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Effect of Ultrasonic Processing on Food Enzymes of Industrial Importance

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1 **Effect of ultrasonic processing on food enzymes of industrial importance**

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Abstract

In the last decade power ultrasound has emerged as an alternative processing option to conventional thermal approaches for pasteurisation and sterilisation of food products. While sonication alone is not adequate for inactivation of various spoilage and harmful enzymes present in food, ultrasound in combination with mild heat treatment and/or pressure has shown potential for both enzyme and pathogen inactivation. Numerous studies have investigated ultrasound for inactivating enzymes such as pectinmethylesterase, polyphenoloxidases and peroxidases responsible for deterioration of fruit & vegetable juice and various enzymes pertinent to milk quality. The efficacy of ultrasound for the inactivation of enzymes in food is outlined in this review along with a description of the inactivation mechanism to elucidate the effect of ultrasound on important enzymes in fruit juices and dairy products.

Keywords: Ultrasound; Enzyme; Inactivation; Dairy; Fruit juices

32 **Introduction**

33 Thermal treatment is the most common and widely employed pasteurisation and
34 sterilisation technique for the inactivation of micro-organisms and enzymes in the
35 food industry. Consumer demands for higher quality products have inspired
36 researchers and the food industry to investigate novel processing technologies to
37 replace traditional processing methods (Awuah, Ramaswamy, & Economides, 2007).
38 The application of the low frequency high power ultrasound (≤ 0.1 MHz, 10-1000
39 W.cm⁻²) in the food industry has been widely investigated over the last decade.
40 Current and potential applications of ultrasound in the food processing industry have
41 been extensively reviewed (Knorr, Zenkar, Heinz, & Lee, 2004; Mason, Paniwnyk, &
42 Lorimer, 1996; McClements, 1995).

43 Power ultrasound has been reported to be sufficient to meet the FDA's
44 mandatory 5 log reduction of food borne pathogens in fruit juices. Ultrasound alone or
45 in combination with mild temperature is reported to be effective against *E. coli* in
46 model fluids (Salleh-Mack & Roberts, 2007) and apple cider (Ugarte-Romero, Feng,
47 Martin, Cadwallader, & Robinson, 2006) and *Listeria monocytogenes* in apple cider
48 (Baumann, Martin, & Feng, 2005). Ultrasound alone or in combination with heat
49 (thermosonication) or pressure (manosonication) or both heat and pressure
50 (manothermosonication) is reported to be effective against various food enzymes
51 pertinent to the dairy and fruit juice industry such as lipoxygenase, peroxidase, and
52 polyphenol oxidase, as well as heat-resistant lipase and protease (López, Sala, de la
53 Fuente, Condon, Raso, & Burgos, 1994; López & Burgos, 1995a,b; Vercet, Lopez, &
54 Burgos, 1997; Villamiel, & de Jong, 2000). Inactivation of pathogenic and spoilage
55 microorganisms or enzymes by sonication is mainly caused by physical (caviation,

56 mechanical effects) and/or chemical (formation of free radicals due to sonochemical
57 reaction) principles.

58 Sonication alone or in combination with thermal processing is reported to be effective
59 against various other enzymes of industrial importance. Coakley, Brown & James
60 (1973) investigated the inactivation of alcohol dehydrogenase, catalase, and lysozyme
61 by exposure to 20 kHz ultrasound in a model solution. They observed an exponential
62 inactivation for alcohol dehydrogenase and lysozyme, however minor effects were
63 observed for catalase. Conversely, Mañas, Muñoz, Sanz, & Condón (2006) reported
64 that sonication at ambient temperature and atmospheric pressure had no significant
65 effect on the activation of lysozyme. However the desired inactivation was achieved
66 at elevated temperatures (60 – 80 °C) and pressure (200 kPa). The enzyme
67 inactivation behaviour in real food systems may be considerably different due to
68 presence of other food components. Kadkhodae & Povey (2008) investigated the
69 inactivation of α -amylase by thermosonication and reported a reduced activation
70 energy (19.27 kJ/mol K) compared to thermal inactivation (109 kJ/mol K). They
71 observed that the activation energy values for ultrasonic treatment were dependent on
72 the emitting face of the probe and gas content of the medium. The effectiveness of
73 ultrasound for control of enzymatic activity is strongly influenced by intrinsic and
74 extrinsic factors such as enzyme concentration, temperature, the pH and composition
75 of the medium. However, in some cases of enzyme inactivation using sonication, it is
76 unclear whether this may attributed solely to the process of enzyme dissociation into
77 subunits as observed with thermal inactivation.

78 Ultrasonic processing of fruit juices has minimal effects on the quality of fruit juices
79 such as orange juice (Velero, Recrosio, Saura, Munoz, Martic & Lizama, 2007),
80 guava juice (Cheng, Soh, Liew, & Teh, 2007) and strawberry juice (Tiwari,
81

82 O'Donnell, Patras, Brunton, & Cullen, 2009a). It is also reported to enhance cloud
83 value and stability of orange juice during storage (Tiwari, O'Donnell,
84 Muthukumarappan, & Cullen, 2009b). Recently, Piyasena, Mohareb & McKellar
85 (2003) and Jiranek, Grbin, Yap, Barnes & Bates (2008) comprehensively reviewed
86 the potential of ultrasound for inactivation of various food borne pathogens. Tiwari et
87 al., (2008) reviewed the effect of ultrasound processing on quality of fruit juices.
88 However, to date the effects of ultrasound on the inactivation of enzymes causing
89 quality deterioration of food have not been comprehensively reviewed. The objective
90 of this paper is to review recent literature on the potential of power ultrasound for the
91 inactivation of enzymes of industrial importance in the dairy and fruit juice industries.

92

93 **Generation of power ultrasound**

94 Ultrasound is a form of vibrational energy in the frequency range of 20–100 kHz with
95 a sound intensity of 10 to 1000 W/cm². Generally, power ultrasound employed in food
96 processing uses lower frequencies (20 to 100 kHz) and causes cavitation with sound
97 intensities of 10 to 1000 W/cm² (Feng and Yang 2005). The ultrasonic transducers
98 convert electrical or mechanical energy to sound energy. There are three types of
99 ultrasonic transducers in common usage including liquid-driven transducers,
100 magnetostrictive transducers and piezoelectric transducers (Mason, 1998), with
101 piezoelectric being the most common. For ultrasonic baths, power is often low in
102 order to avoid cavitation damage to the tank walls and the power density is low due
103 to large volume or processing liquid.

104 When high power ultrasound propagates in a liquid, cavitation bubbles will be
105 generated due to pressure changes. These micro bubbles will collapse violently in the
106 succeeding compression cycles of a propagated sonic wave. This results in regions of

107 high localized temperatures up to 5,000 K and pressure of up to 50,000 kPa, resulting
108 in high shearing effects (Mason, 1991; Piyasena et al., 2003) and a localized
109 sterilization effect.

110 The ultrasound power level or energy transmitted to a food medium can be expressed
111 as ultrasound power (W), ultrasound intensity (W/cm^2), acoustic energy density
112 (W/mL) or cavitation intensity. The sonication treatment and the cavitation activity
113 in a treatment chamber may vary for the same ultrasound intensity if the sample
114 volume and probe location change. Recently, volumetric acoustic energy density
115 (W/cm^3 or W/mL) has been widely employed to indicate the ultrasonic power level.

116 Cavitation intensity can be estimated by measuring hydrogen peroxide (H_2O_2)
117 formation in distilled water during sonication following a catalyzed colorimetric
118 procedure (Mead, Sutherland, & Verrall, 1976). However, the determination of H_2O_2
119 generation during an ultrasound treatment in a food system is complex due to the
120 presence of food components including ions and other colloidal components. To date,
121 no reliable method to measure cavitation activity in a food system has been developed
122 (Raviyan *et al.*, 2005). Tsukamoto et al. (2004) reported that the measurement of
123 ultrasound amplitude is an indication of the ultrasonic cavitation and is also a reliable
124 method for indication of the ultrasound power.

125 Ultrasonic intensity or acoustic energy density can be determined calorimetrically
126 (Mason *et al.*, 1990) using Equations 1-3. The absolute ultrasonic power P is given as:

$$127 \quad P = mc_p \left(\frac{dT}{dt} \right)_{t=0} \quad (1)$$

128 Where, m is the mass, c_p is the specific heat capacity and (dT/dt) is the rate of change
129 of temperature during sonication which can be determined by polynomial curve fitting

130 to the temperature rise vs. time under adiabatic conditions using a standard
131 thermocouple.

132 The intensity of ultrasonic power dissipated from a probe tip with diameter D is given
133 by (Mason *et al.*, 1990)

$$134 \quad UI = \frac{4P}{\pi D^2} \quad (2)$$

135 Acoustic energy density or volumetric energy density can be determined by dividing
136 absolute ultrasound power with the volume (V) of the medium (cm^3 or mL)

$$137 \quad AED = \frac{P}{V} \quad (3)$$

138

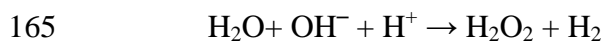
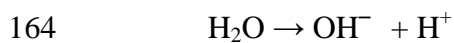
139 **Mechanism of inactivation**

140 In general most studies reported that prolonged exposure periods were
141 necessary to inactivate enzymes using high-intensity ultrasound. However some
142 authors have reported that ultrasound has no impact on certain enzymes while others
143 have demonstrated that acoustic cavitation induced by ultrasound waves both
144 physically and chemically affects enzymes (Kadkhodae & Povey, 2008).
145 Denaturation of protein is mainly responsible for inactivation of enzymes either by
146 free radicals in sonolysis of water molecules ($\text{H}_2\text{O} \rightarrow \text{OH}^- + \text{H}^+$) or shear forces
147 resulting from the formation or collapse of cavitating bubbles (Mason *et al.*, 1994;
148 Suslick, 1988).

149 The intensity of ultrasound applied, strongly influences the effect of sonication on
150 enzyme activity. Researchers (Sakakibara, Wang, Takahashi, Takahashi, & Mori
151 1996; Choi & Kim, 1994) have reported that the activity of free enzymes increases
152 under mild ultrasound irradiation. Selection of appropriate ultrasonic processing
153 parameters can enhance enzymatic assisted processes. Şener, Apar & Özbek (2006)

154 increased the rate of lactose hydrolysis in milk using ultrasound at an acoustic power
155 level of 20 W, duty cycle of 10% and enzyme concentration of 1 mL/L, resulting in a
156 minor loss (25 %) of enzyme activity. Application of ultrasound assists biochemical
157 processes through reduced consumption of enzymes, shorter process times and
158 improved uniformity of treatment (Basto, Tzanov, & Cavaco-Paulo, 2007). Many
159 mechanisms have been proposed for microbial and enzymatic inactivation in foods
160 (Table 1). Cavitation intensity is the most widely reported inactivation mechanism.
161 Cavitation intensity is measured as the rate of H₂O₂ generation, which is formed as
162 follows:

163



166

167 H₂O₂ production is strongly influenced by processing temperature and sample volume
168 (Raviyan, Zhang, & Feng, 2005). Cavitation activity decreases at higher
169 temperatures due to a reduced cavitation threshold, resulting in lower temperatures
170 and pressures upon bubble collapse (Mason & Lorimer, 2002).

171 Reported inactivation mechanisms are directly or indirectly dependent on
172 processing variables such as sonotrode type and geometry, frequency and acoustic
173 energy density. Media properties including treatment volume and gas concentration
174 also affect the efficiency of enzyme inactivation (Kadkhodae & Povey, 2008; Raso,
175 Pagan, Manas, Pagan, & Sala, 1999).

176 Özbek, & Ülgen (2000) reported that ultrasonic inactivation mechanisms are
177 specific to the enzyme under investigation and depend on amino acid composition and
178 the conformational structure of the enzyme. For example manothermosonication is

179 reported to inactivate peroxidase by splitting its prosthetic heme group, as for the
180 mechanism of heat inactivation (Lopez & Burgos, 1995a), whereas lipoxygenase
181 appears to be inactivated by a free radical mediated mechanism (Lopez & Burgos,
182 1995b) and possibly by denaturation of proteins (Mason, 1998). Some enzymes, such
183 as catalase, yeast invertase, or pepsin are resistant to ultrasound (Sala, Burgos,
184 Condon, Lopez, & Raso, 1995).

185 **Fruit juice enzymes**

186 *Pectinmethylesterase*

187 Pectinmethylesterase (PME), an ubiquitous enzyme found in plants, hydrolyses pectin
188 resulting in decreased cloud stability and reduced viscosity due to pectin chain
189 degradation. Ultrasound was reported to inactivate PME in tomato juice and orange
190 juice (Kuldiloke, 2002, López, Vercet, Sanchez, & Burgos, 1998, Vercet, Lopez, &
191 Burgos, 1999 and Vercet, Oria, Marquina, Crelier, & Lopez-Buesa, 2002) in
192 combination with heat and/or pressure. López et al. (1998) reported that the *D*-value
193 of tomato PME was reduced from 45 min for thermal treatment to 0.85 min for
194 manothermosonication at the same temperature (62.5 °C). Raviyan et al. (2005)
195 reported a similar reduction in *D* value from 1571.4 min for thermal treatment to <
196 80 min for thermosonication at the same temperature (50 °C). The *D* value was further
197 reduced from 240.6 min to 1.5 min with an increase in temperature from 50 to 61 °C
198 at a cavitation intensity of 0.007 mg.L⁻¹.min⁻¹ (Raviyan et al. 2005). Wu, Gamage,
199 Vilku, Simons, & Mawson, (2008) reported a reduction in *D* value for PME
200 inactivation at 60 and 65 °C compared to those observed for thermal inactivation.
201 However, they did not observe this synergy at 70 °C, where the *D* values for thermal
202 and thermosonication treatment were similar.
203

204 A number of studies have reported that sonication in combination with either heat or
205 pressure has a synergistic effect on PME inactivation. Raviyan *et al.*, (2005) reported
206 increased inactivation of PME in sonicated tomato juice for a temperature range of 50
207 – 72 °C compared to thermal treatment alone. Increased inactivation was dependent
208 on cavitation intensity which is reported to be temperature dependent. For example,
209 simultaneous applications of heat (72 °C) and ultrasound (frequency of 20 kHz and
210 amplitude of 117 µm) under moderate pressure (200 kPa) increased the inactivation
211 rate of orange juice PME by a factor of 25 in a buffer solution, and by more than a
212 factor of 400 in orange juice (Vercet, Lopez, & Burgos, 1999). Higher inactivation
213 rates in juice could be either due to the presence of co-solutes (substrates or other
214 molecules that physically interact with enzymes) or loss of the protective effect of
215 pectin in orange juice to which PME is bound (Vercet, Lopez, & Burgos, 1999). The
216 effect of pectin on PME inactivation is also reported during orange juice ultrafiltration
217 (Snir *et al.* 1995). Raviyan *et al.*, (2005) reported that the increase in enzyme
218 inactivation during thermosonication is more pronounced at lower temperatures. One
219 possible explanation for this could be that at higher temperatures, increased vapour
220 pressure inside the bubbles introduces a cushioning effect and hence produces less
221 effective bubble collapse (Mason, 1990). Tiwari *et al.* (2008) concluded that
222 sonication alone is not sufficient to inactivate PME. The maximum PME inactivation
223 level reported for orange juice sonicated at the highest acoustic energy density of 1.05
224 W/mL for 10 min was 62% (Figure 1).

225 The reduction of PME activity in sonicated lemon juice resulted in enhanced cloud
226 stability during storage for 18 days at 4 °C compared to thermally processed lemon
227 juice (Knorr *et al.* 2004). The improved cloud stability observed during storage could
228 be due to the mechanical damage of the PME protein structure during sonication.

229

230 ***Polyphenoloxidase***

231 Polyphenoloxidase (PPO) is a copper-containing enzyme that causes enzymatic
232 browning in fresh fruits and vegetables products such as juices. Enzymatic browning
233 is one of the biggest problems faced during the processing of fruits and vegetables
234 (Yemenicioglu & Cemeroglu, 2003). PPO is not an extremely heat stable enzyme, and
235 short exposure to temperatures between 70 and 90 °C is sufficient to inactivate it.
236 Cheng *et al.* (2007) reported an increase in PPO in sonicated (35 kHz; for 30 min)
237 guava juice compared to control. They observed an increase in enzymatic activity
238 possibly due to the processing conditions employed. Cheng *et al.* (2007) employed a
239 standard ultrasonic bath for inactivation studies. Sonication baths are generally of low
240 power in order to avoid cavitation damage to the tank walls, consequently the
241 acoustic energy density is low due to large volume. However, a low ultrasound power
242 level as in this case can enhance the disruption of biological cell walls to facilitate the
243 release of their contents, indeed many ultrasonic horn systems were first marketed as
244 cell disruptors (Mason *et al.*, 1996). Moreover, low power levels can induce
245 stimulation of enzymes whereas, higher power levels inactivate enzymes due to
246 denaturation.

247 A synergistic effect of heat and pressure with ultrasound has been reported for the
248 inactivation of PPO in model buffer systems (Lopez *et al.*, 1994). They reported a
249 linear decrease in log D values for an increase in ultrasound amplitude level over the
250 range 35 – 145 μm . Heat or pressure assisted ultrasonic processing of juice can
251 substantially reduce enzyme resistance and the heat treatment required for
252 inactivation. As discussed earlier, the enzyme inactivation mechanism is complex and

253 depends upon several factors such as fruit juice composition, enzyme type, pH and
254 processing parameters.

256 ***Peroxidases***

257 Peroxidase (POD) is a heme-containing enzyme which can be used to evaluate the
258 efficiency of vegetable blanching (Lopez *et al.*, 1994) because of its relatively high
259 thermal stability. POD which is found in most raw and unblanched fruit and
260 vegetables, is associated with the development of off-flavours and browning
261 pigments. Thermosonication has been reported to reduce the blanching time required
262 for inactivation of POD in watercress; for example to obtain 90% POD inactivation at
263 90 °C, a thermal treatment time of 70 s is necessary compared to 5 s for
264 thermosonication treatment at the same temperature (Cruz, Vieira, & Silva 2006). De
265 Gennaro, Guerrero, Lopez-Malo, & Alzamora (1999) reported first order inactivation
266 kinetics for POD during sonication. This could be due to the cushioning effect of
267 cavitating bubbles which are formed under the tip of sonotrode, acting as a barrier to
268 the solution during sonication (Ratoarinoro, Contamine, Wilhem, Berlan & Delmas,
269 1995). Cruz *et al.*, (2006) reported an increase in POD activity during blanching of
270 watercress (*Nasturtium officinale*) for thermosonication in a temperature range of 40 –
271 80 °C and a decrease in enzymatic activity at a higher temperature range of 82.5 –
272 92.5 °C. They observed a higher rate of inactivation for combined ultrasound and heat
273 treatment compared to heat treatment alone. They reported an increase in the POD
274 enzyme activity due to sonication at low temperatures, which could be related with
275 the change of conformation of the enzyme to a higher enzyme–substrate interaction.
276 Similarly the reduction in enzyme activity at higher temperatures could also be related
277 to the conformation changes in the tertiary structure. Further, the POD enzyme

278 system, found in watercress, is formed by a heat-labile fraction and a heat-resistant
279 fraction. However, thermal inactivation of POD can be either by dissociation of the
280 prosthetic (heme) group from the haloenzyme (active enzyme system),
281 conformational changes in protein or by modification or degradation of the prosthetic
282 group (Lemos, Oliveira, & Saraiva, 2000). Inactivation of POD due to sonication
283 results from conformational changes in protein and by splitting of prosthetic group
284 from haloenzyme (Lopez & Burgos, 1995a). It is difficult to identify the specific
285 enzyme inactivation mechanism during sonication which could be due to a singular or
286 combination of several chemical and physical effects occurring simultaneously (Table
287 1).

288 *Lipoxygenase*

289 Lipoxygenase (LOX) activity in fruit and fruit products is reported to be related to
290 oxidation of fatty acids and pigments. LOX catalyzes the oxidation of polyunsaturated
291 fatty acids containing a cis, cis-1,4-pentadiene system, which produces 9- or 13-cis,
292 trans-hydroperoxides. LOX has been associated with quality deterioration because of
293 its negative effects on pigments such as carotenes during storage, and its role in off-
294 flavour and odour production (King & Klein, 1987; Aguiló-Aguayo, Sobrino-López,
295 Soliva-Fortuny, & Martín-Belloso, 2008). However, in fruit juices a minimum LOX
296 activity may be desirable for long storage periods (Min, Min & Zhang 2003). Thakur
297 & Nelson (1997) reported a 75 to 85% inactivation of LOX in soybeans by
298 ultrasound. Inactivation was strongly dependent on pH, treatment time and ultrasonic
299 frequency. Similarly Lopez and Burgos (1995a) reported that the resistance of LOX
300 against heat and manothermosonication was also pH dependent during sonication over
301 an amplitude range of 0-104 μm and a temperature range of 67.5-76.3 $^{\circ}\text{C}$. pH

302 dependency is mainly due to the profound effects of pH on protein conformation with
303 all enzymes having a maximum stability at an optimum pH .

305 **Dairy Enzymes**

306 Sonication of milk is reported to result in a diversity of physicochemical changes in
307 macromolecules including enzyme inactivation, homogenisation (Villamiel & de
308 Jong, 2000), reduction in fermentation time during yogurt preparation (Wu *et al.*,
309 (2001) and improvement of yoghurt rheological properties (Vercet *et al.*, 2002).
310 Applications of ultrasound in the dairy industry have been reviewed by Villamiel, van
311 Hamerveld, & de Jong (1999). Although many pathogenic and spoilage micro-
312 organisms are easily destroyed under standard heat treatments, many of them produce
313 extracellular lipase and protease, which can withstand UHT treatment (Stead, 1986).
314 These thermoresistant enzymes can reduce the quality and shelf-life of heat-treated
315 milk and other dairy products. The simultaneous application of heat and ultrasound
316 under pressure (manothermosonication) has been found to be more effective than heat
317 treatment alone in the inactivation of heat resistant protease and lipase secreted by *P.*
318 *fluorescens* (Vercet, López, & Burgos 1997). The effect of ultrasound on enzymes
319 involved in the coagulation of milk such as chymosin, pepsin, and several fungal
320 enzymes has been studied in model systems using batch processes. In general, after
321 long (several minutes) ultrasonic treatments, the proteolytic activity of the enzymes
322 investigated decreased. However, when a mixture of milk and chymosin was
323 sonicated, minimal enzyme inactivation was observed (Raharintsoa, Gaulard, & Alais,
324 1977, 1978). It has been reported that enzyme inactivation increases with an increase
325 in solids content and decreases with increase in enzyme concentration (Sala *et al.*,
326 1995; Villamiel, & de Jong, 2000).

327

328 Villamiel & de Jong (2000) outlined the effect of ultrasound on native milk enzymes
329 (Table 2). No effect on milk enzymes was observed when ultrasound was applied
330 without thermal treatment. However inactivation effects were reported when
331 sonication was carried out above 61 °C. Differences observed in the inactivation of
332 the native milk enzymes such as alkaline phosphatase, γ -glutamyltranspeptidase,
333 lactoperoxidase, whey proteins (α -lactalbumin and β -lactoglobulin) in whole and skim
334 milk were attributed to factors relating to the composition of the medium.

335 Villamiel and Jong (2000) reported that the resistance of enzymes to sonication is
336 both enzyme and media specific. Several studies have demonstrated that the effect of
337 ultrasonic waves increases at higher total solids concentration (Santamaria, Castellani,
338 & Levi, 1952; Sala et al., 1995). In skim milk, the concentration of solids is lower
339 than in whole milk resulting in a reduced ultrasonic effect. However, the
340 concentration of enzymes in skim milk (alkaline phosphatase, AP and gamma -
341 glutamyl transpeptidase, GGTP) is also lower than in whole milk leading to a more
342 pronounced effect, as these enzymes are linked to fat globules and can be liberated by
343 the ultrasound effect to the serum phase. Whereas, lactoperoxidase (LPO) is located in
344 the whey, and the main cause of the enhanced decrease of enzyme activity in whole
345 milk than in skim milk by the effect of ultrasound and heat (75.5 °C; 102.3 s) could be
346 due to the higher concentration of solids in the former (Villamiel and Jong, 2000).
347 Ertugay, Yuksel, & Sengul (2003) reported greater inactivation of LPO and AP
348 enzymes which have a significant function in dairy processing at 40 °C compared to
349 20 °C (Table 2).

350 The combination of sonication with heat can assist thermal processing by
351 reducing the thermal resistance of various enzymes. Prolonged exposure to high-

intensity ultrasound has been shown to inhibit the catalytic activity of a number of food enzymes due to the intense pressures, temperatures and shear forces generated by the ultrasonic waves which denature protein. However, in some cases, solutions containing enzymes have been found to have increased activity following short exposures to ultrasound (McClements, 1995). This may be due to the ability of ultrasound to break down molecular aggregates, making the enzymes more readily accessible for reaction, therefore the key enzymes of concern to each food system should be investigated to ascertain the critical control parameters which can be specific to the enzyme, the food system or both.

Inactivation kinetics

As discussed above enzyme inactivation by ultrasound is governed by various intrinsic or extrinsic factors. Predicted kinetic models should be able to establish, appropriate treatment conditions to achieve desired levels of microbial or enzymatic inactivation, facilitating the production of stable and safe foods (Mañas, & Pagán, 2005). The inactivation of enzymes during sonication has been shown to follow first-order kinetics (Equation 4) for PME in tomato juice (Ravian *et al.*, 2005), POD in water cress (Cruz *et al.*, 2006) and POD in a model solution (De Gennero *et al.*, 1999).

$$\log_e \left(\frac{N_t}{N_0} \right) = -kt \quad (4)$$

$$\frac{dN_t}{dN_o} = a \exp(-k_1 t) + (1 - a) \exp(-k_2 t) \quad (5)$$

374 Where, N_0 is the initial enzymatic activity, N_t is the enzymatic activity at time t
375 (min); k (min^{-1}) is the inactivation rate constant; k_1 & k_2 are inactivation rate constants
376 for heat-labile isoenzyme fraction (a) and a heat-resistant isoenzyme fraction ($I-a$)
377 respectively.

378 First order inactivation kinetic models are well established for describing enzyme
379 inactivation during thermal treatments assuming the media is not comprised of
380 multiple isozymes with different thermostabilities (Lopez *et al.*, (1994). Deviations in
381 enzyme inactivation from first order kinetics are due to the formation of enzyme
382 aggregates with different heat stabilities. The monophasic inactivation of enzymes
383 under manothermosonication may be attributed to the well established dissociation
384 effect of ultrasonic waves on aggregates. Similar observations were observed by
385 Vercet *et al.*, (2001) for inactivation of proteases (phospholipase A2, trypsin, α -
386 chymotrypsin) and lipases during manothermosonication. They reported that the
387 biphasic behaviour (Equation 5) observed in thermal inactivation approaches first
388 order kinetics in manothermosonication inactivation. Kinetic mechanisms for
389 inactivation of peroxidase enzymes have been proposed to explain the biphasic course
390 of thermal inactivation of peroxidase (Henley & Sadana, 1985). This phenomenon is
391 generally accepted to be due to the presence of isozymes of different heat stability.

392 Cruz *et al.*, (2006) employed a biphasic inactivation model (Equation 5) for the
393 thermal inactivation of peroxidases in water cress, formed by a heat-labile isoenzyme
394 fraction and a heat-resistant isoenzyme fraction. They showed that the dependencies
395 of k_1 and k_2 on temperature followed the Arrhenius law and first order inactivation
396 during thermosonication. Similar first order inactivation was reported by De Gennaro
397 *et al.* (1999). However the authors did not observe any appreciable increase in the rate

398 constant with respect to increase in power level. They employed an exponential decay
399 curve to model the D value for enzyme inactivation (Equation 6).

$$400 \quad D_t = D_\infty + (D_0 - D_\infty)e^{-\frac{P}{a}} \quad (6)$$

401
402 Tiwari et al. (2008) reported that the fraction conversion model (Equation 7)
403 adequately described the inactivation of PME in orange juice with respect to AED. A
404 fraction conversion model is a special case of the first-order model which can be used
405 when a fraction of the enzyme is not destroyed after prolonged treatment (A_∞) (Van
406 den Broeck et al., 2000; Ly-Nguyen et al., 2003).

$$408 \quad \frac{\log(A_t - A_\infty)}{(A_0 - A_\infty)} = -K_F t \quad (7)$$

409
410 The fraction conversion model adequately described both the inactivation of the heat
411 sensitive portion of the enzyme (thermolabile isoenzyme) along with the thermostable
412 enzyme fraction.

414 **Status review**

415 Although the potential of power ultrasound has been investigated for many food
416 applications, challenges remain prior to widespread adoption of the technology. One
417 of the difficulties reported in the literature is the non-standardised reporting of
418 methodology and control parameters. Comparable reporting in terms of energy
419 density, probe types and sample volumes is required. Generally higher enzyme
420 inactivation is reported for probe type systems compared with ultrasound baths.
421 Ultrasound technology may be employed for many food applications, such as

422 homogenization, crystallisation, extraction etc, however the synergistic effects on
423 enzymes or vice versa are generally not reported. Validation of the technique for
424 enzyme or microbial inactivation needs to deal with the complex nature of food
425 systems, in particular non-Newtonian fluids and particulate matter. Recently,
426 computational fluid dynamic (CFD) simulations have been employed to investigate
427 the influence of fluid properties on the efficacy of various non-thermal food
428 processing techniques, however this approach has not been widely adopted for
429 ultrasound processing to date.

430 Despite promising effects of sonication alone or in combination with heat or pressure,
431 scale-up also remains a significant challenge to industrial adoption. There are few
432 detailed reported industrial scale uses of power ultrasound. For application of power
433 ultrasound on an industrial scale, it is essential to have energy efficient processors.
434 For food applications the design of the probe is paramount, non contact transducers or
435 coated transducers where the construction material is non-reactive, with little or no
436 erosion are required.

437 **Conclusion**

439 Ultrasound alone or in combination with heat and/or pressure can achieve the desired
440 enzyme inactivation by reducing thermal resistance. Sonication efficacy is dependent
441 upon numerous extrinsic and intrinsic control parameters. Ultrasound processing
442 enhances enzymatic reactions at low power levels e.g. α -amylase, invertase and
443 amyloglucosidase for starch, sucrose and glycogen hydrolysis respectively (Barton,
444 Bullock and Weir, 1996) and inactivation of spoilage enzymes e.g. PME, PPO at
445 higher power levels. The lack of standardisation in ultrasound operating frequencies
446 and power levels makes comparisons between different studies difficult.
447 Consequently ambiguity arises within the literature, as these control conditions may

448 not be reported in detail or are reported differently. Although the possibility of
449 deactivating enzymes or microorganisms by ultrasonic processing has been
450 demonstrated under laboratory conditions, industrial adoption of this technology is
451 limited, due to the significant challenges encountered in industrial scale-up. Future
452 research should be focused on the development of non-contact ultrasound transducers
453 or sonication bath systems with variable frequencies and the investigation of the
454 economic feasibility of sonication as a novel food processing and preservation
455 technique.

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