Food Chemistry: X 23 (2024) 101513

Contents lists available at ScienceDirect

Food Chemistry: X

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

Effects of wheat starch content on its flour and frozen dough bread

Zixuan Yang [a,b], Jinling Li [a,b], Zhili Ji [a,b], Shangyuan Sang [d], Xueming Xu [c,*]

[a] *Key Laboratory for Deep Processing of Major Grain and Oil, Ministry of Education, Wuhan Polytechnic University, Wuhan, Hubei 430023, PR China*
[b] *Hubei Key Laboratory for processing and Transformation of Agricultural Products, Wuhan Polytechnic University, Wuhan, Hubei 430023, PR China*
[c] *State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, PR China*
[d] *Zhejiang-Malaysia Joint Research Laboratory for Agricultural Product Processing and Nutrition, Department of Food Science and Engineering, Ningbo University, Ningbo, Zhejiang 315832, PR China*

ARTICLE INFO

Keywords:
Wheat starch
Particle size
Frozen dough
Bread

ABSTRACT

The refined wheat flour was mixed with different types of wheat starch in different addition levels, their microstructure, chemical bonds in the dough and baking characteristics of 0–8 weeks frozen dough bread were studied. With the increase of A-Type starch granules and whole wheat starch, the pores of gluten network first decreased and then increased. Conversely, an increase in B-Type starch granules consistently reduced gluten network porosity. With the increase of whole wheat starch, the content of free sulfhydryl group and hydrophobic interaction decreased gradually. Minimal additions of B-Type granules were found to enhance the specific volume of fresh bread, whereas increased quantities improved the specific volume of frozen dough bread. The addition of a small quantity of A- or B-Type granules enhances the freezing stability of bread. This study provides effective information for elucidating the effects of wheat starch on the frozen dough and bread properties in protein-starch matrix.

1. Introduction

Frozen dough is now widely used in daily standardized and long shelf-life wheat flour products. It should be noted that wheat starch with the highest content (75%) in wheat flour suffers from deterioration during the freezing process, and this deterioration varies with different particle sizes of starch. Wheat starch synthesizes different starch granules in amyloplasts, which are large A-Type starch granules (size between 15 and 40 μm, with an average diameter of about 25 μm and a disc shape), and small B-Type starch granules (granule size of <11 μm, roughly spherical, elliptical, and irregular). In wheat starch, the number of granules of A-Type starch granules (<10%) is much less than the number of granules of B-Type starch granules, but they have the largest percentage of mass fraction (>70%). Previous studies have demonstrated that starch composition affects dough stability (Amritpal et al., 2016; Lu, Zhu, Tao, Wang, & Yang, 2022; Niu, Hou, & Zhao, 2017; Tao, Lu, Zhu, Wang, & Xu, 2023). Other studies have also explored the effects of adding various kinds of powder to wheat flour on dough behavior (Edwards, Dexter, & Scanlon, 2002; Huang & Lai, 2010). Although these studies have investigated the relationship between gluten networks, starch properties, and dough behavior, there are currently no established rules or parameters to accurately predict the gluten-starch interactions and their effect on the final product.

In our previous studies, we found that varying granule sizes of wheat starch had distinct impacts on the deterioration of frozen dough bread during storage (Yang et al., 2022a, b). Building on these findings, we intend to investigate whether adjusting the proportion of wheat starch in flour can improve frozen dough bread and whether selective screening of wheat flour for making frozen dough bread is feasible in the future. The contribution of starch particle size and starch composition to the properties of protein-starch matrix wheat dough in natural flours remains inadequately understood, and little is known about the relationship between starch physicochemical properties and dough properties. There is a scarcity of research investigating the effect of starch properties on dough behavior using wheat flour with different starch granule types and starch contents, especially in the context of frozen dough and its finished bread.

Our research aims to investigate the effects of varying wheat starch contents on the function and final food characteristics of frozen dough. Whole wheat starch or its A-Type and B-Type granules were added to natural wheat flour to explore the effects of different starch compositions on the characteristics of frozen dough and bread. Our research involved the preparation of 9 wheat flour samples, each with a different starch composition. Through exploring the dough properties and frozen

---

[*] Corresponding author.
E-mail address: xmxu@jiangnan.edu.cn (X. Xu).

https://doi.org/10.1016/j.fochx.2024.101513
Received 17 April 2024; Received in revised form 19 May 2024; Accepted 25 May 2024

dough bread properties of these samples, we were able to acquire valuable insights into the effects of wheat starch types and content on the properties of dough and bread.

2. Materials and methods

2.1. Materials

Commercial wheat flour (refined) was obtained from Wilmar Co., Ltd. (Kunshan, China). Sugar, yeast and salt were obtained from the local supermarket in Wuxi, China.

In the following sections, "A", "B" and "W" referred to A-Type, B-Type granules, whole wheat starch, respectively.

2.2. Preparation of wheat starch

Whole wheat starch samples were isolated from fresh dough by the method of Tao, Wang, Wu, Jin, and Xu (2016). The wheat starch slurry is then highly purified into A-Type and B-Type granules by repeated suspension in water. B-Type granules suspended in the upper layer of the water and A-Type granules precipitated in the lower layer. The two types of starch slurry after separation was centrifuged at 3000*g* and dried in an oven at 45 °C, then milled by a grinder and passed through a 100-mesh sieve. All the starch samples were stored in a dryer for further use.

2.3. Compound wheat flour

The formula of compound wheat flour was shown in Table 1. The selection criterion for this formula relies upon the assessment data of A- and B-Type granules and the proportion of wheat starch present in diverse wheat flour varieties, as detailed by Cao et al. (2019). The feasibility of the A- and B-Type granule, whole wheat starch content added to the wheat flour was also calculated and scoped without destroying the overall structure as well as the mixing characteristics of dough.

2.4. Preparation of compound wheat dough

The fundamental dough composition comprises 100% wheat flour, 63% water, 3.5% sugar, and 1% salt, 1.5% yeast. Each batch was prepared using 3000 g of wheat flour as the foundation. Initially, all the solid ingredients were combined in a vertical mixer (SM-25, Sinmag Machinery, Wuxi, China) at a low speed for 1 min, followed by the addition of water. The mixture was then blended at a low speed for 3 min, subsequently increasing to a high speed for 4 min to ensure proper gluten formation. The dough was then enveloped in polyethylene and allowed to rest for 5 min.

Table 1
Flour constitution and explanation for 9 reconstituted frozen whole wheat flour groups.

Samples	Flour constitution
ORIGIN	3000 g Commercial wheat flour
A10	2900 g Commercial wheat flour +100 g A-Type granules
A20	2800 g Commercial wheat flour +200 g A-Type granules
A40	2600 g Commercial wheat flour +400 g A-Type granules
B5	2950 g Commercial wheat flour +50 g B-Type granules
B10	2900 g Commercial wheat flour +100 g B-Type granules
B15	2850 g Commercial wheat flour +150 g B-Type granules
W15	2850 g Commercial wheat flour +150 g whole wheat starch
W30	2700 g Commercial wheat flour +300 g whole wheat starch s
W45	2550 g Commercial wheat flour +450 g whole wheat starch

"A", "B" and "W" refer to A-Type, B-Type granules and whole wheat starches isolated from flour, and the number "5", "10", "15", "20", "30", "40" and "45" refer to the starch weight (g) added in the basic wheat flour (keep total weight 300 g).

2.5. Laser confocal images of compound wheat dough

The cryosectioned specimens of dough (20 μm) were stained utilizing a 0.25% (*w*/w) solution of fluorescein isothiocyanate (FITC) in acetone and a 0.025% (w/w) solution of rhodamine B fluorescent dye. Subsequent to a one-minute incubation period, the samples were rinsed with deionized water, overlaid with a cover slip, and examined using a laser confocal microscope (lsm710, Zeiss, Germany). The magnification of the ocular and objective lens was 10× and 20×, respectively. The excitation/emission wavelengths of FITC and rhodamine B were 488/518 nm and 568/625 nm, respectively.

2.6. Chemical forces and free sulfhydryl groups in compound wheat dough

The dough was frozen at −20 °C overnight, followed by freeze-dried using a Labconco FreeZone (Labconco, USA). The freeze-dried dough was then ground and sieved. In accordance with Guillén et al. (Gómez-Guillén, Borderías, & Montero, 1997), various chemicals were chosen based on the gel's chemical bond binding capacity: 0.05 mol/L NaCl (SA), 0.6 mol/L NaCl (SB), 0.6 mol/L NaCl+1.5 mol/L urea (SC), 0.6 mol/L NaCl+8 mol/L urea (SD). These solutions will partially solubilize the proteins to determine the presence of ionic bonding (differences between proteins dissolved in SB and SA), hydrogen bonds (differences between proteins dissolved in SC and proteins dissolved in SB), and hydrophobic interactions (differences between proteins dissolved in SD and proteins dissolved in SC). The sample (0.2 g) was mixed with 10 mL of each solution, homogenized through centrifugation at 1600×*g* for 15 s, allowed to rest at 4 °C for 1 h, and then centrifuged again at 8000 ×*g* for 20 min. The protein concentration in the supernatant was determined using a BCA protein assay kit.

The assessment of free sulfhydryl groups in dough was conducted in accordance with the method outlined by Su et al. (2019). The Tris-glycine-EDTA (TGE) buffer was composed of 92 mM glycine, 4.1 mM EDTA, and 86 mM Tris-HCl at pH 8.0. The reaction buffer (SDS-TGE) was prepared by dissolving 2.5% (*w/v*) sodium dodecyl sulfate (SDS) in the TGE buffer. The 5,5′-dithio-2-nitrobenzoic acid reagent was solubilized in SDS-TGE buffer, yielding a 5 mg/L colorimetric buffer (DTNB-TGE). Freeze-dried dough (200 mg) was extracted with 5 mL of SDS-TGE buffer and vortexed until completely dissolved. Following the addition of 50 μL of DTNB-TGE buffer, each sample was incubated in darkness for 30 min and subsequently centrifuged at 10,000 ×*g* for 20 min. The absorbance of the supernatant was measured at 412 nm, and a standard curve was generated using reduced glutathione as the reference.

2.7. Baking characteristics of compound wheat flour frozen dough bread

The compound dough was stored at −20 °C for 0, 4, and 8 weeks before making frozen dough bread. The frozen dough was thawed at 25 °C for 2 h. The dough was then placed in a proof cabinet (RD622 + 10F, Sinmag, Wuxi, China) maintained at 37 °C and 80% relative humidity for 90 min, then all the compound frozen dough was baked in an electric oven (MB-622 + 1S + 1B, Sinmag, Wuxi, China) at the upper temperature 170 °C and the lower temperature 210 °C for 15 min.

Each bread was weighed after the 2-h cooling period and the specific volume of bread was evaluated using the rapeseed displacement method. The bread was cut into 12 mm slices with a microtome (SM302NS, Sinmag, Wuxi, China). Two central slices were selected for analysis using a TA-XT2i Texture Analyzer (Stable Micro Systems, Godalming, UK). The Texture Profile Analysis mode was employed, with the following parameters: probe model P/25, induction force of 5 g, compression degree of 40%, pre-test speed of 3.0 mm/s, test speed of 1.0 mm/s, post-test speed of 3.0 mm/s, two compressions, and a 1 s test interval. The sliced bread was scanned using an image scanner (Canon iR-ADV 4225, Japan), and 3 × 3 cm^2 images of the bread's central core was extracted. The cell density (number of cells/cm^2) of bread crumb were obtained by processing and analyzing the images with Image J software at 8-bit grayscale and a resolution of 300 dpi. The color values of the crust were examined using a Chromameter (Konica Minolta CR-400, Japan). The values of L*, a*, b* were recorded. The calculation formula of color difference ΔE* was as follows:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}.$$

2.8. Moisture distribution of compound wheat flour frozen dough bread

A central portion of the bread was obtained, from which a rectangular sample approximately 30 mm in length was excised for subsequent analysis. The slice was then placed in a glass tube for nuclear magnetic resonance (NMR) analysis to minimize the impact of moisture loss on experimental outcomes. The relaxation time (T2) was assessed using the Carr-Purcell-Meiboom-Gill (CPMG) sequence, with the following key parameters: main frequency of 21 MHz, offset frequency of 404 kHz, an inter-scan delay (TW) of 500 ms, echo time (TE) of 0.25 ms, the number of echoes (NECH) set to 800, and the number of scans (NS) set to 8.

2.9. Crystallinity of compound wheat flour frozen dough bread

Bread samples were subjected to freeze-drying, pulverization in a grinding apparatus, and sieving through a 100-mesh screen. X-ray diffraction (XRD) measurements were conducted using a Bruker D8-Advance XRD instrument (Bruker AXS Inc., Karlsruhe, Germany), with radiation settings of 40 kV and 30 mA. Diffraction angles were scanned under the following parameters: a range of 4° to 45°, step size of 0.05, and a step time of 0.5 s. The relative crystallinities of the samples were analyzed in accordance with the method described by Tao et al. (2021) and calculated using JADE 5.0 software.

2.10. Statistical analysis

Each experiment was conducted at least in triplicate. Data were analyzed using one-way analysis of variance (ANOVA), means and standard deviation were compared by Tukey using an SPSS package (version 13.0 for Windows). Differences were considered significance at $p < 0.05$.

3. Results and discussion

3.1. Effect of dough microstructure

To further elucidate the effect of wheat starch on dough microstructure, laser confocal microscopy was employed, as depicted in Fig. 1. Within the dough system, the red part represents a protein-formed network structure, while the green part are starch granules which serve as fillers for network structure (Han, Ma, Li, Zheng, & Wang, 2019). Owing to the encapsulation of starch granules within the gluten framework, protein networks interact with starch granules of varying sizes, thereby influencing the dough's properties. A larger gap within the network structure corresponds to a looser dough network. Gao et al. (2018) posited a negative correlation between porosity and dough mixing performance. As the proportion of A-Type starch granules increased, the gluten structure gradually weakened, and the gluten pores initially decreasing before increasing. This phenomenon might be attributed to the formation of hydrogen bonds between gluten protein chains and hydroxyl groups exposed by starch granules, wherein a minimal amount of A-Type starch granules could fill gluten pores. The higher the A-Type granules content in dough, the larger the gap required within the gluten network to function as a skeleton. However, an excess of A-Type starch granules (A40) rendered the gluten incapable of

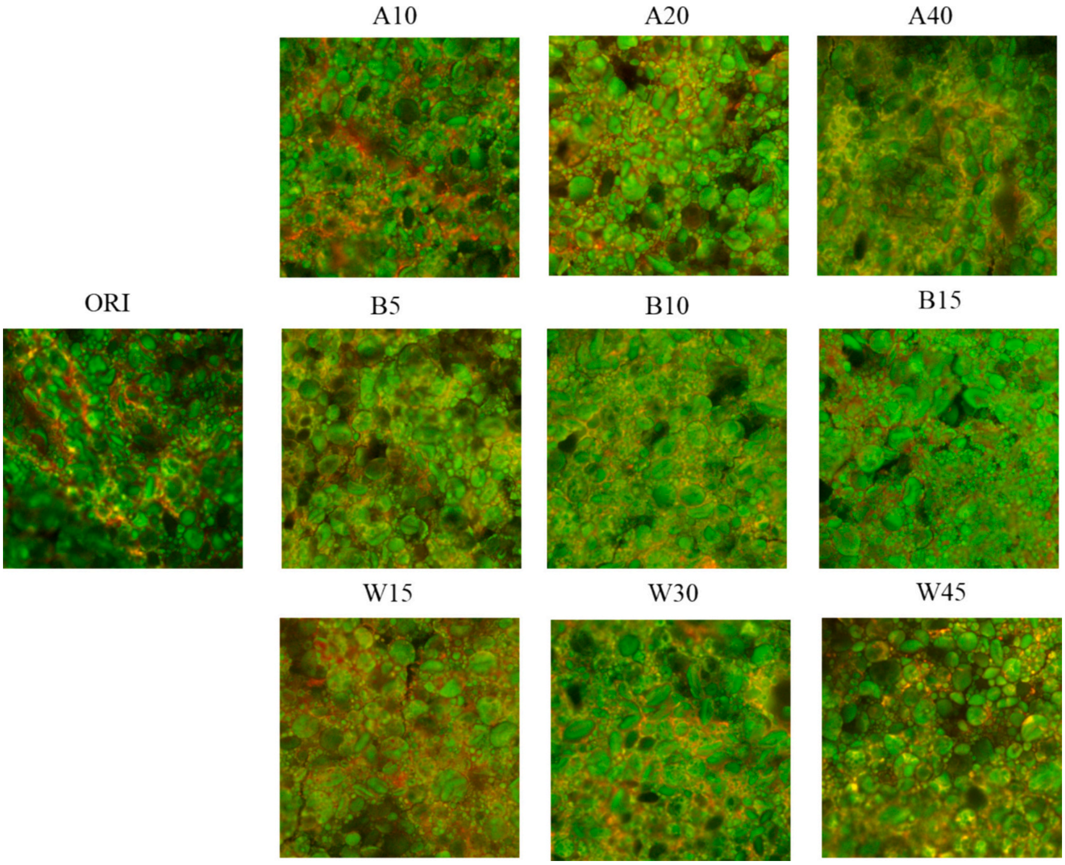

Fig. 1. CLSM images of compound dough with varied starch content. "A", "B" and "W" refer to A-Type, B-Type granules and whole wheat starches isolated from flour, and the number "5", "10", "15", "20", "30", "40" and "45" refer to the starch weight (g) added in the basic wheat flour (keep total weight 300 g).

bearing the load, resulting in significant protein framework damage and increased gluten network breakpoints. Conversely, an increased proportion of B-Type starch granules did not significantly weaken the gluten structure, and gluten pores gradually decreased. This suggested that a higher B-Type granules content could promote the formation of a denser dough structure. Corroborating studies have demonstrated that as the ratio of A-Type to B-Type granules increased, the gluten network structure became more compact in mixed dough system, indicating that A-Type granules could facilitate the formation of tighter dough structures (Li et al., 2021). When the proportion of whole wheat starch increased, the alteration trend resembled that of A-Type granules increments. Initially, the added wheat starch filled gluten pores. However, in W45, the gluten could no longer accommodate the wheat starch volume, resulting in a significant detachment of wheat starch from the gluten network.

3.2. The influence of chemical forces in dough

The formation of dough primarily involves chemical interactions such as ionic bonds, hydrogen bonds, disulfide bonds, and hydrophobic interactions (Cao et al., 2017). Fig. 2 illustrates the variations in free sulfhydryl content and chemical interactions in dough containing different quantities of wheat starch. Fig. 2a displays the free sulfhydryl content in dough composed of distinct wheat starch compositions. As the proportion of A-Type starch granules in the flour increased, only minimal alterations in free sulfhydryl group content were observed. Conversely, an elevation in B-Type granules resulted in the free sulfhydryl content peaking at B5 before subsequently declining. As the whole wheat starch content increased, the free sulfhydryl content gradually decreased and reached the minimum value at W45. These findings contradicted the observation by Li et al. (2021) that B-Type granules yielded more stable dough, while A-Type granules formed less stable dough with gluten. They attributed this primarily to the disparate particle sizes of the two starch types. Smaller B-Type granules could occupy the gluten network's interstices more snugly, facilitating the encapsulation and construction of the gluten network and ultimately fostering the development of a continuous gluten matrix. The discrepancy between these findings might stem from inconsistent raw material selections for the two experimental groups. The wheat starch employed in their study was a composite of A and B-Type granules in varying ratios. In contrast, our experiment incorporated either A and B-Type granules or whole wheat starch into the foundational wheat starch.

The intermolecular forces, such as hydrogen bonds, play a crucial role in the cross-linking of gluten networks in dough, and starch gluten interactions typically occur through van der Waals forces/hydrogen bonds. Quantitatively, it can be observed that hydrophobic interactions contribute the most to the wheat gluten network, followed by hydrogen and ion bonds. Fig. 2b shows the contribution of ionic bonds in various

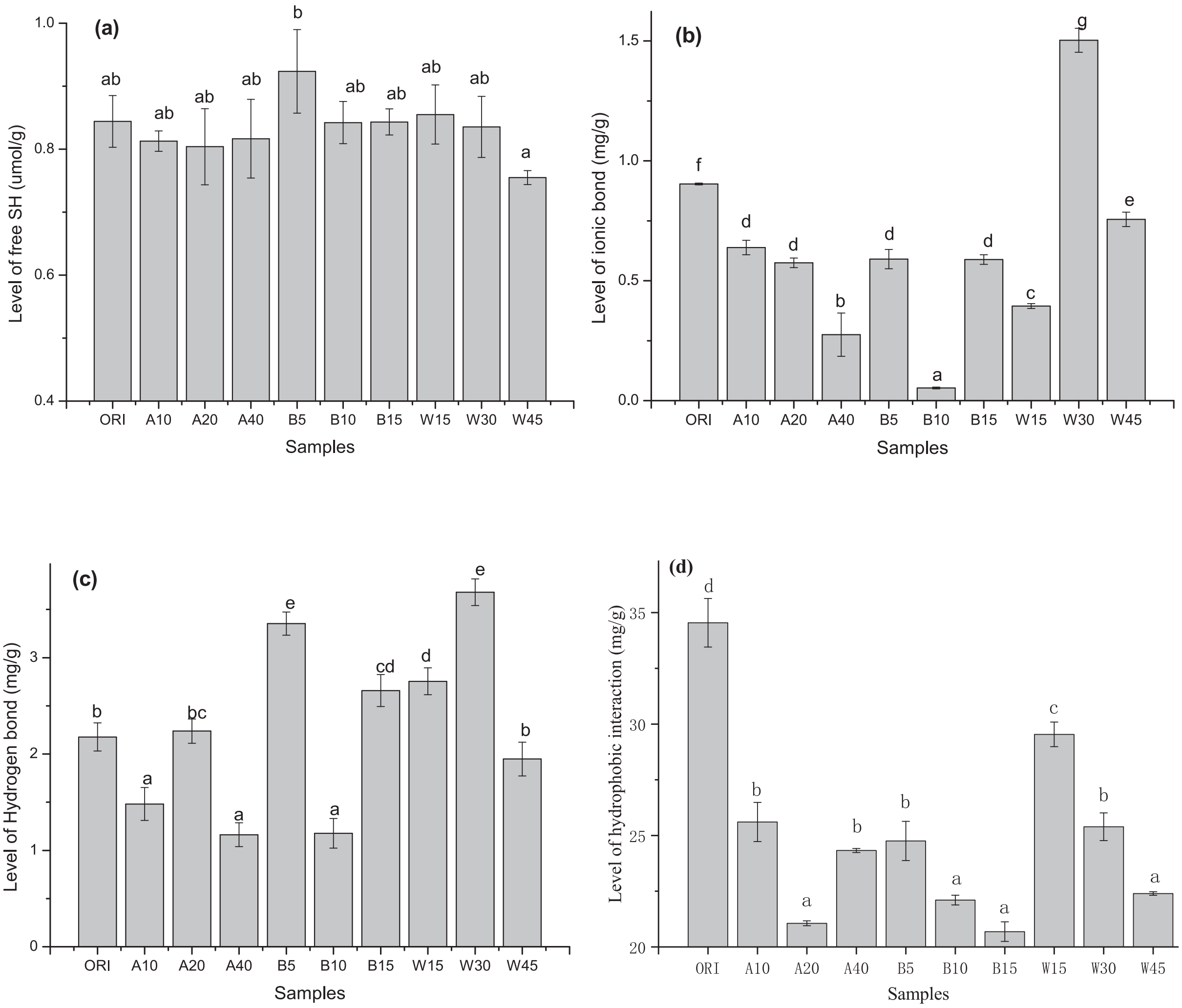

Fig. 2. Sulfhydryl (SH) contents (a), ionic bond (b), hydrogen bond (c) and hydrophobic interaction (d) in compound dough with varied starch content. "A", "B" and "W" refer to A-Type, B-Type granules and whole wheat starches isolated from flour, and the number "5", "10", "15", "20", "30", "40" and "45" refer to the starch weight (g) added in the basic wheat flour (keep total weight 300 g).

dough compositions. Amino acid residues constituting proteins possess a limited number of free side chain groups. In the solution, a majority of amino and carboxyl groups become charged, leading to the formation of ionic bonds in the network structure. As the quantity of A-Type granules in the dough increased, the ionic bonds in dough gradually decreased, suggesting that the incorporation of A-Type granules interfered with the dough network's ion interactions. Wheat starch, especially larger grain size wheat starch, acted as a nonionic polymer, might be located within the gluten network, hindering ion interactions between proteins (Rosell & Foegeding, 2007). This phenomenon could be attributed to the relatively low water binding capacity of A-Type granules, which might sufficiently hydrate the gluten network and enhance water plasticization (Li et al., 2021). Consequently, the ionic strength of the dough decreased as A-Type granules increased. Similarly, with the increase of B-Type granules, the ionic bonds in the dough decreased and reach their minimum value in B10. However, the results varied with the addition of whole wheat starch. Although the ionic bonds in W15 and W45 decreased, they actually increased in W30 achieving a maximum value. This discrepancy might arise when, in the absence of supplemental wheat starch, the dough contained more free water, prompting the facile dissociation of carboxyl and amino groups. Nonetheless, with the inclusion of wheat starch, gluten hydration transformed the free water in the dough into bound water, thereby reducing the ion dissociation capacity of the gluten and decreasing ion bonds. Upon further addition of wheat starch, the binding capacity between wheat starch and gluten protein decreased, and the exposure of ionic bonds escalated. An increased quantity of wheat starch exposed groups with opposing charges in protein molecules, neutralizing the groups with opposite charges on the protein surface and ultimately resulting in a decrease in ionic bonds (Zhang, Mu, & Sun, 2017).

Fig. 2 c illustrates the contribution of hydrogen bonds in dough containing varying levels of wheat starch. Generally, hydrogen bonds predominantly form among water molecules, between bound water and specific functional groups on protein surfaces, and among glutenin molecules. Upon the addition of A-Type granules, there was a noticeable reduction in hydrogen bond content. This might be attributed to the depolymerization of gluten proteins, wherein hydrogen bonds between gluten protein molecules were partially supplanted by those involving water gluten protein molecules and starch gluten protein molecules, consequently leading to a decrease in both dough strength and hydrogen bonds. Conversely, the incorporation of B-Type granules or whole wheat starch resulted in an increased contribution of hydrogen bonding to the dough. This phenomenon could be due to the stable hydrogen bonding force established between the surface hydroxyl groups of B-type starch granules and the hydrophilic amino acids of gluten.

Figs. 2 d shows the contribution of hydrophobic interactions in dough with different amounts of wheat starch addition. The addition of A-Type, B-Type starch granules, or whole wheat starch resulted in lower hydrophobic interactions within the dough as compared to the control dough (ORI). With the increase of A-Type granules, the contribution of hydrophobic interaction in dough first decreased and then increased, reaching its minimum value at A20. In the initial stages of A-Type granules addition, the gluten became fully hydrated, forming more hydrogen bonds, which supplanted hydrophobic bonds, consequently weakening hydrophobic interactions. Upon further addition, non-polar protein groups associated with more free water molecules, leading to the formation of hydrophobic interactions and a slight increase in their presence. The reduction in hydrophobic interactions in dough with whole wheat starch addition was not as pronounced as that observed with the first two types of starch granules, suggesting that the interplay between A- and B-Type starch granules influenced hydrophobic interactions. This observation might be attributed to the distinct surface characteristics of A- and B-Type starch granules, which resulted in varying water absorption capacities.

3.3. The effect on the baking characteristics of frozen dough bread

The baking characteristics of the compound frozen dough bread after 0, 4, and 8 weeks of freezing were measured, and the results were shown in Fig. 3. Fig. 3a illustrated the specific volume change of frozen dough bread during freezing storage. With the extension of freezing time, the specific volume of frozen dough bread demonstrated a declining trend, except that there was no significant difference between the specific volume of A10 and A20 frozen at 4 weeks and that of A10 and A20 frozen at 0 weeks. During the same freezing period, the specific volume of the experiment group supplemented with A-Type granules was consistently lower than that of the ORI group. This implied that the addition of A-Type granules would have a negative impact on the specific volume of frozen dough bread. In the initial week, the specific volume of B5 experienced a slight increase compared to ORI, as the quantity of B-Type granules grew, the specific volume of bread decreased. After 8 weeks of freezing, surpassed that of ORI, and as the B-Type granules continued to rise, the specific volume of bread increased. This indicated that the addition of a small quantity of B-Type granules enhanced the specific volume of fresh bread, while incorporating a larger number of B-Type granules promoted the specific volume of long-term frozen dough bread. Park, Chung, and Seib (2005) also found increasing small starch granules (1–15 μm) can prolong the bread's shelf life. Bread contained 25% to 35% B-Type granules (Soulaka & Morrison, 1985) or 60% B-Type granules had a larger specific volume. These phenomena might be attributed to the optimal surface area and water absorption of 25% to 35% of B-Type granules. As the proportion of whole wheat starch increased, the specific volume progressively decreased, and with the passage of freezing time, the specific volume consistently decreased, which was consistent with the trend of A-Type granules in bread.

Fig. 3b illustrated the alterations in hardness of frozen dough bread with freezing time. With the extension of freezing time, the hardness of frozen dough bread showed an upward trend. Except for the slightly lower B5 at week 0 compared to ORI, the hardness of all other groups was higher than that of ORI. This indicated that incorporating a minimal quantity of B-Type granules enhanced the hardness of freshly baked bread. However, with the extension of freezing time, the increase in the proportion of A-Type, B-Type, and whole wheat starch would lead to an increase in the hardness of frozen dough bread. Roman, Cal, Gomez, and Martinez (2018) found that the bread samples with ratio of 75 A-25B and 25 A-75B exhibited optimal structural properties, characterized by low hardness, high elasticity, and cohesion. Park, Wilson, and Seabourn (2009) also found that flour with different protein contents, there was an optimal range for the addition of B-Type granules, which could produce better bread.

Figs. 3c demonstrated the changes in cell density of frozen dough bread with freezing time. Excluding B10, with the extension of freezing time, an overall decline in cell density became apparent with the extension of freezing time. Upon increasing the proportion of A-Type, B-Type granules, or whole wheat starch, their specific volume exhibited an elevation relative to the cell density of 0RI. At 0-week, B5 possessed the highest cell density, while B10 had the highest cell density after 8 weeks of frozen storage. This indicated that the incorporation of a minimal quantity of B-Type granules could enhance the cell density of fresh bread, whereas the addition of a slightly larger number of B-Type granules resulted in superior pore density for long-term frozen dough bread. This phenomenon could be attributed to the damaged starch and increased surface area of B-Type granules, which in turn augmented water absorption capacity and dough consistency, consequently impeding the expansion of cell during fermentation and baking processes.

Figs. 3d presented the color differences of frozen dough bread crust in relation to freezing time. As freezing time extended, a general decline in the color difference of frozen dough bread crust was observed, with the exception of B5, which experienced a slight increase during 4-week

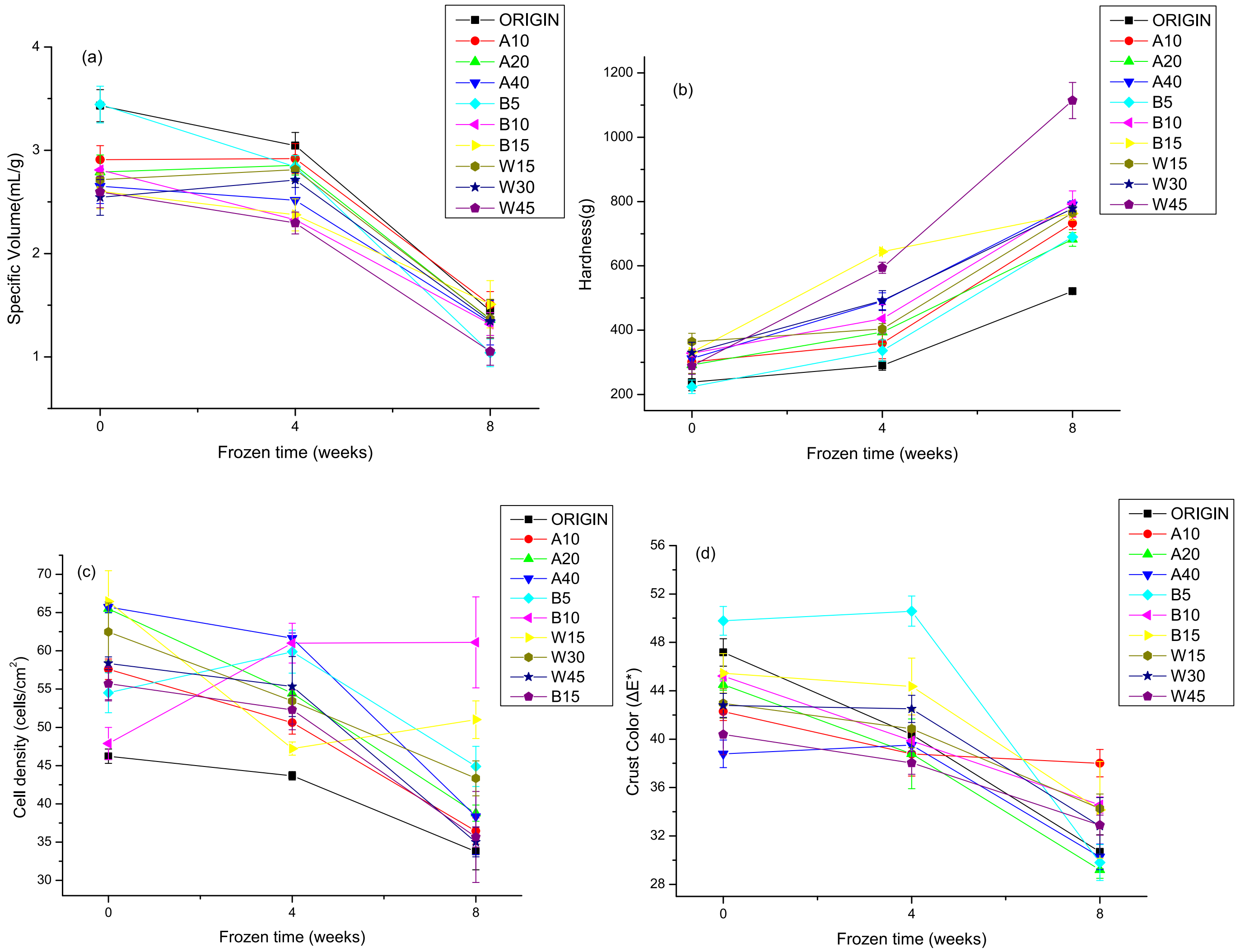

Fig. 3. Specific volume (a), hardness (b), cell density (c) and crust color (d) of compound bread with varied starch content. "A", "B" and "W" refer to A-Type, B-Type granules and whole wheat starches isolated from flour, and the number "5", "10", "15", "20", "30", "40" and "45" refer to the starch weight (g) added in the basic wheat flour (keep total weight 300 g).

frozen storage. At 0-week, the experimental group exhibited a lower bread crust color difference compared to ORI, with the exception of B5. After 8 weeks of freezing, the majority of experimental groups displayed a higher color difference in the crust compared to ORI, with A10 exhibiting the most significant color difference. In summary, the incorporation of a small number of B-Type granules could enhance the color difference of bread crust in short-term frozen dough bread and fresh bread. Conversely, for long-term freezing, the addition of a minimal quantity of A-Type starch granules could increase the color difference of the frozen dough crust. Guo, He, Xia, Qu, and Zhang (2014) also found that granules with large particle size had a positive impact on the specific volume, color, and smoothness of Mantou skin. When flour contained >60% of large starch granules, the resulting Mantou had larger specific volume, improved color, and smoother texture.

3.4. The effect on the moisture distribution of frozen dough bread

The moisture distribution peak area percentages of T_{21}, T_{22}, and T_{23} in the compound frozen dough bread were shown in Figs. 4. T_{21}, T_{22}, and T_{23} represented the binding state of water ranging from closely bound to free. Figs. 4a displayed the distribution of the most tightly bound water in bread. The percentage of T_{21} in the control (ORI) progressively decreased with the prolongation of freezing time, potentially due to the generation of more damaged starch during the freezing process, resulting in higher water holding capacity. Incorporating B-Type starch granules (B5, B15) and a minor quantity of A-Type starch granules (A10) into the flour could increase the percentage of T_{21} in frozen dough bread after 4 weeks of freezing in comparison to ORI. This suggested that that a small number of A- or B-Type granules in short-term freezing for 4 weeks would increase the bound water content in frozen dough bread. However, as the quantity of A-Type granules added increased further, the bound water content was lower than ORI, and the addition of whole wheat starch was consistently remained below ORI. This might be attributed to the dilution and destruction of the gluten network by large starch granules. A-Type granules were not tightly bound to the gluten, leading to a reduction in internal moisture content of the starch granules and an elevation in surface bound moisture content. After 8 weeks of long-term freezing, the T_{21} (bound water) content in all groups remained unchanged or was lower than ORI. In Fig. 4b, the percentage content of T_{22} in ORI progressively increased with the prolongation of freezing time. In both fresh bread and 8 weeks frozen dough bread, an increased proportion of A-Type, B-Type granules and whole wheat starch resulted in a higher T_{22} content than ORI due to gluten dilution. The addition of B-Type granules (B5, B15) and a small number of A-Type granules (A10) after short-term freezing for 4 weeks would result in lower T_{22} content than ORI. In Fig. 4c, T_{23} (free water) content of all

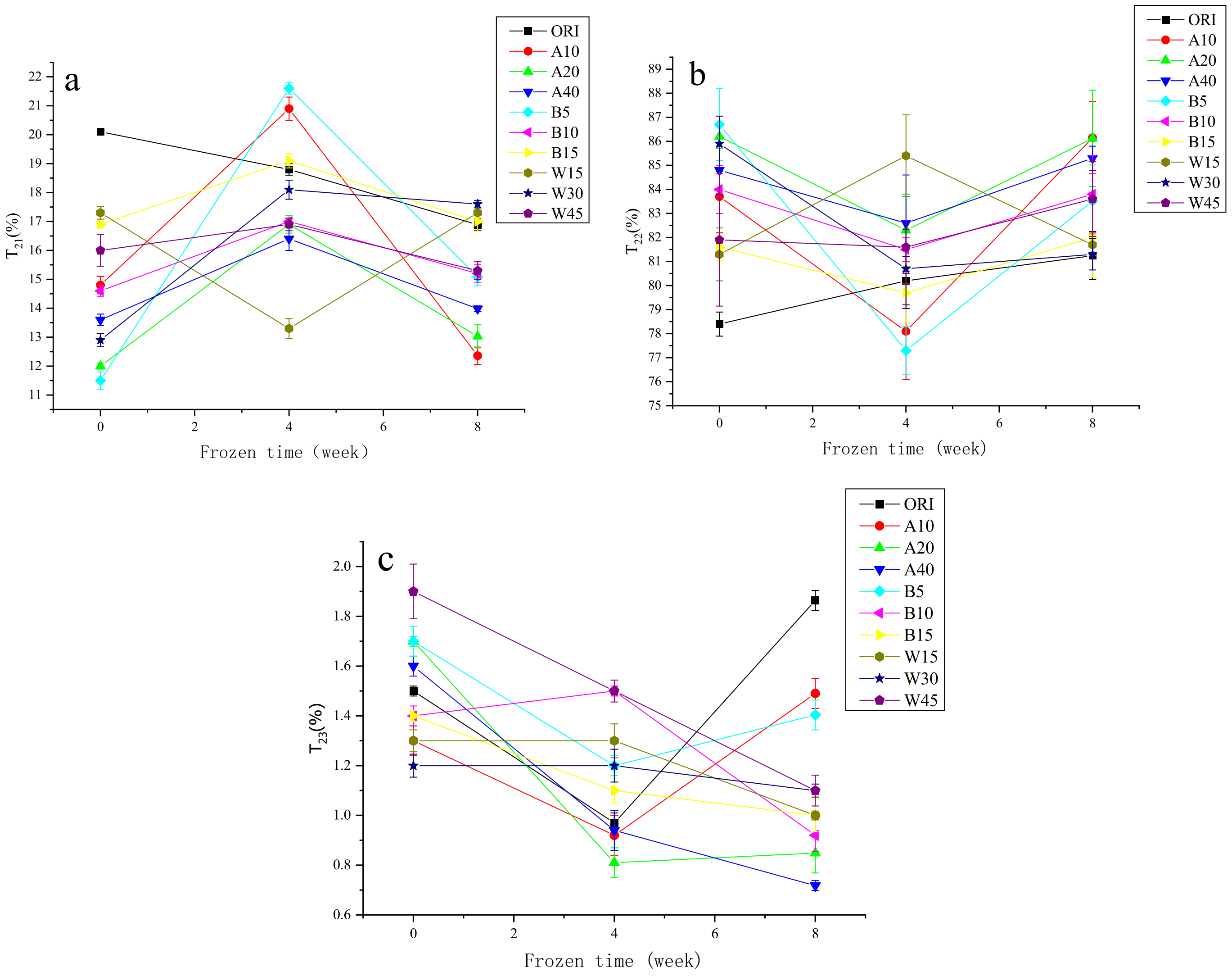

Fig. 4. ^{1}H T_{21}(a), ^{1}H T_{22}(b), ^{1}H T_{23}(c) relative abundance in each proton population of compound frozen dough bread. “A”, “B” and “W” refer to A-Type, B-Type granules and whole wheat starches isolated from flour, and the number “5”, “10”, “15”, “20”, “30”, “40” and “45” refer to the starch weight (g) added in the basic wheat flour (keep total weight 300 g).

experimental groups with additional starch was lower than ORI after long-term frozen storage. However, in fresh bread and short-term frozen storage dough bread, some compound bread caused an increase in free water content compared to ORI, while the T_{23} content of compound bread in long-term freezing was lower than ORI. Considering that more bound water was beneficial for the stability of the dough, the addition of a small amount of A-Type or B-Type granules could improve the freezing stability of the dough.

3.5. *Effect on the starch recrystallization of frozen dough bread*

During the bread cooling process, gelatinized starch molecules spontaneously reorganized, resulting in the reformation of a highly crystallized structure, a phenomenon known as starch retrogradation. The relative crystallinity of compound frozen dough bread was shown in Fig. 5. With the extension of freezing storage duration, the degree of starch recrystallization in the compound frozen dough bread gradually increased, indicating that the aging phenomenon of the bread gradually worsened after the freezing storage process. The deterioration of starch particle structure during the freezing process contributed to higher water absorption of starch, which competed with gluten protein for free water in the bread system and accelerated starch recrystallization. The majority of the compound frozen dough breads have a higher crystallinity than ORI, but the relative crystallinity of B15 notably lower than ORI throughout freezing storage. This suggested that incorporating a large number of B-Type granules during frozen storage might mitigate the recrystallization of frozen dough breads. Conversely, augmenting the proportion of A-Type starch granules or whole wheat starch exacerbated the recrystallization process.

4. Conclusion

Incorporating a small quantity of A-Type granules could effectively fill the pores in the gluten, while the addition of B-Type granules fostered the development of a more compact dough structure in the gluten-starch network. As the proportion of A-Type granules in flour increased, the free sulfhydryl content in the dough remained relatively stable, whereas ionic and hydrogen bonds gradually declined, hydrophobic interactions initially decreased before subsequently increasing. Conversely, as the concentration of B-Type starch granules rose, the content of free sulfhydryl groups in the dough first increased then decreased, accompanied by a reduction in ion bonds and hydrophobic interactions, while hydrogen bonds strengthen. With the incremental addition of whole wheat starch, the content of free sulfhydryl groups and

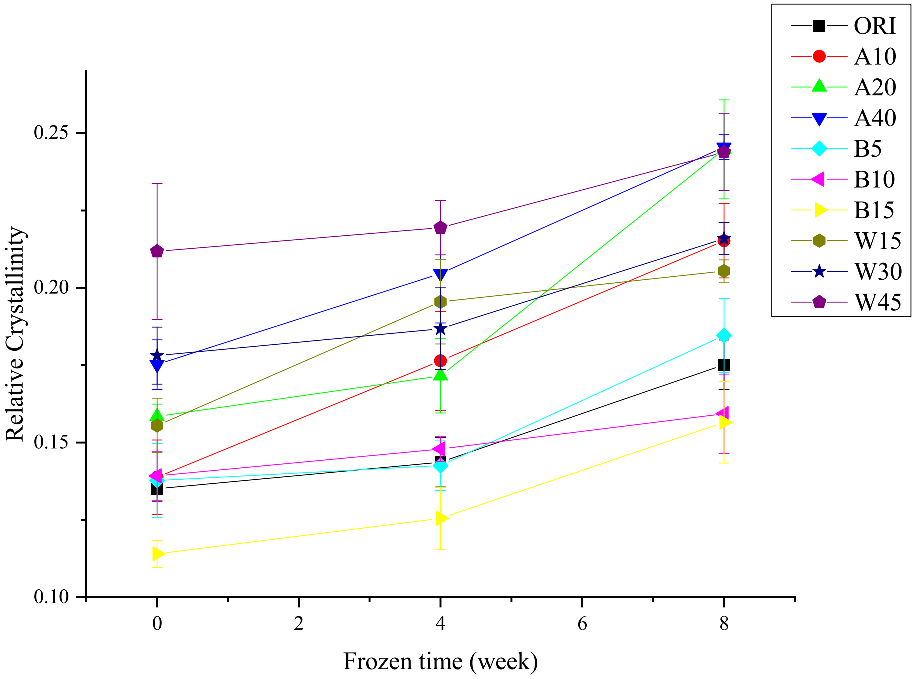

Fig. 5. Relative crystallinities of compound frozen dough bread with varied starch content. "A", "B" and "W" refer to A-Type, B-Type granules and whole wheat starches isolated from flour, and the number "5", "10", "15", "20", "30", "40" and "45" refer to the starch weight (g) added in the basic wheat flour (keep total weight 300 g).

hydrophobic interactions gradually decreased. An increase in the proportion of A-Type granules or whole wheat starch in flour would have a negative impact on the specific volume of frozen dough bread, while an increase in the proportion of B-Type granules would exert a positive effect. Enhancing the proportion of various wheat starches resulted in harder frozen dough bread with higher pore density. During short-term freezing, the introduction of a small quantity of A- or B-Type granules could elevate the bound water content in frozen dough and bread, thereby enhancing the dough's freezing stability. Furthermore, the incorporation of a greater quantity of B-Type granules might mitigate the recrystallization of frozen dough bread. The quality of frozen dough bread can be improved by reasonably selecting the types and proportion of starch granules as well as the starch gluten content in the frozen dough.

CRediT authorship contribution statement

Zixuan Yang: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Jinling Li:** Formal analysis. **Zhili Ji:** Methodology. **Shangyuan Sang:** Resources. **Xueming Xu:** Supervision, Funding acquisition.

Declaration of competing interest

The authors have no conflicts of interest to declare.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the 2023 Open Project of Key Laboratory for Deep Processing of Major Grain and Oil (DZLY2023004), ESI discipline construction project of Wuhan Polytechnic University, Funding for the construction of first-class universities and disciplines in Hubei Province, National Key Research and Development Program of China (2022YFD2100302, 2022YFF1100503), the National Natural Science Foundation of China (No.32001617, 32202076), Zhejiang Provincial Natural Science Foundation of China (LQ23C200003).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101513.

References

Amritpal, K., Khetan, S., Mehak, K., Narpinder, S., Kumar Ahlawat, A., & Mahendru Singh, A. (2016). Physicochemical and rheological properties of starch and flour from different durum wheat varieties and their relationships with noodle quality. *Journal of Food Science and Technology, 53*(4), 2127–2138.

Cao, F.-H., Li, X.-J., Luo, S.-Z., Mu, D.-D., Zhong, X.-Y., Jiang, S.-T., Zheng, Z., & Zhao, Y.-Y. (2017). Effects of organic acid coagulants on the physical properties of and chemical interactions in tofu. *LWT - Food Science and Technology, 85*, 58–65.

Cao, X., Tong, J., Ding, M., Wang, K., Wang, L., Cheng, D., Li, H., Liu, A., Liu, J., Zhao, Z., Wang, Z., & Gao, X. (2019). Physicochemical properties of starch in relation to rheological properties of wheat dough (Triticum aestivum L.). *Food Chemistry, 297*, Article 125000.

Edwards, N. M., Dexter, J. E., & Scanlon, M. G. (2002). Starch participation in durum dough linear viscoelastic properties. *Cereal Chemistry, 79*(6), 850–856.

Gao, X., Liu, T., Ding, M., Wang, J., Li, C., Wang, Z., & Li, X. (2018). Effects of HMW-GS Ax1 or Dx2 absence on the glutenin polymerization and gluten micro structure of wheat (Triticum aestivum L.). *Food Chemistry, 240*, 626–633.

Gómez-Guillén, M. C., Borderías, A. J., & Montero, P. (1997). Chemical interactions of nonmuscle proteins in the network of sardine (Sardina pilchardus) muscle gels. *LWT - Food Science and Technology, 30*(6), 602–608.

Guo, Q., He, Z., Xia, X., Qu, Y., & Zhang, Y. (2014). Effects of wheat starch granule size distribution on qualities of Chinese steamed bread and raw white noodles. *Cereal Chemistry, 91*(6), 623–630.

Han, W., Ma, S., Li, L., Zheng, X., & Wang, X. (2019). Impact of wheat bran dietary fiber on gluten and gluten-starch microstructure formation in dough. *Food Hydrocolloids, 95*, 292–297.

Huang, Y.-C., & Lai, H.-M. (2010). Noodle quality affected by different cereal starches. *Journal of Food Engineering, 97*(2), 135–143.

Li, M., Liu, C., Zheng, X., Hong, J., Bian, K., & Li, L. (2021). Interaction between A-type/B-type starch granules and gluten in dough during mixing. *Food Chemistry, 358*.

Lu, F., Zhu, X.-F., Tao, H., Wang, H.-L., & Yang, Z. (2022). Controlling starch surface characteristics - impact on dough formation in a reconstituted dough system. *LWT, 163*, Article 113591.

Niu, M., Hou, G. G., & Zhao, S. (2017). Dough rheological properties and noodle-making performance of non-waxy and waxy whole-wheat flour blends. *Journal of Cereal Science, 75*, 261–268.

Park, S. H., Chung, O. K., & Seib, P. A. (2005). Effects of varying weight ratios of large and small wheat starch granules on experimental straight-dough bread. *Cereal Chemistry, 82*(2), 166–172.

Park, S. H., Wilson, J. D., & Seabourn, B. W. (2009). Starch granule size distribution of hard red winter and hard red spring wheat: Its effects on mixing and breadmaking quality. *Journal of Cereal Science, 49*(1), 98–105.

Roman, L., Cal, E. D. L., Gomez, M., & Martinez, M. M. (2018). Specific ratio of A-to B-type wheat starch granules improves the quality of gluten-free breads: Optimizing dough viscosity and pickering stabilization. *Food Hydrocolloids, 82*, 510–518.

Rosell, C. M., & Foegeding, A. (2007). Interaction of hydroxypropylmethylcellulose with gluten proteins: Small deformation properties during thermal treatment. *Food Hydrocolloids, 21*(7), 1092–1100.

Soulaka, A. B., & Morrison, W. R. (1985). The bread baking quality of six wheat starches differing in composition and physical properties. *Journal of the Science of Food and Agriculture, 36*(8), 719–727.

Su, X., Wu, F., Zhang, Y., Yang, N., Chen, F., Jin, Z., & Xu, X. (2019). Effect of organic acids on bread quality improvement. *Food Chemistry, 278*, 267–275.

Tao, H., Lu, F., Zhu, X.-F., Wang, H.-L., & Xu, X.-M. (2023). Freezing-induced loss of wheat starch granule-associated proteins affected dough quality: From water distribution, rheological properties, microstructure, and gluten development. *Journal of Cereal Science, 109*, Article 103606.

Tao, H., Lu, F., Zhu, X.-F., Xu, G.-X., Xie, H.-Q., Xu, X.-M., & Wang, H.-L. (2021). Removing surface proteins promote the retrogradation of wheat starch. *Food Hydrocolloids, 113*, Article 106437.

Tao, H., Wang, P., Wu, F., Jin, Z., & Xu, X. (2016). Particle size distribution of wheat starch granules in relation to baking properties of frozen dough. *Carbohydrate Polymers, 137*, 147–153.

Yang, Z., Xu, D., Zhou, H., Wu, F., & Xu, X. (2022a). New insight into the contribution of wheat starch and gluten to frozen dough bread quality. *Food Bioscience, 48*, Article 101777.

Yang, Z., Xu, D., Zhou, H., Wu, F., & Xu, X. (2022b). Rheological, microstructure and mixing behaviors of frozen dough reconstituted by wheat starch and gluten. *International Journal of Biological Macromolecules, 212*, 517–526.

Zhang, D., Mu, T., & Sun, H. (2017). Comparative study of the effect of starches from five different sources on the rheological properties of gluten-free model doughs. *Carbohydrate Polymers, 176*, 345–355.