Precursors of N-Nitrosodimethylamine in Malted Barley. 2. Determination of Dimethylamine

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Dimethylamine (DMA) was quantified in malt and in beer. Commercial malt contained 0.4–9.4 μg/g DMA, and beer contained approximately 10% of the amount found in malt. DMA appears to be biosynthesized during germination and in the early stage of kilning in the malt manufacturing process. Previous work in our laboratory found that the alkaloids hordenine and gramine contributed only a small percentage of the N-nitrosodimethylamine (NDMA) formed by nitrosating malt. Our results from a nitrosation experiment indicate that DMA accounts for a major share of the NDMA formed.

INTRODUCTION

Despite reduced levels of N-nitrosodimethylamine (NDMA) found in beer today compared to levels found over a decade ago (Scanlan et al., 1990), there is considerable interest in identifying NDMA precursors in malt. Hordenine and gramine, thought to be major NDMA precursors in malt, were extracted from malt and quantified by Poocharoen et al. (1992). From a laboratory nitrosation experiment of malt samples, Poocharoen et al. concluded that the role of hordenine and gramine in NDMA formation was relatively minor and that there must be at least one other precursor. They suggested dimethylamine (DMA) as a likely candidate.

Both DMA and trimethylamine have been reported in beer (Drews et al., 1957; Hrdlicka et al., 1964; Singer and Lijinsky, 1976). Drew et al. (1957) found DMA in green malt, kilned malt, wort, and raw barley and suggested that the source of DMA in beer was malt. Slaughter and Uvgard (1971) reported DMA in both malt and beer; amounts of DMA in beer were in the range 0.07–0.69 μg/mL. French et al. (1982) reported DMA in raw barley, green malt, kilned malt, green malt roots, and kilned malt roots.

The purpose of this investigation was to determine the amounts of DMA in malt and beer. Further, we sought to ascertain where DMA is formed during the malting process and to elucidate the role of DMA as a precursor to NDMA in malt.

EXPERIMENTAL PROCEDURES

Samples and Reagents. Three varieties of malt (Klages, Morex, and Piroline) were used in the experiments to determine the amount of DMA biosynthesized at various stages of the malting process. [See Figure 1 in Poocharoen et al. (1992); for more detail about the malting process, see Briggs et al. (1981).] Raw barleys were analyzed for DMA before steeping, and then raw barleys were carried through pilot steeping, germination, and kilning procedures at the Great Western Malting Co., Vancouver, WA. The pilot kilning, freeze dehydration of germinated samples of green malt, and preparation of malt byproducts were as described in Poocharoen et al. (1992).

Sources of chemicals used are listed in Table I.

Extraction and Recovery. Using a modified procedure of Ripley et al. (1982) and French et al. (1982), DMA was extracted from malt samples by the process described in Table II. To estimate recovery, a freeze-dried Idaho Morex malt was spiked with DMA at 2, 4, and 10 μg/g of malt. The added DMA was recovered from the malt using the extraction and derivatization procedure described in Table II. After the DMA was quantified by gas chromatography and the indigenous DMA in the malt was subtracted, the recovery percentage was calculated.

Beer was also analyzed for DMA. Because beer contains a relatively low amount of solids, 10 g of beer and 50 mL of distilled water plus the internal standard were added directly at step 6 in Table II and carried through the rest of the procedure.

Gas Chromatography (GC) and Quantification. Analysis of DMA was carried out on a Varian Model 3700 GC, coupled to a thermionic N-specific detector. A 2 m × 2 mm i.d. nickel column was packed with Ultra-Bond 20M. Flow rates were as follows: helium carrier, 24 mL/min; hydrogen, 3.6 mL/min; air, 150 mL/min. The injection temperature was 250 °C and the column temperature 78 °C. The sample size was 1 μL.

For quantification of DMA in malt, the standard addition curve method, as described by Scanlan et al. (1982) for determining NDMA in malt, was used. Basically, DMA in amounts of 2, 4, 6, and 10 μg/g of malt was added with the internal standard methylethylamine (MEA) to samples of ground, freeze-dried Idaho Morex malt. After extraction, derivatization, and correcting for indigenous DMA, the ratio of DMA peak height to MEA peak height was plotted as a function of the amount of DMA added to the malt samples. Each data point represents the mean of triplicate preparations.

Table I. Commercial Sources of Chemicals

Sigma Chemical Co.	J. T. Baker Co.
gramine	hydrochloric acid (HCl)
hordenine hemisulfate	Burdick and Jackson Co.
Aldrich Chemical Co.	benzene
sarcosine	Eastman Kodak Co.
dimethylamine hydrochloride (DMA)	methylethylamine
trimethylamine hydrochloride	
pentafluorobenzoyl chloride	
gibberellic acid	

Table II. Extraction Procedure

1. *grind* 10-g samples of kilned malt in Osterizer blender and place in a flask
2. *add* 100 mL of 0.1 N HCl and 10 ppm of N-methylethylamine (MEA) as internal standard
3. *shake* for 30 min, using a mechanical shaker
4. *centrifuge* for 10 min at 1000*g*
5. *filter* through folded Whatman 2V filter paper
6. *add* to the filtrate 9 mL of benzene, 5 mL of 2 M K_2CO_3, and 1 mL of 2% pentafluorobenzoyl chloride in benzene to form the pentafluorobenzoyl derivative of DMA
7. *shake* in stoppered flask using a wrist-action shaker for 10 min
8. *transfer* to a 250-mL separatory funnel
9. *centrifuge* the supernatant for 10 min at 1000*g*
10. *remove* clean upper layer which contains the DMA derivative for gas chromatographic analysis

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Table III. Recovery of DMA[a] Added to Idaho Morex Malt

amount added, μg	mean amount from three trials, μg	SD, μg	% recovered[b]
0	1.8	0.1	
2	3.7	0.2	95
4	5.6	0.1	95
10	11.7	0.2	99

[a] Dimethylamine. [b] [(μg recovered−μg in blank)/μg added] × 100 = % recovered.

For quantification of DMA in beer, the standard addition curve method, as described by Marinelli et al. (1981) for determining NDMA in beer, was used.

NDMA analysis was performed on malt samples as described in Poocharoen et al. (1992).

Mass Spectrometry (MS). Confirmation of identity was accomplished using a Finnigan 1015C quadrupole spectrometer interfaced to a Varian 1400 GC by a glass jet helium separator. A Riber 400 data system, including a Digital Equipment Corp. PDP 8/E minicomputer, a Diablo 31 disk system, a Tektronix 4010-1 display terminal, and a Complot DP-1 digital plotter, was used. The GC column, a 2.5 m × 2 mm i.d. nickel tube packed with Ultra-Bond 20M 100/120 mesh, was operated isothermally at 105 °C with a helium flow rate of 17 mL/min and an injector port temperature of 208 °C. MS was performed using a 450-μA filament current, 2.9-kV electron multiplier setting, and mass scans over the range m/z 25–270.

RESULTS

Extraction and Recovery. Indigenous DMA was extracted from five different samples of the same Klages malt. DMA was found at 5.9, 6.0, 6.3, 6.0, and 5.8 μg/g (mean 6.0 μg/g). The small standard deviation (0.19) indicates good reproducibility for the extraction procedure.

Standard solutions of DMA, prepared in 0.1 N HCl, were added to Idaho Morex malt at three concentration levels (2, 4, and 10 μg/g). One sample was left blank. Recovery rates for DMA are shown in Table III. Results demonstrate a high degree of recovery of DMA through the extraction and derivatization procedures.

Quantification. Triplicate samples were prepared at several levels of added DMA over the range 0–10 μg/g, approximating levels that might be found in malt. The ratios of the GC peak heights of DMA to MEA were plotted against added DMA levels. A linear curve was obtained with a correlation coefficient of 0.996. The regression equation was

$$Y = 0.114X - 0.0193 \quad (1)$$

where Y is the ratio of DMA to MEA peak heights and X is the amount of DMA in malt. For beer the regression equation was

$$Y = 1.09X + 0.116 \quad (2)$$

and the correlation coefficient was 0.999.

There was a possibility that some compound other than DMA degraded to DMA under the conditions of analysis. To check that this was not the case, sarcosine, gramine, and hordenine, known to be present in malt, were exposed to the analytical procedure used for DMA. The results verified that none of these compounds contributed to the amounts of DMA reported in this study.

Confirmation of Identities. The identity of DMA extracted from malt was confirmed by comparison with standard DMA using mass spectral analysis. The mass spectrum of the standard DMA derivative and that of the derivative of DMA extracted from malt showed excellent agreement, confirming that the GC peak for DMA from malt was indeed DMA.

Table IV. Amounts of DMA[a] and NDMA[b] Found in Commercial Samples of Klages Malt

	4-day germination			5-day germination	
sample	DMA, μg/g	NDMA, μg/kg	sample	DMA, μg/g	NDMA, μg/kg
1	3.6	1.3	13	12.4	2.0
2	3.1	1.0	14	10.9	3.9
3	3.1	1.1	15	14.1	4.0
4	5.7	1.7	16	12.6	4.3
5	4.8	1.0	17	9.8	4.8
6	7.2	1.3	18	10.1	5.5
7	2.6	0.5	19	10.5	9.4
8	3.8	0.4	20	12.7	4.3
9	4.4	0.8	21	11.4	5.2
10	7.3	2.9	22	9.0	5.5
11	8.2	2.9	23	13.7	5.8
12	5.5	2.0	24	13.0	8.8
mean	4.9	1.4	mean	11.7	5.3

[a] Dimethylamine. [b] *N*-Nitrosodimethylamine.

DMA and NDMA in Barley and Malt. A replicable analytical procedure for quantifying DMA from malt having been established, the method was used to quantify DMA in barleys and malts.

The first experiment was to quantify amounts of DMA and NDMA in 24 samples of commercial Klages malt. The first 12 samples were manufactured from barley grown in southern Idaho that had been germinated for 4 days; the second 12 were 5-day germination malts from barley grown near Klamath Falls, OR. Results are reported in Table IV. The significant increases in both DMA and NDMA over the additional day of germination support previous findings that NDMA precursors are biosynthesized during the germination process (Mangino et al., 1981).

The next experiment was to measure levels of DMA found at various stages of the malting process [see Figure 1 in Poocharoen et al. (1992)]. From three varieties of barley, samples of raw barley, steeped barley, clean malt, and malt byproducts (mostly rootlets) were analyzed. To evaluate the effect of heat during kilning on DMA levels, samples of clean malt and malt byproducts were taken from both freeze-dried and kiln-dried malt. All three barley varieties were treated on the first day of germination with a 0.10 ppb concentration of gibberellic acid, a plant hormone that stimulates germination. Another set of trials at each malting stage for Klages malt was run without the gibberellic acid treatment. Means and standard deviations from the triplicate samples of each set at each malting stage are reported in Table V.

Results are similar to those that Poocharoen et al. (1992) found for *N*-methyltyramine and hordenine at various stages of malting (see their Tables VI and VII). Extremely small amounts of DMA were found in raw and steeped barley. As with the alkaloids, most DMA is formed during germination and is concentrated in the malt byproducts (mostly rootlets). Heat during kilning raised DMA levels in the malt byproducts above those of the freeze-dried samples for all but the Klages malt that was not treated with gibberellic acid. Comparing the two Klages malts, with and without the acid treatment, suggests that the gibberellic acid may have acted to inhibit DMA formation, but additional work would be needed to confirm this possibility.

DMA Levels in Commercial Beer. Since dietary DMA can serve as a precursor for endogenous formation of NDMA, we determined the DMA content of 10 selected brands of 1991-produced commercial beer obtained at retail outlets. The average DMA from 2 samples of each brand of beer ranged from 0.40 to 0.71 μg/g; the mean DMA from all 10 brands was 0.58 μg/g.

Table V. DMA[a] Found at Various Stages of the Malting Process

variety	raw barley, μg/g	steeped barley, μg/g	freeze-dried malt: clean malt, μg/g	freeze-dried malt: byproducts, μg/g	kiln-dried malt: clean malt, μg/g	kiln-dried malt: byproducts, μg/g
Morex	0.8 ± 0.1[b]	0.5 ± 0.1	9.5 ± 0.2	442 ± 30	6.2 ± 0.3	637 ± 10
Piroline	0.6 ± 0.1	0.5 ± 0.1	8.2 ± 0.6	396 ± 14	4.8 ± 0.3	614 ± 38
Klages	1.0 ± 0.1	0.6 ± 0.1	11.0 ± 0.8	453 ± 37	6.6 ± 0.4	571 ± 10
Klages[c]	1.0 ± 0.1	0.6 ± 0.1	17.7 ± 0.5	762 ± 36	6.0 ± 0.2	605 ± 29

[a] Dimethylamine. [b] Mean of results from three samples ± the standard deviation. [c] Without gibberellic acid.

Table VI. NDMA[a] Obtained from Nitrosating Kilned Clean Malts Containing Known Amounts of Hordenine, Gramine, and DMA[b] Compared with Expected NDMA Yields from These Amines

variety	mean amount of DMA in malt samples (3 trials) ± SD, μg/g (dry wt)	NDMA obtained from nitrosation, μg/kg (dry wt)	NDMA, μg/kg (dry wt), expected[c] from: hordenine	gramine	DMA	total NDMA (and percentage[d]) accounted for by known quantities of the three amines, μg/kg (dry wt)
Wintermalt	5.4 ± 0.4	10012	169	2953	8196	11318 (113)
Morex	2.3 ± 0.2	4484	382		3491	3873 (86)
Klages	2.3 ± 0.1	4496	504		3491	3995 (89)
Piroline	5.0 ± 0.2	7878	263		7589	7582 (100)
Steptoe	2.3 ± 0.4	3599	270	2497	3490	6257 (174)

[a] *N*-Nitrosodimethylamine. [b] Dimethylamine. [c] Expected values of NDMA are calculated by multiplying the amount of the amine by the yield of NDMA and adjusting for the respective molecular weights of NDMA and the amine, e.g., for DMA in Wintermalt: [(5400 × 74)/45] × 0.923 = 8196, where 74 and 45 are the respective molecular weights of NDMA and DMA and 92.3% is the average NDMA net yield from DMA under the nitosation conditions used in this study. The amounts of hordenine and gramine in these malts were obtained from Poocharoen et al. (1992). [d] Expected NDMA from hordenine, gramine, and DMA divided by the NDMA obtained, e.g., for the three alkaloids in Wintermalt: [(169 + 2953 + 8196)/10012] × 100 = 113%.

Table VII. Estimated Percentages of NDMA[a] Produced by Nitrosation of Hordenine, Gramine, and DMA[b] in Malt

	hordenine	gramine	DMA
Wintermalt	1.7[c]	29.5	81.9
Morex	8.5	0	77.9
Klages	11.2	0	77.6
Piroline	3.3	0	96.3
Steptoe	7.5	69.4	97.0

[a] *N*-Nitrosodimethylamine. [b] Dimethylamine. [c] NDMA expected from hordenine divided by the total NDMA obtained from nitrosation times 100, e.g., (169/10012) × 100 = 1.7%.

Relative Role of DMA in Producing NDMA. Poocharoen et al. (1992) reported the percentage yields of NDMA in laboratory nitrosated malt from added levels of hordenine, gramine, and DMA-HCl; the yields were 2.7, 87.9, and 92.3%, respectively. They then performed a nitrosation experiment and compared NDMA obtained with amounts expected from the indigenous amounts of hordenine and gramine in malt. It was concluded that there must be at least one NDMA precursor besides hordenine and gramine, and they suggested DMA as a likely candidate.

Using the same kilned, clean malt samples of the five malt varieties used by Poocharoen et al. (1992), we continued the laboratory nitrosation experiment to estimate the amount of NDMA accounted for by hordenine, gramine, and DMA. Results are reported in Table VI. Drawing on the data in Table VI for DMA and the data from Poocharoen et al. (1992, Table IX) for hordenine and gramine, an estimate of the relative amounts of NDMA from these three amines is shown in Table VII. Results clearly indicate that DMA is a major precursor of NDMA in these malt samples.

DISCUSSION

This study adapted a method to extract and quantify DMA from malt and beer. The amounts of DMA found in malt are in general agreement with those reported by French et al. (1982). Using commercial malt samples, considerably more DMA (and NDMA) was found in 5-day-germinated malt samples than in those germinated only 4 days, supporting the hypothesis that NDMA precursors are biosynthesized during the germination process.

An experiment parallel to one done by Poocharoen et al. (1992) measured levels of DMA at various stages of the malting process. Only negligible amounts were found in raw and steeped barley. In the malts, many times more DMA was found in the malt byproducts than in the clean malt. Levels of DMA were higher in three of the four cases for the kiln-dried than for the freeze-dried malt byproducts. These results suggest that DMA is biosynthesized during germination and kilning.

Analysis of various types of commercial beer for DMA content found levels about $^1/_{10}$ of those found in malt. The fact that the dilution factor for beer to malt is about 10 to 1 indicates that no further formation of DMA occurs during the brewing process. Apparently, the DMA level existing in malt is simply carried through to the beer.

The crucial experiment extended the analysis of Poocharoen et al. (1992) on the same malt samples containing known amounts of hordenine, gramine, and DMA. In all cases, the NDMA from DMA was appreciably higher than the combined values from the alkaloids, strongly suggesting that DMA is the primary precursor of NDMA in malt.

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