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N-Nitrosamine in Korean Beer

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Abstract

A total of 47 beer samples, produced in Korea, were analyzed for their N-nitrosamine levels by gas chromatography-thermal energy analyzer. N-nitrosodimethylamine(NDMA) was detected in 35 of 47 samples analyzed. The range of levels was 0~7.2μg/kg while the average was about 0.9μg/kg. The identity of NDMA was confirmed by mass spectrometry. Samples which were found only NDMA before nitrosation were detected to include N-nitrosopyrrolidine(45μg/kg) and N-nitrosomorpholine(4μg/kg) as well as NDMA (837μg/kg).

Key words: N-nitrosodimethylamine, beer, malt

INTRODUCTION

European beer has been reported to contain N-nitrosodimethylamine(NDMA) at an average concentration of 2.7μg/kg(1). Since then, NDMA, a potent carcinogen, has been found in many beers and malt. Goff and Fine(2) reported NDMA ranging from 0.4~7.0μg/kg in 18 brands of beers and found that six of seven brands of scotch wiskey contained levels between 0.3~2.3μg/kg. Sen et al.(3) analyzed the nitrosamine of serval varieties of 22 beers and ales sold in Canada, all but one contained NDMA at levels ranging from 0.4~4.9μg/kg. Scanlan et al.(4) reported the levels of NDMA found ranges from 0~14μg/kg in 25 beer samples.

Barley malt has been the primary contributor of volatile NDMA in malted beverages. The precursors and probable mechanism of NDMA formation in malt and beer were identified by Mangino and Scanlan(5), and Wainright(6). The addition of sulphur during the direct malt drying process significantly reduces the NDMA levels, resulting in decreased levels of NDMA in beer(7,8). Harvery et al.(9) indicated that NDMA levels were significantly less than those found in a previous survey, due to the institution of modification in the malt drying process by North American maltersters. NDMA levels were detected 1μg/kg in 260 beer samples, with an average.

Investigation of raw materials used in the brewing process showed that the use of direct malt driers, in which oxides of nitrogen from the flame come in contact with the malt, seem to be the main factor of NDMA formation. During the malt kilning process, nitrogen oxides formed by combustion of ambient nitrogen come into direct contact with the malt being dried. Nitrogen oxides are implicated in the nitrosation of amines in malt since Challis and Kyrtopoulos(10) showed that N_2O_3 and N_2O_4 are both extremely effective nitrosating agents when formed in neutral or alkaline solution from their component gases. The purpose of our work was to investigate the occurrence of N-nitrosamine in beer produced in Korea.

MATERIALS AND METHODS

Materials

Canned or bottled beer produced in Korea were purchased at the local retail outlets.

In the nitrosation experiments, 20g samples were treated with 0.2g $NaNO_2$ at pH 3.0 in the dark at 27°C for 3hr. The reactions were then quenched with 0.3g of ammonium sulfamate.

Chemicals

N-nitrosamine standards were obtained from Sigma Chemical Co.(St Louis, MO). All other chemical reagents used were of analytical grade. Reagent blanks were analysed from volatile N-nitrosamines and no

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interfering peaks were observed.

Analysis of N-nitrosamine

Samples were steam-distilled by a modification of Hotchkiss et al.(11) as follows: a 25g sample to which 1μg N-nitrosodipropylamine added was steam-distillated on a stream generator and 150ml distillate was collected. The distillate was acidified to pH 1 with sulphuric acid containing sulphamate to inhibit nitrosation, and a small amount of sodium chloride was added.

The distillate was extracted with dichloromethane (DCM, 3×50ml). The combined extracts were dried over anhydrous sodium sulphate, concentrated in a Kuderna-Danish apparatus, and then blown down under nitrogen to a final volume of 1.0~1.5ml. Extracts were analysed by gas chromatography(Hewlett-Packard model 5890A, Hewlett-Packard, Avondale, PA)-thermal energy analyzer(TEA, Model 543, Thermo Electron Corp. Waltham, MA).

Conditions for GC-TEA were as follows : 10ft×2mm *i.d glass column packed with* 10% Carbowax 20M/80-100 chromosorb WHP ; flow rate, 25ml/min ; oven temperature programmed, 110~170°C at 5°C/min ; *injection port temperature*, 180°C ; *pyrolizer temperature*, 550°C ; interface temperature, 220°C ; cold trap temperature, -160°C ; analyzer pressure, 1.9 torr.

GC-MS analysis for NDMA

Samples were extracted using the method described by Hotchkiss(12) and the extract was washed with 30ml pentane three times. Pooled pentane was back-washed with 15ml water twice. The pooled aqueous portions were saturated with sodium chloride and extracted with 40ml DCM three times. The DCM was dried over anhydrous sodium sulfate, concentrated to 4ml in a Kuderna-Danish concentrator, and slowly concentrated to 2ml with N_2. The extract was filtered and chromatographed(500μl injection volume ; 25cm×4.6mm CN 10μm HPLC column ; Alltech Associates ; mobile phase, DCM, 1.0ml/min). Fractions were collected at a 30sec interval and each was analyzed for NDMA. Fractions containing NDMA were pooled, slowly evaporated to 400μl, and rechromatographed on a 15cm×0.46mm Dupont silica HPLC column(5μm ; Alltech Associates) with DCM(1.0ml/min). Fractions were again collected at a 30 sec interval and analyzed by GC-TEA. Fractions containing NDMA were pooled and concentrated *under liquid* N_2 to 5~10μl for GC-MS analysis. Mass spectra were obtained on a Hewlett Packard 5970 GC-Mass Selective Detector(MSD). Separation of NDMA was *achieved on a* 25m×0.32mm Carbowax 20M capillary column with Helium. Two to three μl of concentrated extract was injected into the splitless mode. *Conditions were as follows : injector temperature* 180°C, purge delay 30sec ; oven temperature programmed(initial 40°C, 3.5min hold, rate 10°C/min to 100°C, hold for 10 *min). Scanning was over a mass range of* 25~100m/z ; scan threshold 100 ; solvent delay 3.5min.

RESULTS AND DISCUSSION

The results from analysis of 47 samples of beer are summarized in Table 1. About 75% of collected samples contained detectable levels of NDMA, the only volatile N-nitrosamine found in this study. The average amount of NDMA for canned and bottled samples were 0.8, 0.9μg/kg, and the range was ND-7.2, ND-7.0μg/kg, respectively. The levels shown in Table 1 are uncorrected for recovery, which was approximately 78.2% for NDMA, when chemiluminescence detection for the volatile N-nitrosamine was 0.05μg/kg.

A mass spectrum obtained from a beer containing 2.2μg/kg NDMA is shown in Fig. 1. a(Fig. 1. b is the spectrum of the authentic NDMA). Mass-spectral confirmation was obtained for five of the beer samples containing NDMA of 1.7, 2.2, 7.2μg/kg in canned and 3.7, 4.5μg/kg in bottled, respectively, listed in Table 1. The minimum levels of NDMA necessary for mass-spectral confirmation was 1μg/kg.

Spiegelhalder et al.(1) reported that several brands of beer available in Germany contained an average of 2.7μg/kg of NDMA. Although this level is lower than those found in fried bacon to which nitrite and nitrate are added during its processing, but overall exposure from beer could be much greater because a larger mass of beer may be consumed compared to bacon. Scanlan

Table 1. The levels of N-nitrosodimethylamine in beer produced in Korea

Type	No. of samples	NDMA levels(μg/kg)	
		Average	Range
Can	27	0.8	ND-7.2
Bottle	20	0.9	ND-7.0

ND: not detected
The values are uncorrected for recovery of NDMA

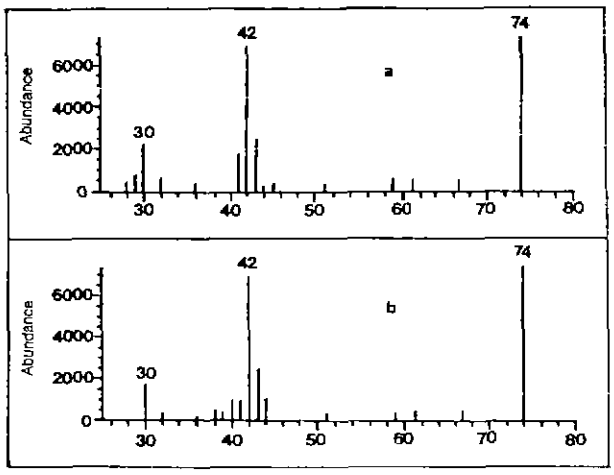

Fig. 1. Mass spectra of N-nitrosodimethylamine from (a) beer containing NDMA of 7.2μg/kg, (b) authentic compound.

et al.(4) also reported that NDMA, the only volatile nitrosamine found, was detected in 23 of 25 beers (USA) analysed. The range of levels was 0~14μg/kg while the mean 5.9μg/kg. Among the cured meats, fried bacon has received considerable attention because it consistently contains trace levels of N-nitrosopyrrolidine (NPYR). It is interesting to compare the amount of volatile nitrosamine ingested by consuming fried bacon and beer. For example, 25g of fried bacon containing 10μg/kg NPYR results in an intake of 0.25μg of NPYR. By comparison 0.95kg of beer containing 5.9μg/kg NDMA results in an intake of 5.6μg of NDMA(4). In addition, animal feeding experiments indicate that, among the volatile nitrosamines, NDMA is one of the more potent carcinogens. Archer and Wishnok(13) estimated that NDMA was six times more potent as a carcinogen than NPYR in the BD rat.

Besides volatile N-nitrosamine, many types of non-volatile N-nitroso compounds were present in malt and beer. Sen et al.(14) reported that the average levels of N-nitrosoproline(NPRO) detected in 11 malt and 28 beer samples were found to be 24.1μg/kg(range 5.6~113.3) and 1.7μg/kg(range trace~6.0), respectively. Only two samples of malt contained trace amounts(<1μg/kg) of N-nitrososarcosine(NSAR). Another report on the NPRO in malt and beer has also been published(15,16).

There are a lot of studies on the formation of N-nitrosamine during the brewing process of beer. Spiegelhalder et al.(1) speculated that the N-nitrosamine was formed during the brewing process and the malt may provide the precursor amine. Scanlan et al.(4) suggested, on the base of preliminary data, that the malt was the source of the N-nitrosamine and that it was formed during the kilning or drying process used in malt manufacture and not during the brewing process. Several investigations have confirmed this suggestion(17). Preussmann et al.(15) insisted that malt was the only source of NDMA and not the formation of N-nitrosamine during the brewing process. Harvery et al.(9) analyzed N-nitrosamine in 120 malt samples, the average NDMA levels of the domestic and imported malts were 5 and 6μg/kg, respectively. Similar results have been reported for New Zealand beer and malt(18). In conclusion, two mechanisms to reduce N-nitrosamine levels of beer may be operating simultaneously; first, the pH at the surface of the malt may be lowered, and second, sulfur oxide reduces nitrogen dioxide to the much poorer N-nitrosating agent nitric oxide. The use of sulfer dioxide has at least two disadvantages; it is itself an atmospheric pollutant and it is corrosive(17).

The results of the nitrosation experiments of bottled beer are presented in Table 2. The two samples which were found only NDMA before nitrosation were detected NDMA, NPYR and N-nitrosomorpholine (NMOR) after nitrosation. The levels of NDMA, NPYR and NMOR were 837, 45 and 4μg/kg on average, respectively. The levels of NPYR and NMOR in samples before nitrosation were not detected, in samples and NMOR after nitrosation were detected highly in the samples. These results suggest that in amines such as pyrrolidine and morpho-

Table 2. Effect of nitrosation with 0.2g sodium nitrite under acidic condition on the levels of N-nitrosamine in Korean beer

(levels, μg/kg)

Beer	Before nitrosation			After nitrosation		
	NDMA	NPYR	NMOR	NDMA	NPYR	NMOR
Sample I	0.9	ND	ND	816	47	5
Sample II	0.6	ND	ND	857	42	3
Average	0.8			837	45	4

ND: not detected

line, high levels were contained in the samples.

Malt contains at least four amines which might serve as precursors to NDMA formation: dimethylamine, trimethylamine, hordenine and gramine. Mangino et al.(19) have presented evidence that the alkaloid gramine is highly susceptible to nitrosation to liberate NDMA *in vitro* under conditions normally used to study the potential of tertiary amines to undergo nitrosation. Mangino and Scanlan(5) studied that nitrosation of the alkaloids hordenine and gramine, potential precursors of NDMA in barley malt. Hordenine and gramine found in barley malt were nitrosated in aqueous acid to determine their potential to form NDMA. At 24°C and pH 3.4, the initial rate of gramine nitrosation to NDMA was equivalent to that seen for dimethylamine. Under the same condition, the rate of hordenine nitrosation to NDMA was no faster than that observed for conversion of trimethylamine to NDMA. These authors concluded that both gramine and hordenine must be considered potential precursors to NDMA in malt. Others(20) have considered hordenine to be the most likely precursor based on its higher concentration in malt. These researchers have shown that hordenine could form NDMA on treatment with oxides of nitrogen.

There are no studies on the occurrence of amines such as pyrrolidine and morpoline in malt and beer. Our experimental results suggest that both pyrrolidine and morpholine may be presented in beer.

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