

Formation of *N*-Nitrosodimethylamine in Direct-Fire Dried Malt^{1,2}

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ABSTRACT

The principal objective of this work was to investigate the formation and establish the identity of amine precursors for *N*-nitrosodimethylamine (NDMA) in direct-fire dried malt. NDMA levels in malt and beer samples were determined using a gas chromatograph coupled with a Thermal Energy Analyzer. A comparison of NDMA levels in nitrosated electric-dried and freeze-dried malt indicated that heat during kilning did not promote formation of precursor amines. Considerable amounts of NDMA were produced from dimethylamine, trimethylamine, hordenine, and gramine, whereas negligible NDMA levels were produced from *N*-methyltyramine, sarcosine, and choline upon aqueous phase nitrosation. Analysis of commercial samples of malt and beer revealed that NDMA levels have been sharply reduced in these commodities during the past several years.

Key words: *Amine precursors, Malt, Nitrosamines, N-nitrosodimethylamine.*

Spiegelhalter et al (10) were the first to report trace levels of *N*-nitrosodimethylamine (NDMA) in beer. Shortly after this report from Germany, workers in other parts of the world demonstrated the general occurrence of low levels (ppb) of NDMA in a variety of beers (5). Investigators quickly found that malt kilned by direct-fire drying was the source of NDMA in beer (1,6,9,11). Presumably, nitrogen oxides in the drying air react with amines in the green malt to form NDMA during direct-fire kilning.

The principal aim of this research was to investigate the formation and to establish the identity of amine precursors for NDMA. Additionally, NDMA levels in samples of beer and malt analyzed in our laboratory over the past several years are compared.

EXPERIMENTAL

Role of Heat in Formation of NDMA Precursors

Two varieties of green malt (Idaho Klages and midwestern Larker) were obtained from a local malster. One portion of each variety of green malt was dried to a moisture content of 3.8% in a Seeger electric pilot malt kiln. A second portion of each variety was dried to a moisture content of 4.2% in a Hull pilot freeze-dehydration unit. The dried malts were cleaned manually over a

precision sieve (slotted, 4%/64 in. × 3/4 in.) to remove rootlets and chaff. Three 25-g samples of both varieties of freeze-dried and electric-dried malt were nitrosated in 0.26 *M* sodium nitrite (pH 3.2) for 18 hr at room temperature. Residual nitrite was then inactivated by lowering the pH of the reaction mixture to pH 1 with concentrated sulfuric acid, followed by addition of 8 g of ammonium sulfamate dissolved in 10 ml of distilled water. The malt mixtures were filtered and the filtrates adjusted with distilled water to a volume of 150 ml and extracted three times with 25 ml of dichloromethane. The dichloromethane fractions were concentrated to 4 ml and analyzed for NDMA using the gas chromatograph Thermal Energy Analyzer (GC-TEA) according to the method of Hotchkiss et al (3) (Table I).

NDMA Formation from Potential Precursors

The potential NDMA precursors (0.1 *M*) listed in Table II were made to react with 0.5 *M* sodium nitrite at 65°C for 16 hr in 20-ml Kimax glass tubes sealed with Teflon-lined caps. Reaction

TABLE I
Comparison of *N*-Nitrosodimethylamine Levels in Freeze-Dried and Electric-Dried Nitrosated Malt^a

Variety	Freeze-Dried	Electric-Dried
Klages	996±220 ^b	719±25 ^b
Larker	847±201 ^b	892±187 ^b

^a Values (micrograms per kilogram) based on dry weight basis (experimental malts intensively nitrosated).

^b N = three repetitions.

TABLE II
Yield (%) of *N*-Nitrosodimethylamine upon Nitrosation of Potential Precursors^a

Amine	pH 4.4 ^b	pH 6.4 ^c
Dimethylamine	78	65
Trimethylamine	8	0.8
Hordenine	11	2
Gramine	76	5
<i>N</i> -Methyltyramine	0.14	...
Sarcosine	0.09	0.15
Choline	0.041	0.0007

^a Precursors (0.1 *M*) amine made to react with 0.5 *M* sodium nitrite at 65°C for 16 hr.

^b Acetate buffer.

^c Citrate-phosphate buffer.

solutions (10 ml) were buffered at pH 4.4 (acetic acid-sodium acetate) and pH 6.4 (citric acid-disodium phosphate) before heating. After cooling, the reaction mixtures were extracted with 2 × 10 ml of dichloromethane, which was subsequently dried over sodium sulfate. The volume of dichloromethane was increased to 25 ml, and NDMA was determined by GC-TEA (3).

NDMA in Beer and Malt

The NDMA content of commercial samples of beer was estimated as described by Scanlan et al (9). Commercial malt samples were analyzed for NDMA by the mineral oil-vacuum distillation procedure (3).

RESULTS AND DISCUSSION

Role of Heat in Formation of NDMA Precursors

The purpose of the electric-dried, freeze-dried malt nitrosation experiment was to determine the role of heat during kilning on the

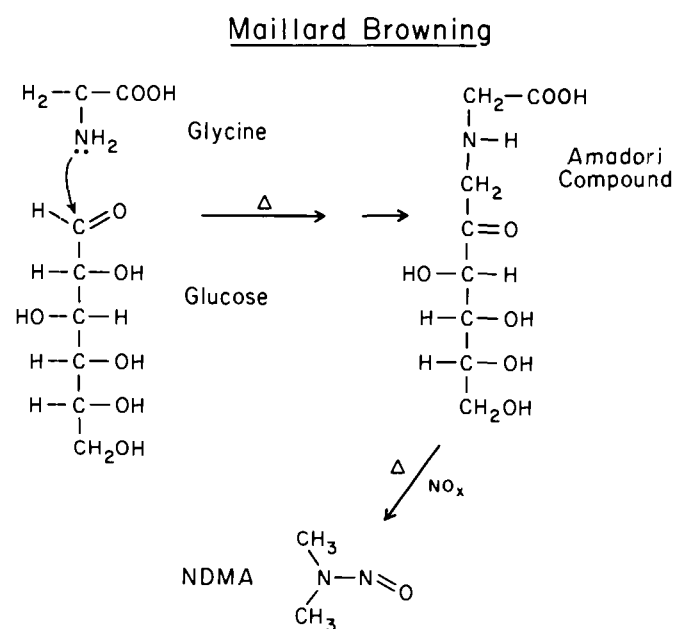


Fig. 1. Potential formation of *N*-nitrosodimethylamine-precursor by Maillard browning reactions.

TABLE III
Comparison of *N*-nitrosodimethylamine Levels ($\mu\text{g}/\text{kg}$)
in Commercial Beers Sampled in 1979 and 1981

	N	Range	Mean
1979 ^a	25	ND ^b -14	5.9
1981	25	ND-0.7	0.2

^aFrom Scanlan et al (9).

^bND = not detected.

TABLE IV
Comparison of *N*-Nitrosodimethylamine Levels ($\mu\text{g}/\text{kg}$) in Direct-Fire
Dried Malt Produced with and without Sulfur

Year and Conditions	N	Range	Mean
April-May 1979			
Without sulfur	7	32-92	58
July-December 1980			
With sulfur	139	0.2-7.0	1.9

formation of amine precursors for NDMA. Through Maillard browning reactions, heat could conceivably promote NDMA precursor formation. For instance, heat-induced Maillard browning reactions between glycine and glucose would lead to the Amadori compound shown in Fig. 1. *N*-Nitrosation, decarboxylation, and fragmentation of the Amadori compound could lead to NDMA.

If heat does promote formation of NDMA precursors, then the nitrosated electric-dried malt would have a higher level of NDMA than the nitrosated freeze-dried malt, because the latter received no heat during drying. The data in Table I show that this was not the case. For both varieties of barley studied, the NDMA levels in the nitrosated freeze-dried malt were similar to or slightly greater than the NDMA levels in the nitrosated electric-dried malt. These results strongly suggest that heat during drying does not promote formation of NDMA precursors. Therefore, the NDMA precursors probably are not formed during kilning but rather during germination of the barley before kilning.

NDMA Formation from Potential Precursors

Several investigators have suggested that the compounds listed in Table II are formed in green malt during germination, and that any one or a combination of these amines might serve as the precursor to NDMA. For example, Widmaier suggested that hordenine and gramine could form NDMA under conditions of direct-fire drying.³ Included in Table II are the yields of NDMA upon nitrosation of these amines at pH 4.4 and pH 6.4. Based on the yields of NDMA, the first four amines must be considered potential precursors, and future work will be required to unambiguously determine which amine or combination of amines, is actually the precursor for NDMA in direct-fire dried malt. Based on the very low yield of NDMA from *N*-methyltyramine, *N*-methyltyramine probably is not a precursor for NDMA. Sarcosine and choline probably are also unlikely candidates as NDMA precursors, although caution should be exercised because reliable quantitative information for these two compounds in green malt is not available. Current research activity in the authors' laboratory is directed toward generating reliable quantitative information for all of the compounds listed in Table II. Such information should greatly aid in determining the exact precursors for NDMA in green malt.

NDMA in Beer and Malt

Levels of NDMA in 25 commercial beers purchased at local retail outlets in January-March of 1979, and again in January-March of 1981 are included in Table III. A comparison of the 1979 with the 1981 data documents the dramatic reductions that occurred in NDMA levels in beer during that two-year period. Recently, similar reductions in the amount of NDMA in beer were also reported in this country by Havery et al (2) and in Germany by Preussmann et al (8).

Table IV shows NDMA levels in samples of direct-fire dried malt produced without sulfur and analyzed in April-May 1979. For comparison, amounts of NDMA in samples of direct-fire dried malt produced with sulfur dioxide and analyzed in July-December of 1980 are also included in Table IV. These data document the very substantial reduction in NDMA in commercial malt that occurred during this period. As reported elsewhere (4,7), these reductions were accomplished by introducing sulfur dioxide into the drying air during the first few hours of the direct-fire drying operation. The data in Table IV provide an example of significant NDMA reduction in malt and beer by using improved processing procedures.

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³Widmaier, 1979. Personal Communication.

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