

Dublin Institute of Technology ARROW@DIT

Articles

School of Food Science and Environmental Health

2009-07-01

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

Sonal Patil Dublin Institute of Technology

Paula Bourke Dublin Institute of Technology, paula.bourke@dit.ie

Bridget Cullen Dublin Institute of Technology, Bridget.Kelly@dit.ie

Jesus Maria Frias Dublin Institute of Technology, Jesus.Frias@dit.ie

Patrick J. Cullen Dublin Institute of Technology, pjcullen@dit.ie

Follow this and additional works at: http://arrow.dit.ie/schfsehart Part of the <u>Food Microbiology Commons</u>

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.

This Article is brought to you for free and open access by the School of Food Science and Environmental Health at ARROW@DIT. It has been accepted for inclusion in Articles by an authorized administrator of ARROW@DIT. For more information, please contact yvonne.desmond@dit.ie, arrow.admin@dit.ie.



This work is licensed under a Creative Commons Attribution Noncommercial-Share Alike 3.0 License



1	(1) Running title: Inactivation of <i>E. coli</i> using power ultrasound
2	
3	
4	The Effects of Acid Adaptation on Escherichia coli
5	Inactivation using Power Ultrasound.
6	
7	SONAL PATIL, PAULA BOURKE*, BRIDGET KELLY, JESUS M. FRÍAS, P. J.
8	CULLEN
9	
10	
11	School of Food Science and Environmental Health, Dublin Institute of Technology,
12	Cathal Brugha Street, Dublin 1, Ireland.
13	
14	(2) Running title: Inactivation of E. coli using power ultrasound
15	
16	
17	* Author for correspondence. Tel: +353-14027594; Fax: +353-14024495; E-mail: Paula.Bourke@dit.ie
18	
19	
20	

21 Abstract

22 Inactivation of *Escherichia coli* in liquids was carried out using power ultrasound. 23 Parameters examined included amplitude levels (0.4µm, 7.5 µm, 37.5 µm), treatment 24 time, cell condition (non-adapted cells, acid adapted cells), liquid media (TSB, model 25 orange juice and model apple juice) and E. coli strain (ATCC 25922, NCTC 12900). 26 The efficacy of ultrasound treatment was found to be a function of amplitude level, 27 treatment time and media (p<0.05). The kinetics of inactivation followed zero order 28 kinetics (R>0.95), with the highest inactivation achieved using an amplitude of 37.5 29 µm. The D-values of E. coli 25922 at all amplitudes in model orange juice were not 30 significantly different than in TSB media. However, at 0.4µm and 37.5 µm amplitude 31 D-values of E. coli 12900 were significantly different in model orange juice compared 32 to TSB media. When efficacy of ultrasound was assessed in model apple juice and 33 phosphate buffered saline treatment times were significantly reduced by comparison 34 with TSB. Inactivation of E. coli was found to be influenced by strain, prior acid 35 adaptation and suspension liquid, but the effect was negated at the higher amplitude 36 levels.

37 Industrial relevance: To facilitate the preservation of unstable nutrients many juice 38 processors have investigated alternatives to thermal pasteurisation, including un-39 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 40 41 have required processors to achieve a 5-log reduction in the numbers of the most 42 resistant pathogens in their finished products. This rule comes after a rise in the 43 number of food borne illness outbreaks and consumer illnesses associated with 44 consumption of untreated juice products. Pathogenic E. coli may survive in acid 45 environments such as fruit juices for long periods. Ultrasound has been identified as

- 46 one possible non-thermal technology to meet the required microbial log reduction.
 47 However it is important to determine if conditions such as acid adaptation and
 48 pathogen strain influence ultrasound efficacy, if the technology is to be adopted by
 49 industry.
- 50 Keywords: Ultrasound, Non thermal technology, E. coli, Acid adaptation

51 **1. Introduction**

52 Over the last decade there has been a shift in food preservation processes from 53 traditional thermal technologies, to non-thermal technologies such as high pressure, 54 pulse electric field and power ultrasound. While heat remains the technique most 55 extensively used for inactivation of micro-organisms in foods, there is growing 56 interest in the development of alternative approaches. This is in response to consumer 57 demand for products which are less organoleptically and nutritionally altered during 58 processing, as well as less reliant on chemical preservation (Gould, 2001). Fruit juices 59 are an important source of bioactive compounds, but techniques used for their 60 processing and subsequent storage may cause alterations in their contents so they may 61 not provide the benefits expected by the consumer. In recent years consumers have 62 increasingly sought ready to use 'fresh-like' products, which are usually refrigerated. 63 This has led the food industry to develop alternative processing technologies, to 64 produce foods with a minimum of nutritional, physicochemical, or organoleptic 65 changes induced by the technologies themselves (Esteve & Frígola, 2007), whilst 66 maintaining microbiological safety profiles. Traditionally, fruit juice processors have 67 relied on thermal pasteurisation and the inherent acidity of their products to assure 68 microbiological safety. However, concerns have arisen regarding their 69 microbiological safety due to a number of outbreaks associated with pathogens 70 including Escherichia coli O157:H7 and Salmonella (Besser et al., 1993; Cook et al., 71 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 72 73 a 5-log reduction in critical pathogen levels (USFDA, 2001).

74 Ultrasound refers to a frequency range of 20 kHz and above, and power ultrasound
75 works at frequencies between 20-100 kHz. The mechanism of microbial inactivation

76 by power ultrasound is through cavitation, the generation and collapse of micro-77 bubbles. Bubble collapse within a liquid medium results in localised temperatures of 78 up to 5500°C and pressures of up to 100 MPa. Consequently the intense local energy 79 and high pressure bring about a localised inactivation effect. The pressure changes 80 that occur from these implosions are the main mechanism for microbial cell disruption 81 (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 82 83 medium influence the degree of cavitation (Sala, Burgos, Condon, Lopez & Raso, 84 1995). Microbial inactivation using ultrasound has been investigated for application to 85 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 86 87 inactivation of E. coli K12 was enhanced using ultrasound at ambient temperatures 88 (Ugarte-Romero, Feng, Martin, Cadwallader & Robinson, 2006). Dehghani (2005) 89 investigated the impact of sonication as a disinfection method for determining the 90 effectiveness of ultrasound waves on the inactivation of E. coli, and showed a strong 91 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 92 93 ultrasound under mild heat conditions (D'Amico et al., 2006). Zenker, Heinz and 94 Knorr (2005) evaluated the effects of continuous flow ultrasound-temperature 95 treatment for bacterial decontamination (E. coli K 12 DH 5 a and Lactobacillus 96 acidophilus) of model suspensions and various liquid food systems including milk, 97 fruit and vegetable juices and compared the energy requirements with conventional 98 thermal treatment.

Bacteria are exposed to stresses in all areas of the food chain. In the case of fruit juiceprocessing, a major stress is the low pH, which may result in the induced acid

101 resistance and enhanced survival of *E. coli* and other pathogens that may subsequently 102 contaminate fruit juices. E. coli O157:H7 is reported to survive in apple, orange, 103 pineapple and white grape juice concentrates for up to 12 weeks (Oyarzabal, 104 Nogueira, & Gombas, 2003). Lever, Wang & Johnson, (1995) recorded an acid-105 adaptive response in E. coli O157:H7 and that the expression of this system augments 106 survival in acidic food products such as apple cider and fermented sausage. 107 Treatment of E. coli O157:H7 with acid has been reported to increase acid resistance 108 after exposure to moderate acid environments (Leyer et al., 1995) and was also shown 109 to confer cross resistance to salt and heat (Rowe & Kirk, 1999). There is potential for 110 survival of pathogenic E. coli in acid environments and there may be effects of prior 111 acid adaptation on resistance to sonication treatment, which has been identified as a 112 gap in current knowledge (Salleh-Mack & Roberts, 2007). Therefore, the objectives 113 of this study were to optimise power ultrasound with regard to the control parameters 114 of amplitude level and treatment time for the inactivation of E. coli. Due to the 115 reported survival of E. coli O157:H7 within acid environments, the effects of prior 116 acid adaptation on the efficacy of sonication was evaluated for both generic and nontoxigenic E. coli O157:H7. 117

118 **2. Materials and Methods**

119 2.1 Experimental Design

120 The parameters examined in this study included amplitude level (0.4µm, 7.5µm, 37.5

121 µm), treatment time, cell condition (non-adapted, acid adapted for 1 h, 4 h, 18 h),

122 media (Tryptic Soya Broth, model orange juice, model apple juice) and *E. coli* strain

123 (generic E. coli ATCC 25922, non-toxigenic E. coli O157:H7 NCTC 12900).

- 124
- 125 2.2 Bacterial strains and growth conditions

126 Two strains of E. coli were used in this study. E. coli ATCC 25922 was obtained 127 from the microbiology stock culture, School of Food Science and Environmental Health, Dublin Institute of Technology. E. coli NCTC 12900 obtained from National 128 129 Collection of Type Cultures, Health Protection Agency, London, UK. Strains were 130 maintained as frozen stocks at -70°C in the form of protective beads (Technical 131 Services Consultants Ltd, UK), which were plated onto tryptic soy agar (TSA, 132 Scharlau Chemie) and incubated overnight at 37°C to obtain single colonies before 133 storage at 4°C. A single colony was inoculated into tryptic soya broth (TSB, Scharlau 134 Chemie) and incubated overnight at 37°C. Working cultures were prepared from this 135 sub-culture, adjusted to 0.5 McFarland turbidity (Biomerieux Inc.) and serially diluted to yield the required concentration of 1×10^6 CFU mL⁻¹ in TSB or model fruit juices. 136

137

138 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 37°C 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 37°C for periods of 1 h, 4 h or18 h.

145

146 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; K₂HPO₄:3H₂O: 2 g.

156 2.5 Power ultrasound treatment

157 Samples (50 ml) were sonicated in a 100 ml glass beaker using a VC750 ultrasound 158 generator (Sonics and Materials, Inc., Newtown, Conn., U.S.A.) fitted with an 159 autoclavable 13 mm diameter ultrasound probe attached to an ultrasound transducer. 160 Samples were processed at a constant frequency of 20 kHz. The measurement of the 161 amplitude is an indication of the ultrasonic cavitation is reported to be a reliable 162 method for indication of the ultrasound power (Tsukamoto, Yim, Stavarache, Furuta, 163 Hashiba & Maeda, 2004). Before and after each experiment, the ultrasound probe was 164 sterilized by washing with Virkon (DuPont), followed by thorough rinsing with sterile 165 water. Amplitude levels of $0.4\mu m$, $7.5\mu m$ and $37.5\mu m$ with pulse durations of 5 s on 166 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 167 168 30°C.

169 2.6 Microbiological Analysis

170 Samples were removed for analysis at 3 min intervals and serially diluted in 171 maximum recovery diluent (MRD, Scharlau Chemie). 0.1 ml aliquots of appropriate 172 dilutions were plated on TSA and incubated at 37°C for 24h. D-values were calculated 173 using linear regression of the survivor curves for each ultrasound treatment.

174 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.

179

180 **3. Results**

181 *3.1 Effect of ultrasound amplitude level on inactivation of E. coli strains*

182 The inactivation of both E. coli populations was found to be dependant on the 183 amplitude levels (p<0.05). During ultrasound treatment, a linear response with 184 exposure time was observed. Total inactivation of E. coli cells was achieved using 185 37.5 µm amplitude (Fig.1 a, b). Both strains of E. coli studied (E. coli ATCC 25922, 186 E. coli NCTC 12900) were found to be sensitive to sonication (p<0.05). An amplitude 187 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 188 at 7.5 µm amplitude resulted in reduction of E. coli ATCC 25922 by 4.4 log cycles 189 190 (Fig. 1a). Similarly, strain NCTC 12900 was reduced by 4.7 log cycles after 191 ultrasound treatment of 15 minutes at 7.5 µm (Figure 1b). D-values for both strains

192 obtained at all amplitudes examined are shown in Tables 1 and 2. D-values decreased 193 with increasing levels of ultrasound amplitude (p < 0.05). At 0.4µm amplitude the D-194 value of E. coli NCTC 12900 was higher than that of strain ATCC 25922. The time 195 required to achieve inactivation by 5 log cycles (t_{5d}) for strain 25922 were 68.6 min, 196 17. 2 min and 11.1 min at 0.4µm, 7.5 µm and 37.5 µm amplitude levels, respectively. 197 For strain NCTC 12900 the t_{5d} values were 76.3 min, 15.2 min and 13.8 min at 0.4µm, 198 7.5 μ m and 37.5 μ m amplitude levels, respectively. Both strains responded similarly 199 to increasing amplitude levels, but at 37.5 µm amplitude level there was a significant 200 difference between D-values of the two strains (p < 0.05).

201 3.2 Effect of acid adaptation on inactivation of E. coli strains

202 Ultrasound treatment at 37.5 µm amplitude of acid adapted E. coli ATCC 25922 (1 h, 203 4 h or 18 h) resulted in 5.7, 4.8 and 4.9 log cycle reductions after 15 minutes of 204 exposure respectively. Strain NCTC 12900 had a similar response with 5.9, 5.8 and 205 5.5 log cycle reductions with 37.5 μ m amplitude for the different conditions 206 respectively. Ultrasound treatment with 7.5 µm amplitude showed a maximum 207 reduction by 4.7 and 3.7 log cycles, with 1 h acid adapted E. coli ATCC 25922 and 208 NCTC 12900, respectively. During 15 min treatment of ultrasound with 0.4µm 209 amplitude, the 1 h acid adapted population of E. coli ATCC 25922 and E. coli NCTC 210 12900 in TSB was reduced by 1.71 and 1.14 log cycles, respectively. In general, 211 regardless of acid adaptation time, the D-values of E. coli decreased as the amplitude 212 level was increased. D-values of the non-adapted control and acid adapted E. coli 213 cultures are outlined in Tables 1 and 2. At 0.4µm amplitude, 1 h acid adaptation of E. 214 *coli* 25922 resulted in lower D-values compared to the control (p < 0.05). However, at 215 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 216

217 adaptation condition compared with control cultures. At 37.5 µm amplitude, prior acid 218 adaptation of E. coli ATCC 25922 for 1 h or 4 h did not significantly affect the D-219 value, however, with 18 h acid adapted cells, the D-value increased, yielding an 220 increased resistance to ultrasound treatment. In the case of E. coli NCTC 12900 there 221 were no significant differences in the inactivation of E. coli with regard to prior acid 222 adaptation at 0.4µm amplitude. However, at 7.5 µm amplitude, increased time of acid 223 adaptation was associated with higher D-values (Table 2). The t_{5d} values for 1 h, 4 h 224 and 18 h acid adapted E. coli 25922 were in the range of 44.1-70.8min, 16-16.7 min 225 and 10.6-14.9 min at 0.4µm- 37.5 µm amplitude, respectively. For 1 h, 4 h and 18 h 226 acid adapted E. coli 12900 the t_{5d} values were in the range of 67.4-12.8 min, 78.9-13 227 min and 67.4-13.5 min, at 0.4µm- 37.5µm amplitude, respectively. Generally 228 ultrasound treatment with 7.5 µm and 37.5 µm amplitude resulted in greater 229 inactivation levels than with 0.4µm amplitude indicating an increased inactivation 230 efficacy at higher amplitude levels.

231 3.3 Ultrasound inactivation of E. coli strains in model orange juice

232 Ultrasound inactivation of both E. coli strains in model orange juice was dependant on 233 the level of amplitude applied (p<0.05). As with TSB, ultrasound treatment in model 234 orange juice gave a linear response with exposure time. Ultrasound amplitudes of 235 7.5µm and 37.5 µm caused total inactivation of E. coli ATCC 25922 within 15 236 minutes. However, in the case of E. coli NCTC 12900, amplitudes of 7.5µm and 37.5 237 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 238 239 to ultrasonication within model orange juice (p<0.05). Using 0.4µm amplitude E. coli 240 ATCC 25922 was reduced by 1 log cycle and E. coli ATCC 12900 by 1.1 log cycles. 241 D-values for both strains at all amplitudes in model orange juice are shown in Tables

242 3 and 4. D-values decreased with increasing levels of ultrasound amplitude (p<0.05).

243 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.

247 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 0.4 μ m amplitude resulted in a 3 log₁₀CFU mL⁻¹ reduction of cells with a corresponding D value of 5.3 minutes. When the amplitude was increased to 7.5 μ m or 37.5 μ m, inactivation was achieved within 6 and 3 minutes respectively.

4. Discussion

254 Ultrasound inactivation of both E. coli strains examined in this study showed a greater 255 than 5 log reduction with increasing level of amplitude in 15 minutes or less. For this 256 work, the level of amplitude employed was taken as an indication of the ultrasonic 257 power intensity. Ultrasound treatment with 7.5 µm or 37.5 µm amplitude displayed a 258 strong influence on the rate of E. coli inactivation in TSB, as shown in Figures 1a and 259 1b. It has been previously reported by several investigators (Baumann, Martin & Feng 260 2005, Villamiel & de Jong, 2000) that ultrasound processing of liquids is most 261 effective in combination with mild heating. However, in this study an ice bath was 262 used to dissipate the heat generated during treatment in order to evaluate the 263 inactivation effects of ultrasound alone. At 37.5 µm amplitude, E. coli ATCC 25922 264 was reduced by 5.9 log cycles and E. coli NCTC 12900 by 5.6 log cycles within 15 265 minutes of ultrasound treatment. This inactivation results from a combination of physical and chemical mechanisms which occur during cavitation. At higher 266

267 amplitude levels, corresponding to higher ultrasound intensities, the inactivation rate 268 was enhanced in both E. coli strains, in accordance with previous studies that found 269 that increasing the acoustic energy density, another indication of ultrasonic power 270 intensity, increased the inactivation of foodborne pathogens (Hua & Thompson, 2000, 271 Ugarte-Romero, Feng and Martin, 2007). There was only a marginal increase in the 272 efficacy of ultrasound at 37.5 µm amplitude levels when compared to 7.5 µm level. 273 Thus, in a processing context, it may be desirable to use 7.5 μ m amplitude, as it was 274 shown previously that the quality parameters of orange juice change as a function of 275 amplitude level and sonication time (Tiwari, Muthukumarappan, O'Donnell & Cullen, 276 2008).

277 It has been reported that acid adaptation prolongs the survival of E. coli O157:H7 in 278 various food systems, including apple cider, sausages (Leyer et al., 1995) and acid 279 fruit juice (Hsin-Yi & Chou, 2001). Acid adaptation responses of foodborne 280 pathogens at different pH conditions were previously examined and pH 5.0-5.5 lead to 281 the highest level of acid resistance for E. coli O157:H7 (Koutsoumanis & Sofos, 282 2004). Consequently, in this study both E. coli strains were subjected to prior acid 283 adaptation at pH 5.0 to examine for any effects on the efficacy of ultrasound 284 treatment. When E. coli ATCC 25922 was acid adapted for 18 h, an increased 285 resistance to ultrasound treatment at 37.5 µm amplitude was observed. However, the 286 non-adapted control strain showed sensitivity to treatment at 7.5µm and 37.5µm 287 amplitude, thus indicating that the longer acid adaptation of 18 h increased the 288 resistance to ultrasound treatment. All prior acid adaptation treatments of E. coli 289 NCTC 12900 increased the resistance of the organism to ultrasound treatment at 7.5 290 µm amplitude but no effect was evident at the other amplitudes. Acid adaptation 291 involves changes in protein expression profiles (Huang, Tsai & Pan, 2007) and 292 membrane lipid composition (Yuk & Marshall, 2004). This could alter the 293 physiological state of the cells enabling them to withstand cavitation effect for a 294 longer duration than the control cells. For both strains, there was a dominant effect 295 where increasing the levels of amplitude (7.5 μ m and 37.5 μ m) of the ultrasound 296 treatment negated any cell condition effects.

297 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 298 299 varying concentrations of soluble solids on the efficacy of ultrasound inactivation of 300 E. coli ATCC 25922, and found that solutions with higher soluble solids required a 301 longer time to achieve a higher inactivation. In this study, this effect was not found for 302 E. coli ATCC 25922 as the D-values for TSB, a complex media, were similar to the 303 D-values for model orange juice, a simpler solution. However, in E. coli NCTC 12900 304 this effect was found at all amplitude levels examined. So, differences in the two E. 305 *coli* strains seem to effect the efficacy of ultrasound treatment in model orange juice. 306 The survival of the non-toxigenic strain of E. coli O157:H7 used in this study was 307 greater than that for the generic strain of E. coli used and this effect was enhanced 308 following acid adaptation for 18h. Although the non-toxigenic strain of E. coli 309 O157:H7 had greater survivial capabilities, the application of power ultrasound 310 resulted in $a > 5\log$ reduction within 15 minutes. Temperatures employed in this 311 study were maintained below 30°C so as to utilize lower processing temperature than 312 that used for thermal pasteurization.

313 **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 317 prior acid stress or adaptation, such as those encountered in acidic products such as 318 fruit juices. Although a higher level of ultrasound amplitude negated the enhanced 319 survival of the acid adapted non-toxigenic strain of E. coli O157:H7, it remains 320 important to take the higher D-values observed into account during process design. 321 Further studies are merited to investigate the mechanism of resistance of acid adapted 322 cells to ultrasound treatment. For fruit juice processing, the parameters such as fruit 323 juice type, presence of pulp, viscosity will be important factors in determining the 324 inactivation rate and treatment time to achieve the desired log reduction. Inactivation 325 of greater than the 5 log level reduction required by the FDA ruling(USFDA, 2001) 326 occurred without the use of extra heating. This is very relevant to the processing of 327 fruit juice as it is desirable to maintain low processing temperature to retain the 328 quality characteristics of fresh juice, and to maintain energy efficiency.

329 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.

333 References

- Baumann, A. R., Martin, S. E., & Feng, H. (2005). Power ultrasound treatment of
- 335 *Listeria monocytogenes* in apple cider. *Journal of Food Protection*, 68, 2333-2340.
- 336 Besser, R. E., Lett, S. M., Weber, J. T., Doyle, M. P., Barrett, T. J., Wells, J. G., &
- 337 Griffin, P. M. (1993). An outbreak of diarrhea and hemolytic uremic syndrome from
- 338 Escherichia coli O157:H7 in fresh-pressed apple cider. The Journal of the American
- *medical Association*, 269, 2217-2220.
- 340 Cook, K. A., Dobbs, T. E., Hlady, W. G., Wells, J. G., Barrett, T. J., Puhr, N. D.,
- 341 Lancette, G. A., Bodager, D. W., Toth, B. L., Genese, C. A., Highsmith, A. K., Pilot,

- 342 K. E., Finelli, L., & Swerdlow, D. L. (1998). Outbreak of Salmonella Serotype
- 343 Hartford Infections Associated With Unpasteurized Orange Juice. The Journal of the
- 344 *American medical Association*, 280, 1504-1509.
- 345 D'Amico, D. J., Silk, T. M., Wu, J. R., & Guo, M. R. (2006). Inactivation of
- 346 microorganisms in milk and apple cider treated with ultrasound. Journal of Food
- 347 *Protection*, 69, 556-563.
- 348 Dehghani, M. H. (2005). Effectiveness of ultrasound on the destruction of *E. coli*.
- 349 *American Journal of Environmental Sciences*, 1 (3), 187-189.
- 350 Esteve M.J., & Frígola A. (2007). Refrigerated fruit juices: quality and safety issues
- 351 Advances in Food Nutrition Research, 52,103-39
- 352 Gould, G. W. (2001). New processing technologies: an overview. Proceedings of the
- 353 *Nutrition Society*, 60, 463-474.
- Hammack, T.S., Amaguana, R.M., & Andrews, W.H. (2001). An improved method
- 355 for the recovery of *Salmonella* serovars from orange juice using universal 356 preenrichment broth. *Journal of Food protection*, 64, 659-663.
- 357 Hsin-Yi, C., & Chou, C. C. (2001). Acid adaptation and temperature effect on the
- 358 survival of *Escherichia coli* O157: H7 in acidic fruit juice and lactic fermented milk
- 359 product. International Journal of Food Microbiology, 70, 189-195.
- Hua, I., & Thompson, J. E. (2000). Inactivation of *Escherichia coli* by sonication at
 discrete ultrasonic frequencies. *Water Research*, 34, 3888-3893.
- 362 Huang, Y. J., Tsai, T. Y., & Pan, T. M. (2007). Physiological response and protein
- 363 expression under acid stress of Escherichia coli O157:H7 TWC01 isolated from
- 364 Taiwan. Journal of Agricultural and Food Chemistry, 55, 7182-7191.
- 365 Koutsoumanis, K. P., & Sofos. J. N. (2004). Comparative acid stress response of
- 366 Listeria monocytogenes, Escherichia coli O157:H7 and Salmonella Typhimurium

- after habituation at different pH conditions. *Letters in Applied Microbiology*, 38, 321368 326.
- 369 Leyer, G. J., Wang, L. L., & Johnson, E. A. (1995). Acid Adaptation of Escherichia-
- 370 Coli O157-H7 increases Survival in Acidic Foods. Applied and Environmental
- 371 *Microbiology*, 61, 3752-3755.
- 372 Oyarzabal, O. A., Nogueira, M. C. L., & Gombas, D.E. (2003). Survival of
- 373 Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella in juice
- 374 concentrates. *Journal of Food Protection*, 66, 1595-1598
- 375 Piyasena, P., Mohareb, E., & McKellar, R. C. (2003). Inactivation of microbes using
- 376 ultrasound: a review. International Journal of Food Microbiology, 87, 207-216.
- 377 Reinders, R.D., Biesterveld, S., & Bijker, P.G.H. (2001). Survival of Escherichia coli
- 378 O157 : H7 ATCC 43895 in a model apple juice medium with different concentrations
- 379 of proline and caffeic acid. *Applied and Environmental Microbiology*, 67, 2863-2866.
- 380 Rowe, M. T., & Kirk, R. (1999). An investigation into the phenomenon of cross-
- 381 protection in *Escherichia coli* O157 : H7. *Food Microbiology*. 16, 157-164.
- 382 Sala, F., Burgos, J., Condo'n, S., Lopez, P., & Raso, J. (1995) in: New Methods of
- 383 Food Preservation, Blackie Academic & Professional, London, pp. 176–204
- 384 Salleh-Mack, S. Z., & Roberts, J. S. (2007). Ultrasound pasteurization: The effects of
- 385 temperature, soluble solids, organic acids and pH on the inactivation of *Escherichia*
- *coli* ATCC 25922. *Ultrasonics Sonochemistry*. 14, 323-329.
- Shinoda, Y., Murata, M., Homma, S., & Komura, H. (2004). Browning and
 decomposed products of model orange juice. *Bioscience Biotechnology and Biochemistry*, 68, 529-536.

- 390 Tiwari, B. K., Muthukumarappan, K., O'Donnell, C. P., & Cullen, P.J. (2008).
- 391 Colour degradation and quality parameters of sonicated orange juice using response
- 392 surface methodology. *LWT- Food Science and Technology*, 41(10), 1876-1883.
- 393 Tsukamoto, I.; Yim, B.; Stavarache, C. E.; Furuta, M.; Hashiba, K.; Maeda, Y.
- 394 (2004). Inactivation of *Saccharomyces cerevisiae* by ultrasonic irradiation. *Ultrasonic*
- 395 Sonochemistry. 11, 61-65.

396 Ugarte-Romero, Feng, E., H. & Martin, S. E. (2007). Inactivation of Shigella boydii

18 IDPH and Listeria monocytogenes Scott A with power ultrasound at different

- acoustic energy densities and temperatures. *Journal of Food Science*, 72, M103M107.
- 400 Ugarte-Romero, Feng, E., H., Martin, S. E., Cadwallader, K. R., & Robinson S. J.
- 401 (2006). Inactivation of Escherichia coli with power ultrasound in apple cider. *Journal*
- 402 *of Food Science*, 71,E102-E108.
- 403 USFDA. U. S. Food & Drug Administration. (2001). Hazard analysis and critical
- 404 control point (HACCP); procedures for the safe and sanitary processing and importing
- 405 of juice; final rule(21 CFR 20). p. 6137-6202. *In*, Federal register, vol. 66.
- 406 Villamiel, M., & de Jong, P. (2000). Inactivation of Pseudomonas fluorescens and
- 407 *Streptococcus thermophilus* in Trypticase (R) Soy Broth and total bacteria in milk by
- 408 continuous-flow ultrasonic treatment and conventional heating. *Journal of Food*409 *Engineering*, 45,171-179.
- 410 Yuk, H. G., & Marshall, D. L. (2004). Adaptation of Escherichia coli O157:H7 to pH
- 411 alters membrane lipid composition, verotoxin secretion, and resistance to simulated
- 412 gastric fluid acid. *Applied and Environmental Microbiology*, 70(6), 3500-3505.

413	Zen	ker, M.	, Heinz,	V., &	k Kno	rr, D. (2005).	Ult	rasound	assiste	ed thermal	processi	ing
414	for	energy	saving	and	mild	preservation	of	liquid	food.	Available	online	at
415	http	://www	.sea-acus	stica.e	s/Sevi	lla02/ult05007	.pdf	f				
416												
417												
418												
419												
420												
421												
422												
423												
424												
425												
426												
427												
428												
429												
430												
431												
432												
433												
434												
435												
436												
437												

438	Figure Captions
439	Figure 1: Effect of amplitude levels on the inactivation of <i>E. coli</i> (a) ATCC 25922,
440	(b) NCTC 12900
441	Figure 2: Effect of media on <i>E. coli</i> ATCC 25922 inactivation using 0.4µm amplitude.
442	
443	
444	
445	
446	
447	
448	
449	
450	
451	
452	
453	
454	
455	
456	
457	
458	
459	
460	
461	
462	







Table 1: D-values and R² values for ultrasound treatment of control and acid-

507 adapted E. coli ATCC 25922

Amplitude	Contro	ol	1 hour	•	4 hou	r	18 hou	r
(µm)								
	D-value	R ²	D-value	R ²	D-value	R ²	D-value	\mathbf{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	$3.44 \pm 0.03^{\circ}$	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
508 Dif	ferent letters indicate	a signific	ant difference at th	ne 0.05 le	evel			
509								
510								
511								
512								
513								
514								
515								
516								
517								
518								
519								
520								
521								
522								
523								

Table 2: D-values and R² values for ultrasound treatment of control and acid-

525 adapted E. coli NCTC 12900

Amplitude	Contro	ol	1 hour		4 hour	•	18 hour	•
(µm)								
	D-value	\mathbf{R}^2	D-value	\mathbf{R}^2	D-value	\mathbf{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
526 Differe	nt letters indicate a	significar	t difference at the (0.05 level				
527								
528								
529								
530								
531								
532								
533								
534								
535								
536								
537								
538								
539								
540								
541								

Table 3: D-values and R² values for ultrasound treatment of *E. coli* ATCC 25922
in TSB and model orange juice

545 Amplitude TSB Model Orange Juice 546 (μ m)						
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	545	Amplitude	TSE	3	Model Orar	nge Juice
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	546	(μm)				
548 0.4 13.73±0.9 0.99 14.85±0.1 0.94 549 7.5 3.44±0.03 0.99 2.92±0.7 0.90 550 37.5 2.23±0.1 0.99 2.45±0.68 0.93 551 552 555 555 555 555 555 556 556 557 558 559 560 561 563 564 565 565 566 565 566 564 565 566	547		D-value	R ²	D-value	R ²
549 7.5 3.44±0.03 0.99 2.92±0.7 0.90 550 37.5 2.23±0.1 0.99 2.45±0.68 0.93 551 552 553 554 555 555 555 556 557 558 559 560 560 561 563 564 563 564 565 565 566 565 566 565	548	0.4	13.73±0.9	0.99	14.85±0.1	0.94
550 37.5 2.23±0.1 0.99 2.45±0.68 0.93 552 553 553 555 556 557 558 559 560 561 562 563 563 564 566 565	549	7.5	3.44±0.03	0.99	2.92±0.7	0.90
551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566	550	37.5	2.23±0.1	0.99	2.45±0.68	0.93
552 553 554 555 556 557 558 559 560 561 562 563 564 565 566	551					
553 554 555 556 557 558 559 560 561 562 563 564 565 566	552					
554 555 556 557 558 559 560 561 562 563 564 565 566	553					
555 556 557 558 559 560 561 562 563 564 565 566	554					
556 557 558 559 560 561 562 563 564 565 566	555					
 557 558 559 560 561 562 563 564 565 566 	556					
 558 559 560 561 562 563 564 565 566 	557					
 559 560 561 562 563 564 565 566 	558					
 560 561 562 563 564 565 566 	559					
 561 562 563 564 565 566 	560					
562 563 564 565 566	561					
563 564 565 566	562					
564 565 566	563					
565 566	564					
566	565					
	566					

567 Table 4: D-values and R² values for ultrasound treatment of *E. coli* ATCC 12900

- 568 in TSB and model orange juice
- 569

Amplitude	TSB	5	Model Orange Juice			
(µm)						
	D-value	\mathbf{R}^2	D-value	\mathbf{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		