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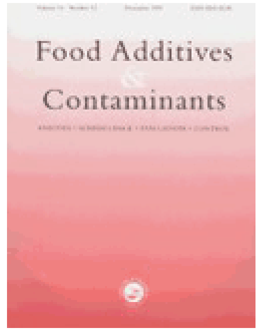

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Current incidence of N-nitrosodimethylamine in beers worldwide

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Abstract

A range of beers (138) from 42 countries were analysed for the presence of N-nitrosodimethylamine (NDMA), using a chemiluminescence technique with a limit of quantification of 0.1 μg/L. The overwhelming majority of samples (79%) did not contain detectable NDMA and only three samples exceeded 0.5 μg/L. No association was found between NDMA content and beer strength, type or geographical origin. It was noted that water can be a potential source of NDMA in beverages.

Keywords: nitrosamines, beer, beverages.

Introduction

The development in the early 1970's of a sensitive method for quantifying N-nitroso groups by thermal energy analysis led to the detection of volatile nitrosamines in a wide range of foods, including cured meats, fish, certain cheeses and also in malt-based beverages such as beer and whisky. In beer, the major nitrosamine is N-nitrosodimethylamine (NDMA), and in the 1970s levels were typically in the range of 1-10 μg/kg (Goff, 1979), although some samples of up to 68 μg/kg have been reported (Walker, 1990). At that time, therefore, beer was considered to be a significant source of

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NDMA in the diet for many people. The NDMA in beer has been considered to be derived entirely from malt, and can be synthesised during malt kilning by a reaction between oxides of nitrogen (from the drying air) and amines (which occur naturally in cereals) (Wainwright, 1986). Once this mechanism was understood, modifications in malting technology were rapidly introduced in order to prevent this reaction occurring. Thus by the early 1980s, levels of NDMA had fallen significantly (Weston, 1983: Osterdahl, 1988). For example, in a survey of UK beers in 1981, only 4% of samples contained more than 5µg/kg NDMA, compared with 14% in a similar survey in 1978 (Ministry of Agriculture Fisheries and Food, UK, 1987). However, the potential for NDMA formation in malt can be more substantially and reliably reduced in the longer term by the use of indirectly fired kilns. Over the last 20 years, such kilns have been widely introduced into commercial maltings. This is likely to result in even lower levels of NDMA in malt and consequently in beer. However, few recent international studies have been conducted. This paper describes a wide-ranging survey of beers currently available in the major beer-drinking markets in the world in order to establish the current levels of NDMA in beer globally.

Materials and Methods

Selection of samples.

The majority of samples were of packaged beer (bottles or cans) and were purchased during 2003. A few samples were provided by the manufacturers from commercial production lines. Many global brands of beer are brewed in several countries, often by other manufacturers under license, using locally produced malts. Care was therefore taken to record the country of origin of each sample according to the country in which it was

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manufactured, rather than the home country of the brand owner. In most cases the major brand of each brewing company was selected, but some speciality products were also included, such as low alcohol, high alcohol and alcohol free beers, Japanese happoshu (low malt beers), South American black beers and UK cask conditioned draught beers,

Reagents and equipment

N-nitrosodimethylamine and N-nitrosodipropylamine were obtained as 5000 µg/ml certified standards (Supelco, UK). Dichloromethane, hexane and ethanol of analytical grade were obtained from Fisher Scientific (Loughborough, UK). ChemElut CE1020 kieselguhr columns were supplied by Varian, Oxford, UK.

Extraction of beer samples

N-nitrosodimethylamine was analysed by the method of Massey et al (1990). The beer was decarbonated by gentle swirling. A 15 ml portion was added to a kieselguhr column together with 1ml of internal standard solution (N-nitrosodipropylamine; 0.05 µg/ml in ethanol) added. The column was eluted with 6 x 10 ml dichloromethane and the eluate passed through a bed of sodium sulphate into a concentration flask. 1 ml of hexane was added to the flask and the contents evaporated to 0.5 ml for GC analysis.

GC analysis

The chromatographic system comprised a HP 5890 Gas Chromatograph (Hewlett Packard, USA) interfaced to a Thermedics 610 Thermal Energy Analyser (TEA) (Woburn, MA, USA) with oxygen gas supply. The analytical column was a 6' x ¼", 4mm i.d. stainless

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steel column packed with Chromosorb WHP 100-120 mesh coated with 10% Carbowax 20M/2% KOH (Supelco, UK). The carrier gas was argon at 40 ml/min and injector and column temperatures were 200°C and 170°C respectively. Samples and standards (10 µl) were injected manually and the concentration of NDMA was calculated from the peak area ratio to the internal standard relative to that of calibration standards. Quantification was achieved by linear regression of calibration standards prepared by analysis of five 5% ethanol solutions fortified at 0.1 - 1.0 µg/L NDMA.

The method used did not detect other volatile nitrosamines such as N-nitrosodiethylamine (NDEA) or N-nitrosopyrrolidine (NPYR). However, other published studies have shown that these nitrosamines are rarely detected in beer *(Lijinsky, 1999; Shin et al, 2005).*

Quality control

A typical analytical batch comprised 10-15 beer samples, one recovery check sample, one reference beer, one water blank and a calibration check standard. The limit of quantification (LOQ), calculated as ten times the standard deviation of a water blank, was 0.1 µg/L and the coefficient of variation (CV) was 17% at 0.5 µg/L. Results were reported corrected for recovery, which was typically in the range 70-80%. Acceptability of a batch was based on Shewhart charts of the results for recovery check and reference samples and results of less than 0.1 µg/L for the water blank. No certified reference materials were available for NDMA in beer: external proficiency testing was maintained through participation in the LGC malt analysis proficiency testing scheme (MAPS). The laboratory and method were accredited to ISO 17025.

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Results and Discussion.

Beer samples.

A total of 138 beers, brewed in 42 different countries, were included in the survey. Approximately half of these were major brands from the ten largest brewing companies in the world (see Table 1). Between them, these ten companies represent 59% of the world's beer production, almost 918 million hectolitres (Barth-Haas group, 2004). The remaining samples included international, national and regional brands.

(Insert Table 1 around here)

The samples also covered the main beer types, including ales, lagers, stouts, strong beers and low or alcohol free beverages.

NDMA content.

Of the 138 samples, 109 (79%) contained no detectable NDMA (that is, less than 0.1μg/L) (Table 2). Only three samples (that is, less than 3%) exceeded 0.5 μg/L. (A limit of 0.5 μg/L has been adopted as a voluntary guideline in many countries, including the UK). The overall mean (calculated by assuming that samples below the limit of detection contained half that limit) was 0.1μg/L and the median value was 0.05 μg/L. The range was from < 0.1 - 1.9 μg/L.

(Insert Table 2 around here)

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The NDMA content of the different beers was evaluated according to beer style, geographic origin or alcohol content. Results are shown in Table 3.

Effect of beer style

There was no significant difference in mean NDMA concentration between the different beer styles. The mean concentration for the dark beers (which includes stouts, porters and black beers) and "other" groups were slightly lower than for the ale and lager groups, but this may reflect the greater number of samples, and consequently the wider range of NDMA concentrations, in these latter two groups.

(Insert Table 3 around here)

Effect of geographic origin

The highest mean values were obtained in the sample sets from the EU and from Asia. As observed with beer styles, this finding may reflect the greater number of samples in these two groups. Mean values from all the other geographical regions were below 0.1µg/L.

Effect of beer strength

There was no indication of an association between alcoholic strength and NDMA concentrations . The highest mean NDMA concentration was found in the medium strength beers and again is probably due to the greater number of samples in this group.

A number of papers published in the 1980s and 1990s have described the reduction in overall NDMA concentrations which followed once the source of NDMA in beer was identified (for a review see Scanlon, 1995). The levels of NDMA found in beers in the current survey are similar to, but slightly lower than, those reported in other more recent

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surveys. Shin et al (2005) found detectable NDMA in 77% of South Korean beers in 1995, with a mean of 0.8 µg/kg, and in 46% of imported beers (mean content 0.5 µg/kg). When the survey was repeated in 2002 the incidence had fallen to 56% of domestic and 44% of imported samples (mean contents 0.3 and 0.6 µg/kg respectively). Yurchenko and Molder (2005) found the mean NDMA content in Estonian beers was close to that in imported beers (0.2 and 0.21 µg/kg respectively).

Potential sources of NDMA in the beers surveyed

The three beers containing NDMA above the voluntary guideline were investigated further in order to try and identify the source of the contamination. Two samples were found to be from Russia, both from the same brewery. Upon further investigation, this brewery was found to be a very small company, operating as much as a tourist attraction than as a commercial brewery. The nine other Russian beers tested were all were below 0.5 µg/L, with a mean of 0.13 µg/L. It was noticeable, however, that only 44% of Russian samples contained no detectable NDMA, compared with 81% for the survey overall. This suggests that the malts being used in Russia may be relatively high in NDMA compared with those being used in the rest of the world. This could reflect atmospheric pollution or could be due to a higher proportion of older directly fired kilns.

Formation of NDMA in water

The highest NDMA concentration (1.9 µg/L) was found in a lager. The contamination in this sample was traced to an ion-exchange resin used to treat constituent water at the brewery site. As far as the authors are aware, this is the first time that NDMA in a beverage has been traced to water.

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NDMA was first identified as a potential disinfection byproduct in 1998, at an aerospace facility in northern California. Another potential route for the contamination of water supplies with NDMA is from the breakdown of certain herbicides and pesticides *(Wolfe et al, 1976; Green & Young, 2006).* Since 1998 surveys of water treatment plants in both the USA and Canada have found that although in the vast majority of sites NDMA was undetectable or below 9 ng/L, these levels were regularly exceeded in certain sites. Since the raw water did not contain detectable NDMA, it seemed probable that it was being formed as a result of treatment processes. *(MOE,1998)*. Other studies have also reported NDMA in waters exposed to ion-exchange resins. Kimoto et al *(1980)* showed that high levels of NDMA (1 - 2 μg/L) could be formed when tap water containing approximately 0.5 - 1.5 mg/L of free chlorine was exposed to a mixed bed deionisation resin. Najm and Trussell *(2002)* suggested that anion exchange resins containing dimethyl-ethanol and trimethyl quaternary amine groups were the most likely to give rise to NDMA. Choi and Valentine *(2002)* and Mitch and Sedlak *(2002)* have also shown that NDMA can be formed by a reaction between dimethylamine (DMA) or ammonia with chlorine or monochloramine. These reactions do not require the presence of nitrite for N-nitrosation.

The exact conditions which allow NDMA to be formed from contact or water with anion exchange resins are still unclear. Although these resins are very widely used, there have only been a few incidents of NDMA release into water. The phenomenon does not appear to be linked to faulty resins or to contaminants present in the resin. Risk factors appear to be the concentration of free chlorine present, the amount of organic material present in the

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raw water, and regeneration procedures which may enhance the release of precursors such as ammonia or DMA.

Conclusions

The results presented in this paper confirm that the levels of NDMA in beer are continuing to fall worldwide. No significant association could be found between NDMA content and beer style, alcoholic strength or geographic origin. Most beers tested do not contain any detectable NDMA, suggesting that beer consumption no longer makes a significant contribution to dietary intake of volatile nitrosamines. However, the results presented here also indicate that water must be considered as a potential source of NDMA in aqueous beverages.

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Table 1. Beer production from the world's leading brewing companies in Dec. 2004

Company	Country	Output (Mill.hl / year)	Percentage of world beer production
Inbev	Belgium	193.4	12.5
Anheuser-Busch	USA	159.7	10.3
SABMiller	UK	148.3	9.6
Heineken	Netherlands	112.6	7.3
Carlsberg	Denmark	92.0	5.9
Molson-Coors	USA/Canada	59.4	3.8
Modelo	Mexico	42.8	2.8
Tsingtao	China	36.9	2.4
BBH	Russia	36.0	2.3
Scottish & Newcastle	UK	30.2	1.9

(Source of data; John Barth & Sons)

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Table 2. NDMA in beers – results of current survey

NDMA content µg/L				
< 0.1	>0.1 – 0.25	> 0.25 – 0.5	> 0.5 – 1.0	> 1.0
109 (79%)	20 (15%)	6 (4%)	2 (2%)	1 (<1%)

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Table 3. NDMA concentrations in different beer types

Beer type		Number of samples	Mean NDMA µg/L
BEER STYLE			
Lager		95	0.105
Ale		27	0.108
Dark beer		13	0.055
Other		3	0.05
GEOGRAPHIC ORIGIN			
EU		52	0.124
Other Europe		8	0.05
Africa		12	0.05
North America		12	0.068
South & Central America		16	0.083
Australia & New Zealand		16	0.054
Asia		23	0.144
ALCOHOL CONTENT (vol/vol)			
Light beers	(<4.0)	9	0.05
Medium strength	(4.0 – 5.5)	99	0.11
Strong beers	(>5.5)	13	0.07

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