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Quality deterioration and improvement of wheat gluten protein in frozen dough

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ABSTRACT

Frozen dough technology can effectively recover sensory quality of frozen flour products. This technique has gradually drawn the research interest in the industrialization of staple food. However, the quality of the final frozen products remains inferior to that of fresh products. This study reviewed the deterioration of gluten network in dough caused by ice recrystallization and various additives. This study also investigated the optimization of the freezing process and other empirical improvement techniques. Suggestions for future research were also provided.

1. Introduction

Frozen dough technology refers to the application of frozen technology in the production of bread, cakes, western pastry, or Chinese pastry, as well as refrigeration for certain period, by thawing and other follow-up processing techniques to obtain the finished product of technology [1]. Frozen dough technology provides several advantages: prolonging food shelf life, preventing starch aging, and facilitating transportation, among others. These benefits greatly promote the development of domestic and foreign baking industry chain business model. Frozen dough technology is an important approach to achieving the industrialization of staple food production. However, the occurrences of starch modification, reduction in yeast fermentation capacity, and decrease in the integrity of the gluten network exhibited in the freezing process. These changes endowed frozen dough products with surfaces that are prone to cracking and collapse, rough, and have an uneven internal structure, increased hardness and chewiness, as well as decreased springiness and resilience [2]. Wheat dough is a complex system with a gluten network as the skeleton. This structure interacts with starch, non-starch polysaccharide, sucrose, salt, and other components to form a complex system. The change in gluten (as a skeleton) protein directly affects the quality of the dough. Gluten protein is divided into two main groups: glutenin and gliadin. Glutenin is soluble in dilute acid and alkali and is a macromolecular polymer formed by multiple peptide chains through intermolecular disulfide (SS) bonds. Gliadin is a single-peptide-chain monomer protein soluble in 70% ethanol. These two types of protein considerably vary in both structure and functionality. Gliadin mainly provides gluten viscosity, and glutenin mainly confers its elasticity. A schematic of gliadin subunits and glutenin subunits is presented in Fig. 1 [3]. To explain the relationship between the complex structure and function of gluten protein, numerous studies have proposed a simplified model. A typical gluten protein molecular model is shown in Fig. 2 [4,5]. In the Fig. 2 (A), gluten peptide chains are cross-linked via interchain disulfide bonds; meanwhile, gliadin is mainly bound to gluten by non-covalent bonding forces. In the Fig. 2 (B), the linear protein is a high-molecular gluten protein subunit (HMS), whereas the globule protein includes a low-molecular-weight glutenin subunit (LMS) and monomeric gliadin. Linear HMSs interact with one another via disulfide bonds. HMS and globular proteins are combined via disulfide bonds and noncovalent bonding forces. The number of linear–linear protein interactions and linear–globular protein interactions mainly depends on the effective length of the peptide chain of HMS [6]. The current study described the deterioration of gluten protein from two aspects: (i) the interaction between gluten protein and water, and (ii) the structural and functional properties of gluten protein.

2. Interaction between gluten and water in frozen storage

2.1. Interaction between gluten and moisture in frozen storage

Wheat protein requires the action of water to form gluten. In the process of the dough mixing, gluten protein molecules are gradually hydrated. The

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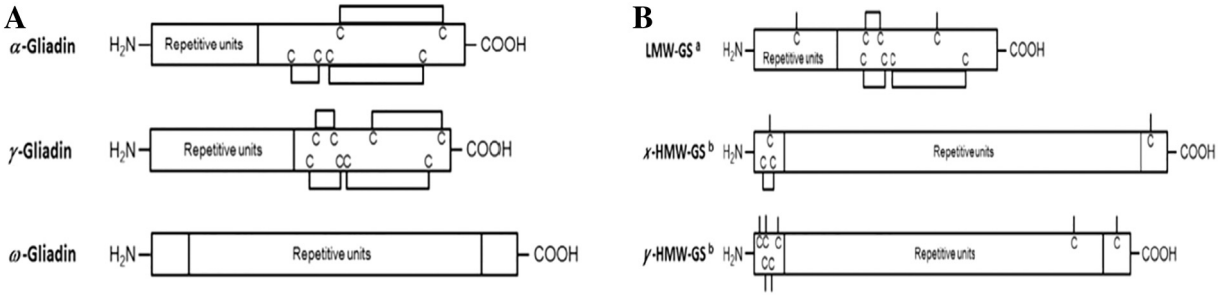


Fig. 1. Schematic of gliadin subunits (A)and glutenin subunits (B) [3].

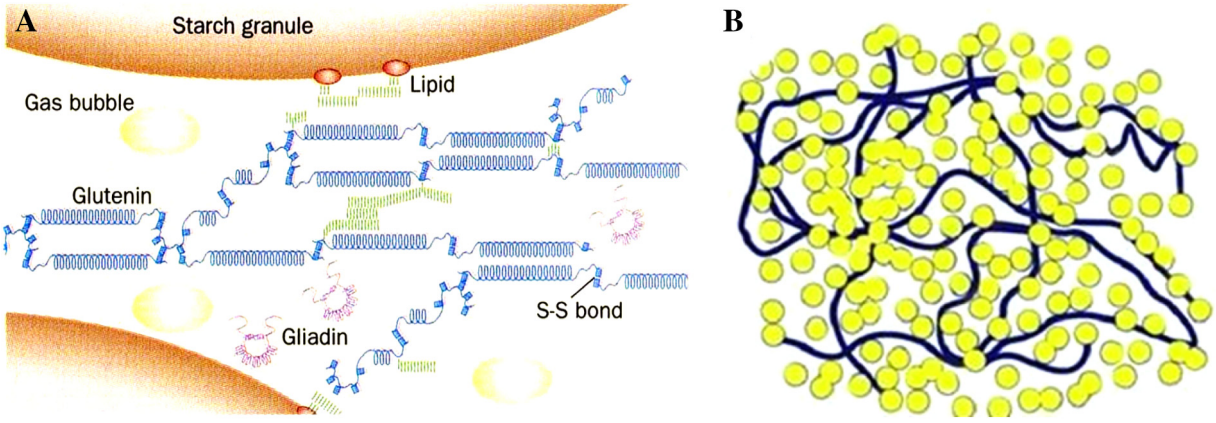


Fig. 2. Structural diagram of gluten proteins in dough (a) and gluten (b) system [4,5].

intermolecular interaction forms a three-dimensional mesh structure. The linear protein forms a ring-and-mesh structure via the disulfide bond. The linear chain and the gliadin are connected by the Van Der Waals force [7]. This structure endows dough with water-retention property, adhesion, and viscous elasticity [8,9]. Thus, the interaction between water and protein in frozen dough needs to be investigated. The first aspect is the change in gluten protein hydration capacity. Schofield et al. [10] reported that each gluten subunit was tightly folded and bonded with one another via hydrophobic interactions, such as aromatic and aliphatic side chains, showing relatively hydrophobic conformation and reduced hydration capacity. Wang et al. [11] found that protein was closely bound with decreased rigid water content, indicating that the moisture in gluten had been redistributed (Fig. 3). Wang et al. [6] also compared the changes in moisture of gluten, glutenin, and gliadin, and changes in gluten and gliadin rigid water and restrictive water were basically the same, indicating that the weakened force of gluten protein water might be mainly caused by increases in non-frozen rigid water and limited. The third is the increase in frozen water content, resulting in ice recrystallization. Esselink et al. [12] found that owing to ice recrystallization, the gluten dehydration occured after 6 weeks of freezing. The starch particles were exposed outside the gluten network structure, and a hollow part was observed. The gluten protein network structure was significantly fractured [13–15]. Kontogiorgos et al. [11] revealed gluten network transformation after refrigeration for 1 d at the microstructural level by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). SEM imaging showed that the gluten network structure were surrounded by homogeneous and irregular voids. TEM results verified the presence of irregular voids mainly attributed to the filling of ice crystals. Small ice crystals aggregate to form large ice crystals in accordance with the principle of freeze concentration. Two mechanisms of ice recrystallization have been identified: (i) enlargement of ice crystals in the capillary tube structure of the gluten network, and (ii) regrowth of larger ice crystals outside the capillary tube [16].

2.2. Effect of adding water on frozen dough products

The amount of water used depends mainly on the water absorption capacity of the flour used. In noodle preparation, if the amount of water was insufficient, the noodles failed to form good gluten tissue; excessive water led to wet and soft noodles, inhibiting rolling [14]. The hardness and breaking load of frozen noodles decreased with an increase in water amount. However, the surface of the noodles with less than 36% water content was rough and prone to cracking. The tensile strength and hardness of the noodles with excess water content decreased. Thus, from a rheological perspective, 36% is the most suitable amount of water. The effect of adding water on the quality of frozen dough buns indicates that when the amount of water is insufficient, the taste of steamed bread is relatively dry, and the surface tends to crack. By contrast, an excessive amount of water hinders the shaping of the steamed bread, affecting ice crystallization and causing more voids for the steamed bread. Adding about 53% of water could improve the quality of the product [17].

3. Changes in the physicochemical properties of gluten protein in frozen storage

3.1. Changes in the foaming nature of gluten protein in frozen storage

Gliadin determines the fluidity, extensibility, and expansibility of dough. The foamability of gluten protein is closely related to that of gliadin. The central domain of gliadin is hydrophilic, whereas the C-terminal is

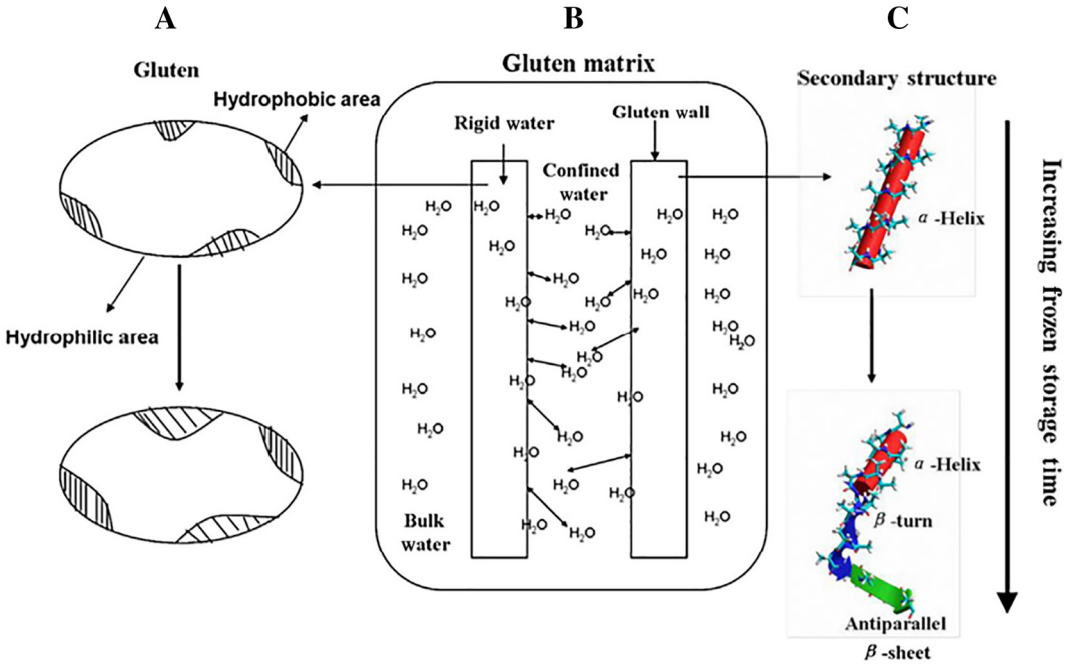


Fig. 3. Schematic of hydrophobic moiety exposure (A), water redistribution (B), secondary structural changes (C) in gluten matrix with the increase frozen storage time [11].

hydrophobic. A decrease in the hydrophobicity of gliadin can reduce the amphipathic property of protein, leading to the deterioration of the foaming property. In this study, the solubility of gliadin in frozen storage did not change significantly, but the foaming volume of gliadin decreased by 26.18% during frozen storage for 45 d, indicating that its foaming properties deteriorated. This deterioration was mainly attributed to the decrease in protein content involved in foam formation. This reduction caused a decrease in foaming volume, affecting the quality of flour products [18]. However, with a change in refrigeration time, the foaming stability was improved. This improvement validates the theory that the foamability of protein is always opposite to its foam stability. Proteins with good foamability generally exhibit poor foam stability. The poorer the foaming ability of protein, the better its foam stability because these qualities rely on different types of molecular properties. Foaming ability depends on the rapid diffusion of protein molecules, reduction of interface tension, and distribution of hydrophobic groups, whereas foam stability is mainly determined by the solubility, hydrophobicity, and flexibility of peptide chains. Foam stability is mainly determined by the rheological properties of the protein solution, such as the water content of protein in the adsorption membrane, protein concentration, membrane thickness, and appropriate intermolecular interaction in protein [19]. Gliadin changes its secondary structure, rearranges its molecular conformation, reduces its flexibility, and increases its surface tension stably in frozen storage; these changes are unfavorable for foaming properties [6].

3.2. Changes in the dynamic rheological characteristics of gluten protein in frozen storage

The dynamic rheology of dough relates to the process and final quality of the product. The three main parameters of dynamic rheological characteristics are as follows: elastic modulus G′, which represents the elastic nature of the substance; viscous modulus G″, which denotes the viscous nature of matter; and loss angle tanδ (tanδ = G″/G′), which reflects the proportion of the viscoelasticity of the system. The higher the value of tanδ is, the greater the proportion of viscosity is in the system, which is characterized by fluidity. The smaller the tanδ value, the greater the proportion of elasticity, and the system is characterized by solidity. The loss angle of the dough, increased by the frequency of scanning [20], indicated that the change in G′ in the dough was larger than that in G″ and a decrease in the content of high polymer in the dough system. The viscous modulus (G″), elastic modulus (G′) and loss angle (tanδ) of gluten protein after frozen storage decreased significantly relative to those of fresh gluten protein, and the longer the frozen storage period, the lower its viscoelasticity [21]. These findings may be attributed to the gradual growth of ice crystals during frozen storage, damaging the gluten matrix and other components. Gluten micelles become thin or broken so as to destroy its network structure and change the viscoelasticity of the dough. The increase in viscoelastic modulus after freezing may result from the changes in the binding ability between the gluten network structure and starch [22]. The maximum tensile resistance of frozen dough was positively correlated with the volume of bread, as determined by empirical rheological experiments [23]. However, predicting the final product quality of frozen dough on the basis of rheological parameters remains inconclusive [6]. Other studies showed that the hardness and swelling capacity of wheat in frozen storage decreased, and the effect on flour with poor gluten quality was more serious.

3.3. Changes in the thermodynamic properties of gluten protein in frozen storage

The thermal properties of gluten reflect the trend in change after gluten protein is heated. Denaturation temperature (Tp) and enthalpy change (ΔH) are the main parameters of protein in thermal denaturation. Wang et al. [6] analyzed the thermodynamic properties of protein during frozen storage. Protein subjected to frozen storage for 60 d exhibited increases in the Tp of gluten protein by 3.37 °C and the Tp of glutenin by 1.58 °C. These results reflect an improvement in the low-temperature stability of the two proteins. After frozen storage, the degradation temperature (Td) of gluten protein decreased by 9.4 °C and glutenin by 5.6 °C, indicating that their high-temperature stability deteriorated. Moreover, no significant change in gliadin Td occurred, suggesting that this change was mainly attributable to glutenin. The ΔH of the three proteins decreased, reflecting a reduction in the ordered structure of proteins. The disorder development of frozen dough is an important reason for deterioration [24].

4. Changes in the molecular composition and spatial structure of gluten protein during frozen storage

4.1. Effect of frozen storage on the molecular weight of gluten protein

During dough processing, the molecular weight distribution of gluten protein determines the physical properties of the dough. Ribotta et al. [25] indicated that glutenin subunits with a molecular weight of 88,700–129,100 Da markedly decreased in molecular weight as frozen storage time was extended under the frozen storage condition of −18 °C. During the process, disulfide bonds in gluten protein were broken, and macromolecular gluten protein (10^5–10^9 Da) was depolymerized; meanwhile, the depolymerization of gluten protein molecules with molecular weights of 3×10^5– 4×10^8 Da intensified after repeated freeze–thaw cycles [26]. The range of the molecular weight distribution of soluble protein did not change as the frozen storage time extended, but the content of soluble protein increased with the prolongation of frozen storage time. Glutenin macropolymer (GMP) depolymerization caused by frozen storage was inversely proportional to glutenin content [6].

4.2. Effect of frozen storage on free sulfhydryl (SH) group and amino groups of gluten protein

The disulfide bond structure of natural wheat gluten is unstable from mature seeds to final product (such as bread). α/β-gliadin and γ-gliadin with monomer structures have 3 and 4 intrachain disulfide bonds, respectively, and polymerized LMW- and HMW-GS have both intrachain and interchain bonds. In addition to the nature of disulfide bonds, the structural state of disulfide bonds also controls the molecular mass distribution of glutenin, which is one of the key factors determining dough quality. Therefore, understanding the function of disulfide bonds is important to understand the structure and properties of gluten proteins. The considerable significance of disulfide bonds can be proved by adding a reducing agent to weaken the dough and adding a mercaptan blocking agent or an oxidizing agent to strengthen the dough.

Studies have shown that free sulfhydryl (SH) group increased by 3.1 μmol/L after frozen storage for 120 d. Two main reasons have been identified. First, frozen storage can reduce free water content, induce freeze concentration effect, and increase the probability of disulfide bond exchange reactions within and between proteins. Second, in frozen storage, ice crystals recrystallize, resulting in partial cleavage of disulfide bonds inside and outside the protein molecule and exposure of sulfhydryl groups inside the molecule, thus increasing the content of free sulfhydryl groups [27]. Glutenin aggregates mainly rely on disulfide bonds to maintain the macromolecular structure, and determine the elasticity and hardness of the dough. Thus, the reduction in glutenin disulfide bond led to the reduction in glutenin macropolymer, which mainly caused the reduction in the elasticity and hardness of the dough [28].

Changes in the free amino and carboxyl contents of gluten protein side chains exert apparent effects on the tensile breaking force, tensile distance, elasticity, hardness, and chewiness of raw and cooked noodles. Gluten protein contains a specific amount of active proteolytic enzymes, such as azocaseinase and haemoglobinase, which can cut off the peptide chain of gluten protein and lead to an increase in free amino group content [29]. Zhao et al. [30] proposed that the content of the free amino group of gluten protein hardly changed during frozen storage for 120 d. This result shows that proteolytic enzyme is inhibited at low temperatures, gluten protein is not hydrolyzed, and breakage of peptide chain is prevented.

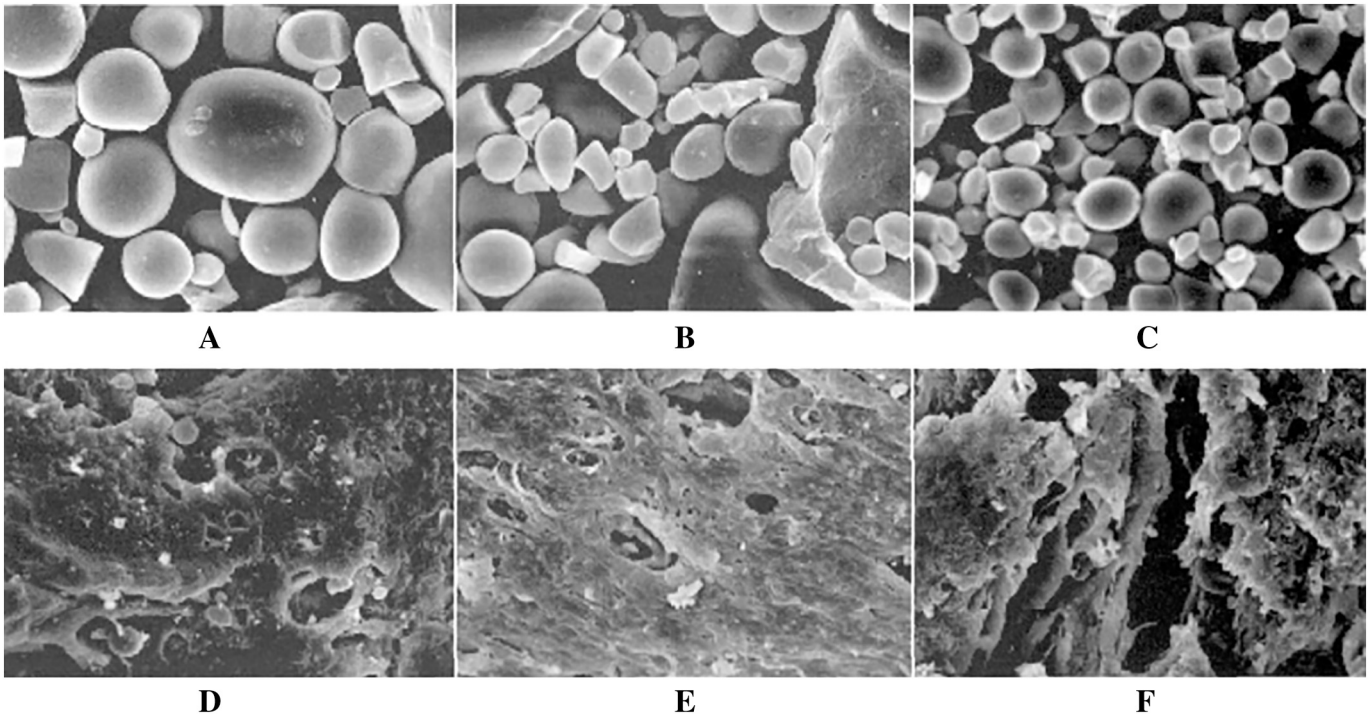
4.3. Effect of frozen storage on protein spatial structure

The secondary structure of the protein refers to the local spatial arrangement and composition of the main chain atom in the polypeptide chain, excluding the structure of the side chain part. The structural units of the protein main chain conformation include α-helices, β-sheets, β-turns, and random coils. The amino acid side chain R in the peptide chain is distributed outside the spiral; its shape, size, and charge affect the formation of the α-helix. Areas where acidic or alkaline amino acids are concentrated are not conducive to α-helix formation because the similar charge and larger areas of R (e.g., phenylalanine, tryptophan, and isolunine) also hinder α-helix formation. Proline is not easily twisted because its α-carbon atoms are located on a five-member ring, and hydrogen bonds are not easily formed. Therefore, the formation of the aforementioned α-helix presents a challenge. The R group of Glycine is H, which occupies a small space, affecting the stability of the helix it contains. The β-sheet is a structure with fairly stretched peptide chains. The plane of the peptide chain folds into serrations, and the adjacent peptide bond planes are at an angle of 110°. The R-side chain of the amino acid residue extends above or below the serrations. The conformation is stabilized by hydrogen bonding between CO and NH, between two peptide chains or two peptide chains in one peptide chain. In protein molecules, the peptide chain often appears to fold back 180°. The conformation at this kink angle is a β-turn (or a β-bend). In the β-turn, a hydrogen bond is formed between the CO of the first amino acid residue and the NH of the fourth residue, thus stabilizing the structure. A random coil is not a determining regular part of the peptide chain structure, and a peptide chain in the irregular arrangement of the peptide bond plane belongs to a loose random coil [31].

Sharadanant et al. [32] found that the more orderly α-helix structure of gluten protein in frozen storage was transformed into antiparallel β-sheet and β-turn [33]. The reductions in α-helix and β-turn are attributed to changes in the hydration environment during freezing. The mechanical force generated by ice crystal growth and the reduction of free water lead to the breakage and reduction of the hydrogen bond maintaining the α-helix and β-turn. The helix and corner structures are destroyed to form small molecular substances. While the free water is reduced, the small molecular substances aggregate with one another under the action of non-covalent bonds, increasing the β-sheet content. The α-helix structure is stable in nature, tough, and elastic. Reduction in the content of the α-helix structure also plays an important role in the reduction of elasticity and hardness of dough. A change in the secondary structure showed that freezing induced the rearrangement of molecular conformation of gliadin, decreased the flexibility of the molecular chain, and lowered adsorption capacity at the gas–liquid interface, leading to the degradation of foaming performance [34]. Zayas et al. [35] proposed that proteins with more random coil structures exhibited stronger flexibility and were easier to be adsorbed to the gas–liquid interface, thus reducing the surface tension and enhancing the foaming performance.

Hydrophobic interaction is one of the important forces to maintain the spatial structure of proteins. The surface hydrophobicity of gluten protein increased with an increase in the number of temperature fluctuations, frozen storage time, and number of frozen circulations [36]. Stable disulfide bonds and non-covalent bonds in frozen storage participate in molecular aggregation behavior, which rearranges the molecular structure of gluten protein and exposes the hydrophobic sites of gluten protein. During frozen storage for 60 d, the surface hydrophobicity index of gluten protein gradually decreased as the frozen storage time extended. This decrease might be caused by the aggregation of gluten molecules; consequently, the hydrophobic groups on the surface of gluten protein were buried and surface hydrophobicity was reduced [37]. Inoue et al. [38] also demonstrated that the exposure of hydrophobic groups increased water fluidity, which was also related to a decrease in the water absorption rate of protein.

Studies have shown that regrowth of ice crystals under repeated freeze–thaw cycles accelerated the destruction of the network structure of the dough and reduced the quality of surface products [25,39,40]. The morphology of starch and gluten clusters after 0, 2, and 4 freeze–thaw cycles under a field-emission scanning electron microscope is presented in Fig. 4 [41]. Zhang et al. [42] used wheat gluten protein as a raw material in the preparation of edible membrane and examined the change in performance under different low-temperature storage conditions. With a change in refrigeration temperature, the performance of wheat gluten protein membrane changed slowly, and the regularity was not apparent. With an extension in the refrigeration and freezing time, the tensile strength increased and then decreased, the elongation rate gradually increased, and the barrier performance gradually weakened.



Notes: (a), (b) and (c) represent the microscopic morphology of starch frozen and thawed 0, 2, and 4 times at a multiple of 5000, respectively; (d), (e) and (f) represent the microscopic morphology of gluten frozen and thawed 0, 2, and 4 times, magnified at 2000×, respectively.

Fig. 4. Microscopic morphology of starch and gluten frozen and thawed 0, 2, and 4 times [41].

4.4. Relationship between the degree of GMP depolymerization, frozen storage time, and gliadin content

In the gluten network structure, linear glutenin can form a highly networked skeleton via the action of the disulfide bond outside the chain and non-covalent force; moreover, spherical gliadin is filled in this structure via non-covalent force. Melnyk et al. [43] proposed that a large amount of gliadin could hinder the formation of a glutenin network, inducing a decrease in the compactness of the glutenin network as gliadin content increased, thus weakening the strength of the gluten network. Therefore, when the gliadin content is high, the gluten network strength is low and more vulnerable to the destruction of ice crystals during frozen storage, resulting in an increase in the degree of GMP depolymerization. A multiple linear regression analysis model was established by Wang et al. [6]. Significance analysis of frozen storage time, gliadin content, and degree of depolymerization showed that the *P* value of the model was less than 0.01, so that the relationship between these parameters could be regarded as statistically significant. The regression coefficient of the model was 94%, indicating that the degree of GMP depolymerization was 94%, as predicted using these two variables.

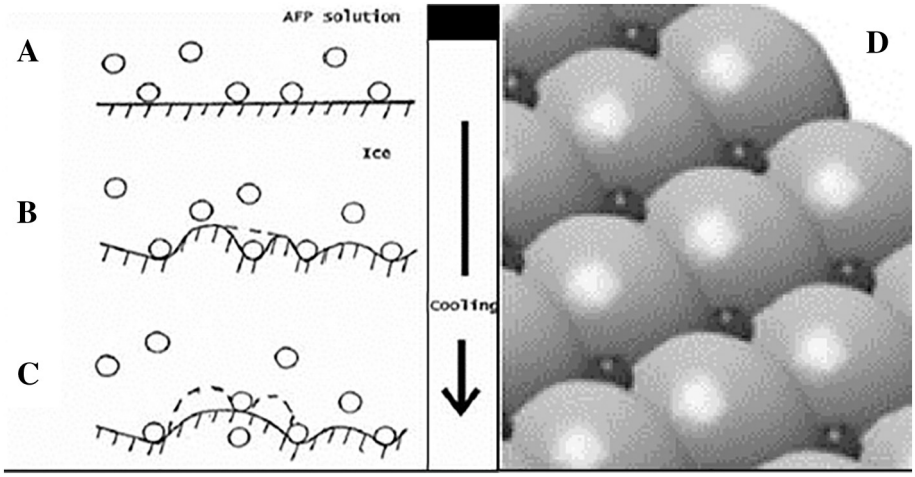
5. Relevant techniques for improving frozen dough

5.1. Regulation of gluten water interaction

The interaction between gluten and water is mainly regulated by controlling the growth of ice crystals, which can be divided into the following aspects.

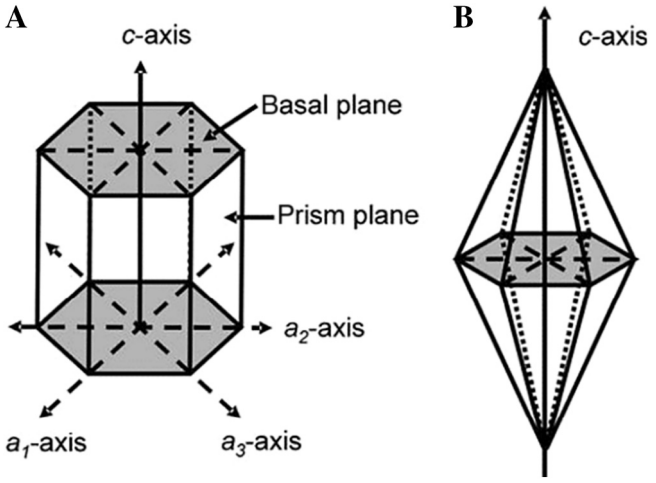
Hydrophilic colloid, of which chemical essence is polysaccharides, contains a large number of hydroxyl groups and easily combines with hydrogen bonds in water. Thus, it exhibits strong hydrophilicity and can compete for water with polymers such as protein and starch, as well as lower the water activity of frozen dough. The effect of hydrophilic colloids on frozen dough depends on its type, amount of addition, as well as the formulation and processing of the frozen dough. Xanthan gum can stabilize the structure of frozen dough via its strong interaction with flour protein. The gum can also increase water absorption in the dough system, air retention of the dough, final volume of bread, and water activity of the bread core. Ferrero [44] observed reduced water mobility when xanthan gum and pectin were used in the study. Guar gum can prolong the shelf life of frozen flour products by preventing their dehydration and shrinkage. Carboxymethyl cellulose creates a mesh structure by interacting with the hydrosol chain, which can control the water loss rate of frozen dough during frozen storage. Carrageenan and konjac-derived glucomannan can be combined with glutenin and bound water to form a hydrophilic compound, so as to improve the water holding capacity of the dough and reduce water migration.

Ice structure protein (ISP or AFP), also known as a thermal hysteresis protein, has three main functional characteristics: thermal hysteresis activity, ice crystal recrystallization inhibition (RI), and the effect of ice crystal morphological modification. The separation of ice crystal formation temperature and ice crystal melting temperature is defined as thermal hysteresis. The most widely accepted thermal hysteresis theory is the so-called absorption–inhibition model [45–47]. According to this model, ISPs exhibit irreversible adsorption on the surface of ice crystal; as ice crystals grow, the ISP molecules on the ice crystal surface combine together. This process results in the gradual formation of hemispherical ice crystals, reducing the curvature of the ice crystal interface. According to the Gibbs-Thomson equation, the increase in the curvature of the ice crystal interface lowers the solution ice point. ISPs can be adsorbed to the surface of the ice crystal to inhibit migration of these ice crystals. This inhibition exerts an RI effect, as described in the Kelvin effect, which inhibits the growth model of the ice crystal (Fig. 5) [48]. In pure water, ice crystals usually stretch in the a-axis parallel to the basal plane, while in the surface direction of the lattice (c-axis) stretch only slightly. In an ISP solution, the growth of ice crystals changes. The interaction between ISP molecules and the surface of ice crystals leads to a change in the sequence of water molecules on the outer layer of the crystal lattice surface. The ice nuclei grew into shapes of spicules and fibers along the c-axis, forming symmetrical bipedal pyramid-shaped ice crystals (Fig. 6) [49]. Adding ISPs separated from carrots to the frozen dough lowered yeast mortality in the frozen melting cycle, inhibited ice crystal growth, and improved the gas-holding capacity of frozen dough [50]. Ding et al. [51] separated ISPs from barley; the



Notes: A–C: The sphere represents ISPs, the solid line represents the surface of ice crystals, and the dotted line represents newly formed ice. A to C illustrate the process of temperature reduction. D: Simulated ice crystal growth surface. Dark dots represent ISP molecules, and light-colored large spheres represent ice crystal surfaces.

Fig. 5. Theoretical model of ice growth inhibition by ISPs binding to ice [48].



Notes: A. Pure water solution, B. ISP solution.

Fig. 6. Ice crystal modified by ISPs during ice growth [49].

apparent specific heat of frozen dough increased, the frozen water content decreased, and the melting temperature increased. Liu et al. [52] expressed three recombinant antifreeze proteins (rAFPs) with different characteristics by using *Pichia pastoris* heterogeneously; using different rAFPs led to an improved compound effect. AFPs could reduce the fluidity of dough moisture during the freeze–thaw cycle and reduce the migration of water [53].

5.2. Strengthening of the gluten protein structure

The gluten structure is mainly reinforced by flour screening and additive addition. With respect to component, the quality of glutenin is more important than the properties of gliadin and starch. Gluten strength is more important than the content. In the frozen dough preparation, high gluten flour is generally selected.

Salt is dissolved in water to accelerate water absorption of flour and facilitate even distribution of water. Simultaneously, sodium ions and chloride ions are distributed around the protein, which can play a role in fixing the water. It is favorable for protein to absorb water quickly to form gluten and tightly adhere to one another, thereby enhancing the elasticity, extensibility, and toughness of gluten. Therefore, the greater the amount of salt added, the higher the tensile strength and hardness of noodles. This finding indicates that salt can enhance the toughness and elasticity of noodles. The optimum salt content for noodles was 2.5% [14].

Emulsifiers possess an amphipathic property, which promotes the crosslinking of gluten protein to form a three-dimensional mesh structure. Emulsifiers can also interact strongly with gluten and starch to form a stable compound and inhibit the starch retrogradation and degeneration of protein in freezing. They enhance the elasticity, toughness, and ductility of dough during modulation. They also improve the rheomorphism and baking properties of frozen dough.

Enzyme preparations, xylanase, amylase, and glucose oxidase are mainly suitable for frozen dough and its products. Enzymes mainly function to decompose flour components, such as starch and xylan. The water released after xylan is decomposed can improve the hydration of the dough. Amylase decomposes broken starch particles that soften the dough. These results enhance the extensibility of the dough. Oxidizing enzyme preparations can promote sulfhydryl bond to form disulfide bond so as to strengthen the chemical structure. Glucose oxidase plays an oxidant role, converting glucose into gluconic acid and simultaneously generating strong oxidant hydrogen peroxide. Glucose oxidase oxidizes the sulfhydryl bond in gluten molecules into the disulfide bond to strengthen gluten and enhance the structural strength of the dough. Transglutaminase can catalyze the acyl transfer between the γ-hydroxylamine group of protein glutamine residue and many primary amine compounds to promote co-valent cross-linking between proteins, thereby enhancing gluten strength.

Soluble dietary fiber (SDF) can improve the tensile properties, farinaceous properties, and aging of steamed bread to a certain extent [54]. Insoluble dietary fiber is covalently linked to many polymers via dual-ferulic acid bridges, reducing solubility. It exhibits high water absorption and has a more complex branch structure. Findings on the influence of dough remain inconclusive. Courtin et al. [55] found that SDF could increase the viscosity, the elasticity, extension resistance, and the hardness of the dough. Rouau et al. [56] reported that the B-WUAX opposite the dough could play a dual role in the improvement and deterioration of the dough. Zhang et al. [57] used a farinograph to analyze whole wheat dough and showed that the wheat bran size was reduced from 609 μm to 278 μm, significantly reducing the formation time. Compared with crude bran or fine wheat bran dough, wheat bran subjected to superfine grinding significantly increases the stabilization time of the dough ($P < 0.05$). Ultrafine wheat bran particles may be less destructive to the formation of the dough gluten network [58]. Ma et al. [59] evaluated the effect of wheat bran dietary fiber (WBDF) on changes in gluten structure and aggregation behavior in gluten (G) and gluten-starch (G + S) systems. Six WBDF levels were established. The correlation between the WBDF level and gluten

depolymerization behavior was provided in the G + S system. The secondary structure of WBDF (Fig. 7) was assumed to induce a change in gluten protein content [59]. The proportion of the β-sheet and antiparallel β-turn between the molecules of the blank control group was increased relative to that of the added group after frozen storage; by contrast, the proportion of random coil, α-helix, and β-turn decreased [60]. The results showed that the addition of wheat bran powder caused the absorption of the water in gluten protein by wheat bran. This effect resulted in the dehydration of gluten protein and an alteration in the secondary structure of gluten protein. As frozen storage time extended, one sulfhydryl bond in the gluten protein of the blank control group and the additive group exhibited an increasing trend; one disulfide bond exhibited a decreasing trend. The extent of change was larger in the additive group than in the blank control group. This result is attributed to the free radicals scavenging ability of hydrophilic groups in wheat bran [61].

Using other additives, Bigne et al. [62] prepared dough with 15%, 25%, and 35% of mesquite instead of wheat flour. The rheological characteristics of the thawed dough at 20 °C for different freezing periods were measured by structural profile analysis, stress relaxation, and dynamic oscillation. ESEM was used to analyze the damage to the structure of the frozen dough. When 15%–25% replacement was added, the composite wheat mesquite dough could be frozen and stored without substantial rheological changes. When the added amount reached 35%, the gluten network structure deteriorated.

5.3. New refrigeration technology

Similar to microwave-assisted freezing, radio frequency-assisted freezing, magnetic freezing, and electrolytic freezing, ultrasonic-assisted freezing (UAF) improved the G″ and G′ of the dough. The technique increased the stability of the dough by migration from weakly combined water to the combined water [63]. The results of FTIR analysis indicated that with ultrasonic-assisted dough proofing, the random coil content in dough increased, the secondary structure of protein transited from order to disorder, and the hardness and flexibility of dough decreased and then increased. The results of SEM observation showed that the microstructure of the dough treated by ultrasound was more uniform, which was conducive to gluten protein extension and gluten protein network formation. UItrasonic-assisted dough proofing can change the moisture distribution, as well as the microstructure and secondary structure of the dough, improving its quality. In one study, UAF at 288 W and 360 W reduced the total freezing time of the dough by more than 11%, decreased the size of ice crystals, and resulted in a relatively high sensory evaluation index [64]. Ultrasonic wave led to a decrease in the degree of supercooling during nucleation of frozen food [65–67]. Kiani et al. [68] demonstrated that UAF could increase the activity of lactic acid bacteria cells and improve the freezing quality at temperature ranging from −4 °C to −20 °C.

Research and development related to auxiliary freezing is still in progress. Further study and a summary of the mechanism of freezing deterioration in the opposite group can contribute to the development of new freezing methods. There are three important aspects worth studying: the combined effect of different auxiliary freezing methods, parameter optimization of existing auxiliary freezing methods, and control of freezing rate and freezing temperature on the basis of the new freezing method. The effects of the frozen melting cycle on the gluten structure and thermal properties of Glu-B1-bit high-molecular-weight gluten subunit (HMW-GS) variant were evaluated using the near-isogenic line NIL1 (Bx6 and By8) and NIL2 (Bx14 and By15). The results showed that the reduction in protein weight was independent of the composition of HMW-GS; moreover, the effect of the freezing thawing cycle on the gluten structure and thermal properties of NIL2 with superior subunits was less than that of NIL1. Thus, NIL2 was more suitable for the preparation of frozen dough than NIL1 [69].

6. Conclusions and prospects

The deterioration of the frozen dough during the freezing process can be suppressed though the flour raw materials, antifreeze additives, antifreeze proteins, refrigeration rate, and auxiliary refrigeration, among others. However, any product improvement should consider operation convenience, adaptability, high value added, and low cost. Moreover, the demand on food nutritional quality should not be ignored.

The AX (arabinoxylan) content in the whole wheat grain is about 4% to 8%, and that in the endosperm or flour is about 1.5% to 2.5%; meanwhile, the proportion of bran reaches 20% to 25%; as a by-product of wheat processing, bran can be used as a good backfilling material for wheat flour products. AX content in wheat tends to increase from the inside out. WUAX (Water unextractable arabinoxylan) comprises a much larger share of the total AX relative to WEAX (Water extractable arabinoxylan), roughly 9:1, in bran. WUAX, an adhesive substance between protein and starch, considerably affects the effective separation of protein and starch. Research on WUAX can contribute to improving the yield of starch and wheat gluten. The WUAX content was correlated with the hardness of endosperm, which

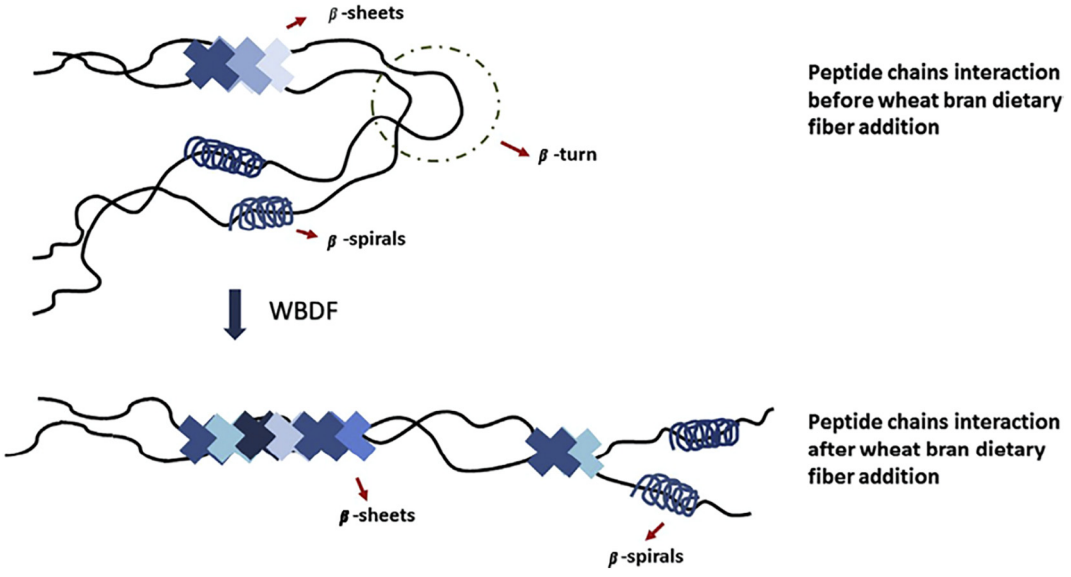


Fig. 7. Schematic description of the proposed hypothesis of wheat bran dietary fiber-induced changes in the secondary structure of gluten [59].

could affect the flour extraction rate and starch crushing rate [70]. WUAX can more efficiently delay aging compared with WEAX, and research on this subject can help lengthen the shelf life of flour products. Conducting more studies on the utilization of insoluble fiber is encouraged to improve the utilization rate of wheat bran and to realize the efficient utilization of cereal by-products.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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