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Influence of yeast and frozen storage on rheological, structural and microbial quality of frozen sweet dough

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ABSTRACT

The aim of the present study was to investigate the effect of yeast content and frozen storage (9 weeks at −40 °C) on the structural and rheological parameters, and fermentative activity of frozen sweet dough. Two types of dough were studied (to estimate dough shelf life): simple yeasted dough (SY) and double yeasted dough (DY). Fermentative activity (yeast viability, gassing power, and dough volume), rheological and textural parameters were assessed for frozen sweet doughs.

These effects were explored by different and complementary methods: Fourier transform infrared (FTIR), dynamic rheology, texture profile analysis (TPA) and differential scanning calorimetry (DSC).

The data showed that the longer the frozen storage time at −40 °C, the higher the decreased of frozen sweet dough quality. The rheological attributes such as hardness, ΔS, springiness, $\tan\delta$ and yeast activity declined significantly during frozen storage. This modification led to lower specific volume of frozen sweet dough during proofing.

The observed changes of the frozen sweet doughs rheological properties after thawing may be attributed to the damage on the gluten cross-linking, mainly produced by the ice crystallization during frozen storage. The storage effect was particularly concentrated in the first 27 days of storage.

1. Introduction

Frozen products are not indefinitely stable; they gradually deteriorate until they reach an unacceptable quality. This quality loss is reflected by reduction in dough volume and an increase in proofing time in comparison with dough prepared by using traditional methods.

The shelf life of frozen dough is estimated for 8–9 weeks if the dough has not been abused during transportation and frozen storage (temperature, formulation, freezing, transportation) (Le Bail et al., 1999).

However, the decrease of frozen dough volume is related to phenomena that occur during freezing and frozen storage: (i) reduced yeast fermentative capacity (ii) loss of the gluten network integrity. This behavior affects the dough machinability, creating a problem in the industrial chain (reducing the dough shelf life), because the dough quality is reduced (resistance losses during proofing) (Selomulyo and Zhou, 2007). The resistance decrease leads to cracking of dough gluten network, the latter resulting in poor gas retention and loss of volume when baking.

These phenomena are probably due to uneven water redistribution in dough matrix during freezing (Giannou and Tzia, 2007). On the other hand, Gormley et al. (2002) presented that the temperature fluctuations during storage and transportation altered frozen dough quality due to the crystal ice recrystallization. Indeed, the gassing power depends on the yeast cells number, strain, physiological state of yeast and fermentable sugars amount (Teunissen et al., 2002).

Freezing and frozen storage affect the viability and activity of yeast cell. The volume of carbon dioxide produced has been used as a measure of yeast activity, since it is primarily produced by yeast. Various studies reported the freezing effect on properties of yeast which is a major concern in the frozen dough manufacturing. Nemeth et al. (1996) demonstrated that yeast viability is strongly influenced by time and dough temperature before freezing.

In the case of frozen dough, it is important that dough temperature before freezing stayed below 20 °C to prevent the fermentation onset (Hino et al., 1987). This phenomenon can be explained by a high sensitivity of yeast cells to damage caused by osmotic pressure in dough matrix.

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The freezing rate can be considered a key indicator in the frozen dough quality because it regulates yeast activity. The freezing rate must be slow enough to avoid the crystals ice formation in the cell, while quick to minimize the cells exposure to the effects of solution concentration caused by water crystallization. Other authors discussed the damage of yeast membranes exposed to high concentrations as well as temperature (Morris and Clarke, 1987).

However, the injuries caused by freezing and storage can be minimized by adopting appropriate measures during formulation and use adapted production process.

The yeast quantity used in frozen doughs, depends on the time of frozen storage, the formulation of the dough and wished fermentation duration after thawing (Brüinsma and Giesenschlag, 1984). To improve freezing performance, some technological parameters, such as freezing rate and freezing temperature must be taken into consideration as well as yeast strain choice and increasing the yeast amount to compensate the loss of fermentative activity. This practice is to add between 50% and 100% more than the standard level according to the frozen storage time. However, over addition of yeast increases the process cost and could cause a yeasty taste in the final product (Lorenz and Kulp, 1995; Neyreneuf and Van Der Plaat, 1991).

Various authors have reported the frozen storage time effect on the dough textural and rheological properties (Gelinas and McKinnon, 2004). During freezing, there are also structural changes in frozen dough due to the mechanical action of ice crystals during freezing and storage, leading to a deterioration of frozen dough network which is manifested by dough strength decrease and poor gas retention during proofing (Berglund et al., 1991).

In addition, the gluten network weakening of frozen dough is attributed to the release of yeast substance reducing such as glutathione (Kline and Sugihara, 1968).

Several authors investigated the individual effects of storage time or temperature on the bread dough properties. El-Hady et al. (1996) studied the yeast content influence on the structural properties of frozen sweet doughs during storage.

According to the literature, no FTIR spectroscopy studies describing the effect of freezing rate and storage time on the rheological properties of frozen dough were published.

A singularity of this work was to investigate effect of yeast content and frozen storage time at glass transition temperature (Tg) on the rheological and textural parameters of frozen sweet dough with 20% sugar and 30% butter, which reduces the water activity of the sweet dough. We also investigated the yeast content effects on the fermentative activity (gas production, yeast viability and specific volume) and rheological behaviors in frozen sweet doughs during frozen storage and these properties were compared to control (0 day) (ii) to define the optimal storage time for sweet doughs.

2. Material and methods

2.1. Sample preparation

Samples of dough have been prepared as described in a previous work (Meziani et al., 2011). Dough experiments were done in duplicate. In order to study the effect of yeast and the freezing time, two types of dough were studied: simple yeasted dough (SY) and double yeasted dough (DY).

2.2. Freezing process and frozen storage

Total dough cylinders were placed in an industrial freezer (Panem, France); the freezer produced air blast temperature at −40 °C. The freezing rate was estimated to be about −0.39 °C/min. Temperature in the dough during freezing process was recorded using a *K* type thermocouple (300 μm diameter Ahlborn France) connected to an Almemo data logger (model 2290-8 V5 AMR). The thermocouples were calibrated using a reference thermometer at ±0.1 °C. At the end of freezing, the dough cylinders were stored at −40 ± 1 °C in the freezer (TT 151 Curtiss, France) for 9 weeks.

2.3. Thawing and preparation of dough for analysis

After frozen storage, frozen dough samples were thawed into two steps according to the method previously described by Meziani et al. (2011). All dough analyses were performed on four frozen doughs samples tested for each dough type during storages period.

2.3.1. Yeast survival determination

After thawing, the number of viable yeasts was determined in the each dough sample, using the direct plate count method (Phimolsiripol et al., 2008). Logarithmic dilution counts were carried after the dilution of 10 g of dough in 90 g of tryptone salt solution (Biokar Diagnostics, France). The diluted suspension (0.1 ml) was cultured on Petri dishes with 15 ml of OGA (Oxytetracycline Glucose Agar) base (Biokar Diagnostics, France). The cells were incubated at 25 °C for 72 h. The developed CFUs were expressed per gram of dough. Triplicates plates were prepared for each of three dough samples per sweet dough (SY and DY).

2.3.2. Gassing power and dough volume evaluation during proofing

The CO_2 production was measured. Thirty grams of dough were put in a vessel (1000 ml volume) immerged into a water bath at 28 °C for 180 min. The vessel was connected to inverted test-tube filled with water at pH 2 and hermetically closed. The CO_2 volume was measured at regular intervals through the displacements of water in the test-tube (Peighambardoust et al., 2010).

The dough volume was determined by the method described by Havet et al. (2000). Dough pieces were placed in a sterilized graduated test-tube (5 cm diameter and 10 cm height). The device was placed in a chamber at 2.8 °C and 85% relative humidity for 180 min. The vertical displacement of the cursor due to the dough volume increase in graduated test-tube during proofing gave the dough volume (ml). All measurements were performed in triplicates using four dough samples per sweet dough (SY and DY).

2.3.3. Infrared measurements

Similarities and differences in secondary structures of sweet doughs according frozen storage time were investigated using Fourier-transform infrared (FT-IR) spectroscopy. The protocol used in this study has been previously described by Meziani et al. (2011). Six independent experiments were done for each sweet dough.

Data analysis was carried out using the OPUS 3.0 software (Bruker, Karlsruhe, Germany) as described by Meziani et al. (2011). This decomposition allowed the determination of the fraction of the various secondary elements in the protein during frozen storage time.

Different zones were identified: water (3350 cm^{-1}); fat (2958 cm^{-1}); Amide I (1650 cm^{-1}); Amide II (1546 cm^{-1}) and Amide III (1245 cm^{-1}). To study the water effect on protein on secondary structure; the amide III band was investigated. The amide III band deconvolution provide various peaks and their frequencies correspond to: α-helix (1330–1295 cm^{-1}), β-turn (from 1295 to 1270 cm^{-1}) and random coil (1270–1250 cm^{-1}) and β-sheet (1250–1220 cm^{-1}) (Cai and Singh, 1999).

2.3.4. Dough rheology

2.3.4.1. Textural analysis. The compression-tension measurement is one of the standard assays used to measure the dough textural properties. The textural parameters were carried out on seven

samples for each type of sweet dough. TPA was used to evaluate freezing treatment effect on dough (Olivera and Salvadori, 2009), using a universal testing machine (LRX-LLOYD tensile-compression) equipped with a 50 mm probe (P/50). The experimental parameters were the following: dough disk: 5 cm in diameter, 2.5 cm thickness and 30 g weight.

The double compression cycle was carried out at test speed of 60 mm/s, with a 40% of compression and a resting period of 60 s. Hardness is defined as the peak force during the first compression cycle and the springiness is defined as the ratio of length of dough detected height during the second compression to that the first compression. Hardness and springiness were measured in the absence of dough adhesiveness by using a plastic film on the dough surface to avoid the distortion induced by the negative peak of adhesiveness (Collar et al., 1999).

Havet et al. (2000) method was used for determining the difference between the maximum force and the strength after the relaxation (ΔS). The experimental samples data were: 5 cm in diameter, 2.5 cm thickness and 30 g weight. The sample was compressed with a 5 N force using to an aluminum probe 5 cm in diameter, a test speed of 1 cm/min and the relaxation was recorded for a 60 s.

To avoid the fermentation effect, all rheological assessments were realized at 8 °C on thermo stated plate connected to an external bath insulated with polystyrene.

2.3.4.2. Rheological measurements. Dynamic rheological measurements were performed with a Kinexus rheometer (Malvern, England) using the method described previously by Meziani et al. (2011).

2.3.5. Tg measurement using DSC

The glass transition temperature was measured using differential scanning calorimetry (Netzcsh DSC 204 F1 Phoenix®, Germany) according to Meziani et al. (2011).

2.3.6. Statistical analysis

Pearson correlation analysis and analysis of variance (ANOVA) were computed using the KyPlot software (Version 2.0 beta 15, 1997–2001 Koichi Yoshioka). All tests were done at the 95% significance level to find out if the effects frozen storage time and yeast amount on the sweet dough properties were significantly different.

3. Results and discussion

3.1. Effect of frozen storage on yeast cells

Frozen storage at −40 °C caused considerable decay in yeast population of sweet doughs, as shown in the Fig. 1.

The reduction kinetics of yeast cells that affect the overall quality of products (bread products in our study) could be obtained from the following equation:

$$-\frac{dN}{dt} = KN^n \quad (1)$$

where N is yeast population number, (t) is variable time, K is death rate constant and n is equation order (can be 0, 1 and 2).

The survival yeast percentage was assessed on two doughs SY and DY ($N_{0DY} = 2 \times N_{0SY}$) stored at −40 °C for 9 weeks. The different reaction orders were studied (0, 1 and 2).

The model equations and their correlation coefficients (R^2) were determined for each reaction order and the slope K was determined from these equations. Meanwhile, analysis of linear regression results showed that the kinetics of decrease in yeast population of both SY and DY doughs obeyed a first order model reaction 1 ($R^2_{SY} = 0.917$ and $R^2_{DY} = 0.906$). The values of yeast loss rate (K) obtained for the doughs SY and DY are 9.00E−03 ± 0.0027and 1.40E−02 ± 0.0013 CFU/days, respectively. The majority of authors recommends to increase or double yeast quantity to compensate this loss in the frozen dough manufacturing (Phimolsiripol, 2009).

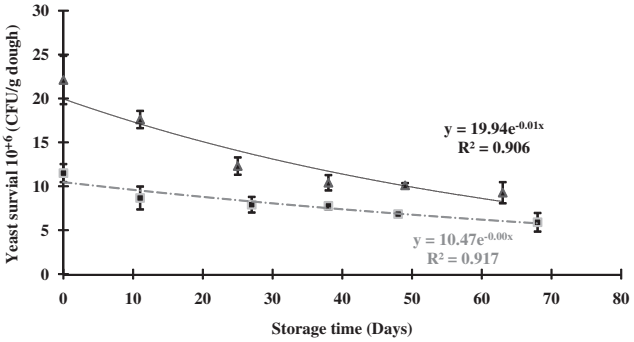

Fig. 1. Yeast survival of SY (■) and DY (▲) frozen sweet doughs with increasing storage time at −40 °C.

The yeast survival results during frozen storage of DY and SY dough is shown in Fig. 1. During frozen storage, the kinetic of yeast viability decrease seemed to follow the same trend for both doughs. After 27 days of frozen storage, the percentage of survival yeast cells decreased by 31% and 44% of SY and DY dough respectively, compared with the unfrozen doughs. For the same time, the yeast survival number on DY was equivalent to the SY dough before freezing. Beyond 27th day, the yeast population decreased slightly until the end of the study, ending with 27% and 42% of SY and DY, respectively.

Statistical analysis showed highly significant correlations ($P < 0.01$) between all the viability parameters with storage time for both doughs ($r = -0.93$ and -0.92 for SY and DY, respectively).

The desiccation and electrolyte release giving to hyperosmotic stress during freezing (Hatano et al., 1996), also could explain partly yeast lysis during first step of frozen storage, this situation gets worse in frozen sweet dough, because the dough recipe contains a high sugar content (20% sugar). In addition, frozen storage allows ice crystals to grow, which results in cellular damage by fracture of the cell membrane and destruction of sub-cellular organization. This recrystallization can occur during prolonged frozen storage and especially during slow thawing at low temperatures (Mazur, 1970).

Yeast cells viability in frozen dough depends on the composition and integrity of the cell membrane. During freezing, the yeast membrane integrity is subjected to high osmotic pressure, to withstand a high content of phospholipids to prevent rupture (Codon et al., 2003). Hohmann (1997) suggested that exposure to hyperosmotic stress leads to rapid dehydration of the cells thus limiting CO_2 production, which results in increases in proofing time. These effects are enhanced in frozen sweet dough, because yeast is exposed to high osmotic pressures and reduced water activity during freezing and frozen storage.

On the other hand, the direct consequence of temperature on yeast cell walls and cytoplasmic cell membranes cannot be excluded; temperature determines the amount of ice present in the matrix. Berglund et al. (1991) suggested that temperature fluctuations were causing especially damaging on the yeast cells. A lower temperature can lead to phase transitions and loss fluidity of the lipid bilayer and alter interactions between the bilayer membrane proteins (Morris and Clarke, 1987).

This present study revealed that the yeast population number of SY dough at 0 days was equivalent to that DY frozen dough stored for 27 days.

Analysis of variance (ANOVA) was applied, within 95% confidence interval, to the results obtained according to frozen storage time, indicated that the content of the yeast influenced significantly the sweet dough quality (shelf life dough); after thawing the higher content increased fermentative activity (CO_2 production, specific volume) due to higher yeast activity during proofing.

3.2. Effect of frozen storage on yeast activity

The effect of frozen storage time of both SY and DY frozen doughs on the CO_2 production (gassing power) and specific volume are shown in Table 1. As a result, gas production decreased significantly with frozen storage time. The gassing power of the DY dough at 0 day was higher of 15% than that of SY dough at the same time. The CO_2 production of SY and DY frozen sweet doughs during the first week of frozen storage declined by about 13% and 15%, respectively, compared to the control.

Table 1
Gassing power and specific volume of SY and DY frozen sweet doughs with increasing frozen storage duration.

Sample	Storage time (days)	Yeast activity	
		Gassing power (ml/g dough)	Specific volume (ml/g dough)
SY	0	**5.01 ± 0.31^{aw}**	**3.02 ± 0.24^{aw}**
	11	4.36 ± 0.11^{ax}	2.73 ± 0.06^{ax}
	27	4.12 ± 0.31^{bx*}	2.59 ± 0.14^{ax}
	38	3.04 ± 0.19^{cy*}	2.38 ± 0.13^{bx*}
	48	2.85 ± 0.13^{cy*}	1.96 ± 0.07^{cx*}
	63	2.39 ± 0.02^{dz*}	1.54 ± 0.18^{cy*}
DY	0	5.88 ± 0.20^{aw}	3.53 ± 0.24^{aw}
	11	5.01 ± 0.09^{aw}	3.24 ± 0.20^{aw}
	27	4.84 ± 0.32^{bx}	3.32 ± 0.01^{aw}
	38	4.28 ± 0.16^{bx*}	2.60 ± 0.16^{bx}
	48	4.59 ± 0.29^{bx*}	2.41 ± 0.05^{bx*}
	63	3.79 ± 0.16^{cy*}	2.30 ± 0.06^{bx*}

In bold: 0 represented the SY control (0 day).
For dough, values with the same following letter do not differ significantly from each other ($P < 0.05$).
^{a,b,c,d} Same letters within each dough do not significantly differ ($P < 0.05$).
^{w,x,y,z} Same letter within each column do not significantly differ ($P < 0.05$).
* Differ significantly with the control 0c (SY dough) ($P < 0.05$).

After 33 days frozen storage, the CO_2 production of SY and DY frozen sweet doughs decreased significantly ($P < 0.05$) by 39% and 27%, respectively, compared to unfrozen doughs.

El-Hady et al. (1996), reported that the total CO_2 production decreased with the increase of storage periods (from 33% compared to unfrozen dough) during four weeks of storage time and this decrease can reach 50% for the same storage period, using three freeze–thaw cycles. At the end of frozen storage (63 days), the gassing power significantly ($P < 0.05$) reduced by 52% and 36% for SY and DY frozen sweet doughs, respectively.

The corresponding dough volume followed the same trend as the gassing power (affected by storage time). Particularly, after 63 days of frozen storage, the specific volume declined significantly ($P < 0.05$) by 49% and 35% for SY and DY frozen sweet doughs, respectively, compared to control (0 day), which was in agreement with (Havet et al., 2000).

It is commonly known that a freezing process followed by storage in frozen condition affects the CO_2 production (El-Hady et al., 1996).

The fermentative activity (CO_2 production and specific volume) of DY sweet dough stored for 27 days is equivalent to the SY sweet dough that is not frozen; this can be explained by maintaining a sufficient yeast population.

The results showed that yeast activity parameters (yeast population, specific volume and gassing power) of both frozen sweet doughs showed a strong negative correlation with frozen storage time.

The frozen storage caused a decrease in yeast cells number which induces a decrease in gas production capacity from residual surviving yeast. The CO_2 production depends on the frequency, the number of yeast cells, the cell activity and the amount of fermentable sugars.

Meric et al. (1995) remarked that in a complex matrix such as dough, yeast cell lysis is not the sole cause of gassing power loss, and nonlethal cryo-damage should also be taken into account. In other words yeast cells may suffer nonlethal damage in frozen dough that precludes them from forming colonies on a plate while retaining the ability to ferment sugars and produce CO_2 in dough.

As shown in Table 1, up to 27 days, the yeast quantity had no significant effect on yeast gassing power. This can be explained by the limitation of substrate (fermentable sugars). Beyond 27 days of frozen storage, the ability of fermentative yeasts is strongly influenced by the duration of storage. These changes are

Table 2
Effect of frozen storage duration on the texture properties (Hardness, springiness and ΔS) of frozen sweet doughs (SY and DY).

Sample	Storage time (days)	Texture analyses		
		Hardness (N)	Springiness	ΔS (N)
SY	0	**60.11 ± 10.62^{aw}**	**0.71 ± 0.12^{aw}**	**5.94 ± 0.28^{aw}**
	11	71.35 ± 15.52^{bx*}	0.68 ± 0.07^{aw}	6.49 ± 0.24^{aw}
	27	73.62 ± 9.23^{bx*}	0.48 ± 0.11^{bx*}	6.74 ± 0.21^{bx*}
	38	75.67 ± 7.76^{bx*}	0.41 ± 0.08^{bx*}	7.23 ± 0.73^{cy*}
	48	78.76 ± 12.29^{cy*}	0.38 ± 0.06^{bx*}	7.93 ± 1.17^{dz*}
	63	90.91 ± 6.98^{dz*}	0.31 ± 0.03^{cy*}	8.54 ± 1.45^{dz*}
DY	0	56.55 ± 9.33^{aw}	0.59 ± 0.02^{aw*}	4.45 ± 0.98^{aw*}
	11	66.18 ± 17.08^{bx}	0.48 ± 0.03^{bx*}	4.74 ± 0.74^{aw*}
	27	66.43 ± 6.76^{bx}	0.31 ± 0.06^{cy*}	5.28 ± 1.24^{bx*}
	38	68.36 ± 5.76^{bx}	0.28 ± 0.02^{cy*}	5.46 ± 0.65^{cy}
	48	72.21 ± 10.19^{cy*}	0.25 ± 0.04^{cy*}	5.58 ± 0.84^{cy}
	63	85.52 ± 7.66^{dz*}	0.22 ± 0.05^{dz*}	5.86 ± 0.65^{dz}

In bold: 0 represented the SY control (0 day).
For dough, values with the same following letter do not differ significantly from each other ($P < 0.05$).
^{a,b,c,d} Same letters within each dough do not significantly differ ($P \leqslant 0.05$).
^{w,x,y,z} Same letter within each column do not significantly differ ($P \leqslant 0.05$).
* Differ significantly with the control 0c (SY dough) ($P \leqslant 0.05$).

manifested by a gradual decrease in specific volume of sweet doughs; this decline is related to both gassing power and yeast population decrease.

The results obtained in this study were consistent with the results of Le Bail et al. (1999) that worked specifically on bread dough, this author to attribute the dough volume decrease to temperature fluctuations. Neyreneuf and Delpuech (1993) suggested that temperature fluctuations accelerate ice recrystallization that reduces fermentative activity of frozen sweet doughs.

3.3. Effects of frozen storage on dough rheology

The three textural parameters (hardness, springiness and ΔS) of the two doughs were significantly affected by frozen storage time (Table 2). The hardness and ΔS values were increased significantly ($P < 0.05$) by 19% and 17%, 9% and 7% for DY and SY doughs compared to control (0 day) during the first week of storage (Table 2). Then they remained constant up to 27 days and increased significantly until the end of storage to reach 50% and 42% (hardness) and 44% and 32% (ΔS) for SY and DY doughs. No significant difference was found between the DY sweet dough stored (during 38 days) and the SY control.

The results of this study are in accordance with Angioloni et al. (2008) that suggested the frozen effect is particularly accented in the early days of frozen storage. Indeed, the hardness and ΔS increase during frozen storage could be interpreted as a reduction in elasticity due to disruption of gluten network bonds caused by mechanical action of ice crystals (Havet et al., 2000). Regarding the springiness of frozen sweet doughs have suffered the same fate as the previous settings. The results in Table 2show a significant decrease ($P < 0.05$) in springiness during 27 days approaching 32% and 47% for SY and DY sweet frozen sweet doughs, respectively, compared to unfrozen sweet doughs, then decreases slightly to stabilize at the end of storage.

The rheological measurement results were expressed as $\tan\delta$ (G''/G'), which indicates the dominant character between elastic or solid in sample. Results of rheological properties grouped in Fig. 2 show the $\tan\delta$ values for both SY and DY frozen sweet doughs at 1 Hz frequency were affected by frozen storage.

As shown by the compression-tension measurements, similar trends were observed with $\tan\delta$ for the two types of sweet doughs (SY and DY). However, for the two types of sweet dough, the rheological behavior was divided in two parts. During the first four weeks of frozen storage, the $\tan\delta$ decrease. $\tan\delta$ was corroborated with elasticity results ($P < 0.05$). After four weeks of storage, $\tan\delta$ increased. This phenomenon was due to the recrystallization of water in the dough matrix. These results were in accordance with the FTIR study.

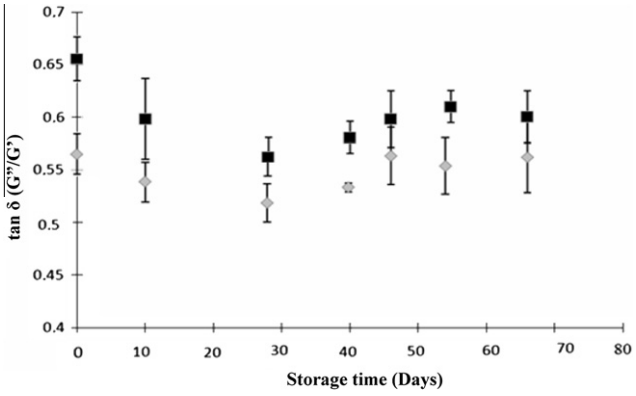

Fig. 2. Evolution of $\tan\delta$ (G''/G') for frozen sweet dough according to storage time: DY (◆) and SY (■).

Leray et al. (2010) studied the storage of wheat dough at two temperatures −18 °C and −30 °C, they observed the modification of rheological parameters (G', G'' and $\tan\delta$) of dough stored at −30 °C resulting changes in dough structure but the rheological properties of dough stored at −18 °C were closer to those of wheat dough (0 day). The authors explained these rheological changes to the storage temperature that covers the glass transition temperature (Tg).

During frozen storage the total ice quantity will not vary unless ice formation was kinetically inhibited during freezing, however ice crystals may undergo changes in shape and size. Migratory recrystallization, also called 'grain growth' and 'Ostwald ripening,' refers to the tendency for large crystals to grow at the small crystals expense (Baier-Schenk et al., 2005). Indeed, the ice crystals growth induces a water redistribution causing mechanical damage to the gluten network of frozen doughs (Selomulyo and Zhou, 2007), which leads to a reduction of gluten cross-linking. On other hand, transmission electron microscope (TEM) micrographs highlight the intracellular ice crystals on the yeast cells during freezing (data not shown). Baier-Schenk et al. (2005) used the scanning electron microscope (SEM) to show the growth of ice crystals during frozen storage.

However, the ice recrystallization accelerated by temperature fluctuations during storage leads to changes in dough matrix by reducing its ability to retain gas (Neyreneuf and Delpuech, 1993).

In addition, the physical state of frozen sweet doughs during frozen storage may affect dough quality. Therefore, it is important to understand phase and state transitions, including glass transition, occurring in sweet doughs at sub-zero temperatures. It has been suggested that the glass transition of frozen dough and its components may affect stability, as the glass transition may control rates of recrystallization of ice and diffusion-controlled-reactions. Laaksonen and Roos (2000) suggested that the glass transition temperatures (Tg) of frozen wheat doughs were between −30 °C and −43.5 °C, depending on the flour used. Räsänen et al. (1998), reported that the glass transition temperatures (Tg) of frozen as less than −30 °C. The difference between the observed values and the one reported by Laaksonen and Roos (2000) could have resulted from the use of different recipes (in this paper water–flour mixture). Sugar, salt and butter (used in our study) have a great effect on freezing properties, decreasing the freezing point.

At this temperature of storage, the dough is close to glassy state thermodynamically unstable and a low energy intake can destabilize and eventually promote the recrystallization water.

In addition, prolonged storage at temperature near the glassy state can expose the sweet dough for a maximum cryoconcentration effect (maximum dehydration of the matrix, because most of the water is frozen around Tg), this phenomenon is amplified by the water diffusion into ice crystals. Bhattacharya et al. (2003) suggested that freezable water does not bind to gluten during dough formation, freezes when the dough is subjected to frozen storage.

Indeed, other authors have shown other effects of freezing as mechanical damage of starch (Berglund et al., 1991). These starch modifications cause a redistribution of the total water present in the sweet dough matrix and changes in proteins (protein depolymerization) (Ribotta et al., 2001), which could also affect the rheological properties of frozen sweet dough. Statistical analysis showed highly significant correlations ($P < 0.01$) between all the textural parameters with storage time for both doughs. As shown in the coefficient values (r) of both sweet doughs, the same correlations are observed for SY and DY sweet doughs.

Dough hardness ($r_{SY} = 0.95$ and $r_{DY} = 0.93$) and ΔS ($r_{SY} = 0.99$ and $r_{DY} = 0.98$) showed high positive correlation with the frozen storage time while a strong negative correlation was found between the dough springiness ($r_{SY} = -0.98$ and $r_{DY} = -0.95$) and

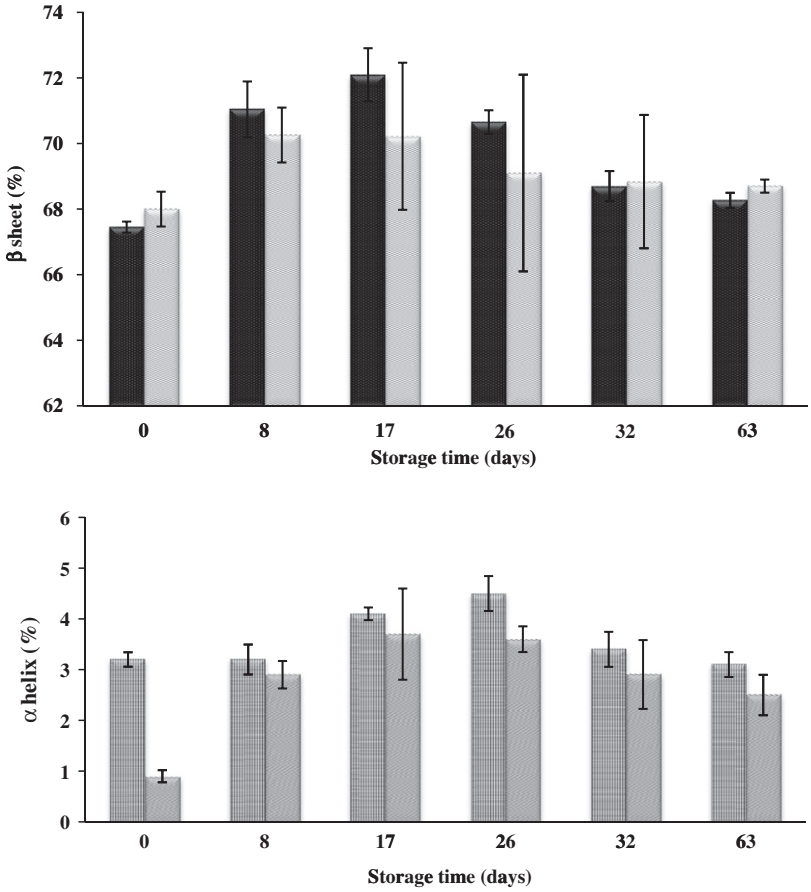

Fig. 3. Conformational changes in the secondary structures of amide III band of SY and DY frozen sweet doughs according to the storage time. The β-sheet and α-helix fractions identified at: 1330–1295 cm^{-1} (α-helix), 1250–1220 cm^{-1} (β-sheet). SY β-sheet (■), DY β-sheet (□), SY α-helix (▦) and DY α-helix (▨).

storage time. These results indicated that all parameters were important for evaluate the frozen sweet dough rheology.

The trends observed with rheological results were corroborated by spectroscopy infrared (FTIR) estimation. The gluten network disruptions can be caused by direct action (mechanical) of crystals ice and indirectly by redistributing water.

On other hand, the presence of dead yeast in dough releases different reducing agents, which can break the S–S linkage in the gluten of the sweet dough, preventing CO_2 retention and affecting dough quality (Codon et al., 2003, Ribotta et al., 2001).

The secondary structures of protein gluten network were studied by infrared spectroscopy. The amide III band was deconvoluted and the different peaks and their frequencies correspond to: α-helix (1330–1295 cm^{-1}) and β-sheet (1250–1220 cm^{-1}) (Cai and Singh, 1999). The quantitative estimation of β-sheet and α-helix fractions of SY and DY sweet doughs are grouped in Fig. 3. Other secondary structures (β-turn and random coil) are not shown in this study because they are not influenced by freezing and frozen storage probably due to the formation of new proteins bonds with other components (Cai and Singh, 1999). However, the β-sheet and α-helix fraction are sensitive to freezing and frozen storage; the β-sheets were partially increased during the first four weeks of storage. This can be interpreted as the formation of a range of new β-sheet together with a shift to less strongly hydrogen-bonded structures (Georget et al., 2006). After four weeks of storage, a slight decrease of secondary structures had been observed. This phenomenon could be explained by the redistribution of water in the dough matrix during the ice recrystallization (Leray et al., 2010).

The secondary structure changes indicated proteins aggregation. The FTIR results revealed a slight increase of secondary structures for both sweet doughs (DY and SY) during the first week of storage. A significant decrease ($P < 0.05$) was observed on α-helix (SY = 28% and DY = 24%) and β-sheet (SY = 2% and DY = 3%) until approximately 4 weeks of frozen storage. These results suggested that α-helix and β-sheet were sensitive to frozen storage. Protein denaturation is governed by various factors including temperature, pH and high ionic strength (Wagner and Anon, 1986). The secondary structure change indicates proteins aggregation.

4. Conclusion

The influence of yeast content and frozen storage time of sweet dough was studied. The frozen storage time had a significant effect on the dough's hardness, ΔS springiness and viscoelasticity.

DY frozen sweet dough (higher yeast content) gave dough with a higher specific volume due to higher yeast activity, whereas the specific volume decreased during frozen storage.

This present study revealed that the yeast content of SY dough (0 day) is equivalent to that DY frozen dough stored for 27 days.

The rheological parameters were not influenced by yeast level. However, hardness and ΔS increased and springiness and $\tan\delta$ decreased since four weeks of frozen storage. These modifications of the rheological properties could be explained by ice crystals growth induces a water redistribution causing mechanical damage to the gluten network of frozen sweet doughs. Indeed, after four weeks of storage a decrease in the amount of α-helix correlated

with content of extended β-sheet fraction has been observed. These results suggest an aggregation of the gluten network proteins. These findings are confirmed by the rheology study, in particular by the slight increase of tan δ after four weeks of storage.

The storage effect was particularly concentrated in the first 27 days of storage. This effect is in accordance with the decreased elasticity and the phenomena accentuated with temperature fluctuations during frozen storage. In this study, the temperature fluctuations comprised between ±2 and ±3.6 °C. Due to the fact that the temperature fluctuations are unavoidable, Phimolsiripol et al. (2008) suggested that variations in temperature must be kept to a minimum or no more than ±3 °C.

To continue this study, it will be interesting to study sensorial and textural attributes after bake of frozen sweet dough during frozen storage.

Different freezing treatment and storage conditions will be conducted to improve the overall sweet dough quality, to minimize the specific volume loss.

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