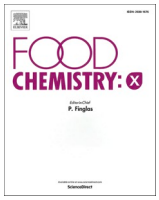
Food Chemistry: X 27 (2025) 102366

Contents lists available at ScienceDirect

Food Chemistry: X

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



# Investigation of the behavior of wheat flour dough under different sheeting-resting cycles and temperatures: Large deformation rheology and gluten molecular interactions

Yu-ling Yang [a,b,c,*], Long Yang [a,b,c], Er-qi Guan [d], Ke Bian [d,*]

[a] College of Food Science and Engineering, Anhui Science and Technology University, Chuzhou, Anhui 233100, PR China
[b] Anhui Provincial Key Laboratory of Functional Agriculture and Functional Food, Chuzhou, Anhui 233100, PR China
[c] Associated Discipline Key Laboratory of Whole Grain Nutrition and High-Value Utilization, Chuzhou, Anhui, 233100, PR China
[d] College of Food Science and Technology, Henan University of Technology, Zhengzhou, Henan, 450001, PR China

ARTICLE INFO

*Keywords:*
Wheat flour dough
Sheeting-resting cycles
Operating temperatures
Rheological behavior
Gluten protein interaction
SDS-PAGE

ABSTRACT

The molecular mechanisms underlying the structural changes in wheat flour dough subjected to repeated sheeting-resting treatments at two temperatures were investigated. The results revealed that alternating calendering and resting processes significantly contributed to the formation of a well-developed and organized gluten network, thereby enhancing the dough's resistance to deformation. Multiple complex treatments in a low-temperature environment promoted the formation of small aggregated proteins and monomeric proteins. In contrast, treatments at a high-temperature environment facilitated the formation of large aggregated proteins, reaching a maximum of 23.58 mg/g. The contribution of ionic and hydrogen bonding was maximum in the dough after two and three alternating treatments in both low- and high-temperature environments, while the strength of hydrophobic interactions remained weak. Furthermore, a combination of noncovalent interactions and sulfhydryl disulfide bond transitions induced notable changes in the structure of the glutenin molecular chain.

## 1. Introduction

Noodles have a broad consumer base, and the market for raw noodles as a traditional food product, shows significant growth potential (Du et al., 2023; Zhang et al., 2022). Sheeting is a critical step in the processing of wheat flour products, particularly in noodle production, where most of the gluten network is formed through sheeting forces. Previous studies have demonstrated that appropriate continuous sheeting of dough results in accompanied by the establishment and destruction of the gluten network, with optimal sheeting passes leading to the full development of the network (Liu et al., 2021; Liu, Jiang, Xu, & Jiang, 2023). Meanwhile, dough that has been subjected to mechanical forces requires resting periods to relieve the strained gluten matrix and improve the dough's overall condition (Feng, Zhang, Wang, & Chen, 2021; Zhang, Ma, Yang, Li, & Sun, 2022). The effectiveness of the dough resting process facilitated a more uniform and compact gluten network distribution, enhancing the texture and cooking properties of the noodles (Obadi, Li, Qi, & Xu, 2023). In contrast to industrial noodle production, which typically involves a single cycle of sheeting or resting, traditional noodle production methods—such as those for Stewed noodles, Lamian noodles, Hand-stretched dried noodles, and Biangbiang noodles—often combine multiple cycles of mechanical forces and resting. The traditional approach is crucial for developing the dough's tensile strength, an essential characteristic for most handmade noodles. For instance, Stewed noodles and Lamian noodles are traditionally produced with a "three times kneading and three times resting" method, promoting better water absorption and yielding dough with enhanced extensibility. However, the process may involve more than three cycles; a five-cycle sheeting-resting operation has also been successfully employed in Stewed noodle preparation (Obadi et al., 2023). A similar approach is used in the production of Hand-stretched dried noodles. In the case of Biangbiang noodles, two resting periods during production ensure that proteins, which have not fully absorbed water, continue to improve the gluten structure, contributing to both production efficiency and product quality. This method also explains the hollow structure of handmade dried noodles, which results from microbial activity

* Corresponding author at: No. 9, Donghua Road, Fengyang County, Chuzhou City, Anhui Province, PR China.
*E-mail address:* 15238635973@163.com (Y.-l. Yang).

https://doi.org/10.1016/j.fochx.2025.102366
Received 24 October 2024; Received in revised form 17 February 2025; Accepted 9 March 2025
Available online 14 March 2025

producing gas during prolonged resting (Lyu, Guo, & Zhu, 2023). Empirically, repeated kneading and resting cycles may even compensate for inherent deficiencies in wheat flour quality, improving the final noodle product.

Numerous studies have investigated the resting conditions of noodle doughs, with most focusing on either a single resting time (typically 0–180 min) or resting temperature (commonly 20–50 °C) (Lu, Guo, & Zhu, 2022; Zhang et al., 2022; Zhang, Ma, Yang, et al., 2022). However, the actual processing of traditional handmade noodles often involves exposure to either hot or cold environments. For example, traditional hollow noodles are typically processed in winter, as the low-temperature environment conditions help prevent the rapid growth of microorganisms that could otherwise occur during extended resting periods (Turksoy, Erturk, & Kokini, 2021). Turksoy et al. examined the patterns of changes in the nonlinear rheological properties of fresh dough for up to five days during aging at 4 °C or 25 °C (Turksoy, Erturk, Bonilla, Turasan, & Kokini, 2020). However, their study focused on Farinograph-produced doughs with much higher moisture content, which differs significantly from low-moisture noodle doughs, as their findings were intended to guide the processing and production of naturally fermented high-moisture bread doughs. There is a notable gap in comparative studies on the rheological properties of noodle doughs processed under low-temperature and high-temperature conditions and their effects on gluten protein structure, particularly in relation to the mechanisms by which calendering-resting cycles influence dough and noodle quality in traditional noodle processing is not clear. Therefore, this study investigates the effects of the combined of sheeting and resting, as essential steps in traditional noodle processing on the rheological properties of low-moisture noodle doughs at different temperatures. The molecular mechanisms of gluten protein aggregation are analyzed to provide insights into dough behavior during processing. The findings aim to contribute to the improvement of noodle quality, offering valuable guidance for the flour-based noodle processing industry.

## 2. Materials and methods

### 2.1. Preparation of dough samples

Dough samples were prepared using a pin mixer (ZKHM-40, Dongfujiuheng Instrument Technology Co., Ltd., Beijing, China) under controlled conditions to ensure consistency. The water content was set to 60n% of the water absorption measured by the Farinograph (Frinograph-E, Brabender, Inc., Duisburg, Germany) and the mixing time was standardized at 7 min. Water was added in three portions at mass ratios of 4:4:2, introduced at 0, 1, and 2 min during the mixing process, respectively (Yang, Guan, Zhang, Li, & Bian, 2019). The resulting loose dough was pressed to produce a sheeted sample (Control) with a thickness of approximately 4 mm. These samples were rested under two specific conditions: at 4 °C with 31 % humidity (referred to as 4SR-1) or at 35 °C with 85 % humidity (referred to as 35SR-1) for 30 min. Following this initial resting, the two samples (4SR-1 and 35SR-1) underwent identical sheeting and resting procedures to create dough samples labeled 4SR-2 and 35SR-2, respectively. This process was repeated to obtain subsequent samples (4SR-3 and 35SR-3), ultimately producing the final samples, 4SR-4 and 35SR-4. All dough samples were subjected to rheological characterization immediately after preparation to prevent structural changes due to time delays. Additionally, the dough samples were freeze-dried, finely ground, and sieved to prepare them for subsequent analyses.

### 2.2. Rheological properties of doughs with large deformation

The large deformation rheological properties of the dough samples were evaluated following the method described by Yang et al. (Yang et al., 2023). A Total Texture Analyzer (TA-XT Plus Instrument, SMS, UK) equipped with an A/KIE test system was employed to assess the uniaxial tensile properties. The testing parameters included a pre-test speed of 2.0 mm/s, a test speed of 0.08 mm/s, a post-test speed of 10.0 mm/s, a stretch distance of 50.0 mm, and a trigger force of 5 g. Each dough sample was measured at least seven times. Key tensile properties, including maximum tensile resistance ($R_{max}$), tensile distance ($E$) and tensile area ($A$), were recorded to evaluate the samples' tensile performance.

The stress relaxation properties of the doughs were assessed using a P50 probe with a Total Texture Analyzer. Fresh dough sheets were prepared using a 3 cm diameter ring mold, and the surface of each sample was coated with vegetable oil to prevent sticking. Measurements were conducted in single compression mode, with the degree of deformation set to 30 %. The pre-test, test and post-test speeds were set at 1.0 mm/s, 1.0 mm/s and 10.0 mm/s, respectively. Once the dough deformation reached 30 %, the strain was held constant, and measurements were recorded over a total duration of 60 s. The data acquisition rate was set at 200 Hz, and the variation of force ($F$, N) with time ($t$, s) was recorded. Each sample was subjected to five replicate measurements. The measured stress relaxation curves were fitted using the Maxwell-Kelvin three-element model, following the equation proposed by $\sigma(t) = \varepsilon_0 E_1 exp(-t/\tau) + \varepsilon_0 E_2$ (Yang, Guan, Zhang, Li, & Bian, 2022). Key parameters, including maximum stress ($\sigma_{max}$, Pa), equilibrium or residual stress ($\sigma_e$, Pa), universal elastic modulus ($E_1$, Pa); high elastic modulus ($E_2$, Pa), viscosity coefficient ($\eta$, Pa·s), relaxation time ($\tau$, s), and degree of deformation ($R$, %) were derived to evaluate the compressive properties of the samples, with the degree of deformation expressed as $R = (\sigma_{max} - \sigma_e) \times 100\%/\sigma_{max}$.

### 2.3. Evaluation of dough and gluten protein structure

The freeze-dried dough samples were polished and fixed onto a sample platform, then coated with a thin layer of gold. The morphological structure of the cross-section was observed using scanning electron microscopy (SEM). Additionally, laser confocal microscopy (CLSM) was employed to analyze the microstructural changes in the dough (Jia, Ma, & Hu, 2020). To evaluate gluten network aggregation, the obtained CLSM images were converted into data using a vascular analysis tool (Angio Tool 6.4 version 0.6 A) to evaluate the aggregation of the gluten network (Zhang, Ma, Yang, et al., 2022).

### 2.4. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was conducted following the method of Liu et al. (Liu et al., 2023) using a 12 % separation gel (pH 8.8) and a 5 % stacking gel (pH 6.8). Briefly, protein sample (containing 8.0 mg of protein) were stirred in 1 mL loading buffer (pH 6.8, 0.05 M Tris-HCl, containing 10 % (*w/v*) SDS, 10 % (*v/v*) glycerol, and 0.1 % (w/v) bromophenol blue). The samples were incubated at room temperature for 2 h, heated in boiling water for 5 min, and then centrifuged at 12,000 rpm for 10 min. Supernatants (18 μL) and protein markers (6 μL, 26616, Thermo Fisher Technology Co., Ltd., Shanghai, China) were loaded into each lane, and electrophoresis was performed at a voltage of 100 V. Additionally, the same samples were extracted with 2 mL of 1.5 % SDS solution (*w/v*). After shaking at room temperature for 2 h and centrifugation at 12,000 rpm for 10 min, the precipitates, containing a mixture of unextractable polymeric protein (UPP) and starch, were retained. The UPP was further reduced with 1.0 mL of buffer containing 1.0 % (w/v) DTT, and changes in the UPP subunit composition were analyzed. The gels were stained with Coomassie Brilliant Blue (R-250) and imaged using a gel image analyzer (WD-9431C, Liuyi Instrument Co., China).

### 2.5. Glutenin macropolymers (GMP) content

To determine the GMP content, 1.0 g of lyophilized dough sample was collected and shaken in 30 mL of 1.5 % (*w/v*) SDS solution for 10

min at room temperature. The mixture was then centrifuged at 12,000 rpm for 30 min, and the supernatant was discarded. The protein content in the residue, representing the GMP content, was quantified using the Kjeltec method (Kjeltec 8400, FOSS, USA). At least three replicates were performed for each dough sample to ensure accuracy.

### 2.6. Solubility determination of gluten proteins in different solution systems

The solubility of gluten proteins was analyzed following the methods Liu and Li et al. (Li et al., 2021; Liu et al., 2021). Samples were dissolved in 1.5 mL of sodium phosphate buffer (0.05 M, pH 6.8) containing 1.5 % (*w/v*) SDS (hereinafter referred to as SDS buffer). After shaking for 2 h at room temperature and centrifugation at 12,000 rpm for 10 min, the supernatant was filtered through a 0.22 μm membrane. The filtered solution was analyzed using the Waters e2695 system equipped with a BioSep-SEC-S 4000 column. The analysis conditions included an injection volume of 20 μL, an elution solvent composed of acetonitrile/water (1:1, *v*/v) containing 0.1 % (*v*/v) trifluoroacetic acid, a flow rate of 1.0 mL/min, a column temperature set at 30 °C, and detection at a wavelength of 214 nm. These parameters were used to determine the composition and proportion of SDS-soluble proteins. Meanwhile, the samples were treated with SDS buffer containing 1.0 % (w/v) DTT to assess the extraction rate of wheat glutenin and gliadin.

### 2.7. Covalent and non-covalent interactions of doughs

To evaluate the covalent interactions in the dough samples, 75 mg of the sample was accurately weighed and dissolved in 10 mL of glycine buffer (pH 8.0) containing 5.0 M guanidine hydrochloride, 8.0 M urea, and 1.5 % EDTA. The free sulfhydryl content was determined by measuring the absorbance at 595 nm using a full-wavelength microplate reader (SpectraMax Plus384, Molecular Devices, Californian, USA). This was achieved by centrifuging the mixture of 5 mL of sample solution and 200 μL of Ellman's reagent and analyzing the supernatant. To determine the total free sulfhydryl content, 2 mL of the sample mixture was reacted with 2 mL of dithiothreitol solution (DTT, 0.5 %, *w/v*) and 10 mL of trichloroacetic acid solution (TCA, 12 %, w/v) for 1 h, followed by centrifugated at 4000 rpm for 10 min. The resulting precipitate was treated with 5 mL of 8.0 M urea and 400 μL of Ellman's reagent for 1 h, then centrifuged at 4000 rpm for 10 min. The absorbance of the supernatant ($A_{412}$) was measured to determine the free sulfhydryl ($N_1$) and total free sulfhydryl level ($N_2$), calculated using the eq. $SH(\mu mol/g) = (A_{412} \times D)/(\varepsilon \times b \times C)$, and the disulfide bond content was subsequently calculated using eq. $SS(\mu mol/g) = (N_2 - N_1)/2$. In these calculations, $\varepsilon$ represents the molar absorption coefficient of DTNB, $b$ is the cell path length, $D$ is the extinction coefficient, and $C$ is the concentration of samples (mg/mL).

For non-covalent interaction analysis, 10 mg of sample was accurately weighed and dissolved in 1.0 mL of phosphate buffer (0.05 M, pH 7.5) with the following conditions: 0.05 M NaCl (PA), 0.6 M NaCl (PB), 0.6 M NaCl +8.0 M urea (PC), and 0.6 M NaCl +8 M urea +2 % SDS (PD). The solutions were allowed to react for 2 h and then centrifuged at 12,000 rpm for 10 min. The supernatant (20 μL) was mixed with 1.0 mL of Coomassie Brilliant Blue G-250 solution (filtered through filter paper), and the absorbance of 200 μL of the mixture was measured at 595 nm using a full-wavelength microplate reader (SpectraMax Plus384, Molecular Devices, Californian, USA). The soluble gluten content was calculated using a standard curve of bovine serum protein and expressed as mg/g of soluble protein per sample (Khoder et al., 2020). Each dough sample was measured in triplicate. The levels of non-covalent interactions among gluten proteins were evaluated based on the differences in solubility across the various solution systems. The contribution of ionic bonds was determined as the difference in soluble gluten protein content between PB and PA. The contribution of hydrogen bonds was calculated as the difference in solubility between PC and PB. Finally, hydrophobic interactions were identified as the difference in solubility between PD and PC (Liu et al., 2021).

### 2.8. Atomic force microscopy (AFM) analysis

The apparent morphology of the dough samples was evaluated using an atomic force microscope (SPM-9700, Shimadzu Corporation, Japan). For sample preparation, 1.0 mg of the samples was dissolved in 0.5 M acetic acid solution, vortexed for 10 min, and centrifuged at 10,000 rpm for 10 min. The resulting supernatant was diluted with the same acetic acid solution to achieve a protein concentration of approximately 0.01 μg/mL. Subsequently, 10 μL of the protein solution was deposited onto a silicon wafer and placed in a constant, wind-free environment to allow the solvent to evaporate completely (Zhang, Ma, Jia, et al., 2022). AFM images were captured in the standard phase mode with a sample image size of 125 μm.

### 2.9. Statistical analysis

All data were expressed as the mean ± standard deviation from a minimum of three independent experiments. Statistical analyses were performed using the SPSS software package (version 13.0 for Windows, SPSS Inc., Chicago, IL). Analysis of variance (ANOVA) was used to assess statistical differences, and Duncan's multiple range test was applied to compare means. A probability value of $P < 0.05$ was considered statistically significant.

## 3. Results and discussion

### 3.1. Rheological properties of dough with large deformation

The uniaxial tensile properties of dough samples subjected to sheeting-resting cycles at different temperatures were analyzed (Fig. 1A, B and C). The results revealed that the tensile strength of the dough decreased sharply, while elongation increased significantly after resting (Fig. 1B, $P < 0.05$). Furthermore, the tensile area decreased significantly at high temperatures (Fig. 1C, $P < 0.05$), whereas at low temperatures, no significant change in the tensile area was observed ($P > 0.05$). During the mixing process, mechanical forces increase the energy stored within the dough. The subsequent resting phase allowed internal stress to dissipate gradually, reducing tensile resistance and enhancing extensibility (Obadi et al., 2023). At higher temperatures, repeated cycles of sheeting and resting resulted in a significant reduction in tensile resistance ($P < 0.05$) with minimal changes in the tensile area. Notably, after three cycles of treatment, the dough sample (35SR-3) exhibited the lowest elongation (36.79 mm). However, elongation increased significantly after the fourth cycle (35SR-4), reaching its maximum value (39.51 mm) (Fig. 1A). This phenomenon can be attributed to the further polymerization and cross-linking of the gluten network during sheeting and relaxation, which enhanced its structural stability and mechanical strength (Obadi et al., 2023; Song et al., 2019). These processes improved the ductility and tensile properties of the gluten network, promoting the continuity and integrity essential for a robust gluten structure. Conversely, at low temperatures, repeated cycles of pressing and resting did not induce significant fluctuations in the maximum tensile resistance of the dough. However, there was a marked reduction in both elongation and tensile area ($P < 0.05$) (Fig. 1B and C). Subsequent sheeting processes at low temperatures facilitated further gluten network formation and allowed the gradual release of internal stresses within the dough. These changes in tensile properties were evidently influenced by the ambient temperature. It was evident that the tensile resistance of the dough decreased significantly after several cycles of sheeting and resting at higher temperatures ($P < 0.05$) (Fig. 1A), while the tensile area remained largely unchanged. Subsequent processing facilitated the further development of the gluten network and the timely release of internal stresses within the dough, resulting in changes in

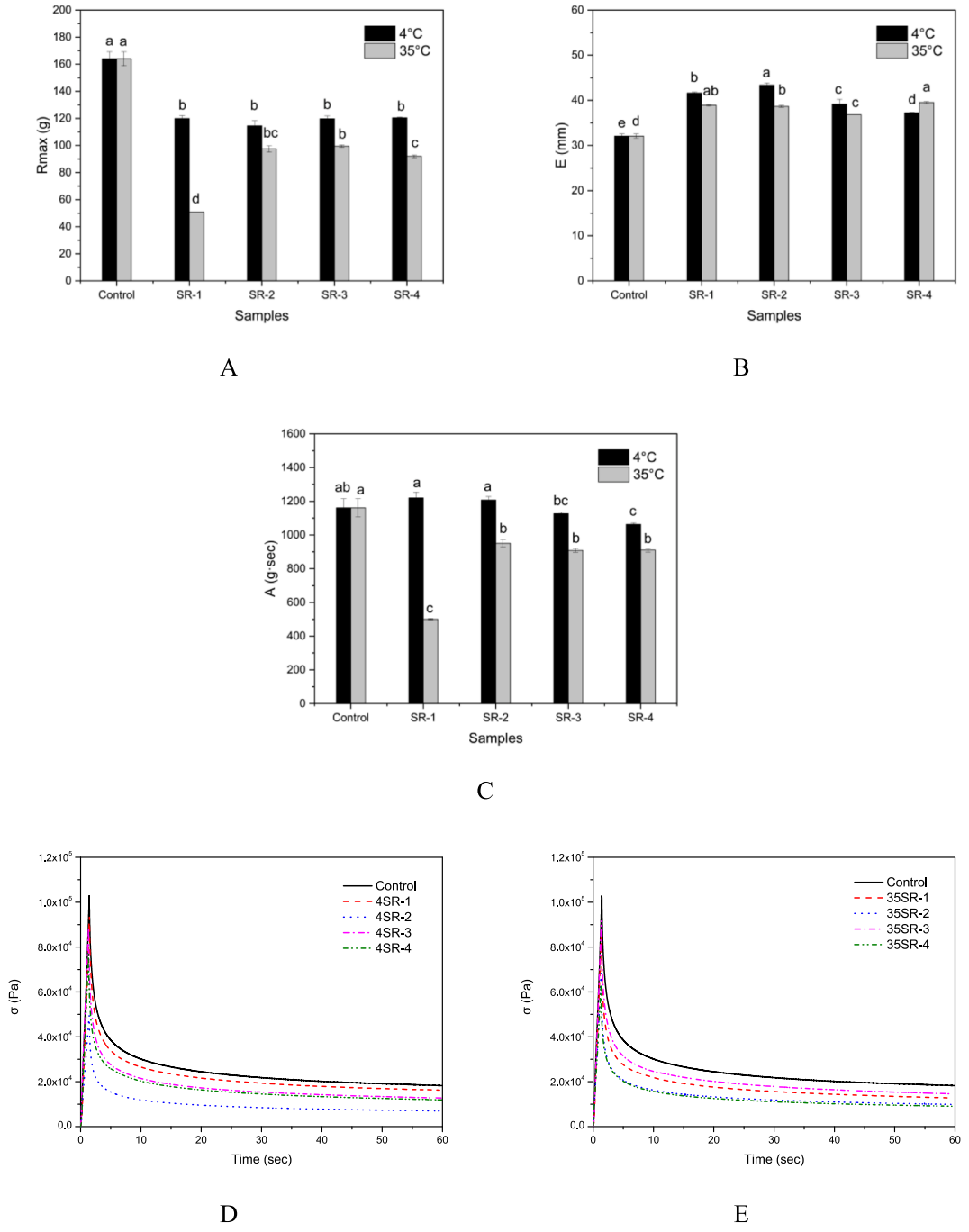



**Fig. 1.** A, The maximum resistance ($R_{max}$) of doughs made in different conditions. B, The extensibility ($E$) of doughs prepared in different ways. C, The area under the curve ($A$) of doughs made in different conditions. D, Stress relaxation curve of doughs sheeted in different conditions in a low-temperature environment (4 °C). E, Stress relaxation curve of doughs prepared in a high-temperature environment (35 °C). Control represents the dough pretreated a simple treatment of the sheeting, the sample of 4SR-1 refers to the dough rested in a low-temperature environment of 4 °C, indicating that the dough has experienced a complete "sheeting-resting" cycle. Samples of 4SR-2, 4SR-3, and 4SR-4 went through 2, 3 and 4 times of "sheeting-resting" cycles in the same temperature environment (4 °C). Similarly, 35SR-1, 35SR-2, 35SR-3, and 35SR-4 were subjected to once, twice, three, four times of "sheeting-resting" cycles in a high-temperature environment (35 °C), respectively. The sample information in the following chart is consistent with the above instructions. Values are expressed as mean ± standard deviation. Different letters on top of the bars indicate significant differences ($P < 0.05$).

tensile properties that were clearly influenced by the ambient temperature. High-temperature pretreatment was observed to cause moisture loss in the dough and disruption of hydrogen bonds within protein molecules. This resulted in dough with stronger tensile resistance but reduced elongation (Litvinov, Faizullin, Zuev, & Weisel, 2012; Yang et al., 2023). These effects explain the changes in tensile behavior observed in dough samples after repeated sheeting-resting treatments at elevated temperatures.

The stress-strain behavior of dough samples subjected to compression testing after undergoing sheeting-resting cycles at different temperatures displayed typical characteristics of stress relaxation testing. As illustrated in Fig. 1D and E, the stress in all dough samples initially increased abruptly to a maximum value as deformation progressed, followed by a rapid decrease after reaching the set strain (80 %). Subsequently, the stress gradually converged to a non-zero steady state as the strain retention time increased. This characteristic stress relaxation

behavior was observed consistently across all samples tested at varying temperatures (Fig. 1D and E). These results suggest that compression-resting cycles significantly influence the mechanical properties of the dough, and the behavior of the samples can be effectively characterized through stress relaxation testing. Relevant parameters derived from these tests are summarized in Table S1. Following a brief resting period, the maximum stress ($\sigma_{max}$), equilibrium or residual stress ($\sigma_e$), viscosity coefficient ($\eta$), universal elastic modulus ($E_1$), and degree of deformation ($R$) increased. Conversely, the relaxation time ($\tau$) and high elastic modulus ($E_2$) decreased. These changes indicate enhanced strength and friction of the polymer chains in the dough, reaching equilibrium within a short period of time. This behavior aligns with previously observed increases in dough strength and the enhancement of covalent interactions after resting (Yang et al., 2022). Resting at low temperatures resulted in an increase in $\sigma_{max}$, indicating that greater force was required to achieve constant deformation. Meanwhile, increased internal viscosity and reduced mobility of polymer chain segments led to lower deformation recovery, with a recovery rate of 81.89 % (Table S1). This contributed to an overall increase in dough stiffness. Doughs rested at low temperatures for 30 min exhibited greater hardness, elasticity, and recovery capacity, as evidenced by higher $\sigma_e$ ($1.59 \times 10^4$ Pa) and $E_2$ ($0.53 \times 10^5$ Pa) values, alongside lower $R$ values (82.98 %) (Table S1). These findings are consistent with prior research on the effects of temperature on dough properties (Yang et al., 2023).

An increase in the number of sheeting-resting cycles resulted in a decrease in $\sigma_{max}$, $\sigma_e$, $E_1$, $E_2$, and $\eta$ of the doughs, indicating that the compounding process reduced both the elasticity and viscosity of the samples. However, compared to the control (81.89 %), the $R$ value of the doughs increased, indicating an enhanced ability to resist external forces (Table S1). The $\tau$ of the dough decreased with multiple resting cycles in a low-temperature environment, while no significant change in $\tau$ was observed under high-temperature conditions ($P > 0.05$). This indicates that low-temperature processing allows the dough to reach a stable state ($\tau$) more quickly. Dough pretreated with two cycles of low-temperature processing (4SR-2) exhibited higher elasticity ($E_1 = 0.82 \times 10^5$ Pa; $E_2 = 0.42 \times 10^5$ Pa), lower viscosity ($\eta = 1.05 \times 10^6$ Pa·s), and a greater degree of deformation ($R = 85.75$ %). Similarly, dough processed at high temperatures after three cycles (35SR-3) demonstrated comparable trends, with the highest $R$ value (85.61 %, $P > 0.05$) (Table S1). At higher numbers of sheeting-resting cycles, doughs subjected to both temperature conditions displayed varying levels of elasticity and viscosity. However, the overall viscoelasticity, as indicated by $R$ value, did not show significant differences ($P > 0.05$). This behavior suggests that the gluten structure was progressively strengthened during the pressing process (Liu et al., 2023). Additionally, changes in the form and content of moisture during processing significantly contributed to the increased strength of the dough structure, likely due to modifications in non-covalent interactions, such as hydrogen bonding (Laurent et al., 2023; Yang et al., 2023). While elasticity and viscosity were influenced by temperature and compounding treatments, the $R$-value remained consistent, reflecting stable viscoelastic properties. These findings highlight the complex interplay of gluten structure, moisture content, and processing conditions in determining dough behavior. Further research is warranted to elucidate the underlying mechanisms and explore practical potential applications in the food industry.

### 3.2. Structural analysis of dough and gluten proteins by SEM and CLSM

The surface morphology of dough samples subjected to the composite calendering-resting process at different temperatures is presented in Fig. 2A. For the fresh dough (Control), starch granules were encapsulated within the gluten protein network, with visible pores in the gluten matrix. The starch granules appeared loose and clearly discernible, consistent with previous findings (Liu, Song, et al., 2021; Song et al., 2019). After a short resting period, the gluten network became slightly loosened but was evenly distributed across the viewing field. As shown in Fig. 2A, an increase in the number of calendering-resting composites resulted in a more continuous, well-defined bundled gluten network, which progressively enshrouded the starch granules more densely. Additionally, the protein matrix adhered more firmly to the surface of the starch granules, making it increasingly difficult to observe the granules in their entirety. This was accompanied by a flattening of the fracture surface of the samples. The observed microstructural changes confirm that the sheeting-resting composite process promotes the cross-linking of gluten fibers, contributing to the formation of tougher and tighter protein networks, as suggested in previous studies (Belton, 1999). It was evident that at higher temperatures, substantial water loss was inevitable, weakening the mechanical resistance of the dough (Fig. 1) (Yang et al., 2023). After freeze-drying, significant fractures were observed in the cross-section of the dough sample (35SR-4), which displayed more pores and a loose structure.

Fresh dough samples were stained to produce CLSM images of gluten protein (Fig. 2B). These images were analyzed to extract quantitative information on the gluten network, ensuring a consistent field of view ($1.05 \times 10^4$ μm$^2$) (Table S2) (Pipintakos, Hasheminejad, Lommaert, Bocharova, & Blom, 2021; Zhang, Ma, Jia, et al., 2022). The data revealed that the resting process promoted the formation and strengthening of the gluten network. This was evidenced by significant increases in gluten area, gluten percentage area, gluten junctions, junction density, total gluten length, and average gluten length ($P < 0.05$), accompanied by decreases in lacunarity, gluten end-points, and end-point rate ($P < 0.05$). These changes were reflected in the gluten network fibers, which appeared thicker and longer (Yang et al., 2023). Repeated sheeting and resting further decreased gluten network lacunarity and significantly increased gluten end-points ($P < 0.05$). These changes contributed to the development of a denser fibrous structure, with starch granules filling the intervals in a more regular and oriented morphology trend. Consequently, repeated sheeting-resting cycles facilitated the orderly arrangement of starch granules and gluten protofibrils, thereby enhancing the structural integrity and strength of dough sheets (Liu et al., 2021; Song et al., 2019).

Meanwhile, dough pretreated at low temperatures did not exhibit significant changes in the gluten area or the number of endpoints ($P > 0.05$). The primary difference was observed in the decreased gluten width (9.19 μm) in the dough subjected to four processing cycles (4SR-4). In contrast, dough treated at higher temperatures (35SR-4) displayed a loose and poorly connected network structure. This was characterized by reductions in gluten area ($43.51 \times 10^5$ μm$^2$) and percentage (41.58 %), gluten junctions (1011) and junction density ($0.97 \times 10^{-3}$), total gluten length ($47.26 \times 10^3$ μm), average gluten length (1.72 μm), and branching rate (0.23). At the same time, the number of endpoints ($2.96 \times 10^{-3}$), endpoint rate ($0.23 \times 10^{-2}$), and lacunarity ($5.95 \times 10^{-2}$) increased (Table S2). These results indicate that repeated calendering-resting at higher temperatures adversely affected the dough structure, leading to a poorly connected gluten network. Nonetheless, higher temperature conditions consistently facilitated gluten network formation, resulting in overall superior gluten strength, as previously reported (Yang et al., 2023). This highlights the complex interplay between temperature, processing cycles, and gluten network development.

### 3.3. Subunit distribution of gluten during sheeting and resting

The molecular weight distribution of proteins and their components after different sheeting-resting cycles and temperatures were analyzed using SDS-PAGE (Fig. 3). The results showed no significant changes in the molecular weight composition of gluten with increasing treatment time (Fig. 3 A and B). However, as the calendering and resting treatments were applied under low- and high-temperature conditions, the intensity of protein bands gradually decreased. This reduction in band intensity was attributed to a decreased extraction rate of protein in 1.5 % SDS (Zhao et al., 2020). The formation of more stable hydrogen bonds between molecules and polymerization induced by SH/SS exchange or

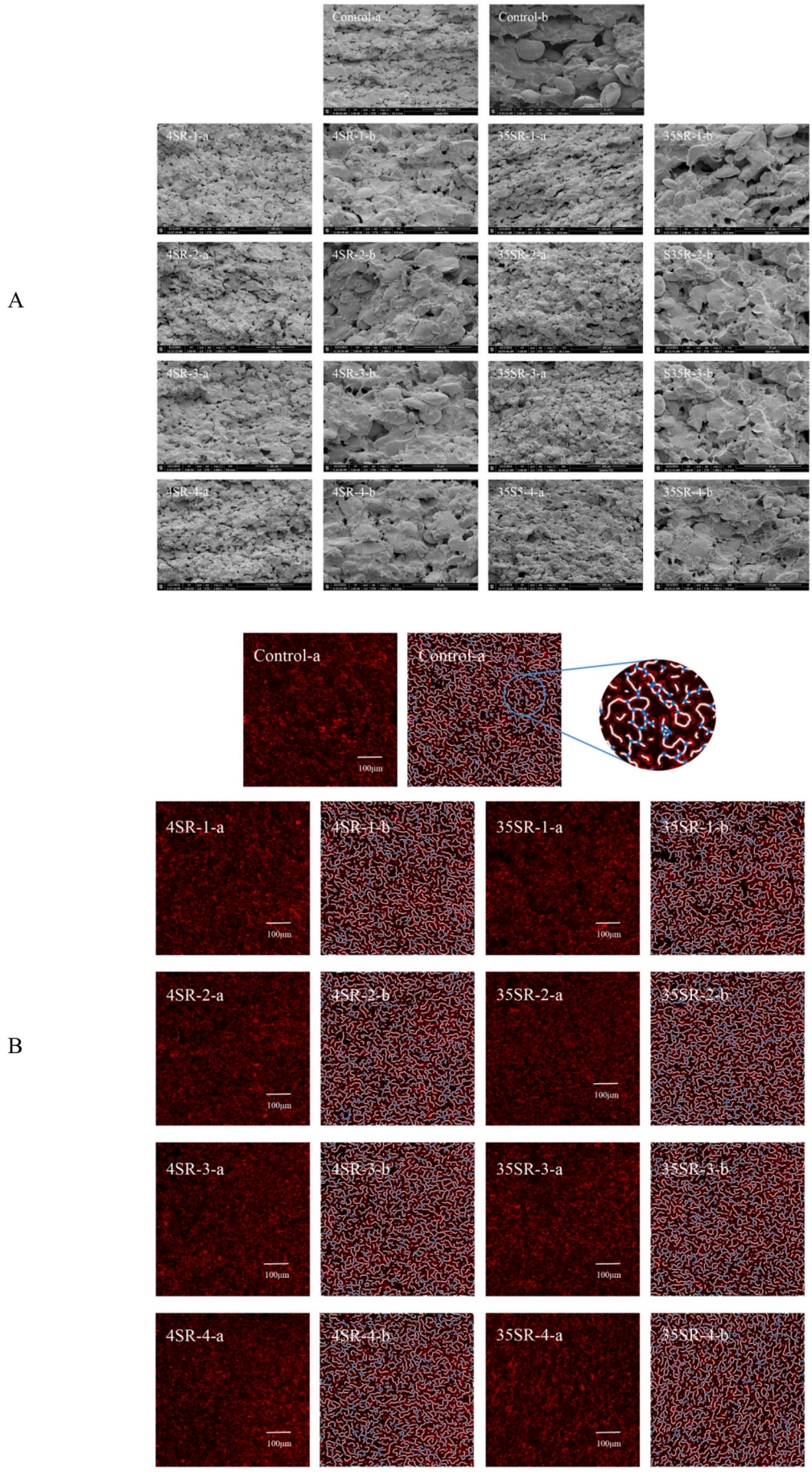



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**Fig. 2.** A, The SEM images of doughs rested in different conditions with the magnification at 150× (a) and 1500× (b), respectively. B, The CLSM images and gluten network analysis of doughs rested in different conditions. a, images of the original CLSM pictures. b, pictures processed with AngioTool with junctions shown in blue, protein skeleton shown in white and protein outline/area shown in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

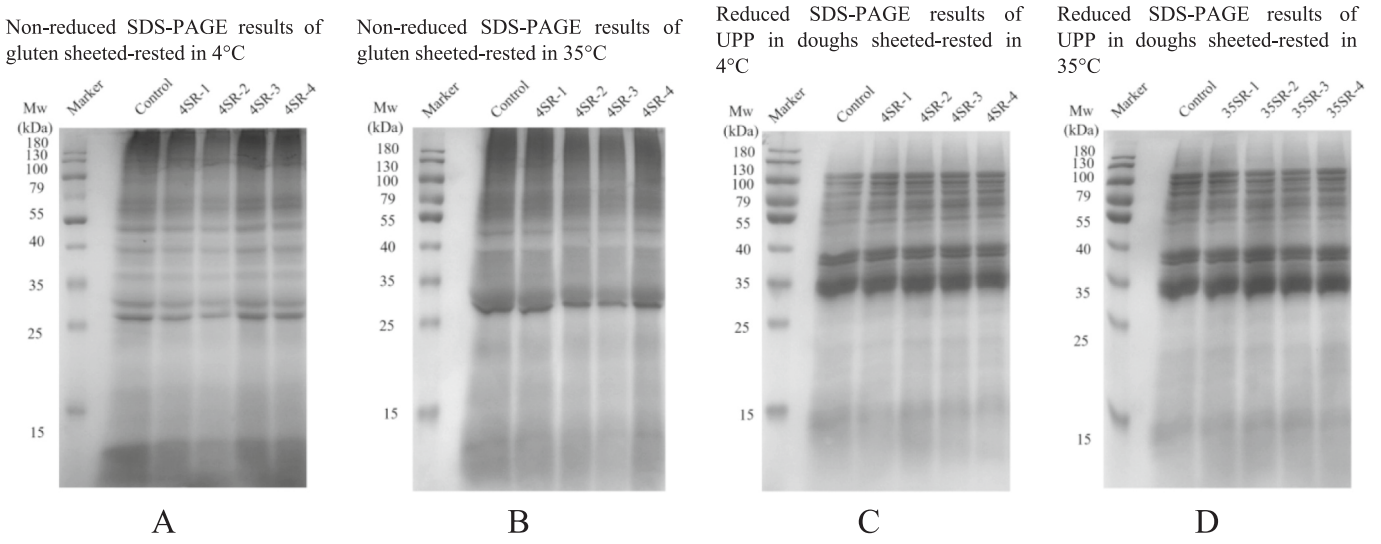


**Fig. 3.** A, Non-reduced SDS-PAGE results of gluten sheeted-rested in 4 °C. B, Non-reduced SDS-PAGE results of gluten sheeted-rested in 35 °C. C, Reduced SDS-PAGE results of UPP in doughs sheeted-rested in 4 °C. D, Reduced SDS-PAGE results of UPP in doughs sheeted-rested in 35 °C.

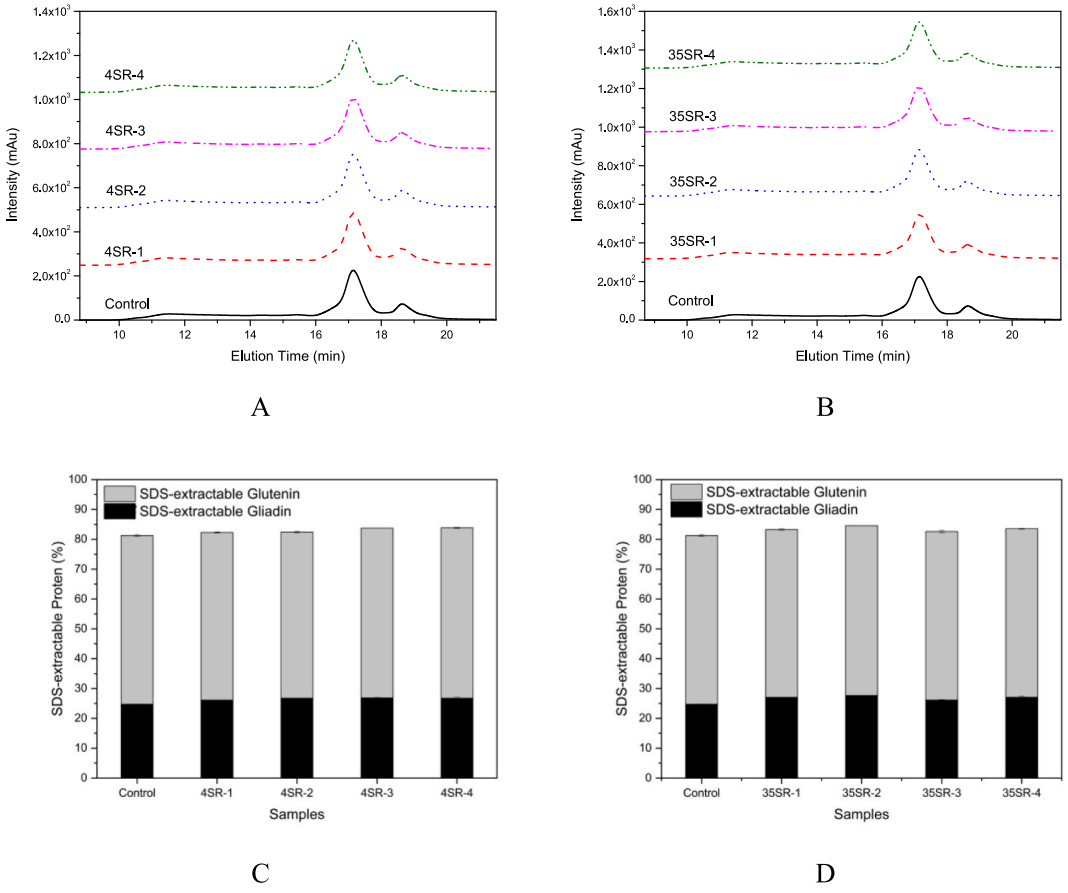


**Fig. 4.** A, SE-HPLC profiles of SDS-extractable protein and SDS-extractable protein of doughs sheeted-rested in a low-temperature environment (4 °C). B, SE-HPLC profiles of SDS-extractable protein and SDS-extractable protein of doughs sheeted-rested in a high-temperature environment (35 °C). C, Protein extractability in sodium dodecyl sulfate containing buffer of doughs sheeted-rested in a low-temperature environment (4 °C). D, Protein extractability in sodium dodecyl sulfate containing buffer of doughs sheeted-rested in a low-temperature environment (35 °C). Values are expressed as mean ± standard deviation.

free SH oxidation during sheeting and resting likely contributed to the reduced extraction rate (Eisenberg & Sawaya, 2017). To further investigate the formation of gluten protein polymers under composite calendering-resting conditions at different temperatures, SDS-insoluble gluten proteins (UPP) were reduced using DTT. After adding the SH-reducing agent, the SDS-PAGE spectra (Fig. 3C and D) showed no significant changes in the number or intensity of bands across different treatment conditions. This suggests that the aggregation of wheat gluten components was primarily driven by the oxidation of free SH groups. Interestingly, the intensity of gluten bands increased after four cycles of treatment in high-temperature environments. Similarly, in low- temperature environments, electrophoretic band intensities were enhanced after three and four cycles of treatment. These observations implied that gluten formation in dough treated by high-intensity calendering and resting was not solely due to free SH oxidation but may involve other reactions. This conclusion was supported by the increased extraction rate in 1.5 % SDS, indicating alternative mechanisms contributing to gluten aggregation.

### 3.4. Molecular weight of gluten protein

SE-HPLC was employed to analyze the molecular weight distribution of wheat gluten proteins subjected to repeated calendering and resting treatments. Based on previous studies and to minimize solvent peak interference, the SE-HPLC spectrum was divided into four regions according to molecular weight: LPP (large polymeric proteins), LMP (larger monomeric proteins), SPP (smaller polymeric proteins), and SMP (smaller monomeric proteins) (Li et al., 2021). The peak areas of each fraction of SDS-extractable proteins were integrated and expressed as percentages (Wang et al., 2023). The results showed no significant shifts in the molecular weight distribution of gluten proteins (Fig. 4 A and B), as indicated by the unchanged positions of gluten peaks (Zhang et al., 2024). However, a short resting period promoted a significant increase in the proportion of LPP and a significant decrease in LMP ($P < 0.05$), while SPP and SMP proportions remained relatively stable ($P > 0.05$). This suggests that resting enhances gluten protein aggregation, evidenced by an increase in polymerized proteins and a decrease in monomeric proteins. The calendering-resting composite process at low temperatures had minimal effect on gluten protein fractions. Across different cycles, the proportions of LPP and LMP remained stable ($P > 0.05$) at 21.52–21.79 % and 50.95–51.15 %, respectively. Notably, dough processed twice in low-temperature conditions (4SR-2) exhibited the highest SPP content (10.65 %) and the lowest SMP proportion (16.56 %) (Fig. 4A). In contrast, high-temperature processing did not significantly affect the SPP percentage ($P > 0.05$), which ranged between 10.52 and 10.66 %. However, dough processed three times at 35 °C (35SR-3) exhibited the highest LPP content (22.92 %) and the lowest SMP proportion (16.40 %) (Fig. 4B). Comparing temperature conditions, dough rested at low temperatures had lower LPP percentages (21.87–22.92 %), but higher LMP percentages (50.03–50.55 %) than dough processed at higher temperatures (Fig. 4A and B). This trend was consistent with non-reduced state SDS-PAGE results (Fig. 3A and B). The findings indicate that elevated temperatures promote gluten aggregation and the formation of larger molecular weight substances. Conversely, repeated treatments at low temperatures had limited effects on gluten polymerization, instead favoring the formation of small-molecule aggregated proteins and monomeric proteins. In high-temperature environments, multiple calendering-resting cycles primarily promoted the formation of large aggregated proteins, with minimal impact effect on monomeric proteins.

To further analyze the degree of cross-linking and polymerization of gluten, reducing agents (DTT) were added to calculate the extraction rates of wheat glutenin and gliadin in the reduced state. As shown in Fig. 4C and D, the resting treatment significantly promoted gluten protein polymerization, as evidenced by an increase in the extractable proportion of glutenin from 24.73 % (Control, $P < 0.05$). Higher ambient temperatures resulted in greater glutenin extractability and lower gliadin extractability under the same processing conditions. In a low-temperature environment, repeated calendering-resting treatments increased the extraction rates of glutenin and gliadin, though these changes were not significant in 4SR-3 and 4SR-4 (Fig. 4C, $P > 0.05$). Similar trends were observed under high-temperature conditions, where the total gluten protein extractability continued to increase, reaching 83.54 % in 35SR-4 (Fig. 4D, $P > 0.05$). The repeated calendering process probably resulted in the decomposition of larger protein agglomerates, which formed during mixing and resting into smaller aggregates, due to the application of considerable mechanical pressure (Zhang, Ma, Yang, et al., 2022). Mechanical stress also induced loosening of the gluten chain structure, resulting in depolymerization and increased proteolysis (Zardetto & Dalla Rosa, 2006), consistent with the observations from SDS-PAGE.

### 3.5. Analysis of GMP content

The content and quality of GMP play a vital part in determining dough properties and the overall quality of wheat flour products. The GMP content under different sheeting-resting treatments is shown in Fig. 5A, comparing treated doughs with fresh dough (Control). After a short resting period, GMP content increased in both temperature environments (35SR-1, $P < 0.05$; 4SR-1, $P > 0.05$). This increase was likely due to the recovery of chemical bonds disrupted during the initial mixing stage, which facilitated GMP polymerization, making it less extractable by SDS. This was reflected in the denser and more uniform gluten network observed (Wang et al., 2021). Meanwhile, hydration during resting further promoted gluten agglomeration (Jia et al., 2022). While continuous sheeting disrupted GMP spherical particles the restorative effect of resting on chemical bonds outweighed the destructive mechanical forces (Song et al., 2019). This facilitated further gluten network development, as indicated by the sustained increase in GMP content in doughs subjected to multiple treatments. Notably, the difference in GMP content between the two temperature conditions widened with increasing treatment cycles, reaching a maximum difference of 3.53 mg/g after four treatments. This disparity likely resulted from the stronger restorative effect of high-temperature resting on damaged chemical bonds, while the disruptive impact of sheeting at low temperatures was more pronounced. Maximum GMP contents of 21.40 mg/g and 23.58 mg/g were observed after two and three compound treatments under cold and hot conditions, respectively. However, further sheeting and resting cycles led to a significant reduction in GMP content by 5.65 % (4SR-3) and 3.56 % (35SR-4) ($P < 0.05$), indicating that the mechanical forces began to overpower the restorative effects of resting. This reduction was likely due to overhydration and the dominance of mechanical disruption in the gluten network at this stage (Jia et al., 2022). Additionally, the activation of proteases in wheat flour, triggered by sulfur-containing amino acids such as cysteine, may have contributed to the decline in GMP content (Lagrain, Brijs, & Delcour, 2006).

### 3.6. Covalent bonds

Disulfide bonds play a crucial role in maintaining the structure and functionality of gluten. The covalent cross-linking of gluten proteins, induced by the combined action of calendering and resting, was examined by monitoring changes in free sulfhydryl (–SH) and disulfide (–SS) bond content (Liang et al., 2024). As illustrated in Fig. 5B and C, the free –SH content in doughs decreased progressively from 2.67 μmol/g, while the –SS bond content increased significantly ($P < 0.05$) from 2.60 μmol/g after a short resting period. This was attributed to the oxidation of free –SH groups into –SS bonds in the presence of oxygen, which facilitated protein adhesion, improved gluten structure, and enhanced dough strength (Feng et al., 2021). The minimum free –SH (2.48 μmol/g) and maximum –SS (2.95 μmol/g) bond contents were observed in dough

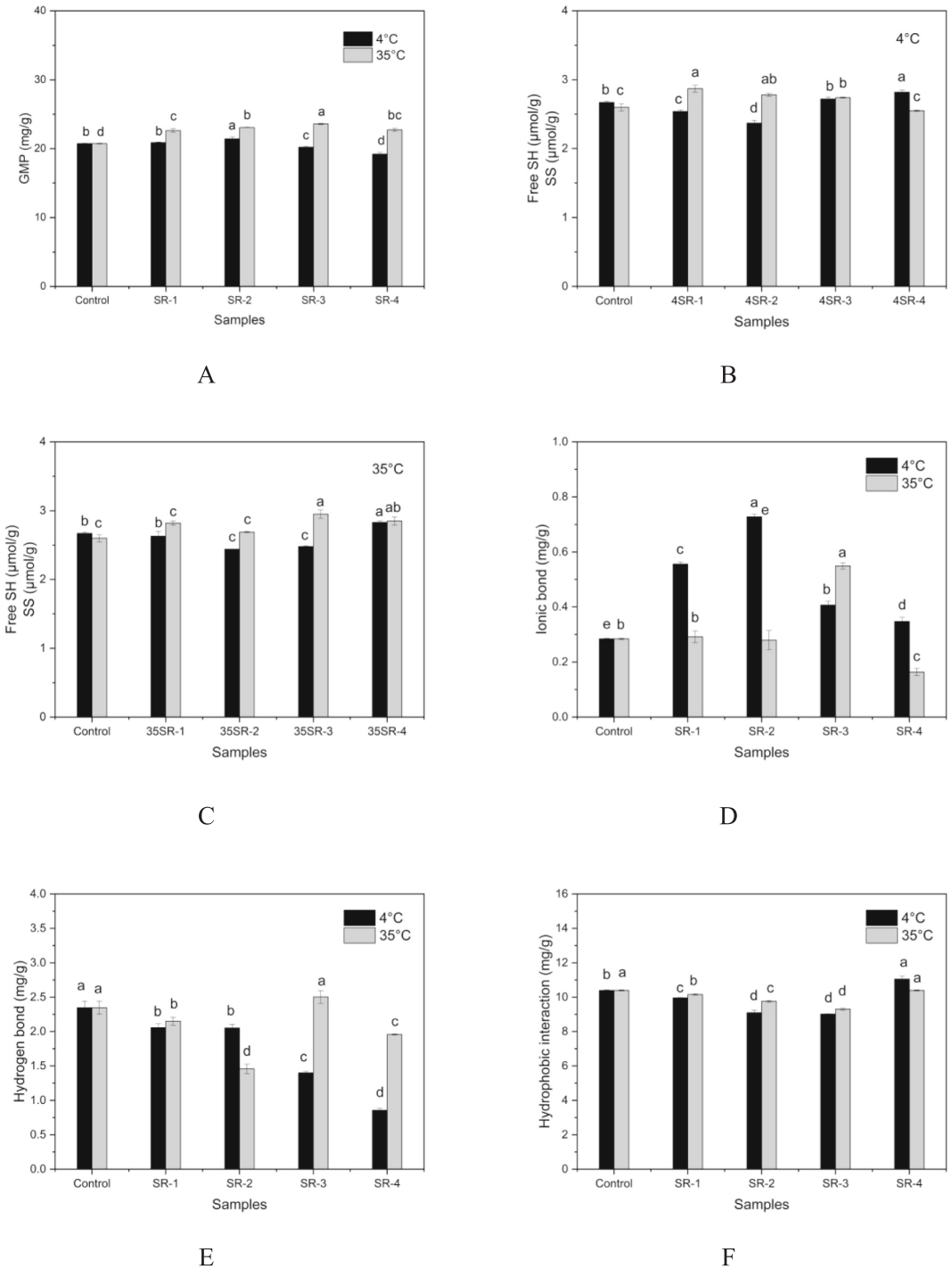


**Fig. 5.** A, GMP content of doughs prepared in different conditions. B, Free sulfhydryl and disulfide bond content of samples pretreated in low-temperature environment (4 °C). C, Free sulfhydryl and disulfide bond content of samples pretreated in a high-temperature environment (35 °C). D, Contributions of ionic bond in doughs made in different conditions. E, Contributions of hydrogen bond in doughs made in different conditions. F, Contributions of hydrophobic interaction in doughs made in different conditions. Values are expressed as mean ± standard deviation. Different letters on top of the bars indicate significant differences ($P < 0.05$).

treated under high-temperature conditions after three calendering-resting cycles (35SR-3). Similarly, for low-temperature conditions, the corresponding values were 2.37 μmol/g and 2.78 μmol/g after two cycles (4SR-2). These results suggest that calendering impedes GMP polymerization via sulfhydryl-disulfide bond exchange, a trend also reflected in GMP content analysis (Fig. 5A). Additionally, oxidation of free –SH groups to form new –SS bonds contributed to increased –SS bond content (Liu et al., 2023). High ambient temperatures significantly facilitated oxidation reactions, as evident in the 35SR-4 sample, which exhibited high free –SH (2.83 μmol/g) and –SS bond content (2.85 μmol/g). In contrast, the lowest –SS bond content (2.55 μmol/g) was observed in the 4SR-4 sample under low-temperature conditions. This could be due to repeated folding and calendering at low temperatures disrupting hydrogen bonds in gluten, leading to the exposure of internal –SH groups (Si et al., 2021). Conversely, high temperatures may reduce –SS bond extractability due to protein folding driven by hydrophobic

aggregation (Wang, Liu et al., 2017). In conclusion, the interplay between mechanical forces and oxygen-driven oxidation shapes the gluten network's covalent interactions. Initially, mechanical disruption dominated the formation of the gluten network, while prolonged resting and repeated calendering progressively enhanced the oxidizing effects by exposing free –SH groups (Sutton et al., 2003).

### 3.7. Solubility of gluten proteins in different solvents

Dough formation is primarily governed by the rupture and reorganization of non-covalent bonds, such as hydrogen bonds and hydrophobic interactions between protein chains, rather than extensive covalent bond rupture (Iwaki, Aono, Hayakawa, Fu, & Otobe, 2020; Yang et al., 2023). To evaluate the role of non-covalent interactions, the solubility of gluten proteins in different solvent systems was measured for doughs subjected to repeated sheeting-resting operations (Fig. 5D, E, and F). After a short resting period at lower temperatures, the ionic bonding contribution of the dough increased significantly ($P < 0.05$), indicating that cold resting facilitated gluten network development by promoting gluten aggregation, making it less extractable in phosphate buffer. The ionic bonding strength continued to increase with additional sheeting-resting cycles at low temperatures, peaking at two cycles (4SR-2) with an ionic bonding level of 0.73 mg/g. This suggests that short resting combined with mild sheeting enhances ionic interactions, resulting in higher solubility of gluten proteins in highly concentrated NaCl solutions. Under high-temperature conditions, the dough with the maximum ionic bonding contribution (0.55 mg/g) was observed after three cycles of sheeting-resting treatments (35SR-3). However, the disruption of ionic bonds became apparent with multiple calendering and rousing, as mechanical sheeting increased the proportion of salt-soluble proteins and peptides (Wang, Zou, Gu, & Yang, 2018). This facilitated the solubilization of gluten proteins in salt solutions and significantly increased ionic bonding levels.

In addition, the hydrogen bonding contribution in doughs (4SR-1 and 35SR-1) decreased after 30 min of resting compared to the control dough (2.35 mg/g). Previous studies have shown that ripening allows water to maximize contact with protein colloidal particles, fully hydrating them through hydrogen bonding (Feng et al., 2021). However, the present study observed the opposite trend, likely due to intermittent calendering treatments creating unresolved internal tension within the dough, which slowed the bonding of gluten to water molecules (Obadi et al., 2023). It was found that weak mechanical forces (no more than 5 sheeting cycles in one direction) did not significantly enhance hydrogen bonding in gluten proteins ($P > 0.05$), while short resting periods had a weakening effect. After two cycles of re-calendering and waking, the dough processed at high temperatures exhibited a lower hydrogen bonding contribution (1.46 mg/g), whereas dough at low temperatures showed a much higher contribution (2.05 mg/g). Notably, in low-temperature environments, hydrogen bonding was continuously inhibited, decreasing to as low as 0.86 mg/g (4SR-4). This inhibition was attributed to weak interactions among water, gluten protein, and starch (Donmez, Pinho, Patel, Desam, & Campanella, 2021). Consequently, the increase in free –SH content was not solely due to the breakage of –SS bonds but also resulted from the disruption of hydrogen bonds, which exposed internal protein groups. In contrast, a sudden increase in hydrogen bonding levels (up to 71.96 %) was observed after three cycles of treatments at high temperatures (35SR-3, $P < 0.05$). This was attributed to reduced internal tension within the dough and the stronger facilitating effect of ripening, which enhanced water binding to protein and starch molecules. This process promoted the formation of hydrogen bonds, thereby improving the dough's structural integrity (Feng et al., 2021).

It has been shown that during dough resting, water gradually penetrates protein particles, leading to the exchange of hydrophilic groups and the weakening of hydrophobic interactions. This effect was evident after a short resting period, as reflected by decreased hydrophobic interaction levels in the dough samples (4SR-1: 9.96 mg/g; 35SR-1: 10.16 mg/g, $P < 0.05$). Subsequent calendering induced gluten rearrangement and repetitive protein folding, resulting in the burial of hydrophobic residues. This burial likely reduced the exposure of hydrophobic sites, further suppressing hydrophobic interactions (Wang et al., 2018; Zhang, Ma, Yang, et al., 2022). The joint inhibitory effect of calendering and resting on hydrophobic interactions was demonstrated by the lowest hydrophobic interaction strengths observed in the dough samples: 9.10 (4SR-2) in low-temperature environments and 9.31 mg/g (35SR-3) in high-temperature environments. Meanwhile, hydrophobic interactions were enhanced in dough subjected to three or four cycles of sheeting-resting in low-temperature environments and four cycles in high-temperature environments. This finding contrasts with Sutton et al. (Sutton et al., 2003), who suggested that continuous calendering exposes additional hydrophobic sites, with subsequent resting processes re-masking these residues by relieving internal dough stress. The observed discrepancy may arise from differences in experimental conditions or variations in the balance between protein folding and hydrophobic residue exposure. In addition, CLSM analysis indicated that dough samples treated with multiple cycles of sheeting and resting exhibited larger gaps in the gluten network (higher lacunarity). This structural change contributed to the easier solubilization of gluten proteins in SDS solutions, promoting partial depolymerization of gluten proteins. The increased solubility of alcohol-soluble proteins in SDS ultimately weakened the strength of the gluten network, further disrupting its integrity (Liu, Song, et al., 2021; Song et al., 2019).

### 3.8. Molecular morphology of gluten chains

Atomic force microscopy, in combination with traditional food science methods, provides a unique tool for studying mechanisms at the cellular and molecular levels (Pipintakos et al., 2021). As an imaging tool, AFM can reveal high-resolution protein aggregation structures, elucidating the molecular chain structure of gluten in solution (Koehler et al., 2024). Diluted acetic acid serves as an effective solvent for glutenin, preserving its original chain structure. The surface morphology of gluten proteins in doughs subjected to composite calendering-resting treatments at different temperatures is presented in Fig. 6. After a short period of rousing, gluten chains exhibited stacking and increased height, with measured heights of 9.83 nm (4SR-1) and 9.16 nm (35SR-1) in low- and high-temperature environments, respectively. The similarity in morphology was attributed to protein chain aggregation facilitated by hydration and resting (Chen et al., 2019; Zhang, Ma, Jia, et al., 2022). Two cycles of calendering-resting treatments led to a slight increase in gluten width, while the height of the gluten protein chains remained unchanged at lower temperatures but continued to increase in higher-temperature environments. At lower temperatures, gluten protein chains showed a more homogeneous morphology after three composite treatments, characterized by decreased steepness in the chains. Conversely, at 35 °C, calendering-resting treatments not only increased the height of gluten chains (ranging from 9.38 to 10.19 nm) but also contributed to the formation of large-particle polymers. Particularly, three cycles of treatment (35SR-3) resulted in the formation of homogeneous, large-grained gluten protein polymers. Previous studies have shown that mechanical pressure between rollers breaks gluten molecules into smaller, uniformly sized chains, leading to gradual decreases in width and height, enhancing the homogeneity of the gluten molecular chains and reinforcing the gluten network structure (Khuzwayo, Taylor, & Taylor, 2020). These findings align with CLSM results. In contrast, after four cycles of treatment at 35 °C (35SR-4), the uniformity of gluten particles decreased, and particle steepness increased, a trend more pronounced than in dough treated under cold conditions (4SR-4). Excessive or continuous calendering disrupted gluten molecules into smaller fragments, with dynamic structural changes (breakage and reorganization) altering the morphology of gluten chains and the overall gluten network (Zhang et al., 2024). Moreover, the ambient operating

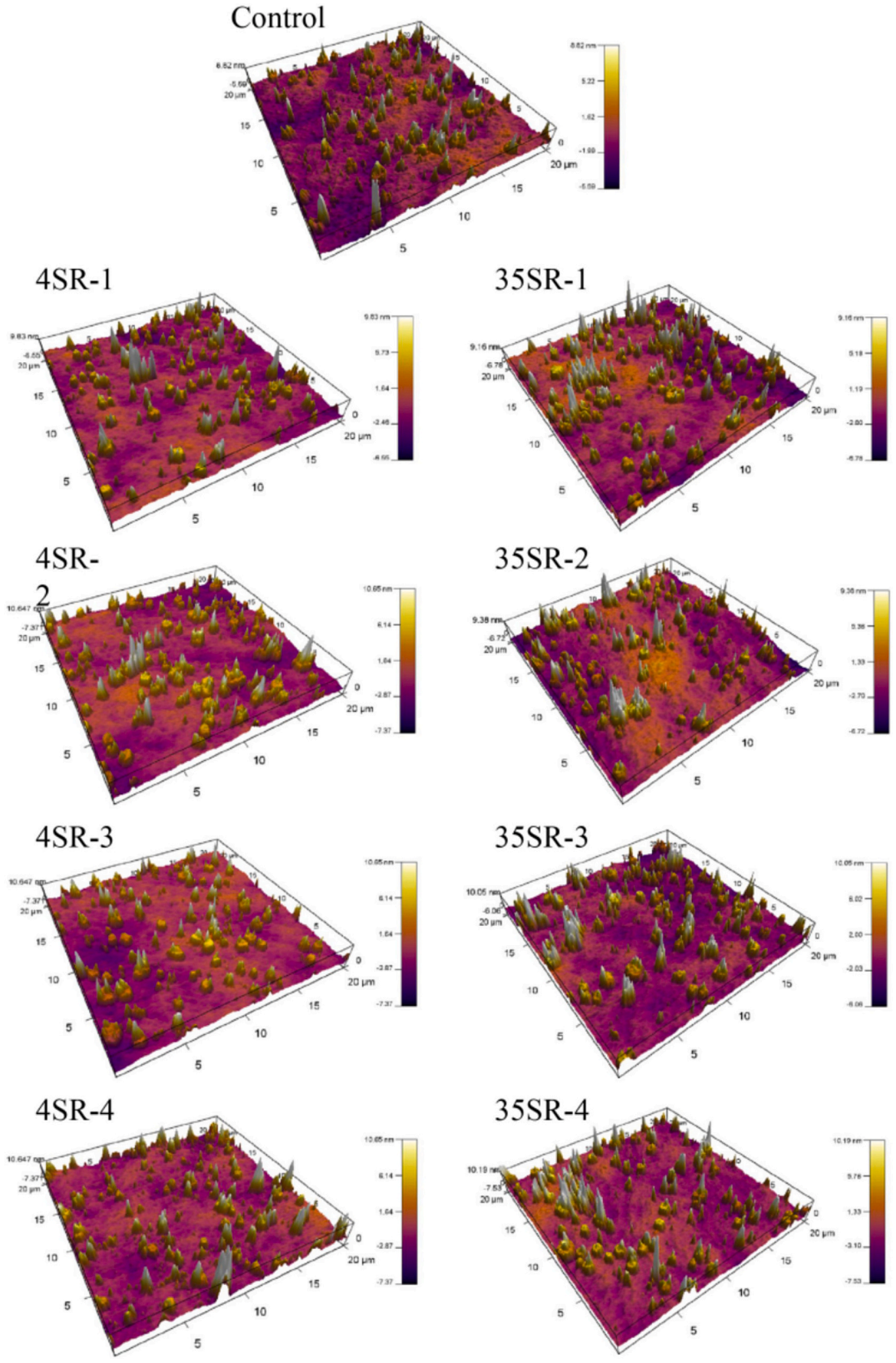


**Fig. 6.** Representative AFM images of dough samples sheeted-rested in different temperatures.

temperature played a critical role in influencing the molecular morphology transformation of gluten chains (Yang et al., 2023).

### 3.9. Discussions on the behavior of wheat flour dough at different sheeting-resting cycles and temperatures

The molecular mechanisms underlying the changes in the rheological properties of dough after composite calendering-resting treatments at two ambient temperatures (4 °C or 35 °C) were discussed. Previous studies indicate that gluten in fresh dough exists in a naturally curled "loop" structure. When subjected to mechanical forces, gluten chains stretch and rearrange into more orderly "train" structures. After a short relaxation period, the transformation of free sulfhydryl groups in the dough was facilitated (Fig. 6), while hydrogen bonding and hydrophobic interactions were weakened (Fig. 2). These changes enhanced the dough's overall processing properties, as evidenced by increased *R*-values. The recoiling of gluten chains or the occurrence of interchain linkages contributed to the formation of large gluten protein polymers. Repeated stretching and self-relaxation cycles promoted alternating stretching and contraction of the gluten chain, resulting in increased interstitial space (Fig. 3) and reduced internal dough viscosity (Fig. 1 and Table S1). These changes made gluten proteins more extractable (Fig. 4 and Fig. 5) and significantly improved dough processability. However, in low-temperature environments, the improvement slowed and became limited with prolonged operational cycles (Fig. 1 and Table S1). Nonetheless, prolonged operational cycles in high-temperature environments significantly enhanced non-covalent interactions within the dough, while the contribution of covalent interactions to viscoelasticity diminished (Fig. 6). The gluten molecular chains experienced rupture, reduced homogeneity, and stacking (Fig. 6). Moreover, high-temperature treatments increased the number of holes in the dough profile, resulting in greater lacunarity (Fig. 2), which softened the dough and made it more prone to deformation (Fig. 1). Based on physical and chemical characterization, a schematic diagram (Fig. 7) illustrates the macroscopic effects of repeated mechanical force application and relaxation. This visualization highlights the interplay between structural rearrangements and dough properties under different operational conditions.

## 4. Conclusion

This study investigated the molecular mechanisms underlying changes in the rheological properties of dough subjected to multiple calendering-resting treatments at two ambient temperatures (4 °C or 35 °C). After a brief relaxation period, fresh dough exhibited transformations of free sulfhydryl groups into disulfide bonds, along with weakened hydrogen bonding and hydrophobic interactions, and enhanced ionic bonding. These changes contributed to an increase in the proportion of SDS-soluble polymerized proteins. Repeated stretching and self-relaxation cycles caused gluten chains to extend, increasing lacunarity, reducing internal viscosity, and improving dough strength. Low-temperature environments slowed sulfhydryl oxidation by suppressing relaxation effects, while high temperatures promoted water exchange, thereby enhancing hydrogen bonding and hydrophobic interactions. However, the enhancement of dough processability through repeated calendering and rising was found to be limited. Extended exposure to high-temperature environments significantly enhanced non-covalent interactions, while diminishing the contribution of covalent interactions to dough the dough's viscoelastic properties. Prolonged mechanical forces eventually fractured gluten molecular chains, reducing their homogeneity and inducing a stacking phenomenon among gluten particles. This effect was particularly pronounced in the increased number of holes and expanded gaps within the dough profile under high-temperature environments, which negatively impacted its processability. Based on physicochemical characterizations, a schematic diagram was presented to elucidate the effects of combined mechanical forces and relaxation treatments on gluten structure from a macroscopic perspective. Future work should focus on evaluating noodle quality and

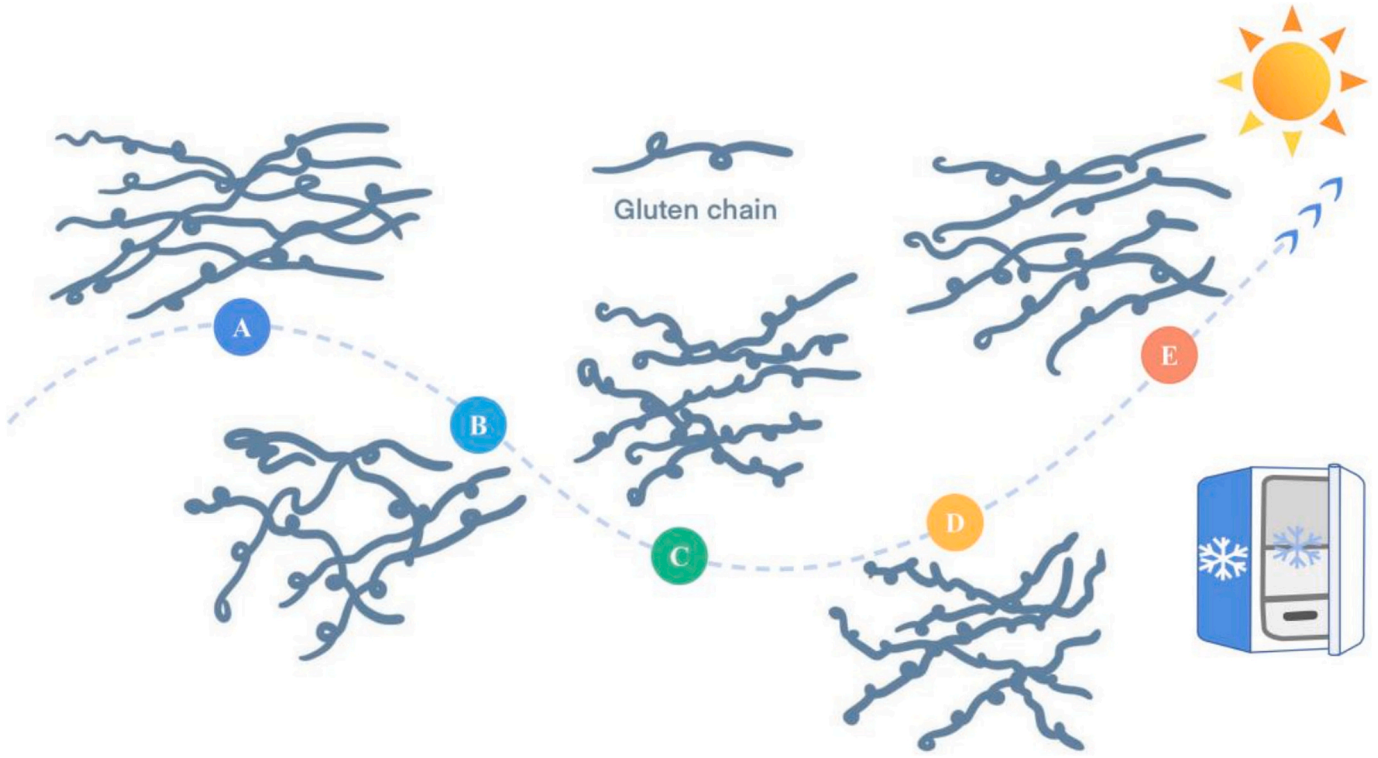


**Fig. 7.** A schematic diagram is presented to elucidate the effects of repeated force action and relaxation from a macroscopic point of view based on physical and chemical characterization. Where, serial numbers A-E refer to the morphology of the gluten chain after simple calendering, short time relaxation, repeated calendering and resting, cycling multiple treatments in a low-temperature environment and a high-temperature environment for a long time, respectively.

establishing a correlation between dough characteristics and product quality, thereby providing practical guidance for noodle production.

## CRediT authorship contribution statement

**Yu-ling Yang:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Long Yang:** Writing – original draft, Conceptualization. **Er-qi Guan:** Writing – original draft, Supervision, Methodology. **Ke Bian:** Resources, Project administration, Methodology, Formal analysis, Conceptualization.

## Declaration of competing interest

We have confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He is responsible for communicating with the other authors about progress, submissions of revisions, and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from *15238635973@163.com*.

## Acknowledgements

The research was supported by National Key R&D Program of China (2021YFD2100903), National Modern Agricultural (Wheat) Industry Technology System Construction Program (CARS-03), National Natural Science Foundation of China (U1604235), Anhui Science and Technology University Talent Introduction Project (SPYJ202305).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102366.

## Data availability

Data will be made available on request.

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