Evaluation of dual-frequency multi-angle ultrasound on physicochemical properties of tofu gel and its finished product by TOPSIS-entropy weight method

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\begin{abstract}

The effects of dual-frequency (40 + 20 kHz) and multi-angle ultrasound (0°, 30°, 45°) on the coagulation state, network structure, flavor and protein conformation of tofu gel were studied. The results showed that the gel flavor of 40 + 20 kHz 0° group was the best and fluorescence intensity was low. The gel flavor in the 40 + 20 kHz 30° group was better than the group without ultrasound, and hydrophobic interaction and disulfide bond content was the largest. Meanwhile, the degree of protein cross-link was increased. The gel in 40 + 20 kHz 45° group had tightly gel state, high thermal stability, but poor flavor. Combined with The Order Preference by Similarity to Ideal Solution (TOPSIS)-entropy weight method, the 40 + 20 kHz 30° group, was the best ultrasonic treatment of gel. It can change the interaction between proteins, promote protein cross-link, and form a uniform and dense gel network. Finally, the hardness and moisture content of finished tofu were increased significantly, and the quality was improved.

\end{abstract}

1. Introduction

Tofu contains 50 % soybean proteins and 27 % oil, and the remaining constituents are carbohydrates and minerals, so it is also called “boneless meat” [1]. It is beneficial to human health and can be digested and absorbed easily, which is deeply loved by consumers. However, due to the weak and fragile texture, tofu is not easy to store and transport, and it cannot satisfy the market demand. Therefore, the texture is the important index of tofu quality, including hardness, and the overall acceptability of tofu is affected [2]. In addition, tofu is essentially a highly gelatinous food made from proteins, so its texture and moisture content (MC) are primarily determined by the interactions of the proteins in the gel network, which can be altered by changes of environmental conditions including solvent composition and mechanical forces [3]. Covalent and non-covalent interactions are the main effects of the gelation process, such as disulfide bonds and hydrophobic interactions, and the density of the gel network can be affected by the degree of cross-link between proteins. A dense and ordered gel network structure can improve the gel hardness and MC. MC is the quantitative indicator of the amount of water retained in the gel network, showing a similar trend with gel hardness; thus, well-structured gels can effectively retain water in the matrix.

In recent years, ultrasonic technology is a popular green processing technology because of safe, non-toxic, and simple operation. It is well known that the ultrasonic cavitation effect is mainly promoted due to the growth, movement, and collapse of bubbles in the liquid environment. At the same time, the extreme environment is generated by microjet, turbulence phenomena, and increased temperature. It has been reported that ultrasonic cavitation can change the structure of proteins, and then the physicochemical properties of tofu gel and its finished product. Khattar et al. [4] reported that the sonicated soybean protein exhibited better water absorption and higher gel hardness. Zhang et al. [5] studied the effect of ultrasound on Transglutaminase (TGase) cross-linked whey protein, it was found that ultrasonic treatment exposed more TGase reaction sites, increased the degree of enzymatic cross-link, and the gel network became denser and stronger. He et al. [6] believed

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that ultrasonic modification could promote the unfolding of the α-helix structure, leading to the exposure of internal groups, further enhancing hydrophobic interactions and hydrogen bonds between protein molecules, leading to the formation of smaller pores and denser structures. Furthermore, it is found that simultaneous synergistic ultrasonic effects from two or more frequencies can reduce the cavitation threshold, promote nucleation and collapse of bubbles, and enhance the cavitation effect [7]. Therefore, compared with single-frequency ultrasound, multi-frequency ultrasound is easier to start, and intensity of the ultrasonic field is stronger. Research groups of Jiangsu University believed that dual-frequency ultrasound increased the thermal stability of myofibrillar proteins [8] and promoted the interaction between sodium caseinate and zein [9]. In fact, due to ultrasonic directivity, effects of the angles between the transducers are different during food processing. The dual-frequency multi-angle ultrasonic processing on physicochemical properties of raw soymilk, and soybean protein has been preliminarily studied, but the protein interaction mechanism and tofu gel properties were not involved [10].

TOPSIS approach is a good way to handle a multi-attribute decision issue with restricted options: the optimum option is the one that is closest to the positive ideal scheme and farthest away from the negative ideal scheme [11]. The entropy weight method determines index weight by evaluating the index value. The larger the index weight, the greater its role in the evaluation system and the more information it provides. The combination of TOPSIS method and entropy weight method can evaluate the importance of indexes and the feasibility of the scheme on the effect of ultrasonic processing for tofu.

Therefore, the purpose of this study was to evaluate the influence of dual-frequency multi-angle ultrasound on protein interaction in tofu gel by using TOPSIS-entropy weight method, based on gel molecular weight, particle size, zeta potential, thermal stability, and flavor changes. Finally, the most suitable processing parameters were chosen to improve the texture and MC of finished tofu. It may provide a new production decision to expand the application of ultrasound and develop tofu with an improved texture.

2. Materials and methods

2.1. Materials

The soybeans were purchased from Shanxi Jinsui Agriculture and Forestry Comprehensive Development Co., Ltd. (Shanxi, China). Calcium sulfate was a food-grade additive purchased from Angel Yeast Co., Ltd. (Hubei, China). β-mercaptoethanol was bought from Macklin Biochemical Co., Ltd. (Shanghai, China). BeyoGelTM Plus Precast PAGE Gel (Tris-Gly, 4–12 %, 10 well), pretained color protein molecular weight marker (10–180 kDa), and protein loading buffer (5 × , DTT) were purchased from Beyotime Institute of Biotechnology (Shanghai, China). A 2.5 % glutaraldehyde fixed solution was purchased from Yida Technology Co., Ltd. (Fujian, China). All other reagents were analytical grade, purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preparation of tofu gel

Sericifum and scum mixture was prepared according to the method by Zhang et al. [10], and dual-frequency multi-angle ultrasonic processing was carried out on the basis of sweeping-ultrasonic mode with sweeping period of 100 ms. The center frequency was dual-frequency of 40 + 20 kHz (LULP20, CHEER-SONIC Ultrasonic Equipment Co. Ltd., China); when two energy-gathered ultrasonic transducers were deviated from the vertical baseline at 0° (40 + 20 kHz 0°), 30° (40 + 20 kHz 30°) and 45° (40 + 20 kHz 45°), respectively, and multi-angle ultrasonic processing was obtained. The total ultrasonic power was 160 W. The total ultrasonic time was 20 min, and the intermittent ratio was 1:2. The temperature of the whole system was controlled at 8 ± 1 °C through ice-water bath. The group without ultrasound was the control group (Control).

After ultrasonic treatment, two layers of gauze were filtered to remove okara to make tofu gel. The raw soymilk was boiled for 5 min, cooled to 80 °C, calcium sulfate was added, thoroughly mixed, and kept at 80 °C for 20 min. After complete heat preservation, the tofu gel was obtained when cooled to room temperature [12].

During the processing, the whey separated from the upper layer. The remaining solid gel was freeze-dried into gel powders (Epsilon 2-6D LSC plus, Martin Christ, Germany) to determine physicochemical indexes.

2.3. Preparation of tofu

After the heat preservation, the fresh tofu gel (Section 2.2) was pressed with the weight of 1.7 kg for 30 min to get the finished tofu product.

2.4. Characterisation of gel state and its flavor

2.4.1. Appearance of gel state

Thirteen mL raw soymilk were added to the glass bottle and cooled to room temperature after boiling, solidification, and heat preservation (Section 2.2). And then, the glass bottle was turned upside down and photographed against a black background in a small photo studio. Thus, the appearance photos of gel state were observed.

The gel strength in bottles was measured by a texture analyzer (TA. XT Plus Stable Micro System, UK). P/0.5 probe was selected with a triggering force of 5 g, and a compression ratio kept at 50 %. The speeds before, during, and after the test were 2, 1, and 1 mm/s, respectively.

2.4.2. Volatile profile

Two g fresh gel were kept at 45 °C for 10 min to volatilize smell fully. The electronic nose system (PEN 3.5, AIRSENSE Analytics GmbH, Germany) was equipped with a sensor array consisting of 10 semiconductor of metal oxide chemical sensor elements. Different sensors corresponded to several sensitive substances. The experimental cleaning time, sensor zero time and analysis time were set as 180 s, 5 s, and 120 s, respectively. The chamber flow rate and initial injection flow rate were 400 mL/min. Principal component (PCA) analysis was performed on the electronic nose data using the supporting WinMust software.

A gas chromatography-mass spectrometry system (GC–MS, TQ8040, Shimadzu, Japan) equipped with Rtx-Wax (30 m × 0.25 mm × 0.25 μm) was used to determine the content of volatile compounds in the gel. Five gel samples and 10 μL of 0.5 μg/μL 2-methyl-3-heptanone were mixed in 20 mL solid-phase micro-extraction bottle and sealed by the lid with silicone gaskets of PTFE. The sample in the bottle was incubated at 40 °C for 5 min, extracted for 30 min, and then desorbed at 250 °C for 4 min. Helium gas was used as a carrier gas, and the flow rate was 1 mL/min. The sampling method was not shunt, and the interface and ion source temperature were 250 °C and 230 °C, respectively. Scan mode was used with an interval of 0.3 s in a range of 30–550 m/z [13]. Compounds were initially identified by NIST 17 standard mass spectrometry database (NIST, Gaithersburg, MD, USA) and http://webbook.nist.gov/, and confirmed by calibration of the retention index (RI) of n-alkanes (C7–C30). The compound contents were determined by the ratio of the peak area of the compound to the internal standard (2-methyl-3-heptanone).

2.5. Determination of molecular weight, particle size, zeta potential, and thermal stability of tofu gel

2.5.1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

It was modified according to the method described by Li et al. [14]. Electrophoresis was performed using a vertical electrophoresis tank (Mini-protein, Bio-RAD Laboratories, Inc., USA). Tris-HCl buffer (1 M, pH 6.8) was used to prepare 15 mg/mL of gel solution and mixed by
shaking. The protein was fully hydrated at 40 °C for 2 h and centrifuged at 9000 × g for 10 min (5424 centrifuge, Eppendorf, Germany) to collect the supernatant. And then, the supernatant was mixed with loading buffer (contained a small amount of DTT), and the ratio of the mixed solution was 4:1. Subsequently, the solution was heated to 95 °C for 5 min and cooled to room temperature before centrifugation. Next, 10 μL centrifuged supernatant with 60 μg protein was added to the lane. The molecular weight of pre-staining (5 μL) was used as the standard molecular weight. Electrophoresis was performed at 15 V (30 min) and 120 V (1 h). The prepared gel was dyed (0.125 % Coomassie bright blue dissolved by ethanol; glacial acetic acid; water = 5:1:4) and decolorized (ethanol; glacial acetic acid; water = 1:2:17). Image analysis was performed by Image J software (National Institutes of Health, USA).

2.6.1. Surface hydrophobicity (S2)

2.6. Characterization of tofu gel change

2.6.2. Free sulfhydryl content (FSC)

The method of Fan et al. [15], 0.96 g lyophilized gel was dissolved in 40 mL phosphate buffer (0.01 M, pH = 7.50), bathed in water at 40 °C for 2.5 h, cooled to 4 °C, centrifuged at 11000 × g for 10 min (5804 R centrifuge, Eppendorf, Germany), and the insoluble substance was removed. And then, the supernatant was diluted 12 times, 6 times, 4 times, 3 times, and 2.4 times by phosphate buffer to obtain gel solution with gradient concentration (2, 4, 6, 8, 10 mg/mL). 40 μL ANS (8-aniline-1-naphthalene sulfonic acid, 8 mM, dissolved in 0.01 M, pH 7.0 phosphate buffer) was evenly mixed with 4 mL of diluted gel solutions. The reaction was conducted in the dark for 15 min. Fluorescence intensity was determined by a fluorescence spectrophotometer (Cary Eclipse, Varian Inc., USA). The excitation and emission wavelengths were set at 470 nm and 390 nm, respectively. The slope was the protein surface hydrophobicity with gel concentration (mg/mL) as abscissa and fluorescence intensity as ordinate.

2.6.3. Chemical interaction

2.6.4. Secondary structure

A small amount of lyophilized gel was dissolved in water, bathed at 40 °C for 20 min, and centrifuged at 11000 × g and 4 °C for 10 min. After centrifugation, the gel concentration of the supernatant was adjusted to 0.21 mg/mL with deionized water. A circular dichroism spectrometer (JASCO J-815, JASCO Corporation, Japan) was used for circular dichroism scanning. The scanning wavelength range, bandwidth, and response time were set to 190 ~ 260 nm, 1 nm, and 1 s, respectively. The data were generated by scanning for 3 times.

2.6.5. Tertiary structure

The lyophilized gel was weighed and dissolved in deionized water to formulate a concentration of 1 mg/mL and bathed it at 40 °C for 20 min. The supernatant was then collected by centrifugation at 11000 × g for 10 min at 4 °C. The parameters of Card-F98 spectrofluorometer (Shanghai Lengguang Technology Co., Ltd., China) were set as follows: excitation wavelength = 280 nm, emission wavelength = 300 ~ 450 nm, speed = 300 nm/min, scan interval = 0.1 nm. Both excitation bandwidth and emission bandwidth were 10 nm. Scan mode was high gain.

2.7. Determination of physicochemical properties of tofu

2.7.1. Moisture content (MC)

The tofu of the same quality (w1) was placed in a glass drying dish (w2) and dried in an oven until it reached a constant weight (w3). The formula for calculating the MC was as follows:

\[
MC = \frac{w_1 + w_2 - w_3}{w_1} \times 100\%
\]

2.7.2. Color

Fresh tofu samples were covered with plastic wrap, and the colorimeter (CR-400, Konica Minolta, Japan) was attached to the wrap surface to measure the color. The L’, a’, and b’ displayed on the instrument represented lightness, redness, and yellowness values, respectively. Whiteness value (w’) was calculated in accordance with the method carried out by Zhang et al. [10].

2.7.3. Texture

The measurement of tofu texture distribution and gel strength was the same in Section 2.4.1. The sample size was 2 × 1 cm (diameter × height) for texture determination.

2.7.4. Low-Field nuclear magnetic resonance (LF NMR)

Water distribution in tofu was observed by LF NMR analyzer (NM120-060VJS-I, Niumag Analytical Instrument Co., Ltd., China). Approximately 4 g of tofu samples were put into glass tubes with a diameter of 40 mm and placed vertically in the magnetic field center. The center frequency and pulse width of the gel were obtained by FID sequence, and the sample was scanned by CP2M sequence. Sequence parameters were set as: SF1 = 21 MHz, O1 = 139146.38 Hz, P1 = 7.52 μs, P2 = 14.48 μs, TD = 704080, SW = 200 kHz, TW = 3500 ms, TE = 0.206 ms, NECH = 17000, NS = 4. Each sample type was continuously
tested for 3 times, and the average value was taken.

2.7.5. Microstructures analysis

The tofu was cut into cubes with side lengths less than 5 mm, fixed in 2.5 % glutaraldehyde solution for 12 h, immersed in phosphate buffer (0.1 M, pH 7.2) for 20 min, and then dehydrated with 30 %, 50 %, 70 %, 80 %, 90 % and 100 % of ethanol solution for 20 min. Pure isoamyl acetate was used to completely replace ethanol (substituted twice for 20 min each). The samples were freeze-dried, sprayed with gold, and observed by scanning electron microscope (SEM) (S-3400 N, Hitachi ltd., Japan) at 5 kV voltage.

2.8. Statistical analysis

All experiments were performed at least three times, and the mean ± standard deviation of the data was calculated using Excel (Microsoft Corp., USA) and Prism 8.0 software (GraphPad Inc., USA). Differences between groups (p less than 0.05) were assessed by Duncan’s in SPSS 26.0 software (SPSS Inc., Chicago, IL, USA). Origin 2019 (OriginLab Corporation., Northampton, MA, USA) software described cluster analysis graph.

3. Results and discussion

3.1. Effects of dual-frequency multi-angle ultrasound on gel state and flavor

3.1.1. Appearance of gel state

The condensation states of different tofu gels are shown in Fig. 1A-1. Soymilk was heated, solidified, insulated, and then inverted. The effect of ultrasound on the hardness of tofu gel was studied by the condensation state of gel in a glass bottle. By comparison, the gel in Control group was not uniform and cohesive. It was dispersed, broken with existing residual liquid, which slowly flowed to the lower part after inversion. Although the 40 + 20 kHz 0° and 30° groups can form more complete gels, they also sank slowly after inverted. In addition, it was found that the gel volume left at the top of 40 + 20 kHz 0° group was much larger than that of 40 + 20 kHz 30° group, therefore, the gel hardness of 40 + 20 kHz 30° was stronger. The gel hardness of the 40 + 20 kHz 45° group was the strongest, adhering to the bottle wall and showing a white and uniform state, and it did not sink after inversion. TPA measurements were performed on gel in glass bottles. As shown in Fig. 1A-2, it was found that ultrasound significantly improved the gel strength, and the peak value was 154.07 g in the 40 + 20 kHz 45° group, followed by the 40 + 20 kHz 30° group was 136.24 g. The observation in Fig. 1A-1 was verified.

3.1.2. Volatile profile

The electronic nose was a kind of aroma detection technology with a bionic olfactory function. PCA analysis can distinguish the overall flavor changes of tofu gel in different groups. As shown in Fig. 1B, PC1 and PC2 explained 91.43 % of the total variables, effectively reflecting the original data. The Control group was significantly separated from the ultrasonic groups, indicating that the ultrasonic treatments obviously changed the flavor properties of tofu gel. Furthermore, significant separation was observed between different ultrasonic groups with varying flavors of gel. It was noteworthy that the distance between the 40 + 20 kHz 45° group and the Control group was the longest along the PC1 axis because of the most differences. GC–MS was an analytical method combining the advantages of the high separation ability of gas chromatography and high identification ability of mass spectrometry, which could be used to detect volatile and semi-volatile compounds in foods [17]. Among the detected volatile components, esters, alcohols, aldehydes, ketones, acids, and furans were the main volatile components in the gel. Esters and ketones had the most significant number of compounds, 15 and 7, respectively. Next were alcohols, including 5 compounds. Both numbers of aldehydes and acids were 4. There were only 2 furans, which were 2-amyl furan and 2-ethyl furan.

Fig. 1C shows that esters and ketones are the most volatile

Fig. 1. Effects of dual-frequency multi-angle ultrasound on gel state and flavors. (A-1) Gel state, (A-2) Gel strength, (B) PCA diagram, (C) Total content of major flavor compounds, and (D) Cluster analysis of most compounds, blue represents the lowest content, while a red represents the highest content.)
compounds in the tofu gel, and their contents reduced as the ultrasound was added. In addition, the ultrasonic groups increased the contents of aldehydes and acids but with little effect on alcohols and furans. Firstly, ultrasonic cavitation could reduce enzyme activity; It was reported that thermosonication at 60 °C for 60 min reduced lipoxygenase activity to 95 % of the initial value [18]. Therefore, ultrasonic modification reduced the production of flavor compounds in the gel by reducing oxidase activity. Previous studies also found that proteins in foods could bind to or absorb flavor compounds, affecting flavor perception during consumption [19]. It was believed that ultrasound promoted protein particle aggregation, and its active site was covered, reducing the adsorption capacity of myofibrillar to flavor compounds [20]. Thus, ultrasonic groups can change the flavor of tofu gel through soybean protein conformation. Moreover, ultrasound induces the generation of free radicals, such as hydroxyl free radicals, superoxide free radicals, hydrogen-free radicals, and so on. These free radicals might promote oxidation reactions and affected flavor changes.

Esters were indispensable volatile substances in tofu and were reported in other bean products. Esters with fruity and floral aromas improved the flavor of bean paste [21]. Carbonyl compounds, including aldehydes, ketones, and acids, produced due to catalyzed degradation of polyunsaturated fatty acid derivatives (PUFA) by lipoxygenase, were the key factor of soybean flavor. Hexanal was a critical compound formed during the degradation of PUFA. It had a cut-grassy taste and a very low threshold in water (0.0045 Ppb) [22], which negatively affected tofu flavor. 2-heptanone had a fruity, pungent, cinnamon flavor and was detected in fermented bean sauce, cooked soybean, or soymilk. As shown in Fig. 1D, the overall content of ketones is high, but their aromatic contribution may be small due to the high threshold (3 Ppb) [22]. A total of 4 acids were identified (valeric acid, nonanoic acid, acetic acid, and hydroxymethyl-2-hydroxy-2-methyl-propionic acid). In general, acids exhibited unpleasant tastes, such as cheese, fat, sweat, sour, rancid, and pungent, which affected product flavor. The end-product of enzymatic oxidation of linoleate was 1-octene-3-ol, which exhibited a mushroom flavor and was not accepted by consumers [13]. Furans were mainly produced by Maillard and Strecker reactions, with caramel, sweet and toasty flavors [17], can give final tofu products pleasant flavor. As observed in Fig. 1D, the 40 + 20 kHz 0° group had the lowest hexanal content and no 1-octene-3-ol. The content of esters, furans, and other compounds beneficial to flavor was higher than that of other ultrasonic groups, while the content of acids was lower than that of other groups, so the gel flavor was the best. Meanwhile, the hexanal content of 40 + 20 kHz 30° group was lower than that of Control group, and it did not contain 1-octene-3-ol, but beneficial compounds such as esters and furans were higher. Hence, the gel flavor of 40 + 20 kHz 30° group was better than Control.

![Fig. 2](image-url) Effects of dual-frequency multi-angle ultrasound on gel molecular weight, particle size, zeta potential and thermal stability. (A) SDS-PAGE, (B) Particle size, (C) Zeta potential, and (D) Thermal stability, the internal histogram refers to the weight loss at each stage.
3.2. Effects of ultrasound on gel molecular weight, particle size, zeta potential, and thermal stability

3.2.1. SDS-PAGE

SDS-PAGE electrophoresis is used to observe the changes of soybean protein subunits in tofu gel after ultrasonic treatments, as shown in Fig. 2A. It was found that ultrasonic modification had no significant effect on the electrophoretic profile of molecular weight of protein subunits in the gel. The four treatment groups showed similar band distribution without additional high molecular weight compounds forming. Soybean protein was mainly composed of globulin with centrifugal sedimentation coefficient of 11S and β-conglycinin with a centrifugal sedimentation coefficient of 7S, both of which were closely related to the quality of tofu gel. 7S consisted of α (MW 75 kDa), α’ (MW 80 kDa) and β (MW 50 kDa) subunits, while 11S mainly consisted of acidic (MW 28–42 kDa) and alkaline (MW 18–20 kDa) subunits [23].

Besides, the higher the ratio of 11S, the higher the hardness of tofu gel. The hardness of soybean products was mainly affected by 11S protein content. Fig. 2A shows that the electrophoretic bands of tofu gel are mainly in the range of 34–43 kDa, which is an acidic subunit of 11S. ImageJ software was used to compare the gray values of the bands in the ultrasonic groups and the untreated group, which were 16889.619, 21446.154, 21946.326 and 19748.33, respectively. It was found that the gray values of the bands in the ultrasonic groups increased, and the gray value in the 40 + 20 kHz 30’ group was the largest, indicating that there were a large number of soybean protein aggregates. It was proved indirectly the protein cross-link degree was the highest in 40 + 20 kHz 30’ group.

3.2.2. Particle size and zeta potential

The particle size of protein solution reflected the aggregation of protein. As shown in Fig. 2B, the hydrodynamic radius and particle size distribution graph directly reflect the particle size of different samples. All samples showed discontinuous bimodal particle size distribution. Ultrasonic treatments changed the particle size of the gel, widened the particle distribution, and increased the number of large-size polymers. The number of large-size polymers was the largest at 40 + 20 kHz and 30’ group, and next was 40 + 20 kHz 0’ group. It was also consistent with the SDS-PAGE gray value results. Compared with the Control group, the hydrodynamic radius of 40 + 20 kHz 30’ group was the largest, increasing by 15.83 %. Calcium ions could bind to deprotonated phosphate groups and catalyze cross-link reactions between protein molecules to produce more polymers or aggregates. Zhang et al. [5] reported that ultrasonic treatment exposed more TGAse reaction sites and formed more high molecular weight polymers. Therefore, the increase in gel particle size after ultrasonic treatment might be due to the high shear force generated by ultrasonic cavitation that disrupted the chemical interaction within the molecule, that was explained in Section 3.3.2, and exposed the active sites inside the protein, which helped Ca\(^{2+}\) cross-link with proteins to form larger aggregates.

Fig. 2C shows the zeta potential of lyophilized gel. Zeta potential decreased after ultrasonic treatments. The absolute potential value of the 40 + 20 kHz 30’ group was the largest, which was 21.89 % higher than that of the Control group; followed by the 40 + 20 kHz 0’ group, which was 16.29 % higher than the Control group. Ultrasound also changed the charge distribution by disrupting the balance between hydrophilic and hydrophobic groups. Zhang et al. [24] proposed that proteins with high zeta potential (negative or positive) was electrically stable, while proteins with low zeta potential tended to coagulate or flocculate. After ultrasonic treatments, protein surface groups were exposed, the charge distribution was changed, the protein particles interacted to form aggregates, and size increased. At 40 + 20 kHz 30’ group, the zeta potential was the smallest and it aggregated into large particles. This also corresponded to the particle size result (Fig. 2B).

3.2.3. Thermogravimetric analysis (TGA)

TGA provided the weight loss curve of tofu gel with temperature change. With the increase in temperature, the sample changed in different degrees, including evaporation of free water, gel degradation, and low molecular compounds degradation, etc. The weight loss during the whole stage is shown in the histogram (Fig. 2D). The initial weight loss from 35.00 °C to 199.33 °C was mainly related to the loss of free and bound water during heating. After ultrasonic treatment, the gel initial weight loss rate was lower because ultrasound promoted the cross-link reaction of proteins, resulting in the dense gel network, better retention of water, and less water loss. A further increase in temperature resulted in the breaking of covalent bonds, disulfide bonds, and O–N and O–O bonds. Gel samples degraded, evaporated, and loosed most weight at the melting point. As the temperature continued to increase, the gel continued to decompose until 482.667 °C. The temperature of 199.33 to 482.667 was the main weight loss stage for the gel. The weight loss rate of the Control group in the stage reached 71.579 %, which was significantly higher than that of the ultrasonic group. Higher weight loss during gel degradation stage corresponded to lower thermal stability. The lower thermal stability of the Control group meant that lower temperatures could induce protein denaturation and aggregation, showing poor gel performance. Hoque et al. [25] studied the high thermal stability of gelatin/mung bean protein isolate mixture membrane; they found that the more hydrophobic bonds between protein and gelatin molecules, the better the structure of the membrane network, the stronger the heat resistance. Fig. 3B also shows that the hydrophobic interaction in the ultrasonic group is significantly higher than that in the Control group. The results indicated that ultrasonic treatment increased the thermal stability of the gel and exhibited good gel properties.

3.3. Mechanism of tofu gel changes

3.3.1. Surface hydrophobicity (S0ANS) and free sulfhydryl content (FSC)

S0ANS reflected the distribution of hydrophobic amino acid residues on the protein surface, which was related to the functional characteristics of the protein. ANS fluorescence probe was commonly used to measure. The effects of different ultrasonic treatments on S0ANS of tofu gel are studied in Fig. 3A. The results showed that after ultrasonic treatments, S0ANS of the gel decreased significantly from 9.4965 ± 0.0991 to 6.3074 ± 0.0432, especially at 40 + 20 kHz 30’ group. It might be ultrasonic treatments increased the cross-link between protein molecules, promoted the formation of protein polymers, and hid the hydrophobicity region, which was also similar to the results of Guo et al. [26]. TGAse treatment reduced S0ANS of peanut milk protein because protein cross-link was also promoted, and protein dispersion and surface area reduced. There was an association between protein thermal aggregates, and exposed hydrophobic groups were re-encapsulated.

The sulfhydryl groups exposed to the protein surface were called free sulfhydryl groups, which was readily react with Ellman’s reagent. As shown in Fig. 3A, the effects of different ultrasonic treatments on FSC of tofu gel are studied. After ultrasonic modification, the FSC of the gel reduced, and the effect at 40 + 20 kHz 30’ group was the most obvious, reducing by 39.20 %. This might be that ultrasound promoted aggregation of smaller granular proteins, buried of sulfhydryl groups (SH) and reduced free sulfhydryl groups. Wang et al. [27] found that high temperature could reduce the FSC of wheat gluten gels due to the formation of disulfide bond (S–S). S–S was sensitive and easily converted into mercapta derivatives during oxidized, and S–S was the main product [28]. The exchange between −SH and S–S was a dynamic process [26]. The transformation of −SH to S–S caused the formation of protein aggregates. The decrease of FSC revealed the formation of covalent cross-link between proteins, protein aggregation and the number reduction of monitorable active sulfhydryl groups. High intensity ultrasound also promoted the reaction of free radicals (OH and H) to produce hydrogen peroxide, which could oxidize sensitive functional
groups, such as -SH.

3.3.2. Chemical interaction

Coagulants caused different texture of tofu, mainly due to the presence of various forces that stabilized the gel network, including non-covalent forces such as ionic bond, hydrogen bond, hydrophobic interaction, and covalent forces such as disulfide bond. Specific agents could destroy chemical forces, therefore, the magnitude of the chemical forces involved was inferred by comparing the solubility of gels in different reagents. As shown in Fig. 3B, NaCl, urea and β-mercaptoethanol are used to investigate the effect of ultrasonic treatments on the chemical interaction of tofu gel. Compared with the values of ionic bond, hydrogen bond, hydrophobic interaction, and disulfide bond, it was found that hydrophobic interaction and disulfide bond were the main chemical forces of tofu gel. Water-protein and protein–protein interactions could be improved by heat-induced dissociation, depolymerization, unfolding and trap more water when the gel was heated. After ultrasonic modification, the molecular force level of hydrophobic interaction and disulfide bond in tofu gel increased. The ultrasonic modification promoted the exposure of the hydrophobic region but buried -SH inside the protein to external environment, and protein aggregation was generated through the hydrophobic interaction and disulfide bonds. Wang et al. [29] reported that hydrophobic interaction could promote the gelation of proteins to form uniform network structure. Sulfhydryl and disulfide bond were important chemical bonds to maintain protein molecular stability and change functional properties, and the total amount of sulfhydryl groups in protein was fixed. As FSC decreased, the corresponding disulfide bond increases, and disulfide bond content reaches the maximum at 40 + 20 kHz 30° group in Fig. 3B, corresponding to the result in Fig. 3A. The more disulfide bonds in the gel, the more stable the protein gel structure was. It is found from Fig. 3B that the protein solubility of ionic and hydrogen bonds is very small, less than 1.5 mg/g, and has no significant effect on maintaining stable gel conformation. However, hydrogen bonds and ionic bonds were still necessary forces for protein gel formation, and they were associated with protein conformation and gel structure changes. For example, the rearrangement of hydrogen bonds during gelation leaded to a transition from α-helix to β-sheet structure [30], and the reduction of ionic bonds or increase of hydrogen bonds leaded to an increase in gel hardness [29].

3.3.3. Secondary structure

CD of tofu gel after ultrasonic treatments is shown in Fig. 3C. CD had a typical negative peak between 190 ~ 260 nm, and a peak value between 200 ~ 210 nm, showing a typical β-sheet secondary structure [31]. As shown in Fig. 3C, 40 + 20 kHz 0° ultrasound treatment reduces the peak value with blue shift, indicating a decrease in the content of β-sheet. The calculation results in Fig. 3D also show that β-sheet content is the lowest in 40 + 20 kHz 0° group and α-helix content is the lowest in 40 + 20 kHz 30° group. Compared with the α-helix, the more β-sheet with loose structure meant easier interaction, and these interactions promoted protein aggregation [16]. However, higher β-sheet content might induce poor gel water retention. Cheng et al. [32] found that the caffeic acid-modified myofibril gel had the most β-sheet content, but the gel water retention was poor. The changes in the α-helix and β-sheet contents of the gel structure were attributed to ultrasonic cavitation forces. The stronger acoustic cavitation of dual-frequency ultrasound destroyed the interaction between protein molecules, as well as the effects of temperature, pressure and shear force caused by acoustic cavi- tation, which all caused changes in the secondary structure of the protein.

3.3.4. Tertiary structure

When the excitation wavelength was 280 nm, the fluorescence spectrum was mainly ascribed to the microenvironmental polarity
change of tryptophan and tyrosine residues, which was a classic method to monitor tertiary structure changes. As shown in Fig. 3E, the fluorescence intensity of the gel samples decreases after ultrasonic treatments compared to Control. The fluorescence intensity of 40 + 20 kHz 30' group did not change significantly, indicating that 40 + 20 kHz 30' treatment on the proteins in the gel was not enough to destroy the tertiary structure. The fluorescence intensity of the 40 + 20 kHz 0' group was the lowest, indicating that the 40 + 20 kHz 0' treatment had a great impact on the protein in the gel, with tertiary structural changes and tryptophan residues reaching a more subtle microenvironment, which also corresponded to the results of CD. When treated with 40 + 20 kHz 0', the tryptophan residues in the gel were hidden, leading to a decrease of fluorescence intensity. Zhou et al. [33] suggested that the decrease in fluorescence intensity was also related to disulfide and hydrogen bonds, because they were tryptophan fluorescence quenchers.

3.4. Selecting the optimal method based on the TOPSIS-entropy weight method

Indexes were divided into two types: cost indexes and efficiency indexes. The smaller the result, the better the index was the cost index, including S0,ANS, FSC, ionic bond, weight loss rate in water evaporation stage, weight loss rate in gel degradation stage, etc. The larger the result, the better the index was the efficiency index, including hydrogen bond, hydrophobic interaction, disulfide bond, particle size, and zeta potential. The weight of each index is shown in Table 1. It was found that the hydrophobic interaction had the greatest weight with the greatest impact on the gel quality, followed by disulfide bonds and FSC. The weight of S0,ANS and ionic bonds were the least, and the influence on the gel quality were small. Table 2 is the ranking of gel quality under different processing conditions by TOPSIS method. The findings indicated that the ultrasonic group had a considerably higher C value than the control group and that the maximum value was at 40 + 20 kHz 30', indicating that the latter was the best treatment technique and had the best gel quality performance. As a result, the 40 + 20 kHz 30' group was selected to produce the final tofu product.

3.5. Performance evaluation of final tofu product

3.5.1. Moisture content (MC), color and texture

MC was an important index of tofu and represented the ability of tofu gel matrix to immobilize water molecules. MC of tofu under different processes is shown in Fig. 4A. The results showed that the water holding capacity of tofu after ultrasonic increased by 10.78 % compared with the untreated group. On one hand, this might be because cavitation promoted the exposure of the surface-active groups of protein molecules, leading to the formation of a denser, uniform three-dimensional network, so smaller pores could bind more water and increase water holding capacity(WHC) [15]. On the other hand, ultrasonic cavitation could partly affect soybean protein, which prompted conformational change and increased cross-link, contributing to positive effects of ultrasonic on MC in tofu.

As shown in Fig. 4B, the w' (3.07 %) and L' (4.43 %) of tofu after ultrasonic treatment are significantly reduced, while the a' and b' remain unchanged. Previous studies found that the use of ultrasound could cause cell destruction and release intracellular substances. These compounds acted as substrates in the oxidation process and affected the product’s color. The principle of the chromometer was based on Lambert-Beer law, and the composition and molecular arrangement of the medium affected the molar absorption coefficient. Ultrasonic cavitation changed the interaction inside tofu and involved the arrangement of fillers in the matrix. The water dispersed within the gel affected the reflectivity of the gel. Therefore, the improvement of tofu MC might be the reason for the change in surface color.

Gel hardness and MC were the key parameters for evaluating gel quality. As shown in Fig. 4C, ultrasound increases the hardness of tofu to 464.17 g, 89.06 % higher than that of the Control group (p < 0.05), while the springiness and cohesiveness of tofu remain unchanged (p > 0.05). Wang et al. [24] homogenized for 4 cycles at a pressure of 80 MPa, the hardness of mung bean tofu (5 cm × 5 cm × 1 cm) was the largest, but it still did not reach 300 g, which was lower than the hardness of the 40 + 20 kHz 30' group. Fan et al. [15] showed that with the increase of ultrasonic power from 500 W to 600 W, the hardness of tofu (1 cm × 1 cm × 1 cm) increased from 305.77 g to 656.75 g, and the WHC was less than 75 %. By comparison, the hardness of tofu in the 40 + 20 kHz 30' group was found to be lower than that in the 600 W treatment, while the MC reached 79.47 %. Zuo et al. [35] found that when boiling time of raw soymilk was extended to 15 min under atmospheric pressure, the hardness of tofu (1.5 cm × 1.5 cm × 1.5 cm) increased to 452.10 g, slightly lower than that of the 40 + 20 kHz 30' group. The hardness of tofu was related to the dense three-dimensional network inside, and the dense and ordered structure could increase the texture properties of the gel. Li et al. [14] found that hindering the formation of protein aggregates during the gelation process would make the gel network loose and porous, leading to a decrease in the hardness of tofu gel. Previous studies showed that disulfide bonds and hydrophobic interactions were positively correlated with gel hardness [36]. Combined with Fig. 3B, the content of disulfide bonds and hydrophobic interactions in the 40 + 20 kHz 30' group is significantly higher than that in the Control group, increasing the texture properties. In addition, ultrasonic treatment promoted more intermolecular cross-link and increased MC, thus improving the texture properties.

3.5.2. Low-field nuclear magnetic resonance (LF NMR)

LF NMR might be used to study the mobility and proportion of water molecules and characterize the internal structure of tofu without destroying the structure of the sample. Changes in T2 relaxation time and corresponding ratios are shown in Fig. 4D and Fig. 4E. Four obvious peaks were observed, namely, T21, T22, T23 and T24, and their peak area

| Number | Index                  | Index type | Weight |
|--------|------------------------|------------|--------|
| 1      | Surface hydrophobicity | Cost index | 0.075  |
| 2      | Free sulfhydryl content| Cost index | 0.118  |
| 3      | Ionic bond             | Cost index | 0.079  |
| 4      | Weight loss rate rate  | Cost index | 0.881  |
| 5      | Weight loss rate rate  | Cost index | 0.085  |
| 6      | Hydrogen bond          | Efficiency index | 0.083  |
| 7      | Hydrophobic interaction| Efficiency index | 0.160  |
| 8      | Disulfide bond         | Efficiency index | 0.148  |
| 9      | Particle size          | Efficiency index | 0.088  |
| 10     | Zeta potential         | Efficiency index | 0.083  |

Table 1 Weight of tofu gel indexes.

| Group       | D+     | D−     | C1     | Rank |
|-------------|--------|--------|--------|------|
| Control     | 0.3292 | 0.0003 | 0.0010 | 4    |
| 40 + 20 kHz 0' | 0.1915 | 0.1861 | 0.4928 | 3    |
| 40 + 20 kHz 30' | 0.0527 | 0.3123 | 0.8556 | 1    |
| 40 + 20 kHz 45' | 0.1956 | 0.2092 | 0.5168 | 2    |

D+ is the distance between each evaluation index and the positive ideal solution, D− refers to the distance between each evaluation index and the negative ideal solution, and C1 represents the relative proximity.

Table 2 The final ranking of gel quality under different processing conditions by TOPSIS method.
network structure was accompanied by stronger hardness and better capture of water molecules. The microstructure changes further explained the improvement of tofu physical properties (MC, texture). It emphasized the structure-function relationship [40].

4. Conclusions

Compared with untreated gels in the Control group, dual-frequency and multi-angle ultrasonic treatment could promote protein cross-link aggregation, change the covalent and non-covalent interactions of gels, and induce a more uniform formation denser gel network. After a comprehensive evaluation by the TOPSIS-entropy weight method, the optimal ultrasonic condition was 40 + 20 kHz 30° treatment. A significant increase in the hardness and MC of tofu was achieved under the selected conditions, with a slight decrease in the w′, and the gel flavor was improved than the control. As a result, the 40 + 20 kHz 30° treatment produced the optimal tofu quality. This work established a theoretical and technological foundation for improving the ultrasonic processing and utilization of gel.

CRediT authorship contribution statement

Lei Zhang: Data curation, Writing – original draft, Funding acquisition. Xue Wang: Data curation, Writing – original draft. Wenjuan Qu: Supervision. Ao Zhang: Data curation, Writing – original draft. Hafida Wahia: Writing – review & editing. Xianli Gao: Supervision. Haile Ma: Supervision. Cunshan Zhou: Supervision.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultrasochem.2022.106196.

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