Limited Alcalase hydrolysis improves the thermally-induced gelation of quinoa protein isolate (QPI) dispersions

Xueyang Wang\textsuperscript{a}, Lirong Cheng\textsuperscript{b}, Haifeng Wang\textsuperscript{c}, Zhi Yang\textsuperscript{a,\ast}

\textsuperscript{a} School of Food and Advanced Technology, Massey University, Auckland, 0632, New Zealand
\textsuperscript{b} The Riddet Institute, Massey University, Palmerston North, 0745, New Zealand
\textsuperscript{c} Collaborative Innovation Centre of Seafood Deep Processing, Zhejiang Province Joint Key Laboratory of Aquatic Products Processing, Institute of Seafood, Zhejiang Gongshang University, Hangzhou, 310018, China

\begin{abstract}
Gelation is critical in many food applications of plant proteins. Herein, limited hydrolysis by Alcalase was used to promote thermally induced gelation of quinoa protein isolates (QPI). Mechanical properties of various QPI gels were characterised by small and large oscillatory shear deformation rheology while the microstructural features were observed by confocal laser scanning microscopy (CLSM). Both the gel strength and microstructure are strongly related to the hydrolysis time. The maximum gel strength (~100 Pa) was achieved after Alcalase hydrolysis for 1 min, which was ~20 folds higher than that of untreated QPI. Extended hydrolysis up to 5 min progressively decreased the gel strength. A string-like interconnected protein network was formed after proteolysis. The change of gel strength with hydrolysis time correlated well to the $G'_{20^\circ C}/G'_{90^\circ C}$ value and results of intrinsic fluorescence and surface hydrophobicity. The $G'_{20^\circ C}/G''_{90^\circ C}$ value is sensitive to hydrogen bonds formation while the intrinsic fluorescence and surface hydrophobicity are associated with protein unfolding and exposure of hydrophobic groups. Therefore, both hydrogen bonding and hydrophobic interactions are critical in improving the gel strength of QPI hydrolysates. Finally, FTIR analysis revealed that protein secondary structures are affected by the proteolysis and formation of inter-molecular hydrogen bonds between polypeptides. This study provides an efficient strategy for improving thermally induced gelation of QPI and enables a deep understanding of QPI gelation mechanism induced by Alcalase hydrolysis.
\end{abstract}

\section{Introduction}

In recent years, research on alternative proteins such as protein isolates obtained from plant resources has gained increasingly attention from both academia and industry because production of plant proteins is regarded as more sustainable than that of animal and dairy proteins as well as a significant increase in vegan, vegetarian, and flexitarian populations (Grossmann and McClements, 2021). Gelation is regarded as one of the most important techno-functional properties of food proteins, which plays critical roles in manufacturing a wide range of food products such as sausages, meat analogues, and yoghurt (Clark et al., 2001). However, compared to animal and dairy proteins, plant proteins usually exhibit inferior gelation properties due to their poor solubilities and/or lack of covalent interactions (e.g. disulfide bonds) (Akharume et al., 2021). Therefore, developing strategies to enhance gelation properties of plant proteins is highly desirable.

Most plant proteins are globular proteins (Kumar et al., 2022). Gelation of globular proteins occurs when the proteins unfold and create interactions through non-covalent and covalent interactions to form aggregates. When protein concentrations exceed the critical protein concentration for gelation, a three-dimensional cross-linking network was formed, finally leading to the formation of gels. There are various methods that can induce the gelation of plant proteins including heat treatment (Nicolai and Chassenieux, 2019; Yang et al., 2022), high hydrostatic pressure (HHP) processing (Luo et al., 2021), addition of acids and salts (Langton et al., 2020), and enzymatic cross-linking (e.g. transglutaminase) (Xing et al., 2020). Among these methods, thermodinduced gelation is most commonly used due to its ease of operation and availability for pilot and industrial scales.

Enzymatic hydrolysis is another method that can affect the gelation property of globular proteins and most studies focused on whey proteins. It has been reported that whey protein isolate (WPI) or its main products such as sausages, meat analogues, and yoghurt (Clark et al., 2001).

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component β-lactoglobulins can form gels when subjected to hydrolysis by *Bacillus licheniformis* protease (BLP) (Spotti et al., 2017), α-chymotrypsin (Rabiey and Britten, 2009), and Alcalase (Doucet et al., 2003; Otte et al., 1996; Otte et al., 2000). Gelation kinetics and final gel strength are significantly affected by preheat treatment of proteins, concentrations of proteins and enzymes, pH, ionic strength, and temperature (Otte et al., 1999).

Compared to WPI, there are far less studies available in the literature on the effect of enzymatic hydrolysis on the gelation of plant proteins; and most often, soy protein was studied (Vogelsang-O’Dwyer et al., 2022). Lamsal, Jung, and Johnson (2007) studied the impact of hydrolysis with bromelain on the heat gelation (95 °C) properties of defatted soy flour, soy protein concentrate, and soy protein isolate at pH 7. A substantial decrease in storage modulus (G') was observed in all hydrolysed samples and G' decreased as the degree of hydrolysis (DH) increased. In another study related to peanut protein isolate (PNI), Jiang et al. (2021) found that the limited Alcalase hydrolysis of PNI can significantly improve the gel formation below 70 °C. Previous studies suggest that around ~70–90% of saponins can be removed from quinoa seeds using similar water washing methods (El Hazzam et al., 2020; VEGA-GALVEZ, San Martin, Sanders, Miranda, & Lara, 2010). Then, the quinoa seeds were ground into flour with a bench-top coffee grinder (Breville, New Zealand) before passing through a 500 μm sieve to obtain fine quinoa powder. After that, defatting was achieved by mixing the quinoa flour with petroleum ether at a ratio of 1:10 (w/v) under magnetically stirring for 5 h at 20 °C. The defattting process was repeated twice by changing new petroleum ether every 5 h. The similar defattting method has been used in previous studies of quinoa protein extractions (Luo et al., 2022; Shen et al., 2021). After drying, the defatted quinoa flour (~200 g) was dispersed into ~2 L Milli-Q water containing 500 mM NaCl for 2 h and the pH was adjusted to 8 using 1 M NaOH. Thereafter, the supernatant containing soluble proteins was obtained after centrifugation at 10,000 g for 30 min at 20 °C (Sigma centrifuge, Harz, Germany). Afterwards, the pH of the supernatant was adjusted to pH 4.5 (close to isoelectric point of quinoa proteins) in order to precipitate proteins. The proteins were recovered by centrifugation again at the same condition and then thoroughly washed with Milli-Q water 4 times to remove salt. Finally, the protein precipitates were resolubilised in Milli-Q water containing 0.02 wt% sodium azide at pH 7 before being lyophilized (Labconco, MO, USA) to obtain dry powders for further uses. The QPI powder contains 92.0 w/w% protein, 1.5 w/w% fat, 3.0 w/w% ash and 3.5 w/w% moisture as determined by the Nutrition Laboratory of Massey University (Palmerston North, New Zealand). The protein content of QPI was determined using a Kjeldahl autoanalyzer (Foss Tecator, Sweden) according to the AOAC method 2001.11. The protein content was calculated using a nitrogen conversion factor of 6.25 for quinoa protein (Tavano et al., 2022).

### 2. Materials and Methods

#### 2.1. Materials

Quinoa seeds for quinoa protein extractions were kindly provided by Kiwi Quinoa Inc. (Tahupe, New Zealand). All chemicals including petroleum ether, NaOH, NaCl, HCl, SDS, isopropanol, Alcalase 2.4 L (from *Bacillus licheniformis*, P4860, EC number 3.4.21.62, ≥2.4 U/g), low viscosity mineral oil (M5904), fast green dye, 8-anilino-1-naphthalene sulphonate (ANS), and sodium azide were purchased from Sigma-Aldrich (St. Louis, MO, USA). The o-phthaldialdehye (OPA) reagent solution was purchased from Thermofisher Scientific (Waltham, MA, USA). All chemicals were of analytical grade. Chemicals for SDS-PAGE including the Laemmli sample buffer (4 ×), Tris/Glycine/SDS running buffer (10 ×), β-mercaptoethanol, and Coomasie Brilliant Blue R-250 staining solution were purchased from Bio-Rad (Hercules, CA, USA). Milli-Q water was used throughout the sample preparations.

#### 2.2. Preparation of quinoa protein isolate (QPI)

The QPI was extracted according to our previous studies (Yang et al., 2022; Zhang et al., 2021). Briefly, quinoa seeds were thoroughly washed with water (quinoa/water = 1:10 (w/w)) at room temperature (~20 °C) for 5 times before drying in a fume hood overnight. Previous studies suggest that around ~70–90% of saponins can be removed from quinoa seeds using similar water washing methods (El Hazzam et al., 2020; VEGA-GALVEZ, San Martin, Sanders, Miranda, & Lara, 2010). Then, the quinoa seeds were ground into flour with a bench-top coffee grinder (Breville, New Zealand) before passing through a 500 μm sieve to obtain fine quinoa powder. After that, defatting was achieved by mixing the quinoa flour with petroleum ether at a ratio of 1:10 (w/v) under magnetically stirring for 5 h at 20 °C. The defattting process was repeated twice by changing new petroleum ether every 5 h. The similar defattting method has been used in previous studies of quinoa protein extractions (Luo et al., 2022; Shen et al., 2021). After drying, the defatted quinoa flour (~200 g) was dispersed into ~2 L Milli-Q water containing 500 mM NaCl for 2 h and the pH was adjusted to 8 using 1 M NaOH. Thereafter, the supernatant containing soluble proteins was obtained after centrifugation at 10,000 g for 30 min at 20 °C (Sigma centrifuge, Harz, Germany). Afterwards, the pH of the supernatant was adjusted to pH 4.5 (close to isoelectric point of quinoa proteins) in order to precipitate proteins. The proteins were recovered by centrifugation again at the same condition and then thoroughly washed with Milli-Q water 4 times to remove salt. Finally, the protein precipitates were resolubilised in Milli-Q water containing 0.02 wt% sodium azide at pH 7 before being lyophilized (Labconco, MO, USA) to obtain dry powders for further uses. The QPI powder contains 92.0 w/w% protein, 1.5 w/w% fat, 3.0 w/w% ash and 3.5 w/w% moisture as determined by the Nutrition Laboratory of Massey University (Palmerston North, New Zealand). The protein content of QPI was determined using a Kjeldahl autoanalyzer (Foss Tecator, Sweden) according to the AOAC method 2001.11. The protein content was calculated using a nitrogen conversion factor of 6.25 for quinoa protein (Tavano et al., 2022).

#### 2.3. Enzymatic hydrolysis of QPI by Alcalase

QPI was dispersed in Milli-Q water containing 50 mM NaCl to make a protein solution of 8 wt%. The pH was adjusted to 8 with 1 M NaOH and the QPI dispersion was kept gentle stirring overnight at 20 °C to allow protein hydration. The pH was regularly checked and adjusted if necessary. Subsequently, the QPI solution (~5 g) was placed in a water bath set at 50 °C until the temperature equilibrates to 50 °C followed by adjusting the pH to 8. Enzymatic hydrolysis was initiated by adding Alcalase enzyme solution to the QPI solution at an enzyme to protein ratio of 2:100 (E/S, v/w) (Chen and Campanella, 2022). At the desired hydrolysis times (1 min, 3 min, and 5 min), aliquots of QPI hydrolysates were immediately heated at 90 °C for 20 min to stop the enzymatic reaction (Nieto-Nieto et al., 2014). Hydrolysates were freeze-dried and stored for further characterisations. The QPI sample (pH 8) subjected to the same heat treatment (90 °C, 20 min) but in the absence of enzyme was regarded as a control.

#### 2.4. Degree of hydrolysis (DH)

The degree of hydrolysis (DH) was determined following the method of Chen and Campanella (2022) and Nielsen et al. (2001) using the o-phthaldialdehye (OPA) method. Briefly, QPI samples were dissolved to reach appropriate concentrations and then 400 μL QPI solutions were added into 3 mL of OPA reagent solution. The mixtures were left at 20 °C for exactly 2 min followed by measuring the absorbance at 340 nm by a UV–Vis spectrophotometer (UV-1700, Shimadzu, Japan). To obtain a completely hydrolysed sample for estimation of total number of amides groups, the QPI was incubated with 6 M HCl at 110 °C for ~18 h. OPA reagent added with 400 μL Milli-Q water or 400 μL unhydrolyzed QPI sample was treated as the blank and control, respectively. DH was calculated using the equation below.

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where Abs (QPH), Abs (QPI), Abs (Total), and Abs (Blank) represent the absorbance of QPI hydrolysates, unhydrolyzed QPI, QPI incubated with 6 M HCl, and the blank, respectively.

2.5. Measurement of zeta potential and pH of QPI hydrolysates

The zeta potentials and pH of untreated and Alcalase treated QPI samples were determined at a concentration of 1 wt% and at 20 °C. The zeta-potentials were measured by a Malvern Zetasizer Nano-ZS90 instrument with a disposable folded capillary cell (DTS1070, Malvern Instruments, UK) (Cheng et al., 2022; Nieto-Nieto et al., 2014). The pH was determined with a HALO Bluetooth wireless pH electrode (HI13302, Hanna instruments, USA).

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

SDS-PAGE was conducted according to Laemmli (1970) and our previous studies (Luo et al., 2021; Yang et al., 2022). The SDS-PAGE was run under the reducing condition, and 0.15% (20 μL) QPI dispersions was mixed with Laemmli sample buffer (4 × 6 μL) and 0.8 μL of β-mercaptoethanol. Subsequently, the mixture was boiled for 10 min. The sample (10 μL) was carefully loaded on the well of a commercial Tris/Glycine precast gel (Bio-Rad, USA) containing 4% stacking gel and 15% resolving gel. A PowerPac Basic was used to conduct electrophoresis at a fixed voltage of 150 V in a running buffer consisting of Tris/Glycine/SDS running buffer (Bio-Rad, USA). The gels were stained with Coomassie Brilliant Blue solution followed by destaining with 10% isopropanol/glacial acetic acid solution under gentle shaking. Protein bands were identified by comparing with a protein molecular weight marker (precision plus protein dual xtra standards). A ChemiDoc XRS imaging station (Bio-Rad, MO, USA) was used to scan the gels.

2.7. Confocal laser scanning microscopy (CLSM)

CLSM was used to probe microstructural features of thermally induced gels made from unhydrolyzed and hydrolysed QPI. Briefly, servo drops of 1% fast green dye was added into the QPI dispersions and then vortex mixed for ~10 s at 20 °C. The Alcalase solution was added into the stained QPI solution followed by mixing well by inverting or flicking the tube (~10 s). Thereafter, the mixture was transferred into a glass slide with a cavity and was covered with a coverslip. To prevent water evaporation during incubation, nail polish was used to seal edges of the coverslip (Wang et al., 2019). The samples were first incubated in an oven at 50 °C for 1 min, 3 min, and 5 min, respectively and then immediately transferred to another oven at 90 °C for 20 min. The strained QPI sample in the absence of Alcalase but only incubated at 90 °C for 20 min was used as a control. All the samples were cooled down to ~20 °C before observations under a confocal laser scanning microscope (Leica TCS SP5, Leica Microsystems) equipped with an oil immersion objective lens (100×) at a wavelength of 630 nm. The Image J software (NIH, MD, USA) was used to process all images.

2.8. Intrinsic fluorescence spectroscopy

The intrinsic fluorescence spectra were recorded following the method of Jiang et al. (2021) using a fluorescence spectrophotometer (Shimadzu RF-6000, Kyoto, Japan). The QPI samples were dissolved to make a protein solution of 0.02 wt% and fluorescence spectra were recorded in an emission range from 300 to 500 nm with an excitation wavelength of 290 nm.

2.9. Determination of surface hydrophobicity (H0)

Surface hydrophobicity (H0) was determined according to Luo et al. (2022) with slight modifications. For each QPI sample, dispersions at the concentrations of 12.5, 25, 50, 100, and 200 μg/mL were prepared with 10 mM Tris-HCl buffer at pH 7.0 separately. Afterwards, 8.0 mM of 8-anilino-1-naphthalene sulphonate (ANS) acting as a fluorescence probe was added into 3 mL of each QPI solution and incubate at 20 °C for 20 min in a dark. A fluorescence spectrophotometer (Shimadzu RF-6000, Kyoto, Japan) was used to record fluorescence intensities at 390 nm and 470 nm for excitation and emission, respectively with a fixed slit width of 5 nm. The surface hydrophobicity was calculated as the initial slope from the linear regression analysis of peak fluorescence intensities (background subtracted) versus QPI concentrations (Kato and Nakai, 1980). QPI samples in the absence of ANS were used as the background and results were reported as means of duplicate measurements of two replicated samples.

2.10. Fourier transform infrared spectroscopy (FTIR)

FTIR was used to probe protein secondary structure alternations of QPI subjected to Alcalase hydrolysis. A compact ATR FTIR spectrometer (ALPHA II, Bruker, Germany) was used to collect FTIR spectra in a range between 4000 and 500 cm−1 at 4 cm−1 resolutions. The spectra were background subtracted before further processing. A total of 64 scans with duplicate samples were averaged. Peak deconvolutions of the Amide I region (1590-1710 cm−1) were carried out using the PeakFit software (Systat software Inc, USA) to determine percentages of protein secondary structure. The Savitzky–Golay filter function were used to smooth the spectrum. Then Gaussian function was used for data fitting until the fit converged with R2 value greater than 0.99 (Byler and Susi, 1986). The component peaks from secondary structures were identified after peak deconvolutions (β-sheet: ~1628 cm−1, random coil: ~1647 cm−1, α-helix: ~1661 cm−1, and β-turn: ~1683 cm−1). The percent contribution of each specific secondary structure component was determined by calculating the area of each component peak divided by the total area of amide I band before the deconvolution (Sow and Yang, 2015).

2.11. Rheological characterisations

Small oscillatory deformation rheology was used to in situ monitor the thermally induced gelation of various QPI samples by a rotational stress-controlled rheometer (DHR-3, TA instruments, DE, USA) equipped with a stainless-steel parallel plate geometry (diameter 20 mm, gap 1 mm). The Alcalase solution was added into the QPI solution followed by mixing well by inverting or flicking the tube (~10 s). Subsequently, aliquots of QPI samples were carefully pipetted onto the bottom plate setting at 50 °C. To prevent water evaporation during rheological measurements, low viscosity mineral oil was added around the premier of the sample. The total time used before initiating rheological measurements (including mixing enzyme, loading sample, and applying mineral oil) was controlled as ~20 s. The rheological measurements were conducted in the following steps: (1) temperature was maintained at 50 °C for 0 min (control), 1 min, 3 min, and 5 min, respectively, this step was used for enzymatic hydrolysis; (2) thereafter, the temperature was increased from 50 °C to 90 °C at 40 °C/min and maintained at 90 °C for 20 min, this step was used to inactive the enzyme as well as promoting gelation; (3) after this step, the temperature was decreased from 90 °C to 20 °C at 40 °C/min and equilibrated at 20 °C for 15 min; (4) subsequently, a frequency measurement was conducted at a fixed strain of 1% with the frequency between 0.01 and 10 Hz at 20 °C; (5) Finally, a strain sweep measurement was conducted at a constant frequency of 1 Hz and the strain amplitude varied from 0.1% to 1000% at 20 °C. In steps (1−3), a fixed strain (1%) and frequency (1 Hz) was used to monitor the gelation kinetics and all rheological measurements were repeated at
least twice.

2.12. Statistical analyses

All the characterisations were determined in duplicate (intrinsic fluorescence, SDS-PAGE), or triplicate (DH, pH, zeta-potential, rheology, and surface hydrophobicity) and the results are reported as mean ± standard deviations (SD). The Least Significance Difference (LSD) test was used for mean comparison through Analysis of Variance (ANOVA) using the SPSS software (v. 21.0, Chicago, USA). The significance level is $P < 0.05$.

3. Results and discussion

3.1. Hydrolysis of QPI

The degree of hydrolysis (DH) for the QPI hydrolysed by Alcalase is shown in Table 1. Previous studies indicated that the DH is strongly dependent on protein species, enzyme to substrate (E/S) ratios, and enzymatic hydrolysis conditions (e.g. temperature, ionic strength, etc) (Sun, 2011; Vogelsang-O’Dwyer et al., 2022). The DH values obtained in the current study were lower than some previous studies on QPI hydrolysed by Alcalase (Chirinos et al., 2020; Li et al., 2018). For example, in the study of Chirinos et al. (2020), a QPI dispersion (2.5% w/v) was subjected to Alcalase hydrolysis up to 4 h using a E/S (Alcalase:quinoa proteins) ratio of 1/6.25, which is about 8 times of what we used in the present study. The higher DH (~35%) found in their study suggested that a higher dosage of the Alcalase and prolonger hydrolysis time induced more extensive hydrolysis. However, when the same enzyme dosage (E/S = 1:50) was used, comparable DH values (~6% and ~3% as determined by OPA and pH-Stat methods, respectively) were reported for the pea protein isolate hydrolysed by Alcalase for 3 min (Cheng et al., 2022). And a significant improvement in the thermally induced gelation property of these pea protein hydrolysates was observed. Limited hydrolysis may be sufficient to induce conformational and structural alterations of proteins, thus promoting protein-protein interactions, aggregation, and further gelation under heating. By contrast, more extensive hydrolysis may induce significant reductions of molecular mass thus imparting negative effects on the gelation (Vogelsang-O’Dwyer et al., 2022).

The pH decreased progressively from ~7.82 (control) to ~7.31 after 5 min Alcalase hydrolysis (Table 1), which can be attributed to the release of hydrogen ions due to the cleavage of peptide bonds and production of acidic amino acids (Kumar et al., 2016; Li et al., 2021; Nielsen et al., 2001). The Zeta-potentials of all QPI samples indicating their surface charges, are all negative, which is expected because the pH values are well above the isoelectric point of QPI (pl ~4.5) (Dakhili et al., 2019). Furthermore, the absolute zeta potential values were found to decrease slightly with increasing hydrolysis time from around –26.2 mV (control) to –21.7 mV at 5 min (Table 1). This could be due to the fact that the pH of QPI hydrolysates decreased toward the pl during the hydrolysis thus reducing net surface charges of the protein. However, small changes of the Zeta potentials were found in the QPI up to 5 min of Alcalase hydrolysis, suggesting electrostatic interactions between protein molecules are not significantly changed. Similar findings have been reported in Alcalase treated rice bran proteins (Thammarathip et al., 2016) and trypsin catalysed lentil proteins (Avramenko et al., 2013).

To probe changes in QPI protein profiles when subjected to Alcalase hydrolysis, reduced SDS-PAGE has been conducted (Fig. 1). There are few major protein bands present in the untreated QPI, namely 11S globulin (~45–50 kDa) consisting of acid subunits (~30–35 kDa) and basic subunits (20–25 kDa), and 2S albumin (10–15 kDa). In the presence of a reducing agent, most of the 11S globulin dissociated to the acidic and basic subunits. This is consistent with previous studies (Luo et al., 2022; Shen et al., 2021; Yang et al., 2022). After the Alcalase hydrolysis, major protein bands became progressively faded with the hydrolysis time and meanwhile smaller molecular weight fractions with Mw < 15 kDa are generated. They are probably partially digested proteins or peptides. This finding agrees well with previous studies on Alcalase hydrolysis of PPI (Cheng et al., 2022), peanut protein (Jiang et al., 2021), and fava bean protein (Liu et al., 2019); confirming that the protein hydrolysis by Alcalase is non-specific (Zhu et al., 2010).

3.2. Microstructural characteristics of various QPI gels revealed by CLSM

Microstructures of various QPI gels were observed by CLSM, and micrographs are shown in Fig. 2. The protein stained by fast green is green, while the serum voids are black. For the control sample, some large protein aggregates can be observed which agrees with previous CLSM studies of heat induced QPI (Yang et al., 2022) and pea protein isolate (Cheng et al., 2022) gels. The presence of aggregates is attributed to the poor solubility of QPI and heat induced denaturation and aggregation of proteins (Luo et al., 2022). In the case of QPH gels, an interconnected protein network structure can be observed, with some large aggregates embedded within gel matrix. Furthermore, the size of large protein aggregates become smaller when the hydrolysis time is increased from 1 min to 5 min. It is apparent that limited enzymatic hydrolysis (e.g. 1 min) could promote the aggregation and network formation of QPI thus leading to a strong gel strength. On the other hand, excessive hydrolysis (e.g. 5 min) results in a looser and less compact network structure, which may lead to inferior gel strength.

Table 1

Degree of hydrolysis (DH), pH, and Zeta potential for Alcalase treated QPI as a function of hydrolysis time.

| Samples (min) | DH (%) ± SD | pH ± SD | Zeta potential (mV) ± SD |
|--------------|-------------|---------|-------------------------|
| Control (0 min) | 2.10 ± 0.05 | 7.82 ± 0.05 | -26.2 ± 0.33 |
| 1 min | 2.44 ± 0.25 | 7.61 ± 0.04 | -24.8 ± 0.75 |
| 3 min | 3.00 ± 0.22 | 7.43 ± 0.05 | -23.4 ± 0.43 |
| 5 min | 3.30 ± 0.22 | 7.31 ± 0.03 | -21.7 ± 0.33 |

All data were represented as the means and standard deviations of triplicate determinations. Superscript (a-d) indicate significant differences ($P < 0.05$) in the same column.
3.3. Intrinsic fluorescence and surface hydrophobicity

To understand the gelation enhanced by the limited Alcalase hydrolysis, conformational and structural alterations of proteins were probed. Intrinsic fluorescence spectra have been used to reflect the degree of exposure of aromatic amino acids (e.g. tryptophan) to surrounding solvent, thus indicating alternations of protein tertiary conformation (Cheng et al., 2022; Xu et al., 2016). The fluorescence spectra for all the QPI samples are shown in Fig. 3A. The Alcalase hydrolysis significantly increased the fluorescence intensity, and the maximum intensity can be found in 1 min hydrolysed sample. This could be due to the fact that more buried hydrophobic amino acids were exposed to water after the hydrolysis, thus emitting higher fluorescence (Ma et al., 2022; Sponton et al., 2014). Moreover, further increasing in hydrolysis time induced a reduction of fluorescence intensity. Extensive enzymatic hydrolysis may promote hydrophobic interactions of the polypeptides with higher extent of hydrolysis, and those exposed hydrophobic groups could be re-buried in the interior of aggregates. This may reduce the number of hydrophobic groups available leading to reduction of fluorescence intensity (Zhao et al., 2011). A similar observation has been found in a previous study of rice glutelin subjected to trypsin hydrolysis (Xu et al., 2016). They found that the fluorescence intensity of rice glutelin was significantly increased after trypsin hydrolysis. The fluorescence intensity was increased with the DH up to DH 2% and then decreased when the DH was further increased to DH 5%. In addition, enzymatic hydrolysis shifted the fluorescence emission maximum wavelength ($\lambda_{\text{max}}$) from ~331 nm to ~334 nm (red shift), indicating the exposure of Trp residues and/or alternations in tertiary conformation (Ma et al., 2022). Similar observations have been found in previous studies of pea proteins subjected to Alcalase hydrolysis (Cheng et al., 2022), SPI subjected to papain hydrolysis (Ma et al., 2022), and peanut proteins subjected to Alcalase hydrolysis (Jiang et al., 2021).

Surface hydrophobicity ($H_0$) is another important structural parameter to indicate the extent of protein unfolding and conformational changes (Alizadeh-Pasdar and Li-Chan, 2000). The surface hydrophobicity for all the QPI samples is shown in Fig. 3B. In agreement with the intrinsic fluorescence measurement, $H_0$ reached the maximum upon 1 min hydrolysis and then progressively decreased with further increase in hydrolysis time. Similar findings have been reported in previous studies on fava bean protein isolate (Liu et al., 2019) and rice glutelin (Xu et al., 2016) subjected to Alcalase hydrolysis. Limited Alcalase hydrolysis could enhance the $H_0$ by inducing protein unfolding and exposing more of initially buried hydrophobic groups. However, extended hydrolysis led to further breakdown of hydrophobic areas and/or aggregation of extensively hydrolysed polypeptides, thereby decreasing the $H_0$ (Avramenko et al., 2013; Liu et al., 2019; Xu et al., 2016). Finally, both the intrinsic fluorescence and $H_0$ findings correlated well with rheology results, indicating hydrophobic interactions also play important roles in promoting gel strength of hydrolysed QPI.

3.4. FTIR spectra analysis

FTIR analysis was used to probe protein secondary structure changes of QPI as affected by the Alcalase hydrolysis. The percentage of α-helix, β-sheet, β-turn, and the random coil was determined through the peak deconvolution of the Amide I region (Table 2). Compared with the control QPI, Alcalase hydrolysis induced a significant decrease in the α-helix and β-turn contents and great increase in the β-sheet and random coil contents. This indicated that the Alcalase hydrolysis affected the secondary structure of QPI. It has been suggested that protein secondary structures are partially deconstructed by the enzymatic hydrolysis and newly exposed intra-molecular hydrogen bonds could form inter-
molecular hydrogen bonds between polypeptide chains, leading to a significant reduction in the $\alpha$-helix content and a significant increase in the $\beta$-sheet content (Jiang et al., 2021). In addition, an increase in the random coil contents after Alcalase hydrolysis could be attributed to the protein unfolding, resulting in more flexible and extended protein conformations (Tatham et al., 1985). Similar results have been reported in a circular dichroism (CD) study of Alcalase hydrolysed peanut protein isolate (Jiang et al., 2021) and a FTIR study of papain hydrolysed SPI (Ma et al., 2022).

### 3.5. Rheological characterisations of various QPI gels

The changes in microstructural characteristics and protein conformations of quinoa protein hydrolysates could lead to changes in their gelation behaviour, thus rheological measurements were performed to confirm this. The development of $G'$ and $G''$ for various QPI gels formed during different enzymatic hydrolysis time followed by the heat treatment (90 °C, 20 min) is plotted in Fig. 4. The $G'$ and $G''$ represent the elastic and viscous component of QPI gels, respectively.

For all the QPI samples, heating from 50 to 90 °C induced significant increases in $G'$, which could be due to extensive protein denaturation, unfolding and aggregation at the elevated temperature above the denaturation temperature of QPI (~70–85 °C) (Dakhili et al., 2019). Similar behaviours have been observed in previous studies on heat induced gelation of QPI (Kaspchak et al., 2017; Patole et al., 2022; Yang et al., 2022). When the temperature is decreased to 20 °C, all the QPI gels displayed increases in both $G'$ and $G''$, and these increases being more prominent in enzymatic treated samples. The ratio of $G'_20 °C / G'_90 °C$ has been used to indicate the formation of non-covalent bonds, particularly hydrogen bonds in plant protein gels during cooling and a high $G'_20 °C / G'_90 °C$ value is associated with a higher amount of hydrogen bond interaction (Andlinger et al., 2021; Tanger et al., 2022). The $G'_20 °C / G'_90 °C$ values for all QPI gels are shown in Fig. 4 E. The $G'_20 °C / G'_90 °C$ value increased from ~1.4 for the QPI gel to ~2.8–4.0 for the QPH gels and it reaches the maximum for the 1 min Alcalase hydrolysed QPI.

### Table 2

Secondary structure content of QPI dispersions subjected to Alcalase hydrolysis at different times determined by FTIR.

| Samples    | $\alpha$-helix (%) | $\beta$-sheet (%) | $\beta$-turn (%) | Random coil (%) |
|------------|--------------------|-------------------|-----------------|-----------------|
| Control (0 min) | 38.6 ± 0.02        | 15.3 ± 0.01       | 22.3 ± 0.01     | 23.8 ± 0.02 |
| 1 min      | 29.2 ± 0.01        | 29.3 ± 0.02       | 17.1 ± 0.02     | 24.4 ± 0.02 |
| 3 min      | 28.7 ± 0.02        | 29.6 ± 0.03       | 16.8 ± 0.03     | 24.9 ± 0.02 |
| 5 min      | 28.2 ± 0.02        | 30.6 ± 0.02       | 16.1 ± 0.02     | 25.2 ± 0.03 |

All data were represented as the means and standard deviations of triplicate determinations. Superscript (a-d) indicate significant differences ($P < 0.05$) in the same column.

Fig. 4. The evolution of $G'$ (solid symbols) and $G''$ (empty symbols) for quinoa protein isolate (QPI) solutions at 8 wt% in the absence of Alcalase (A) and in the presence of Alcalase (E/S = 1:200) for proteolysis at 50 °C for 1 min (B), 3 min (C), and 5 min (D) followed by thermally induced gelation. Temperature profiles were indicated as dashed lines. (E) Ratio of $G'_20 °C / G'_90 °C$ for all quinoa protein isolate (QPI) and quinoa protein hydrolysates (QPH) gels.
sample. This suggests that the limited Alcalase hydrolysis promotes high amount of non-covalent interactions such as hydrogen bond, which may result in a higher gel strength (Sun and Arntfield, 2012).

At the end of temperature sweep measurements, the frequency sweep and strain sweep measurement were used to characterise the small and large deformation rheological properties of various QPI gels and the results are shown in Fig. 5A and B. In terms of the frequency sweep measurement, a small strain (1%) was used throughout the measurement which well fell into the Linear Viscoelastic Region (LVR). For all the QPI samples, $G'$ is larger than $G''$ in the entire frequency range and both $G'$ and $G''$ are only weakly dependent on the frequency, which are indicative of typical gel characteristics (Mitchell, 1980). Similar dependence of $G'$ and $G''$ with frequency have been observed in various thermally induced plant protein gels including quinoa (Yang et al., 2020), pea (Tanger et al., 2022), and potato (Katzav et al., 2020) to name a few. The complex modulus $G^*$ (1Hz) taking into account of contributions from both $G'$ and $G''$ to the gel strength was plotted in Fig. 5C to allow better comparisons among different QPI samples. In comparison with control, the gel strength is significantly increased in all QPH gels and a maximum gel strength of ~100 Pa is achieved in the sample subjected to Alcalase hydrolysis for 1 min. Interestingly, prolonged enzymatic hydrolysis (e.g. at 5 min) reduces the gel strength to ~30 Pa. This could be due to a significant reduction of protein molecular weight and generation of small peptides after prolonged enzymatic hydrolysis as revealed by SDS-PAGE (Nieto-Nieto et al., 2014), which agrees with previous studies for gels obtained from quinoa (Galante et al., 2020) and soy protein hydrolysates (Fan et al., 2005). The fact that the trend of $G^*$ (1Hz) and $G'_{20}^{\circ}/G''_{90}^{\circ}$ being the same further confirms that the vital role of noncovalent interaction (e.g. inter-molecular hydrogen bond) in the formation of gels, which agree with the finding obtained from the FTIR analysis.

Findings of the present study agreed well with previous studies, showing that hydrolysis time and DH play critical roles in determination of gel strength (Vogelsang-O’Dwyer et al., 2022). Zhao et al. (2011) studied the effect of Alcalase hydrolysis on gelation properties of peanut protein isolate (PNPI). Various PNPI gels were prepared by heating 10 wt% PNPI solutions at 95 °C for 30 min then cooling to 25 °C. They found that the hydrolysates with DH 2.1% showed a highest gel strength (~374 Pa) while more extensive hydrolysis i.e. with DH 5.4% caused a considerable decrease in gel strength (~3.7 Pa), which is even lower than the control (~5.5 Pa, only heated but without adding Alcalase). The limited enzymatic hydrolysis (DH ~2.1%) greatly improved gel and aggregate formation by unfolding the conformation of PPI and exposure the sulfhydril and disulfide bond buried inside the protein molecules as revealed by intrinsic fluorescence and surface hydrophobicity determinations. However, at higher DH the aggregates might be further hydrolysed resulting in weakening of protein-protein interactions and breakdown of the gel network. A similar behaviour was found in another study working on heat induced gelation of soy protein isolate (SPI) (12 wt%, treated at 95 °C for 10 min) as affected by bromelain hydrolysis (Lopes-da-Silva & Monteiro, 2019). It showed that the SPI subjected to 1 min hydrolysis resulted in a formation of a strongest gel ($G'\sim 1012$ Pa) and the $G'$ progressively decreased to ~194 Pa after 6 min hydrolysis which is also lower than that of control ($G'\sim 627$ Pa).

In terms of large deformation rheology, the $G'$ and $G''$ are nearly independent of applied strain until reaching certain strain amplitude and above which $G'$ and $G''$ considerably decreased with further increase in strain. The $G'$ decreased more rapidly than $G''$, which eventually leading to cross over of $G'$ and $G''$. The stress (flow point) is defined as the stress at this crossover point. Beyond this point, $G''$ dominate $G'$ suggesting the breakdown of gel structures and samples began to flow. The stress at flow points for all the QPI gel samples was plotted in Fig. 5D. It can be observed that they follow a similar trend as the $G^*$ (1Hz) indicating the small and large deformation properties of QPI gels are similar.

4. Conclusions

This study demonstrates that limited Alcalase hydrolysis can

![Fig. 5. The $G'$ (solid symbols) and $G''$ (empty symbols) as a function of frequency (A) and strain amplitude (B) for various quinoa protein isolate (QPI) and quinoa protein hydrolysates (QPH) measured at 20 °C; (C) the complex modulus $G^*$ (1 Hz) and stress at flow points (D) for various QPI and QPH gels. (Alcalase hydrolysis for 0 min (●), 1 min (●), 3 min (●), and 5 min (●)).](image-url)
significantly increase the mechanical strength of thermally induced QPI gels and the gel strength is highly related to the hydrolysis time or DH. The significant increase in the mechanical strength of thermally induced QPI gels via enzymatic hydrolysis for potential industrial production of food products such as sausages, meat substitutes, and tofu-like products. Finally, future work can be conducted to investigate the impact of enzymatic hydrolysis on the cold gelation of QPI via addition of acid/salt or subjected to high hydrostatic pressure processing.

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.

Data availability

Data will be made available on request.

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References

Akbarame, F.U., Aluko, R.E., Adejei, A.A., 2021. Modification of plant proteins for improved functionality: a review. Compr. Rev. Food Sci. Food Saf. 20 (1), 198–224.

Abadzeh-Pasdar, N., Li-Chun, E.C., 2000. Comparison of protein surface hydrophobicity measured at various pH values using three different fluorescent probes. J. Agric. Food Chem. 48 (2), 328–334.

Andlinger, D.J., Bornkessel, A.C., Jung, I., Schroeter, B., Szmimova, I., Kulozik, U., 2021. Microstructures of potato protein hydrogels and aerogels produced by thermal crosslinking and supercritical drying. Food Hydrocolloids 112, 106305.

Avramenko, N.A., Low, N.H., Nickerson, M.T., 2013. The effects of limited enzymatic hydrolysis on the physicochemical and emulsiﬁcation properties of a lentil protein isolate. Food Res. Int. 51 (1), 162–169.

Bahmanyar, F., Hoseini, S.M., Mirmoghtadaie, L., Shojaei-Alibadi, S., 2021. Effects of replacing soy protein and bread crumb with quinoa and buckwheat flour in traditional beef burger formulation. Meat Sci. 172, 108305.

Byler, D.M., Sani, H., 1986. Examination of the secondary structure of proteins by deconvolved FTIR spectra. Biopolymers 25 (3), 469–487.

Chen, D., Campaenalla, G., 2022. Limited enzymatic hydrolysis induced protein gelation at low protein concentration with less heat requirement. Food Hydrocolloids 107547.

Cheng, L., Ye, A., Yang, Z., Gilbert, E.P., Knot, R., de Campo, L., Storer, B., Hemar, Y., Singh, H., 2022. Small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS) study on the structure of sodium caseinate in dispersions and at the oil-water interface: effect of calcium ions. Food Struct. 32, 100276.

Chirinos, R., Pedreschi, R., Velazquez-Sanchez, M., Aguilar-Galvez, A., Campos, D., 2020. Protein antioxidant and protein emulsifying properties of enzymatically hydrolysed quinoa (Chenopodium quinoa) and kiwifruit (Amaranthus caudatus) proteins. Cereal Chem. 97 (5), 949–957.

Clark, A.H., Kavanaugh, G.M., Ross-Murphy, S.B., 2001. Globular protein gelation-theory and practice. Food Hydrocolloids 15 (4), 381–404.

Dakhili, S., Abdelazizadeh, L., Hoseini, S.M., Shojaei-Alibadi, S., Mirmoghtadaie, L., 2019. Quinoa protein: composition, structure and functional properties. Food Chem. 299, 125161.

Doucet, D., Otter, D.E., Gauthier, S.F., Foegeding, E.A., 2003. Enzyme-induced gelation of extensively hydrolyzed whey proteins by Alcalase: peptide identification and determination of enzyme specificity. J. Agric. Food Chem. 51 (21), 6300–6308.

El Hazzam, K., Hafja, J., Sobeh, M., Mhada, M., Taourite, M., El Kacimi, K., Yari, A., 2020. An insight into saponins from Quinoa (Chenopodium quinoa Willd): a review. Molecules 25 (5), 1095.

Fan, J., Saito, M., Yanyan, Z., Szesze, T., Wang, L., Tatsumi, E., Li, L., 2005. Gel-forming ability and radical-scavenging activity of soy protein hydrolysate with transglutaminase. J. Food Sci. 70 (1), 387–392.

Galante, M., De Flavisi, R., Boeris, V., Speleini, D., 2020. Effects of the enzymatic hydrolysis treatment on functional and antioxidant properties of quinoa protein acid-induced gels. LWT (Lebensm.-Wiss. -Technol.) 118, 108845.

Grossmann, L., McClements, D.J., 2021. The science of plant-based foods: approaches to create nutritious and sustainable plant-based cheese analogs. Trends Food Sci. Technol. 118, 207–229.

Guo, H., Hao, Y., Yang, X., Ren, G., Richel, A., 2021. Exploration on bioactive properties of quinoa protein hydrolysates and peptides: a review. Crit. Rev. Food Sci. Nutr. 1–16.

Jiang, S., Zhang, J., Li, S., Zhang, C., 2021. Effect of enzymatic hydrolysis on the formation and structural properties of peanut protein gels. Int. J. Food Eng. 17 (3), 176–182.

Kaschak, E., Oliveira, M.A. S.d., Simas, F.F., Franco, C.R.C., Silveira, J.L.M., Mafra, M.R., Igarashi-Mafra, L., 2017. Determination of heat-set gelation capacity of a quinoa protein isolate (Chenopodium quinoa) by dynamic oscillatory rheological analysis. Food Chem. 232, 263–271.

Kato, A., Nakai, S., 1980. Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. Biochim. Biophys. Acta Protein Struct. 624 (1), 13–20.

Katzav, H., Chirug, L., Okun, Z., Davidovich-Pinhas, M., Shipgelman, A., 2020. Comparison of thermal and high-pressure gelation of potato protein isolates. Foods 9 (8), 1041.

Kumar, D., Chatti, M.K., Singh, R., Mehta, N., Kumar, P., 2016. Enzymatic hydrolysis of camel milk casein and its antioxidant properties. Dairy Sci. Technol. 96 (3), 391–404.

Kumar, M., Tomar, M., Potkule, J., Reetu, Punia, S., Dhakane-Lad, J., Singh, S., Dhumal, S., Chandra Pradhan, P., Bhusan, R., Anirha, T., Alajal, O., Alhariri, A., Amronica, R., Kennedy, J.F., 2022. Functional characterization of plant-based protein to determine its quality for food applications. Food Hydrocolloids 123, 106986.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227 (5259), 680–685.

Lamsal, B.P., Jung, S., Johnson, L.A., 2007. Rheological properties of soy protein hydrolysates obtained from limited enzymatic hydrolysis. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft - Technol.) 40 (7), 1215–1223.

Langton, M., Ehsanzamir, S., Karkehabadi, S., Feng, X., Johannson, M., Johannson, D.P., 2020. Gelation of faba bean proteins-Effect of extraction method, pH and NaCl. Food Hydrocolloids 103, 105622.

Li, J., Yang, Z., Lin, X., Wu, S., Li, G., Li, N., Otter, D., Zuo, F., Hartinger, C., Corke, H., 2021. In-silico SASSI investigation of whey protein isolate hydrolyzed by bromelain. Colloids Surf. A Physicochem. Eng. Asp. 631, 127662.

Li, X., Da, S., Li, C., Xue, F., Zang, T., 2018. Effects of high-intensity ultrasound pretreatment with different levels of power output on the antioxidant properties of alcalase hydrolyzates from Quinoa (Chenopodium quinoa Willd) protein isolate. Cereal Chem. 95 (4), 518–526.

Liu, C., Bhattarai, M., Mikkenos, K.S., Heinonen, M., 2019. Effects of enzymatic hydrolysis of fava bean protein isolate by alcalase on the physical and oxidative stability of oil-in-water emulsions. J. Agric. Food Chem. 67 (23), 6625–6632.

Lopes-da-Silva, J.A., Monteiro, S.R., 2019. Gelling and emulsifying properties of soy protein isolate hydrolysates. Food Hydrocolloids 123, 215–223.

Luo, L., Cheng, L., Zhang, R., Yang, Z., 2022. Impact of high-pressure homogenization on physico-chemical, structural, and rheological properties of quinoa protein isolates. Food Struct. 32, 100265.

Lundberg, B., Palmer, J., Hemar, Y., Yang, Z., 2021. Impact of high hydrostatic pressure on the gelation behavior and microstructure of quinoa protein isolate dispersions. ACS Food Science & Technology 1 (11), 2144–2151.
Ma, Z., Li, L., Wu, C., Huang, Y., Teng, F., Li, Y., 2022. Effects of combined enzymatic and ultrasonic treatments on the structure and gel properties of soybean protein isolate. LWT (Lebensm.-Wiss. & Technol.) 158, 113123.
Mitchell, J., 1980. The rheology of gels. J. Texture Stud. 11 (4), 315–337.
Nicolaï, T., Chassenieux, C., 2019. Heat-induced gelation of plant globulins. Curr. Opin. Food Sci. 27, 18–22.
Nielsen, P.M., Petersen, D., Dambmann, C., 2001. Improved method for determining food protein degree of hydrolysis. J. Food Sci. 66 (5), 642–646.
Nieto-Nieto, T.V., Wang, Y.X., Oztızek, L., Chen, L., 2014. Effects of partial hydrolysis on structure and gel properties of oat globular proteins. Food Res. Int. 55, 418–425.
Otte, J., Jo, Y.Z., Skriver, A., Qvist, K.B., 1996. Effects of limited proteolysis on the microstructure of heat-induced whey protein gels at varying pH. J. Dairy Sci. 79 (5), 782–790.
Otte, J., Lomholt, S., Halkier, T., Qvist, K., 2000. Identification of peptides in aggregates formed during hydrolysis of β-lactoglobulin B with a Gla and Asp specific microbial protease. J. Agric. Food Chem. 48 (6), 2443–2447.
Otto, J., Schumacher, E., Ipsem, R., Ju, Z.Y., Qvist, K.B., 1999. Protease-induced gelation of unheated and heated whey proteins: effects of pH, temperature, and concentrations of protein, enzyme and salts. Int. Dairy J. 9 (11), 801–812.
Patele, S., Cheng, L., Yang, Z., 2022. Impact of incorporations of various polysaccharides on rheological and microstructural characteristics of heat-induced quinoa protein isolate gels. Food Biophys. 17, 314–323.
Rahay, L., Britten, M., 2009. Effect of whey protein enzymatic hydrolysis on the rheological properties of acid-induced gels. Food Hydrocolloids 23 (8), 2302–2308.
Ran, X., Lou, X., Zheng, H., Gu, Q., Yang, H., 2022a. Improving the texture and rheological qualities of a plant-based fishball analogue by using konjac glucomannan to enhance crosslinks with soy protein. Innovat. Food Sci. Emerg. Technol. 75, 102910.
Ran, X., Yang, Z., Chen, Y., Yang, H., 2022b. Konjac glucomannan decreases metabolite release of a plant-based fishball analogue during in vitro digestion by affecting amino acid and carbohydrate metabolic pathways. Food Hydrocolloids 129, 107623.
Shen, Y., Tang, X., Li, Y., 2021. Drying methods affect physicochemical and functional properties of quinoa protein isolate. Food Chem. 339, 127823.
Sow, L.C., Yang, H., 2015. Effects of salt and sugar addition on the physicochemical properties and nanostructure of fish gelatin. Food Hydrocolloids 45, 72–82.
Sponton, O.E., Perez, A.A., Carrara, C., Santiago, L.G., 2014. Effect of limited enzymatic hydrolysis on linoleic acid binding properties of β-lactoglobulin. Food Chem. 146, 577–582.
Spotti, M.J., Tarhan, O., Schaffer, S., Corvalan, C., Campanella, O.H., 2017. Wheat protein gelation induced by enzymatic hydrolysis and heat treatment: comparison of creep and recovery behavior. Food Hydrocolloids 63, 696–704.
Sun, X.D., 2011. Enzymatic hydrolysis of soy proteins and the hydrolysates utilisation. Int. J. Food Sci. Technol. 46 (12), 2447–2459.
X. Wang et al. Current Research in Food Science 5 (2022) 2061–2069
Sun, X.D., Arntfield, S.D., 2012. Molecular forces involved in heat-induced pea protein gelation: effects of various reagents on the rheological properties of salt-extracted pea protein gels. Food Hydrocolloids 28 (2), 925–332.
Tanger, C., Müller, M., Andlinger, D., Kulsoz, U., 2022. Influence of pH and ionic strength on the thermal gelation behaviour of pea protein. Food Hydrocolloids 123, 106903.
Tatham, A., Miflin, B., Shewry, P., 1985. The beta-turn conformation in wheat gluten proteins: relationship to gluten elasticity. Cereal Chem. 62 (5), 405–412.
Tavano, O.L., Amistà, M.D.M., Del Cirillo, G., Rodrigues, M.C.M., Bono Nishioka, A.M., Valadares, L.A., Siqueira, B.M., Gomes, R.A.d.S., Parolini, M.T., Silva Junior, S.L.d., 2022. Isolation and evaluation of quinoa (Chenopodium quinoa Willd.) protein fractions: A nutritional and bio-functional approach to the globulin fraction. Curr. Res. Food Sci. 5, 1028–1037.
Thamnarathip, P., Jiangchud, K., Jiangchud, A., Vardhanabhuti, B., 2016. Functional properties of protein hydrolysates from Riceberry rice bran. Int. J. Food Sci. Technol. 51 (5), 1110–1119.
Vega-Gálvez, A., San Martin, R., Sanders, M., Miranda, M., Lara, E., 2010. Characteristics and mathematical modeling of convective drying of quinoas (Chenopodium quinoa Willd.): influence of temperature on the kinetic parameters. J. Food Process. Prev. 34 (6), 945–963.
Vilcacundo, R., Martínez-Villaluenga, C., Hernández-Ledesma, B., 2017. Release of dipeptidyl peptidase IV, α-amylase and α-glucosidase inhibitory peptides from quinoa (Chenopodium quinoa Willd.) during in vitro simulated gastrointestinal digestion. J. Funct. Foods 35, 531–539.
Vogelsang-O'Dwyer, M., Sahin, A.W., Arendt, E.K., Zannini, E., 2022. Enzymatic hydrolysis of pulse proteins as a tool to improve techno-functional properties. Foods 11 (9), 1307.
Wang, Y., Eastwood, B., Yang, Z., de Campo, L., Knott, R., Prosser, C., Carpenter, E., Hemar, Y., 2019. Rheological and structural characterization of acidified skimmed milk and infant formulae made from cow and goat milk. Food Hydrocolloids 96, 161–170.
Xing, G., Giussafoto, C.V.L., Carpenterieri, A., Pasquino, R., Dong, M., Marinelli, L., 2020. Gelling behaviour of bio-tofu coagulated by microbial transglutaminase combined with lactic acid bacteria. Food Res. Int. 134, 109200.
Xu, X., Liu, W., Liu, C., Luo, L., Chen, L., Luo, S., McClements, D.J., Wu, L., 2016. Effect of limited enzymatic hydrolysis on structure and emulifying properties of rice gluten. Food Hydrocolloids 61, 251–260.
Yang, Z., de Campo, L., Gilbert, E.P., Knott, R., Cheng, L., Steer, B., Lin, X., Luo, L., Patole, S., Hemar, Y., 2022. Effect of NaCl and CaCl2 concentration on the rheological and structural characteristics of thermally-induced quinoa protein gels. Food Hydrocolloids 124, 107350.
Yü, M., Lin, S., Ge, R., Xiong, C., Xu, L., Zhao, M., Fan, J., 2022. Buckwheat self-assemblying peptide-based hydrogel: preparation, characteristics and forming mechanism. Food Hydrocolloids 125, 107378.
Zhang, R., Cheng, L., Luo, L., Hemar, Y., Yang, Z., 2021. Formation and characterisation of high internal phase emulsions stabilised by high-pressure homogenised quinoa protein isolate. Colloids Surf. A Physicochem. Eng. Asp. 631, 127688.
Zhao, G., Liu, Y., Zhao, M., Ren, J., Yang, B., 2011. Enzymatic hydrolysis and their effects on conformational and functional properties of peanut protein isolate. Food Chem. 127 (4), 1438–1443.
Zhou, Y., Yang, H., 2020. Enhancing tilapia fish myosin solubility using proline in low ionic strength solution. Food Chem. 320, 126665.
Zhu, Z., Qiu, N., Yi, J., 2018. The beta-turn conformation in wheat gluten proteins: relationship to gluten elasticity. Cereal Chem. 62 (5), 405–412.