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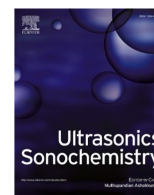

Study of ultrasonic treatment on the structural characteristics of gluten protein and the quality of steamed bread with potato pulp

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A R T I C L E I N F O

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A B S T R A C T

Physicochemical properties and microstructure of gluten protein, and the structural characteristics of steamed bread with 30 % potato pulp (SBPP) were investigated by ultrasonic treatments. Results showed that 400 W ultrasonic treatment significantly ($P < 0.05$) increased the combination of water and substrate in the dough with 30 % potato pulp (DPP). The contents of wet gluten, free sulfhydryl (S—H), and disulfide bond (S—S) were influenced by ultrasonic treatment. Moreover, UV-visible and fluorescence spectroscopy demonstrated that the conformation of gluten protein was changed by ultrasonic treatment (400 W). Fourier transform infrared (FT-IR) illustrated that the β-sheet content was significantly ($P < 0.05$) increased (42 %) after 400 W ultrasonic treatment, and the surface hydrophobicity of gluten protein in SBPP increased from 1225.37 (0 W ultrasonic treatment) to 4588.74 (400 W ultrasonic treatment). Ultrasonic treatment facilitated the generation of a continuous gluten network and stabilized crumb structure, further increased the specific volume and springiness of SBPP to 18.9 % and 6.9 %, respectively. Those findings suggested that ultrasonic treatment would be an efficient method to modify gluten protein and improve the quality of SBPP.

1. Introduction

Steamed bread is a typical staple food, accounting for 40 % of wheat consumption in China [1]. The reduction of nutrition content such as fiber and minerals of refined flour, which is attributed to the milling process, has become a critical issue in wheat products [2]. In order to improve the nutritional values of steamed bread, several studies were investigated to find the replacement materials for wheat flour, such as potato, buckwheat, and pumpkin [3–6].

Potato (*Solanum tuberosum* L.) is the fourth largest crop in the world after rice, wheat, and corn, with an annual production of 376 million tons [7,8]. Potato is a typical staple crop which is rich in nutrition such as dietary fiber, potassium, and vitamins. Meanwhile, it is positive for human health, e.g., antioxidant, anti-inflammatory, lipid regulation, and obesity prevention [9]. Therefore, the substitution of potatoes for wheat flour in steamed bread is potential to expand the consumer market for potatoes and enhance the nutritional values of steamed bread. In the past few years, the introduction of potato flour instead of wheat flour has been widely used in the preparation of staple food to improve its nutrition values [10,11]. However, the preparation of potato flour was associated with a large amount of energy consumption and pollution [12]. In contrast, the addition of fresh potato pulp reduced pollution and energy consumption and improved the nutrients, along with the novel eating properties.

Gluten protein has a distinct three-dimensional network attributed to the interaction forces, including the interchain and intermolecular disulfide bonds of glutenin and gliadin [13]. The viscoelasticity and extensibility properties of wheat dough were ascribed to the gluten protein, which also determined the quality of steamed bread [14]. The gluten aggregation properties led to the formation of the gluten network [15]. Previous study demonstrated that the ≥ 30 % addition of PP significantly reduced the disulfide content, β-sheet, and α-helix structures, which destroyed the gluten structure in wheat-based dough [16]. Therefore, it's crucial to find an effective way to improve the structure characteristics of gluten protein in dough with PP addition.

Ultrasound technology is extensively applied for food analysis and

Abbreviations: CSB, Chinese steamed bread; PP, potato pulp; SBPP, steamed bread with 30% potato pulp addition; DPP, dough with 30% potato pulp addition.

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storage, attributing to its green and non-pollution [17]. Cavitation effect, shear force, and intense pressure produced by ultrasound facilitated the food quality, including color, texture, flavor, and safety [18]. The ultrasound-assisted freezing prevented the damage of gluten's molecular structure. [19]. The multi-frequency ultrasound (28/40 and 28/40/80 kHz) influenced the structure of wheat gluten protein and reduced the wheat gluten protein content [20]. The ultrasonic treatment influenced the functional characteristics of gluten protein according to change the molecular structural formula, microstructure, and nanomachines characteristics [21]. Previous study has demonstrated that the qualities of steamed bread with 50 % sweet potato pulp addition were improved by the ultrasonic treatment (22.5 W/L), whereas the influence of ultrasonic-treated gluten protein on the qualities of steamed bread has not been understood [22]. Therefore, exploring the influence of ultrasonic treatment on gluten protein is essential for expanding the application of pulp in wheat-based products.

The present study focused on the modification mechanism of ultrasonic treatment on the gluten protein in DPP and the effect on the quality of SBPP. The physiochemical and microstructure of gluten protein were investigated, including water distribution of dough, wet gluten content, chemical bonds, protein secondary structure, and microstructure. Moreover, the crumb structure, specific volume, and texture properties of steamed bread were also studied. The objective of this study would present insight into the modification of gluten protein by ultrasonic technology and its mechanism on the structural characteristics of SBPP, thus providing accumulated relevant data for the potato pulp product.

2. Materials and methods

2.1. Materials

Wheat flour (73.1 % starch, 13.1 % moisture and 10.9 % protein, Yunhai Flour factory, Zibo, China). Fresh potatoes and instant dry yeast were purchased from the local market. Potato pulp (PP, 12.3 % starch, 79.5 % moisture, and 1.9 % protein) was prepared by homogenizing potatoes (peeled and cubed) with ascorbic acid (0.2 %, w/w) in a blender. All chemicals were analytical grade.

2.2. Preparation of dough and steamed bread

The formula for dough was based on the previous experiment, 300.00 g wheat flour, 2.55 g instant dry yeast, and 170.40 mL water [23]. Wheat flour was replaced by potato pulp (PP) at a 30 % (w/w) level in dough, and the 20.3 % water added for consistency of water content. The ingredients, including wheat flour, PP, water, and instant dry yeast were mixed and kneaded manually for 3 min to complete dough development. The dough fermented in the BRF-18C fermentation tank (Guangzhou zhanzhuo Equipment Co., Ltd., Guangzhou, China) at 32 °C and 80 % relative humidity for 60 min was the first fermentation stage.

The first fermentation stage of the dough with ultrasonic treatment was performed as follows: the dough was placed in a plastic bag and inserted a straw into the bag to keep the gas exchange. Then, the package was tied and put into an ultrasonic bath (KQ-500 DE, Kunshan Ultrasonic Instrument Co., Ltd., Kunshan, China) for ultrasonic treatment (0, 200, 300, 400, and 500 W) at 32 °C for 40 min [22].

The product was rolled 7 times on the sheeter, cut into a 100 g portion and rounded manually into a round shape. The second fermentation stage was performed in the fermentation tank (32 °C and 80 % relative humidity) for 15 min, following 20 min steam for and 60 min cooling at room temperature (25 °C and 50 % relative humidity) before test.

2.3. Analysis of moisture distribution

After first fermentation stage, the dough (8 ± 0.01 g) was wrapped to prevent the evaporation of water. The relaxation measurements of dough were performed by LF NMR (MesoMR23-060H-I, Niumag, Shanghai, China) following the report of Zhang with slight modification [24]. The parameters are as follows: echo time (TE) = 1 ms, sampling points (TD) = 119992, sampling interval time (TW) = 2000 ms, scanning frequency (SF) = 20 MHz, accumulative frequency (NS) = 8.

2.4. Wet gluten content

The contents of wet gluten in dough were obtained by the Approved Method 38–10 (AACC, 2000) after the first fermentation stage. The dough (m_1, g) was washed with sodium chloride solution (2 %, w/v) for 15 min until starch and all soluble matter were removed. The gluten was centrifuged by a centrifuge (DL-5-B, Shanghai Anting Scientific Instrument Factory Co., Ltd., China) at 1800 g for 15 min to remove the excess solution. Repeat several times until no starch-iodine reaction was observed in washing solution, and the weight of residue was wet gluten (m_2, g). The content of wet gluten was calculated as follows:

$$Wet\ gluten\ content\ (\%) = m_2/m_1 \times 100\%$$

2.5. Intrinsic mechanism of gluten protein with ultrasonic treatment

The gluten protein in 2.4 was dehydrated in the FD-8 freeze dryer (Boyikang Lab Instrument Co., ltd., Beijing, China) at −50 °C for 36 h. Then the samples were pulverized and passed through a 100-mesh sieve.

2.5.1. Free sulfhydryl (S—H) and disulfide bonds (S—S) determination

The contents of free sulfhydryl groups (S—H) and disulfide bonds (S—S) in gluten protein were obtained by the Ellman's method, which was published by Patrick with slight modification [25]. The samples were mixed with the guanidine hydrochloride (1:63, w/w), and set to a constant volume of 10 mL with Tris-Gly buffer (pH 8). 1 mL of sample solution was added with 5 mL of Tris-Gly-8 M Urea buffer, then reacted with 40 μL of Ellman's reagent (5, 5-dithiodinitrobenzonic acid, 4 mg/mL) in the dark for 20 min at 40 °C. The absorbance (412 nm) was measured by a microplate reader (Multiskan Sky, USA).

The S—S bonds of sample were measured as follows: The sample solution was added with 10 M Urea buffer and β-ME (10:50:1, v/v/v) at 40 °C for 20 min, then mixed with trichloroacetic acid (5 mL, 12 %) and incubated at 40 °C for 60 min. The mixture solution was centrifuge at 1800 g for 15 min. The precipitate was washed with trichloroacetic acid (5 mL, 12 %), then the final precipitate was incubated in the mixture of 8 M Urea and Ellman's reagent (100:1, v/v) at 25 °C for 30 min. And the absorbance of samples at 412 nm were measured.

The concentration of gluten protein was obtained by the Biuret method, and the contents of S—H and S—S were expressed as μmol SH/g protein and μmol SS/g protein, respectively.

2.5.2. UV–visible spectroscopic analysis

The gluten protein powder was mixed with phosphate buffer (1 % w/v, pH 7.4). Sample solutions were centrifuged at 1800 g for 30 min, and the ultraviolet spectrum (260–400 nm) of supernatant was measured by the microplate reader (Multiskan Sky, USA).

2.5.3. Fluorescence spectroscopic analysis

The fluorescence spectroscopic analysis of gluten protein was detected by the spectrofluorometer (RF-6000, Shimadzu, Japan) according to the method of Zhang [26].

2.5.4. Protein secondary structure determination

The infrared spectrograms of gluten protein were collected through a spectrophotometer (Nicolet 5700, Thermo Nicolet Corporation, USA)

[27]. The spectra of gluten protein were scanned in the wavelength interval of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}, and the spectra of the amide I band (1700–1600 cm^{-1}) was analyzed by using OMNIC 32 and deconvolved with PeakFit4.12.

2.5.5. Surface hydrophobicity of gluten protein

The surface hydrophobicity of gluten protein was determined by the ANS method using a spectrofluorometer (RF-6000, Shimadzu, Japan) [28]. The gluten protein was diluted with the phosphate buffer solution (0.01 M, pH 7.4) to a concentration interval of 0.001 to 1 mg/mL. The concentration of sample solution was measured by the Biuret method. Diluted protein solution was mixed with ANS solution (80:1, v/v, 8 mM). The relevant parameters are as follows: the excitation and emission wavelengths were 390 and 470 nm, respectively, and the slit width was set to 5 nm. The surface hydrophobicity index (H_0) was indicated as the slope of the plot of the fluorescence intensity against the protein concentration.

2.5.6. Microstructure of gluten protein

The microstructure of gluten protein was investigated by the scanning electron microscope (Quanta 250, FEI, USA) at a magnification of 5000 x. Different samples of dehydrated gluten protein were cut into 0.1 mm thickness slices. The structure micrographs of gluten protein were obtained at the accelerating voltage of 20 kV.

2.6. Steamed bread characteristics

2.6.1. Specific volume analysis

The specific volumes (mL/g) of steamed bread after 1 h of cooling were obtained by the method of millet replacement.

2.6.2. Texture profile analysis (TPA)

The texture profile analysis (TPA) method of cooled steamed bread was performed by the Texture Analyzer (TA-XT Plus, Stable Micro-systems, UK). Samples were cut into a thickness of 2 cm from the middle part of steamed bread, then measured using a probe of P/36R. The parameters were as follows: the per-test, test, and post-test speed were 3.0 mm/s, 1.0 mm/s, and 5.0 mm/s, respectively.

2.6.3. Crumb structure evaluation

The crumb structures of steamed bread were performed by the published method [29]. The samples were sliced (3×3 cm^2 area, 2.0 cm thickness) and acquired an image in RGB color and bmp format with a resolution of 300 dpi. The image was analyzed by Image J software. The porosity (%) and average cell area (mm^2) of steamed bread were calculated.

2.7. Statistical analysis

All experiments were performed in six times, expressed as average ± standard deviation, and analyzed by SPSS 22. The statistical differences ($P < 0.05$) of the results were determined by one-way analysis of variance (ANOVA) and Duncan's multiple range tests.

3. Results and discussion

3.1. Moisture distribution of dough

The water content of the dough was more than 60 %, which was a critical factor in the generation of steamed bread. The fermentation of dough was accompanied by the migration of water. Low-field nuclear magnetic resonance (LF NMR) extensively investigated the distribution and migration of water at dough [12,30–32]. The T_2 relaxation distribution curve of dough as shown in Fig. 1, three major populations were T_{21} (0.01–0.49 ms), T_{22} (0.60–33.70 ms), and T_{23} (41.50–155.23 ms), indicating bond water, immobilized water, and free water, respectively.

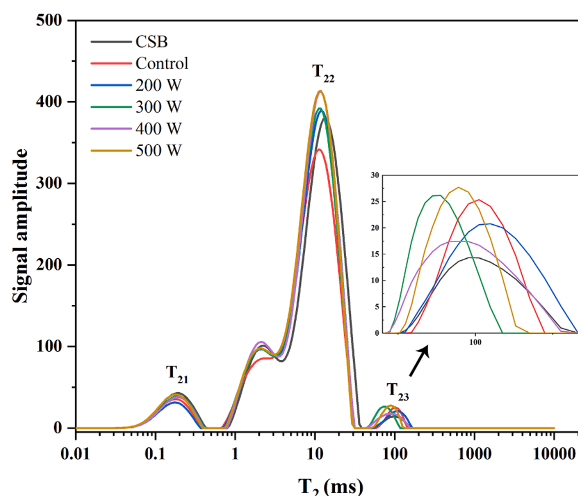

Fig. 1. Moisture distribution of PP dough with different ultrasonic power treatments (0 W, 200 W, 300 W, 400 W, 500 W).

The T_{22} was the most abundant population, which had the most intense signal amplitude, demonstrating that the immobilized water was the dominant moisture from in the dough.

The variation of water state (T_2) and proton signal intensities (S_2) in dough with different ultrasonic power treatments was presented in Table 1. Generally, the short relaxation time of T_2 indicated low water mobility and tight bond between water and substrate [33]. The T_{22} showed a parabolic increment following the increase of ultrasonic power. Comparing to the CSB and Control, 400 W ultrasonic treatment reduced the T_{22} by 2.47 % and 0.76 %, and improved the S_{22} by 2.04 % and 0.99 % ($P < 0.05$), respectively, which resulted that the stabilization of immobilized water was related to ultrasonic treatment. The ultrasonic treatment increased the frozen dough's water holding capacity [26]. The addition of PP significantly decreased ($P < 0.05$) the bond water content (4.90–6.03 %) in the dough (Table 1). The starch limits water migration in the dough as an inert filling component, the gluten network might the major factor leading to the decrease of bond water (S_{21}) [34,35]. PP was a barrier for the aggregation of gluten protein in DPP, causing the reduction of bond water. The results indicated that ultrasonic treatment increased the tight bond between water and substrate in the DPP.

Table 1
Effect of ultrasonic treatment on water distribution of dough.

Ultrasonic power (W)	T_2 (%)			S_2 (%)		
	T_{21}	T_{22}	T_{23}	S_{21}	S_{22}	S_{23}
CSB	0.32 ± 0.02^a	27.99 ± 0.96^{ab}	71.69 ± 0.978^b	7.23 ± 0.22^a	91.09 ± 0.23^b	1.68 ± 0.03^a
Control	0.34 ± 0.05^a	26.28 ± 1.10^{abc}	73.38 ± 1.15^{ab}	5.60 ± 1.15^b	92.14 ± 0.91^{ab}	2.26 ± 0.24^a
200	0.36 ± 0.09^a	25.84 ± 1.50^{bc}	73.80 ± 1.59^{ab}	4.90 ± 1.10^b	93.00 ± 0.08^a	2.10 ± 1.05^a
300	0.33 ± 0.08^a	25.15 ± 1.90^c	74.52 ± 1.98^a	6.03 ± 0.27^{ab}	92.01 ± 0.31^{ab}	1.96 ± 0.08^a
400	0.39 ± 0.07^a	25.52 ± 0.58^c	74.10 ± 0.52^a	5.08 ± 0.44^b	93.13 ± 0.72^a	2.12 ± 0.14^a
500	0.35 ± 0.05^a	28.18 ± 0.60^a	71.44 ± 0.63^b	5.17 ± 1.27^b	92.77 ± 1.25^a	2.06 ± 0.15^a

a-c Means different samples in the same column were significant differences ($P < 0.05$).

3.2. Mechanism investigation of gluten protein with ultrasonic treatment

3.2.1. Content of wet gluten, free S—H and S—S in gluten protein

As illustrated in Fig. 2, the CSB contained 4.92 % wet gluten more than the Control. The gluten network was discontinuous with high addition of PP, which diluted the gluten protein [36]. However, the wet gluten content of DPP was significantly increased by ultrasound treatment at 400 W (1.56 %, $P < 0.05$). The results were due to the enhancement of ultrasonic treatment on the intermolecular contact of gluten protein in the dough, and the content of chemical bonds, which improved the stability of gluten network [37]. On the contrary, excessive ultrasonic power destroyed the aggregation structure of gluten protein, reducing the content of wet gluten. The alteration of wet gluten content was associated with the ultrasonic treatment and affected the final quality of SBPP.

The free sulfhydryl (S—H) and disulfide bond (S—S) content of gluten with ultrasonic treatment were shown in Fig. 2. The Free S—H and S—S largely influenced the structural characteristics of wheat gluten. The contents of S—H and S—S in Control were 0.97 μmol SH/g protein and 20.98 μmol SS/g protein less than the CSB ($P < 0.05$), respectively. This difference was induced by the interaction between dietary fiber of PP and gluten, which lead to the structural change of gluten protein [38]. The S—S between the two cysteine residues played a critical role in the stability of gluten [39]. The parabolic trend of S—S content was observed after ultrasonic treatment in sample, which showed the same trend of wet gluten. The S—S content of gluten was significantly increased ($P < 0.05$) from 23.70 μmol SS/g protein in the Control to 35.63 μmol SS/g protein in group with 400 W ultrasonic power treatment (Fig. 2). Therefore, the stability of gluten protein was efficiency improved by ultrasonic treatment. In contrast, excessive ultrasonic power accelerated the destruction of structural characteristics of gluten protein.

3.2.2. UV–visible spectroscopic analysis of gluten protein

UV–visible spectroscopic analysis is a critical method for investigating protein structure. Fig. 3A indicated that UV–visible spectra (260–400 nm) of gluten protein with ultrasonic treatments, exhibiting the maximum absorption wavelength at 280 nm. The absorption in 240–300 nm was attributed to the aromatic side chains of phenylalanine (Phe), tryptophan (Trp), and tyrosine (Tyr) [40,41]. The ultraviolet absorption intensity significantly increased after the PP addition (Fig. 3A). The addition of PP destroyed the gluten network and exposed the polar groups. In addition, the ultraviolet absorption intensity was increased after 400 W ultrasonic treatment (Fig. 3A). The gluten protein was unfolded and exposed chromophore groups by the ultrasonic treatment [20]. In short, the structural property of gluten protein was changed by ultrasonic treatment.

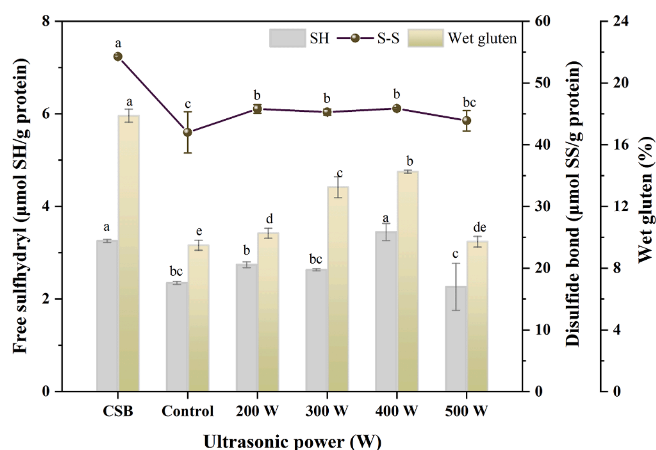

Fig. 2. Changes of wet gluten, free sulfhydryl, and disulfide bond content with different ultrasonic power treatments. [a–e] Means different letters were significant differences ($P < 0.05$).

3.2.3. Fluorescence spectroscopic analysis of gluten protein

Fluorescence spectroscopy was a practical technique to follow the spatial conformation of protein. The change of fluorescence intensity in aromatic amino acid (tryptophan, tyrosine, and phenylalanine) attributed to environment polarity was associated with protein denaturation [42]. The fluorescence intensity of gluten with ultrasonic treatment was shown in Fig. 3B. The maximum fluorescence intensity was lower in group with ultrasonic treatment than CSB. The maximum absorption (λ^{max}) was a hypsochromic shift from the CSB of 340 nm to 338 nm for the group with 400 W ultrasonic power treatment (Fig. 3B). The shift of λ^{max} demonstrated that the changes of the protein conformational. Previous study suggested that the protein conformational was changed in ultrasonic treatment, which is attributed to the changes in the spatial arrangement and interactions of amino acid [26]. The results evidenced that the conformational of gluten protein was modified by ultrasonic treatment to a certain extent.

3.2.4. Fourier transform infrared (FT-IR) analysis of gluten protein

The FT-IR spectra (400–4000 cm^{-1}) of gluten protein with different ultrasonic treatments was recorded for the intensive study of protein structure (Fig. 4A). Amide I region (1700–1600 cm^{-1}) was caused by the H—O—H bending vibration and C═O stretching [43]. The peak intensity of amide I region for the Control was lower than that in the CSB, which was attributed to the interference of PP on the gluten structure (Fig. 4A). However, the peak strength was further reduced by the broken of hydrogen bond, which was the main force maintaining the amide I region, after ultrasonic treatment [44]. The ultrasound process, accompanied by thermogenesis, stretched the gluten protein structure and increased the internal hydrogen bond, leading to the increase of peak strength in the amide I region (especially at 400 W ultrasonic treatment) [45]. The wavenumber of 3005 cm^{-1} and 3099 cm^{-1} was attributed to O—H stretching. Comparing with the CSB, the peak intensity (3100–3000 cm^{-1}) of gluten protein was significantly decreased in the Control, which was caused by the increase of hydrogen bond between water and gluten protein after the PP addition [23]. The hydrophilic groups in gluten protein were exposed, which was attributed to cavitation in ultrasonic treatment (200 W), resulting in the reduced peak strength [46]. However, the excessive ultrasonic treatment destroyed the structural of gluten protein (Fig. 4A).

The secondary structure was associated with the formation of gluten network and the strength of gluten protein [47]. The influence on the protein secondary structure of gluten after different ultrasonic power treatments was shown in Fig. 4B. The corresponding contents of secondary structure of gluten protein followed the decrement in the order of β-sheet, β-turn, random coil, and α-helix, demonstrating that β-sheet was the predominantly from in gluten protein [48]. Also, the contents of α-helix and β-sheet were associated with the stable protein conformation [47]. Compared to CSB, the total proportion of α-helix and β-sheet of gluten protein secondary structure was significantly decreased from 58 % to 54 % in the Control, since the destructive effect of the addition of the PP on continual gluten network (Fig. 4B). The total proportion of α-helix and β-sheet in the secondary structure was significantly higher in the group with 400 W ultrasonic treatment than the Control, therefore, the ultrasonic treatment (400 W) had a positive effect on the gluten network.

3.2.5. Surface hydrophobicity of gluten

As an important interaction among protein molecules, the surface hydrophobicity has become a critical indicator for evaluating the protein properties of stability, function, and microstructure [49]. The effect on the surface hydrophobicity of gluten with ultrasonic treatments was shown in Fig. 5. The surface hydrophobicity of the Control (1255.37) was significantly higher ($P < 0.05$) than the CSB (629.02), which could

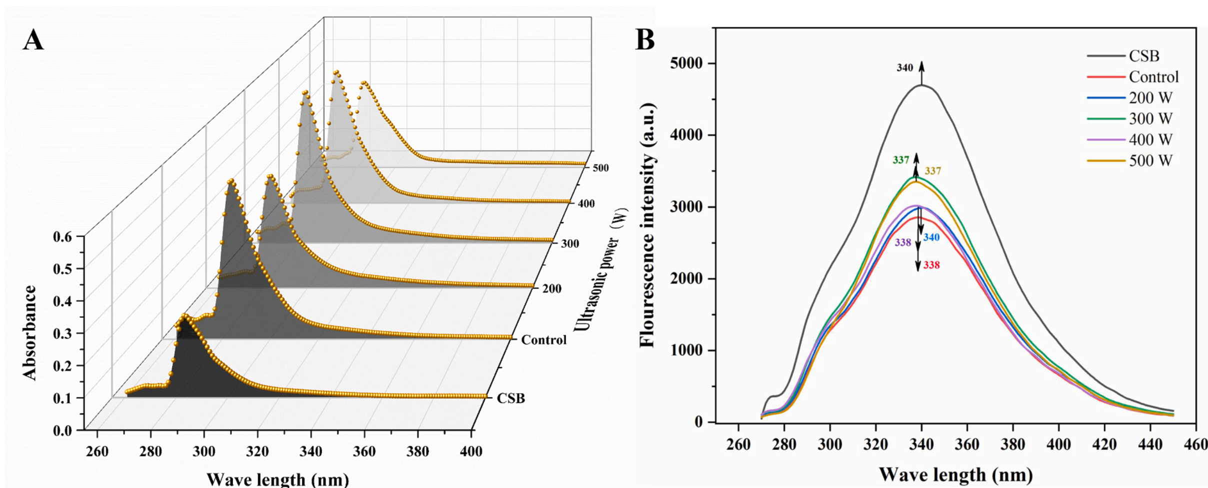

Fig. 3. The UV–visible spectra and fluorescence spectra of gluten with different ultrasonic power. A, UV–visible spectra, B, fluorescence spectra.

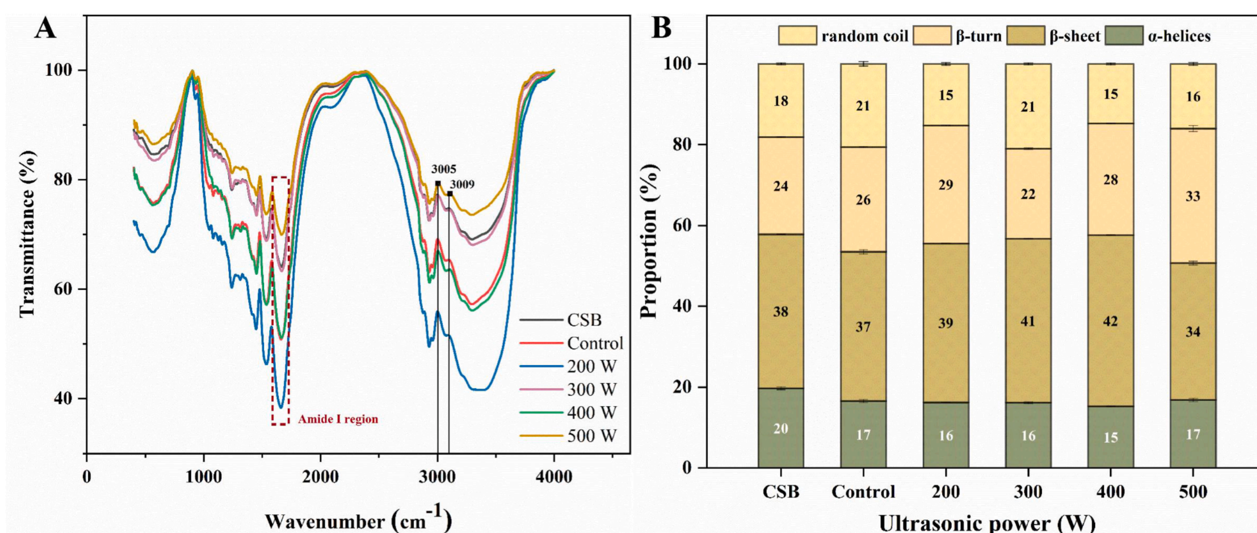

Fig. 4. The gluten protein structure as affected by ultrasonic treatment, A, FT-IR spectra (400–4000 cm^{-1}). B, protein secondary structure.

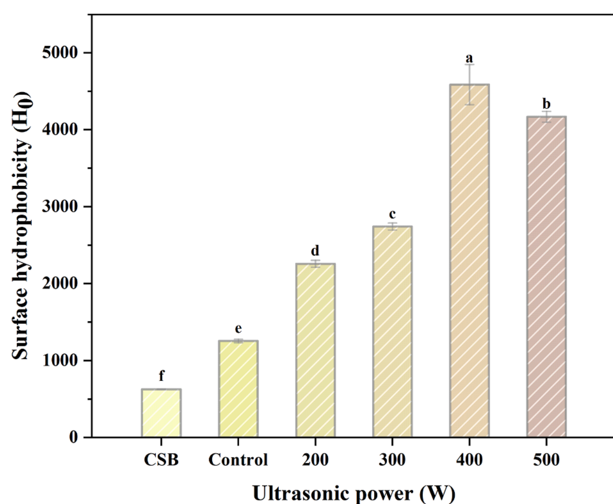

Fig. 5. The effect of ultrasonic power on surface hydrophobicity of gluten. [a–f] Means different letters were significant differences ($P < 0.05$).

be found evidences from the destroyed network and the exposed internal hydrophobic groups of gluten protein within the addition of PP (Fig. 5). The turbulence, shearing force, and microfluidization effect of ultrasound cavitation and exposure of the hydrophobic groups and region increased the surface hydrophobicity (Fig. 5) [50]. However, excessive ultrasonic power decreased the surface hydrophobic by damaging the hydrophobic groups [51]. As a result, the ultrasonic treatment facilitated the unfolding of gluten protein.

3.2.6. *Scanning electron microscopy (SEM) analysis of gluten protein*

The gluten protein showed a typical three-dimensional network structure (Fig. 6) [52]. The gluten protein of CSB group existed in a dense and ordered structure (Fig. 6A). The Control showed the loose and irregular structure of gluten protein, the destruction of PP on the gluten network was responsible for this structure change (Fig. 6B). The continuous and uniform network structure of gluten protein was shown after ultrasonic treatment (400 W), which was due to the effect of ultrasonic treatment on intermolecular interactions such as hydrogen bond and van der Waals forces in the peptide chain of gluten protein [53]. In addition, the formation of gluten network was consistent with the results of the S—S content above-mentioned (section 3.2.1). The results demonstrated that the microstructures of gluten protein in DPP

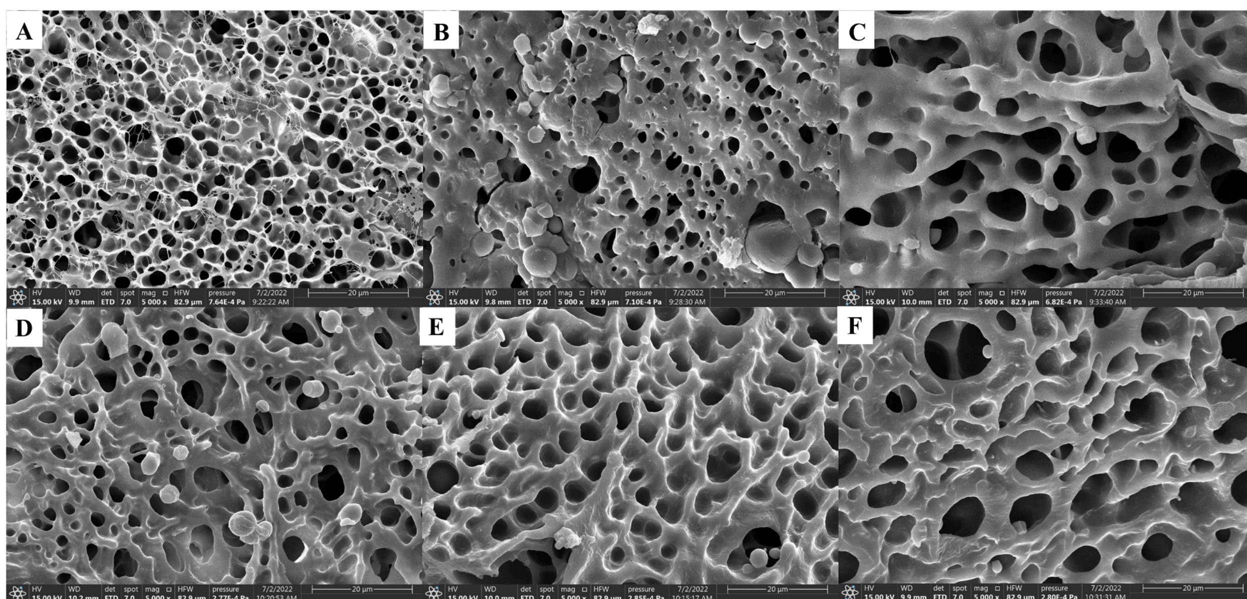

Fig. 6. The effect of ultrasonic power on the microstructure of gluten. (A) CSB; (B) Control; (C) 200 W; (D) 300 W; (E) 400 W; (F) 500 W.

were modified by ultrasonic treatment.

3.3. Steamed bread characteristics

3.3.1. Specific volume and texture profile analysis (TPA)

The specific volume is a critical characteristic of steamed bread, and directly influence the orientation of consumer choice. The strong gluten network is essential for the desired properties (such as volume and texture) of steamed bread. The addition of 30 % PP significantly ($P < 0.05$) reduced the specific volume by 16.60 % (Table 2). The specific volume of group with 400 W ultrasonic treatment was 18.96 % more than the Control ($P < 0.05$), even exhibiting no significant difference ($P > 0.05$) between that of CSB. The ultrasonic treatment enhanced the gluten network strength of SBPP, improving the elasticity and the specific volume of stead bread [54]. This result demonstrated that ultrasonic treatment was beneficial in developing the specific volume of SBPP.

Soft texture is one of the desirable properties of steamed bread [55]. The hardness and gumminess of steamed bread increased by the ultrasonic treatment from 2537.13 g to 2833.59 g and 2024.22 to 2181.05, respectively. The PP addition induced the gluten dilution and reduced the gas hold capacity of SBPP, which could be verified by SEM observation [56]. The PP addition reduced the cross-linking of gluten protein, weakened the gluten network, and then reduced the springiness and cohesiveness of SBPP [5]. The hardness was reduced by 5.28 % in SBPP after 400 W ultrasonic treatment than Control, and the springiness and cohesiveness were increased by 6.90 % and 2.60 %, respectively, resulting that ultrasonic treatment could improve the quality of steamed bread. Previous results reported that ultrasonic treatment improved the texture properties of apricot skins bread [57].

The appearances of dough were founded in Fig. 7, where the destructive effect of the PP addition was illustrated with an apparent coarse appearance in Control. The uniform and smooth surfaces of SBPP were obtained with the increasing ultrasonic power (200–400 W), while the excessive ultrasonic power (500 W) adversely affected the quality of SBPP. In addition, the crumb of SBPP showed a continual and compact structure after the ultrasonic treatment, especially the uniform pores and the tight internal structure that appeared in the group with 400 W ultrasonic treatment. It was suggested that ultrasonic treatment effectively improved the appearance of SBPP.

3.3.2. Crumb structure

The structure of steamed bread appeared irregularly and porously following the addition of PP (Fig. 8A). Previous study demonstrated that the destruction of gluten protein decreased the gas hold capacity after the addition of PP, therefore, the larger holes were generated in the development of steamed bread [58]. The macroporous structure of crumb from SBPP was reduced after ultrasonic treatment, and the surface was more uniform and continuous than the Control. Those results were attributed to the improvement of gas hold capacity which was related to the stability and strength of gluten protein in section 3.2.1. There was no significant difference in porosity among the ultrasonic treatment groups. The average cell area of SBPP was significantly ($P < 0.05$) decreased after the 400 W ultrasonic treatment (Fig. 8B). The results demonstrated that ultrasonic treatment (400 W) could stabilize air cells and prevent cell coalescence in SBPP.

Table 2
Effect of ultrasonic treatment on physical properties of steamed bread.

Ultrasonic power (W)	Specific volume (mL/g)	Texture					
		Hardness (g)	Springiness (mm)	Cohesiveness	Gumminess	Chewiness (g*mm)	Resilience
CSB	2.53 ± 0.01^{a}	2537.13 ± 49.76^{d}	0.91 ± 0.04^{ab}	0.80 ± 0.00^{a}	2024.22 ± 46.47^{bc}	1847.62 ± 100.50^{bc}	0.44 ± 0.01^{a}
Control	2.11 ± 0.01^{c}	2833.59 ± 175.24^{bc}	0.87 ± 0.05^{b}	0.77 ± 0.01^{c}	2181.05 ± 156.48^{b}	1897.13 ± 215.56^{abc}	0.39 ± 0.01^{c}
200	2.14 ± 0.02^{c}	3014.86 ± 109.09^{ab}	0.89 ± 0.02^{ab}	0.78 ± 0.00^{bc}	2345.30 ± 79.02^{a}	2077.79 ± 108.64^{ab}	0.41 ± 0.01^{b}
300	2.23 ± 0.10^{b}	3078.77 ± 119.32^{a}	0.88 ± 0.05^{ab}	0.79 ± 0.01^{ab}	2423.61 ± 87.92^{a}	2124.27 ± 165.38^{a}	0.42 ± 0.01^{b}
400	2.51 ± 0.01^{a}	2683.83 ± 216.66^{cd}	0.93 ± 0.02^{a}	0.79 ± 0.01^{a}	2131.70 ± 144.32^{bc}	1986.00 ± 136.08^{abc}	0.44 ± 0.02^{a}
500	2.49 ± 0.02^{a}	2503.76 ± 129.99^{d}	0.93 ± 0.01^{a}	0.79 ± 0.01^{ab}	1972.07 ± 101.33^{c}	1836.33 ± 96.56^{a}	0.44 ± 0.01^{a}

$^{a–d}$Means different samples in the same column were significant differences ($P < 0.05$).

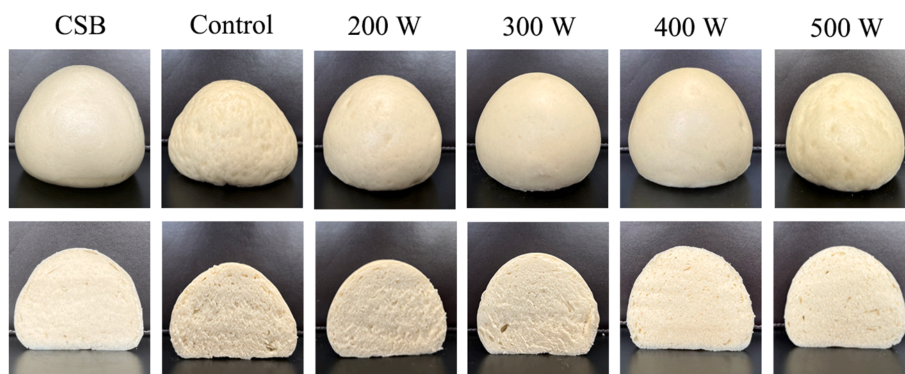

Fig. 7. Effect of ultrasonic treatment on the appearance of steamed bread.

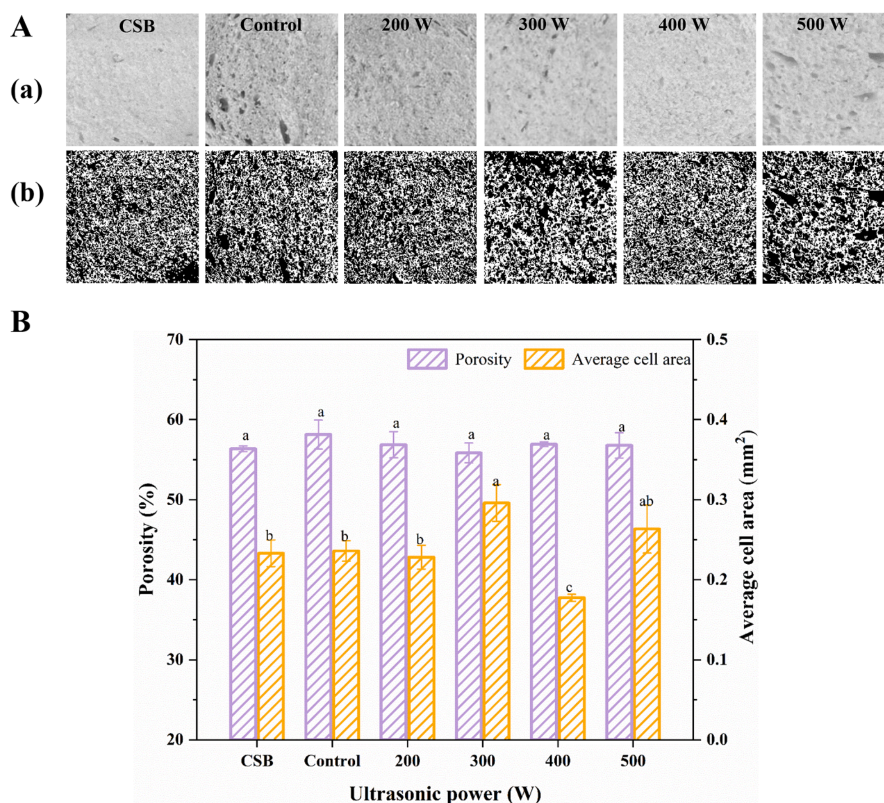

Fig. 8. Effect of ultrasonic treatment on the crumb microstructure of steamed bread with different ultrasonic power treatment A, thresholding images of cell structure, (a) gray level images; (b) binary images. B, The crumb structure parameters of steamed bread.

4. Conclusion

In this study, the physicochemical properties and microstructure of gluten and the quality of SBPP were investigated by ultrasonic treatments at different ultrasonic power. It was found that the stability and strength of gluten protein in DPP were significantly enhanced by ultrasonic treatment at 400 W, including the tight bonding between water and substrate and the increment of wet gluten and disulfide bond. In addition, the possible modified mechanism of ultrasonic treatment on the conformational of gluten protein was illustrated by UV–visible spectroscopic and fluorescence spectroscopy. The stabilized and integrity internal structures of gluten protein were suggested by FT-IR and SEM. Furthermore, the satisfactory appearances of SBPP, including the specific volume and internal structure, were obtained by ultrasonic treatment. Therefore, those results suggested that the ultrasonic treatment could potentially facilitate the generation of potato pulp products.

CRediT authorship contribution statement

Jialin Song: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Lijun Jiang:** Investigation. **Mingming Qi:** Investigation. **Luxia Li:** Investigation. **Mei Xu:** Investigation. **Yueming Li:** Investigation. **Dongliang Zhang:** Writing – review & editing. **Chenjie Wang:** Writing – review & editing. **Shanfeng Chen:** Conceptualization, Writing – review & editing, Project administration, Funding acquisition. **Hongjun Li:** Conceptualization, Writing – review & editing, Project administration, Funding acquisition.

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

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References

[1] F. Zhu, R. Sakulnak, S. Wang, Effect of black tea on antioxidant, textural, and sensory properties of Chinese steamed bread, Food Chemistry 194 (2016) 1217–1223.
[2] P. Wang, C. Hou, X. Zhao, M. Tian, Z. Gu, R. Yang, Molecular characterization of water-extractable arabinoxylan from wheat bran and its effect on the heat-induced polymerization of gluten and steamed bread quality, Food Hydrocolloids 87 (2018) 570–581.
[3] Y. Xiao, C. Yang, H. Xu, J. Zhang, L. Zhang, Study on the change of flavonoid glycosides to aglycones during the process of steamed bread containing tartary buckwheat flour and antioxidant, α-glucosidase inhibitory activities evaluation in vitro, LWT - Food Science and Technology 145 (2021), 111527.
[4] L. Zhou, T.-H. Mu, M.-M. Ma, R.-F. Zhang, Q.-H. Sun, Y.-W. Xu, Nutritional evaluation of different cultivars of potatoes (Solanum tuberosum L.) from China by grey relational analysis (GRA) and its application in potato steamed bread making, Journal of Integrative, Agriculture 18 (2019) 231–245.
[5] P.-H. Hsieh, Y.-M. Weng, Z.-R. Yu, B.-J. Wang, Substitution of wheat flour with wholegrain flours affects physical properties, sensory acceptance, and starch digestion of Chinese steam bread (Mantou), LWT - Food Science and Technology 86 (2017) 571–576.
[6] M. Ebrahimi, S.M. Ali, Noori, A. Sadeghi, O.e., Coban,, J., Zanganeh,, S.M., Ghodsmofidi,, Z., Malvandi,, M., Raeisi,, Application of cereal-bran sourdoughs to enhance technological functionality of white wheat bread supplemented with pumpkin (Cucurbita pepo) puree, LWT - Food Science and Technology 158 (2022), 113079.
[7] Z. Zhao, T. Mu, H. Sun, Microbial characterization of five Chinese traditional sourdoughs by high-throughput sequencing and their impact on the quality of potato steamed bread, Food Chemistry 274 (2019) 710–717.
[8] K.J. Chen, J.D. Wood, I.K. Mohammed, S. Echendu, D. Jones, K. Northam, M. N. Charalambides, Mechanical Characterisation and modelling of the rolling process of potato-based dough, Journal of Food Engineering 278 (2020), 109943.
[9] M.M. Seppänen, K. Fagerstedt, The role of superoxide dismutase activity in response to cold acclimation in potato, Physiologia Plantarum 108 (2000) 279–285.
[10] L. Cheng, X. Wang, Z. Gu, Y. Hong, Z. Li, C. Li, X. Ban, Effects of different gelatinization degrees of starch in potato flour on the quality of steamed bread, International Journal of Biological Macromolecules 209 (2022) 144–152.
[11] X. Liu, T. Mu, H. Sun, M. Zhang, J. Chen, M.L. Fauconnier, Influence of different hydrocolloids on dough thermo-mechanical properties and in vitro starch digestibility of gluten-free steamed bread based on potato flour, Food Chemistry 239 (2018) 1064–1074.
[12] G. Shen, L. Zhang, T. Hu, Z. Li, A. Chen, Z. Zhang, H. Wu, S. Li, X. Hou, Preparation of potato flour by freeze-thaw pretreatment: Effect of different thawing methods on hot-air drying process and physicochemical properties, LWT - Food Science and Technology 133 (2020), 110157.
[13] Y.-H. Wang, Y.-R. Zhang, Y.-Y. Yang, J.-Q. Shen, Q.-M. Zhang, G.-Z. Zhang, Effect of wheat gluten addition on the texture, surface tackiness, protein structure, and sensory properties of frozen cooked noodles, LWT - Food Science and Technology 161 (2022), 113348.
[14] J. Pico, M.P. Reguilón, J. Bernal, M. Gómez, Effect of rice, pea, egg white and whey proteins on crust quality of rice flour-corn starch based gluten-free breads, Journal of Cereal Science 86 (2019) 92–101.
[15] C.-C. Wang, Z. Yang, X.-N. Guo, K.-X. Zhu, Effects of insoluble dietary fiber and ferulic acid on the quality of steamed bread and gluten aggregation properties, Food Chemistry 364 (2021), 130444.
[16] Y. Cao, M. Zhang, S. Dong, P. Guo, H. Li, Impact of potato pulp on the processing characteristics and gluten structures of wheat flour dough, Journal of Food Processing and Preservation 44 (2020) e14473.
[17] F. Chemat, N. Rombaut, A. Meullemiestre, M. Turk, S. Perino, A.-S. Fabiano-Tixier, M. Abert-Vian, Review of Green Food Processing techniques, Preservation, transformation, and extraction, Innovative Food Science & Emerging Technologies 41 (2017) 357–377.
[18] A.C. Soria, M. Villamiel, Effect of ultrasound on the technological properties and bioactivity of food: a review, Trends in Food Science & Technology 21 (7) (2010) 323–331.
[19] Y. Zhang, Y. Li, H. Wang, A.O. Oladejo, H. Zhang, X. Liu, Effects of ultrasound-assisted freezing on the water migration of dough and the structural characteristics of gluten components, Journal of Cereal Science 94 (2020), 102893.
[20] H. Zhang, G. Chen, M. Liu, X. Mei, Q. Yu, J. Kan, Effects of multi-frequency ultrasound on physicochemical properties, structural characteristics of gluten protein and the quality of noodle, Ultrasonics Sonochemistry 67 (2020), 105135.
[21] Y. Zhang, H. Ma, B. Wang, W. Qu, Y. Li, R. He, A. Wali, Effects of Ultrasound Pretreatment on the Enzymolysis and Structural Characterization of Wheat Gluten, Food Biophysics 10 (2015) 385–395.
[22] M. Zhang, W. Suo, Y. Deng, L. Jiang, M. Qi, Y. Liu, L. Li, C. Wang, H. Zheng, H. Li, Effect of ultrasound-assisted dough fermentation on the quality of dough and steamed bread with 50% sweet potato pulp, Ultrasonics Sonochemistry 82 (2022), 105912.
[23] Y. Cao, M. Zhang, S. Dong, P. Guo, H. Li, Impact of potato pulp on the processing characteristics and gluten structures of wheat flour dough, Journal of Food Processing and Preservation 44 (6) (2020).
[24] Z. Yanyan, L. Yinli, L. Ying, Z. Hua, Effects of multiple freeze–thaw cycles on the quality of frozen dough, Cereal Chemistry 95 (2018) 499–507.
[25] P.S. Patrick, H.E. Swaisgood, Sulfhydryl and Disulfide Groups in Skim Milk as Affected by Direct Ultra-High-Temperature Heating and Subsequent Storage1, Journal of Dairy Science 59 (1976) 594–600.
[26] Y. Zhang, B. Wang, W. Wang, H. Wang, X. Liu, H. Zhang, Study on the mechanism of ultrasonic treatment impact on the dough's fermentation capability, Journal of Cereal Science 100 (2021), 103191.
[27] X. Ke, D. Cc, C. Zsab, C. Xlab, C. Yzab, C. Hwab, Z. Hua, Understanding how starch constituent in frozen dough following freezing-thawing treatment affected quality of steamed bread, Food Chemistry 366 (2021), 130614.
[28] A.G.B. Wouters, E. Fierens, I. Rombouts, K. Brijs, C. Blecker, J.A. Delcour, Air-water interfacial properties of enzymatically hydrolyzed wheat gluten in the presence of sucrose, Food Hydrocolloids 73 (2017) 284–294.
[29] A. Pérez-Nieto, J.J. Chanona-Pérez, R.R. Farrera-Rebollo, G.F. Gutiérrez-López, L. Alamilla-Beltrán, G. Calderón-Domínguez, Image analysis of structural changes in dough during baking, LWT - Food Science Technology 43 (2010) 535–543.
[30] X. Hu, L. Cheng, Y. Hong, Z. Li, C. Li, Z. Gu, Impact of celluloses and pectins restrictions on gluten development and water distribution in potato-wheat flour dough, International Journal of Biological Macromolecules 206 (2022) 534–542.
[31] Y. He, J. Guo, G. Ren, G. Cui, S. Han, J. Liu, Effects of konjac glucomannan on the water distribution of frozen dough and corresponding steamed bread quality, Food Chemistry 330 (2020), 127243.
[32] J. Yin, L. Cheng, Y. Hong, Z.-F. Li, C.-M. Li, X.-F. Ban, Z.-B. Gu, Use of two-stage dough mixing process in improving water distribution of dough and qualities of bread made from wheat–potato flour, Journal of Integrative, Agriculture 20 (2021) 300–310.
[33] X. Zhu, P. Yuan, T. Zhang, Z. Wang, D. Cai, X. Chen, Y. Shen, J. Xu, C. Song, D. Goff, Effect of carboxymethyl chitosan on the storage stability of frozen dough: State of water, protein structures and quality attributes, Food Research International 151 (2022), 110863.
[34] M. Geertrui, Bosmans, Bert, Lagrain, Lomme, J., Deleu, Ellen, Fierens, Assignments of Proton Populations in Dough and Bread Using NMR Relaxometry of Starch, Gluten, and Flour Model Systems, Journal of Agricultural and Food Chemistry 60 (2012) 5461–5470.
[35] X. Chang, X. Huang, X. Tian, C. Wang, J.H. Aheto, B. Ernest, R. Yi, Dynamic characteristics of dough during the fermentation process of Chinese steamed bread, Food Chemistry 312 (2020), 126050.
[36] Y. Cao, F. Zhang, P. Guo, S. Dong, H. Li, Effect of wheat flour substitution with potato pulp on dough rheology, the quality of steamed bread and in vitro starch digestibility, LWT - Food Science and Technology 111 (2019) 527–533.
[37] S. Li, Y. Xue, Y. Zhang, H. Ma, G.K. Mahunu, Effects of ultrasound and ultrasound assisted alkaline pretreatments on the enzymolysis and structural characteristics of rice protein, Ultrasonics Sonochemistry 31 (2016) 20–28.
[38] Y. Zhou, S. Dhital, C. Zhao, F. Ye, J. Chen, G. Zhao, Dietary fiber-gluten protein interaction in wheat flour dough: Analysis, consequences and proposed mechanisms, Food Hydrocolloids 111 (2021).
[39] X. Hu, L. Cheng, Y. Hong, Z. Li, C. Li, Z. Gu, An extensive review: How starch and gluten impact dough machinability and resultant bread qualities, Food Science and Nutrition (2021) 1969535.
[40] F.-X. Schmid John Wiley & Sons, Ltd eLS 1 Wiley.
[41] C. Zhou, H. Ma, Q. Ding, L. Lin, X. Yu, L. Luo, C. Dai, A.-E.-G.-A. Yagoub, Ultrasonic pretreatment of corn gluten meal proteins and neutrase: Effect on protein conformation and preparation of ACE (angiotensin converting enzyme) inhibitory peptides, Food and Bioproducts Processing 91 (2013) 665–671.
[42] J. Jia, H. Ma, W. Zhao, Z. Wang, W. Tian, L. Luo, R. He, The use of ultrasound for enzymatic preparation of ACE-inhibitory peptides from wheat germ protein, Food Chemistry 119 (2010) 336–342.
[43] S. Li, M. Li, H. Cao, X. Guan, Y. Zhang, K. Huang, Y. Zhang, The intervening effect of l-Lysine on the gel properties of wheat gluten under microwave irradiation, Food Chemistry: X 14 (2022), 100299.
[44] Q. Wang, Y. Li, F. Sun, X. Li, P. Wang, J. Sun, J. Zeng, C. Wang, W. Hu, J. Chang, M. Chen, Y. Wang, K. Li, G. Yang, G. He, Tannins improve dough mixing properties through affecting physicochemical and structural properties of wheat gluten proteins, Food Research International 69 (2015) 64–71.
[45] L. Huang, X. Ding, C. Dai, H. Ma, Changes in the structure and dissociation of soybean protein isolate induced by ultrasound-assisted acid pretreatment, Food Chemistry 232 (2017) 727–732.
[46] O.F. Eze, A. Chatzifragkou, D. Charalampopoulos, Properties of protein isolates extracted by ultrasonication from soybean residue (okara), Food Chemistry 368 (2022), 130837.

[47] L. Xiong, B. Zhang, M. Niu, S. Zhao, Protein polymerization and water mobility in whole-wheat dough influenced by bran particle size distribution, LWT - Food Science and Technology 82 (2017) 396–403.
[48] A. Marti, J.E. Bock, M.A. Pagani, B. Ismail, K. Seetharaman, Structural characterization of proteins in wheat flour doughs enriched with intermediate wheatgrass (Thinopyrum intermedium) flour, Food Chemistry 194 (2016) 994–1002.
[49] X.-S. Wang, C.-H. Tang, B.-S. Li, X.-Q. Yang, L. Li, C.-Y. Ma, Effects of high-pressure treatment on some physicochemical and functional properties of soy protein isolates, Food Hydrocolloids 22 (2008) 560–567.
[50] J.A. Resendiz-Vazquez, J.A. Ulloa, J.E. Urías-Silvas, P.U. Bautista-Rosales, J. C. Ramírez-Ramírez, P. Rosas-Ulloa, L. González-Torres, Effect of high-intensity ultrasound on the technofunctional properties and structure of jackfruit (Artocarpus heterophyllus) seed protein isolate, Ultrasonics Sonochemistry 37 (2017) 436–444.
[51] Y. Wang, Z. Zhang, R. He, B.K. Mintah, M. Dabbour, W. Qu, D. Liu, H. Ma, Proteolysis efficiency and structural traits of corn gluten meal: Impact of different frequency modes of a low-power density ultrasound, Food Chemistry 344 (2021), 128609.
[52] E.B. Ceresino, E. Johansson, H.H. Sato, T.S. Plivelic, R. Kuktaite, Morphological and structural heterogeneity of solid gliadin food foams modified with transglutaminase and food grade dispersants, Food Hydrocolloids 108 (2020), 105995.
[53] J. Jin, H. Ma, K. Wang, A.-E.-G.-A. Yagoub, J. Owusu, W. Qu, R. He, C. Zhou, X. Ye, Effects of multi-frequency power ultrasound on the enzymolysis and structural characteristics of corn gluten meal, Ultrasonics Sonochemistry 24 (2015) 55–64.
[54] L. Tebben, Y. Shen, Y. Li, Improvers and functional ingredients in whole wheat bread: A review of their effects on dough properties and bread quality, Trends in Food Science & Technology 81 (2018) 10–24.
[55] W. Li, W. Cao, P. Wang, J. Li, Y. Yan, Selectively hydrolyzed soy protein as an efficient quality improver for steamed bread and its influence on dough components, Food Chemistry 359 (2021), 129926.
[56] R. Cui, F. Zhu, Changes in structure and phenolic profiles during processing of steamed bread enriched with purple sweetpotato flour, Food Chemistry 369 (2021), 130578.
[57] J.-L. Yao, Q.-A. Zhang, M.-J. Liu, Utilization of apricot kernel skins by ultrasonic treatment of the dough to produce a bread with better flavor and good shelf life, LWT - Food Science and Technology 145 (2021), 111545.
[58] Y. Cao, L. Jiang, W. Suo, Y. Deng, H. Li, Influence of emulsifiers and enzymes on dough rheological properties and quality characteristics of steamed bread enriched with potato pulp, Food Chemistry 360 (2021), 130015.