

Editorial





Effects of the high power ultrasound on microorganisms in fruit juices

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Pasteurization of fruit juices is very effective against pathogens and several spoilage microorganisms. Thermal processing affects sensory and nutritional properties. Demands for nutritious and safe foods have resulted in increased interest in non thermal preservation techniques. The U.S. Food and Drug Administration (FDA) requests the potential to meet 5log microbial reductions, in order to have safe product. Ultrasonication is a non thermal method of food processing that has the advantage of preserving fruit juices without/or minor causing the common side effects associated with conventional heat treatments.

Ultrasound is typically divided into three regions of frequency. Power ultrasound is in the region from 16-100kHz (1 Hertz is 1 cycle/ sec), high-frequency ultrasound is from 100kHz-1MHz and diagnostic ultrasound is from 1-10MHz. Power ultrasound (20-100kHz) can provide the mechanical effect of cavitation in liquid systems which can alter physical and chemical properties of food depending on the type of material involved. When ultrasound waves pass through a medium, a series of compression and rarefaction waves on the molecules of the medium are produced. This enforces a sinusoidal acoustic pressure (Pa) in addition to the hydrostatic pressure acting on the medium. If a large negative pressure (sufficiently below ambient) is applied to the liquid so that the distance between the molecules exceeds the critical molecular distance necessary to hold the liquid intact, the liquid will break down and the cavitation bubbles will be formed. These bubbles are formed from the gas nuclei within the fluid and are distributed throughout the liquid. After a period of a few cycles, the bubbles will grow into a critical size which makes them unstable and collapse violently. Power ultrasound is technique that is used in processing of fruit and vegetable juices. Several mechanisms of inactivation of microorganisms have been proposed. The mechanical stress of ultrasound on cells suspended in liquid medium (hydrodynamic events: acoustic cavitation and cavitation induced microstreaming) and radical formation are proposed mechanisms for microbial inactivation. Physical and chemical factors affect fruits, vegetables and plant tissues by causing the excessive accumulation of reactive oxygen species (ROS). Reactive oxygen species can cause damage to membranes and other essential macromolecules such as proteins, DNA, and lipids.1

Fruit juices are spoiled primarily because of growth of acid tolerant and osmophilic microorganisms. Thermal pasteurization is commonly used in fruit juice industries for the preservation of fruit juices. Due to thermal treatment, there are changes in essential nutrients and in physicochemical and organoleptic properties. Nonthermal pasteurization methods such as power ultrasound have been employed in fruit juices processing to overcome the negative effects of thermal pasteurization. Power ultrasound has been identified as potential technology to meet the US Food and Drug Administration (FDA) requirement of 5log reduction of *Escherichia coli* in fruit juices by thermosonication treatment. High power ultrasound causes bubble cavitation in a liquid due to pressure changes. These resultant microbubbles collapse violently in the succeeding compression cycles

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of propagated ultrasonic waves resulting in localized high temperature up to 5000K, pressures up to 50,000kPa, and high shearing effects causing breakdown of cell walls, disruption of cell membranes, and damage of DNA of microorganisms.² The simultaneous application of ultrasound and mild heat in fruit juice processing industry has the greatest potential and benefits of juice quality preservation. For a shorter processing time, it can be categorized as minimal processing for freshness and health purposes.³

Generally, cavitation is more effective on gram-positive bacteria, spores, spherical-shaped and small round cells. Gram-positive bacteria are more resistant to ultrasound than gram-negative because of their thicker cells wall (tightly adherent layer of peptidoglycans) which gives them a better protection against ultrasound effects. Bacterial spores and fungi are more resistant to ultrasound than vegetative bacteria. Spores are more difficult to be destroyed than vegetative cells which are in phase of growth.

Bevilacqua et al.4 reported the use of ultrasound (US) as a suitable strategy to control the growth of spoiling yeasts in fruit juices. In a first phase, US technique was tested towards Saccharomyces cerevisiae inoculated in different juices (strawberry, orange, apple, pineapple and red-fruits): the treatment was performed by modulating the level of the power (20-60%), the duration of the treatment (2-6min) and the pulse (2-6s), according to a fractional design. Then, the best treatment was applied against some other spoiling yeasts (Pichia membranifaciens, Wickerhamomycesanomalus, Zygosaccharomycesrouxii, Zygosaccharomycesbailii, norvegica). The results showed that the effect of US was mainly influenced by the power and the duration of the treatment. The highest reduction of S. cerevisiae was found in the following combinations of the design: power 60%, time 4min and pulse 2s, and power 60%, time 6min and pulse 6s; these results were confirmed for the other spoiling yeasts. US and citrus extract used could be combined to prolong the shelf life of the red-fruit juice and control the growth of Z. bailii.

Gabriel⁵ established the inactivation kinetic parameters of some pathogenic bacteria including *Escherichia coli* O157:H7, *Salmonella enterica serotypes*, and *Listeria monocytogenes*; and spoilage yeasts namely, *Debaryomyceshansenii*, *Clavisporalusitaniae*, *Torulasporadelbrueckii*, *Pichia fermentans*, and *Saccharomyces cerevisiae* in orange juice. Juice with microorganisms was subjected to multi-frequency Dynashock power ultrasound treatment. All test



organisms exhibited a biphasic inactivation behavior with a sigmoidal inactivation curve consisted of an initial inactivation lag, followed by logarithmic linear inactivation. Injury accumulation in the inactivation lag phase was established in acid-adapted bacteria. The time necessary to reduce initial inoculated populations by 5log cycles (99.999%), T5D values, significantly increased with acid adaptation. The T5D of *E. coli*, *S. enterica*, and *L. monocytogenes* increased from 37.64, 36.87, and 34.59, respectively; to 54.72, 40.38, and 37.83min, respectively, after acid exposure. Temperature increase due to heat propagation during ultrasound treatment decreased the resistance of the test bacteria. The cocktail of *E. coli* O157:H7 had significantly greater resistance towards ultrasound treatment (T5D=54.72min) than any of the individual strain (T5D=41.48-47.48min) in the mix. Similar results were found in the composited (T5D = 60.02 min) and individual species (T5D=20.31-59.04min).

Pineapple, grape and cranberry juice were thermo-sonicated (24kHz, 400W, 120μm) at 40°C, 50°C and 60°C during 10min at continuous and pulsed mode.⁶ Inactivation of *Saccharomyces cerevisiae* was tested from 0 to 10min. Survivor's curves were fitted with Weibull distribution, four parameter model and modified Gompertz equation. The acoustic energy was also calculated. *S. cerevisiae* was inactivated in the treatments at 60°C, with the continuous mode being more effective. Grape juice showed total inactivation (7-log) after 10min. The modified Gompertz equation showed the best fit. Energy analysis showed that pineapple juice (4287.02mW/mL) required a higher amount of energy; grape juice showed the lowest value (3112.13mW/mL). Because of the abovementioned results, these authors concluded that ultrasound represents a viable option for juice pasteurization.⁶

Finally, Pala et al.7 studied the effects of ultrasound treatment at various amplitudes (50, 75, and 100%) and times (0, 6, 12, 18, 24, and 30min) on Escherichia coli ATCC 25922 (a surrogate for E. coli O157:H7) and Saccharomyces cerevisiae ATCC 2366 in pomegranate juice. More than a 5-log inactivation of E. coli ATCC 25922 and a 1.36-log inactivation of S. cerevisiae ATCC 2366 were achieved after 30 min of ultrasound treatment at 100% amplitude. The log-linear and Weibull models were successfully used to estimate the microbial inactivation as a function of ultrasound treatment time (R₂=0.97). US processing achieved more than a 5-log reduction in E. coli ATCC 25922 which meets the U.S. Food and Drug Administration guidelines regarding pathogen reduction in fruit juices. The effect of ultrasound treatment on S. cerevisiae was more limited, resulting in only a 1.36-log reduction under the maximum processing conditions, which is why additional treatments such as mild heat, pressure, or a natural antimicrobial are required to control yeast in sonicated juice.

Methods and results from mentioned case studied showed that ultrasound treatment can we very useful tool in inactivation of microorganisms. Application of ultrasound showed great potential to be used as processing method, as time reducing technique, less chemicals usage, low energy consumption. Therefore, for high power ultrasound we can use term green non-thermal food processing technique.

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Conflict of interest

The author declares no conflict of interest.

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