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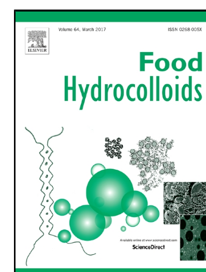

Applications of ultrasound for the functional modification of proteins and nanoemulsion formation: A review

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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.

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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

26 **Keywords**: Ultrasonic processing, Proteins, Functional properties, Emulsifying performance,
27 Nanoemulsion fabrication, Sonoreactor design

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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

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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.).

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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

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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

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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

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

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

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

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).

207 **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.

210 *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

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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 (≥ 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

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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,

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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

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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

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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,

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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

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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 (Abismaїl, *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

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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

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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 (Abismaïl, *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

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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 (Abismaïl, *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.

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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),

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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).

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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

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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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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.

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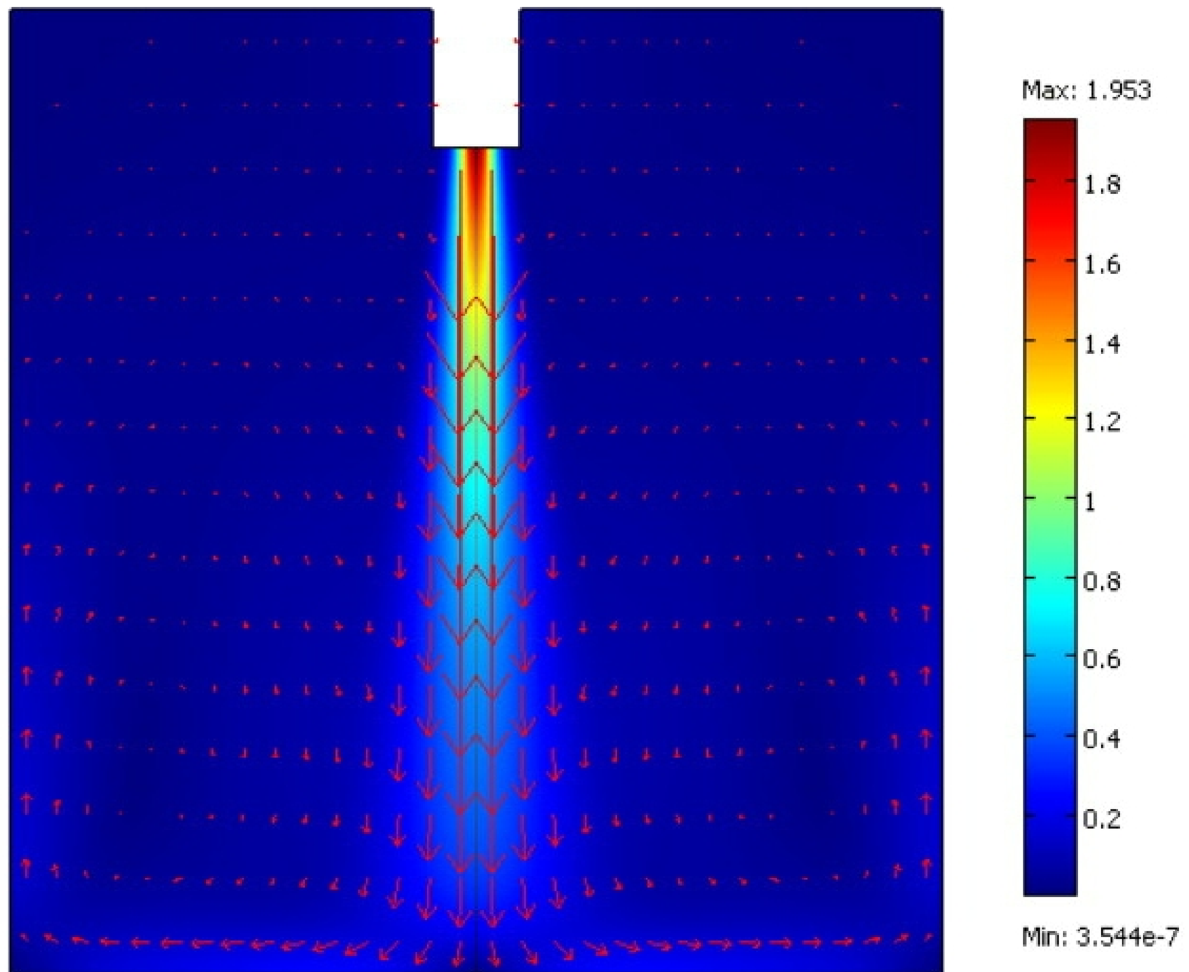
977 **Figures**

978 Fig. 1.



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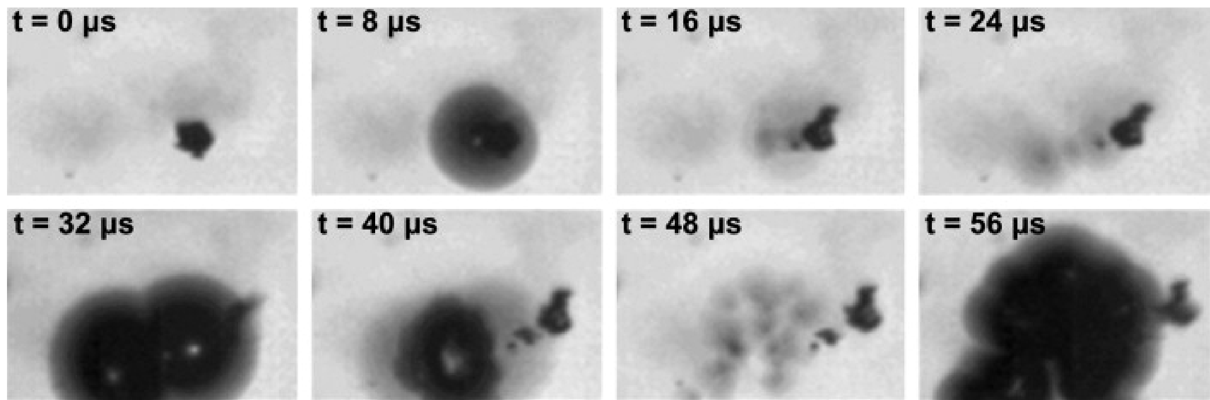
981 Fig. 2.



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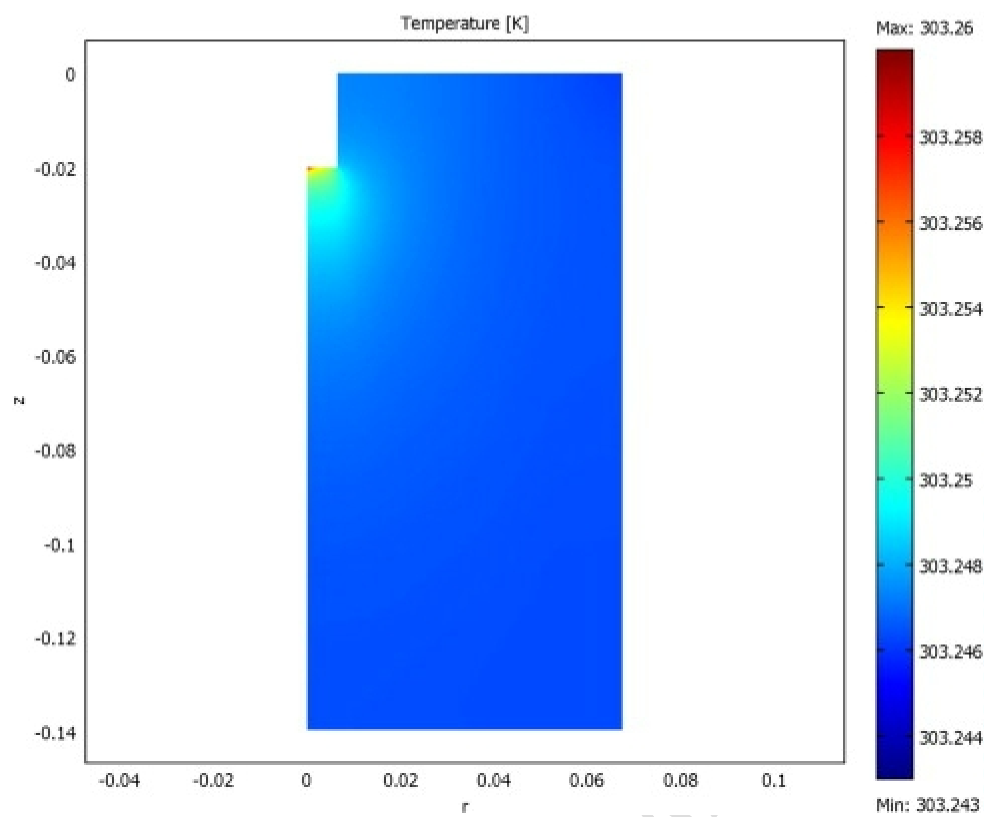
984 Fig. 3.



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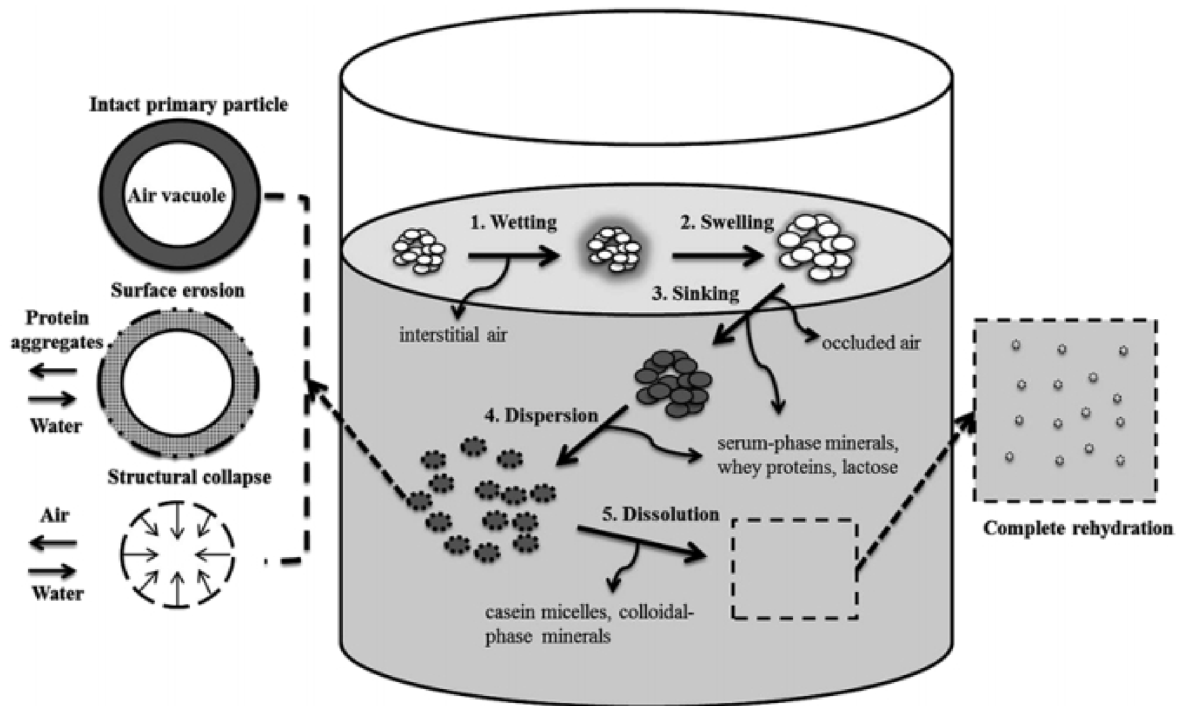
987 Fig. 4.



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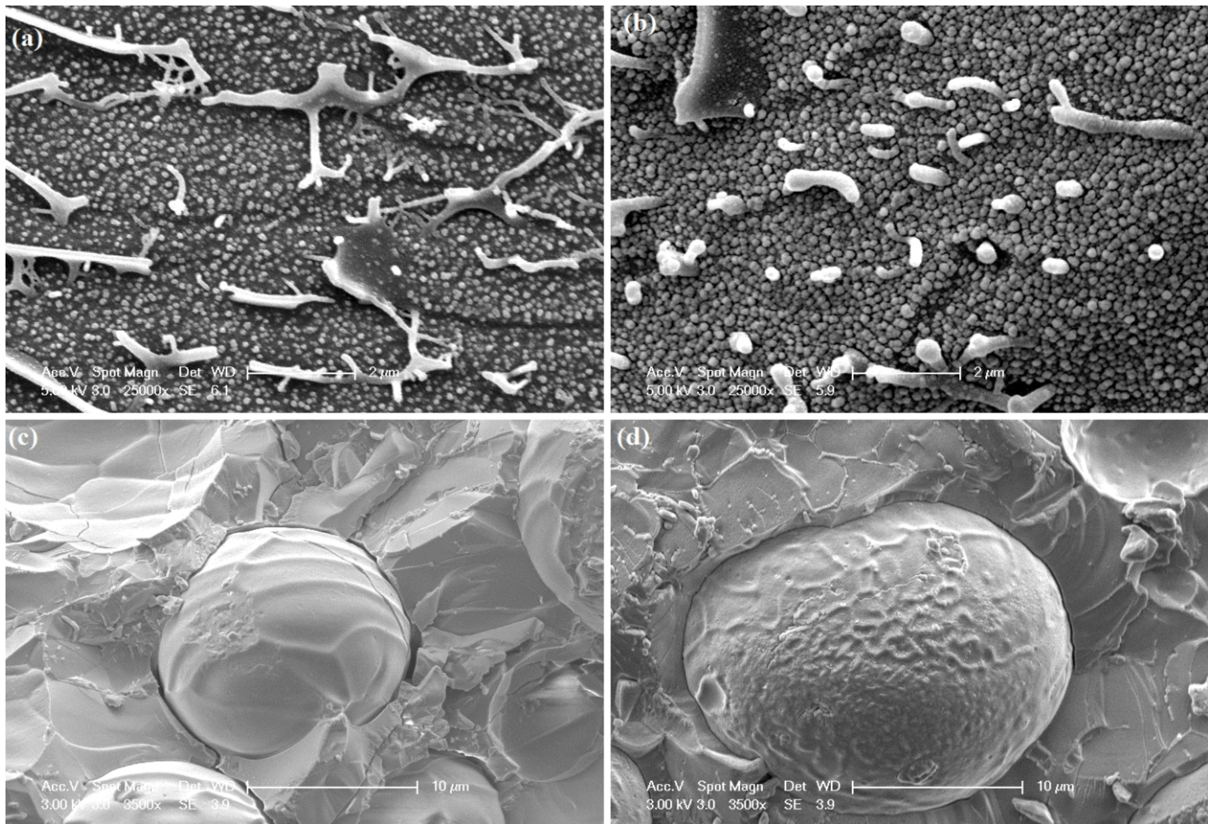
990 Fig. 5.



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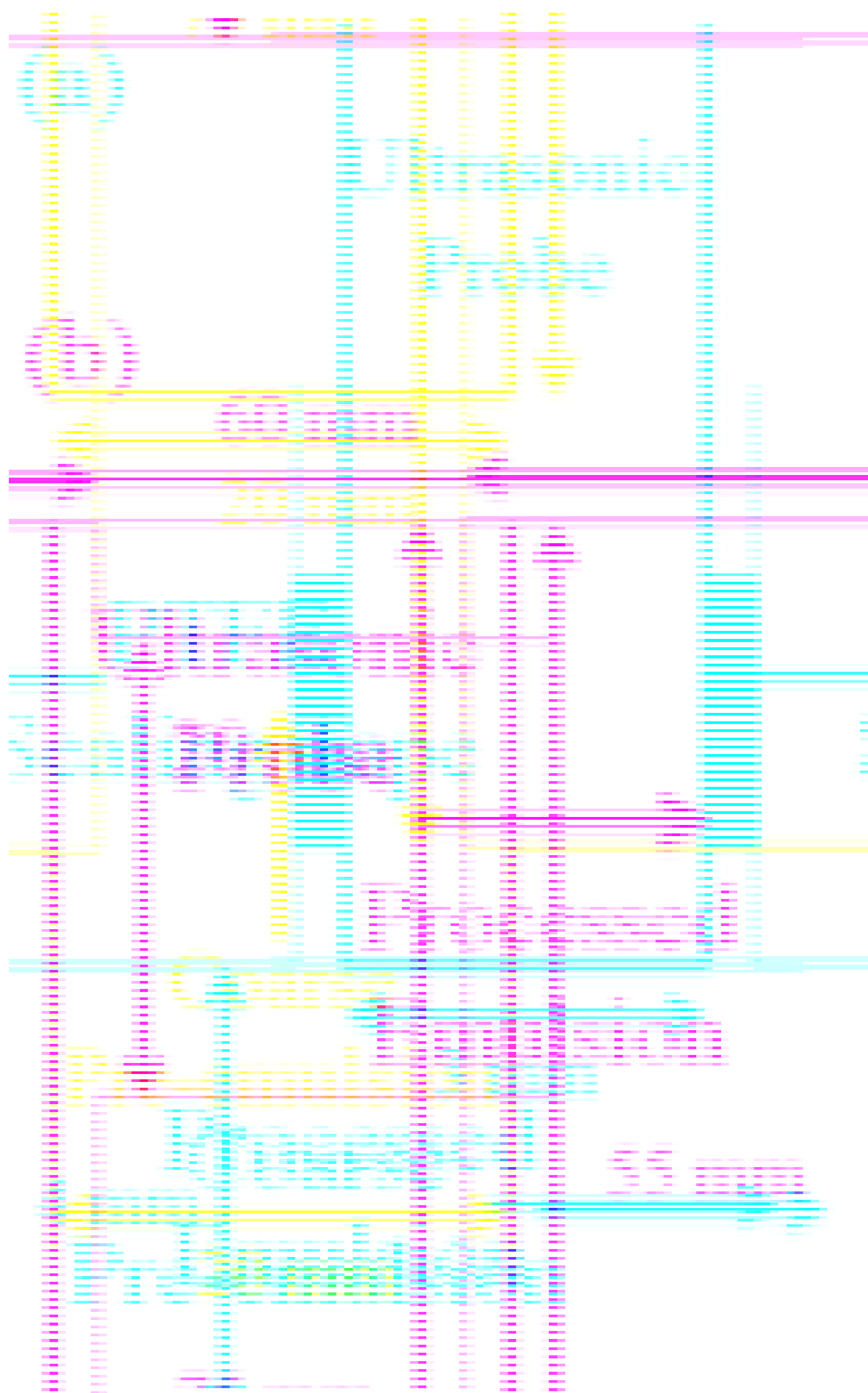
993 Fig. 6.



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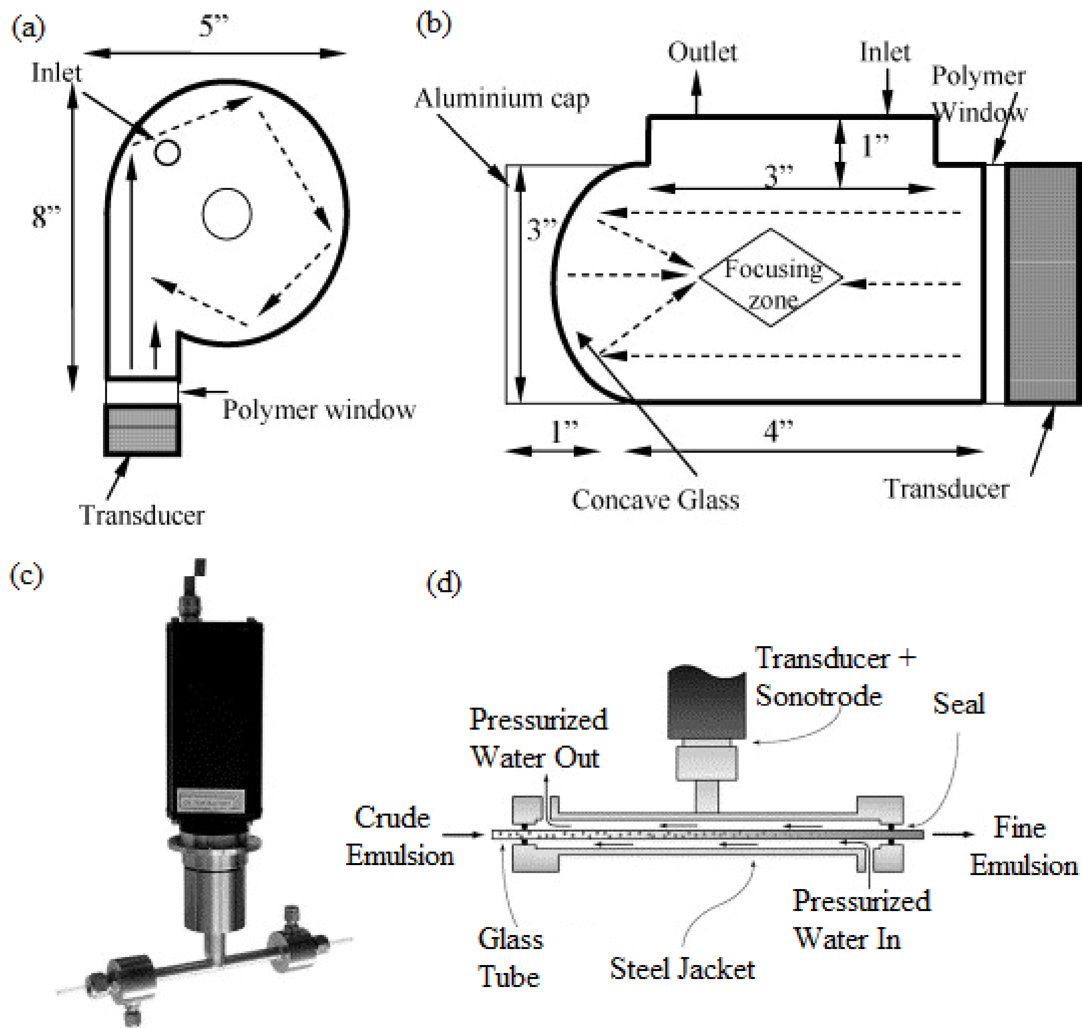
996 Fig. 7.



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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)

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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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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})
Intramolecular bonds present within peptide chains	C-N (peptide bond)	285
	C=N	615
	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

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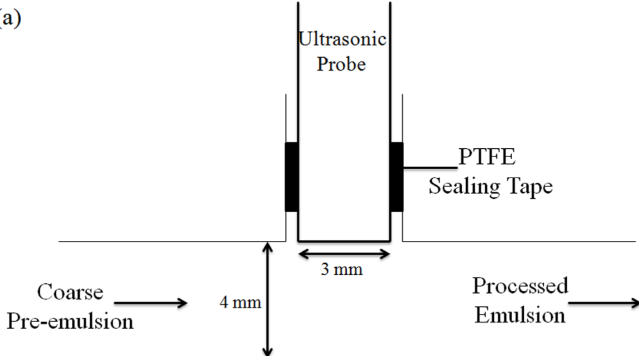
(a)



(b)

60 mm
Ultrasonic
Probe
20 mm
Processed
Emulsion
55 mm
75 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