Effect of Formulation on the Quality of Frozen Bread Dough

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ABSTRACT

The main objective of this work was to determine the influence of formulation on the stability of bread dough during frozen storage. Bread doughs containing gluten and trehalose were submitted to mechanical freezing at -30°C and stored frozen for 45 days. Two types of instant yeast were tested: (A) for sweet doughs and (B) for savoury doughs. Specific volume was significantly affected by the yeast type, type A showing better effect than type B. Frozen storage of the doughs negatively affected the specific volume, crumb hardness and technological score of the bread. The addition of 10% trehalose had a beneficial effect on the cell survival rate for both the yeasts.

Key words: Breadmaking, frozen dough, formulation

INTRODUCTION

Various factors are related to the quality of bread produced from frozen dough stored for long periods. The effect of freezing on the yeast properties has been widely studied. Several researches showed that the yeast viability is strongly influenced by the fermentation process prior to freezing (Hino et al., 1987; Báguena et al., 1991; Salas-Mellado, 1992), by freezing and thawing velocities (Gélinas et al., 1993; Gehrke et al., 1992; Autio and Sinda, 1992; Murakami et al., 1994) and by the duration of frozen storage (Berglund and Shelton, 1993; Wang and Ponte, 1995). The yeast type and its properties also play important role in yeast viability and in the quality of the product (Gélinas et al., 1993; Murakami et al., 1994; Van Dam and Hille, 1992; Inoue et al., 1994). In commercial productions of frozen dough, higher amounts of yeast are used, and also no-time bakery procedures, low preparation temperatures and tunnel freezing, in order to minimize the above mentioned effects. The influence of storage time and the structural conditions of the gluten are important factors in the quality of products made from frozen dough. The structure of the gluten protein matrix appears to break up during prolonged storage and repeated cycles of freezing/thawing (Berglund et al., 1991; Wang and Ponte, 1995; Autio and Sinda, 1992), resulting in a weakening of the strength properties of the dough, loss of gas retention and deterioration of product quality (Inoue and Bushuk, 1991; Inoue and Bushuk, 1992; Autio and Sinda, 1992; Inoue et al., 1994; Czuchajowska and Paszczyńska, 1996). The extent of these adverse effects can be reduced by using very strong flours (Inoue and Bushuk, 1992), by the addition of gluten (Wang and Ponte, 1994; Wang and Ponte, 1995) or by the use of additives such as sodium or calcium stearoyl lactlate (SSL and CSL) or the diacetyl-tartrate ester of monoglyceride (DATEM) (Wolt and

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D’Appolonia, 1984; Inoue et al., 1995; El-Hady et al., 1995; Nemeth et al., 1996). Other studies have shown that the type and level of oxidant (Inoue and Bushuk, 1991; Salas-Mellado, 1992), enzymes (Himmelstein, 1984), mixing and moulding procedures (Dubois and Blockowsky, 1986; Gélinas et al., 1995; Nemeth et al., 1996) and a variety of other ingredients can influence the quality of frozen dough (Davis, 1981; Hosomi et al., 1992; Addo, 1997).

The capacity of the yeast to maintain a high fermentative capacity after long periods of low temperature storage is affected by technological and cellular parameters. Trehalose is considered to be an important physiological compound in the resistance of the yeast to cold (Almeida and Pais, 1996). This dissacharide has been related to the resistance of the yeast to various types of stress (Smewing, 1995; Oda et al., 1986; Gadd et al., 1987; Hino et al., 1990; Van der Plaat, 1974; Van Dijck et al., 1995; Hottinger et al., 1987; Mackenzie et al., 1988), demonstrating that a high intracellular trehalose content is an advantage for good stability to freezing/thawing after prolonged frozen storage. Research in the area by Méric et al. (1995) is of special interest. This group worked with bakery yeast, studying the evolution of trehalose during the preparation of frozen dough, finally concluding that a content of 4-5% (based on the dry weight of the yeast) at the moment of freezing, was sufficient to protect the yeast cells during the sequence of operations comprising pre-fermentation, freezing, frozen storage and thawing. This research led us to include this sugar in the formulation, for studying its protective effect on the yeast cells.

The objective of this study was to test the effect of trehalose and gluten as well as the type of yeast in the formulation of bread dough and its stability during frozen storage.

MATERIALS AND METHODS

Raw material, ingredients and dough formulation

The basic formulation (flour based) was the following: flour (100%), water (farinographic absorption less than 2%), instant dry yeast (1.3%), salt (2%), sugar (2%), hydrogenated vegetable fat (2%), sorbitan monoooleate (0.3%), ascorbic acid (120 ppm), vital gluten (2 and 4%) and D+trehalose (5 and 10%, based on the yeast dry weight). In each basic formulation, two types of instant dry biological ferment (Fleischmann Royal) were used: for sweet dough (A) and for savoury dough (B). Seven formulations were chosen for each type of yeast (Table 1), varying in their gluten and trehalose contents, the standard formulation (F₁) containing no additives.

Dough preparation, freezing, thawing and bread making

For each formulation (1 kg), the ingredients were mixed in a laboratory dough mixer for 8 minutes, low speed to obtained optimal dough development. The dough was cut, kneaded and moulded into the form of 80g buns, which were frozen in a mechanical tunnel with an air temperature of -30°C, until the temperature at the centre of the buns, measured by thermocouples was -15°C. The frozen dough buns were removed from the tunnel, and stored in polyethylene bags in a domestic freezer at -15°C for 45 days.

After preparing the doughs, zero time samples, corresponding to the unfrozen dough, were removed. Further samples were removed after 1, 10 and 45 days of frozen storage, thawed in an incubator at 30°C for 1 hour, fermented at 30°C for 1 hour and 35 minutes and finally baked at 200°C for 20 minutes. The buns obtained were cooled at room temperature for at least one hour and evaluated with respect to volume, crumb hardness and scores for technological characteristics.

The flowsheet for the dough and bun preparations can be seen in Fig. 1.

Determination of rheological characteristics of the dough

After thawing, the dough samples at 0, 1, 10 and 45 days were analysed using the TAXT-2 texturometer equipped with the probe used for the determination of extensibility according to Smewing (1995). The average area of the curve for 10 determinations of dough microextensibility was registered as the strength of the dough, expressed in newtons (N).

Determination of yeast viability

After thawing, the number of viable yeasts was determined in the 0, 1, 10 and 45 day dough samples, using the direct plate count method. The microbiological counts were carried out after 4 days of incubation at 30°C and the survival rate
calculated from the number of viable yeasts after 45 days as a percentage (%) of the number present on the first day.

**INGREDIENTS**
- MIXING (8 min., low speed)
- CUTTING, KNEADING, MOULDING (80g buns)
- FREEZING (tunnel with forced air, -30°C)
- FROZEN STORAGE OF DOUGH (freezer, -15°C)
- SAMPLING (0, 1, 10 and 45 days)
- THAWING (incubator 30°C, 1h)
- FERMENTATION (incubator 30°C, 1h 35min)
- BAKING (200°C, 20min)
- COOLING (room temperature, 1h)

**BREAD EVALUATION**

**Table 1 - Formulations of bread dough for freezing**

<table>
<thead>
<tr>
<th>Ingredients [%]</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Water</td>
<td>58.9</td>
<td>58.9</td>
<td>58.9</td>
<td>58.9</td>
<td>58.9</td>
<td>58.9</td>
<td>58.9</td>
</tr>
<tr>
<td>Yeast</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Salt</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Sugar</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Fat</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>SMO</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>120.0</td>
<td>120.0</td>
<td>120.0</td>
<td>120.0</td>
<td>120.0</td>
<td>120.0</td>
<td>120.0</td>
</tr>
<tr>
<td>Vital gluten</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Trehalose a</td>
<td>-</td>
<td>5.0</td>
<td>-</td>
<td>5.0</td>
<td>10.0</td>
<td>5.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

SMO: Sorbitan monooleate; a: ppm; b: based on the yeast dry weight

**Statistical analysis**

Three effects were considered in this study:

a) Effect of formulation: Seven formulations studied for each yeast were those presented in Table 1.

b) Effect of the yeast: Two types of dry instant yeast were used, denominated as A for sweet dough, and B for savoury dough.

c) Effect of storage time: Dough samples and their respective bread were analysed after zero (non-frozen dough), 1, 10 and 45 days of frozen storage at –15°C.

The responses (dependant variables) were: specific volume, crumb hardness and technological characteristics scores of the bread. Extensibility and average curve area for 10 determinations on each dough sample were measured.

The results for specific volume, hardness and bread scores, as well as the data for extensibility were analysed statistically using the analysis of

**Determination of bread volume and hardness**

The specific volume of the buns obtained from the doughs after 0, 1, 10 and 45 days of frozen storage was determined from the ratio of the volume obtained by seed displacement and the weight of the bread. Crumb hardness was determined by the compression of two 15 mm thick slices of bread, after 30 minutes cooling on the TAXT2 texturometer using the cylindrical 30 mm probe and expressing the results in newtons.

**Determination of the technological characteristics scores of the bread**

The scores were awarded to the bread for the external characteristics as follows: crust colour (10), break and shred (5), symmetry (5), and for the internal characteristics: crust characteristics (5), crumb colour (10), cellular crumb structure (10), and crumb texture (10), given a maximum total of 55 points, according to El-Dash (1978).
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variance (Anova) applying a computer programme (SAS v. 5.0). The statistically significant differences ($p \leq 0.05$) between the different effects were determined by the LSD (Least Significant Difference) test.

RESULTS AND DISCUSSION

The analysis of variance of the values obtained, when the effects of formulation, yeast and storage time were considered, showed that all three effects were important. The effects of formulation, yeast and storage time were significant for the specific volume, crumb hardness and technological characteristics scores of the bread. No significant differences were detected for dough extensibility due to the effects of formulation, yeast and storage time.

Effect of formulation

When the average values for bread specific volume were compared, considering that formulation was the main effect, formulation F7 stood out from the others; others forming groups within which they did not differ from one another (Table 2). Highest value for specific volume was that obtained for the buns prepared using formulation F7, followed by those from formulations F6 and F4; formulations F5, F3, F1 and F2 resulted in lower values. These results show good correlation between specific volume and the amount of added gluten. Highest values for specific volume were obtained for the formulations with the highest amounts of added gluten. The formulations with no added gluten (F1 and F2) showed the lowest specific volumes. This demonstrated the beneficial effect of gluten. One interesting result was the lack of differentiation between formulations F6 and F4, despite different amounts of added gluten (4% and 2% respectively). This could be related to the positive effect of trehalose, which, in both cases, was 5%. Trehalose showed a protective effect on the yeast cells with respect to cold stress (Thevelein, 1984; Van Laere, 1989), which could have let the yeasts in the doughs containing trehalose more resistant to the effects of freezing, thus producing bread with higher specific volumes than those containing no trehalose.

When the average values for bread hardness were compared for all the formulations including the values for both types of yeast, there were significant differences for crumb hardness between the different dough formulations. The formulations with high levels of added gluten showed the best performance with respect to crumb hardness, showing lower values (Table 2). Formulations F4 and F3, with low levels of gluten, presented intermediate hardness values and formulations F1 and F2, with no gluten, showed the highest values for crumb hardness. Observing the values for specific volume and hardness together (Fig. 2), a direct interrelation between the formulations with added gluten and trehalose (F6 and F7) was found, showing the highest values for specific volume and lower values for hardness. The beneficial effect of gluten to the dough has been observed by other researchers (Inoue and Bushuk, 1991; Wang and Ponte, 1994; Czuchajowska and Paszczyńska, 1996). The behaviour of formulation F5 deserved some attention, since despite a high level of trehalose, it did not show any clearly beneficial effect, being placed in an intermediary position between the rich formulations (F6 and F7) and the poor formulations (F1 and F2).

Figure 2 - Effect of dough formulation in bread specific volume and hardness

Effect of yeast

The specific volume of the bread was the only response affected by the type of yeast used. The buns made with the yeast for sweet dough (A) showed a mean average specific volume (3.70 mL/g) higher than buns prepared with the yeast for savoury dough (3.37 mL/g). This may have been due to the fact that yeast A, being a yeast suitable for sweet dough, with characteristics of osmotolerance, could have better resisted the
operations of freezing, thawing and frozen storage. The good performance of dry active yeasts has been discussed by El-Hady et al. (1996), who showed that the dried yeast was more stable during frozen storage for 12 weeks, when compared to compressed yeasts.

Table 2 - Average values\(^a\) for specific volume and crumb hardness for the different formulations.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Specific volume [cc/g]</th>
<th>Crumb hardness [N]</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>3.36a</td>
<td>6.53a</td>
</tr>
<tr>
<td>F2</td>
<td>3.35a</td>
<td>6.04a</td>
</tr>
<tr>
<td>F3</td>
<td>3.43a</td>
<td>5.37b</td>
</tr>
<tr>
<td>F4</td>
<td>3.54b</td>
<td>4.90b,c</td>
</tr>
<tr>
<td>F5</td>
<td>3.44a</td>
<td>5.59b</td>
</tr>
<tr>
<td>F6</td>
<td>3.71b</td>
<td>4.24b,c</td>
</tr>
<tr>
<td>F7</td>
<td>3.93c</td>
<td>4.91b,c</td>
</tr>
</tbody>
</table>

\(^a\) Average values for each formulation, considering both yeasts A and B. The averages with the same letter do not present significant differences (p \(\leq\) 0.05, LSD)

The crumb hardness and bread scores, as also the dough extensibility, were not influenced by the type of yeast used, since the results of this effect did not present statistically significant differences.

**Effect of storage time**

The specific volume, crumb hardness and technological characteristics scores were influenced by the time of frozen storage of the doughs. The dough extensibility was not influenced by this effect. A significant reduction in specific volume was observed for the bread elaborated with frozen dough stored for 1, 10 and 45 days when compared with the non-frozen dough, as can be seen in Table 3.

Table 3 - Average values\(^a\) for specific volume, hardness and bread scores with respect to storage time.

<table>
<thead>
<tr>
<th>Storage Time [days]</th>
<th>Specific Volume [cc/g]</th>
<th>Crumb Hardness [N]</th>
<th>Bread Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0(^b)</td>
<td>4.17a</td>
<td>4.04a</td>
<td>45.85a</td>
</tr>
<tr>
<td>1</td>
<td>3.70b</td>
<td>4.80a</td>
<td>45.32a</td>
</tr>
<tr>
<td>10</td>
<td>3.47c</td>
<td>5.96b</td>
<td>44.67b</td>
</tr>
<tr>
<td>45</td>
<td>2.82d</td>
<td>6.66b</td>
<td>43.91b</td>
</tr>
</tbody>
</table>

\(^a\) Average values for buns from all the formulations with both yeasts. \(^b\) non-frozen dough. Values with the same letter did not present significant differences (p \(\leq\) 0.05, LSD)

The average values for specific volume showed a progressive decrease during frozen storage, signifying a loss of bread quality. On the other hand, an increase in crumb hardness with increasing storage time was observed for all the formulations studied. This has also been observed by Berglund and Shelton, (1993), who studied doughs frozen at -23°C and stored for 20 weeks. They showed that long storage periods resulted in an increase in the values for hardness, associated with a decrease in bread volume.

The storage time also influenced the scores conferred on the buns for their technological quality. A fall in the scores and an increase for hardness with storage time was noted. The statistical analysis demonstrated that the freezing process and frozen storage for one day had little effect on these characteristics, because there was no significant difference between the non-frozen dough and that frozen and stored frozen for one day. The highest changes occurred between 1 and 10 days of storage; further storage up to 45 days not greatly affected the hardness characteristics and bread scores when compared with the values obtained for doughs stored frozen for 10 days.

![Figure 3](image-url) - Effect of storage time in bread specific volume and crumb hardness: 1: non-frozen dough; 2: 1 day of frozen storage; 3: 10 days of frozen storage; 4: 45 days of frozen storage.

Fig. 3 shows the results for specific volume and bread hardness with respect to storage time, showing clearly the decrease in specific volume and increase in hardness of the bread, resulting in a fall in bread quality as a result of the loss of
stability of the doughs during frozen storage, which could partly be due to the effect of the growth of ice crystals in the doughs during frozen storage (Reid, 1983).

Dough extensibility as measured by the texturometer showed no significant differences with any of the effects studied. These results seem to show that the chosen methodology was not the most appropriate, since the test was not sensitive to the rheological changes which must have occurred in the doughs due to the formulations and frozen storage.

**Yeast Viability**
The cell survival rates in doughs elaborated with yeasts A and B after 45 days of storage are shown in Table 4. It could be seen that the cell survival rates were higher in the doughs elaborated with yeast A than in those elaborated with yeast B, demonstrating that the yeast for sweet dough (A) was more resistant to freezing and frozen storage than the yeast for savoury dough (B), as already mentioned in item 3.2. The better response of yeast A could be due to higher osmotolerance as compared to yeast B on account of being adapted for sweet dough, as related by Van Dam and Hille (1992). The osmotolerance property allowed yeast A to present a greater resistance against freezing, thawing and frozen storage.

As can be observed from Fig 4, that register the yeast survival rate have been presented by the different bread formulations arranged in increasing trehalose content, formulations containing yeast type B showed a direct relation between the two factors which was not shown by yeast type A formulations. The fall in microbial counts after freezing and frozen storage has been reported by others also (Berglund and Shelton, 1993; Wang and Ponte, 1995).

The formulations containing no trehalose (F1 and F3) presented the lowest cell survival rates, and those with 10% trehalose the highest. Of the formulations with low amounts of trehalose, only formulation 6 showed a much lower survival rate, however there was no obvious reason for this. It would be necessary to measure gas production by the yeasts to elucidate why a high gluten content negatively influenced cell growth in this formulation.

The yeast survival rates in doughs prepared with both types of yeast (A and B) were higher than 90% with 10% trehalose in the formulation, showing the protective effect of this dissacharide to the cold stress experienced by the yeast cells. This has also been reported by D’Amore et al. (1991), Oda et al. (1986) and Van Dijk et al. (1995).

**Table 4 - Yeast counts and survival rates in the different formulations.**

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Zero day Count [x 10^4]</th>
<th>Count on Day 45 [x 10^4]</th>
<th>Survival Rate [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1A^a</td>
<td>923</td>
<td>744</td>
<td>84</td>
</tr>
<tr>
<td>F1B^a</td>
<td>842</td>
<td>546</td>
<td>65</td>
</tr>
<tr>
<td>F3A^a</td>
<td>888</td>
<td>740</td>
<td>83</td>
</tr>
<tr>
<td>F3B^a</td>
<td>853</td>
<td>533</td>
<td>62</td>
</tr>
<tr>
<td>F2A^b</td>
<td>958</td>
<td>756</td>
<td>79</td>
</tr>
<tr>
<td>F2B^b</td>
<td>841</td>
<td>574</td>
<td>68</td>
</tr>
<tr>
<td>F4A^b</td>
<td>950</td>
<td>763</td>
<td>80</td>
</tr>
<tr>
<td>F4B^b</td>
<td>866</td>
<td>615</td>
<td>71</td>
</tr>
<tr>
<td>F6A^b</td>
<td>963</td>
<td>616</td>
<td>64</td>
</tr>
<tr>
<td>F6B^b</td>
<td>808</td>
<td>426</td>
<td>53</td>
</tr>
<tr>
<td>F5A^b</td>
<td>1033</td>
<td>957</td>
<td>93</td>
</tr>
<tr>
<td>F5B^b</td>
<td>907</td>
<td>903</td>
<td>99</td>
</tr>
<tr>
<td>F7A^c</td>
<td>798</td>
<td>746</td>
<td>93</td>
</tr>
<tr>
<td>F7B^c</td>
<td>808</td>
<td>740</td>
<td>92</td>
</tr>
</tbody>
</table>

A: yeast for sweet dough; B: yeast for savoury dough; a: no added trehalose; b: 5% of trehalose; c: 10% of trehalose; d: c.f.a./g dough

**Figure 4 - Effect of dough formulation in yeast survival rate for yeast type A and B**

**CONCLUSIONS**

The dough formulation significantly influenced the specific volume and crumb hardness of the bread. The buns prepared with formulation F7, containing high levels of gluten and trehalose, were those which presented the highest specific volume,
significantly different from those made with the other formulations, which presented smaller values.

The addition of 10% trehalose in dough formulation produced higher survival yeast rates after frozen storage of 45 days for both yeast type used in this work. High levels (4%) of gluten showed good performance on bread crumb hardness. The type of yeast significantly affected the specific volume and the yeast for sweet dough showed better performance when compared with yeast for savoury dough. Frozen storage time of 45 days resulted in decrease of bread specific volume and bread score of technological characteristics and an increase of bread crumb hardness.

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