Nitrate, Nitrite, and Volatile Nitrosamines in Whey-Containing Food Products

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The levels of nitrate, nitrite, and volatile nitrosamines in whey-containing food products were determined in 231 samples purchased at retail outlets of Belo Horizonte, Minas Gerais, Brazil. Nitrite was determined spectrophotometrically and nitrate after reduction to nitrite with spongy cadmium. Volatile nitrosamines were determined by vacuum distillation followed by gas chromatography interfaced with a thermal energy analyzer. Every analyzed sample contained nitrate, with levels ranging from 4.9 to 1250 mg/kg. Sixty percent of the samples showed detectable levels of nitrite that varied from 1.1 to 4.6 mg/kg. The levels of nitrate detected in one serving of some of the products exceeded the acceptable daily intake recommended by WHO for children of less than 10 kg body weight. *N*-Nitrosodimethylamine and *N*-nitrosopyrrolidine were present at very low levels in 44% of the food samples, and *N*-nitrosopiperidine was present in one sample.

Keywords: *Whey; nitrate; nitrite; nitrosamine*

INTRODUCTION

The use of nitrate in the manufacture of certain types of Brazilian cheese has been considered a simple and cost effective way to prevent fermentative reactions in cheese by the butyric acid bacteria *Clostridium tyrobutyricum, Clostridium butyricum*, and *Clostridium sporogenes* (Gray et al., 1979; Moraes, 1981; Abreu et al., 1986). As a consequence, Brazilian cheesemakers are allowed to add 20 g of nitrate/100 L of milk in the manufacture of certain types of cheese (Brazil, 1991). Dutch and Canadian cheesemakers are also permitted to add 15 and 20 g of nitrate/100 L of milk, respectively, in the manufacture of certain cheese (Gray et al., 1979).

The practice of adding nitrate to cheese milk is sometimes criticized on the basis that it may cause a health hazard. The ingestion of 8–15 g of nitrate can cause severe gastroenteritis with abdominal pain, blood in stool and urine, weakness, and collapse. Chronic ingestion of smaller doses can cause dyspepsia, mental depression, and headache (Magee, 1983). Nitrate can also be reduced to nitrite *in vivo*. Nitrite can interact with hemoglobin, forming methemoglobin by oxidation of ferrous iron to the ferric state, preventing or reducing the ability of blood to transport oxygen, a condition described as methemoglobinemia (Phillips, 1971; Tannenbaum, 1984; Bruning-Fann and Kaneene, 1993; Jones, 1993). Nitrite is also involved in the formation of nitrosamines, compounds known to be carcinogenic in experimental animals. Some of them are also mutagenic, embryopathic, and teratogenic (Gray et al., 1979). Children might be especially susceptible to the toxic effect of these compounds as they have low body weight, immature enzymatic systems, and lower gastric acidity.

Levels of nitrate and nitrite in cheese have been well documented (Przybylowski et al., 1975; Goodhead et al., 1976; Abreu et al., 1986). Goodhead et al. (1976) detected nitrate levels of 56 mg/kg in Gouda cheese immediately after it was prepared from milk containing 15 g of nitrate/100 L. After 6 weeks of storage, nitrate levels had decreased to 30 mg/kg. The nitrite levels were very low; a maximum of 1 mg/kg was detected.

According to Devoyod (1976), Arora (1980), and Abreu et al. (1986), cheese itself should not be a potential risk since 67% of the nitrate and nitrite added to cheese milk are leached out into the whey. The whey, therefore, could constitute a greater potential for health risk, especially due to the fact that it is concentrated and spray-dried which could lead to increased nitrate and nitrite levels and also to the formation of nitrosamines.

The use of cheese whey as an ingredient in processed foods has increased significantly in the past two decades (Vitti, 1981). Therefore, special attention should be directed toward food products for children containing cheese whey as an ingredient (Devoyod, 1976; Woollard, 1986). The purpose of this study was to survey levels of nitrate, nitrite, and volatile nitrosamines in whey-containing food products.

MATERIALS AND METHODS

Reagents and Samples. All chemicals were of analytical grade. Samples of whey-containing food products (total of 231) were purchased in retail outlets of Belo Horizonte, Minas Gerais, Brazil. The food products included powdered modified milk (containing 55% demineralized cheese whey), non-fat powdered milk (containing 10% cheese whey), seven different brands of chocolate-flavored and one of strawberry-flavored instant drink mixes, and a dairy beverage. Three samples were obtained from seven different production lots and analyzed in triplicate.

Nitrate and Nitrite. Determination of nitrate and nitrite was performed according to the recommendations of AFNOR (1974). The ions were extracted from the sample at 60 °C with a solution of borax. Turbid extracts were cleared with potassium ferrocyanide and zinc acetate. Nitrite was determined directly and nitrate after reduction to nitrite with spongy cadmium (El Assaf et al., 1982). For the color formation, nitrite was reacted with sulfanilic acid and naphthylethylene-

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Table 1. Levels of Nitrate and Nitrite in Whey-Containing Food Products

		nitrate levels[a] (mg/kg)			nitrite levels[a] (mg/kg)		
product	no. of samples analyzed	no. of samples positive	range	mean	no. of samples positive	range	mean
powdered modified milk	21	21	7.2–10.8	9.0 ± 1.4	2	nd–1.1	1.1 ± 0.0
powdered non-fat milk	21	21	28.9–102	52.0 ± 26.0	9	nd–4.6	2.5 ± 1.7
dairy beverage	21	21	4.9–9.0	7.3 ± 1.1	0	nd	–
strawberry-flavored instant mix	21	21	39.5–935	333 ± 354	17	nd–1.5	1.3 ± 0.2
chocolate-flavored instant mix							
brand A	21	21	54.2–717	253 ± 239	21	1.1–1.9	1.4 ± 0.2
brand B	21	21	32.3–1250	530 ± 464	15	nd–1.6	1.2 ± 0.2
brand C	21	21	39.5–717	378 ± 273	7	nd–1.3	1.2 ± 0.1
brand D	21	21	28.9–60.9	38.4 ± 9.4	6	nd–1.5	1.3 ± 0.2
brand E	21	21	75.6–867	343 ± 305	21	1.3–2.0	1.7 ± 0.2
brand F	21	21	38.5–717	244 ± 298	18	nd–1.9	1.5 ± 0.2
brand G	21	21	244–782	532 ± 159	21	1.1–2.0	1.5 ± 0.3
total	231	231			137		

[a] Mean ± standard deviation. Results are expressed as potassium nitrate and sodium nitrite. nd, not detected (<1.6 mg/kg for nitrate; <1.1 mg/kg for nitrite).

diamine shortly before detection at 538 nm. Levels of sodium nitrite were calculated by using the standard curve: concentration = $-0.0014 + 0.0112X$ ($r^2 = 0.999$). Levels of potassium nitrate were calculated by multiplying sodium nitrite levels (from the reduction of nitrate) by the factor 1.4638, which corrects for the different salts used.

Volatile Nitrosamines. Volatile nitrosamines were extracted by vacuum distillation as described by Scanlan et al. (1982), except that 50 g of sample and 80 mL of mineral oil were used. The distillate extracts were quantitatively analyzed on a gas chromatograph (GC) interfaced with a thermal energy analyzer (TEA). Identification and quantification of the nitrosamines were done by injecting known amounts of nitrosamine standards containing *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodipropylamine (NDPA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP), and *N*-nitrosopyrrolidine (NPYR). NDPA was used as the internal standard and was added to the samples before extraction. The standard solution was also used to calibrate the instrument (Scanlan et al., 1982).

GC-TEA conditions were as follows: column: 6 ft × 6 mm i.d. packed with 15% Carbowax 20 M–terephthalic acid (TPA) on Chrom P 60–80 mesh; column temperature, 120 °C/10 min, increase to 180 °C at 4 °C/min, and keep at 180 °C for 30 min; injection port temperature, 190 °C; carrier gas, He at 30 mL/min; TEA furnace temperature, 475 °C; vacuum with oxygen, 1.0 Torr; trap temperature, −150 °C.

Safety. Nitrosamines are carcinogens in many animals species, and extreme care should be exercised in handling these compounds.

RESULTS AND DISCUSSION

Levels of Nitrate and Nitrite. Among the samples analyzed, all of them contained nitrate at levels that varied from 4.9 to 1250 mg/kg expressed as potassium nitrate, and 60% of them contained nitrite at levels ranging from 1.1 to 4.6 mg/kg expressed as sodium nitrite (Table 1). The relatively large variability in nitrate and nitrite mean levels in the food products was due to significant difference ($p \leq 0.05$, Student–Newman–Keuls test) among the different production lots (data not shown). It was observed that nitrate occurred in much larger amounts than nitrite, which is consistent with the relative stability of these two ions.

On the basis of a review of the ingredients present in the analyzed food samples, whey is most likely the main source of nitrate and nitrite. In fact, some commercially available samples of dehydrated cheese whey were analyzed and observed to contain levels of nitrate and nitrite varying from 644 to 937 and from 1.1 to 1.3 mg/kg, respectively. Abreu et al. (1986) also detected nitrate levels of 143.4 mg/L of whey from the processing of Prato cheese from milk containing 20 g of nitrate/100 L. Sen and Lee (1979) observed that whey powders may contain up to 1760 ppm levels of sodium nitrate.

Table 2. Levels of Nitrate and Nitrite in One Serving of Whey-Containing Food Products

	levels (mg) per serving[a]	
product	nitrate	nitrite
powdered modified milk	0.3	tr
powdered non-fat milk	2.6	0.1
dairy beverage	9.0	tr
strawberry-flavored instant mix	29.1	0.1
chocolate-flavored instant mix		
brand A	21.7	0.1
brand B	51.9	0.1
brand C	28.7	0.1
brand D	2.5	0.1
brand E	34.7	0.1
brand F	29.4	0.1
brand G	31.3	0.1

[a] Nitrate and nitrite levels per serving (200 mL) were calculated from levels of these ions in the dry product, according to directions on the label of each product. tr, trace.

According to the World Health Organization, the acceptable daily intake (ADI) values for nitrate and nitrite are 5.0 and 0.2 mg/kg of body weight, respectively (WHO, 1978). To evaluate the risk associated with the presence of these ions in the investigated food products, nitrate and nitrite levels in the dry products were used to calculate nitrate and nitrite levels if products were reconstituted according to directions on the label (Table 2) and compared to the ADI.

The levels of nitrite in the reconstituted products were low. However, nitrate levels were high in some of the products.

Powdered modified milk is a formula type product intended for use by infants. After reconstitution, one serving was calculated to have 0.3 mg of nitrate. Considering the number of servings consumed per day according to body weight, total daily ingestion would be below the ADI value established by WHO.

The consumption of powdered non-fat milk and dairy beverage would not be a concern even if very young children consumed them. However, when considering the flavored instant drink mixes, health concerns are pertinent. For instance, if children of up to 10 kg of body weight consume one serving of the product with the highest nitrate level, they would be exceeding the ADI value. Children of 15 and 20 kg of body weight consuming one serving of this same product would be ingesting 70 and 52%, respectively, of the ADI. Furthermore, the consumption of two or more servings of this product or one serving of it along with other

Table 3. Levels of Nitrosamines in Whey-Containing Food Products

product	nitrosamine levels[a] (μg/kg)		
	NDMA	NPIP	NPYR
powdered modified milk	nd	nd	nd
powdered non-fat milk	nd	nd	nd
strawberry-flavored instant mix	nd	nd	nd
chocolate-flavored instant mix			
brand A	0.05	nd	0.08
brand B	nd	nd	nd
brand C	0.05	nd	0.62
brand D	nd	nd	nd
brand E	0.18	0.52	0.93
brand F	0.05	nd	0.43

[a] Two samples of each product were analyzed. nd, not detected (<0.05 μg/kg for NDMA; <0.08 μg/kg for NPIP and NPYR).

significant sources of nitrate in the diet (such as vegetables or even some water sources) could have the daily intake increased to levels above the ADI values recommended by WHO.

Levels of Volatile Nitrosamines. Nitrosamines were present in 44% of the samples analyzed (Table 3). NDMA and NPYR were present in 44% of the samples, while NPIP was detected in one of the samples. However, the levels detected were very low, below those that can be confirmed by mass spectrometry. It should be recognized that the levels of volatile nitrosamines in Table 3 would become considerably lower when the product is diluted for consumption.

All of the nitrosamines detected in this study have also been found in other dairy products (Elgersma et al., 1978; Stephany et al., 1978; McIntyre and Scanlan, 1993). According to Weihrauch and Schwartz (1974) several secondary amines, such as dimethylamine, pyrrolidine, and piperidine, have been found in whey. Therefore, it is likely that the limiting factor in nitrosamine formation is the amount of nitrite, which is low.

No correlation could be established between nitrosamine levels and nitrate or nitrite levels or the presence of any other specific ingredient in the food products. Similar results were observed by Stephany et al. (1978) in various types of Dutch cheese.

Although NDMA has been found to be a potent carcinogen in animal feeding studies, the hazard posed to humans by ingestion of foods containing trace (ppb) levels is unknown. Therefore, until more is known about the effects of exposure to very low doses, it seems prudent to minimize human exposure to *N*-nitroso compounds (Libbey et al., 1979).

Cheesemakers who add nitrate and/or nitrite to cheese milk need to be aware that subsequently generated whey probably contains undesirable compounds compared to cheesemaking without these additives. To overcome this problem, there are at least two alternatives. One approach would be elimination of nitrate and nitrite from cheese whey by using techniques such as demineralization. Another possibility would be to replace the use of nitrate by nisin or nisin-producing starters, lysozyme, hydrogen peroxide, or techniques such as salting of the curd or bactofugation (Gray et al., 1979; Conner, 1993).

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