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Applications of ultrasound for the functional modification of proteins and nanoemulsion formation: A review

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Highlights

- Fundamentals of low frequency high power ultrasound are outlined.

- Functional modification of proteins from ultrasonic processing is described.

- The factors involved in ultrasonic emulsification are critically discussed.

1 **Applications of ultrasound for the functional modification of proteins and**

2 **nanoemulsion formation: A review**

3

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8   **Abstract:**

9   This review surveys the most recent developments in low frequency, high power ultrasound for

10   the functional modification of proteins derived from a number of food sources (*e.g.* dairy, animal,

11   cereal, legume, tuber and fruit), and subsequently for the fabrication of nano-sized emulsion droplets.

12   Aside from an overview of the fundamentals of ultrasound, including a cursory outline of ultrasonic

13   cavitation, heat generation and acoustic energy determination via calorimetry, examples of ultrasound

14   treatment for improvements in the dissolution, hydration, hydrophobicity, emulsifying and rheological

15   performance of proteins are described. Ultrasound possesses the industrial capability to improve the

16   functional properties of proteins, and this review emphasises the improvement to the surface active

17   properties of proteins, which is attributed to decreases in protein aggregate size and increases in

18   hydrophobicity, demonstrating increased molecular mobility. Finally, the utilisation of ultrasound for

19   the fabrication of nanoemulsions is assessed with a particular focus on the intrinsic relationship between

20   process configuration (*i.e.* batch or continuous), processing parameters (*i.e.* acoustic power and

21   residence time) and emulsion formulation (*i.e.* emulsifier type and concentration). A better

22   understanding of the effect of industrially relevant high molecular weight biopolymers (*i.e.* proteins)

23   within ultrasonic emulsification processes would increase the utilisation of ultrasound as a fabrication

24   technique for nano-sized emulsion droplets.

25

28   **1. Introduction**

29   Low frequency, high power ultrasound, commonly referred to as power ultrasound, has

30   gained significant interest over the past decade as it possesses a wide range of uses within a

31   myriad of sectors making it a versatile processing technology, for the alteration, generation and

32   modification of microstructures. As a consequence, due to ultrasonic cavitation, it is capable

33   of mechanically altering the structure of proteins in solution without the use of additives

34   (chemical or biological) or excess heat, and increasing specific surface area in emulsion

35   systems for the generation nano-sized emulsion droplets (McClements, 1995; O'Brien, 2007).

36   Proteins are ingredients utilised within a wide range of formulations due to both their

37   nutritional value and functionality (O'Sullivan & O'Mahony, 2016). The term 'functionality'

38   as applied to food ingredients describes any property other than nutritional attributes that

39   contribute to an ingredient's beneficial aspects within a formulation (Damodaran, 1997).

40   Proteins are highly functional molecules within food systems capable of the stabilisation of oil

41   droplets and air bubbles, formations of gel structures and the enhancement of viscosity

42   (O'Connell & Flynn, 2007; Walstra & van Vliet, 2003). This functionality is due to the

43   complex chemical makeup of these molecules owing to their unique amino acid sequences

44   (Beverung *et al.*, 1999). Improvement to the functional properties of proteins is of great interest

45   so as to increase their commercial value, and improve utilisation of these high value

46   ingredients, which is conventionally achieved through either molecular weight modification

47   (*i.e.* proteolysis or aggregation), or conjugation/complexation with other biopolymers (Drapala

48   *et al.*, 2015; Grigorovich *et al.*, 2012; Kurukji *et al.*, 2015; Malaki Nik *et al.*, 2010; Mulcahy

49   *et al.*, 2016; O'Sullivan *et al.*, 2016).

50   As for emulsion formation, traditionally it is achieved industrially through the

51   implementation of homogenisers, usually two stages, operating at pressures up to 25 MPa

52 (McClements, 2005). Numerous technologies have shown the capacity for the fabrication of

53 nano-sized emulsion droplets, such as microfluidics, high and ultrahigh pressure valve

54 homogenisers, and membrane emulsification (crossflow and rotary) (Lee & Norton, 2013;

55 Lloyd, *et al.*, 2014). However, industry is reluctant to readily adopt these technologies due to

56 the associated capital expenditure and scalability issues.

57   Amongst the forthcoming technologies for the functional modification of proteins and

58 generation of nano-sized emulsion droplets, power ultrasound, also commonly referred to as

59 high intensity ultrasound, has garnered particular interest due in part to the mechanical nature

60 of this process (*i.e.* ultrasonic cavitations). Traditionally, the functionality of proteins is altered

61 by aggregation (*i.e.* increasing molecular weight), proteolysis (*i.e.* reducing molecular weight)

62 or conjugation with other entities (*e.g.* Maillard reaction with reducing sugars). Power

63 ultrasound offers the possibility of altering protein structures without the use of additives or

64 excessive thermal treatments, simplifying the processing of these ingredients and generating a

65 'cleaner' packaging label for consumers. With adequate sonoreactor design (*i.e.* chamber

66 volume and volumetric flow rate selection), and high throughput, cost effective generation of

67 nano-sized emulsion droplets is readily achievable (Gogate & Kabadi, 2009; Gogate, *et al.*,

68 2011).

69   The aim of this review is to outline the fundamentals of ultrasound and critically

70 assesses applications of ultrasound treatment for the functional modification of proteins in

71 aqueous solution (*e.g.* solubility, hydrophobicity, rheological behaviour, emulsifying

72 performance, etc.) and the generation of nano-sized emulsion droplets. A particular focus has

73 been placed on the industrial relevance of ultrasonic processing within the food industry, as a

74 cost effective, mechanical method for the generation, alteration and modification of food

75 microstructures (*e.g.* emulsifications, lipid crystallisation, structural modification of

76 biopolymers, etc.).

77 **2. Fundamentals of ultrasound**

78   Ultrasound is an acoustic wave above the threshold of human auditory perception (> 16

79 kHz). Acoustic waves are the propagation of mechanical waves of pressure and displacement

80 through a medium, as longitudinal waves, exhibiting compressions (high pressure regions) and

81 rarefactions (low pressure regions). Longitudinal waves are waves whereby the displacement

82 of the medium is in the same direction as the wave (Mansfield & O'Sullivan, 1998).

83   Ultrasound can be further classified in two distinct categories based on the frequency

84 range, high frequency (100 kHz – 1 MHz), low intensity (< 1 W cm^{-2}) ultrasound, utilised most

85 commonly for the analytical evaluation of the physicochemical properties of food (Chemat *et*

86 *al.*, 2011; Demirdöven & Baysal, 2008), and low frequency (20 – 100 kHz), high intensity (10

87 – 1000 W cm^{-2}) ultrasound recently employed for the alteration, generation and modification

88 of foods, either physically or chemically (McClements, 1995). The acoustic power intensity

89 (I_a; W cm^{-2}) is defined as the acoustic power (P_a; W) per unit area of ultrasound emitting

90 surface (S_A; cm^{-2}). This review will focus solely upon low frequency, high power ultrasound,

91 and hereafter will refer to it as simply power ultrasound.

92   The effects of power ultrasound on food structures are attributed to ultrasonic

93 cavitation, the rapid formation and collapse of gas bubbles, generated by localised pressure

94 differentials occurring over short periods of times (a few microseconds). These ultrasonic

95 cavitations cause localised regions of intense hydrodynamic shear forces and a rise in

96 temperature at the site of bubble collapse (up to 5000°C), contributing to the observed effects

97 of power ultrasound (Güzey *et al.*, 2006; O'Brien, 2007; O'Donnell *et al.*, 2010).

98   Acoustic waves are generated from the conversion of electrical energy into mechanical

99 energy. A transducer, a device which converts energy from one form to another, is employed

100 to produce acoustic waves. In acoustics, transducers are commonly referred to as tips. More

101   specifically, the tip, a part of the sonotrode, is the point from which the acoustic waves emanate.

102   The piezoelectric material (*e.g.* quartz or lithium sulphate zirconate titanates) within the

103   transducer oscillates in response to electrical energy, leading to mechanical vibrations in the

104   tip. When the tip is submerged in liquids, the mechanical energy at the tip is delivered to the

105   medium as the tip vibrates generating acoustic waves (Martini, 2013; Soria & Villamiel, 2010;

106   Trujillo & Knoerzer, 2011a).

107   Ultrasonic emanation from the tip of the sonotrode is referred to as acoustic streaming

108   (Nyborg, 1953; Tjøtta, 1999).There are two main acoustic streaming theories which describe

109   this phenomena mathematically, those developed by Rayleigh (Rayleigh, 1896), Nyborg

110   (Nyborg, 1953) and Westervelt (Westervelt, 1953), referred to as the RNW theory, and that

111   proposed by Lighthill, the Stuart streaming theory (Lighthill, 1978). The RNW theory is only

112   applicable to laminar systems, whereas the Stuart streaming theory is applicable to systems

113   demonstrating acoustic jets (*i.e.* turbulent), resulting from high power acoustic beams from

114   transducers, a computationally developed example of which is shown in Fig. 1 (Lighthill, 1978;

115   Stuart, 1963). Ultrasonic processing utilised within the food industry for the development of

116   microstructures and functional modification of food ingredients is usually power ultrasound

117   processing which is most adequately modelled and explained by the Stuart streaming theory

118   (McClements, 1995; Trujillo & Knoerzer, 2011a).

119   *2.1. Ultrasonic cavitations*

120   High power ultrasonic waves generate several different types of cavitation bubbles due

121   to pressure changes during wave propagation (Servant *et al.*, 2001). Cavitation bubbles are

122   formed at acoustic intensities greater than that of the cavitation threshold. The cavitation

123   threshold pressure required to initiate cavitations is a strong function of stream width and

124   acoustic power, and once triggered bubble generation increases with increasing acoustic power

125   (Leighton, 1995; Neppiras, 1980). Fig. 2 shows the formation and collapse of ultrasonic

126   cavitations over a 56 µs timescale. It can be seen that over a 16 µs timeframe, cavitations are

127   formed, and their subsequent implosion occurs, highlighting that this phenomena occurs over

128   very short periods of time, < 20 µs in the majority of instances (Trujillo & Knoerzer, 2011). As

129   time progresses, and more acoustic energy is provided to the system, the number of ultrasonic

130   cavitations increases, as can be seen from 32 µs onward.

131   Cavitation bubbles disperse (*i.e.* reflect or scatter) and attenuate (*i.e.* gradual reduction

132   of ultrasonic intensity) ultrasonic waves due to the acoustic impedance differential between the

133   liquid and gaseous phases. When an acoustic wave moves from one medium to another (*i.e.*

134   from liquid to gaseous bubbles) differences in the speed of sound and compressibility between

135   the two phases induces an impedance mismatch (McClements, 1995; O'Brien, 2007). As a

136   consequence, the acoustic wave is either partially or completely scattered by the bubble. The

137   cavitation locus is situated in an area close to the tip of the sonotrode, whereby this region

138   yields the highest levels of acoustic intensity, and thus an area of increased formation of

139   cavitations. Therefore, the attenuation in this region is quite high and dominated by acoustic

140   scattering (Martini, 2013), decaying exponentially with respect to distance from sonotrode tip,

141   almost completely dissipated at distances as low as 2 cm (Chivate & Pandit, 1995; Kumar *et*

142   *al.*, 2006; Kumaresan *et al.*, 2006), highlighting the importance of adequate sonotrode

143   positioning for effective processing of liquid medium (Gogate *et al.*, 2011; Gogate *et al.*,

144   2003).

145   *2.2. Heat generation*

146   Ultrasonic processing of fluid systems yields heat generation due to a number of factors

147   which occur as a consequence of the transmission of an acoustic wave through the medium,

148   including molecular absorption, dissipation of turbulence, dispersion of acoustic waves by

149   gaseous bubbles and viscous losses. The acoustic energy transmitted to the medium manifests

150   as both kinetic energy (*i.e.* bulk motion) and thermal energy (*i.e.* heat). The kinetic energy

151   transmitted to the medium is dissipated as heat due to viscous losses (Tjøtta, 1999; Zisu *et al.*,

152   2010).

153   In ultrasonic processes where the attenuation coefficient, β, is high (*i.e.* a high number

154   of ultrasonic cavitations) it can be assumed that the acoustic energy is rapidly converted to

155   thermal energy in the locus of the sonotrode tip, from which the acoustic waves emanate

156   (Lighthill, 1978). The validity of this assumption is true for systems exhibiting high attenuation

157   coefficients where dissipation of acoustic energy occurs at the transducer, and additionally

158   where the kinetic energy disperses at the sonotrode tip. Chivate & Pandit, (1995) confirmed

159   that acoustic energy dissipates completely within close proximity of the sonotrode tip,

160   approximately 2 cm, and it was found that the majority of kinetic energy (> 80 %) is dissipated

161   in the form of thermal energy in a small volume (< 2 % of a 2 L batch volume) in the locus of

162   the transducer (Kumar *et al.*, 2006; Kumaresan *et al.*, 2006).

163   Trujillo & Knoerzer, (2011a) employed a computational approach to investigate the

164   distribution of temperature in a batch ultrasonic process, as shown in Fig. 3. Fig. 3 highlights,

165   that there is a higher temperature in the immediate proximity of the sonotrode tip, owing to the

166   aforementioned cavitation mediated ultrasonic attenuation, which to a large extent, limits

167   transmission of energy from the sonotrode tip.

168   *2.3. Acoustic energy determination*

169   The determination of the acoustic energy input into a volume of liquid is a topic under

170   investigation, however a satisfactory description of the solution has thus far to be elucidated,

171   even though the fields of sonochemistry and ultrasonic cavitation have been under investigation

172   for several decades. The electrical consumption of the ultrasonic process and the acoustic

173 power under non-cavitational conditions are attainable, however acoustic power measurements

174 within the cavitational regime are lacking (Margulis & Margulis, 2003).

175   As acoustic energy is transmitted to a liquid medium via the sonotrode tip, this acoustic

176 energy is dissipated as absorbed acoustic energy, manifesting as thermal energy, and

177 unadsorbed energy. The absorbed acoustic energy is the active component of total acoustic

178 energy involved in the processing. Acoustic power intensity, I_a, can be estimated from the

179 following:

180    $I_a = \frac{kf^2U}{\rho c}$ (1)

181   Where f is the frequency of sound (Hz), U is the voltage of the transducer (V), k is a

182 conversion of coefficient dependent on the transducer type, ρ is the density of the liquid

183 medium (kg m^{-3}) and c is the speed of the acoustic wave in a given medium (m s^{-1}). The product

184 of density and speed of sound (*i.e.* ρc) is known as the acoustic resistance (Margulis &

185 Margulis, 2003). Under non-cavitational conditions the acoustic energy can be estimated

186 accurately using *Eq.* 1, whilst in the cavitational regime the acoustic resistance is significantly

187 reduced. The reduction of both the speed of sound and bulk density of the medium by the

188 presence of cavitation bubbles within the medium depresses the accuracy of the acoustic

189 intensity determination from *Eq.* 1. The underlying principles involved in the formation of and

190 interactions between cavitation bubbles are not fully understood, hence the reliability of the

191 acoustic resistance term and consequently *Eq.* 1 as an effective method for the estimation of

192 the acoustic intensity within the cavitational regime is dubious (Leighton, 1995; Margulis &

193 Margulis, 2003; O'Brien, 2007).

194   The drawbacks associated with *Eq.* 1 are mitigated against by the usage of a

195 calorimetric method for the determination of absorbed energy (*cf. Eq.* 2), whereby the acoustic

196   resistance term is neglected. The main assumption for the determination of acoustic energy via

197   calorimetry is that all absorbed acoustic energy is converted to thermal energy.

198    $$I_a = \frac{P_a}{S_A} = \frac{m\, c_p\left(\frac{dT}{dt}\right)}{S_A}$$ (2)

199   Where P_a is the absorbed acoustic power (W), S_A is the surface area of the tip of the

200   transducer (cm^2; *i.e.* ultrasound emitting surface), m is the mass of ultrasound treated medium

201   (g), c_p is the specific heat capacity of the medium (J/gK) and dT/dt is the rate of change of

202   temperature with respect to time, starting at $t = 0$ (^{o}C s^{-1}). As energy emitted from the sonotrode

203   tip, it is absorbed within close proximity to the tip due to cavitational attenuation, the energy

204   is dissipated as heat, allowing for estimation of the acoustic energy absorbed without the

205   necessity to account for cavitation bubbles (*i.e.* the acoustic resistance term) (Jambrak *et al.*,

206   2008; Margulis & Margulis, 2003).

3. Physicochemical alteration of food proteins via ultrasonic processing

208   From the literature, the application of ultrasonic treatment has been related to proteins

209   derived from dairy, animal, cereal, legume, tuber and fruit sources, see Table 1.

3.1. Dissolution effects of ultrasonic processing

211   Dissolution of powder ingredients is essential for functional utilisation within a given

212   formulation system, and depending upon the specific powder, its rehydration can be

213   challenging. Broadly, high protein systems are difficult to reconstitute, with certain protein

214   fractions exacerbating this, for example, casein-dominant high-protein content powders

215   (Crowley *et al.*, 2015; O'Sullivan *et al.*, 2017). Upon addition of a powder to water, there are

216   5 stages in its complete dissolution, schematically represented in Fig. 4 for a high-protein dairy

217   powder: (1) Wetting, (2) Swelling, (3) Sinking, (4) Dispersion and (5) Dissolution (Crowley *et*

218   *al.*, 2016). The key stages where power ultrasound could affect the rehydration process is that

219   of dispersion, the fragmentation of wetted powder particles, and dissolution, the complete

220   breakdown of granular structure and release of constituent molecules (Vos *et al.*, 2016).

221   Ultrasound treatment offers improved rates of dissolution and solubilisation of poorly

222   soluble dairy protein powders in comparison to conventional dissolution methodologies (*i.e.*

223   low/high shear mixing or high pressure homogenisation) (Chandrapala *et al.*, 2014; McCarthy

224   *et al.*, 2014; O'Sullivan *et al.*, 2016). McCarthy *et al.*, (2014) demonstrated that the high levels

225   of hydrodynamic shear associated with ultrasonic cavitations disrupt agglomerates of powder

226   imparting greatly increased rates of solubilisation in comparison to conventional overhead

227   mixer dispersion methodologies employed for dairy powders possessing a high degree of

228   micellar casein (MC), whilst Chandrapala *et al.*, (2014) observed that the most effective

229   methodology for the dissolution of dairy powders possessing a high MC content ($\geq$ 80 wt. %)

230   was high pressure homogenisation (single stage at either 80 or 200 bar), with ultrasonic

231   processing being an intermediate methodology for dissolution, followed by low/high shear

232   mixing. Enhancement of dissolution of MPC in this case may be achieved by operating at an

233   increased ultrasonic amplitude (50% was employed by Chandrapala *et al.*, (2014), whilst 100%

234   was utilised by McCarthy *et al.*, (2014)) and/or optimal positioning of the ultrasonic horn so as

235   to achieve the maximum effect of the ultrasonic sound beam (*i.e.* minimisation of dead-zones)

236   (Gogate *et al.*, 2011).

237   The available literature is limited to studies on the effect of ultrasonic processing for

238   dairy powders for dissolution purposes. Be that as it may, there is a growing interest within the

239   food industry for the use of plant derived protein ingredients rather than animal sourced

240   systems, for a variety of reasons, such as nutritional profile, functional properties and

241   commercial rationale (Gonzalez-Perez & Arellano, 2009). Ultrasound processing of plant

242   protein systems could offer potential benefits for dissolution of powders, as ultrasound has

243 been shown to be capable to reduce aggregate size of plant proteins in aqueous solution, as

244 discussed in the following section.

245 *3.2. Size effects of ultrasonic processing*

246 Ultrasound treatment reduced the size of aggregated caseins in aqueous solution

247 (phosphocasein, calcium caseinate, milk protein concentrate from retentate and milk protein

248 concentrate reconstituted from powder), from micron-sized entities (5 - 30 μm) to nano-sized

249 species (~200 nm) (Madadlou, *et al.*, 2009; McCarthy, *et al.*, 2014; Shanmugam, *et al.*, 2012;

250 Yanjun, *et al.*, 2014; Zisu, *et al.*, 2010), the expected size for casein micelles (O'Connell &

251 Flynn, 2007). This size reduction is attributed to the high shear forces associated with ultrasonic

252 cavitations in liquid mediums (Trujillo & Knoerzer, 2011). Be that as it may, prolonged

253 ultrasound treatment led to growth in aggregate size toward the micron-scale, related to whey-

254 whey or casein-whey protein interactions as a consequence of both protein denaturation and

255 deceased solubility attributed to elevated temperatures from ultrasound treatment (McCarthy,

256 *et al.*, 2014; Shanmugam, *et al.*, 2012). Sonication of whey protein (suspensions, concentrates,

257 isolates, and from retentate) similarly reduced the size of protein aggregates due to disruption

258 of non-covalent interactions, to sizes ~100 nm (*i.e.* hydrogen bonding, hydrophobic and

259 electrostatic interactions) (Arzeni, *et al.*, 2012; Chandrapala, *et al.*, 2011; Jambrak, *et al.*, 2014;

260 Martini, *et al.*, 2010; Zisu, *et al.*, 2010), yet similarly displayed growth of particle size

261 attributed to increases in temperature, resulting in protein denaturation and aggregation

262 (Gülseren, *et al.*, 2007).

263 Furthermore, the ultrasound treatment of proteins derived from legume sources (pea

264 protein, soy protein, black bean protein and mung bean protein) and wheat protein displayed a

265 significant reduction in aggregate size (> 20 μm) to entities which were submicron (~200 nm),

266 thus enhancing the solubility of traditionally poorly soluble plant protein solutions

267 (Charoensuk, *et al.*, 2014; Jiang, *et al.*, 2014; O'Sullivan, Beevers, *et al.*, 2015; O'Sullivan,

268 Murray, *et al.*, 2016; O'Sullivan, Park, *et al.*, 2016b; Zhang, *et al.*, 2011). However, ultrasound

269 treatment of egg white proteins (Arzeni, *et al.*, 2012; Krise, 2011) exhibited growth in

270 aggregate size, from submicron (~500 nm) to micron sized entities (~100 μm), attributed to

271 thermal denaturation of protein due to increases in temperature from prolonged ultrasonic

272 treatment. Be that as it may, size reduction of egg white protein aggregates is achievable if the

273 temperature is maintained well below denaturation temperatures (~40 ^{o}C) (O'Sullivan, Murray,

274 *et al.*, 2016). Sonication of rice protein isolate, lupin protein concentrate and zein demonstrated

275 no significant differences in size, associated with insufficient provided acoustic energy to

276 disrupt disulphide bonding maintaining the denatured aggregate structure (O'Sullivan, Murray,

277 *et al.*, 2016; O'Sullivan, Park, *et al.*, 2016a; Ren, *et al.*, 2015). Size reduction of protein

278 aggregates in aqueous solution from ultrasound treatment is associated with the disruption of

279 associative non-covalent interactions which maintain protein aggregate structure in aqueous

280 solutions.

281 *3.3. Molecular structure effects of ultrasonic processing*

282 Even though ultrasound treatment has been shown to possess the capability of reducing

283 the size of proteins in aqueous solution and enhance dissolution, it does not appear to cause

284 scission of the primary structure for a large number of proteins, including milk protein

285 concentrate (Yanjun *et al.*, 2014), whey protein suspensions (Martini, *et al.*, 2010), soy protein

286 isolate (Hu, *et al.*, 2013), pea protein isolate (O'Sullivan, Murray, *et al.*, 2016), wheat gluten

287 (Zhang *et al.*, 2011), black bean protein isolate (Jiang *et al.*, 2014), potato protein isolate

288 (O'Sullivan, Park, *et al.*, 2016a), gelatin (O'Sullivan, Murray, *et al.*, 2016) and egg white

289 protein (Krise, 2011), as ultrasound treatment provides insufficient energy to cause scission of

290 the primary acid sequence (*i.e.*, peptide bond). Krise, (2011) observed a minor shift in the

291 molecular weight distribution of egg white protein and attributed this to scission of disulphide

292 bonds between cysteine residues present in egg white protein (Mine, 2002). The bond energy

293 associated with the disulphide bond is less than that of the peptide bond maintaining the

294 primary structure of proteins (*cf.* Table 2), nevertheless, the majority of ultrasonic energy is

295 utilised in the disruption of the associative non-covalent interactions maintaining the protein

296 associate structure, rather than disruption of covalent linkages. However, a significant

297 reduction in the molecular weight of α-lactalbumin (Jambrak, *et al.*, 2010) and whey protein

298 concentrate/isolate (Jambrak, *et al.*, 2014), generating peptide species possessing molecular

299 weights within a range of 4.5 to 8 kDa, was observed from pixel intensity plots generated from

300 SDS-PAGE gels. Based on the acoustic intensity provided in both of these trials, the maximum

301 and minimum of which were 1 W cm^{-2} and 48 W cm^{-2}, respectively, insufficient energy is

302 provided to disrupt the peptide bonds, especially at the high concentrations of protein tested

303 (up to 10 wt. %), and further testing should be conducted to further elucidate these results, such

304 as high performance liquid chromatography (HPLC), circular dichroism (CD) or nuclear

305 magnetic resonance (NMR) spectroscopy. The acoustic energy employed provided sufficient

306 energy to disrupt hydrogen bonding, reducing aggregate size (as observed in these studies),

307 with insufficient energy provided to achieve scission of covalent linkages.

308 *3.4. Viscosity effects of ultrasonic processing*

309 Sonication of protein solutions has been shown to either reduce the bulk viscosity, in

310 the cases of calcium caseinate (Zisu, *et al.*, 2010), milk protein concentrate (Yanjun, *et al.*,

311 2014; Zisu, *et al.*, 2010), whey protein from retentate (Zisu, *et al.*, 2010), soy protein isolate

312 (Hu, *et al.*, 2013) and egg white protein (Arzeni *et al.*, 2012), or to yield no difference in bulk

313 viscosity, as for skimmed milk powder (Shanmugam, *et al.*, 2012) and α-lactalbumin (Jambrak,

314 *et al.*, 2010). For the case of soy protein, a reduction from 1 to 0.2 Pa.s at a shear rate of 100

315 s^{-1} and concentration of 12.5 wt. % was observed (Hu, *et al.*, 2013), and for whey protein (from

316 retentate) a reduction from 0.065 to 0.055 Pa.s at 100 s^{-1} for a 33 wt. % solution was

317 demonstrated. The reduction in bulk viscosity is attributed to the reduction in aggregate size as

318   a consequence of ultrasonic cavitations. The spatial distance between adjacent protein

319   aggregates is increased upon size reduction via ultrasound treatment, increasing the critical

320   overlap concentration, c^*, for a given protein solution, and thus, decreasing the bulk viscosity

321   with respect to increasing protein concentration (Lefebvre, 1982; Morris *et al.*, 1981).

322   *3.5. Emulsifying effects of ultrasonic processing*

323   Proteins which have been treated with power ultrasound have shown improvements in

324   both emulsion formation and stability, for milk protein concentrates (O'Sullivan, Arellano, *et*

325   *al.*, 2014; Yanjun, et al., 2014), egg white protein (O'Sullivan, Murray, *et al.*, 2016), bovine

326   gelatin (O'Sullivan, Murray, *et al.,* 2016), soy protein isolate (Chen, *et al.*, 2012), pea protein

327   isolate (O'Sullivan, Murray, *et al.*, 2016), potato protein isolate (O'Sullivan, Park, *et al.*,

328   2016a), wheat protein (O'Sullivan, Park, *et al.*, 2016b; Zhang, *et al.*, 2011) and walnut protein

329   (Jincai, *et al.*, 2013). Yanjun *et al.*, (2014) reported a significant increase in both EAI (*i.e.*,

330   emulsion activity index) and ESI (*i.e.*, emulsion stability index) for emulsions prepared with

331   MPC, from 3.5 to 6 m^2 g^{-1}, and from 50 to 80 min, respectively. In addition, O'Sullivan,

332   Murray, *et al.*, (2016) observed significant enhancements in both emulsion formation and

333   stability for emulsions prepared with bovine gelatin. At a protein concentration of 0.1 wt. %

334   emulsions prepared with untreated and ultrasound treated bovine gelatin yielded emulsion

335   droplet sizes of 1.75 μm and 1 μm, respectively, and moreover emulsions prepared with

336   ultrasound treated bovine gelatin were stable throughout a 28 day stability study, whereas their

337   untreated counterparts were unstable at concentrations < 1 wt. %, leading to growth in emulsion

338   droplet size.

339   These improvements in emulsion formation and stability for ultrasound treated proteins

340   were associated with increases in hydrophobicity, which occurred as hydrophobic protein

341   residues within the interior of the untreated aggregate became revealed upon treatment with

342   ultrasound, and improved interfacial packing at the emulsion droplet interface. O'Sullivan,

343   Park, *et al.*, (2016a) observed a significant reduction in the hydrodynamic volume of potato

344   protein isolate which is associated to an increase in the hydrophobicity of proteins (Khan, *et*

345   *al.*, 2012), accounting for the observed enhancements in emulsion formation and stability in

346   this instance. In addition, ultrasound treatment of whey protein (Arzeni, *et al.*, 2012; Gülseren,

347   *et al.*, 2007), soy protein (Arzeni, *et al.*, 2012; Hu, *et al.*, 2013), black bean protein (Jiang, *et*

348   *al.*, 2014) and egg white protein (Arzeni, *et al.*, 2012) increased the hydrophobicity, and the

349   rate of protein adsorption to and interfacial packing at the oil-water interface, as measured by

350   interfacial tension. These differences were measured for the cases of milk protein isolate

351   (O'Sullivan, *et al.*, 2014), bovine gelatin (O'Sullivan, Murray, *et al.*, 2016), pea protein isolate

352   (O'Sullivan, Murray, *et al.*, 2016) and soy protein isolate (Chen, *et al.*, 2012), further

353   accounting for improvements in emulsion formation and stability. O'Sullivan, Murray, *et al.*,

354   (2016) reported reductions in the equilibrium value of interfacial tension (*i.e.*, rapeseed oil and

355   water) for both bovine gelatin and soy protein isolate, from 5 to 2.5 mN m^{-1}, and from 6 to 3.5

356   mN m^{-1}, respectively. Furthermore, O'Sullivan, Murray, *et al.*, (2016) visualised the improved

357   interfacial packing using cryo-SEM for ultrasound treated bovine gelatin in comparison to

358   untreated bovine gelatin. Ultrasound treatment of bovine gelatin reduced the size of the

359   untreated fibres (*cf.* Fig. 5a) to smaller fibrils (*cf.* Fig. 5b), whereby this reduction in fibre size

360   of bovine gelatin after sonication allowed for improved packing at the oil-water interface (*cf.*

361   Fig. 5d), in comparison to emulsions prepared with untreated bovine gelatin (*cf.* Fig. 5c)

362   (O'Sullivan, Murray, *et al.*, 2016).

363   Ultrasound treatment of a range of dairy proteins (whey protein concentrate, milk

364   protein from retentate and calcium caseinate) utilising large scale sonoreactors demonstrated

365   the capacity for ultrasound to modify the rheological behaviour (*i.e.*, reduction in bulk

366   viscosity) of these proteins at pilot scale and was attributed to a reduction in protein aggregate

367   size (Zisu *et al.*, 2010). This work highlights the potential applicability of ultrasound for the

368 functional modification of proteins at larger scales, whilst more work is required to fully

369 implement this technology industrially (Gogate & Kabadi, 2009; Gogate, *et al.*, 2011).

370 **4. Nanoemulsion fabrication from ultrasound and the associated parameters**

371   Power ultrasound is a well-established technique for the formation of emulsions from

372 either coarse pre-emulsions (*i.e.* $d_{3,2} > 50$ μm) or discrete continuous and dispersed phases

373 (Bondy & Söllner, 1935), consistently yielding nano-sized emulsion droplets (Leong, *et al.*,

374 2009). The resultant microstructure of emulsions is dependent upon formulation and the

375 emulsification processing conditions. Processing configuration (*i.e.* batch or continuous

376 processing methodologies) and associated parameters (*i.e.* acoustic power, residence time, etc.)

377 have been extensively investigated, yet the fundamental influence of emulsion formulation

378 with industrial relevant emulsifiers (*i.e.* high molecular weight biopolymers), geometric

379 configuration to optimise contact time and the intrinsic interactions between processing and

380 formulations have yet to be fully explored.

381   Increasing the contact time of a coarse pre-emulsion within the acoustic field can

382 decrease the emulsion droplet size to a minimum size, provided the residence time of the

383 emulsion within the acoustic field is sufficient and there is sufficient emulsifier present for

384 droplet coverage (Maa & Hsu, 1999). For batch processing methodologies increasing the

385 processing time decreases the emulsion droplet size (Abismail, *et al.*, 1999; Cucheval & Chow,

386 2008; Delmas, *et al.*, 2011; Jafari, *et al.*, 2007; Jena & Das, 2006; Kaltsa, *et al.*, 2013; Kentish,

387 *et al.*, 2008; Kiani & Mousavi, 2013; Leong, *et al.*, 2009; O'Sullivan, Murray, *et al.*, 2015;

388 O'Sullivan & Norton, 2016; Ouzineb, *et al.*, 2006; Ramisetty & Shyamsunder, 2011;

389 Shanmugam, *et al.*, 2012; Tang, *et al.*, 2013). Similarly increasing the residence time of

390 emulsions for continuous processing, by decreasing the flow rate, decreases emulsion droplet

391 size (Behrend, *et al.*, 2000; Behrend & Schubert, 2001; Freitas, *et al.*, 2006; Kentish, *et al.*,

392 2008; O'Sullivan, Murray, *et al.*, 2015; O'Sullivan & Norton, 2016; Tang, *et al.*, 2013). For

393 both configurations, nano-sized emulsion droplets (~200 nm) were achieved. Nevertheless,

394 prolonged residence time within the acoustic field can lead to growth in droplet size due to re-

395 coalescence of emulsion droplets (*i.e.* over processing) in systems possessing insufficient

396 emulsifier (Jafari, *et al.*, 2008; O'Sullivan, Murray, *et al.*, 2015; O'Sullivan & Norton, 2016).

397 Despite the size reduction of emulsion droplets as a function of increasing residence

398 time, the same trend is not observed when considering droplet size distribution (DSD).

399 Typically, the DSD initially increases as a function of ultrasonic processing time, followed by

400 a decrease (Abismail *et al.*, 1999; Leong *et al.*, 2009). This behaviour is more pronounced for

401 batch processing in comparison to continuous configurations, whereby there is a larger

402 propensity for stagnant zones. Other emulsification technologies exhibit more uniform size

403 distributions, often with minimal change in distribution width as a function of processing time,

404 as demonstrated for valve-homogenisation and microfluidization approaches, in comparison to

405 ultrasonic emulsification (Heffernan *et al.*, 2011; Lee & Norton, 2013).

406 The acoustic energy transmitted from the tip of the sonotrode to the medium is highly

407 localised (as low as 1 cm from the sonotrode; Chivate & Pandit, 1995) due to attenuation (*i.e.*

408 dispersion of acoustic waves from cavitation bubbles). Ultrasonic cavitation bubbles are highly

409 unstable entities yielding implosions creating highly localised regions of hydrodynamic shear

410 within close proximity of the tip (Kumar, *et al.*, 2006; Kumaresan, *et al.*, 2006). These

411 ultrasonically induced implosions from cavitations result in the disruption of micron-sized oil

412 droplets (> 50 μm) and facilitate the formation of nano-sized emulsion droplets (~200 nm).

413 Batch processing of emulsions utilising ultrasound is often inefficient due to the nature of the

414 emulsification process, whereby less than 2 % of the medium of a given volume experiences

415 acoustic energy due to acoustic attenuation (Kumar, *et al.*, 2006; Kumaresan, *et al.*, 2006), and

416 the turbulent forces generated by the acoustic streaming transfer the coarse emulsion from the

417 bulk to within the vicinity of the tip, whereby emulsification occurs. Depending on the volume

418 of coarse emulsion being processed and the surface area of the tip via batch configuration this

419 can be a time consuming process, in comparison to continuous processing methodologies,

420 which typically demonstrate smaller chamber volumes relative to tip surface area, examples of

421 which are shown in Fig. 6 and 7. Fig. 6b and 7a show configurations where the path of fluid

422 flow through the system may potentially bypass the ultrasound, owing to the geometrical

423 configuration of the chamber. Conversely, Fig. 6a, 7b, 7c and 7d depict setups where fluid flow

424 is focused to a specific location, where there is a high probability of ultrasonic cavitations, thus,

425 maximising the efficiency of the process.

426   Continuous processing configurations operate at lower residence times in comparison

427 to batch processing (< 1 s), yet are capable of achieving comparable droplet sizes due to

428 minimisation of chamber volume to maximise the volume of coarse emulsion within the

429 acoustic field (*cf.* Fig. 6). By optimisation of the geometry, whereby the course of emulsion is

430 pumped directly into the tip of the sonotrode, maximum droplet breakup can be achieved (*cf.*

431 Fig. 6). The residence time for continuous processing is dictated by the flow rate of emulsion,

432 whereby reduction of flow rate increases the contact time, allowing for a greater reduction in

433 the droplet size (Freitas, *et al.*, 2006; Kentish, *et al.*, 2008; O'Sullivan, Murray, *et al.*, 2015;

434 O'Sullivan & Norton, 2016; Tang, *et al.*, 2013).

435   The rate of droplet breakup can be improved by increasing the acoustic power

436 transmitted to the coarse emulsion for both batch processing (Abismail, *et al.*, 1999; Cucheval

437 & Chow, 2008; Delmas, *et al.*, 2011; Higgins & Skauen, 1972; Kaltsa, *et al.*, 2013; O'Sullivan,

438 Murray, *et al.*, 2015; O'Sullivan & Norton, 2016) and continuous processing configurations

439 (Freitas, *et al.*, 2006; O'Sullivan, Murray, *et al.*, 2015; O'Sullivan & Norton, 2016). However

440 the minimum achievable droplet is dictated by the formulation of the emulsion (Maa & Hsu,

441 1999). For example, when comparing droplet sizes of emulsions prepared with 0.1 and 0.75

442 wt. % Tween 80, the achieved droplet sizes were 1 μm and 150 nm, respectively, highlighting

443   that sufficient emulsifier is necessary to achieve nano-sized emulsion droplets (O'Sullivan,

444   Murray, *et al.*, 2015). Thus, increasing the acoustic power minimises the processing time

445   required to achieve the minimum droplet size, dictated by emulsion formulation.

446   The resultant droplet size of emulsions fabricated via ultrasonic processes is dictated

447   by the formulation of the emulsion (*i.e.* emulsifier type and concentration, dispersed phase type

448   and volume fraction, presence of stabilisers, etc.), whilst the processing parameters determine

449   the rate at which the resultant droplet is formed (Jafari, *et al.*, 2007). The majority of studies

450   conducted utilise model emulsifier systems (*i.e.* low molecular weight surfactants), whereby a

451   high degree of purity can be guaranteed. These surfactants include Tween 40 (Kentish, *et al.*,

452   2008), Tween 60 (Abismail, *et al.*, 1999), Tween 80 (O'Sullivan, Murray, *et al.*, 2015) and

453   Span 80 (Leong, *et al.*, 2009). Increasing the emulsifier concentration decreases the droplet

454   size to a minimum size given optimal processing conditions to achieve the minimal droplet

455   size. Few studies have been conducted whereby industrial applicable ingredients are utilised,

456   such as multi-component protein sources as the emulsifying agent. Kaltsa *et al.*, (2013),

457   Heffernan *et al.*, (2011), O'Sullivan, Murray, *et al.*, (2015) and O'Sullivan & Norton, (2016)

458   employed whey protein concentrate, sodium caseinate, milk protein isolate and pea protein

459   isolate, respectively, as the emulsifying agent in oil-in-water emulsions. Submicron emulsion

460   droplets have been prepared from these dairy proteins, whereby Kaltsa, *et al.*, (2013) and

461   Heffernan, *et al.*, (2011) solely utilised batch processing, achieving ~600 and ~200 nm sized

462   emulsion droplets, respectively. O'Sullivan, Murray, *et al.*, (2015) and O'Sullivan & Norton,

463   (2016) comparatively assessed both batch and continuous configurations, highlighting the

464   efficiency of continuous processing, as acoustic energy is utilised more efficiently in lower

465   processing volumes associated with the chamber of the continuous configuration. In both cases,

466   submicron emulsion droplets, ~200 nm, were achieved with sufficient emulsifier and adequate

467   processing.

468   Power ultrasound has demonstrated a capacity for alteration of the functionality of

469   proteins, and the efficient fabrication of emulsions, both acting through ultrasonic cavitations.

470   However, to the author's knowledge, only one study is available comparing the effects of

471   ultrasonic processing upon protein functionality as an emulsifier for pre- (*i.e.*, unadsorbed) and

472   post-emulsification (*i.e.*, interfacial) (O'Sullivan, Beevers, *et al.*, 2015). Milk protein isolate

473   and pea protein isolate were employed as the emulsifying agents in this study, and emulsions

474   were prepared via microfluidiser (100 MPa for 1 pass). This study highlighted that emulsions

475   prepared with ultrasound treated milk protein isolate post-emulsification yielded smaller

476   emulsion droplets (12 μm) in comparison to emulsions prepared with either untreated or

477   ultrasound treated pre-emulsification milk protein isolate (27.5 μm and 20 μm, respectively) at

478   a concentration of 0.1 wt. % (O'Sullivan, Beevers, *et al.*, 2015). Emulsions prepared with

479   ultrasound treated pea protein isolate yielded smaller droplets in comparison to their untreated

480   counterparts, yet no significant differences were observed between ultrasound treated pea

481   protein pre- and post-emulsification, attributed to the highly aggregated nature of pea protein

482   in comparison to that of milk protein isolate (O'Sullivan, Beevers, *et al.*, 2015). The aggregated

483   nature of pea protein, which is also typically observed in other plant derived protein ingredients

484   upon solubilisation, is associated with a combination of isolation of the proteins components

485   from the initial raw material and subsequent dehydration to produce a powder, yielding systems

486   with hydrophobic exteriors and hydrophilic interiors (Boye, *et al.*, 2010; O'Sullivan, Murray,

487   *et al.*, 2016).

488   From an industrial perspective, the most practical method for the implementation of

489   ultrasound within a production environment is the continuous processing configuration,

490   primarily due to the higher throughputs. Irrespective of configuration, the implementation of

491   ultrasound within the food industry has been limited for a number of reasons: including pitting

492   of the sonotrode tip (*i.e.* the gradual erosion of the tip material due to mechanical vibrations),

493 deposition of tip debris within the processed medium and poor performance of current

494 ultrasound geometric configurations (*i.e.* dead zones). Freitas, *et al.*, (2006) developed a

495 configuration for continuous processing of emulsions, whereby the ultrasonic probe was

496 welded to the steel jacket (*cf.* Fig. 7c, d). Additionally the space in between the jacket and the

497 glass tube, through which the medium passed, contained pressurised water which behaved as

498 an acoustic conductor. This methodology prevents direct contact of the sonotrode with the

499 medium being processed, hence removing the potential for contamination from ultrasonic

500 pitting. Nevertheless, a fundamental understanding of energy transfer through the acoustic

501 medium needs to elucidated. O'Sullivan, Murray, *et al.*, (2015) compared the effect of

502 continuous processing at both lab and pilot scale, demonstrating that the pilot scale continuous

503 configuration is dependent upon the ultrasonic amplitude (*i.e.* acoustic power), unlike the lab

504 scale, due to bypassing of elements of pre-emulsion from the acoustic field at lower ultrasonic

505 amplitudes, highlighting the necessity for optimisation of processing conditions at larger scales

506 to efficiently achieve nanoemulsions.

507   The design of conventional continuous configurations is under investigation and

508 continual development (Gogate *et al.*, 2011, 2003). The primary design criteria for the

509 development of continuous ultrasonic processes are the operating conditions (*i.e.* acoustic

510 power and processing time) and geometric parameters (sonotrode location, chamber volume,

511 tip location within the chamber, etc.). Be that as it may, several other factors must be taken into

512 consideration during the development and design of continuous ultrasonic systems: such as the

513 hydrodynamic conditions within the acoustic field, variance due to the presence of discrete

514 entities within the liquid medium (*i.e.* gaseous bubbles, immiscible liquid droplets, solid

515 particles or high molecular weight biopolymers), the degree of acoustic attenuation chiefly due

516 to the non-homogenous nature of food systems, and ratio of frequency irradiation to power

517 dissipation within the locus of the tip of the sonotrode (Gogate *et al.*, 2011, 2003).

518 **5. Conclusions and future trends**

519 Even though low frequency, high power ultrasonic processing is a well-established

520 technology within the food industry, numerous advances have been achieved in understanding

521 the fundamental mechanisms for the functional modification of the physicochemical properties

522 of proteins for specific applications and the factors associated with the efficient generation of

523 nano-sized emulsion droplets in recent years. Ultrasound offers the potential for the functional

524 modification of proteins through mechanical means, without the use of chemical or biological

525 (*i.e.* enzymes) additives.

526 Ultrasonic treatment of proteins is related to physicochemical changes in structure,

527 manifesting as: modifications to the functional attributes of proteins, reduction of bulk

528 viscosity, increases of hydrophobicity and improvements in emulsion formation and stability.

529 Ultrasound treatment of proteins in solutions affects the associative behaviour of proteins,

530 disrupting the non-covalent forces which maintain protein aggregate structure, and reducing

531 aggregate size.

532 Power ultrasound has shown to be an effective emulsification methodology, either

533 utilising batch or continuous configurations, for the formation of nano-sized droplets. The

534 development of nano-sized droplets is related to a combination of process parameters (*i.e.*

535 acoustic power and contact time), geometric considerations (*i.e.* sonotrode location within the

536 chamber, chamber geometry, etc.) and emulsion formulation (*i.e.* emulsifier type and

537 concentrations, dispersed phase volume fraction, etc.). Emulsion formation within the acoustic

538 field is attributed to the high levels of hydrodynamic shear generated by ultrasonic cavitations

539 within close proximity to the tip of the sonotrode. Increasing the residence time which the

540 coarse pre-emulsion has within the acoustic field decreases the emulsion droplet size, to a

541 minimum droplet size as determined by the emulsion formulation. In addition, increasing the

542 acoustic power increases the rate by which this minimal droplet size is achieved. Nevertheless,

543 further investigations of emulsification implementing ultrasound are required to develop

544 optimised geometries for maximum droplet breakup, utilisation of industrial relevant

545 ingredients (*i.e.* high molecular weight biopolymers) and the intrinsic interactions between

546 emulsion formulation and operating conditions (*i.e.* microstructural engineering).

547 Lastly, it is worth mentioning that although numerous advances have been made in

548 understanding the effects of power ultrasound upon proteins in aqueous solution and for the

549 fabrication of nanoemulsions, this understanding is predominately at lab scale. Although

550 studies are being conducted for both the ultrasound treatment of proteins and emulsion

551 generation at pilot scale, further work is required to fully understand the specific design criteria

552 to allow the effective utilisation of this versatile technology within the food industry.

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955

956 **Figure legends**

957 **Fig. 1.** Velocity distribution for acoustic streaming as predicted by Stuart Streaming, with chart

958 bar indicating the magnitude of velocity (m s^{-1}), adapted from Trujillo & Knoerzer, (2011a),

959 rights of use acquired from Elsevier.

960 **Fig. 2.** Images of oscillating cavitation bubbles, formation of cavity can be seen at t = 8 μs,

961 and cavity collapse at t = 16 μs. Frame rate: 125,000 fps. Image taken from Wagterveld, *et al.*,

962 (2011), rights of use acquired from Elsevier.

963 **Fig. 3.** Temperature profile distribution of an ultrasonic probe, after a 10 minute timescale,

964 with chart bar indicating temperature range (K). Image taken from Trujillo & Knoerzer,

965 (2011a), rights of use acquired from Elsevier.

966 **Fig. 4.** Schematic representation of rehydration of agglomerated high-protein dairy powder,

967 showing the 5 stages of powder rehydration. Image taken from Crowley *et al.*, (2016), rights

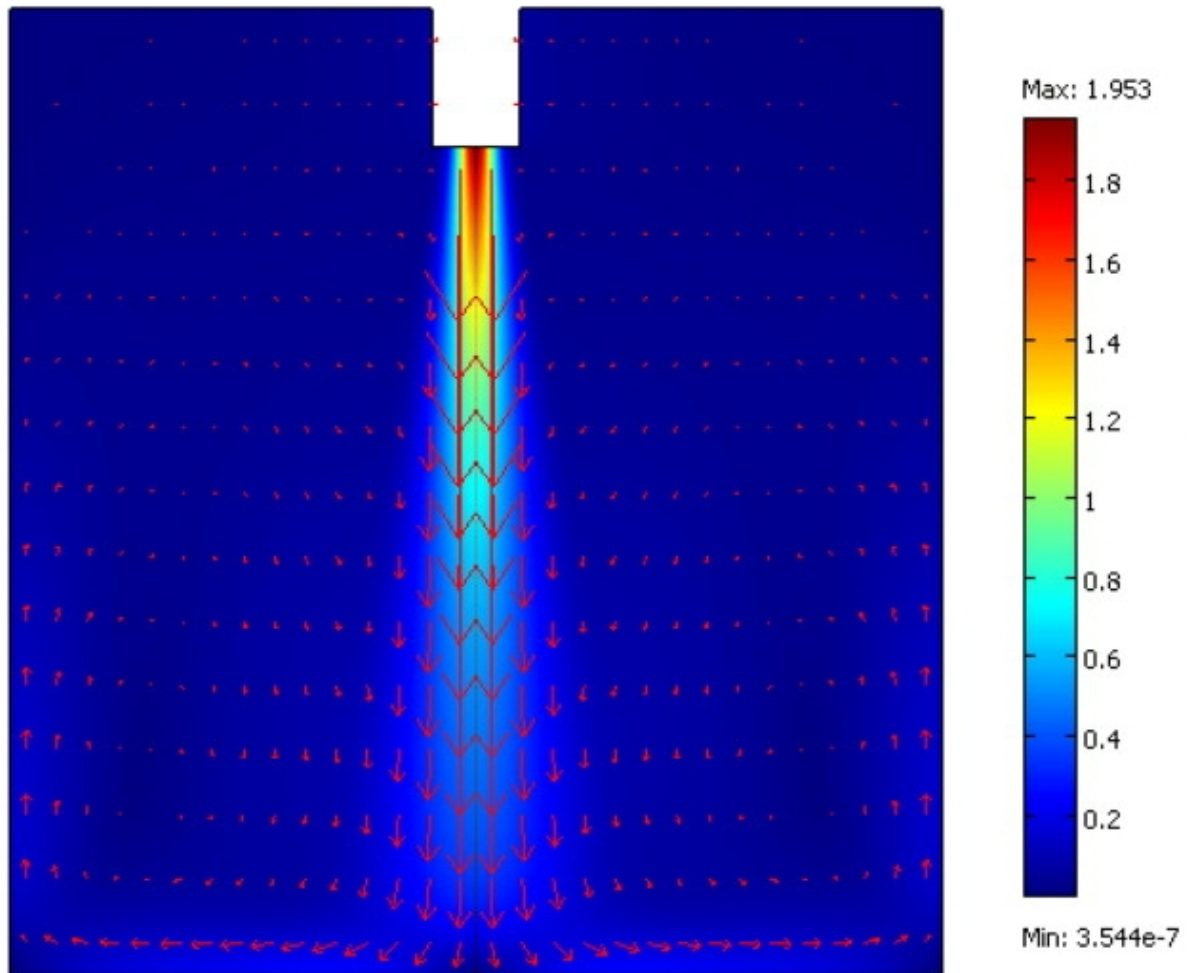968 of use acquired from Springer.

969 **Fig. 5.** Cryo-SEM micrographs of (a) 1% untreated bovine gelatin solution, (b) 1% ultrasound

970 treated bovine gelatin solution, (c) 1% untreated bovine gelatin stabilised emulsion and (d) 1%

971 ultrasound treated bovine gelatin stabilised emulsion. Scale bars are 2 μm and 10 μm for

972 solutions and emulsions, respectively. Image adapted from O'Sullivan, Murray, *et al.*, (2016).

973 **Fig. 6.** Schematic of continuous emulsification configurations for (a) lab scale and (b) pilot

974 scale processing. Image adapted from O'Sullivan, Murray, *et al.*, (2015).

975 **Fig. 7.** Examples of continuous ultrasonic configurations. Images taken from Gogate, *et al.*,

976 (2011) and Freitas, *et al.*, (2006), rights of use acquired from Elsevier.
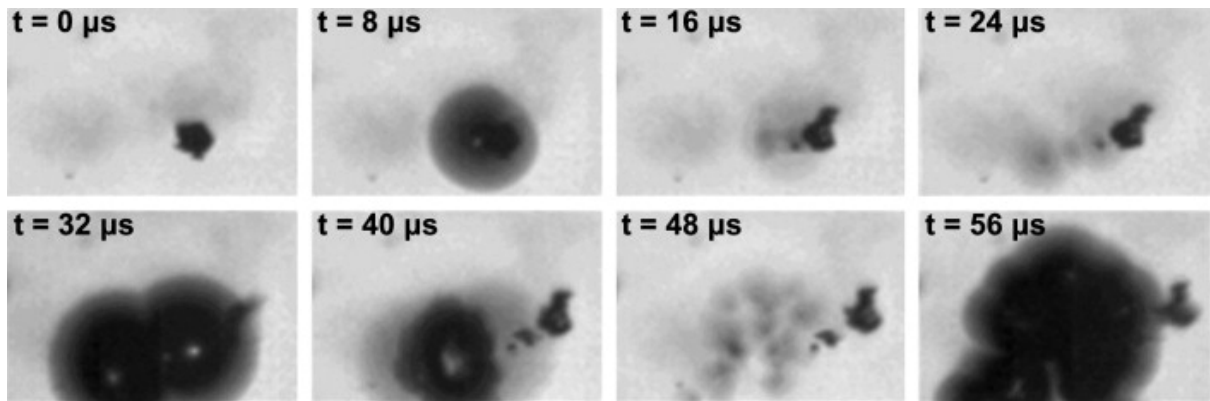
977   **Figures**
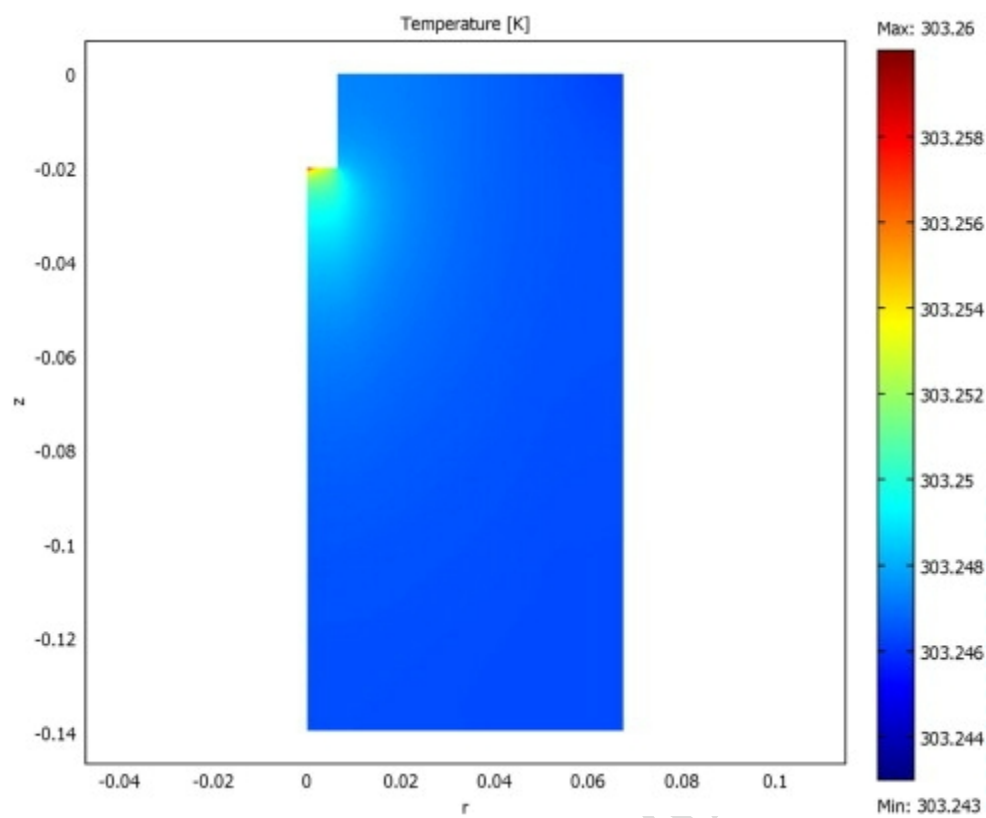
978   Fig. 1.

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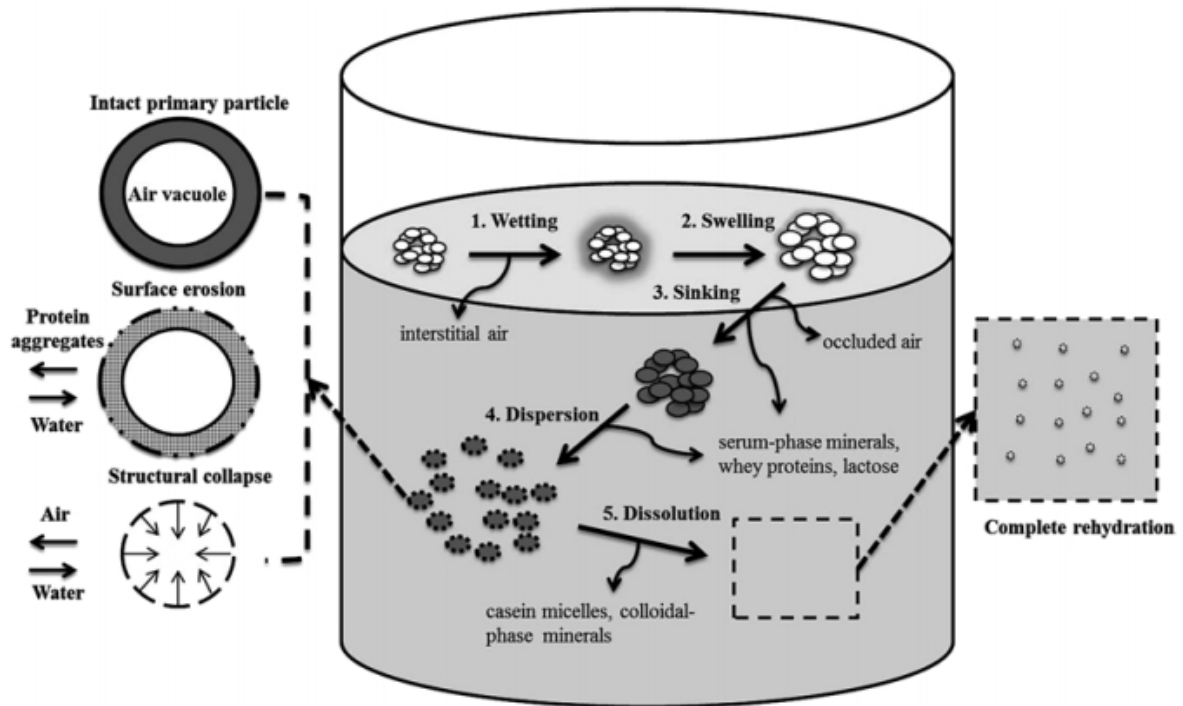
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981   Fig. 2.

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984   Fig. 3.
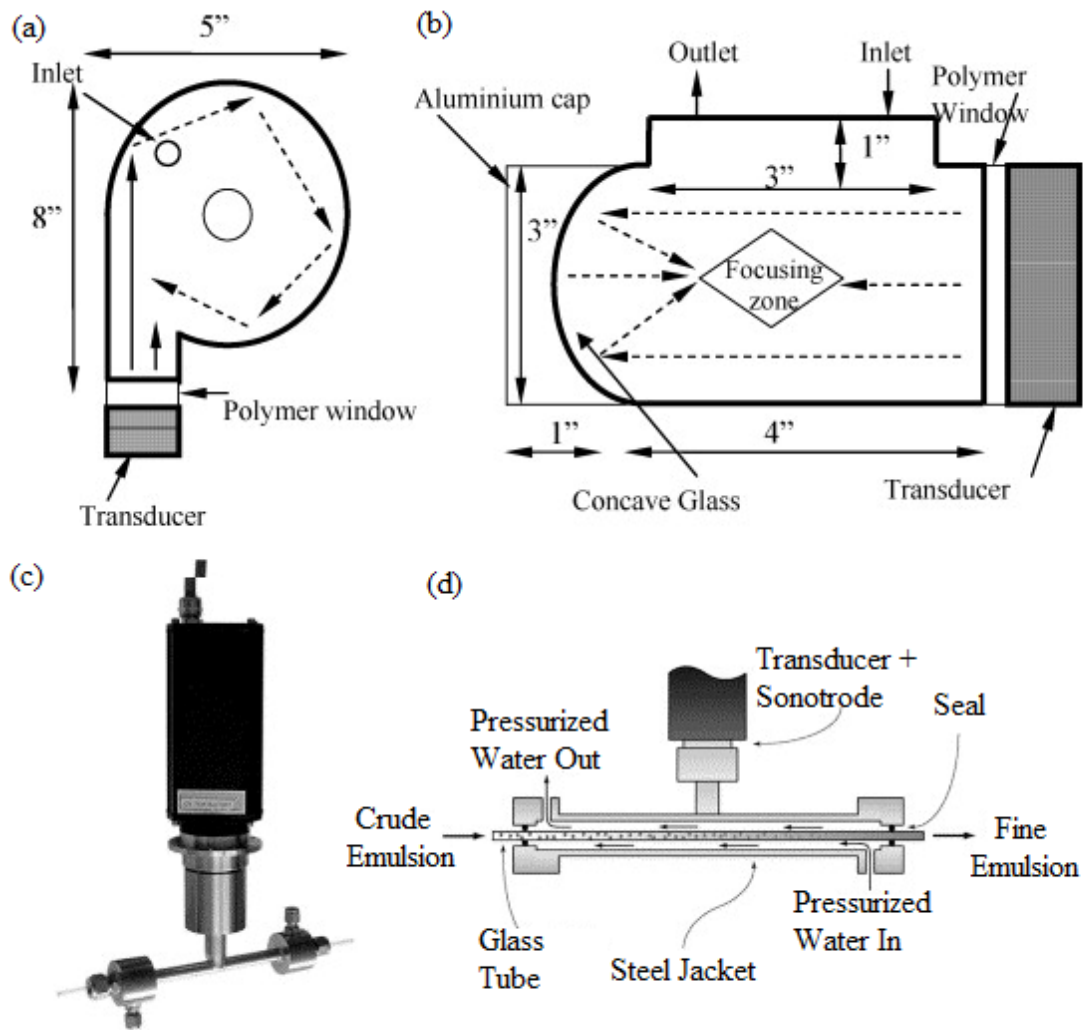

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986

987   Fig. 4.

988
989

990   Fig. 5.
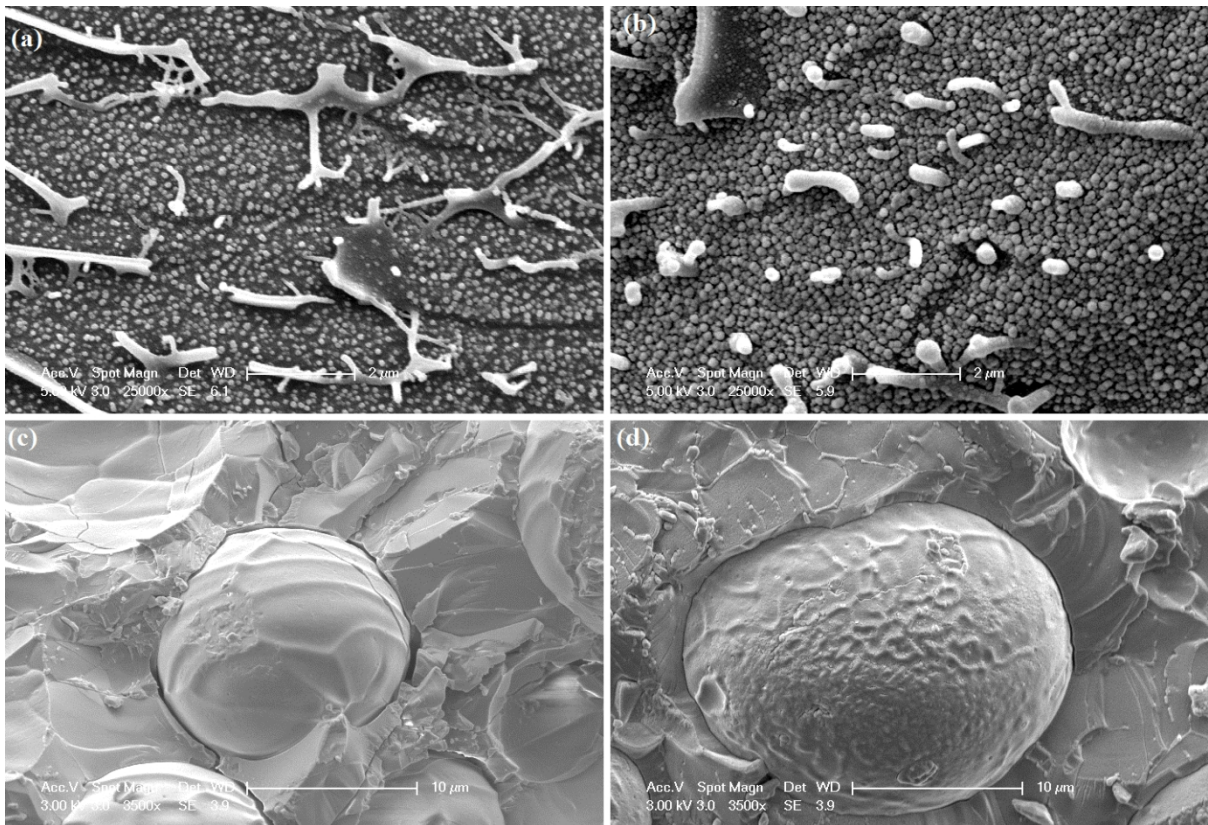
991

992

993   Fig. 6.

994

995

996   Fig. 7.

(a)

Inlet

5"

8"

Polymer window

Transducer

(b)

Aluminium cap

Outlet   Inlet   Polymer Window

1"

3"

3"

Focusing zone

1"   4"

Concave Glass   Transducer

(c)

(d)

Transducer + Sonotrode   Seal

Pressurized Water Out

Crude Emulsion

Fine Emulsion

Glass Tube   Steel Jacket   Pressurized Water In
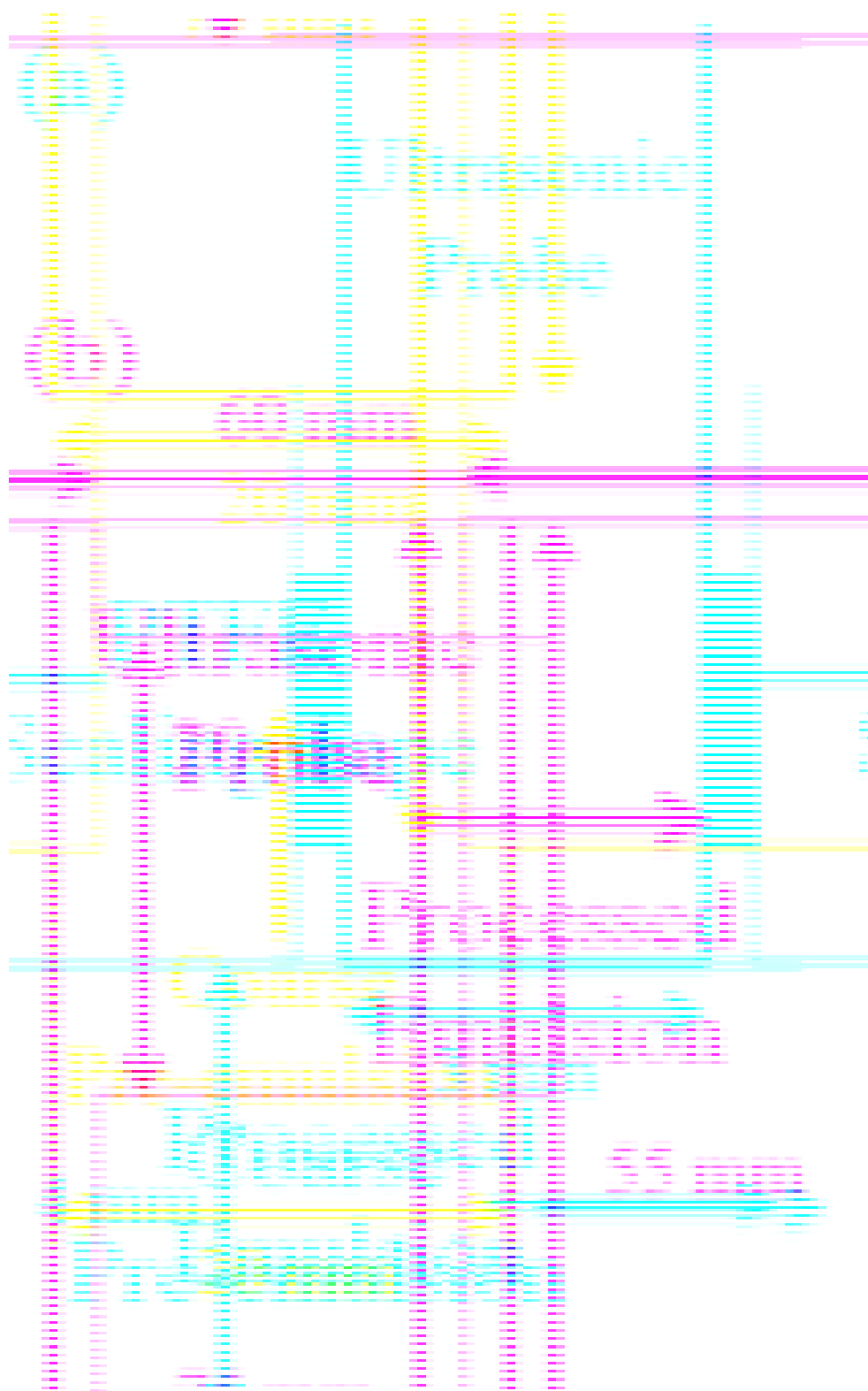
997

998

999 **Tables**

1000 **Table 1.**

1001 Examples of studies examining the effect of ultrasonic treatment related to dairy, animal,

1002 cereal, legume, tuber and fruit protein sources.

Protein source		Reference
Dairy	Micellar casein	Madadlou, *et al.*, (2009)
	Sodium caseinate	O'Sullivan, Arellano, *et al.*, (2014); O'Sullivan, *et al.*, (2014), de Figueiredo Furtado *et al.*, (2017); de Figueiredo Furtado *et al.*, (2016)
	Calcium caseinate	Zisu, *et al.*, (2010)
	Milk protein concentrates/ isolates (including retentates and skim powders)	Chandrapala, *et al.*, (2014); McCarthy, *et al.*, (2014); O'Sullivan, Arellano, *et al.*, (2014); O'Sullivan, Beevers, *et al.*, (2015); Shanmugam, *et al.*, (2012); Uluko, *et al.*, (2013); Yanjun, *et al.*, (2014); Zisu, *et al.*, (2010)
	Whey protein concentrates/ isolates (including retentates, BSA and α-lactalbumin)	Arzeni, *et al.*, (2012), Barukčić, *et al.*, (2014), Chandrapala, *et al.*, (2011), Gülseren, *et al.*, (2007), Güzey, *et al.*, (2006), Guzey & Weiss, (2001), Jambrak, *et al.*, (2008), Jambrak, *et al.*, (2010), Jambrak, *et al.*, (2014), Martini, *et al.*, (2010), O'Sullivan, Arellano, *et al.*, (2014), Zisu *et al.*, (2010), Shen *et al.*, (2016), Abadía-García *et al.*, (2016)
Animal	Egg white proteins	Arzeni, *et al.*, 2012; Arzeni, Pérez, *et al.*, (2012); Krise, (2011); O'Sullivan, *et al.*, (2016); Zhou, *et al.*, (2015), Xiong *et al.*, (2016)
	Gelatin (bovine and piscine)	O'Sullivan, Murray, *et al.*, (2016)
Cereal	Rice	Li, *et al.*, (2015, 2016); O'Sullivan, *et al.*, (2016)
	Wheat	O'Sullivan, *et al.*, (2016b); Zhang, *et al.*, (2011)
	Corn	Ren, *et al.*, (2015), Zhou *et al.*, (2016)
	Millet	Nazari *et al.*, (2016)
Legume	Soy protein concentrates/ isolates (including flakes)	Arzeni, *et al.*, (2012); Chen, *et al.*, 2012; Hu, *et al.*, (2013); Jambrak, *et al.*, (2009); Karki, *et al.*, 2010; O'Sullivan, Murray, *et al.*, (2016); O'Sullivan, Park, *et al.*, (2016b), Wang *et al.*, (2017), Liu *et al.,*, (2016), Zhou *et al.*, (2016)
	Pea protein isolate	O'Sullivan, Beevers, *et al.*, (2015); O'Sullivan, Murray, *et al.*, (2016), McCarthy *et al.*, (2016)
	Black bean protein isolate	Jiang *et al.*, (2014)
	Mung bean protein isolate	Charoensuk *et al.*, (2014)
	Lupin protein concentrate	O'Sullivan, Park, *et al.*, (2016a)

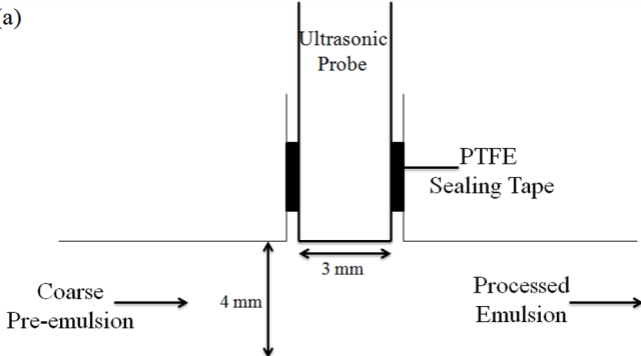|		
Tuber	Potato protein isolate	O'Sullivan, Park, *et al.*, (2016a)
Fruit	Walnut protein	Jincai *et al.*, (2013)
	Peanut protein	Chen *et al.*, (2016), Huang *et al.*, (2016)

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1004

1005 **Table 2.**

1006 Bond energy (kJ mol^{-1}) associated with intra- and intermolecular bonds present in proteins
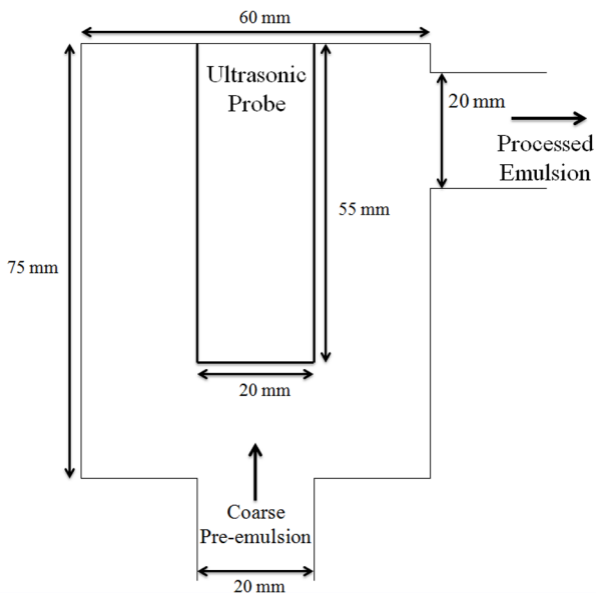
1007 (McMurry, 2011).

	Typical bonds present in proteins	Bond energy (kJ mol^{-1})
	C-N (peptide bond)	285
	C=N	615
Intramolecular bonds present within peptide chains	C-C	348
	N-H	391
	C-H	413
	C=O	799
Intermolecular bonds occurring between amino acids	Hydrogen bonding	4 – 13
	S-S	226

1008

(a)

Ultrasonic Probe

PTFE Sealing Tape

Coarse Pre-emulsion

Processed Emulsion

4 mm

3 mm

(b)

60 mm

Ultrasonic Probe

20 mm

Processed Emulsion

75 mm

55 mm

20 mm

Coarse Pre-emulsion

20 mm

Untreated BG Emulsion

Acc.V Spot Magn Det WD ├──────┤ 10 μm
3.00 kV 3.0 2000x   SE   3.9
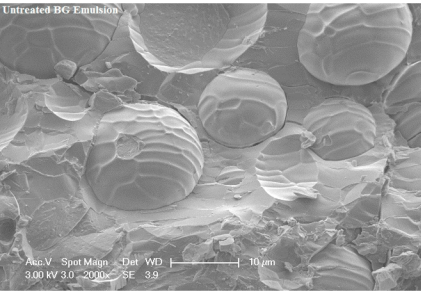
Ultrasound Treated BG Emulsion

Acc.V Spot Magn Det WD ├──────┤ 10 μm
3.00 kV 3.0 2000x   SE   3.9