



## Combined effects of freezing rate, storage temperature and time on bread dough and baking properties

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### ABSTRACT

This study compares the effects of freezing temperature and rate as well as storage temperature and time on the quality of frozen dough. Yeasted bread dough was frozen using four freezing rates (19–69 °C/h), then stored at –10, –20, –30, or –35 °C for up to 180 days. Dough strength diminished with longer storage time and higher storage temperatures. Cryo-SEM showed that dough stored at –30 and –35 °C had the least damaged gluten network. NMR studies showed that more rapidly frozen dough, and that stored at lower temperatures had lower transverse relaxation ( $T_2$ ) times (9–10 ms). However, dough stored at –20 °C displayed the highest yeast activity among samples. Bread loaf volume decreased with storage time, and bread made from dough stored at –20 °C showed the highest loaf volume. Breads produced from –30 and –35 °C stored dough displayed less change in the texture profile during storage as well as less change in  $T_2$  values. Response surface analysis showed that optimal properties occurred at freezing rates of around 19–41 °C/h and storage temperatures of –15 to –20 °C.

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### 1. Introduction

Bread made from frozen dough has become an increasingly popular alternative to that made directly from unfrozen dough. Frozen dough can be manufactured in large quantities off-site, and then shipped to local restaurants or retail operations, saving on both equipment and labor costs. In recent years, the quality of these products has improved owing to advances in technology and formulation, but there is room for additional improvement. Problems associated with frozen dough include long proof time, low volume, poor texture, and variable performance (Kenny, Wehrle, Dennehy, & Arendt, 1999). Some of the poorer quality can be attributed to diminished yeast activity, the characteristics of the yeast and their survival after freezing (Baguena, Soriano, Martinez-zanaya, & Debarber, 1991; El-Hady, ElSamahy, Seibel, & Brummer, 1996; Hino, Takano, & Tanaka, 1987; Hsu, Hosoney, & Seib, 1979; Ribotta, Leon, & Anon, 2003; Wolt & D'Appolonia, 1984). In order to improve performance, frozen dough processors may add extra yeast, use short or no-time dough processing procedures, mix ingredients at relatively low temperatures, or incorporate new strains of freeze-tolerant yeasts.

During freezing, there are also deleterious effects on dough structure. Electron microscopy studies (Esselink, Aalst, Maliepaard, Henderson, et al., 2003) showed a breakdown of the reticular gluten network when subjected to freezing and thawing, and related these to changes in the rheological properties of the dough. Ice recrystallization and water migration in the dough also affects the dough structure (Bache & Donald, 1998; Rojas, Rosell, Benedito de Barber, Pérez-Munuera, & Lluch, 2000). Extended frozen storage alters the gluten protein matrix (Berglund, Shelton, & Freeman, 1991; Varriammarston, Hsu, & Mahdi, 1980), results in dough weakening, and causes less gas retention (Autio & Sinda, 1992; Hsu et al., 1979; Inoue & Bushuk, 1991). In addition, injury to yeast membranes caused by freezing and thawing release cellular chemicals that have deleterious effects on the dough structure. However, researchers have different opinions on the effects of specific leachates, mainly reducing compounds such as glutathione, on the gluten network (Casey & Foy, 1995; Inoue & Bushuk, 1991; Wolt & D'Appolonia, 1984).

While several researchers have studied the effects of storage time or temperature on yeast viability and dough structure (El-Hady et al., 1996; Havet, Mankai, & Le Bail, 2000; Mazur, 1961), less has been done on the influence of freezing rate. Especially in frozen dough preparations, where freezing and sometimes prolonged frozen storage intervene between dough formation and bread baking, several factors still have not been fully investigated (Giannou & Tzia, 2007). In the present study, we examined the

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combined effects of freezing rate, storage temperature and storage time on dough and bread quality. The dough properties were evaluated based on dough extensibility and adhesiveness, and the viability of yeast assayed through measurements of gas production. Both volume and firmness were measured in the finished breads. Direct examination of the dough in the frozen state was accomplished using cryogenic scanning electron microscopy (cryo-SEM), allowing visualization of the ultra-structure of gluten-starch association and the state of gluten strands in the network. We also used time-domain NMR to investigate changes in water dynamics (Esselink, Aalst, Maliepaard, & Duynhoven, 2003; Roman-Gutierrez, Guilbert, & Cuq, 2002; Ruan et al., 1999).

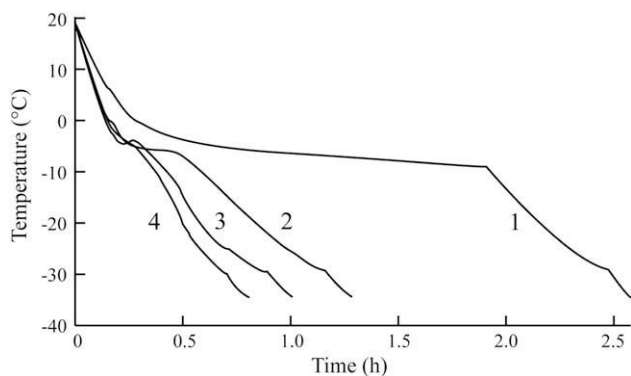
## 2. Materials and methods

### 2.1. Dough preparation and freezing

Bread dough was prepared from enriched, bleached hard winter wheat flour (Organic Select Artisan Flour, King Arthur Company, Norwich, Vermont) with 11.5 g/100 g protein content; Baker's dry yeast (Fleischmann's Yeast, Quebec, Canada); Dominion pure cane sugar (Dixie Crystal, Savannah, GA); non-iodized salt (Morton International, Inc., Chicago, IL); and soybean oil (Wesson Vegetable Oil, ConAgra Foods, Omaha, Nebraska). The basic formulation was (per 100 g flour weight): water (60 g/100 g), sugar (5 g/100 g), oil (5 g/100 g), yeast (3 g/100 g), and salt (2 g/100 g). The yeast was prehydrated with the water, and then all of the dough ingredients were placed in a 6-quart, 575 W mixer (Kitchen Aid Professional 600 Series, St. Joseph, MI) and mixed for 2 min at 120 rpm with a paddle, and for 8 min at 178 rpm with a dough hook. Once the dough was formed, it was separated into samples of 50 g, and shaped by hand into approximately 50 mm diameter circular slabs.

Dough pieces were frozen in a built-in forced-air blast freezer located at the UGA Department of Food Science and Technology in Athens, GA. As part of the experimental design, the freezer was adjusted to give four different freezing rate schemes, as shown by the temperature-time plots in Fig. 1. Specific freezing times and rates depend on the conditions and final temperature. The average rate (and time) to reach  $-30^{\circ}\text{C}$  were: Rate 1:  $19^{\circ}\text{C/h}$  (2.53 h); Rate 2:  $41^{\circ}\text{C/h}$  (1.18 h); Rate 3:  $55^{\circ}\text{C/h}$  (0.88 h); and Rate 4:  $72^{\circ}\text{C/h}$  (0.67 h). In terms of the time to go from the initial freezing point to  $-30^{\circ}\text{C}$ , these times were: Rate 1: 2.27 h; Rate 2: 0.95 h; Rate 3: 0.62 h; and Rate 4: 0.45 h.

After freezing to the desired temperature, dough samples were vacuum-packaged in plastic bags and placed at four different frozen storage temperatures ( $-10$ ,  $-20$ ,  $-30$  or  $-35^{\circ}\text{C}$ ), and stored for 30,



**Fig. 1.** Freezing protocols for frozen bread dough. Average rate (and time) to reach  $-30^{\circ}\text{C}$ . Rate 1:  $19^{\circ}\text{C/h}$  (2.53 h); Rate 2:  $41^{\circ}\text{C/h}$  (1.18 h); Rate 3:  $55^{\circ}\text{C/h}$  (0.88 h); and Rate 4:  $72^{\circ}\text{C/h}$  (0.67 h).

60, 90, and 180 days. At each sampling period, 3 replications of the 16 treatments were withdrawn from the freezers and placed in an environmental chamber (Model HEC10R, HotPack, Warminster, PA) at  $8^{\circ}\text{C}$  and 35% RH in order to thaw. Thawed dough pieces were removed from the package, then placed in a chamber at  $36^{\circ}\text{C}$  and 85% RH for proofing. Dough samples were baked in a 5-rack gas convection oven (Blodgett BLD-DFG100, Burlington, Vermont) at  $180^{\circ}\text{C}$  for 15 min. After baking, bread samples were allowed to cool for approximately 1 h prior to subsequent measurements.

### 2.2. Dough extensibility and adhesiveness

Extensibility of the dough was measured using a texture analyzer (TA-XT2i, Texture Technologies Corp., Scarsdale, NY) with a modified Kieffer extensibility rig and 5 kg load cell. Approximately 50 g dough samples were molded into strips approximately 7 mm in diameter and 60 mm in length. All samples were left to rest on a grooved plate at  $8^{\circ}\text{C}$  for 20 min and 90% RH prior to testing (Anderssen, Bekes, Gras, Nikolov, & Wood, 2004). The dough was pulled at a crosshead speed of 3.3 mm/s. The resistance to extension (maximum force) and extensibility (distance to break) were calculated from the force–deformation curves. One advantage of the Kieffer dough extensibility rig is that it uses a micro-extension method involving a very small sample size. It correlates highly with methods such as the extensigraph as indicated by baking performance (Kieffer, Wieser, Henderson, & Graveland, 1998; Sharadanant & Khan, 2003; Suchy, Lukow, & Ingelin, 2000).

Adhesiveness was measured using the texture analyzer with a modified Chen–Hoseney stickiness rig, with conditions as described by Chen and Hoseney (1995). The sample was placed in a cylindrical cell on the base of the texture analyzer, which was then enclosed by a lid with a perforated hole. A small amount of dough was extruded through the hole. The upper cylindrical probe was brought in contact with the exposed dough to adhere to it, and the probe was pulled away from the base at a speed of 1.7 mm/s. Both the maximum force and the area under the force–deformation curve required to separate the probe from the test sample were used as measures of adhesiveness.

### 2.3. Cryo scanning electron microscopy

A Jeol JSM-5410 scanning electron microscope with a CT-500C cryo-unit (Oxford Instruments, Oxfordshire, UK) was used to investigate the microstructure of the frozen bread dough. Each frozen dough sample was placed on the cryo-specimen holder, placed in liquid nitrogen, and then transferred to the cryo-unit in the frozen state. The dough specimens were fractured, sublimated (10 min at  $-70^{\circ}\text{C}$ ) and sputter coated with gold (4 min at 0.2 kPa). The prepared dough specimen was transferred to the microscope where it was observed at 15 kV and  $-120^{\circ}\text{C}$ . Micrographs were taken at 500, 1000 and 2000 $\times$  magnification.

### 2.4. NMR measurements of dough

Proton relaxation measurements were made using a 20 MHz Proton ( $^1\text{H}$ ) NMR spectrometer (Resonance Instruments, Whitney, UK). Approximately 5 g dough samples were taken from the center of the thawed dough. Three replicates were taken from each piece of dough. Each sample was placed in a 10 mm diameter glass tube then covered with parafilm. The glass tube was placed in a  $10^{\circ}\text{C}$  bath for 20 min. After 20 min, the glass tubes with dough specimens were placed in 18 mm diameter NMR tubes. Transverse ( $T_2$ ) relaxation curves were developed using the CPMG pulse sequence:  $90x-(\tau-180y-\tau\text{-echo})n$ . Acquisition parameters were set to a  $90^{\circ}$  pulse of 4.2  $\mu\text{s}$  and a recycle delay of 2 s. A pulse spacing ( $\tau$ ) of

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