Effect of Freezing and Frozen Storage of Doughs on Bread Quality

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The effects of freezing and storage in frozen conditions on bread quality, crumb properties, and aggregative behavior of glutenins were analyzed. The effect of different additives on bread quality was also studied. The results obtained showed that freezing and storage at −18 °C decreased the bread quality. Samples stored in frozen conditions supplemented with diacetyl-tartaric acid ester of monoglycerides, gluten, and guar gum produced breads of greater volume and more open crumb structure than those prepared with the base formulation (without additives). All additives analyzed increased the proof time. Crumb firmness increased with dough frozen storage and bread aging time at 4 °C. A decrease in the amount of glutenin subunits of high molecular mass was observed by electrophoresis analysis of the SDS-soluble proteins aggregates extracted from the frozen dough. This result suggested that the protein matrix of bread underwent depolymerization during storage in frozen conditions.

Keywords: *Dough; bread; freezing; frozen storage; glutenins*

INTRODUCTION

The quality of bread baked from frozen doughs (which were stored in frozen conditions gradually) deteriorates during storage at sub-zero temperatures. The loss of baking quality has been ascribed to dough weakening and a reduction of both yeast viability and activity (*1*−*3*).

Dough weakening during frozen storage and successive freeze−thaw cycles has been attributed to (1) the release of reducing substances from yeast during freezing that may reduce gluten proteins, (2) water redistribution provoked by a modification in the water-binding capacity of dough constituents, and/or (3) a reduction of gluten cross-linking (*4* and *5*). Results obtained by Berglund et al. (*6*) showed that the formation of ice-crystals in nonfermented doughs stored for 24 weeks led to a disruption of the gluten matrix rendering a network separated from starch granules.

The main consequences related to these phenomena occurring in doughs that have been frozen and stored in frozen conditions are the production of bread with lower loaf volume, longer fermentation times, and stronger alteration of textural properties (*1, 7*−*10*).

The inclusion of additives in bread formulation may overcome the aforementioned problems. For example, the supplementation of vital gluten to relatively weak doughs improved loaf volume and breadcrumb and decreased fermentation times (*11*). On the other hand, the ester monoglyceride derived from diacetyl-tartaric acid (DATEM) reduced bread rigidity and improved loaf volume of bread obtained from frozen and fresh (unfrozen) doughs (*9, 12*−*14*). Guar gum is frequently used in combination with locust bean gum and carrageenan gum as a stabilizer in ice creams, filled pies, and other frozen products (*15*).

Despite the latest improvements in technology for the production of bakery products, more experimental evidence is still necessary to elucidate the physicochemical transformations undergone by dough components during freezing and frozen storage.

The aim of this work was to contribute to the present knowledge about the effects of freezing and storage in frozen conditions on bread quality, crumb properties, and the aggregative behavior of glutenins. The effect of different additives on bread quality was also analyzed.

MATERIALS AND METHODS

Baking Procedure. The dough base formulation used in this study comprised 100% flour (protein 13.2%, water 11.8%, and ash 0.7%), 3% compressed yeast, 1.8% sodium chloride, 0.2% sodium propionate, 0.015% ascorbic acid, and 63% of water. Additives used in the different formulations were gluten 1.5%, guar gum 0.5%, soybean flour 10%, and DATEM 0.5% (flour based). Doughs with higher amounts of yeast (5%) were used as other additive.

Ingredients corresponding to the base formulation and to those formulations containing additives were mixed in an Argental L-20 mixer (Argental, Argentina). Yeast and salt were previously dissolved in water and the remaining ingredients were added as solids. The resulting dough was rested for 15 min in a fermentation cabinet at 30 °C and 70% RH. The bulk dough was sheeted in a Mi-Pan vf roller (Argentina) containing two rolls of 50 × 12.7 cm. The dough was then divided into 80-g pieces and hand-molded. Six dough pieces were immediately proofed at 30 °C (96% RH) until optimum development and baked at 200 °C for 18 min (control). Other samples were placed on individual trays and wrapped on polyethylene bags for immediate freezing. Freezing and frozen storage took place at −18 °C in a freezer (Frare, Argentina) and stored at the same temperature for 4, 30, and 60 days. After the corresponding storage time, samples were thawed for 1 h at 30 °C (70% RH), proofed at 30 °C (96% RH) until optimum development, and baked. This recipe and breadmak-

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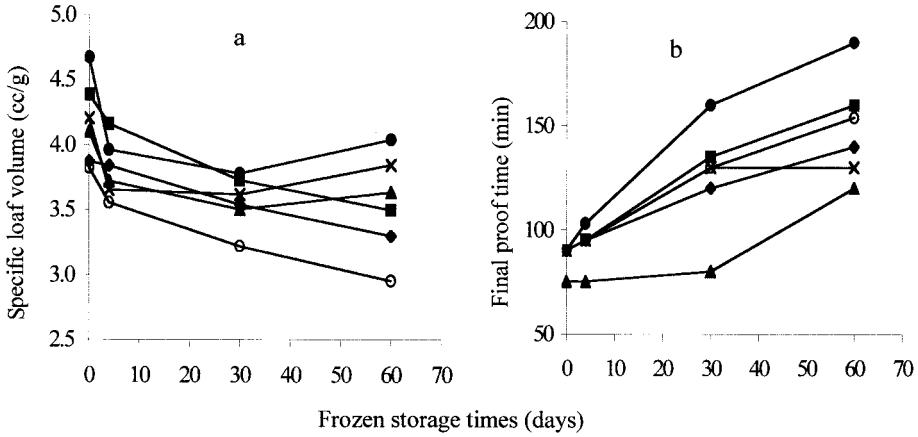

Figure 1. Effect of freezing and frozen storage time on specific loaf volume (a) and final proof time (b) of dough. Dough without additive, (—◆—). Dough with gluten, (—■—); guar gum, (—×—); DATEM, (—●—); soy bean flour, (—○—); and 5% of yeast, (—▲—).

ing process are currently employed in our country in the preparation of bread, and its simplicity allows a clear observation of changes occurring during the processing of frozen dough.

Bread Properties. *Bread Volume.* Bread loaf specific volumes were determined by rapeseed displacement and weight 24 h after baking. Form ratio was measured as maximum height/width in each piece.

Characterization of Crumb Structure. For each bread loaf, two slices were obtained from the central region and photographed with a Nikon 90 camera (Nikon, Japan) (macro lens, blue filter 80B). Illumination was generated with four lamps (photoflood, 500 W) placed at 70 cm from the samples. Pictures were scanned and analyzed with an Image-Pro PLUS analyzer (version 3.0 for Windows 95/NT) (Media Cybernetics, Silver Spring, CA). The program automatically selected bread regions (gas cells) on the basis of the intensity of gray color. Results were expressed as gas cells per area unit of crumb (% gas cell).

Analysis of Hardening of Crumb. For this assay, dough was prepared as indicated previously and divided into two lots. One of the lots was frozen and stored at −18 °C for 60 days. Then, samples were thawed, fermented, and baked as described in a previous section. The second lot (control) was fermented and baked immediately after the preparation step. In either case, bread pieces were stored at 4 ± 1 or 20 ± 2 °C. At timed intervals, 3 bread pieces were cut into 2 slices (2 cm wide and 5 cm diameter) and the ends were discarded. Each slice was submitted to a compression test in an Instron Model 1011 electromechanical testing system (Instron Corp., Canton, MA), under specific conditions: compression cell, 50 N; crosshead speed, 100 mm/min; maximum deformation, 50%; grip dimension, 1.4 cm diameter. The hardness of the crumb was reported as the force in N required to compress samples to 25% of their original width (5 mm). Six determinations were made per point and average values were reported.

Protein Analysis. *Preparation of Glutenins.* Dough was prepared according to the recipe described previously. Ground, freeze-dried, frozen dough samples (stored 2, 3, and 4 months at −18 °C) were extracted with a buffer solution (dough/buffer 1:30) of pH 6.8 containing 0.063 M Tris-HCl, 2% (w/v) SDS, 10% (v/v) glycerol. and 0.01% (w/v) bromophenol blue (*16*). The resulting suspension was kept at room temperature for 2.5 h, and was shaken every 15 min. Then it was heated in a boiling-water bath for 3 min and cooled at room temperature. After sedimentation, the supernatant containing the glutenins was separated.

Multistacking SDS−PAGE. A multistacking sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE), prepared according to Laemmli (*17*), was devised to characterize the glutenin fraction.

Two stacking gels of 4 and 8% (w/v) acrylamide concentration (0.108 and 0.216% w/v bisacrylamide concentration, respectively), and one resolving gel of 12% (w/v) acrylamide (0.48% (w/v) bisacrylamide), were prepared. A Mini Protean II Dual Slab cell (Bio-Rad Laboratories, Hercules, CA) was employed to perform electrophoretic runs working at constant voltage (150 V) until the front reached the end of the gel (approximately 90 min). Gels of 0.75-mm width were prepared for analytical purposes, and gels of 1.5-mm width were used for preparative analysis.

SDS−PAGE. After the multistacking SDS−PAGE run, the gel was divided into three parts: cut 1, corresponding to the stacking of 4% acrylamide; cut 2, corresponding to the stacking of 8% acrylamide; and cut 3, corresponding to the resolving gel. Proteins were eluted from the gel by incubation in buffer pH 6.8 (0.063 M Tris-HCl), 1.5% (w/v) SDS, 3% mercaptoethanol, 10% (v/v) glycerol, and 0.01% (w/v) blue bromophenol for 48 h at room temperature, while being shaken constantly. The resulting mixtures were placed in a water bath at 100 °C for 10 min. Protein composition was analyzed by SDS−PAGE (stacking gel of 4% (w/v) acrylamide and resolving gel of 12% (w/v) acrylamide). Runs were performed in the same equipment as described above. The proteins employed as molecular mass markers were myosin 200 000; β-galactosidase 116 250; phosphorylase b 97 400; serum albumin 66 200; ovalbumin 45 000; carbonic anhydrase 31 000; trypsin inhibitor 21 500; lysozyme 14 400; and aprotinin 6500 (SDS−PAGE MW standards, broad range, Bio-Rad Laboratories, Hercules, CA).

Protein Quantification. Gels were analyzed by densitometry in an image master VDS (Pharmacia Biotech Inc., Uppsala, Sweden) using the software image master VDS). A blank lane was used to obtain the background signal. The volume of protein band (integrated optical density, IOD) was represented by the expression

$$\text{IOD} = [\text{mean intensity (Im)} - \text{background (Ib)}] \times \text{band area}$$

The proportions of glutenins relative to total protein in the corresponding lane were quantified as IOD from each band/total IOD of the lane.

At least three determinations per point were made and average values were determined.

Statistical Analysis. The data obtained were statistically evaluated by variance analysis (ANOVA). The comparison of means was done by the Turkey test at a level of 0.05. Both were carried out using the statistical analysis package SYSTAT (*18*).

RESULTS AND DISCUSSION

Effect of Dough Freezing and Storage at −18 °C on Bread Quality. *Loaf Volume and Fermentation Time.* Figure 1a shows loaf volumes from bread obtained from fresh and frozen doughs. Loaf volume decreased with the storage time at sub-zero temperatures in bread prepared with the base formulation (no additives). A higher volume was achieved with nonfrozen samples.

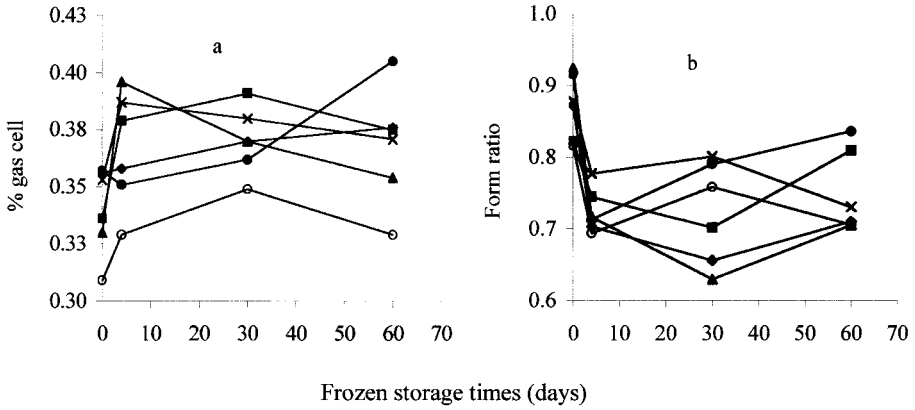

Figure 2. Effect of freezing and frozen storage time on gas cells per area unit of crumb (a) and form ratio (b) of dough. Dough without additive, (—◆—). Dough with gluten, (—■—); guar gum, (—×—); DATEM, (—●—); soy bean flour, (—○—); and 5% of yeast (—○—).

These results agree with those previously obtained by other authors (*1* and *14*).

Fresh doughs supplemented with additives rendered breads with higher volumes in comparison with those obtained with the base formulation, except when soybean flour was employed, probably due to an excess of this sort of flour in the formulation. Those differences were more remarkable when DATEM was employed as an additive.

Samples which were frozen and stored at −18 °C showed the same behavior as fresh samples. A higher deterioration was observed during the first 4 days of storage. After the whole period of storage, doughs supplemented with additives (except soybean flour) originated breads with greater volumes than those prepared with the base formulation. The addition of DATEM and guar gum yielded the best results in this regard.

Figure 1b depicts the proofed times obtained for each formulation both for fresh samples and for frozen samples stored at −18 °C. Fresh dough supplemented with additives had increased fermentation time. The formulation comprising the higher proportion of yeast resulted in decreased fermentation time. The fermentation time of frozen samples prepared with the base formulation without additives increased during the whole period of storage. Again, those doughs with a higher proportion of yeast showed lower fermentation times, with a strong deterioration over 60 days. Additives produced an increase in the fermentation time (compared to controls) of the rest of the doughs. This difference was more important when using DATEM.

It is commonly known that a freezing process followed by storage in frozen condition affects the gassing power of yeast (*19*−*21*). This effect is more evident in doughs supplemented with additives: additives strengthen the dough matrix and, consequently, a higher gas pressure is necessary to produce an increment in the loaf volume.

Bread Crumb Properties. Figure 2a and b shows the gas cells per area unit of crumb (% gas cell) and the form ratio of bread prepared with fresh and frozen doughs with and without additives. Bread crumbs from frozen doughs showed higher values with regard to gas cell area than the corresponding crumbs from nonfrozen doughs, suggesting the presence of structures with a higher proportion of gas. Bread supplemented with soybean flour showed more compact structures (lower percentage of gas cells) regardless of the storage time. Crumb structures in breads prepared with additives were more open than those of the controls if storage was less than 40 days.

The form ratio provides useful information related to the dough elasticity. Results indicated that fresh doughs produced bread with higher form ratios than frozen doughs which were stored at −18 °C regardless of the storage time and the formulation analyzed (Figure 2b). All additives improved the ability of doughs to retain their form during thawing, fermentation, and baking, in contrast to control samples (without additives and higher yeast content). This last result indicated that the tested additives improved the quality of breads prepared with frozen doughs through a mechanism different from that for the yeast survival, probably strengthening the gluten structure.

Crumb Firmness. Figure 3a and b shows the evolution of crumb firmness of bread prepared with doughs formulated without additives, fresh and frozen (frozen stored for 60 days), and finally kept after baking at 4 ± 1 and 20 ± 2 °C. In all cases, firmness increased with the storage time, but some differences were observed over the fifth day (at 4 and 20 °C) between bread obtained from fresh and frozen doughs. After 7 days of storage, those breads prepared with frozen doughs presented higher firmness at 4 °C than at 20 °C. At shorter times of storage, those breads obtained from frozen doughs showed a higher firmness than those prepared with a fresh dough. This result was observed at both aging temperatures. Apparently, at longer times of storage there is a less important influence of freezing than of the aging temperature. However, on the basis of the bread formulation employed in this study, the acceptability of the final product is lost within 3 days. Studies carried out in our laboratory indicated that a higher retrogradation of amylopectin is produced in breads prepared from frozen doughs, stored in frozen conditions, and aged at 4 °C in comparison to that of breads obtained from nonfrozen doughs or frozen doughs which were aged at 20 °C (*22*). A higher degree of amylopectin retrogradation would be one of the explanations for the increase in bread firmness.

Effect of Dough Freezing and Storage in Frozen Conditions on Proteins from the Bread Matrix. SDS-soluble protein aggregates extracted from the dough (as described in Materials and Methods) were separated, in nonreducing conditions, into three fractions on the basis of their migration in a multistacking polyacrylamide gel (Figure 4). A high proportion of protein aggregates of different sizes were recovered in

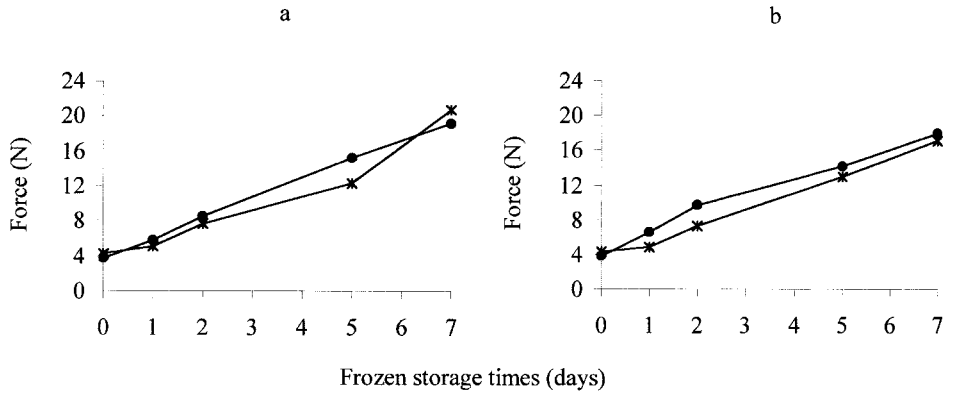

Figure 3. Influence of freezing and frozen storage time on bread firmness. Bread prepared with fresh dough (●) and frozen dough stored for 60 days (*), and bread aging at 4 ± 1 °C (a) and 20 ± 2 °C (b).

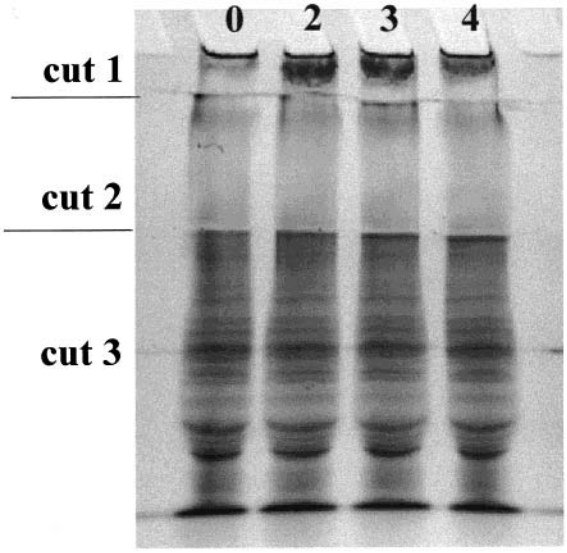

Figure 4. Multistacking SDS−PAGE of total extract of dough proteins without reducing agent. Line 0: fresh dough (not frozen). Lines 2, 3, and 4: dough frozen stored (−18 °C) for 2, 3, and 4 months, respectively. Cut 1: stacking gel (4% acrylamide). Cut 2: stacking gel (8% acrylamide). Cut 3: resolving gel (12% acrylamide).

cut 1 and 2 of the gel (4% and 8% of polyacrylamide, respectively) and in the origin of the resolving gel. Densitometric analysis did not show significant differences ($p > 0.05$) among the electrophoretic patterns corresponding to samples of bread stored at −18 °C for different times.

Protein aggregates separated in the aforementioned conditions were then eluted from each cut of the gel and separated by SDS−PAGE in order to analyze probable changes in the polypeptide composition. Results are shown in Figure 5. A large proportion of protein species of high molecular mass and a lower proportion of intermediate molecular mass composed the protein aggregates. No subunits of low molecular mass (<30 000) were present. These results agreed with the fact that only glutenin fractions of high and low molecular weight are able to form glutenin aggregates stabilized by disulfide bonds (inter and intrachain) (*23*).

Protein aggregates retained in cut 2 contained a higher amount of glutenin subunits of intermediate molecular mass, whereas those retained in cut 1 had a higher amount of high molecular mass subunits. Densitometric analysis corresponding to cut 1 for different samples (Figure 6a and b) indicated that as the storage time increased, the amount of glutenin subunits of high molecular mass decreased. Those subunits were referred as r1, r2, and r3 with molecular masses of 129 100, 105 900, and 88 700, respectively. On the other hand, the amount of low-molecular-mass subunits increased, identified as r5 (64 700), r6 (46 900), r8 (40 200), and polypeptides in the end of the gel (front). These results suggest that, in the protein matrix, a depolymerization took place during storage in frozen conditions, and that this depolymerization increased with time. This fact was supported by the highly significant correlation between the ratio of glutenin subunits of high molecular mass to total protein content and the storage time (r1, −0.992; r2, −0.969; r3, −0.977; r5, 0.999; r6, 0.951; r8, 0.998; and front, 0.995). These phenomena may cause a loss in gas retention capacity during fermentation, reflected by the lower loaf volumes and the increase in the fermentation time.

Similar analysis performed with proteins belonging to cut 2 (Figure 7a and b) showed an increase of glutenin subunits of high molecular mass (r1, r2, and r3; r = 0.978, 0.993, and 0.968 respectively) and glutenin subunits of medium molecular mass (r4, r5, and r6; r = 0.939, 0.999, and 0.929, respectively) with the increasing storage time. Besides, a decrease of protein species corresponding to a molecular mass of 38 900 and 36 700 (identified as r9 and r10, $r = -0.918$ and −0.912) was detected. The larger SDS-soluble protein aggregates, recovered in cut 1 and 2, were formed by subunits of high molecular mass (>30 000). These results agreed with the fact that high-molecular-mass subunits of glutenin are able to form glutenin aggregates stabilized by disulfide bonds (inter- and intrachain) (*23*). This increase of glutenin subunits of high and medium molecular mass could be explained by polymerization of the biggest aggregates retained in cut 1. Depolymerization could originate smaller aggregates, rich in high- and medium-molecular-mass glutenins that were retained in the 8% acrylamide gel. The proportion of glutenins of high molecular mass to the total protein content (bands r1, r2, and r3) increased with storage time at −18 °C.

Protein subunits extracted in the resolving gel had a low proportion of glutenin subunits of high molecular mass (65 000−130 000) and a high amount of proteins of molecular mass between 60 000 and 30 000. This result indicated that larger glutenin subunits formed big aggregates in the dough. There was no clear tendency in the variation of the amount of protein separated in cut 3 with freezing and storage time.

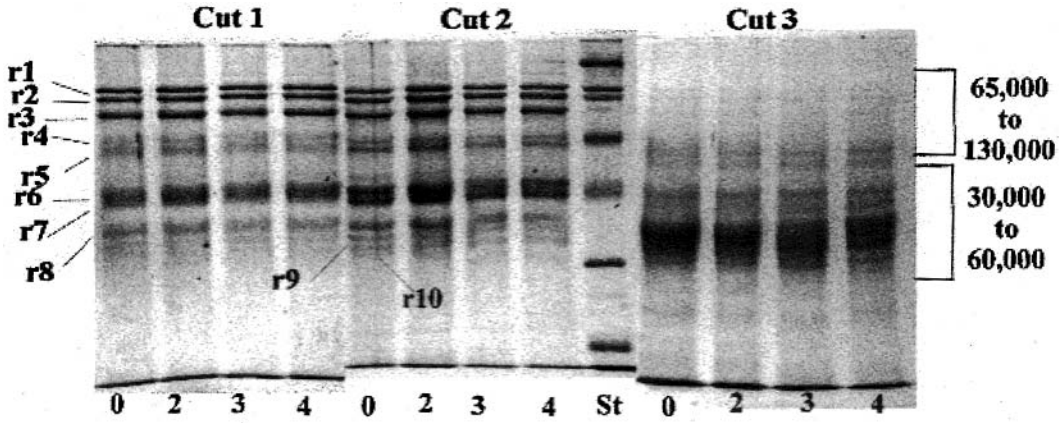

Figure 5. SDS−PAGE (stacking 4% and resolving 12% acrylamide) of reduced proteins from multistacking SDS−PAGE gels (cuts 1, 2, and 3). Line 0: fresh dough (not frozen). Lines 2, 3, and 4: dough frozen stored (−18 °C) for 2, 3, and 4 months, respectively. Line St: molecular mass markers. Arrows r1−r10: bands analyzed by densitometry. Numbers labeled at right of the gel photo corresponded to molecular mass regions analyzed.

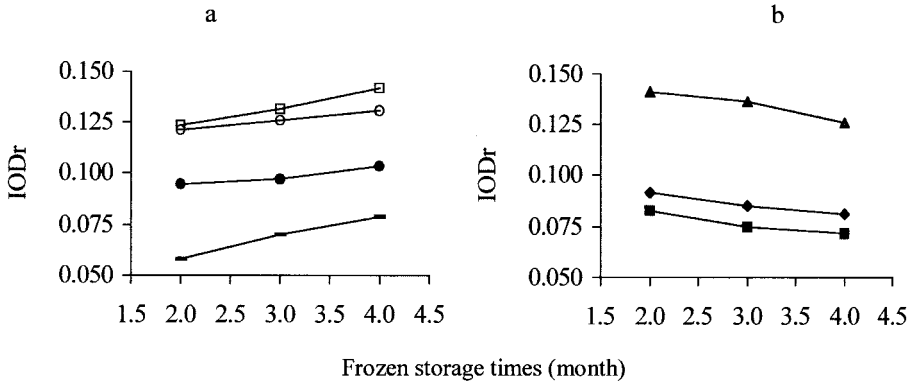

Figure 6. Effects of freezing and frozen storage time on reduced proteins from cut 1 (4% acrylamide). Band molecular mass: ◆ = 129 100, ■ = 105 900, and ▲ = 88 700 (a); and □ = 64 700, ● = 46 900, ○ = 40 200, and — = front (b).

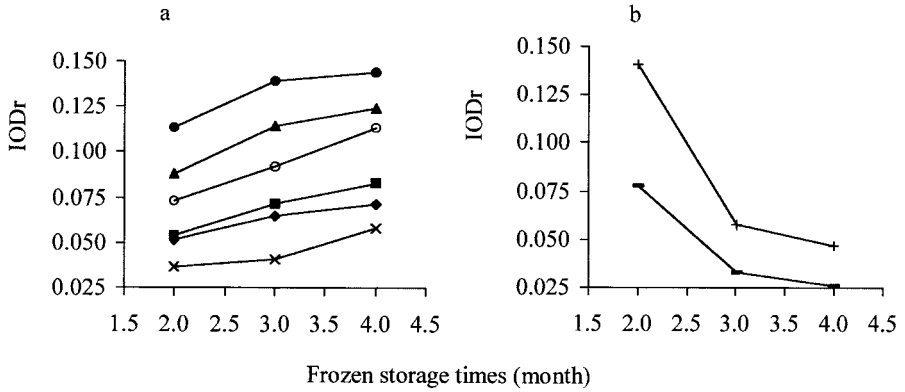

Figure 7. Effects of freezing and frozen storage time on reduced proteins from cut 2 (8% acrylamide). Band molecular mass: ◆ = 129 100, ■ = 105 900, ▲ = 88 700, × = 69 900, ○ = 64 700, ● = 46 900 (a); and — = 38 900 and + = 36 700 (b).

It is commonly known that glutenin subunits, but not gliadins, are able to establish intra- and intermolecular disulfide bonds. This difference allows the formation of a glutenin macropolymer (GMO) which plays a special role in the maintenance of gluten structure (*24*−*26*). Different studies have showed that GMP is composed by weak aggregates of glutenins subunits that could be broken during the mixing of the dough, leading to the liberation of oligomers and dimers of a defined composition. Some low-molecular-weight (LMW) (especially type B) and x-high-molecular-weight (HMW) glutenins could be depolymerized if doughs were extensively mixed (*24*−*26*). Water redistribution, ice recrystallization, and an increase in the amount of freezable water may affect gluten structure and may be one of the reasons for the depolymerization of glutenin aggregates of high molecular mass observed in this work. This depolymerization rendered protein species of molecular mass (65−40 kDa) similar to that of the LMW glutenins more likely to be broken during mixing.

CONCLUSIONS

Freezing and storage of dough at −18 °C generated loss in bread quality reflected by a lower loaf volume, longer fermentation time, an increment in the proportion of gas cells, and less elasticity in bread dough.

The supplementation of DATEM, gluten, and guar gum improved loaf volume and form ratio. However,

none of the additives employed in this work shortened the fermentation time. After aging, those breads prepared from frozen doughs were harder than those prepared with fresh dough. This effect was more evident at 4 °C and is related to a faster retrogradation of amylopectin.

A depolymerization of glutenin aggregates was observed during storage at −18 °C and was enhanced during the time in which the dough was kept in frozen conditions. We observed a disappearance of high-molecular-weight glutenins (129 100−88 700) in those macromolecular aggregates comprising the gluten network, strongly dependent on the storage at −18 °C.

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