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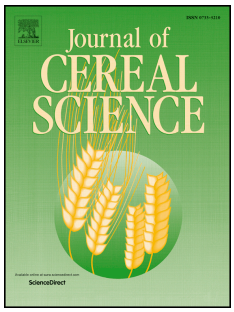

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The effect of ultrasound upon the physicochemical and emulsifying properties of wheat and soy protein isolates

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Abstract:

The effect of ultrasound upon the physicochemical and emulsifying performance of wheat protein isolate (WhPI) and soy protein isolate (SPI) was investigated. Protein solutions (0.1 – 3 wt. %) were sonicated with an acoustic intensity of ~34 W cm^{-2} for 2 min. The physicochemical properties were assessed in terms of changes in protein aggregate size, hydrodynamic volume and molecular structure. The emulsifying performance of ultrasound treated WhPI and SPI was compared to their untreated counterparts, and a low molecular weight surfactant, Tween 80, for comparative purposes. Ultrasonic processing significantly reduced the aggregate size of both proteins, whilst no reduction in the primary structure molecular weight profile was observed in both instances, ascribed to insufficient energy to hydrolyse the peptide bond. Emulsions prepared with both untreated proteins yielded submicron emulsion droplets (~150 nm) at concentrations ≥ 0.75 wt. %. Emulsions fabricated with both sonicated proteins at concentrations < 0.75 wt. % demonstrated significantly ($P < 0.05$) smaller emulsion droplets and long term emulsion stability in comparison to their untreated counterparts. This effect is consistent with the observed reduction in the equilibrium value of interfacial tension between untreated and ultrasound treated proteins.

Keywords: *Triticum aestivum*, *Glycine max*, Ultrasound, Submicron emulsions

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1. Introduction

Proteins are ubiquitously utilised as functional ingredients within the food and pharmaceutical industries for emulsification, foaming, gelation and viscosity enhancement. The functionality of proteins is due to the chemical make-up of these molecules, their unique amino acid sequences (Walstra & van Vliet, 2003). Proteins are of particular interest in food formulations as emulsifying agents, due to their ability to adsorb and form viscoelastic films at oil-water interfaces (O'Connell & Flynn, 2007). Proteins provide several advantages for emulsion droplet stabilisation, such as protein-protein interactions at the interface, and electrostatic and steric stabilisation mechanisms due to the charged and bulky nature of these biopolymers, in comparison to low molecular weight surfactants (O'Sullivan, *et al.*, 2014).

Ultrasound is a mechanical pressure wave with a frequency greater than 20 kHz, the threshold for human auditory detection. Low frequency (20 – 100 kHz), high power ultrasound (10 – 1,000 W cm^{-2}), commonly referred to as power ultrasound, is utilised for the alteration, generations or modification of food microstructures (O'Sullivan, *et al.*, 2014). The effects of power ultrasound upon food microstructures are attributed to ultrasonic cavitations, generated by localised pressure differentials over short periods of time (a few microseconds). Ultrasonic cavitations yield localised regions of high hydrodynamic shear and rises in temperature at the site of bubble collapse (~5000 ^{o}C) accounting for the observed effect of power ultrasound (O'Sullivan, *et al.*, 2016).

Ultrasound treatment has been related to the physicochemical modifications of food proteins. However, few studies detail the effect of ultrasound upon cereal proteins, other than that of Zhang *et al.*, (2011) for wheat gluten and O'Sullivan, *et al.*, (2016) for rice protein isolate, both demonstrated that the acoustic energy provided insufficient energy to reduce the molecular weight profile of these cereal proteins. Zhang *et al.*, (2011) studied the effect of

ultrasound upon the rheologically behaviour of wheat gluten, both the storage (G') and loss (G") modulii decreased, and additionally the foaming capacity and emulsifying performance, both were enhanced. O'Sullivan, *et al.,* (2016) reported no significant reduction in aggregate size of rice protein isolate, ascribed to insufficient energy to achieve scission of disulphide bonds maintaining the structure of denatured aggregates. However, the effect of ultrasound treatment upon the physicochemical structure of wheat protein and relation to submicron emulsion formation and long term stability with respect to protein concentration has yet to be investigated.

Wheat protein isolate (WhPI) is of particular interest to the food industry, as it is the second most cultivated cereal crop (725 million metric tonnes) after maize (1,100 million metric tonnes), and followed by rice (496 million metric tonnes) (FAO, 2015). WhPI is a highly functional ingredient utilised commonly within baked and process foods (Ahmedna *et al.*, 1999). WhPI is extracted from *Triticum aestivum* and is primarily cultivated in the EU, China, India and USA (FAO, 2015). The major protein fractions in WhPI are polymeric glutenins and monomeric gliadins, with minor fractions of albumins and globulins (Kuktaite *et al.*, 2004).

Soy protein isolate (SPI) a food ingredient of great importance, as it is the largest commercially available legume protein source owing to its high nutritional value, current low cost, and a highly functional ingredient due to its emulsifying and gelling capabilities (Achouri *et al.,* 2012; Molina *et al.*, 2002; Sorgentini *et al.*, 1995). SPI, extracted from *Glycine max*, is an oilseed legume grown primarily in the United Sates, Brazil, Paraguay and Uruguay (Gonzalez-Perez & Arellano, 2009). The major protein fractions in oilseed legumes are albumins (2S) and globulins, the dominant fractions of which are glycinin (11S; 300-360 kDa) and β-conglycinin (7S; 150-190 kDa) (Shewry *et al.*, 1995).

In this work, wheat protein isolate (WhPI) and soy protein isolate (SPI) were investigated in order to assess the significance of power ultrasound for the improvement of emulsifying performance. The objectives of this research were to discern the effects of ultrasound treatment upon WhPI and SPI in terms of differences to physicochemical properties, measured in terms of aggregate size, molecular structure and hydrodynamic volume. Additionally, the emulsifying efficacy of WhPI and SPI before and after ultrasound treatment was assessed in terms of initial emulsion droplet size, long term stability and interfacial tension. Oil-in-water emulsions were prepared with either untreated or ultrasound treated WhPI and SPI at different concentrations, and compared between them and to a low molecular weight surfactant, Tween 80.

2. Materials and methodology

2.1. Materials

Wheat protein isolate (Prolite® 100; WhPI) and soy protein isolate (Pro-Fam® 781; SPI) were both kindly provided by Archer Daniels Midland (ADM; Decatur, USA). The protein content of WhPI and SPI was 90 wt. % and 86 wt. %, respectively. The pH of WhPI and SPI at a protein concentration of 1 wt. % was 4.2 ± 0.1 and 6.9 ± 0.1, whereby WhPI possessed a cationic charge (17.4 ± 0.4 mV) and SPI an anionic charge (-35.5 ± 0.6 mV). Tween 80 and sodium azide were purchased from Sigma Aldrich (UK). The oil used was commercially available rapeseed oil. The water used in all experiments was passed through a double distillation unit (A4000D, Aquatron, UK). All materials were used with no further purification or modification of their properties.

2.2. Methods

2.2.1. Preparation of emulsifier solutions

WhPI, SPI and Tween 80 were dispersed in water to obtain solutions within a protein concentration range of 0.1 – 3 wt. %, and Tween 80 was soluble at the range of concentrations, whereas WhPI and SPI possessed an insoluble component regardless of hydration time. Sodium azide (0.02 wt. %) was added to the solution to mitigate against microbial activity.

2.2.2. Ultrasound treatment of protein solutions

An ultrasonic processor (Viber Cell 750, Sonics, USA) with a 12 mm diameter stainless steel probe was used to ultrasound treat 50 ml aliquots of protein solution in 100 ml plastic beakers, which were placed in an ice bath to reduce heat gain. The protein solutions were sonicated with a frequency of 20 kHz and amplitude of 95% (wave amplitude of 108 μm at 100% amplitude) for up to 2 min. This yielded an ultrasonic power intensity of ~34 W cm^{-2}, which was determined calorimetrically by measuring the temperature rise of the sample as a function of treatment time, under adiabatic conditions. The acoustic power intensity, I_a (W cm^{-2}), was calculated as follows (Margulis & Margulis, 2003):

$$I_a = \frac{P_a}{S_A}\ , where \quad P_a = m.c_p\left(\frac{dT}{dt}\right) \tag{1}$$

Where P_a (W) is the acoustic power, S_A is the surface area of the ultrasound emitting surface (1.13 cm^2), m is the mass of ultrasound treated solution (g), c_p is the specific heat of the medium (4.18 kJ/gK) and dT/dt is the rate of temperature change with respect to time, starting at t = 0 (^oC/s).

The temperature of protein solutions was measured before and after sonication by means of a digital thermometer (TGST3, Sensor-Tech Ltd., Ireland), with an accuracy of ± 0.1 °C. Prior to ultrasound treatment, the temperature of protein solutions were within the range of 5 – 10 ^{o}C. After ultrasonic irradiation, the temperature raised to approximately ~45 °C.

2.2.3. Characterisation of untreated and ultrasound treated protein solutions

2.2.3.1. Microstructure characterisation

The size of untreated and ultrasound treated WhPI and SPI were measured by laser diffraction using the Mastersizer 2000 (Malvern Instruments, UK). Protein size is reported as a size distribution. The protein size distributions are reported as the average of three repeat measurements.

2.2.3.2. Molecular structure characterisation

The molecular structure of untreated and ultrasound treated WhPI and SPI was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), using a Mini-Protean 3 Electrophoresis System (Bio-Rad, UK). 100 μL of protein solution at 1 wt. % concentration were added to 1 mL of native sample buffer (Bio-Rad, UK) in 2 mL micro tubes and sealed. A 10 μL aliquot was taken from each sample and loaded onto a Tris-acrylamide gel (Bio-Rad, UK; 4-20% Mini Protean TGX Gel, 10 wells). A protein standard (Bio-Rad, UK; Precision Plus Protein^{TM} All Blue Standards) was used to determine the molecular weight of the samples. Gel electrophoresis was carried out initially at 55 V (I > 20 mA) for 10 min, then at 155 V (I > 55 mA) for 45 min in a running buffer (Bio-Rad, UK; 10x Tris/Glycine/SDS Buffer). The gels were removed from the gel cassette and stained with

Coomassie Bio-safe stain (Bio-Rad, UK) for 1 hr and de-stained with distilled water overnight.

2.2.3.3. Hydrodynamic volume characterisation

The intrinsic viscosity (*i.e.* hydrodynamic volume) of untreated and ultrasound treated WhPI and SPI were determined by a double extrapolation to a zero concentration method, as described by Morris *et al.*, (1981), using the models of Huggins' and Kraemer, as follows:

Huggins, (1942): $$\frac{\eta_{sp}}{c} = [\eta] + k_H[\eta]^2 c \quad (2)$$

Kraemer, (1938): $$\frac{\ln \eta_{rel}}{c} = [\eta] + k_K[\eta]^2 c \quad (3)$$

Where η_{sp} is the specific viscosity (viscosity of the solvent, η_0 / viscosity of the solution, η), c the protein concentration (w/v%), $[\eta]$ the intrinsic viscosity (dL/g), k_H the Huggins constant. η_{rel} is the relative viscosity (viscosity of the solution, η / viscosity of the solvent, η_0) and k_K is the Kraemer constant.

The concentration ranges used for the determination of the intrinsic viscosity of WhPI and SPI was 1 – 2.5 wt. % and 1.5 – 3 wt. %, respectively. The validity of the regression procedure is confined within a discrete range of η_{rel}, $1.2 < \eta_{rel} < 2$. The upper limit is due to the hydrodynamic interaction between associates of protein molecules, and the lower limit is due to inaccuracy in the determination of very low viscosity fluids. A value of η_{rel} approaching 1 indicates the lower limit (Morris *et al.*, 1981).

The viscosity of the protein solutions was measured at 20 °C using a Kinexus rheometer (Malvern Instruments, UK) equipped with a double gap geometry (25 mm diameter, 40 mm height). For the determination of intrinsic viscosity by extrapolation to infinite dilution, there must be linearity between shear stress and shear rate, which indicates a

Newtonian behaviour region on the range of shear rate used in the measurements. The Newtonian plateau region of WhPI and SPI solutions at the range of concentrations used was found within a shear rate range of 25 - 1000 s^{-1} (data not shown). Thus, the values of viscosity of the protein solutions and that of the solvent (distilled water) were selected from the flow curves data at a constant shear rate of 250 s^{-1} (within the Newtonian region), which were subsequently used to determine the specific viscosity, η_{sp}, the relative viscosity, η_{rel}, and the intrinsic viscosity, [η]. Three replicates of each measurement were made.

2.2.4. Preparation of oil-in-water emulsions

10 wt. % dispersed phase (rapeseed oil) was added to the continuous aqueous phase containing either untreated or sonicated proteins, or Tween 80 at different concentrations, ranging from 0.1 - 3 wt. %. An oil-in-water pre-emulsion was prepared by emulsifying this mixture at 8000 rpm for 2 min using a high shear mixer (SL2T, Silverson, UK). Submicron oil-in-water emulsions were then prepared by further emulsifying the pre-emulsion using an air-driven microfluidiser (M110S, Microfluidics, USA), at 100 MPa for 1 pass. The initial temperature of pre-emulsions was 5 ^{o}C to minimise the potential for protein aggregation from the high processing pressures. The final temperatures of emulsions prepared after homogenisation was ~30 ^{o}C.

2.2.5. Characterisation of oil-in-water emulsions.

2.2.5.1. Droplet size measurements

The droplet size of the emulsions was measured by laser diffraction using a Mastersizer 2000 (Malvern Instruments, UK) immediately after emulsification. Emulsion droplet size values are reported as the volume-surface area mean diameter (Sauter diameter; $d_{3,2}$). The stability of the emulsions was assessed by droplet size measurements over 28 days,

where emulsions were stored under refrigeration conditions (4 ^{o}C) throughout the duration of the stability study. The droplet sizes and error bars are reported as the mean and standard deviation, respectively, of measured emulsions prepared in triplicate.

2.2.5.2. Interfacial tension measurements

The interfacial tension between the aqueous phase (pure water, protein solution, or surfactant solution) and oil phase (rapeseed oil) was measured using a tensiometer K100 (Krűss, Germany) with the Wilhelmy plate method. The Wilhelmy plate has a length, width and thickness of 19.9 mm, 10 mm and 0.2 mm, respectively and is made of platinum. The Wilhelmy plate was immersed in 20 g of aqueous phase to a depth of 3 mm. Subsequently, an interface between the aqueous phase and oil phase was created by carefully pipetting 50 g of the oil phase over the aqueous phase. The test was conducted over 3,600 s and the temperature was maintained at 20 °C throughout the duration of the test. The interfacial tension values and the error bars are reported as the mean and standard deviation, respectively, of three repeat measurements.

2.3. Statistical analysis

Student's t-test with a 95% confidence interval was used to assess the significance of the results obtained. t-test data with $P < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Effect of ultrasound treatment on the physicochemical properties of WhPI and SPI

The effect of ultrasound treatment on the aggregate size of WhPI and SPI was initially investigated. 1 wt. % WhPI and SPI solutions were sonicated for 2 min, with a frequency of 20 kHz and an ultrasonic amplitude of 95%. Protein size distributions for untreated and ultrasound treated WhPI and SPI are shown in Fig. 1. Untreated WhPI (*cf.* Fig. 1a) exhibited

a bimodal size distribution, a nano-sized peak of ~200 nm and a micron-sized peak of ~50 μm, whereas untreated SPI (*cf.* Fig. 1b) solely displayed a micron-sized peak of ~10 μm. A significant reduction ($P < 0.05$) in the micron-sized aggregates of WhPI (*cf.* Fig. 1a) was observed, whilst only partially disrupted. The partial breakup of these micron-sized aggregates is ascribed to disruption of associative non-covalent interactions (hydrophobic forces and electrostatic interactions), whilst insufficient acoustic energy is provided to reduce the remaining micron aggregate irrespective of processing time (data not shown). The residual micron sized aggregates are denatured wheat protein entities formed due to the processing of this isolate and are maintained by disulphide bonds. Similarly in the case of SPI, a significant reduction ($P < 0.05$) in the size of the micron-sized peak to the nano scale (~200 nm) is observed, whilst insufficient acoustic energy is provided to completely disrupt the micron sized entity, for the same reasons as previously described for WhPI. The acoustic energy provided from the ultrasound treatment is insufficient to reduce these disulphide bonds (-S-S-; 226 kJ mol^{-1}) present within the denatured aggregates, whilst sufficient to disrupt associative non-covalent interactions (4 – 13 kJ mol^{-1}) (O'Sullivan, *et al.*, 2016).

The molecular structures of untreated and ultrasound treated WhPI and SPI were subsequently investigated. Protein solutions at a concentration of 1 wt. % were irradiated with ultrasound for 2 minutes with an acoustic intensity ~ 34 W cm^{-2}. Electrophoretic profiles for untreated and ultrasound treated WhPI and SPI, and a molecular weight standard are shown in Fig. 2. As can be seen from the results in Fig. 2, there is no significant reduction ($P > 0.05$) in the molecular weight profile of WhPI or SPI after ultrasound treatment. These results are in agreement with those presented by Zhang *et al.*, (2011) who reported no differences in the molecular structure of wheat gluten after ultrasound treatment (900 W at 100% amplitude for 10 min). Insufficient acoustic energy is provided to achieve proteolysis of the peptide bond (-C-N-; 285 kJ mol^{-1}), or scission of disulphide bonds (-S-S-; 226 kJ mol^{-}

[1]) (Chandrapala, *et al.*, 2012; Zisu, *et al.*, 2011). The majority of acoustic energy is utilised for the disruption of associative non-covalent interactions maintaining aggregate structure (O'Sullivan, *et al.*, 2016).

Intrinsic viscosity, [η], was determined from the fitting of the Huggins' and Kraemer equations to the experimental viscosity data, for untreated and ultrasound treated WhPI and SPI solutions at different concentrations, as shown in Fig. 3. Intrinsic viscosity is a measure of a solvents capacity to achieve hydration of a polymer and provides information about the hydrodynamic volume (Behrouzian *et al.*, 2014). Ultrasound treatment of WhPI and SPI induced a significant ($P < 0.05$) reduction in the intrinsic viscosity, and thus a significant reduction in the hydrodynamic volume. These results are consistent with the reduction in aggregate size as measured by laser diffraction (*cf.* Fig. 1). Cole *et al.*, (1984) reported intrinsic viscosity values of α-gliadin ranging between 0.95 – 1.85 dL g^{-1}, owing to differences in solvent quality (*i.e.* solvent conditions), and Prakash, (1994) reported intrinsic viscosity values of 0.46 dL g^{-1} glycinin (11S; soy globulin). These values differ to the results presented in this work for both untreated proteins, and these differences are ascribed to the complexity of WhPI and SPI solutions, which is composed of a mixture of protein fractions, rather than the single component α-gliadin and glycinin used by Cole *et al.*, (1984) and Prakash, (1994), respectively. Additionally, the solvent used in the work of Cole *et al.*, (1984) was guanidine hydrochloride at concentrations ranging from 1.1 – 5.9 M, whilst in this work untreated WhPI was dissolved in distilled water.

Intrinsic viscosity of proteins in solution can give a measure of the degree of hydrophobicity (Tanner & Rha, 1980). The intrinsic viscosity of proteins in solution depends on its conformation and thus on its levels of hydration, which is a result of the amount of hydrophobic residues concealed within the interior of protein associates in solution. Furthermore, Khan *et al.*, (2012) reported that a decrease of intrinsic viscosity resulted in

dehydration of amphiphatic biopolymers associates, increasing the hydrophobicity of these biopolymers, hence reducing the energy required for adsorption at oil-water interfaces. Therefore, the reported decrease in intrinsic viscosity of WhPI and SPI induced by ultrasonic treatment, expresses an increase in the degree of hydrophobicity of these proteins.

3.2. Comparison of the emulsifying performance of untreated and ultrasound treated WhPI and SPI

Oil-in-water emulsions were prepared with 10 wt. % rapeseed oil and a continuous phase containing either untreated or ultrasound treated WhPI or SPI, or Tween 80, at different concentrations (0.1 – 3 wt. %). The emulsions were passed through a microfluidiser at 100 MPa for a single pass, and droplet sizes as a function of emulsifier type and concentration are shown in Fig. 4. The emulsion droplet sizes were measured immediately after emulsification, and all exhibited unimodal droplet size distributions.

Emulsions fabricated with ultrasound treated WhPI (*cf.* Fig. 4a) and SPI (*cf.* Fig. 4b) at concentrations < 0.75 wt. % yielded a significant ($P < 0.05$) reduction in emulsion droplet size in comparison to their untreated counterparts. The decrease in emulsion droplet size after ultrasonic processing at concentrations < 0.75 wt. % is consistent with the aforementioned significant ($P < 0.05$) reduction in protein size (*i.e.* increase in surface area-to-volume ratio) upon ultrasound treatment which allows for enhanced adsorption of protein at the oil-water interface, as reported by Damodaran & Razumovsky, (2008). Furthermore, the significant increase in the hydrophobicity (*i.e.* reflected in a reduction in the intrinsic viscosity; *cf.* Fig. 3) would lead to an increased rate of protein adsorption to the oil-water interface, reducing the interfacial tension, thus improved facilitation of emulsion droplet break-up. The reported submicron emulsion droplet sizes for untreated WhPI are comparable to those measured by

Day *et al.*, (2009), in the order of ~300 nm for emulsions containing deamidated wheat protein (4 wt. %).

The reported emulsion droplet sizes for WhPI and SPI (*cf.* Fig. 5) are smaller than that of the untreated proteins (*cf.* Fig. 1). Be that as it may, the protein sizes of the untreated proteins represent aggregates of protein molecules rather than discrete protein fractions. α-gliadin and glycinin have hydrodynamic radii (R_h) of approximately 2.5 nm and 12.5 nm, respectively (Blanch *et al.*, 2003; Peng *et al.*, 1984), in comparison to the micron sized entities presented in Fig. 1. This disparity in size is due to the preparation of these isolates, whereby a combination of high shear and elevated temperatures result in the formation of insoluble aggregated material, in comparison to soluble native protein fractions. Proteins in aqueous solution associate together to form aggregates due to both hydrophobic and electrostatic interactions (O'Connell & Flynn, 2007), however in the presence of a hydrophobic dispersed phase (*i.e.* rapeseed oil) the protein molecules which comprise these aggregates dissociate and adsorb to the oil-water interface (Beverung *et al.*, 1999), accounting for the production of submicron emulsion droplets demonstrated in this study.

The observed emulsion droplet size data (*cf.* Fig. 4) can be explained by considering the interfacial tension of the presented systems. Fig. 5 shows the interfacial tension between water and rapeseed oil, for untreated and sonicated WhPI and SPI, and Tween 80 at a concentration of 0.1 wt. %. In order to assess the presence of impurities of the systems the interfacial tension between distilled water and rapeseed oil was measured. The interfacial tension of all systems decreased as a function of time (*cf.* Fig. 5), and this behaviour is ascribed to the nature of the dispersed phase and to a lesser extent the emulsifier utilised. Gaonkar, (1989) reported the time dependant characteristic of interfacial tension for commercial vegetable oils with water, attributed to the adsorption of surface active impurities within the oil to the oil-water interface. Moreover, Gaonkar, (1989) demonstrated that after

purification of vegetable oils the time dependency of interfacial tension was no longer exhibited.

The interfacial tension values obtained for both ultrasound treated WhPI and SPI were significantly lower ($P < 0.05$) than that of their untreated counterparts, and furthermore lower than values obtained with Tween 80. These results are consistent with the obtained emulsion droplet sizes (*cf.* Fig. 4), and validates the hypothesis that aggregates of sonicated protein adsorb at an increased rate at the oil-water interface due to the higher surface area-to-volume ratio (*cf.* Fig. 1) and elevated hydrophobicity (*i.e.* reduced intrinsic viscosity; *cf.* Fig. 3), significantly reducing the interfacial tension, enhancing emulsion droplet breakup during emulsification and fabricating smaller emulsion droplets, in comparison to untreated proteins.

The stability of emulsions prepared with untreated and ultrasound treated WhPI and SPI was investigated over a 28 day period. In addition, emulsions prepared with Tween 80 were assessed for comparative purposes. Fig. 6 shows the development of emulsion droplet size ($d_{3,2}$) as a function of time for emulsions prepared with untreated and ultrasound irradiated WhPI and SPI, as well as with Tween 80, at a concentration of 0.1 wt. %.

Emulsions prepared with untreated WhPI (*cf.* Fig. 6a) exhibited a growth in emulsion droplet size at emulsifier concentrations < 0.75 wt. %, whilst emulsions prepared with higher concentrations (≥ 0.75 wt. %) of untreated WhPI were stable for the duration of the 28 day stability study (data not shown). Nevertheless, it can also be observed that emulsions prepared with ultrasound treated WhPI (*cf.* Fig. 6a) were resistant to coalescence for the 28 days of the study, and possessed the same stability as Tween 80 (*cf.* Fig. 6). This behaviour was exhibited at all concentrations of ultrasound treated WhPI (data not shown). This enhanced emulsion stability of ultrasound treated WhPI in comparison to untreated WhPI is attributed to an increase in the hydrophobicity (*i.e.* decrease in the intrinsic viscosity) and

improved interfacial packing of ultrasound treated WhPI observed by a decrease in the equilibrium interfacial tension value (*cf.* Fig. 5a). Similar to emulsions prepared with WhPI, emulsions prepared with untreated SPI (*cf.* Fig. 6b) were unstable at emulsifier concentrations < 0.75 wt. %, whereas ultrasound treated stabilised SPI emulsions (*cf.* Fig. 6b) were stable at all concentrations over the 28 days of this study. This stability was observed for all concentrations explored in this study (≥ 0.5 wt. %) of ultrasound treated SPI (data not shown). Emulsions prepared with higher concentrations (≥ 0.5 wt. %) of untreated SPI were stable over the duration of the stability study (data not shown).

4. Conclusions

This study demonstrated the capacity of ultrasonic processing (20 kHz, ~34 W cm^{-2} for 2 min) of WhPI and SPI to significantly ($P < 0.05$) reduce aggregate size and hydrodynamic volume, whilst no significant ($P > 0.05$) reduction in the primary molecular structure of the proteins was observed. This reduction in protein aggregate size, yet no reduction in primary molecular structure of WhPI and SPI is ascribed to sufficient energy to disrupt associative non-covalent interactions (3 – 14 kJ mol^{-1}), whereas insufficient acoustic energy is provided to disrupt covalent interaction within the peptide chain, disulphide linkages (-S-S-; 226 kJ mol^{-1}) and peptides bonds (-C-N-; 285 kJ mol^{-1}).

Emulsions prepared with sonicated WhPI and SPI at concentrations < 0.75 wt. % yielded smaller emulsion droplets in comparison to their untreated counterparts at the same concentrations. This behaviour is attributed to the reduction in protein aggregate size (*i.e.* enhanced mobility through the bulk) and an increase in hydrophobicity (*i.e.* reflected by a decrease in the intrinsic viscosity) of ultrasound treated WhPI and SPI. Furthermore, emulsions prepared with both ultrasound irradiated WhPI and SPI exhibited improved emulsion stability against coalescence for 28 days at all concentrations. This enhancement is

attributed to an improved interfacial packing, observed by a lower equilibrium interfacial tension.

Thus, ultrasound is capable of enhancing the emulsifying performance WhPI and SPI, in terms of both emulsion formation and long term stability, and moreover, possesses the capacity for improving the solubility of previously poorly soluble cereal (WhPI) and leguminous (SPI) proteins.

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Fig. 1. Protein size distributions for: (a) untreated WhPI (solid line) and ultrasound treated WhPI immediately after processing (dashed line) and (b) untreated SPI (solid line) and ultrasound treated SPI immediately after processing (dashed line).

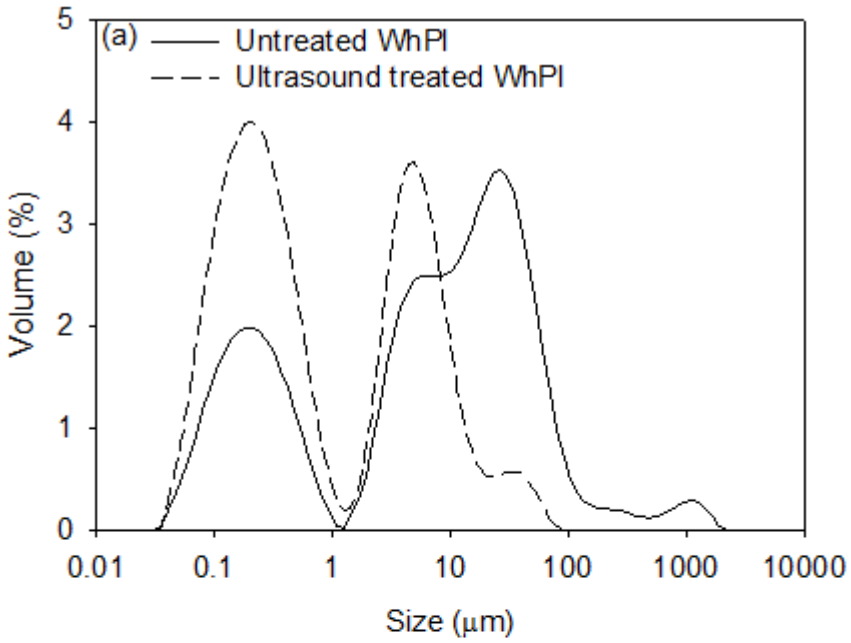
Fig. 2. SDS-PAGE electrophoretic profiles of protein solutions: (a) molecular weight standard (10 – 250 kDa), (b) untreated WhPI, (c) ultrasound treated WhPI, (d) untreated SPI and (e) ultrasound treated SPI.

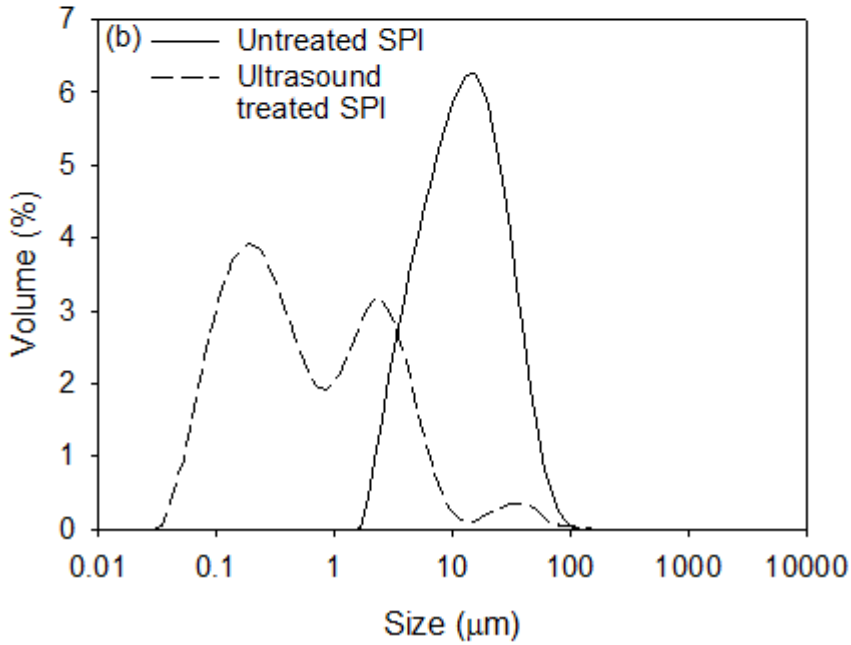
Fig. 3. Fittings of the Huggins' (●) and Kraemer (○) equations to the viscosity data of (a) untreated WhPI, (b) ultrasound treated WhPI, (c) untreated SPI and (d) ultrasound treated SPI.

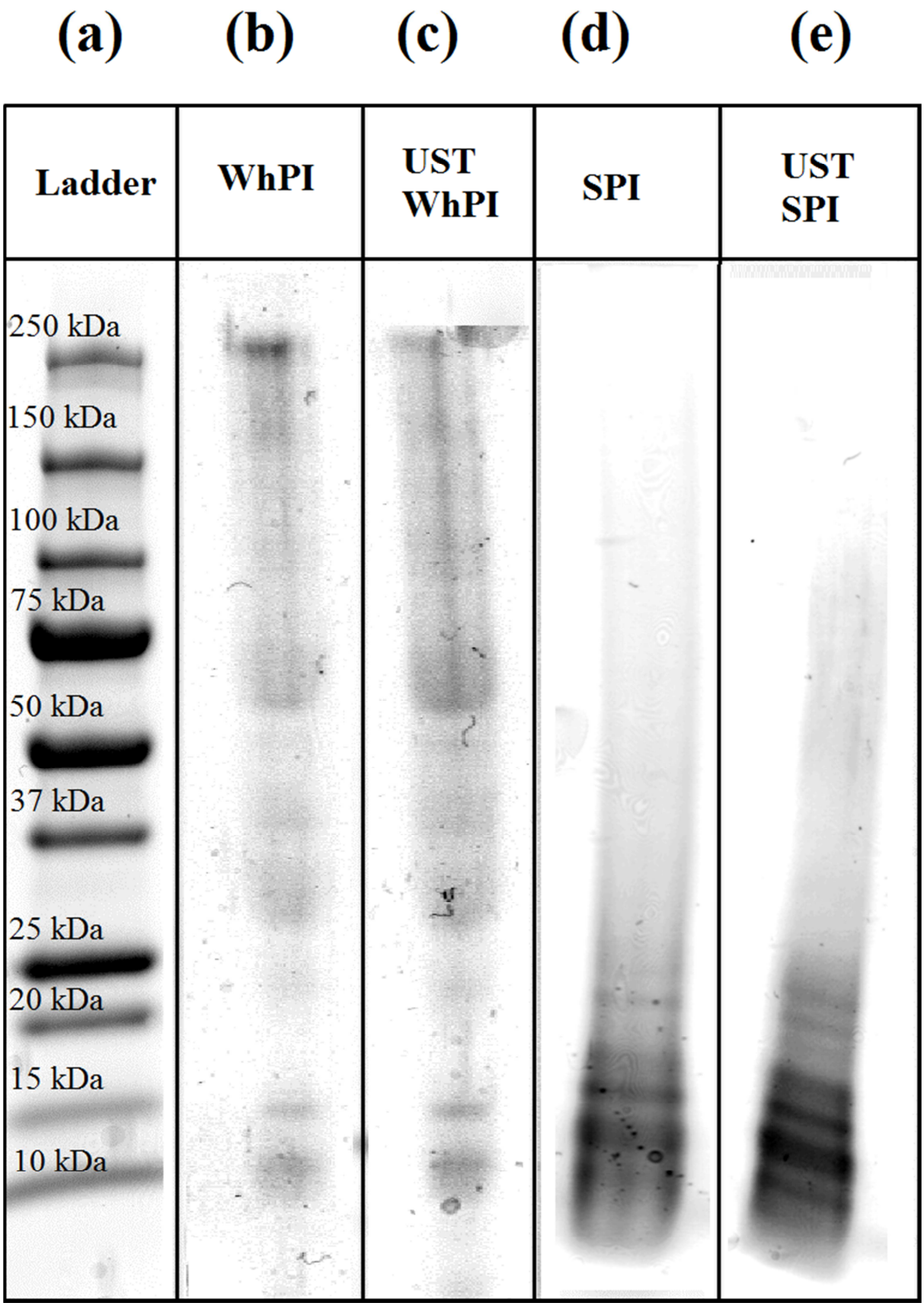
Fig. 4. Emulsion droplet size ($d_{3,2}$) as a function of concentration (0.1 – 3 wt. %) of: (a) untreated WhPI (●), ultrasound treated WhPI (○) and Tween 80 (▼) and (b) untreated SPI (●), ultrasound treated SPI (○) and Tween 80 (▼).

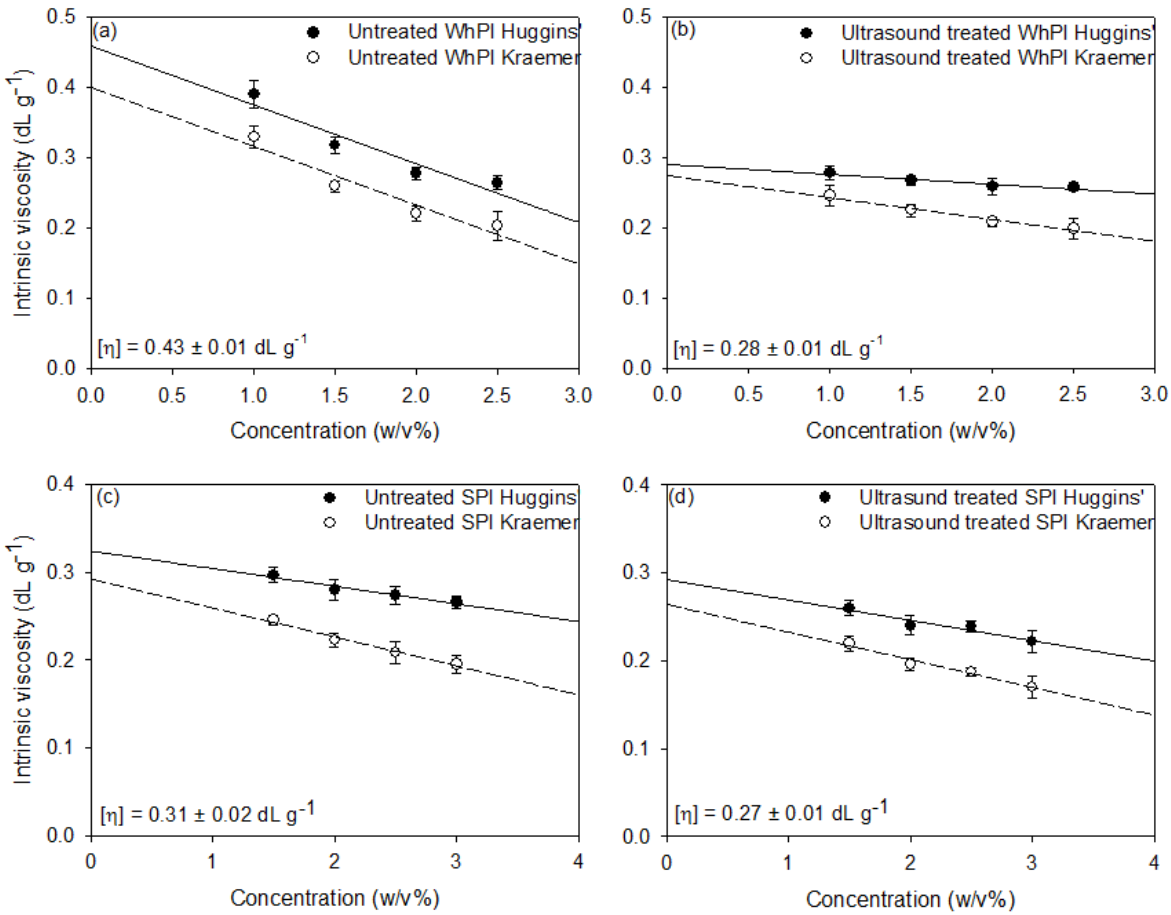
Fig. 5. Interfacial tension between: (a) untreated WhPI (●), ultrasound treated WhPI (○), Tween 80 (▼) and distilled water (Δ) and rapeseed oil, and (b) untreated SPI (●), ultrasound treated SPI (○), Tween 80 (▼) and distilled water (Δ) and rapeseed oil. The concentration of all emulsifiers was 0.1 wt. %.

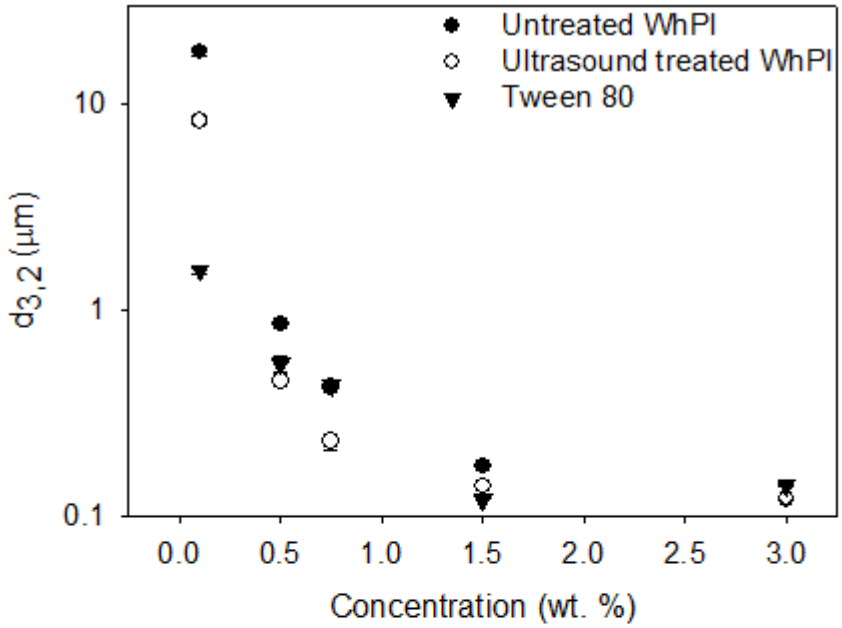
Fig. 6. Effect of emulsifier type on droplet size as a function of time for emulsions stabilised by: (a) untreated WhPI (●), ultrasound treated WhPI (○) and Tween 80 (▼), and (b) untreated SPI (●), ultrasound treated SPI (○) and Tween 80 (▼). The concentration for all emulsifiers was 0.1 wt. %.

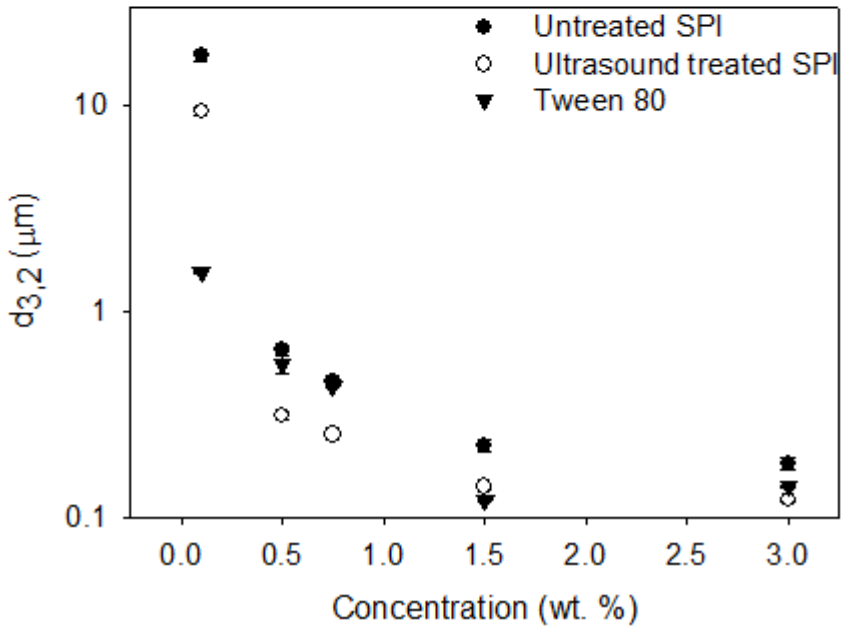

(a)
Untreated WhPI
Ultrasound treated WhPI
Volume (%)
Size (μm)
0
1
2
3
4
5
0.01
0.1
1
10
100
1000
10000

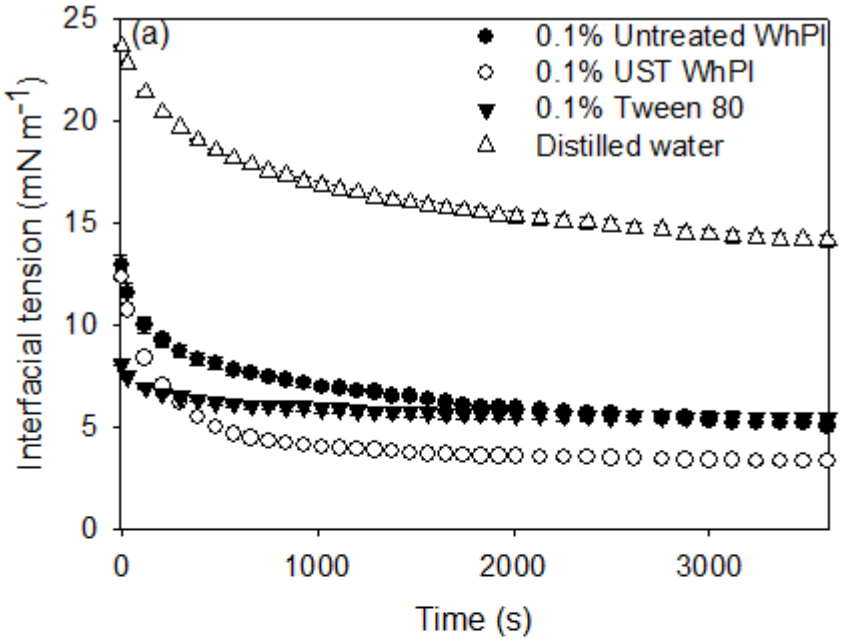

(b)
Untreated SPI
Ultrasound treated SPI
Volume (%)
Size (μm)
0
1
2
3
4
5
6
7
0.01
0.1
1
10
100
1000
10000

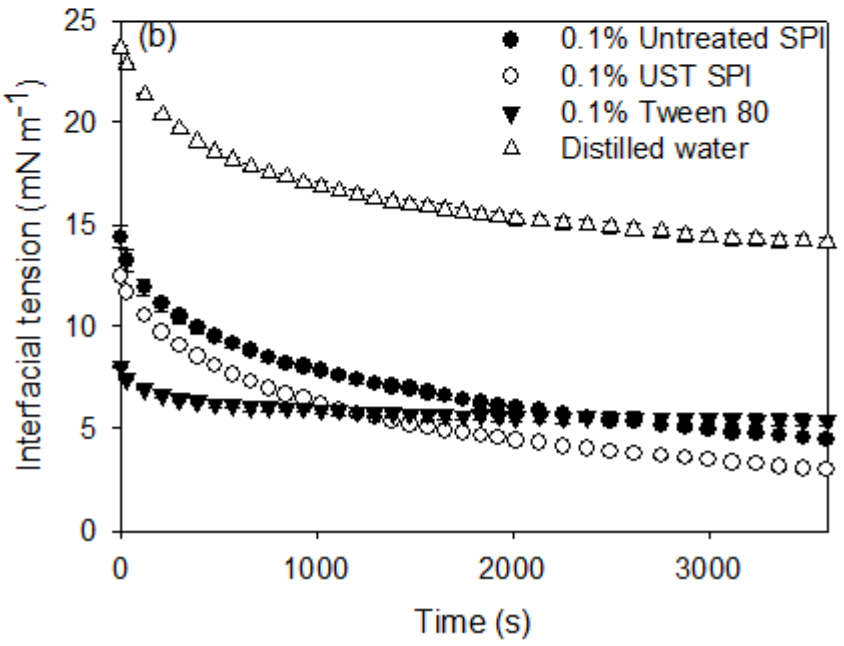

(a)
(b)
(c)
(d)
(e)
Ladder
WhPI
UST WhPI
SPI
UST SPI
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa

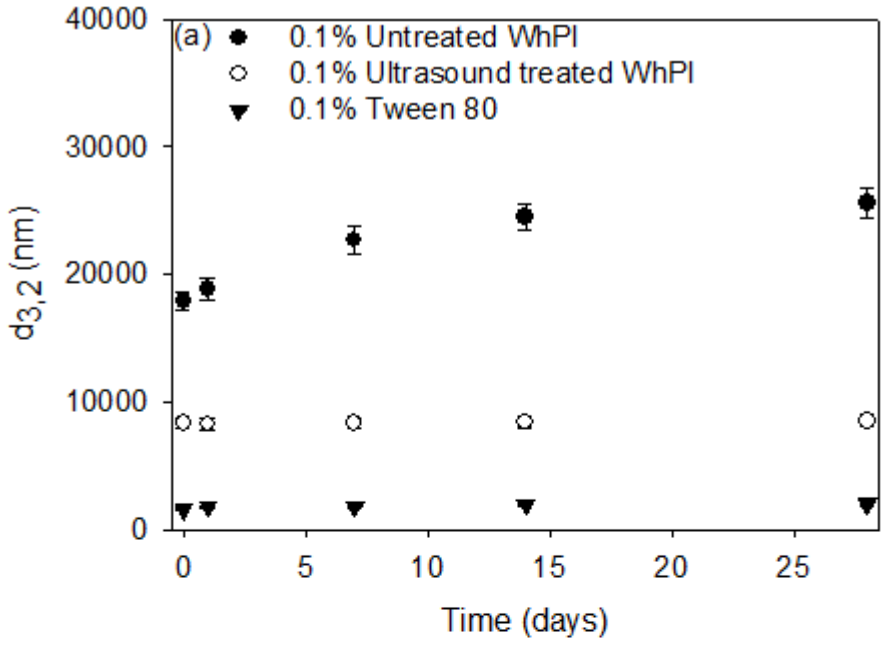


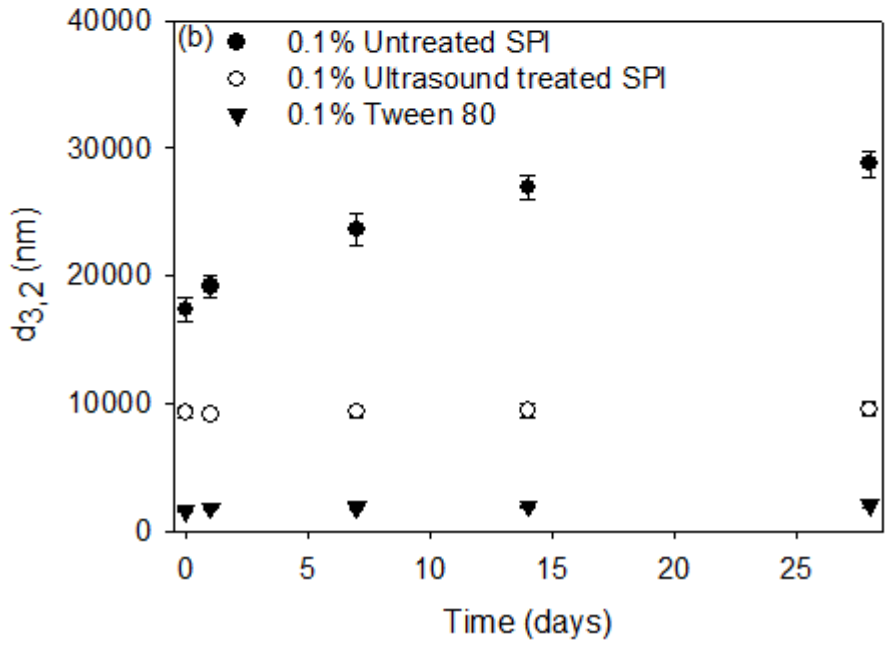

Untreated WhPI
Ultrasound treated WhPI
Tween 80
$d_{3,2}$ (μm)
10
1
0.1
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Concentration (wt. %)


Untreated SPI
Ultrasound treated SPI
Tween 80
$d_{3,2}$ (μm)
10
1
0.1
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Concentration (wt. %)


(a)
0.1% Untreated WhPI
0.1% UST WhPI
0.1% Tween 80
Distilled water
Interfacial tension (mN m-1)
Time (s)
0
5
10
15
20
25
0
1000
2000
3000


(b)
0.1% Untreated SPI
0.1% UST SPI
0.1% Tween 80
Distilled water
Interfacial tension (mN m-1)
Time (s)
0
5
10
15
20
25
0
1000
2000
3000


(a)
0.1% Untreated WhPI
0.1% Ultrasound treated WhPI
0.1% Tween 80
d3,2 (nm)
Time (days)
0
10000
20000
30000
40000
0
5
10
15
20
25


(b)
0.1% Untreated SPI
0.1% Ultrasound treated SPI
0.1% Tween 80
d3,2 (nm)
Time (days)
0
10000
20000
30000
40000
0
5
10
15
20
25

Highlights:

- Ultrasonic effect on properties of WhPI and SPI was assessed.
- Power ultrasound (~34 W cm^{-2}) reduced aggregate size of both proteins.
- SDS-PAGE confirmed UST had no effect on the molecular weight of proteins.
- UST WhPI and SPI produced smaller O/W emulsion droplets.