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# Changes in protein solubility, fermentative capacity, viscoelasticity and breadmaking of frozen dough

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**The use of frozen dough remedied availability of fresh bread. However, bread elaborated from frozen dough has less volume and texture is firmer. This study evaluates how storage affects the protein solubility, fermentative capacity and viscoelasticity of frozen dough. In addition to examining the effects of storage on the quality of the final baked bread. Dough was frozen at a rate of -0.146°C/min and stored at -18°C for 42 days. Protein solubility was measured using the SE-HPLC method. A dynamic measurement method was used to determine the viscoelastic parameters of dough: storage and loss modulus (G´ and G´´), and phase angle (δ). The most drastic changes in the frozen dough occurred during the first seven days of storage. The weakening of frozen dough correlated with the hydrolysis of insoluble polymeric proteins, which is associated with the increase in the concentration of the protein soluble polymer. The viscous (δ) of the frozen dough increased to 25.88% after 28 days of storage, and the soluble polymeric protein concentration increased by 10.12% in this period. Frozen dough should be stored for fewer than 21 days; time in which the loaf volume of bread made from frozen dough was approximately 40.84% smaller than that of fresh bread dough formulation.**

**Key words:** French type bread, frozen dough, protein solubility, baking quality, viscoelasticity.

## INTRODUCTION

Frozen bread dough was developed with the goal of obtaining products that are similar to "fresh" bread made according to a traditional recipe. However, developing an adequate freezing step in the continuous process of bread-making, still presents a number of challenges. The diminished loaf volume of bread produced from frozen dough in comparison to bread made from fresh dough remains a challenge for the bread-making industry. The reduction in the volume of bread made from frozen dough can be attributed to decrease in yeast viability and

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changes in the structure of the dough (Gant et al., 1990; Esselink et al., 2003; Cauvain and Young, 2008).

Wheat-based bread dough is a viscoelastic material that exhibits both viscous and elastic behavior. Wheat is the only cereal grain that has the ability to retain gas and that can be converted into a spongy product called bread. The gluten network that facilitates this ability forms as a result of the increased hydrophobic interactions, and disulfide bonds between the protein polymers found within the flour (gliadins, GLI and glutenins, GS) as well as noncovalent disulfide bonds (Godón and Herard, 1984; Shewry et al., 1995; Weiser, 2007; Kontogiorgos, 2011).

Proteins can be isolated on the basis of their solubility in alcohol-water solutions. The GLI fractioninclude monomers linked by noncovalent disulfide bonds. GS fraction comprise a heterogeneous group of high-molecular-weight polypeptides linked by disulfide bonds (Shewry et al., 1995; Weiser, 2007; Kontogiorgos, 2011). Pro-teins in the GLI fraction give the dough extensibility and viscous, whereas GS mainly influence functional properties of the dough such as strength and elasticity (Lu and Grant, 1999). The GS fraction is composed of a mix of high- and low-molecular weight (HMW and LMW, respectively) GS (Weiser, 2007; Kontogiorgos, 2011). The HMW-GS in wheat flour contribute greatly to the elastic behavior of wheat dough (Shewry et al., 1995; Lefebvre and Mahmoudi, 2007). Lu and Grant (1999) found that the GS fraction has a substantial effect on the baking characteristics of dough that has been frozen; the GLI and starch fractions affect the baking characteristics of the dough to a lesser extent. These authors suggest using strong flours (those with high levels of GS) to produce frozen dough. Borneo and Khan (1999) found a direct and inverse relationship between soluble polymeric protein fractions and albumin+globulin (A+G) with loaf volume.

Each protein fraction has a different degree of solubility, and the solubility of each fraction varies depending on the stage of the bread-making process. In general, the solubility of various proteins decreases during mixing and fermentation. During mixing, the polymers bind covalently to water and subsequently produce a continuous macromolecular viscoelastic material. During fermentation, an oxidative process that involves the crosslinking of these polymers occurs (Hoseney et al., 1979; Borneo and Khan 1999; Cuq et al., 2003). Dough freezing results in further changes to the gluten-based polymeric structures coupled with changes in protein solubility that occurs during bread preparation. Lei et al. (2012) used size chromatography (SEC) to determine the degree of depolymerization of the frozen gluten proteins stored at -18°C. They found a 40.83% decrease of the GS-HMW fraction in the gluten stored for 120 days. These authors discuss that there is a greater degree of depolymerization in the gluten protein fractions of higher molecular weight and are increasing during frozen storage. These changes on gluten contribute to viscoelastic changes in the dough that disrupt its ability to be baked. The impacts of these changes can increase with storage time, and they often result in the comparative weakening of frozen dough evidenced by the results of viscoelastic tests, diminished fermentative capacity, and decreased loaf volume associated with the use of frozen dough.

The freezing process results in mechanical damage to the gluten network that result from the formation of ice crystals. Ice crystal formation provokes ruptures in the gas cell membrane (Gant et al., 1990), and the increase in crystal size within the gas cells results in water redistribution that ultimately causes dehydration of the dough (Esselink et al., 2003) and in protein that changes the original structure of the dough. During storage, changes in the number, sizes and shapes of the ice crystals occur; this phenomenon is called of "re-crystallization" (Baier-Schenk et al., 2005a). Kontogiorgos et al. (2008) agreed that both the formation of ice crystals and the re-crystallization phenomenon cause disruptions in the structure of the gluten network and change its morphology. During thawing (rehydration), the process of water transfer occurs in reverse, and the water molecules bind to different sites on the dough proteins than those they initially occupied, thereby changing the conformation of the dough.

The structural changes in the dough that occur during freezing and storage modify the viscoelastic properties of the dough and thereby alter its behavior during baking. During storage, both the elastic and viscous (G' and G'') moduli of frozen dough decay over time, and the viscous behavior prevails in comparison to that of fresh dough (Ribotta et al., 2004), which reduces the degree to which frozen dough is able to retain gas (Selomulyo and Zhou, 2007). The loss of elastic behavior in frozen dough is attributed to the fragmentation of the polymeric protein chains.

SDS gel electrophoresis has provided evidence of increased protein solubility in frozen dough that was thought to result from glutenin degradation (Kennedy, 2000; Ribotta et al., 2001). Leray et al. (2010) concluded that, during storage, changes in the viscoelasticity of the dough occur and are related to the observed reduction in the volume of the final baked bread (Ribotta et al., 2001).

In relation to the frozen dough, changes that occur in wheat dough during freezing and storage have been evaluated using empirical and fundamental rheological methods (Bhattacharya et al., 2003; Giannou and Tzia, 2007; Angiolini et al., 2008; Leray et al., 2010). It has also been shown that the freezing conditions (Aibara et al., 2005) may alter the structure of frozen dough, and the effect of ingredients and additives (Sharadanant and Khan, 2006; Selomulyo and Zhou, 2007) have also been studied. The freezing-induced weakening of the dough can be attributed to damage to the gluten network that result from the formation (Shelton and Freeman, 1991) and growth (Ribotta et al., 2004; Selomulyo and Zhou,

2007) of ice crystals, but there is still a need for information about the interrelationship between the changes in the protein solubility and viscoelasticity that occur in frozen dough and the quality of the bread made from it (Kennedy, 2000; Ribotta et al., 2001; Sharadanant and Khan, 2006). To date, there is no clear understanding of the ways in which the effects of both freezing process and storage time on the protein solubility and viscoelastic properties of frozen dough affect the quality of the baked product.

Currently, no research has been done employing to identify changes in proteins during frozen storage of dough. In addition, there is no evidence of the degree of hydrolysis of proteins detected by the technique of SE-HPLC and how it affects changes in the viscoelasticity of frozen dough, which are reflected in poor baking quality.

The aim of the present study was to evaluate the way in which storage time affects the protein solubility and viscoelasticity of frozen bread dough in addition to the quality of the baked product.

## MATERIALS AND METHODS

### Raw materials

We used a formulation for French type bread to prepare the frozen dough. Commercial high-protein flour (13.64%, dry basis) was supplied by Molino la Fama S.A. de C.V. The remaining ingredients were: salt (Sea of Cortez, Sales del Valle SA de CV), shortening (Inca, Food Capullo, S. de R. L de CV), instant yeast (Nevada, SAFMEX SA de CV/FERMEX SA de CV) and white bread improver (Magimix 40, SAFMEX SA de CV / FERMEX SA de CV). The yeast used remained under freezing conditions in order to increase its cryotolerance and preserve their fermentative power (Wolt and D'Appolonia, 1984; Ribotta et al., 2003).

### Flour quality evaluation

Proximate composition of the flour was determined by the methodology of A.A.C.C. (2000): protein content (method 46-13), ash content (method 08-03), and moisture content (method 44-40). The water absorption, stability and the development time of the dough were evaluated using the farinographic method (54-21) proposed by the A.A.C.C. (2000). The extensibility and deformation energy of the dough were tested using the alveographic method (53-30) established by the A.A.C.C. (2000).

### Processes for freezing and thawing the dough

#### *Formulation*

Preliminary, we tested three formulations of ingredients for frozen dough in which was varied only the yeast content (2, 3 and 5%, dry basis), and the rest ingredients remaining constant. The frozen dough formulation that resulted in baked bread with similar in quality to fresh bread included the following ingredients: high-protein flour (13.54%) (100%, weight basis flour), salt (1.5%), lyophilized yeast (3%), shortening (5%), white bread improver (2%) and 200 ml of water (the appropriate water volume calculated using a farinograph).

#### *Dough preparation*

The dough was prepared according to the method described by Magaña-Barajas et al. (2011). Briefly: the dry ingredients were mixed in a blender (MFG Lincoln, NE, USA) for 1 min, after which the dough was mixed for 3 min upon incorporating appropriate volume of water obtained via the farinograph.

#### *Molding*

Fifty-gram dough samples were rounded and set aside for 5 min, after which they were manually molded into bread loaves. In addition, larger dough samples (315 g) were used to evaluate the fermentative capacity of the dough and were rounded and molded for bread-making according to the same procedure.

#### *Preproofing*

The dough samples were preproofed for 10 min in a controlled environment (30°C, 85% relative humidity) using a proofing cabinet (MFG National brand, Lincoln, NE, USA).

#### *Freezing and storage*

Preproofed dough samples were frozen using a slow-rate freezing method that appears to minimize damage to the gluten structure and yeast viability of the dough (El-Hady et al., 1996; Codón et al., 2003). The loaves were frozen in a freezer at a temperature of -18°C (Frigidaire brand, model GLFC1526FW, Mississauga, Ont., Canada). The total freezing time was 5 h and 44 min; dough was frozen at a rate of -0.146°C/min. Samples were stored at -18°C for up to 42 days. The freezer was calibrated during 24 h by monitoring the temperature using a thermocouple (Digi Sens). Every 7 days, frozen dough samples were removed from the freezer and subjected to a series of evaluations.

#### *Thawing*

The dough samples were thawed under refrigeration conditions (4°C) (Ribotta et al., 2001, 2003; Karaoğlu et al., 2008). The thawing time and rate were determined by measuring temporal changes in the temperature of the dough using a thermocouple (Digi Sens). Thawing occurred at a rate of 0.062°C/min, and the samples reached the equilibrium temperature (4°C) after thawing for 4 h and 15 min.

#### *Proofing*

The thawed dough was fermented for 50 min in a proofing cabinet. The temperature in the proofing cabinet was 30°C and the relative humidity was 85% (MFG National brand, Lincoln, NE, USA).

#### *Frozen dough evaluations*

Assessments of the protein solubility, fermentative capacity, viscoelasticity and baking quality of frozen, thawed and fermented dough samples were carried out in triplicate. Samples of the frozen dough were assessed after each storage period (0, 7, 14, 21, 28, 35 or 42 days).

#### *Protein solubility*

For each storage time, 300 mg samples of dough were used to eva-

luate the changes in protein solubility. Protein solubilities were determined via molecular exclusion liquid chromatography (SE-HPLC). Soluble proteins were extracted using a 50% propanol solution. An SE-HPLC system (Varian ProStar equipment, Model 410, Palo Alto CA) with a diode array detector (Varian, Palo Alto CA) and an autosampler (Varian, Palo Alto CA) was used for all of the analyses. Detections using a chromatography column were performed at a wavelength of 210 nm (Biosep-SEC-S-S4000, Phenomenex, Torrence, CA). The mobile phase was an acetonitrile/water (50:50) mixture with 0.1% TFA (Lookhart et al., 2003). The flow rate was 0.5 ml/min, the temperature of the column was 40°C and, the conditions remained isocratic. The chromatograms were evaluated, and each peak represented one of the protein fractions: the soluble polymeric protein (SPP) fraction, the gliadin (GLI) fraction, and the albumin and globulin (A+G) fraction. The aforementioned proteins are listed by the order in which they were excluded.

#### *Fermentative capacity*

The fermentative capacities of the dough were determined by placing 315 g samples into a rheofermentometer (Chopin, type Rheo F3), and the protocol provided in the equipment manual was followed. The results were read after 3 h of fermentation at a constant temperature (28.5°C). Values for the volume of the total gas production ($CO_2$T, ml) and the volume of retained gas ($CO_2$R, ml) were obtained.

#### *Viscoelasticity*

Dough viscoelasticity was evaluated using a 2.6 g sample of thawed proofed dough. A controlled deformation rheometer (Rheometrics Scientific brand, model, RSF III, Piscataway, NJ, USA) equipped with parallel plates that were 25 mm in diameter was used for this purpose, and a Peltier system was used to maintain a sample temperature of 25°C. The dough was maintained on the appropriate plate with a 2 mm separation between the plates. Any leftover dough was removed from the apparatus, and the part of the sample that was exposed to the environment was covered with petroleum jelly to prevent dehydration. The sample was allowed to stand for 15 min in order to set. Oscillatory tests in a frequency sweep were measured at 0.1% strain in a linear regime; the frequency range was 0.1 to 100 rad/s (Magaña-Barajas et al., 2011). The storage modulus (G ', Pa), loss modulus (G'', Pa) and phase angle (δ, °) parameters were calculated using an appropriate software program (RSI Orchestrator, Rheometrics Scientific).

#### *Bread quality of frozen dough*

The molded thawed dough was fermented in a proofing cabinet with a fermentation temperature of 30°C and a relative humidity of 85% (MFG National brand, Lincoln, NE, USA). After fermentation, bread samples were obtained by baking the dough for 12 min at 250°C in a Partlow oven (National brand, MFG, Lincoln, NE, USA). Fully baked loaves were cooled for two hours at a temperature of 25°C (Magaña-Barajas et al., 2011) after the specific volume and crumb firmness of the bread was measured.

#### *Specific volume*

The displacement principle was used to determine the loaf volume of the bread; volume measurements were made using rapeseed and a volume meter (National brand MFG Company, PUP) that had been calibrated to a volume of 400 $cm^3$. Each loaf was weighed using a balance (OHAUS, 2610 g capacity), and the specific volume of the bread was obtained using its volume/weight ratio.

#### *Firmness*

The maximum firmness of the bread crumbs were evaluated using a universal testing machine (Instron Corp, model 4465, USA), and a modified version of A.A.C.C. method 74-09 (A.A.C.C., 2000); the modifications have been described by Magaña-Barajas et al. (2011). The modification of the method consisted of using geometry referenced to a 30 mm diameter. The sample bread crumbs were obtained by slicing the bread into pieces that were 25 mm thick. Square samples with side lengths of 30 mm were extracted from the center of the crumb and were used in the subsequent firmness evaluation. The measured parameter was the maximum force (kg-f) of the bread after two hours of storage.

#### *Experiment design and statistical analysis*

A randomized experimental design was performed in which the independent variable factor was the time over which the frozen dough was stored, and the levels of the variable were: 0, 7, 14, 21, 28, 35 or 42 days. To determine the effects of storage time on the various measured parameters, analyses of variance (ANOVA) were conducted. A 95% significance level was chosen to indicate significant differences. Tukey tests with the same statistical significance level were conducted to identify differences between specific experimental manipulations. In addition, simple correlations (r) among the various evaluations were made. The ANOVA was conducted using the Statistical Analysis Software System (SAS Institute, Inc. Cary, NC, 2002).

## RESULTS

### Flour quality evaluation

Flour with high protein content (13 to 15%) is recommended for production of frozen dough (Mesas and Alegre, 2002). Table 1 shows the results of the physicochemical and rheology of the flour quality. The flour showed high protein content, farinogram water absorption and alveogram extensibility (P/L) values 13.64%, 63.84% and 1.9. These parameter values are consistent for a high-quality bread flour (Mesas and Alegre, 2002), that is suitable for the production of frozen dough to make French type bread.

### Frozen dough evaluation

#### *Protein solubility*

Several researchers associate frozen dough deterioration with the degradation of protein fractions (Kennedy, 2000; Ribotta, et al., 2001; Li et al., 2012). Figure 1 shows chromatograms of bread dough after 0 and 42 days of storage at -18°C. The chromatograms were obtained using an SE- HPLC technique and outline three soluble protein fractions. Peak I corresponds to the soluble polymeric protein (SPP) fraction; peak II corresponds to the gliadin (GLI) fraction; and peak III corresponds to a fraction

**Table 1.** Physicochemical and rheological characteristics of the flour used to make french type bread.

| Determination | Value |
|---|---|
| *Proximal* | |
| Moisture[1] (%) | 11.11 |
| Protein[1] (%) | 13.64 |
| Ash[1] (%) | 0.94 |
| *Farinograph* | |
| Water absorption (%) | 63.84 |
| Stability (min) | 7.50 |
| Development time (min) | 7.17 |
| *Alveograph* | |
| Extensibility, P/L | 1.91 |
| General strength, W ($10^{-4}$J) | 248.50 |

1, Dry basis; P, Maximum height of the curve or stretch resistance; L Length of the curve or dough extensibility; W, Strain energy.

containing two proteins that are not related to gluten, albumin and globulin (A+G) (Borneo and Khan, 1999).

### *Soluble protein*

Figure 2 shows changes in solubilities of the soluble protein fractions (calculated as the areas under the curve) that occurred during the storage of frozen dough. Figure 2 shows that the average of soluble polymeric protein (SPP) solubility in frozen dough increased by approximately 8.09% during the first 14 days of storage. In general, after this time period (21 to 42 days) the average of SPP content of the frozen dough increase more slow. This reveals the degradation of high molecular weight glutenin results in gluten weakening.

The increase in degradation of SPP suggests that there may be structural breakdowns in protein polymers that promote the formation of new smaller and/or more soluble polymers or the reassociation of more soluble polymers to a new polymer with similar molecular weight at SPP. This coincided with the negative correlation found between SPP and GLI (r=-0.96) content, which indicates that higher levels of SPP hydrolysis were associated with increases in the amount of GLI. These results are in agreement with Lei et al. (2012) who observed a decrease of higher molecular weight proteins during storage of the frozen gluten. It could indicate that there is degradation in gluten of frozen dough. The general increase in SPP degradation explains the observed changes in the elastic behavior of the dough and in the quantity of retained gas.

Dough elasticity has been attributed to the SPP fraction, and the type(s) of SPP subunits in the dough determine its functionality (Field et al., 1983 in Tatham et al. 1995). Cornec et al. (1993) used SE-HPLC to characterize sub-fractions of fresh gluten by evaluating their individual rheology and relationships to the viscoelasticity of the dough. Gluten subunits can be classified into three groups; the HMW-GS group contains the gluten subunits that are responsible for dough elasticity. The elasticity conferred by the HMW-GS group appears to result from three aspects of these protein subunits. The first relates to their potential to form cysteine residue cross-links. The second is their spiral structure, and the third aspect is their high capacity to form intra- or intermolecular hydrogen bonds due to high levels of residual glutamine (Field et al., 1983 in Shewry et al., 1995). Belton (1999) describes a new model for the elasticity of the HMW-GS, indicating that viscosity is due to the high density of attached groups by hydrogen bonds provided by the long chain polymer itself. At the end, there are cysteine residues. The chains are joined together in the absence of water.

When hydrated promoted protein interactions by hydrogen bonds, without promoting the breakdown in existing hydrogen bonds. There will be a balance between interchain bonds and bonds with water. This promotes the formation of the region of loops and train region. This region is likely related to the β sheet formation. With increasing hydration of the region, the loop increases thereby decreasing the train region. The structure can be deformed first by the loops then by the train region. When this occurs the entropy of the loops is lost due to the formation of inter-chain hydrogen bonds, and is partially substituted by increasing the entropy of the hydrogen bonds of water released**.** Because HMW-GS subunits appear to determine the elasticity and baking quality of the dough, maintaining a certain quantity of these subunits in frozen dough designed for French type bread would be ideal (Shewry et al., 1995. Baier-Schenk et al. (2005b) used laser scanning microscopy to observe changes that occurred in gluten that had been isolated during freeze-thaw cycles. This group observed changes in the gluten fibrils that resulted from the water fusion-mediated cryo-concentration of protein polymers. Although the observed changes appeared to be reversible during thawing, the distribution of water in the resulting dough had changed by the end of the freeze-thaw cycle. Some authors explain that the degree to which the structural rearrangement of gluten is reversible depends on the origination of the structure and the types of links between the polymers that are present in it (Evans et al., 1996; Goff et al., 1999; Lozinsky et al., 2000 in Baier-Shenck et al*.,* 2005b).

Figure 2 also shows changes in the solubility of the Gliadin (GLI) fraction of frozen dough during storage. In general, during the first 21 days was observed a decrease on GLI fraction. This can be explained as function of a possible association between this polymer and other one to form a new polymer with similar molecular weight to the SPP. After this period, this fraction was reduced by an average of 10.55%, indicating the degradation of these polymers. Borneo and Khan (1999) evaluated changes in protein solubility in fresh dough during the baking process,

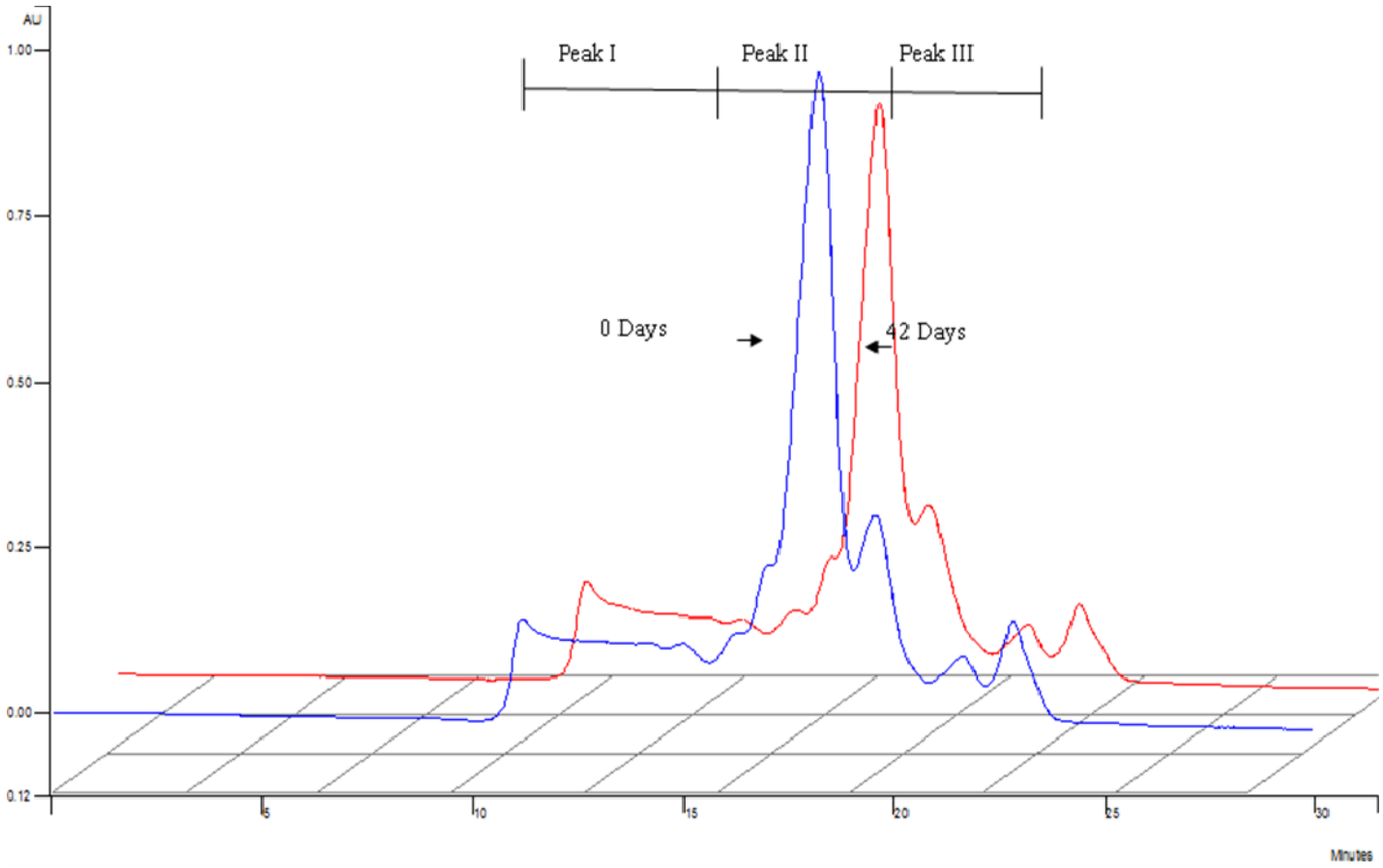


**Figure 1.** Chromatograms of french type bread dough at 0 and 42 days of storage at -18ºC.

and only they found evidence of changes in the SPP and A+G fractions. They interpret their findings as suggesting that large branching polymers composed of higher molecular weight subunits are more susceptible to changes during the baking process than smaller polymers, and the aforementioned changes are reflected in protein solubility; these changes appear to be similar to the changes that occur during the process of freezing and thawing dough.

Figure 2 shows also the changes in solubility of the albumin and globulin (A+G). In general, this coincides with changes in GLI fraction solubility. This reveals the degradation of gluten polymers associated with weakening of dough, and the poor breadmaking quality of frozen dough. Fluctuations in SPP, GLI and A+G contents of frozen dough evaluated by SE-HPLC after various period of frozen storage demonstrate the occurrence of the dissociation and/or reassociation of protein polymers during freezing and storage steps associated with increase of viscoelastic behavior of frozen dough. In general, the solubilities from all of the soluble protein fractions changed after 21 days of storage, which shows that a restructuring of protein polymers occurs in frozen bread dough. More specifically, a shift in the direction of the mass balance occurred that was oriented toward soluble protein degradation, and the size of this shift increased over time. Ribotta et al. (2001) observed a similar trend when using electrophoresis to evaluate changes in the concentration of various proteins in frozen dough protein that had been stored for as long as 7 days.

Clearly, the observed increase in the presence of non-gluten polymers in the frozen dough that occurred during storage corresponds to the weakening of the frozen dough, and this increase is likely also related to the loss of bread quality. In general, the SPP fraction (0.73%) was more affected by the freezing and storage of the dough than the other fractions was (0.63% GLI, ≈ A+G). Sharadanant and Khan (2003) used an SDS (sodium dodecyl sulfate) technique to study protein concentrations, and they also observed a direct relationship between soluble protein concentrations and storage. Shewry et al. (1995) mentioned a loss of dough elasticity that appears to occur when the SPP fraction dissociates into monomers because of the activity of reducing agents such as b-mercaptoethanol and dithiothreitol.

The frozen dough have intensified their use in recent decades, however, the low quality of their products has been associated with several factors. One of them is

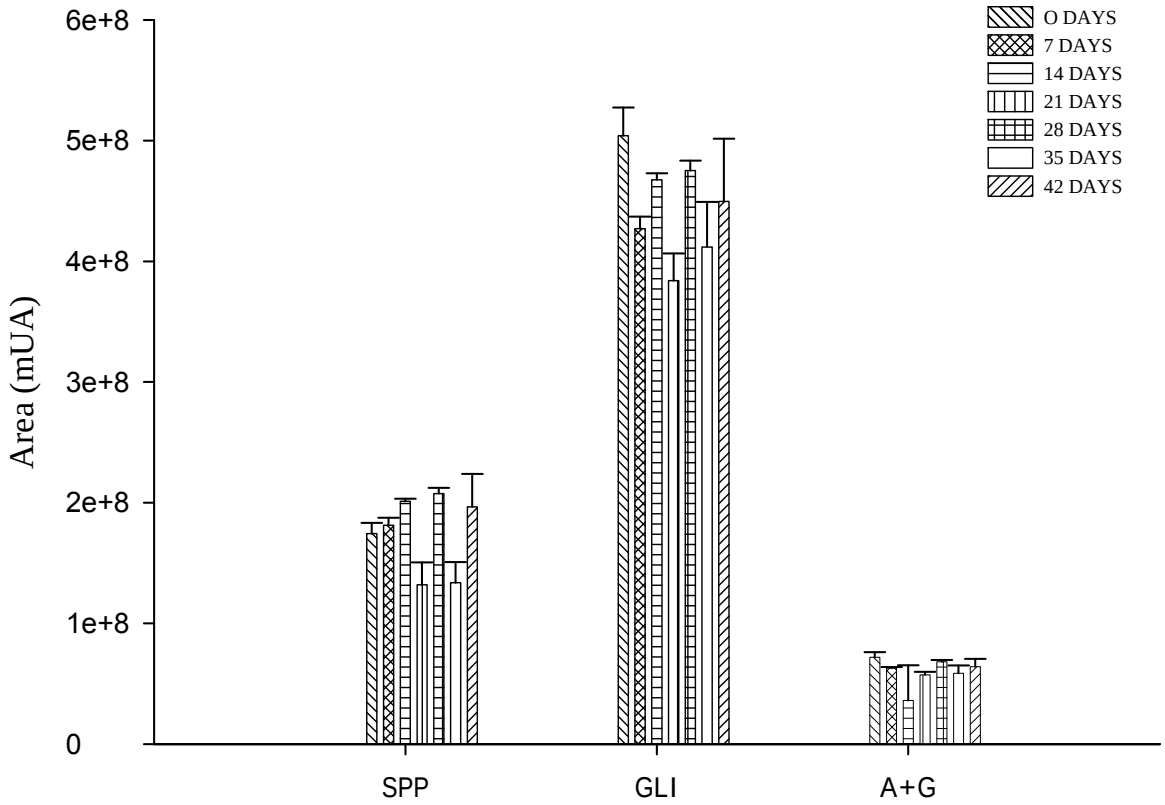


**Figure 2.** Changes in the solubilities of the soluble protein fractions in frozen french type bread dough during storage. SPP, soluble polymeric protein (peak I); GLI, gliadin (peak II); A+G, albumin + globulin (peak III). Bars indicate standard deviations.

attributed to possible breakings in the gluten protein polymers. However, there is little evidence of this. With our study, it was established that the SE-HPLC technology is suitable for detecting changes in frozen dough protein during storage. The main protein fraction affected was SPP, which is expected to be associated with the possible loss of the elastic behavior of dough and the low baking quality.

### *Fermentative capacity*

The low amount of $CO_2$ produced and retained in frozen dough is attributed to the weakening of the dough relevant mainly to the gluten network, and a deficient activity of the yeast. Changes in the fermentative capacity of frozen dough with respect to the storage time are shown in Figure 3. The variability of both the total gas production and the gas retention ($TCO_2$ and $RCO_2$, respectively) of the frozen dough during storage were evaluated using a rheofermentometer. Storage time had a significant effect ($p < 0.01$) on both parameters.

During the first 7 days of storage (day 0 to day 7), the fermentative capacity values of the frozen dough were reduced by 20% (for $TCO_2$) and 15% (for $RCO_2$). Between 7 and 42 days of storage, there were slight decreases in both parameters and the fermentative capacity of the frozen dough was still reduced compared to 0 days. During mixing, gas cells form in the dough, and the resulting increase in gas production results in a spongy product called bread. The freezing process apparently modifies the structure of the bread dough, thereby alters the fermentative capacity of it. The gas pressure exerted on the damaged dough exceeds its capacity to support gas cell formation, resulting in diminished bread volume. The observed decrease in the fermentative capacity of the dough used in the present study, particularly in terms of the $TCO_2$ (14.43%) value, was less substantial than that reported by El-Hady et al., (1996) (54% after 28 days of storage). This difference is most likely due to differences in the specific formulation and type of flour used in each study.

Some authors (Kontogiorgos, et al., 2008; Phimolsiripol et al., 2008) have attributed the observed reductions in gas retention to damage on the gluten network, damage caused by recrystallization. After damage to the gluten network occurs, the diffusion of both nutrients and the byproducts of yeast metabolism is limited (Yi and Kerr, 2009). This results in an increase in the crumb firmness of the fully baked bread and a decrease in the volume of it (Aibara et al., 2001). The observed reduction in $RCO_2$ also coincided with the measured protein degradation,

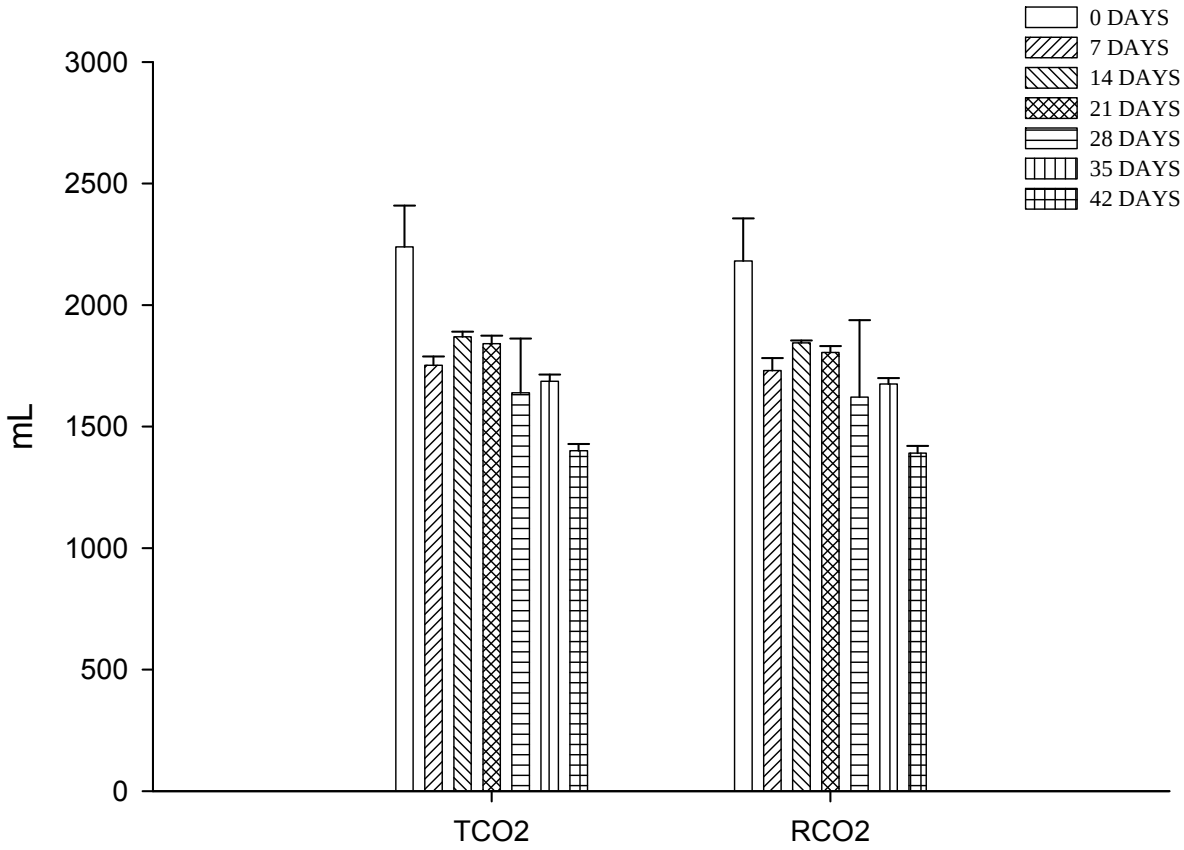


**Figure 3.** Changes in the fermentative capacity of frozen french type bread dough during storage. TCO2, total gas production; RCO2, retained gas. Bars indicate standard deviations.

particularly with the degradation of SPP, so a lower bread volume was expected. A conclusion is that the relationship between low levels of $RCO_2$ and decreased $TCO_2$ affect the specific volume of bread baked from frozen dough more than they affect its maximum crumb firmness.

The most dramatic changes in the fermentation capacity of the frozen dough were observed during the first 28 days of storage and appeared to coincide with the breaking and regrouping of protein chains. Both gas production and retention were adversely affected by increases in storage time, which further demonstrates the loss of the fermentative capacity associated with the use of frozen dough. These results agree with the findings of Aibara et al. (2001), who observed reduced $CO_2$ production by frozen yeast that had been stored at sub-zero temperatures that were reflected in the volume and firmness of the finished bread. An attempts to preserve and extend the freshness of French type bread dough by storing it at sub-zero temperatures affects both the viability of yeast and the viscoelastic structure characteristic of the gluten network. The temperature decrease creates pressure gradients in the dough that put osmotic pressure on the yeast and thereby compromises their ability to function normally. Protein hydrolysis that occurs in the frozen dough also coincides with its poor fermentative capacity. During freezing and storage, the polymer chains in the dough break down, which is demonstrated by the observed increase in the degradation of soluble protein. This weakening of the dough reduces its gas retention capacity.

### *Viscoelasticity*

Loss of the integrity of frozen dough has been reported as a general viscous behavior increase during storage (Ribotta et al., 2004). All of the viscoelastic parameters of the frozen dough were significantly affected ($p < 0.01$) by storage time. The values of the viscoelastic parameters used to evaluate the effect of storage time on the viscoelasticity of the frozen dough were recorded at a frequency 5 rad/s; the viscoelastic properties behave linearly at this frequency. Figures 4a, b, and c show the values of the storage modulus (G´), loss modulus (G´´) and phase angle (δ), respectively, obtained from the frozen dough after various periods of storage.

Figures 4a and 4b show that all of the storage durations had similar effects on the viscous (G´´) and elastic (G´) moduli of the frozen dough. Increases in the test

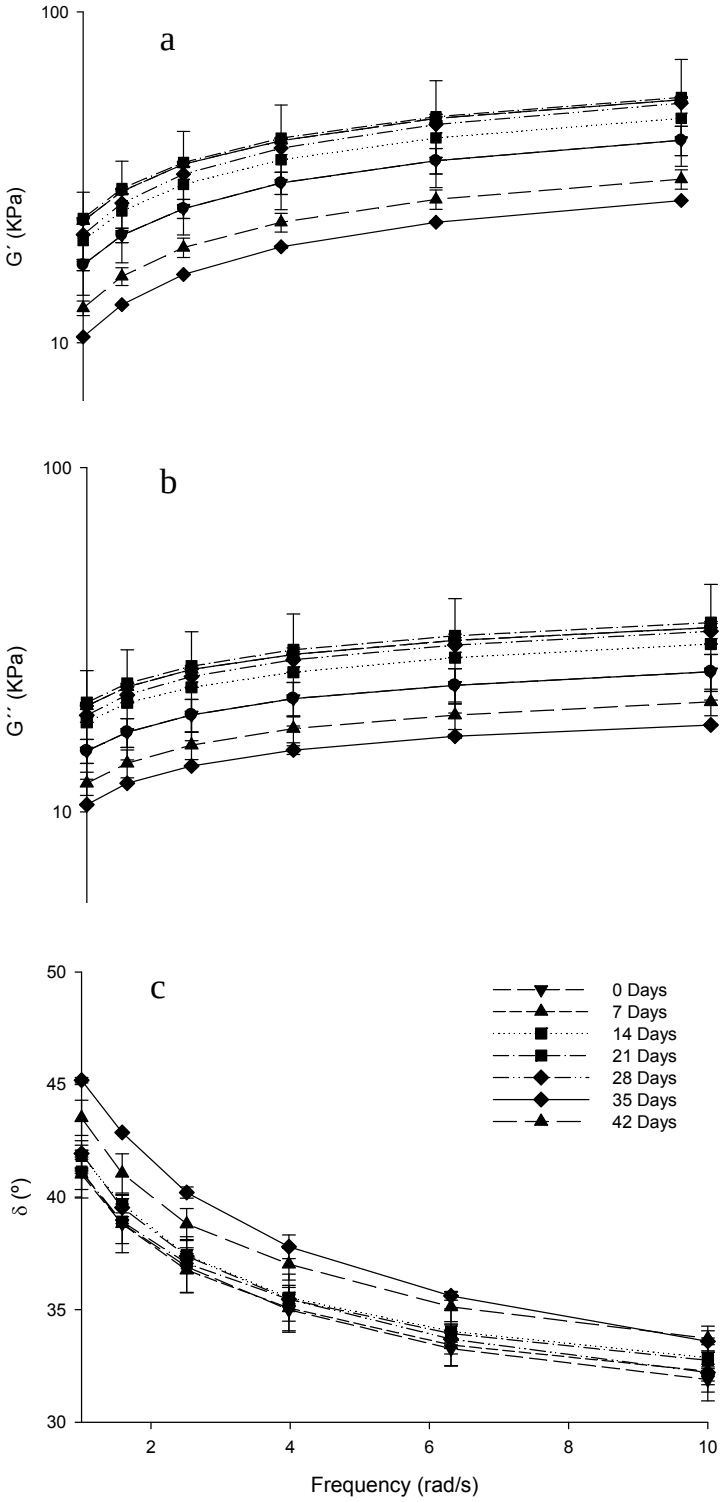


**Figure 4.** Behavior of the: a) storage modulus (G´), b) loss modulus (G´´), and c) phase angle (δ) of frozen french type bread dough during storage. Bars indicate standard deviations.

frequency were accompanied by increases the values of storage (G´) and loss (G´´) moduli. The G´ values were greater than the G´´ values, which agree with the findings of other authors (Ribotta et al., 2004; Angiolini et al., 2008; Leray et al., 2010). Moreover, both Figures (4a and 4b) show that for any frequency, the intermediate values

of both moduli were observed after 0 days of storage (G´=30.41 KPa and G´´=21.32 KPa, at a frequency of 5 rad/s). Furthermore, the largest increases in the G´ and G´´ values were observed between 0 and 7 days of storage (34.13 and 34.38% increases for G´ and G´´, respectively), coinciding with changes on SPP protein solubility.

From day 7 to 14, both the G´ and G´´ moduli of the frozen dough decreased to half of the respective values that had been obtained from frozen dough that had only been stored for seven days. However, the values were still greater than the values observed in dough that had been stored for 0 days (Figures 4a and 4b). From day 14 to day 21, the stored frozen dough had G´ values that were similar to the G´ values that had been obtained from frozen dough that had been stored for 7 days; the same trend was observed in the relevant values of G´´ (Figures 4a and 4b). This observation is consistent with both the increase in protein degradation and the reduction in gas retention capability that have been discussed in the preceding sections. Additionally, this observation shows that the reorganization of protein polymers that occurs during freezing affected the viscoelasticity of the dough.

Frozen dough that had been stored from 35 to 42 days showed lower values in two of the measured viscoelastic parameters (G´ and G´´). For this storage duration, the observed results of the viscoelastic evaluation of the dough were in agreement with those of other researchers who observed decreases in both G´ and G´´ during storage (Kenny et al., 1999; Leray et al., 2010). Between 28 and 42 days of storage, the average decreases in these parameters were 57.22 and 42.97% for the G´ and G´´ moduli, respectively. Progressive decreases in the values of G´ and G´´ during storage were expected to occur after day 35 (Ribotta et al., 2004; Angiolini et al., 2008; Leray et al., 2010), but the expected decreases occurred after 28 days of storage in this case.

The *Phase angle (δ)* value was used as a measure of the viscous behaviour of the frozen bread dough (Figure 4c). The structure of the frozen dough deteriorates during storage, and a clear increase in the value of δ with storage time can be observed. This increase in the δ value is consistent with the hydrolysis of the glutenin polymer and the low fermentative capacity of frozen dough. Frozen dough had the most elastic behavior at 0 days of storage (δ=35°), and this value was higher than that of dough made with fresh dough formulation (2% yeast and 2% shortening, data not show) (Magaña-Barajas et al., 2011), which concurs with the findings of other authors (Ribotta et al., 2004; Leray et al., 2010).

The decrease in the elastic behavior of frozen dough associated with structural changes in its gluten network may be caused by mechanical damage that occurs during the formation and growth of ice crystals. This damage results in smaller loaves. Phimolsiripol et al. (2008) further suggested that the damage to the gluten network was associated with decreased rates of gas retention.

Using transmission electron microscopy, Jiang et al. (2008) observed that the changes in the morphology of the gluten structure that occurred during freezing were caused by the formation of ice crystals and the re-crystallization phenomenon. This observation supported the notion of a direct relationship between structural changes in protein polymers that occur during freezing and the relatively poor quality of bread baked from frozen dough (Ribotta et al., 2001; 2004).

The viscous character of frozen dough increased between 0 and 42 days of storage, which is also in agreement with several researchers (Autio and Sinda, 1992; Ribotta et al., 2004, Angiolini et al., 2008). The transition of water from liquid to a glassy state and an increase in crystal size occurs during storage breakdown of protein polymers. This weakens the structure of the frozen dough and thereby increases the viscous behavior of it.

Thus, the weakening of the dough is associated with both an increase in SPP degradation and a loss of gas retention capacity. The most substantial loss of elastic behavior (25.88%) occurred after 28 days of storage, and most likely resulted in the most profound changes in the baking characteristics of the dough. Moreover, this temporal effect coincided with the observed loss of fermentation capacity, which is consistent with the findings of Leray et al. (2010).

The effect that freezing process has on bread dough is largely reflected in both the observed decreases in the G´ and G´´ moduli and the increase in the δ of frozen dough. Taken together, these effects indicate that the dough has been damaged, and they are consistent with Leray et al. (2010).

The weakening of the frozen dough during storage was expected and was reflected in the increase in the viscous behavior of the dough at the expense of the associated decrease in elastic behavior. In general, ice crystals tend to breakdown of various protein structures that alter the viscoelastic properties of the dough may be related to the observed loss of baking quality. The observed increase in the δ of the frozen dough (Autio and Sinda, 1992; Angiolini et al., 2008) and the loss in the extensibility of it (Yi and Kerr, 2009) that occur during storage associated with the development a gluten network containing more ruptures and fewer continuous, disintegrated remains of starch granules.

This result in the weakening of the dough was shown in the present study.

### *Bread quality of frozen dough*

The poor quality of bread obtained from frozen dough bread is associated with the loss of dough structure, increasing its effect during extended storage time. Figures 5 and 6 show the average values of the specific volume (SV) and the maximum crumb firmness (MF) of bread baked from frozen dough. Structural changes in

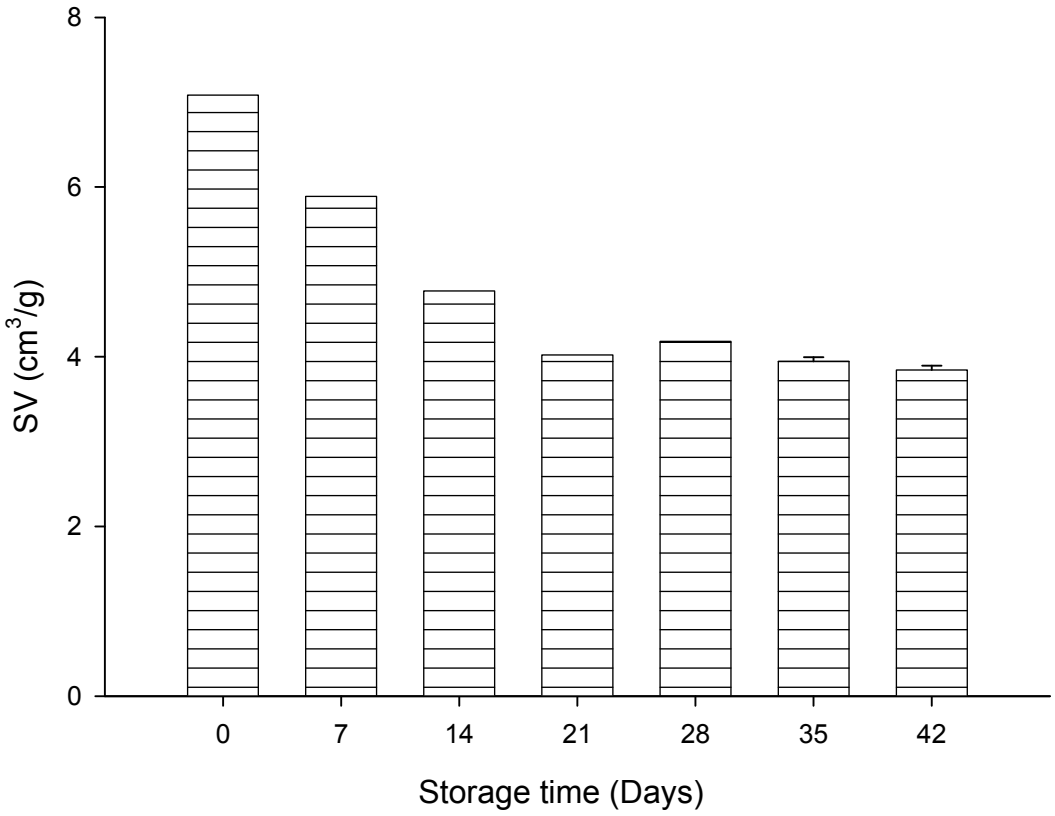


**Figure 5.** Effect of storage duration on the loaf volume of bread made from frozen french type bread dough. SV, specific volume. Bars indicate standard deviations.

the protein polymers and variations in the viscoelasticity of the frozen dough that occur during storage can result in variations in bread quality. Two research groups (El-Hady et al., 1996; Gianno and Tzia, 2007) have shown that the loaf volume is the main quality of bread affected by the liquid-glassy transition of water that occurs during the freezing process. The ANOVA showed that storage time had a significant ($p < 0.01$) effect on both of the bread quality parameters evaluated.

### *Specific volume (SV)*

Bread volume made of frozen dough, is considered the main quality parameter that is affected. Figure 5 presents the effect of storage time on SV. Bread made with dough with 0 days of storage had the highest SV (7.10 $cm^3/g$) of the breads made from dough that had been stored for any of the durations we tested. The largest decrease in the SV (10.47%) occurred between 0 and 7 days of storage. The isolation of proteins led to an increase in the viscous behavior of frozen dough that had been stored for 7 days (0.28%, $\delta = 35.10°$). Because the gas pressure exceeded the rate of gas cell rupture in the weakened frozen dough, some of the gas produced during baking was released, thereby reducing the SV of the bread. This observation is consistent with Kenny et al. (1999) who suggested that the poor gas retention capacity of the frozen dough was associated with damage to the gluten network. The results are also consistent with Gabric et al. (2011). They observed an inverse relationship between the SV of bread made from frozen dough and the duration of dough storage, and they attributed the loss of SV to the phenomena of the coalescence and disproportionation (Kokelaar and Prins, 1995) of the gas cells in the dough. Those authors related the observed reduction in the SV of bread made from frozen dough with breaks in the structure of the gluten network and $CO_2$ diffusion within the frozen dough. Borneo and Khan (1999) identified relationship between the bread volume and the SPP and GLI protein fractions ($r=0.73$ and $r=-0.64$, respectively) found in fresh dough. Lu and Grant (1999) increased the content of the GS fraction in frozen dough and found that doing so had a positive effect on the bread volume.

The SV of the bread made from frozen dough in the present study decreased by 46.05% between 0 and 21 days of storage. During days 21 to 28 and 28 to 42 of storage, bread made from frozen dough had a SV decrease of 1.5%. Kenny et al. (1999) reported that rolls that were smaller in volume than the frozen dough itself exhibited more viscous behavior. This progressive loss in

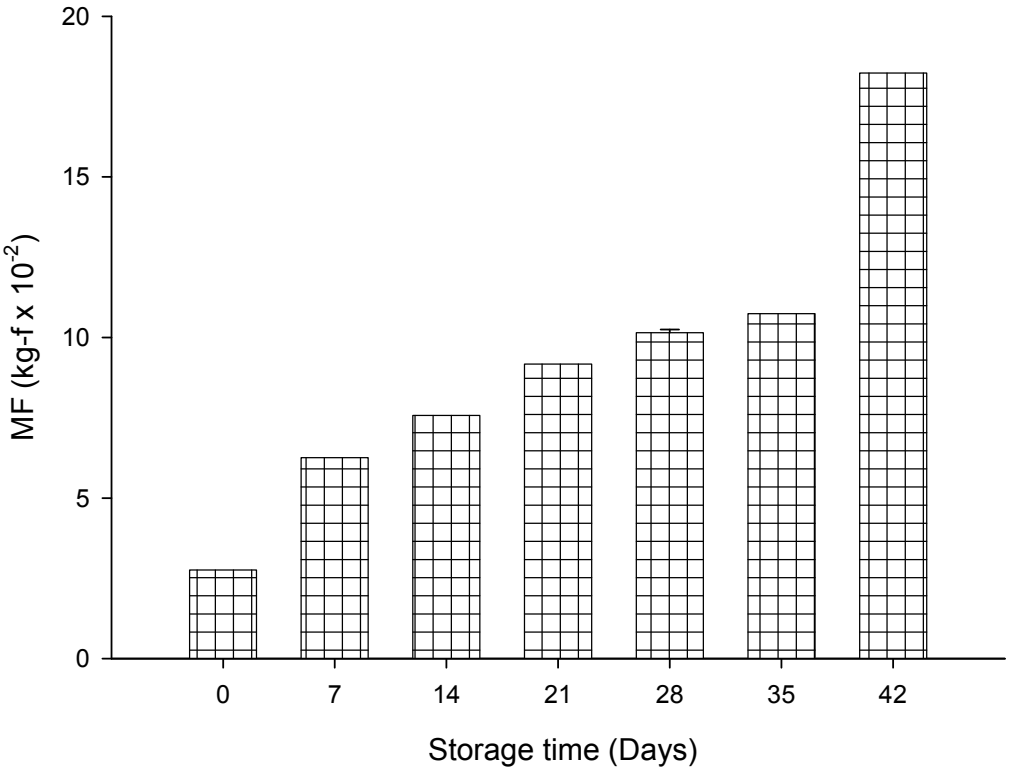


**Figure 6.** Effect of storage duration on the crumb firmness of bread made from frozen french type bread dough. MF, maximum firmness. Bars indicate standard deviations.

the SV of bread made from frozen dough is consistent with the observed protein degradation and weakening of the dough during storage. It also concurs with the observations of Matuda et al. (2008), who concluded that the compressive stress caused by the ice-water transition caused a reduction in both the number and size of the gas cells. Further, Ribotta et al. (2001) suggested that a reduction in gas retention efficiency in frozen bread dough was associated with the depolymerization of the gluten proteins. In the present study, SV losses associated with the storage of frozen dough appear to result from the increased viscous behavior of the dough caused by the degradation of proteins within it and the poor gas retention capacity of it.

Mezaize et al. (2010) observed a 24% reduction in the SV of bread made from frozen dough in comparison to bread made from fresh dough. In the present investigation, a lower average difference of 10.47% was observed and can be attributed to the formulation and flour that were used. We identified a significant correlation between the SV and $RCO_2$ ($r = 0.79$, $p < 0.05$). Reductions in the concentrations of certain protein polymers that occur during both the freezing and storage of frozen dough cause distinct structural reorganization of the native protein polymers. This reorganization contributes to the formation of less elastic gas cells, so the resulting bread is less likely to harbor large amounts of gas and therefore has a lower SV. These results are also consistent with Yi and Kerr (2009), who observed that the SV (specific volume) of bread tends to diminish with the duration of dough storage. However, in one study, a loaf in which the SV increased after storage at -18°C was observed, and the increase was thought to be associated with the decreased extensibility of the dough (which is thought to determine the ability of the dough to retain gas during fermentation (Sharadanant and Khan, 2003).

### *Firmness*

The decrease in cell size of gas and its heterogeneous distribution in the crumb are parameters related to damage occurring during freezing of the dough, which is reflected in an increase in bread firmness. Figure 6 shows the effect of storage on the maximum crumb firmness (MF) of bread made from frozen dough. Bread made from dough with 0 days of storage had both the softest MF ($2.68 \times 10^{-2}$ kg-f) and the highest SV.

Moreover, the greatest increase in MF occur between 0 and 7 days of storage about a factor of two occurred, but this value is similar at MF of bread made with the formulation of fresh bread (2% yeast and 2% shortening, data not show) (Magaña-Barajas et al., 2011). The MF is inversely related to the SV. During the first 7 days of sto-

rage, structural changes occur in the gluten network of frozen dough and result in three-dimensional changes in it. The prevailing view is that these changes likely results in a reduction in the formation of gas cells that contributes to the observed increase in MF. Between 7 and 14 days of storage, there was an increase of 20% of the MF, the same occur during 14 and 21 days of storage. During 21 and 28 days of storage, there was an average MF increase of 10%. The next seven days the MF there were not a significative change. At 42 days of storage there was an average increase in MF about a factor of seven.

The MF was the baking quality parameter that was most affected by the storage of the frozen dough, and this observation is in accordance with the negative correlation between $RCO_2$ and MF ($r = -0.90$, $p < 0.01$). This correlation results from the low gas retention capacity of the frozen dough, which is also associated with the structural rearrangement of the proteins that contributed to the observed MF increases. In bread made from frozen dough, both the capacity to retain gas and the amount of retained gas decrease, thereby reducing the interstitial spaces within the matrix formed by gluten and other ingredients and ultimately resulting in a bread material that is denser than fresh bread. Therefore, compressing bread made from frozen dough requires more force than compressing fresh bread, and the measured MF increases. Some authors have also observed an increase in the MF that results from the storage of frozen bread dough (Ribotta et al., 2004; Phimolsiripol et al., 2008; Yi and Kerr, 2009). One group (Ribotta et al., 2004) suggested that the observed increase in bread firmness that was associated with storage resulted from a high degree of glutenin depolymerization that was also linked to the retrogradation of starch present in frozen dough.

The bread made from frozen dough that had been stored for more to 7 days had a lower SV and higher MF than bread made from fresh dough. These changes were associated with protein degradation, a decline in the fermentative capacity of the dough and increased viscous behavior during storage at -18°C, and the greatest differences among the dough were observed for these three measures.

## Conclusions

The technique of SE-HPLC found that freezing and storing bread dough causes protein polymer restructuring which affects fermenting capacity (gas retention), viscoelasticity and consequently the baking quality of the bread. Stress caused by freezing the dough increased both the size and number of water crystals that formed during storage, which then weakened the network of gluten. The observed decay in the elastic behavior of the frozen dough is related to the breakdown of gluten polymers; this decay is indicated by the increase in protein solubility, particularly that of the soluble polymeric protein fraction. Damage to the structure of the gluten network in the frozen dough also lowered the capacity of the dough to retain gas produced during fermentation. Thus, freezing French type bread dough results in a lower loaf volume and increased crumb firmness.

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## Conflict of Interests

The author(s) have not declared any conflict of interests.

### REFERENCES

Aibara S, Mishimura K, Esaki K (2001). Effects of shortening on the loaf volume of frozen dough bread. Food Sci. Biotech. 10: 521-528.

Aibara S, Ogawa N, Hirose M (2005). Microestructures of bread dough and the effects of shortening on frozen dough. BBB. 69: 397-402.

Angiolini A, Balestra F, Pinnavaia GG, Dalla Rosa M (2008). Small and large deformation test for the evaluation on frozen dough viscoelastic behavior. J. Food Eng. 87: 527-531.

Autio K, Sinda E (1992). Frozen doughs: rheological changes and yeast viability. Cereal Chem. 69: 409-413.

Baier-Schenk A, Handschin S, Conde-Petit B (2005a). Ice in prefermented frozen bread dough—an investigation based on calorimetry and microscopy. Cereal Chem. 82: 251-255.

Baier-Schenk A, Handschin S, Von Shönau M, Bitterman AG, Bächi T, Conde-Petit B (2005b). In situ observation of the freezing process in wheat dough by confocal laser scanning microscopy (CLSM): formation of ice and changes in the gluten network. J. Cereal Sci. 42: 255-260.

Belton PS (1999). Mini review on the elasticity of wheat gluten. J. Cereal Sci. 29: 102-107.

Berglund PT, Shelton DR, Freeman TP (1991). Frozen bread dough ultrastructure as affected by duration of frozen storage and freeze-thaw cycles. Cereal Chem. 68: 105-107.

Bhattacharya M, Langstaff TM, Berzonsky WA (2003). Effect of frozen storage and freeze-thaw cycles on the rheological and baking properties of frozen dough. Food Res. Int. 36: 365-372.

Borneo R, Khan K (1999). Protein changes during various stages of breadmaking of four spring wheats: quantification by size-exclusion HPLC. Cereal Chem. 76: 711-717.

Cauvain S, Young L (2008). Bakery Food Manufacture and Quality. Water Control and Effects. 2da. Ed. Ames, Iowa. ISSN: 9781405176132 140517613X

Codón AC, Rincón AM, Moreno-Mateos MA, Delgado-Jarana J, Rey M, Limón C, Rosado IV, Cubero B, Peñate X, Castrejón F, and Benítez T (2003). New Saccharomyces cerevisiae baker´s yeast displaying enhanced resistance to freezing. Journal of Agricultural Food Chem. 51: 483-491.

Cornec M, Popineu Y, Lefebvre J (1993). Characterization of gluten subfraction by SE-HPLC and dynamic rheological analysis in shear. J. Cereal Sci. 19: 131-139.

Cuq B, Abecassis J, Guilbert S (2003). State diagrams to help describe wheat bread process. Int. J. Food Sci. Technol. 38: 759-766.

El-Hady EA, El-Samahy SK, Seibel W, Brümmer JM. (1996). Changes in gas production and retention in non-prefermented frozen wheat doughs. Cereal Chem. 73: 472-477.
Esselink FJ, Van Aalst H, Maliepaard M, Van Duynhoven PM (2003). Long-term storage effect in frozen dough by spectroscopy and microscopy. Cereal Chem. 80: 396-403.
Field JM, Shewry PR, Miflin BJ. (1983). Solubilization and characterization of wheat gluten proteins: correlation between the amount of aggregated proteins and baking quality. J. Sci. Food Agric. 34: 370-377.
Gabric D, Ben-Aissa F, Le-Bail A, Monteau JY, Curic D (2011). Impact of process conditions on the structure of pre-fermented frozen dough. J. Food Eng. 105: 361-366.
Gant Z, Angold RE, Wiliams MR, Ellis PR, Vaughan JG, Galliard T (1990). The microstructure and gas retention of bread dough. J. Cereal Sci. 12: 15-24.
Giannou V, Tzia C (2007). Frozen dough bread: quality and textural behavior during prolonged storage-prediction of final product characteristics. J. Food Eng. 79: 929-934.
Godón B, Herard J (1984). Extractability of wheat proteins. I. Effects on the protein associations of the mixing of dough in presence of various active compounds [molecular interactions]. Sci. Aliment. 4: 287-303.
Hoseney RC, Hsu KH, Junge RC (1979). A simple spread test to measure the rheological properties of wheat flour dough. Cereal Chem. 56: 153-160.
Jiang B, Kontogiorgos V, Kasapis S, Goff HD (2008). Rheological investigation and molecular architecture of highly hydrated gluten networks at subzero temperatures. J. Food Eng. 89: 42-28.
Karaoğlu MM, Kotancilar HG, Gerçekaslam KE (2008). Quality and textural behavior of par-baked and rebaked cake during prolonged stored. Int. J. Food Sci. Technol. 44: 93-99.
Kennedy CJ (2000). Freezing process foods: in Kennedy CJ (ed), managing frozen foods. Woodhead Publishing, Cambridge, pp. 143-149.
Kenny S, Wherle K, Dennehy, Arendt EK (1999). Correlations between empirical and fundamental rheology measurements and baking performance of frozen bread dough. Cereal Chem. 76: 421-425.
Kokelaar JJ, Prins A (1995). Surface rheological properties of bread dough components in relation to gas bubble stability. J. Cereal Sci. 22: 53-61.
Kontogiorgos V, Goff HD, Kasapis S (2008). Effect of aging and ice structuring proteins on the physical properties of frozen flour-water mixtures. Food Hydrocolloid. 22:1135-1147.
Kontogiorgos V (2011). Microstructure of hydrated gluten network. Food Res. Int. 44: 2582-2586.
Lefebvre J, Mahmoudi N (2007). The pattern of the linear viscoelastic behavior of wheat flour dough as delineated from the effects of water content and high molecular weight glutenin subunits composition. J. Cereal Sci. 45: 49-58.
Lei Z, Li L, Guo-Qin L, Bing L (2012). Effect of frozen storage on molecular weight, size distribution and conformation of gluten by SAXS and SEC-MALLS. Molecules. 17: 7169-7182.
Leray G, Oliete B, Mezaise S, Chevallier S, De Lamballerie M (2010). Effects of freezing and frozen storage conditions on the rheological properties of different formulations of non-yeasted wheat and gluten free bread dough. J. Food Eng. 10: 70-76.
Lookhart GL, Beans SR, Bietz JA (2003). HPLC of gluten monomeric proteins. In: wheat gluten proteins analysis. P.R. Shewry G.L., Lookhart, eds. A.A.C.C. International: St. Paul, MN.
Lu W, Grant LA (1999). Role of flour fractions in breadmaking quality of frozen dough. Cereal Chem. 76: 663-667.
Magaña-Barajas E, Ramírez-Wong B, Torres PI, Sánchez-Machado DI, López-Cervantes J (2011). Efecto del contenido de proteína, grasa y levadura en las propiedades viscoelásticas de la masa y la calidad del pan tipo francés. Interciencia. 36: 248-255.
Matuda TG, Chevallier S, Pessôa Filho P, Lebail A, Tadini CC (2008). Impact of guar and xantan gums on proofing and calorimetric parameters of frozen bread dough. J. Cereal Sci. 48: 741-746.
Mezaize S, Chevallier S, Le-Bail A, De Lamballerie M (2010). Gluten-free frozen dough: influence of freezing on dough rheological properties and bread quality. Food Res. Int. 43: 2186-2192.
Phimolsiripol Y, Siripatrawan U, Tulyathan V, Cleland D (2008). Effects of freezing and temperature fluctuations during frozen storage on frozen dough and bread quality. J. Food Eng. 84: 48-56.
Ribotta PD, León AE, Añón MC (2001). Effect of freezing and frozen storage of doughs on bread quality. J. Agric. Food Chem. 49: 913-918.
Ribotta PD, León AE, Añón MC (2003). Effects of freezing in frozen dough. Cereal Chem. 80: 454-458.
Ribotta PD, León AE, Añón MC (2004). Effect of emulsifier and guar gum on micro structural, rheological and baking performance of frozen bread dough. Food Hydrocolloids. 1: 305-313.
Selomulyo VO, Zhou W (2007). Frozen bread dough: effects of freezing storage and dough improvers. J. Cereal Sci. 45:1-17.
Sharadanant R, Khan K (2003). Effect of hydrophilic gums on frozen dough. Cereal Foods World. 40: 827-831.
Sharadanant R, Khan K (2006). Effect of hydrophilic gums on the quality of frozen dough: electron microscopy, protein solubility, and electrophoresis studies. Cereal Chem. 83: 411-417.
Shewry PR, Tatham AS, Barro F, Barcelo P, Lazzeri P (1995). Biotechnology of breadmaking: unraveling and manipulating the multi-protein gluten complex. Biotechnology. 1185-1190.
Wolt MJ, D´Appolonia BL (1984). Factors involved in the stability of frozen dough. I. the influence of yeast reducing compounds on frozen-dough stability. Cereal Chem. 61: 209-212.
Yi J, Kerr WL (2009). Combined effects of freezing rate, storage temperature and time on bread dough and baking properties. Food Sci. Tech. 42: 1474-1483.