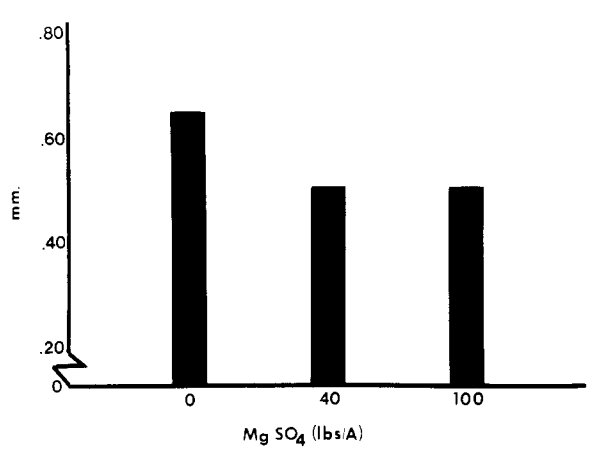

Figure 4. Effect of $MgSO_4$ application on the actual deformation (mm) of Katahdin potato tubers (year 3).

portant since iron deficiency is probably the most prevelant deficiency state affecting human populations, and it is generally higher in developing countries where the population relies heavily on vegetable foods and where infections and excessive sweating are common (Underwood, 1977).

Firmness. Magnesium fertilization resulted in tubers which exhibited significantly ($p < 0.01$) less deformation than controls in response to pressure testing (Figure 4). Firmness of fresh potatoes is important since baking and chipping quality are dependent upon it. A mealy texture of the cooked tuber is associated with the specific gravity and dry matter content of the raw potato (Smith, 1977). Since resistance of raw tuber tissue to a pressure force is significantly correlated with specific gravity and texture (Lujan and Smith, 1964), a firmer potato suggests a mealier texture and is associated with better baking and processing qualities. A firmer potato is also less susceptible to bruising and enzymatic darkening.

An elevation in the calcium and magnesium content of fertilized tubers (Table III) may be related to the firmer texture since the presence of both elements in cell walls has been associated with metal bridges between pectin molecules and a firmer texture in cooked tubers (Bartolome and Hoff, 1972).

Since fertilization with magnesium sulfate results in altered chemical composition of potato tubers, levels of fertilization should be controlled in order to produce tubers of desirable quality.

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Isolation and Identification of *N*-Nitrosothiazolidine in Fried Bacon

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An unidentified possible nitrosamine observed in extracts from fried bacon obtained by the mineral oil distillation procedure, which elutes after *N*-nitrosopyrrolidine when analyzed by gas chromatography–thermal energy analyzer, was shown to be *N*-nitrosothiazolidine (NTHZ). This substance had the same gas chromatographic retention time and low-resolution mass spectrum as standard NTHZ. Preliminary investigations indicate that most, but not all, of it is produced artifactually as a result of analysis when residual nitrite is present prior to analysis.

During the analysis of fried bacon for volatile nitrosamines, primarily *N*-nitrosodimethylamine (NDMA) and -pyrrolidine (NPYR), by the mineral oil distillation procedure (Fine et al., 1975), an unknown peak was occasionally observed on the gas chromatography–thermal energy analyzer (GC–TEA) chromatogram. This possible new nitrosamine was brought to our attention by the Food Safety Inspection Service (FSIS). The component re-

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sponsible for the TEA peak was photolabile when exposed to UV light at 365 nm, suggesting it was a potential nitrosamine (Doerr and Fiddler, 1977). The unknown compound has a relative retention time approximately twice that of NPYR on a Carbowax 20M-TPA column when the GC–TEA analysis was performed isothermally at 170 °C. A large unidentified peak with a similar relative retention time has been observed in cured meat product extracts when they were analyzed by vacuum distillation GC–TEA methods (Sen et al., 1979; Kühne and Mirna, 1981).

This paper reports the isolation and identification of a nitrosamine, heretofore not previously found in food products.

EXPERIMENTAL SECTION

Reagents. Nitrosothiazolidine (NTHZ) [**note**: precaution should be exercised in the handling of nitrosamines since they are potential carcinogens] was synthesized and purified by a general procedure reported previously (Pensabene et al., 1972). Neutral alumina (Bio-Rad) was activated for 3 h at 190 °C and then deactivated to activity 3 (6% water). Silicic acid was activated for 4 h at 110 °C and then deactivated with 5% H_2O. Hexane, dichloromethane (DCM; "Distilled in Glass" solvents from Burdick and Jackson Laboratories), and all other chemicals, including thiazolidine, were purchased from commercial suppliers and used without further purification.

Bacon Sampling. The fried cure pumped and dry cured bacon samples were obtained from the Food Safety and Inspection Service monitoring program of commercial producers, and the unfried samples from local retail stores. Bacon samples were ground and fried as described by Pensabene et al. (1979).

Nitrosamine Analysis. *Mineral Oil Distillation Procedure.* A modification of the procedure described by Fine et al. (1975) was employed to analyze for NTHZ in the fried bacon. A 25-g ground fried bacon sample was placed in a 500-mL distillation flask equipped with a thermometer well. Twenty-five milliliters of mineral oil and 2 mL of 0.2 N NaOH were added, and the sample was distilled under vacuum (0.5 mmHg) until the temperature reached 140 °C. The distillate, collected in a glass trap immersed in liquid nitrogen, was quantitatively transferred to a 125-mL separatory funnel and extracted with 15 mL of DCM. The trap washing and extraction steps were repeated twice, and the combined DCM extracts were dried by passage through anhydrous sodium sulfate and concentrated to 1.0 mL in a Kuderna-Danish apparatus. If necessary, the procedure was repeated or scaled up to 100 g, and the samples were combined to provide sufficient NTHZ for mass spectral confirmation.

Direct Extraction Column Chromatographic Procedure. The procedure was as described by Pensabene and Fiddler (1982). In it, a 32 mm × 35 cm chromatographic column containing a glass wool plug was packed with mixtures of 6 N H_3PO_4–Celite, Celite–fried bacon–sodium sulfate, and sodium sulfate. The column was then eluted with 5% DCM in pentane and the eluate concentrated. The concentrate was diluted with hexane and placed on a column containing basic alumina (Camag) hydrated with 1.5% H_2O. The column was washed with hexane and the NTHZ eluted with DCM. When sufficient sample was available, the procedure was scaled up from 10 to 20 or 30 g of fried bacon, depending on the lipid content of the fried bacon, and repeated as many times as was necessary so that the combined samples provided sufficient NTHZ for MS confirmation. Samples 8 and 9 (Table I) represented extracts from 240 and 285 g of fried bacon, respectively.

Determination. The concentration of NTHZ was determined quantitatively by using a Varian-Aerograph Model 2700 gas chromatograph (GC) containing a 2.7 m × 3.2 mm stainless steel column packed with Carbowax 20M-TPA on 60–80-mesh Gas-Chrom P and interfaced with a thermal energy analyzer (TEA). The helium flow rate was 35 mL/min, the injector port temperature was 200 °C, and the oven temperature was either programmed from 120 to 220 °C at 4 °C/min or operated isothermally at 170 °C. The TEA was operated under conditions similar to those reported previously (Pensabene et al., 1980).

Table I. *N*-Nitrosothiazolidine in Fried Bacon Samples Confirmed by GC-MS

	ppb	
sample	mineral oil distillation[a]	direct extraction column
1[b]	149	
2[b]	692	
3[c]	89	32
4[c]	298	18
5[c]	245	13
6[c]	219	26
7[c]	107	19
8[c]		20[a]
9[c]		19[a]

[a] Confirmed by GC-MS. [b] Dry cured. [c] Cure pumped.

Sample Cleanup. *Mineral Oil Distillation Procedure.* Prior to mass spectral confirmation, the extract from individual fried bacon samples was diluted with 20 mL of hexane and added to an 11 mm × 30 cm water-cooled chromatographic column containing 5 g of activity 3 neutral alumina plus 1 g of anhydrous Na_2SO_4 in hexane. The column was washed with 50 mL of 10% DCM in pentane (flow rate of 1–2 mL/min) and the NTHZ eluted with 50 mL of DCM–pentane (1:1). The sample was first concentrated to 1.0 mL with the Kuderna-Danish apparatus and then to approximately 40–80 μL under vacuum (50–90 mmHg).

Direct Extraction Column Chromatographic Procedure. Extracts (1 mL) were added to an 11 mm × 39 cm water-cooled chromatographic column containing 2 g silicic acid and 15 mL of pentane. The column was washed with 100 mL each of 10% and 30% DCM in pentane and the NTHZ eluted with 150 mL of 50% DCM in pentane. The eluate was concentrated in a Kuderna-Danish apparatus to 1 mL. The extract then underwent further cleanup by the above procedure (alumina column) prior to GC–MS analysis.

Confirmation. NTHZ was analyzed by use of a Hewlett-Packard Model 5992B low-resolution quadrupole gas chromatograph–mass spectrometer (GC–MS) fitted with a capillary interface system. A 30 m × 0.5 mm glass capillary column coated with UCON 5100 was used. The helium flow rate was 3.5 mL/min, and the injector port temperature was 150 °C. The oven was maintained at 20 °C for 2 min and then programmed from 20 to 160 °C at 10 °C/min. When the GC–MS isolation valve was opened, 0.75 mL/min helium entered the MS, and the remainder was vented to the atmosphere. The eluting peak was scanned from m/z 29 to 170 at a speed of 330 amu/s. Under these conditions, NTHZ had a retention time of 26.6–27.0 min. When the analysis was performed on the same day, the retention times of the sample and NTHZ standard were identical.

RESULTS AND DISCUSSION

In the extracts of a number of fried bacon samples analyzed by the mineral oil distillation procedure, a TEA responsive peak other than NDMA and NPYR has been

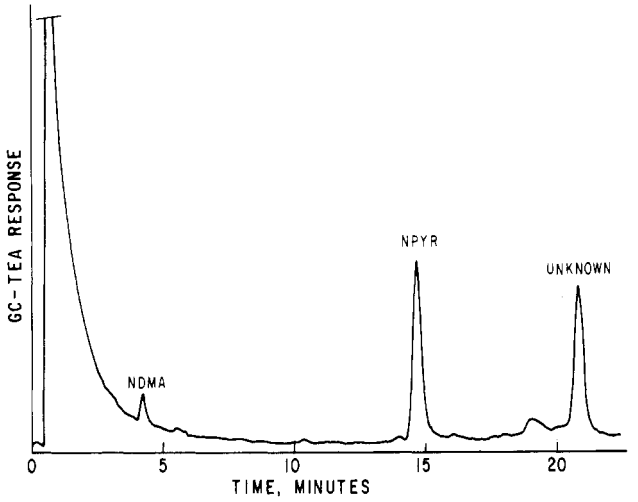

Figure 1. GC–TEA chromatogram of the mineral oil distillation extract of fried bacon containing the unknown peak.

occasionally detected. This peak was not observed when the fried bacon was analyzed by a distillation procedure under basic aqueous conditions, as employed in the FDA nitrosamine multidetection method (Fazio et al., 1972), presumably because it decomposed under these conditions. Figure 1 shows a representative GC–TEA chromatogram of a bacon extract containing NDMA, NPYR, and the unknown peak when the GC oven was temperature programmed. Under these conditions, the unknown peak had a relative retention time 1.4 times that of NPYR.

Cure pumped and dry cured bacon samples believed to contain this unknown compound were obtained from local retail stores (unfried) or FSIS (fried). The compound was isolated, concentrated, and then analyzed by GC–MS. The mass spectra of sample 1 listed in Table I and of a NTHZ standard (parts A and B, respectively) are shown in Figure 2. There were 10 major ions with abundances that varied from 15% to 100% of the base peak abundance (m/z 88), and these were m/z 30, 35, 42, 45, 46, 59, 60, 61, 88, and 118. In sample 1 (Figure 2A), nine of these ions differed from those of the NTHZ standard (Figure 2B) by 3% or less and one by 10%. Similarly for samples 2-8 in Table I, nine of the ions varied by less than 7% and one by 6–12%, and for sample 9, six of the ions varied by 4% or less and four by 10–13%. The mass spectra and the retention time of these compounds indicate that the standard and sample were identical. The concentration of NTHZ in nine samples is shown in Table I. The extracts of these samples were analyzed by GC–MS, and all showed several minor extraneous ions which did not interfere with the confirmation of the identity of the nitrosamine. Each sample was compared to the NTHZ standard on the same day since there were variations in the relative proportions of the ions over a period of time. When a new column chromatographic method was employed on samples 3–7, considerably lower NTHZ values were obtained as indicated in Table I. These samples were not confirmed by GC–MS because the amount of sample was inadequate. However, after analysis by the direct extraction chromatographic method, combined extracts of a single bacon (sample 8) and combined extracts of several bacon samples (sample 9) were confirmed for NTHZ by GC–MS. Therefore, NTHZ was shown to be present in extracts from fried bacon analyzed by both of the analytical methods. Krull et al. (1978) discussed a number of experiments that can be employed to determine if nitrosamines were artifactually formed by the analytical method. These experiments were performed on the new direct extraction

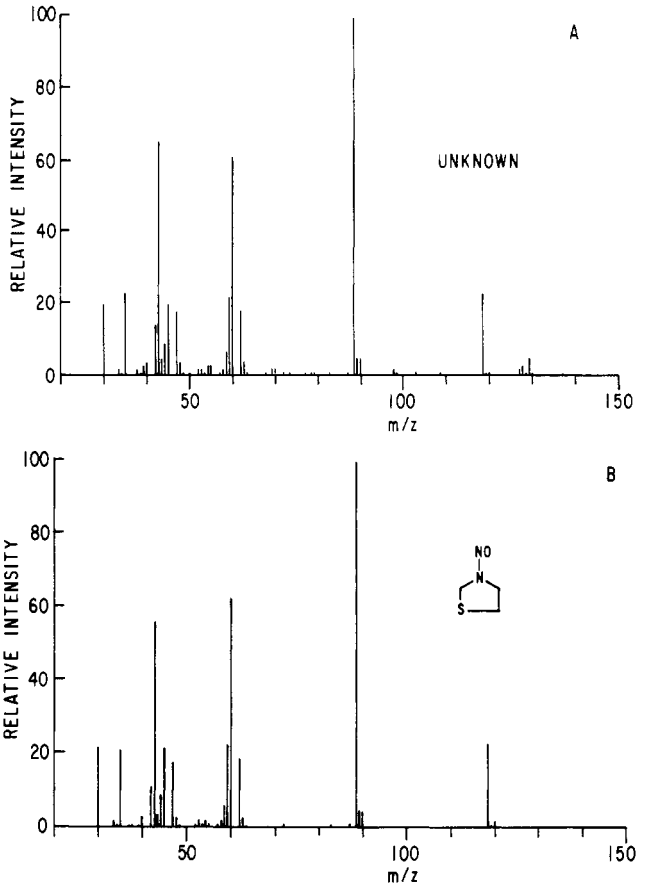

Figure 2. Mass spectra of NTHZ isolated from fried bacon (A) and the NTHZ standard (B).

column chromatographic and mineral oil distillation methods. Addition of 100 ppm of sodium nitrite and/or 1000 ppm of cysteamine or 100 ppm of thiazolidine to fried nitrite-free bacon prior to analysis by this column method yielded no detectable levels of NTHZ. All of the above reactants yielded detectable levels of NTHZ when analysis was performed by the mineral oil distillation method with approximately 45 and 450 ppb of NTHZ detected when 100 ppm of thiazolidine and a mixture of 100 ppm of sodium nitrite and 1000 ppm of cysteamine, respectively, were added to the nitrite-free bacon prior to analysis. Addition of 100 ppm of thiazolidine to fried commercial processed bacon containing 42 ppm of residual nitrite prior to analysis by the mineral oil distillation and the new column chromatographic methods yielded 2500 and 12 ppb of NTHZ, respectively. These two analytical methods gave 10 and 5 ppb of NTHZ, respectively, on this bacon sample without thiazolidine addition. These results suggest that most, but not all, of the NTHZ found in fried bacon reported in Table I was artifactually formed as a result of the mineral oil distillation method, which has the potential to form nitrosamines from their precursors when heated to high temperatures. The artifactual production of NPYR and of NDMA and NPYR in fried bacon as a result of the mineral oil distillation method has been reported by Pensabene et al. (1982) and Hotchkiss et al. (1980), respectively. The column chromatographic–TEA method yields recoveries of approximately 90% in samples fortified with 10 ppb of NTHZ or its analogue *N*-nitrosothiomorpholine internal standard (Pensabene and Fiddler, 1982).

Thus far, no report has appeared on the carcinogenic properties of NTHZ in laboratory animals. However, NTHZ is of interest since it is reported to be a direct-acting mutagen according to the Ames *Salmonella* test (Mihara

and Shibamoto, 1980; Sekizawa and Shibamoto, 1980).

The presence of NTHZ in fried bacon is not altogether unexpected. Thiazolidine, the parent amine, has not been reported in foods; however, it has been obtained by a cysteamine–D-glucose–water Browning model system reaction (Sakaguchi and Shibamoto, 1978). The expected precursors for thiazolidine formation, cysteamine, and formaldehyde should be present in bacon. Cysteamine can result from the decarboxylation of cysteine and formaldehyde by the fragmentation of endogenous or added sugar and to a lesser extent the oxidation of pork lipids, both of which are known to produce carbonyl compounds. Nitrite used for curing could serve as the nitrosating precursor for NTHZ formation. The presence of structurally related *N*-nitroso-5-methyl-1,3-oxazolidine has been reported in one brand of cooling, cutting, and hydraulic fluid (Stephany et al., 1978). The nitrosamine was presumably formed by a similar reaction to that of NTHZ, in which 1-amino-2-propanol instead of cysteamine reacted with formaldehyde and nitrite.

In conclusion, we have evidence that the NTHZ is present in fried bacon, a product intentionally browned for consumption. However, most of the nitrosamine found in the mineral oil distillation method was artifactually formed when residual nitrite was present in the sample prior to analysis.

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Phytotoxic Compounds from *Melilotus alba* (White Sweet Clover) and Isolation and Identification of Two New Flavonoids

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Coumarin derivatives and flavonoids from a hot MeOH extract of white sweet clover flowers (*Melilotus alba*) inhibited the growth of tomato and radish seedling roots. Studies indicate that ortho-substituted hydroxyphenyl aliphatic acids caused greater root growth inhibition than the corresponding para-substituted isomers and that the root growth inhibition increased as the length of the aliphatic chain increased. Blockage of the *o*-hydroxyl group eliminates the phytotoxic properties. Two new flavonoids were isolated: kaempferol 3-*O*-galactosyl-(1→6)-glucoside 7-*O*-rhamnorhamnoside (melitin) and quercetin 3-rhamnosyl-(1→6)-galactoside 7-*O*-rhamnoside (clovin). These compounds were characterized from MS, UV, ^{1}H NMR, and ^{13}C NMR spectra along with enzymatic hydrolysis. These flavonoids showed little phytotoxicity.

Previous chemical studies on *Melilotus alba* (white sweet clover) have dealt with coumarins and sugar derivatives of coumarins. Haskins and Gorz (1959) and Kosuge (1961) found that bound coumarin was present as coumarinic acid β-glucoside. Haskins and Gorz (1961) observed that the conversion of *trans*-coumarins to the cis form was photochemical rather than enzymatic. Huisman and Kosuge (1970) showed that coumarin and related compounds occur primarily as β-D-glucosides. He further demonstrated that in the reduction of *o*-(β-D-glucosyl)cinnamic acid to the corresponding hydrocinnamic acid the glucose remains attached, indicating free coumarin was not an important intermediate. Blaim and Preszlakowska (1969) analyzed the seed of sweet clover for glycosides and found evidence of a saponin. Torck et al. (1971) found robinin and assumed the presence of quercetin 3-rhamnogalactoside from sweet clover flowers. They tested these flavonoids against 14 bacteria and found the kaempferol showed the greatest antibacterial activity.

The phytotoxic properties of the water extract of sweet clover have been tested on the germination and growth of corn by McCalla and Duley (1948), who found variations

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