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2009-07-01

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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.

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

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

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

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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

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
82 and amplitude of ultrasound waves, as well as temperature and viscosity of the liquid
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
86 mL^{-1} with ultrasound in apple cider (D'Amico, Silk, Wu & Guo, 2006) and the
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
92 *Listeria monocytogenes* were reduced by 5 log CFU mL^{-1} when processed with
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 α 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.
99 Bacteria are exposed to stresses in all areas of the food chain. In the case of fruit juice
100 processing, 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). Leyer, 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 non-
117 toxigenic *E. coli* O157:H7.

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
128 Health, Dublin Institute of Technology. *E. coli* NCTC 12900 obtained from National
129 Collection of Type Cultures, Health Protection Agency, London, UK. Strains were
130 maintained as frozen stocks at -70ºC 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 37ºC to obtain single colonies before
133 storage at 4ºC. A single colony was inoculated into tryptic soya broth (TSB, Scharlau
134 Chemie) and incubated overnight at 37ºC. Working cultures were prepared from this
135 sub-culture, adjusted to 0.5 McFarland turbidity (Biomerieux Inc.) and serially diluted
136 to yield the required concentration of 1 x 10^6 CFU mL^{-1} in TSB or model fruit juices.
137

138 *2.3 Acid adaptation of bacterial cultures*

139 Acid-adapted cells were prepared using the protocol by Leyer et al. (1995) with some
140 modifications. Cultures of the appropriate *E. coli* strain, grown from a single colony
141 in 5 mL TSB at 37ºC for 18h, were harvested by centrifugation (5000rpmX12min)
142 and washed twice with sterile phosphate buffered saline (PBS, Oxoid, U.K). The
143 pellet was re-suspended in 10 ml TSB (pH 5.0, adjusted with 1N HCl) and incubated
144 at 37ºC for periods of 1 h, 4 h or18 h.
145

146 *2.4 Model orange juice and Model apple juice*

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

150 1.0 g; ascorbic acid: 30 mg; L-serine: 7.0 mmol; L-asparagine: 5.4 mmol, L-alanine:
151 1.9 mmol; L-arginine: 0.75 mmol; L-glutamic acid: 0.54 mmol; L-proline: 0.42 mmol.
152 Model apple juice (MAJ) was prepared in the laboratory as per the method described
153 by Reinders, Biesterveld and Bijker, (2001). The composition of MAJ per 1000 ml
154 was as follows: fructose: 66 g; glucose: 22 g; sucrose: 27 g; sorbitol: 6.0 g; malic acid:
155 6.0 g; sodium citrate: 0.07 g; K_2HPO_4:$3H_2O$: 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 0.4µm, 7.5µm and 37.5 µm 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
167 heat generated during ultrasound treatment, and temperatures were maintained below
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 37ºC for 24h. D-values were calculated
173 using linear regression of the survivor curves for each ultrasound treatment.

174 *2.7 Statistical analysis*

175 Statistical analysis was performed using SPSS 15.0 (SPSS Inc., Chicago, U.S.A).
176 Data represent the means of experiments performed in duplicate and replicated at least
177 twice. Means were compared using ANOVA followed by LSD testing at $p < 0.05$
178 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
188 12900 by 1.1 log cycles (Fig. 1b) within 15 minutes. Ultrasonication for 15 minutes
189 at 7.5 µm amplitude resulted in reduction of *E. coli* ATCC 25922 by 4.4 log cycles
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 µm and 37.5 µm 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 µm 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*
216 ATCC 25922 (Table 1). At 7.5 µm amplitude, there was no significant effect of

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
238 *E. coli* studied (*E. coli* ATCC 25922, *E. coli* NCTC 12900) were found to be sensitive
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
244 between D-values obtained in TSB and model orange juice. However, for *E. coli*
245 NCTC 12900, there were significant differences between D-values obtained in TSB
246 and model orange juice at all level of amplitudes.

247 *3.4 Ultrasound inactivation of E. coli ATCC 25922 in model apple juice*

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

253 **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
266 physical and chemical mechanisms which occur during cavitation. At higher

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 µm 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 µm and 37.5 µm) of the ultrasound
296 treatment negated any cell condition effects.
297 Ultrasound inactivation of bacteria has been found to be dependent upon the solution
298 which is under study. Salleh-Mack & Roberts, (2007) investigated the effect of
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 > 5log reduction within 15 minutes. Temperatures employed in this
311 study were maintained below 30ºC so as to utilize lower processing temperature than
312 that used for thermal pasteurization.

313 **5. Conclusion**

314 The results of this study indicate that power ultrasound treatment has potential for
315 inactivation of key microorganisms of concern in fruit juice processing. Ultrasound
316 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**

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

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438 **Figure Captions**

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

440 (b) NCTC 12900

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

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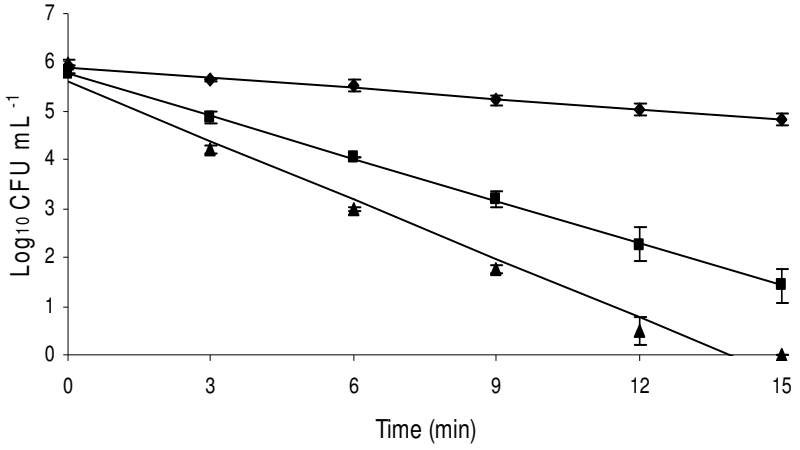
465 (a)



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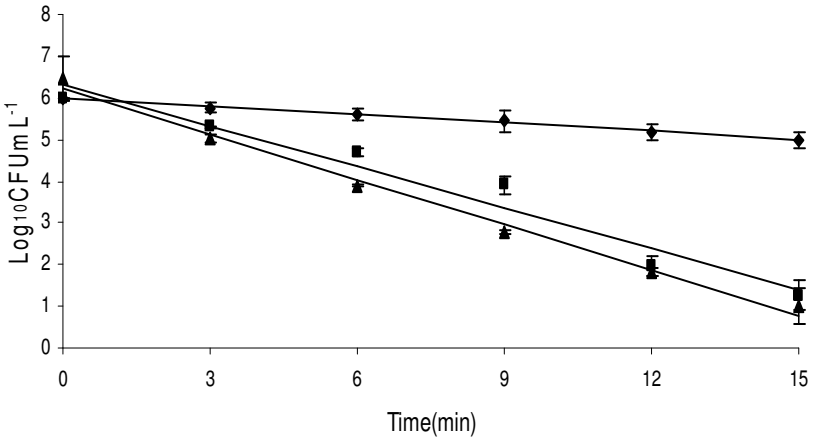

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480 **Figure 1: ♦ 0.4µm amplitude, ■ 7.5 µm amplitude and ▲ 37.5 µm amplitude**

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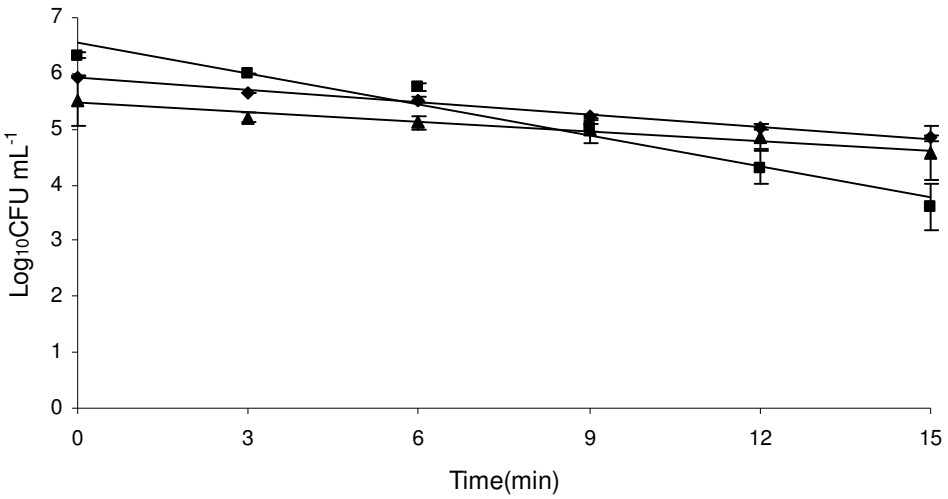

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490 **Figure 2: ♦ TSB, ■ Model apple juice and ▲ Model orange juice**

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506 **Table 1: D-values and R^2 values for ultrasound treatment of control and acid-**

507 **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

508 Different letters indicate a significant difference at the 0.05 level

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524 **Table 2: D-values and R^2 values for ultrasound treatment of control and acid-**

525 **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

526 Different letters indicate a significant difference at the 0.05 level

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542 **Table 3: D-values and R^2 values for ultrasound treatment of *E. coli* ATCC 25922**

543 **in TSB and model orange juice**

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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

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567 **Table 4: D-values and R^2 values for ultrasound treatment of *E. coli* ATCC 12900**

568 **in TSB and model orange juice**

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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