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A Note on Autoradiography of Tritium-Labeled Galactolipids in Dough and Bread[1]

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ABSTRACT

Tritium-labeled galactosyldidecanoylglycerol was synthesized. Sections 5 μ thick prepared from dough and bread containing the labeled galactolipid were studied by autoradiography. In the dough, the galactolipid was distributed in gluten and, to some extent, on the starch surface. In baked bread, most of the galactolipid appeared in the starch.

Solubility studies of gluten proteins have shown that wheat flour glycolipids are bound to glutenin protein by hydrophobic and to gliadin protein by hydrophilic bonds (1). Infrared spectroscopy indicated feasibility of hydrogen bonds between glycolipids and gelatinized starch and gluten components (2). In addition, Van der Waals bonds between glycolipid and gluten components were indicated. Nuclear magnetic resonance spectra pointed to hydrophobic bonding with glutenin. The investigations on isolated starch or gluten with glycolipids pointed to the possibility of starch-glycolipid-gluten interactions in dough. The present study was to investigate, by autoradiography, whether such interactions take place in dough and bread containing both starch and proteins.

MATERIALS AND METHODS

Trans-2-decenoic acid was purchased from Aldrich Chemical Co., Milwaukee, Wis. Then, 170 mg. (1 mmole) was dissolved in 1 ml. ethylacetate containing 3 curies of tritium gas (New England Nuclear Corp., Boston, Mass.) and 25 mg. of 10% palladium on carbon. The mixture was stirred for 30 min. Hydrogen was added

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to the system; the measured uptake of hydrogen was 24 ml. (1 mmole). The solvent was removed by vacuum distillation. The residue was redissolved in 10 ml. methanol and evaporated again in vacuo to yield 100 mg. decanoic acid with 500 μc.

Fifty milligrams of the labeled decanoic acid was diluted with 120 mg. untreated decanoic acid. Fatty acid chloride was prepared as described by Bauer (3) and distilled in vacuo in a bulb tube. Synthesis of labeled galactosyldidecanoylglycerol (rac-1) was carried out with 137 mg. labeled decanoylchloride as described elsewhere (4). The yield was 13.6 mg. (6.7% of tritium-labeled galactosyldidecanoylglycerol with 13.6 μc. Galactosyldidecanoylglycerol was selected for the investigation because the compound is a much better bread improver than glycolipids with long-chain saturated fatty acids (5).

Five milligrams tritium-labeled galactosyldidecanoylglycerol, with an activity of 5 μc, and 30 mg. shortening were dissolved in 5 ml. ethyl ether and poured into a mortar which was preheated to 60°. The ether was evaporated instantaneously. One gram flour, 60 mg. sucrose, 20 mg. sodium chloride, 30 mg. nonfat dry milk, 5 mg. yeast food, 20 mg. yeast, and 0.6 ml. water were added. The dough was mixed for 10 min. with a spatula. The mortar was covered with a wet cloth, allowed to stand at 25° for 90 min., and punched at 60 min. It was then placed for 50 min. in a proof cabinet at 37°. The fermented dough was transferred to a test tube (15 mm. in diameter) and heated in a glycerol bath at 130° for 30 min. The resulting "bread" had a thin, light-brown crust. Grain and texture were comparable to those of normal bread.

To eliminate artifacts from fixation and embedding (6), we used a sectioning procedure that minimizes diffusion, redistribution, and leaching of the labeled glycolipid. Some diffusion artifacts may occur after the mounted wet sections are thawed and with a liquid emulsion at 40°. We believe that such changes are small as the label is rather insoluble.

Small pieces of dough (immediately after mixing and after fermentation) and of bread were placed on the freezing table of a microtome (A O Spencer automatic clinical microtome with freezing attachment) that had been precooled with dry ice. The pieces were attached to the table with a few drops of water. Microtomy was made in a cold room at −15°C. Knife and dough temperatures were kept below −30°C. by placing chunks of dry ice under the freezing table. Above that temperature no satisfactory sections could be made. The sections, 5 μ thick, were transferred by a brush with about 10 camel hairs, from the knife to a microscope slide covered with a thin layer of glycerol.

After standing for a day in a box at room temperature, the slides were preheated to about 60°C. and were dipped into a Kodak N-2 nuclear track emulsion at about 40°C. The emulsion was allowed to dry for 2 hr. in a box at room temperature, and the box with slides was stored in the refrigerator at 4°C. for 6 days. To develop the photographic emulsion, the slides were dipped consecutively for 5 min. into Eastman Developer D-19, twice for 15 sec. into distilled water, for 10 min. into Kodak Rapid Fixer with Hardener, and washed for 25 min. in running water. All operations beginning with dipping into the emulsion were made in complete darkness. The sections were stained with iodine by dipping the slides for 10 sec. in 10% Lugol iodine solution. Then the sections were examined under a Leitz-Wetzlar microscope. Pictures were taken with the Orthomat microscope attachment camera

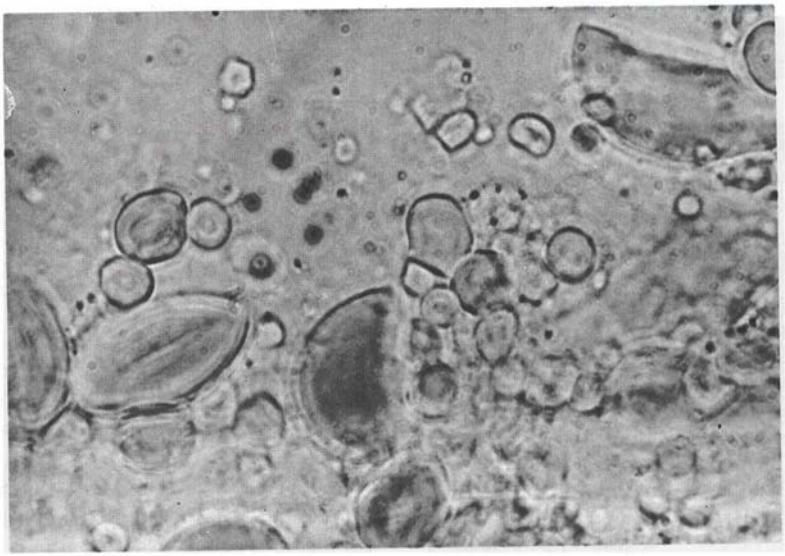

Fig. 1. Autoradiograph of dough containing tritium-labeled glycolipid. Starch is stained with iodine. Original magnification 1,000X.

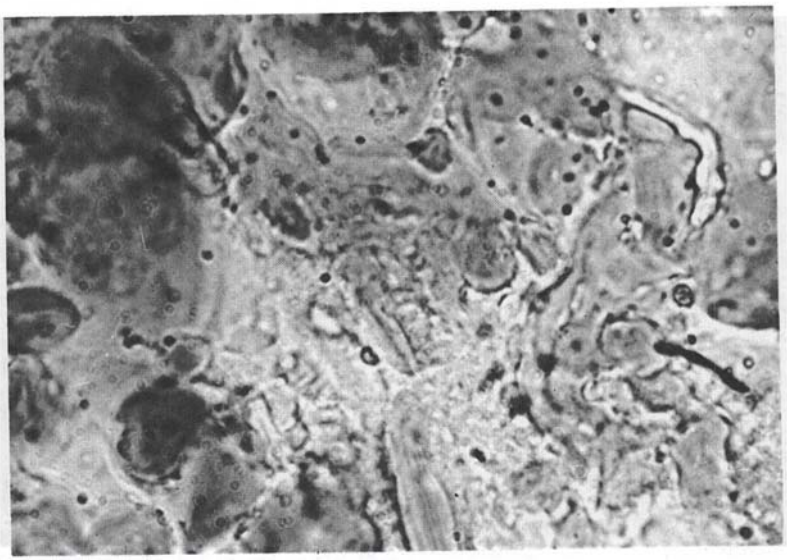

Fig. 2. Autoradiograph of bread containing tritium-labeled glycolipid. Starch is stained with iodine. Original magnification 1,000X.

at a magnification of 1,000X. For comparison, nonradioactive dough and bread were processed and compared.

RESULTS AND DISCUSSION

Sections of fermented dough and bread containing tritium-labeled galactosyldidecanoylglycerol are shown in Figs. 1 and 2. The picture of fully fermented dough was similar to that of mixed dough. The microscopic pictures are basically similar to those published by other authors. The starch granules (stained blue) are embedded in an unstained protein matrix. In the dough, black points produced by tritiated lipid are distributed both in the gluten matrix and in the starch granule. In the baked bread, most of the black points appeared in the gelatinized starch. It would seem, therefore, that in dough the glycolipid is associated with the protein and to a limited extent with compact starch granules that have a relatively small available surface area for hydrogen bonding. During gelatinization, apparently a considerable interaction with gelatinized starch takes place.

Within the first 6.5 min. of baking, the internal temperature of a commercial white pan bread reaches about 63°C. (7). At that time, yeast action is killed, carbon dioxide is fully released, and maximum expansion due to oven spring is reached. Yet, at that stage essentially no starch gelatinization has taken place, and protein denaturation is limited. It would seem, therefore, that in the dough, glycolipids interact with gluten protein, according to the scheme proposed by Hoseney et al. (1). A limited interaction with starch granules is also indicated. Our preliminary investigations with labeled glycolipid indicate that increase in loaf volume during oven spring can be attributed to formation of a complex between gluten proteins and glycolipids, and probably with starch. The oven spring, which no other wheat lipid, emulsifier, or shortening can produce (8), seems due to this specific complex which presumably seals gas cells during heat-denaturation of gluten and prevents the gas from escaping. In the baked bread, a complex between glycolipids and starch would seem of significance and could be responsible for the improved freshness retention of bread baked with glycolipids.

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