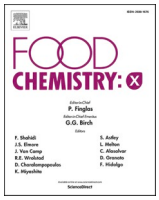
Food Chemistry: X 15 (2022) 100448

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Evolution of the morphological, structural, and molecular properties of gluten protein in dough with different hydration levels during mixing

Ruobing Jia [a], Mengli Zhang [a], Tianbao Yang [b], Meng Ma [a,b], Qingjie Sun [a], Man Li [a,*]

[a] School of Food Science and Engineering, Qingdao Agricultural University, Qingdao 266109, Shandong Province, PR China
[b] Beltsville Agricultural Research Center, United States Department of Agriculture-Agricultural Research Services, Beltsville 20705, United States

ARTICLE INFO

Keywords:
Hydration level
Dough
Mixing
Gluten evolution
Molecular chain
Network structure

ABSTRACT

To understand the formation process of dough with different hydration levels upon mixing and the response of dough rheology, the dynamic evolution of gluten protein was tracked and quantified at morphological, structural, and molecular levels. Both macroscopical and microscopic distribution images showed that partial and full hydration induced quick formation of a more compact gluten network compared with limited hydration. Gluten network in highly hydrated samples was more susceptible to the formation and collapse induced by mechanical force. SE-HPLC results indicated significant depolymerization of glutenin macropolymer (GMP) in fully and partially hydrated samples. Sufficient mixing was accompanied by the increase of ionic and hydrogen bonds, while excessive mixing increased exposure of free -SH. Higher hydration level induced more ordered secondary structure. Correlation and principal component analysis revealed the patterns and dynamics of gluten evolution during dough formation with different hydration levels, and their contribution to the changes in dough modulus.

1. Introduction

As a widely grown food crop, wheat has steadily become one of the most consumed human food sources over the centuries (Ma et al., 2020). Wheat flour is the main raw material for various staple foods including noodles, bread, steamed bread et al. Protein, as one of the key components of wheat flour, plays a vital role in both the food production process and the presentation of the final quality (Han et al., 2021). Gluten protein accounts for 80 %–85 % of total wheat proteins and is a key component that determines the quality of wheat-based products (Gao et al., 2020). The processing parameters and conditions are important factors affecting the eating quality, and any changes in the processing parameters will influence the quality and stability of the final products. Mixing is an essential step during dough-making and the manner in which it is performed determines the dispersion of ingredients, their interactions, and, in turn, the processing efficiency and quality of the wheat products (Carini et al., 2010). The introduction of water and mechanical force allows the wheat flour to be hydrated and energized during the mixing process. Mixing involves the exchange between sulfhydryl groups (—SH) and disulfide bonds (S—S), which also affects the formation of the gluten network and glutenin macropolymer polymerization (GMP) (Feng et al., 2021). Both the rheological and structural properties of dough could be significantly impacted by the mixing time. Insufficient mixing could cause uneven spatial distribution of gluten proteins on the surface of the dough. Excessively long mixing resulted in a reduction or partial aggregation of gluten, and induced softer, stickier dough than optimum mixed ones (Gómez et al., 2011; Liu et al., 2015). Water is known to play a critical role in the quality and storage stability of food. It can interact with other molecules to influence their configuration, plasticity, and functionality (Carini et al., 2010). The hydration capacity of wheat flour often defines its quality and ability to form a viscoelastic dough and the quality of the dough affects the product's functional properties and the culinary product's quality (Berton et al., 2002). Therefore, different wheat products have different requirements for the degrees of hydration of the dough and the amount of added water. For example, biscuits require a certain degree of hardness and chewiness, noodles are usually elastic and chewy, and bread requires a fluffy and porous structure to make it soft and elastic. These different characteristics could be adjusted by the hydration levels of the dough to meet people's needs for taste (Guibert-Martin et al., 2017; Li et al., 2017; Mastromatteo et al., 2013; Yue et al., 2019).

Currently, the connections among gluten network structure/properties and dough rheology and the eating quality of wheat products have been widely studied. However, most of them focused on the analysis of

* Corresponding author.
E-mail address: manliqau@163.com (M. Li).

https://doi.org/10.1016/j.fochx.2022.100448
Received 22 July 2022; Received in revised form 7 September 2022; Accepted 13 September 2022
Available online 14 September 2022

the macroscopic and microscopic characteristics of the final products and revealed the principle and effect of quality regulation. Few studies explored the dynamic distribution and evolution of the key component (gluten protein) during processing. In addition, in dough systems with different hydration levels, such as fully hydrated (bread), partially hydrated (steamed bread), and limitedly hydrated (biscuits and noodles) dough, the changes in gluten proteins and their regulation mechanisms on the quality of dough and the final products are different. Moreover, the existing studies still lack the quantitative characterization and calculation of gluten protein morphology, distribution, structural transformation, and the correlation analysis with macro-quality changes.

In this research, we systematically investigated and tracked the dynamic changes in the morphological distribution, structure, and molecular evolution of gluten protein in dough as affected by different hydration levels during mixing. Meanwhile, its regulation on the formation of gluten network and the rheological properties of dough were also systematically explored. The dynamic morphological distribution and evolution, and the structural, conformational, and molecular transformation of gluten proteins were extracted to calculate and evaluate the correlation with the changes in macroscopical rheology of wheat dough. This study visually reveals the formation processes of gluten networks in doughs with different hydration levels during mixing. And it provides new ideas to precisely control dough processing and the quality of wheat products.

2. Materials and methods

2.1. Materials

Specially refined wheat flour (with contents of carbohydrates, protein, and fat 73.8 %, 12.2 %, 1.0 %, respectively) was purchased from Jinshahe Group Co., ltd., Hebei, China. All of the chemical reagents were of analytical grade and can be used without further purification.

2.2. Sample preparation for mixed wheat dough with different hydration levels

The amount of water added was determined based on the results displayed by the Farinograph experiment (Model JFZD, Beijing, China). The prepared dough samples consisted of 34 mL, 50 mL, 62 mL distilled water, and 100 g wheat flour (12.8 % moisture content) respectively to make limitedly, partially, and fully hydrated dough. The Farinograph with a mixing bowl (50 g) was used to mix wheat flour and water, and record them as three groups of 34 (limitedly hydrated), 50 (partially hydrated), and 62 (fully hydrated). For each group of samples, three time points (34-1, 34-5, 34-15, 50-1, 50-5, 50-15, 62-1, 62-5, 62-15) were selected to indicate mixing for 1 min, 5 min, and 15 min. The mixing times were selected from the dough before formation, the stable period and after weakening (overmixed) as detected in the Farinograph experiment. Each sample was prepared in triplicate. It is worth noting that because of the low hydration level, only dough crumbles can be formed after mixing with 34 mL of distilled water. Some of each sample was freeze-dried by a vacuum freeze dryer (CHRIST, Osterode, Germany) and then ground into powder.

2.3. Evolution of gluten distribution in dough

Fast Green (FCF) was used as a specific dye to explore the evolution of the distribution of gluten protein phase. According to Auger et al. (2008), the dye (0.896 mg per gram of protein) was dissolved in the corresponding water for each sample. In order to reduce the error of multiple experiments, dough sampling was performed at intervals. The sampling process did not cause a significant change in dough morphology in the visual. A metal spoon was used to sample the dough while not disturbing the dough surface. The prepared sample was placed in a white weighing paper, and the surface of the dough was lightly pressed with a glass sheet to make it smooth. A digital electron microscope (JHOPT, China) was used to take photographs of dough samples. All the pictures were binarized by ImageJ under the same processing conditions. The white and black areas represented starch granules and gluten respectively. Each sample was tested in triplicate.

2.4. CLSM analysis of gluten network structure

The dough samples were analyzed using a confocal laser scanning microscopy (CLSM) based on the method reported by Silva et al. (2013). The samples were preembedded with Leica Tissue Freezing Medium and fixed at low temperature (-80 °C). The samples were cut into 20 μm thick sections using a freezing microtome (Leica CM 1850, Germany) at −20 °C. The sections were stained with Rhodamine B (0.025 %) for 1 min on the glass slides, distilled water was used to rinse the sections to remove excess dye. Then the samples were covered with a coverslip and placed upside down on the microscope. The images of the samples were taken at 40× magnification, 1024 × 1024 pixels, and 100 Hz frequency. The observation was conducted at the excitation and emission wavelength of 568 and 625 nm. The software Angio Tool was applied to analyze CLSM images, calculating and quantifying the evolution of the gluten network. Each sample was repeated in triplicate.

2.5. SEM analysis

The freshly mixed samples were fixed with glutaraldehyde solution (2.5 %) for 12 h and washed 3–5 times with 0.1 M phosphate-buffered saline. The lyophilized samples were fixed at the conductive sample stage and evenly sprayed with gold particles. The microstructure of the gluten network (triplicates per sample) was observed using scanning electron microscopy (SEM, Model 7500F, Japan) at 600× magnification.

2.6. Size exclusion-HPLC analysis

SE-HPLC results of the samples were obtained by an LC system (LC-20AT, Shimadzu, Kyoto, Japan) equipped with an ultraviolet detector. According to the method of Wang et al. (2018). The freeze-dried sample (15 mg) was dispersed in 1 mL phosphate buffer (0.05 M, pH 7.0, containing 1 % sodium dodecyl sulfate). In order to evaluate the extractability under reducing conditions, the sample (10 mg) was dissolved with the phosphate buffer containing 1 % dithiothreitol (DTT). The soluble protein in the sample solution was fully extracted by vortexing for 1 h and centrifuged at 10,000*g* for 10 min. 20 μL supernatant was filtered through a 0.45 μm microporous filter to inject into the TSK G4000-SWXL analysis column (Tosoh Biosep, Japan). The flow rate of the phosphate buffer was 0.7 mL/min with the wavelength of the ultraviolet detector set as 214 nm and the thermostat temperature set as 30 °C.

2.7. Secondary structure analysis

Based on the method of Wang et al. (2018), the infrared spectra of different samples were recorded on a Fourier transform infrared (FT-IR) spectrometer (Model NEXUS-870; Thermo Nicolet Corp., Madison, USA) with a single reflection diamond attenuated total reflection (ATR) accessory and a mercury-cadmium-telluride (MCT) detector. Bands in the region of 1650–1660 cm^{-1} can be attributed to α-helices; 1665–1671 cm^{-1} was attributed to β-turns; random coil, 1640–1650 cm^{-1}; β-sheets, 1612–1614 cm^{-1} and 1687–1690 cm^{-1}.

The Freeze-dried sample powders were placed on the ATR crystal and measured with 32 scans, resolution of 4 cm^{-1}, and scan range of 400–4000 cm^{-1}. Before applying the sample to the crystal, the background spectra was recorded using a blank ATR and automatically subtracted from the measured spectrum of sample. The 1600–1700 cm^{-1} spectra was analyzed by Omnic (version 8.2.0) and Peak Fit software

(Version 4.12).

2.8. Determination of free sulfhydryl (-SH) contents

The free -SH content determination was based on the method reported by Rombouts et al. (2014) with minor modifications. The sample (0.4 g) was dissolved in 10 mL buffer A (8 M urea, 1 % w/v SDS, 3 mM EDTA, 0.2 M Tris-HCl, pH 8.0). The mixture was shaken at room temperature (25 °C) for 1 h and centrifuged at 12,000 g for 20 min. Then 0.1 mL buffer B (0.2 M Tris-HCl, 10 mM DTNB, pH 8.0) was added to the supernatant and violently shaken to get a homogeneous mixture. After incubation in the dark for 20 min, the samples were measured at 412 nm. The free -SH content (F_{SH}) was calculated as:

$$FSH\ (\mu mol/g) = \frac{73.53A412 \times D}{\rho}$$

where the constant $73.53 = 10^6/1.36 \times 10^4$, 1.36×10^4 is the molar absorption coefficient of DTNB (L/mol); A_{412} indicates the absorption value at 412 nm; D is the dilution ratio; ρ indicates the sample concentration (mg/mL).

2.9. Measurement of gluten solubility in specific solvents

The determination of chemical interactions was performed based on the method of Wang et al. (2017) with minor modifications. The specific solvents used to dissolve proteins in phosphate buffer solution (0.05 M, pH 7.0) were as follow: 0.05 M NaCl (*P*1), 0.6 M NaCl (*P*2), 0.6 M NaCl and 1.5 M urea (*P*3), and 0.6 M NaCl and 8 M urea (*P*4). The lyophilized dough powder (100 mg) was added to 10 mL specific solvents and mixed for 1 h at 25 °C, followed by centrifugation at 10,000*g* for 20 min. Folin-phenol colorimetry was used to measure the soluble protein concentration in the supernatant and the results were expressed as mg/mL. The difference in soluble protein between P1 and P2 indicates ionic bond, the difference between P3 and P2 indicates hydrogen bond, while the difference between P4 and P3 indicates hydrophobic interaction (Zhang et al., 2022a).

2.10. Determination of GMP gel weight

Glutenin was mainly in the form of polymer in gluten. The gluten fraction that was insoluble in SDS solution had a larger molecular weight and was called GMP. After high-speed centrifugation, the GMP was covered on the starch layer as a colloidal substance in the SDS solution, so it was called colloidal protein (Don et al., 2003). The wet weight of GMP referred to the weight of the transparent gelatinous substance in the upper layer of the precipitate after extraction with the SDS solution, which was used to reflect the content of GMP and evaluate the quality of GMP (Ma et al., 2019). The GMP gel was extracted based on the method presented by Zhang et al. (2022b) with some modifications. 1.4 g of freeze-dried powder sample was evenly suspended in 28 mL of 1.5 % SDS and centrifuged at 20,000 g for 30 min at 25 °C. The precipitated gelatinous substance was carefully collected and weighed.

2.11. Dynamic rheological measurements

The frequency sweep is widely used to measure the viscoelasticity of polymer solids, melts, and solutions. The viscosity and elastic properties of the dough can be measured by evaluating its amplitude-dependent and frequency-dependent behavior, which plays an important role in the quality of wheat products (Yang et al., 2021). Storage modulus (G′) and loss modulus (G″) are two key indicators of frequency sweep, reflecting the degree of dough elasticity and viscosity, respectively (Iuga et al., 2020). The tanδ could be used to indicate the polymer content and degree of polymerization, which is expressed by the ratio of G″ to G′. A higher tanδ value indicates less agglomeration on the surface of the dough, a lower polymer content, and a lower elastic ratio. Conversely, the proportion of molecules with a large degree of polymerization is lower (Lopes-da-Silva et al., 2007).

Dynamic rheological properties of hydrated dough samples were measured based on the method of Han et al. (2021) using a controlled stress rheometer (MCR102, Anton Paar, Austria). Dough samples with different mixing times and different hydration levels were prepared and relaxed for 20 min at 25℃ before analysis. Placed the rested dough on the circular stage with a gap of 1 mm and selected the PP50 probe. After sample loading, the excessive edge was gently scraped off and coated with a thin layer of silicone oil to prevent moisture evaporation. The sample was allowed to stand for 3 min before the test. Storage modulus (G′), loss modulus (G″), and tanδ (G″/G′) were recorded at a constant strain amplitude of 0.5 %and a frequency range of 0.1 to 100 Hz under the oscillatory frequency sweep pattern.

2.12. Statistical analysis

The data used for analysis were measured at least three times, the mean value and standard deviation were calculated. SPSS processing software (Version 22.0, SPSS Inc., Chicago, IL, USA) by one-way analysis of variance (ANOVA) and Duncan's test was used to verify significant differences. $P < 0.05$ was regarded as significant. Correlation analysis (CA) and principal component analysis (PCA) were carried out using RStudio (version 1.4.1717 based on R version 4.1.0), and graphs were performed using Origin (Origin Lab).

3. Results and discussion

3.1. Macroscopic gluten phase distribution and evolution during dough mixing at different hydration levels

As shown in Fig. 1A, the 34 % hydrated dough sample was unevenly mixed due to the low moisture content, so there were some starch granules and unhydrated flour particles. Under the same degree of hydration, the network of gluten protein gradually appeared with continuous mixing, and the distribution of starch became more uniform (Liu et al., 2020). The degree of hydration was essential for forming the gluten network structure. In the cases of high hydration levels (50 % and 62 %), the distribution of the gluten phase tended to be uniform with the mixing time increased. These results suggested that both mixing and hydration level profoundly influenced the formation of the gluten network, which makes the following research meaningful.

3.2. Evolution of gluten network morphology at different hydration levels during mixing

CLSM is an effective tool to get even better insights into protein network by visualizing the main structural factors of dough (Bernklau et al., 2016). The CLSM images obtained from the freshly made dough samples and fitting images of the gluten network are shown in Fig. 1B. The red area represents protein, which can be used to indicate the formation and distribution of gluten.

The formation of a three-dimensional network structure of gluten in wheat dough could be observed in all samples, with starch granules randomly distributed in the gluten network matrix. The gluten network in dough samples with 50 % (Fig. 1B-b) and 62 % (Fig. 1B-c) hydration levels were uniformly distributed and formed a continuous and compact protein matrix. The large space between the protein fibrils was interspersed with aggregated starch granules. In contrast, the gluten network of dough prepared with a hydration level of 34 % showed an overall loose structure. This might be because the flour particles were not fully hydrated under low moisture conditions, thus the gluten network was insufficiently developed (Liu et al., 2015). It could be seen from these results that the formation and distribution of gluten network were influenced by the different hydration degrees of dough, and might affect

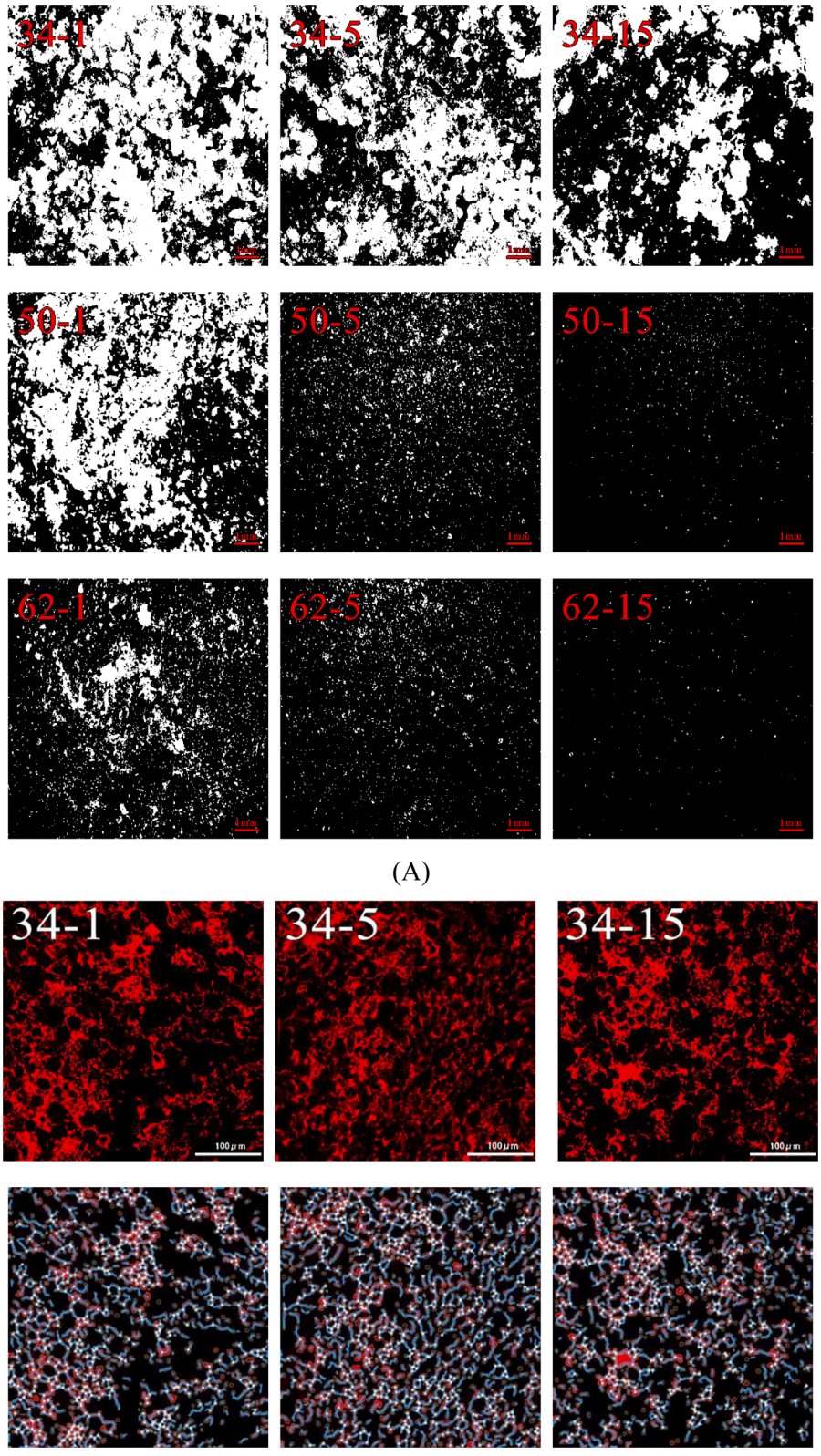
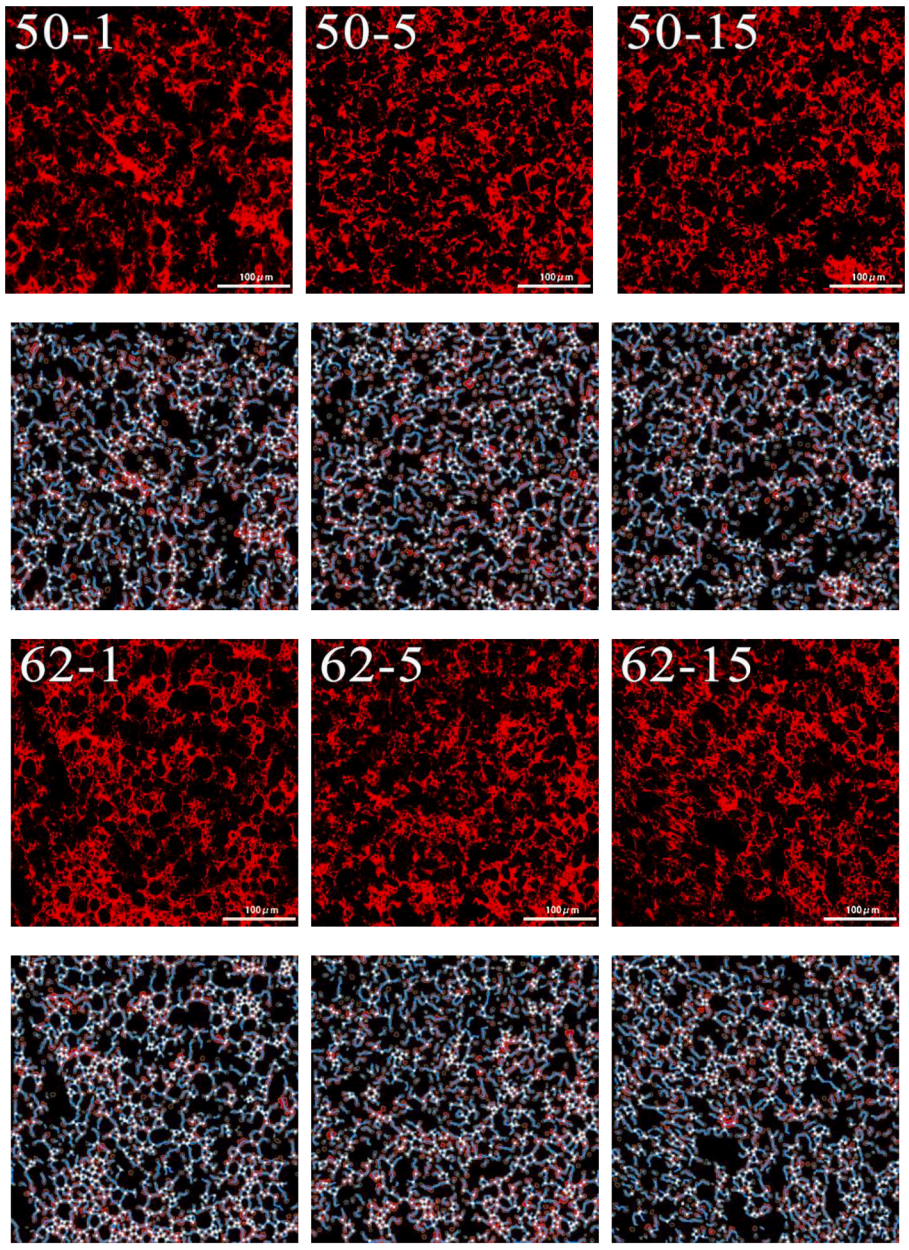


Fig. 1. Dynamic evolution of gluten network morphology: A). The grayscale 8-bit images of Fast Green stained dough samples by ImageJ binarization and analysis; B) CLSM original images and the corresponding Angio Tool analysis images; C) quantitative calculation of the CLSM micrographs. 34-1, 34-5, and 34-15 represent the dough with hydration level of 34 % mixing for 1 min, 5 min, and 15 min, respectively; 50-1, 50-5, and 50-15 represent the hydration level of 50 % mixing for 1 min, 5 min, and 15 min, respectively; 62-1, 62-5, and 62-15 represent the hydration level of 62 % mixing for 1 min, 5 min, and 15 min, respectively. d, e, f, g, and h indicate the total number of junctions, protein width, branching rate, lacunarity, and protein percentage area (%), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

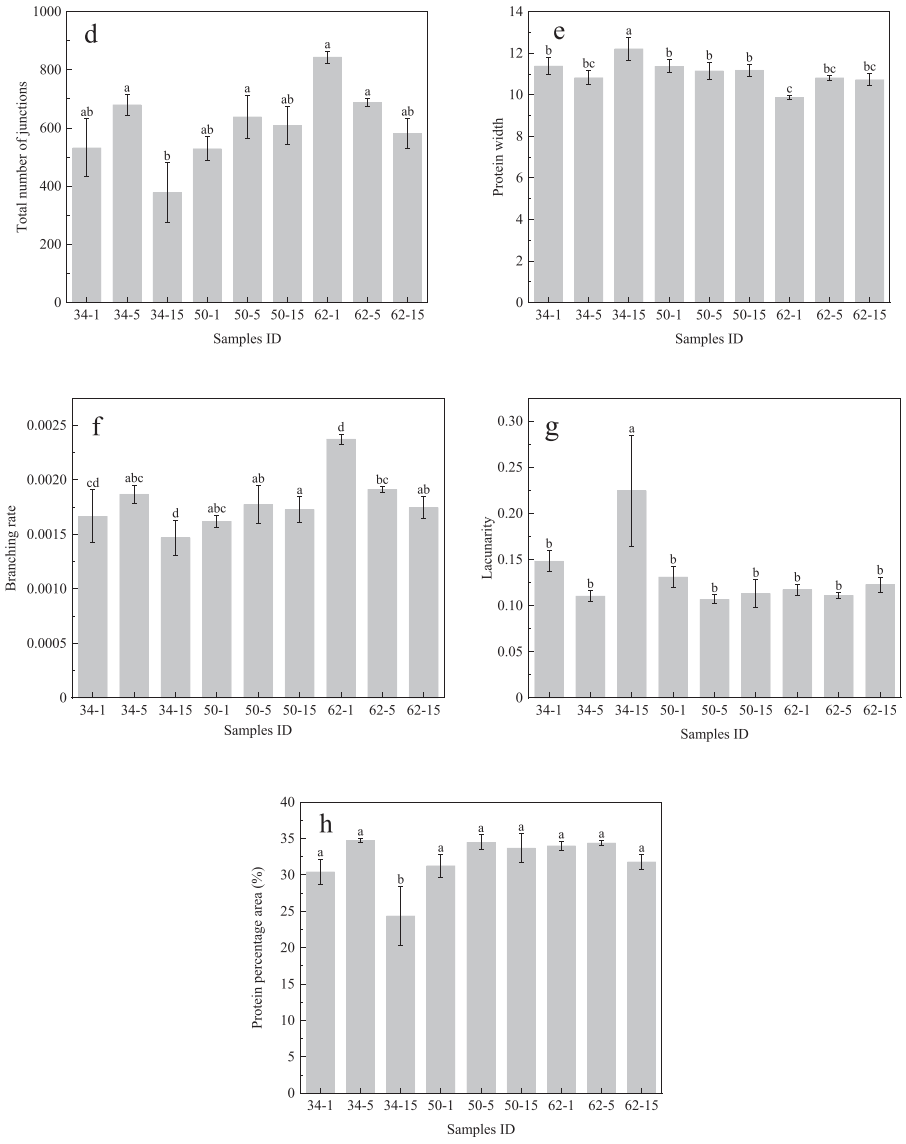


(B)

Fig. 1. (*continued*).

the density and strength of the dough. By evaluating the CLSM images, the dough mixed for 5 min showed a well-developed gluten network structure compared to 1 min and 15 min samples at the same hydration level. However, uneven distribution of gluten fibrils and large gaps were observed in doughs mixed for 1 and 15 min. This situation was more pronounced in dough with a hydration level of 34 %. This could be explained that insufficient mixing time led to inadequate development of the gluten network, and the shearing action significantly damaged the gluten network during long-term mixing (Gómez et al., 2011).

To further detect the gluten network structure of the dough accurately, the CLSM images were processed by Angio Tool software. The quantitative indicators of the total number of junctions, protein width, branching rate, lacunarity, and protein percentage area (%) are shown in Fig. 1C. There was no noticeable difference in the protein percentage area of all samples, except for 34–15 which had a lower percentage area. Bernklau et al. (2016) reported that the protein percentage area linearly decreased with the increase of water addition in a certain concentration range (59.2–89.9 mL per 100 g flour). We did not obtain similar results, which may be due to the amount of water added in this study being within the appropriate range for dough formation, while in the extreme case of higher water concentration, like pre-doughs were formed owing to dilution and plasticization of water, resulting in the decrease of protein area. The total number of junctions was significantly lowered in 34-15 samples ($P < 0.05$), and the lacunarity significantly increased ($P < 0.05$). Furthermore, a higher number of junctions represents compact protein cross-links which affects the stability of the dough. The longer mixing time (15 min) destroyed the cross-links between the protein due to the destruction of disulfide bonds or other non-covalent bonds. Higher lacunarity indicates that the gluten network is loose due to starch granules of different sizes incorporated (Gao et al., 2020), which affects the dough quality. There was no significant difference in porosity for all samples except 34-15 which had higher porosity. The branching rate is



(C)

Fig. 1. (*continued*).

highly related to the uniformity of gluten distribution (Bernklau et al., 2016). The branching rate of the dough after mixing for 15 min was lower, and the uniformity of the gluten network was affected by the mixing time. Therefore, it could be speculated that hydration level and mixing affected the dough properties mainly by increasing the uniformity and cross-linking of the gluten network.

3.3. Changes in microstructure

Scanning electron microscopy (SEM) can be used to observe the microstructure of samples with different levels of hydration and mixing degrees (Han et al., 2021). The SEM images of the samples with different hydration levels during different mixing processes are shown in Fig. 2. When the hydration level was 34 %, starch cannot be well embedded in a loose and weak gluten network because of the limited addition of water. The dough with a high degree of hydration (50 %, 62 %) showed a continuous gluten network structure, with the dispersed starch granules embedded in the gluten matrix. The microstructure of the properly mixed dough (5 min) exhibited a continuous gluten network, while the excessive mixing (15 min) caused the gluten network to break, with increasingly exposed starch granules. It might be because the mechanical force of the mixing blade damaged the dough and caused the collapse of gluten network structure. The uneven contact between wheat flour and water, and the incomplete development of the gluten network were observed in the dough with insufficient mixing (1 min) (Obadi et al., 2022). Starch granules cannot be well wrapped in the gluten network due to the sparse distribution of the gluten network. The effect of mixing degree on the microstructure of the sample was more obvious in the low-hydrated dough.

3.4. Evolution of SDS solubility and molecular weight distribution profiles of proteins at different hydration levels during mixing

During the processing of dough, wheat proteins undergo complicated changes of depolymerization and repolymerization, which determine the final quality of dough products. These changes might lead to differences in dough rheological, textural, and cooking properties (Li et al., 2018). Gluten proteins are susceptible to mixing and hydration, and

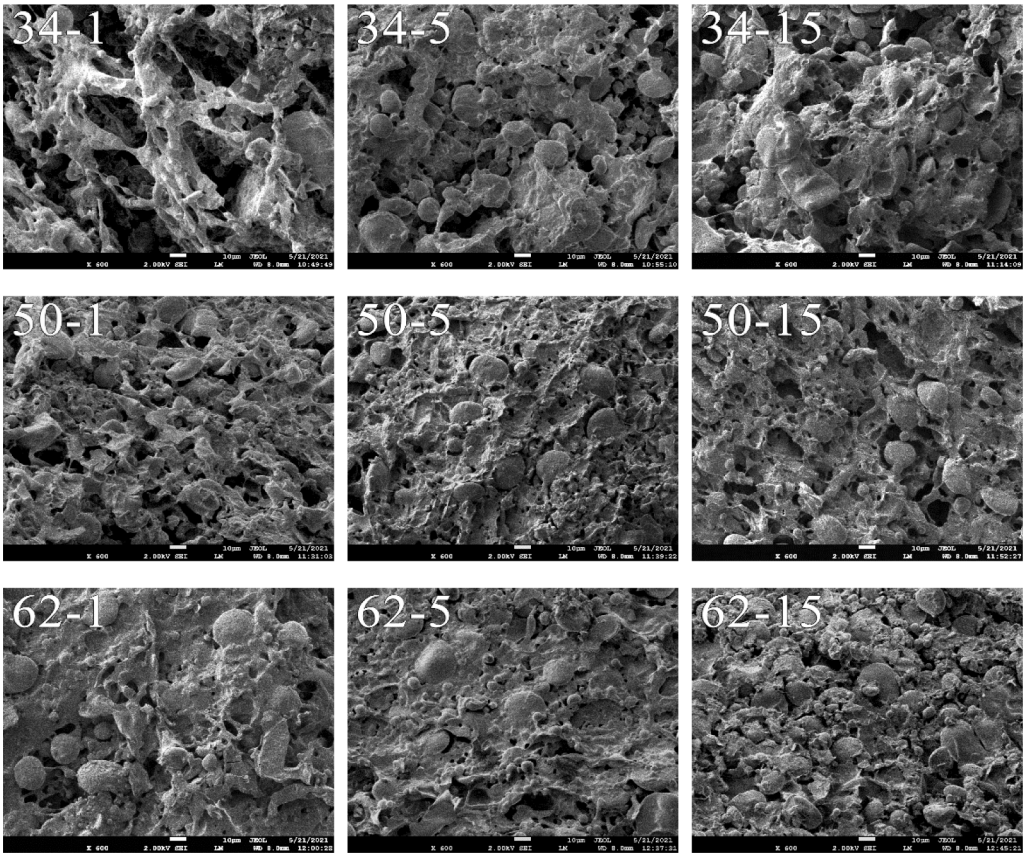


Fig. 2. The images of scanning electron microscope. 34-1, 34-5, and 34-15 represent the dough samples with hydration level of 34 % mixing for 1 min, 5 min, and 15 min, respectively 50-1, 50-5, and 50-15 represent the hydration level of 50 % mixing for 1 min, 5 min, and 15 min, respectively; 62-1, 62-5, and 62-15 represent the hydration level of 62 % mixing for 1 min, 5 min, and 15 min, respectively.

which lead to disulfide bond or non-covalent bond-linked aggregates as well as conformational changes. These are crucial for the development of the gluten polymer. Both reduced and non-reduced SE-HPLC profiles were used to analyze the evolution of molecular weight distribution of gluten proteins induced by hydration and mixing degree (Fig. 3). The SE-HPLC profile of wheat proteins could be recorded as four parts, P1 stood for large glutenin polymers, P2 for medium glutenin polymers, P3 for monomeric proteins, while P4 for peptides and amino acids (Wang et al., 2014).

The proportion of SDS unextractable glutenin macropolymers (GMP) is an important factor determining the processing quality of dough, and non-reduced curves could indirectly indicate the changes in GMP. As shown in Fig. 3a, during mixing, compared with the dough with a hydration level of 34 %, the peak area of the curves, specifically the peak of large glutenin polymers (P1) of the highly hydrated dough (50 % and 62 %) were more obviously increased with the extension of mixing time. The depolymerization of GMP component was the main reason for the increase in peak area. These results indicated that dough with high hydration level was more susceptible to the mechanical force induced by mixing, which might be due to the enhanced protein mobility and conformational changes promoted by hydration (Wang et al., 2016). Therefore, excessive mixing would also destroy large gluten aggregates and further increase the protein extractability in SDS. In addition, with respect to *P*3, slight aggregation of the monomeric proteins might also occur (decreased peak area) in dough with a high degree of hydration after mixing for 5 min and 15 min. The peak area of the extractable protein was almost the same under different hydration levels and mixing times as shown in the reduced elution profiles (Fig. 3b), indicating that these aggregations were mainly induced by disulfide bonds.

3.5. Secondary structure analysis

As one of the important indexes of the conformation of gluten protein, secondary structure is of great significance for the evaluation of gluten network structure formation (Li et al., 2018). Peak Fit software was used to fit the reconstituted spectra in the amide I region and to obtain the content of the corresponding secondary structure by calculating the area ratio.

Mechanical mixing can change the balance of the gluten network structure. The secondary structure content of dough samples with different hydration levels during mixing is shown in Table S1. For insufficiently-developed (mixing 1 min), well-developed (mixing 5 min), and over-mixed (mixing 15 min) dough under the same degree of hydration, the β-sheets increased first and then decreased, while the α-helixes decreased first and then increased. During the dough formation, α-helices and β-sheets represented the formation of ordered structures (Wang et al., 2016), indicating that moderate mixing time promoted a more ordered gluten network structure. Compared with mechanical mixing, the influence of hydration level on the gluten conformation of the dough was more obvious. Under the same degree of mixing, the samples with high hydration levels (50 % and 62 %) had a higher-ordered structure (α-helices and β-sheets) area percentage. The secondary structure changed more obviously in the highly hydrated sample during the mixing process. This might be because the presence of water enhanced the hydrogen bonds in gluten and its subfractions (Wellner et al., 2005), which increased the content of α-helices and β-sheets.

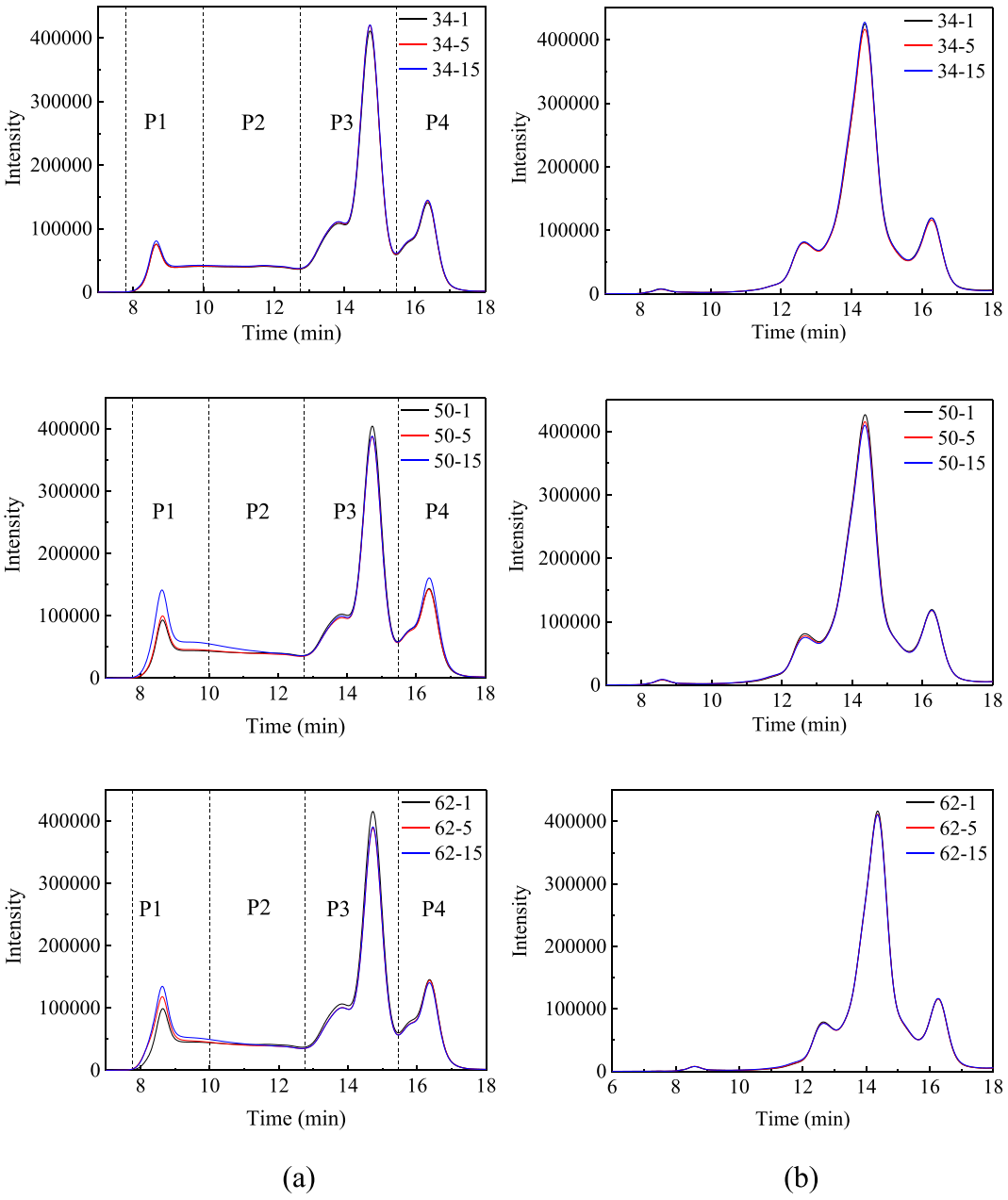


Fig. 3. Changes in non-reduced (a) and reduced (b) SE-HPLC profiles of protein in dough with different hydration levels during mixing.

3.6. Changes in free sulfhydryl (-SH) contents

Free -SH groups content always changes with the breaking of the covalent bonds (disulfide) and the formation of new bonds. The cross-linking of gluten proteins is usually confirmed by the content of free sulfhydryl groups during the formation of gluten network structure, which affects the quality of the final wheat products. The changes in free -SH content in dough samples with different hydration levels during mixing are summarized in Fig. 4a. During the mixing process, the content of -SH decreased (5 min) and then increased (15 min) with the extension of mixing time. Thiol/disulfide exchange existed in wheat flour under mechanical action and hydration, while excessive mechanical force could break the gluten disulfide bonds and oxidize the thiol radical moieties (Morel et al., 2002). The degree of change in free -SH content of the low hydration sample (34 %) was lower than higher samples (50 % and 62 %). It might be because when the moisture content was insufficient, and some starch granules failed to embed in the gluten protein matrix, resulting in a less continuous gluten network with fewer disulfide bond (SS) connections (Martínez & Gómez, 2017). The content of free -SH in samples with high hydration level changed more obviously. Compared with wheat flour (6.27 μmol/g), the -SH content in the dough with 52 % and 62 % hydration level after mixing for 5 min were 4.81 and 4.11 μmol/g, respectively, while the values after 15 min slightly increased to 4.90 and 4.72 μmol/g, respectively. When the water mobility was higher, more water was distributed in the hydrated region of gluten and starch, so the stability of gluten protein deteriorated (Zhang et al., 2018). We might suspect that when the degree of hydration was higher (more than 50 %), the stability of the dough gluten structure would become worse, and thiol/disulfide exchange was more likely to occur under mixing. Moreover, the change of free -SH content was relevant to the content of polymerized protein, which influenced the final quality of the product.

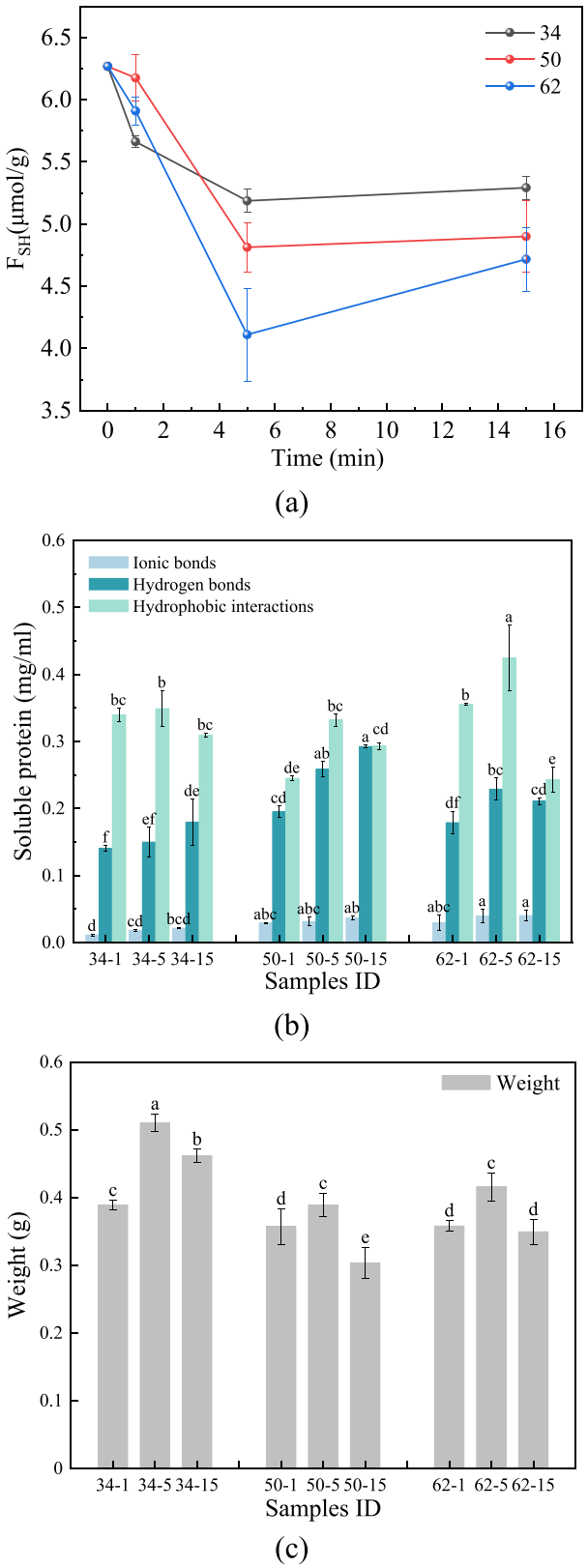


Fig. 4. Changes in free sulfhydryl (-SH) contents (a), non-covalent interactions (b), and glutenin macropolymer (GMP)wet weight (c) in dough samples with different hydration levels during mixing.

3.7. Dynamic changes in non-covalent interactions

Apart from the SS bonds, the development of the gluten network also depends on non-covalent bonds, such as ionic bonds, hydrogen bonds, and hydrophobic interactions. Non-covalent interactions can be assessed by measuring the solubility of gluten in different solutions. The first type of reagent was a general salt buffer solution, such as phosphate buffer (containing NaCl), which was used to extract proteins. And the second type of reagent could destroy the non-covalent interactions by the addition of reagents, such as urea, which could break the hydrophobic interactions and hydrogen bonds through different concentrations.

The solubility of gluten proteins in different reagents are shown in Fig. 4b. Compared with hydrogen bonds and hydrophobic interactions, ionic bonds are weaker in all samples, indicating fewer ionizable amino acid side chains in gluten proteins (Wieser, 2007). The strength of the dough is related to the abundance of hydrogen bonds (Wang et al., 2022). With the increase of mixing time, the hydrogen bond interactions increased (34 % and 50 % samples). This might be because the flour and water were in full contact during the mixing process, and the interaction between proteins enhanced the binding between protein and water, as well as hydrogen bonds between proteins. The sample with a hydration degree of 50 % had stronger hydrogen bonding interaction than 34 %. This might be because 50 % gluten protein fibers contain more water to facilitate hydrogen bond interactions. However, when the hydration level was increased to 62 %, the dough strength decreased and the non-covalent interactions were broken by excessive mixing, resulting in a reduction in hydrogen bonds (Marti et al., 2016). Hydrophobic interactions contribute to the stabilization of the gluten structure (Wang et al., 2020). As the hydration level increased, the changes in hydrophobic interactions during mixing were more pronounced. This indicated that the dough became more unstable in structure. The hydrophobic interaction presented a trend of first increasing and then decreasing under different mixing levels. At the initial stage of mixing, the hydrophilic groups on the surface of gluten proteins were first bound to water. Moderate mixing (5 min) promoted gluten protein aggregation and enhanced hydrophobic interactions. Excessive mixing (15 min) disrupted the gluten network structure, resulting in exposure of hydrophobic groups on the surface of gluten protein and weakened hydrophobic interactions (Feng et al., 2020).

3.8. Changes in GMP wet weight

The changes in GMP wet weight of each sample are summarized in Fig. 4c. With the extension of mixing time, the GMP wet weight changed significantly and showed a trend of first increasing (5 min) and then decreasing (15 min). This indicated that GMP wet weight increased during the mixing process, and the GMP structure was destroyed after excessive mixing (15 min). According to the results of SEM, the change of GMP wet weight was positively correlated with the uniformity of gluten network. It might be that the decrease of GMP wet weight which caused by glutenin depolymerization, made more possibilities of rearrangement of protein and starch granules, and led to instability of the gluten network (Wang et al., 2016). The GMP wet weight under high hydration levels (50 % and 62 %) was relatively lower, which could be due to the more obvious depolymerization of gluten polymer during mixing in the high moisture dough system.

3.9. Dynamic rheological properties

The change in dynamic rheological measurement of different samples is shown in Fig. 5. The G′ of all samples was always higher than G″, indicating that all dough samples exhibited solid properties. Due to the strong intermolecular cross-linking effect in the dough, G′ and G″ showed an increasing trend with increasing frequency (Shewry et al., 2000).

Under the same hydration level, with the increase of mixing time, the

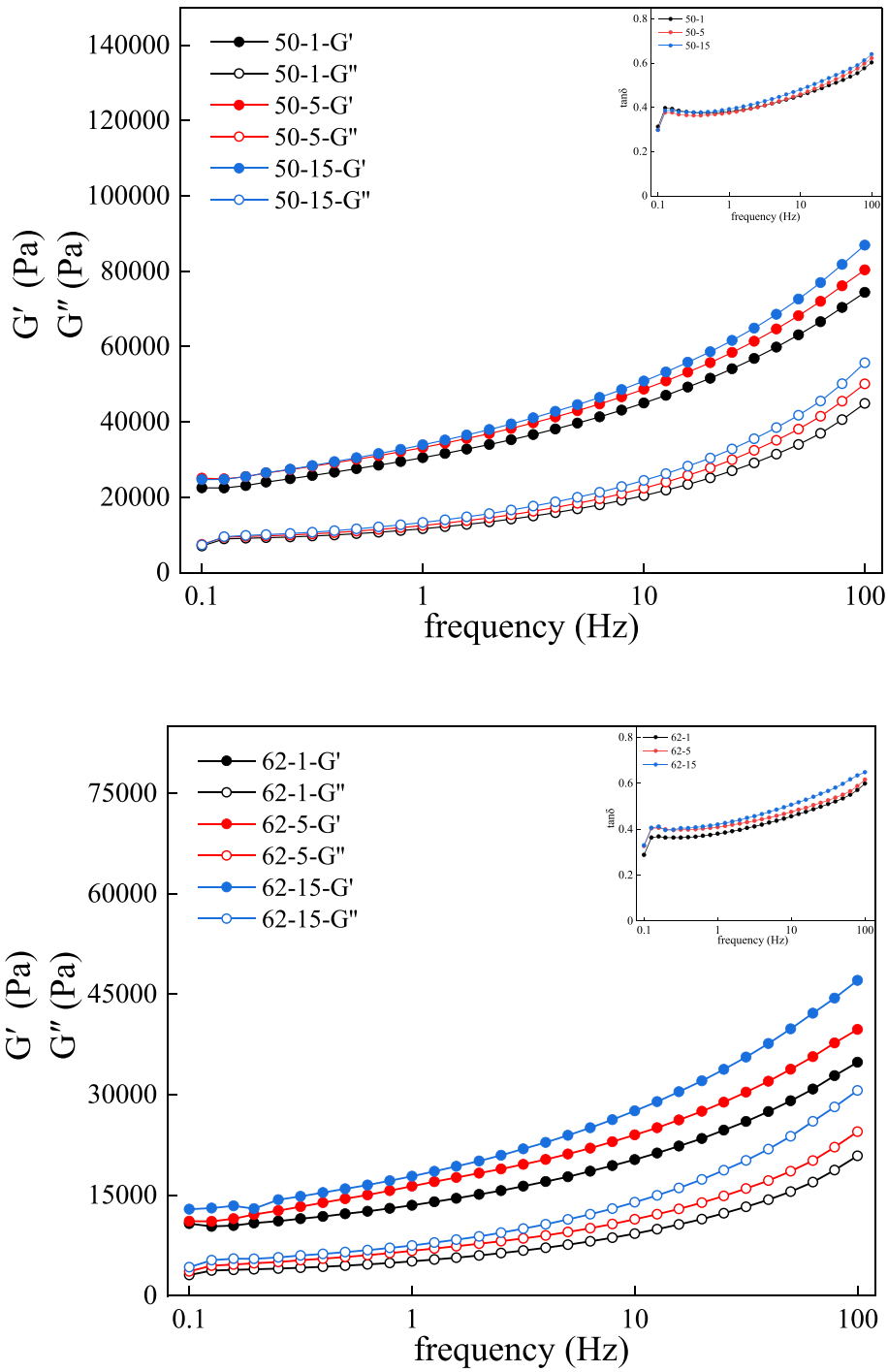


Fig. 5. Frequency scanning of dough with different hydration levels and mixing times. The illustration represents the variation of tanδ with frequency.

G′ and G″ of dough sample presented an increasing trend. This might be because the action of moderate mechanical force would cause a certain degree of aggregation of macromolecular polymers, and the increase in tanδ of the samples also indicated the decrease of the macromolecular polymer in the samples. It proved the previous result of SE-HPLC.

Similar to the effect of mixing, hydration level also caused a change in storage and loss modulus, and the difference between G′ and G″ caused by hydration was even more obvious. With the increase of hydration degree from 50 % to 62 %, both G′ and G″ of the dough showed a significant downward trend, the tanδ of 62 % dough sample was also lower than 50 %. For example, when the frequency reached 10 Hz, the G′ of the sample with 50 % hydration level in 5 min was 48689 Pa, while the sample with 62 % was only 24002 Pa. The potential reason could be that the higher hydration level has made the gluten network looser. These results show that reasonable mixing time and water addition were of great significance for the preparation of dough products.

3.10. Correlation analysis (CA) and principal component analysis (PCA)

The indicators of the changes in gluten network were characterized and quantified, and the correlation between rheological property changes and the morphological, structural, and molecular evolution of gluten protein in the dough with different hydration levels during mixing was analyzed (Fig. 6).

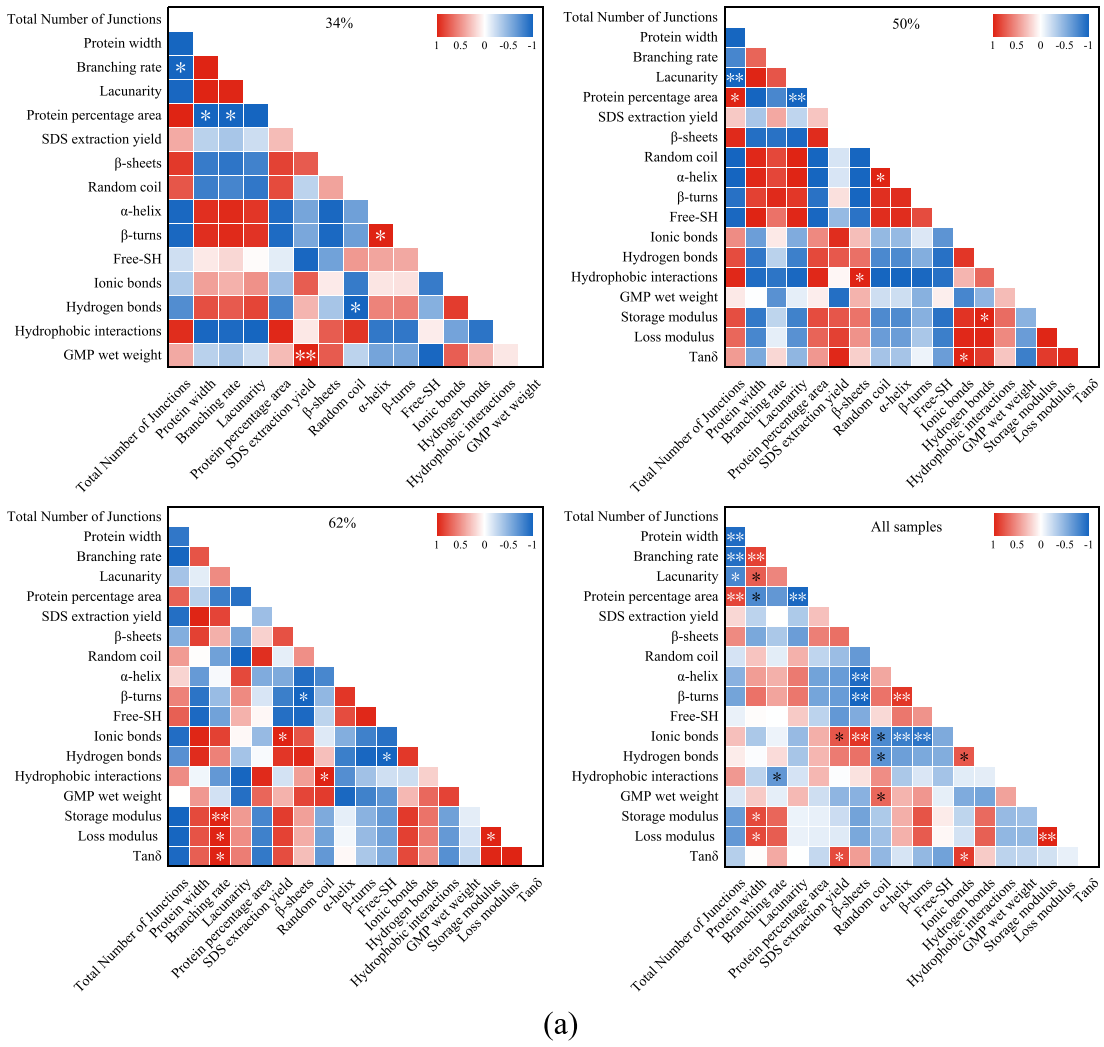
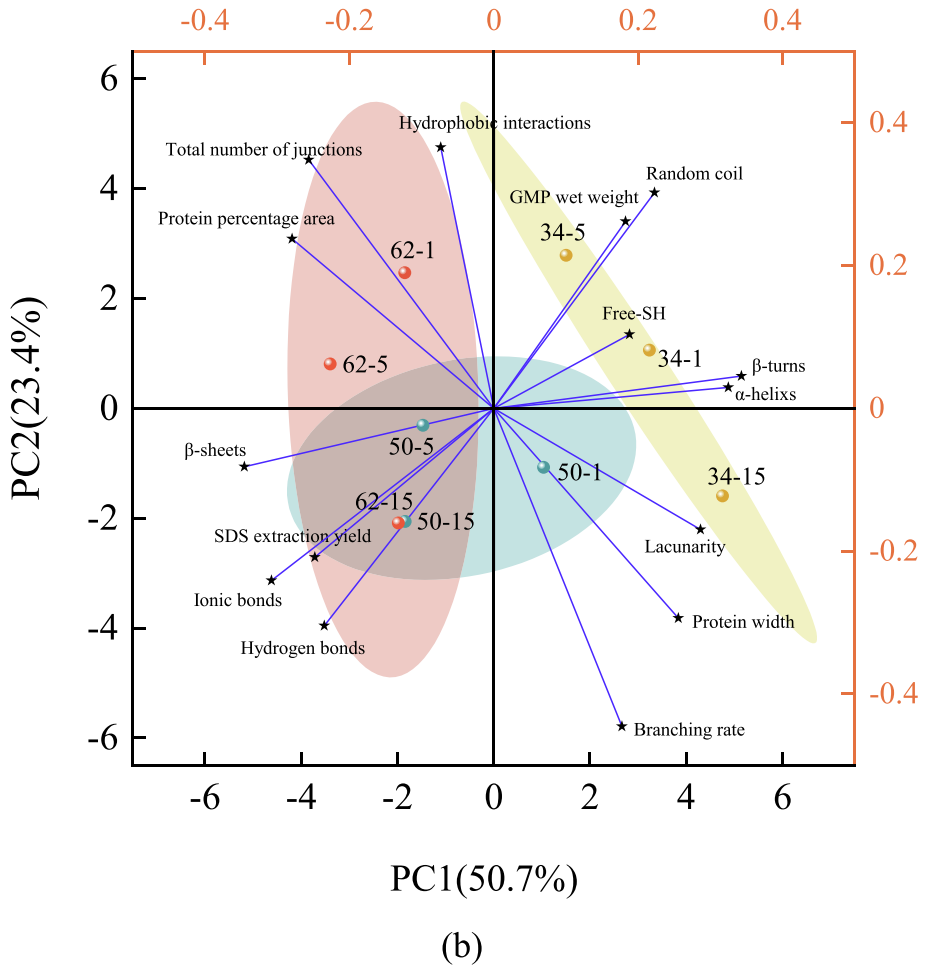


Fig. 6. Correlation analysis and Principal Component Analysis. a) Distribution of correlations between factors. * $P \leq 0.05$; ** $P \leq 0.01$. b) PCA score plot. PC1, principle component 1; PC2, principle component 2.

For limitedly hydrated dough samples, GMP wet weight showed a significant positive correlation with SDS extraction yield, there was a correlation between intermolecular interactions and conformation. For partially hydrated dough samples, lacunarity and total number of junctions, lacunarity, and protein percentage area showed a significant negative correlation, while intermolecular interactions were correlated with conformation and rheological properties. For fully hydrated dough samples, a significant correlation was detected between storage modulus and branching rate, rheological properties were more strongly correlated with protein morphological changes. Molecular transitions were correlated with conformational and protein morphological changes.

Generally, the storage and loss modulus of the dough showed a highly similar degree of correlation with the micro-indicators (Fig. 6a). The G′ and G″ were positively correlated with protein width, branching rate, and hydrogen bonds interaction, while showed a negative correlation with the total number of junctions. The tanδ value exhibited a significantly positive correlation with SDS extractable protein and ionic bonds interaction. Based on the correlation analysis (CA) of all samples, it could be observed that conformational changes were strongly correlated with molecular transformation. This suggested that changes in intermolecular interactions and chemical bonds might contribute to the conformation changes. Indicating the regulation of the microscopic properties of gluten proteins on the macroscopic dough rheological properties. The correlation of GMP and SDS extraction yields was more pronounced in limitedly hydrated dough samples. This indicated that the addition of water had an important effect on the depolymerization of GMP in the dough sample and led to a further decrease in the viscoelasticity of the dough. In terms of bond energy conversion, correlations of secondary structures and hydrogen bonds, free -SH and hydrophobic interactions are more pronounced at high hydration levels. It further shows that hydration will affect the rheological properties of the dough, and thus affects the macroscopic quality of the product.

Principal component analysis (PCA) is a commonly used method of linear dimensionality reduction, in which variables with strong correlations are grouped, and variables with opposite correlations are distributed at both ends of the line passing through the origin. The coordinates of each variable in Fig. 6b correspond to the correlation and directionality with PC1 and PC2, respectively. It could be observed that compared with low hydration samples (34 %), highly hydrated samples got a lower score in the direction of PC1, while the discrimination of samples with different mixing degrees was not obvious. This further suggested that dough with high hydration levels responded more quickly to mixing. With the increase of mixing time, 50 % and 62 % hydrated samples presented less difference in both PC1 and PC2 scores, and the 50–15 and 62–15 samples were almost in the same location. It indicated that with the increase of mixing degree, the discrepancy



(b)

Fig. 6. (*continued*).

between the characteristics of 50 % and 62 % hydrated dough samples gradually decreased. Additionally, classification analysis showed that for most indicators, the influence of the hydration level was more obvious than that of mixing degree. It could also be found that most variables of the 50 % hydrated sample were more highly correlated, which also showed that appropriate hydration degree was more conducive to the stability of the dough.

4. Conclusion

The dynamic evolution of gluten proteins the dough with different hydration levels upon mixing was systematically investigated and quantified, to reveal the formation process of gluten network structure in limitedly, partially, and fully hydrated dough and the respective response of dough rheology. Hydration levels had an effect on the morphology, structure, and molecular properties of the dough gluten network during mixing. The gluten network in highly hydrated samples was more susceptible to the formation and collapse induced by the mechanical force during mixing. More significant depolymerization of GMP in fully and partially hydrated samples was detected during mixing compared with limitedly hydrated dough. Sufficient mixing induced the increase in ionic bonds and hydrogen bonds, while excessive mixing caused more exposure of free -SH groups. The conversion of bond energy was more obvious with the addition of water. Higher hydration level induced more ordered secondary structure, while mixing resulted in the exchange of α-helix and β-sheet structure. Based on CA and PCA, compared with the mixing process, the degree of hydration had a more obvious influence on dough properties. Dough with higher hydration levels responded more quickly to mixing. Gluten evolution and dynamic rheology were significantly correlated with dough modulus. This study revealed the mechanisms underlying hydration induced discrepancy in the formation process of dough suitable for different wheat-based products.

CRediT authorship contribution statement

Ruobing Jia: Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Mengli Zhang:** Validation, Investigation, Methodology. **Tianbao Yang:** Writing – review & editing. **Meng Ma:** Validation, Investigation. **Qingjie Sun:** Validation, Supervision. **Man Li:** Software, Data curation, Conceptualization, Funding acquisition, 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.

Data availability

The authors do not have permission to share data.

Acknowledgments

This work was supported by the National Natural Science Foundation

of China (Grant No. 32072135), the Young Elite Scientists Sponsorship Program by CAST (Grant No. 2019QNRC001), and the Program for Youth Science Innovation in Colleges and Universities of Shandong Province (Grant No. 2020KJF005).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100448.

References

Auger, F., Morel, M. H., Lefebvre, J., Dewilde, M., & Redl, A. (2008). A parametric and microstructural study of the formation of gluten network in mixed flour-water batter. *Journal of Cereal Science, 48*(2), 349–358.

Bernklau, I., Lucas, L., Jekle, M., & Becker, T. (2016). Protein network analysis – A new approach for quantifying wheat dough microstructure. *Food Research International, 89*(Pt 1), 812–819.

Berton, B., Scher, J., Villieras, F., & Hardy, J. (2002). Measurement of hydration capacity of wheat flour: Influence of composition and physical characteristics. *Powder Technology., 128*(2), 326–331.

Carini, E., Vittadini, E., Curti, E., Antoniazzi, F., & Viazzani, P. (2010). Effect of different mixers on physicochemical properties and water status of extruded and laminated fresh pasta. *Food Chemistry, 122*(2), 462–469.

Don, C., Lichtendonk, W., Plijter, J. J., & Hamer, R. J. (2003). Glutenin macropolymer: A gel formed by glutenin particles. *Journal of Cereal Science, 37*(1), 1–7.

Feng, W., Ma, S., & Wang, X. (2020). Quality deterioration and improvement of wheat gluten protein in frozen dough. *Grain & Oil Science and Technology, 3*(1), 29–37.

Feng, Y., Zhang, H., Wang, J., & Chen, H. (2021). Dynamic changes in glutenin macropolymer during different dough mixing and resting processes. *Molecules, 26*(3), 541.

Gao, X., Tong, J., Guo, L., Yu, L., Li, S., Yang, B., & Cao, X. (2020). Influence of gluten and starch granules interactions on dough mixing properties in wheat (Triticum aestivum L.). *Food Hydrocolloids, 106*, Article 105885.

Gómez, A., Ferrero, C., Calvelo, A., Añón, M. C., & Puppo, M. C. (2011). Effect of mixing time on structural and rheological properties of wheat flour dough for breadmaking. *International Journal of Food Properties, 14*(3), 583–598.

Guibert-Martin, S., Jury, V., Bouchet, B., Roellens, G., Lioret, P., & Le-Bail, A. (2017). Impact of the baking protocol on the structure of French crêpes. *Journal of Food Engineering, 196*, 183–192.

Han, C. W., Ma, M., Yang, T. B., Li, M., & Sun, Q. J. (2021). Heat mediated physicochemical and structural changes of wheat gluten in the presence of salt and alkali. *Food Hydrocolloids, 120*, Article 106971.

Iuga, M., Boestean, O., Ghendov-Mosanu, A., & Mironeasa, S. (2020). Impact of dairy ingredients on wheat flour dough rheology and bread properties. *Foods, 9*(6), 828.

Li, M., Ma, M., Zhu, K. X., Guo, X. N., & Zhou, H. M. (2017). Delineating the physico-chemical, structural, and water characteristic changes during the deterioration of fresh noodles: Understanding the deterioration mechanisms of fresh noodles. *Food Chemistry, 216*, 374–381.

Li, M., Sun, Q. J., Han, C. W., Chen, H. H., & Tang, W. T. (2018). Comparative study of the quality characteristics of fresh noodles with regular salt and alkali and the underlying mechanisms. *Food Chemistry, 246*, 335–342.

Liu, R., Xing, Y., Zhang, Y., Zhang, B., Jiang, X., & Wei, Y. (2015). Effect of mixing time on the structural characteristics of noodle dough under vacuum. *Food Chemistry, 188*, 328–336.

Liu, S., Sun, Y., Obadi, M., Jiang, Y., Chen, Z., Jiang, S., & Xu, B. (2020). Effects of vacuum mixing and mixing time on the processing quality of noodle dough with high oat flour content. *Journal of Cereal Science, 91*.

Lopes-da-Silva, J. A., Santos, D. M. J., Freitas, A., Brites, C., & Gil, A. M. (2007). Rheological and nuclear magnetic resonance (NMR) study of the hydration and heating of undeveloped wheat doughs. *Journal of Agricultural and Food Chemistry, 55*(14), 5636–5644.

Ma, M., Han, C. W., Li, M., Song, X. Q., Sun, Q. J., & Zhu, K. X. (2019). Inhibiting effect of low-molecular weight polyols on the physico-chemical and structural deteriorations of gluten protein during storage of fresh noodles. *Food Chemistry, 287*, 11–19.

Ma, M., Sun, Q. J., Li, M., & Zhu, K. X. (2020). Deterioration mechanisms of high-moisture wheat-based food - A review from physicochemical, structural, and molecular perspectives. *Food Chemistry, 318*, Article 126495.

Marti, A., Bock, J. E., Pagani, M. A., Ismail, B., & Seetharaman, K. (2016). Structural characterization of proteins in wheat flour doughs enriched with intermediate wheatgrass (Thinopyrum intermedium) flour. *Food Chemistry, 194*, 994–1002.

Martínez, M. M., & Gómez, M. (2017). Rheological and microstructural evolution of the most common gluten-free flours and starches during bread fermentation and baking. *Journal of Food Engineering, 197*, 78–86.

Mastromatteo, M., Guida, M., Danza, A., Laverse, J., Frisullo, P., Lampignano, V., & Del Nobile, M. A. (2013). Rheological, microstructural and sensorial properties of durum wheat bread as affected by dough water content. *Food Research International, 51*(2), 458–466.

Morel, M. H., Redl, A., & Guilbert, S. (2002). Mechanism of heat and shear mediated aggregation of wheat gluten protein upon mixing. *Biomacromolecules, 3*(3), 488–497.

Rombouts, I., Jansens, K. J., Lagrain, B., Delcour, J. A., & Zhu, K. X. (2014). The impact of salt and alkali on gluten polymerization and quality of fresh wheat noodles. *Journal of Cereal Science, 60*(3), 507–513.

Obadi, M., Zhang, J., He, Z., Zhu, S., Wu, Q., Qi, Y., & Xu, B. (2022). A review of recent advances and techniques in the noodle mixing process. *Lwt, 154*.

Shewry, P. R., Popineau, Y., Lafiandrax, D., & Belton, P. (2000). Wheat glutenin subunits and dough elasticity: Findings of the EUROWHEAT project. *Trends in Food Science & Technology, 11*(12), 433–441.

Silva, E., Birkenhake, M., Scholten, E., Sagis, L. M. C., & van der Linden, E. (2013). Controlling rheology and structure of sweet potato starch noodles with high broccoli powder content by hydrocolloids. *Food Hydrocolloids, 30*(1), 42–52.

Wang, K. Q., Luo, S. Z., Zhong, X. Y., Cai, J., Jiang, S. T., & Zheng, Z. (2017). Changes in chemical interactions and protein conformation during heat-induced wheat gluten gel formation. *Food Chemistry, 214*, 393–399.

Wang, L., Zhang, Y., Xu, F., & Chen, J. (2022). Effects of ultrasound-assisted resting on the tensile properties and gluten network formation of wheat noodle dough. *Journal of Food Measurement and Characterization*.

Wang, P., Chen, H., Mohanad, B., Xu, L., Ning, Y., Xu, J., & Xu, X. (2014). Effect of frozen storage on physico-chemistry of wheat gluten proteins: Studies on gluten-, glutenin- and gliadin-rich fractions. *Food Hydrocolloids, 39*, 187–194.

Wang, P., Zou, M., Gu, Z., & Yang, R. (2018). Heat-induced polymerization behavior variation of frozen-stored gluten. *Food Chemistry, 255*, 242–251.

Wang, X. Y., Guo, X. N., & Zhu, K. X. (2016). Polymerization of wheat gluten and the changes of glutenin macropolymer (GMP) during the production of Chinese steamed bread. *Food Chemistry, 201*, 275–283.

Wang, W., Shen, M., Jiang, L., Song, Q., Liu, S., & Xie, J. (2020). Influence of Mesona blumes polysaccharide on the gel properties and microstructure of acid-induced soy protein isolate gels. *Food Chemistry, 313*, Article 126125.

Wellner, N., Mills, E. N. C., Brownsey, G., Wilson, R. H., Brown, N., Freeman, J., & Belton, P. S. (2005). Changes in protein secondary structure during gluten deformation studied by dynamic fourier transform infrared spectroscopy. *Biomacromolecules, 6*(1), 255–261.

Wieser, H. (2007). Chemistry of gluten proteins. *Food Microbiology, 24*(2), 115–119.

Yang, Y. L., Guan, E. Q., Zhang, L. L., Pang, J. Y., Li, M. M., & Bian, K. (2021). Effects of vacuum degree, mixing speed, and water amount on the moisture distribution and rheological properties of wheat flour dough. *Journal of Food Science, 86*(6), 2421–2433.

Yue, Q., Liu, C., Li, L., Zheng, X., & Bian, K. (2019). Effects of fermentation on the rheological characteristics of dough and the quality of steamed bread. *Journal of Food Processing and Preservation, 43*(10), 14115.

Zhang, D., Mu, T., & Sun, H. (2018). Effects of starch from five different botanical sources on the rheological and structural properties of starch-gluten model doughs. *Food Research International, 103*, 156–162.

Zhang, M. L., Ma, M., Jia, R. B., Yang, T. B., Sun, Q. J., & Li, M. (2022a). Delineating the dynamic transformation of gluten morphological distribution, structure, and aggregation behavior in noodle dough induced by mixing and resting. *Food Chemistry, 386*, Article 132853.

Zhang, M. L., Ma, M., Yang, T. B., Li, M., & Sun, Q. J. (2022b). Dynamic distribution and transition of gluten proteins during noodle processing. *Food Hydrocolloids, 123*, Article 107114.