Frozen Bread Dough Ultrastructure as Affected by Duration of Frozen Storage and Freeze-Thaw Cycles[1]

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ABSTRACT

Cryogenic preparation of frozen bread dough permitted examination of changes in ultrastructure by low-temperature scanning electron microscopy (SEM). Doughs examined after mixing and frozen doughs, thawed during SEM preparation, exhibited a reticular pattern. After mixing and 24 hr frozen storage, starch granules appeared firmly embedded in the gluten network, which was mostly intact. Some starch granules exhibited internal damage and were more separated from the gluten matrix after 24 weeks of frozen storage and freeze-thaw cycles. When doughs were kept frozen during SEM preparation, the reticular pattern was not apparent. After 24 weeks frozen storage and freeze-thaw cycles, doughs that were kept frozen during SEM preparation had less free water distributed throughout the sample. In these doughs, less water appeared to be associated with the gluten matrix and starch. Patches of ice were observed in samples kept frozen during SEM preparation. The observed changes in ultrastructure may help explain the extended proof times and reduced loaf volumes of frozen bread dough.

Frozen bread dough is used by more than 50% of in-store supermarket bakeries as well as by retail customers. The major shortcoming of frozen dough is its short shelf life (six to eight weeks). With extended frozen storage, proof times of frozen bread dough increase. Another major problem is the loss of quality that results from mishandling in transportation and storage.

Scanning electron microscopy (SEM) has been widely used to study the ultrastructure of food products; a number of researchers have used it to examine the ultrastructure of doughs and breads (Aranyi and Hawrylewicz 1969; Moss 1974; Khoo et al 1975; Cumming and Tung 1975, 1977; Varriano-Marston 1977; Evans et al 1981; Junge et al 1981; Parades-Lopez and Bushuk 1982).

Varriano-Marston et al (1980) hypothesized that ice crystallization itself could contribute to the weakening of the three-dimensional protein network responsible for gas retention in dough, which could cause weakening of the dough and contribute to excessively long proof times. They acknowledged that excessive proof times were, at least in part, due to destruction of yeast during freezing, resulting in decreased gas production. They hypothesized that the structural components of dough, particularly protein, must also be drastically altered by the recrystallization processes. On examining frozen doughs, they found that freezing and thawing increased the number of disrupted yeast cells in doughs and altered the protein network. It should be noted that their frozen dough samples were freeze-dried in preparation for SEM, and thus water was removed from the dough. They concluded that their findings supported earlier research (Hanafusa 1969) showing that conformational changes occur in proteins as a result of freeze-thaw cycles. Varriano-Marston and co-workers (1980) emphasized the role that water plays in the maintenance of protein structure.

Since the traditional preparation of frozen dough samples for SEM has required the removal of volatile constituents such as water, changes may have occurred in the dough ultrastructure. SEM of cryogenically prepared samples is a promising method of examining water distribution and ultrastructural changes in frozen dough. New developments in low-temperature SEM (LT-SEM) allow samples to be examined in a frozen, fully hydrated state. Although LT-SEM requires sublimation of surface ice, Moor and Muhlethaler (1963) found that etching (very brief freeze-drying) of frozen yeast cells revealed the fine structures in the fracture plane and did not deform structures. LT-SEM presents an ideal way to determine the extent of ice-crystal growth and structural damage during storage of frozen foods. The use of cryopreparation of frozen dough samples and subsequent SEM examination can indicate the location of ice or water and clearly demonstrate the ultrastructure of gluten and/or starch in frozen doughs subjected to varying frozen storage times or freeze-thaw cycles.

The objective of this study was to use the new technique of LT-SEM to describe ultrastructural features of bread doughs after mixing and of frozen doughs subjected to various frozen storage times and freeze-thaw cycles.

MATERIALS AND METHODS

Dough for examination by SEM was prepared by a no-time dough formula, molded, and examined within 2 hr or frozen at −23°C. Doughs were examined by SEM shortly after mixing, after frozen storage of 24 hr and 24 weeks, and after one and three freeze-thaw cycles. Each freeze-thaw cycle was accomplished by freezing the mixed and molded doughs at −23°C for three weeks. Bagged doughs were then allowed to thaw partially in controlled conditions of 22°C and 40% rh for 2 hr. These doughs were then refrozen for a minimum of two days. This procedure was repeated for each freeze-thaw cycle. Doughs were sampled by the following three procedures:

1. Samples were taken from the freshly mixed and molded dough, which was refrigerated up to 2 hr and placed on the specimen holder at room temperature (22°C).
2. Samples were taken from the interior of frozen loaves and placed on the specimen holder at room temperature, at which time at least partial thawing took place.
3. Samples were cut from the interior of the frozen loaf with a precooled cutting tool, kept frozen on dry ice, and placed on a cooled specimen holder. All samples were attached to the specimen holder with Tissue-Tec O.C.T. Compound (Miles Scientific, Naperville, IL) and mixed with Aquadag (Tousimis Research Corp., Rockville, MD).

The International Electron Optics EMscope SP2000A cryo-unit (I.E.O. Inc., Houston, TX) was used for cryogenic preparation of all samples for SEM. The cryo-unit allows the sample to be examined in a fully hydrated state, which eliminates the need for dehydration or fixation of the sample. The samples were rapidly brought to a lower temperature by plunging the specimen holder into nitrogen slush at −196°C in the integral freezing chamber. Even the fresh samples were frozen in liquid nitrogen before being examined while still in the hydrated condition. The

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alternative would have required dehydration, which is known to alter gluten ultrastructure. A shroud was closed over the frozen specimens before their transfer under vacuum onto the cold stage of the cryo-unit. The preparation chamber was maintained at −160°C or colder by means of vacuum and a stage cooled by liquid nitrogen. The shroud on the specimen holder was opened, and the sample was fractured with a cooled macro knife assembly.

The fractured sample was again shrouded and transferred under vacuum onto the cryostage of the scanning electron microscope (JEOL JSM 35, JEOL USA Inc., Peabody, MA). Samples were quickly observed and then sublimed at −65°C for 6 min (previously determined as optimal sublimation time) in the cryostage. After being sublimed, the samples were either examined and photographed or shrouded and returned to the EMscope cryo-unit under vacuum for sputter coating. Before being coated, the samples were heated under the radiant head for 60 sec to sublime any frost that may have formed during transfer. Samples were sputter coated with gold at 0.1 Torr and at 30 mA for 3 min. Coated samples were shrouded and transferred under vacuum to the cryostage of the SEM, examined, and photographed.

RESULTS AND DISCUSSION

The three sampling techniques used to prepare doughs for LT-SEM produced uniquely different appearances in the micrographs. A previous study (Berglund et al 1990) found that when doughs were prepared for LT-SEM at room temperature, the predominant ultrastructural characteristic was the reticular pattern of the specimen. However, when specimens were kept frozen during the entire process, this was not evident.

In fresh doughs that were examined within 2 hr after mixing (Fig. 1A), starch granules appeared to be securely embedded in the gluten network, and little evidence was seen of ice damage to the internal portion of the starch granules or the gluten. Broad, flat sheets of gluten with little reticulate or marring were apparent in the freshly mixed dough (Fig. 1B).

Doughs frozen 24 hr before examination were characterized by intact, undamaged starch granules (Fig. 1C). The gluten matrix appeared mostly intact but had some ice-crystal damage, which was probably a result of freezing for a 24-hr period.

After 24 weeks of frozen storage, the gluten matrix appeared less continuous, more ruptured, and separated from the starch granules (Fig. 1D). The gluten strands were also thinner. Because a less uniform gluten matrix would retain gas poorly, these structural features may help explain the decreased loaf volume and increased proof times of frozen doughs stored for long periods. The angular pattern of ice-crystal formation was apparent on the surface of the intact starch granules. This pattern was formed when the solutes were forced to the margin of the developing ice crystals (Lewis and Pawley 1981).

Doughs subjected to either one or three freeze-thaw cycles exhibited considerable damage to the gluten network (Fig. 2), including separation of starch granules from the gluten network. The gluten network was frequently characterized by an extensive reticular pattern (Fig. 2A). The damage appeared to be much greater than that observed in freshly mixed doughs and doughs frozen for 24 hr, even though all doughs were frozen during preparation for LT-SEM. Frozen bread doughs stored for long periods (24 weeks) and/or subjected to freeze-thaw cycles, exhibited considerable disruption or marring of the gluten network. Varriano-Marston et al (1980) found that conformational

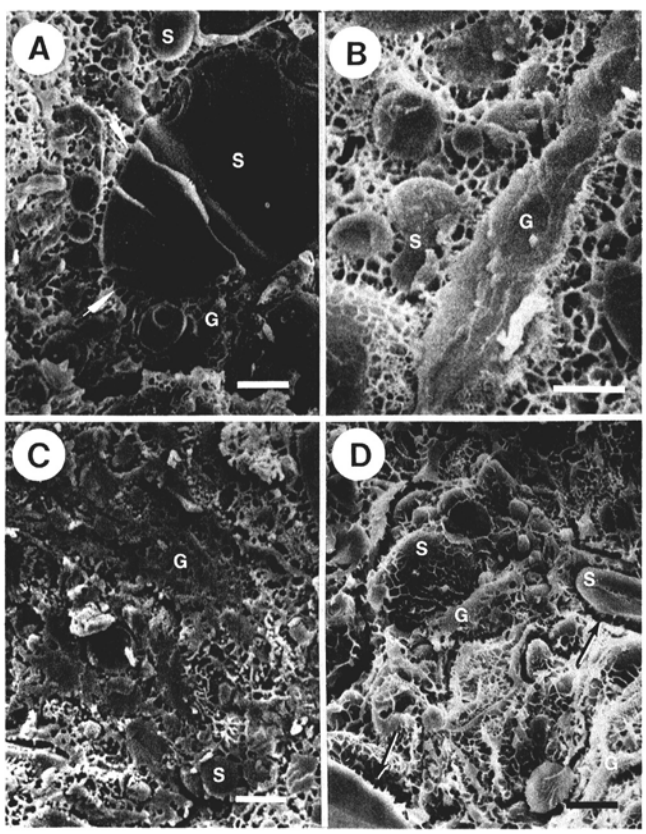

Fig. 1. Scanning electron micrographs of doughs sampled at room temperature. **A,** Fresh dough. Arrows show gluten (**G**) attached to starch granules (**S**). **B,** Fresh dough. Intact gluten and starch granules firmly embedded in gluten matrix. **C,** Dough frozen 24 hr. Gluten matrix mostly intact but with some disruption due to freezing. **D,** Dough frozen 24 weeks. Gluten less continuous and more ruptured. Arrows indicate separation of gluten from starch granules. Angular pattern resulting from the formation of ice crystals is apparent on the surface of the intact starch granules. This pattern is formed when the solutes are forced to the margin of the developing ice crystals. Bars = 5 μm.

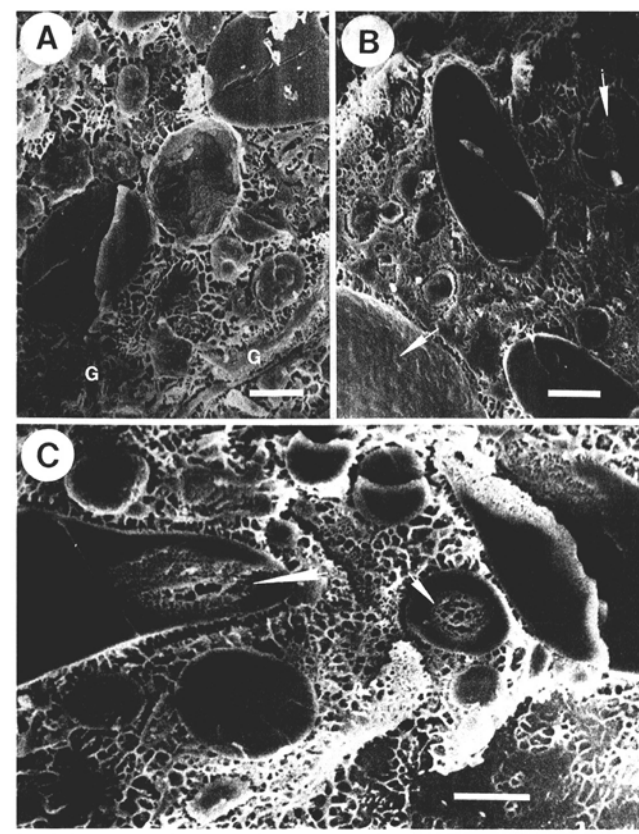

Fig. 2. Scanning electron micrographs of frozen doughs sampled at room temperature. **A,** Doughs subjected to one freeze-thaw cycle. Gluten matrix (**G**) shows some evidence of freezing damage. **B,** Dough subjected to three freeze-thaw cycles. Arrow at upper right indicates starch damage. Arrow at lower left indicates a large space, probably resulting from the accumulation of carbon dioxide formed by the yeast. **C,** Dough subjected to three freeze-thaw cycles. Arrows indicate internal starch damage. Bars = 5 μm.

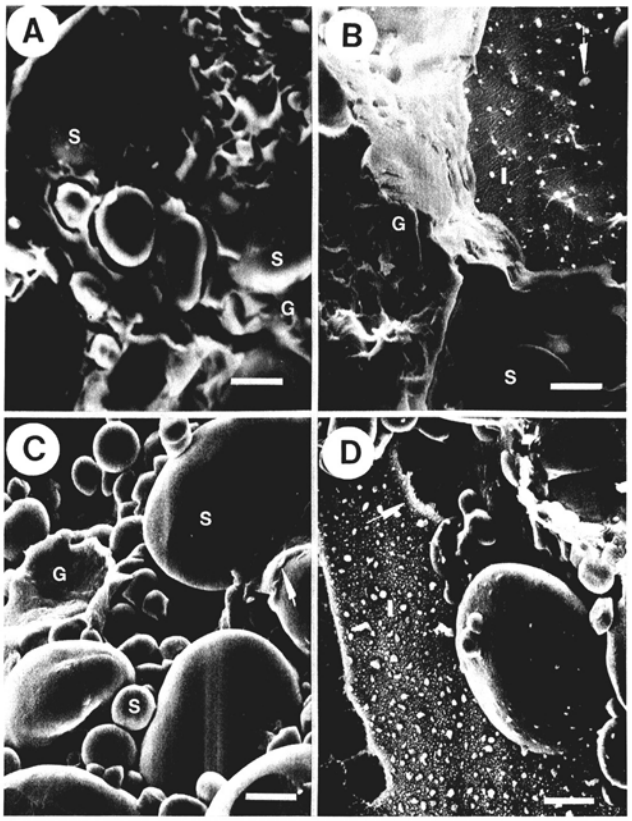

Fig. 3. Scanning electron micrographs of doughs kept frozen during sampling. **A,** Dough frozen 24 hr. Gluten matrix (**G**) is attached to larger starch granules (**S**) and is mainly continuous. **B,** Doughs frozen 24 weeks. Ice (**I**) found separated from starch and gluten. Arrow indicates the probable solutes on exposed surface of ice. **C,** Doughs subjected to three freeze-thaw cycles. Arrow indicates attachment of gluten and starch. Many starch granules were separated from gluten. **D,** Doughs subjected to three freeze-thaw cycles. Arrow shows concentration of solutes at eutectic point. Many areas of ice were separated from gluten and starch. Bars = 5 μm.

changes occurred in the protein network as a result of freezing and thawing.

Doughs examined by SEM showed large ice masses that were formed during recrystallization. Perhaps these structural changes contributed to a decreased ability of the gluten to retain gas during proofing. Additionally, very large spaces with smooth-walled edges were occasionally evident (Fig. 2B). These spaces were identified even before sublimation, indicating that they were filled with gas, not water. The gas was probably carbon dioxide formed by the yeast.

Internal damage to starch granules was also noted (Figs. 2B and 2C). Starch granules that were damaged internally by ice were generally intact on their outer surfaces. Starch that was physically damaged by milling would allow penetration of water into a crack or fracture on the surface or outer edge of the granule, which may not be visible at all planes. Additional starch damage may also contribute to less desirable dough structure. Tipples (1969) reported that damaged starch caused a linear increase in water absorption by flour since the damaged starch acts like a sponge in the presence of water. Increased damage to starch granules may account for the redistribution of the water. With more damaged starch in the dough, it is possible that the starch granules draw water away from the gluten matrix.

In doughs frozen 24 hr and kept frozen throughout the SEM sample preparation, the larger starch granules appeared to be more firmly embedded in the gluten matrix (Fig. 3A). Patches of ice were also commonly observed in many areas of this sample.

Fewer areas of free water (ice) were found in doughs frozen 24 weeks and kept frozen during SEM preparation. However, large patches of ice (Fig. 3B) were occasionally observed. It is possible that over long periods of frozen storage, the doughs underwent a freeze-drying process, reducing the water within the gluten matrix and starch. The water that was apparent seemed to be concentrated in large pools recognized as ice. These areas were characterized by a speckled appearance caused by the concentration of solutes on the exposed surface (Fig. 3B). Similar areas were sublimed at higher temperatures to confirm that they were indeed frozen water (Berglund et al 1990).

In doughs subjected to freeze-thaw cycles and kept frozen during preparation for SEM, the starch granules appeared separated from the gluten matrix (Figs. 3C and 3D). The starch granules and gluten appeared almost as free floating entities. It seemed that freeze-thawing drew water out of the matrix. The remaining water was separated, not part of the gluten or starch network but pooled as large ice crystals (Fig. 3D).

CONCLUSIONS

Changes in water distribution occurred during extended frozen storage and freeze-thaw cycles. The observed changes in the ultrastructure of the starch granules and gluten may contribute to the extended proof times and reduced loaf volumes of frozen bread doughs.

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