditions after any treatment. The success of a combined treatment should be correlated with the degree of sub-lethal injury caused by the hurdles in the microbial population (García et al., 2005). A basic theory in microbiology establishes that when a cell is surrounded by aggressive environmental conditions, the cell will try to survive by natural mechanisms, but if the adverse conditions are extended, their metabolism is exhausted with the subsequent cellular death (Leistner, 2000). However, under suitable conditions, injured cells might repair themselves during storage, potentially compromising product quality or stability (Chapman et al., 2013). There are few reports about sub-lethal injury by TS on food-borne pathogens on juices. Kiang et al. (2013) studied the thermosonicated mango juice at 50 °C (10 min) and 60 °C (5 min), and reported 99.95% and 99.99% of sub-lethal injury for *Escherichia coli* and *Salmonella enteritidis* respectively. Also the evidence showed that TS could increase the microbial inactivation rate, caused sub-lethal injury and ensure the stability of fruit juice (Halpin, Duffy, Cregenzán-alberti, Lyng, & Noci, 2014). Therefore, it can be concluded that TS may cause sub-lethal injury.

4. Effect of thermosonication on endogenous enzymes

Endogenous enzymes as pectinmethylesterase (PME), polyphenoloxidase (PPO), peroxidases (POD) and lipoxygenase (LOX) in fruits are released during processing of juices, and their presence may affect the quality of these products in different ways as loss of viscosity, development of off-flavors and browning pigments (O'Donnell, Tiwari, Bourke, & Cullen, 2010). Some authors proposed that ultrasound alone applied at temperatures <50 °C is not effective for inactivation against several of these food enzymes because they are heat-resistant (Butz & Tauscher, 2002; Dias et al., 2015; Tiwari et al., 2009a).

Table 2 shows some effects of TS on enzyme inactivation in fruit and vegetable juices. It is clear that sensitivity to treatments can vary between enzymes. TS has shown better and significant results in comparison to thermal treatment or ultrasound alone on PME inactivation in juice (Jabbar et al., 2015; Siwach & Kumar, 2012). Additionally, Terefe et al. (2009) mentioned that inactivation of PME and polygalacturonase (PG) by TS showed an apparent first-order kinetics. Wu et al. (2008) indicated that free radicals generated by sonolysis did not play a major role, because the inactivation range increased with temperature, while free radical production decreased, thus the heat and mechanical damage lead to enzyme inactivation. Inactivation of PME in thermosonicated samples may contribute to maintain the degree of methoxylation in pectin at its original state, avoiding losses of consistency and syneresis in juices (Aadil et al., 2015). Optimization of PME inactivation with TS is a novel and promising method to cut down the required temperature and time, in comparison with thermal pasteurization (Koshani, Ziaee, Niakousari, & Golmakani, 2014).

PPO and POD are usually inactivated by thermal treatments, which demand a large amount of energy and may affect quality losses (O'Donnell et al., 2010). Abid et al. (2014) reported that the combination of ultrasonication (20 kHz frequency and 525 W power) and heat (60 °C during 10 min) achieved the inactivation of these enzymes at rates of 93.85% and 91%, respectively compared with the control samples in apple juice. Similar results by Jabbar et al. (2015) in carrot juice (20 kHz, 525 W, 60 °C, 10 min) for both enzymes were found. Also, Sulaiman, Soo, Farid, and Silva (2015) mentioned that inactivation of PPO in pear, apple and strawberry purees, followed first-order kinetics during TS (24 kHz and 1.3 W g^{-1} of AED and 10 min). Baltacıoğlu, Bayindirly, & Severcan (2017) investigated the effect of TS (24 kHz; 210 μm; 30 min; 60 °C) on the structure of mushroom PPO by FTIR spectroscopy; they reported that PPO inactivation was manly caused by a global conformation change of the enzyme structure and not by a simple change in the active site. Compared with conventional thermal inactivation and ultrasound treatment alone, the time required to decrease the initial PPO activity by 90% was shorter for TS (Dias et al., 2015; Fonteles et al., 2012).

The inactivation of enzymes is attributed to a synergistic effect between heat and mechanical damage by TS causing protein denaturation by depolymerization and change in conformation of their tertiary structure, which is related to the reduction of specific enzymatic activity (Aadil et al., 2015). Micro-streaming that occurs by sonication can disrupt Van der Waals interactions and hydrogen bonds in the polypeptide chains (Feng et al., 2008). Inactivation mechanisms are specific to each enzyme and depend on their amino acid composition and their conformational structure (Islam, Zhang, & Adhikari, 2014). Also, the presence of free radicals produced during sonolysis of water molecules can attack specific sites such as disulfide bonds, that can destabilize enzyme conformation; or these radicals can oxidize amino acid residues such as tryptophan, tyrosine, histidine and cysteine, that are involved in the catalytic activity and stability of several enzymes (Cheng, Zhang, & Adhikari, 2013; Terefe et al., 2009).

Different intrinsic and extrinsic control parameters are responsible for the efficiency of TS on enzyme inactivation: the type of sonotrode, its geometry, frequency used, acoustic energy density, treatment volume, pH, enzyme concentration, the presence of enzymatic activity inhibitors and concentration of dissolved gas. In addition, the presence of microorganisms also can affect the effectiveness of TS (Adekunte et al., 2010b; O'Donnell et al., 2010; Tiwari, Muthukumarappan, O'Donnell, & Cullen, 2008). Under heat stress, microorganisms accumulate several low molecular weight organic compounds (osmolytes), which are capable of increasing the thermal stability of proteins, and protect enzymes against inactivation by TS (Earnshaw et al., 1995). Also, it is necessary to consider that enzymes can display different response to TS treatment as mentioned by Rojas, Hellmeister Trevilin, Augusto, and Esteves (2016). It should be noted that the difference in reported data is mainly attributed to the fruit variety, their degree of ripening, heating techniques, extraction, preparation and assay method used to quantify enzyme activity (Table 2).

TS was proposed by different authors as an alternative to blanching for enzyme inactivation in fruits, vegetables (Cruz, Vieira, & Silva, 2008, Cruz, Vieira, & Silva, 2006, Cruz, Vieira, & Silva, 2007;

Table 2
Effect of thermosonication on enzymatic inactivation in fruit and vegetable juices.

Type of juice	Enzyme	Ultrasonic equipment	[a]Experimental conditions	Results	Reference
Purple cactus pear	PME	Probe of 13 mm diameter at 1500 W[b]	20 kHz; 80%; 25 min, 50 °C	Decrease in PME activity	Cruz-Cansino et al. (2015)
Carrot	POD PPO PME LOX	Probe of 13 mm diameter at 750 W[b]	20 kHz; 2.1 W mL^{-1}; 10 min; 60 °C	Inactivation more than 90% in all enzymes	Jabbar et al. (2015)
Grape	PME PPO POD	Ultrasound water bath at 420 W	28 kHz; 294 W; 60 min; 60 °C	Inactivation of PME, PPO and POD were 91%, 90% and 89%, respectively	Aadil et al. (2015)
Orange	PME	Probe of 3 mm diameter at 200 W[b]	24 kHz; 80 W; 9.8 min; 63 °C	Enzyme inactivation of 91%	Koshani et al. (2014)
Apple	PPO POD PME	Probe of 13 mm diameter at 750 W[b]	20 kHz; 0.3 W mL^{-1}; 10 min; 60 °C	Inactivation of PPO, POD and PME were 93.85%, 91% and 92.9%, respectively	Abid et al. (2014)
Mosambi	PME	Ultrasound water bath at 400 W	50 kHz; 400 W; 20 min; 80 °C	Enzyme inactivation of 96.8%	Siwach and Kumar (2012)
Orange	PME	Ultrasound water bath at 100 W	30 kHz; 100 W, 20 min; 55 °C	Enzyme Inactivation of 96%	Walkling-Ribeiro et al. (2009b)
Tomato	PME PG	Probe of 10 mm diameter at 200 W[b]	20 kHz; 75 μm; 40 W; 4 min; 75 °C	Almost complete inactivation of PME, and about 72% inactivation of PG	Terefe et al. (2009)
Tomato	PME	Probe of 22 mm diameter at 400 W[b]	24 kHz; 75 μm; 400 W; 8 min; 65 °C	Enzyme Inactivation of 98.9%	Wu et al. (2008)

[a] Optimal experimental condition: Frequency (kHz); Amplitude (μm or %); Processing time (min); Final temperature (°C); Ultrasonic intensity (W); Acoustic Energy Density (W mL^{-1}); PME = Pectinmethylesterase; POD = Peroxidase; PPO = Polyphenoloxidase; PG = Polygalacturonase; LOX: Lipoxigenase.
[b] Ultrasonic equipment coupled on circulating water bath.

Alexandre, Santos-Pedro, Brandao, & Silva, 2011; Cruz et al., 2011; Gamboa-Santos, Montilla, Soria, & Villamiel, 2012; Gamboa-Santos et al., 2013) and mushrooms (Cheng et al., 2013); these authors reported a minor impact on sensory attributes than the ones produced by heat blanching and suggested that TS could have better feasibility than heat for blanching in industry.

5. Effect of thermosonication on quality parameters of fruit and vegetable juices

The presence of bioactive compounds such as ascorbic acid, carotenoids and phenolic compounds in fruit and vegetable juices define the nutritional value of these products. Thus, the effect of the TS treatments on the above compounds is of great importance. The impact of TS on bioactive compounds of fruit and vegetable juices is presented below.

5.1. Ascorbic acid

Retention of ascorbic acid (AA) in juices has been employed as an indicator of quality. It is assumed that their shelf life ends when the original concentration of AA decreases by 50% (Abid et al., 2014). Some authors have applied TS on juices from different fruits and vegetables (Table 3) at different treatment conditions to evaluate its effect on AA.

The AA losses in juices were less than 15% when TS was employed in comparison with the traditional thermal processes, in which losses were up to 90% (Ordóñez-Santos & Vázquez-Riascos, 2010). Martínez-Flores, Garnica-Romo, Bermúdez-Aguirre, Pokhrel, and Barbosa-Cánovas (2015) reported the complete stability of AA in carrot juice during 20 days of storage at 4 °C when 24 kHz, 120 μm, 2 min and 58 °C were applied. Cavitation was directly responsible because of the extreme physical conditions, which occur within the bubbles during the cavitational collapse at microscale. Conversely, Cheng, Soh, Liew, and Teh (2007) mentioned that heat and cavitation helped to eliminate dissolved oxygen from the medium, thus it delayed AA degradation.

Production of hydroxyl radicals by water sonolysis may be involved in AA degradation; interactions between these free radicals and ascorbic acid may occur at the gas–liquid interface causing oxidation (Adekunte et al., 2010b; Martínez-Flores et al., 2015; Rawson et al., 2011; Valdramidis, Cullen, Tiwari, & O'Donnell, 2010). Under TS AA degradation followed first order kinetics and was dependent on the amplitude, acoustic energy density, temperature and exposure time (Abid et al., 2014; Cruz et al., 2008; Gómez-López, Orsolani, Martínez-Yépez, & Tapia, 2010). In some cases, degradation occurred because AA provides a protective effect on other compounds such as carotenoids, phenolic compounds or anthocyanins, or by the presence of oxygen, causing AA degradation in juices (Tiwari et al., 2009b.

5.2. Carotenoids and polyphenols

Table 4 shows reports of optimal conditions for fruit juice processing using TS, in which the highest retention of compounds of interest was monitored when TS was applied. Nafar, Emam-Djomeh, Yousefi, and Hashemi (2013) found that the importance of independent variables on the effect on bioactive compounds could be ranked in the following order: ultrasound frequency > temperature > exposure time.

The increase in total phenolic compounds and carotenoids by TS might be caused by mechanical disruption of the cell wall and ultimate release of these compounds. Ultrasound is well known for extracting some components caused by its disrupting effect on cell walls (Costa et al., 2013; Lieu & Le, 2010). The disruption mechanism is dependent on the structure of the cell walls and the lignin content because phenolic compounds can form links with various compounds of cell walls such as polysaccharides or proteins (Bychkov, Ryabchikova, Korolev, & Lomovsky, 2012). Other authors proposed that such increase was caused by a response of plant cells to stress suffered during cavitation or microbial invasion, which enlarged their antioxidant capacity by increasing the synthesis of these compounds (Hsieh & Ko, 2008; Martínez-Flores et al., 2015).

Meanwhile, some authors found that temperature, amplitude level and processing time could influence the loss of phenols, flavonoids, flavonols, anthocyanins and lycopene (Rawson et al., 2011;

Table 3
Effect of thermosonication on vitamin C content in different fruit and vegetable juices.

Type of juice	Ultrasonic equipment	Experimental conditions[a]	Results	Reference
Purple cactus pear	Probe of 13 mm diameter at 1500 W[b]	20 kHz; 1200 W; 25 min; 50 °C	Significant Increase compared to control	Cruz-Cansino et al. (2015)
Carrot	Probe of 13 mm diameter at 750 W[b]	20 kHz; 525 W; 10 min; 60 °C	Retention of 90% approximately	Jabbar et al. (2015)
Carrot	Probe of 22 mm diameter at 400 W[b]	24 kHz; 120 μm; 10 min; 58 °C	Retention of 100% after 20 days of storage	Martínez-Flores et al. (2015)
Apple	Probe of 13 mm diameter at 750 W[b]	20 kHz; 0.30 W mL^{-1}; 5 min; 40 °C	Retention about 96%	Abid et al. (2014)
Jamun	Probe unspecified features[b]	20 kHz; 80%; 5 min; 80 °C	Retention about 96%	Shaheer et al. (2014)
Watermelon	Probe of 19 mm diameter at 1500 W[b]	20 kHz; 30 μm; 5 min; 30 °C	Retention about 94%	Rawson et al. (2011)
Tomato	Probe of 19 mm diameter at 1500 W[b]	20 kHz; 61 μm; 10 min; 45 °C	Retention about 68% compared with control	Adekunte et al. (2010b)
Orange	Probe of 19 mm diameter at 1500 W[b]	20 kHz; 61 μm; 10 min; 30 °C	Reduction less than 15%	Valdramidis et al. (2010)
Orange	Ultrasound water bath 100 W	30 kHz; 100 W; 20 min; 55 °C	Retention about 98%	Walkling-Ribeiro et al. (2009b)
Strawberry	Probe of 19 mm diameter at 1500 W[b]	20 kHz; 0.81 W mL^{-1}; 10 min; 40 °C	Reduction less than 15%	Tiwari et al. (2009b)

[a] Optimal experimental condition: Frequency (kHz); Amplitude (μm or %); Processing time (min); Final temperature (°C); Ultrasonic intensity (W); Acoustic Energy Density (W mL^{-1}).
[b] Ultrasonic equipment coupled on circulating water bath.

Table 4
Effect of thermosonication on carotenoids and polyphenols content in different fruit and vegetable juices.

Type of juice	Bioactive compounds	Ultrasonic equipment	Experimental conditions[a]	Results	Reference
Purple cactus pear	Phenolic compounds	Probe of 13 mm diameter at 1500 W[b]	20 kHz; 80%; 25 min; 50 °C	Increase at 40% approximately	Cruz-Cansino et al. (2015)
Carrot	Phenols Flavonoids Tanins Carotenoids	Probe of 13 mm diameter at 750 W[b]	20 kHz; 2.1 W mL^{-1}; 10 min; 60 °C	Reduction less than 10% in all compounds	Jabbar et al. (2015)
Grape	Carotenoids; phenolic compounds and flavonols	Ultrasound water bath at 420 W	28 kHz; 294 W; 60 min; 60 °C	Significant increase compared to the control	Aadil et al. (2015)
Carrot[a]	Phenolic compounds and total Carotenoids	Probe of 22 mm diameter at 400 W[b]	24 kHz; 120 μm; 2 min; 58 °C	Increase at 3.44% in total carotenoids and 9.74% in phenolic compounds as compared to fresh juice	Martínez-Flores et al. (2015)
Apple	Total phenolics, flavonoids and flavonols	Probe of 13 mm diameter at 750 W[b]	20 kHz; 0.30 W mL^{-1}; 5 min; 40 °C	When temperature increase, phenolic compounds content decreases (up 40 °C)	Abid et al. (2014)
Black mulberry	Anthocyanins	Probe of 13 mm diameter at 750 W[b]	20 kHz; 100%; 6 min; 50 °C	Reduction less than 3% compared with the control	Dinçer and Topuz (2014)
Jamun	Anthocyanins	Probe unspecified features[b]	20 kHz; 80%; 5 min; 80 °C	Retention about 73%	Shaheer et al. (2014)
Strawberry	Anthocyanins	Probe of 12 mm diameter at 600 W[b]	20 kHz; 90 μm; 9 min; 40 °C	Retention more than 106% compared with pasteurized juice	Herceg et al. (2013a)
Red grape	Anthocyanins	Probe at 200 W[b]	67.5 kHz; 200 W; 50 °C; 30 min	Reduction less than 5% compared with the control	Nafar et al. (2013)
Strawberry	Anthocyanins	Probe of 12 mm diameter at 600 W[b]	20 kHz; 60 μm; 3 min; 55 °C	Reduction less than 2% compared with the control	Dubrovic et al. (2011)
Watermelon	Lycopene and total phenols	Probe of 19 mm diameter at 1500 W[b]	20 kHz; 61 μm; 2 min; 45 °C	Retention about 83% of lycopene and 106% of total phenols compared with the control	Rawson et al. (2011)
Grape	Total phenolics	Ultrasound water bath at 360 W	35 kHz; 360 W; 13 min; 74 °C	Retention more than 120% compared with the control	Lieu and Le (2010)
Strawberry	Anthocyanins	Probe of 19 mm diameter at 1500 W[b]	20 kHz; 0.81 W mL^{-1}; 10 min; 40 °C	Retention more than 90% after 10 days of storage at 4 °C.	Tiwari et al. (2009b)

[a] Optimal experimental condition: Frequency (kHz); Amplitude (μm or %); Processing time (min); Final temperature (°C); Ultrasonic intensity (W); Acoustic Energy Density (WmL^{-1}).
[b] Ultrasonic equipment coupled on circulating water bath.

Shaheer et al., 2014). Some theories about degradation of these compounds propose that it may be caused by the collapse of microscopic bubbles produced by cavitation and the corresponding formation of free radicals; therefore, antioxidants act on these free radicals and decrease their own concentration (Dubrovic et al., 2011; Tiwari, Patras, Brunton, Cullen, & O'Donnell, 2010).

5.3. Physicochemical properties

The vegetables and fruit juices must retain their physicochemical properties after TS. Table 5 shows the effect of TS applied on fruit and vegetables juices investigated by several authors.

Most of the authors report no significant changes in the pH values, titratable acidity (TA), total soluble solids and electric conductivity when juices were treated with TS. Acidity is an important property related to stability of fruit juices and is dependent on the type of fruit from which it is made. However, the slight changes in pH or TA could be attributable to the content of some compound as organic acids, polyphenols, etc. (Sharma, Kaur, Sarkar, Singh, & Singh, 2009), formation of some chemical products by sonolysis of water molecules (OH^-, H_2O_2, H^+, among others) on the fruit juice, as it was shown by Kruus (2000) when ultrasound waves were applied in aqueous media.

The color can serve as an indicator of microbial quality during processing and storage of fruit and vegetable juices (Herceg et al., 2013a). Furthermore, changes in color by TS on juices have been

Table 5
Effect of thermosonication on physicochemical properties of different fruit and vegetable juices.

Type of juice	Ultrasonic equipment	Experimental conditions[a]	Results	Reference
Purple cactus pear	Probe of 13 mm diameter at 1500 W[b]	20 kHz; 80%; 25 min; 50 °C	pH, TA, TSS, NEBI and CI did not change; slightly decrease in L and increase in a and b	Cruz-Cansino et al. (2015)
Carrot	Probe of 13 mm diameter at 750 W[b]	20 kHz; 2.1 W mL^{-1}; 10 min; 60 °C	pH, TA and TSS did not change; increase in $L^* a^* b^*$ chroma parameters. Change in color (TCD = 9.36)	Jabbar et al. (2015)
Grape	Ultrasound water bath at 420 W	28 kHz; 294 W; 60 min; 60 °C	EC decreased when temperature increased; NEBI increased; attributes of color increased	Aadil et al. (2015)
Carrot	Probe of 22 mm diameter at 400 W[b]	24 kHz; 120 μm; 2 min; 58 °C	pH and TA did not change compared to the control	Martínez-Flores et al. (2015)
Apple	Probe of 13 mm diameter at 750 W[b]	20 kHz; 0.30 W mL^{-1}; 5 min; 40 °C	pH, TA and TSS did not change; slightly increase in $L^* a^* b^*$ parameters	Abid et al. (2014)
Black mulberry	Probe of 13 mm diameter at 750 W[b]	20 kHz; 100%; 6 min; 50 °C	pH and TA did not change, slightly increase in $L^* a^* b^*$ parameters	Dinçer and Topuz (2014)
Jamun	Probe unspecified features[b]	20 kHz; 80%; 5 min; 80 °C	pH, TA, TSS and reducing and total sugars did not change	Shaheer et al. (2014)
Strawberry	Probe of 12 mm diameter at 600 W[b]	20 kHz; 120 μm; 9 min; 55 °C	Decreased on L^*, a^* and b^* values compared to untreated sample	Herceg, Markhov et al. (2013a); Herceg, Lelas et al. (2013b)
Red grape	Probe at 200 W[b]	67.5 kHz; 200 W; 50 °C; 30 min	pH, TA, TSS did not change; slight change in color	Nafar et al. (2013)
Pineapple, grape and cranberry	Probe of 22 mm diameter at 400 W[b]	24 kHz; 120 μm; 10 min; 60 °C	Decrease in pH and change in color	Bermúdez-Aguirre and Barbosa-Cánovas (2012)
Watermelon	Probe of 19 mm diameter at 1500 W[b]	20 kHz; 60 μm; 10 min; 45 °C	Increase in lightness and negative change in TCD	Rawson et al. (2011)
Orange	Probe of 19 mm diameter at 1500 W[b]	20 kHz; 60 μm; 10 min; 30 °C	Increased in NEBI with processing temperature	Valdramidis et al. (2010)
Tomato	Probe of 19 mm diameter at 1500 W[b]	20 kHz; 61 μm; 10 min; 45 °C	pH values, TA and TSS did not change; but color showed changes influenced by the amplitude level and treatment time	Adekunte et al. (2010b)
Orange	Ultrasound water bath at 100 W	30 kHz; 20 min; 55 °C	pH, TSS, conductivity and NEBI did not change; significant increase in L*, and minor decrease in a* and increase in b*	Walkling-Ribeiro et al. (2009b)
Strawberry	Probe of 19 mm diameter at 1500 W[b]	20 kHz; 0.81 W mL^{-1}; 10 min; 40 °C	pH, TSS and TA were influenced by the extrinsic processing variables investigated, but did not report differences	Tiwari et al. (2009b)

NEBI: Non-Enzymatic Browning Index; TA: titratable acidity (%); EC: Electrical conductivity; TSS: total soluble solids (°Brix); TCD: Total Color Difference; CI: Color index.
[a] Optimal experimental condition: Frequency (kHz); Amplitude (μm or %); Processing time (min); Final temperature (°C); Ultrasonic intensity (W); Acoustic Energy Density (WmL^{-1}).
[b] Ultrasonic equipment coupled on circulating water bath.

reported, and these changes may be caused by different reasons. In some cases color changes can be caused by mechanical disruption of the cell wall and further the release of colored compounds as it has been found in carotenes of carrot juice (Martínez-Flores et al., 2015); lycopene in watermelon juice (Rawson et al., 2011) and phenolic compounds in grape juice (Lieu & Le, 2010) when compared with fresh or pasteurized samples.

Also, the degradation of compounds responsible for the color in tomatoes (Adekunte et al., 2010b), and red grape (Tiwari et al., 2010) juices have been studied. During long-time exposure to TS, high temperature and amplitude, Maillard reaction may also occur; therefore, it is necessary to find the optimum operating conditions of TS to avoid an adverse effect on the quality of fruit and vegetable juices (Jaeger, Janositz, & Knorr, 2010). Other authors described that changes in color were attributable to reactions promoted by cavitation, which controls various physical, chemical, or biological reactions as oxidation of some compounds by free radicals or by the presence of oxygen (Tiwari et al., 2008), by isomerization of some compounds (Sun, Ma, Ye, Kakuda, & Meng, 2010), or by non-enzymatic and enzymatic browning (Bermúdez-Aguirre & Barbosa-Cánovas, 2012; Islam et al., 2014).

Cloudiness is an important quality parameter that affects the appearance of the juice and is related to the presence of suspended particles (Ertugay & Baslar, 2014). There are only few reports about the effects of TS on the viscosity, turbidity/cloudiness and sedimentation values of fruit and vegetable juices (Table 6). However, an increase in viscosity has been reported in grape juice (Aadil et al., 2015), carrot juice (Martínez-Flores et al., 2015) and tomato juice (Wu et al., 2008); turbidity in black mulberry juice (Dinçer & Topuz, 2014) and sedimentation in apple juice (Ertugay & Baslar, 2014). The increase in turbidity, viscosity and sedimentation of fruit and vegetables juices may be attributable to factors such as: particle size, pectin, cellulose, hemicellulose, proteins and lipids in suspension (Aadil et al., 2015). TS reduces the size of the particles present in a liquid, providing better uniformity, stability and affects the transmittance (Fonteles et al., 2012). Particles are converted to soluble colloidal forms (Ertugay & Baslar, 2014). Depending on the experimental conditions of TS in fruit juice, parameters as viscosity, turbidity and sedimentation can be affected either momentarily or permanently (Soria & Villamiel, 2010). Additionally, the presence of ions as K^+, Na^+, Ca^{2+} and Mg^{2+} may contribute to the agglomeration process of colloidal particles in juices. Divalent ions such as calcium and magnesium promote gel formation due to cross-linking of pectin chains, which is favored by the presence of high sugar concentrations (Dahdouh et al., 2015). This kind of treatment provides a positive effect on the viscosity and consistency in milk, yogurt and cheese (Almanza-Rubio, Gutiérrez-Méndez, Leal-Ramos, Sepulveda, & Salmeron, 2016; Erkaya, Başlar, Şengül, & Ertugay, 2015; Riener, Noci, Cronin, Morgan, & Lyng, 2009; 2010).

5.4. Changes in sensory properties and shelf-life

The FDA (2004) in its "Guidance for Industry: Juice, Hazard Analysis and Critical Control Points (HACCP)" established the safety criteria of the products processed by emerging technologies. For processing of unpasteurized fruit juices, this guideline takes into consideration only the reduction of pathogenic microorganisms in a range of five log cycles of vegetative microbial cells, but not the

Table 6
Effect of thermosonication on viscosity and turbidity/cloudy properties in fruit and vegetable juices.

Type of juice	Ultrasonic equipment	Experimental conditions[a]	Results	Reference
Purple cactus pear	Probe of 13 mm diameter at 1500 W[b]	20 kHz; 80%; 25 min; 50 °C	Reduction of viscosity compared with control and did not change un physical stability at the end of storage	Cruz-Cansino et al. (2015)
Grape	Ultrasound water bath at 420 W	28 kHz; 294 W; 60 min; 60 °C	Cloud value and viscosity increased	Aadil et al. (2015)
Carrot	Probe of 22 mm diameter at 400 W[b]	24 kHz; 120 μm; 2 min; 58 °C	Slightly increase of viscosity compared to the control	Martínez-Flores et al. (2015)
Black mulberry	Probe of 13 mm diameter at 750 W[b]	20 kHz; 100%; 6 min; 50 °C	Significant Increased turbidity in thermosonicated sample compared with control	Dinçer and Topuz (2014)
Apple	Probe of 22 mm diameter at 400 W[b]	24 kHz; 100%; 10 min; 60 °C	Thermosonicated sample present better cloud value, stability and homogenization than the pasteurized sample	Ertugay and Baslar (2014)
Tomato	Probe of 22 mm diameter at 400 W[b]	24 kHz; 75 μm; 400 W; 8 min; 65 °C	All samples treated had greater apparent viscosity than the control.	Wu et al. (2008)

[a] Optimal experimental condition: Frequency (kHz); Amplitude (μm or %); Processing time (min); Final temperature (°C); Ultrasonic intensity (W); Acoustic Energy Density (WmL^{-1}).
[b] Ultrasonic equipment coupled on circulating water bath.

time and storage conditions in which the juice is considered safe for consumption. For this reason, it is necessary to assess conditions of storage and time during which fruit and vegetable juices processed by TS might be considered safe.

Martínez-Flores et al. (2015) analyzed carrot juice during 20 days after TS (24 kHz, 120 μm amplitude at 58 °C for 10 min). Samples were kept under refrigeration (4 °C) without direct exposure to light and the growth of enterobacteria, aerobic mesophiles, yeasts and molds after evaluation time were 2.02, 3.1 and 4.56 log cycles, respectively. These values represent microbial counts still acceptable for consumption, while untreated samples showed signs of spoilage after 10 days of storage. Cruz-Cansino et al. (2015) evaluated the shelf-life and effect on physicochemical, microbial and antioxidant properties of purple cactus pear juice after TS (20 kHz, 1200 W; 48 °C, 15 min) during 28 days of storage at 4 °C. They reported that after 28 days of storage of thermosonicated juice had only minor changes in color parameters and better stability in microbial counts, PME activity and antioxidant properties than control and thermally pasteurized juices. Ferrario et al. (2015) achieved an extended shelf life in apple juice of 15 days using combinations of ultrasound (20 kHz), heat (44 °C) during 10 min and light pulses (20 s), but mentioned that after the time of storage the microbial population was able to recover only by 1.1 log-cycles. Therefore, it is necessary to assess the eventual existence of sub-lethal cell damages induced by the combination of treatments.

Walkling-Ribeiro, Noci, Cronin, Lyng, and Morgan (2009a) studied changes in orange juice treated with lab-scale TS (30 kHz at 55 °C for 10 min) before exposure to pulsed electric fields (40 kV/cm for 100 μs) (TS/PEF) or after been subjected to pilot-scale conventional pasteurization (94 °C for 26 s) (HTST). Consumer acceptability was evaluated (6 female, 21 male) and shelf-life measured by total bacterial counts, conductivity, soluble solids, pH, and color attributes on day 0, 14, 28, 84 and 168 at 25 °C. For sensory attributes, panelists found no significant differences ($P < 0.05$) between HTST and TS/PEF juices in terms of color, aroma, acidity, sweetness, flavor and overall acceptability, but some panelists detected a metallic flavor in TS/PEF juice. After 168 days of storage, the conductivity, pH and soluble solids indicated no significant changes in TS/PEF or HTST juices, but there was a slight decrease in lightness for TS/PEF juice at the end of storage, compared with HTST. Likewise, mesophile counts were below 3.0 log cycles. Meanwhile, Simunek et al. (2013) examined the influence of TS (24 kHz, 600 W, 12–60 μm with a 12.7 mm diameter probe for 3, 6 and 9 min at 20, 40 and 60 °C) and pasteurization (80 °C for 2 min) on the changes in the aroma profile and sensory attributes of juice and nectar apple. The results showed that samples treated by TS lead to the formation of new compounds (which are not present in the untreated sample) or the disappearance of compounds that are present in the untreated samples. Changes in the composition of aromatic compounds derive from the extreme physical conditions that occur inside the cavitation bubbles after their collapse. TS caused a statistically significant decrease in all tested sensory parameters (color, aroma, taste and overall quality), but without reaching sensory rejection of the product. Panelists did not find a metallic taste. Possible changes in the odor and taste of juices treated with ultrasound can be attributed to the rapid isomerization of compounds and oxidation. The discrepancy between the results could be explained by the nature of the different fruit juices investigated (Tiwari et al., 2010).

6. Conclusions

Evidence shows that TS is a viable technology for fruit and vegetable juice processing, compared with conventional thermal treatments. Additive effects between ultrasound and heat have the potential to ensure stable product in compliance with standards set by FDA-HACCP and is an effective treatment for inactivation of enzymes, to conserve nutrients and bioactive compounds present in juices. TS offers some advantages over the conventional thermal process in terms of sensory and nutritional quality of juices. This type of technology represents a rapid, efficient and reliable alternative to improve the quality and extend the shelf life of fruit and vegetable juices. However, additional research is needed to improve the processing equipment, coupled with industrial development to achieve a wider application of this combined process in the beverage industry.

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