RESEARCH PAPERS

Survey of Fluid and Nonfat Dry Milks for N-Nitrosamines[1,2]

L. LAKRITZ and J. W. PENSABENE
Eastern Regional Research Center[3]
Philadelphia, PA 19118

ABSTRACT

A limited survey of milk and dairy products was undertaken to determine the presence of volatile N-nitrosamines, a class of compounds carcinogenic to a broad range of animal species. Nitrosamines were detected and quantitated by a gas liquid chromatograph interfaced with a Thermal Energy Analyzer. Detectable N-nitrosodimethylamine, the only nitrosamine found, ranged from .05 ppb for whole milk to .30 ppb for nonfat dry milk. No N-nitrosodimethylamine was detected in raw milk, whereas 11 of 21 samples of pasteurized milk contained apparent N-nitrosodimethylamine at a mean .10 ppb. N-nitrosodimethylamine also was detected, but not confirmed by mass spectrometry, in evaporated and condensed milk with means .32 ppb and .58 ppb and in 9 of 10 nonfat dry milk samples with a mean of 1.69 ppb. Upon reconstitution condensed milk and nonfat dry milk contained N-nitrosodimethylamine comparable to that in pasteurized milk. No N-nitrosodimethylamine was detected in cultured milk products such as buttermilk and yogurt.

INTRODUCTION

Nitrosamines have been found in a variety of foods including dairy products (7). Newell and Sisken (13) detected N-nitrosodimethylamine (NDMA) in raw milk at an average 1.2 ppb, and El-Aaser et al. (3) found unspecified and unconfirmed nitrosamines in two of ten milk samples at 3.2 and 9.7 ppb (calculated as N-nitrosopyrrolidine). Nitrosamines, particularly NDMA, have been detected in trace quantitites in cheese, notably European products, where nitrate is added to prevent "late blowing". In the manufacture of Gouda and Edam cheeses the addition of 150 mg $NaNO_3$/liter of cheese milk is considered essential for effective inhibition of Clostridia (5).

Nitrosamines in food have generated concern because this class of compounds is carcinogenic in a broad range of animal species (10). Nitrosamines are formed principally by the reaction of secondary amines with nitrite, and both these constituents are in milk. A number of amines, including dimethylamine (DMA), have been identified in cheese and milk (17). While concentrations of nitrite and nitrate reported in liquid milk have been low (<1 ppm), they have been higher in milk as a result of the introduction of nitrogen oxides during the drying process (11). The use of direct-dried malt in the brewing of beer has been implicated as the probable source of nitrosamines in that product (15). A previous examination of the effects of direct and indirect drying of nonfat dried milk on the possible formation of nitrosamines did not demonstrate their presence; however, the minimum detectable in that study was 10 ppb (14). We are reporting a survey for nitrosamines in fresh milk, other fluid milk products, and dried milk with a procedure that can detect nitrosamines below .5 ppb.

MATERIALS AND METHODS

Sample Preparation

Fluid Milk. A 100-g milk sample (whole, low fat, skim, buttermilk, or yogurt selected at random from stores) was placed in a 1-liter single neck, round bottom flask. An internal standard containing .25 μg/ml N-nitrosomethylethylamine (NMEA) and N-nitrosohexamethyleneimine (NHMI), equivalent to 2.5 ppb, were added. After addition of 50 ml of 5 N

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[1] NOTE: Precaution should be exercised in the handling of nitrosamines since they are potential carcinogens.

[2] Reference to brand or firm name does not constitute endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

[3] Agricultural Research, Science and Education Administration, US Department of Agriculture.

NaOH and 8 g $Ba(OH)_2$, the sample was distilled. The aqueous distillate was collected, 15 g of NaCl and 10 ml of 6 N HCl were added, and the reagents were extracted three times with 125 ml of dichloromethane (DCM). The combined DCM extracts were washed with 25 ml of 5 N NaOH, dried by passage through anhydrous Na_2SO_4, and concentrated to 1.0 ml in a Kuderna-Danish apparatus on a steam bath. The mean recoveries of the internal standards were 94 ± 7% for NMEA and 86 ± 7% for NHMI.

Evaporated/Condensed Milk

A 25-g milk sample was diluted with 25 g of distilled water and spiked with the previously described internal standards. The sample then was treated the same as fluid milk, except that the aqueous distillate was extracted three times with 100 ml of DCM. The mean recoveries of the internal nitrosamine standards were 96 ± 6% for NMEA and 93 ± 7% for NHMI.

Instant Nonfat Dry Milk

Ten grams instant nonfat dry milk (NDM) were dissolved in 90 g water and treated identically to the evaporated and sweetened condensed milk samples after dilution. Average recoveries of the internal nitrosamine standards were 104 ± 9% for NMEA and 89 ± 8% for NHMI.

Nitrosamine Determination

The concentrations of volatile nitrosamines were determined quantitatively by gas-liquid chromatography (GLC)-Thermal Energy Analyzer (TEA) under conditions similar to those described by Fine et al. (4), except that a −117°C liquid nitrogen-ethanol slurry cold trap was employed. The Thermal Energy Analyzer is a chemiluminescent detector specific for nitrogen oxide liberated by the pyrolysis of nitrosamines, thereby being selective for this class of compounds. The minimum detectable NDMA was .05 ppb for whole milk, .10 ppb for evaporated/condensed milk, and .3 ppb for nonfat dry milk powder. No attempt was made to confirm the NDMA in any of the samples by mass spectrometry. We have been unable to confirm concentrations of nitrosamines < 5 ppb because of insufficient concentrations in the sample size or because of the presence of interfering background components. Therefore, the NDMA reported herein can be considered only apparent or presumed.

To preclude the possibility of nitrosamine formation as a result of the analytical procedure, experiments were conducted in which 10 ppm nitrosamine-free piperidine was added to a number of the samples prior to analysis. No corresponding N-nitrosopiperdine was detected.

RESULTS AND DISCUSSION

Concentrations of apparent NDMA in fresh pasteurized whole milk, other fluid milk products, and nonfat dry milk are in Table 1. In 21 samples of pasteurized milk (whole,

TABLE 1. Volatile nitrosamines in milk and milk-derived products.

Product	No. samples positive/total	Apparent N-nitrosodimethylamine (ppb)	
		Range	Mean[a]
Raw	0/3	ND[b]	....
Whole (pasteurized)	3/10	.05–.17	.10
Low fat (pasteurized)	3/5	.05–.18	.09
Skim (pasteurized)	5/6	.05–.17	.10
Evaporated	7/10	.11–.48	.32 (.16)[c]
Sweetened condensed	2/2	.56–.60	.58 (.26)[c]
Nonfat dry milk	9/10	.45–4.2	1.69 (.17)[c]
Buttermilk	0/5	ND	....
Yogurt	0/3	ND	....

[a]Positive samples.

[b]ND, none detected.

[c]After reconstitution.

low fat, skim), 11 contained NDMA in a range of .05 to .18 ppb. No other volatile nitrosamines were detected. Three samples of fresh raw milk were assayed to determine whether nitrosamines were present prior to pasteurization; none were detected.

Formation of nitrosamines is dependent on the amine and nitrite concentrations, the pH, and temperature. Several amines have been identified in whole and evaporated milk including methyl-, dimethyl-, trimethyl-, ethyl-, and butylamine, pyrrolidine, piperidine, and morpholine (1, 12, 16, 17). Scant quantitative data are available on these amines. The concentration of dimethylamine in milk is normally in the range of 1 ppm or less. However, dietary factors can influence the amine composition of milk. Mehta et al. (12) found up to 57 ppm methylamine in milk from cows which were on a pasture of winter wheat for 8 days. The concentration of amines also increases with the removal of water, as in evaporated and powdered milk. Singer and Lijinsky (16) reported .2 ppm dimethylamine in whole milk and 3 ppm in evaporated milk. Kawamura et al. (8) found two and four times more secondary amines, calculated as DMA, in condensed and modified milk powder, respectively, than in fluid milk. In our tests of seven brands of canned evaporated and sweetened condensed milk, 9 of 12 samples yielded higher NDMA than did fluid milk, with means .32, and .58 ppb vs. .10 ppb in the pasteurized milk samples containing NDMA. This slight increase may be from the higher temperatures and longer heating times in the preparation of evaporated and sweetened condensed milks. In 10 nonfat dry milk samples, 9 contained .45 to 4.2 ppb NDMA, dry weight, with a mean concentration of 1.69 ppb NDMA. This was higher than the mean concentration in other fluid milk products tested.

Two of the dried milk powders containing the highest NDMA, 2.0 and 4.2 ppb, were subjected to ultraviolet photolysis by the procedure described by Doerr and Fiddler (2). The TEA responsive peak having the GLC retention time coincident with a NDMA standard disappeared, suggesting the peak was actually a nitrosamine. In the production of nonfat dry milk, the elevated temperatures could favor degradation of phospholipids to yield higher precursor amines, and direct gas-drying introduces nitrogen oxides which can serve as nitrosating agents. Our results suggest that nitrosamine formation does not occur to a great extent. In addition to the samples in Table 1, two samples of powdered goats' milk also were tested; no NDMA was detected in one sample, and .11 ppb was detected in the other.

Low pH (3 to 4) favors nitrosamine formation; therefore, it is possible that cultured milk products, pH ~4.4, may contain higher nitrosamines than milk. Five different brands of buttermilk and three samples of plain cultured yogurt were analyzed with no nitrosamines detected. The possibility of nitrosamines in these products cannot be excluded, because these compounds may form and then degrade during the fermentation, as hypothesized by others (6, 9).

In conclusion, milk products were tested for volatile nitrosamines by a procedure with lower limit of detection for NDMA ranging between .05 and .3 ppb. Only nonfat dried milk contained apparent N-nitrosodimethylamine greater than 1 ppb. When the recommended dilution of 1:10 was taken into account, the nitrosamine concentrations were comparable to those in fluid pasteurized milk.

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