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Exploring the effects of heat, ultrasound, and high hydrostatic pressure on the structural, functional, and rheological properties of whey protein isolate

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Abstract

This study aimed to investigate the effects of heat (HE), ultrasound (US), and high hydrostatic pressure (HHP) on the structural and functional properties of whey protein isolate (WPI). The structural modifications were characterized by determining surface hydrophobicity, scanning electron microscopy (SEM), circular dichroism (CD) spectroscopy, and steady shear rheological measurements. The functional performance was evaluated by studying adsorption kinetics, interfacial dilatational rheology, emulsifying activity index (EAI), and emulsion stability index (ESI). The results showed that all treatments increased surface hydrophobicity, with HE having the greatest impact (529.78 ± 11.81) , followed by HHP (402.35 ± 10.44) and US (245.48 ± 2.55). Each treatment also resulted in distinct changes in conformation and functionality. Specifically, US decreased viscosity, increased molecular flexibility (as evidenced by an increase in α-helix content to 20.9%), and resulted in the formation of an elastic interfacial film. It also accelerated both interfacial adsorption and rearrangement, yielding the highest EAI (1.22 ± 0.11 m²/g) and ESI (257.33 ± 13.49 min). HE treatment caused protein unfolding and aggregation into larger, more viscous structures, resulting in mobile interfacial films and moderate improvement in emulsification. HHP led to mild unfolding without altering viscosity, but reduced adsorption dynamics and relatively enhanced interfacial elasticity, ending up in diminished emulsifying performance (0.75 ± 0.12 m²/g). These findings provide a deep understanding of how different physical treatments affect the structure and function of WPI and can be used to tailor protein functionality for food emulsification applications.

Keywords: WPI, Ultrasound, High hydrostatic pressure, Surface hydrophobicity, Interfacial rheology

Introduction

Proteins are a crucial component of food, whether they occur naturally or are added as an ingredient. In addition to their nutritional value, proteins also play a vital role in texturizing and structuring food due to their functional properties, such as foaming, gelling, and emulsifying abilities. The emulsifying activity of proteins is linked to their amphiphilic nature, which involves the coexistence of both hydrophilic and hydrophobic groups in a single molecule. This enables them to reduce the interfacial tension between oil and water by adsorbing at the interface of the two phases, allowing the emulsion to form (McClements, 2004). Whey proteins, which are derived from whey, a byproduct of the cheese-making process, are a popular source of proteins often utilized in the processing of a wide variety of foods. This is due to their unique functional properties, as well as their high digestibility and biological value (Kheto et al., 2025). However, native WPI has inherent limitations that hinder its performance in complex food systems. These include moderate interfacial activity and foaming stability, as well as poor resistance to environmental stresses such as changes in pH, ionic strength, and thermal processing. These limitations are primarily due to its compact globular structure, which restricts molecular flexibility and inhibits effective adsorption and rearrangement at oil-water and air-water interfaces. As a result, its functional performance is suboptimal. To address these issues, it is crucial to improve both the structural properties, such as surface hydrophobicity and molecular flexibility, and the functional properties, including solubility, emulsifying capacity, and foaming stability. This will expand the potential uses of WPI in food formulations (Khojasteh et al., 2025; Minj & Anand, 2020). Heat (HE) treatment, as the most conventional unit operation widely used in the food industry, has been shown to affect these proteins, leading to unfolding and aggregation (Sobhaninia et al., 2017). All whey protein fractions, including β-lactoglobulin, α-lactoalbumin, and bovine serum albumin, are prone to structural changes, which are commonly irreversible when exposed to temperatures above 70 °C (Dziuba et al., 2021). The type and extent of these variations depend on several factors, such as temperature and time of exposure, pH, ionic strength, and total protein concentration (Zhang et al., 2015). Previous research has shown that heating WPC at a temperature of 80 °C results in the formation of aggregates of varying size, depending on the pH and duration of the process, with the largest particles being produced at around the isoelectric point (Sajedi et al., 2014). These protein aggregates have been found to have a higher emulsifying capacity compared to the native protein (Fan et al., 2021).

US treatment is a nonthermal processing method that has been proven to decrease the size of protein particles without altering their primary structure (Loushigam et al., 2025). Furthermore, it has been demonstrated to significantly enhance their emulsifying activity by increasing their hydrophobicity, improving their interfacial packing, and boosting their ability to effectively reduce interfacial tension (O'Sullivan et al., 2014). However, the effects of US treatment may vary for different types of proteins, depending on their nature, environmental conditions, and the power or frequency of the US source (Jadhav et al., 2024; Qian et al., 2023).

High hydrostatic pressure (HHP) treatment is another nonthermal technique that has been found to disrupt hydrophobic and electrostatic interactions, resulting in changes to protein structure. However, the extent of these variations depends on various factors, including the physicochemical properties of the protein surrounding environment, the level of pressure applied, as well as the time and temperature of the process (Dehnad et al., 2023). HHP treatment may also affect the functionality of proteins, particularly their emulsifying activity. Nevertheless, inconsistent results have been reported, suggesting that different proteins may respond differently to HHP treatment (Denda & Hayashi, 1992). For example, while whey proteins and β-lactoglobulin have shown a significant decrease in their emulsifying activity, ovalbumin and soy proteins have exhibited noticeable improvements in this feature (Galazka et al., 1996; Galazka et al., 1995). These changes, which are specific to each protein, are linked to pressure-induced structural alterations and aggregation (Galazka et al., 2000).

Although there have been numerous studies on the effects of HE, US, and HHP treatments on proteins, including whey proteins, the underlying mechanisms are still not fully understood. Therefore, it is necessary to comprehensively investigate these changes from the molecular level to the macroscopic scale in order to gain a better understanding of how these processes affect the structure-function relationship. In our previous study, we examined the influence of these thermal and non-thermal techniques on the key structural features of whey proteins and their links to isoelectric point and solubility (Khojasteh et al., 2025). In this current work, our main objective is to study the impact of these methods on the interfacial functionality of whey proteins, which is a crucial factor in determining their successful industrial applications. To achieve this goal, we conducted a variety of analyses, including as measuring surface hydrophobicity, interfacial dynamic moduli, and interfacial tension, examining microstructure, and studying structural changes using circular dichroism (CD) spectroscopy, and correlated the results to changes in emulsifying activity. These findings are expected to provide a foundational framework for the rational design of multi-step or sequential processing strategies to tailor WPI functionality for specific food applications.

Material and Methods

Materials

Whey protein isolate (WPI) with the following specifications was provided by Davisco International Inc. (Minneapolis, Minnesota, USA): 96.2% protein, 1.54% ash sulfate, 5% moisture, and less than 0.5% lactose. 8-Anilinonaphthalene-1-sulfonic acid (ANS), hydrochloric acid (HCl), sodium azide, and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used in this study were of analytical-grade. Solutions were prepared using deionized water.

Methods

Preparation of WPI solution

WPI powder was gradually added to deionized MilliQ water to make a 5% w/w solution and mixed at 500 rpm for two hours at ambient temperature. It was then kept refrigerated at 4 °C overnight for complete hydration. The pH of the WPI solution was adjusted to 7.0 using either 0.1 N NaOH or 0.1 N HCl solution before being subjected to HE, US, and HHP treatment. Sodium azide (0.02% w/w) was added to inhibit microbial growth.

HE, US, and HHP treatment of WPI

The WPI solution, with a concentration of 5% w/w, was subjected to HE, US, and HHP treatment, following the methods described in our previous study (Khojasteh et al., 2025). Briefly, for the HE treatment, the solution was heated at a temperature of 90 °C in a jacketed vessel connected to a water bath (EH-19, Julabo, USA) for 30 minutes while being gently agitated at 500 rpm. The US treatment was performed at a temperature of 25 ± 0.1 °C for 20 minutes using an ultrasonic processor (VCX 750 Sonics & Materials, Inc., USA) operating at 20 kHz and 750 W, equipped with a 13 mm diameter titanium cylindrical probe. The HHP treatment was conducted at a pressure of 500 MPa for 20 minutes at ambient temperature, using an HHP device with a 60 mL chamber designed and built by the Research Institute of Food Science and Technology (Mashhad, Iran).

Determination of surface hydrophobicity

The surface hydrophobicity of WPI was determined using the fluorescent probe 8-anilino-1-naphthalene sulfonic acid (ANS), following the method described by Mohammadian et al. (2019). WPI solutions with concentrations ranging from 0.002 to 0.01% were prepared in phosphate-buffered saline (PBS) solution (0.01 M) at pH 7.0. Next, 4 mL of each solution was mixed with 20 μL of ANS solution (0.008 M in 0.01 M PBS). The mixture was thoroughly vortexed and then incubated in complete darkness for 15 minutes. The fluorescence intensity of each sample was measured at an excitation wavelength of 390 nm and an emission wavelength of 470 nm using a Jasco FP-6200 spectrofluorometer. The fluorescence intensity was plotted against protein concentration, and the initial slope of the curve was taken as an indicator of surface hydrophobicity (Mohammadian et al., 2019).

Circular dichroism (CD) spectroscopy

To analyze the secondary structure composition of the WPI samples, their far UV spectra were recorded using a J-810 CD spectropolarimeter (Jasco International Co., Ltd., Tokyo, Japan). The lyophilized samples were dissolved in deionized water at a pH of 7.0 to reach a concentration of 0.25 mg/mL. The measurements were carried out by collecting the CD spectra of the solutions in the far-UV region (190-260 nm) at ambient temperature using a quartz cuvette with a path length of 1 mm. The scan speed was set to 50 nm/min with a 4 s response time, 1 nm bandwidth, and 0.1 nm data pitch. The signal average was performed on at least four scans, and the baseline was corrected by subtracting the spectrum of deionized water at pH 7.0 as a blank. The ellipticity data were converted to a concentration-independent parameter of mean residue ellipticity using Equation 1:

$$[\theta]_\lambda = \frac{MRW \times \theta_\lambda}{10 \times l \times c} \quad (1)$$

where $[\theta]_\lambda$ (deg cm^2 dmol^{-1}) is the mean residue ellipticity at wavelength λ, θ_λ is the observed ellipticity (millidegree) at wavelength λ, *MRW* is the mean residue molecular weight of WPI (*Mw*/*n*; *Mw* and *n* denote molecular weight and the number of residues, respectively), *c* (mg/mL) is the WPI concentration, and *l* (cm) is the optical path length of the cuvette. The MRW of WPI was taken as 114.63 g/mol (Spotti et al., 2017).

The composition of the secondary structural elements was determined using the Spectra Manager software, ver. 1.53.02 (Jasco International Co., Ltd., Tokyo, Japan) (Chen et al., 2019).

Scanning electron microscopy (SEM)

The surface morphology of WPI particles was examined using a scanning electron microscope (Zeiss Supra 40VP, Carl Zeiss NTS GmbH, Germany). To prepare the samples, WPI solutions were dehydrated in a freeze dryer (FDU-8606, OPERON, South Korea) at a pressure of 0.2 mbar and a temperature of -70 °C for 48 hours. The resulting dried powders were then mounted onto an aluminum stub using double-sided conductive carbon tape and sputter-coated with a thin layer of gold under vacuum for 120 seconds. Images were captured at an acceleration voltage of 20 kV and magnifications of 500 and 1000×, and were analyzed using ImageJ software, version 1.4.3.67 (National Institute of Health, Bethesda, MD, USA).

Steady shear rheological measurements

The flow behavior of WPI solutions was studied using a Discovery Hybrid rheometer (DHR-2, TA Instruments, New Castle, DE, USA) equipped with a Peltier concentric cylinder system at a concentration of 5% w/w and a pH of 7.0. Measurements were taken at 25 °C and a shear rate ranging from 1 to 100 s^{-1}. The collected data was then fitted to the Power law model using the following equation:

$$\sigma = K\dot{\gamma}^{n} \tag{2}$$

where K, $\dot{\gamma}$, and n represent the consistency coefficient (mPa s^{n}), shear rate (s^{-1}), and flow behavior index (dimensionless), respectively.

Surface tension measurement

The surface tension of WPI solutions was measured at a concentration of 5% w/w and a pH of 7.0 using a tensiometer (K100, Krüss GmbH, Germany) and the Du Noüy ring method. The tensiometer was calibrated with deionized Milli-Q water at a temperature of 25 °C with a surface tension of 72.1 mN/m. Before taking each measurement, the glass measuring cup was meticulously washed with concentrated sulfuric acid, rinsed with deionized Milli-Q water, and then air-dried. The ring was also washed with deionized Milli-Q water, flushed with absolute ethanol, and burned in a flame. Surface tension was measured after 5 minutes of equilibration and continued up to 180 minutes until it reached a plateau.

Interfacial dilatational rheological measurements

Surface dilatational properties were determined using a drop tensiometer (OCA 25, DataPhysics Instruments, Filderstadt, Germany) equipped with an oscillating drop generator (ODG 20, DataPhysics). A 15 µL droplet of 0.01% (w/w) native or treated WPI was formed at the tip of a stainless-steel needle (0.51 mm diameter) and submerged in a quartz-glass cuvette (1 × 1 × 1 cm^3) filled with MCT oil. After allowing for a 3-h equilibration period to ensure the formation of a quasi-stable interfacial film, a strain sweep test was conducted by manually applying a deformation amplitude ranging from 0.027 to 0.27 mm (as interfacial area) at a fixed oscillation frequency of 0.01 Hz. Each deformation was applied for five cycles of oscillation, followed by a 90 second rest period to allow the interfacial film to recover. This was followed by frequency sweep of 0.01–0.1 Hz at a fixed deformation amplitude of 0.13 mm, which had previously been determined to be within the linear viscoelastic regime. A 90 s rest period was applied between each frequency increment to allow the interfacial layer to re-equilibrate (Subasi et al., 2022).

Determination of emulsifying properties

To evaluate the emulsifying properties of WPI, 12 mL of sunflower oil was gradually added to 48 mL of WPI solution (0.5% w/w) on a magnetic stirrer. The mixture was then homogenized using an Ultra-Turrax T25 homogenizer (IKA Werke GmbH & Co., Staufen, Germany) at 12,000 rpm for 3 minutes to create an emulsion. Samples of 50 µL were taken immediately and 10 minutes after the emulsion was prepared and mixed with a 10 mL of 0.1% sodium dodecyl sulfate solution with a pH of 7.0. The mixture was vortexed for 15 seconds and its absorbance was measured at a wavelength of 500 nm using a UV-vis spectrophotometer (UV-160A, Shimadzu, Japan). The emulsifying activity index (EAI) and emulsion stability index (ESI) were then calculated using the following equations (S. Jiang et al., 2018):

$$EAI\left(\frac{m^2}{g}\right) = \frac{2 \times 2.303 \times A_0 \times D_f}{(1-\varphi) \times m_p} \tag{3}$$

$$ESI\ (min) = \frac{A_0 \times \Delta t}{A_0 - A_{10}} \tag{4}$$

where A_0 and A_{10} represent the absorbance of the diluted emulsion at 500 nm immediately and at $\Delta t = 10$ minutes after preparation, respectively. D_f is the dilution factor, M_p is the mass of protein (g), and φ is the volume fraction of oil in the emulsion.

Statistical analysis

All measurements were conducted in triplicates. The experimental data were analyzed using one-way analysis of variance (ANOVA) with IBM SPSS Statistics software version 24.0 (IBM Corp., Armonk, NY, USA) at a significance level of $p < 0.05$. The means were compared using Duncan's multiple range test at $\alpha = 0.05$. The data were reported as mean ± standard deviation.

Results and discussion

Surface hydrophobicity

The surface hydrophobicity of a protein is a measure of the amount of exposed hydrophobic amino acids in its three-dimensional structure (Zhang et al., 2021). This indicates the presence of hydrophobic regions within the protein molecule (Alves et al., 2022), which determine its amphiphilic nature. Changes in the protein's hydrophobicity can affect its functional properties, such as emulsifying and foaming abilities (Tan et al., 2021).

Figure. 1 shows the surface hydrophobicity of native and treated WPI at pH 7.0. It is evident that all treatments significantly increased the surface hydrophobicity of WPI ($p < 0.05$) compared to its native state, with the order of HE > HHP > US. The dramatic increase in the surface hydrophobicity of HE-treated WPI from 245.48±2.55 to 529.78±11.81 is in alignment with previous reports (Gao et al., 2019). The exact mechanism underlying this rise is unclear; however, it is likely the result of thermal disruption of noncovalent bonds that maintain the tertiary and secondary structures of WPI molecules. Upon heating, the internal energy of protein molecules, including vibrational and rotational energies, increases, while the surface tension of water decreases. These events impose destabilizing effects on the protein tertiary and secondary structures, leading to unfolding and exposure of hydrophobic side chain residues. Among the three fractions of β-lactoglobulin, α-lactalbumin, and bovine serum albumin, the former undergoes the most structural changes and exhibits higher hydrophobicity relative to the other two fractions. It is worth noting that at elevated temperatures and prolonged heating times, the formation of dimers, trimers, and other higher oligomers of WPI fractions, particularly β-lactoglobulin, may occur, resulting in a decrease in hydrophobicity. However, this was not observed, possibly because the changes in hydrophobicity as a function of temperature were not monitored.

The US treatment increased the surface hydrophobicity of WPI to 282.82±2.05. This can be attributed to the high shear forces generated by the pulsation and collapse of bubbles in the acoustic cavitation field, which are strong enough to change the protein tertiary and secondary structures by breaking noncovalent weak bonds and unfolding the molecule, exposing the originally concealed hydrophobic amino acid residues (Shi et al., 2020; Vargas et al., 2021b). Additionally, the $OH^{\bullet}$ free radicals produced during the sonolysis of water may oxidize amino acids, such as tyrosine, leading to covalent crosslinks that further alters the hydrophobicity (Kadkhodaee & Povey, 2008). These findings are consistent with other studies that have shown an increase in the hydrophobicity of proteins when exposed to US (Vargas et al., 2021a; Yan et al., 2021).

Similarly, the application of HHP caused a noticeable increase in the surface hydrophobicity of WPI to 402.35 ± 10.43 (Kato & Nakai, 1980). This increase has also been observed in previous studies (Lee et al., 2006; Lim et al., 2008; Liu et al., 2005). It is linked to the penetration of water molecules inside the protein structure due to the reduction of water-protein interfacial energy, resulting in the breakage of the hydrophobic core and unfolding of the protein (Cheung et al., 2006), with a greater impact on the tertiary rather than the secondary structure. However, it has been reported that the surface hydrophobicity of myofibrillar protein decreases when the applied pressure surpasses 400 MPa, likely due to protein aggregation. This shows that the impact of HHP on the surface hydrophobicity is determined by the type of protein, as well as the pressure level and physicochemical properties of the surrounding environment (Liu et al., 2021).

Secondary structure

CD spectroscopy is a highly sensitive method used to detect changes in protein secondary structure. Figure. 2 shows the far-UV CD spectrum of native WPI, as well as WPI treated with HE, US, and HHP. The native WPI exhibited a maximum at 197 nm, a broad minimum spanning from 208 to 222 nm, and an isodichroic point at 202 nm, suggesting a predominance of β-sheets over helical conformation. The proportions of the secondary structure elements were calculated using the Spectra Manager TM II software package and are presented in Figure. 3. The native WPI had a distribution of 17.3% α-helix, 35.2% β-sheets, 18.3% β-turn, and 29.2% random coil (Figure. 3), showing slight deviations relative to the profiles reported by previous studies, which may be attributed to differences in the source of protein (Chen et al., 2019; Dai et al., 2016; Qin et al., 2016; Tomczyńska-Mleko et al., 2014).

After being treated with HE, the CD spectrum of the WPI underwent significant changes. The positive band shifted to 194 nm and the isodichroic point displaced to 198 nm. The negative peak also became broader and less intense compared to the native WPI. Although the spectrum still showed characteristics of a β-sheet rich protein, the decrease in intensity of the minimum band suggests a reduction in α-helix conformation. The blue shift of the isodichroic point indicates a possible α-helix to random coil or folding to unfolding transition, which has been previously reported during heating (Wooster & Augustin, 2007; Zhou et al., 2017). Figure. 3 illustrates that heating decreased the α-helix content to 15.2% and increased the β-sheet and random coil content to 40.9% and 33.1%, respectively. These results are consistent with other studies (Tomczyńska-Mleko et al., 2014). It has been observed that HE treatment of WPI leads to an increase in unordered structures and β-sheets at the expense of α-helices (Joshi et al., 2014), although there is also evidence that heating can induce a β-sheet to α-helix transition (Viseu et al., 2007). This discrepancy may be attributed to the aggregation of unfolded proteins through inter- and intramolecular interactions, such as disulfide linkages, hydrophobic interactions, and electrostatic forces (Momen et al., 2018; Narayanan & Dias, 2013). It is important to note that the secondary structural changes induced by HE on proteins are influenced by both the temperature and duration of heating. When heated mildly above the denaturation temperature, both α-helices and β-sheets become destabilized, causing the protein structure to unfold and leading to an increase in the random coil motifs. However, when heated severely at elevated temperatures for a prolonged period of time, the α-helices decrease even further, while the β-sheet content significantly increases due to the formation of stable β-sheet aggregates, although the abundance of random coils also continues to increase (Joeres et al., 2023; Pang et al., 2011).

It is worth noting that HE treatment significantly decreased β-turn content, which can be attributed to the disruption of hydrogen bonds and the promotion of structural transitions into irregular random coils and β-sheets, as has been reported by others (Li et al., 2021).

Similar to HE, HHP also caused a shift in the positive band to 195 nm and the isodichroic point to 198 nm, with both positive and negative peaks having lower intensity compared to the spectrum of HE-treated WPI. This suggests an increase in β-sheets and random coils, and a decrease in α-helix elements. Figure. 3 shows a reduction in α-helix content to 14.7%, while β-sheets and random coils increased to 40.9% and 32.1%, respectively. These findings indicate that α-helices are more susceptible to HHP than β-sheets, which may undergo a transition to permanent unfolding due to infiltration of water molecules. The increase in the content of β-sheet structures is likely a result of degradation of intermolecular β-sheets and the formation of intramolecular β-sheet structures through protein aggregation (Cepero-Betancourt et al., 2020; Yin et al., 2022).

It should be noted that, while this study found that HHP treatment of WPI increased the β-sheets content at the expense of α-helices, other researchers have reported the opposite effect (Baier et al., 2015; Ma et al., 2023). Similar results have been observed for HHP-treated β-lactoglobulin, which has been attributed to the conversion of native β-sheets to non-native α-helices due to the protein structure transitioning to a molten globule state (Yang et al., 2001). However, it appears that the molten globule intermediate state did not occur under the HHP conditions used in this study, likely due to aggregation caused by intra- and intermolecular disulfide crosslinking or hydrophobic interactions during HHP treatment or depressurization process (Aouzelleg et al., 2004; Funtenberger et al., 1995; Iametti et al., 1997; Pittia et al., 1996) as reported in our previous study (Khojasteh et al., 2025).

In contrast to HE and HHP, the application of US increased the α-helix content of WPI to 20.9% and reduced β-sheets to 31.3%. However, there was also a slight increase in random coil conformation to 30.8% (Figure. 3). This was confirmed by a significant rise in the intensity of the positive band at 198 nm, a red shift of the isodichroic point to 203 nm, and an increase in the intensity of the broad negative band. These findings are consistent with the data reported by other researchers (Aouzelleg et al., 2004; Chandrapala et al., 2012; Chandrapala et al., 2011). The changes in secondary structure composition indicate that WPI fractions, particularly β-lactoglobulin, may have adopted a molten globule state. It is believed that the denatured whey proteins, with open structure, underwent a transition from a native β-sheet to a non-native α-helix when refolding. This intermediate state between ordered and unordered structures appears to occur at high enough ultrasound power and prolonged sonication time. These findings are in consistent with previous studies that have reported a decrease in β-sheets and an increase in α-helices in α-lactalbumin (Chandrapala et al., 2011; Zhao et al., 2020) and β-lactoglobulin (Chandrapala et al., 2011) when sonicated at high power for a reasonable amount of times. However, at low ultrasound power or sonication time, either no changes in the secondary structure have been observed (Frydenberg et al., 2016), or, interestingly, the opposite effect, has been found, with an increase in β-sheets and random coils at the expense of α-helices, (Khatkar et al., 2018; Ortiz-Deleón et al., 2024; Silva et al., 2018; Vargas et al., 2021a). This could be mainly due to weak to mild acoustic cavitation, which imposes almost no chemical modification to the protein structure but can disrupt intramolecular hydrogen bonds and lead to unfolding through the mechanical shearing effects and shockwaves produced by the vibration and collapse of bubbles (Hamada et al., 1996; Vargas et al., 2021a).

Microstructure

The changes in the morphology of WPI particles were studied to understand how HE, US, and HHP affect their microarchitecture attributes, such as size, surface appearance, and homogeneity, by impacting the tertiary and secondary structures of protein molecules. SEM images of native WPI particles, as shown in Figure. 4, revealed that they are globular in shape with a dense and smooth surface, small size, and a uniform and narrow size distribution, consistent with previous studies (Paul et al., 2023; Yu et al., 2021). However, HE treatment resulted in the formation of larger particles with irregular shapes and rough surfaces compared to the untreated sample. This is associated with the breakage of intramolecular hydrogen bonds and the formation of disulfide linkages, resulting in the denaturation of protein molecules and subsequent aggregation of β-lactoglobulin, either alone or together with α-lactalbumin, through hydrophobic interactions and other noncovalent bonds (Čurlej et al., 2022; Li et al., 2021; Zhao et al., 2020). As a result, more intricate aggregates with larger sizes and higher polydispersity are formed (Wagner et al., 2020).

In contrast, US-treated WPI showed smaller particles compared to the native protein. This is likely due to the size-reducing effect of acoustic cavitation, which disrupts weakly bound molecular aggregates including dimers, trimers, and other oligomers. Additionally, changes in the protein conformation (Guo et al., 2023; Meng et al., 2021) and subsequent noncovalent and covalent inter- and intramolecular interactions may lead to the formation of particles with a broader size distribution ranging from small to large loose aggregates (Z. Jiang et al., 2018; Vargas et al., 2021a) with irregular shape and rough surface, as also reported previously (Mushtaq et al., 2025).

HHP treatment resulted in irregularly shaped and tightly agglomerated lamellar structures with porous surfaces, reflecting the profound changes in the inter- and intramolecular interactions (Cepero-Betancourt et al., 2020; Liu et al., 2021; Zhao et al., 2022). This can be attributed to the unfolding and denaturation of protein molecules and conformational variations, bringing the hydrophobic residues from the interior to the surface, eventually leading to aggregation, as illustrated in Figure. 4 (Zhao et al., 2022). The dense lamellar structure of particles, as seen in Figure. 4, is likely due to the compressing effect of HHP treatment (Ma et al., 2023).

Steady shear rheological properties

Changes in the viscosity of protein solutions can serve as an indicator of their conformational changes, which are commonly interpreted as a sign of denaturation (Fu et al., 2025). Upon treatment with HE and HHP, the apparent viscosity of WPI solution increased, while exposure to US resulted in a slight decrease. This variation in viscosity is linked to the size of WPI particles, and thus it was more noticeably increased as the larger aggregates were formed as a result of a higher degree of denaturation, as was the case in the HE-treated sample. Additionally, the viscosity may also increase due to the entrapment of water within the loose aggregates (Joyce et al., 2018; O'Loughlin et al., 2012). Despite the differences in viscosity among the treatments, all solutions exhibited Newtonian behavior (Figure. 5). The viscometric data were well fitted to the Power law model with a determination coefficient (R^2) greater than 0.99 over the shear rate range of 1-100 s^{-1} (Table 2). The flow behavior index, *n*, was approximately equal to 1 for all samples, indicating that they can be classified as Newtonian fluids (He & Vardhanabhuti, 2021). Their consistency index, *K*, which is a measure of viscosity (Ahmadi et al., 2017), was found to vary in the order of HE > HHP > native > US. It should be noted that various factors, such as size, shape, flexibility, and charge of the protein, interactions between protein molecules and solvent, and hydrodynamic volume, significantly influence changes in the rheological behavior of the solution (Zhang & Liu, 2017).

As mentioned earlier, the viscosity of WPI solutions notably increased after HE treatment. This can be attributed to the denaturation of protein molecules, which disrupts their tertiary and secondary structures, leading to unfolding (Čurlej et al., 2022; Li et al., 2021) and subsequent protein-protein interactions through -SH groups, -SH/S-S interchange, and hydrophobic bonds, resulting in aggregation (Čurlej et al., 2022; Guo, 2019; Zhang et al., 2022) and the formation of large particles that increase the resistance of the solution against flow (Mohammadian et al., 2019). This increase in viscosity due to HE treatment has also previously been reported by other researchers (S. Jiang et al., 2018; Zhang et al., 2019).

On the other hand, the slight decrease in the viscosity of US-treated WPI solution can be related to the smaller particle size. This reduction in size is a consequence of US-induced structural changes, leading to a decrease in particle hydration and a smaller hydrodynamic volume, ultimately resulting in a decrease in viscosity. In addition, the US modification of the WPI molecular structure, which increases surface hydrophobicity and forms covalently and noncovalently bound particle associations, can cause entrapped water to be repled out, further contributing to the drop in viscosity (Kang et al., 2022; Vargas et al., 2021a). Previous studies have also shown a correlation between the reduction in viscosity and the US-induced decrease in protein particle size (Ma et al., 2024; Vargas et al., 2021a).

Similar to HE, HHP treatment also increased the viscosity of the WPI solution, which can be associated with the increase in particle size and surface hydrophobicity. The HHP-forced penetration of water inside the protein molecules, which increases the degree of hydration, may also contribute to the overall rise of viscosity. These findings are consistent with the results reported by other researchers (Kanno et al., 1998; Liu et al., 2005).

Dynamic surface tension and adsorption kinetics

To gain insight into the effects of HE, US, and HHP treatments on the functionality of WPI as a surface-active compound, its adsorption kinetics were studied. The adsorption of a protein molecule at the interface and the rate at which the surface tension is reduced, can be influenced by various parameters, such as size, conformation, hydrophobicity, and environmental conditions (Schröder et al., 2017). The adsorption process at the interface involves three steps: diffusion (migration of protein molecules from the bulk solution to the interfacial layer), penetration or adsorption, and rearrangement of the protein structure to a more energetically favorable conformation (Jin et al., 2022; Zhan et al., 2021).

The rates of protein adsorption (k_p) and rearrangement (k_r) were determined using a first-order kinetics equation (Graham & Phillips, 1979):

$$ln\frac{\sigma_t - \sigma_\infty}{\sigma_0 - \sigma_\infty} = -kt \qquad (5)$$

where σ_0, and σ_∞ are the initial (t = 0) and equilibrium surface tensions, and σ_t is the surface tension at any specific moment. The first-order rate constant is represented by k.

Plotting $\ln[(\sigma_t - \sigma_\infty)/(\sigma_0 - \sigma_\infty)]$ against t reveals two distinct linear regions, as shown in Figure 6. The slope of the first segment implies the molecular penetration rate constant (k_P), while the slope of the second segment denotes the molecular rearrangement rate constant (k_r) (Panizzolo et al., 2014). The values of these constants are presented in Table 2. As can be seen, k_r for all WPI samples, regardless of whether they were native or treated, was noticeably higher than k_p, highlighting the importance of protein conformational rearrangements in dynamic adsorption behavior and the formation of the interfacial layer (Tian et al., 2020; Zhu et al., 2024). The adsorption and unfolding rate constants followed the order of HE > US > native > HHP, while for k_r the order was US > native > HE > HHP.

The higher penetration rate of HE-treated WPI at the interface can be attributed to its greater hydrophobicity (Fan et al., 2021; Zhou et al., 2021). This decreases the energy barrier to adsorption and increases the adsorption kinetics (Arzeni et al., 2012; Moghadam et al., 2020). The relatively low zeta potential of HE-treated WPI (Khojasteh et al., 2025) also contributes to its high penetration rate by diminishing the electrostatic energy barrier to adsorption (Wang et al., 2020), making it easier for the particles to interact with the interface. However, despite their high penetration rate, HE-treated WPI particles did not show a noticeable rearrangement rate. This is likely due to their high amount of intramolecular disulfide bonds, as reported in our previous study (Khojasteh et al., 2025), which limits their flexibility and makes it challenging for them to change their structure at the interface (Liu & Tang, 2016; Wong et al., 2012). Furthermore, the secondary structure of the protein also plays a role in its rearrangement rate (Perticaroli et al., 2013; Perticaroli et al., 2014). It has been shown that β-sheet motifs are quite stable and rigid, meaning that the internal dynamics do not change with small atomic fluctuations or cleavage of noncovalent bonds (Sljoka, 2022). Therefore, given that HE-treated WPI contains a relatively high amount of β-sheet, it is expected to have a decreased rearrangement rate at the interface (Perticaroli et al., 2014).

The US-treated WPI, despite having lower surface hydrophobicity than HHP-treated sample and similar zeta potential to the native species (Khojasteh et al., 2025), displayed a greater k_p than both of them. This suggests that its smaller size plays a significant role in decreasing the energy barrier to adsorption (O'sullivan et al., 2016; Wang et al., 2022). However, the contribution of surface electric charge and surface hydrophobicity to the penetration can not be neglected (Shi et al., 2020; Xiong et al., 2019). Compared to all other samples, whether native or HE- and HHP-treated, the US-treated WPI showed a higher k_r. This is likely associated with the higher content of α-helix elements in the secondary structure of US-treated WPI (Figure. 3), which enables it to rearrange more easily at the interface due to its flexiblity and less rigidity (Chang, Chen, et al., 2023; Perticaroli et al., 2014; Xiong et al., 2018; Zhou et al., 2021).

The lower k_p and k_r observed for HHP-treated WPI relative to native, HE- and US-treated samples may result from its unique structural and conformational changes, which could potentially affect its interfacial characteristics, including the rate of adsorption and rearrangement (Carullo et al., 2021). The large size of the HHP-treated WPI particles and their high number of intramolecular disulfide bonds (Khojasteh et al., 2025) may limit molecular flexibility (Liu & Tang, 2016; Wong et al., 2012), potentially negatively impacting the adsorption kinetics. In addition, the remarkable alteration of the secondary structures, with a rise in stiff β-sheet motifs at the expense of flexible α-helices (Figure. 3) may increase the molecular rigidity and hinder the rearrangement of protein molecules at the interface (Perticaroli et al., 2014).

Interfacial dilatational properties

The viscoelasticity of the interfacial layer formed by WPI at the oil-water interface was investigated using small deformation surface dilatational rheology. The results of the amplitude sweep showed that all WPI adsorbed layers were elastic in nature, with the order of elasticity as HHP ≳ US > native > HE (Figures 7a and 7b). The higher elasticity of the adsorbed layer of HHP- and US-treated WPI could possibly be due to the stronger intermolecular interactions at the interface compared to the native WPI. Conversely, the opposite was observed for HE-treated WPI, implying a more loosely packed protein aggregates. This could be associated with the significantly high hydrophobicity of HE-treated WPI particles (Figure 1), causing them to preferentially interact with the oil phase. This, along with their large size (Figure 4), diminishes the frequency of interparticle interactions and thus weakens the interfacial layer. Additionally, the surface electric charge also contributes to the extent of intermolecular charge, resulting in strong repulsion with increasing its magnitude, and further weakening of the interfacial layer. As shown in our previous study (Khojasteh et al., 2025), the zeta potential significantly decreased upon HHP treatment of WPI, which presumably led to strengthening the interfacial adsorbed film by promoting the attractive forces and hence increasing the number of crosslinks. The initial increase of E_d at very low amplitudes, despite being at a quasi-equilibrium state, may be likely related to the adsorption and rearrangement of proteins.

In Figure 7c, it is evident that the surface dilatational complex modulus of all interfacial layers increased with frequency, indicating a response that scales with the applied deformation rate . The frequency dependence of the complex dilatational modulus was characterized by the Power law model, $|E^*|=k\omega^n$. The fits adequately described the data with an R^2 in the range of 0.92 to 0.94, indicating a tradeoff between the strength (k) and frequency sensitivity (n) of the interfacial layer (Table 3). The log-log plot of E* versus ω gave sigmoid-shaped curves for all WPI adsorbed films, typical of an interfacial layer with a viscoelasticity governed by diffusion exchange. This implies that the particles are pushed into the bulk upon compression of the interface, and are adsorbed upon creation of new surface area during extension. According to the Lucassen-van den Tempel model, a slope of n = 0 indicates a fully elastic response of the interface, which approaches 0.5 when diffusion exchange dominates (Lucassen & Van Den Tempel, 1972). The native WPI-adsorbed film exhibited a slope of 0.12, which is lower than that of treated WPI interfacial layers. This is due to partial unfolding of protein molecules at the interface, resulting in interactions between exposed hydrophobic groups and the formation of disulfide linkages, which act as strong barriers to desorption and reorganization of the interfacial film. On the other hand, the HE-treated WPI film displayed the highest degree of frequency dependency with an n = 0.24, suggesting a more mobile adsorbed layer. This could be due to the rigid and highly aggregated structure of HE-treated WPI particles. The lower number of surface free thiol groups in the aggregates, as reported in our previous study (Khojasteh et al., 2025), significantly reduces the number of interparticle crosslinks, allowing them to readily slide along their own contour. Additionally, the larger size of particles may cause them to loosely pack at the interface, reducing the extent of interaggregate junctions and enhancing the degree of freedom. The US- and HHP-treated WPI interfacial films also showed a similar extent of frequency dependency to the HE-treated one. The surprising behavior for the adsorbed US-treated WPI film, despite having the the highest abundance of surface free sulfhydryl groups in the particles, could be attributed to their higher hydrophilicity, which results in unfavorable interactions with the oil phase and thus reversible adsorption (Khojasteh et al., 2025). However, considering the value of n ranging from 0.12 to 0.24, which is less than 0.5, it can be concluded that WPI particles, once adsorbed at the interface, form a disordered soft-solid-like structure with a low degree of exchangeability with the bulk phase, but dominated by in-plane interactions. This is confirmed by the value of the loss tangent (tan δ), which, despite being frequency dependent with a magnitude following the order of US > HE ≳ HHP > native, it was lower than 1 across the entire frequency domain applied (Figure 7d). The loss tangent showed an increase with increasing frequency, indicating that the dilatational response is governed by diffusion relaxation at low frequencies and viscous relaxation at high frequencies.

The US-treated WPI interfacial film showed the highest k value, followed by HHP-, native, and HE-treated ones, respectively (Table 3). This suggests that the US-treated WPI formed the strongest, but also most dynamically reorganizing film, whereas HHP-treated and native WPI led to more structured, solid-like interfaces. The HE-treated WPI film was still solid-like, but with a lower small-amplitude interfacial strength. These differences in the rheological properties of the interfacial layers result from the structural variations of the corresponding film-forming WPI particles.

Comparison of the dilatational rheological results to interfacial tension versus time analyses shows that adsorption and rearrangement play complementary roles. The initial adsorption rate constant (k_p) was the largest for the HE-treated WPI. In contrast, the rearrangement rate constant (k_r) was the largest for the US-treated WPI, consistent with its higher k and E^* as a stronger lateral network develops through molecular reorganization. Native WPI exhibited intermediate k_p and k_r, consistent with their intermediate k and low n.

Emulsifying property

The emulsifying property of a protein, as a surface-active compound, is often characterized by measuring its emulsifying activity index (EAI) and emulsion stability index (ESI). The EAI represents the total surface area stabilized per unit weight of protein, indicating the ability of protein molecules to adsorb at the oil-water interface. The ESI reflects the degree of droplets resistance to coalescence once formed and stabilized by the protein (Wang et al., 2024; Xie et al., 2023). Figure. 8 shows the EAI and ESI of native and treated WPI at pH 7.0. As is evident, the US-treated WPI exhibited significantly higher EAI and ESI compared to the other samples ($p < 0.05$). This can be related to the smaller particle size of US-treated WPI (Figure. 4) and lower apparent viscosity (Figure. 5) of its corresponding solution, which promote the diffusion rate of particles from the bulk solution to the interface. Additionally, the considerably high k_p also appears to enhance the EAI (Li et al., 2014; O'Sullivan et al., 2014; O'sullivan et al., 2016; O’Sullivan et al., 2015; Shi et al., 2020). The notably higher k_r of US-treated WPI indicates more structural flexibility of the particles, which can be attributed to an increase in α-helical conformation. This enhanced flexibility allows for faster rearrangement of the particles, facilitating the rapid formation of the interfacial film. This, in turn, leads to more effective stabilization of the droplets once they are formed, resulting in a higher ESI (Chang, Lan, et al., 2023; Khojasteh et al., 2025; Yang et al., 2023). These findings are consistent with previous results showing improved emulsifying properties of US-treated WPI (Shen et al., 2017; Shi et al., 2020).

The HE-treated WPI, while having the highest k_p among all samples, was found to have a slightly lower EAI than the US-treated WPI. This could be due to the larger particle size (Figure. 4) and higher viscosity of the solution (Figures. 5), which slow down their diffusion to the interface. However, the significantly higher surface hydrophobicity of the particles seems to compensate for this effect, improving their oil-water interfacial activity and thus the EAI. On the other hand, HE treatment increases the rigidity of protein aggregates through the induction of inter- and intramolecular β-sheet motifs, which hinders the rearrangement of the adsorbed particles (Table 2). This also strengthens and stabilizes the interfacial film, improving ESI (Dissanayake & Vasiljevic, 2009; S. Jiang et al., 2018; Raymundo et al., 1998; Zhou et al., 2021). It has been shown that the low surface-to-volume ratio of large aggregates makes it challenging for them to orient at the interface. The high viscosity also slows down the rearrangement process, preventing the migration of proteins throughout the oil-water interface, but it also contributes to a stronger interfacial layer, resulting in higher emulsion stability (Dickinson, 2010; S. Jiang et al., 2018; Lu et al., 2022; Voutsinas et al., 1983).

In contrast to US and HE, HHP treatment led to the lowest EAI and ESI (Figure. 8). The impact of HHP treatment on EAI may vary depending on the type of protein and the operating conditions, and may either increase or decrease it (Qin et al., 2013). For example, it has been reported that HHP treatment of rice bran protein hydrolysates increased both EAI and ESI up to a pressure of 200 MPa, but had an inverse effect at 300 MPa (S. Wang et al., 2021). HHP treatment of WPI, on the other hand, showed a reduction in its emulsifying property (Lee et al., 2006), which supports the findings of this study. According to Le Chatelier's principle, during the compression period, proteins experience a volume shrinkage which results in significant changes in their conformation and tertiary structure (C. Wang et al., 2021). These alterations adversely affect the capacity of proteins to adsorb and reorient at the oil-water interface. HHP treatment may also cause a decrease in the solubility of proteins, which is a key factor for their emulsifying activity (Dissanayake & Vasiljevic, 2009; Krešic et al., 2006; Qin et al., 2013).

Conclusion

This study aimed to investigate the impact of heat (HE), high power ultrasound (US), and high hydrostatic pressure (HHP) on the interfacial behavior and emulsifying properties of WPI. The results showed that HE treatment at 90 °C for 30 min disrupted tertiary and secondary structures, leading to the exposure of hydrophobic residues and the formation of large, viscous aggregates. This resulted in a slower diffusion rate, moderate emulsifying activity, and reduced the interfacial elasticity. On the other hand, US treatment at 20 kHz for 20 min, decreased particle size, increased surface hydrophobicity, and promoted α-helical conformation. These changes led to rapid adsorption, efficient interfacial reorganization, and the formation of an elastic interface, resulting in the highest emulsifying activity and emulsion stability. However, HHP treatment at 500 MPa for 20 min induced moderate unfolding without significantly altering viscosity, but weakened the interfacial film, slowing both penetration and rearrangement steps and ultimately reducing emulsification performance. Based on the unique effects of each treatment on the structure, bulk and interfacial rheological properties, and adsorption kinetics of WPI, it is concluded that by combining HE, US, and HHP treatments, it may be possible to overcome their limitations and improve overall emulsifying properties to meet final requirements.

CRediT Authorship Contribution Statement

Sara Keshvari Khojasteh: Investigation, Formal analysis, Data curation, Writing-original draft, Visualization. **Rassoul Kadkhodaee:** Supervision, Conceptualization, Methodology, Validation, Resources, Writing-review & editing, Project administration, Funding acquisition. **Christos Ritzoulis:** Supervision. **Behdad Shokrollahi Yancheshmeh:** Formal analysis, Data curation. **Mohammad Amin Mohammadifar:** Supervision. **Zahra Emam-Djomeh:** Supervision. **Bahareh Emadzadeh:** Supervision

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References

Ahmadi, Z., Razavi, S. M. A., & Varidi, M. (2017). Sequential ultrasound and transglutaminase treatments improve functional, rheological, and textural properties of whey protein concentrate. *Innovative Food Science & Emerging Technologies*, *43*, 207-215. https://doi.org/https://doi.org/10.1016/j.ifset.2017.08.013

Alves, A. C., Martha, L., Casanova, F., & Tavares, G. M. (2022). Structural and foaming properties of whey and soy protein isolates in mixed systems before and after heat treatment. *Food Science and Technology International*, *28*(6), 545-553. https://doi.org/https://doi.org/10.1177/10820132211031756

Aouzelleg, A., Bull, L. A., Price, N. C., & Kelly, S. M. (2004). Molecular studies of pressure/temperature-induced structural changes in bovine β-lactoglobulin. *Journal of the Science of Food and Agriculture*, *84*(5), 398-404. https://doi.org/https://doi.org/10.1002/jsfa.1669

Arzeni, C., Martínez, K., Zema, P., Arias, A., Pérez, O., & Pilosof, A. (2012). Comparative study of high intensity ultrasound effects on food proteins functionality. *Journal of Food Engineering*, *108*(3), 463-472. https://doi.org/https://doi.org/10.1016/j.jfoodeng.2011.08.018

Baier, D., Purschke, B., Schmitt, C., Rawel, H. M., & Knorr, D. (2015). Effect of high pressure–low temperature treatments on structural characteristics of whey proteins and micellar caseins. *Food chemistry*, *187*, 354-363. https://doi.org/https://doi.org/10.1016/j.foodchem.2015.04.049

Carullo, D., Barbosa-Cánovas, G., & Ferrari, G. (2021). Changes of structural and techno-functional properties of high hydrostatic pressure (HHP) treated whey protein isolate over refrigerated storage. *LWT*, *137*, 110436. https://doi.org/https://doi.org/10.1016/j.lwt.2020.110436

Cepero-Betancourt, Y., Opazo-Navarrete, M., Janssen, A. E., Tabilo-Munizaga, G., & Pérez-Won, M. (2020). Effects of high hydrostatic pressure (HHP) on protein structure and digestibility of red abalone (Haliotis rufescens) muscle. *Innovative Food Science & Emerging Technologies*, *60*, 102282. https://doi.org/https://doi.org/10.1016/j.ifset.2019.102282

Chandrapala, J., Zisu, B., Kentish, S., & Ashokkumar, M. (2012). The effects of high-intensity ultrasound on the structural and functional properties of α-Lactalbumin, β-Lactoglobulin and their mixtures. *Food Research International*, *48*(2), 940-943. https://doi.org/https://doi.org/10.1016/j.foodres.2012.02.021

Chandrapala, J., Zisu, B., Palmer, M., Kentish, S., & Ashokkumar, M. (2011). Effects of ultrasound on the thermal and structural characteristics of proteins in reconstituted whey protein concentrate. *Ultrasonics sonochemistry*, *18*(5), 951-957. https://doi.org/https://doi.org/10.1016/j.ultsonch.2010.12.016

Chang, L., Chen, B., & Rao, J. (2023). Synergistic effect of pH-shift and controlled heating on improving foaming properties of pea vicilin and its adsorption behavior at the air-water interface. *Food Hydrocolloids*, *145*, 109022. https://doi.org/https://doi.org/10.1016/j.foodhyd.2023.109022

Chang, L., Lan, Y., Chen, B., & Rao, J. (2023). Interfacial, and emulsifying properties nexus of green pea protein fractions: Impact of pH and salt. *Food Hydrocolloids*, *140*, 108652. https://doi.org/https://doi.org/10.1016/j.foodhyd.2023.108652

Chen, W., Ma, X., Wang, W., Lv, R., Guo, M., Ding, T.,…Liu, D. (2019). Preparation of modified whey protein isolate with gum acacia by ultrasound maillard reaction. *Food Hydrocolloids*, *95*, 298-307. https://doi.org/https://doi.org/10.1016/j.foodhyd.2018.10.030

Cheung, J. K., Shah, P., & Truskett, T. M. (2006). Heteropolymer collapse theory for protein folding in the pressure-temperature plane. *Biophysical journal*, *91*(7), 2427-2435. https://doi.org/https://doi.org/10.1529/biophysj.106.081802

Čurlej, J., Zajác, P., Čapla, J., Golian, J., Benešová, L., Partika, A.,…Jakabová, S. (2022). The effect of heat treatment on cow's milk protein profiles. *Foods*, *11*(7), 1023. https://doi.org/https://doi.org/10.3390/foods11071023

Dai, Q., Zhu, X., Yu, J., Karangwa, E., Xia, S., Zhang, X., & Jia, C. (2016). Mechanism of formation and stabilization of nanoparticles produced by heating electrostatic complexes of WPI–dextran conjugate and chondroitin sulfate. *Journal of agricultural and food chemistry*, *64*(27), 5539-5548. https://doi.org/https://doi.org/10.1021/acs.jafc.6b01213

Dehnad, D., Emadzadeh, B., Ghorani, B., & Rajabzadeh, G. (2023). High hydrostatic pressure (HHP) as a green technology opens up a new possibility for the fabrication of electrospun nanofibers: Part I-improvement of soy protein isolate properties by HHP. *Food Hydrocolloids*, *140*, 108659. https://doi.org/https://doi.org/10.1016/j.foodhyd.2023.108659

Denda, A., & Hayashi, R. (1992). Emulsifying properties of pressure-treated proteins. *Colloques-Insitut National De La Sante Et De La Recherche Medicale Colloques Et Seminaires*, 333-333.

Dickinson, E. (2010). Food emulsions and foams: Stabilization by particles. *Current Opinion in Colloid & Interface Science*, *15*(1-2), 40-49. https://doi.org/https://doi.org/10.1016/j.cocis.2009.11.001

Dissanayake, M., & Vasiljevic, T. (2009). Functional properties of whey proteins affected by heat treatment and hydrodynamic high-pressure shearing. *Journal of Dairy Science*, *92*(4), 1387-1397. https://doi.org/https://doi.org/10.3168/jds.2008-1791

Dziuba, J., Minkiewicz, P., & Darewicz, M. (2021). Milk proteins. In *Handbook of dairy foods analysis* (pp. 65-90). CRC Press.

Fan, Y., Peng, G., Pang, X., Wen, Z., & Yi, J. (2021). Physicochemical, emulsifying, and interfacial properties of different whey protein aggregates obtained by thermal treatment. *LWT*, *149*, 111904. https://doi.org/https://doi.org/10.1016/j.lwt.2021.111904

Frydenberg, R. P., Hammershøj, M., Andersen, U., Greve, M. T., & Wiking, L. (2016). Protein denaturation of whey protein isolates (WPIs) induced by high intensity ultrasound during heat gelation. *Food chemistry*, *192*, 415-423. https://doi.org/https://doi.org/10.1016/j.foodchem.2015.07.037

Fu, Y., Wang, J., Liu, X., Ni, Y., Niu, Z., Qiu, L.,…Wei, C. (2025). Effect on heat treatment on the physical stability, interfacial tension and in vitro digestion of whey protein-stabilized ω-6/ω-3 fatty acid balanced pumpkin seed oil complex condensate. *Food Hydrocolloids, 160*, 110728. https://doi.org/https://doi.org/10.1016/j.foodhyd.2024.110728

Funtenberger, S., Dumay, E., & Cheftel, J. (1995). Pressure-induced aggregation of β-lactoglobulin in pH 7.0 buffers. *LWT-Food Science and Technology*, *28*(4), 410-418. https://doi.org/https://doi.org/10.1016/0023-6438(95)90025-X

Galazka, V. B., Dickinson, E., & Ledward, D. (2000). Effect of high-pressure on surface behaviour of adsorbed films formed from mixtures of sulfated polysaccharides with various proteins. *Innovative Food Science & Emerging Technologies*, *1*(3), 177-185. https://doi.org/https://doi.org/10.1016/S1466-8564(00)00035-7

Galazka, V. B., Dickinson, E., & Ledward, D. A. (1996). Effect of high pressure on the emulsifying behaviour of β-lactoglobulin. *Food Hydrocolloids*, *10*(2), 213-219. https://doi.org/https://doi.org/10.1016/S0268-005X(96)80037-3

Galazka, V. B., Ledward, D. A., Dickinson, E., & Langey, K. R. (1995). High pressure effects on emulsifying behavior of whey protein concentrate. *Journal of Food Science*, *60*(6), 1341-1343. https://doi.org/https://doi.org/10.1111/j.1365-2621.1995.tb04587.x

Gao, F., Zhang, X., Wang, H., Sun, X., Wang, J., & Wang, C. (2019). Comparison of dry-and wet-heat induced changes in physicochemical properties of whey protein in absence or presence of inulin. *Food science and biotechnology*, *28*(5), 1367-1374. https://doi.org/https://doi.org/10.1007/s10068-019-00577-w

Graham, D. E., & Phillips, M. (1979). Proteins at liquid interfaces: III. Molecular structures of adsorbed films. *Journal of Colloid and Interface Science*, *70*(3), 427-439. https://doi.org/https://doi.org/10.1016/0021-9797(79)90050-X

Guo, M. (2019). *Whey protein production, chemistry, functionality, and applications*. John Wiley & Sons. https://doi.org/https://doi.org/10.1002/9781119256052

Guo, N., Ye, S., Zhou, G., Zhang, Y., Zhang, F., Xu, J.,…Wang, Z. (2023). Effect of ultrasound treatment on interactions of whey protein isolate with rutin. *Ultrasonics sonochemistry, 95*, 106387. https://doi.org/https://doi.org/10.1016/j.ultsonch.2023.106387

Hamada, D., Segawa, S.-i., & Goto, Y. (1996). Non-native α-helical intermediate in the refolding of β-lactoglobulin, a predominantly β-sheet protein. *Nature structural biology*, *3*(10), 868-873. https://doi.org/https://doi.org/10.1038/nsb1096-868

He, Y., & Vardhanabhuti, B. (2021). Improved heat stability of whey protein isolate by glycation with inulin. *Dairy*, *2*(1), 135-147. https://doi.org/ https://doi.org/10.3390/dairy2010013

Iametti, S., Transidico, P., Bonomi, F., Vecchio, G., Pittia, P., Rovere, P., & Dall'Aglio, G. (1997). Molecular modifications of β-lactoglobulin upon exposure to high pressure. *Journal of agricultural and food chemistry*, *45*(1), 23-29. https://doi.org/https://doi.org/10.1021/jf960330w

Jadhav, H. B., Das, M., Das, A., Geetha, V., Choudhary, P., Annapure, U., & Alaskar, K. (2024). Enhancing the functionality of plant-based proteins with the application of ultrasound–A Review. *Measurement: Food*, 100139. https://doi.org/https://doi.org/10.1016/j.meafoo.2024.100139

Jiang, S., Cheng, J., Jiang, Z., Geng, H., Sun, Y., Sun, C., & Hou, J. (2018). Effect of heat treatment on physicochemical and emulsifying properties of polymerized whey protein concentrate and polymerized whey protein isolate. *LWT*, *98*, 134-140. https://doi.org/https://doi.org/10.1016/j.lwt.2018.08.028

Jiang, Z., Yao, K., Yuan, X., Mu, Z., Gao, Z., Hou, J., & Jiang, L. (2018). Effects of ultrasound treatment on physico-chemical, functional properties and antioxidant activity of whey protein isolate in the presence of calcium lactate. *Journal of the Science of Food and Agriculture*, *98*(4), 1522-1529. https://doi.org/https://doi.org/10.1002/jsfa.8623

Jin, H., Sun, Y., Pan, J., Fang, Y., Jin, Y., & Sheng, L. (2022). Adsorption kinetics of ovalbumin and lysozyme at the air-water interface and foam properties at neutral pH. *Food Hydrocolloids*, *124*, 107352. https://doi.org/https://doi.org/10.1016/j.foodhyd.2021.107352

Joeres, E., Drusch, S., Töpfl, S., Juadjur, A., Psathaki, O. E., Heinz, V., & Terjung, N. (2023). Formation of amyloid fibrils from ovalbumin under Ohmic heating. *Heliyon*, *9*(11). https://doi.org/https://doi.org/10.1016/j.heliyon.2023.e22061

Joshi, V., Shivach, T., Yadav, N., & Rathore, A. S. (2014). Circular dichroism spectroscopy as a tool for monitoring aggregation in monoclonal antibody therapeutics. *Analytical chemistry*, *86*(23), 11606-11613. https://doi.org/https://doi.org/10.1021/ac503140j

Joyce, A. M., Kelly, A. L., & O'Mahony, J. A. (2018). Controlling denaturation and aggregation of whey proteins during thermal processing by modifying temperature and calcium concentration. *International Journal of Dairy Technology*, *71*(2), 446-453. https://doi.org/ https://doi.org/10.1111/1471-0307.12507

Kadkhodaee, R., & Povey, M. J. (2008). Ultrasonic inactivation of Bacillus α-amylase. I. Effect of gas content and emitting face of probe. *Ultrasonics sonochemistry*, *15*(2), 133-142. https://doi.org/https://doi.org/10.1016/j.ultsonch.2007.02.005

Kang, Z.-L., Shang, X.-Y., Li, Y.-P., & Ma, H.-J. (2022). Effect of ultrasound-assisted sodium bicarbonate treatment on aggregation and conformation of reduced-salt pork myofibrillar protein. *Molecules*, *27*(21), 7493. https://doi.org/https://doi.org/10.3390/molecules27217493

Kanno, C., Mu, T.-H., Hagiwara, T., Ametani, M., & Azuma, N. (1998). Gel formation from industrial milk whey proteins under hydrostatic pressure: effect of hydrostatic pressure and protein concentration. *Journal of agricultural and food chemistry*, *46*(2), 417-424. https://doi.org/https://doi.org/10.1021/jf970652f

Kato, A., & Nakai, S. (1980). Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. *Biochimica et biophysica acta (BBA)-Protein structure*, *624*(1), 13-20. https://doi.org/https://doi.org/10.1016/0005-2795(80)90220-2

Khatkar, A. B., Kaur, A., Khatkar, S. K., & Mehta, N. (2018). Characterization of heat-stable whey protein: Impact of ultrasound on rheological, thermal, structural and morphological properties. *Ultrasonics sonochemistry*, *49*, 333-342. https://doi.org/https://doi.org/10.1016/j.ultsonch.2018.08.026

Kheto, A., Adhikary, U., Dhua, S., Sarkar, A., Kumar, Y., Vashishth, R.,…Saxena, D. (2025). A review on advancements in emerging processing of whey protein: Enhancing functional and nutritional properties for functional food applications. *Food Safety and Health*, *3*(1), 23-45. https://doi.org/https://doi.org/10.1002/fsh3.12067

Khojasteh, S. K., Kadkhodaee, R., Ritzoulis, C., Emam-Djomeh, Z., & Emadzadeh, B. (2025). A comparative study on the effects of heat, ultrasound and high hydrostatic pressure on the structural and physicochemical properties of whey proteins. *Applied Food Research*, 101168. https://doi.org/https://doi.org/10.1016/j.afres.2025.101168

Krešic, G., Lelas, V., Herceg, Z., & Režek, A. (2006). Effects of high pressure on functionality of whey protein concentrate and whey protein isolate. *Le Lait*, *86*(4), 303-315. https://doi.org/https://doi.org/10.1051/lait:2006012

Lee, W., Clark, S., & Swanson, B. (2006). Functional properties of high hydrostatic pressure-treated whey protein. *Journal of food processing and preservation*, *30*(4), 488-501. https://doi.org/https://doi.org/10.1111/j.1745-4549.2005.00081.x

Li, C., Huang, X., Peng, Q., Shan, Y., & Xue, F. (2014). Physicochemical properties of peanut protein isolate–glucomannan conjugates prepared by ultrasonic treatment. *Ultrasonics sonochemistry*, *21*(5), 1722-1727. https://doi.org/https://doi.org/10.1016/j.ultsonch.2014.03.018

Li, H., Zhao, T., Li, H., & Yu, J. (2021). Effect of heat treatment on the property, structure, and aggregation of skim milk proteins. *Frontiers in nutrition*, *8*, 714869.

Lim, S.-Y., Swanson, B., Ross, C., & Clark, S. (2008). High hydrostatic pressure modification of whey protein concentrate for improved body and texture of lowfat ice cream. *Journal of Dairy Science*, *91*(4), 1308-1316. https://doi.org/https://doi.org/10.3168/jds.2007-0391

Liu, F., & Tang, C.-H. (2016). Soy glycinin as food-grade Pickering stabilizers: Part. I. Structural characteristics, emulsifying properties and adsorption/arrangement at interface. *Food Hydrocolloids*, *60*, 606-619. https://doi.org/https://doi.org/10.1016/j.foodhyd.2015.04.025

Liu, H., Xu, Y., Zu, S., Wu, X., Shi, A., Zhang, J.,…He, N. (2021). Effects of high hydrostatic pressure on the conformational structure and gel properties of myofibrillar protein and meat quality: A review. *Foods, 10*(8), 1872. https://doi.org/https://doi.org/10.3390/foods10081872

Liu, X., Powers, J. R., Swanson, B. G., Hill, H. H., & Clark, S. (2005). Modification of whey protein concentrate hydrophobicity by high hydrostatic pressure. *Innovative Food Science & Emerging Technologies, 6*(3), 310-317. https://doi.org/https://doi.org/10.1016/j.ifset.2005.03.006

Loushigam, G. L., Sari, T., Badgujar, P. C., Dige, M. S., & Pareek, S. (2025). Extraction and functional properties modification of guar protein isolates by ultrasound: A promising alternative protein. *Food Structure, 44*, 100420.

Lu, J., Xu, X., & Zhao, X. (2022). Interfacial rheology of alkali pH-shifted myofibrillar protein at O/W interface and impact of Tween 20 displacement. *Food Hydrocolloids, 124*, 107275. https://doi.org/https://doi.org/10.1016/j.foodhyd.2021.107275

Lucassen, J., & Van Den Tempel, M. (1972). Dynamic measurements of dilational properties of a liquid interface. *Chemical Engineering Science, 27*(6), 1283-1291. https://doi.org/https://doi.org/10.1016/0009-2509(72)80104-0

Ma, H., Liu, D., Cheng, Y., Raynaldo, F. A., Dabbour, M., Chao, J.,…Yang, S. (2024). Ultrasound viscous reduction effects on the proteolysis of soy protein isolate at a limited degree of hydrolysis: Changes in the functional characteristics and protein structure. *Ultrasonics sonochemistry, 104*, 106847. https://doi.org/https://doi.org/10.1016/j.ultsonch.2024.106847

Ma, Y., Xu, J., Guo, R., Teng, G., Chen, Y., & Xu, X. (2023). In vitro gastrointestinal model for the elderly: Effect of high hydrostatic pressure on protein structures and antioxidant activities of whey protein isolate. *Food Bioscience, 52*, 102452. https://doi.org/https://doi.org/10.1016/j.fbio.2023.102452

McClements, D. J. (2004). Protein-stabilized emulsions. *Current Opinion in Colloid & Interface Science, 9*(5), 305-313. https://doi.org/https://doi.org/10.1016/j.cocis.2004.09.003

Meng, Y., Liang, Z., Zhang, C., Hao, S., Han, H., Du, P.,…Liu, L. (2021). Ultrasonic modification of whey protein isolate: Implications for the structural and functional properties. *LWT, 152*, 112272. https://doi.org/https://doi.org/10.1016/j.lwt.2021.112272

Minj, S., & Anand, S. (2020). Whey proteins and its derivatives: Bioactivity, functionality, and current applications. *Dairy, 1*(3), 233-258. https://doi.org/https://doi.org/10.3390/dairy1030016

Moghadam, M., Salami, M., Mohammadian, M., Delphi, L., Sepehri, H., Emam-Djomeh, Z., & Moosavi-Movahedi, A. A. (2020). Walnut protein–curcumin complexes: fabrication, structural characterization, antioxidant properties, and in vitro anticancer activity. *Journal of Food Measurement and Characterization, 14*(2), 876-885. https://doi.org/https://doi.org/10.1007/s11694-019-00336-9

Mohammadian, M., Salami, M., Momen, S., Alavi, F., Emam-Djomeh, Z., & Moosavi-Movahedi, A. A. (2019). Enhancing the aqueous solubility of curcumin at acidic condition through the complexation with whey protein nanofibrils. *Food Hydrocolloids, 87*, 902-914. https://doi.org/https://doi.org/10.1016/j.foodhyd.2018.09.001

Momen, S., Salami, M., Emam-Djomeh, Z., Hosseini, E., Sheibani, N., & Moosavi-Movahedi, A. A. (2018). Effect of dry heating on physico-chemical, functional properties and digestibility of camel whey protein. *International Dairy Journal, 86*, 9-20. https://doi.org/https://doi.org/10.1016/j.idairyj.2018.06.015

Mushtaq, M., Ayoub, A., Banday, J. A., Rashid, A., Rasheed, N., & Gani, A. (2025). Nano-reduction of whey protein using ultra-sonication as a novel approach to improve its applicability in food industry. *Ultrasonics sonochemistry*, 107230. https://doi.org/https://doi.org/10.1016/j.ultsonch.2025.107230

Narayanan, C., & Dias, C. L. (2013). Hydrophobic interactions and hydrogen bonds in β-sheet formation. *The journal of chemical physics*, *139*(11), 09B640_641. https://doi.org/https://doi.org/10.1063/1.4821596

O'Sullivan, J., Arellano, M., Pichot, R., & Norton, I. (2014). The effect of ultrasound treatment on the structural, physical and emulsifying properties of dairy proteins. *Food Hydrocolloids*, *42*, 386-396. https://doi.org/https://doi.org/10.1016/j.foodhyd.2014.05.011

O'sullivan, J., Murray, B., Flynn, C., & Norton, I. (2016). The effect of ultrasound treatment on the structural, physical and emulsifying properties of animal and vegetable proteins. *Food Hydrocolloids*, *53*, 141-154. https://doi.org/https://doi.org/10.1016/j.foodhyd.2015.02.009

O'Loughlin, I. B., Murray, B. A., Kelly, P., Fitzgerald, R. J., & Brodkorb, A. (2012). Enzymatic hydrolysis of heat-induced aggregates of whey protein isolate. *Journal of agricultural and food chemistry*, *60*(19), 4895-4904. https://doi.org/https://doi.org/10.1021/jf205213n

O'Sullivan, J., Beevers, J., Park, M., Greenwood, R., & Norton, I. (2015). Comparative assessment of the effect of ultrasound treatment on protein functionality pre-and post-emulsification. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *484*, 89-98. https://doi.org/https://doi.org/10.1016/j.colsurfa.2015.07.065

Ortiz-Deleón, A. M., Román-Guerrero, A., Sandoval-Castilla, O., & Cuevas-Bernardino, J. C. (2024). Characterization of O/W emulgels based on whey protein-alginate-inulin coacervates: Influence of temperature and ultrasound as protein preconditioning process. *International Journal of Biological Macromolecules*, *265*, 131260. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2024.131260

Pang, Z.-H., Zhu, J., Wu, W.-J., Wang, F., Ren, F.-Z., Zhang, L.-D., & Guo, H.-Y. (2011). The secondary structure of heated whey protein and its hydrolysates antigenicity. *Spectroscopy and Spectral Analysis*, *31*(11), 3055-3059. https://doi.org/ https://doi.org/10.3964/j.issn.1000-0593(2011)11-3055-05

Panizzolo, L. A., Mussio, L. E., & Añón, M. C. (2014). A kinetic model for describing the effect of proteins on the air-water interface tension. https://doi.org/https://doi.org/10.17265/2159-5828/2014.06.003

Paul, A., Martin, F., Simard, B., Scher, J., Gaiani, C., le Floch-Fouere, C.,…Burgain, J. (2023). Deciphering the segregation of proteins in high-protein dairy powders after spray-drying. *Journal of Dairy Science*, *106*(2), 843-851. https://doi.org/https://doi.org/10.3168/jds.2022-22133

Perticaroli, S., Nickels, J. D., Ehlers, G., O'Neill, H., Zhang, Q., & Sokolov, A. P. (2013). Secondary structure and rigidity in model proteins. *Soft Matter*, *9*(40), 9548-9556. https://doi.org/https://doi.org/10.1039/C3SM50807B

Perticaroli, S., Nickels, J. D., Ehlers, G., & Sokolov, A. P. (2014). Rigidity, secondary structure, and the universality of the boson peak in proteins. *Biophysical journal*, *106*(12), 2667-2674. https://doi.org/https://doi.org/10.1016/j.bpj.2014.05.009

Pittia, P., Wilde, P. J., Husband, F. A., & Clark, D. C. (1996). Functional and structural properties of β-lactoglobulin as affected by high pressure treatment. *Journal of Food*

Science, *61*(6), 1123-1128. https://doi.org/ https://doi.org/10.1111/j.1365-2621.1996.tb10944.x

Qian, S., Lan, T., Zhao, X., Song, T., Cao, Y., Zhang, H., & Liu, J. (2023). Mechanism of ultrasonic combined with different fields on protein complex system and its effect on its functional characteristics and application: A review. *Ultrasonics sonochemistry*, *98*, 106532. https://doi.org/https://doi.org/10.1016/j.ultsonch.2023.106532

Qin, X., Liu, G., Zheng, W., He, J., Zhu, Z., & Lamikanra, O. (2016). Heat stability improvement of whey protein isolate via glycation with maltodextrin without control of the relative humidity. *Rsc Advances*, *6*(48), 41785-41792. https://doi.org/https://doi.org/10.1039/C5RA26137F

Qin, Z., Guo, X., Lin, Y., Chen, J., Liao, X., Hu, X., & Wu, J. (2013). Effects of high hydrostatic pressure on physicochemical and functional properties of walnut (Juglans regia L.) protein isolate. *Journal of the Science of Food and Agriculture*, *93*(5), 1105-1111. https://doi.org/https://doi.org/10.1002/jsfa.5857

Raymundo, A., Franco, J., Gallegos, C., Empis, J., & Sousa, I. (1998). Effect of thermal denaturation of lupin protein on its emulsifying properties. *Food/Nahrung*, *42*(03-04), 220-224. https://doi.org/https://doi.org/10.1002/(SICI)1521-3803(199808)42:03/04<220::AID-FOOD220>3.0.CO;2-Q

Sajedi, M., Nasirpour, A., Keramat, J., & Desobry, S. (2014). Effect of modified whey protein concentrate on physical properties and stability of whipped cream. *Food Hydrocolloids*, *36*, 93-101. https://doi.org/https://doi.org/10.1016/j.foodhyd.2013.09.007

Schröder, A., Berton-Carabin, C., Venema, P., & Cornacchia, L. (2017). Interfacial properties of whey protein and whey protein hydrolysates and their influence on O/W emulsion stability. *Food Hydrocolloids*, *73*, 129-140. https://doi.org/https://doi.org/10.1016/j.foodhyd.2017.06.001

Shen, X., Shao, S., & Guo, M. (2017). Ultrasound-induced changes in physical and functional properties of whey proteins. *International Journal of Food Science and Technology*, *52*(2), 381-388.

Shi, R., Liu, Y., Hu, J., Gao, H., Qayum, A., Bilawal, A.,…Hou, J. (2020). Combination of high-pressure homogenization and ultrasound improves physiochemical, interfacial and gelation properties of whey protein isolate. *Innovative Food Science & Emerging Technologies*, *65*, 102450. https://doi.org/https://doi.org/10.1016/j.ifset.2020.102450

Silva, M., Zisu, B., & Chandrapala, J. (2018). Influence of low-frequency ultrasound on the physico-chemical and structural characteristics of milk systems with varying casein to whey protein ratios. *Ultrasonics sonochemistry*, *49*, 268-276. https://doi.org/https://doi.org/10.1016/j.ultsonch.2018.08.015

Sljoka, A. (2022). Structural and functional analysis of proteins using rigidity theory. *Sublinear Computation Paradigm: Algorithmic Revolution in the Big Data Era*, 337-367. https://doi.org/https://doi.org/10.1007/978-981-16-4095-7_14

Sobhaninia, M., Nasirpour, A., Shahedi, M., & Golkar, A. (2017). Oil-in-water emulsions stabilized by whey protein aggregates: Effect of aggregate size, pH of aggregation and emulsion pH. *Journal of Dispersion Science and Technology*, *38*(9), 1366-1373. https://doi.org/https://doi.org/10.1080/01932691.2016.1224719

Spotti, M. J., Tarhan, Ö., Schaffter, S., Corvalan, C., & Campanella, O. H. (2017). Whey protein gelation induced by enzymatic hydrolysis and heat treatment: Comparison of creep and recovery behavior. *Food Hydrocolloids, 63,* 696-704. https://doi.org/https://doi.org/10.1016/j.foodhyd.2016.10.014

Subasi, B. G., Yildirim-Elikoğlu, S., Altay, İ., Jafarpour, A., Casanova, F., Mohammadifar, M. A., & Capanoglu, E. (2022). Influence of non-thermal microwave radiation on emulsifying properties of sunflower protein. *Food chemistry, 372,* 131275. https://doi.org/https://doi.org/10.1016/j.foodchem.2021.131275

Tan, M., Xu, J., Gao, H., Yu, Z., Liang, J., Mu, D.,…Zhao, Y. (2021). Effects of combined high hydrostatic pressure and pH-shifting pretreatment on the structure and emulsifying properties of soy protein isolates. *Journal of Food Engineering, 306,* 110622. https://doi.org/https://doi.org/10.1016/j.jfoodeng.2021.110622

Tian, Y., Zhang, Z., Zhang, P., Taha, A., Hu, H., & Pan, S. (2020). The role of conformational state of pH-shifted β-conglycinin on the oil/water interfacial properties and emulsifying capacities. *Food Hydrocolloids*, *108*, 105990. https://doi.org/https://doi.org/10.1016/j.foodhyd.2020.105990

Tomczyńska-Mleko, M., Kamysz, E., Sikorska, E., Puchalski, C., Mleko, S., Ozimek, L.,…Wesołowska-Trojanowska, M. (2014). Changes of secondary structure and surface tension of whey protein isolate dispersions upon pH and temperature. *Czech Journal of Food Sciences*, *32*(1), 82-89. https://doi.org/https://doi.org/10.17221/326/2012-CJFS

Vargas, S. A., Delgado-Macuil, R.-J., Ruiz-Espinosa, H., Rojas-López, M., & Amador-Espejo, G.-G. (2021a). High-intensity ultrasound pretreatment influence on whey protein isolate and its use on complex coacervation with kappa carrageenan: Evaluation of selected functional properties. *Ultrasonics sonochemistry*, *70*, 105340. https://doi.org/https://doi.org/10.1016/j.ultsonch.2020.105340

Vargas, S. A., Delgado-Macuil, R., Ruiz-Espinosa, H., Rojas-López, M., & Amador-Espejo, G. (2021b). High-intensity ultrasound pretreatment influence on whey protein isolate and its use on complex coacervation with kappa carrageenan: Evaluation of selected functional properties. *Ultrasonics sonochemistry, 70*, 105340. https://doi.org/https://doi.org/10.1016/j.ultsonch.2020.105340

Viseu, M. I., Melo, E. P., Carvalho, T. I., Correia, R. F., & Costa, S. M. (2007). Unfolding kinetics of β-lactoglobulin induced by surfactant and denaturant: A stopped-flow/fluorescence study. *Biophysical journal*, *93*(10), 3601-3612. https://doi.org/https://doi.org/10.1529/biophysj.106.101667

Voutsinas, L. P., Cheung, E., & Nakai, S. (1983). Relationships of hydrophobicity to emulsifying properties of heat denatured proteins. *Journal of Food Science*, *48*(1), 26-32. https://doi.org/ https://doi.org/10.1111/j.1365-2621.1983.tb14781.x

Wagner, J., Biliaderis, C. G., & Moschakis, T. (2020). Whey proteins: Musings on denaturation, aggregate formation and gelation. *Critical reviews in food science and nutrition*, *60*(22), 3793-3806. https://doi.org/https://doi.org/10.1080/10408398.2019.1708263

Wang, C., Yin, H., Zhao, Y., Zheng, Y., Xu, X., & Yue, J. (2021). Optimization of high hydrostatic pressure treatments on soybean protein isolate to improve its functionality and evaluation of its application in yogurt. *Foods*, *10*(3), 667. https://doi.org/https://doi.org/10.3390/foods10030667

Wang, J., de Figueiredo Furtado, G., Monthean, N., Dupont, D., Pédrono, F., & Madadlou, A. (2020). CaCl2 supplementation of hydrophobised whey proteins: Assessment of protein particles and consequent emulsions. *International Dairy Journal*, *110*, 104815. https://doi.org/https://doi.org/10.1016/j.idairyj.2020.104815

Wang, M., Ettelaie, R., & Sarkar, A. (2024). Enzymatic hydrolysis of legume proteins: Lessons on surface property outcomes. *Current Opinion in Food Science*, 101259. https://doi.org/https://doi.org/10.1016/j.cofs.2024.101259

Wang, S., Wang, T., Sun, Y., Cui, Y., Yu, G., & Jiang, L. (2021). Effects of high hydrostatic pressure pretreatment on the functional and structural properties of rice bran protein hydrolysates. *Foods*, *11*(1), 29. https://doi.org/https://doi.org/10.3390/foods11010029

Wang, Y., Li, B., Guo, Y., Liu, C., Liu, J., Tan, B.,…Jiang, L. (2022). Effects of ultrasound on the structural and emulsifying properties and interfacial properties of oxidized soybean protein aggregates. *Ultrasonics sonochemistry*, *87*, 106046. https://doi.org/https://doi.org/10.1016/j.ultsonch.2022.106046

Wong, B. T., Zhai, J., Hoffmann, S. V., Aguilar, M.-I., Augustin, M., Wooster, T. J., & Day, L. (2012). Conformational changes to deamidated wheat gliadins and β-casein upon adsorption to oil–water emulsion interfaces. *Food Hydrocolloids*, *27*(1), 91-101. https://doi.org/https://doi.org/10.1016/j.foodhyd.2011.08.012

Wooster, T. J., & Augustin, M. A. (2007). Rheology of whey protein–dextran conjugate films at the air/water interface. *Food Hydrocolloids*, *21*(7), 1072-1080. https://doi.org/https://doi.org/10.1016/j.foodhyd.2006.07.015

Xie, H., Wei, X., Liu, X., Bai, W., & Zeng, X. (2023). Effect of polyphenolic structure and mass ratio on the emulsifying performance and stability of emulsions stabilized by polyphenol-corn amylose complexes. *Ultrasonics sonochemistry*, *95*, 106367. https://doi.org/https://doi.org/10.1016/j.ultsonch.2023.106367

Xiong, T., Xiong, W., Ge, M., Xia, J., Li, B., & Chen, Y. (2018). Effect of high intensity ultrasound on structure and foaming properties of pea protein isolate. *Food Research International*, *109*, 260-267. https://doi.org/https://doi.org/10.1016/j.foodres.2018.04.044

Xiong, Y., Li, Q., Miao, S., Zhang, Y., Zheng, B., & Zhang, L. (2019). Effect of ultrasound on physicochemical properties of emulsion stabilized by fish myofibrillar protein and xanthan gum. *Innovative Food Science & Emerging Technologies*, *54*, 225-234. https://doi.org/https://doi.org/10.1016/j.ifset.2019.04.013

Yan, S., Xu, J., Zhang, S., & Li, Y. (2021). Effects of flexibility and surface hydrophobicity on emulsifying properties: Ultrasound-treated soybean protein isolate. *LWT*, *142*, 110881. https://doi.org/https://doi.org/10.1016/j.lwt.2021.110881

Yang, J., Dunker, A. K., Powers, J. R., Clark, S., & Swanson, B. G. (2001). β-Lactoglobulin molten globule induced by high pressure. *Journal of agricultural and food chemistry*, *49*(7), 3236-3243. https://doi.org/https://doi.org/10.1021/jf001226o

Yang, J., Huang, F., Huang, Q., Ma, D., Chen, Y., Peng, D.,…Geng, F. (2023). Physical and emulsifying properties of pea protein: influence of combined physical modification by flaxseed gum and ultrasonic treatment. *Food Science and Human Wellness*, *12*(2), 431-441. https://doi.org/https://doi.org/10.1016/j.fshw.2022.07.045

Yin, X., Fan, C., Zhang, L., Hu, H., Peng, B., & Zhang, Z. (2022). Structural and rheological behavior of β-lactoglobulins influenced by high hydrostatic pressure–From a single molecule to the aggregates. *Food Hydrocolloids*, 107622. https://doi.org/https://doi.org/10.1016/j.foodhyd.2022.107622

Yu, M., Le Floch-Fouéré, C., Pauchard, L., Boissel, F., Fu, N., Chen, X. D.,…Lanotte, L. (2021). Skin layer stratification in drying droplets of dairy colloids. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *620*, 126560. https://doi.org/https://doi.org/10.1016/j.colsurfa.2021.126560

Zhan, F., Li, J., Youssef, M., & Li, B. (2021). Enhancement of foam stability parallel with foamability of the foam stabilized by sodium caseinate-based complex: Octenyl succinate starch acting a dual role. *Food Hydrocolloids*, *113*, 106479. https://doi.org/https://doi.org/10.1016/j.foodhyd.2020.106479

Zhang, J., Wang, J., Li, M., Guo, S., & Lv, Y. (2022). Effects of heat treatment on protein molecular structure and in vitro digestion in whole soybeans with different moisture content. *Food Research International*, *155*, 111115. https://doi.org/https://doi.org/10.1016/j.foodres.2022.111115

Zhang, R., Zhang, Z., Zhang, H., Decker, E. A., & McClements, D. J. (2015). Influence of emulsifier type on gastrointestinal fate of oil-in-water emulsions containing anionic dietary fiber (pectin). *Food Hydrocolloids*, *45*, 175-185. https://doi.org/https://doi.org/10.1016/j.foodhyd.2014.11.020

Zhang, T., Zhao, Y., Tian, X., Liu, J., Ye, H., & Shen, X. (2021). Effect of ultrasound pretreatment on structural, physicochemical, rheological and gelation properties of transglutaminase cross-linked whey protein soluble aggregates. *Ultrasonics sonochemistry*, *74*, 105553. https://doi.org/https://doi.org/10.1016/j.ultsonch.2021.105553

Zhang, X., Sun, X., Gao, F., Wang, J., & Wang, C. (2019). Systematical characterization of physiochemical and rheological properties of thermal-induced polymerized whey protein. *Journal of the Science of Food and Agriculture*, *99*(2), 923-932. https://doi.org/https://doi.org/10.1002/jsfa.9264

Zhang, Z., & Liu, Y. (2017). Recent progresses of understanding the viscosity of concentrated protein solutions. *Current opinion in chemical engineering*, *16*, 48-55. https://doi.org/https://doi.org/10.1016/j.coche.2017.04.001

Zhao, F., Liu, X., Lian, M., Yang, Y., Li, C., Xu, H.,…Wang, W. (2022). Effects of high hydrostatic pressure on physicochemical and functional properties of soybean protein isolate. *Food Science and Technology*, *43*. https://doi.org/https://doi.org/10.1590/fst.95422

Zhao, X., Cheng, M., Zhang, X., Li, X., Chen, D., Qin, Y.,…Wang, C. (2020). The effect of heat treatment on the microstructure and functional properties of whey protein from goat milk. *Journal of Dairy Science*, *103*(2), 1289-1302. https://doi.org/https://doi.org/10.3168/jds.2019-17221

Zhou, B., Tobin, J. T., Drusch, S., & Hogan, S. A. (2021). Dynamic adsorption and interfacial rheology of whey protein isolate at oil-water interfaces: Effects of protein concentration, pH and heat treatment. *Food Hydrocolloids*, *116*, 106640. https://doi.org/https://doi.org/10.1016/j.foodhyd.2021.106640

Zhou, L., Wu, F., Zhang, X., & Wang, Z. (2017). Structural and functional properties of Maillard reaction products of protein isolate (mung bean, Vigna radiate (L.)) with dextran. *International journal of food properties*, *20*(sup2), 1246-1258. https://doi.org/ https://doi.org/10.1080/10942912.2017.1338727

Zhu, Q., Wang, H., Li, Y., Yu, H., Pei, J., Chen, H., & Chen, W. (2024). Dynamic interfacial adsorption and emulsifying performance of self-assembled coconut protein and fucoidan mixtures. *International Journal of Biological Macromolecules*, *276*, 133928. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2024.133928