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Application of Thermosonication Treatment in Processing and Production of High Quality and Safe-to-Drink Fruit Juices

Norazlin Abdullah, Nyuk Ling Chin*

Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia,43400 UPM Serdang, Selangor, Malaysia

Abstract

The demand for higher quality and freshness in fruit juices with absence of additives and preservatives has encouraged the use of ultrasound technology in juice processing. Thermosonication is a novel and good alternative technique to replace the conventional heat treatment process. It has potential in enhancing quality and safety of the fruit juices. It is more energy-efficient than conventional thermal technology. It is also more efficient, safe and reliable than ultrasound application alone by itself to achieve the limit of food-borne pathogens destruction. This review covers the effects of ultrasound-heat combined application on fruit juices quality and safety aspects.

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Keywords: Thermosonication; fruit juice; ultrasound; juice yield; microorganism

1. Introduction

Fruits are highly perishable and have to be processed into juices to ensure year-round continuous supply. The highest juice quality is required to meet consumer needs and juice safety aspects are important considerations for prolonging shelf life. Fruit juices are one of the food products which are thermally sensitive and susceptible to chemical, physical and microbiological changes. The processing methods may affect the quality and safety of the fruit juices. Today, the use of non-thermal food processing technologies is available to meet the demands of natural and healthy fruit juice drinks with minimal damage of its natural nutritional and organoleptic properties. The

* Corresponding author. Tel.: +603-89466353; fax: +603-89464440.
E-mail address: chinnl@upm.edu.my

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conventional fruit juices processing process is thermally inclined and results in some nutritional compounds loss such as flavonoids (Igual et al., 2011) and carotenoids (Fratianni et al., 2010). Its production efficiency is about 60 to 80% of fruit juice yield with rapid drop if old fruits are used. Risks of darker juice and containing excessive suspended solids and unwanted flavours also occur (Rutledge, 1996). It accounts for 90% of the total energy consumption (Sandhu et al., 2012) and requires prolonged processing time of 15 to 45 minutes(Horváth-Kerkai, 2006). The usage of ultrasound as an alternative technology to the conventional fruit juices processing technologies has attracted interest of many for its benefits in decreasing processing time, reducing energy consumption, increasing efficiency and improving shelf life and quality of fruit juices. These improvements are possible due to the properties of instantaneous transfer of acoustic energy into fruit juices. The ultrasonic treatment can penetrate fruit cell walls and release cell contents trapped in the fruit tissues.

Thermal preservation such as pasteurisation and sterilisation are commonly used to destroy microorganisms and inactivate enzymes in fruit juices. These extreme heat treatments at temperature of more than 80 °C may cause undesirable changes in various properties of fruit juices including physical, chemical, biological and organoleptic such as nutrients, colour and flavour (Bhattacherjee et al., 2011; Piasek et al., 2011; Giner et al., 2013; Mena et al., 2013). The thermosonication technology, which combines moderate heat of 37 to 75 °C with ultrasound treatment, is a potential alternative processing technique to enhance inactivation of enzymes and microbial (Ugarte-Romero et al., 2007; Terefe et al., 2009; Lee et al., 2013). The US Food and Drug Administration requires fruit juices to meet a minimum of 5-log reduction in pertinent microorganisms to control the spread of food-borne illnesses (USFDA, 2001; Lee et al., 2013). To achieve the USFDA requirement, food-borne pathogens inactivation with ultrasound undergoes long processing time leading to high production cost and small throughput. Therefore, the combination of low frequency ultrasound with mild heat will help in reducing processing temperature and time by 16 and 55%, respectively, minimising the negative effects on fruit juices quality and makes the processing more economically feasible (Koshani et al., 2014). Thermosonication involves a lower processing temperature than the conventional thermal processing to attain the same lethality values as the conventional method. The enzymes and microbial inactivation by thermosonication treatment is attributed to heat and cavitation, which is the phenomenon of formation, growth and explosion of bubbles in a liquid. The cavitation causes disruption of cell membrane and production of free radicals by both temperature and pressure changes.

This paper reviews on the current knowledge and applications of ultrasound on its own and in thermosonication treatment for fruit juices processing. It includes discussion on effects of thermosonication treatment in preserving juices quality, i.e. juice yield, ascorbic acid and colour, as well as destruction of enzymes and contaminants in fruit juices.

2. Basic principles of ultrasound

Ultrasound is sound wave transmitted with frequency higher than audible frequency of 20 kHz (Butz and Tauscher, 2002). The ultrasound equipment usually has frequencies from 20 kHz to 10 MHz (López-Malo et al., 2005). The application of ultrasound in food industry consists of low and high energy ultrasound. The low energy ultrasound has intensities of less than 1 Wcm^{-2} and frequencies of more than 100 kHz. It can be used in non-destructive analytical measurements and monitoring of composition and physicochemical properties of food during processing and storage for quality control purposes such as those in detection of honey adulteration (Alissandrakis et al., 2010), particle sizing (Lefebvre et al., 2013) and emulsion stability (Kaci et al., 2014). High energy ultrasound, which is also known as power ultrasound has intensities higher than 1 Wcm^{-2} and frequency range of 20 to 100 kHz. The power ultrasound is useful in invasive applications, which gives impact to physical, chemical and biological properties of foods in processing, preservation and safety such as milk homogenisation (Bosiljkov et al., 2009), juice yield enhancement (Lieu and Le, 2010) and microbial inactivation (Gao et al., 2014). There are two different ultrasonication techniques, which are submergence in an ultrasonic bath (Wu et al., 2008; Walkling-Ribeiro et al., 2009) or direct application to the fruit juices using a probe sonicator (Valdramidis et al., 2010; Dubrović et al., 2011; Rawson et al., 2011; Šimunek et al., 2013).

Fig. 1 illustrates the formation, growth and collapse of bubbles during a cavitation phenomenon. Gas bubbles are produced in liquid media by ultrasonic waves prior to acoustic cavitation phenomenon, which is the interaction between ultrasonic waves, liquid and dissolved gas when ultrasound passes through a liquid medium. Pressure changes around the dissolved gas nuclei leads to oscillations, where the dissolved gas and solvent vapour disperse in and out of the oscillating bubbles. The quantity of gas and vapour that enters the bubbles during this expansion period is beyond the quantity that diffuses out of the bubbles during the compression stage of bubbles oscillations. The bubbles then grow in successive cycles to an unstable size, burst in the compression phase and release very high heat and pressure around the collapsing bubbles to break the compounds in the liquid and give localised sterilisation effect. At this point, particle dispersion and cell disruption occur.

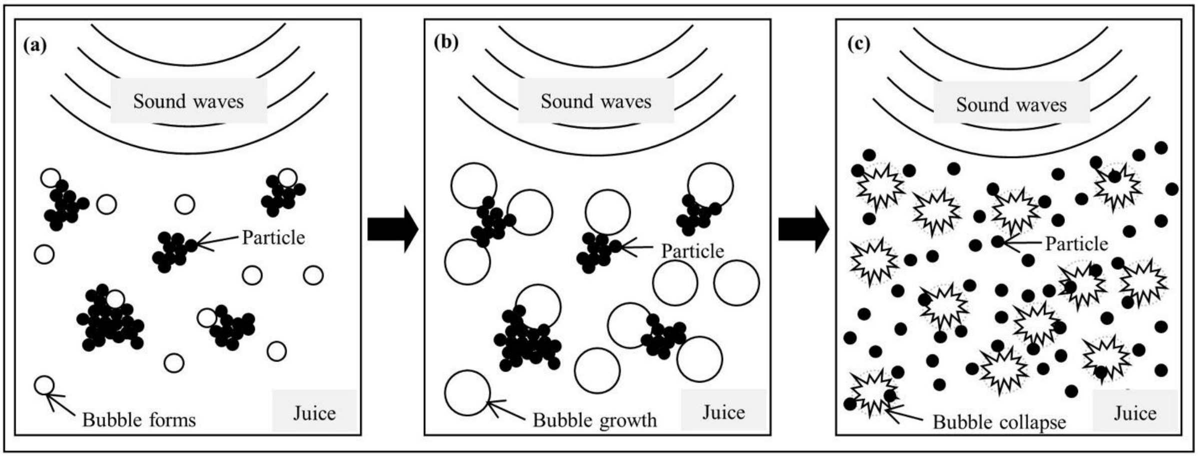

Fig. 1. Cavitation phenomenon. (a) bubbles formation in juice by sound waves; (b) bubbles growth to the maximum size and (c) bubbles collapse, and particle dispersion and cell disruption occurrence.

3. Thermosonication treatment in fruit juice production

Fruit juice quality is also often degraded by its enzymes and microbial activities such as the quality loss problems in apple juice due to enzymatic browning and microbial growth. Ultrasound processing of juices is reported to have a minimal degradation effect on the key quality parameters of fruits juices such as vitamin C content, colour, cloud stability and viscosity. The thermosonication treatment method is able to inactivate the enzymes and destroy the microorganisms at lower temperature and shorter time to give lower losses of ascorbic acid, total phenolics, flavonoids and flavonols (Abid et al., 2014).

3.1. Effects of thermosonication treatment on fruit juice quality

Table 1 summarises the effects of thermosonication treatment on fruit juice quality and its references. Juice production measured as yield is maximised to increase production capacities in beverage processing industries. In general, juice yield increases to an optimum following temperature before decreasing as heat leads to viscosity increase and a weak intensity of bubble collapse at higher vapour pressure of a thermosonication process. Thermosonication treatment process is often optimised in terms of temperature for enough violent cavitation bubbles for juice extraction (Patist and Bates, 2008; Holtung et al., 2011). The maximum temperature of 74 °C and sonication time of 13 minutes gave prediction response of grape juice yield extraction of 82.3%, which is 12.9% higher than the yield extraction value of untreated juice (Lieu and Le, 2010). The blackcurrant juice yield of more than 45% were achieved at optimal thermosonication temperature of 55 °C (Holtung et al., 2011).

Ascorbic acid is a form of vitamin C that can be gained from fruits as it has potential to keep optimum human immunisation system especially to protect against cancer and infectious disease (Chambial et al., 2013; Landete, 2013). It is not a stable compound and is easily oxidised under severe conditions such as prolonged heating and excessive heat treatment. Moderate processing temperature is needed to ensure higher retention of ascorbic acid as it is a highly heat sensitive compound. The optimised thermosonication treatment which had enough violent cavitation bubbles formed produced less ascorbic acid degradation compared to the conventional thermal treatment for fruit juices processing (Rawson et al., 2011; Abid et al., 2014). The ascorbic acid retention of about 96% in watermelon juice happened at treatment temperature of 29 °C and sonicator amplitude of 25 μm for 5 minutes of processing time (Rawson et al., 2011). Apple juice has ascorbic acid retention of about 96% when undergoing thermosonic treatment at temperature of 40 °C and sonication time of 5 minutes (Abid et al., 2014). As oxygen influences the stabilisation of ascorbic acid in fruit juices, sonication also aids in removing dissolved oxygen during cavitation to get higher ascorbic acid content (Cheng et al., 2007).

Colour is an important quality parameter for consumer preferences in choice of fruit juices. Lightness of juice becomes stable when enzyme activity and partial precipitation of insoluble particles in the juice are low. Conventional thermal treatment causes darkening of juice, while thermosonication treatment promotes juice lightness (Walkling-Ribeiro et al., 2009). Thermosonication is able to replace hot break treatment in tomato juice production for minimal colour change, development of unpleasant flavour and nutrient losses (Wu et al., 2008). Prolonged thermosonication treatment time on watermelon juice causes increment of its lightness (Rawson et al., 2011). As fruits and vegetables contain various useful colour pigments for health benefits and attractive colourants, thermosonication treatment of its juices can help to preserve these compounds. Two types of colour pigments that are usually found in fruits are lycopene, which is the red pigment and anthocyanin, which is the class of purple, red and blue pigments (Tiwari et al., 2009; Dubrović et al., 2011; Rawson et al., 2011). The range of lycopene retention 98 to 106.68% in watermelon juice was achieved in thermosonication treatment for 5 minutes at temperature range of 25 to 30 °C, amplitude of 24.4 to 40 μm and frequency of 20 kHz (Rawson et al., 2011). Higher anthocyanin retention of more than 98% in strawberry juice was observed when being treated at 40 °C for 3 minutes at amplitude of 60 μm and frequency of 20 kHz (Dubrović et al., 2011).

Thermosonication treatment has been used to inactivate various enzymes such as polyphenoloxidase (Cheng et al., 2007), peroxidase (Ercan and Soysal, 2011; Gamboa-Santos et al., 2012), pectin methylesterase (Terefe et al., 2009; Gamboa-Santos et al., 2012; Koshani et al., 2014) and polygalacturonase (Terefe et al., 2009). These endogenous enzymes are released during processing and must be inactivated as quickly as possible to avoid food spoilage. Thermosonication is useful in acting against the thermo-resistant enzymes where it is difficult to denature by thermal treatment alone and the use of extreme heat could lead to adverse changes in juice quality (Tribess and Tadini, 2006; Wu et al., 2008; Terefe et al., 2009; de Carvalho et al., 2013; Koshani et al., 2014). Since enzymes are more thermo resistant than microorganisms in citrus juices, the inactivation of enzymes promises the achievement of required number of microbial destruction for spoilage prevention (Torres et al., 2008).

The polyphenoloxidase (PPO) and peroxidase (POD) are two major enzymes causing browning that need to be inactivated for colour preservation (Abid et al., 2014). PPO is a copper-containing enzyme and primarily considered to cause browning in fruits and vegetables resulting in less attractive appearance and nutritional loss. It catalyses the oxidation of monophenolic compounds to *o*-diphenols and *o*-dihydroxy compounds to *o*-quinones, which are then polymerised to dark-coloured pigments (Van Loey et al., 2001). The inactivation of PPO and POD in apple juice were significantly higher in thermosonication treatment with probe sonicator at 60 °C for 10 minutes which were 93.85% and 91%, respectively (Abid et al., 2014). Ultrasonic treatment alone could give significant decrease in PPO and POD activities in cantaloupe melon juice (Fonteles et al., 2012). It was found that the time required to decrease initial PPO activity by 90% is shorter for thermosonication inactivation compared to the time duration for conventional thermal inactivation.

Pectin methylesterase (PME) de-esterifies pectin molecules with a lower degree of esterification (Fachin et al., 2003). Polygalacturonase (PG) catalyses the hydrolytic cleavage of the polygalacturonic acid chain by introducing water across the oxygen bridge resulting to viscosity loss and phase separation during storage (Terefe et al., 2009; Tu et al., 2013). Tomato juice consistency is mainly determined by the pectin breakdown during processing, either by chemical conversions or by the action of endogenous pectin-degrading enzymes that are PME and PG (Verlent et al., 2004; Wu et al., 2008). Both PME and PG are synergistic in pectin breakdown leading to browning of fruits and vegetables damaged tissues and loss of quality by the oxidation of phenolic compounds (Fachin et al., 2003; Chisari et al., 2007). The degree of polymerisation of pectic substances affects juice viscosity. Pectin reduction leads to a large decrease in tomato juice viscosity (Terefe et al., 2009). Low viscosity of juice causes less fouling of heat exchanger and pumping process gets easier. These enzymes in tomato are traditionally inactivated by thermal treatment, which are hot break and cold break (Terefe et al., 2009). The hot break process involves rapid heating of the pulp to temperature range of 95 to 102 °C immediately after or during crushing, while the cold break process involves heating of the pulp to temperature range of 60 to 71 °C. Although the hot break process causes complete enzymes inactivation and high juice consistency, the severe heat treatment leads to loss of juice colour, flavour and nutritional value. The cold break process only causes partial enzymes inactivation and low juice consistency. Thermosonication is a potential alternative for cold break and hot break treatments of tomato juice (Wu et al., 2008). Thermosonication could enhance the inactivation rates of both PME and PG in fruit juices leading to improvement of the juice rheological properties. PME inactivation of 98.5% and 98.9% in tomato juice was achieved by thermosonication at 60 °C and 65 °C, respectively (Wu et al., 2008). PME was almost inactivated and PG was 90% inactivated after 18 minutes of thermosonication treatment of tomato juice at frequency of 20 kHz, amplitude of 64 µm and temperature of 75 °C (Terefe et al., 2009). Thermosonic treated products had a greater apparent viscosity than conventional heat treated products (Šimunek et al., 2013). A lower activity of PME will improve cloud stability in juice (Tiwari et al., 2009). The inactivation of PME activity at a medium temperature range and higher viscosity are attributed to mechanical effects of thermosonication (Wu et al., 2008).

Table 1. Effects of thermosonication on the juice quality.

Quality parameter	Effects of thermosonication treatment	Previous findings of thermosonication treated juice
Juice yield	Increases juice production capacity.	1) Grape juice yield extraction of 82.3% (Lieu and Le, 2010). 2) Blackcurrant juice yield extraction of more than 45% (Holtung et al., 2011).
Ascorbic acid content	Removes dissolved oxygen during cavitation to produce less degradation of ascorbic acid.	1) Ascorbic acid retention of about 96% in watermelon juice (Rawson et al., 2011). 2) Ascorbic acid retention of about 96% in apple juice (Abid et al., 2014).
Juice colour	Promotes juice lightness.	1) Lycopene retention of 98 to 106.68% in watermelon juice (Rawson et al., 2011). 2) Anthocyanin retention of more than 98% in strawberry juice (Dubrović et al., 2011).
Enzymes inactivation	1) Preserves juice colour by inactivation of polyphenoloxidase (PPO) and peroxidase (POD). 2) Improves juice rheological properties by inactivation of pectin methylesterase (PME) and polygalacturonase (PG).	1) PPO and POD inactivation in apple juice were 93.85% and 91%, respectively (Abid et al., 2014). 2) PME inactivation in tomato juice were more than 98.5% (Wu et al., 2008). 3) PME and PG in tomato juice was almost inactivated and 90% inactivated, respectively(Terefe et al., 2009).

3.2. Effects of thermosonication treatment on fruit juice safety

The lethal effect of ultrasound is reported to be very much dependent on type of microorganism and processing parameters and sonication medium (Cheng et al., 2007). Thermosonication treatment is able to increase microbial inactivation rate in fruit juice. Microbial destruction effectiveness is influenced by the amplitude of ultrasound waves, processing time, treatment temperature, volume of juice processed and juice composition because the microorganisms do not react in the same way to ultrasound treatment. Sufficient intensity is required to disrupt biological structures and cause death of cell. The mechanism of microbial killing is mainly caused by the thinning of cell membranes, localised heating and pressure increase and production of free radicals. The treatment with combination of heat and ultrasound could give extensive cell damage and breakage on *E. coli* K12 cells (Lee et al., 2009). Prolonged treatment time and high acoustic energy density are needed to achieve the 5-log decrement in the number of pathogenic cells (Lee et al., 2009). There is a temperature limit for which thermosonication above the optimum temperature will not lead to any additional killing compared to thermal treatment because of the cushioning effect and reduction of cavitation activity at elevated temperatures, increment of vapour pressure and surface tension decrement (Ugarte-Romero et al., 2007).

Previous authors have observed the reduction of potential pathogens number like *Shigella boydii* (Ugarte-Romero et al., 2007), *Listeria monocytogenes* (Ugarte-Romero et al., 2007; Gastélum et al., 2012), *Staphylococcus aureus* (Walkling-Ribeiro et al., 2009) and *Escherichia coli* (Lee et al., 2013). Details on these microorganisms and their destruction efficiency after thermosonication treatment are presented in Table 2. *Shigella boydii* is a gram-negative food-borne pathogen and could survive in pH range of 3.3 to 4.1 of fruit juices (Bagamboula et al., 2002; Ugarte-Romero et al., 2007). The thermosonication treatment has successfully achieved the USFDA recommendation to reduce *S. boydii* up to 5-log cycle. *Listeria monocytogenes* is a gram-positive and heat resistant food-borne pathogen, which easily grown in pH of 3.8 to 9.8 and temperature of 0.5 to 45 °C (Bermúdez-Aguirre et al., 2011; Gastélum et al., 2012). It could contaminate nearly all kind of foods including minimally processed fruit juices due to its ubiquitous presence. Listeriosis is a food-borne illness most commonly caused by *L. monocytogenes* leading to meningitis and miscarriage in human (Mor-Mur and Yuste, 2010). Under the same acoustic power density of 1.43 W/mL, *L. monocytogenes* is more resistant to sonication than *S. boydii*. This led to the application of slightly higher temperature of 65 °C to achieve 5-log reduction of *L. monocytogenes* with shorten time to 2.5 minutes (Ugarte-Romero et al., 2007). *Staphylococcus aureus* is a gram-positive bacteria with cocci shape, which can survive in pH environment of 4 to 10 and cause staphylococcal food poisoning (Brennan, 2006; Rodriguez-Caturla et al., 2012). To achieve 5-log cycle reduction of *S. aureus*, thermosonication was combined with pulsed electric fields technology (Walkling-Ribeiro et al., 2009). *Escherichia coli* is a gram-negative bacteria which is pH sensitive and cannot survive in extremely alkaline and acidic conditions (Sharma and Beuchat, 2004; Brennan, 2006). It could be destroyed up to 5-log cycle by thermosonication treatment (Lee et al., 2013). The lethal effect of thermosonication is not the same for all microorganisms because the efficiency of microbial inactivation is also influenced by their morphological differences such as type, shape or diameter of the microorganisms. Generally, cavitation of sonication is more effective on gram-positive bacteria, spores, spherical-shaped and small round cells (Brennan, 2006). Gram-positive bacteria are more resistant to ultrasound than gram-negative because of their thicker cells wall that give them a better protection against ultrasound effects. Differences in cell sensitivity could be caused by the more tightly adherent layer of peptidoglycans in gram-positive cells. 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. The cocci or spherical-shaped cell is more resistant to ultrasound than bacilli or rod-shaped cell because of the relationship of cell surface and volume. Cells with larger surface area are more sensitive to ultrasound than the small and round cells. In summary, the effectiveness of ultrasound in treating the microorganisms is different depending on the species and its cell wall structures.

Table 2. Microorganisms morphology and its lethal effect of thermosonication.

Microorganism	Shape	Cell wall structure	Frequency (kHz)	Acoustic power density (W/mL)	Time (min)	Temperature (°C)	Microbial reduction (log cycle)	Reference
S.boydii	Bacilli	Gram negative	22.3	1.43	10	37	5	Ugarte-Romero et al.(2007)
L.monocytogenes	Bacilli	Gram positive	22.3	1.43	2.5	65	5	Ugarte-Romero et al.(2007)
S.aureus	Cocci	Gram positive	30	ND	20	55	3.3	Walkling-Ribeiro et al.(2009).
E. coli	Bacilli	Gram negative	20	3	3.8	59	5	Lee et al.(2013)

ND means data was not determined.

4. Conclusions

The simultaneous application of ultrasound and mild heat in fruit juice processing industry has the greatest potential and numerous benefits of juice quality preservation and safe processing. The cavitation effects of ultrasound functions to reduce juice yield, ascorbic acid content and colour loss and gives improvement in terms of the enzymes inactivation and microbial destruction. For a shorter processing time, it can be categorised as minimal processing for freshness and health purposes.

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