Effects of ultrasound on the techno-functional properties of milk proteins: A systematic review

Sajad Shokri, Fardin Javanmardi, Mehrdad Mohammadi, Amin Mousavi Khaneghah

ABSTRACT

Techno-functional properties of proteins, including foaming capacity, water holding capacity, solubility, emulsifying properties, and gelling formation, are known to play an important role in food processing technologies. This study aimed to understand if ultrasound can influence these properties. Scopus, Web of Science, PubMed, Google Scholar, ProQuest, and FSTA databases were searched to find all related articles from 2000 to 2021. The results showed that ultrasound can modify these properties. However, further studies are required to reach conclusive results that permit the employment of ultrasound to improve the techno-functional properties of milk proteins.

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- Milk proteins
- Techno-functional properties
- Systematic review

1. Introduction

Proteins are known as macronutrients that, in addition to their nutritional role and supply of essential acids to the body, they have different functional properties in the food matrix [1]. Functional properties include the physical and chemical properties of proteins that determine the behavior of proteins during food processing. These properties include foaming capacity, water holding capacity, solubility, emulsifying properties, and gelling formation. Functional properties of proteins are affected by pH, temperature, ionic strength, presence of other ingredients, presence of reducing agents, and physical, chemical, or enzymatic modifications [2]. During food processing, some processes alter the functional properties of proteins due to changes in protein structure, unfolding, aggregation or flocculation, and hydrophobic group exposures. These factors alter the techno-functional properties of proteins, which ultimately lead to changes in food texture [3].

Milk proteins are widely used in the food industry due to their high nutritional value and functional properties. They are suitable for water binding, foaming, emulsifying, and gelling purposes [4]. Also, milk proteins, especially whey proteins, are used in many products such as pasta, ice cream, cakes, and desserts. Therefore, these proteins must be stable during mixing, heating, and drying and maintain their structure without compromising food quality [5].

Today, due to consumers' increasing demand for high-quality food products, emerging technologies such as ultrasound, high-pressure treatment, pulsed electric fields, microwave, irradiation, and radiofrequency have grown significantly in food science [6,7]. These technologies aim to maintain the quality and safety of the product with high nutritional properties and minimal processing [8]. Ultrasound, among others, has attracted particular attention due to its broad applicability in food industries. Ultrasound has been used for several purposes, such as emulsification [9] and extraction [10]. Ultrasound also has a great potential in food safety and can improve and facilitate food processes without compromising food quality [11–15].

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Several traditional reviews have been conducted on the effect of ultrasound on the physical and chemical properties of milk proteins [8,16–18]. To the best of our knowledge, no systematic review has been performed on this subject. A comprehensive study has not yet been conducted that collect all the studies on this subject and extract and analyze the results. Therefore, this study aimed to extract all the articles from different databases using systematic search and appropriate keywords. After combining the results, we aimed to determine the effectiveness of ultrasound on each of the functional properties of milk proteins.

2. Search strategy

2.1. Searching methods and strategy

This study conducted a comprehensive search to find all related articles in Scopus, Web of Science, PubMed, Google Scholar, ProQuest, and FSTA (Food Science and Technology Abstracts) databases. First, synonyms and related keywords were selected for the “ultrasound” and “milk protein” components using Mesh terms at PubMed and Emtree at Embase database. Therefore, the following keywords were selected: “Milk protein*” OR “whey protein” OR casein* OR “sodium caseinate” OR “dairy protein*”; “Emerging technologies” OR “Emerging processing” OR “Emerging techniques” OR ultrasound OR ultrasonic* OR sonication OR “non-thermal processing” OR “non-thermal techniques” OR “non-thermal processing.”

Fig. 1. Scheme of study design describing the systematic review and meta-analysis process based on PRISMA.
Table 1

| Study | Country | US condition | Protein type | Sonication time (min) | Particle size (µm) | Significancy |
|-------|---------|--------------|--------------|-----------------------|-------------------|-------------|
|       |         |              |              | Control | Treatment | Control | Treatment |
| Gordon and Pilosof (1) | Argentina | A high-intensity ultrasonic processor | WPI | 0 | 0.733 nm | – | – |
|       |         | Frequency: 20 kHz; 13 mm probe | (7.5% w/w) | 2 | 0.359 S | – | – |
|       |         | Frequency: 20 kHz; 13 mm probe | 5 | 0.252 S | – | – |
|       |         | Frequency: 20 kHz; 13 mm probe | 10 | 0.26 S | – | – |
|       |         | Frequency: 20 kHz; 13 mm probe | 20 | 0.207 S | – | – |
| O’Sullivan, Beever (2) | United Kingdom | Ultrasonic processor Viber Cell 750 W | MPI | 2 | 44 | – | – |
|       |         | Frequency: 20 kHz | (1% w/w) | 20 | 0.327 | – | – |
| Arzeni, Martinez (3) | Argentina | Ultrasonic processor Viber Cell 750 W | WPC | 20 | 0.259 S | – | – |
|       |         | Frequency: 20 kHz | (10% w/w) | 0.138 S | – | – |
|       |         | Amplitude: 20% | 13 mm probe | 0.11 S | – | – |
| Sun, Chen (4) | China | Ultrasonic horn with a maximum net output power of 600W | MPC | 0 | 28.45 | – | – |
|       |         | Frequency: 20 kHz | (5% w/w) | 0.5 | 0.13 S | – | – |
|       |         | Amplitude: 50% | 1 | 0.11 S | – | – |
|       |         | | 2 | 0.11 S | – | – |
|       |         | | 5 | 0.13 S | – | – |
|       |         | | 20 | 0.1 S | – | – |
| Jambrak, Mason (5) | Croatia | High power intensity ultrasonic (600W); A vibrating titanium tip probe 1.2 cm; Ultrasonic intensity: 43–48 W/cm² | WPI (95 %) (10% aqueous suspensions) | 0 | 508 | – | – |
|       |         | Frequency: 20 kHz; Ultrasonic intensity: 43–48 W/cm² | 15 | 264 S | – | – |
|       |         | | 30 | 285 S | – | – |
|       |         | | 0 | 324 | – | – |
|       |         | | 15 | 4.67 S | – | – |
|       |         | | 30 | 1.04 S | – | – |
| Shen, Zhao (6) | China | An ultrasonic processor VCX800; 13 mm high grade titanium alloy probe; Intensity: 107 W/cm² | WPI | 0 | 47.19 | – | – |
|       |         | Frequency: 20 kHz; pH = 3; pH = 7; pH = 11; 120 W | (10% w/v) | 5 | 42.64 S | – | – |
|       |         | | 10 | 39.95 S | – | – |
|       |         | | 20 | 38.2 S | – | – |
|       |         | | 40 | 34.34 S | – | – |
| Gao, Ma (7) | China | DY–1200Y ultrasonicator with a maximum power of 1200 W; Titanium probes (0.6 cm diameter for 120 and 360 W; 2 cm diameter for 600 W)  | WPI (50 mg/mL) | 30 | NR | – | – |
|       |         | Frequency: 20 kHz; pH = 3; pH = 7; pH = 11; 120 W; pH = 3; 360 W; pH = 7; 600 W; pH = 11 | 0.397 | – | – |
|       |         | | 0.253 | – | – |
|       |         | | 0.226 | – | – |
|       |         | | 0.221 | – | – |
|       |         | | 0.222 | – | – |
|       |         | | 0.23 | – | – |
|       |         | | 0.191 | – | – |
|       |         | | 0.234 | – | – |
|       |         | | 0.22 | – | – |
|       |         | | 0.189 | – | – |
| Yao, Xia (8) | China | Sonication in a 150 mL beaker immersed in the ice-water (25 ± 2°C); A titanium probe with a 0.636 cm diameter | WPI | 0 | 0.246 | – | – |
|       |         | Frequency: 20 kHz; A titanium probe with a 0.636 cm diameter | (100 g/L) | 20 min; 200 W | 0.219 S | – | – |
|       |         | | 20 min; 400 W | 0.202 S | – | – |
|       |         | | 20 min; 600 W | 0.134 S | – | – |
|       |         | | 20 min; 800 W | 0.133 S | – | – |
|       |         | | 40 min; 200 W | 0.205 S | – | – |
|       |         | | 40 min; 400 W | 0.185 S | – | – |
|       |         | | 40 min; 600 W | 0.118 S | – | – |
|       |         | | 40 min; 800 W | 0.114 S | – | – |

(continued on next page)
Table 1 (continued)

| Study                        | Country | US condition                                                                 | Protein type                                                                 | Sonication time (min) | Particle size (μm) | Significance |
|------------------------------|---------|-------------------------------------------------------------------------------|-------------------------------------------------------------------------------|-----------------------|-------------------|--------------|
| Martini and Walsh (9)        | USA     | Misonix Sonicator 3000; 3.2 mm titanium microtip; Intensity: 15 W             | Whey protein (10% w/v) (whey suspension) pH=3.5                               | 15                    |                   |              |
|                              |         |                                                                               |                                                                               |                       |                   |              |
|                              |         |                                                                               |                                                                               |                       |                   |              |
|                              |         |                                                                               |                                                                               |                       |                   |              |
|                              |         |                                                                               |                                                                               |                       |                   |              |
| Furtado, Mantovani (10)      | Brazil  | An ultrasonic processor QR 750 W; Titanium probe with 13 mm diameter; Power: 300 W | Sodium caseinate (1/3 w/v)                                                   | 0                     | 12.1              |              |
|                              |         |                                                                               |                                                                               |                       |                   |              |
|                              |         |                                                                               |                                                                               |                       |                   |              |
|                              |         |                                                                               |                                                                               |                       |                   |              |
| Shammugam, Chandrapala (11)  | Australia | 450 W ultrasonic horn; Frequency: 20 kHz; 12 mm diameter; A titanium probe | Sodium caseinate 20 W                                                        | 0                     | 0.178             | NR           |
|                              |         |                                                                               |                                                                               |                       |                   |              |
|                              |         |                                                                               |                                                                               |                       |                   |              |
|                              |         |                                                                               |                                                                               |                       |                   |              |
| Liu, Juliano (12)            | Australia | Ultrasound transducers; Frequency: 20 kHz; Titanium probe with 13 mm diameter | Casein (10% w/w) at pH= 6.7                                                  | 0                     | 0.202             |              |
|                              |         |                                                                               |                                                                               |                       |                   |              |
|                              |         |                                                                               |                                                                               |                       |                   |              |
|                              |         |                                                                               |                                                                               |                       |                   |              |
| Shen, Shao (13)              | China   | An ultrasonic processor (VCX800); Frequency: 20 kHz; A 13 mm grade titanium    | WPI (10% w/w) at pH= 8                                                       | 0                     | 0.19              |              |
|                              |         | alloy probe                                                                   |                                                                               |                       |                   |              |
|                              |         |                                                                               |                                                                               |                       |                   |              |
|                              |         |                                                                               |                                                                               |                       |                   |              |
|                              |         |                                                                               |                                                                               |                       |                   |              |

S: Statistically significant difference compared to control group; NS: Statistically not significant difference compared to control group; NR: Not reported.

Applied syntaxes in the databases such as Scopus: TITLE-ABS (“Milk protein”*) OR TITLE-ABS(“whey protein”) OR TITLE-ABS(protein*) AND TITLE-ABS(whey)) OR TITLE-ABS(casein*) OR TITLE-ABS(“sodium caseinate”) OR TITLE-ABS(caseinate) OR TITLE-ABS(sodium)) OR TITLE-ABS(“dairy protein”*) AND TITLE-ABS(“Emerging technologies”) OR TITLE-ABS(“Emerging processing”) OR TITLE-ABS(“Emerging techniques”) OR TITLE-ABS(ultrasound) OR TITLE-ABS(ultrasonic*) OR TITLE-ABS(sonication) OR TITLE-ABS(non-thermal processing) OR TITLE-ABS(non-thermal techniques) OR TITLE-ABS(“non-thermal processing”) AND (PUBYEAR > 1999 AND PUBYEAR < 2022); Web of Science: ((TS = (“Milk protein”*) OR TS = (“whey protein”) OR TS = (protein*) AND TS = (whey)) OR TS = (“casein”) OR TS = (“sodium caseinate”) OR TS = (caseinate) AND TS = (sodium)) OR TS = (“dairy protein”*) AND TS = (“Emerging technologies”) OR TS = (“Emerging processing”) OR TS = (Emerging techniques) OR TS = (“Emerging ultrasonic*) OR TS = (sonication) OR TS = (“non-thermal processing”) OR TS = (“non-thermal processing”) AND (PY = 2000–2021)); PubMed:”(Milk protein”*[tiab] OR “whey protein”*[tiab] OR (protein*[tiab] AND whey*[tiab]) OR casein*[tiab] OR “sodium caseinate*[tiab] OR caseinate*[tiab] AND sodium*[tiab]) OR “dairy protein”*[tiab] AND (“Emerging technologies*[tiab] OR “Emerging Ultrasound”*[tiab] OR ultrasound*[tiab] OR ultrasonic*[tiab] OR sonication*[tiab] OR non-thermal processing*[tiab] OR “non-thermal techniques”*[tiab]) AND (2000/01:2021/08/30(dp))). The syntax for other databases is given in Supplementary 1.

2.2. Eligibility criteria

All selected articles were reviewed based on title, abstract, and full text, and related articles were selected according to inclusion criteria. The inclusion criteria were: 1) reporting functional properties include particle size, solubility, water holding capacity, viscosity or consistency, gelling strength, and emulsifying properties; 2) control group is defined; 3) studies were conducted from 2000 to 2021; 4) no language limitation; 5) ultrasound should be used alone. In addition, the following criteria were considered as exclusion criteria: 1) studies did not have a control group; 2) combination of thermal and non-thermal methods with ultrasound; 3) studies were conducted under 2000 (Fig. 1).
databases. According to the inclusion and exclusion criteria of the study, 85 articles were finally selected, and in the next step, the full text of these articles was reviewed. Finally, 22 articles were selected. The data of these 22 selected articles were extracted. The data status showed high heterogeneity in the studies methods as the results showed that different ultrasound conditions, protein percentage, frequencies, amplitude, and sonication time had been considered in different studies. Therefore, it was not possible to combine and meta-analyze the results.

Table 2
Effect of ultrasound on solubility of milk proteins.

| Study                  | Country      | US condition                                                                 | Protein type | Sonication time (min) | Amplitude (%) | Solubility (%) | Significancy |
|------------------------|--------------|-------------------------------------------------------------------------------|--------------|-----------------------|---------------|----------------|--------------|
| Arzeni, Martínez (3)   | Argentina    | Ultrasonic processor Vibre Cell 750 W Frequency: 20 kHz 13 mm high grade titanium alloy probe | WPC (10% w/w) | 20                    | 20            | 66.74          | 64.53 NS     |
| Zhang, Pang (14)       | China        | A high-intensity ultrasonic processor Frequency: 20 kHz 13 mm ultrasonic probe | Micellar casein concentrate (3.4% w/w) | 0          | –              | 81.43         | – S         |
| Sun, Chen (4)          | China        | Ultrasonic horn with a maximum net output power of 600 W Frequency: 20 kHz | MPC (5% w/w) | 0.5                  | 50            | 35.63          | – –         |
| Jambrek, Mason (15)    | Croatia      | A high-intensity ultrasonic (600 W) Frequency: 20 kHz Probe with a vibrating titanium tip | WPI (10% w/w) | 0                    | –              | 66.8           | – –         |
| Nazari, Mohammadifar (16) | Iran        | Ultrasonic processor with an output power of 100 W; Frequency: 20 kHz A 3 mm diameter titanium sonotrode probe | MPC (10% w/w) | 5                    | 20            | 65.96          | – –         |
| Shen, Zhao (6)         | China        | An ultrasonic processor VCX800; 13 mm high grade titanium alloy probe; Intensity: 107 W/cm² | WPI (10% w/v) | 5                    | 20            | 2.91           | – –         |
| Jambrek, Mason (17)    | Croatia      | High intensity and low frequency ultrasonic; Probe with a vibrating titanium tip 1.2 cm Power: 600 W Intensity: 39–44 W/cm² | α-lactalbumin (10% w/w) | 0                    | –              | 80.6           | – –         |
| Shen, Shao (13)        | China        | An ultrasonic processor (VCX800); Frequency: 20 kHz | WPI (10% w/w) | 0                    | 10            | 75.29          | – –         |

HWP: Whey Protein Hydrolysate; S: Statistically significant difference compared to control group; NS: Statistically not significant difference compared to control group; NR: Not reported.
3.1. Effect of ultrasound on the particle size of milk proteins

The particle size of milk proteins (MPs) plays a vital role in their functional properties, i.e., smaller particle size has a higher surface area which can exhibit a higher propensity for protein solubility, leading to improved protein emulsifying gelling and foaming properties [19,20]. The ultrasound (US) potential to modify protein functionalities has been well documented in recent years. However, no clear trends are reported on the effect of the US on the particle size of MPs. Most of the studies used in this review have indicated that (controlled or moderate) US

Table 3

| Study          | Country | US condition                        | Protein type     | Sonication time (min) | Emulsifying properties | Significance |
|----------------|---------|-------------------------------------|------------------|-----------------------|------------------------|-------------|
|                |         |                                     |                  |                       | Control | Treatment | Control | Treatment |
|                |         |                                     |                  |                       | EAI (m^2/g) | ESI (min) |          |           |
|                |         |                                     |                  |                       |          |          |          |           |
| Zhang, Pang (14) | China   | A high-intensity ultrasonic processor | Micellar casein concentrate | 0 | 6.47 | – | 55.08 | – | – |
|                |         | Frequency: 20 kHz                     |                  | 0.5 | – | 6.92 | 60.85 | S-S |
|                |         | 13 mm ultrasonic probe                |                  | 1 | – | 7.83 | 68.58 | S-S |
|                |         | Power density: 58 W/L                 |                  | 2 | – | 8.15 | 75.81 | S-S |
|                |         |                                       |                  | 5 | – | 7.99 | 77.09 | S-NS |
| Sun, Chen (4) | China   | Ultrasonic horn at power of 600 W     | MPC              | 0 | 4.23 | – | 4.33 | – | – |
|                |         | Frequency: 20 kHz                     |                  | 0.5 | – | 4.43 | 4.29 | NS |
|                |         | Amplitude: 50%                        |                  | 1 | – | 5.51 | 5.79 | S |
|                |         |                                       |                  | 2 | – | 6.05 | 5.26 | S |
|                |         |                                       |                  | 5 | – | 6.06 | 5.19 | S |
| Bi, Ge (18)   | China   | Ultrasound processor                  | Casein           | 0 | 74.15 | – | 15.37 | – | – |
|                |         | Frequency: 20 kHz; (8% w/v)           |                  | 10 | 80.05 | 17.42 | S-S |
|                |         | A 0.636 cm diameter titanium probe;   |                  | 20 | 94.1 | 20.67 | S-S |
|                |         | Intensity: 120–127 W/cm^2             |                  | 30 | 85.39 | 20.82 | S-S |
|                |         |                                       |                  | 40 | 78.65 | 19.59 | S-S |
| Gao, Ma (7)   | China   | DY-1200Y ultrasonicator with a maximum power of 1200 W; Frequency: 20 kHz; Titanium probes (0.6 cm diameter for 120 and 360 W, 2 cm diameter for 600 W) | WPI (50 mg/mL) pH–3; pH–3; 120 W | 30 | 83.15 | 17.05 |
| Yao, Xia (8)  | China   | Sonication in a 150 mL beaker immersed in the ice-water (25 ± 2 °C); Frequency: 20 kHz; A titanium probe with a 0.636 cm diameter | WPI (100g/L) | 0 | 58.64 | – | 40% | – | – |
| Furtado, Mantovani (10) | Brazil | An ultrasonic processor QR 750 W; Titanium probe with 13mm diameter; Frequency: 20 kHz; Power: 300 W | Sodium caseinate (1% w/w) | 0 | 30% | – | 19.5 | – | – |
| Shen, Shao (13) | China  | An ultrasonic processor (VCX800); Frequency: 20 kHz; A 13 mm high-grade titanium alloy probe; | WPI (10% w/w) | 0 | 3.18 | – | 62.33 | – | – |
|                |         |                                       |                  | 10 min; 31 W/ cm^2 | 3.58 | 71.53 | S-S |
|                |         |                                       |                  | 20 min; 31 W/ cm^2 | 4.12 | 84.62 | S-S |
|                |         |                                       |                  | 10 min; 69 W/ cm^2 | 4.97 | 97.4 | S-S |

S: Statistically significant difference compared to control group; NS: Statistically not significant difference compared to control group; NR: Not reported.
treatment induces a decrease in the particle size of MPs via structural disruption of the proteins (Table 1). This is due to the disrupting effects of acoustic cavitation on hydrophobic and electrostatic interactions such as hydrogen bonds and van der Waals forces among protein three-dimensional complex structures, which can dissociate protein molecules to generate smaller fragments [21,22].

For example, Gordon and Pilosof [23] found a significant reduction in whey protein isolate (WPI) particle size from 0.733 µm in untreated samples to 0.359, 0.252, 0.260, 0.207 µm after 2, 5, 10, and 20 min US treatment at an amplitude of 20% (114 µm) with an ultrasonic processor, operating at 20 kHz. Similarly, Jambrak Mason [22] reported a 244 µm and a 223 µm reduction in WPI particle size, following ultrasonication (20 kHz; intensity 43–48 W/cm²) for 15 and 30 min, respectively. The effect of US on WPI particle size was lower when they used a bath sonicator, i.e., the primary particle size of 508 µm decreased to 334.59 µm after 15 min and 313.22 µm after 30 min ultra-sonication at 40 kHz (intensity: 1 W/cm²).

An induced size reduction by US has also been reported for whey protein concentrate (WPC), e.g., a reduction from 0.327 to 0.259 after 20 min [24] and 324 µm to 1.04 µm after 30 min [22] ultra-sonication at 20 kHz for probe sonicator and from 324 µm to 1.67 µm after 30 min at 40 kHz for bath sonicator [22]. Similarly, a decreasing effect of US treatment on MPs particle size was also observed for milk protein concentrate (MPC) from 28.45 to 0.10–0.13 µm depending on sonication time [25] and milk protein isolate (MPI) from 44 µm to 0.13 µm [26]. The reducing effects of ultrasonication on MPs particle size depend on ultrasound processing conditions. In general, it has been proposed that higher intensity decreases the MPs particle size up to a certain level. These studies suggested that the decrease in protein size after US treatment is due to cavitational forces that induce sono-physical processes such as micro-streaming, micro-streamers, micro-jets, and hydrodynamic shock waves due to cavitation bubbles, leading to the turbulent fluid movement and a microscale velocity gradient in the proximity of cavitational bubbles which break up the molecular structure of the proteins and reduce their size.

In contrast, ultra-sonication with relatively high intensity, e.g., using a higher amplitude for a more extended period, for example, >40 min sonification in the case of bovine serum albumin [27], increases sulf-hydryl group content that was previously buried within the interior of the protein due to partial unfolding of protein molecules which can react with themselves or be oxidized to form bigger aggregates [28]. Another explanation is the re-aggregation of disordered proteins via hydrophobic bonds [1,29–31]. For instance, de Figueiredo Furtado, Mantovani [32] observed a significant increase in sodium caseinate particle size from 12.10 µm to 20.5, 19.6, and 26.3 µm after US treatment for 2, 4, and 6 min, respectively, at 20 kHz with a power input of 300 W, whereas, Nguyen and Anema [33] reported a reduction in the micelle size of casein after 30 min ultra-sonication at 22.5 kHz with a power input of 50 W. Similar results were also reported by Liu, Juliano [34], who observed a significant increase in the particle size of casein after 15 min ultra-sonication at 1600 kHz.

### 3.2. Effect of ultrasound on the solubility of milk proteins

The protein solubility contributes to most industrial application functionalities, such as emulsifying, foaming, gelling, and viscosity functions. At a defined (given) pH, the protein solubility depends on the degree of protein denaturation and aggregation, protein surface hydrophobicity, and the size of protein molecules [35,36]. Previous studies have demonstrated ultrasound treatment as a practical approach to modify these factors. Hence it has been used to improve MPs solubility up to a certain level, depending on US conditions (Table 2). The improving effects of US on MPs solubility can be attributed mainly to conformational changes in the (partial unfolding of) protein structure consequent ultrasonic cavitational effect, that helps to expose the buried hydrophilic groups to the surrounding water, allowing higher protein-water interactions, and hence improved the protein solubility [21,28,37].

Nazari, Mohammadifar [1] showed that ultra-sonication (20 kHz; 3 mm diameter titanium sonotrode probe) increases MPC solubility; the solubility increased linearly with increasing ultrasound time and amplitude, i.e., in a constant time, MPC solubility increased with the increase in ultrasound amplitude and vice versa. Several other studies on ultrasound treatment of MPs reported a similar trend of increase in the solubility of WPI [38,39], WPC [39], whey protein hydrolysate [39], α-lactalbumin [40], MPC [25], and micellar casein concentrate [37] subsequent ultrasound treatment. Decreased particle size and partial unfolding of proteins during ultra-sonication increases charged groups including NH⁺ and COO– on the surface of the protein, this also increases protein–water interactions via electrostatic forces (leads to the dispersion of protein and thus improves solubility) [25], which significantly improves the protein solubility. The role of increased temperature after ultra-sonication in facilitating protein dissolution should not be neglected.

Nevertheless, there is some evidence indicating that MPs exposure to a very high-intensity ultrasound or a low-intensity treatment for a long time decreases the MPs solubility remarkably. In these conditions, some covalent bonds in the protein break and more hydrophobic groups appear, which make proteins more susceptible to water, thus creating an opportunity for re-aggregation of dissociated proteins through hydrophobic bonds to form large aggregates, which in turn reduce solubility (so the solubility decreases.) [28,41]. For example, a relatively high-intensity US treatment of 107 W/cm² at 20 kHz reduced WPI solubility from 2.91% to 2.10–2.84%, depending on the ultra-sonication time [42]. Arzeni, Martinez [24] also reported a decrease in WPC solubility from 66.47% to 64.53% after 20 min ultra-sonication at 20 kHz with 20% amplitude.

However, before US treatment of MPs, as an efficient treatment, the protective effects of the other components present in the sample should also be taken into account, e.g., Jambrak, Mason [39] detected a significant decrease in the solubility of WPC treated by 20 kHz probe with 40 and 500 kHz baths, attributing the result to the considerable amount of lactose in WPC, similarly displayed by other disaccharides that present a protective effect during pressurization [43].

### 3.3. Effect of ultrasound on emulsifying properties of milk proteins

Emulsifying properties of a protein are its ability to form and stabilize a homogeneous emulsion in an oil–water system via being adsorbed at the oil–water interface [8,44]. This function of proteins is determined by emulsifying activity index (EAI) and emulsion stability index (ESI), which list the ability of proteins to be adsorbed and their capacity to remain at the interface of water and oil during processing and storage, respectively. MPs’ emulsifying properties are related to the protein size and conformation, their surface hydrophobicity, protein solubility, oil/water ratio, and pH [8,21,44–46].

Research has well-proven that ultra-sonication can influence emulsifying properties of MPs via affecting these properties, i.e., a positive effect of ultra-sonication on both EAI and ESI of WPI [38,45,47], casein [32,37,48] and MPC [25] has been demonstrated (Table 3). Gao, Ma [45] showed a significant increase in EAI and ESI of WPI under US treatment with different power inputs including 120, 360, and 600 W at different pHs. The improving effect of US on WPI emulsifying properties depended on the level of power input and pH; at acidic (pH = 3) or basic (pH = 11) pHs, the EAI of WPI decreased with increasing the power input, whereas, at a natural pH, this trend was reversed. Inversely, ESI improved as power input increased at pH = 7 and pH = 3, whereas at pH = 11, the ultra-sonication effect was the opposite.

It is worth mentioning that, regardless of the change in EAI of WPI under US, the alkaline-treated WPI had a higher EAI than those of acid-treated and neutral WPI probably due to more negative charges at alkaline conditions, changing the electrostatic interactions between
protein molecules, resulting in evenly dispersed protein molecules in oil–water emulsions [46]. Yao also reported similar trends in changing both EAI and ESI of WPI [47], whereas Shen, Shao [38] reported a gradual increase in EAI and ESI of WPI with increasing ultrasound time and intensity when the treatment was carried out at 10 min; 31 W/cm$^2$, 20 min; 31 W/cm$^2$, 10 min; 69 W/cm$^2$, 20 min; 69 W/cm$^2$.

Maximizing the emulsification properties of MPs by moderate ultrasound treatment has also been shown for casein [48], micellar casein concentrate [37], sodium caseinate [32], and MPC [25], e.g., EAI and ESI for sodium caseinate reached their maximum after 4 min ultrasound (20 kHz; actual power input of 300 W, amplitude 30 %) compared to untreated and ultrasonicated sodium caseinate for 2 min, and 6 min under the same condition [32].

The improved emulsifying properties of MPs can be directly attributed to the changes in the protein secondary and tertiary structures as the α-helix and β-sheets are affected by ultra-sonication, which allows effective adsorption of ultrasound treated protein at the oil–water interface [8,21,41,49,50]. The ultra-sonication can reduce the particle size of proteins, which increases the surface area-to-volume ratio, thus enhancing the emulsifying properties of MPs [45,50]. The ultrasonicated proteins also exhibit a higher solubility, where more proteins are available at the oil–water interface during emulsification [25]. Additionally, the US treatment increases the proteins’ surface hydrophobicity by unfolding protein which reduces the tension at the oil–water interface, thereby increasing the rate of protein absorption and making the protein films rigid through hydrophobic interactions [30,51], resulting in a facilitated emulsification activity of ultrasonicated milk proteins. Furthermore, emulsion homogenization can be enhanced by using high-intensity ultrasound to pre-treatment the proteins [52].

### Table 4

| Study | Country | US condition | Protein type | Protein concentration (%) | Amplitude (%) | Sonication time (min) | Foaming capacity (%) | Significance |
|-------|---------|--------------|--------------|---------------------------|--------------|----------------------|----------------------|--------------|
| Tan, Chin (19) | Malaysia | High-Intensity Ultrasound at 400 W; Frequency: 20 kHz; A probe with vibrating titanium tip of 2.54 cm | WPC | 200 g/kg | 60 | 0 | 1100 | – |
| Jambrak, Mason (15) | Croatia | A high-intensity ultrasonic (600 W) Frequency: 20 kHz Probed with a vibrating titanium tip 1.2 cm | WPI | 10% w/w | – | 0 | 132 | – |
| Nazari, Mohammadifar (16) | Iran | Ultrasonic processor with an output power of 100 W; Frequency: 20 kHz; A 3 mm diameter titanium sonotrode probe | MPC | 10% w/w | – | 0 | 271 mL | – |
| Ahmadi, Razavi (20) | Iran | Misonix sonicator with 550 W; Frequency: 20 kHz 10 mm probe | WPC | 10% w/w | – | 0 | 89.99 | – |
| Jambrak, Mason (17) | Croatia | High intensity and low frequency ultrasonic; Probe with a vibrating titanium tip 1.2 cm; Power: 600 W | α-lactalbumin | 10% w/w | – | 0 | 113 | – |

S: Statistically significant difference compared to control group; NS: Statistically not significant difference compared to control group; NR: Not reported.
Nevertheless, a re-aggregation of unfolded proteins often occurs through hydrophobic bonds due to increased surface hydrophobicity after ultrasound treatment, which may cause a decrease in both EAI and ESI MPs [37]. Thus, to improve MPs’ emulsifying properties using ultrasound treatment, there must be a balance between exposure of hydrophobic groups and aggregation of protein molecules.

### 3.4. Effect of ultrasound on foaming capacity of milk proteins

The MPs are surface-active agents due to their amphiphilic nature, possessing both hydrophilic and hydrophobic groups in their structure, enabling them to be adsorbed at fluid interfaces. The protein adsorption at the fluid interfaces, i.e., air–water interface for foam systems and oil–water interface for emulsions, decreases the interfacial tension, which is critical for stabilizing the foam and emulsion systems. For example, in a foam system, MPs can stabilize air bubbles being diffused throughout the air–liquid colloidal system by forming a highly viscoelastic film at the boundary of the air–liquid interface. The foaming properties of proteins are closely related to their particle size, surface hydrophobicity [53], and structural flexibility [25,30,39,54] (Table 4).

It has been reported that controlled ultrasound treatment of MPs can improve their foaming properties. However, the excessive use of ultrasonication can cause a considerable loss in their foaming properties due to protein aggregates formation induced by ultrasound treatment. For example, Ahmadi Razavi [55] reported a slight increase in foaming capacity (FC) of WPC following US (20 kHz 10 mm probe, 550 W) for 2.5 and 5 min from 89.99% to 92.12% and 91.81%, respectively, whereas 7.5 min treatment caused a significant decrease in FC of WPC to 86.96%. Partially similar results were reported by Jambrak, Mason [39]. Using ultra-sonication treatment, it is possible to decrease the particle size of protein molecules, which leads to an increase in MPs adsorption at the air–liquid interface that can reduce interfacial tension [57,58], thus improving the foaming properties of MPs. Furthermore, improved foaming properties of MPs by ultrasound treatment can be attributed to the mechanical effects of cavitation that induce changes in the conformation of the protein structure, i.e., partially denaturation and unfolding in the structure of MPs (structural changes on α-helix and β-sheets or changes in the secondary, and tertiary structures) [20].

The conformational changes after ultrasound treatment reveal hydrophobic groups and regions from the interior of the protein, which results in an increased interaction of protein at the air–liquid interface, creating more stable foams with more rigidity [30,40]. As emphasized earlier, to improve MPs’ functional properties by ultrasound treatment, an optimized condition is necessary since ultrasound treatment at very low or very high intensities may even destroy MPs’ functionalities. For instance, FC of native MPC decreased after US treatment (20 kHz, amplitude 60%, 400 W) depending on exposure time; the highest effect was observed at 25 min of ultrasonication followed by 15 min and 10 min treatments, respectively [56]. Similar improving effects of ultrasound treatment on foaming properties were reported for WPC, WPI, and whey protein hydrolysate by Jambrak, Mason [39]. Using ultra-sonication treatment, it is possible to decrease the particle size of protein molecules, which leads to an increase in MPs adsorption at the air–liquid interface that can reduce interfacial tension [57,58], thus improving the foaming properties of MPs. Furthermore, improved foaming properties of MPs by ultrasound treatment can be attributed to the mechanical effects of cavitation that induce changes in the conformation of the protein structure, i.e., partially denaturation and unfolding in the structure of MPs (structural changes on α-helix and β-sheets or changes in the secondary, and tertiary structures) [20].

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### 3.5. Effect of ultrasound on gel strength of milk proteins

MPs’ gelling properties play an important role in the textural properties of different food products such as yogurts, cheeses, and meat products. A protein gel forms when the protein structure is partially unfolded, in which intermolecular interactions such as hydrophobic

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**Table 5**  
Effect of ultrasound on gel strength of milk proteins.

| Study          | Country    | US condition                                                                 | Protein type | Amplitude (%) | Sonication time (min) | Gel strength (g) | Significancy |
|----------------|------------|-------------------------------------------------------------------------------|--------------|---------------|-----------------------|-----------------|--------------|
| Bi, Ge (18)    | China      | Ultrasound processor                                                         | Casein       | –             | 0                     | 141.39          | –            |
| Frequency: 20 kHz;  
A 0.636 cm diameter titanium probe;  
Intensity: 120–127 W/cm² | 10         | 156.65 g S                                                                 |
| 20             | 165.63 g S |
| 30             | 153.96 g S |
| 40             | 143.19 g S |
| Tan, Chin (21) | Malaysia   | High intensity ultrasound probe at 400 W; Frequency: 20 kHz;                | WPC (10% w/w) | 20            | 5                     | 122.26          | –            |
|                |            | A titanium probe with a 0.636 cm diameter                                   |              |               | 15                     | 122.65          | S            |
|                |            |                                                                               |              |               | 25                     | 123.97          | S            |
|                |            |                                                                               |              |               | 40                     | 122.87          | S            |
|                |            |                                                                               |              |               | 15                     | 133.92          | S            |
|                |            |                                                                               |              |               | 25                     | 140.55          | S            |
|                |            |                                                                               |              |               | 60                     | 129.72          | S            |
| Yao, Xia (8)   | China      | Sonication in a 150-ml beaker immersed in the ice-water (25 ± 2 °C);        | WPI          | –             | 0                     | 47.46           | –            |
| Frequency: 20 kHz;  
A titanium probe with a 0.636 cm diameter | (100g/L)   |                                                                               |              | 20 min; 200 W | 207.79 g S        |
|                |            |                                                                               |              | 20 min; 600 W | 419.55 g S        |
|                |            |                                                                               |              | 20 min; 800 W | 251.47 g S        |
|                |            |                                                                               |              | 40 min; 200 W | 227.64 g S        |
|                |            |                                                                               |              | 40 min; 400 W | 363.97 g S        |
|                |            |                                                                               |              | 40 min; 600 W | 238.23 g S        |
|                |            |                                                                               |              | 40 min; 800 W | 250.14 g S        |

S: Statistically significant difference d to control group; NS: Statistically not significant difference compared to control group; NR: Not reported.
increasing intermolecular interactions due to the unfolding of the protein. Ultrasound treatment promotes stronger whey protein gels through a positive effect on the strength of the MP gel. Tan Chin [60] found that the strength of WPI increased, up to a certain level, as ultrasound power input increased for both 20- and 40-min treatment times, i.e., the maximum gel strength of WPI was achieved at 600 W and 400 W for 20 min, whereas longer ultrasonic treatment times resulted in weakened gel strength. Similarly, Bi, Ge [61] observed a significant increase in WHC of proteins [20,21,25,28,30,37,51]. These changes following ultrasound treatment lead to increased intermolecular hydrophobic interactions and SS-bonds, the latter due to oxidation of free SH groups by hydroxyl radicals generated through sonolysis of water molecules [27], to make larger protein aggregates, which eventually can increase the physical entrapment of water molecules by protein aggregates, thus improving WHC of MPs.

As shown in Table 5, ultrasound treatment also remarkably increased the gel strength of WPI, an increase in gel strength of WPI ranging from 748.00% depending on US condition [47] due to splitting whey proteins functional properties [20,21,25,28,30,37,51]. These changes following ultrasound treatment lead to increased intermolecular hydrophobic interactions and SS bonds, the latter due to oxidation of free SH groups by hydroxyl radicals generated through sonolysis of water molecules [27], to make larger protein aggregates, which eventually can increase the physical entrapment of water molecules by protein aggregates, thus improving WHC of MPs.

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3.6. Effect of ultrasound on water holding capacity of milk proteins

The water holding capacity (WHC) of proteins reflects their ability to stabilize water molecules in a protein–water interaction system through capillary effects in various food systems. WHC is a critical parameter of gel properties in some food products, i.e., yogurt, which influences viscosity, thickening, and juiciness. As discussed earlier, generation and collapse of cavitation bubbles due to interaction between the ultrasonic waves, liquid, and dissolved gas during ultrasound waves propagation (passing) through a medium result in protein unfolding, therefore exposing buried hydrophobic and sulfhydryl groups of proteins, increasing surface hydrophobicity and free SH which influence the proteins functional properties [20,21,25,28,30,37,51]. These changes following ultrasound treatment lead to increased intermolecular hydrophobic interactions and SS bonds, the latter due to oxidation of free SH groups by hydroxyl radicals generated through sonolysis of water molecules [27], to make larger protein aggregates, which eventually can increase the physical entrapment of water molecules by protein aggregates, thus improving WHC of MPs.

It is worth mentioning, though, that the effect of ultra-sonication on WHC is treatment condition-dependent, i.e., at an excessive ultrasound treatment, vigorous ultrasound-induced micro-streaming, micro-streamers, micro-jets, and hydrodynamic shock dissociates the protein aggregates into smaller fragments, that eventually negatively affects WHC of proteins [21,61]. For example, as shown in Table 6, Vargas, Delgado-Macuil [21] reported a significant increase in WHC of transglutaminase cross-linked whey protein solubles aggregates by high-intensity ultrasound (20 kHz) for 10–40 min. However, no further increase was observed when the time increased from 10 to 40 min, indicating the fact that there is a balance between the protein aggregation and US treatment. Cheng, Donkor [62] observed a significant increase in WHC of WPC (10%) following ultra-sonication (20 kHz) from 79.53% for untreated samples to 84.71, 89.11, 89.37, 90.67% for 5, 10, 15, and 20 min for US treated samples, respectively. Shen reported similar results Shen, Shao [38] for WHC of WPI (10%) where the US treatments at

### Table 6

| Study       | Country | US condition                        | Protein type | Amplitude | Sonication time (min) | Water Holding Capacity (%) | Significance |
|-------------|---------|-------------------------------------|--------------|-----------|-----------------------|-----------------------------|-------------|
| Cheng, Donkor (22) | China   | Mono-frequency ultrasound; Frequency: 20 kHz; (10% W/W) | WPC          | 40        | 0                     | 85.71                       |             |
| Shen, Zhao (6) | China   | An ultrasonic processor VCX800; Frequency: 20 kHz; 13 mm high grade titanium alloy probe; Intensity: 107 W/cm$^2$ | WPI          | 40        | 0                     | 85.71                       |             |
| Bi, Ge (18) | China   | Ultrasonic processor | Casein Frequency: 20 kHz; A 0.63 cm diameter titanium probe; Intensity: 120–127 W/cm$^2$ |  |  |  | 48.98 |             |

S: Statistically significant difference compared to control group; NS: Statistically not significant difference compared to control group.
Table 7
Effect of ultrasound on viscosity and consistency of milk proteins.

| Study                | Country         | US condition                                                                 | Protein type      | Protein concentration (%) | Amplitude (%) | Sonication time (min) | Viscosity or consistency (mPa) | Significancy |
|----------------------|-----------------|-------------------------------------------------------------------------------|-------------------|---------------------------|--------------|-----------------------|-------------------------------|--------------|
| Arzeni, Martínez (3) | Argentina       | Ultrasonic processor Viber Cell 750 W; Frequency: 20 kHz; 13 mm high grade   | WPC               | 10 w/w                    | 20           | 20                    | 2.94a 1.93                    |              |
|                      |                 | titanium alloy probe                                                         |                   |                           |              |                       |                               |              |
| Tan, Chin (19)       | Malaysia        | High-Intensity Ultrasound at 400 W; Frequency: 20 kHz; A probe with vibrating | WPC               | 200 g/kg                  | 20           | 0                     | 8.44 (Pa.s)                   | –            |
|                      |                 | titanium tip of 2.54 cm                                                        |                   |                           |              |                       |                               |              |
| Martini and Walsh (9) | USA             | Misonix Sonicator 3000; 3.2 mm titanium microtip; Intensity: 15 W             | Whey protein      | 10 w/v                    | –            | 15                    | (Pa.s) (Pa.s)                  | –            |
|                      |                 |                                                                                |                   |                           |              |                       |                               |              |
| Shanmugam, Chandrapala (11) | Australia | 450 W ultrasonic horn; Frequency: 20 kHz; 12 mm diameter; 41 W               | Sodium caseinate  | –                         | –            | 0                     | 0.99                          | –            |
|                      |                 |                                                                                |                   |                           |              |                       |                               |              |
| Jambrak, Mason (17) | Croatia         | High intensity and low frequency ultrasonic; Probe with a vibrating          | α-lactalbumin     | 10 w/w                    | –            | 0                     | 6 mPa.s                       | –            |
|                      |                 | titanium tip 1.2 cm; Power: 600 W; Intensity: 39-44 W/cm²                   |                   |                           |              |                       |                               |              |
|                      |                 |                                                                                |                   |                           |              |                       |                               |              |
| Jambrak, Mason (17) | Croatia         | High intensity and low frequency ultrasonic; Probe with a vibrating          | α-lactalbumin     | 10 w/w                    | –            | 0                     | 0.042a                        | –            |
|                      |                 | titanium tip 1.2 cm; Power: 600 W; Intensity: 39-44 W/cm²                   |                   |                           |              |                       |                               |              |
|                      |                 |                                                                                |                   |                           |              |                       |                               |              |
| Shen, Shao (13)      | China           | An ultrasonic processor (VCX8000); Frequency: 20 kHz; A 13 mm high-grade   | WPI               | 10 w/w                    | –            | 0                     | 5.64                          | –            |
|                      |                 | titanium alloy probe                                                         |                   |                           |              |                       |                               |              |
|                      |                 |                                                                                |                   |                           |              |                       |                               |              |
| Shen, Shao (13)      | China           | An ultrasonic processor (VCX8000); Frequency: 20 kHz;                        | WPI               | 10 w/w                    | –            | 0                     | 3.87                          | –            |

(continued on next page)
20 kHz with an intensity of 107 W/cm² for 5–40 min increased the WHC from 85.71% to 86.79%, 86.79%, 95.88%, 95.68% after 5, 10, 20, and 40 min ultra-sonication. US treatment (20 kHz, intensity: 120–127 W/cm², for 10, 20, 30, and 40 min) also caused a remarkable increase in WHC of casein (8% w/v) ranging 13.71–39.11% depending on treatment time [48].

3.7. Effect of ultrasound on viscosity and consistency of milk proteins

The MPs are widely used in the food industries as functional/nutritional ingredients mainly due to their potential to contribute to the physical and functional attributes of the food products valued by consumers and the industry. Techno-functional properties of MPs are mainly associated with their complex three-dimensional structure [1,21,32,37], e.g., a larger molecular size with close-packed globular structure limits hydration properties of the protein, thus influencing its functional properties such as the protein solubility, emulsifying capability, and the flow behavior (viscosity and consistency) in a dispersed proteins system [1].

According to Tanner and Rha [63], the viscosity of a protein solution depends on (protein molecular properties such as size and shape and [24]) the degree of protein surface hydrophobicity which is closely related to the confirmation of the protein and thus on the level of protein hydration. Accordingly, the MPs structural modification could lead to better protein hydration properties due to exposing buried hydrophobic hydration. Accordingly, the MPs structural modification could lead to the degree of protein surface hydrophobicity which is closely related to the confirmation of the protein and thus on the level of protein hydration [28].

O’Sullivan Arelanno [64] reported that the changes in the viscosity of MPI, sodium caseinate, and WPI induced by the ultrasound is related to the change in the protein surface hydrophobicity since Abbas Iram [65] observed a decrease in intrinsic viscosity led to the dehydration of amphiphilic biopolymer micelles and increased the hydrophobicity of the biopolymer. As shown in Table 7, the decreasing effects of US on MPs viscosity have also been reported by others, i.e., Arzeni, Martinez [24] reported a decrease in WPC viscosity (10% w/w solution) from 2.94 mPa.s to 1.93 mPa.s under US treatment at 20 kHz for 20 min with an amplitude of 20%. US treatment also decreased the WPI viscosity i.e. sonication at 20 kHz for 10 min and 20 min with an intensity of 31 W/cm² and 69 W/cm² (20 % and 30 % amplitudes) decreased the WPI viscosity (10% w/w solution) by 0.13–0.83 mPa.s depending on US condition [38]. Shanmugam, Chandrapala [59] also reported a decrease in sodium caseinate viscosityShanmugam, Chandrapala [66] Shanmugam, Chandrapala [65] Shanmugam, Chandrapala [64] Shanmugam, Chandrapala [62] Shanmugam, Chandrapala [60].

The decreasing effects of ultrasound treatment on MPs solutions viscosity were explained by a change in the protein structure and the disruption of large protein aggregates. However, a slight but not significant change in α-lactalbumin viscosity was observed after ultrasoundation at different frequencies, including 20, 40, and 500 kHz, and at different times, including 15 min and 30 min [40] since α-lactalbumin does not contain many large insoluble aggregates. There is some evidence indicating that ultrasound can also increase the MPs viscosity. For example, as reported by Tan, Chin [56], ultra-sonication (20 kHz, 20% amplitude, probe with vibrating titanium tip of 2.54 cm) of WPC solution (200 g/kg) (whey protein foams) caused a significant increase in the solution viscosity from 8.44 Pa.s to 8.86, 9.49, and 10.21 Pa.s after 5, 15, and 25 min treatment, respectively. These results were explained by fracturing bubbles in the ultrasound-treated whey protein foam system into smaller bubbles, resulting in a more stable foam. A significant increase in viscosity following sonication also was observed for whey suspension after 15 min sonication at pH values of 3.5, 4.5, and 7.5 [67].

4. Conclusion

This systematic review showed that the methodological heterogeneity in ultrasound studies is very high. A variety of ultrasound treatment conditions, i.e., different: power input, frequency, amplitude, treatment time, sample volume, and protein properties, were used in the included studies. Hence, the meta-analysis of the results was not possible. For this reason, the results were reported descriptively and discussed. Therefore, it was not possible to provide conclusive results for each result. The results showed that the improving effects of ultrasound on each of the functional properties of milk proteins is entirely dependent on the ultrasound treatment condition and the type of ultrasoundated protein. Therefore, further studies are required to reach conclusive results concerning the employment of ultrasound to improve the techno-functional properties of milk proteins.

| Study | Country | US condition | Protein type | Protein concentration (%) | Amplitude (Sonication time (min)) | Viscosity or consistency (mPa.s) | Significance |
|-------|---------|--------------|--------------|---------------------------|----------------------------------|---------------------------------|--------------|
|       |         | Control      | Treatment    |                           |                                   |                                 |              |
| A 13 mm high-grade titanium alloy probe; | 20 | 20 min; 31 W/cm² | 3.50° | NS |
| | 30 | 10 min; 69 W/cm² | 3.43° | S |
| | 30 | 20 min; 69 W/cm² | 3.75° | NS |

Table 7 (continued)

Shanmugam, Chandrapala [65] Shanmugam, Chandrapala [64] Shanmugam, Chandrapala [62] Shanmugam, Chandrapala [60].

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.

CRediT authorship contribution statement

Sajad Shokri: Investigation, Data curation, Resources, Conceptualization, Methodology, Writing – original draft. Fardin Javanmardi: Investigation, Data curation, Resources, Conceptualization, Methodology, Writing – original draft. Mehrdad Mohammadi: Supervision, Writing – review & editing. Amin Mousavi Khaneghah: Supervision, Writing – review & editing.

Declaration of Competing Interest

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F.T. Saricaoglu, O. Gul, A. Besir, I. Atalar, Effect of high pressure homogenization X. Tao, Y. Cai, T. Liu, Z. Long, H. Wang, X. Qu, Effects of ultrasonic pretreatment and glycosylation on functional properties of casein grafted with glucose, J. Food Process. Preserv. 41 (5) (2017) e13177, https://doi.org/10.1111/jfpp.2017.41.issue-510.1111/jfpp.13177.

H.B. Wijayanti, A. Brodkorb, S.A. Hogan, E.G. Murphy, Thermal denaturation, aggregation, and methods of prevention, Elsevier, Whey proteins, 2019, pp. 185–247.

K.S. Ojha, B.K. Tiwari, C.P. O’Donnell, in: Effect of ultrasound technology on food and nutritional quality, Elsevier, 2018, pp. 207–240.

J. O’Sullivan, M. Park, J. Beevers, The effect of ultrasound upon the physicochemical and emulsifying properties of whey and soy protein isolates, J. Cereal Sci. 69 (2016) 77–84.

Weiss J, Kristbergsson K, Kjartansson GT. Engineering food ingredients with high-intensity ultrasound. ultrasound technologies for food and bioprocessing. Springer; 2011. p. 239-85.

X. Tao, Y. Cai, T. Liu, Z. Long, L. Huang, X. Dong, Q. Zhao, M. Zhao, Effects of pretreatments on the structure and functional properties of okara protein, Food Hydrocolloids 90 (2019) 394–402.

A.R. Jambrak, V. Lelas, T.J. Mason, G. Krešić, M. Badanjak, Physical properties of ultrasound treated soy proteins, J. Food Eng. 93 (4) (2009) 386–393.

Z. Ahmadi, S.M.A. Razavi, M. Varidi, Sequential ultrasound and transglutaminase treatments improve functional, rheological, and textural properties of whey protein concentrate, Innov. Food Sci. Emerg. Technol. 43 (2017) 207–215.

M.C. Tan, N.L. Chin, Y.A. Yusof, J. Abdullah, Effect of high power ultrasonic treatment on whey protein foaming quality, Int. J. Food Sci. Technol. 51 (3) (2016) 617–624.

F.T. Saricaoglu, O. Gul, A. Besir, I. Atalar, Effect of high pressure homogenization (HPH) on functional and rheological properties of hazelnut meal proteins obtained from hazelnut oil industry by-products, J. Food Eng. 233 (2018) 98–108.

A. Martínez-Velasco, C. Lobato-Calleros, B.E. Hernández-Rodríguez, A. Román-Guerrero, J. Alvarez-Ramírez, E.J. Vernon-Carter, High intensity ultrasound treatment of faba bean (Vicia faba L.) protein: Effect on surface properties, foaming ability and structural changes, Ultrasound. Sonochem. 44 (2018) 97–105.

J.A. Resendiz-Vazquez, J.A. Ulloa, J.E. Urtas-Silvas, P.U. Bautista-Rosas, J. C. Ramírez-Ramírez, P. Rosas-Ulloa, L. González-Torres, Effect of high-intensity ultrasound on the technofunctional properties and structure of jackfruit (Artocarpus heterophyllus) seed protein isolate, Ultrasound. Sonochem. 37 (2017) 436–444.

M.C. Tan, N.L. Chin, Y.A. Yusof, F.S. Taip, J. Abdullah, Gel strength and stability characterization of ultrasound treated whey protein foams, Agric. Agric. Sci. Procedia 2 (2014) 144–149.

Krešić G, Režek Jambrak A, Lelas V, Hercog Z. Influence of innovative technologies on rheological and thermophysical properties of whey proteins and guar gum model systems. Mjelkarski: casopis za unapređenje proizvodnje i prerade mlijeka. 2011;61(1):64–78.

Y. u. Cheng, P.D. Donkor, X. Ren, J. Wu, K. Agyemang, I. Ayim, H. Ma, Effect of ultrasound pretreatment with mono-frequency and simultaneous dual-frequency on the mechanical properties and microstructure of whey protein emulsion gels, Food Hydrocolloids 89 (2019) 434–442.

R. Tanner, ChoKyun Rha, in: Rheology, Springer US, Boston, MA, 1980, pp. 277–283, https://doi.org/10.1007/978-1-4684-3743-0_53.

J. O’Sullivan, M. Arellano, R. Pichot, I. Norton, The effect of ultrasound treatment on the structural, physical and emulsifying properties of dairy proteins, Food Hydrocolloids 42 (2014) 386–396.

K. Abbas, B. Imam, P. Seemab, M. Khalid, S. Mohammad, S. Mohammad, Surface tension, density and viscosity studies on the associative behaviour of oxyethylene-oxybutylene diblock copolymers in water at different temperatures. International, J. Org. Chem. 2012 (2012).

A. Shammasgah, J. Chandrapala, M. Ashokkumar, The effect of ultrasound on the physical and functional properties of skim milk, Innov. Food Sci. Emerg. Technol. 16 (2012) 251–258.

S. Martini, M.K. Walsh, Sensory characteristics and functionality of sonicated whey, J. Food Res. Int. 49 (2) (2012) 694–701.