Effects of Thermosonication on Escherichia coli O157:H7 and Salmonella Enteritidis as A Function of pH and Temperature

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***Abstract*—The effects of thermosonication and thermal treatment on Escherichia coli O157:H7 and Salmonella Enteritidis suspended in citrate-phosphate buffer of pH 3 to pH 6 were studied at 30, 40 and 50 ℃. Application of ultrasound and low pH was found to enhance the inactivation of the pathogens; with the highest reduction of the pathogens was achieved by thermosonication at 50 ℃ in pH 3 buffer. Approximately 4 log cycle reduction of E. coli O157:H7 and 6 log cycle reduction of *S.* Enteritidis were obtained after being thermosonicated for 15 minutes at 50 ℃.**

***Index Terms*—Escherichia coli, non-thermal processing, *Salmonella*, thermal treatment, ultrasound**

I. Introduction

Nowadays, consumers demand for minimally processed food products is very high, due to the fact that processing may reduce the nutritional value of processed foods. This leads to the production and marketing of lightly pasteurized or freshly squeezed juices in the markets [1]. Unfortunately, there are reports showed that the rise of foodborne illness outbreaks was associated partially with the increase in consumption of minimally processed products, such as fruit juices [2].

Pasteurization and sterilization are the conventional technologies used to ensure microbiological safety in foods; but the extensive treatment with long treatment time or high temperature may cause the loss of nutrients, formation of undesirable flavours and degradation of functional properties [3], [4]. Non-thermal processing technologies which are normally less energy-intensive, more cost-efficient and environmentally friendly than the thermal processing are currently being developed. Examples of non-thermal processing are pulsed electric field processing, ultrasound processing, high pressure processing, just to name a few. These types of technologies have the ability to inactivate microorganisms present in foods while minimizing the amount of heat applied [5]. Non-thermal technologies, the technologies that work under mild treatment conditions such as high-intensity ultrasound, have been proposed to be able to serve as an alternative to juice thermal processing [5], [6]. It was reported that ultrasound processing enables the treatment to be carried out below thermal pasteurization's temperature with minute changes in food quality such as nutrients, flavours and vitamins; whilst having the ability to kill microorganisms [3], [7].

Ultrasound application in food industry is generally categorized into either low-intensity or high-intensity ultrasound. Low-intensity ultrasound (power level less than 1 W cm^{-2}) is able to generate information regarding physicochemical properties and it is a non-destructive technique while high-intensity ultrasound (power level from 10-1000 W cm^{-2}) can alter the properties of foods [8]. When high-intensity ultrasound is applied to a liquid medium, cavities will be formed. The materials present in the vicinity of the cavities will be affected by the intense hydrodynamic shock waves and pressure pulses due to the collapsing of cavities [9]. Thus, high-intensity ultrasound has the ability to physically disrupt material structure due to intense pressure, shear and temperature gradients created during cavitation [8].

The efficiency of ultrasound processing can be enhanced when it is combined with heat, pressure or pH adjustment [5], [10]. With such combinations, one may achieve similar log reduction but of shorter treatment time [1].

The optimum growth for *Escherichia coli* O157:H7 and *Salmonella* Enteritidis are around pH 7.0 and pH 6.5-7.5, respectively. Although *E. coli* O157:H7 and *Salmonella* spp. are commonly associated with products of animal origin; however, they are involved in outbreaks which are linked to orange juice and apple juice [11], [12]. On the other hand, *E. coli* O157:H7 also have the ability to survive for up to 56 days under acidic conditions and for 12 weeks of storage at -23 ℃ in apple, orange, banana, white grape and pineapple concentrates [13], [14].

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Besides that, *S*. Enteritidis has been shown to be able to grow on papaya at pH 4.87 [15]. Thus, cross-contamination is likely to occur if the improperly composted manure is being used as fertilizer, soil amendment or in irrigation water because *Salmonella* spp. and *E. coli* O157:H7 can be found in animal faeces [16]. Hence, the abovementioned cross-contamination facts have drawn our attention to study the survival of survival of *E. coli* O157:H7 and *Salmonella* spp. under acidic conditions in thermosonication treatment.

The aim of this study was to evaluate the effects of thermosonication and thermal treatment on *E. coli* O157:H7 and *Salmonella* Enteritidis suspended in citrate-phosphate buffer as a function of pH levels.

II. Materials and Methods

A. Citrate-Phosphate Buffers Preparation

Citrate-phosphate buffers with various pH levels of pH 3, pH 4, pH 5 and pH 6 were prepared using citric acid (SYSTERM, Shah Alam, Malaysia) and sodium phosphate dibasic heptahydrate (Merck, Darmstadt, Germany).

B. Bacterial Culture and Inoculum Preparation

The initial *E. coli* O157:H7 and *S*. Enteritidis (obtained from Medical Research Institute of Malaysia) inoculum were prepared by transferring a loopful of the stock culture to 100 mL Tryptic soy broth (TSB; Merck, Darmstadt, Germany) and 100 mL of Nutrient broth (NB; Merck, Darmstadt, Germany), respectively. The pathogens were then grown on an incubator shaker at 37 ℃ and 100 rpm for 18 hours.

C. Thermosonication and Thermal Treatment

Thermosonication was carried out at 25 kHz and 200 W in an ultrasonic Elma® cleaning bath (Model TI-H-10, Germany). During the course of the treatment, sweep mode was chosen to ensure a homogenous sound field distribution. Initial bacterial concentration of at least 10^9 CFU mL^{-1} of both *E. coli* O157:H7 and *S.* Enteritidis was inoculated into citrate-phosphate buffer separately. The inoculated buffers were then treated at three different treatment temperatures of 30, 40 and 50 ℃. Samples were collected and transferred to Butterfield's phosphate-buffered dilution water at 3 minutes interval for a total of 15 minutes. Thermal treatments were also carried out at the same temperatures without the application of ultrasound in order to serve as a parallel control. All the experiments were performed in triplicate.

D. Bacterial Enumeration

The samples collected were serially diluted in Butterfields's phosphate-buffered dilution water and pour-plated in duplicate on Tryptone glucose yeast extract agar (TGYEA; Himedia, Mumbai, India) for *E. coli* O157:H7 and Nutrient agar (NA; Merck, Darmstadt, Germany) for *S.* Enteritidis. All of the plates were incubated at 37 ℃ for 24 hours before enumeration. Replicate counts were averaged.

E. Determination of Decimal Reduction Time and Thermal Resistance Constant

Decimal reduction time (*D*-value) which is the time needed for survival count to decrease by 1 log cycle was calculated using linear regression method from the graph of log CFU ml^{-1} versus treatment time. Decimal reduction time curves (DRTC) were used for thermal resistant constant, *z*-value calculations. DRTC were drawn by plotting log D-value against their respective treatment temperatures. The *z*-value denotes the increases in temperature needed for *D*-value to reduce by 1 log cycle and it was calculated using linear regression method.

III. Results and Discussion

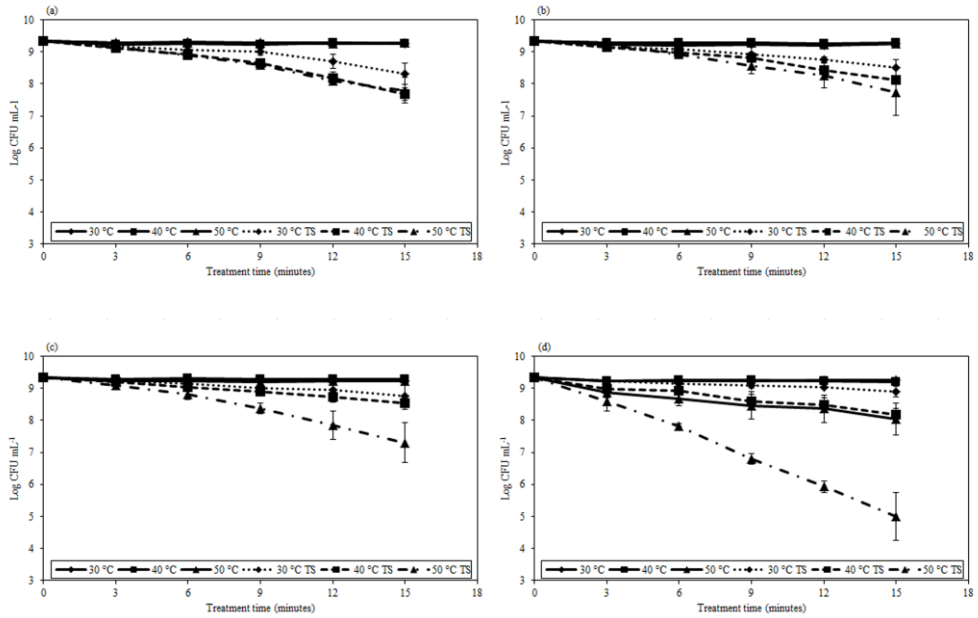

Figure 1. Inactivation of *Escherichia coli* O157:H7 in citrate-phosphate buffer as affected by thermal treatment and thermosonication (TS) at (a) pH 6, (b) pH 5, (c) pH 4 and (d) pH 3. Data are the mean of three replicate treatments; the error bars represent the standard deviations of the mean.

Fig. 1 and Fig. 2 show the effects of thermal and thermosonication treatments on *E. coli* O157:H7 and *S.* Enteritidis survival in citrate-phosphate buffer at various pH levels. From Fig. 1, it is noted that the application of ultrasound always leads to a higher inactivation of *E. coli* O157:H7 compared to their counterpart which is the thermal treatment alone. These observations are substantiated by the *D*-values calculated and presented in Table I. For samples underwent thermal treatment only, when temperature of the buffer was increased from 30 to 50 ℃, no significant change in inactivation on *E. coli* O157:H7 was observed at all pH studied, except pH 3 where marginal enhancement in inactivation was seen. Similar effects but happened at a higher number of log reduction was observed in those samples subjected to thermosonication. At pH 3, approximately 4 log cycle of reduction was obtained in thermosonication carried out at 50 ℃; whilst thermal treatment only leads to approximately 1 log cycle reduction after 15 minutes treatment. Similar observation was reported in Reference [17], [18].

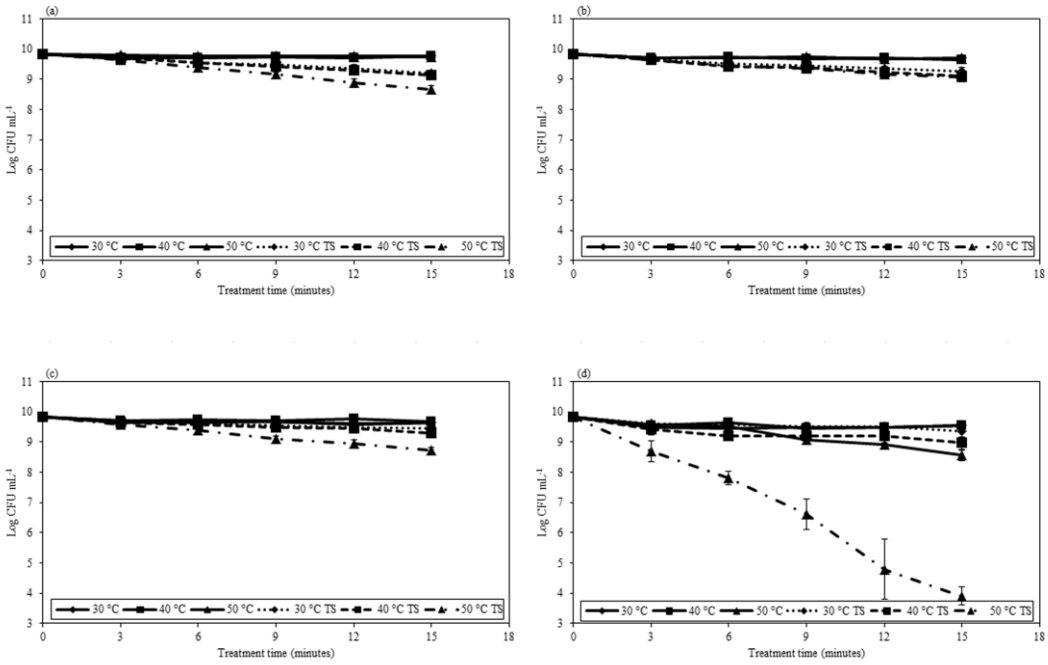

Figure 2 Inactivation of *Salmonella* Enteritidis in citrate-phosphate buffer as affected by thermal treatment and thermosonication (TS) at (a) pH 6, (b) pH 5, (c) pH 4 and (d) pH 3. Data are the mean of three replicate treatments; the error bars represent the standard deviations of the mean.

For *S.* Enteritidis (Fig. 2), application of ultrasound and increase in treatment temperature were found to show similar inactivation enhancement as observed in *E. coli* O157:H7, and the *D*-values are showed in Table I. Highest *S.* Enteritidis reduction was attained at pH 3 after 15 minutes of thermosonication with approximately 6 log cycle reduction and *D*-value of 2.47 minutes compared to approximately 1 log cycle reduction and *D*-value of 12.06 minutes for thermally treated sample at 50 ℃. The increase in treatment medium acidity has been shown to be able to lower salmonella and *E. coli* resistance to heat and ultrasound [10], [18]-[20]. However, *S.* Enteritidis survived better than *E. coli* O157:H7 when the treatments medium pH was varied from pH 6 to pH 4 at 50 ℃. Besides that, *S.* Enteritidis also has higher *D*-values compared to *E. coli* O157:H7 in thermosonicated samples. Nevertheless, the *D*-values for both pathogens increases when the treatment temperature rises from 30 to 50 ℃ in pH 6 for thermally treated samples. This may be due to the growth of the pathogens or higher thermal resistance of the pathogens when they were suspended in medium close to neutral pH. *E. coli* O157:H7 has been reported to be only fully inactivated after being heated at 55 ℃ for at least one hour; *Salmonella* spp. inoculated on alfalfa seeds have been shown to be able to survive after 10 days of dry heat treatment at 55 ℃ [21], [22].

Table II tabulates the *z*-values calculated for both *E. coli* O157:H7 and *S.* Enteritidis. As shown in Table II, *z*-values are lower in thermosonicated samples and the values increase with progressive increase in pH except for *S.* Enteritidis treated at pH 6. This implies that application of ultrasound lower the amount of temperature increment needed in order for the *D*-values to decrease by 90 % and hence shorter treatment time. The lowering of *z*-value after the application of ultrasound has also been reported for *Saccharomyces cerevisiae* [23].

The combination of ultrasound with thermal treatment has been shown to enhance inactivation of both pathogens studied. A synergistic effect between ultrasound and heat had previously been reported [17]. The enhanced killing in thermosonication is due to the heat causing changes in cells outer membrane susceptibility, which leads to the reduction in resistance to ultrasound [17]. Apart from that, ultrasound also able to change the cell membrane permeability, which in turn will lead to increase sensitivity to high temperature [23]. After thermosonication, the cells suffered from erosion

and disruption of membrane, perforation and lysis of cells causing the leakage of intracellular contents [24].

TABLE I. DECIMAL REDUCTION TIME (D-VALUE) OF ESCHERICHIA COLI O157:H7 AND SALMONELLA ENTERITIDIS IN CITRATE-PHOSPHATE BUFFER TREATED WITH THERMAL AND THERMOSONICATION (TS)

pH	Treatment condition*	*D*-value (minutes)	
		Escherichia coli O157:H7	*Salmonella* Enteritidis
3	30 ℃	294.12	75.76
	40 ℃	163.93	63.29
	50 ℃	12.79	12.06
	30 ℃ TS	35.71	39.37
	40 ℃ TS	13.72	20.83
	50 ℃ TS	3.42	2.47
4	30 ℃	232.56	109.89
	40 ℃	294.12	200
	50 ℃	178.57	84.75
	30 ℃ TS	26.88	44.25
	40 ℃ TS	18.83	31.35
	50 ℃ TS	7.31	13.74
5	30 ℃	185.19	175.44
	40 ℃	312.5	116.28
	50 ℃	169.49	133.33
	30 ℃ TS	19.08	27.4
	40 ℃ TS	12.41	21.88
	50 ℃ TS	9.35	20.08
6	30 ℃	212.77	303.03
	40 ℃	344.83	312.5
	50 ℃	270.27	357.14
	30 ℃ TS	15.77	24.75
	40 ℃ TS	9.23	22.12
	50 ℃ TS	9.39	12.56

TABLE II. THERMAL RESISTANCE CONSTANT (*Z*-VALUE) OF ESCHERICHIA COLI O157:H7 AND SALMONELLA ENTERITIDIS IN CITRATE-PHOSPHATE BUFFER TREATED WITH THERMAL AND THERMOSONICATION

pH	Treatment condition	*z*-value (℃)	
		Escherichia coli O157:H7	*Salmonella* Enteritidis
3	Thermal	14.68	25.06
	Thermosonication	19.65	16.64
4	Thermal	175.44	178.57
	Thermosonication	35.34	39.37
5	Thermal	526.32	166.67
	Thermosonication	64.52	149.25
6	Thermal	192.31	277.78
	Thermosonication	88.50	68.03

The main bactericidal effect of ultrasonication is due to cavitation. Cavitation also can cause micromechanical shocks which can disarray the cellular structural and functional components thus lead to cell lysis [25]. The region created during cavitation has extremely high pressure and temperature of up to 50000 kPa and 5500 ℃, respectively [5]. Although, microorganisms may have the ability to resist high pressures but they are unable to withstand the quick alternating pressures produced during ultrasonication [18]. Cavitation will cause formation of free radicals which attack on cells and formation of liquid jets which has the ability to puncture the cell wall [9], [23].

IV. CONCLUSION

Combination of ultrasound and thermal treatment enhanced the reduction of both *E. coli* O157:H7 and *S.* Enteritidis. Generally, thermosonication at lower pH level and higher temperature will lead to higher inactivation. Hence, thermosonication has the possibility to be used in fruit juice pasteurization in order to achieve the 5 log cycle reduction for the most pertinent microorganism as required by FDA due to the low pH of fruit juices. Thermosonication can accelerate the rate of pasteurization of liquid foods; hence shorten the duration and intensity of thermal treatment and the negative effects on foods.

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