to nitrosomethomyl and *S*-methyl *N*-hydroxythioacetimidate were removed from the plates and the radioactivity of the silica gel was determined by liquid scintillation counting. The counts per minute (disintegrations per minute, dpm) for the 1.0-ppm methomyl spikes into ham and hot dog are given in Table III. Table III also shows the calculation of the maximum possible amount of nitrosomethomyl which could have been formed in these experiments. The maximum possible level of nitrosomethomyl in either meat is <1 ppb, which equals <0.1% of the applied methomyl.

DISCUSSION

For these tests aimed at examining the likelihood of pesticide interaction (specifically methomyl) with nitrites in cured meats, careful attempts were made to select realistic parameters. The temperature was body temperature. The incubation times (1 and 3 hr) bracketed normal residence times in the human stomach. The mass of solid matter (100 g of meat) to liquid (150 ml) was close to typical. The pH of the cured meat macerates was 2 both before and after the incubations. The cured meats were purchased commercially in local stores and were analyzed directly to ensure that they contained residual nitrite in the 16–20 ppm range. The amount of methomyl added (1 ppm based on solid content) represents a high level of consumed residue, since the highest tolerances for methomyl are in the 1–5 ppm range; most methomyl tolerances are <0.2 ppm. Also, there would be an immediate dilution of residue in the stomach with other foods containing no methomyl at all, e.g. a cured meat. Therefore, these studies, with their negative findings, indicate that there is very little likelihood that nitrites in the diet can combine with methomyl to form detectable amounts of a nitroso compound in the human stomach.

Extensive recovery studies of radiolabeled nitrosomethomyl added at various stages of the experimental procedure demonstrated the validity of the analytical method which is capable of detecting 1 ppb of nitrosomethomyl. The recovery studies also showed that nitrosomethomyl is inherently unstable and readily degrades to methomyl and *S*-methyl *N*-hydroxythioacetimidate under the experimental conditions.

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N-Nitrosamines: Absence from Sauerkraut and Silage

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Volatile *N*-nitrosamines were not detected in corn silage and sauerkraut prepared in the laboratory. Nitrosamines were not formed if the corn was supplemented with 250 ppm of nitrate nitrogen and the cabbage was supplemented with 250 or 1250 ppm of nitrate nitrogen prior to the fermentation. Nitrosamine synthesis did occur in sauerkraut if both a secondary amine and nitrite were added. Thus, formation of volatile nitrosamines is unlikely even if the silage and sauerkraut are prepared from nitrate-rich plants.

The carcinogenicity and teratogenicity of *N*-nitrosamines assume particular importance in view of their isolation from foodstuffs such as cooked bacon (Fazio et al., 1973; Sen et al., 1973), frankfurters (Wasserman et al., 1972), and fish (Ender et al., 1964). They also may be formed in the mammalian intestine following ingestion of the secondary amine and nitrite precursors (Alam et al., 1971; Lane and Bailey, 1973; Lijinsky and Greenblatt, 1972). The synthesis of these toxicants requires the simultaneous occurrence of the two precursors, typically nitrite and a secondary amine, and usually an acid pH, although microorganisms may promote the synthesis at neutral pH values (Hawksworth and Hill, 1971; Ayanaba and Alexander, 1973).

Two characteristics of silage and sauerkraut suggest that nitrosamines might be formed in these fermented products. These properties are the acid pH of the final material and the occasionally high nitrate levels in the original crop used for silage formation or the cabbage. Nitrate levels of 313 to 2328 ppm have been noted in corn (Wilson, 1943), but the level varies with growth conditions and the rate of nitrate fertilization (Wright and Davison, 1964). Nitrate nitrogen levels of 0.2 to 0.3% (dry weight basis) are quite common in cabbage leaves and heads used for making sauerkraut (N. H. Peck, personal communication). The microbial conversion of a portion of this nitrate to nitrite during the fermentation might lead to spontaneous nitrosamine formation.

The present study was designed to determine whether *N*-nitrosamines might be formed in sauerkraut and silage and to establish factors affecting their production.

MATERIALS AND METHODS

Silage. Silage was prepared in the laboratory from corn chopped to about 9 mm length with a New Holland 770 chopper. Samples (100 g) were tightly packed in test tubes, 3.6 × 22 cm, as described by Wang and Burris (1960) and the tubes were sealed with parafilm. To simulate silage pre-

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pared with nitrate-rich corn, KNO_3 was added to half of the samples to augment the nitrate nitrogen concentration by 250 ppm. The tubes were incubated at 30° for 4 weeks.

Sauerkraut. Sauerkraut was prepared using standard commercial methods. For this purpose, 2.25% (w/w) NaCl was added to the shredded cabbage. The salted cabbage was placed in vats, 26 cm diameter × 30 cm high, 8 kg wet weight per vat. The cabbage was covered with black plastic, and the plant material was compressed with bags containing a 2.25% NaCl solution. The fermentation was allowed to proceed at 25° for 3 weeks.

A variation of the method of Keybets et al. (1970) was used for the extraction. A 100-g portion (wet weight) was blended with 150 ml of 6 *N* NaOH until the suspension was homogenous. A few drops of Antifoam A (Dow Corning) were added, and the suspension was refluxed for 30 min. While still warm, the suspension was filtered through cheesecloth, and the filtrate was then steam distilled. One-half of the volume of the filtrate was collected as distillate. A small portion of the distillate was assayed for nitrosamines by the method of Daiber and Preussmann (1964).

About 0.1 g of NaCl was added to the remaining distillate, which was then extracted with an equal volume of dichloromethane. The dichloromethane layer was collected, and any residual water was removed by passage through Na_2SO_4. The dichloromethane extraction was repeated twice, and the pooled dichloromethane fractions were concentrated in a Kuderna-Danish evaporative concentrator. Samples of the concentrated liquid were placed on silica gel G thin-layer chromatography plates (Eastman Chemicals, Rochester, N.Y.), and constituents were separated using a hexane–ether–dichloromethane (4:3:2) solvent system. The presence of nitrosamines on the chromatograms was detected with the Griess reagents (Keybets et al., 1970) and by the method of Preussmann et al. (1964). Only spots giving positive reactions by both methods were considered to be nitrosamines. By this extraction procedure, 0.1 ppm of *N*-nitrosodimethylamine (as nitroso-*N*) could be detected. A minimum of three samples was examined for each product and treatment.

The nitrate was extracted from the plant tissues by the procedure of Greweling (1966). The nitrate was then reduced to ammonia, and the solution was steam distilled and assayed for ammonia by the method of Bremner (1965).

Warning. Since *N*-nitrosamines are carcinogenic, teratogenic, and acutely toxic, care should be taken in their handling. Work with these chemicals should be carried out in a fume hood, and precautions should be taken to avoid their contact with the skin.

RESULTS AND DISCUSSION

The original corn contained 2.3 ppm of nitrate nitrogen, so that chopped corn supplemented with the inorganic nitrogen contained 252 ppm of nitrate nitrogen. None of the samples of silage prepared either with or without added nitrate contained volatile nitrosamines in quantities sufficient to be detected by the method employed.

Sauerkraut was prepared from the Roundup variety of cabbage, a variety used for sauerkraut manufacture in New York. Prior to the start of fermentation, KNO_3 was added to three vats of the fermenting cabbage to give a final concentration of 255 and 1255 ppm of nitrate nitrogen (wet weight). The unamended cabbage contained 4.6 ppm of nitrate nitrogen on a wet weight basis. Volatile nitrosamines were not detected in any of the amended or unamended samples by the procedures employed.

Several new varieties of low-water cabbage are being developed at the New York State Agriculture Experiment Station at Geneva, N.Y. Because some of these are likely to be used in the future for commercial sauerkraut production, varieties 346, 322, 307, and 325 were used to make sauerkraut, and the final fermented products were tested for their nitrosamine content. The nitrate nitrogen contents of varieties 346 and 325 were 76 and 60 ppm on a wet weight basis, respectively. Analysis of the final product of the fermentation of these four varieties failed to reveal the presence of nitrosamines.

To determine if nitrosamines could indeed be formed in sauerkraut, triplicate samples of freshly fermented cabbage were amended with nitrite and/or dimethylamine to give final concentrations of 100 ppm of $NaNO_2$ nitrogen, 100 ppm of dimethylamine, and 100 ppm of dimethylamine plus 100 ppm of $NaNO_2$ nitrogen. One sample was unamended. The sauerkraut was incubated at 25°, and after 24 and 48 hr, the fermented product was extracted and analyzed. *N*-Nitrosodimethylamine was not detected in any sample incubated for 24 hr. *N*-Nitrosodimethylamine was not evident in samples receiving no additions or treated with either dimethylamine or nitrite and incubated for 48 hr; on the other hand, 0.14 ppm of *N*-nitrosodimethylamine was observed in sauerkraut receiving both dimethylamine and nitrite and incubated for 48 hr.

The fact that volatile nitrosamines could not be detected in any sample of silage or sauerkraut, whether or not they were amended with nitrate or nitrite, suggests that nitrosamine synthesis probably will not occur even in products prepared from nitrate-rich plants. It is possible that nitrosamines would have been detected if more sensitive analytical procedures had been used. However, inasmuch as high concentrations of both nitrite and dimethylamine had to be added before 0.14 ppm of *N*-nitrosodimethylamine was formed in sauerkraut, it appears that the formation of the carcinogen is unlikely owing not to the absence of suitable conditions for nitrosation but rather to a low level of the requisite precursors.

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