Impacts of sonication and high hydrostatic pressure on the structural and physicochemical properties of quinoa protein isolate dispersions at acidic, neutral and alkaline pHs

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https://doi.org/10.1016/j.ultsonch.2022.106232
Received 7 October 2022; Received in revised form 15 November 2022; Accepted 19 November 2022
Available online 21 November 2022

ARTICLE INFO

Keywords:
Quinoa protein isolates
Sonication
High hydrostatic pressure
Physicochemical properties

ABSTRACT

Herein, 1 wt% quinoa protein isolate (QPI) was exposed to sonication using a 20 kHz ultrasonicator equipped with a 6 mm horn (14.4 W, 10 mL, up to 15 min) or high hydrostatic pressure (HHP, up to 600 MPa, 15 min) treatments at pH 5, pH 7, and pH 9. The changes to physicochemical properties were probed by SDS-PAGE, FTIR, free sulfhydryl group (SH), surface hydrophobicity (H), particle size and solubility. As revealed by SDS-PAGE, substantial amounts of 11S globulin participated in the formations of aggregates via S–S bond under HHP, particularly at pH 7 and pH 9. However, protein profiles of QPI were not significantly affected by the sonication. Free SH groups and surface hydrophobicity were increased after the sonication treatment indicating protein unfolding and exposure of the embedded SH and/or hydrophobic groups. An opposite trend was observed in HHP treated samples, implying aggregation and reassociation of structures under HHP. HHP and sonication treatments induced a decrease in ordered secondary structures (random coil and β-turn) accompanied with an increase in disordered secondary structures (α-helix and β-sheet) as probed by FTIR. Finally, the sonication treatment induced a significant improvement in the solubility (up to ~3 folds at pH 7 and ~2.6 folds at pH 9) and a reduction in particle sizes (up to ~3 folds at pH 7 and ~4.4 folds at pH 9). However, HHP treatment (600 MPa) only slightly increased the solubility (~1.6 folds at pH 7 and ~1.2 folds at pH 9) and decreased the particle size (~1.3 folds at pH 7 and ~1.2 folds at pH 9). This study provides a direct comparison of the impacts of sonication and HHP treatment on QPI, which will enable to choose the appropriate processing methods to achieve tailored properties of QPI.

1. Introduction

With the rapid growth of global population, last decades have seen a great increase in food protein demand [1]. Production of plant proteins is regarded to be more sustainable due to a higher conversion rate and less resource needed compared to those from animal sources. Proteins from plants are regarded as novel and promising food ingredients and have been increasingly used to fabricating dairy and meat substitutes [2]. Quinoa (Chenopodium quinoa Willd) is a pseudocereal that originates from South America. Quinoa has a high protein content of ~25% (based on dry matter) in its seeds depending on varieties. The main storage proteins in quinoa seeds are 11 S globulins and 2 S albumins which accounts for ~37% and ~35% of the total proteins, respectively [3]. Quinoa proteins have a balanced amino acid profile containing all the nine essential amino acids. Further, quinoa proteins are gluten free, which make it a nutritious protein source for Celiac disease patients, who usually have inadequate nutritional intake due to the restrictions in food choices [4]. Moreover, QPI is regarded as a less allergenic ingredient compared to traditional plant protein ingredients such as soy protein isolate (SPI) [5,6].

Despite holding a great promise as future protein ingredients, plant proteins including quinoa proteins commonly show inferior techno-functionalities (e.g., solubility) compared to milk proteins, limiting their wide applications in food products. Extensive efforts have been
recently devoted to modifying plant proteins and improving their technical performances including biotransformation-enzymatic hydrolysis/crosslinking or fermentation, chemical modifications (e.g., Maillard reaction and pH-shifting) [7], as well as physical modifications (e.g., heat treatment and extrusion cooking) [8]. Non-thermal processing technologies including sonication and high hydrostatic pressure (HHP) are standing out due to relative low cost, ease of implementation, and availability at pilot and industry scale [9].

HHP has been widely applied in the food industry as a non-thermal pasteurization technique, and it has been also used to modify the structures and properties of food biomacromolecules such as proteins and starch [10]. It could induce unfolding, denaturation, and gelation (when the concentration of protein exceeds the critical gelation concentration) of globular proteins such as soy protein [11], and kidney bean protein [12], to name a few. For example, Ahmed et al. (2018) reported that the HHP treatment (200 to 600 MPa, 15 min) could significantly improve the water holding and gelation capacities of kidney bean protein isolates at pH 8 [12]. Sonication is another non-thermal processing technique that are widely employed in the modification of plant proteins [13]. The high shear forces, micro-jetting and shockwaves generated by the sonication cavitation collapse could lead to the disruption of large protein aggregates and the enhancement of protein solubilities [9]. In a recent study from Mir et al. (2019), 8 % w/v QPI dispersions were sonicated at 20 kHz, 500 W and 25 % amplitude for 5, 15, 25, and 35 min, respectively. The study showed that the gelling behaviour, emulsifying capability, water solubilities are enhanced whereas the particle size and turbidity are decreased with the increase in sonication time [14].

Sonication and HHP studies on quinoa proteins are scarce compared to other plant proteins. Further, the pH of protein dispersions which is critical to tuning their aggregation behaviours and electrostatic interactions were rarely considered in previous studies. This study is an extension of our previous HHP study of concentrated (10 w/w%) QPI (HHP 002 R1, Russell Hobbs, New Zealand). A Multivac high-pressure unit (HHP 002 R&D, Multivac, Wolfertschwenden, Germany) was used to conduct HHP treatments with water as the pressurising medium. The samples were treated at 250 and 600 MPa at room temperature for 15 min. The temperature of samples was recorded throughout the HHP treatment and never exceeded 33 °C. The pressure was released to atmospheric pressure instantaneously after the treatment is completed. The treated samples were kept sealed until further characterisations. All QPI samples were made in duplicates.

2. Materials and methods

2.1. Materials

Quinoa seeds were kindly provided by Kiwi Quinoa Inc. (Taihape, New Zealand). According to the supplier, the quinoa seeds used for protein extractions contain 17.5 % w/w protein, 4.5 % w/w fat, 64.0 % w/w carbohydrates, and 11.0 % w/w dietary fibre based on a dry matter basis.

Bradford reagent and bovine γ-globulin standard for determination of protein solubility and chemicals for performing SDS-PAGE (including the 4 × Laemmli sample buffer, β-mercaptoethanol, 10 × Tris/Glycine/SDS running buffer, Coomassie Brilliant Blue R-250 staining solution) were obtained from Bio-Rad (Hercules, CA, USA). Milli-Q water was used throughout all sample preparations.

2.2. Extraction of quinoa protein isolates (QPI) from quinoa seeds

The QPI was extracted from quinoa seeds using an alkaline extraction and isoelectric precipitation method [8]. Briefly, quinoa flour obtained from grinding of quinoa seeds were defatted using petroleum ether. Thereafter, defatted quinoa flour was dispersed in 0.5 M NaCl solution at pH 8 under stirring for protein extraction. Starch and cell wall polysaccharides/fibres were separated from the solubilized protein fractions using centrifugation. The supernatant obtained were precipitated when the pH was adjusted to 4.5 which is close to the isoelectric point of quinoa proteins. Precipitated proteins were recovered after centrifugation and washed several times with Milli-Q water to remove salts. Finally, the precipitated proteins were redispersed in Milli-Q water containing 0.02 w%/v sodium azide and pH was adjusted to neutral (pH ~7) before freeze-drying to powder. The protein content was ~90 % based on a dry matter basis.

2.3. High hydrostatic pressure (HHP) treatment of QPI dispersions

A stock QPI dispersion (1.25 wt%) was prepared by the dispersion of QPI powder in Milli-Q water containing 0.02 wt% sodium azide by magnetic stirring at room temperature (~20 °C) for ~24 h to allow full hydration. The stock solution was then divided into three aliquots and the pH was adjusted to 5, 7, and 9, using 1 M HCl and 1 M NaOH and then the dispersions were left under stirring for another ~12 h. The pH was regularly checked and adjusted if needed. Finally, the QPI dispersions were topped up with Milli-Q water to achieve a final QPI concentration of 1 wt%. The HHP treatment was conducted under the same conditions as in our previous study [15]. In brief, QPI dispersions with different pH (~5 mL) were carefully transferred into LDPE plastic pouches (Thomas Scientific, USA) to avoid air bubbles. The QPI dispersions were firstly vacuum sealed with a vacuum sealer (Type C300, Multivac, Germany) and then double sealed with a food vacuum sealer (RHVS1, Russel Hobbs, New Zealand). A Multivac high-pressure unit (HHP 002 R&D, Multivac, Wolfertschwenden, Germany) was used to conduct HHP treatments with water as the pressurising medium. The samples were treated at 250 and 600 MPa at room temperature for 15 min. The temperature of samples was recorded throughout the HHP treatment and never exceeded 33 °C. The pressure was released to atmospheric pressure instantaneously after the treatment is completed. The treated samples were kept sealed until further characterisations. All QPI samples were made in duplicates.

2.4. Sonication treatment of QPI dispersions

Sample preparations for the sonication treatment are the same as described in the section 2.3. QPI dispersions (10 mL each) were transferred to 20 mL glass vials and sonicated for 5 and 15 min, respectively in a 30 s on/30 s off mode using a 20 kHz ultrasonicator equipped with a 6 mm horn with a dial power of 950 W (JY92-IIIN, Ningeo Scientz Biotechnology Co., Ltd, China). The actual sonication power was determined as 14.4 W using a calorimetric method by determining the increase in temperature of sonicated water with time [16]. To prevent overheating, glass vials containing samples were kept in a water–ice bath throughout the sonication treatment to maintain the temperature below 35 °C. All QPI samples were made in duplicates.

2.5. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

Samples including the control (untreated), sonication (15 min) and HHP (600 MPa) treated were selected for SDS-PAGE analysis. The SDS-PAGE analysis was conducted under both non-reducing and reducing conditions according to Laemmli et al. (1970) [15,17] using a commercial, precast Mini-protein TGX (Tris/Glycine) gel (Bio-Rad, USA) consisting of 4 % stacking gel and 15 % resolving gel. The running buffer was used was diluted 10 times from 10 × tris/glycine/SDS running buffer with Milli-Q water (Bio-Rad). The gels were stained with Coomassie Brilliant Blue R-250 staining solution (Bio-Rad) for 60 min and
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destained using a destaining solution (10 % Isopropanol and 10 % Acetic acid glacial in Milli-Q water) under shaking. The destaining solution was changed every half hour until the gel background became transparent, the gels were stored in Milli-Q water at 4 °C. A ChemiDoc XRS imaging system (Bio-Rad, USA) was used to scan the gels. Quantification of band density was performed using the software Image Lab (Bio-Rad, USA).

2.6. Determination of free sulphydryl group (SH)

Contents of free sulphydryl group in various untreated and HHP and sonication treated QPI dispersions were determined according to previous studies [18]. Briefly, 10 μL of Ellman’s reagent (4 mg/mL DTNB in Tris-glycine buffer) was mixed with 0.25 mL 1 % QPI dispersions and 1.25 mL Tris-glycine buffer (0.086 M Tris, 0.09 M glycine, 0.004 M EDTA). The mixtures were vortexed for 30 s and incubated for 15 min at room temperature (~20 °C) before the absorbance measurement. The blank was prepared with the same protocol, but samples were replaced by Milli-Q water. The absorbance at 412 nm was measured by a UV-vis spectrophotometer (Shimadzu 2000, Kyoto, Japan).

2.7. Determination of surface hydrophobicity (Hs)

Surface hydrophobicity was measured using a fluorescence spectrophotometer (RF-6000, Shimadzu, Kyoto, Japan) according to a previously reported method [19] with slight modifications. Each sample was diluted to the concentrations 12.5, 25, 50, 100 and 200 μg/L in 10 mM Tris-HCl buffer (pH 7.0) separately with or without adding a fluorescence probe-8-anilino-1-naphthalene sulfonate (ANS). The reaction was conducted at 20 °C for 20 min in the dark. Fluorescence spectra were recorded at 470 nm (emission) and 390 nm (excitation) with a slit width of 5 nm. QPI samples in the absence of ANS was used as backgrounds. The index of surface hydrophobicity was expressed as the initial slope of 5 nm. QPI samples in the absence of ANS was used as backgrounds. FTIR spectra was recorded at 470 nm (emission) and 390 nm (excitation) with a slit width of 5 nm. QPI samples in the absence of ANS was used as backgrounds. FTIR spectra was recorded at 470 nm (emission) and 390 nm (excitation) with a slit width of 5 nm. QPI samples in the absence of ANS was used as backgrounds.

2.8. Fourier transform infrared spectra (FTIR) analysis

Sonication and HHP treated QPI samples were freeze-dried and ground to powder by a pestle and mortar. FTIR spectra was recorded using a compact ATR-FTIR spectrometer (ALPHA II, Bruker, Germany) in a range of scan between 4000 and 500 cm⁻¹ with the scattering angle of 173° and a fixed wavelength of 633 nm at 20 °C. Samples were allowed to sediment on the bench overnight, then the supernatants were collected and diluted with Milli-Q water for the DLS measurements with a refractive index and viscosity of water of 1.33 and 0.89 mPa s, respectively. Each sample was measured 20 times and the z-average size is reported.

2.9. Solubility determination

1 % QPI samples were centrifuged at 1000 × g for 10 min at 20 °C using a benchtop centrifuge (Pico17 microcentrifuge, Thermofisher scientific, USA). Thereafter, the supernatant was transferred to new Eppendorf tubes and diluted with Milli-Q water to reach the absorbance in a range from 0.2 to 0.8. The diluted supernatants (20 μL) were firstly mixed with 1.48 mL of Bradford reagent (Bio-Rad, USA) and then absorbance of each sample at 595 nm was measured using a UV-vis spectrophotometer (Shimadzu 2000, Kyoto, Japan). A standard curve of absorbance versus protein concentrations was made using a Bovine γ-globulin standard (Bio-Rad, USA). All measurements were conducted in triplicate. The solubility was calculated by the ratio of protein content of supernatant to the original protein concentration in the QPI suspension before centrifugation.

2.10. Particle size measurements of soluble proteins

The particle size (hydrodynamic diameter z-average) of various untreated and treated QPI dispersions was determined with dynamic light scattering (DLS) using ZetaSizer Nano ZS (Malvern instruments, UK) with the scattering angle of 173° and a fixed wavelengths of 633 nm at 20 °C. Samples were diluted with Milli-Q water to reach the absorbance of 0.1 at 633 nm. The mixtures were vortexed for 30 s and incubated for 15 min at room temperature (~20 °C) before the absorbance measurement. The blank was prepared with the same protocol, but samples were replaced by Milli-Q water. The absorbance at 412 nm was measured by a UV-vis spectrophotometer (Shimadzu 2000, Kyoto, Japan).

3. Results and discussion

3.1. QPI protein profiles as revealed by SDS-PAGE analysis

SDS-PAGE analysis was conducted to determine any alterations in the protein profiles after sonication or HHP treatments and results are shown in Fig. 1. Under nonreducing conditions, untreated QPI exhibits a complex protein profile with predominant protein bands appearing at ~55 kDa and ~45 kDa; these are attributed to chenopodin or 11 S globulin [20]. Smaller proteins (molecular weight smaller than 15 kDa) are suggested to be 2 S albumin [3]. Further, untreated QPI dispersions at different pH display similar protein profiles, which is consistent with previous SDS-PAGE studies on QPI at pH 6.5, 8.5, and 10.5 [21].

The HHP treatment (600 Mpa, 15 min) induced a significant reduction in band densities of 11 S globulin at all three pHs, which could be explained by the denaturation and aggregation of 11 S globulin induced by HHP. Similar observations were found in previous HHP studies of QPI [15] and potato protein isolates [22]. It has been suggested that HHP treatment could induce unfolding of globular proteins and promote formations of protein aggregates via various non-covalent (i.e., hydrophobic interactions and hydrogen bonding) and covalent interactions (i.e., disulphide bonds) [11,23]. Under reducing conditions (Fig. 1B), large protein aggregates which accumulated in the loading wells during the non-reducing SDS-PAGE analysis disappeared and all the QPI dispersion exhibit similar protein profiles, which confirms the formation of disulphide bonds in HHP induced QPI aggregates. Protein bands located at ~37 kDa (acidic subunit) and ~25 kDa (alkaline subunit) also become more obvious under the reducing condition, suggesting two subunits of 11 S globulin are linked through disulphide bonds. In addition, the extent of 11 S globulin band fading increased with the increase in pH and the HHP treatment of QPI at pH 9 resulting in less 11 S globulin remaining in the gel (Fig. 1C). This finding agrees well with previous studies on soy proteins [24] and faba bean protein isolates [25]. This could be due to the oxidation of the SH groups to S-S interchange reactions being much more favourable under alkaline conditions [26].

Compared to HHP treatment, sonication treatment did not induce significant alterations to the protein profiles at all the three pHs (Fig. 1A and 1C), which agrees well with a previous sonication study of QPI [14] confirming that sonication does not hydrolyse these proteins. However, the large molecular weight protein aggregates observed in the loading wells of lane 7 (pH 7, sonication treated 15 min) and lane 9 (pH 9, sonication treated 15 min) under non-reducing conditions are vanishing in the presence of the reducing agent may indicate that sonication induced the formations of large protein aggregates via disulphide bonds (Fig. 1). Previous studies suggested that highly reactive free radicals generated from water sonolysis could react with amino acids containing sulphur groups (Cys and Met), leading to the oxidation of free -SH groups and the formation of disulphate bonds [20]. However, the fading of major QPI protein bands (i.e., 11 S globulin) is not observed. This could be due to compensation from higher solubilities and smaller particles sizes of the sonicated QPI samples, thereby increasing their solubilities in the sample buffer used in the non-reducing SDS-PAGE [14].
3.2. QPI protein secondary structural changes revealed by FTIR analysis

FTIR spectra of untreated and sonication or HHP treated QPI samples are shown in Fig. 2. All samples display characteristic absorbance features of proteins, namely the Amide I region (peak \( \sim 1630 \text{ cm}^{-1} \)) originated from C=O stretching, or hydrogen bonding coupled with COO– group; the Amide II region (peak \( \sim 1520 \text{ cm}^{-1} \)) mainly attributed to the NH bend coupled with CN stretch; and the Amide III region (peak \( \sim 1230 \text{ cm}^{-1} \)) due to NH bend stretch coupled with CN stretch [12,27]. These features and the range of characteristic peaks are comparable with previous FTIR studies of QPI [14]. Compared to the untreated QPI sample, the sonication or HHP treatment did not induce peak shifting in the Amide I region, however, a shift in peak \( \sim 1520 \text{ cm}^{-1} \) (Amide II region) to higher wavenumbers can be observed in some sonication or HHP treated samples. For example, the HHP (600 MPa) treatment at pH 5 induced a shift of the Amide II peak from \( \sim 1510 \text{ cm}^{-1} \) to \( 1525 \text{ cm}^{-1} \). Similarly, the sonication treatment (5 min) at pH 9 induced a shift of the
Amide II peak from ~1518 cm\(^{-1}\) to 1532 cm\(^{-1}\). A similar shift in the Amide II peak was reported in a previous FTIR study of kidney bean protein isolate after HHP treatment (600 MPa, 15 min) [12] and it has been suggested to occur due to the unfolding of the protein tertiary structure [28]. The red shift (an increase in the wavenumber of amide II band peaks) is also observed in a recent sonication study (600–2000 W, 5–30 min) of walnut protein isolates, which could be attributed to the change in interactions between protein molecules and structural unfolding due to ultrasonication [29].

In order to better understand the impact of sonication or HHP treatment on the secondary structures of QPI, the deconvolution of the amide I region (1590 cm\(^{-1}\) to 1710 cm\(^{-1}\)) was conducted, and the percentage of each structural component (\(\alpha\)-helix, \(\beta\)-turn, \(\beta\)-sheet, and random coil) is illustrated in Fig. 3. In general, changes of all the secondary structures have similar trends at three pHs: percentages of disordered secondary structures (\(\beta\)-turn and random coil) increased after the sonication/HHP treatment, whereas a decrease in ordered secondary structures (\(\alpha\)-helix and \(\beta\)-sheet) was observed in the sonication and HHP treated samples. This indicated that both sonication and HHP treatments could induce the unfolding of protein structures, in agreement with previous studies [30,31]. However, changes in secondary structures of QPI are more prominent in the sonication treated samples particularly at pH 9. For example, the content of \(\beta\)-sheet at pH 9 is decreased from ~35.9 % (untreated) to ~29.7 % after the sonication treatment for 15 min, while the HHP treatment (600 MPa, 15 min) only leads to a reduction of ~36.6 %. Changes in other secondary structures follow a similar trend.

It has been suggested that cavities in proteins and hydrogen bonds are the most compressible parts of proteins, and pressurisation could induce the collapse of the cavities due to water penetrations and decreasing the distance between hydrogen bonds [32]. These effects enhance intermolecular interactions but destabilise the protein tertiary structures, leading to a partial or complete unfolding of proteins [23]. Under sonication treatment, proteins unfolding, weakening of non-covalent interactions and cleavages of disulfide bonds could also occur as a result of strong shear cavitation forces and micro-streaming generated during ultrasonication [9]. Using circular dichroism to probe the secondary structures of quinoa proteins, Li et al. (2018) found that the content of \(\alpha\)-helix and random coil significantly decreased and increased, respectively, after a sonication treatment at HHP treatment [33]. In another sonication study on soybean proteins, Hu et al. (2013) reported that the percentage of \(\beta\)-sheet is reduced from 60 % to 38.2 % while the percentage of random coil increased from 32.3 % to 38.9 % [31]. In a HHP study of patatin, Elahi et al. (2017) found that the percentage of \(\alpha\)-helix progressively decreased from 24.2 % (untreated) to 4.1 (550 MPa) while the content of random coil increased from 30.3 % to 37.2 % [30]. Different extents of changes in secondary structures could be due to the differences in plant proteins and different treatment conditions used in these studies.

Further, at the same pH, sonication treatment induced much more significant alterations in protein secondary structures than the HHP treatment, particularly at higher pHs (7 and 9). This could be due to the effects of high shear forces, microstreaming, and microjetting exerted by the cavitation during the sonication treatment. Further, the high pH could increase electrostatic repulsions between protein molecules and enhance interactions between proteins and water molecules, thus promoting the unfolding of protein structures and extension of protein chains. The synergistic effect of ultrasonication and high pH on unfolding of plant proteins have been well documented on pea proteins [34] and barley proteins [35], for examples. It has been suggested that the change in protein secondary structures is commonly accompanied with the exposure of embedded hydrophobic regions [36]. Therefore,

**Fig. 3.** Percentages of the secondary structure (\(\alpha\)-helix (A), \(\beta\)-sheet (B), \(\beta\)-turn (C), and random coil (D)) in untreated and HHP or sonication treated QPI dispersions at pH 5, 7, and 9. Mean values between samples at same treatment with different uppercase letters (A–C) are significantly different. Mean values between samples at same pH with different lowercase letters (a–c) are significantly different.
the contents of free SH groups and surface hydrophobicity of QPI is further investigated in the following sections.

3.3. Determination of free SH groups and surface hydrophobicity

The content of free SH groups and surface hydrophobicity of untreated and the sonication /HHP treated QPI samples is shown in Fig. 4. For the untreated QPI samples, higher pHs (7 and 9) resulted in a significant increase in the free SH content, which could be due to diminished protein–protein attractions and greater extents of structural unfolding as supported by FTIR [24]. At all pHs, the content of free SH groups and surface hydrophobicity are increased after the sonication treatment. For example, the content of free SH group is increased from ~22 µmol/g (untreated) to ~28 µmol/g (sonication, 10 min) at pH 9. The increase of SH content and surface hydrophobicity might be attributed to the exposure of embedded SH and other hydrophobic groups to the surface of QPI molecules, the enhanced solubility, or the cleavages of S–S bonds induced by sonication [31,34]. Similar observations have been made in previous sonication studies of various plant protein samples including QPI [33], soy protein isolates [31], and pea proteins [24]. It is worth noting that the oxidation of free SH groups by the free radicals during the sonication and the formation of S–S bonds via SH/SS interchange reactions under alkaline conditions might also occur [24], but probably to a smaller extent, so that the exposure of embedded SH and hydrophobic groups is dominated leading to the overall increase of free SH content.

Contrary to sonication treatment, HHP treatment induced a decrease in the free SH content at all three pHs. At pH 5 and 7, the free SH content progressively decreased when the pressure is decreased from 250 MPa to 600 MPa. For example, the content of free SH group decreased from ~7.7 µmol/g (untreated) to ~6.1 µmol/g (600 MPa, 15 min) and ~22.2 µmol/g (untreated) to ~8.3 µmol/g (600 MPa, 15 min) at pH 7 and pH 9, respectively. This might be due to the formations of S–S linkages via SH/SS interchange reactions and protein aggregation [30]. This observation is also supported by the SDS-PAGE results that 11S globulin participated in the formations of large protein aggregates via S–S bonds (Fig. 1). The aggregation of proteins induced by HHP treatment is also reflected in the decrease in the surface hydrophobicity (Fig. 4B). The index of surface hydrophobicity is decreased from ~35299 (untreated) to ~121888 (600 MPa, 15 min), ~283940 (untreated) to ~257824 (600 MPa, 15 min), and ~172962 (untreated) to ~126646 (600 MPa, 15 min) at pH 5, pH 7, and pH 9, respectively. Aggregation of unfolded protein leads to the formation of protein aggregates with large molecular weight and subsequent decrease of the surface hydrophobicity [31]. Further, the decrease in the surface hydrophobicity is most significant at pH 5 because the electrostatic repulsions between proteins are minimized at the isoelectric point, thus the most extensive protein aggregation is expected at this pH. This observation agrees well with our previous study on HHP treatment of 10 wt% QPI [15].

In a HHP study of patatin, Elahi et al. (2017) found that the content of free SH groups and surface hydrophobicity is firstly increased from atmospheric to 350 MPa and 450 MPa, respectively, and then decreased when the pressure is further increased to 550 MPa [30]. A similar trend has been reported in a HHP study of soy protein isolate, but the maximum of the free SH group content was reached at 300 MPa before decreasing when the pressure is increased up to 550 MPa [37]. The authors attributed this to the initial unfolding of proteins at intermediate pressures followed by the subsequent protein aggregation and reassociation at higher pressures. In another HHP study of soy protein isolates, Torrezan, Tham, Bell, Frazier and Cristianini [38] reported that free SH contents are lower in all HHP (198–702 MPa) treated samples (in comparison with the untreated one). The discrepancy between our findings and previous studies could be due to different HHP treatment conditions (pressure, time, and temperature) and different structures of these plant proteins.

3.4. Protein solubilities and particles sizes as affected by the sonication or HHP treatment

The solubility and particle size of various untreated and sonication/ HHP treated QPI dispersions are shown in Fig. 5. Among the untreated samples, the QPI dispersion at pH 5 shows the lowest solubility (~4%) and largest particle size (~1200 nm), while at pH 9 the solubility markedly increased to (~33 %) with a small reduction in the particle size to ~800 nm. This finding is consistent with previous studies that proteins typically exhibit minimum solubility and largest particle size at or close to their isoelectric point [39]. The sonication treatment induced a significant increase in solubility at pH 7 and pH 9, while the change of solubility is marginal at pH 5. Comparing to the untreated sample, sonication treated for 15 min increased the solubilities to ~45 % and ~82 % at pH 7 and pH 9, respectively. Similar observations have been found in the sonication studies of QPI [14,20], soy protein isolate [31], and canola protein isolate [40]. Various extents of increase in solubilities of proteins have been reported in previous studies, which depends on the sonication conditions and sample characteristics. However, it is generally believed that protein interactions (e.g., hydrophobic interactions and hydrogen bonding) can be disrupted by the sonication treatment, leading to smaller particle sizes, large surface areas for water accessibility, and higher solubilities. This is confirmed with the particle size results showing that the particle size is decreased from ~750 nm (untreated) to ~210 nm (sonication, 15 min) at pH 7, and from ~800 nm (untreated) to ~160 nm (sonication 15 min) at pH 9. The unfolding
of proteins and the loss of their internal native structure were also confirmed with FTIR analysis. The change in particle size and solubility by sonication treatment is not obvious at pH 5, which could be due to strong electrostatic attractions close to its isoelectric point (pH $\approx 4.5$). It has been suggested that turbulent flow and high shear force exerted by ultrasound cavitations could facilitate creating large interfacial area between air and water thereby disrupting hydrogen bonds and hydrophobic interactions of the protein molecules. Thus techno-functional properties of proteins such as solubilities and some physicochemical characteristics, such as particle size, can be affected [43,44,45].

Contrary to sonication treatment, the solubility of QPI increased only slightly after HHP (600 MPa) treatment at pH 7 and pH 9. The solubility increased from $\sim 14.7\%$ (untreated) to $\sim 22.8\%$ (600 MPa, 15 min) and $\sim 33.8\%$ (untreated) to $\sim 39.8\%$ (600 MPa, 15 min) at pH 7 and pH 9, respectively. However it did not induce changes in solubility at pH 5 without significantly affecting the protein profiles as revealed by SDS-PAGE. Due to the substantial protein aggregation at pH 5 (close to the isoelectric point of QPI), the changes in particle size and solubility are marginal. Further, the sonication treatment induced the exposure of interior SH and/or hydrophobic groups via proteins unfolding which is reflected in the increase in free SH content and surface hydrophobicity. In contrast, HHP treatment induced significant changes in the QPI protein profiles where 11 S globulins tend to form aggregates via S–S bonds especially at pH 9. This is also supported by a decrease in the free SH content and surface hydrophobicity after the HHP treatment. FTIR revealed that both HHP and sonication treatments induce an increase in the disordered secondary structure ($\beta$-sheet and random coil) resulting from the transformations from the ordered secondary structures ($\alpha$-helix and $\beta$-turn). In addition, other than pH, some other process parameters such as ultrasonication power/pulses/ times, the level/duration of applied pressure, the treatment temperature, as well as the presence of other food constituents and additives (e.g., salts and polysaccharides) may also have an impact on QPI dispersions being treated [41]. Further research is needed to clarify the effect of these different parameters. Finally, this study demonstrated that tailored QPI properties can be achieved by selecting different processing techniques and different pHs. Further studies can be conducted to reveal the effects of sonication and HHP on techno-functional properties (e.g., gelation, emulsifying, and foaming) of the treated QPI dispersions.

### 4. Conclusions

In summary, this study shows that sonication and HHP treatments have different influences on microstructural characteristics and physico-chemical properties of QPI dispersions, and these influences are pH dependent. Sonication treatment significantly reduce the particle size and improve the solubility of QPI at pH 7 and pH 9 without significantly affecting the protein profiles as revealed by SDS-PAGE. Due to the substantial protein aggregation at pH 5 (close to the isoelectric point of QPI), the changes in particle size and solubility are marginal. Further, the sonication treatment induced the exposure of interior SH and/or hydrophobic groups via proteins unfolding which is reflected in the increase in free SH content and surface hydrophobicity. In contrast, HHP treatment induced significant changes in the QPI protein profiles where 11 S globulins tend to form aggregates via S–S bonds especially at pH 9. This is also supported by a decrease in the free SH content and surface hydrophobicity after the HHP treatment. FTIR revealed that both HHP and sonication treatments induce an increase in the disordered secondary structure ($\beta$-sheet and random coil) resulting from the transformations from the ordered secondary structures ($\alpha$-helix and $\beta$-turn). In addition, other than pH, some other process parameters such as ultrasonication power/pulses/times, the level/duration of applied pressure, the treatment temperature, as well as the presence of other food constituents and additives (e.g., salts and polysaccharides) may also have an impact on QPI dispersions being treated [41]. Further research is needed to clarify the effect of these different parameters. Finally, this study demonstrated that tailored QPI properties can be achieved by selecting different processing techniques and different pHs. Further studies can be conducted to reveal the effects of sonication and HHP on techno-functional properties (e.g., gelation, emulsifying, and foaming) of the treated QPI dispersions.

### CRediT authorship contribution statement

**Lan Luo:** Investigation, Formal analysis, Data curation, Writing – original draft. **Zhi Yang:** Conceptualization, Supervision, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Funding acquisition, Project administration. **Haifeng Wang:** Formal analysis, Data curation. **Muthupandian Ashokkumar:** Writing – review & editing. **Yacine Hemar:** Conceptualization, Data curation, Supervision, Writing – review & editing.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgements

This work was funded by the College of Sciences Massey University Research Fund (MURF) to Z.Y. We appreciate the support from the Kiwi...
Quinoa company (Taihape, NZ) for kindly providing the quinoa seeds used in this work. We thank Associate Professor John Harrison from Massey University who kindly provided us with access to the fluorescence spectrophotometer and Dr. Jon Palmer who provided us access to the HUP unit.

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