Comparison of Two Sample Preparation Procedures for Low-Temperature Scanning Electron Microscopy of Frozen Bread Dough

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ABSTRACT

New developments in low-temperature scanning electron microscopy (LT-SEM) allow samples to be examined in a frozen, fully hydrated state. We studied the effect of sample preparation procedure for LT-SEM on resulting ultrastructure of frozen bread dough. Frozen doughs that thawed during SEM preparation exhibited a reticular pattern that was not apparent when samples remained frozen. The procedure used for preparing samples to be examined by LT-SEM is critical in determining the ultrastructural nature of frozen products.

Scanning electron microscopy (SEM) has been widely used to study the ultrastructure of food products. However, most sample preparation procedures require dehydration, freeze-drying, or exposure to fixatives or buffers that can produce artifacts, mask surface detail, or cause alteration of ultrastructure. Several researchers have examined dough ultrastructure by electron microscopy following either freeze-drying or chemical fixation (Aranyi and Hawrylczewicz 1969; Moss 1972, 1974; Khoo et al 1975; Cumming and Tung 1975, 1977; Varriano-Marston 1977; Varriano-Marston et al 1980; Evans et al 1981; Junge et al 1981; Paradis-Lopez and Bushuk 1982).

Water is an important structural and chemical component of frozen foods and, therefore, it is desirable to examine these products with a minimum of alteration. Cryogenic preparation of samples for low-temperature (LT)-SEM is a promising method to examine water distribution and microstructural changes within a frozen dough with SEM. By cooling the specimen in nitrogen slush prior to fracturing with a cooled knife, samples may be examined in a frozen, fully hydrated state.

Perhaps some of the differences in ultrastructure of bread doughs described in the literature have resulted from sampling technique rather than differences in formulation, mixing method, freezing parameters, and length of frozen storage. Ultrastructural characteristics may be better studied utilizing the new technique of LT-SEM if consistent procedures are rigorously maintained.

MATERIALS AND METHODS

Frozen doughs were prepared by a no-time dough formula, molded, and frozen at −23°C. Frozen doughs were sampled by the following two procedures: 1) samples were taken from the interior of frozen loaves and immediately placed on the specimen holder at room temperature (22°C), during which time there was at least a partial thawing; or 2) samples were cut from the interior of the frozen loaf with a precooled cutting tool and kept frozen with dry ice during placement on a cooled specimen holder. The dry ice was used only to keep the specimens completely frozen during the mounting procedure. Both samples were attached to the specimen holder with Tissue-Tek O.C.T. Compound (Miles Scientific, Naperville, IL) mixed with Aquadag (Toasitms Research Corp., Rockville, MD). The samples were then cryogenically prepared using the International Electron Optics EMscope SP2000A cryo-unit (I.E.O., Inc., Houston, TX). All samples were frozen by plunging the specimen holder into nitrogen slush at −196°C in the integral freezing chamber. A shroud was closed over the frozen specimens prior to their transfer under vacuum onto the cold stage of the cryostage. A shrouded specimen holder provides further cryogenic protection against excessive warming and contamination with condensing vapors during transfer from the preparation chamber to the electron microscope, which has a cold stage maintained at about −180°C. The preparation chamber was maintained at −160°C or colder by means of vacuum and a liquid nitrogen cooled stage. The shroud on the specimen holder was opened and the sample was fractured with a cooled macroknife assembly. The fractured samples were again shrouded and transferred under vacuum onto the cryostage of the JEOL JSM 35 scanning electron microscope. Thawed samples were observed, sublimed at −65°C for 6 min on the cryostage, and photographed. Frozen samples were photographed after 2, 6, and 10 min sublimation on the cryostage. The samples were examined and photographed at 10 kilovolts without metallic coating.

RESULTS AND DISCUSSION

The two sampling techniques used to prepare doughs for LT-SEM produced frozen bread doughs with significantly different

Fig. 1. Frozen bread dough thawed during specimen mounting. The reticular pattern resulted from the redistribution of water and solutes during subsequent freezing in liquid nitrogen. Bar = 5 μm (2,000X).
When specimens were removed from the center of frozen loaves with a precooled cutting tool and kept frozen with dry ice for 30 sec to 1 min while being mounted onto a precooled stub, there was no evidence of a reticular network (Figs. 2-5) even when samples were sublimed at room temperature. When the frozen dough specimens were sublimed for 2 min (Fig. 2) the presence of ice still masked most ultrastructural detail. After 6 min of sublimation (Fig. 3), the starch granules and gluten matrix were more apparent. The speckled appearance on the small patches of remaining ice was probably due to solutes on the surface. An additional 4 min of sublimation (10 min total) removed the ice to a greater depth (Fig. 4). With the ice sublimed, the ultrastructure of the bread dough became apparent. No reticulation was apparent even when these specimens were examined at higher magnification (Fig. 5).

The rapid thawing that occurs when small frozen dough specimens are mounted at room temperature results in a redistribution of the water in the specimen. Subsequent freezing in liquid nitrogen causes a recrystallization of the water that forces the solutes into a reticular pattern and alters the ultrastructure of the dough. Thus, some ultrastructural differences of bread doughs described in the literature may be a result of sampling technique, rather than differences in treatments. To avoid this artifact, it is therefore essential to keep samples frozen during all stages of processing and microscopic examination. Studies to determine what affects the quality of frozen bread doughs are in progress.

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LITERATURE CITED


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