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Comparison of quinoa proteins modified by thermal acid hydrolysis, enzymatic hydrolysis, and ultrasonication as Pickering emulsifiers☆

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ABSTRACT

The poor functionality of seed storage proteins is the major factor limiting their practical utilization. Although thermal acid hydrolysis, enzymatic treatment, and ultrasonication enhance quinoa protein functionality, their effects are rarely compared under identical conditions. Herein, quinoa protein isolate (QPI) was extracted from air-classified quinoa flours without the defatting step, as this step greatly reduces extraction efficiency, and subsequently modified via thermal acid hydrolysis, moderate tryptic hydrolysis, and ultrasonication. The solubility, morphology, surface and emulsifying properties of QPI, acid-hydrolyzed QPI (AQPI), enzyme-hydrolyzed QPI (EQPI) and ultrasonication-modified QPI (UQPI) were evaluated to elucidate how these modifications influenced QPI performance as a Pickering emulsifier under identical conditions. The results showed that the solubility and wettability of all modified QPIs were improved. Both QPI and modified QPIs were able to form oil-in-water emulsions with an average droplet size of ~13 μm. The interfacial protein concentration increased linearly with emulsifier concentration from 0.5 to 2 % *w/w*, with AQPI and EQPI exhibiting higher adsorption capacity than QPI and UQPI, which was ascribed to a contact angle closer to 90° (i.e., a balanced hydrophilic-hydrophobic interface). Over 14 days of storage, all emulsions stabilized by QPI and modified QPIs remained resistant to flocculation and coalescence due to high protein adsorption, with EQPI providing the smallest droplet size increase (12.5 to 15.3 μm). Increasing the concentration of QPI and modified QPIs improved the creaming stability of all emulsions. This study demonstrates that these modification methods, particularly moderate tryptic hydrolysis, effectively tailored quinoa proteins for Pickering emulsions.

1. Introduction

Currently, animal-based proteins account for up to 70 % of human dietary protein intake [1]. However, the limiting factors such as resource constraints, environmental issues, health concerns, and animal welfare have become significant challenges facing animal-based proteins [2]. In recent years, plant-based proteins have been recognized as ideal alternative protein sources due to their sustainability, cost-effectiveness and easy access [3–5]. Edible quinoa seeds are a nutritionally complete food that can meet basic human dietary needs and are often referred to as a 'super grain' [6]. Particularly, they are rich in gluten-free storage proteins that provide all essential amino acids and exhibit high digestibility, holding significant potential as an alternative protein source [7–9].

Emulsions are two-phase systems generated by dispersing one liquid as fine droplets into another immiscible liquid. Pickering emulsions, in contrast to surfactant or emulsifier-stabilized systems, rely on colloidal particles that adsorb at the interface to form physical barriers against destabilization. Various types of colloidal particles, such as nanoparticles, nanofibrils and nanocages, have been used as effective Pickering stabilizers [10–15]. Protein-based particles are considered ideal candidates for stabilizing Pickering emulsions because of their

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amphiphilicity, abundant availability, and full edibility [16]. The Pickering emulsions can promote the oral delivery of oil-soluble nutrients/bioactives and facilitate the development of novel food structures [17,18]. In this context, identifying food-grade protein particles that can effectively form and stabilize emulsions warrants further study [19–21].

Quinoa proteins have relatively low aqueous solubility, a property common to many seed storage proteins [22,23]. They mainly exist as aggregates of different sizes in aqueous solutions. The protein-oil-water three-phase contact angle of quinoa protein isolate (QPI) is ~130.5°, demonstrating that quinoa proteins display significant hydrophobicity on their surfaces [24]. During storage, smaller aggregates (100–200 nm) progressively coagulate into larger ones, resulting in kinetic instability [25]. With further storage, quinoa protein aggregates undergo both dissociation and precipitation, accompanied by a reduction of surface hydrophobicity [25]. This suggests that: (1) individual protein molecules exhibit lower surface hydrophobicity than their aggregated forms; (2) the surface hydrophobicity of aggregates varies significantly, leading to co-precipitation among aggregates with higher surface hydrophobicity. Thus, overcoming the low aqueous solubility of seed storage proteins, including quinoa proteins, to enable their effective use has posed ongoing challenges for the food industry. Modification of quinoa proteins into effective Pickering emulsifier provides a strategy to address this challenge.

Plant-based proteins are often modified using techniques such as ultrasonication, enzymatic hydrolysis and thermal acid hydrolysis. High-intensity sonication generates high shear forces and macro-turbulence through creating bubbles and their violent collapse in liquid medium [26]. This acoustic cavitation induced by sonication process can alter physicochemical and functional properties of proteins by reducing the size of protein aggregates and breaking the structure of proteins [27–29]. A growing consensus on how ultrasonic treatment influences the properties of quinoa proteins has been reached, i.e., high-intensity ultrasonication enhances their solubility [30–32]. However, discrepancies in physicochemical properties, including particle size, zeta potential, and surface hydrophobicity, are observed among studies, largely due to variations in quinoa protein extraction and characterization methods [30–33]. Proteolytic enzymes hydrolyze proteins by targeting specific cleavage sites between amino acid residues, breaking peptide bonds and fragmenting protein molecules into smaller peptides [34]. Enzymatic hydrolysis modifies the structural properties of proteins by reducing their average molecular weight, exposing embedded hydrophobic regions, and releasing more ionizable groups [35,36]. However, excessive hydrolysis can result in the loss of protein functionality due to the total breakdown of protein structures [37,38]. For example, limited Alcalase hydrolysis (enzyme-to-protein ratio of 1:50, 1 min, pH 8, 50 °C) significantly improves the gelling capacity of quinoa proteins [39]. By contrast, excessive Alcalase hydrolysis (4 % *w/w* Alcalase, 4 h, pH 9, 50 °C) greatly reduces their emulsifying capacity [40]. Thermal acid hydrolysis disrupts protein molecules into peptides and amino acids. With precise manipulation, it can induce protein reassociation, forming aggregates of varying morphologies and sizes and modifying their functional properties [24,41,42]. For example, thermal treatment at 90 °C and pH 1.3 for 8 h can induce fibrillization of quinoa proteins, which significantly enhances their solubility and strengthens quinoa protein emulsion gels via acting as active fillers [24,41]. However, in these studies, quinoa proteins were extracted using different procedures and their physicochemical and functional properties were investigated under varying conditions, which does not allow for direct comparisons of the modification effects.

Recently, green and sustainable food production has gained increasing global attention. In typical seed storage protein extraction via the alkaline solubilization–acid precipitation method, defatting is a crucial step to disrupt the seed matrix, enhance the accessibility of protein to extraction solvents, and improve protein quality [43,44]. On the other hand, this step increases extraction costs, causes environmental and safety concerns, decreases extraction efficiency, and limits large-scale production for the seeds with low oil content [45]. Therefore, developing green protein extraction method from seeds without defatting (e.g., milling and subsequent air-classification, and enzyme-assisted extraction) has also emerged as a key challenge in the food industry [46,47].

In this study, QPI was extracted from crude quinoa protein powder obtained by milling and air classification using the alkaline solubilization–acid precipitation method, omitting the conventional defatting step. QPI was further modified by thermal acid hydrolysis, enzymatic hydrolysis, and ultrasonication. Native and modified QPIs were examined under identical conditions to elucidate how different modification methods influenced solubility, particle size, zeta potential, oil-protein-water three-phase contact angle, and protein morphology. Oil-in-water Pickering emulsions were subsequently prepared using QPI and its modified forms. Their emulsifying properties were characterized by quantifying interfacial protein adsorption and monitoring changes in droplet size, microstructure, and creaming index over a 14-day storage period. The aim of this study was to pave the way for precisely modifying the physicochemical and functional properties of quinoa proteins.

2. Materials and methods

2.1. Materials

Quinoa seeds (Geli No. 1) were purchased from a local company in Qinghai Province, China. Trypsin (T819002) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Soybean oil was purchased from a local grocery. All other reagents were purchased from Shanghai Macklin Biochemical Technology Co., Ltd., and were of analytical grade.

2.2. Quinoa protein extraction

QPI was extracted using the method of alkaline solubilization and acid precipitation without defatting [23]. Briefly, quinoa seeds were first ultrafine-milled and then subjected to air classification to obtain crude quinoa protein powder. The protein content of the crude powder was 25.5 % (Table S1). Next, the crude powder was dispersed in deionized water at a ratio of 1:10 (*w/w*). The pH of the mixture was adjusted to 9.0, with stirring at 300 rpm for 90 min using a magnetic stirrer. Following this, the mixture was centrifuged at 8000 rpm and 20 °C for 15 min using a high-speed centrifuge (Hitachi CR 21 GIII, Hitachi, Japan). The pH of the supernatants was then adjusted to 4.5, resulting in protein precipitation. The precipitates were collected by centrifuging at 8000 rpm and 20 °C for 15 min, and washed using deionized water 2–3 times. After washing, the precipitates were redispersed in deionized water, neutralized to pH 7.0, and freeze-dried for 48 h to obtain QPI. QPI was stored in a desiccator at room temperature for further use.

2.3. Preparation of modified QPI

QPI was dispersed in deionized water at a 1:20 (*w/w*) ratio and stirred at 300 rpm for 2 h using a magnetic stirrer. The pH of the dispersions was adjusted to 1.3 prior to heating. The dispersions were then heated in a water bath at 90 °C with shaking at 100 rpm for 12 h [41]. After that, the dispersions were immediately cooled to room temperature using running water, and neutralized to pH 7.0. The dispersions were dialyzed in deionized water for 8 h. Finally, the dialysates were freeze-dried to obtain acid-hydrolyzed QPI (AQPI).

QPI was mixed with trypsin solution (4 mg/mL) to achieve an enzyme-to-protein ratio of 1:50 (*w/w*). The mixture was stirred thoroughly, and the pH was adjusted to 8.0. Then, the mixture was heated at 50 °C for 30 min in a water bath. Based on the preliminary experiments, a moderate enzymatic hydrolysis was achieved after 30 min of treatment. Thereafter, enzyme reactions were terminated immediately by heating at 90 °C for 15 min. Finally, the dispersions were neutralized to

pH 7.0, and freeze-dried to obtain enzyme-hydrolyzed QPI (EQPI).

QPI dispersions (1:20 *w/w*, QPI:water) were subjected to ultrasonication using an ultrasonic cell disruptor (VC 800, SONICS, USA) at 300 W for 20 min. After sonication, the dispersions were freeze-dried to obtain ultrasonication-modified QPI (UQPI). All modified QPIs were stored in a desiccator at room temperature for further use.

2.4. Determination of protein purity

QPI or modified QPIs were added to deionized water containing 1 % *w/w* sodium dodecyl sulfate (SDS) and 4 mM dithiothreitol (DTT) at a ratio of 1:100 (*w/w*). The dispersions were adjusted to pH 10.0 and stirred for 2 h to ensure complete dissolution of protein aggregates. The Lowry method was employed to determine the protein concentration of the test solutions [48]. The protein purity was calculated according to the following equation:

$$\varphi = \frac{C \times V}{W_0} \times 100\%$$

In this equation, C is the protein concentration of the protein solutions, V is the volume of the protein solutions, and W_0 is the weight of QPI or modified QPIs added in the protein solutions.

2.5. Determination of protein solubility

At first, 0.2 g of QPI or modified QPIs was added into 10 mL of deionized water to achieve a protein concentration of 20 mg/mL. Then, the mixture was stirred at 200 rpm for 30 min using a magnetic stirrer and neutralized to pH 7.0. The resulting dispersions were centrifuged at 8000 rpm and 20 °C for 15 min. Finally, the protein concentration of the supernatants was determined using the Lowry method and the solubility was calculated as the following equation:

$$S = \frac{C \times V}{W_0 \times \varphi}$$

In this equation, C is the protein concentration of the supernatants, W_0 is the weight of QPI or modified QPI added in the protein dispersions, and φ represents protein purity.

2.6. Measurement of three-phase contact angle

The contact angle was measured using a contact angle measuring device (OCA50, DataPhysics, Germany). QPI or modified QPIs were pressed to form thin tablets using a powder tablet press machine. The tablets were placed at the bottom of a glass container filling with soybean oil. A single drop of water was deposited onto the surface of each tablet. The interactions between the water droplets and the tablets were recorded using a high-speed camera. The contact angle was determined by analyzing the tangent angle at the point where the droplet contacted the tablet surface, based on the droplet contour captured by the camera.

2.7. Determination of particle size and zeta-potential

The particle size and zeta-potential of QPI and modified QPIs were measured using a Malvern Zetasizer Nano (ZEN3700, Malvern Panalytical, Malvern, UK). The refractive index of protein and water was set as 1.45 and 1.33, respectively [49,50]. The dispersions containing 1 % *w/w* QPI or modified QPIs were centrifuged at 8000 rpm and 20 °C for 15 min. The supernatants were diluted to a protein concentration of 1 mg/mL. The diluted supernatants were used for the measurement.

2.8. Transmission electron microscopy (TEM)

The samples prepared for the particle size measurement were used for TEM imaging. The samples were dropped onto a 200-mesh copper grid coated with a 5 nm carbon support film, and subsequently stained with a 1 % uranyl acetate solution. The images were captured using a TEM (JEM-F200, JEOL Ltd., Japan) operated at an acceleration voltage of 200 kV.

2.9. Preparation of Pickering emulsions

QPI and modified QPI dispersions were prepared by dispersing QPI or its modified forms into deionized water to achieve concentrations of 0.5, 1 and 2 % *w/w*. The Pickering emulsions were prepared by homogenizing the mixture of 30 g of soybean oil and 70 g of dispersions using an Ultra Turrax (S18N-19G, IKA, KG, Staufen, Germany) at 16,000 rev/min for 5 min. To prevent bacterial growth, 0.02 % (w:v) of sodium azide was added to fresh emulsions.

2.10. Emulsion characterization

2.10.1. Determination of the creaming index

Fresh Pickering emulsions were stored in glass tubes at room temperature. The creaming of oil droplets was monitored by a digital camera over 14 days. The creaming index (CI) was expressed as the following equation:

$$CI = \frac{H_s}{H_t} \times 100\%$$

In this equation, H_s is the height of serum layer and H_t is the total emulsion height.

2.10.2. Quantification of interfacial protein adsorption

Fresh Pickering emulsions were mixed with 2 % *w/w* SDS solution at a ratio of 1:9 (*w/w*). The particle size was determined by a Mastersizer 3000 (Malvern Instruments, Malvern, UK), which was reported as the Sauter mean diameter ($D_{3,2}$). The mixture was centrifuged at 20000 rpm for 30 min. The protein concentration of the serum phase was determined by the Lowry method, which represented the protein concentration of initial emulsions (C_0). Fresh Pickering emulsions were centrifuged at 20000 rpm for 30 min at 20 °C. The serum phase was removed carefully using a syringe, and filtered through a 0.45 μm cellulose acetate membrane. The protein concentration of the filtrates was determined by the Lowry method, which represented unadsorbed protein concentration (C_u). The interfacial protein concentration (Γ) was calculated using the equation below:

$$\Gamma = \frac{(C_0 - C_u)(1 - \phi)}{6\phi} * D_{3,2}$$

In this equation, ϕ is the oil volume fraction of emulsions.

2.10.3. Measurement of oil droplet size

The particle size of the emulsions was measured after 0 and 14 days of storage using a Mastersizer 3000. The refractive indices of the aqueous and oil phases were set as 1.33 and 1.46, respectively [49,50]. The particle size was expressed as the volume-weighted average diameter ($D_{4,3}$).

2.10.4. Confocal laser scanning microscopy (CLSM)

The microstructure of the Pickering emulsions after 0 or 14 days of storage was observed using a CLSM (AXR NSPARC, Nikon, Japan) with a 40× objective lens. A staining dye was prepared by mixing a 0.4 % (*w/v*) Fast Green solution and a 0.1 % (*w/v*) Nile red acetone solution at a 1:1 (*v/v*) ratio. The dye was mixed with the Pickering emulsions at a 1:9 (*v/v*) ratio. The oil phase was stained by Nile red and excited by a 488 nm argon laser; the protein phase was stained by Fast Green, and excited by a 633 nm He—Ne laser. The stained emulsions were transferred to a concave slide, covered with a coverslip, and sealed with a nail polish. The emission spectra above 650 nm and above 505 nm were collected for the protein and oil phases, respectively.

2.11. Statistical analysis

Data were presented as mean ± standard deviation (SD) and analyzed using SPSS Statistics 25 software. Analysis of variance (ANOVA) followed by the least significant difference (LSD) post-hoc tests was used to determine if there were any significant differences between groups at $p < 0.05$.

3. Results and discussion

3.1. The solubility of QPI and modified QPIs

Protein solubility reflects the thermodynamic equilibrium between proteins and water, and it plays a crucial role in determining the emulsifying properties of proteins [51]. In this study, QPI and modified QPIs were freeze-dried, and their relative solubility at neutral pH was evaluated to allow direct comparison under identical conditions. As shown in Fig. 1, the solubility of QPI was 53.3 %, which was in agreement with the values reported in previous studies [22,23]. The solubility after thermal acid hydrolysis (pH 1.3, 90 °C, 12 h), enzymatic hydrolysis (trypsin, 50 °C, 30 min) and ultrasonication (300 W, 20 min) was 57.8 %, 65.3 % and 59.4 %, respectively. The results demonstrated that these modification methods significantly improved the solubility of quinoa proteins. Particularly, moderate enzymatic hydrolysis had the greatest effect on enhancing the aqueous solubility of quinoa proteins.

3.2. Morphology of QPI and modified QPIs

TEM was used to assess how thermal acid hydrolysis, enzymatic hydrolysis, and ultrasonication affected the morphology of quinoa proteins in aqueous solutions (Fig. 2). TEM images revealed that the soluble QPI retained in the supernatants of the centrifuged dispersions was mainly composed of irregular aggregates or particles smaller than 200 nm. After 12 h of incubation at 90 °C and pH 1.3, worm-like fibrous structures were observed, while the presence of irregular aggregates was reduced. This was consistent with our previous finding that fibril-like structures were formed by incubating quinoa protein dispersions at pH 1.3 and 90 °C for 8 h. The formation of fibrils under thermal acid hydrolysis has also been observed in other seed storage proteins, such as pea, soybean, lentil, and mung bean proteins [42,52–54]. However, the diameter and contour length of acidic heating-induced fibrils are determined by the intrinsic structure of seed storage proteins [42,55]. In the image of EQPI, individual small particles were rarely observed, whereas large particles (> 200 nm) were clearly present, suggesting both protein hydrolysis and protein aggregation occurred. In the UQPI image, spherical small particles with relatively uniform size were observed, which was ascribed to the turbulence and shear forces generated during ultrasonication, resulting in the physical disruption of larger protein aggregates [56].

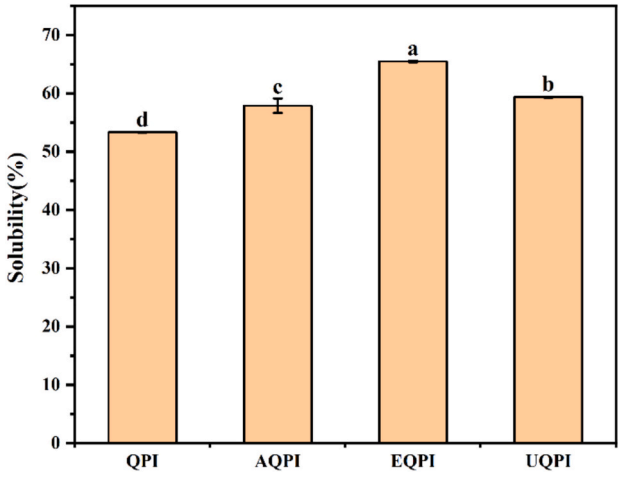

Fig. 1. The solubility of quinoa protein isolate (QPI) and modified QPIs. AQPI, EQPI and UQPI represent acid-hydrolyzed, enzyme-hydrolyzed, and ultrasonication-modified QPIs, respectively. Different lowercase letters indicate significant differences among samples at $p < 0.05$.

3.3. Particle size and zeta-potential of QPI and modified QPIs

The average particle size and zeta potential of QPI and modified QPIs, measured after centrifugation, are presented in Fig. 3. The average particle size of QPI was 272.6 nm (Fig. 3A), confirming that QPI mainly existed as aggregates in aqueous solutions. Although the solubility of AQPI increased significantly compared with that of QPI, the average particle size of AQPI showed only a slight decrease, possibly because heat-induced acid hydrolysis transformed quinoa protein irregular aggregates into soluble fibrils [55,57]. Ultrasonication led to the largest decrease in the average particle size of QPI, which was consistent with the observation in TEM images. Interestingly, the average particle size of EQPI supernatants was 497.5 nm, suggesting that moderate enzymatic hydrolysis resulted in the formation of larger aggregates, which was also confirmed by the TEM image. In this context, limited enzymatic hydrolysis could expose embedded hydrophobic groups and thiol groups, leading to protein aggregation, even though some segments had been removed from protein molecules. This was strongly supported by a previous study, which highlighted the key finding that limited Alcalase hydrolysis enhanced surface hydrophobicity, forming stronger gel network [39].

Electrostatic repulsion is a key factor that governs protein-protein interactions. The zeta potential of QPI was about −36 mV (Fig. 3B), which was similar to that of whey proteins [58]. However, whey proteins have much higher aqueous solubility, suggesting that protein aggregation induced by hydrophobic interactions could be the dominating factor leading to the poor solubility of quinoa proteins [25,59,60]. Both heat-induced acid hydrolysis and enzymatic hydrolysis significantly increased the absolute magnitude of the zeta potential. By contrast, ultrasonic treatment did not significantly change the magnitude of the surface charge of QPI. This demonstrated that the former two modification methods exposed more embedded charged groups compared to physical modification induced by ultrasonication, leading to enhanced aqueous solubility.

3.4. Surface wettability

The protein-oil-water three-phase contact angle reflects the surface wettability of proteins. The particles with balanced wettability for both oil and water phases, i.e., those with contact angles closer to 90°, are more likely to serve as effective stabilizers for Pickering emulsions [61]. The contact angle images of a water droplet on protein films in oil are shown in Fig. 4. The contact angle of QPI was 128.3°, indicating that QPI had a highly hydrophobic surface; this explained why QPI had low aqueous solubility. After thermal acid hydrolysis, enzymatic hydrolysis and ultrasonication, the contact angle of QPI decreased to 105.7°, 101.0°, and 119.4°, respectively, indicating that all modification methods improved the surface wettability of QPI at the oil-water interface. This improvement was more pronounced for AQPI and EQPI compared to UQPI, suggesting that chemical and biological modifications could more significantly alter the surface group distribution of QPI and subsequently improve its surface properties compared to physical modification. Additionally, the changes in contact angle of the modified QPIs aligned with their zeta potential trend.

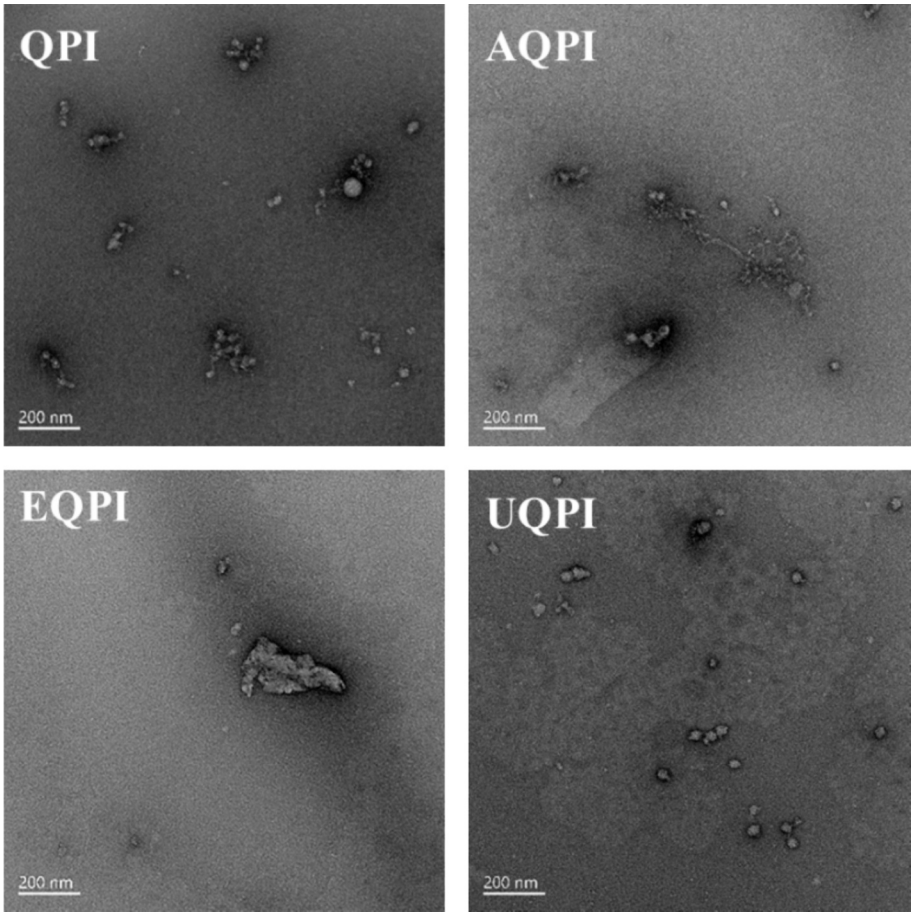

Fig. 2. The transmission electron microscopy (TEM) images of quinoa protein isolate (QPI) and modified QPIs. AQPI, EQPI and UQPI represent acid-hydrolyzed, enzyme-hydrolyzed, and ultrasonication-modified QPIs, respectively.

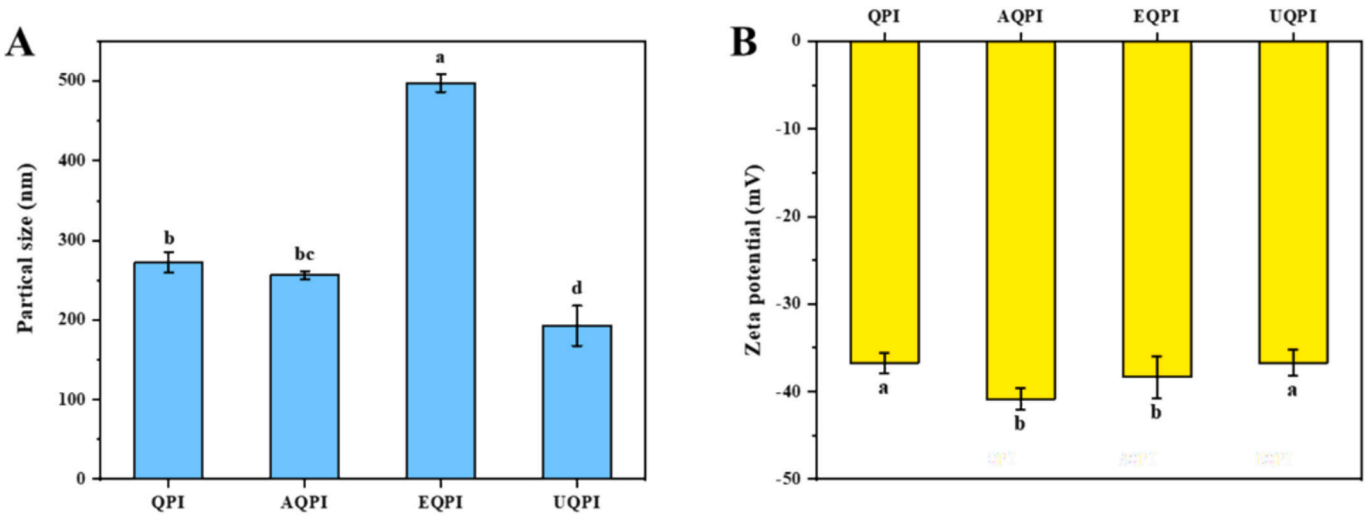

Fig. 3. The average particle size (A) and zeta potential (B) of quinoa protein isolate (QPI) and modified QPIs. AQPI, EQPI and UQPI represent acid-hydrolyzed, enzyme-hydrolyzed, and ultrasonication-modified QPIs, respectively. Different lowercase letters indicate significant differences among samples at $p < 0.05$.

3.5. Emulsifying properties of QPI and modified QPIs

3.5.1. Interfacial adsorption of QPI and modified QPIs at oil droplet surfaces

The interfacial protein concentrations of the Pickering emulsions stabilized by QPI and modified QPIs are illustrated in Fig. 5. As shown in Fig. 5, the interfacial protein concentration linearly increased with emulsifier concentration from 0.5 to 2 % *w/w* regardless of emulsifier type (i.e., QPI, AQPI, EQPI and UQPI). In this context, the emulsions stabilized by higher emulsifier concentrations would form a denser and thicker interfacial protein layer [62]. At all emulsifier concentrations, the emulsions stabilized by AQPI and EQPI had a significantly higher

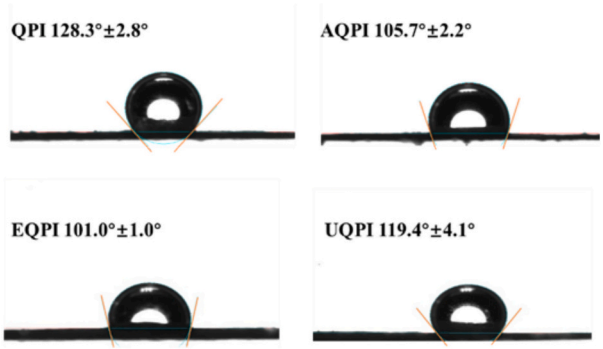

Fig. 4. The contact angle images of a water droplet on protein films in oil. QPI represents quinoa protein isolate; AQPI, EQPI and UQPI represent acid-hydrolyzed, enzyme-hydrolyzed, and ultrasonication-modified QPIs, respectively.

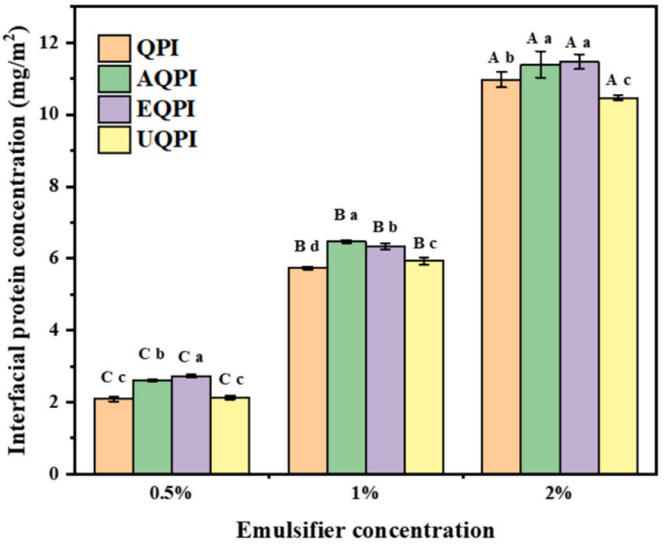

Fig. 5. The interfacial protein concentration of the emulsions stabilized by QPI, AQPI, EQPI and UQPI. QPI represents quinoa protein isolate; AQPI, EQPI and UQPI represent acid-hydrolyzed, enzyme-hydrolyzed, and ultrasonication-modified QPIs, respectively. Different capital letters indicate significant differences among QPI, AQPI, EQPI or UQPI samples at different concentrations, while different lowercase letters indicate significant differences among QPI, AQPI, EQPI and UQPI samples at the same concentration ($p < 0.05$).

interfacial protein concentration compared to those stabilized by QPI and UQPI. This could be explained by the three-phase contact angle of AQPI and EQPI being closer to 90° than QPI and UQPI. Previous research has shown a strong correlation between the interfacial protein adsorption at the oil-water interface and protein wettability [63]. The interfacial protein concentration of the emulsions stabilized by AQPI was lower than that reported in the previous study, which was ascribed to a higher oil volume fraction (30 % vs. 10 % *w/w*) [24]. Additionally, the balanced wettability for both oil and water phases of QPI and its modified forms resulted in a much higher interfacial protein concentration compared with milk proteins at the similar protein concentrations [64,65].

3.5.2. Evolution of the droplet size of the emulsions during storage

The droplet size of the emulsions represents the emulsifying capacity of emulsifiers. The volume-mean diameter ($D_{4,3}$) of the Pickering emulsions stabilized by QPI, AQPI, EQPI and UQPI is shown in Fig. 6. The $D_{4,3}$ of all fresh emulsions containing 30 % *w/w* oil was about ~13 μm, which was much smaller than that of the emulsions stabilized by

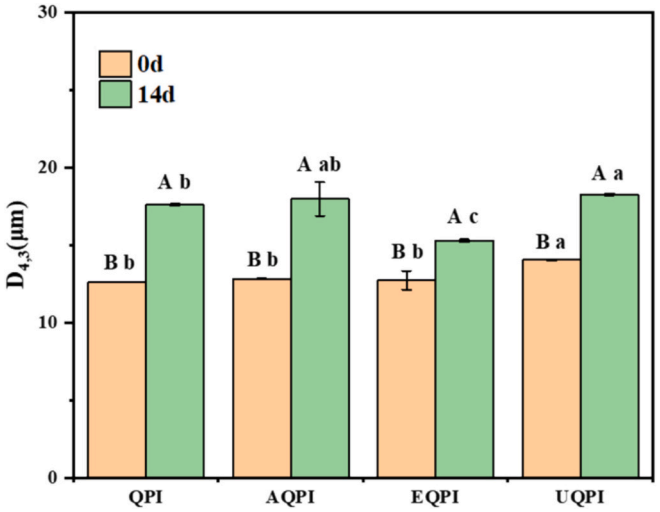

Fig. 6. The volume mean diameter ($D_{4,3}$) of the Pickering emulsions stabilized by 2 % *w/w* QPI and modified QPIs at 0 or after 14 days of storage. Different capital letters indicate significant differences between 0 and 14 days samples within the same group (QPI, AQPI, EQPI or UQPI), while different lowercase letters indicate significant differences between QPI, AQPI, EQPI and UQPI samples after 0 or 14 days of storage ($p < 0.05$). QPI represents quinoa protein isolate; AQPI, EQPI and UQPI represent acid-hydrolyzed, enzyme-hydrolyzed, and ultrasonication-modified QPIs, respectively.

whey protein aggregates of 235 nm (i.e., ~80 μm) under the similar preparation conditions [66]. In theory, quinoa protein aggregates have a relatively high desorption energy from the oil-water interface according to the following Eq. [67]:

$$\Delta G = -\pi R^2 \gamma((1 - |\cos\theta|))^2$$

For example, desorption energy of quinoa aggregates from the oil-water interface (200 nm in diameter, $\gamma = 10$ μN/m, $\theta = 100° - 130°$) is −0.4 to -2.14×10^{-15} J ($>>$ kT). Whey proteins and their aggregates formed at neutral pH exhibit a lower contact angle (~26.2°) [65], resulting in reduced desorption energy and consequently poorer emulsifying capacity compared to quinoa protein aggregates. After 14 days of storage, the droplet size of all emulsions had a small increase, indicating that QPI and modified QPIs could effectively protect oil droplets against coalescence and flocculation. Even though the moderate enzymatic hydrolysis caused protein aggregation, the increase in the droplet size of the emulsion stabilized by EQPI after storage was the smallest, which was ascribed to the higher desorption energy at the oil-water interface. The $D_{4,3}$ of fresh UQPI-stabilized emulsions was significantly larger than that of other emulsions. The smaller particle size of UQPI compared to QPI, AQPI and EQPI, along with higher contact angle of UQPI than AQPI and EQPI, could reduce its ability to stabilize oil droplets ranging from 10 to 20 μm [68].

3.5.3. Microstructural changes of the emulsions during storage

CLSM images of the emulsions after 0 or 14 days of storage are shown in Fig. 7. The emulsions stabilized by QPI and modified QPIs consisted of uniformly distributed oil droplets ranging from 10 to 20 μm, which was consist with the results of the droplet size. No significant flocculation or coalescence of oil droplets was observed in all emulsions after 14 days of storage. This confirmed that the interfacial layers formed by QPI and modified QPIs could effectively protect oil droplets against destabilization through the steric and electrostatic repulsion.

3.5.4. Creaming stability of the emulsions

The creaming index (CI) is an important indicator to evaluate the stability of emulsions against phase separation. The visual appearance of the emulsions stabilized by 0.5–2 % *w/w* QPI and modified QPIs after 1,

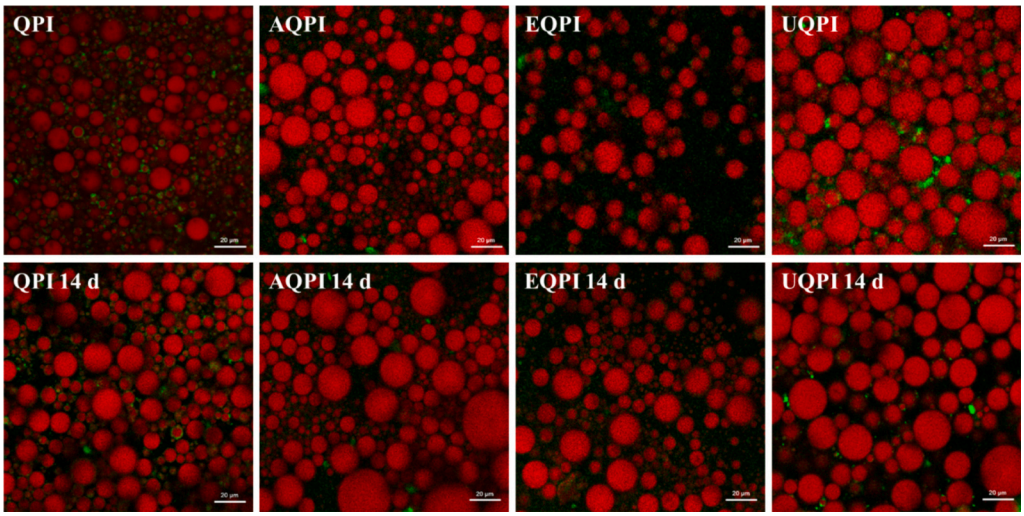

Fig. 7. The confocal laser scanning microscopy (CLSM) images of the Pickering emulsions stabilized by 2 % *w/w* quinoa protein isolate (QPI) and modified QPIs after 0 or 14 days of storage. The oil volume fraction of the emulsions is 30 % *w/w*. Red and green colors represent oil and water phases, respectively. AQPI, EQPI and UQPI represent acid-hydrolyzed, enzyme-hydrolyzed, and ultrasonication-modified QPIs, respectively. The scale bar is 20 μm.

7 and 14 days of storage and the CI values are shown in Fig. 8 and Table 1, respectively. After 1 day of storage, all emulsions stabilized by different concentrations of QPI and modified QPIs experienced severe creaming. At all protein concentrations, the CI value of all emulsions showed a significant increase after 7 days of storage. However, the CI value did not change significantly with further storage, suggesting that the 3-dimensional droplet network could be formed in the cream phase to inhibit the motion of oil droplets as indicated in CLSM images (Fig. 7). Although there were some differences in the CI values among the emulsions stabilized by QPI and modified QPIs at the same protein concentration, the overall differences were not significant. Even modifying quinoa proteins into fibrous structures did not significantly improve the creaming stability of the emulsions, which could be attributed to the short contour length of seed storage protein fibrils formed under heat-induced acid hydrolysis [18,41,42]. By comparing the CI values of the emulsions stabilized by QPI *vs.* modified QPIs, it was evident that, the emulsion stability was improved significantly as the protein concentration increased from 0.5 % to 2 % *w/w*. This was likely because a more saturated interfacial layer promoted stronger particle-particle interactions and excess protein particles in the continuous phase of the emulsions facilitated the formation of a three-dimensional network that restricted the movement of oil droplets [69]. Zhang et al. [70] also observed the similar phenomenon in their study on the Pickering emulsions stabilized by wheat gliadin, where the increase of protein concentration effectively reduced the creaming index.

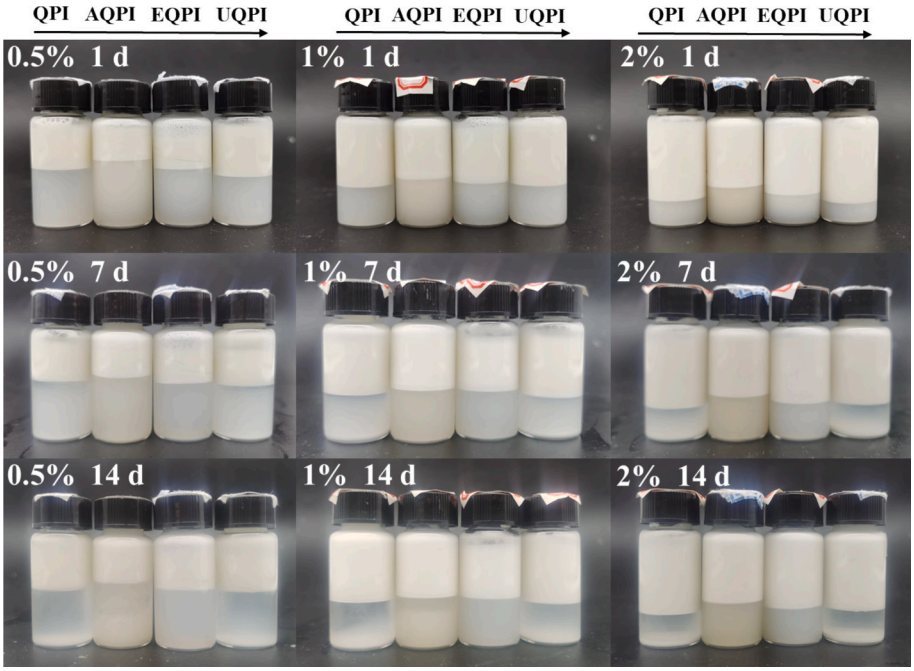

Fig. 8. The visual appearance of the Pickering emulsions stabilized by 0.5–2 % *w/w* quinoa protein isolate (QPI) and modified QPIs prepared with different concentrations (0.5 %, 1 %, 2 %) after 1, 7 and 14 days of storage. The upper layer represents the creamy phase, while the lower layer represents the separated serum phase. AQPI, EQPI and UQPI represent acid-hydrolyzed, enzyme-hydrolyzed, and ultrasonication-modified QPIs, respectively.

Table 1
The creaming index of the Pickering emulsions stabilized by 0.5–2 % *w/w* QPI and modified QPIs after 1, 7 and 14 days of storage.

		1 d	7 d	14 d
QPI	**0.5 %**	$0.55^{A\,b}$	$0.57^{A\,a}$	$0.57^{A\,a}$
	1 %	$0.36^{B\,b}$	$0.42^{B\,a}$	$0.42^{B\,a}$
	2 %	$0.21^{C\,b}$	$0.27^{C\,a}$	$0.28^{C\,a}$
AQPI	**0.5 %**	$0.57^{A\,b}$	$0.61^{A\,a}$	$0.61^{A\,a}$
	1 %	$0.39^{B\,b}$	$0.47^{B\,a}$	$0.47^{B\,a}$
	2 %	$0.25^{C\,b}$	$0.36^{C\,a}$	$0.36^{C\,a}$
EQPI	**0.5 %**	$0.52^{A\,b}$	$0.59^{A\,a}$	$0.60^{A\,a}$
	1 %	$0.36^{B\,b}$	$0.45^{B\,a}$	$0.46^{B\,a}$
	2 %	$0.24^{C\,b}$	$0.32^{C\,a}$	$0.31^{C\,a}$
UQPI	**0.5 %**	$0.5^{A\,b}$	$0.57^{A\,a}$	$0.57^{A\,a}$
	1 %	$0.33^{B\,b}$	$0.40^{B\,a}$	$0.40^{B\,a}$
	2 %	$0.18^{C\,b}$	$0.28^{C\,a}$	$0.28^{C\,a}$

QPI represents quinoa protein isolate; AQPI, EQPI and UQPI represent acid-hydrolyzed, enzyme-hydrolyzed, and ultrasonication-modified QPIs, respectively. Different capital letters indicate significant differences between samples at different concentrations, while different lowercase letters indicate significant differences between samples after 0, 7 and 14 days of storage ($p < 0.05$).

4. Conclusions

This study investigated how thermal acid hydrolysis, enzymatic hydrolysis, and ultrasonication modified fundamental properties of quinoa proteins and subsequently influenced their emulsifying properties. Thermal acid hydrolysis converted proteins into fibril-like structures. Moderate enzymatic hydrolysis led to both protein aggregation and breakdown. Ultrasonication broke quinoa protein aggregates into more uniformly-distributed aggregates. All these modifications led to enhanced aqueous solubility and wettability (i.e., the protein-oil-water three-phase contact angle was closer to 90°). QPI and modified QPIs effectively formed oil-in-water emulsions, serving as efficient Pickering emulsifiers. Acid and enzymatic hydrolysis achieved more remarkable improvement in protein wettability, with increased interfacial adsorption of proteins at oil droplet surfaces. During 14 days of storage, the emulsions stabilized by QPI and modified QPIs were largely stable against flocculation and coalescence, with EQPI achieving the highest stability (i.e., the smallest increase in droplet size). Evident creaming was observed in all emulsions due to the large oil droplet size. However, increasing the concentration of QPI or modified QPI significantly improved their creaming stability. Future research may further explore how interactions between unabsorbed protein particles or aggregates and emulsified oil droplets influence emulsion stability.

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CRediT authorship contribution statement

Fenglin Yang: Writing – original draft, Visualization, Validation, Investigation, Data curation. **Bingyi Li:** Methodology, Investigation. **Aiqian Ye:** Writing – review & editing. **Qing Guo:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

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.

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Data availability

Data will be made available on request.

References

[1] G.T. Tonsor, J.L. Lusk, T.C. Schroeder, Market potential of new plant-based protein alternatives: insights from four US consumer experiments, Appl. Econ. Perspect. Policy 45 (1) (2023) 164–181.
[2] G. Andreani, G. Sogari, A. Marti, F. Froldi, H. Dagevos, D. Martini, Plant-based meat alternatives: technological, nutritional, environmental, market, and social challenges and opportunities, Nutrients 15 (2) (2023) 452.
[3] A.G.A. Sá, Y.M.F. Moreno, B.A.M. Carciofi, Plant proteins as high-quality nutritional source for human diet, Trends Food Sci. Technol. 97 (2020) 170–184.
[4] F.U. Akharume, R.E. Aluko, A.A. Adedeji, Modification of plant proteins for improved functionality: a review, Compr. Rev. Food Sci. Food Saf. 20 (1) (2021) 198–224.
[5] F. Janssen, A. Pauly, I. Rombouts, K.J. Jansens, L.J. Deleu, J.A. Delcour, Proteins of amaranth (Amaranthus spp.), buckwheat (Fagopyrum spp.), and quinoa (Chenopodium spp.): a food science and technology perspective, Compr. Rev. Food Sci. Food Saf. 16 (1) (2017) 39–58.
[6] A.M.M. Filho, M.R. Pirozi, J.T.D.S. Borges, H.M. Pinheiro Sant'Ana, J.B.P. Chaves, J.S.D.R. Coimbra, Quinoa: nutritional, functional, and antinutritional aspects, Crit. Rev. Food Sci. Nutr. 57 (8) (2017) 1618–1630.
[7] Y. Wu, Q. Guo, Effect of whey protein on the formation, structure and gastrointestinal breakdown of quinoa flour-based composite gels, Food Hydrocoll. 160 (2025) 110775.
[8] C. Mota, M. Santos, R. Mauro, N. Samman, A.S. Matos, D. Torres, I. Castanheira, Protein content and amino acids profile of pseudocereals, Food Chem. 193 (2016) 55–61.
[9] S. Dakhili, L. Abdolalizadeh, S.M. Hosseini, S. Shojaee-Aliabadi, L. Mirmoghtadaie, Quinoa protein: composition, structure and functional properties, Food Chem. 299 (2019) 125161.
[10] M. Sarker, N. Tomczak, S. Lim, Protein Nanocage as a pH-switchable Pickering emulsifier, ACS Appl. Mater. Interfaces 9 (12) (2017) 11193–11201.
[11] H. Chen, H. Dai, H. Zhu, L. Ma, Y. Fu, X. Feng, Y. Sun, Y. Zhang, Construction of dual-compartmental micro-droplet via shrimp ferritin nanocages stabilized Pickering emulsions for co-encapsulation of hydrophobic/hydrophilic bioactive compounds, Food Hydrocoll. 126 (2022) 107443.
[12] Y. Zhao, F. Zhang, M. Chen, F. Liu, B. Zheng, W. Miao, H. Gao, R. Zhou, Cellulose nanofibrils-stabilized food-grade Pickering emulsions: clarifying surface charge's contribution and advancing stabilization mechanism understanding, Food Hydrocoll. 152 (2024) 109920.
[13] S. Lv, H. Zhou, L. Bai, O.J. Rojas, D.J. McClements, Development of food-grade Pickering emulsions stabilized by a mixture of cellulose nanofibrils and nanochitin, Food Hydrocoll. 113 (2021) 106451.
[14] S. Cui, D.J. McClements, X. He, X. Xu, F. Tan, D. Yang, Q. Sun, L. Dai, Interfacial properties and structure of Pickering emulsions co-stabilized by different charge emulsifiers and zein nanoparticles, Food Hydrocoll. 146 (2024) 109285.
[15] B. Zeng, Z. Chen, Y. Lou, Y. Tian, Y. Jin, X. Fu, Preparation and characterization of ovomucin-xanthan gum stabilized Pickering emulsions: microstructure, rheology, and stability, Food Chem. 489 (2025) 145004.
[16] Y. Tian, X. Lv, D.-H. Oh, J.M. Kassem, M. Salama, X. Fu, Emulsifying properties of egg proteins: influencing factors, modification techniques, and applications, Compr. Rev. Food Sci. Food Saf. 23 (5) (2024) e70004.
[17] H. Hou, Y. Zhang, Y. Liu, Q. Zeng, Q. Li, X. Fang, T. Guo, H. Yuan, S. Zeng, T. Meng, Pickering emulsion co-delivery system: stimuli-responsive biomineralized particles act as particulate emulsifiers and bioactive carriers, Colloids Surf. B: Biointerfaces 241 (2024) 114029.
[18] X. Xu, Y. Zhang, M. Han, Q. Guo, Whey protein fibrils enhance fat-related texture of emulsion systems: translating structural changes to textural perception, Food Hydrocoll. 146 (2024) 109208.
[19] Y. Chevalier, M.-A. Bolzinger, Emulsions stabilized with solid nanoparticles: Pickering emulsions, Colloids Surf. A Physicochem. Eng. Asp. 439 (2013) 23–34.
[20] E. Dickinson, Use of nanoparticles and microparticles in the formation and stabilization of food emulsions, Trends Food Sci. Technol. 24 (1) (2012) 4–12.
[21] X. Guan, H. Jiang, J. Lin, T. Ngai, Pickering emulsions: microgels as alternative surfactants, Curr. Opin. Colloid Interface Sci. 73 (2024) 101827.
[22] G.A. Ruiz, W. Xiao, M. van Boekel, M. Minor, M. Stieger, Effect of extraction pH on heat-induced aggregation, gelation and microstructure of protein isolate from quinoa (Chenopodium quinoa Willd), Food Chem. 209 (2016) 203–210.
[23] S. Liu, Y. Xie, B. Li, S. Li, W. Yu, A. Ye, Q. Guo, Structural properties of quinoa protein isolate: impact of neutral to high alkaline extraction pH, Foods 12 (13) (2023) 2589.
[24] B. Li, Y. Wu, A. Ye, Q. Guo, Emulsifying properties of quinoa protein fibrillar aggregates and their influence on quinoa protein emulsion gel formation, Int. J. Biol. Macromol. 285 (2025) 138234.
[25] Y. Xie, M. Han, Y. Wu, X. Xu, Q. Guo, Deciphering the mechanism underlying poor aqueous solubility of extracted quinoa proteins, Int. J. Biol. Macromol. 282 (2024) 137270.
[26] X. Zhu, R.S. Das, M.L. Bhavya, M. Garcia-Vaquero, B.K. Tiwari, Acoustic cavitation for Agri-food applications: mechanism of action, design of new systems, challenges and strategies for scale-up, Ultrason. Sonochem. 105 (2024) 106850.

[27] Z. Zhu, W. Zhu, J. Yi, N. Liu, Y. Cao, J. Lu, E.A. Decker, D.J. McClements, Effects of sonication on the physicochemical and functional properties of walnut protein isolate, Food Res. Int. 106 (2018) 853–861.
[28] J. Jin, O.D. Okagu, A.E.A. Yagoub, C.C. Udenigwe, Effects of sonication on the in vitro digestibility and structural properties of buckwheat protein isolates, Ultrason. Sonochem. 70 (2021) 105348.
[29] L. Liu, Y. Miao, C. Hu, L. Gao, W. He, H. Chu, T. Zhang, C. Li, W. Guo, Effect of ultrasonic frequency on the structural and functional properties of pea protein isolation, J. Sci. Food Agric. 104 (3) (2024) 1431–1440.
[30] L. Luo, Z. Yang, H. Wang, M. Ashokkumar, Y. Hemar, Impacts of sonication and high hydrostatic pressure on the structural and physicochemical properties of quinoa protein isolate dispersions at acidic, neutral and alkaline pHs, Ultrason. Sonochem. 91 (2022) 106232.
[31] A. Vera, M.A. Valenzuela, M. Yazdani-Pedram, C. Tapia, L. Abugoch, Conformational and physicochemical properties of quinoa proteins affected by different conditions of high-intensity ultrasound treatments, Ultrason. Sonochem. 51 (2019) 186–196.
[32] C. Yang, X. Zhu, J. Huang, Y. Wei, L. Wen, F. Yang, F. Yang, W. Liu, Harnessing ultrasonic power to optimize quinoa byproduct protein for sustainable utilization, LWT–Food Sci. Technol. 207 (2024) 116629.
[33] N.A. Mir, C.S. Riar, S. Singh, Structural modification of quinoa seed protein isolates (QPIs) by variable time sonification for improving its physicochemical and functional characteristics, Ultrason. Sonochem. 58 (2019) 104700.
[34] F. Li, A. Leier, Q. Liu, Y. Wang, D. Xiang, T. Akutsu, G.I. Webb, A.I. Smith, T. Marquez-Lago, J. Li, J. Song, Procleave: predicting protease-specific substrate cleavage sites by combining sequence and structural information, Genom. Proteom. Bioinform. 18 (1) (2020) 52–64.
[35] F.-F. Liu, Y.-Q. Li, C.-Y. Wang, Y. Liang, X.-Z. Zhao, J.-X. He, H.-Z. Mo, Physicochemical, functional and antioxidant properties of mung bean protein enzymatic hydrolysates, Food Chem. 393 (2022) 133397.
[36] C. Yang, W. Liu, X. Zhu, X. Zhang, Y. Wei, J. Huang, F. Yang, F. Yang, Ultrasound-assisted enzymatic digestion for efficient extraction of proteins from quinoa, LWT-Food Sci. Technol. 194 (2024) 115784.
[37] X. Shuai, L. Gao, Q. Geng, T. Li, X. He, J. Chen, C. Liu, T. Dai, Effects of moderate enzymatic hydrolysis on structure and functional properties of pea protein, Foods 11 (15) (2022) 2368.
[38] Z.-u. Islam, N.A. Mir, A. Gani, Effect of controlled enzymatic treatment on the physicochemical, structural and functional properties of high-intensity ultrasound treated album (*Chenopodium album*) protein, Food Hydrocoll. 144 (2023) 108940.
[39] X. Wang, L. Cheng, H. Wang, Z. Yang, Limited Alcalase hydrolysis improves the thermally-induced gelation of quinoa protein isolate (QPI) dispersions, Curr. Res. Food Sci. 5 (2022) 2061–2069.
[40] R.E. Aluko, E. Monu, Functional and bioactive properties of quinoa seed protein hydrolysates, J. Food Sci. 68 (4) (2003) 1254–1258.
[41] B. Li, Y. Xie, Q. Guo, Thermal acid hydrolysis modulates the solubility of quinoa protein: the formation of different types of protein aggregates, Food Hydrocoll. 151 (2024) 109825.
[42] T. Li, J. Zhou, M. Peydayesh, Y. Yao, M. Bagnani, I. Kutzli, Z. Chen, L. Wang, R. Mezzenga, Plant protein amyloid fibrils for multifunctional sustainable materials, Adv. Sustainable Syst. 7 (4) (2023) 2200414.
[43] G. Náthia-Neves, A.T. Getachew, C. Jacobsen, Enhancing lupin protein extraction and functionality through defatting: impact of different solvents, Food Chem. 495 (2025) 146378.
[44] J. Yue, Z. Gu, Z. Zhu, J. Yi, J.-B. Ohm, B. Chen, J. Rao, Impact of defatting treatment and oat varieties on structural, functional properties, and aromatic profile of oat protein, Food Hydrocoll. 112 (2021) 106368.
[45] T.A. Russin, J.I. Boye, Y. Arcand, S.H. Rajamohamed, Alternative techniques for defatting soy: a practical review, Food Bioprocess Technol. 4 (2) (2011) 200–223.
[46] G. Avila Ruiz, A. Arts, M. Minor, M. Schutyser, A hybrid dry and aqueous fractionation method to obtain protein-rich fractions from quinoa (Chenopodium quinoa Willd), Food Bioprocess Technol. 9 (9) (2016) 1502–1510.
[47] A. Akyüz, S. Ersus, Optimization of enzyme assisted extraction of protein from the sugar beet (Beta vulgaris L.) leaves for alternative plant protein concentrate production, Food Chem. 335 (2021) 127673.
[48] Q. Guo, T.H. Mu, Emulsifying properties of sweet potato protein: effect of protein concentration and oil volume fraction, Food Hydrocoll. 25 (1) (2011) 98–106.
[49] X. Shen, Q. Guo, Fabrication of robust protein-based foams with multifunctionality by manipulating intermolecular interactions, Green Chem. 23 (20) (2021) 8187–8199.
[50] D. Wilby, N.W. Roberts, Optical influence of oil droplets on cone photoreceptor sensitivity, J. Exp. Biol. 220 (Pt 11) (2017) 1997–2004.
[51] Y. Ladjal-Ettoumi, H. Boudries, M. Chibane, A. Romero, Pea, chickpea and lentil protein isolates: physicochemical characterization and emulsifying properties, Food Biophys. 11 (1) (2016) 43–51.
[52] C. Liu, D. Wu, P. Wang, D.J. McClements, S. Cui, H. Liu, F. Leng, Q. Sun, L. Dai, Study on the formation mechanism of pea protein nanofibrils and the changes of structural properties of fibril under different pH and temperature, Food Hydrocoll. 150 (2024) 109735.
[53] Y. Zheng, Y. Gao, Y. Chang, C. Sun, Y. Fang, Concentration-regulated fibrillation of soy protein: structure and in vitro digestion, J. Agric. Food Chem. 71 (29) (2023) 11170–11179.
[54] A. Herneke, C. Lendel, D. Johansson, W. Newson, M. Hedenqvist, S. Karkehabadi, D. Jonsson, M. Langton, Protein Nanofibrils for Sustainable Food–Characterization and Comparison of Fibrils from a Broad Range of Plant Protein Isolates, ACS Food Science & Technology 1 (5) (2021) 854–864.
[55] X. Xu, J. Yang, M. Han, F. Yang, Q. Guo, Unraveling the molecular pathways of protein fibrillation under thermal acid hydrolysis, Small 21 (29) (2025) 2504082.
[56] A. Gani, Z. ul Ashraf, N. Noor, I. Ahmed Wani, Ultrasonication as an innovative approach to tailor the apple seed proteins into nanosize: effect on protein structural and functional properties, Ultrason. Sonochem. 86 (2022) 106010.
[57] S. Mykolenko, R. Mezzenga, Sustainable protein and amyloid fibrils production from tofu wastewater, Food Hydrocoll. 163 (2025) 111072.
[58] K. Engelhardt, M. Lexis, G. Gochev, C. Konnerth, R. Miller, N. Willenbacher, W. Peukert, B. Braunschweig, pH effects on the molecular structure of β-lactoglobulin modified air–water interfaces and its impact on foam rheology, Langmuir 29 (37) (2013) 11646–11655.
[59] K.N. Ryan, E.A. Foegeding, Formation of soluble whey protein aggregates and their stability in beverages, Food Hydrocoll. 43 (2015) 265–274.
[60] K.N. Ryan, B. Vardhanabhuti, D.P. Jaramillo, J.H. van Zanten, J.N. Coupland, E. A. Foegeding, Stability and mechanism of whey protein soluble aggregates thermally treated with salts, Food Hydrocoll. 27 (2) (2012) 411–420.
[61] B.P. Binks, Particles as surfactants—similarities and differences, Curr. Opin. Colloid Interface Sci. 7 (1–2) (2002) 21–41.
[62] X.-S. Qin, Z.-G. Luo, X.-C. Peng, Fabrication and characterization of quinoa protein nanoparticle-stabilized food-grade Pickering emulsions with ultrasound treatment: interfacial adsorption/arrangement properties, J. Agric. Food Chem. 66 (17) (2018) 4449–4457.
[63] A. Sethuraman, M. Han, R.S. Kane, G. Belfort, Effect of surface wettability on the adhesion of proteins, Langmuir 20 (18) (2004) 7779–7788.
[64] J.A. Hunt, D.G. Dalgleish, Adsorption behaviour of whey protein isolate and caseinate in soya oil-in-water emulsions, Food Hydrocoll. 8 (2) (1994) 175–187.
[65] F. Jiang, Y. Pan, D. Peng, W. Huang, W. Shen, W. Jin, Q. Huang, Tunable self-assemblies of whey protein isolate fibrils for Pickering emulsions structure regulation, Food Hydrocoll. 124 (2022) 107264.
[66] M. Destribats, M. Rouvet, C. Gehin-Delval, C. Schmitt, B.P. Binks, Emulsions stabilised by whey protein microgel particles: towards food-grade Pickering emulsions, Soft Matter 10 (36) (2014) 6941–6954.
[67] B.P. Binks, P.D.I. Fletcher, Particles adsorbed at the oil–water Interface: a theoretical comparison between spheres of uniform wettability and "Janus" particles, Langmuir 17 (16) (2001) 4708–4710.
[68] B.P. Binks, S.O. Lumsdon, Pickering emulsions stabilized by monodisperse latex particles: effects of particle size, Langumir 17 (15) (2001) 4540–4547.
[69] F.-Z. Zhou, X.-H. Yu, D.-H. Luo, X.-Q. Yang, S.-W. Yin, Pickering water in oil emulsions prepared from biocompatible gliadin/ethyl cellulose complex particles, Food Hydrocoll. 134 (2023) 108050.
[70] Y. Zhang, H. Wang, Y. Hou, J. Song, W. Shang, P. Zhang, S. Hou, M. Tan, Pickering emulsion stabilized by gliadin nanoparticles for astaxanthin delivery, J. Food Eng. 345 (2023) 111417.