2009-07-01

The Effects of Acid Adaptation on Escherichia Coli Inactivation Using Power Ultrasound

Sonal Patil
Technological University Dublin

Paula Bourke
Technological University Dublin, paula.bourke@tudublin.ie

Bridget Cullen
Technological University Dublin, Bridget.Kelly@tudublin.ie

See next page for additional authors

Recommended Citation

Patil, S et al: The effects of acid adaptation on Escherichia coli inactivation using power ultrasound. Innovative Food Science and Emerging Technologies, Vol.10, Issue 4, pp. 486-490.

Funder: Irish Department of Agriculture and Food as part of the National Development Plan 2000-2006.

Authors

Sonal Patil, Paula Bourke, Bridget Cullen, Jesus Maria Frias, and Patrick Cullen

(1) Running title**: Inactivation of *E. coli* using power ultrasound**

The Effects of Acid Adaptation on *Escherichia coli* Inactivation using Power Ultrasound.

SONAL PATIL, PAULA BOURKE*, BRIDGET KELLY, JESUS M. FRÍAS, P. J. CULLEN

School of Food Science and Environmental Health, Dublin Institute of Technology,
Cathal Brugha Street, Dublin 1, Ireland.

(2) Running title**: Inactivation of *E. coli* using power ultrasound**

* Author for correspondence. Tel: +353-14027594; Fax: +353-14024495; E-mail: Paula.Bourke@dit.ie

Abstract

Inactivation of *Escherichia coli* in liquids was carried out using power ultrasound. Parameters examined included amplitude levels (0.4μm, 7.5 μm, 37.5 μm), treatment time, cell condition (non-adapted cells, acid adapted cells), liquid media (TSB, model orange juice and model apple juice) and *E. coli* strain (ATCC 25922, NCTC 12900). The efficacy of ultrasound treatment was found to be a function of amplitude level, treatment time and media ($p<0.05$). The kinetics of inactivation followed zero order kinetics ($R>0.95$), with the highest inactivation achieved using an amplitude of 37.5 μm. The D-values of *E. coli* 25922 at all amplitudes in model orange juice were not significantly different than in TSB media. However, at 0.4μm and 37.5 μm amplitude D-values of *E. coli* 12900 were significantly different in model orange juice compared to TSB media. When efficacy of ultrasound was assessed in model apple juice and phosphate buffered saline treatment times were significantly reduced by comparison with TSB. Inactivation of *E. coli* was found to be influenced by strain, prior acid adaptation and suspension liquid, but the effect was negated at the higher amplitude levels.

Industrial relevance: To facilitate the preservation of unstable nutrients many juice processors have investigated alternatives to thermal pasteurisation, including un-pasteurised short shelf life juices with high retail value. This trend has continued within the European Union. However within the US recent regulations by the FDA have required processors to achieve a 5-log reduction in the numbers of the most resistant pathogens in their finished products. This rule comes after a rise in the number of food borne illness outbreaks and consumer illnesses associated with consumption of untreated juice products. Pathogenic *E. coli* may survive in acid environments such as fruit juices for long periods. Ultrasound has been identified as one possible non-thermal technology to meet the required microbial log reduction. However it is important to determine if conditions such as acid adaptation and pathogen strain influence ultrasound efficacy, if the technology is to be adopted by industry.

Keywords: Ultrasound, Non thermal technology, *E. coli*, Acid adaptation

1. Introduction

Over the last decade there has been a shift in food preservation processes from traditional thermal technologies, to non-thermal technologies such as high pressure, pulse electric field and power ultrasound. While heat remains the technique most extensively used for inactivation of micro-organisms in foods, there is growing interest in the development of alternative approaches. This is in response to consumer demand for products which are less organoleptically and nutritionally altered during processing, as well as less reliant on chemical preservation (Gould, 2001). Fruit juices are an important source of bioactive compounds, but techniques used for their processing and subsequent storage may cause alterations in their contents so they may not provide the benefits expected by the consumer. In recent years consumers have increasingly sought ready to use 'fresh-like' products, which are usually refrigerated. This has led the food industry to develop alternative processing technologies, to produce foods with a minimum of nutritional, physicochemical, or organoleptic changes induced by the technologies themselves (Esteve & Frígola, 2007), whilst maintaining microbiological safety profiles. Traditionally, fruit juice processors have relied on thermal pasteurisation and the inherent acidity of their products to assure microbiological safety. However, concerns have arisen regarding their microbiological safety due to a number of outbreaks associated with pathogens including *Escherichia coli* O157:H7 and *Salmonella* (Besser et al.,1993; Cook et al., 1998; Hammack, Amaguana, & Andrews, 2001). In 2001, the U.S. Food and Drug Administration (FDA), published a final rule requiring fruit juice producers to achieve a 5-log reduction in critical pathogen levels (USFDA, 2001).

Ultrasound refers to a frequency range of 20 kHz and above, and power ultrasound works at frequencies between 20-100 kHz. The mechanism of microbial inactivation by power ultrasound is through cavitation, the generation and collapse of micro-bubbles. Bubble collapse within a liquid medium results in localised temperatures of up to 5500°C and pressures of up to 100 MPa. Consequently the intense local energy and high pressure bring about a localised inactivation effect. The pressure changes that occur from these implosions are the main mechanism for microbial cell disruption (Piyasena, Mohareb & McKellar, 2003). A number of parameters such as frequency and amplitude of ultrasound waves, as well as temperature and viscosity of the liquid medium influence the degree of cavitation (Sala, Burgos, Condon, Lopez & Raso, 1995). Microbial inactivation using ultrasound has been investigated for application to a range of liquid foodstuffs. Levels of *E. coli* O157:H7 were reduced by 5 log CFU mL^{-1} with ultrasound in apple cider (D'Amico, Silk, Wu & Guo, 2006) and the inactivation of *E. coli* K12 was enhanced using ultrasound at ambient temperatures (Ugarte-Romero, Feng, Martin, Cadwallader & Robinson, 2006). Dehghani (2005) investigated the impact of sonication as a disinfection method for determining the effectiveness of ultrasound waves on the inactivation of *E. coli*, and showed a strong influence of ultrasound on the rate of *E. coli* disruption in water. In milk, levels of *Listeria monocytogenes* were reduced by 5 log CFU mL^{-1} when processed with ultrasound under mild heat conditions (D'Amico et al., 2006). Zenker, Heinz and Knorr (2005) evaluated the effects of continuous flow ultrasound-temperature treatment for bacterial decontamination (*E. coli* K 12 DH 5 α and *Lactobacillus acidophilus*) of model suspensions and various liquid food systems including milk, fruit and vegetable juices and compared the energy requirements with conventional thermal treatment.

Bacteria are exposed to stresses in all areas of the food chain. In the case of fruit juice processing, a major stress is the low pH, which may result in the induced acid resistance and enhanced survival of *E. coli* and other pathogens that may subsequently contaminate fruit juices. *E. coli* O157:H7 is reported to survive in apple, orange, pineapple and white grape juice concentrates for up to 12 weeks (Oyarzabal, Nogueira, & Gombas, 2003). Leyer, Wang & Johnson, (1995) recorded an acid-adaptive response in *E. coli* O157:H7 and that the expression of this system augments survival in acidic food products such as apple cider and fermented sausage. Treatment of *E. coli* O157:H7 with acid has been reported to increase acid resistance after exposure to moderate acid environments (Leyer et al., 1995) and was also shown to confer cross resistance to salt and heat (Rowe & Kirk, 1999). There is potential for survival of pathogenic *E. coli* in acid environments and there may be effects of prior acid adaptation on resistance to sonication treatment, which has been identified as a gap in current knowledge (Salleh-Mack & Roberts, 2007). Therefore, the objectives of this study were to optimise power ultrasound with regard to the control parameters of amplitude level and treatment time for the inactivation of *E. coli.* Due to the reported survival of *E. coli* O157:H7 within acid environments, the effects of prior acid adaptation on the efficacy of sonication was evaluated for both generic and non-toxigenic *E. coli* O157:H7.

2. Materials and Methods

2.1 Experimental Design

The parameters examined in this study included amplitude level (0.4µm, 7.5µm, 37.5 µm), treatment time, cell condition (non-adapted, acid adapted for 1 h, 4 h, 18 h), media (Tryptic Soya Broth, model orange juice, model apple juice) and *E. coli* strain (generic *E. coli* ATCC 25922, non-toxigenic *E. coli* O157:H7 NCTC 12900).

2.2 Bacterial strains and growth conditions

Two strains of *E. coli* were used in this study. *E. coli* ATCC 25922 was obtained from the microbiology stock culture, School of Food Science and Environmental Health, Dublin Institute of Technology. *E. coli* NCTC 12900 obtained from National Collection of Type Cultures, Health Protection Agency, London, UK. Strains were maintained as frozen stocks at -70ºC in the form of protective beads (Technical Services Consultants Ltd, UK), which were plated onto tryptic soy agar (TSA, Scharlau Chemie) and incubated overnight at 37ºC to obtain single colonies before storage at 4ºC. A single colony was inoculated into tryptic soya broth (TSB, Scharlau Chemie) and incubated overnight at 37ºC. Working cultures were prepared from this sub-culture, adjusted to 0.5 McFarland turbidity (Biomerieux Inc.) and serially diluted to yield the required concentration of 1 x 10^6 CFU mL^{-1} in TSB or model fruit juices.

2.3 Acid adaptation of bacterial cultures

Acid-adapted cells were prepared using the protocol by Leyer et al. (1995) with some modifications. Cultures of the appropriate *E. coli* strain, grown from a single colony in 5 mL TSB at 37ºC for 18h, were harvested by centrifugation (5000rpmX12min) and washed twice with sterile phosphate buffered saline (PBS, Oxoid, U.K). The pellet was re-suspended in 10 ml TSB (pH 5.0, adjusted with 1N HCl) and incubated at 37ºC for periods of 1 h, 4 h or18 h.

2.4 Model orange juice and Model apple juice

Model orange juice (MOJ) with a pH of 3.0 was prepared as per the method described by Shinoda , Murata, Homma & Komura (2004). The composition of MOJ per 100 ml was as follows: sucrose: 5.0 g; glucose: 2.5 g; fructose: 2.5 g; citric acid:

1.0 g; ascorbic acid: 30 mg; L-serine: 7.0 mmol; L-asparagine: 5.4 mmol, L-alanine: 1.9 mmol; L-arginine: 0.75 mmol; L-glutamic acid: 0.54 mmol; L-proline: 0.42 mmol. Model apple juice (MAJ) was prepared in the laboratory as per the method described by Reinders, Biesterveld and Bijker, (2001). The composition of MAJ per 1000 ml was as follows: fructose: 66 g; glucose: 22 g; sucrose: 27 g; sorbitol: 6.0 g; malic acid: 6.0 g; sodium citrate: 0.07 g; $K_2HPO_4{:}3H_2O$: 2 g.

2.5 Power ultrasound treatment

Samples (50 ml) were sonicated in a 100 ml glass beaker using a VC750 ultrasound generator (Sonics and Materials, Inc., Newtown, Conn., U.S.A.) fitted with an autoclavable 13 mm diameter ultrasound probe attached to an ultrasound transducer. Samples were processed at a constant frequency of 20 kHz. The measurement of the amplitude is an indication of the ultrasonic cavitation is reported to be a reliable method for indication of the ultrasound power (Tsukamoto, Yim, Stavarache, Furuta, Hashiba & Maeda, 2004). Before and after each experiment, the ultrasound probe was sterilized by washing with Virkon (DuPont), followed by thorough rinsing with sterile water. Amplitude levels of 0.4µm, 7.5µm and 37.5 µm with pulse durations of 5 s on and 5 s off were applied for up to 15 minutes. An ice bath was used to dissipate the heat generated during ultrasound treatment, and temperatures were maintained below 30°C.

2.6 Microbiological Analysis

Samples were removed for analysis at 3 min intervals and serially diluted in maximum recovery diluent (MRD, Scharlau Chemie). 0.1 ml aliquots of appropriate dilutions were plated on TSA and incubated at 37ºC for 24h. D-values were calculated using linear regression of the survivor curves for each ultrasound treatment.

2.7 Statistical analysis

Statistical analysis was performed using SPSS 15.0 (SPSS Inc., Chicago, U.S.A). Data represent the means of experiments performed in duplicate and replicated at least twice. Means were compared using ANOVA followed by LSD testing at $p < 0.05$ level.

3. Results

3.1 Effect of ultrasound amplitude level on inactivation of E. coli strains

The inactivation of both *E. coli* populations was found to be dependant on the amplitude levels ($p<0.05$). During ultrasound treatment, a linear response with exposure time was observed. Total inactivation of *E. coli* cells was achieved using 37.5 µm amplitude (Fig.1 a, b). Both strains of *E. coli* studied (*E. coli* ATCC 25922, *E. coli* NCTC 12900) were found to be sensitive to sonication ($p<0.05$). An amplitude of 0.4µm reduced *E. coli* ATCC 25922 by 1.2 log cycles (Fig. 1a) and *E. coli* ATCC 12900 by 1.1 log cycles (Fig. 1b) within 15 minutes. Ultrasonication for 15 minutes at 7.5 µm amplitude resulted in reduction of *E. coli* ATCC 25922 by 4.4 log cycles (Fig. 1a). Similarly, strain NCTC 12900 was reduced by 4.7 log cycles after ultrasound treatment of 15 minutes at 7.5 µm (Figure 1b). D-values for both strains obtained at all amplitudes examined are shown in Tables 1 and 2. D-values decreased with increasing levels of ultrasound amplitude ($p<0.05$). At 0.4µm amplitude the D-value of *E. coli* NCTC 12900 was higher than that of strain ATCC 25922. The time required to achieve inactivation by 5 log cycles (t_{5d}) for strain 25922 were 68.6 min, 17. 2 min and 11.1 min at 0.4µm, 7.5 µm and 37.5 µm amplitude levels, respectively. For strain NCTC 12900 the t_{5d} values were 76.3 min, 15.2 min and 13.8 min at 0.4µm, 7.5 µm and 37.5 µm amplitude levels, respectively. Both strains responded similarly to increasing amplitude levels, but at 37.5 µm amplitude level there was a significant difference between D-values of the two strains ($p < 0.05$).

3.2 Effect of acid adaptation on inactivation of E. coli strains

Ultrasound treatment at 37.5 µm amplitude of acid adapted *E. coli* ATCC 25922 (1 h, 4 h or 18 h) resulted in 5.7, 4.8 and 4.9 log cycle reductions after 15 minutes of exposure respectively. Strain NCTC 12900 had a similar response with 5.9, 5.8 and 5.5 log cycle reductions with 37.5 µm amplitude for the different conditions respectively. Ultrasound treatment with 7.5 µm amplitude showed a maximum reduction by 4.7 and 3.7 log cycles, with 1 h acid adapted *E. coli* ATCC 25922 and NCTC 12900, respectively. During 15 min treatment of ultrasound with 0.4µm amplitude, the 1 h acid adapted population of *E. coli* ATCC 25922 and *E. coli* NCTC 12900 in TSB was reduced by 1.71 and 1.14 log cycles, respectively. In general, regardless of acid adaptation time, the D-values of *E. coli* decreased as the amplitude level was increased. D-values of the non-adapted control and acid adapted *E. coli* cultures are outlined in Tables 1 and 2. At 0.4µm amplitude, 1 h acid adaptation of *E. coli* 25922 resulted in lower D-values compared to the control ($p< 0.05$). However, at longer acid-adaptation times of 4 h and 18 h, this effect was not evident in *E. coli* ATCC 25922 (Table 1). At 7.5 µm amplitude, there was no significant effect of adaptation condition compared with control cultures. At 37.5 µm amplitude, prior acid adaptation of *E. coli* ATCC 25922 for 1 h or 4 h did not significantly affect the D-value, however, with 18 h acid adapted cells, the D-value increased, yielding an increased resistance to ultrasound treatment. In the case of *E. coli* NCTC 12900 there were no significant differences in the inactivation of *E. coli* with regard to prior acid adaptation at 0.4µm amplitude. However, at 7.5 µm amplitude, increased time of acid adaptation was associated with higher D-values (Table 2). The t_{5d} values for 1 h, 4 h and 18 h acid adapted *E. coli* 25922 were in the range of 44.1-70.8min, 16-16.7 min and 10.6-14.9 min at 0.4µm- 37.5 µm amplitude, respectively. For 1 h, 4 h and 18 h acid adapted *E. coli* 12900 the t_{5d} values were in the range of 67.4-12.8 min, 78.9-13 min and 67.4-13.5 min, at 0.4µm- 37.5µm amplitude, respectively. Generally ultrasound treatment with 7.5 µm and 37.5 µm amplitude resulted in greater inactivation levels than with 0.4µm amplitude indicating an increased inactivation efficacy at higher amplitude levels.

3.3 Ultrasound inactivation of E. coli *strains in model orange juice*

Ultrasound inactivation of both *E. coli* strains in model orange juice was dependant on the level of amplitude applied ($p<0.05$). As with TSB, ultrasound treatment in model orange juice gave a linear response with exposure time. Ultrasound amplitudes of 7.5µm and 37.5 µm caused total inactivation of *E. coli* ATCC 25922 within 15 minutes. However, in the case of *E. coli* NCTC 12900, amplitudes of 7.5µm and 37.5 µm resulted in a 2.5 log reduction and a 2.7 log reduction respectively. Both strains of *E. coli* studied (*E. coli* ATCC 25922, *E. coli* NCTC 12900) were found to be sensitive to ultrasonication within model orange juice ($p<0.05$). Using 0.4µm amplitude *E. coli* ATCC 25922 was reduced by 1 log cycle and *E. coli* ATCC 12900 by 1.1 log cycles. D-values for both strains at all amplitudes in model orange juice are shown in Tables

3 and 4. D-values decreased with increasing levels of ultrasound amplitude ($p<0.05$). In the case of *E. coli* ATCC 25922, there were no significant differences observed between D-values obtained in TSB and model orange juice. However, for *E. coli* NCTC 12900, there were significant differences between D-values obtained in TSB and model orange juice at all level of amplitudes.

3.4 Ultrasound inactivation of E. coli ATCC 25922 in model apple juice

In this study *E. coli* cells previously grown in TSB were resuspended in model apple juice and treated with varying amplitude levels. Ultrasound treatment at 0.4µm amplitude resulted in a 3 $\log_{10}$CFU mL^{-1} reduction of cells with a corresponding D value of 5.3 minutes. When the amplitude was increased to 7.5 µm or 37.5 µm, inactivation was achieved within 6 and 3 minutes respectively.

4. Discussion

Ultrasound inactivation of both *E. coli* strains examined in this study showed a greater than 5 log reduction with increasing level of amplitude in 15 minutes or less. For this work, the level of amplitude employed was taken as an indication of the ultrasonic power intensity. Ultrasound treatment with 7.5 µm or 37.5 µm amplitude displayed a strong influence on the rate of *E. coli* inactivation in TSB, as shown in Figures 1a and 1b. It has been previously reported by several investigators (Baumann, Martin & Feng 2005, Villamiel & de Jong, 2000) that ultrasound processing of liquids is most effective in combination with mild heating. However, in this study an ice bath was used to dissipate the heat generated during treatment in order to evaluate the inactivation effects of ultrasound alone. At 37.5 µm amplitude, *E. coli* ATCC 25922 was reduced by 5.9 log cycles and *E. coli* NCTC 12900 by 5.6 log cycles within 15 minutes of ultrasound treatment. This inactivation results from a combination of physical and chemical mechanisms which occur during cavitation. At higher amplitude levels, corresponding to higher ultrasound intensities, the inactivation rate was enhanced in both *E. coli* strains, in accordance with previous studies that found that increasing the acoustic energy density, another indication of ultrasonic power intensity, increased the inactivation of foodborne pathogens (Hua & Thompson, 2000, Ugarte-Romero, Feng and Martin, 2007). There was only a marginal increase in the efficacy of ultrasound at 37.5 µm amplitude levels when compared to 7.5 µm level. Thus, in a processing context, it may be desirable to use 7.5 µm amplitude, as it was shown previously that the quality parameters of orange juice change as a function of amplitude level and sonication time (Tiwari, Muthukumarappan, O'Donnell & Cullen, 2008).

It has been reported that acid adaptation prolongs the survival of *E. coli* O157:H7 in various food systems, including apple cider, sausages (Leyer et al., 1995) and acid fruit juice (Hsin-Yi & Chou, 2001). Acid adaptation responses of foodborne pathogens at different pH conditions were previously examined and pH 5.0-5.5 lead to the highest level of acid resistance for *E. coli* O157:H7 (Koutsoumanis & Sofos, 2004). Consequently, in this study both *E. coli* strains were subjected to prior acid adaptation at pH 5.0 to examine for any effects on the efficacy of ultrasound treatment. When *E. coli* ATCC 25922 was acid adapted for 18 h, an increased resistance to ultrasound treatment at 37.5 µm amplitude was observed. However, the non-adapted control strain showed sensitivity to treatment at 7.5µm and 37.5µm amplitude, thus indicating that the longer acid adaptation of 18 h increased the resistance to ultrasound treatment. All prior acid adaptation treatments of *E. coli* NCTC 12900 increased the resistance of the organism to ultrasound treatment at 7.5 µm amplitude but no effect was evident at the other amplitudes. Acid adaptation involves changes in protein expression profiles (Huang, Tsai & Pan, 2007) and membrane lipid composition (Yuk & Marshall, 2004). This could alter the physiological state of the cells enabling them to withstand cavitation effect for a longer duration than the control cells. For both strains, there was a dominant effect where increasing the levels of amplitude (7.5 μm and 37.5 μm) of the ultrasound treatment negated any cell condition effects.

Ultrasound inactivation of bacteria has been found to be dependent upon the solution which is under study. Salleh-Mack & Roberts, (2007) investigated the effect of varying concentrations of soluble solids on the efficacy of ultrasound inactivation of *E. coli* ATCC 25922, and found that solutions with higher soluble solids required a longer time to achieve a higher inactivation. In this study, this effect was not found for *E. coli* ATCC 25922 as the D-values for TSB, a complex media, were similar to the D-values for model orange juice, a simpler solution. However, in *E. coli* NCTC 12900 this effect was found at all amplitude levels examined. So, differences in the two *E. coli* strains seem to effect the efficacy of ultrasound treatment in model orange juice. The survival of the non-toxigenic strain of *E. coli* O157:H7 used in this study was greater than that for the generic strain of *E. coli* used and this effect was enhanced following acid adaptation for 18h. Although the non-toxigenic strain of *E. coli* O157:H7 had greater survivial capabilities, the application of power ultrasound resulted in a > 5log reduction within 15 minutes. Temperatures employed in this study were maintained below 30ºC so as to utilize lower processing temperature than that used for thermal pasteurization.

5. Conclusion

The results of this study indicate that power ultrasound treatment has potential for inactivation of key microorganisms of concern in fruit juice processing. Ultrasound treatment alone can be effective for inactivation of *E. coli* that has been exposed to prior acid stress or adaptation, such as those encountered in acidic products such as fruit juices. Although a higher level of ultrasound amplitude negated the enhanced survival of the acid adapted non-toxigenic strain of *E. coli* O157:H7, it remains important to take the higher D-values observed into account during process design. Further studies are merited to investigate the mechanism of resistance of acid adapted cells to ultrasound treatment. For fruit juice processing, the parameters such as fruit juice type, presence of pulp, viscosity will be important factors in determining the inactivation rate and treatment time to achieve the desired log reduction. Inactivation of greater than the 5 log level reduction required by the FDA ruling(USFDA, 2001) occurred without the use of extra heating. This is very relevant to the processing of fruit juice as it is desirable to maintain low processing temperature to retain the quality characteristics of fresh juice, and to maintain energy efficiency.

Acknowledgement

Funding for this research was provided under the National Development Plan, through the Food Institutional Research Measure, administered by the Department of Agriculture, Fisheries & Food, Ireland.

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Figure Captions

Figure 1: Effect of amplitude levels on the inactivation of *E. coli* (a) ATCC 25922, (b) NCTC 12900

Figure 2: Effect of media on *E. coli* ATCC 25922 inactivation using 0.4µm amplitude.

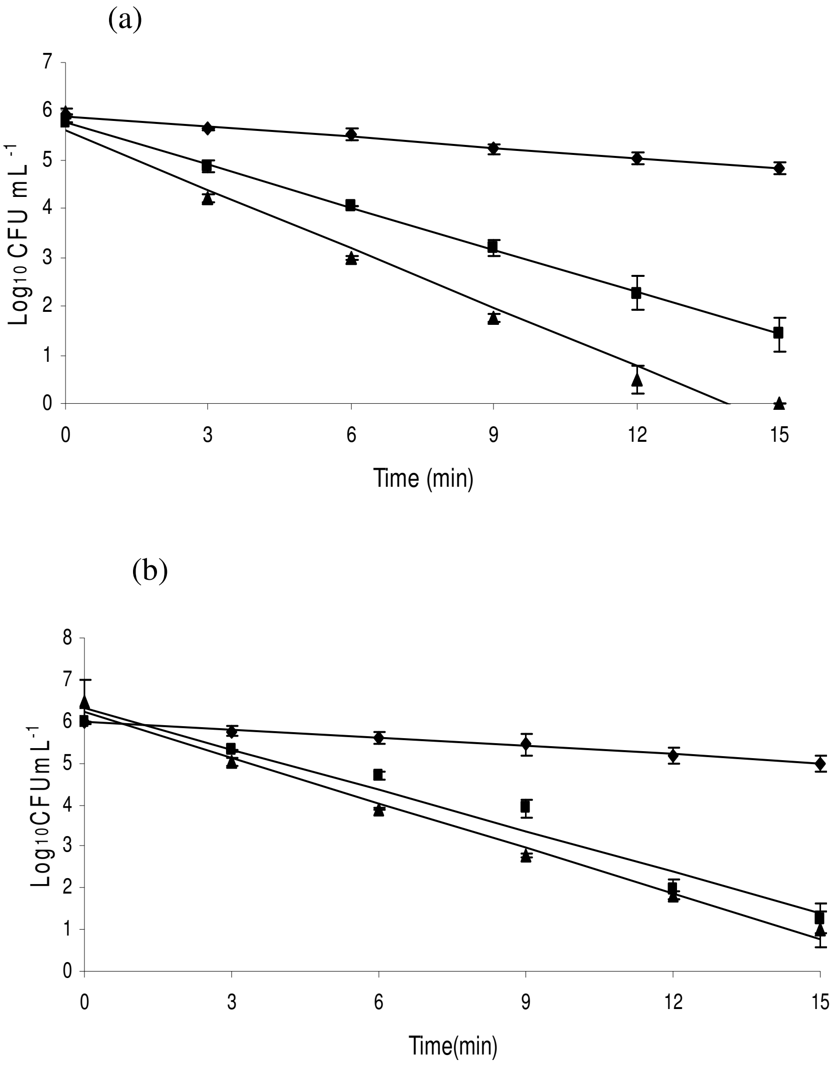

Figure 1: ♦ 0.4μm amplitude, ■ 7.5 μm amplitude and ▲ 37.5 μm amplitude

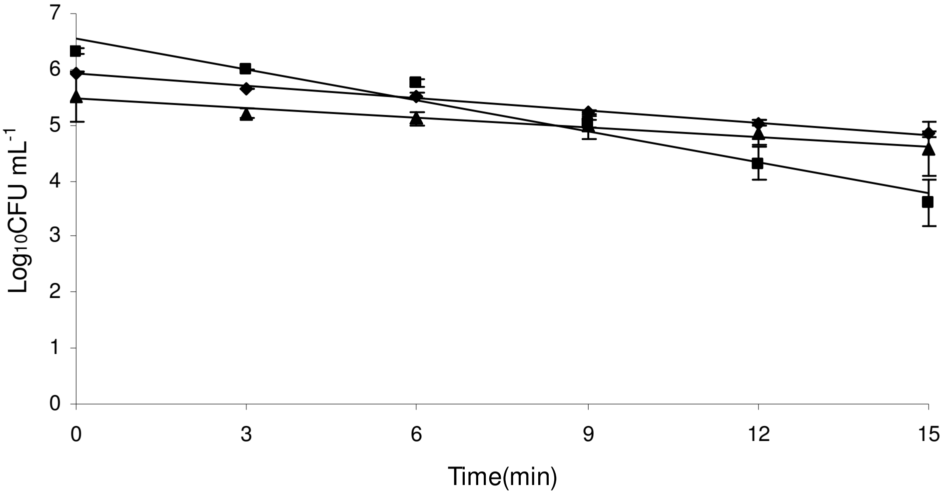

Figure 2: ♦ TSB, ■ Model apple juice and ▲ Model orange juice

Table 1: D-values and R^2 values for ultrasound treatment of control and acid-adapted *E. coli* ATCC 25922

Amplitude (μm)	Control		1 hour		4 hour		18 hour	
	D-value	**R^2**	**D-value**	**R^2**	**D-value**	**R^2**	**D-value**	**R^2**
0.4	13.73±0.9^{a}	0.99	8.83±0.03^{b}	0.99	12.46±0.1^{a}	0.97	14.16± 1.0^{a}	0.97
7.5	3.44±0.03^{c}	0.99	3.21± 0.22^{c}	0.98	3.29± 0.1^{c}	0.99	3.34±0.03^{c}	0.99
37.5	2.23±0.1^{d}	0.99	2.12± 0.16^{d}	0.98	2.43± 0.3^{d}	0.96	2.98± 0.17^{e}	0.98

Different letters indicate a significant difference at the 0.05 level

Table 2: D-values and R^2 values for ultrasound treatment of control and acid-adapted *E. coli* NCTC 12900

Amplitude (µm)	**Control**		**1 hour**		**4 hour**		**18 hour**	
	D-value	**R^2**	**D-value**	**R^2**	**D-value**	**R^2**	**D-value**	**R^2**
0.4	15.26±0.1^{a}	0.99	13.47±0.12^{a}	0.99	15.78±1.5^{a}	0.98	13.48±1.1^{a}	0.97
7.5	3.05±0.3^{b}	0.95	4.02±0.2^{c}	0.99	4.15±0.08^{cd}	0.99	4.48±0.09^{de}	0.99
37.5	2.75±0.1^{f}	0.99	2.55±0.09^{f}	0.98	2.60±0.09^{f}	0.99	2.69±0.09^{f}	0.99

Different letters indicate a significant difference at the 0.05 level

Table 3: D-values and R^2 values for ultrasound treatment of *E. coli* ATCC 25922 in TSB and model orange juice

Amplitude (µm)	**TSB**		**Model Orange Juice**	
	D-value	**R^2**	**D-value**	**R^2**
0.4	13.73±0.9	0.99	14.85±0.1	0.94
7.5	3.44±0.03	0.99	2.92±0.7	0.90
37.5	2.23±0.1	0.99	2.45±0.68	0.93

Table 4: D-values and R^2 values for ultrasound treatment of *E. coli* ATCC 12900 in TSB and model orange juice

Amplitude (µm)	TSB		Model Orange Juice	
	D-value	**R^2**	**D-value**	**R^2**
0.4	15.26±0.1	0.99	6.56±0.3	0.92
7.5	3.05±0.3	0.95	6.14±0.1	0.99
37.5	2.75±0.1	0.99	5.4±0.2	0.97