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RESEARCH

Impact of Ultrasound Emulsification on the Physicochemical Properties of Emulsions Stabilised by Quinoa Protein Isolates at Different pHs

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Abstract
Ultrasonication (20 kHz, 19.9 W/10 mL sample) was used to form O/W emulsions stabilised by quinoa protein isolate (QPI) particles at 3 wt%. Effects of pH (3, 5, 7, 9) and oil volume fractions (20%, 40%, and 60%) on rheological properties and microstructural characteristics of emulsions were investigated. All emulsions show viscoelastic behaviours and form a network structure comprising aggregated oil droplets and QPI particles. Emulsions stabilised by QPI at pH 5 showed largest droplet sizes and lowest gel strength due to extensive aggregation of proteins around the isoelectric point (pI ~ 4.5). The gel strength (G'(1 Hz)) were enhanced when the oil volume fraction increased and reached ~ 1100–1350 Pa at 60% oil volume fraction at different pH. This could be attributed to a tighter packing of oil droplets at 60% oil. Confocal laser scanning microscopy (CLSM) and transmission electron microscopy (TEM) revealed that interdroplets bridging and voids filling of QPI particles between oil droplets are critical in formation of aggregated emulsions network. Emulsions stabilised by QPI at pH 7 and 9 possessed thinner interfacial layers compared to those at pH 3 and 5. Finally, this study shows a potential of using ultrasonication to prepare gel-like emulsions stabilised by QPI, broadening applications of quinoa proteins in making dairy substitutes with semi-solid textural characteristics.

Keywords Emulsion gels · High intensity ultrasound · Viscoelasticity · Microstructures · Quinoa protein isolates

Introduction

Recently, ultrasonication emulsification (UE) has gained increasingly interests from both academia and industry for preparation of emulsions [1–3]. Compared to other commonly used emulsification methods such as high shear mixing and high pressure homogenisation, UE has demonstrated several advantages-flexibility of implementation (batch or continuous modes), ease of cleaning, resulting in small and nanoscale oil droplet size, and possibilities for upscaling to pilot or industrial scale [4]. The UE has been used to prepare emulsions that are stabilised by natural biopolymers or food ingredients such as whey protein isolate (WPI) [5], pasteurised homogenised skim milk [6], and chitosan particles [7]. However, only liquid emulsions with low viscosities were obtained due to low volume fraction of oil (i.e. < 20%) used in these studies. In few recent UE studies, aggregated emulsions with high viscosities or a gel-like viscoelasticity were formed when higher volume fraction of oil (i.e. 30–60%) was used in the presence of nanometre sized colloidal particles such as milk protein concentrate (MPC 70) [8], soy protein isolate [9] and inorganic nanoparticles such as TiO_2 [2].

Quinoa (*Chenopodium quinoa Willd*) is an ancient pseudocereal originating from South America, which contains 12–23% protein depending on varieties [10]. Quinoa protein isolates (QPI) have been recently explored as a promising food protein ingredient as it contains all nine essential amino acid and exhibits excellent techno-functional properties such as gelation and emulsification [11–14]. Until now, almost all the QPI stabilised emulsions reported in literature are high internal phase emulsions (HIPE) with

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high oil volume fractions (e.g. >74%) that were prepared by the high shear mixing such as ultraturrax [15–18]. The HIPE stabilised by QPI exhibited several advantages such as good stability during storage and a solid-like elastic behaviour with gel-like network microstructures, which are important for their potential applications as plant based mayonnaise or salad dressing, for examples. However, the high oil volume fraction required for oil structuring in the HIPE might potentially cause a health related issue due to excessive oil consumptions [19]. In addition, large oil droplet size (20–100 μm) obtained from lower energy emulsification methods such as ultraturrax typically led to a weak gel strength [20]. To overcome these issues, ultrasonication seems a promising way to form aggregated or gelled emulsions using lower oil volume fractions while achieving a desirable gel strength.

Due to an inferior aqueous solubility and large particle size of the QPI, various pretreatments such as high pressure homogenisation [8] or ultrasonication [17] were applied to reduce the particle size of QPI prior to the emulsification. Previous studies have demonstrated that the pH values exerted significant impacts on physicochemical properties and microstructural characteristics of protein stabilised emulsions [21, 22]. Liang et al. (2013) prepared pea protein isolate (PPI) stabilised emulsions at various pH values (3, 5, 7, and 9). They found that the PPI exhibited the least emulsifying ability at pH 5 which is close to its isoelectric point. The emulsifying ability of the PPI is the greatest at pH 3 followed by neutral and alkaline pH [21]. The pH values can alter the conformation of proteins (e.g. particulate or polymetric) thus affecting their interfacial behaviour and emulsification performance [22, 23], which in turn modulates the rheological and microstructural characteristics of emulsions [24].

Until now, studies on preparation of O/W emulsions by UE using emerging protein resources such as QPI were very limited. Furthermore, the pHs of protein that can significantly affect emulsification performances was rarely considered in previous studies. Therefore, in this study high-power ultrasonication was employed to prepare aggregated emulsions (oil volume fraction = 20%, 40%, and 60% v/v) stabilised solely by QPI suspensions at various pH values (3, 5, 7, and 9). The rheological properties, microstructural characteristics, and stability (up to 21 days) were investigated, and the aggregation mechanism was briefly discussed. We believe that this study is the first to investigate the properties of UE induced aggregated emulsions stabilised by the QPI.

Materials and Methods

Materials

Quinoa seeds were kindly donated by KiwiQuinoa Inc. (Taihape, New Zealand). Soybean oil used for emulsion preparation was purchased from local supermarket (New World, Auckland, New Zealand). Chemicals including NaOH, HCl, NaCl, sodium azide, sodium dodecyl sulfate (SDS), petroleum ether, fast green, and nile red were purchased from Sigma-Aldrich (St Louis, MO, USA) which were of analytical grade. Milli-Q water (Millipore, USA) was used for all sample preparations.

Preparation of Quinoa Protein Isolate (QPI)

QPI was extracted following pH shifting procedures. Quinoa seeds were grounded using a bench-top coffee grinder (Breville, New Zealand) into flour. Then the flour was passed through a 500-micron sieve to remove any large particles. After that, the flour was mixed with petroleum ether (1:10 (w/v)) and the suspension was kept under magnetically stirring for 5 h in a fume hood at room temperature for fat removing. The defatting procedure had been repeated twice and then the defatted quinoa flour was obtained by gravity sedimentation. The sediment was dried on a plastic tray in the fume hood at ~20 °C for overnight. The defatted flour (200 g) was added into 2 L of 0.5 M NaCl solution containing 0.04% sodium azide and the pH was adjusted to 8 using 1 M NaOH solution every hour for 3 h. After stirring for overnight the suspension was centrifuged at 20,000 g for 15 min at 20 °C twice using centrifuge (sigma 3-18KS, Osterode am Harz, Germany) and the supernatant was collected. The supernatant was adjusted to pH 4.5 using 1 M HCl every hour for 2 h. Afterwards, the suspension was centrifuged at 20,000 ×*g* at 20 °C for 15 min. The residual NaCl concentration in the washing solutions was determined by monitoring the electric conductivity using an EC meter (HI98304, HANNA instruments, UK). The pellets were thoroughly washed with Milli-Q water four times by centrifugation until the electricity was measured lower than 80 μS/cm. The protein pellets were then collected and redispersed in 100 mL of Milli-Q water containing 0.04% sodium azide. The suspension was adjusted to pH 7 and under stirring overnight. Finally, the suspension was frozen at -20 °C for 24 h and a freeze dryer (Labconco, MO, USA) was used to lyophilize the protein solutions to QPI powder for four days. The dried protein isolate was collected and ground to powder by a mortar and pestle. Then, the QPI powder was transferred in a plastic bottle sealed with parafilm and put into a desiccator at 20 °C for further uses. The proximate analyses of QPI were performed at the

Nutrition Laboratory of Massey University (Palmerston North, New Zealand). The fat, protein, moisture, and ash contents of the QPI powder were 2.9 wt%, 91.6 wt%, 2.7 wt%, and 2.8 wt%, respectively.

Ultrasonication and Characterisation of QPI Suspensions

Ultrasonication Treatment of QPI Suspensions

QPI suspensions (3w/w%) were prepared using Milli-Q water containing 0.04% sodium azide to avoid the growth of microorganism. The QPI suspension was then mixed by a rotor-stator homogeniser (T10 basic, IKA, Staufen, Germany) at 15,000 rpm for 5 min. After that, the suspension was kept under magnetically stirring at ~300 rpm for overnight at 20 °C for fully hydration. Then, the suspension was sonicated at 19.9 W for up to 25 min using a 20 kHz ultrasonicator (JY92-IIN, Ningbo Scientz Biotechnology Co.,Ltd) fitted with a 6 mm titanium probe. Suspension was kept in an ice water bath to prevent protein denaturation during the sonication treatment. The energy consumption during the ultrasound treatment was determined by a calorimetric method [25].

Particle Size Measurements

The particle size (z-average) of QPI suspensions before and after sonicated for 5 min, 10 min, 15 min, 20 and 25 min were determined using Zetasizer (Nano-ZS, Malvern Instruments Ltd., UK) with the scattering angle of 173° and a fixed wavelength of 633 nm at 20 °C. Refractive indices of QPI particles and water were 1.456 and 1.33, respectively. The viscosity of dispersion medium (water) was set at 0.89 mPa s and each sample was measured 25 times [26]. Finally, the z-average size is reported.

Zeta-potential Measurements

Zeta-potential of the QPI suspensions at pH 3, 5, 7 and 9 was determined by the Zetasizer (Nano-ZS, Malvern Instruments Ltd., UK). Samples were diluted 100 × using pH-adjusted Milli-Q water. Refractive indices of QPI particles and water were 1.456 and 1.33, respectively. The samples were measured in triplicates at 20 °C.

Preparation of O/W Emulsions by Ultrasonication

The pH of the QPI suspension was adjusted to 3, 5, 7 and 9 using 1 M HCI or 1 M NaOH solution. The pH was regularly checked, and the QPI suspension was kept under magnetically stirring for overnight at 20 °C. After that, the oil-QPI mixture (oil volume fractions = 20%, 40%, and 60% v/v) was pre-homogenised using a rotor-stator homogeniser (T10 basic, IKA, Staufen, Germany) at 10,000 rpm for 7 s. Finally, the pre-homogenised emulsions were subjected to US treatments using a 20 kHz ultrasound equipment (JY92-IIN, Ningbo Scientz Biotechnology Co.,Ltd) with a 6 mm probe for 5 s at a constant power of 19.9 W determined by a calorimetric method by measuring the increase in temperature of sonicated water at the same volume with time [25]. The 6 mm probe was selected as it is appropriate for processing 10 mL of emulsion samples according to manufacturer's instruction. The sonication time (5s) was selected based on preliminary experiments and a previous study [8] so that 5 s is sufficient to form a homogeneous emulsion and the formed emulsion is not disrupted under prolonged sonication.

Physicochemical and Structural Characterizations of Emulsions

Confocal Laser Scanning Microscopy (CLSM)

Confocal laser scanning microscopy (Leica DM60000B) with a 40 × objective lens was used to observe microstructures of UQPI stabilised emulsions. Fast green and nile red were used to stain protein and oil phases, respectively at the volume ratio of dye to protein and oil phases 1:500 (v/v) and 1:5000 (v/w), respectively [16]. Then, the stained emulsion samples were transferred onto concave microscopy slides with a glass coverslip. The laser wavelengths that fast green and nile red were excited at were 644 nm and 561 nm, respectively.

Transmission Electron Microscopy (TEM)

Emulsion samples for TEM observations were prepared according to Cheng, Ye [27] with slight modifications. In brief, samples were firstly fixed and embedded into alginate tubes and cured at 60 °C for 48 h. The embedded samples were then sectioned to a thickness of ~90 nm with an ultramicrotome (Leica EM UC7, Germany). After that, the sections were placed on 3 mm copper grids and stained with lead citrate. Finally, the samples were observed under a transmission electron microscope (FEI Tecnai G2 biotwin, FEI, the Netherlands) at an acceleration voltage of 60 kV with magnifications of 8.2 k and 60 k, respectively.

Oil Droplet Size Measurements

The oil droplet size of emulsions before and after storage at 20 °C for 15 days was determined in triplicates at room temperature (~25 °C) using a Mastersizer 2000 (Malvern

Instruments, Worcestershire, UK). To break oil flocs and determine individual oil droplet size, 1 mL of emulsion was mixed with 1 mL of 2% sodium dodecyl sulfate (SDS) solution and leave overnight. All the measurements were repeated twice at room temperature. Refractive indices of dispersant and soybean oil were 1.33 and 1.47, respectively. The mean particle diameter for oil droplets was represented as the volume mean diameter (D [4, 3]= $\Sigma n_i d_i^4 / n_i d_i^3$) and Sauter means diameter (D [3, 2]= $\Sigma n_i d_i^3 / n_i d_i^2$) where n_i is the number of particles with a diameter d_i.

Rheological Properties

A stress-controlled rheometer (DHR-3, TA instruments, USA) with a parallel plate geometry (40 mm in diameter, 1 mm gap) was used for rheological measurements. The sample was loaded on the bottom plate carefully using a spatula. The storage modulus G' and loss modulus G'' were recorded according to the following procedures: (1) firstly, a time sweep measurement was conducted at 0.5% strain and 1 Hz for one hour for structural rebody. (2) Secondly, a frequency sweep was conducted at a constant strain of 0.5% while the frequency was varied from 0.01 to 100 Hz. (3) Finally, a strain sweep was conducted at a constant frequency 1 Hz with the strain amplitude varying from 0.1 to 1000%. All the measurements were conducted in triplicate at 20 ℃.

Statistical Analyses

All the experiments were performed in triplicate, and the results were reported as mean ± standard deviations (SD). The mean comparison was conducted through analysis of variance (ANOVA) via the Least Significance Difference (LSD) test using the SPSS software (version 21.0, Chicago, USA). The significance level was set at $P < 0.05$.

Results and Discussion

QPI Particle Size and Zeta-potential as Affected by US Treatment and pH

Preliminary experiments showed that QPI without treatment cannot form stable emulsions due to large protein aggregate size (> 3 μm) and inferior surface properties [14, 16]. In addition, despite a previous study suggested that non-sonicated QPI particles (2wt%) could from a stable emulsion (oil volume fraction of ~ 66.7%) using ultraturrax; however, the emulsion gel strength is much weaker compared to the one prepared using sonicated QPI particles [28]. Therefore, the US treatment was employed to reduce the particle size of QPI suspension prior to preparation of emulsions [26]. As shown in Fig. 1A, sonication at 19.9 W for 25 min significantly reduced the z-average particle size of QPI suspension from ~ 3500 nm to ~ 300 nm. The particle size significantly decreased with US treatment time up to 5 min and then it nearly reached a plateau at 25 min. Therefore, the 25 min US treated QPI sample was selected for further studies. A significant reduction in particle size induced by US treatment has been widely reported in other plant protein suspensions. Zhang, Zuo [29] found that the particle size of QPI suspension was significantly decreased to ~ 132 nm at a sonication density of 10 kJ/mL. O'Sullivan, Murray [30] found that US treatment (power density 34 $W \cdot cm^{-2}$) for 2 min could substantially decreased the particle size of PPI suspensions from ~ 5000 nm to ~ 200 nm. Various extents of decrease in particle sizes have been reported in previous studies, which could be related to the sample characteristics and sonication conditions. The size reduction of protein particles could be due to the breakdown of non-covalent bonds in protein large aggregates induced by extensive shear forces, micro-jetting, and turbulence generated by ultrasonic cavitations [26, 31].

The emulsification performance of food proteins is also strongly determined by the pH and surface charges as protein diffusion to the oil-water interface could be affected by these factors [21]. The effect of pH on zeta-potential (surface charges) and particle sizes of US treated QPI suspensions are shown in Fig. 1B C, respectively. The absolute zeta potential values of QPI suspensions tend to approach to zero at the pH around 5 and significantly increased at pH 3 (~ 28 mV) and pH 7 and pH 9 (~ -33 mV). This finding is consistent with previous studies that the isoelectric point of QPI is around pH 4.5-5 [32, 33].

The sonicated QPI suspension at pH 5 shows the largest particle size (~ 1650 nm) followed by at pH 3 (~ 500 nm), indicating the aggregation of sonicated QPI at acidic pH. While at pH 7 and pH 9, the particle size of sonicated QPI remained ~ 300 nm. This observation agrees well with previous studies that proteins typically exhibit the largest particle size at or close to their isoelectric point due to weakened electrostatic repulsions between adjacent protein molecules [15, 26]. The low charge on the surface of protein molecules also promotes protein aggregation through other non-covalent interactions including hydrophobic interactions, van der Waals and hydrogen bonding interactions [34]. The high absolute zeta-potential values of the QPI at pHs far away from the isoelectric point could be due to protein unfolding and exposure of highly charged amino acids [35].

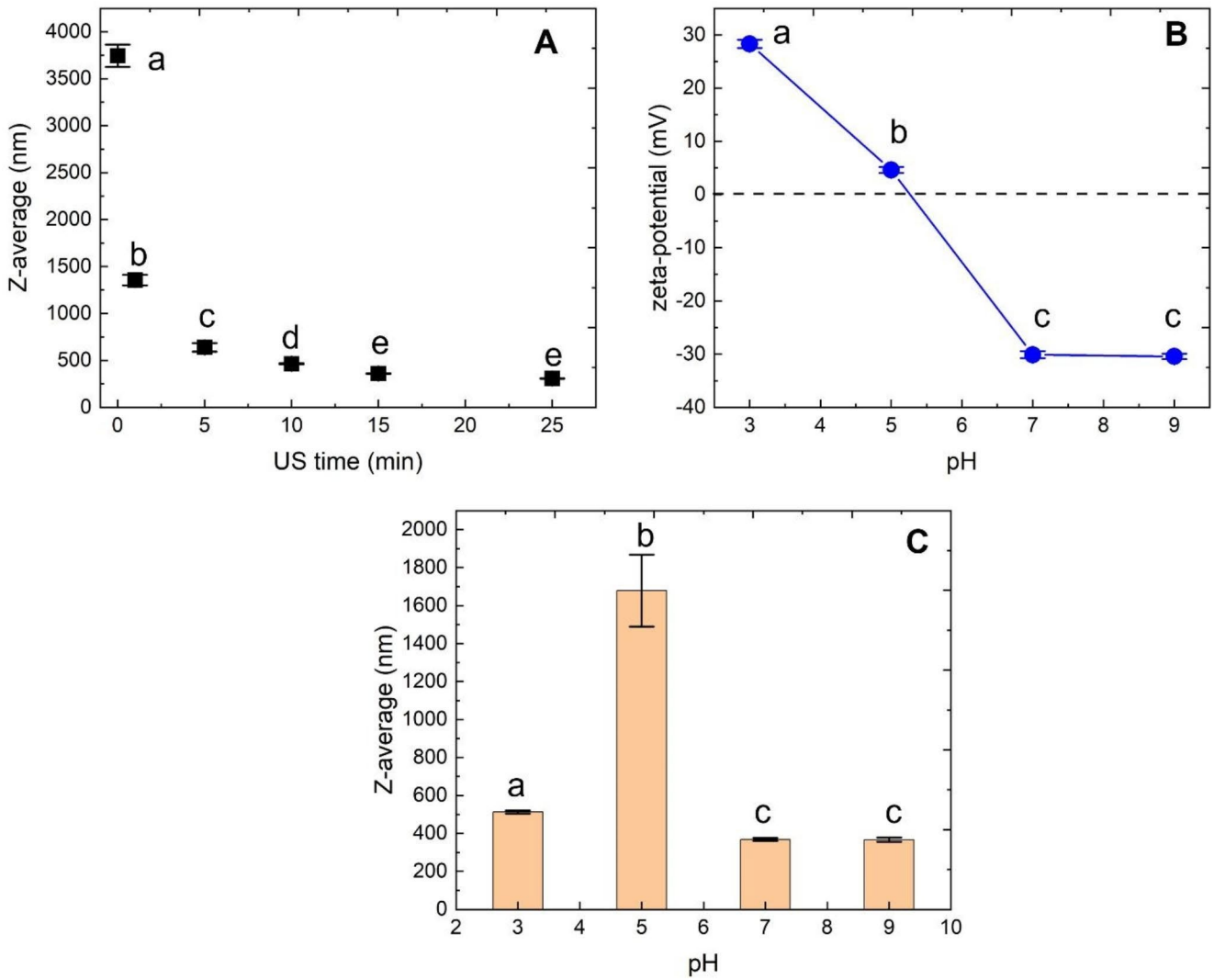

Fig. 1 (**A**) Particle size (Z-average) of quinoa protein isolates (QPI) suspensions at pH 7 as a function of ultrasonication (US) treatment time. The effect of pH value on the particle size (**B**) and zeta-potential (**C**) of US treated QPI suspensions. Different lowercase letters (a, b, c) indicate the significant difference at the $P < 0.05$ level due to the differences in US treatment time (**A**) or differences in pH values (**B** and **C**)

Effect of pH and Oil Volume Fraction on Microstructural Characteristics of QPI Stabilised Emulsions

The microstructural characteristics of QPI stabilised emulsions containing 20%, 40%, and 60% oil at various pHs were evaluated by CLSM and confocal micrographs are shown in Fig. 2 where the protein phase are shown in green and oil droplets appear in red. For all the samples, proteins indeed stabilised oil-water interfaces and large aggregate network of both oil droplets and protein particles can be observed. This indicates the robustness of utilizing ultrasonication to prepare O/W emulsions stabilised by sonicated QPI particles. The formation of aggregate network structure has been observed in previous studies of protein stabilised emulsions prepared by high intensity emulsification techniques such as ultrasonication and high pressure microfluidisation. Zhang, Luo [8] showed that the aggregated emulsion stabilised with MPC 70 can be formed by ultrasonication when the oil volume fraction ≥ 35%. In another study of formation of emulsions by ultrasonication, Li, Martin [2] demonstrated that stable aggregated emulsion gels can be formed by using various food and non-food particles such as bovine micellar casein and graphene oxide. Liu, Zhang [36] prepared gel-like emulsions stabilised by WPI using microfluidisation at the oil volume fraction varying from 30 to 60%. The formation of aggregated emulsions could be due to interdroplets bridging and QPI void-filling effects, which can be observed in Fig. 2. It can be also found that the microstructure of emulsions is significantly affected by the pH of QPI suspension and the oil volume fraction. Protein interfacial layers seem thicker and the protein aggregates in the continue

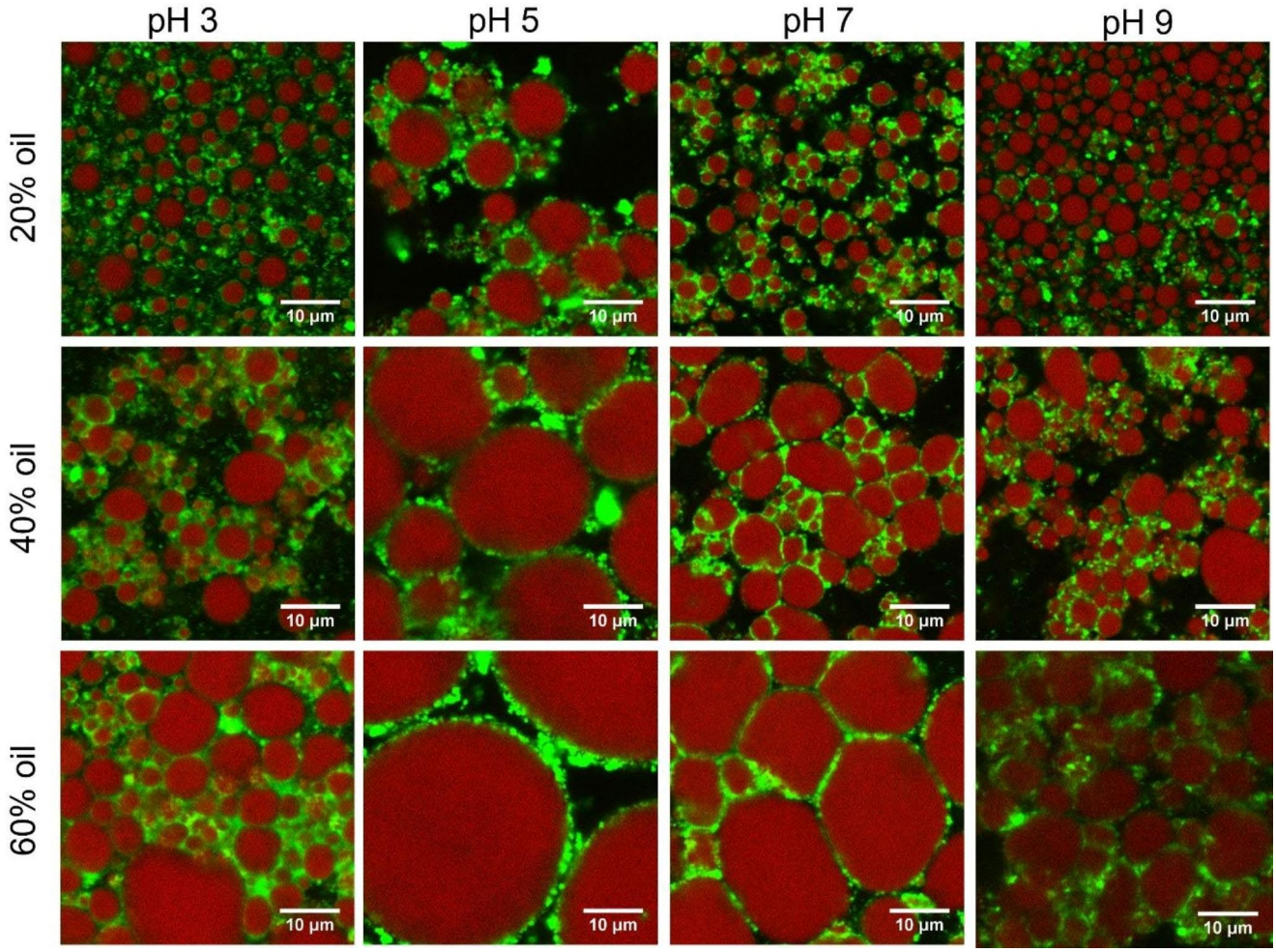

Fig. 2 Confocal micrographs of emulsions stabilised by sonicated quinoa protein isolates (QPI) particles at varied pH values of pH 3–10 containing 20, 40 and 60 v/v% oil. Scale bars represent 10 µm in length. Proteins appear as green, whereas the oil phase appear as red

phase are larger at pH 3 and 5 than at pH 7 and 9. At pH 5 in particular, QPI tend to form large protein aggregates at the oil-water interface as well as in the continuous phase due to low solubilities close to the isoelectric point. At the same oil volume fraction, emulsions stabilised by the QPI at pH 5 showed the largest oil droplet size compared to other pHs, suggesting an inferior emulsification performance of QPI at or close to the isoelectric point. Similar observations had been found in previous studies of emulsions formed by commercial potato, rice and pea proteins at pH 3.5, 5, and 7 using a conventional rotor-stator type mixer (Ultraturrax) [37]. The severe aggregation of proteins close to the isoelectric point could prevent the exposure of buried hydrophobic groups, enhance protein-protein interactions, and make a slower diffusion of proteins to oil-water interfaces, impeding protein absorption and rearrangement at the interfaces [38, 39].

At all the pHs of QPI suspensions, oil droplet size showed a significant increase with an increase in the oil volume fraction. This could be due to the fact that at fixed QPI concentration (3 wt%), the QPI particles available for the stabilisation of oil-water interfaces decreased, resulting in the formation of large oil droplets. The similar observations have been made in emulsions stabilised by sorghum kafirin particles at the oil volume fraction varying from 30 to 70% [40] and emulsions stabilised by commercial potato, rice and pea proteins at the oil volume fraction varying from 10 to 40% [37]. In addition, as the oil volume fraction increased, oil droplets tend to become more closely packed. For QPI stabilised emulsions at pH 7 and 9 containing 60% oil, some oil droplets were even compressed and exhibited polyhedron-type shapes. This has been commonly observed in high internal phase emulsions (oil volume fraction $\geq$ 74%) stabilised by QPI particles [16] and soy proteins [41], for examples. However, it is worth noting that most of high internal phase emulsions were prepared using low intensity emulsification method such as ultraturrax. It has been suggested that the interdroplets bridging and colloidal

particle void filling under extensive turbulence by ultrasonication could induce distortion and rearrangement of oil droplets thus leading to the compress packing of oil droplets with polyhedron-type shapes [2, 8]. This is confirmed by the TEM observation of QPI stabilised emulsions as shown in Fig. 3. TEM images show more extensive oil droplets aggregation at 60% oil than 20% oil. Furthermore, QPI particles formed a cluster-like network at the oil-water interface as well as in the continuous phase. The large protein aggregates (particularly at pH 5) were found on both oil-water interfaces and in the continuous phase. The distance between oil droplets seems to be smaller at pH 7 than at pH 3 and pH 5, indicating more closely and compressed packing of oil droplets that are in line with CSLM observations.

Effects of pH and Oil Volume Fraction on Oil Droplet Sizes of QPI Stabilised Emulsions

In order to further characterise emulsion structures and validate CLSM findings, droplet sizes D [4, 3] and D [3, 2] were determined by static light scattering (SLS) and results are shown in Fig. 4 (A, B). Generally, both D [4, 3] and D [3, 2] showed a similar dependence with pH of QPI particles and oil volume fractions. At the same oil volume fraction, oil droplets stabilised by QPI at pH 5 were the largest followed by at pH 3 and pH 7, while the oil droplets had the smallest size at pH 9. For example, for emulsions containing 20% oil, the oil droplet size D [4, 3] was ~ 10 μm, ~ 5 μm, ~ 3.5 μm and ~ 2.5 μm for oil droplets stabilised by QPI at pH 5, pH 3, pH 7, and pH 9, respectively. At the same pH, the oil droplet size showed a substantial increase as the oil volume fraction increased. For example, for droplets stabilised by QPI at pH 7, the D [4, 3] of oil droplets increased from ~ 3.5 μm at 20% oil volume fraction to ~ 23 μm at 60% oil volume fraction. Overall, the oil droplet size determined by SLS agreed well with CLSM observations. The smaller oil droplet size found at pH 7 and pH 9 could be due to their high surface charges (absolute zeta-potential values), enhanced electrostatic repulsions and solubilities, leading to high molecular structural flexibility and emulsification capability [37]. In addition, all of emulsions have been stored at 20 °C for 15 days to evaluate their stabilities by

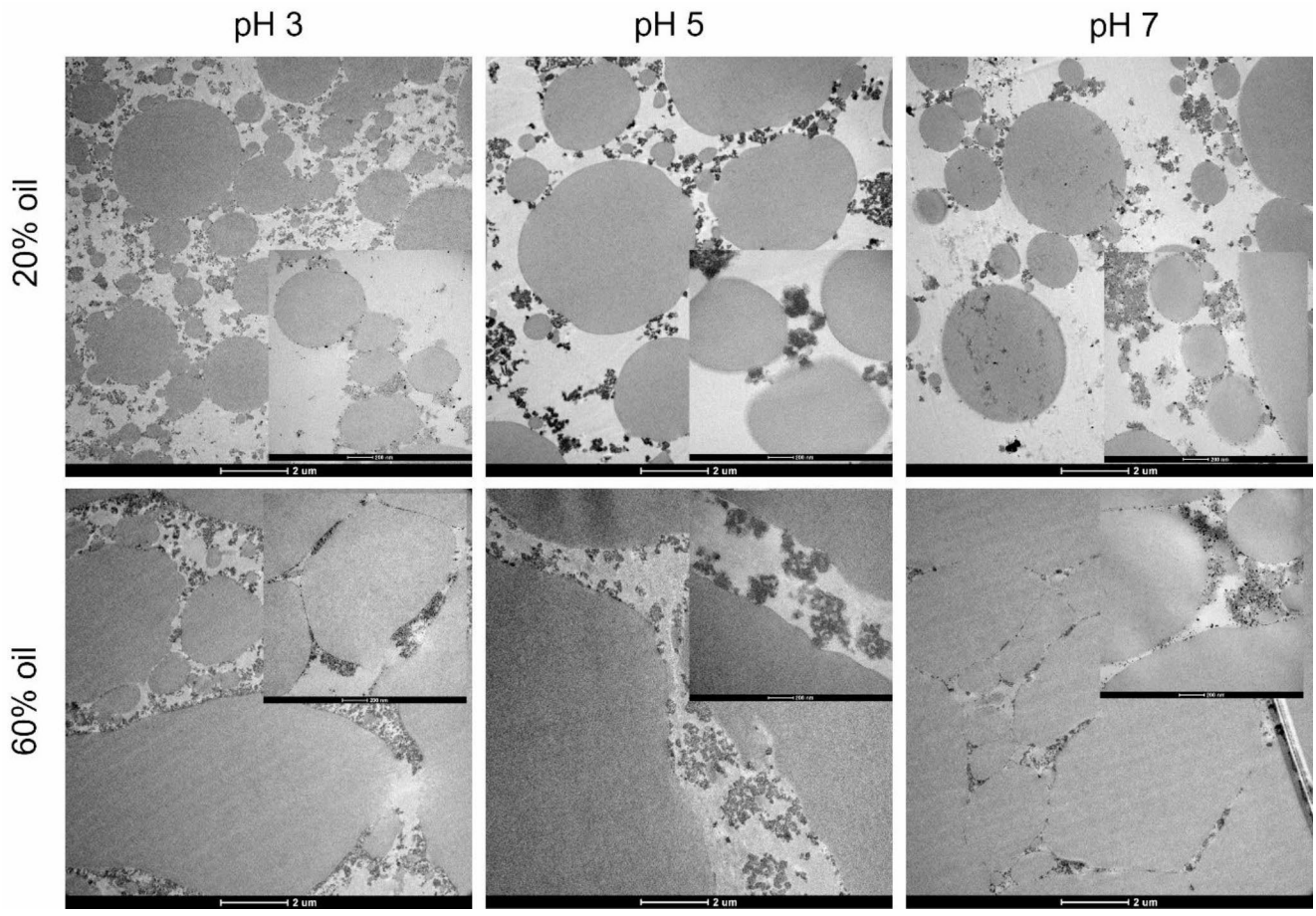

Fig. 3 TEM images of emulsions stabilised by sonicated quinoa protein isolates (QPI) particles at pH 3, pH 5, and pH 7 containing 20 and 60 v/v% oil. Scale bars represent 2 μm. Inset images are the corresponding samples at a higher magnification. Scale bars of inset images represent 200 nm in length

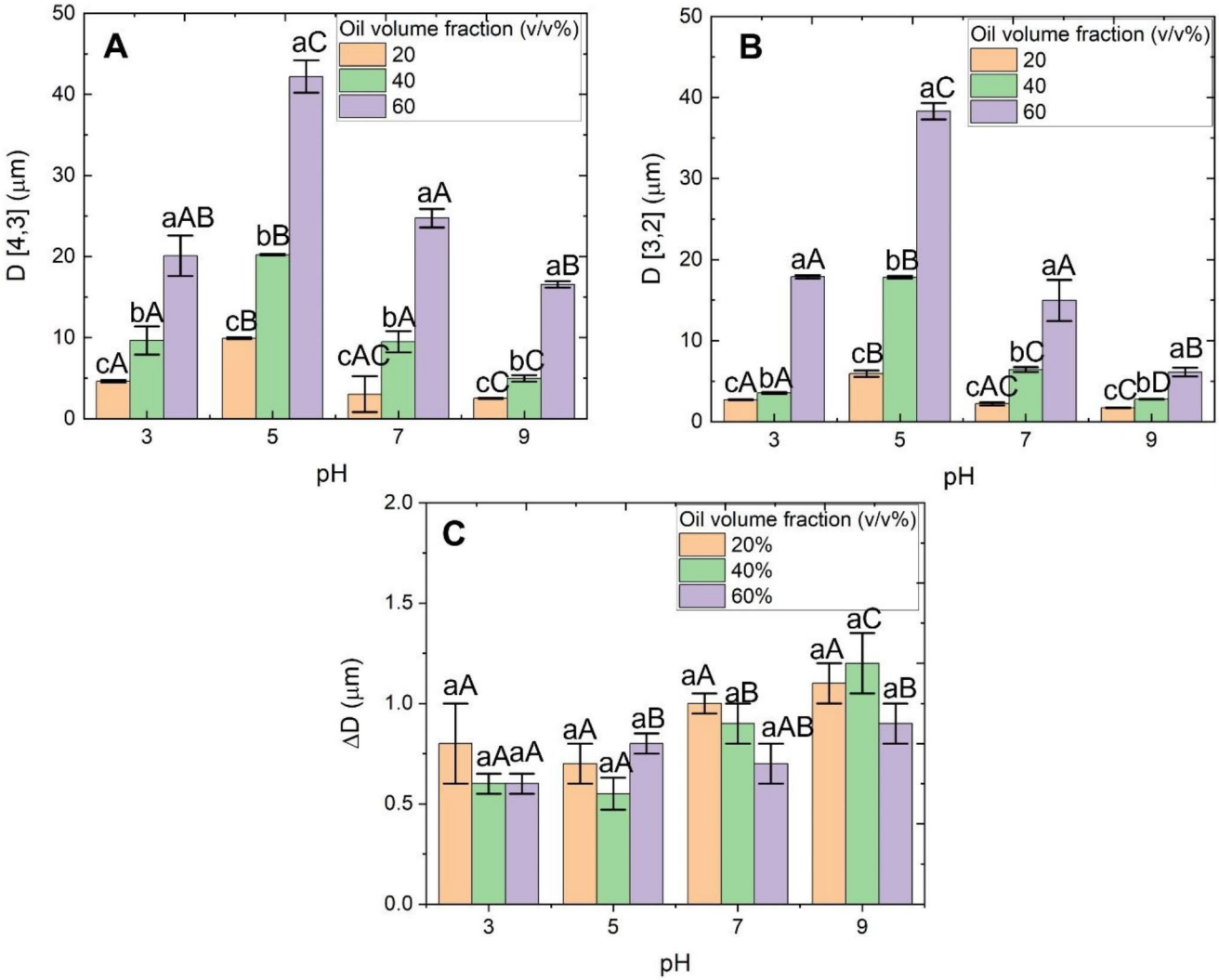

Fig. 4 The volume means diameter D [4, 3] (**A**) and Sauter means diameter D [3, 2] (**B**) of oil droplets of emulsions stabilised by sonicated quinoa protein isolates (QPI) particles at varied pH values of pH 3–9 containing 20, 40 and 60 **v**/v% oil. (**C**) the droplet sizes changes D [4, 3] (ΔD) of oil droplets of emulsions before and after storage at 20 °C for 15 days stabilised by sonicated quinoa protein isolates (QPI) particles. Mean values between samples containing the same amount of oil with different uppercase letters (**A**, **B**, **C**, **D**) are significantly difference. Mean values between samples at same pH with different lowercase letters (a, b, c, d) are significantly different

observations and determinations of oil droplet size changes (ΔD) (Fig. 4C). All emulsions showed outstanding storage stability without gravity separation, creaming and phase separation. There was a little change (< 1.5 μm) in droplet size of all QPI stabilised emulsions during storage, which could be attributed to excellent emulsifying ability of sonicated QPI particles.

Effects of pH and Oil Volume Fraction on Rheological Properties of QPI Stabilised Emulsions

The highly aggregated nature of oil droplets and network formation may result in formation of gel-like emulsion or emulsion gels. Small deformation oscillatory rheology was used to study viscoelastic properties of all emulsion samples. The storage modulus (G') and loss modulus (G'') as a function of frequency for emulsion samples are shown in Fig. 5 (left hand-side). As shown in frequency sweep results, all the samples displayed that the G' was higher than G'' in the whole frequency range. Further, both G' and G'' were only slightly dependent on frequency, indicating a typical solid-like gel behaviour for all samples [13, 42]. This was confirmed by a tube-inversion test (inset images in Fig. 5) that most of emulsion samples showed gel-like and self-supporting behaviour that can adhere to the bottom of the glass tubes after inversion. However, some samples such as oil droplets stabilised by QPI at pH 5 containing 20% and 40% oil, they flowed after tube inversion which could be due to weak gel strength. The flowability of emulsions is related to the movement of oil droplets against one another

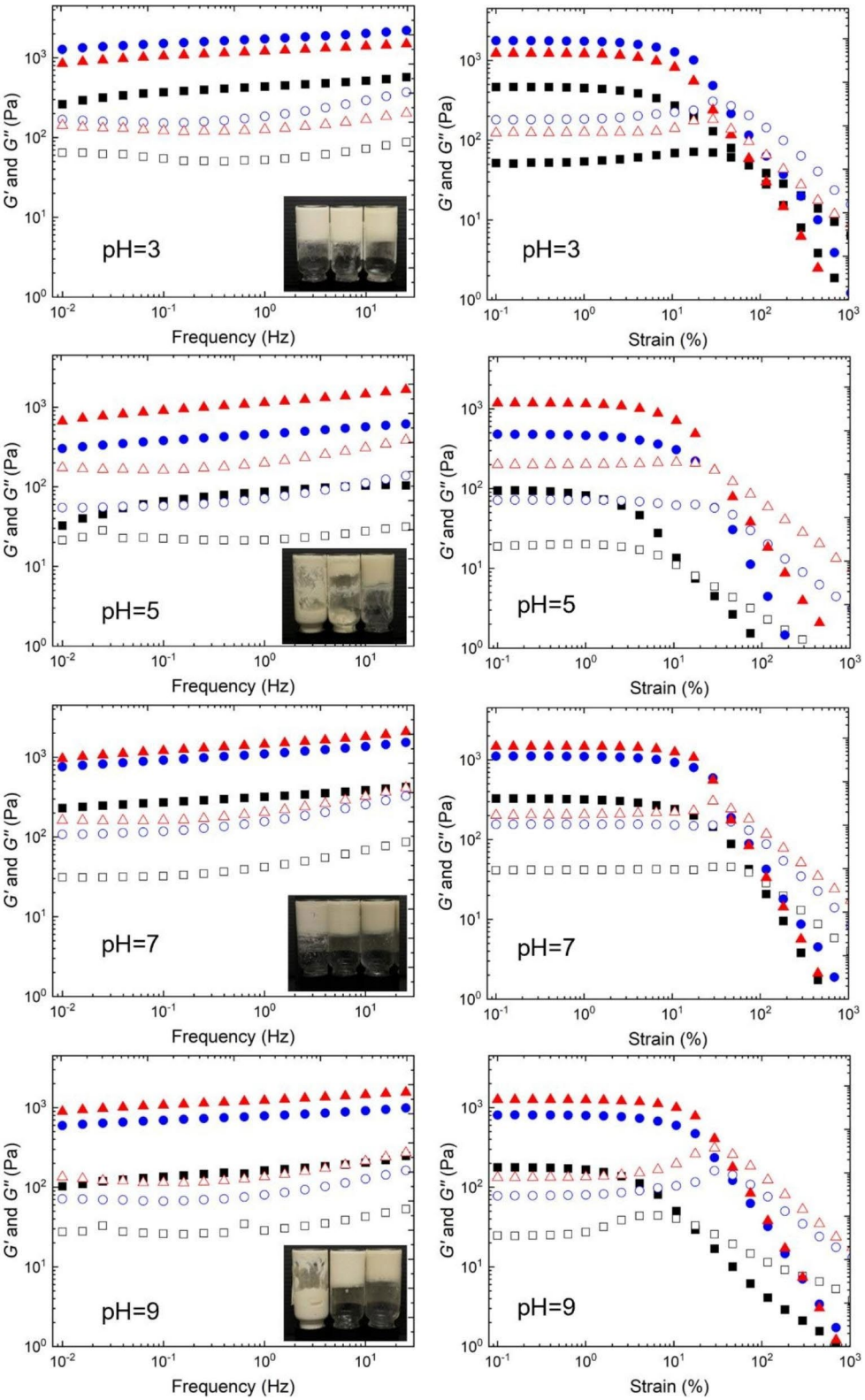

Fig. 5 The storage modulus (G') and loss modulus (G'') as a function of frequency (left-hand side) and strain amplitude (right-hand side) for emulsions stabilised by sonicated quinoa protein isolates (QPI) particles at varied pH values of pH 3–9 containing 20% (■), 40% (●), and 60% (▲) oil. Inset images: visual appearance of various emulsion samples. From left to right: emulsions containing 20, 40 and 60 v/v% oil

[2]. Therefore, the gel-like behaviour of the aggregated emulsion could be attributed to the network formation of oil droplet and protein particles, leading to the constrains of oil droplets movement in the network, which was revealed by CLSM and TEM observations (Figs. 2 and 3). To compare the gel strength among all the samples, the G' at 1 Hz are plotted in Fig. 6A. At pH 5, pH 7 and pH 9, G'(1 Hz) showed an increasing trend with an increase in oil volume

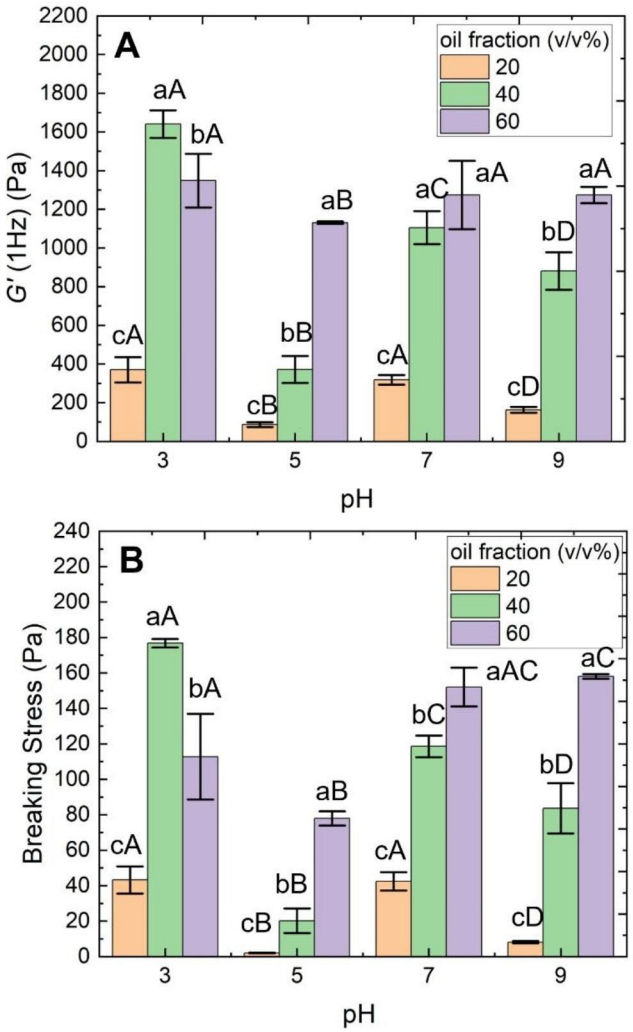

Fig. 6 (**A**) G' at 1 Hz obtained from frequency sweep results and (**B**) breaking stress of emulsions stabilised by sonicated QPI particles at varied pH values of pH 3–9 containing 20%, 40% and 60% oil. Mean values between samples containing the same amount of oil with different uppercase letters (A, B, C, D) are significantly different. Mean values between samples at same pH with different lowercase letters (a, b, c, d) are significantly different

fraction. For example, the G'(1 Hz) of oil droplets stabilised at pH 5 substantially increased from ~90 Pa at 20% oil volume fraction to ~1150 Pa at 60% oil volume fraction. The greater gel strength found at a high oil volume fraction could be attributed to the tighter packing of oil droplets as observed in CLSM and TEM micrographs. This behaviour agrees well with previous rheological studies of kafirin particles stabilised emulsions with oil volume fraction varied from 30 to 70% [40].

However, the G'(1 Hz) of emulsions at pH 3 firstly increased from ~350 Pa at 20% oil volume fraction to ~1600 Pa at 40% oil volume fraction and then decreased to ~1300 Pa at 60% oil volume fraction. As the phase inversion (O/W to W/O) was not observed, the decrease in G'(1 Hz) at 60% oil at pH 3 could be due to inhomogeneities of samples and/or differences in protein aggregation at the oil-water interface and in the continuous phase. It has been suggested that the rheology of emulsion is not only related to the oil droplet size and interfacial layer structures but also depends on the excess protein particles remaining in the continuous phase [16, 43]. This is indeed reflected in the complex rheological properties of emulsions as affected by pH. At low oil volume fractions (20% and 40% oil), oil droplets stabilised by QPI at pH 5 exhibited the lowest G'(1 Hz) value compared to other pHs. This behaviour may have been caused by being close to the isoelectric point of the protein (pI ~ 4.5), which generates the formation of protein aggregates, thus inducing the loose packing of oil droplets with the largest oil droplet size at pH 5. This could be responsible for the lowest gel strength at low oil volume fractions (20% and 40% oil volume fractions). However, oil droplets tend to become tighter packings at all pHs when the oil volume fraction is 60%. Under this circumstance, the rheological property of emulsion seems to be dominated by the interactions and packing of oil droplets. Therefore, all the emulsion exhibited strong gel strength (i.e. G'(1 Hz) > 1000 Pa) at 60% oil.

Large deformation rheological properties of all emulsion samples were studied by strain sweep measurements. G' and G'' as a function of strain amplitude are demonstrated in Fig. 5 (right hand-side). For all the samples, both G' and G'' were independent of strain amplitude at small strains, indicating a linear viscoelastic behaviour. This also indicated that 0.5% strain amplitude used for frequency sweeps measurements, was within the linear viscoelastic region (LVR). When the strain was increased, G' decreased for all emulsion samples, suggesting a structural broken down and shear thinning behaviour. In terms of G'', most samples exhibited a monotonous decrease as the strain was increased. However, for the emulsion stabilised by QPI at pH 9 containing 20% oil, the G'' was first increased and then decreased with the increase in strain amplitude. The overshoot of G'' could be related to dissipation of deformation energy due to rearrangement of oil droplets before the final break down of emulsion structures [16, 44]. The similar behaviour was reported in large deformation rheological studies of concentrated emulsions stabilised by canola protein isolates [45] and multiwalled carbon nanotubes [44]. When the strain amplitude was further increased, the decrease of G'' was faster than G', leading to a crossover of G' and G''. The stress at which $G' = G''$ occurred was defined as the breaking stress. Beyond this point, the G'' is greater than G', suggesting the disintegration of gel network and samples became fluid-like [46]. To compare large deformation rheological properties among all emulsion samples, the breaking stress are plotted and shown in Fig. 6B. Overall, the breaking stress showed similar trends with pH and oil volume

fractions as G'(1 Hz), indicating the large deformation rheology results agreed well with small deformation rheology findings.

Conclusions

This work demonstrates that rheological and microstructural characteristics of US formed QPI emulsions can be tuned by changing pH of QPI suspensions and oil volume fractions. Oil droplets stabilised by QPI suspensions at pH 5 resulted in the largest size and loose packing of oil droplets, resulting in the lowest gel strength especially at 20% and 40% oil volume fractions. At all pHs, increasing oil volume fractions led to a significant increase in gel strength, which could be due to denser and compacter packing of oil droplets. Microscopies observations revealed that formation of aggregate emulsion network is due to the interdroplets bridging and void filling of protein particles between oil droplets. This work also shows the advantage of using US compared to other low energy emulsification method such as Ultraturrax (UT) to form gel-liked emulsions. For example, in order to achieve emulsions with a high gel strength (e.g. G'(1 Hz) > 1000 Pa), 3 wt% QPI emulsions prepared by US requires much smaller oil volume fraction (e.g. 60%) than emulsions prepared by UT (≥ 80%) [8, 15]. This could be useful for reducing the intake of oil consumption without compromising the gel strength of emulsions. Finally, this study broadens the application potentials of plant proteins such as QPI as stabilisers to form gel-like emulsions in making plant based foods such as spread cheese and salad dressings. Future studies can be conducted to further explore applications of these emulsion gels for 3D printing and encapsulation of flavour compounds and nutraceuticals [47].

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Data Availability Data will be available upon request.

Declarations

Competing Interests The authors declare no competing interests.

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