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Effect of multiple-frequency ultrasound-assisted transglutaminase dual modification on the structural, functional characteristics and application of Qingke protein

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ABSTRACT

Qingke protein rich in restricted amino acids such as lysine, while the uncoordination of ratio of glutenin and gliadin in Qingke protein has a negative impact on its processing properties. In this study, the effect of multiple-frequency ultrasound combined with transglutaminase treatment on the functional and structural properties of Qingke protein and its application in noodle manufacture were investigated. The results showed that compared with the control, ultrasound-assisted transglutaminase dual modification significantly increased the water and oil holding capacity, apparent viscosity, foaming ability, and emulsifying activity index of Qingke protein, which exhibited a higher storage modulus G' ($P < 0.05$). Meanwhile, ultrasound combined with transglutaminase treatment enhanced the cross-linking degree of Qingke protein ($P < 0.05$), as shown by decreased free amino group and free sulfhydryl group contents, and increased disulfide bond content. Moreover, after the ultrasound-assisted transglutaminase dual modification treatment, the fluorescence intensity, the contents of α-helix and random coil in the secondary structure of Qingke protein significantly decreased, while the β-sheet content increased ($P < 0.05$) compared with control. SDS-PAGE results showed that the bands of Qingke protein treated by ultrasound combined with transglutaminase became unclear. Furthermore, the quality of Qingke noodles made with Qingke powder (140 g/kg dual modified Qingke protein mixed with 860 g/kg extracted Qingke starch) and wheat gluten 60–70 g/kg was similar to that of wheat noodles. In summary, multiple-frequency ultrasound combined with transglutaminase dual modification can significantly improve the physicochemical properties of Qingke protein and the modified Qingke proteins can be used as novel ingredients for Qingke noodles.

1. Introduction

In face to the severe status quo (e.g., population growth, environmental concerns, and global climate changes), there is a growing interest and urgent need to seek sustainable protein sources. Qingke (*Hordeum vulgare Linn. var. nudum* Hook.f.), as a functional cereal, is a cultivar of hulless barley that grows at high altitude. Strong ultraviolet radiation and seasonal drought make it contain more abundant dietary fiber, β-glucan, minerals, and other nutrients than other grains. In particular, Qingke is rich in protein, which has made it draw more and more attention [1,2]. As the major component of Qingke, Qingke protein is composed of glutenin, globulins, gliadins, and albumins, highly similar to the composition of wheat protein. Moreover, Qingke protein contains eight essential amino acids required by humans, especially lysine, which is lacking in other grain proteins, making Qingke protein not only satisfy nutritional demands of consumers, but also provide new ingredient for

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development of functional foods [3,4]. However, Qingke protein contains high levels of glutenin, but low levels of gliadins with hydrophilicity, making it insufficient in functional properties such as insufficient water and oil holding capacity, and apparent viscosity, etc. [5]. These defects in functional properties of Qingke protein directly limit its application in food. Therefore, it is urgent to find a safe and effective mean to improve the functional properties of Qingke protein and enhance its application in food industry.

The application of natural proteins is limited by their inherent properties mentioned above, which cannot meet the requirements of modern food industry. Fortunately, the deficiency of functional properties of Qingke protein can be improved by modification. At present, the modification methods mainly include physical, chemical, and enzymatic methods [6–9]. The physical and enzymatic methods allowed to be used in food processing are obviously safer than chemical methods. For example, transglutaminase (TGase) has been extensively utilized to enhance the functional properties of proteins by promoting the cross-linking of peptides and proteins, such as enhancement of thermal stability of wheat protein [10] and improvement of emulsifying activity and stability index of whey protein isolate [6]. Furthermore, to improve the level of TGase-mediated cross-linking, some trials have been undertaken in attempts to expose more target sites, involving approaches such as heat treatment [11], superfine grinding treatment [8], the addition of reducing agents [12], and ultrasound treatment [13]. Ultrasound is a new, environmentally friendly, and sustainable physical technology and has been used in combination with TGase in food processing [14]. Zhang et al. [13] treated whey protein with ultrasound combined with TGase and found that ultrasound improved the degree of TGase-mediated cross-linking, thereby improving its rheological and gelation properties. Hu et al. [15] processed soybean protein isolate with high-intensity ultrasound combined TGase to increase the hydrophobicity of soybean protein and improve the stability of hydrogels. Clearly, the differences in structure and composition of proteins from different sources may lead to differences in modification effects under the same modification conditions. Our previous study [7] proved that gluten protein treated by multiple-frequency ultrasound had stronger water holding capacity and protein network structure stability compared with gluten protein treated by single-frequency ultrasound. However, the effect of multiple-frequency ultrasound-assisted transglutaminase dual modification on the structural, functional of Qingke protein is still unclear. Therefore, further exploration is needed to determine whether multiple-frequency ultrasound combined with TGase can improve Qingke protein functional properties.

Traditionally, wheat flour has been widely used for noodle production due to the abundant gluten components in wheat. However, exogenous grain components such as germinated mung bean powder [16], oats, aleurone [10], and date fiber [17] are frequently added to food formulae to improve noodles' nutritional quality. Nevertheless, there is little systematic information about completely replacing wheat with other grains to produce noodles. Therefore, if Qingke protein is successfully modified, taking Qingke as the main raw material and mixed with a small amount of gluten protein to produce noodles will maximize the nutritional value of noodles and enrich the species of noodles, and this could provide a critical method for preparing nutritional food and enhance the quality of current whole cereal products.

The purpose of this study was to explore whether multiple-frequency ultrasound combined with TGase can improve the functional properties of Qingke protein such as water and oil holding capacity, and apparent viscosity; second, to explore the correlation between functional and structural properties of Qingke protein from the changes of free sulfhydryl groups (F-SH), disulfide bonds, free amino acid, secondary structure, and tertiary structure groups after multiple-frequency ultrasound combined with TGase treatment. Finally, modified Qingke protein, extracted Qingke starch, and a small amount of gluten protein were mixed to produce Qingke noodle, and the best modification combination under the minimum amount of gluten protein was investigated.

2. Materials and methods

2.1. Materials and chemicals

Whole grain Qingke (*Hordeum vulgare* Linn. var. *nudum* Hook. f. Himara 22$^{\#}$) was provided by Xigaze Agricultural Science Research Institute (Tibet, China). Gluten protein was obtained from Yuxiang Food Co., Ltd. (Zhengzhou, China). Salt was purchased from Yonghui Supermarket (Chongqing, China). Molecular weight protein markers and 5-Dithiobis-2-nitrobenzoic acid (DTNB) were purchased from Yamei Biotechnology Co., Ltd. (Shanghai, China) and Sigma-Aldrich., Ltd. (St. Louis, Missouri, USA), respectively. All other chemicals were of analytical grade.

2.2. Raw material pretreatment

2.2.1. Extraction of Qingke protein and starch

Qingke grains were ground into powder and filtered through a 40-mesh sieve. Qingke protein was extracted according to the methods reported by Nieto-Nieto et al. [18] with some modifications. Qingke starch was extracted according to the methods reported by Wang et al. [19]. Qingke powder (100 g) was dispersed in 2 L pure water and the pH was adjusted to 10.5 by 4 g/L NaOH solution. The suspension was stirred using a RW20 blender (IKA Company, Baden-Württemberg, German) for 2 h at 40 °C, and then centrifuged at 5000 rpm for 10 min (5810, Eppendorf Company, Hamburg, Germany). The supernatant was collected to further extract Qingke protein, and the sediment was washed three times with ultrapure water to obtain wet Qingke starch. The wet starch was dried in an oven at 40 °C for 24 h after removing the upper layer for later use. The pH of the supernatant was adjusted to 4.5 with HCl, followed by centrifugation at 5000 rpm for 10 min. Then, the precipitate was washed three times with ultrapure water and freeze-dried using a vacuum freeze drier (Alpha 2-4 LSC plus, Christ Company, Osterode, Germany) for 48 h to obtain Qingke protein.

2.2.2. Treatment of Qingke protein with combined ultrasound and TGase

Based on our preliminary results, 1 L of 80 g/L Qingke protein suspension was treated with ultrasound reactor (Jiangda Wukesong Biotechnology Co., Ltd., Zhenjiang, China) at either 0 kHz, 40 kHz, 28/40 kHz, or 28/40/50 kHz at 25 °C for 10 min and 9 U/g TGase (the optimal amount of TGase) was added into the ultrasound-pretreated protein suspension and reacted in a water bath at 45 °C for 180 min [20]. The treated samples were then freeze-dried, ground, and sealed for later use.

2.2.3. Preparation of Qingke protein gel

According to the methods reported by Yu et al. [21], Qingke protein suspensions (200 g/L) were stirred at a speed of 300 rpm for 10 min, incubated at 100 °C for 30 min in a boiling water bath, then cooled to room temperature. Samples were then stored at 4 °C for 24 h to obtain gels for further analysis.

2.2.4. Preparation of Qingke dough and noodles

Since Qingke powder cannot form a soft and elastic dough without gluten protein, the flour was made by mixing Qingke powder (140 g/kg modified Qingke protein was mixed with 860 g/kg extracted Qingke starch) and an appropriate amount of gluten protein (Table 1). 120 g/kg gluten protein was added to untreated Qingke powder to serve as a Qingke control sample, and untreated wheat flour (with 122.90 ± 3.80 g/kg gluten protein) served as a wheat control. Dough and noodles were prepared according to our previously reported methods [7] using a dough mixer (Guangzhou Hongyang Casting Co., Ltd.) and an electric pasta machine (Zhejiang Tianxi Kitchenware Co., Ltd.) with slight modifications. According to our preliminary results, 100 mL of 20 g/L salt water was added to the wheat control and 130 mL of 20 g/L salt water was added to the Qingke control and Qingke samples. The fresh

Table 1
The mixing ratio of gluten content and mixing Qingke powder during noodle processing.

Group	Sample	Ultrasound frequency (kHz)	Mixing Qingke powder content (g/kg)	Gluten content (g/kg)
Control	Wheat control	–	–	–
	Qingke control	–	880	120
TGase	TG-100	–	900	100
	TG-90	–	910	90
	TG-800	–	920	80
Multiple-frequency ultrasound combined with TGase	S-TG-100	40	900	100
	S-TG-90	40	910	90
	S-TG-80	40	920	80
	D-TG-80	28/40	920	80
	D-TG-70	28/40	930	70
	D-TG-60	28/40	940	60
	T-TG-80	28/40/50	920	80
	T-TG-70	28/40/50	930	70
	T-TG-60	28/40/50	940	60

noodles were sealed in plastic bags and stored at room temperature for use in determining noodle quality.

2.3. Determination of Qingke protein function properties

2.3.1. Water holding capacity (WHC) and oil holding capacity (OHC)

The WHC and OHC were determined using the methods described previously by Liu et al. [22] with some modifications. The Qingke protein sample (1.0 g) and 10 mL of distilled water or oil were placed in a pre-weighed centrifuge tube, incubated at 25 °C for 30 min, and then centrifuged at 2000 rpm for 30 min. The free water or oil was removed, and the excess water or oil in the upper phase was drained using a filter paper for 10 min. Then, the centrifuge tube and the sediment were weighed.

2.3.2. Rheological properties

Qingke protein suspension apparent viscosity measurement was performed according to the methods reported by Hu et al. [23] with slight modifications. Apparent viscosity characterization of Qingke protein dispersions (200 g/L) was performed at 25 °C using a MCR302 rotational rheometer (Anton Paar GmbH, Graz, Austria) loaded with a parallel plate (40 mm diameter and 1 mm gap) and with a shear rate range of 0.1–100 s^{-1}.

Thereafter, according to the methods reported by Yu et al. [21], a MCR302 rotational rheometer (Anton Paar GmbH, Graz, Austria) loaded with a parallel plate (25 mm diameter and 1 mm gap) was used for frequency sweeps of the gel, and the samples were subjected to a frequency sweep from 0.1 to 100 rad/s at 25 °C with a strain of 0.5 %. According to the Power-Law model, the data were fitted into Eqs. (1) and (2).

$$G' = K'\omega^{n'} \tag{1}$$

$$\tau = \eta/E_1 \tag{2}$$

Where G' is the storage modulus (Pa), G'' is the loss modulus (Pa), ω is the angular frequency (rad/s), K' and K'' (Pa·sn) are consistency indices, and n' and n'' are flow behavior indices.

2.3.3. Protein solubility

According to the methods reported by Zhu et al. [24], protein solubility was quantified using a BCA protein quantification kit (Jiancheng Bioengineering Institute Co., Ltd., Nanjing, China). The absorbance was measured at 562 nm using a T6P ultraviolet spec-trophotometer (Puxi general Instrument Co., Ltd., Beijing, China). The solubility was calculated according to the Eq. (3).

$$\text{Solubility} = (A_s - A_b)/(A_{stan} - A_b) \times C \times D \tag{3}$$

Where A_s, A_b, and A_{stan} are the absorbances of the samples, blank, and standard solution, respectively, C is the standard solution concentration, and D is the dilution factor.

2.3.4. Foaming properties

Qingke protein suspension (20 g/L) was homogenized using a T10 homogenizer (IKA, Staufen, Germany) for 60 s to incorporate air and induce foaming. According to the methods reported by our previous research [25], the foaming ability (FA) and foaming stability (FS) were measured by recording the foaming volume at t = 0 (initial foam volume) and 30 min (final foam volume), respectively.

2.3.5. Emulsifying properties

According to the method reported by Agyare et al. [26], 6 mL Qingke protein suspension (10 g/L, dissolvent was phosphate buffer) was mixed with 2 mL canola oil and homogenized using a T10 homogenizer for 60 s. 50 μL Emulsion was taken from the bottom of the container at standing for 0 min and 10 min, respectively, and evenly mixed with 5 mL sodium dodecyl sulfate (1 g/L, SDS). Then, the absorbance was measured at 500 nm against 1 g/L SDS solution blank using a T6P ultraviolet spectrophotometer. Emulsifying activity index (EAI, m^2/g) and emulsifying stability index (ESI, min) were calculated according to the Eqs. (4)–(5).

$$EAI = (2T \times A_0 \times D)/(C \times \Phi \times 10^4) \tag{4}$$

$$ESI = (\Delta t \times A_0)/(A_{10} - A_0) \tag{5}$$

Where T is 2.302, D is the dilution factor (100), C is the protein concentration (g/L) before emulsification, Φ is the canola oil volume fraction (v/v) of the emulsion (0.25), Δt is 10 (min), A_0 and A_{10} are the absorbances of the samples standing for 0 and 10 min, respectively.

2.4. Determination of Qingke protein structural properties

2.4.1. Free sulfhydryl groups (F-SH) and disulfide bonds

According to the methods reported by Zhang et al. [6], 0.5 mL Qingke protein suspension (10 g/L), 2.5 mL Tris-glycine-8 M urea buffer (8 M urea fully dispersed in 1 L Tris-glycine buffer), and 0.02 mL Ellman's reagent (400 mg DTNB dissolved in Tris-glycine buffer and adjusted to a final volume of 100 mL) were rapidly mixed and incubated at room temperature for 25 min. The absorbance was measured at 412 nm using a T6P ultraviolet spec-trophotometer (Puxi general Instrument Co., Ltd., Beijing, China). The free sulfhydryl group content was calculated according to Eq. (6).

$$SH(\mu mol/g) = \frac{73.53 \times A_{412} \times D}{C} \tag{6}$$

Where C is the protein sample concentration (mg/mL) and D is the dilution factor (100).

200 μL Qingke protein solution (10 mg/mL), 1.0 mL Tris-glycine-10 M urea buffer (10 M urea fully dispersed in 1 L Trimethylol aminomethane-glycine buffer), and 20 μL β-mercaptoethanol were mixed and incubated at room temperature for 1 h, and then 10 mL trichloroacetic acid (120 g/L, TCA) was added and incubation was continued at room temperature for 1 h, followed by centrifugation at 5000 rpm for 10 min. The precipitate was washed twice with TCA solution and dissolved in 3.0 mL Tris-glycine-8 M urea buffer, followed by

mixing with 40 μL DTNB and incubation at room temperature for 25 min. The absorbance was measured at 412 nm. The total sulfhydryl group content was calculated according to Eq. (6) and the disulfide bond content was calculated according to Eq. (7).

$$disulfide\ bonds(\mu mol/g) = (T - SH - F - SH)/2 \quad (7)$$

2.4.2. Free amino groups

The free amino group content of samples was determined by the o-phthalaldehyde (OPA) method [13]. 10 mg Qingke protein was dissolved in 1 mL HCl (3.65 g/L) and stirred using a RW20 blender for 1 h, and then centrifuged at 7500 rpm for 10 min. The supernatant (200 μL) was mixed with 4 mL of OPA reagent and incubated for 2 min at 35 °C. The absorbance was measured using a spec-trophotometer at 340 nm.

2.4.3. Fourier transform infrared spectroscopy (FTIR)

A previously described FTIR method [27] was used with slight modifications. A Qingke-potassium bromide sample (2 mg Qingke protein mixed with 98 mg potassium bromide) was ground to powder in an agate mortar and scanned from 4000 to 500 cm^{-1} using a Spectrum100 Fourier transform infrared spectrophotometer (PerkinElmer, Waltham, Massachusetts, USA). 64 and 32 scans were collected from background and samples, respectively, at a resolution of 4 cm^{-1}.

2.4.4. Intrinsic fluorescence

The 5 g/L Qingke protein suspension (dissolvent was phosphate buffer) was shaken at room temperature for 1 h and then centrifuged at 7500 rpm for 10 min. 2 mL of supernatant was scanned from 220 nm to 500 nm using a F-2500 fluorescence spectrophotometer (Hitachi Ltd., Tokyo, Japan). The excitation wavelength was 280 nm, the emission width was 5 nm, and the slit width was 5 nm.

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

SDS-PAGE was performed according to the methods reported by Li et al. [28] with slight modifications. 5 mg Qingke protein was mixed with 300 μL electrophoretic buffer solution (4×) and incubated for 5 min in boiling water, cooled, and then centrifuged at 10000 rpm for 10 min. 10 μL supernatant was added to the gel and electrophoresis was performed using the Mini-PROTEAN Tetra system (Bio-Rad, Hercules, CA, USA) in constant-current mode (5 % concentrated gel run at 80 V and 12 % separation gel run at 120 V). After electrophoresis was complete, the gel was stained with Coomassie blue (R250) for 2 h and photographed.

2.5. Determination of Qingke dough and noodle quality

2.5.1. Texture profile analysis (TPA)

The dough was molded into a cylindrical shape with a height of 20 mm and a diameter of 40 mm. The textural parameters were determined using a TA/XT Plus texture analyzer (Stable Micro System, UK) with a P3/6R probe at a test speed of 1.0 mm/s, a compression ratio of 50 %, and a pressure of 5.0 g. Each sample was measured 6 times.

2.5.2. Stress relaxation test

Stress relaxation was determined using a P/50 probe at a test speed of 1.0 mm/s, a pressure of 5.0 g, a compressive deformation of 10 % (ε_0), and a release time of 90 s. A Maxwell model was selected to analyze stress relaxation (σ(t)) [29], which was fitted into Eq. (8), and τ was calculated in Eq. (9).

$$\sigma(t) = \varepsilon_0 E_1 \exp(-t/\tau) + \varepsilon_0 E_2 \quad (8)$$

$$\tau = \eta/E_1 \quad (9)$$

Where E_1 is the first element of Hooke's elastic modulus, E_2 is residual stress, t is time, η is the damping coefficient, and τ is the relaxation time. Each sample was measured 6 times.

2.5.3. Noodle cooking quality

Noodle cooking quality was determined according to the methods reported in our previous research [7].

2.6. Statistical analysis

Results are expressed as mean ± standard deviation (SD). Data were analyzed by one-way analysis of variance using SPSS 16.0 (SPSS Inc, Chicago, IL, USA). Duncan's test was used to check the significance of the differences between mean values, and $P < 0.05$ was considered to be significant.

3. Results and analysis

3.1. Effect of multiple-frequency ultrasound-assisted transglutaminase dual modification on Qingke protein functional properties

3.1.1. WHC and OHC

WHC and OHC represent a protein's ability to interact with water and oil, respectively. It can be seen from Fig. 1 that the WHC and OHC of Qingke protein were significantly improved by combined ultrasound and TGase treatment ($P < 0.05$) in the following order: triple-frequency ultrasound combined with TGase > dual-frequency ultrasound combined with TGase > single-frequency ultrasound combined with TGase > TGase. Presumably, after TGase treatment, Qingke protein network structure and flow resistance were enhanced, thereby improving the binding ability of protein to water and oil [30]. After pretreatment with ultrasound, the protein network was loosed by ultrasound, which enhanced the TGase-mediated cross-linking and increased the exposure of polar amino acid side chains and lipophilic sites, thus enhancing WHC and OHC of the protein [7]. The protein treated by dual- and triple-frequency ultrasound combined with TGase had higher WHC and OHC ($P < 0.05$), indicating that multiple-frequency ultrasound had a better effect on TGase-mediated cross-linking and protein network conformation compared with single-frequency ultrasound pretreatment.

3.1.2. Rheological properties

Apparent viscosity is affected by complex factors such as the size and shape of protein molecules, interactions between the protein and solvent, hydrodynamic volume, and molecular elasticity in the hydrated state [13]. As shown in Fig. 2a, the apparent viscosity of all samples decreased with increasing shear rates. This may be due to the structure of the aggregated protein was destroyed partially, which led to the

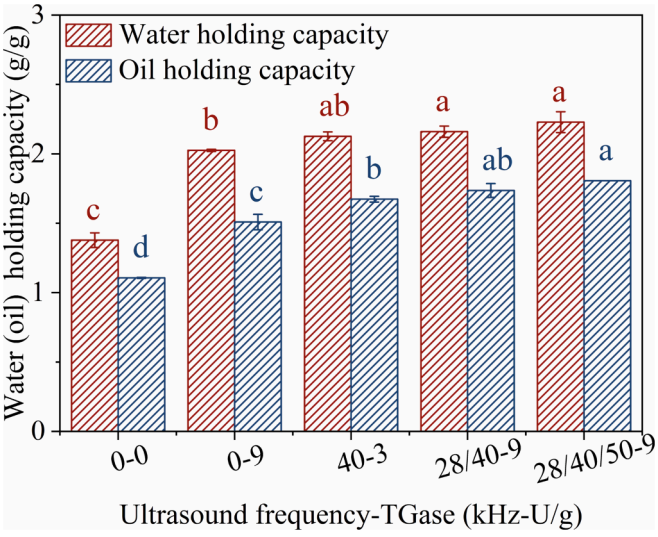

Fig. 1. Effect of multiple-frequency ultrasound-assisted transglutaminase dual modification on the water (oil) holding capacity of Qingke protein.

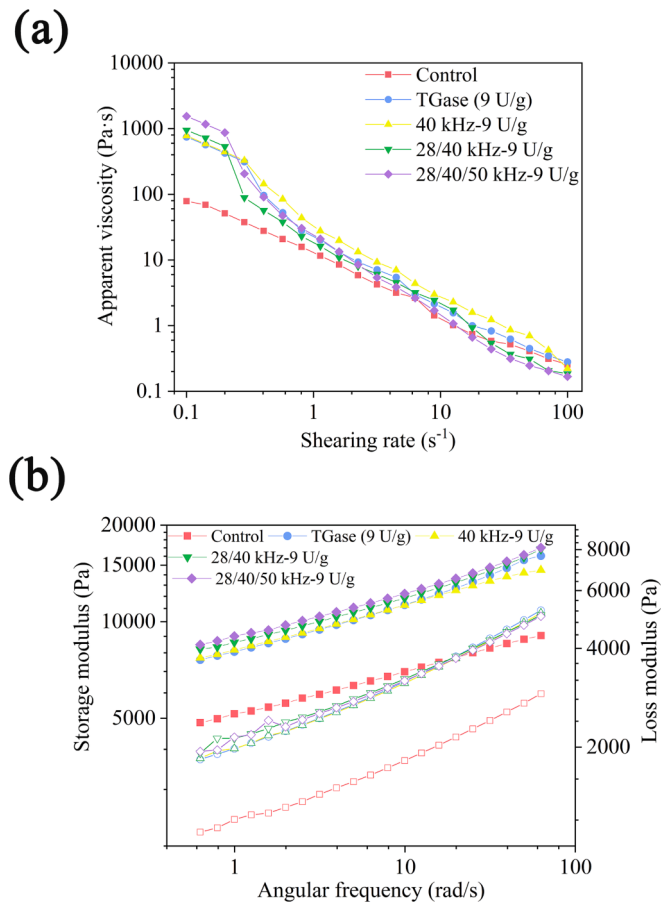

Fig. 2. Effect of multiple-frequency ultrasound-assisted transglutaminase dual modification on the apparent viscosity (a) and gel frequency sweeps (b) of Qingke protein.

reduction of flow resistance and thereby decreasing the apparent viscosity [6]. At the shear rate of 0.1 s^{-1}, TGase treatment significantly increased Qingke protein apparent viscosity compared with the control. Meanwhile, Qingke protein treated by ultrasound combined with TGase had higher apparent viscosity compared to TGase treatment alone, and the apparent viscosity increased with increasing ultrasound frequency combination. Moreover, after triple-frequency ultrasound combined with TGase treatment, the apparent viscosity of Qingke protein reached the highest value (1540 Pa·s). This result may be attributed to the fact that TGase and ultrasound promoted the formation of high-molecular-weight protein polymers, Moreover, the non-covalent interactions between the Qingke protein were destroyed by ultrasound treatment, leading to exposure of more binding sites and facilitating TGase binding to substrates [13,15]. Thus, flow resistance of the protein network was increased and water mobility was decreased, resulting in a higher apparent viscosity.

To better understand the rheological properties of Qingke protein gel, frequency sweeps were carried out at a temperature of 25 °C. G′ and G″ are related to the elasticity and viscosity of the protein, respectively [30]. Fig. 2b and Table A.1 show that all the samples exhibited bigger G' than G'', indicating that these Qingke protein gel samples exhibited the solid-like behavior properties. It was found that G' and G'' of gel increased significantly, while n' and n'' of gel decreased after TGase treatment compared to the control ($P < 0.05$). Compared with TGase treatment alone, single-frequency ultrasound combined with TGase had no significant effect on the elasticity and viscosity of Qingke protein gel ($P > 0.05$), but dual- or triple-frequency ultrasound combined with TGase treatment significantly increased the Qingke protein gel elasticity and viscosity and decreased n' ($P < 0.05$). A low n' indicates a high degree of crosslinking and a highly stable and tight protein network structure [21]. Therefore, it can be speculated that ultrasound combined with TGase treatment can promote the aggregation of Qingke protein and make the protein form a denser and uniform network in the process of gelation. According to the finding of Qin et al. and Yuan et al. [31,32], the cavitation and microfluidic effects of ultrasound treatment caused the protein chain to unfold and the molecules to move rapidly. The exposed hydrophobic groups contribute to the formation of a more consolidated gel network, and the exposure of certain targeted regions may also promote TGase-mediated cross-linking.

3.1.3. Protein solubility

Solubility is the most practical measure of protein denaturation and aggregation [23]. As shown in Fig. 3a, the solubility of Qingke protein significantly decreased after TGase treatment compared to the control ($P < 0.05$), and no significant difference was observed between TGase alone treatment and combined treatment of TGase with ultrasound ($P > 0.05$). Zhang et al. [6] showed that a low solubility was associated with the formation of cross-linked proteins and Karki et al. [33] showed that protein solubility was also related to charge and intermolecular forces between proteins. Therefore, it can be speculated that TGase-catalyzed cross-linking enhances protein intermolecular forces such as hydrophobic interactions and hydrogen bonding, thus leading to the formation of insoluble protein aggregates. The conformational changes of proteins induced by ultrasound may expose hydrophobic, hydrophilic, and non-polar binding sites in the protein's internal structure and promote the cross-linking of TGase [32], resulting in a decrease in solubility. However, there was no significant change probably due to the restriction of the cross-linking sites.

3.1.4. FA and FS

The molecular factors such as rapid diffusion of the molecule to the air/water interface, amphiphilic structure and flexibility affect the ability of a protein to form and stabilize foam [34]. As shown in Fig. 3b, TGase treatment significantly increase the FA and FS of Qingke protein ($P < 0.05$). Compared with TGase treatment alone, ultrasound combined with TGase had no significant effect on the FS of Qingke protein ($P > 0.05$), while the combination of triple-frequency ultrasound and TGase significantly improved the FA of Qingke protein ($P < 0.05$). Previous study [20] showed that TGase-catalysed cross-linking led to an increase in polymers and viscosity of protein and reduced the FA of proteins, which was inconsistent with our study. A plausible explanation is that TGase-mediated cross-linking improves protein amphiphilicity, facilitates protein unfolding on the water surface, thereby improving FA and FS [34]. Simultaneously, another hypothesis is that ultrasound pretreatment resulted in a more uniform distribution of protein particles and improved the surface activity of proteins at the solution-air interface [20]. Under the same condition of TGase treatment, the FA of samples pretreated with triple-frequency ultrasound was increased by 15.38 % compared with that of TGase treatment alone, suggesting that multiple-frequency ultrasound and TGase have a synergistic effect to improve the FA of Qingke protein.

3.1.5. ESI and EAI

EAI and ESI represent the formation capacity and stability of protein emulsifying, respectively, which are critical for protein functions in food composition. As shown in Fig. 3c, TGase treatment significantly improved the ESI and EAI of Qingke protein emulsions ($P < 0.05$), and after combined with ultrasound, the EAI was significantly increased ($P < 0.05$), while ESI showed no significant change ($P > 0.05$). Similar to foaming properties, the significant improvement in emulsifying properties may be due to the increased amphiphilicity of proteins caused by TGase treatment, which promotes protein-lipid interaction and facilitates the anchoring of protein molecules at the oil–water interface, resulting in reduced interfacial tension and increased EAI. In addition, the higher molecular weight of crosslinked Qingke protein changes the

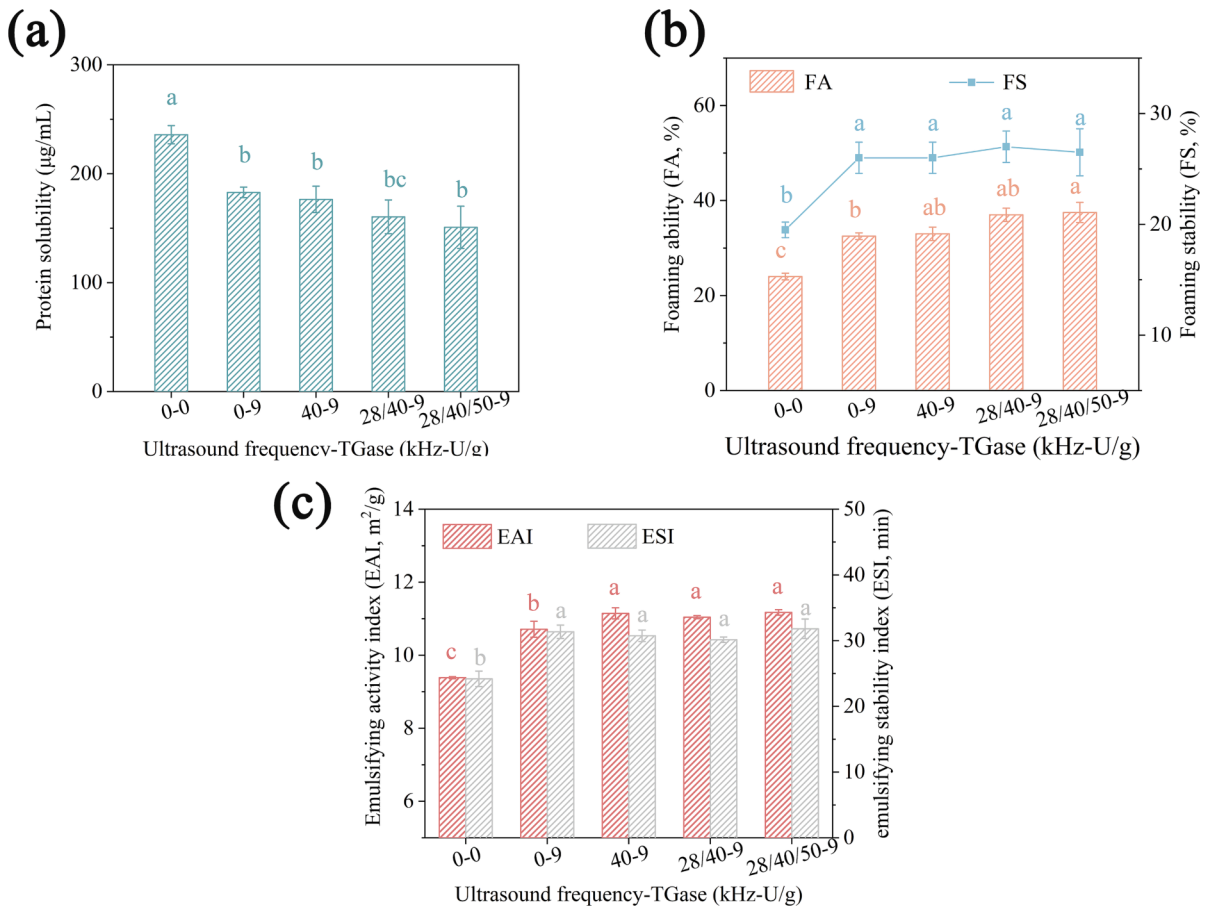

Fig. 3. Effect of multiple-frequency ultrasound-assisted transglutaminase dual modification on the solubility (a), foaming properties (b), and emulsifying properties (c) of Qingke protein.

electrostatic energy and interfacial energy of the emulsifying, thus improving ESI [8]. After combined with ultrasound, the increase of EAI may be due to the fact that ultrasound leads to a larger proportion of small soluble protein adsorbed to the oil–water interface, while ESI has no significant change, which may be caused by the extensive protein unfolding and aggregation caused by ultrasound [24]. To explain the changes of functional properties, the structural properties of the modified Qingke protein were measured.

3.2. *Effect of multiple-frequency ultrasound-assisted transglutaminase dual modification on Qingke protein structural properties*

3.2.1. *Free amino groups*

Fig. 4a shows the effect of ultrasound combined with TGase treatment on the free amino groups of Qingke protein. The content of free amino groups of Qingke protein was significantly reduced after TGase treatment. The content of free amino groups was not significantly changed by single-frequency ultrasound pretreatment compared with TGase treatment alone, but it was significantly reduced when combined with dual- or triple-frequency ultrasound. This result was similar to that of whey protein treated by ultrasound combined with TGase treatment [13]. Djoullah et al. [35] shown that the decrease of free amino groups content was related to the increase of protein cross-linking degree. Therefore, corresponding to previous speculation, the reduction of free amino groups may be due to TGase-mediated intermolecular and/or intramolecular cross-linking of proteins. In addition, ultrasound may destroy the non-covalent interaction between protein molecules and expose more active regions inside the protein [13]. While the mechanical physical and chemical effects caused by the cavitation effect can reduce the size of Qingke protein particles, thereby enhancing the cross-linking effect of TGase and increasing the consumption of free amino groups. Obviously, multiple-frequency ultrasound pretreatment promotes protein cross-linking much more than single-frequency ultrasound.

3.2.2. *F-SH groups and disulfide bonds*

As shown in Fig. 4b, with the addition of TGase, the contents of total sulfhydryl and F-SH groups in Qingke protein decreased significantly, while the content of disulfide bonds increased significantly ($P < 0.05$), consistent with the findings of Mattice & Marangoni [36]. Compared with TGase treatment alone, the combined ultrasound and TGase treatment significantly decreased the content of the Qingke protein F-SH group while increased the content of disulfide bonds ($P < 0.05$). Notably, treatment of triple-frequency ultrasound combined with TGase decreased the content of Qingke protein F-SH by 16.14 % and increased the content of disulfide bonds by 0.92 %. Some literature [6] showed that the decrease of F-SH group content was due to that TGase promoted the oxidation of F-SH groups to turn into new disulfide bonds, and formed crosslinking products through the extensive aggregation of disulfide bonds. Gao et al. [14] showed an increase in F-SH content after ultrasound treatment of soybean milk and a decrease in F-SH content after ultrasound combined with TGase treatment, which was similar to our finding. The results may be due to the high intensity shock waves, shear force, and turbulence produced by ultrasound, which led to partially unfolded Qingke protein, exposing F-SH group on protein surface, thereby promoting TGase-mediated cross-linking, which facilitates the transformation of F-SH groups to disulfide bonds [37]. The dual- and triple-frequency ultrasound cavitation intensities were higher

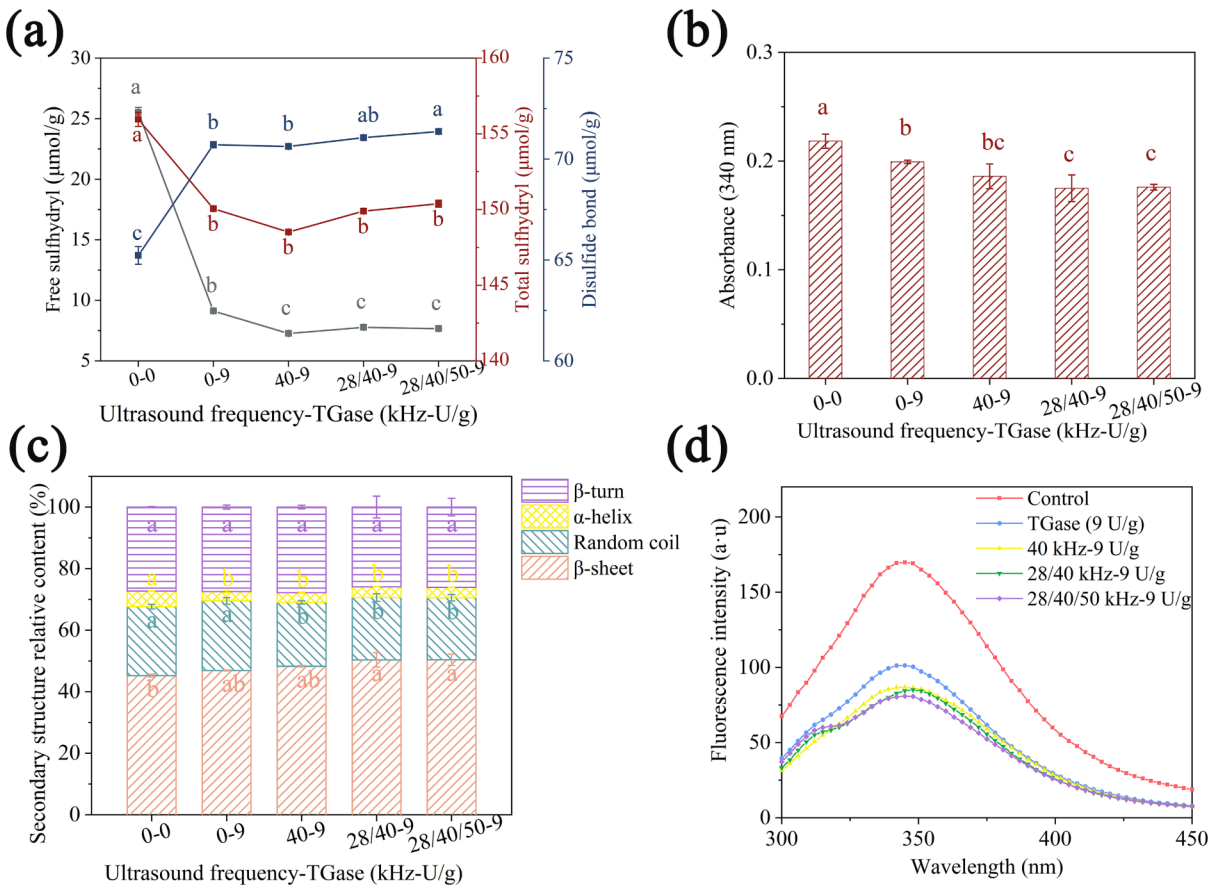

Fig. 4. Effect of multiple-frequency ultrasound-assisted transglutaminase dual modification on the free sulfhydryl groups (F-SH), disulfide bonds (a), free amino groups (b), secondary structure (c), and fluorescence intensity (d) of Qingke protein.

than that of single-frequency ultrasound treatment, consistent with our previous findings.

3.2.3. FTIR analysis

The FTIR amide I band (1700–1600 cm^{-1}) is mainly affected by the dipole coupling transition, which is sensitive to the main protein chain structure and is often used to analyze protein secondary structure transformation [38]. By fitting the amide I band's second derivative spectrum, β-sheet (1610 ~ 1640 cm^{-1}), random coil (1640 ~ 1650 cm^{-1}), α-helix (1650 ~ 1660 cm^{-1}), and β-turn (1660 ~ 1670 cm^{-1}) peaks could be separated, and their peak areas represent their secondary structure contents [39].

The relative β-sheet content of Qingke protein treated by TGase alone increased, while the α-helix content decreased significantly ($P < 0.05$) compared to the control (Fig. 4c), similar to whey protein after TGase treatment [8]. The Qingke protein random coil content significantly decreased ($P < 0.05$), while the β-sheet content significantly increased in Qingke protein treated by dual- or triple-frequency ultrasound combined with TGase ($P < 0.05$) compare with TGase treatment alone. Presumably, TGase may destroy the stability of hydrogen bonds necessary for the regular arrangement of the α-helix structure, and the expanded polypeptide chain exposes more hydrogen bonds, rearranges the hydrophobic groups, and reconnects to form β-sheets when the molecules gather [11]. Additionally, ultrasound combined with TGase treatment may induce the random coils in Qingke protein to form β-sheets, thereby increasing the secondary structure order and promoting Qingke protein cross-linking, thus building a stable three-dimensional network [40]. Gao et al. [14] showed that β-sheets had a greater hydration ability than α-helices, thus, an increase in the β-sheet ratio is beneficial to the protein gelation, improving its functional properties like WHC.

3.2.4. Intrinsic fluorescence analysis

The intrinsic fluorescence spectra of proteins mainly characterize the interactions of fluorescent amino acid residues, such as tryptophan, tyrosine, phenylalanine, and other amino acid residues, which can be used to determine protein tertiary structure conformational changes [7]. The fluorescence emission maximum (λ_{max}) of samples was 345 nm and tended to move towards 348 nm when treated with dual-frequency ultrasound combined with TGase, and the Qingke protein fluorescence intensity (FI) at λ_{max} after different treatments was as follows: triple-frequency ultrasound combined with TGase < dual-frequency ultrasound combined with TGase < single-frequency ultrasound combined with TGase < TGase (Fig. 4d). The λ_{max} is usually associated with the environment of tryptophan and tyrosine residues located in the protein molecule. The λ_{max} motion indicated that the tertiary structure of protein had a conformational change, which affected the environment of tryptophan and tyrosine [6]. The decrease in FI at λ_{max} may be due to the peptides of different sizes, side chain groups, and hydrophobic residues in Qingke protein were crosslinked by TGase, forming polymers with different polar environments and masked fluorescent amino acid residues [6]. Compared with TGase treatment alone, Qingke protein pretreated by ultrasound had lower FI, indicating that the combined ultrasound and TGase treatment may induce stronger protein polymerization, leading to a greater degree of fluorescent amino acid residues concealment [41].

3.2.5. SDS-PAGE

As shown in Fig. 5, the molecular weight distribution of untreated Qingke protein was mainly concentrated in 10–15 kDa and 25–70 kDa.

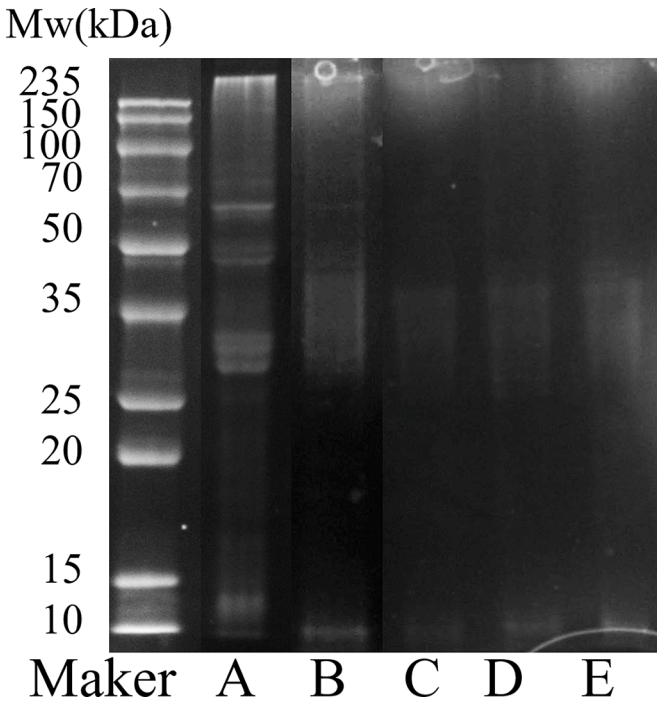

Fig. 5. Effect of multiple-frequency ultrasound-assisted transglutaminase dual modification on the SDS-PAGE bands of Qingke protein. (A: untreated; B: 0 kHz-9 U/g; C: 40 kHz-9 U/g; D: 28/40 kHz-9 U/g; E: 28/40/50 kHz-9 U/g).

The Qingke protein treated with TGase formed a weak and unclear band compared with the control group, consistent with the soybean protein research results [36]. Compared with TGase treatment alone, the Qingke protein bands from samples treated with combined ultrasound and TGase were more unclear. This result may be attributed to the fact that TGase-induced Qingke protein cross-linking leads to formation of high molecular weight aggregates, and the high molecular weight subunits cannot enter the gel, causing blurred bands. At the same time, Hu et al. [15] reported a similar finding that high-intensity ultrasound treatment led to partial expansion and exposure of functional groups within the protein. Therefore, it was inferred that ultrasound treatment destroyed non-covalent interactions between protein molecules, expanded partial protein molecules, and exposed some functional groups previously buried within the protein molecules, thus improved cross-linking, and led to formation of a large number of polymers and lighter bands [42]. Next, this study tried to use modified Qingke protein, extracted Qingke starch, and a small amount of gluten to make Qingke noodles in order to realize the application of modified Qingke protein.

3.3. *Effect of multiple-frequency ultrasound-assisted transglutaminase dual modification on Qingke dough and noodle quality*

3.3.1. *Dough textural characteristics*

In order to retain Qingke's nutritional characteristics while achieving a quality similar to wheat noodles, we combined ultrasound treatment with TGase to produce Qingke noodles with a small amount of gluten protein. Hardness and gumminess of dough reflect the sensory quality of noodles during consumption. The hardness of the Qingke control was significantly higher than that of the wheat control ($P < 0.05$), which can be attributed to a higher amount of dietary fiber from Qingke (Table 2) [43]. There was a significant increase in Qingke dough hardness after TGase treatment compared to the Qingke control ($P < 0.05$) while no significant difference in gumminess ($P > 0.05$) when the gluten protein content was 90–100 g/kg. Compared to the group treated with TGase alone, there were significant increases in Qingke dough hardness when dual- or triple-frequency ultrasound was combined with TGase when the gluten protein content was 80 g/kg ($P < 0.05$). Among the conditions tested, triple-frequency ultrasound combined with TGase treatment of samples with 60 g/kg added gluten protein resulted in a

Table 2
Quality parameters of Qingke dough and noodles with less gluten protein treated by combined ultrasound and TGase.

TPA parameters			Stress relaxation parameters			Cooking quality		
Sample	Hardness (g)	Gumminess	Relaxation time τ(s)	Residual stress E_2(N/m^2)	Damping coefficient η(N/m^2/s)	Optimum cooking time (min)	Water absorption (%)	Dry matter loss rate (%)
Wheat control	1249.17 ± 34.63^{e}	511.88 ± 20.57^{ab}	7.71 ± 1.53^{b}	580.95 ± 20.15^{bcd}	8120.07 ± 395.8^{abc}	4.83 ± 0.29^{e}	139.86 ± 5.43^{a}	5.21 ± 0.16^{c}
Qingke control	1488.06 ± 78.38^{d}	517.23 ± 43.19^{ab}	8.75 ± 0.93^{b}	492.95 ± 43.76^{d}	8139.47 ± 549.76^{abc}	5.78 ± 0.25^{d}	109.18 ± 4.72 ^{g}	7.04 ± 0.23^{a}
TG-100	1934.85 ± 95.02^{ab}	545.41 ± 54.08^{ab}	7.98 ± 0.98^{b}	766.95 ± 95.65^{a}	10030.72 ± 1296.44^{ab}	6.17 ± 0.17^{bc}	114.42 ± 2.12^{ef}	4.57 ± 0.14^{c}
TG-90	1832.75 ± 77.58^{abc}	556.03 ± 34.52^{ab}	8.30 ± 0.83^{b}	680.55 ± 97.35^{ab}	9065.12 ± 2573.76^{abc}	6.50 ± 0.01^{a}	122.65 ± 11.22^{cde}	5.17 ± 0.25^{c}
TG-80	1398.84 ± 57.31^{de}	477.36 ± 70.43^{b}	7.70 ± 0.81^{b}	512.60 ± 54.87^{d}	6701.81 ± 537.10^{c}	6.36 ± 0.13^{abc}	128.49 ± 4.87^{bcd}	5.70 ± 0.17^{b}
S-TG-100	1951.06 ± 164.70^{abc}	511.15 ± 51.46^{ab}	9.38 ± 0.95^{ab}	743.80 ± 84.18^{a}	11003.57 ± 2179.64^{a}	6.50 ± 0.01^{a}	119.76 ± 9.71^{de}	5.00 ± 0.14^{c}
S-TG-90	1722.25 ± 111.54^{c}	489.64 ± 26.23^{ab}	7.49 ± 0.36^{b}	655.85 ± 19.72^{abc}	8584.56 ± 766.59^{abc}	6.47 ± 0.01^{ab}	124.33 ± 4.60^{bcde}	5.28 ± 0.32^{b}
S-TG-80	1480.20 ± 172.93^{d}	381.43 ± 18.34^{c}	11.37 ± 0.39^{a}	527.30 ± 31.39 ^{cd}	9250.39 ± 245.90^{abc}	6.47 ± 0.05^{ab}	130.11 ± 5.24^{abcd}	5.73 ± 0.21^{b}
D-TG-80	1984.52 ± 130.51^{a}	599.08 ± 5.62^{a}	7.12 ± 0.83^{b}	679.35 ± 46.08^{ab}	9544.76 ± 1370.27^{abc}	6.25 ± 0.25^{abc}	131.64 ± 6.50^{abc}	4.33 ± 0.15^{d}
D-TG-70	1777.41 ± 85.51^{abc}	516.40 ± 36.89^{ab}	8.37 ± 0.40^{b}	590.90 ± 8.06^{bcd}	8584.56 ± 1468.59^{abc}	6.33 ± 0.25^{abc}	134.48 ± 3.61^{ab}	5.25 ± 0.10^{c}
D-TG-60	1586.00 ± 236.84^{d}	370.58 ± 46.24^{c}	8.48 ± 2.75^{b}	572.35 ± 32.59^{bcd}	7637.25 ± 766.59^{bc}	6.11 ± 0.10^{c}	126.26 ± 3.03^{bcd}	5.73 ± 0.21^{b}
T-TG-80	1946.61 ± 120.40^{a}	571.18 ± 32.88^{ab}	7.28 ± 0.01^{b}	735.75 ± 67.45^{a}	8933.91 ± 1154.86^{abc}	6.28 ± 0.09^{abc}	130.64 ± 1.26^{abc}	4.97 ± 0.21^{c}
T-TG-70	1644.54 ± 161.05^{bc}	545.73 ± 7.47^{ab}	8.52 ± 0.71^{b}	580.55 ± 32.88^{bcd}	8275.13 ± 121.19^{abc}	6.06 ± 0.20^{c}	134.36 ± 2.58^{ab}	5.16 ± 0.21^{c}
T-TG-60	1717.94 ± 121.42^{c}	516.97 ± 91.69^{ab}	8.46 ± 1.13^{b}	559.45 ± 24.40^{bcd}	8216.67 ± 32.65^{abc}	6.11 ± 0.10^{c}	127.85 ± 4.66^{bcd}	5.25 ± 0.40^{c}

Notes: Lowercase letters represent significant differences among different samples ($P < 0.05$).

higher hardness ($P < 0.05$) and similar gumminess compared to the wheat control. Yang et al. [10] suggested that the increased hardness and gumminess may be due to the cross-linking reaction catalyzed by TGase, which promoted the formation of a continuous and uniform Qingke protein network such that starch and fibers were well dispersed in the network structure. At the same time, it was also related to the interaction between gluten protein and Qingke protein and starch. As previously described, ultrasound combined with TGase treatment can promote the cross-linking reaction between Qingke proteins, and ultrasound may change the protein conformation, promote the distribution of starch in the protein structure and the cross-linking effect of gluten protein [7]. Triple-frequency ultrasound combined with TGase treatment has better dough quality, resulting in a Qingke dough texture similar in quality to wheat dough despite having less gluten protein.

3.3.2. Dough stress relaxation characteristics

Protein plays an important role in dough's stress relaxation behavior, so the stress relaxation parameters of Qingke dough after various treatments were measured next (Table 2). The relaxation time (τ) is the result of the interaction between elastic behavior and viscous behavior [44]. Overall, there were no significant changes in τ in our study ($P > 0.05$), suggesting that the combination of ultrasound with TGase had no significant effect on the dough's anti-deformation ability. The residual stress (E_2) reflects the dough's elastic behavior, while the damping coefficient (η) is positively correlated with the dough's initial viscosity [45]. Compared with the wheat control, Qingke control has a lower E_2 ($P < 0.05$), but there was no significant difference in η between them ($P > 0.05$). This may be because the dietary fiber filling material in the Qingke control disrupted the gluten network's compactness and structural integrity [17], thus reduced the dough's ability to recover from elastic deformation. After the addition of TGase, E_2 and η were significantly higher in Qingke dough with 90–100 g/kg gluten protein compared to the Qingke control ($P < 0.05$). There was no significant change in E_2 in the Qingke dough treated by single-frequency ultrasound, but E_2 was significantly higher in Qingke dough after dual- or triple-frequency ultrasound treatment combined with TGase compared to dough treated by TGase alone ($P < 0.05$) when the gluten protein content was 80 g/kg. Interestingly, there were no significant differences ($P > 0.05$) in E_2 or η between the wheat control and the Qingke dough with less gluten protein content (60–70 g/kg) after dual- or triple-frequency ultrasound treatment combined with TGase. As our previous study demonstrated [7], dual- and triple-frequency ultrasound disturb protein significantly more than single-frequency ultrasound, therefore, Qingke dough treated by dual- or triple-frequency ultrasound combined with TGase had a higher degree of polymerization and enhanced internal binding forces, while still maintaining good stress relaxation characteristics when 60–70 g/kg gluten protein was added.

3.3.3. Noodle cooking quality

The optimal cooking time, water absorption rate, and dry matter loss rate of the Qingke control were significantly different from those of the wheat control ($P < 0.05$) (Table 2). This is due to the Qingke control's high dietary fiber content, which destroyed the gluten network continuity, thus increasing the optimal cooking time and dry matter loss rate. In addition, Qingke has a higher protein content than wheat, which was negatively correlated with noodle water absorption rate [16]. Compared with the Qingke control, TGase treatment significantly increased the optimal cooking time and water absorption rate, and decreased the dry matter loss rate of Qingke noodles with a gluten content of 80–100 g/kg ($P < 0.05$). Compared to Qingke noodles treated by TGase alone, single-frequency ultrasound combined with TGase treatment did not significantly improve the Qingke noodle cooking quality ($P > 0.05$). However, with less gluten protein (60–80 g/kg), the Qingke noodle water absorption rate after dual- or triple-frequency ultrasound treatment combined with TGase significantly increased and dry matter loss rate decreased ($P < 0.05$) compared to the Qingke control. There was no significant difference in cooking quality between wheat control and Qingke samples treated by dual- or triple-frequency ultrasound combined with TGase when 70 g/kg gluten protein was added ($P > 0.05$). An increase of the optimal cooking time may be attributed to the Qingke protein cross-linking induced by TGase, which increased the dough's internal network structure compactness [44]. Meanwhile, the starch was wrapped completely in the dough, thus reducing the noodles' dry matter loss rate and improving their water absorption [10]. Obviously, multi-frequency ultrasound pretreatment enhanced the gluten structure of Qingke noodles treated with TGase, so that it could achieve the cooking quality of wheat noodles with less gluten protein added.

4. Discussion

In this study, multiple-frequency ultrasound combined with TGase treatment significantly improved the functional properties of Qingke protein. In order to explain the potential reasons for this improvement, we speculated based on the changes in the structural properties of Qingke protein. To intuitively reveal the relationships between Qingke protein functional properties and structural properties under the combined ultrasound and TGase treatment, correlation heatmap analysis is shown in Fig. 6a. WHC was negatively correlated with free amino group and α-helix ($P < 0.05$), significantly negatively correlated with F-SH ($P < 0.01$), extremely significantly negatively correlated with FI ($P < 0.001$), and significantly positively correlated with disulfide bonds ($P < 0.01$). OHC was negatively correlated with F-SH ($P < 0.05$), significantly negatively correlated with free amino group and FI ($P < 0.01$), and positively correlated with disulfide bonds and β-sheet ($P < 0.05$). The apparent viscosity was negatively correlated with free amino group ($P < 0.05$) and positively correlated with β-sheet ($P < 0.05$). G' was negatively correlated with free amino group and α-helix ($P < 0.05$), significantly negatively correlated with F-SH ($P < 0.01$), extremely significantly negatively correlated with FI ($P < 0.001$), and extremely

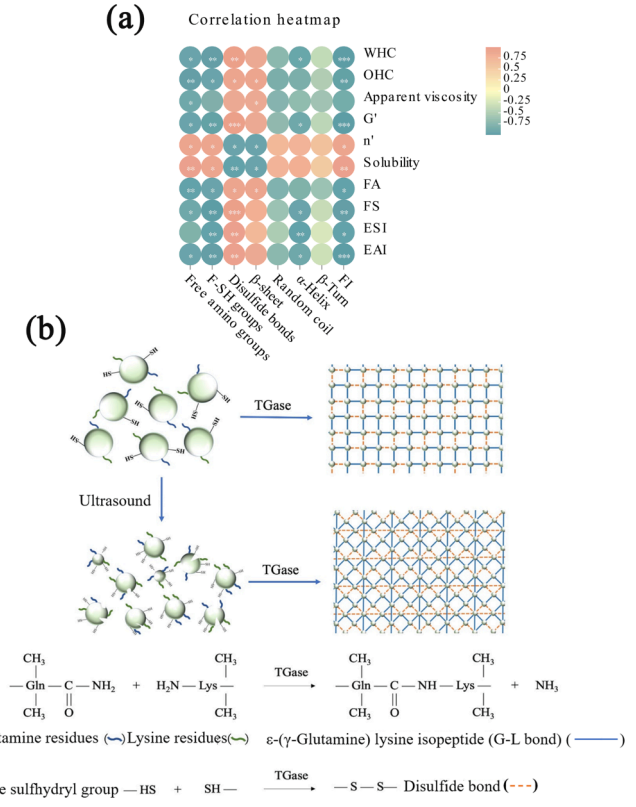

Fig. 6. Correlation heatmap of functional and structural properties of Qingke protein (a) and a schematic summarizing the mechanisms (b).

significantly positively correlated with disulfide bonds ($P < 0.001$). n' was negatively correlated with disulfide bonds and β-sheet ($P < 0.05$) and positively correlated with free amino acid, F-SH, and FI ($P < 0.05$). The protein solubility was negatively correlated with β-sheet ($P < 0.05$), significantly negatively correlated with disulfide bonds ($P < 0.01$), positively correlated with F-SH ($P < 0.05$), and significantly positively correlated with free amino group and FI ($P < 0.01$). FA was negatively correlated with F-SH and FI ($P < 0.05$), significantly negatively correlated with free amino group ($P < 0.01$), and positively correlated with disulfide bonds and β-sheet ($P < 0.05$). FS was negatively correlated with free amino group and α-helix ($P < 0.05$), significantly negatively correlated with F-SH and FI ($P < 0.01$), and extremely significantly positively correlated with disulfide bonds ($P < 0.001$). ESI was negatively correlated with FI ($P < 0.05$), significantly negatively correlated with F-SH and α-helix ($P < 0.01$), and significantly positively correlated with disulfide bonds ($P < 0.01$). EAI was negatively correlated with free amino group and α-helix ($P < 0.05$), significantly negatively correlated with F-SH ($P < 0.01$), extremely significantly negatively correlated with FI ($P < 0.001$), and significantly positively correlated with disulfide bonds ($P < 0.01$).

A schematic summarizing the mechanisms underlying how combined ultrasound and TGase treatment improves Qingke functional properties is presented in Fig. 6b. Presumably, TGase catalyzed Qingke protein intermolecular and/or intramolecular cross-linking to promote network structure formation [10,46]. The shear, cavitation, and turbulence effects caused by ultrasound led to a partial expansion of Qingke protein structure, which not only changed protein conformation, but also increased the number of TGase cross-linking action sites, improving the degree of cross-linking between TGase and the Qingke protein [15]. Cross-linking reaction under combined ultrasound and TGase treatment disrupted the tertiary structure of Qingke proein and enhanced fluorescent amino acid residues masking [6], resulting in a decrease in FI. It also destroyed the stability of the hydrogen bonds that make the regular arrangement of the α-helix structure, and the expanded polypeptide chain exposed more hydrogen bonds and rearranged the hydrophobic groups, so that the molecules were reconnected into β-sheet when the molecules gather [11]. On the other hand, the exposure of hydrophobic groups increased the close binding of Qingke protein to water, thus increasing WHC. The exposure of lipophilic sites increased oil retention and emulsifying properties, and enhanced the interaction with protein interface and foaming properties [34]. Some scholars [6] believe that ultrasound combined with TGase treatment promotes the transformation of F-SH groups to disulfide bonds, through polymerization of extensive disulfide bonds to form polymer protein products, improving the degree of polymerization. This results in a decrease in both protein solubility and free amino groups, while increasing the apparent viscosity and rheological behavior of Qingke protein. In addition, compared with single-frequency ultrasound, dual- and triple-frequency ultrasound may have stronger mass transfer effects, higher degrees of cavitation, and stronger protein structure modifications that provided more action sites for TGase [7].

This work demonstrated that combined ultrasound and TGase treatment can significantly improve Qingke protein functional properties and explored the underlying mechanism from the changes in Qingke protein structural properties, Moreover, the modified Qingke protein and extracted Qingke starch were used to produce Qingke noodles with less gluten content, and the quality was similar to that of wheat noodles.

5. Conclusion

Multiple-frequency ultrasound-assisted TGase dual modification treatment can significantly improve the functional properties of Qingke protein, including the improvement of WHC and OHC, the enhancement of rheological properties, and the optimization of foaming and emulsifying properties. This may be attributed to the reduction of free amino groups and F-SH, as well as the increase of disulfide bonds, making the Qingke protein to aggregate. The dense structure generated by ultrasound combined with TGase and the exposure of some hydrophobic groups and lipophilic sites resulted in improvement of WHC and OHC of proteins, along with the improvement of foaming and emulsifying properties. In addition, cross-linking made the fluorescent amino acid residues to be masked in the protein polymer, resulting in FI decline, α-helix and random coil to convert to β-sheet. When exploring the application of modified Qingke protein, it was found that the noodles made with Qingke protein treated by dual- and triple-frequency ultrasound combined with TGase had a quality similar to that of wheat noodles when 60–70 g/kg gluten was added. In conclusion, multiple-frequency ultrasound-assisted TGase dual modification treatment is an effective means to improve the functional properties of Qingke protein. However, some chemical reagents were used in the production of Qingke noodles in this study, and the products' safety has not yet been evaluated, thus, more in-depth investigation and sensory evaluation of the noodles is needed.

CRediT authorship contribution statement

Aijun Li: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft. **Zehang Guo:** Formal analysis, Methodology, Software, Supervision, Writing – review & editing. **Zhirong Wang:** Investigation, Supervision, Writing – review & editing. **Qingqing Yang:** Conceptualization, Data curation. **Leyan Wen:** Conceptualization, Data curation. **Xuwen Xiang:** Formal analysis, Investigation. **Jianquan Kan:** Funding acquisition, Project administration, Resources, Writing – review & editing.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2023.106317.

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