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Volatile N-Nitrosamines in Selected Italian Cheeses

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Volatile N-nitrosamines are a small part of the larger class of nitroso compounds, which are widespread and often carcinogenic food contaminants. Since Freund's discovery (1937) of human liver damage due to exposure to N-nitrosodimethylamine and Magee and Barnes' demonstration of the toxic and carcinogenic action of this nitrosamine in animals (1956), many studies have set up to measure environmental contamination with these molecules. Beer and meats were found to be an important exogenous source, but other foods such as fish, vegetables and dairy products also made significant contribution to the total dietary intake of N-nitrosamines (MAFF, 1987 and 1992; Gangolli, 1994). Only few research projects have dealt with the measurement of nitrosamines in cheese (Goodhead *et al.,* 1976; Gough *et al.,* 1977; Sen *et al.,* 1978 ; Elgersma *et al.,* 1978; Stephany *et al.,* 1978; Pedersen *et al.,* 1980, Mavelle *et al.,* 1991). The Italian food market has rarely been studied and no recent data are available about N-nitrosamines in cheeses in Italy (Cantafora *et al,* 1975; Cerutti *et al.,* 1975).

We measured the content of N-nitrosamines in several cheeses available in Italy, using a simultaneous distillation-extraction procedure, followed by high resolution gas chromatography-thermal energy analysis (GC-TEA) (Gavinelli *et al.,* 1986).

MATERIALS AND METHODS

Eleven types of cheese (9 Italian, 2 Dutch) were bought in Milan, Italy at local retail stores or supermarkets, either wrapped in suitable food paper, or in vacuum packs. The nitrosamine analysis was performed in duplicates on each piece of cheese after discarding the crust and the edible part immediately below it.

Standard volatile nitrosamines N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodipropylamine (NDPA), N-

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nitrosodibutylamine (NDBA), N-nitrosopiperidine (NPIP), N-nitrosopyrrolidine (NPYR), N-nitrosomorpholine (NMOR), and N-nitrosobutylpropylamine (NBPA) were purchased from Sigma Chemical Co., St. Louis, MO, USA; Tween 80 was purchased from Aldrich Chimica, Milan, Italy, and antibumping granules from BHD Italia srl, Milan. All other reagents were of the purest grade available. All reagents were tested to exclude the presence of any interfering substance or nitrosamine.

The analysis was carried out following the method described by Gavinelli *et al.* (1986 and 1988) modifying it slightly to fit the fatty matrix of cheese better. Briefly, 20 g of cheese were homogenised in a mixture containing 57 ml glycerol and enough distilled water to bring total water content (natural moisture content plus added water) to 18 g. In order to inhibit unwanted nitrosation, 1.2 g α-tocopherol, 600 mg sulfamic acid, and 5 ml 0.14 M sulfuric acid were added before homogenisation. The homogenate, 15 g sodium chloride, 0.5 ml Tween 80, antibumping granules, and 250 ng internal standard N-nitrosobutylpropylamine (NBPA) were added to a two-neck round-bottom distillation flask. The distillation flask was slowly heated in order to avoid strong foam formation, and the temperature inside the sample was kept at 130°C. The extraction chamber of the Likens and Nickerson (1964) microsteam distillator-extractor was filled with 2.5 ml distilled water and 2.5 ml dichloromethane; 5 ml dichloromethane and antibumping granules were kept boiling in the collecting tube by partially dipping it in a 50°C water-bath.
Distillation took 150 minutes and was followed by 20 minutes of solvent reflux. The dichloromethane from the collecting flask and the distillation chamber were combined; 150 µl iso-octane were added. The volume was then reduced to about 150 µl under a gentle air stream, and 2 µl of the sample were injected into the GC-TEA system. Because of the presence of known amounts of internal standard, the measurement of the exact volume of the samples could be avoided.

Recovery studies were done on Fontina cheese by adding increasing amounts (5-25 µg/kg) of the seven nitrosamines.

Analyses were carried out using a DANI 3800 gas chromatograph interfaced with a Thermal Energy Analyser (TEA 543: Thermo Electron, Waltham, MA, USA). The injector temperature was 2OO°C, while the wall-coated fused silica capillary column, CP WAX 52 CB, 25 m x 0.32 mm i.d., 1.2 mm film thickness (Chrompack Italia, Cernusco S/N) was heated from 60°C to 160°C at a rate of 25°/min, staying at 130°C for 2 min. The GC-TEA interface and pyrolyzer temperatures were 250 and 500°C respectively.

RESULTS AND DISCUSSION

Table 1 summarises the moisture and fat content and ripening age of the types of cheese considered.

Table 1. Characteristics of eleven cheeses analysed

CHEESE	MOISTURE % on fresh cheese	FAT % on dry matter	AGE
Mozzarella	55	44	3 days
Fontina	35	45	3 months
Provolone	37	44	2 months
Taleggio	46	50	1 month
Grana Padano	23	26	15 months
Parmigiano Reggiano (Parmesan cheese)	23	32	24 months
Gorgonzola	40	48	45 days
Processed cheese	55	35	
Processed cheese with added polyphosphates	55	35	
Edam	35	40	*
Gouda	40	40	*

* imported cheese, age not declared

As increasing amounts of the seven nitrosamines were added to the cheese, the percentage of recovery varied depending on the molecule considered, but it was constant over the concentration range (5-25 µg/kg) of each: NDMA: 48 ± 8% (mean ± SE); NDEA: 64 ± 12%; NDPA: 75 ± 14%; NDBA: 82 ± 14%; NPIP: 57 ± 10%; NPYR: 20 ± 4%; NMOR: 16 ± 4 %. The detection limits were ≤ 0.35 µg/kg for NDMA, ≤ 0.34 µg/kg for NDEA, ≤ 0.20 µg/kg for NDPA, ≤ 0.20 µg/kg for NDBA, ≤ 0.24 µg/kg for NPIP, ≤ 1.67 µg/kg for NPYR, ≤ 5.0 µg/kg for NMOR.

The most frequently detected nitrosamine turned out to be NDBA, which was present in five of the nine Italian cheeses, with concentrations ranging from 0.63 to 0.86 µg/kg (Table 2). NDMA was found in three of the Italian samples with concentrations of 0.38 - 0.84 µg/kg; Grana Padano showed only trace amounts of this nitrosamine (0.35 µg/kg).
The two Dutch cheeses, in which the addition of nitrate to the cheese-milk is allowed in the Netherlands, were analysed as a comparison to the Italian ones. Their content of NDBA was 1.00 and 1.72 µg/kg; NDMA had a concentration of 0.73 and 0.39 µg/kg. Edam was the only cheese in which NDPA could be detected.

The presence of NDBA in cheese has been rarely reported (Stephany *et al.,* 1978); the frequency with which we found this nitrosamine might be due to the high extraction yield of the method rather than to any contamination. Gavinelli *et al.* (1988) using the same apparatus, noticed NDBA in meat products to which nitrate and/or nitrite had been added, and demonstrated the extreme sensitivity of the method towards this nitrosamine. Groenen *et a*/. (1987) observed N-nitrosamine formation in

Table 2. N-nitrosamine content of eleven types of cheese (μg/kg).

	NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
MOZZARELLA	nd	nd	nd	nd	nd	nd	nd
FONTINA	nd	nd	nd	0.95	nd	nd	nd
PROVOLONE	nd	nd	nd	nd	nd	nd	nd
TALEGGIO	nd	nd	nd	0.63	nd	nd	nd
GRANA PADANO	0.35	nd	nd	nd	nd	nd	nd
PARMIGIANO REGGIANO (Parmesan cheese)	nd	nd	nd	nd	nd	nd	nd
GORGONZOLA	0.84	nd	nd	0.65	nd	nd	nd
PROCESSED CHEESE	0.40	nd	nd	0.64	nd	nd	nd
PROCESSED CHEESE WITH ADDED POLYPHOSPHATES	0.38	nd	nd	0.86	nd	nd	nd
EDAM	0.73	nd	1.64	1.00	nd	nd	nd
GOUDA	0.39	nd	nd	1.72	nd	nd	nd

Values are the mean of two replicates.

NDMA, N-nitrosodimethylamine; NDEA, N-nitrosodiethylamine; NDPA, N-nitrosodipropylamine; NDBA, N-nitrosodibutylamine; NPIP, N-nitrosopiperidine; NPYR, N-nitrosopyrrolidine; NMOR, N-nitrosomorpholine.

nd = not detectable (limits of sensitivity of the method: NDMA ≤ 0.35 μg/kg; NDEA 5 0..34 μg/kg; NDPA ≤ 0.20 μg/kg; NDBA ≤ 0.20 μg/kg; NPIP ≤ 0.24 ug/kg; NPYR ≤ 1.67 ug/kg; NMOR ≤ 5.0 μg/kg.

cheese during the sample preparation procedure when no nitrosation inhibitors were added; however, this artefact happened only at pH above 10. In our experiments the sample had been acidified and enough α-tocopherol was added to avoid this. We found that increasing amounts of α-tocopherol from 1.2 g did not affect the results of our analyses (data not shown).

The traces of NDMA detected in Grana Padano could have been caused by the presence of formaldehyde, a nitrosation catalyst (Mirvish, 1975) whose addition during the Grana cheese-making process was still permitted by Italian law when the analysis was run. Parmigiano

Reggiano, a very similar cheese in which formaldehyde is not allowed, showed no trace of nitrosamine.

Even if the content of the nitrosamines analysed seemed higher in the Dutch cheeses, further studies are needed to assess the correlation between the added nitrate and nitrosamine formation.

Although the nitrosamine content in cheese is low, this food contributes to the daily intake of these mutagenic and carcinogenic molecules which have been detected in all food classes. Because of the health threat of nitrosamines, it is recommendable to limit their presence in any foodstuff by avoiding the addition of their precursors nitrate and nitrite or of catalysts.

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