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Effects of freezing rate and terminal freezing temperature on frozen croissant dough quality

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ABSTRACT

Using frozen ready-to-bake dough is a very common practice in the industrial croissant production. However, the freezing process during the preparation frozen croissant dough can deteriorate its quality. In this study, we investigated the effects of the freezing rate (FR) and terminal freezing temperatures on the volume and firmness of croissants by analyzing frozen dough for yeast viability, thermal property changes, and internal microstructure integrity. Croissant dough samples were frozen at rates ranging from −0.72 to −3.56 °C min^{-1} down to final temperatures of −20, −40, and −55 °C. Our results showed that the ice crystals normally forming in the dough during freezing, causing a lower yeast viability and croissants quality, were of smaller size when a rapid FR $\geq$ −3.19 °C min^{-1} was used. Furthermore, a freezing termination temperature lower than −20 °C induced more yeast cell death, thereby deteriorating croissant quality. Therefore, we suggest that the croissant dough freezing process should be conducted with an appropriate FR down to a suitable terminal temperature. Consequently, our results are helpful to understand how the freezing procedure affects ice crystal formation and yeast viability in the frozen dough matrix and our findings can be applied to enhance bread quality in the frozen dough industry.

1. Introduction

Frozen bread dough is widely used in the baking industry, mainly due to its convenience. Dough freezing can reduce processing time and labor intensity, increase products shelf life, improve productivity, and facilitate distribution to distant locations (Chen et al., 2013). Because of these advantages, the freezing technology has been used for several viennoiseries, such as croissants (Le-Bail, Nicolitch, & Vuillod, 2010). However, the freezing process used to prepare the frozen dough can induce many negative effects on the quality of baked products compared to those baked from unfrozen dough (Ribotta, León, & Añón, 2001). Particularly, damaged gluten networks and reduced yeast viability are regarded as the main culprits for quality deterioration in bread prepared from frozen dough (Ribotta, León, & Añón, 2003). Incidentally, a number of studies have focused on how to preserve gluten networks and yeast viability from freezing injuries by sustaining the yeast gas productivity and the gas retention capacity of the gluten networks.

Many researchers have suggested that the ice crystal formation in the freezing dough could result in two negative effects. First, the ice crystals formed within yeast cells can have cell membranes be damaged during the freezing procedure and ultimately decrease their viability (Muldrew & McGann, 1990). Additionally, if the ice crystals only form in the dough matrix (and not in or through the cells), the concentration of salts, sugars, and other molecules can increase the osmotic pressure as these molecules become more

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concentrated in the water present outside of the cells. This results in a water outflow from the yeast cytoplasm and leading to cell death (Randez-Gil, Sanz, & Prieto, 1999). Second, the ice crystal formation and growth can physically damage the gluten networks (Baier-Schenk et al., 2005). Therefore, ice crystal formation should be properly controlled to obtain good croissants from frozen dough, which could be accomplished by tightly controlling the freezing conditions (Yi & Kerr, 2009a). Notably, the freezing rate (FR) is one of the parameters used to regulate ice crystal size in the food freezing process (Petzold & Aguilera, 2009), and many studies explored the relationship between the FR and baked products quality (Le-Bail et al., 2010). Moreover, the storage temperature is well known for its important influence on ice crystal growth during frozen storage and on bread quality after baking (Phimolsiripol, Siripatrawan, Tulyathan, & Cleland, 2008).

A slower freezing process can result in larger ice crystals in the dough than rapid freezing, and larger ice crystals are more disruptive for the gluten networks. Damaged gluten networks prevent the proofing dough from properly retaining the CO_2 gas released by the yeast cells, which can reduce the overall loaf volume of bread prepared from frozen dough. Contrastively, the slower freezing process better preserves yeast viability in the dough (Selomulyo & Zhou, 2007). Therefore, to produce good breads, the optimal FR should be determined empirically from a complex consideration of both yeast viability and gluten network integrity.

The FR is a very significant factor affecting the quality of bread baked from frozen dough, as referred to earlier. Nevertheless, as far as we know, there was no study for frozen croissant dough focusing on determining the optimal dough FR. Moreover, even in many studies for freezing process of general frozen bread doughs, the researchers have only focused on the suitable temperature for long time storage not mentioned the appropriate terminal temperature for freezing process (Yi & Kerr, 2009a, 2009b).

Despite the fact that frozen dough ice crystals nucleate and grow during the freezing stage, analyses using cooling curves under differential scanning calorimetry (DSC) were rarely used in studies assessing ice crystal formation relative to the FR. Actually, many researchers thoroughly studied the frozen dough ice crystal formation process using DSC heating curves (Bot, 2003; Chen, Jansson, Lustrup, & Swenson, 2012; Kontogiorgos, Goff, & Kasapis, 2008). Consequently, in this study, the freezing process was controlled by the FR and terminal freezing temperature of the croissant dough. Lastly, we investigated the ice crystal formation phenomenon in intact dough using DSC thermograms. Additionally, we analysed the internal structure of the dough using SEM micrographs during the cooling part of the freezing procedure to evaluate the beneficial effects of a controlled freezing method on croissant quality.

2. Material and methods

2.1. Samples preparation

Two kinds of commercial flours and white sugar were gifted by Samyang Genex Co., Korea; flour 1 was composed of 72.5% carbohydrates, 12.5% protein, 0.6% fat, 14% water, and 0.4% ashes and flour 2 was composed of 75% carbohydrates, 9.5% protein, 0.6% fat, 14.5% water, and 0.4% ashes. Fresh compressed yeast (Ottugi Fresh Yeast Gold, Ottugi Co., Ltd., Korea), eggs, unsalted butter (Samyang Genex Co., Korea), butter for rolling (Pastry Sheet Gold, Samyang Genex Co., Korea), and refined salt (Beksul, CJ Cheiljedang Co., Korea) were obtained from the local market. A dough was prepared using the following recipe: 275 g of flour 1, 150 g of flour 2, 40 g of white sugar, 6.25 g of refined salt, 20 g of fresh yeast, 100 g of eggs, 162.5 g of water, 12.5 g of unsalted butter, and 250 g of cold butter for rolling. The flours, sugar, salt, and yeast were blended for 2 min using a mixer at speed 2 (Kenwood titanium major kitchen machine, KM020, UK). Then, water and eggs were added, and the mixture was blended for 2.5 min at speed 1 before adding butter and the dough was kneaded for 3 min at speed 1. Then, the dough was placed in a 4 °C refrigerator for the first 30 min of resting. For dough layering, the cold butter spread was placed on the dough, shaped in a 30 cm by 30 cm square after resting, and wrapped with the pressed out part of the dough. The dough with the cold butter was folded three times and allowed to rest in a refrigerator for 5 min (second resting). After the second resting, the dough folding procedure was repeated twice, with resting steps of 15 and 30 min. After the fourth resting, the dough was rolled and cut into isosceles triangle shaped (base, 10 cm; height, 15 cm; and thickness, 3 mm) and each triangle was rolled until the dough overlaps three times.

Freezing system was composed of two parts: a freezer (FD-170-SF, Unique Daesung Co., Ltd., Gyeonggi, Korea) and an adiabatic box. The styrofoam adiabatic box had two large holes on opposite sides, one of which was occupied by an electric fan, and a sample loading rack at center of the box. The FR of the dough was controlled by a combination of two means, namely the temperature of the cold air of the freezer, and the convection induced by the electric fan. Additionally, the terminal dough freezing temperature was defined as the point when the temperature at the center of the dough reached either −20, −40, or −55 °C. During the freezing process, the temperature change at the center of the dough was monitored using a data logger (Agilent 34970A, Agilent Technologies Inc., Santa Clara, CA, USA). The FR of the samples was calculated on the basis of the freezing profiles showed in the Fig. 1, using the definition provided by the International Institute of Refrigeration (Bøgh-Sørensen, 2006):

$$\mathrm{FR} = (T_t - T_i)/\Delta t$$

where, T_t is the sample terminal temperature, T_i is the sample initial temperature, and Δt is the time difference to reach T_t from T_i. When the dough reached its pre-defined T_t, a frozen sample was packaged in a polyethylene bag and stored for a day in a freezer set at −18 °C.

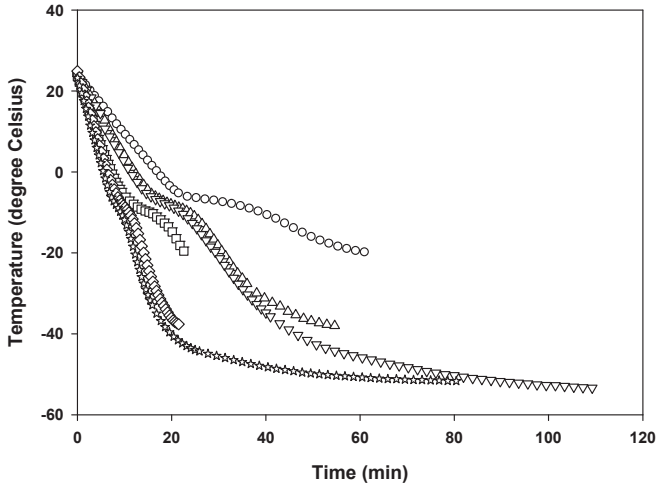

Fig. 1. Temperature profiling at the center of the croissant dough during the freezing procedure and sample coding names. The samples were coded as follows: FR1, −0.72 °C min^{-1} (open circle; −0.24 °C min^{-1} in zone of maximum ice crystal formation); FR2, −1.43 °C min^{-1} (open triangle; −0.50 °C min^{-1} in the zone); FR3, −1.50 °C min^{-1} (open inverted triangle; −0.52 °C min^{-1} in the zone); FR4, −1.84 °C min^{-1} (open square; −0.61 °C min^{-1} in the zone); FR5, −3.19 °C min^{-1} (open diamond; −1.53 °C min^{-1} in the zone); and FR6, −3.56 °C min^{-1} (open star; −2.01 °C min^{-1} in the zone).

The frozen croissant dough samples were thawed at 25 °C for 90 min and proofed for 60 min in a chamber kept at 35 °C with 85% relative humidity. The proofed dough samples were baked using an electric oven (MA921SBT, LG DIOS, Seoul, Korea) at 180 °C for 20 min. After baking the croissants, they were cooled for 1 h prior to subsequent analyses.

2.2. Determination of croissant quality

The croissant volume was measured using a laser-based Volscan Profiler 600 (Stable Micro Systems, UK). On the basis of loaf volume and weight of the croissants, a specific volume value was calculated.

A compression test was conducted to determine the croissant firmness using a texture analyser (TA-XT2i, Texture Technologies, Scarsdale, NY, USA) based on a method modified from AACC 74-09 (AACC, 1986). The croissant samples were placed at the center of the base in the texture analyser. The measurement was performed using an aluminum cylindrical probe (diameter, 50 mm) to press the sample until reaching 40% of deformation at a speed of 1.7 mm s^{-1}. The maximum force obtained from the force-deformation curve was used as the firmness value.

2.3. Croissant dough yeast viability measurement

Yeast viability was defined as the number of viable yeasts in the dough, and measured using a modified version of the AACC 42–50 (AACC, 2000). The frozen dough was taken out of the storage freezer and thawed at 25 °C for 90 min. The thawed dough was placed in a Filter-bag (Labplas, Canada), diluted tenfold with peptone water (2 mg mL^{-1}), and homogenised for 2 min using a stomacher WS-400 (Shanghai Zhisun Equipment Co. Ltd., Shanghai, China). Then, the samples were appropriately re-diluted with peptone water (2 mg mL^{-1}). One milliliter of the diluted suspension was cultured on Sabouraud Dextrose Agar (Difco, Detroit, MI, USA) and incubated for 48 h in a chamber at 30 °C before the yeast viability of the frozen dough was calculated.

2.4. Thermal characterisation of croissant dough and yeast

The thermal characteristics of the croissant dough and compressed yeast were determined by DSC (Diamond DSC, Perkin Elmer, Waltham, MA, USA). Prior to the freezing procedure, dough samples weighing between 14.8 and 24.2 mg were collected from the central part of the dough and from the fresh yeast, respectively. Samples were placed in a hermetic aluminum pan, which was then sealed. An empty pan was used as the reference. The DSC scanning of the dough immediately started at 25 °C and were conducted using six FR (−0.72, −1.43, −1.50, −1.84, −3.19, and −3.56 °C min^{-1}; referred to as FR1–6, respectively) until −20 °C was reached. On the basis of the thermograms, the values for the onset (T_{onset}), peak (T_{peak}), and end (T_{end}) temperatures were determined and the time values between each onset (t_{onset}) and end (t_{end}) exothermic peak were extracted using the Pyris 7 DSC data processing software (Perkin Elmer, Waltham, MA, USA) to characterise the formation of ice crystals in the dough. In addition, a DSC scan of the yeast was performed from 25 to −30 °C with a FR6.

2.5. Observation of the croissant dough microstructure

The internal microstructure of the frozen croissant dough was observed using a SEM S-3500N (Hitachi Science Systems Ltd., Hitachinaka, Japan). Dough fragments were obtained from the center of the frozen dough using a hammer. These steps were performed in the freezing room to avoid thawing. Observations were conducted at an accelerating voltage of 20 keV with a Robinson backscattered electron detector, which was maintained at −20 °C using a Deben Coolstage Peltier Stage (Deben UK Ltd., Suffolk, UK).

2.6. Statistical analyses

All results were analysed using a Tukey's significant difference test performed with the IBM SPSS Statistics software version 21.0 (IBM Co., Armonk, NY, USA). All data represent an average of at least three independent experiments or measurements.

3. Results and discussion

3.1. Freezing procedure for frozen croissant dough preparation

Bread dough prepared with wheat flour, fat, sugar, salt, yeast, and water would freeze at subzero temperatures due to the freezing point depression. The actual freezing temperature slightly differs according to changes in the ingredient contents and the degree of dough fermentation (Sharadanant & Khan, 2003). Additionally, the zone of maximum ice crystal formation (ZMICF) for dough can be observed on the time-dependent freezing curves, when almost all water molecules in the dough are frozen (Kiani & Sun, 2011). This zone was assessed on all temperature profiles obtained during our croissant dough freezing procedure (Fig. 1). Representative temperature profiles show that the water in the dough did not freeze at a fixed temperature point, but crystallised over temperatures ranging from −6.1 to −11.8 °C. Evidently, a faster FR resulted in a shorter ZMICF.

Bald introduced an empirical model for the relationship between the FR and crystal size as follows (Kiani & Sun, 2011):

$$D = (\pi U r_c/2)\sqrt{\rho/k}(dT/dt)^{-0.5}$$

where, D is the crystal size, dT/dt is the FR, U is the crystal growth velocity, r_c is the ice nucleus critical radius, ρ is the frozen phase density, and k is the frozen phase thermal conductivity. According to this equation, the crystal size is inversely proportional to the square root of the FR. In other words, accelerating the FR would influence the decrease of crystal size. As observed in Fig. 1, the FR values in the ZMICF decreased from each initial rate to −0.24, −0.50, −0.52, −0.61, −1.53, and −2.01 °C min^{-1}, respectively. Resultingly, the ice crystal size in the frozen dough might decrease sequentially from FR1 to FR6, which could influence the internal structure of the dough (Cauvain, 1998).

3.2. Effects of the dough freezing rate and terminal temperature on baked croissant quality

The specific volume and firmness were measured to examine the effects of the FR and T_t on the quality of croissants prepared from frozen dough. The specific volume, representing as one of the quality values, was determined as shown in the Fig. 2a. The specific volume of samples frozen at the FR1–4 until −20 °C was similar to the volume of croissants prepared from unfrozen dough (fresh). However, the samples frozen at the FR5 and FR6 until −20 °C had lower values than the fresh. Additionally, lower T_t appeared to reduce the volume of the croissants ($p < 0.05$). Many researchers suggested that frozen dough inflation could be weakened by a rapid FR (Le-Bail et al., 2010) and a storage temperature below −20 °C for an extended time period (Yi & Kerr, 2009b). Based on the FR, whereas previous results appear consistent with ours, they slightly differ in terms of storage temperature. Our data showed that lower

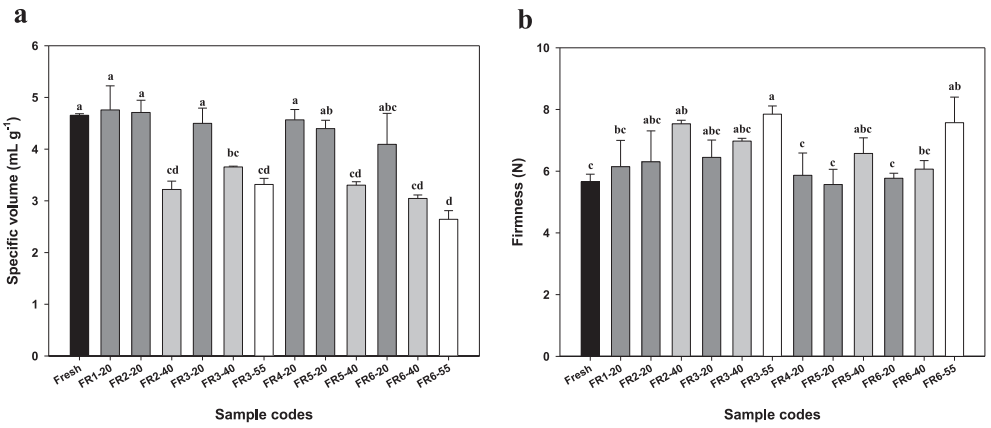

Fig. 2. (a) Specific volume and (b) firmness of croissants baked from the dough samples frozen under different conditions and stored at −18 °C for a day. The samples were coded as follows: Fresh (croissant sample baked from unfrozen dough); FR1-20 (T_t, terminal freezing temperature, −20 °C; FR, freezing rate, −0.72 °C min^{-1}); FR2-20 (T_t, −20 °C; FR, −1.43 °C min^{-1}); FR2-40 (T_t, −40 °C; FR, −1.43 °C min^{-1}); FR3-20 (T_t, −20 °C; FR, −1.50 °C min^{-1}); FR3-40 (T_t, −40 °C; FR, −1.50 °C min^{-1}); FR3-55 (T_t, −20 °C; FR, −1.50 °C min^{-1}); FR4-20 (T_t, −20 °C; FR, −1.84 °C min^{-1}); FR5-20 (T_t, −20 °C; FR, −3.19 °C min^{-1}); FR5-40 (T_t, −40 °C; FR, −3.19 °C min^{-1}); FR6-20 (T_t, −20 °C; FR, −3.56 °C min^{-1}); FR6-40 (T_t, −40 °C; FR, −3.56 °C min^{-1}); and FR6-55 (T_t, −55 °C; FR, −3.56 °C min^{-1}). The different letters, ranging from a to d, were considered significantly different at $p < 0.05$.

storage temperatures (−40 and −55 °C) at the end of the freezing procedure, even when kept under 2 h, significantly reduced the bread volume.

Firmness data, used as the other croissant quality index, are shown in Fig. 2b. Previous research suggested that dough freezing resulted in an increased firmness of the bread (Ribotta, Perez, León, & Añón, 2004). Specifically, the dough stored at −30 and −35 °C over 30 days was significantly affected in terms of firmness (Yi & Kerr, 2009a). In our study, all croissant firmness values for every FR conditions increased proportionally with decreasing the freezing croissant dough T_t, despite the short frozen storage time that was kept under 2 h. Contrastively, the FR seemed to only influence firmness insignificantly. According to previous studies, specific volume and firmness as bread quality values could be affected by several parameters, such as the forming ice crystals (Baier-Schenk et al., 2005), yeast viability (Ribotta et al., 2003), and gluten networks (Ribotta et al., 2004), which are described in more details in the following sections.

3.3. Influence of dough freezing temperature and terminal temperature on yeast viability

Yeast viability in frozen dough significantly affects loaf volume, bread firmness, and bread porosity because the viable yeasts can produce CO_2 to inflate the dough and form gas pores during the proofing step (Ribotta et al., 2003). Furthermore, reducing compounds liberated from dead yeast cells such as glutathione can damage the gluten network bonds during thawing, resulting in a loss of the gas retention capacity of the dough (Selomulyo & Zhou, 2007; Wolt & D'APPOLONLA, 1984). Resultingly, yeast viability is a key factor directly or indirectly influencing bread quality. We assessed yeast viability in croissant dough samples frozen under several conditions (Fig. 3). While the yeast viability in the FR1–5 dough samples frozen to −20 °C was similar to the fresh dough value, the FR6 samples frozen to −20 °C showed a lower viability value than fresh dough ($p > 0.05$). Particularly, we observed that yeast viability dropped drastically under the low dough freezing T_t conditions (−40 and −55 °C). These results implied that freezing the dough too rapidly and to temperatures that are too low could induce the yeast cell death in the dough.

Yeast cells in the freezing dough matrix undergo fatal stresses such as chilling injury, intracellular ice crystal formation, and high osmotic pressure (Randez-Gil et al., 1999). Moreover, the ice crystal

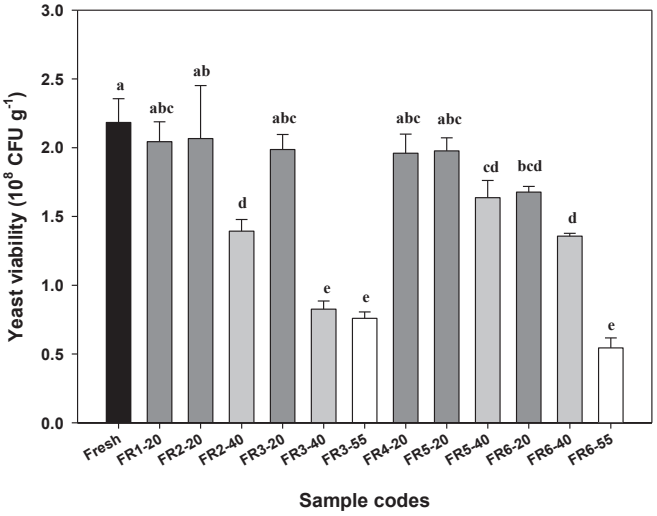

Fig. 3. The number of viable yeast in the frozen croissant dough after freezing and 1 day of storage. The samples were coded as follows: Fresh (unfrozen dough sample); FR1-20 (T_t, terminal freezing temperature, −20 °C; FR, freezing rate, −0.72 °C min^{-1}); FR2-20 (T_t, −20 °C; FR, −1.43 °C min^{-1}); FR2-40 (T_t, −40 °C; FR, −1.43 °C min^{-1}); FR3-20 (T_t, −20 °C; FR, −1.50 °C min^{-1}); FR3-40 (T_t, −40 °C; FR, −1.50 °C min^{-1}); FR3-55 (T_t, −20 °C; FR, −1.50 °C min^{-1}); FR4-20 (T_t, −20 °C; FR, −1.84 °C min^{-1}); FR5-20 (T_t, −20 °C; FR, −3.19 °C min^{-1}); FR5-40 (T_t, −40 °C; FR, −3.19 °C min^{-1}); FR6-20 (T_t, −20 °C; FR, −3.56 °C min^{-1}); FR6-40 (T_t, −40 °C; FR, −3.56 °C min^{-1}); and FR6-55 (T_t, −55 °C; FR, −3.56 °C min^{-1}). The different letters, ranging from a to e, were considered significantly different at $p < 0.05$.

volume increase or the nucleation of small ice crystals in the yeast plasma membrane could pierce the membrane, resulting in the extraction of their cytoplasmic content and death (Seki, Kleinhans, & Mazur, 2009). Because all of these cellular stress responses might be caused by the ice crystal formation in the dough, the croissant dough thermal properties were minutely examined using DSC to detect ice crystal formation during the freezing process.

3.4. Croissant dough ice crystal formation during the freezing procedure

The DSC method was used to mimic dough freezing and verify the effects of the FR on the croissant dough thermal characteristics.

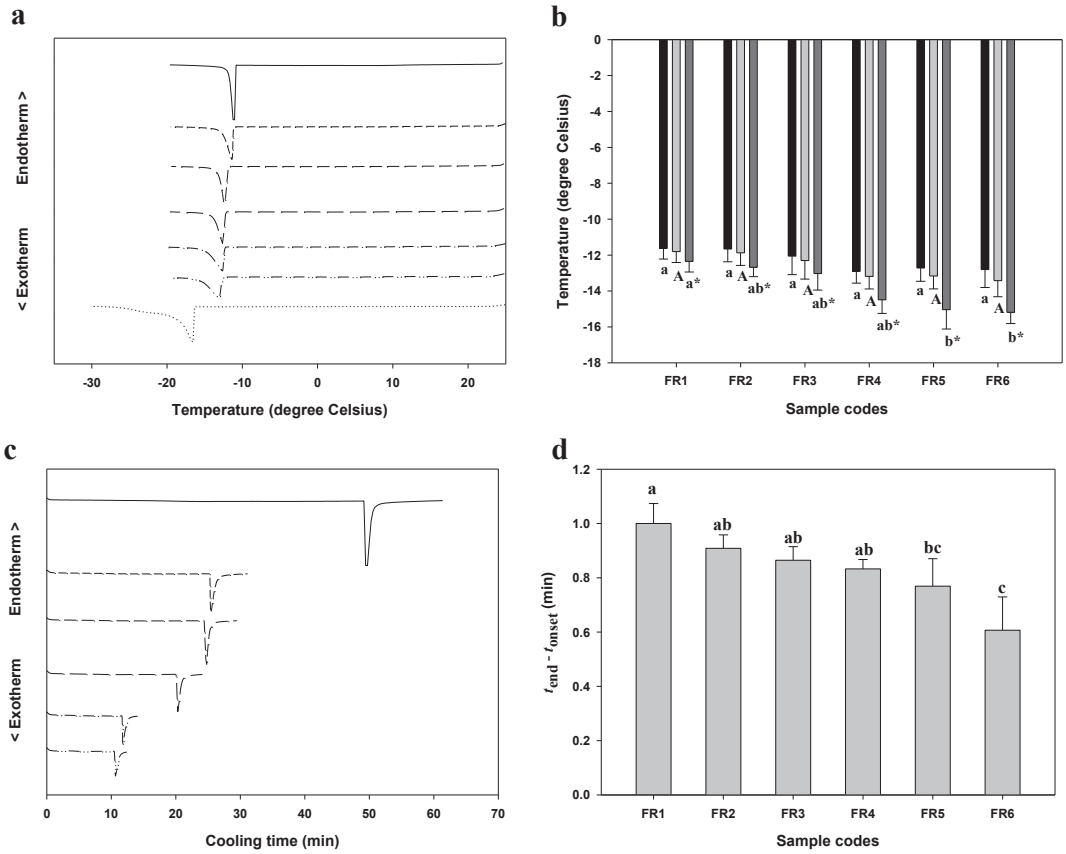

Fig. 4. (a) Thermograms (versus temperature) of croissant dough (solid line, FR1; short dashed line, FR2; medium dashed line, FR3; long dashed line, FR4; short-short dashed line, FR5; and medium-medium dashed line, FR6) and fresh compressed yeast (short-long dashed line) during DSC freezing (T_{peak}, the temperature of the maximum exothermic on the unimodal peak; T_{onset} and T_{end}, the upper and lower temperatures on the peak, respectively), and (b) temperature values (T_{onset}, left bars; T_{peak}, middle bars; and T_{end}, right bars) for ice crystals formation. (c) Thermograms (versus time) of croissant dough (solid line, FR1; short dashed line, FR2; medium dashed line, FR3; long dashed line, FR4; short-short dashed line, FR5; and medium-medium dashed line, FR6) during DSC freezing (t_{onset} and t_{end}, the lower and upper times on the unimodal peak, respectively), and (d) the time range ($t_{end} - t_{onset}$) of ice crystal formation. The sample were coded as follows: FR1, −0.72 °C min^{-1}; FR2, −1.43 °C min^{-1}; FR3, −1.50 °C min^{-1}; FR4, −1.84 °C min^{-1}; FR5, −3.19 °C min^{-1}; and FR6, −3.56 °C min^{-1}. The different letters in (b) and (d) were considered significantly different at $p < 0.05$.

Dough thermograms under the measurement were recorded as shown in Fig. 4a. In each thermogram, the T_{peak} represents the temperature of the maximum exothermic on unimodal peak, and T_{onset} and T_{end} are the upper and lower temperatures, respectively (Fig. 4b). Based on these results, the phase transition temperature under the DSC was observed between −11.6 and −15.2 °C, which is lower than the −6.1 to −11.8 °C range previously observed in the experimental freezing dough profiling section. Even if the temperature range for dough ice crystal formation was not different among the samples, as shown in the profiling section, the T_{end} values were grouped as follows: FR1 sample, FR2 to FR4 samples, and FR5 and FR6 samples ($p < 0.05$). The difference between the practical dough temperature profiling and the DSC mimicking method may result from the hermetic condition of the pan and the unavoidably biased simulation of the ZMICF under DSC freezing at constant FR.

The thermograms relative to the temperature (Fig. 4a) were converted to graphs relative to time (Fig. 4c), and the corresponding values of the T_{onset} and T_{end} were t_{onset} and t_{end}, respectively. The time gap between t_{onset} and t_{end} might correspond to the time when almost the water molecules of the dough were crystallised during DSC freezing (Fig. 4d) (Montenegro, Antonietti, Mastai, & Landfester, 2003). This time was shortened proportionally with the FR elevation ($p < 0.05$). Miyawaki and coworkers explained that the necessary time (t) for an ice crystal to grow up to a size D was represented by $t = D/U$, where U is the crystal growth velocity (Miyawaki, 2001; Miyawaki, Abe, & Yano, 1992). In other words, the crystal size is proportional to the time required for the ice crystal to form. Therefore, a faster freezing period results in smaller ice crystals forming in the dough, which is consistent with our croissant freezing profiles and our suggestions for other researchers (Chevalier, Le-Bail, & Ghoul, 2000). In summary, we conclude that the FR determines ice crystal size in the dough.

Yeast viability was not only influenced by the FR but also by the T_t, which could be explained using a DSC thermogram of compressed yeast (Fig. 4a). Ice crystals in the yeast formed from −16.3 to −27.7 °C. The large exothermic peak seen on the thermogram could be divided into a major sharp peak and a minor wide peak. The major and minor peaks may be attributed to ice crystal forming in the intercellular and intracellular space, respectively (Seki et al., 2009). This result suggests that the water molecules in the yeast cytoplasm were mainly frozen at temperatures below −20 °C. Considering that cytoplasmic ice crystal formation induces yeast cell death, it is logical that a lower yeast viability and worse croissant quality were observed in samples frozen to −40 and −55 °C compared with those frozen to −20 °C.

3.5. Microstructural morphologies of frozen croissant dough

SEM is generally used to observe the dough microstructure and assess the changes occurring during the freezing procedure.

Accordingly, we used SEM to observe the microstructural changes of the dough frozen in each of the tested freezing condition. The analysis was performed at a constant temperature of −20 °C to avoid the thawing of the frozen dough. SEM revealed a typical dough structure, including gluten network, voids, and starch granules (Fig. 5). Because SEM was performed before the proofing process, no spherical voids were detected in the images. Instead, in all images, angular voids were identified within the structures composing starch granules and gluten networks, which may represent the spaces where ice crystals were present (Zounis, Quail, Wootton, & Dickson, 2002). Indeed, the size of the angular voids could be considered as the size of the ice crystals formed during the dough freezing process. The angular voids size differences between the FR1-20 (Fig. 5a) and FR6-20 (Fig. 5d) samples were verified. However, the difference among the FR1-20 (Fig. 5a), FR2-20 (Fig. 5b), and FR4-20 (Fig. 5c) samples was unclear. These results are in accordance with our DSC results (Fig. 4d) in terms of the statistical grouping of the samples. In other words, the FR can determine the ice crystal size in the frozen dough, as predicted by the dough freezing profiles and DSC results. Furthermore, because the size of the angular voids in the FR6-20 (Fig. 5d) sample was similar to that of the FR6-55 (Fig. 5e) sample, it appears that the ice crystal size was dominantly affected by the FR but not by the T_t of the freezing dough.

It was recognized that yeast viability in frozen croissant dough was influenced by the FR and the freezing T_t. The FR can determine the ice crystal size, thereby determining the distribution of the ice crystals in the dough because of the restricted amount of water present. Assuming an even distribution of the yeast cells in the dough, a more even distribution of the ice crystals resulting from their smaller size may affect a larger surface of dough around the ice crystals. This could eventually cause more cell death from chilling stress, osmotic pressure, and crystal formation on the yeast cell membranes (Seki et al., 2009). Furthermore, a T_t below the freezing temperature of the yeast cytoplasmic water could also induce cell death by increasing the internal pressure of the cells (Mazur, 1984). Yeast viability largely affects croissant quality values such as specific volume and firmness. Therefore, the croissant dough freezing process should be performed using a moderately rapid FR with temperatures kept above the freezing point of the yeast cells internal water to obtain the best croissants produced from the frozen dough.

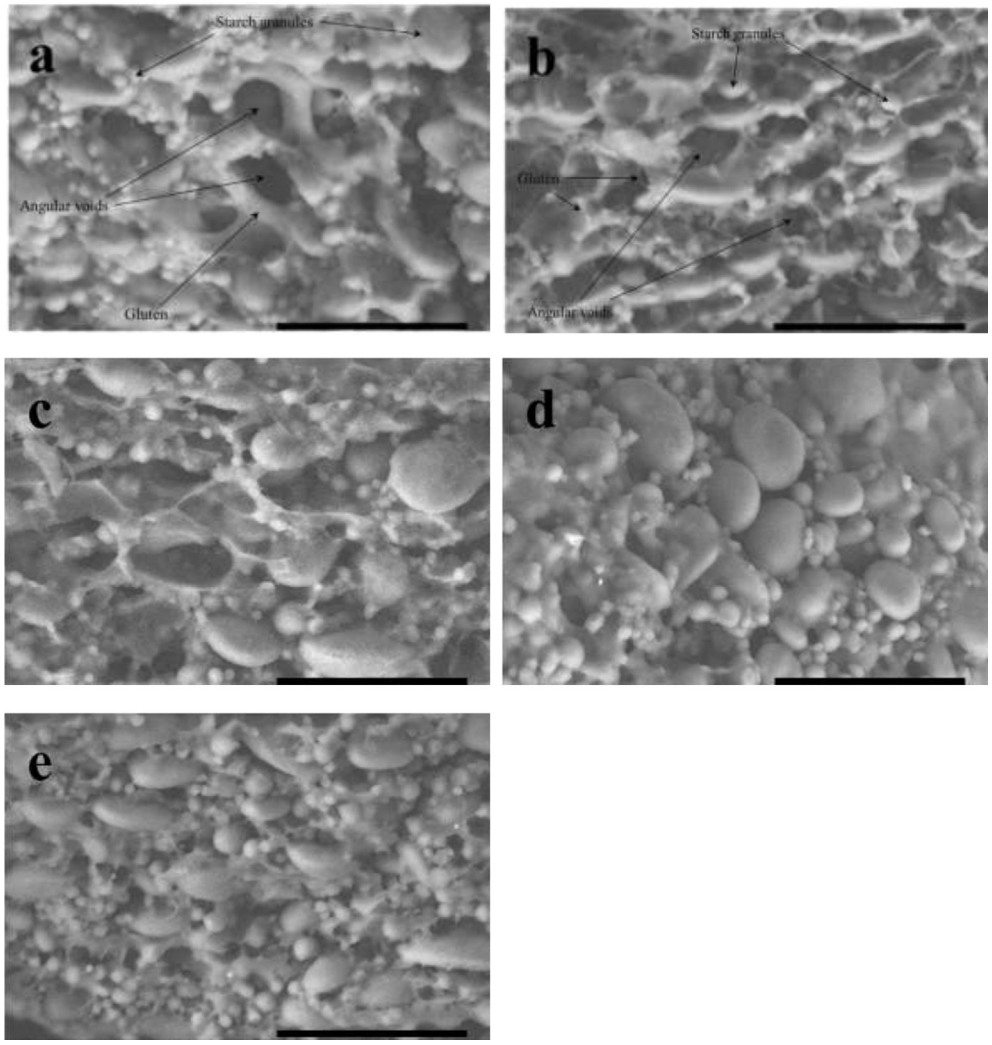

Fig. 5. Microstructural images of frozen croissant dough pieces sampled at each freezing conditions after storage at −18 °C for a day. (a) FR1-20 (T_t, terminal freezing temperature, −20 °C; FR, freezing rate, −0.72 °C min^{-1}); (b) FR2-20 (T_t, −20 °C; FR, −1.43 °C min^{-1}); (c) FR4-20 (T_t, −20 °C; FR, −1.84 °C min^{-1}); (d) FR6-20 (T_t, −20 °C; FR, −3.56 °C min^{-1}); and (e) FR6-55 (T_t, −55 °C; FR, −3.56 °C min^{-1}). The scale bars represent 50 μm.

4. Conclusions

Our results suggest that the FR in frozen croissant dough production is a dominant determining factor of ice crystals size in the dough. Because ice crystal size affects yeast viability, the FR influences croissant quality values, such as specific volume and firmness. Additionally, we verified that the T_t influences the fate of yeast cells within a short time, which also affects croissant quality parameters. Therefore, to obtain high-quality croissant produced from frozen dough, the freezing procedure should be performed at a suitable FR and the temperature should be maintained above the yeast cytoplasmic water crystallisation point. Our results showed that the optimal FR was $-3.19\ ^{\circ}C\ min^{-1}$ (FR5) and that the optimal T_t was $-20\ ^{\circ}C$. Consequently, our results could improve the current technical knowledge for optimal frozen croissant dough preparation conditions and should be applied to enhance bread quality in the frozen dough industry.

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References

AACC. (1986). *Approved methods of the AACC* (8th ed.). St. Paul, MN: American Association of Cereal Chemists (Methods 74-09).
AACC. (2000). *Approved methods of the AACC* (10th ed.). St. Paul, MN: American Association of Cereal Chemists (Methods 42-50).
Baier-Schenk, A., Handschin, S., Von Schönau, M., Bittermann, A. G., Bächi, T., & Conde-Petit, B. (2005). 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. *Journal of Cereal Science, 42*(2), 255–260.
Bøgh-Sørensen, L. (2006). *Recommendations for the processing and handling of frozen foods* (4th ed., pp. 8–10). Paris: International Institute of Refrigeration (IIR).
Bot, A. (2003). Differential scanning calorimetric study on the effects of frozen storage on gluten and dough. *Cereal chemistry, 80*(4), 366–370.
Cauvain, S. P. (1998). Improving the control of staling in frozen bakery products. *Trends in Food Science and Technology, 9*(2), 56–61.
Chen, G., Jansson, H., Lustrup, K. F., & Swenson, J. (2012). Formation and distribution of ice upon freezing of different formulations of wheat bread. *Journal of Cereal Science, 55*(3), 279–284.
Chen, G., Öhgren, C., Langton, M., Lustrup, K. F., Nydén, M., & Swenson, J. (2013). Impact of long-term frozen storage on the dynamics of water and ice in wheat bread. *Journal of Cereal Science, 57*(1), 120–124.
Chevalier, D., Le-Bail, A., & Ghoul, M. (2000). Freezing and ice crystals formed in a cylindrical food model: part I. Freezing at atmospheric pressure. *Journal of Food Engineering, 46*(4), 277–285.
Kiani, H., & Sun, D. (2011). Water crystallization and its importance to freezing of foods: a review. *Trends in Food Science and Technology, 22*(8), 407–426.
Kontogiorgos, V., Goff, H. D., & Kasapis, S. (2008). Effect of aging and ice-structuring proteins on the physical properties of frozen flour–water mixtures. *Food Hydrocolloids, 22*(6), 1135–1147.
Le-Bail, A., Nicolitch, C., & Vuillod, C. (2010). Fermented frozen dough: impact of pre-fermentation time and of freezing rate for a pre-fermented frozen dough on final volume of the bread. *Food and bioprocess technology, 3*(2), 197–203.
Mazur, P. (1984). Freezing of living cells: mechanisms and implications. *American Journal of Physiology-Cell Physiology, 247*(3), C125–C142.
Miyawaki, O. (2001). Analysis and control of ice crystal structure in frozen food and their application to food processing. *Food Science and Technology Research, 7*(1), 1–7.
Miyawaki, O., Abe, T., & Yano, T. (1992). Freezing and ice structure formed in protein gels. *Bioscience, Biotechnology and Biochemistry, 56*(6), 953–957.
Montenegro, R., Antonietti, M., Mastai, Y., & Landfester, K. (2003). Crystallization in miniemulsion droplets. *The Journal of Physical Chemistry B, 107*(21), 5088–5094.
Muldrew, K., & McGann, L. E. (1990). Mechanisms of intracellular ice formation. *Biophysical journal, 57*(3), 525.
Petzold, G., & Aguilera, J. M. (2009). Ice morphology: fundamentals and technological applications in foods. *Food Biophysics, 4*(4), 378–396.
Phimolsiripol, Y., Siripatrawan, U., Tulyathan, V., & Cleland, D. J. (2008). Effects of freezing and temperature fluctuations during frozen storage on frozen dough and bread quality. *Journal of Food Engineering, 84*(1), 48–56.
Randez-Gil, F., Sanz, P., & Prieto, J. A. (1999). Engineering baker's yeast: room for improvement. *Trends in biotechnology, 17*(6), 237–244.
Ribotta, P. D., León, A. E., & Añón, M. C. (2001). Effect of freezing and frozen storage of doughs on bread quality. *Journal of Agricultural and Food Chemistry, 49*(2), 913–918.
Ribotta, P. D., León, A. E., & Añón, M. C. (2003). Effects of yeast freezing in frozen dough. *Cereal chemistry, 80*(4), 454–458.
Ribotta, P. D., Perez, G. T., León, A. E., & Añón, M. C. (2004). Effect of emulsifier and guar gum on micro structural, rheological and baking performance of frozen bread dough. *Food Hydrocolloids, 18*(2), 305–313.
Seki, S., Kleinhans, F. W., & Mazur, P. (2009). Intracellular ice formation in yeast cells vs. cooling rate: predictions from modeling vs. experimental observations by differential scanning calorimetry. *Cryobiology, 58*(2), 157–165.
Selomulyo, V. O., & Zhou, W. (2007). Frozen bread dough: effects of freezing storage and dough improvers. *Journal of Cereal Science, 45*(1), 1–17.
Sharadanant, R., & Khan, K. (2003). Effect of hydrophilic gums on frozen dough. I. Dough quality. *Cereal chemistry, 80*(6), 764–772.
Wolt, M. J., & D'APPOLONLA, B. L. (1984). Factors involved in the stability of frozen dough. I. The effects of yeast type, flour type, and dough additives on frozen-dough stability. *Cereal chemistry, 61*(3), 213–221.
Yi, J., & Kerr, W. L. (2009a). Combined effects of dough freezing and storage conditions on bread quality factors. *Journal of Food Engineering, 93*(4), 495–501.
Yi, J., & Kerr, W. L. (2009b). Combined effects of freezing rate, storage temperature and time on bread dough and baking properties. *LWT-Food Science and Technology, 42*(9), 1474–1483.
Zounis, S., Quail, K. J., Wootton, M., & Dickson, M. R. (2002). Studying frozen dough structure using low-temperature scanning electron microscopy. *Journal of Cereal Science, 35*(2), 135–147.