Quality of bread baked from frozen dough –effects of rye, and sugar content, kneading time and proofing profile

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Abstract

The objectives of this study were to evaluate whether proofing profile influences volume and crumb firmness in bread baked from frozen dough, and whether rye or sugar content and different kneading times affect the microstructure of the frozen dough. Microscopy was used to explain the differences.

Wheat doughs mixed with rye ("rye") and with sugar ("sweet") were frozen after 3 different proofing times (0, 18, and 38 min) and visualized with confocal laser scanning microscopy and 3-dimensional micro-computed tomography. The baked breads were evaluated for volume and texture. Breads from un-proofed frozen dough allowed to proof after thawing showed the highest volume (4.0 cm^3/g) and the softest crumb texture. The pre-proofed sweet bread had firmer crumbs and lower volume (2.5-3.0cm^3/g) than the pre-proofed rye bread (2.7-3.7cm^3/g). Reasons for the differences in quality parameters between the rye and sweet breads were investigated by studying the different influences of kneading time and sugar content on

fresh and frozen dough. The gluten network was found to be more homogeneously distributed in doughs with longer kneading times and lower sugar content, and less well distributed and more lumped in frozen than in fresh dough.

1. Introduction

The “in-store baked” market segment is steadily increasing. Freezing has been an important advance for this industry, allowing improved working hours and making it easier for many companies to produce “freshly baked” bread in stores, bars, restaurants, etc. However, while freezing has helped to increase the shelf life of bakery products, low temperature storage is known to negatively affect dough, damage the structure of the bread, and reduce the final bread quality (Bárcenas and Rosell, 2006; Selomulyo and Zhou, 2007; Yi and Kerr, 2009). When the dough is subjected to temperatures below zero, free water leaks and forms ice crystals that may grow and damage the gluten network during storage (Bárcenas and Rosell, 2006; Meziani, Jasniewski, Ribotta, Arab-Tehrany, Muller, Ghoul and Desobry, 2012b; Naito, Fukami, Mizokami, Ishida, Takano, Koizumi and Kano 2004; Yi and Kerr, 2009). These ice crystals may also affect the yeast membrane leading to decreased viability (Naito et al, 2004; Yi & Kerr, 2009). These effects produce unanticipated texture, pore size distribution (density), volume, and consumer acceptance.

Unfermented frozen doughs need time for thawing and for proofing prior to baking. This method is often used for goods such as sweet buns and pizza dough. (Le Bail, Nicolitch and Vuillod, 2010; Meziani, Ioannou, Jasniewski, Belhaj, Muller, Ghoul and Desobry, 2012a). In what is often referred to as ‘pre-fermented frozen dough’ or ‘partially fermented dough’ the dough ingredients are mixed and left to proof; the fermentation is then interrupted by freezing the dough (Le-Bail et al., 2010). An investigation of pre-proofed doughs (Lucas, Grenier, Bornet, Challois and Quellec, 2010) found that those subjected to proofing before freezing showed deformation, higher proportions of large bubbles, lower specific volume and height, and densification of the bottom part contributing to a greater collapse of the dough. All of these effects seemed to be attributable to the compression of the gases in the dough during cooling, a subsequent rupture of the dough between the air bubbles, and their resulting coalescence (Lucas et al., 2010). The greatest collapse was observed in doughs with the longest fermentation time previous to freezing that were thawed before baking (Lucas et al., 2010).

Huang, Kim, Li and Rayas-Duarte, (2008) described baking frozen sweet dough as a challenge. When the dough contains sugar, its osmotic pressure increases and the yeast cells may dehydrate more quickly, decreasing gas production, and leading to a lower final volume (Huang et al., 2008; Meziani, 2012a). Meziani, Kaci, Jacquot, Jasniewski, Ribotta, Muller, Ghoul and Desobry, (2012c) also mentioned in their study that sugar increases the development of yeast before freezing. Another reported result of having sugar in frozen bread dough is the change it induces in rheological characteristics, including the final rigidity of the bread caused by the formation of ice crystals (Meziani, Jasniewski, Gaiani, Ioannou, Muller, Ghoul and Desobry 2011). However, it has also been reported that sweet doughs require a longer mixing process than plain white doughs to develop both the matrix of gluten and the porous structure of the dough (Calderón-Dominguez, Neyra-Guevara, Farrera-Rebollo, Arana-Errasquín and Mora-Escobedo,2003; Tlapale-Valdivia, Chanona-Pérez, Mora-Escobedo, Farrera-Rebollo, Gutiérrez-López and Calderón-Domínguez, 2010). Mixing is an important operation. During this process the structure of the dough and resulting bread is formed, the visco-elastic properties of the gluten are developed, and air is incorporated into the dough (Dobraszczyk and Morgenstern, 2003).

Rye is known to be the cereal with the highest dietary fibre, found mainly as arabionoxylan, fructan, and β-glucan (Andersson, Åman, Wandel and Frølich, 2010; Rakha, Åman and Andersson, 2010). In addition to its health aspects, rye flour has been noted to affect baking processes and it has an important delaying effect on both staling and starch retrogradation in bread due to the great amount of water-holding arabinoxylan contained in its cell walls (Rakha et al., 2010).

The aim of this study was to evaluate whether proofing profile influenced volume and crumb firmness in bread baked from frozen stored dough. Possible reasons for the different effects on quality parameters of the breads were explored though investigating the structure of doughs with various kneading times, sugar content, and freezing protocols.

2. Material and Methods

2.1. Preparation of sweet and rye dough, pre-proofing, and freezing regimes

The doughs for the two different types of bread, rye (wheat flour and rye) and sweet (wheat flour with sugar), were prepared by Fazer Bakery in Umeå 10 weeks before the study was carried out. The recipes are shown in Table 1. The wheat flour used was "bakery wheat flour" from Nord Mills with a protein content of 11.2-14.2% and ash content of max 0.7% of dry substance. The doughs were formed into small (60 g) buns, frozen on trays for 24 hours (-20 ºC was reached after 30min), put in plastic bags, placed in boxes at −30 ºC, and transported, frozen, to the laboratories at SIK, Gothenburg and SLU, Uppsala, where they were stored at −20 ºC until the analyses were performed. Both dough types were prepared in common industrial conditions following the same steps: ingredient scaling, mixing (2 min), kneading (9 min), resting, dough scaling, and shaping. The dough rested (5 min) and was later cut and shaped. The formed buns were treated in three different ways before freezing: one group was not proofed at all but frozen directly (sample A), another group was half-proofed (rye: 19 min; sweet: 18 min) (37ºC, 50% RH) (sample B), and the third group was fully proofed (rye: 38 min; sweet 36 min) (37ºC, 50% RH) (sample C). Dough ingredients are shown in Table 1 and proofing treatments in Table 2. Proofing times were screened in a pre-test, where the time of the fully proofed was the time that gave the largest volume increase after baking.

2.2. Baking

The non-proofed doughs were allowed to thaw for 45 minutes (23ºC, 50% RH) before full proofing (37ºC, 50% RH) in a proofing chamber and baked at 230 ºC for 15 minutes (rye) and at 200 ºC for 12 minutes (sweet) (sample A). Half-proofed doughs were treated in two different ways: sample B was thawed and half-proofed before baking and after 5 minutes conditioning sample B* was baked directly under the same conditions as the non-proofed doughs. Fully proofed doughs were also treated in two ways: sample C was thawed before baking and sample C* was conditioned for 5 minutes before direct baking.

2.3. Preparation of mini doughs in ReoMixer

Three different concentrations of sugar (96g/kg, 44g/kg, and 1.0g/kg) were used in the sweet doughs prepared in a 10-g ReoMixer (Reomix Instruments, Lund, Sweden) which measures the shear torques during the kneading. The three sweet doughs were kneaded for three different times: 2.5 minutes (under-kneaded), 5 minutes (close to optimal kneading), and 10 minutes (over-kneaded). Each recipe was prepared on 4 different occasions using each of the kneading times. The recipes of the mini doughs are shown in Table 1. The starting recipe was the 9.3% (96g/kg) sugar recipe used in the previous analysis of different proofing profiles. Because yeast was not included in the mini dough recipes, the remaining ingredients were increased somewhat over the starting recipe. Likewise, the content of the other ingredients were increased somewhat in the doughs with less sugar. To obtain the right consistency of the doughs with less sugar, it was also necessary to add somewhat more water to them.

2.4. Mass and volume measurements

The dough samples that were withdrawn from the freezer for proofing and baking were weighed using a ±0.01 g precision scale while frozen. The baked samples were weighed once more 1 hour after baking. At that point, the volume of the buns was measured by rapeseed displacement according to the AACC's Method 10-05 (2001). The results are presented as specific volume (cm^3/g).

2.5. Texture analysis

The bread texture was measured 1 hour after baking using a texture analyser Instron, 5542 (Instron, Norwood, MA, US) with a cylindrical specimen of 20 mm diameter, a compressive strain of 40%, an extension of 1.7 mm/sec, and a compressive load of 0.01N. The bread slices were approximately 1.5 cm thick. Three buns of each treatment were kept in plastic bags at room temperature and their texture was measured after 2 days using the same procedure just described.

2.6. Macroscopic structure and 3D-micro-CT

Vertical slices of the breads were photographed with a Nikon D70 camera (Nikon Nordic AB, Solna, Sweden) to visualize the shape of the baked buns.

The macroscopic structure of the doughs was analysed by 3D-micro-CT using X-ray tomography equipment (GE | phoenix V | xm 240, Wunstorf, Germany). The analysis included whole buns made from both rye and sweet dough using three different processes: non-proofed, half-proofed, and fully proofed before freezing. The samples were thawed for about 1 hour before the analyses. The images were taken at 45 µm resolution. These images were obtained during a fast cycle of 3 minutes and a longer one of about 21 minutes. The images were analysed with FIJI, ImageJ 1.47 (Maryland, US).

2.7. Microstructural investigations of dough

The microstructural analyses of the sweet and rye dough were carried out on the frozen dough. The frozen doughs were prepared in a cryostat, Leica CM 1900 (Leica Ltd, Nussloch, Germany) at −15ºC, sliced into 40-µm thick samples and put on objective glasses. The samples were air-fixed in 30% dry formaldehyde for 1.5 hours and then stained. Akriflavine was used to stain the starch granules, Texas red® the protein, and BODIPY® the fat. The microstructural analysis of the dough prepared in the ReoMixer was carried out on both fresh and frozen stored dough prepared on two different occasions. A Leica TCS SP2 (Leica Ltd, Heidelberg, Germany) confocal laser scanning microscope was used. The light source was an argon laser with an emission maximum of 488 nm and a HeNe laser with an emission maximum of 594 nm. The emitted signals were recorded in wavelength intervals of 502–550 nm (Akriflavine and BODIPY®) and 608–673 nm (Texas Red®). HCX APO water objectives with 20× and 63× magnification and numerical apertures of 0.5 and 0.90, respectively, were used.

The yeast cells were visualized using a LIVE/DEAD®BacLight™ viability kit containing CYTO®9 and propidium iodide. CYTO®9 and propidiumjodid were detected in the wavelength intervals of 502–554 nm and 619–657nm, respectively, and an HCX PL APO CS objective with 63× magnification and numerical aperture 1.20 was used.

2.8. Chemicals used for microstructure staining

Akriflavine (1g/l in ethanol) was used to stain the starch granules, Texas red®, Texas Red sulfonychloride, (10g/l in water) the protein, and BODIPY®, BODIPY FL C16 (4,4- difluoro-5,7-dimethyl-4-bara-3a,4a-diaza-s-inda-cene-3-hexadecanoic acid) (0.2g/l in methanol) the fat phase. The yeast cells were visualized using a LIVE/DEAD®BacLightTM viability kit, where the Live cells is stained with ingredient CYTO®9 (green) and dead cells is stained with propidium iodide (red). The dyes were purchased from Invitrogen (Eugene, Oregon, USA).

2.9. Image analysis

To obtain a quantitative measure of the distribution of the gluten protein in the dough structure the distribution of the area fraction of protein was determined with the images divided into $5 \times 5 = 25$ smaller parts. The area fraction was determined on 10 different images of each dough using Matlab, (Mathworks®, Natick, MA, US). This measurement of the distribution assumes that a structure with a well-distributed protein network will have almost the same area fraction in all 25 parts, resulting in a narrow normal distribution of the protein. When the protein is less homogeneously distributed the normal distribution will be broader and there will be more parts with a very low fraction of protein.

2.10. Statistics

The specific volume and the Young´s Modulus were presented as ranges with one standard deviation around the mean. To assess the statistical significance of the differences between treatments, a one-way analysis of variance (ANOVA) test was applied. The null hypothesis being that all treatment means are equal. For all tested cases, (rye and sweet for specific volume, rye and sweet day 1 and 3 for Young´s Modulus), the ANOVA analysis lead to a rejection of the null hypothesis ($P < 10^{-3}$ in all cases) using Matlab, (Mathworks®, Natick, MA, US).

3. Results and Discussion

3.1. Effect of proofing conditions of frozen rye and sweet dough on volume and firmness of bread

Quality bread is often described as soft and voluminous. Decreased volume is one of the major quality problems of using frozen dough, and the decrease occurs continuously during frozen storage (Eckardt, Öhgren, Alp, Ekman, Åström, Chen, Swenson, Johansson and Langton, 2013).

In this study rye and sweet wheat flour breads made from fully and half pre-proofed dough were baked in the oven either after thawing or complete proofing (samples B and C) or directly from the freezer (samples B* and C*). These were compared with bread made from dough that was un-proofed before freezing and thawed and proofed after frozen storage (sample A). The different proofing treatments of the dough are shown in Table 2. Figure 1 shows the specific volumes of the rye and sweet breads made using different proofing before and after frozen storage. The largest volume of the rye bread was obtained when the dough was either allowed to proof fully after frozen storage (sample A) or was half-proofed before and half-proofed after frozen storage (sample B). Dough fully proofed before frozen storage resulted in flatter buns with a lower volume (samples C and C*). Somewhat higher volume was obtained when the buns were allowed to thaw before baking (sample C). Buns half-proofed before freezing and baked without thawing (sample B*) were higher than buns fully proofed before freezing (samples C and C*), but had the same low total volume. Gabric, Ben Aissa, Le Bail, Monteau and Curic, 2011 also showed that an increased degree of pre-proofing resulted in a reduced bread volume. There was a larger difference between the reference (sample A, thawed and fully proofed after freeze storage) and the other samples that was proofed before freeze storage (sample C, C*, B, B*) in sweet buns compared to the rye buns. The sweet bun that was frozen and stored un-proofed (sample A) was the only sweet bun that was truly approved and did also significantly differ from the other treatments in specific volume. The other sweet buns had much lower volume and a pyramidal form, especially those that were allowed to thaw (sample C*) and half proof (sample B*) after frozen storage (Figure 1). Different superscript letters in the figures indicate significantly different values of the specific volume at $P < 10\text{-}3$.

198 Texture quality measured by Young´s Modulus (Table 3) was similar for both rye and sweet bread when
199 measured freshly baked directly after cooling except for the buns that had been stored as un-proofed dough
200 (sample A) that showed a significant softer crumb compared to the other treatments. However, there were
201 larger differences between the breads after storage in plastic bags at room temperature for 2 days. The
202 staling of the bread strongly depended on the processing conditions used. The Young´s Modulus after 2
203 days was lowest in sample A for both rye and sweet bread. The sweet bread staled faster than the rye bread.
204 It is clear that bread that was pre-proofed before freezing (samples B and C) had a higher tendency to faster
205 staling than bread made from un-proofed dough before freezing. Of all the buns, those made from fully
206 proofed dough (sample C) staled fastest, as measured by texture firmness. These results were consistent
207 between rye and sweet bread. Different superscript letters in the columns indicate significantly different
208 values of the Young´s Modulus at $P < 10^{-3}$.

209 **3.2. Structure formation of frozen rye and sweet doughs**

210 The doughs' structures were investigated on different levels to understand why the sweet breads decreased
211 more in volume and had a harder texture than the rye breads after frozen storage of the dough. The
212 macrostructure inside the dough was investigated using 3D-micro-CT (Figure 2a-b). Using this technique
213 cross-sections of the whole buns were visualized, with the dough shown in yellow and air bubbles in blue.
214 In Figure 2a-b the un-proofed rye and sweet doughs, respectively, are shown before baking. The difference
215 between them is clear already: the rye dough has more air bubbles more homogeneously distributed than
216 the sweet buns, and some regions of the sweet buns have no pores at all. The sweet buns also show a flatter
217 form, which may indicate that the bun collapsed during freezing or thawing.

218 Figure 2c (rye) and 2d (sweet) shows the microstructure of the lamellas in the dough and the distribution
219 of the gluten protein (red) in relation to the starch granules (green). The rye dough has a well-distributed
220 gluten protein network that surrounds the wheat starch granules, while the protein in the sweet dough is

more often distributed in lumps that are not as connected as in the rye dough. We do not know whether the sweet dough forms this gluten microstructure when it is fresh or during frozen storage.

Another difference between the rye and sweet doughs, other than the difference in sugar content, is that the sweet dough contains more fat. In Figure 2e-f the protein networks are shown in red and the fat in green. We see here again the well-distributed protein-gluten-protein network in the rye dough and the less homogeneously distributed gluten–protein network in the sweet dough. The sweet dough has much more fat than the rye dough, but the fat seems well-distributed in both doughs and in domains of similar sizes. Thus, it seems either that fat is more easily distributed in dough than gluten or that its distribution is less likely to change during frozen storage.

An important aspect of working with frozen dough, whether pre-proofed or not, is estimating the condition of the yeast. Frozen rye and sweet dough were therefore examined using the fluorescent dye LIVE/DEAD, which distinguishes between living and dead cells. In Figure 2g-h living cells are shown as green spheres and dead cells as red. Cells that are green with a red spot inside are assumed to be not yet dead, but in poor condition. Clearly, more living cells remain in the sweet dough (about 50%) than in the rye (about 10%) after frozen storage, which seems logical since the yeast has more food in the sweet dough and the sugar may protect the yeast. The smaller volume of the sweet buns is therefore not due to the yeast. However, the degeneration of the yeast in the freezer is a contributing cause to the generally lower volume of bread baked from frozen stored dough than bread baked from fresh dough. We also found that the longer the frozen storage, the more live yeast cells die or deteriorate. After analysis at different intervals during frozen storage, most of yeast cells were found to have died early in the frozen storage. In fresh sweet dough about 80% of yeast cells are living, but after 2 weeks of freezing the proportion of living cells drops to about 60%.

3.3. Effect of kneading, sugar content, and frozen storage on dough microstructures

To better understand why the volume of sweet bread decreases more than that of rye after frozen storage as dough, we investigated the effects of sugar content and kneading time on the distribution of the gluten

structure. In Figure 3a, the wheat granules are shown in green and the gluten in orange on 12 different doughs varying in sugar content and kneading time. The dough containing 1.0, 44, and 96g/kg sugar contained the same proportions of other ingredients as the sweet dough described in the previous sections. The reference dough (ref) contained only wheat flour and water and no sugar. Figure 3a shows that longer kneading time with the same sugar content produced a more developed gluten network. This is in accord with earlier studies by Calderón-Dominguez et al. (2003) and Tlapale-Valdivia et al. (2010). We can also see that given the same kneading time, the gluten network is best distributed in doughs with the lowest sugar content and least homogeneous with the highest sugar concentration. Most clear is the difference in gluten distribution at different sugar contents at the kneading times defined as close to optimal, i.e. the ones framed in red in Figure 3a. Figure 3a also shows poor distribution of gluten at high sugar contents may be compensated for by longer kneading.

To obtain a more quantitative measurement of the distribution of the gluten protein, a calculation of the area fraction was performed on the images divided into 5 × 5 squares. The distribution is shown as the relative intensity (0-80%) versus area fraction (0-1). When gluten proteins are well distributed the distribution will be narrowly and normally distributed, i.e. the distribution of gluten protein and starch are roughly equal in the 25 squares. When the gluten distribution is less homogeneous, many squares show a low proportion of protein resulting in a broader distribution on the left (low fraction of protein) in the diagrams in Figure 3a. These results show by the shift to the left of the distribution that values of the protein area fraction are lower with higher sugar content.

The gluten network distribution is also influenced by frozen storage. In Figure 3b, the microstructure after different kneading times of the reference dough is shown before and after 6 weeks of frozen storage. The fresh reference dough has a very well-distributed gluten network, but that becomes less homogeneous after frozen storage when large areas without gluten appear. Part of the explanation of why the quality of sweet bread is more negatively influenced by freezing than non-sweet bread is likely that the higher sugar content causes additional coarseness.

3.4. Impact of water and ice

Understanding how the water content behaves during freezing and frozen storage is probably the key to understanding the different final textures of various breads. Since the water exists first as a liquid and then as ice states, it will shift during freezing and thawing, with accompanying changes in the number and size of ice crystals in proportion to the volume of free movable water. Ideally, the dough should contain as much non-frozen water as possible, which can be achieved by adding substances that lower its freezing point or by distributing the water in such small confined areas that it will not freeze (Chen, Swenson, Van der Meulen and Villman, 2013). This study showed that staling was faster in breads that were frozen and stored as proofed dough than in breads from dough frozen and stored un-proofed. This may be because pre-proofed dough contains more large voids in which large ice crystals can form than un-proofed dough. One reason why dough retains more water during frozen storage than bread at room temperature is that the dough has a greater number of small pores in which the water can remain either un-frozen or in small crystals even after long-term frozen storage (Chen et al., 2013).

3.5. The influence of water and ice on the dough structure and components

Extra-cellular ice crystals destroy the yeast and the gluten structure, while moving water during all parts of the process results in either less retained water or poorly distributed water in the dough or the bread. It is generally recommended to use as low freezing temperature as possible to speed freezing and avoid the formation of ice crystals or at least make them as small as possible. However, dough is an exception to this general rule because it contains yeast, which is a living organism. There is a risk in quick freezing that water will not be transported out of the yeast cell quickly enough, which increases the risk of ice crystals forming within the cells.

Degeneration of gluten results, as would be expected, in less gluten in the dough and higher gluten content is known to decreases the rate of staling (Callejo, Gil, Rodríguez and Ruiz, 1999). Therefore, reducing degeneration of (or retaining) gluten content would be expected to reduce staling. In general,

bread made from dough stored proofed in the freezer had both lower volume and a harder crumb than that stored un-proofed. One reason for this is that the proofed dough buns partially collapsed during frozen storage. The results of this study showing more collapse with longer proofing times before frozen storage is consistent with earlier studies by Lucas et al. (2010). This collapse of the bubble structure may be attributable to the underdevelopment of the gluten network, which was also the case for the sweet breads. However, the rye dough was also affected during the frozen storage. This study showed that the gluten network contracts during frozen storage, as shown by others who have noted that gluten is degraded by depolymerisation during freezing (Wang, Chen Mohanad, Xu, Ning, Xu, Wu, Yang, Jin and Xu, 2014). Increasing the concentration of gluten is a well-known method of strengthening the gluten network, while changing and prolonging the mixing and kneading time are less common. Enzymes are also now available on the market to facilitate the linkage of gluten to form long elastic threads.

Adding substances to lower the freezing point keeps the water in an un-frozen state for a longer time, but these substances can influence the texture by affecting the distribution of gluten, as was shown in this study by the addition of sugar. Another way to keep the water in the product during frozen storage is to bind it to highly water-absorbing substances such as fibres. We saw that the staling rate was lower in the rye breads than in the sweet breads. The high content of arabinoxylan in the rye bread had a positive effect on retarding the rate of staling (Rakha et al., 2010).

4. Conclusions

Bread from both rye and sweet doughs had larger volume if they were baked from frozen stored un-proofed dough compared to frozen stored proofed dough. In addition, sweet breads baked from pre-proofed frozen stored dough were pyramidal shaped. Staling was also significantly higher for bread that was stored as fully proofed dough in the freezer.

The importance shown in this study was the different demands on doughs arising with increasing sugar content:

318 • The higher the content of sugar in the dough, the longer kneading time is necessary to obtain a
319 well-distributed and homogeneous gluten network.

320 • Frozen storage involves contraction of the gluten network resulting in a less well-distributed
321 gluten structure.

322 Thus, increasing the kneading time of the dough, especially for the sweet dough, might help to improve the
323 quality of bread made from frozen dough.

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