Influence of Nitrate Levels Added to Cheesemilk on Nitrate, Nitrite, and Volatile Nitrosamine Contents in Gruyere Cheese

M. Beatriz A. Glória,[†] Silvana R. Vale,[†] Otacílio L. Vargas,[‡] James F. Barbour,[§] and Richard A. Scanlan*,[§]

Departamento de Alimentos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Av. Olegário Maciel 2360, Belo Horizonte, Minas Gerais, Brazil 30180-112, Instituto de Laticínios Cândido Tostes, Epamig, Juiz de Fora, Minas Gerais, Brazil 36045-560, and Department of Food Science and Technology, Oregon State University, Corvallis, Oregon 97330

The influence of different nitrate levels added to cheesemilk on residual nitrate, nitrite, and volatile nitrosamine contents in cheese was investigated. Nitrate levels of 0 (control), 20, 80, and 160 g/100 L were added to cheesemilk. Gruyere cheese was manufactured, ripened, and analyzed for nitrate, nitrite, and nitrosamines. Nitrite was determined spectrophotometrically and nitrate after reduction to nitrite with spongy cadmium. Volatile nitrosamines were recovered by vacuum distillation and determined by gas chromatography/thermal energy analysis. Nitrate levels in the cheese increased with the amount of nitrate added to cheesemilk. No correlation was observed between added nitrate and residual nitrite levels. Positive correlation was observed between the level of nitrate added to cheesemilk and the levels of *N*-nitrosodimethylamine and *N*-nitrosodiethylamine in the cheese.

Keywords: *Gruyere cheese; nitrate; nitrite; nitrosamine*

INTRODUCTION

The practice of adding potassium or sodium nitrate in the manufacture of certain types of cheeses has been considered one of the most successful methods of preventing late blowing and gassy defects in cheese (Gray *et al*., 1979; Moraes, 1981; Zerfiridis and Manolkidis, 1981; Abreu *et al*., 1986). As a consequence, Dutch cheesemakers are allowed to add 15 g of nitrate per 100 L of milk. Brazilian, Canadian, and Danish regulations permit the use of 20 g of nitrate per 100 L of milk in the manufacture of certain cheeses (Gray *et al*., 1979; Government of Brazil, 1991).

Levels of nitrate and nitrite in cheese have been well documented (Przybylowski *et al*., 1975; Goodhead *et al*., 1976; Zerfiridis and Manolkidis, 1981; Abreu *et al*, 1986). Goodhead *et al*. (1976) detected nitrate levels of 5.6 mg/100 g in Gouda cheese immediately after it was manufactured from milk containing 15 g of nitrate/100 L. After 6 weeks of storage, nitrate levels had decreased to 3.0 mg/100 g. According to Munksgaard and Werner (1987), nitrate in cheese is reduced to nitrite by xanthine oxidase present in milk or by nitrate reductase produced by microorganisms. In cheese, nitrite levels are very low; a maximum of 0.1 mg/100 g has been detected. Nitrite is very unstable, being quickly reduced to other compounds (Hotchkiss, 1989a).

A number of volatile nitrosamines have been reported to occur sporadically at low levels in cheese. Although they have not been found in all cheeses analyzed, *N*-nitrosodimethylamine (NDMA) and *N*-nitrosodiethylamine (NDEA) are most commonly found (Sen *et al*., 1978; Stephany *et al*., 1978; Gray *et al*., 1979; Osterdahl, 1988; Ellen, 1990; Scanlan, 1995). Reports have been contradictory on the relationship between nitrate added to cheesemilk and nitrosamine content in cheese (Goodhead *et al*., 1976; Gough *et al*., 1977; Elgersma *et al*., 1978) as nitrosamines have been found also in cheeses to which no nitrate was added (Sen *et al*., 1978; Gough *et al*., 1977; Zerfiridis and Manolkidis, 1981). Undoubtedly some of the attempts to correlate nitrosamine formation with nitrate addition were confounded by lack of sensitivity in nitrosamine analysis at the time those studies were conducted (Crosby *et al*., 1972). For instance, a comprehensive investigation (Goodhead *et al*., 1976) in which Gouda cheese manufactured with various amounts of nitrate failed to establish a relationship between nitrate addition and nitrosamine formation, but the nitrosamine detection limit was 1 μg/kg at that time. Gray *et al*. (1979) have discussed extensively the problems associated with lack of analytical sensitivity and nitrosamine detection in cheese.

The purpose of this study, therefore, was to investigate NDMA and NDEA formation using a relatively sensitive nitrosamine detection system (0.05 μg/kg detection limit) in Gruyere cheese manufactured with different levels of nitrate.

MATERIALS AND METHODS

Cheese Manufacture. Gruyere cheese was manufactured at the Instituto de Laticínios Cândido Tostes in Juiz de Fora, Minas Gerais, Brazil. Sodium nitrate levels of 0 (control), 20, 80, and 160 g/100 L were added to cheesemilk. Higher than legal amounts of nitrate (20 g/100 L) were deliberately used to help elucidate the role of nitrate in nitrosamine formation. Each experiment was performed in triplicate, and two samples were taken from each production lot. The sodium nitrate was prepared by standard synthesis from sodium hydroxide and nitric acid.

Nitrate and Nitrite. Determination of nitrate and nitrite was performed according to an AFNOR (1974) method. 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 naphthylethylenediamine shortly

* Author to whom correspondence should be addressed.

[†] Universidade Federal de Minas Gerais.

[‡] Instituto de Laticínios Cândido Tostes.

[§] Oregon State University.

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Table 1. Levels[a] of Nitrate, Nitrite, and Volatile Nitrosamines in Gruyere Cheese Manufactured from Milk with Different Levels of Sodium Nitrate

$NaNO_3$ added (g/100 L) to cheesemilk	levels[b] (mg/100 g) in cheese		nitrosamine levels[c] (μg/kg) in cheese	
	KNO_3	$NaNO_2$	NDMA	NDEA
0	0.43 ± 0.05	0.04 ± 0.02	0.09 ± 0.01	0.04 ± 0.01
20	1.33 ± 0.88	0.09 ± 0.06	0.11 ± 0.06	0.13 ± 0.03
80	11.04 ± 0.13	0.10 ± 0.07	0.25 ± 0.02	0.23 ± 0.04
160	20.80 ± 2.42	0.03 ± 0.01	0.34 ± 0.05	0.38 ± 0.05

[a] Mean value ± standard deviation. [b] Values are means of six determinations. [c] Values are means of three determinations.

before detection at 538 nm. Levels of sodium nitrite were calculated by using the standard curve equation $Y = -0.0014 + 0.0112X$ ($r^2 = 0.999$; Y = absorbance at 538 nm; X = concentration). 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.

Volatile Nitrosamines. Volatile nitrosamines were extracted by vacuum distillation as described by Hotchkiss *et al.* (1980), except that 50 g samples and 80 mL of mineral oil were used. This sample size allowed a detection limit of 0.05 μg/kg. Extracts of the distillates were quantitatively analyzed on a gas chromatograph (GC) interfaced with a thermal energy analyzer (TEA). Identification and quantification of the nitrosamines was accomplished by injecting known amounts of nitrosamine standards containing NDMA and NDEA. *N*-Nitrosodipropylamine was used as internal standard and was added to the samples before extraction. The standard solution was also used to calibrate the instrument. GC/TEA conditions were as follows: column, 2 m × 1.6 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 maintain 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. Precaution should be exercised in handling nitrosamines since they are potent carcinogens in many animal species.

RESULTS AND DISCUSSION

Levels of Nitrate and Nitrite. Nitrate levels detected in the cheese samples are indicated in Table 1. No significant difference ($p \leq 0.05$, Tukey test) was observed on nitrate levels in cheese manufactured with 0 (control) and 20 g of nitrate in 100 L of cheesemilk. Significant difference was observed for the samples to which nitrate was added at levels >20 g/100 L. Regression analysis indicated significant correlation ($r = 0.9944$; $p \leq 0.01$) between nitrate levels added to cheesemilk and residual nitrate contents in Gruyere cheese. This result is contradictory to reports by Sen *et al.* (1978); however, in their study only levels up to 20 g of nitrate were added to 100 L of cheesemilk. Nitrate was detected in cheese to which no nitrate was added. According to Elgersma *et al.* (1978), milk contains traces of nitrate, which might explain this observation.

It was observed that nitrite occurred in much smaller amounts than nitrate, which is consistent with the relative stability of these two ions and the inherent reducing properties of the cheese (Oliveira *et al.*, 1995). Nitrite levels in the cheese samples were very low, regardless of the nitrate level added to the cheesemilk. The fate of nitrite in cheese is not clearly understood. It can be converted to nitric oxide, nitrous oxide, or nitrogen by the reducing activity of certain strains of lactobacilli (Fournaud *et al.*, 1964). It is also involved in the formation of nitrosamines (Gray *et al.*, 1979; Hotchkiss, 1989a,b).

No significant correlation ($p \leq 0.05$) was observed between residual nitrite levels and nitrate levels added to cheesemilk or nitrate detected in Gruyere cheese. Similar results were reported by Goodhead *et al.* (1976) during the analysis of Gouda cheese prepared from milk containing levels up 60 g of nitrate/100 L.

Levels of Volatile Nitrosamines. NDMA and NDEA were detected in Gruyere cheese at very low levels (Table 1). A positive correlation was observed between the level of nitrate added to cheesemilk and the levels of NDMA ($r = 0.9864$; $p \leq 0.05$) and NDEA ($r = 0.9897$; $p \leq 0.05$) detected in the cheese. Significant correlation was observed between nitrate and NDMA levels in the cheese ($r = 0.9912$; $p \leq 0.01$) and between nitrate and NDEA levels in the cheese ($r = 0.9713$; $p \leq 0.05$). Significant correlation was also observed between NDMA and NDEA levels in the cheese ($r = 0.9741$; $p \leq 0.05$).

We fully recognize that the levels of NDMA and NDEA in Table 1 are very small and that the amounts of nitrate used are higher than those allowed for addition to cheesemilk. Nevertheless, the data in Table 1 do represent a strong case for a role of nitrate in nitrosamine formation. The low levels of nitrosamines in Table 1 help explain why earlier studies using less sensitive methods for nitrosamine detection produced confounding results.

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