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Scientific Article

Solubility of Part-Baked Bread Gluten Protein During Frozen Storage

Solubilidad de las proteínas del gluten en pan pre-horneado durante el almacenamiento en congelación

Ana Irene Ledesma-Osuna[1], Beatriz Montaño-Leyva[1], José Luis Espinoza-Acosta[2], Mariela Menchaca-Armenta[1], María Fernanda Amaya-Villalva[1], Guadalupe Amanda López-Ahumada, and Jesús Enrique Gerardo-Rodríguez[1]*

[1]Universidad de Sonora. Departamento de Investigación y Posgrado en Alimentos. Hermosillo, Sonora 83000, México
[2]Universidad Estatal de Sonora (UES). Navojoa, Sonora, México

*Correspondencia: Correo electrónico: jesusenrique.gerardo@unison.mx (Jesús Enrique Gerardo Rodríguez)
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Abstract

This study investigates how freezing rate and part-baking time affect the solubility of gluten proteins in bread, aiming to improve production in the frozen bakery industry. Bread samples were part-baked for 3 and 6 min, frozen at either slow (0.15 °C/min) or fast (1.45 °C/min) rates and stored at -20 °C for up to 56 days. Every 14 days, samples were analyzed to measure the solubility of low and high molecular weight glutenin (LMW-GLU and HMW-GLU), gliadins, and free thiol content. Statistical analysis (ANOVA) revealed that part-baking time, freezing rate, and frozen storage time significantly influenced protein behavior ($P < 0.01$). LMW-GLU solubility decreased 71.2 % with longer part-baking and frozen storage, while HMW-GLU levels increased 1.5 % under the same conditions, indicating protein aggregation. Free thiol content decreased 14.5 % (0.35 mol SH/g protein), with longer part-baking but was better retained with fast freezing rate. Overall, fast freezing proved to be more effective at preserving protein and bread quality, offering valuable insights for optimizing industrial frozen bread production processes.

Keywords: **gluten network, free thiol, protein denaturalization, freezing, part-baked bread**

Resumen

Este estudio investiga cómo la velocidad de congelación y el tiempo de pre-horneado afectan la solubilidad de las proteínas del gluten en el pan, con el objetivo de mejorar la producción de la industria de panificación. Las muestras de pan se pre-hornearon durante 3 y 6 min, se congelaron a velocidad lenta (0,15 °C/min) o rápida (1,45 °C/min) y se almacenaron a -20 °C durante 56 días. Cada 14 días, se analizaron las muestras para medir la solubilidad de las gluteninas de bajo y alto peso molecular (GLU-LMW y GLU-HMW), las gliadinas y el contenido de tioles libres. El análisis estadístico (ANOVA) reveló que el tiempo de pre-horneado, la velocidad de congelación y el tiempo de almacenamiento en congelación influyeron significativamente en el comportamiento de las proteínas ($P < 0.01$). La solubilidad del GLU-LMW disminuyó 71.2 % con el pre-horneado y el almacenamiento en congelación prolongados, mientras que los niveles de GLU-HMW aumentaron 1.5 % en las mismas condiciones, lo que indica agregación proteica. El contenido de tioles libres disminuyó con un pre-horneado más prolongado 14.5 % (0.35 mol SH/g proteína), pero se conservó mejor con una congelación rápida. En general, la congelación rápida demostró ser más eficaz para preservar las proteínas y la calidad del pan, lo que ofrece información valiosa para optimizar los procesos de producción industrial de pan congelado.

***Palabras clave*: red de gluten, tioles libres, desnaturalización de la proteína, congelación, pan pre-horneado**

1. Introduction

Frozen dough bakery products and frozen part-baked bread have gained increasing popularity (Halagarda, 2017). Among these two categories, the part-baked bread segment alone was valued at approximately US$5.59 billion in 2024 and is projected to grow to US$9.33 billion by 2032, representing a compound annual growth rate (CAGR) of 6.38 % over the period from 2025 to 2032 (Datamintelligence, 2025). This success is attributed to several advantages over traditional breadmaking methods, including a wider distribution area, improved efficiency, and reduced time and labor in bread production. However, this process presents challenges, such as ice crystal formation, water redistribution, loss of yeast viability, and protein denaturation.

Unlike frozen dough, part-baked bread process benefits from a completed fermentation stage, preventing loss of yeast viability. Nevertheless, these factors still affect the functional properties of dough, resulting in quality deterioration in the final product. One of the most impacted quality parameters is the dough's fermentative capability, which declines due to reduced gluten network strength and stability, impairing gas retention (Castillo-Arias et al., 2024). Gluten is composed of glutenin (20 %) and gliadin (80 %), which are responsible for the viscoelastic properties of dough. Despite being present in a lower proportion, glutenin plays a crucial role in determining gluten quality, as they form a structural backbone through terminal linkages via disulfide bonds in gluten proteins. Glutenin consists of low molecular weight glutenin subunits (LMW-GLU) and high molecular weight glutenin subunits (HMW-GLU), with the latter ranging from approximately 77 to 160 kDa. HMW-GLU has been strongly correlated with dough quality (Ding et al., 2024). Osborne, (1907) classified wheat proteins based on their solubility in different solvents, categorizing gliadin and LMW-GLU as soluble in alcohol, while HMW-GLU is soluble in acidic solutions.

During frozen storage, ice crystals grow while the overall water balance remains unchanged. Souza-Guimarães et al. (2025) observed ruptures in gas cell membranes, likely caused by mechanical damage to the gluten network due to ice crystal formation. The freezing rate significantly influences gluten structure and yeast viability. Slow freezing rates (<1 °C/min) promote large ice crystal formation, disrupting the gluten network and reducing CO_2 retention capacity (Su et al. 2024). Conversely, fast freezing rates (>1 °C/min) generate smaller crystals during nucleation, causing less damage to gluten proteins. These deterioration processes lead to gluten network weakening, disulfide bond rupture, and undesirable protein modifications (Bai et al., 2022). An adequate freezing rate regulates recrystallization and water distribution, facilitating a rapid transition to the glassy phase. This phase creates a highly viscous medium (10^8-10^{12} Pa·s), significantly reducing molecular mobility and restricting chemical reactions (Roos, 2021). The glass transition temperature is a key parameter in food preservation, as it marks the point at which chemical and enzymatic processes stop. This temperature is influenced by various factors, including moisture content and the molecular composition of the food (Mahato et al., 2019).

To mitigate the adverse effects of freezing on part-baked bread, various strategies have been explored, including modifications to process parameters, dough formulation adjustments, and the use of additives (Gaikwad & Arya, 2018). On the other hand, the evaluation of free thiol content (SH) has represented an important indicator of reduction of disulfide bonds on products obtained by these strategies, principally frozen dough and part-baked bread (Wei et al., 2025). This study aims to evaluate the physicochemical changes through the assessment of solubility and free thiol content in gluten proteins (glutenin and gliadin) of dough induced by part-baking time, freezing rate, and frozen storage time.

2. Materials and methods

2.1 Raw material

Wheat of Borlaug variety was obtained from Yaqui Valley, Sonora, and milled using an experimental mill (Brabender Quadrumat Jr. Mill Quadruplex; Brabender Instruments, South Hackensack, NJ, USA) to obtain refined flour. It was reposed for 15 days and sieved to particle size of 200 μm. The characterization was carried out with the following AACC 2000 methods: moisture content (44-01.01), protein content (46-12.01), and ash content (08-01.01). Empirical rheological properties of the flour were evaluated using a farinograph (Brabender Instruments, model 810143, South Hackensack, NJ, USA) (54-21.02), and an alveograph (Chopin Instruments, Villeneuve-La-Garenne, France) (54-30.02). Fresh yeast was provided by Azteca S.A. of C.V from Guadalajara México. Salt and shortening were obtained from a local store in Hermosillo, México.

2.2 Dough preparation

To assess gluten proteins in bread formulation, the following ingredients were used: wheat flour (100 %), shortening (5 %), fresh yeast (3 %), and salt (1.5 %). The ingredients were mixed (National MFG brand mixer, Lincoln NE, US) for 4 min. The amount of water incorporated into the formulation was 61.2 % which was determined based on farinograph analysis. The fermentation was

conducted in a controlled chamber (National MFG brand, Lincoln, NE, US) at 30 °C and 85 % relative humidity (RH) for 60 min following the procedure described by Gerardo-Rodriguez et al. (2021). Subsequently, the fermented dough was divided into 50 g portions and shaped manually to form pieces of bread.

2.3 Part-baking and freezing of dough

The formed dough was part-baked for 3 and 6 min in a convection oven (National MFG, Lincoln NE, US) and 85 % RH at 250 °C. Part-baking times were determined considering different stages of baking, through preliminary experiments. The part-baked bread was cooled for 1 h until 25 °C and packed in hermetic bags. The samples were frozen at -20 °C with slow freezing rate (0.15 °C/min) in a freezer (Frigidaire, model GLFC1526FW, Mississauga, Ont., Canada), or a fast freezing rate (1.45 °C/min) in an ultra-freezer (Thermo Fisher Scientific LCC, model UXF40086A62, Ashville, NC USA) for 56 days. The freezing rate was determined by using a thermocouple attached at the center of the dough to measure the time necessary to reach -20 °C. The samples were freeze-dried every 14 days for evaluation.

2.4 LMW-glutenin and gliadin solubility

Soluble proteins were extracted twice using 1 mL of 50 % propanol solution. Samples were vortexed and centrifuged for 5 min (Rani et al., 2023). Each extraction (supernatant) was collected to analyze protein solubility using a high-performance liquid chromatography (HPLC) system (Agilent 1260 Infinity Quaternary LC System, mod. 240; Palo Alto, CA, USA) equipped with an Agilent 1260 Infinity diode array detector. Protein detection was performed using a BIOSEP-SEC-S 4,000 column (Phenomenex, Torrence, CA) with 5 μm particle size, 7.8-mm diameter, and 200-mm length. The mobile phase was acetonitrile/water (50:50 v/v) with 0.1% trifluoroacetic acid (Lookhart et al., 2003). The column temperature during analysis was 40 °C, and the flow rate was 0.5 mL/min. The chromatograms detected at 210 nm exhibited three elution peaks: the first peak corresponding to soluble polymeric proteins, and the last two peaks associated with monomeric proteins. The analyzed protein fractions included soluble polymeric proteins and gliadins.

2.5 Insoluble protein quantification

The precipitate obtained from the extraction of soluble protein was washed twice with 50 % propanol and dried to form pellets. This fraction corresponded to insoluble proteins (HMW-glutenin). Subsequently, the pellets were pulverized to quantify total protein by Dumas method (AACC, 46-30.01) using a nitrogen determinant (LECO brand model FP-528, Michigan, USA) with a conversion factor of N x 5.7.

2.6 Free thiols content

The free thiol (SH) content of protein in freeze-dried part-baked bread samples was analyzed following the method used by Marti et al. (2017) with modifications. Briefly, 60 mg of samples were

suspended in a solution containing 50 % isopropanol, 80 mM Tris HCl, and Ellman's reagent. Absorbance was recorded at A_{412} and results were expressed as μmol thiol/g protein of flour. Analyses were conducted in triplicate independent experiments.

2.7 Experimental design and statistical analysis

A factorial design of 2x3x5 was used. The factors and levels were freezing rate (slow 0.15 °C/min or fast 1.45 °C/min), part-baking time (fresh dough, 3 and 6 min) and frozen storage time (fresh dough, 14, 28, 42 and 56 days). An analysis of variance (ANOVA) with a confidence level of 95 % was performed. The Tukey test was carried out for differences between specific means. Experimental design and statistical analysis were performed using the software SAS 9.4.

3. Results and discussion

3.1 LMW-glutenin and gliadin solubility

The ANOVA results in the effects of freezing rate, part-baking time, and frozen storage time on the solubility of low molecular weight glutenin (LMW-GLU) and gliadins (GLI) indicate a significant effect ($P<0.01$) of part-baking time, followed by the interaction between freezing rate and frozen storage time, on both glutenin and gliadins.

The impact of part-baking time and frozen storage time on the solubility of LMW-GLU and GLI in part-baked bread is shown in Table 1. Across all frozen storage time, LMW-GLU and GLI decrease with increasing part-baking time. In fresh dough (not stored), LMW-GLU decreased by 31.5 % and 71.2 % after 3 and 6 min of part-baked time, respectively. Similarly, after 14 days of frozen storage, LMW-GLU decreased by 14.2 % and 91.2 % by 3 and 6 min of part-baked time, respectively. This trend remains consistent on 28, 42, and 56 days of frozen storage. This decline is likely attributed to heat-induced denaturation of LMW-GLU proteins. However, no clear tendency was evident regarding the effect of frozen storage time. The weakening of frozen dough during prolonged frozen storage has been evidenced in other studies by viscoelastic tests, which indicate a reduction in fermentative capacity and lower loaf volume in bread made from frozen dough (Carboni et al., 2022).

Regarding the effects of freezing rate on LMW-GLU solubility in part-baked bread, a slower freezing rate was associated with higher solubility at 3 min of part-baking, suggesting reduced glutenin damage. In dough stored for 14 days, yeast cells remain viable, reducing the harmful effects of freezing since intracellular ice crystal formation is minimal. However, as frozen storage progresses, water redistribution leads to dough dehydration, enlarging ice crystals in gas cells and altering the original protein structure (Armenta-Aispuro et al., 2024). This process, known as recrystallization, modifies the number, size, and shape of ice crystals (Jiang et al., 2025), limiting water availability and impairing the formation of inter- and intramolecular bonds. Thus, hydrophilic interactions between proteins become weaker, contributing to further degradations of the gluten network (Li et al., 2023).

After 3 min of part-baking, some yeast viability remains; however, by 6 min, the opposite effect was observed, with rapid freezing proving more beneficial in the absence of yeast. The effects of part-baking time and frozen storage time on gliadin solubility in part-baked bread is shown in Table 1.

Longer part-baking times result in greater solubility loss, decreasing 7.1 and 12 % at 3 and 6 min of part-baking time respectively for not stored dough. This trend was consistent throughout all frozen storage time. This reduction is likely associated with gliadins forming hydrophobic bonds with other proteins, including glutenin, leading to reduced functionality and the formation of a rigid crumb structure. Solubility remains relatively stable in fresh dough and 3 min of part-baking but decreases after 6 min. This suggests that minimal damage occurs in fresh dough, whereas extended thermal treatment induces significant protein denaturation.

Regarding the effects of freezing rate and frozen storage time on gliadin solubility in part-baked bread, fast freezing enhances protein solubility over frozen storage time, whereas slow freezing maintains a constant solubility level. These findings suggest that a slow freezing rate appears to better preserve glutenin stability, making it a potentially favorable option for bread production. On the other hand, SDS gel electrophoresis carried out by Yu et al. (2020) showed an increase in soluble proteins in frozen dough, indicating ongoing protein degradation during frozen storage, however, in this study this tendency was not evidenced.

Tabla 1. Efecto del tiempo de pre-horneado, velocidad de congelación y tiempo de almacenamiento en congelación sobre la proteína soluble (glutenina de bajo peso molecular y gliadina) del pan pre-horneado.
Table 1. Effect of part-baking time, freezing rate and frozen storage time on soluble protein (LMW-glutenin and gliadin) of part-baked bread.

Frozen storage (days)	Freezing rate (°C/min)	Part-baking (min)	LMW-GLU ($x10^{10}$)	GLI ($x10^{10}$)
Fresh dough		0	1.84 ± 0.15^{c}	3.68 ± 0.01^{d}
		3	1.26 ± 0.06^{f}	3.42 ± 0.17^{fg}
		6	0.53 ± 0.07^{k}	3.24 ± 0.01^{h}
14	0.15	0	2.04 ± 0.04^{b}	3.76 ± 0.03^{b}
		3	1.75 ± 0.02^{c}	3.61 ± 0.03^{e}
		6	0.18 ± 0.01^{m}	3.22 ± 0.02^{h}
	1.45	0	1.34 ± 0.40^{e}	3.66 ± 0.11^{d}
		3	0.53 ± 0.13^{k}	3.58 ± 0.04^{e}
		6	0.32 ± 0.02^{l}	3.11 ± 0.02^{i}
28	0.15	0	2.07 ± 0.04^{b}	3.81 ± 0.07^{a}

		3	1.11 ± 0.05^{fg}	3.62 ± 0.01^{de}
		6	0.33 ± 0.02^{l}	2.88 ± 0.31^{j}
	1.45	0	1.48 ± 0.12^{d}	2.94 ± 0.08^{j}
		3	1.11 ± 0.18^{f}	3.10 ± 0.02^{i}
		6	0.31 ± 0.02^{l}	2.51 ± 0.26^{k}
42	0.15	0	1.77 ± 0.16^{c}	3.68 ± 0.01^{d}
		3	1.03 ± 0.03^{h}	3.49 ± 0.05^{f}
		6	0.63 ± 0.01^{k}	2.09 ± 0.03^{m}
	1.45	0	1.70 ± 0.10^{c}	3.59 ± 0.02^{e}
		3	0.91 ± 0.06^{i}	3.37 ± 0.01^{g}
		6	0.67 ± 0.01^{j}	2.00 ± 0.11^{m}
56	0.15	0	1.65 ± 0.09^{cd}	3.46 ± 0.11^{fg}
		3	1.55 ± 0.08^{d}	3.35 ± 0.17^{gh}
		6	0.50 ± 0.01^{k}	2.33 ± 0.16^{kl}
	1.45	0	1.47 ± 0.03^{e}	3.38 ± 0.05^{g}
		3	1.63 ± 0.01^{d}	3.71 ± 0.01^{c}
		6	0.96 ± 0.06^{i}	3.39 ± 0.29^{gh}

The values of LMW-GLU and GLI represent the area under the curve on the SEC-HPLC chromatograms. LMW-GLU: Low molecular weight glutenin; GLI: Gliadin.
Values with different letters in the same column are significative different (P<0.05)

3.2 Insoluble protein quantification

The ANOVA results indicate a significant effect (P<0.01) of part-baking time, followed by frozen storage time, on high molecular weight glutenin (HMW-GLU) concentration.

Fig. 1 illustrates the impact of part-baking time and frozen storage time on HMW-GLU content. In fresh dough, there is approximately 6 % of HMW-glutenin (insoluble protein), whereas at 3 and 6 min of part-baking, a slight increase in insoluble protein concentration (up to 1.5 %) was observed. This phenomenon may be explained by the partial unfolding of α-helix structures into β-sheet and random coils structures before exposure to elevated temperatures causing thermal denaturation and transforming soluble protein into insoluble complexes (Fan et al., 2024). During part-baking, the temperature enhances hydrophobic interactions and promotes the formation of insoluble protein complexes, a process further intensified by moisture loss. This trend persists across all frozen storage time. During protein extraction, previously soluble protein material may have become insoluble due to heat exposure, thereby increasing its measured concentration at 3 and 6 min of part-baking. Increased hydrophobic interactions may reduce the cross-linking of unfolded HMW-GLU proteins, thereby weakening the gluten network (Yang et al., 2025).

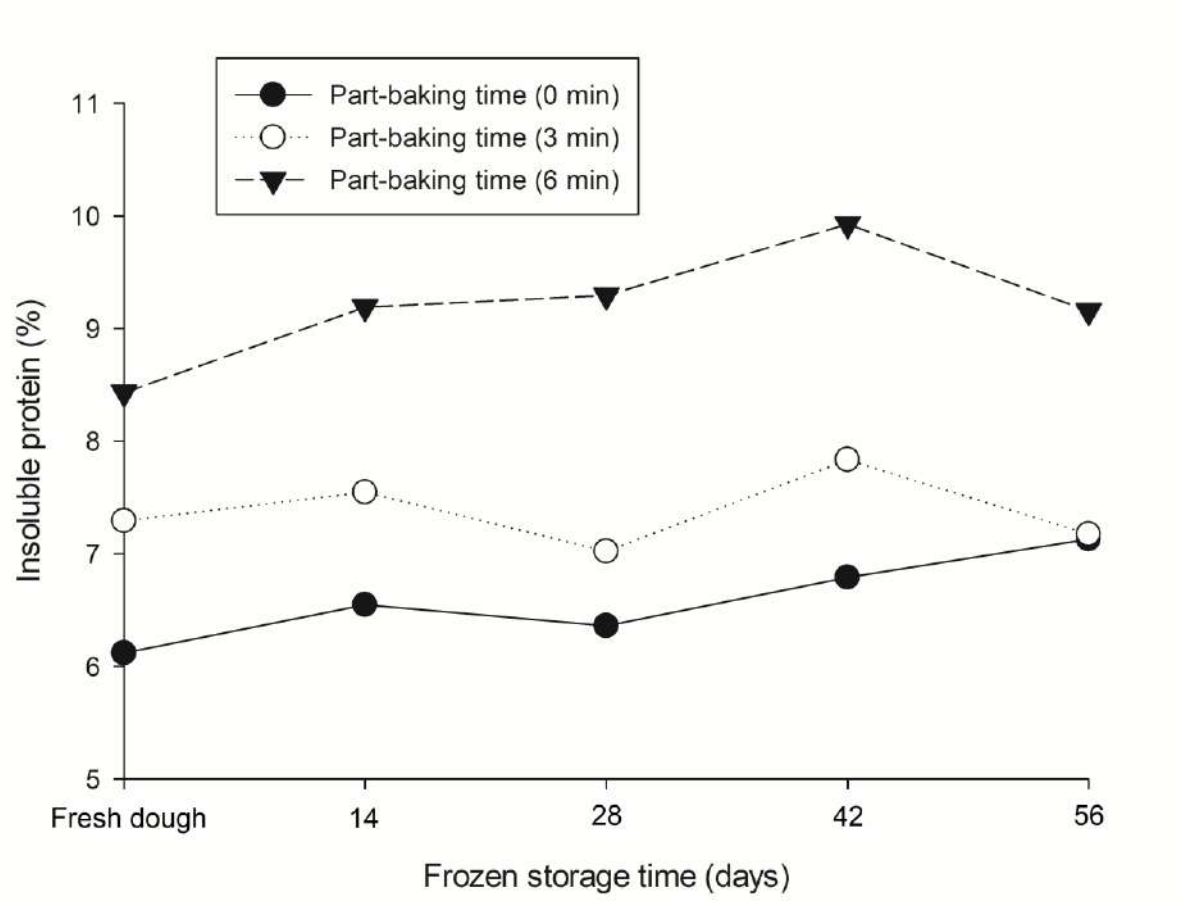

Figure 1. Effect of part-baking time and frozen storage time on the insoluble protein (HMW-GLU) of part-baked bread.

Figura 1. Efecto del tiempo de pre-horneado y el tiempo de almacenamiento en congelación sobre la proteína insoluble (HMW-GLU) del pan pre-horneado.

Conversely, when gluten proteins are exposed to high temperatures, a deamination process occurs, leading to peptide chain folding and structural deformation. Additionally, according to another studies, the gelatinization of starch granules further compacts the protein structure by reinforcing the folded conformation (Chen et al. 2021).

Fig. 2 depicts the effect of freezing rate and frozen storage time on insoluble proteins in part-baked bread. The freezing rate did not show a significant influence on HMW-GLU content ($P<0.05$). Higher proportions of insoluble protein were observed at fresh dough and 28 days of frozen storage, whereas lower levels were found at 14, 42 and 56 days. Under slow freezing conditions, HMW-GLU levels remained constant throughout the frozen storage period. This observation contrasts with previous studies that reported reductions due to freezing and oxidation-reduction reactions (Zhang et al. 2024). In the present study, an increase in insoluble protein during frozen storage was observed only under fast freezing conditions, suggesting that fast freezing rate may be more effective in preserving glutenin functionality.

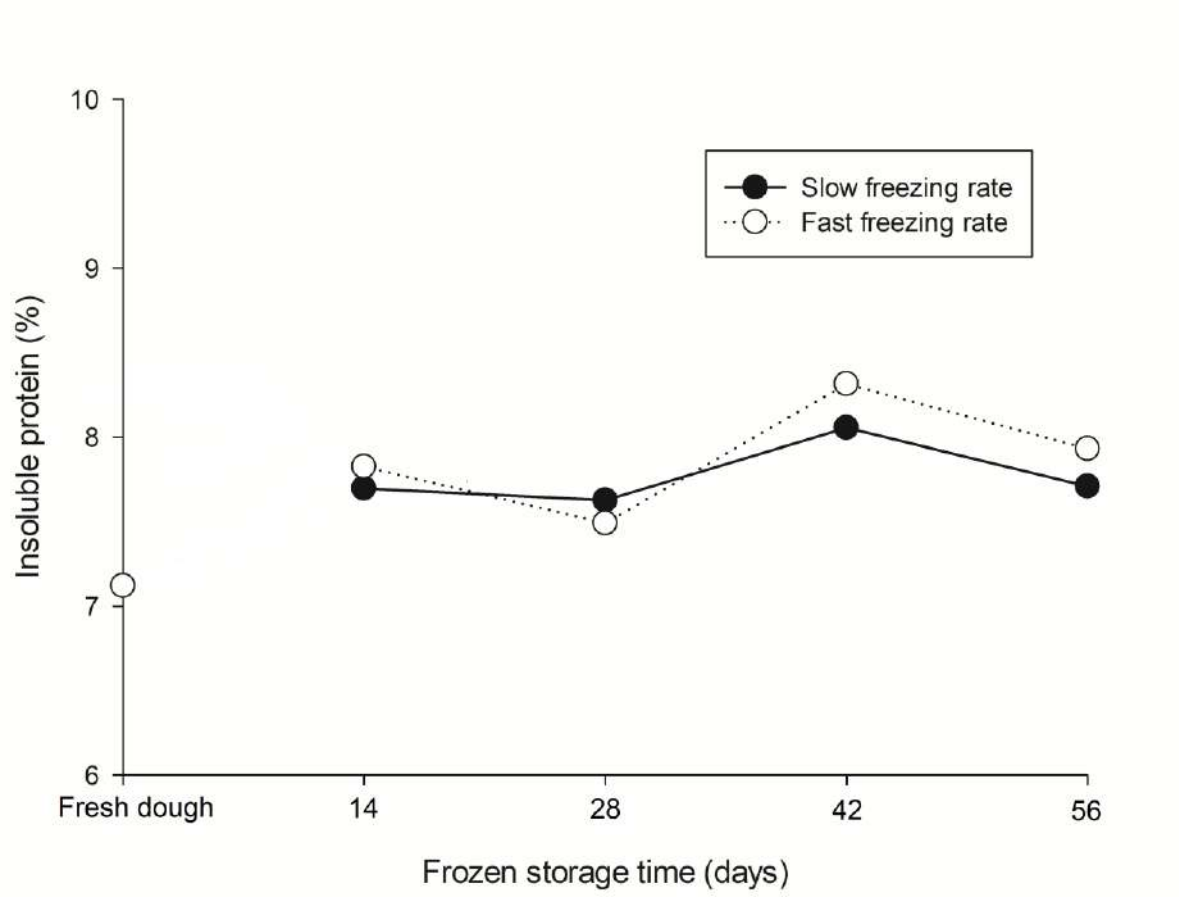

Figure 2. Effect of freezing rate and frozen storage time on the insoluble protein (HMW-GLU) of part-baked bread.
Figura 2. Efecto de la velocidad de congelación y el tiempo de almacenamiento en congelación sobre la proteína insoluble (HMW-GLU) del pan pre-horneado.

3.3 Free thiols content

The ANOVA results indicate a significant effect ($P<0.01$) of freezing rate on protein free thiol content (SH), followed by part-baking time and frozen storage time.

Fig. 3 illustrates the influence of part-baking time and frozen storage time on the concentration of SH groups in part-baked bread proteins. Variations in free thiol content provide insight into the disruption or formation of disulfide bonds, which play a crucial role in gluten aggregation and structural integrity (Li et al., 2019). In fresh dough, the highest SH content is observed; however, a notable decrease of 4.2 % and 14.5 % was observed at 3 and 6 min, respectively, reaching its lowest value after 6 min of part-baking. Additionally, free thiol content tends to decline with increasing frozen storage time, with the highest levels recorded with fresh dough and the lowest at 42 and 56 days.

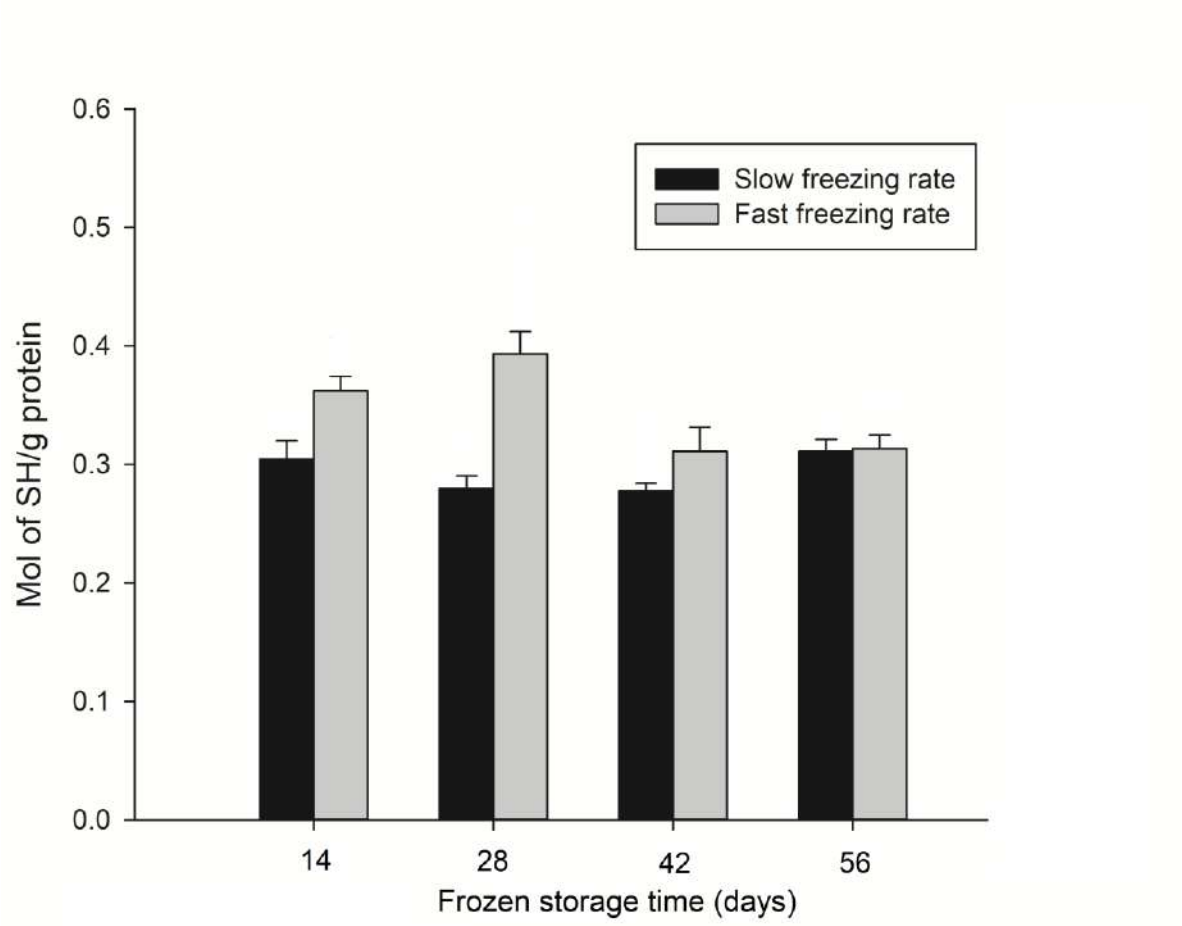

Figure 3. Effect of part-baking time and frozen storage time on the free thiols content of protein of part-baked bread.

Figura 3. Efecto del tiempo de pre-horneado y del tiempo de almacenamiento en congelación sobre el contenido de tioles libres de las proteínas del pan pre-horneado.

The stability of the gluten network depends on the number of disulfide bonds, even though free thiols represent only 2 % of the total amino acids in wheat proteins (Wieser et al., 2023). Studies indicate that exposure to high temperatures during baking promotes proteins denaturation, causing them to aggregate into hydrophobic complexes, reducing functionality and impairing the ability to revert to their original state. Moreover, heat-induced pH changes also lead to protein fragmentation, precipitation, and polymer breakdown. During this process, glutathione molecules present in flour and yeast are oxidized to glutathione disulfide, a reaction reported to have minimal impact on dough rheology (Hong et al., 2023). During frozen storage, ice crystal formation damages proteins and disrupts disulfide bonds, resulting in an increase in the number of free thiol groups per gram of protein (Shu et al., 2022). However, as frozen storage time progresses, water redistribution due to freezing promotes hydrophobic interactions between proteins, leading to the formation of insoluble complexes, which make free thiol groups unavailable for bond formation.

Fig. 4 shows the effect of freezing rate and frozen storage time on free thiol content in part-baked bread proteins. Higher levels of free thiols were observed at 14, and 28 days of frozen storage using fast freezing rate. The opposite effect was observed at 42 days, and no difference was detected at 56 days. Taylor et al. (2016) reported that most reductions in free thiol content facilitated by glutathione occur within the first few minutes of kneading.

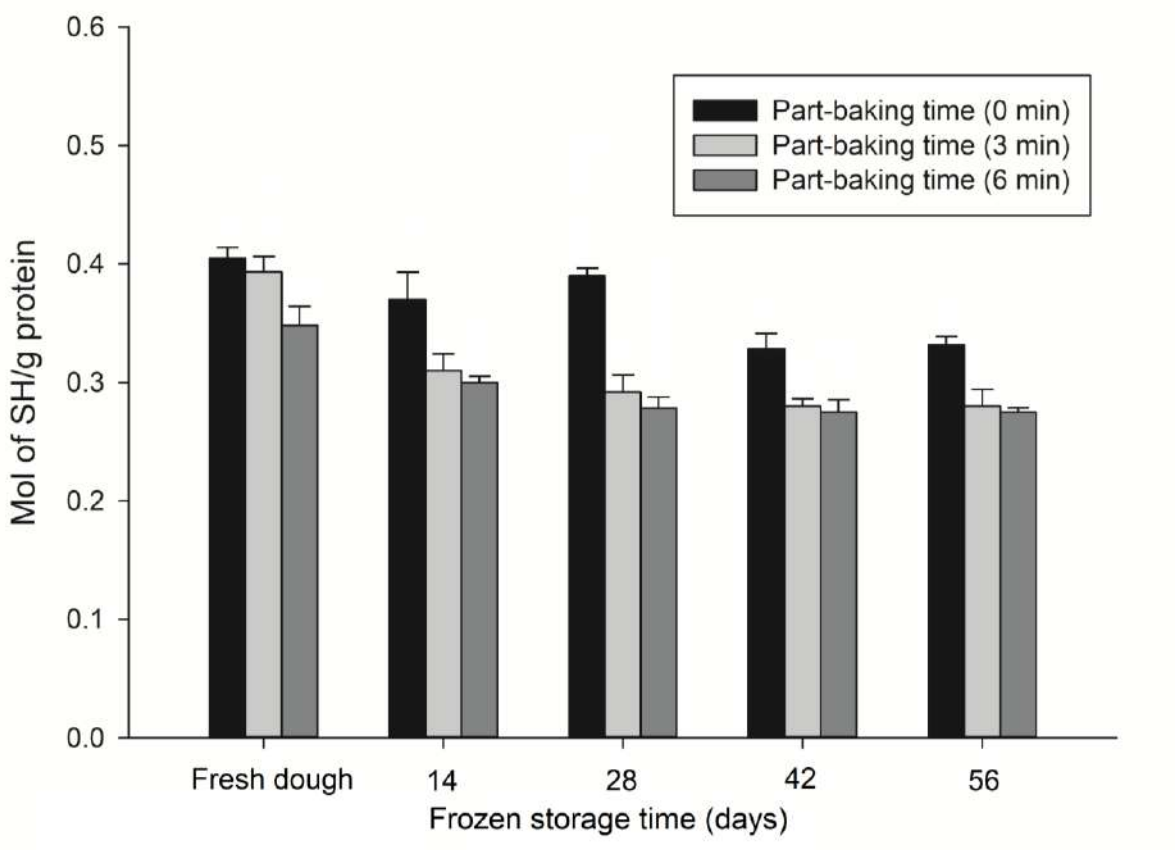

Figure 4. Effect of part-baking time and freezing rate on the free thiols content of proteins of part-baked bread.
Figura 4. Efecto del tiempo de pre-horneado y la velocidad de congelación sobre el contenido de tioles libres de las proteínas del pan pre-horneado

As explained in Fig. 3, part-baking reduces free thiol content due to protein denaturation, making SH groups unavailable for bond formation. Previous studies suggest that fast freezing leads to less disulfide bond breakdown, resulting in fewer SH groups. However, the present findings show higher free thiol content under fast freezing at short frozen storage. This effect may be attributed to ice crystal formation during the nucleation stage, which limits water redistribution and reduces protein denaturation. Although Baratto et al. (2016) found that wheat flour supplemented with ascorbic acid improved dough plasticization by increasing dry yeast concentration, further research is needed to fully understand the impact of freezing conditions on the SH groups availability.

4. Conclusions

This study demonstrated that part-baking time, freezing rate, and frozen storage time significantly influence the solubility and structural stability of gluten proteins in part-baked bread. At all frozen storage times, the percentage of LMW-glutenin and gliadin was highest with fresh dough but decreased at 3 and 6 min of part-baking. Conversely, insoluble protein concentration increased at 3 and 6 min of part-baking, likely due to heat-induced protein insolubility. The freezing rate played a key role in protein preservation. Fast freezing better preserves soluble protein levels and free thiol groups, particularly at early frozen storage. Conversely, slow freezing was more effective in preserving glutenin stability over extended frozen storage. Free thiol content decreased with longer frozen storage, likely due to structural damage caused by ice crystal formation and subsequent hydrophobic interactions. Part-baking time also contributed to this decline, as protein denaturation made sulfhydryl (SH) groups unavailable for bond formation. Overall, short part-baking times combined with fast-freezing is recommended to better preserve protein functionality in part-baked bread, providing valuable insight for improving the quality and shelf-life of frozen bakery products.

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Conflict of interest

The authors declare no conflict of interest.

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