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Title: Determination of Dimethylamine in Barley Malt

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Dimethylamine (DMA), in addition to the alkaloids hordenine and gramine, has been suggested as a potential precursor of nitrosodimethylamine (NDMA) in malt. Analytical procedures were adopted and applied to extract and quantify DMA from malt, green malt, malt roots and raw barley. Ground malt was extracted at room temperature with 0.1 N HCl, and the extract was derivatized with 2% pentafluorobenzoyl chloride in benzene. After purification, the derivatized DMA was quantitated by gas chromatography with a thermionic N-specific detector. The method yielded a 96.3 per cent recovery for DMA, and exhibited a linear relationship between DMA concentration and GC peak height within a given range. The identity of the DMA derivative was confirmed by mass spectrometry.

Commercial barley samples of 4 and 5-day germination of Klages variety were analyzed. The mean value for the 4-day germination Klages was 4.9, and for the 5-day, 11.7 μg/gm. The DMA level of 5-day germination Klages were significantly higher than the 4-day ($P < 0.05$).

Levels of DMA in samples at each stage of the malting process were determined. DMA was found with a mean and range of 0.9 (0.6-1.0); 0.6 (0.5-0.6); 11.6 (8.2-17.7); 513 (396-762); 5.9 (4.8-6.6); and 607 μg/gm (571-637 μg/gm) on a dry weight basis for, respectively, raw barley, steeped barley, green malt, green malt roots, kilned malt and kilned malt roots. It was found that roots contained the highest levels of DMA. These results indicate that DMA is biosynthetically formed during germination.

The nitrosation of 5 varieties of green and kilned malts (Winter, Morex, Klages, Piroline and Steptoe) was carried out to determine the significance of DMA as a precursor for NDMA. The amount of NDMA from DMA was compared to the amounts of NDMA from hordenine and gramine for each sample. In every sample the amount of NDMA from DMA was larger than the combined amount of NDMA from hordenine and gramine. The results strongly suggest that DMA is the primary precursor of NDMA in malt.

# Determination of Dimethylamine in Barley Malt

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## * DEDICATION *

The author wishes to express his deep appreciation to his family for their endless encouragement and understanding. This thesis is dedicated to his father, Jaebong; his mother, Nanhee; and his wife, Amelia Hye-Suk.

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## I. INTRODUCTION

Because N-nitroso compounds have been shown to be carcinogenic (Magee and Barnes, 1956), the presence of nitrosamines in foods has received a great deal of attention in recent years.

N-Nitrosodimethylamine (NDMA) was first detected in European beers. Spiegelhalder et al. (1979) reported that 70 per cent of 158 samples of European beers examined contained NDMA. The mean value found for NDMA was 2.7 ppb and the highest value obtained was 68 ppb. In 1980, Scanlan et al. examined a variety of commercially available beers produced in the United States; NDMA was detected in 23 of the 25 beers tested and the mean value found was 5.9 ppb. It was estimated that the daily intake of NDMA from beer for the American public was 0.97 µg/person (Goff and Fine, 1979). In 1954, Barnes and Magee reported the toxicological properties of NDMA. Single exposures of rats to high doses of NDMA caused severe liver necrosis, and long-term, chronic feeding of NDMA to rats was shown to cause malignant tumors of the liver and kidney (Magee and Barnes, 1956). Lijinsky and Taylor (1977) intensively studied the carcinogenicity of NDMA and N-nitrosodiethylamine (NDEA) in several species of animals, and they reported that both compounds induced tumors in all species tested.

The discovery that NDMA was present in a high percentage of

beer samples led to investigations as to the origin of this compound in beer (Kann et al., 1980; Spiegelhalder et al., 1980; Hardwick et al., 1981). They analyzed barley malt, raw materials used in brewing, and beer at different stages of brewing, and discovered that malt was the source of NDMA.

Hordenine, an alkaloid of malt biosynthesized during the germination of barley, was reported as a potential NDMA precursor (Kann et al., 1980; Spiegelhalder et al., 1980; Hardwick et al., 1981). Another malt alkaloid, gramine, had been found to occur in the shoots of germinating barley (Schneider and Wightman, 1974). Gramine could also be a source of NDMA since it was shown to be extremely susceptible to nitrosation to form NDMA (Mangino and Scanlan, 1982). These studies revealed that hordenine and gramine might be potential precursors of NDMA in barley malt. Mangino and Scanlan (1982) have reported the yield of NDMA from nitrosation under laboratory conditions, and concluded that dimethylamine (DMA), trimethylamine (TMA), hordenine and gramine must be considered potential precursors of NDMA in direct-fired malt. TMA is probably not a major NDMA precursor in malt, since it is less reactive to nitrosation when compared to DMA (Mangino and Scanlan, 1982), and furthermore, TMA has not yet been found in malt. Expected values of NDMA from hordenine and gramine alkaloids in malt, however, were far less than the total values for NDMA obtained from the nitrosation of the same malt (Poocharoen, 1984). DMA has been suggested as a potential precursor of NDMA in malt

(Drews et al., 1957; Koike et al., 1972; French et al., 1982). It was decided therefore to investigate the role of DMA as a source of NDMA in malt.

## Purpose of the Research

Preliminary investigation on the precursors of NDMA in malt indicated that, in addition to the alkaloids, hordenine and gramine, DMA might be a potential precursor of NDMA. The following objectives were set for this study:

1. To adopt an analytical method for the extraction and determination of DMA from malt.
2. To confirm the identity of DMA found in samples by mass spectrometry.
3. To quantitate the levels of DMA in raw barley, steeped barley, green malt and finished malt.
4. To compare the values of NDMA obtained from the nitrosation of malt with the expected values of NDMA from hordenine, gramine and DMA.

# II. LITERATURE REVIEW

## A. Introduction

N-Nitroso compounds are important because of their carcinogenicity and mutagenicity; it has been shown that N-nitroso compounds are a major class of chemical carcinogens which are the most broadly acting and very potent carcinogens and lead to a wide variety of tumors in many animals (Magee and Barnes, 1956; Mirvish, 1975; Lijinsky, 1977; Walters, 1977). The chemistry of N-nitrosamines has been intensively studied by many investigators (Sander et al., 1968; Anselme, 1979).

Amongst other amines in malt and beer, dimethylamine may be important as a precursor for NDMA (Drews et al., 1957; Barnes et al., 1954; Koike et al., 1972; French et al., 1982). Little is known, however, of the distribution and contribution of DMA in malt. Therefore, estimation of DMA in malt is necessary in view of the possible formation of NDMA by reaction of DMA with nitrous acid (Sander et al., 1968; Mangino et al., 1982). In this review, formation, occurrence, and precursors for nitrosamines, and also methods for measurement of DMA in malt will be reviewed.

## B. Formation of Nitrosamines

Nitrosamines are formed by chemical reactions between nitrosating agents and nitrosatable amines, The formation of N-nitrosamines from secondary and tertiary amines will be discussed in

this section. Several possible mechanisms for the formation of the most common nitrosamines will be presented.

## Nitrosation of Secondary Amines

Formation of N-nitroso compounds from nitrosation of secondary amines may be important environmentally, since these amines occur in food as a result of biosynthesis, fermentation and cooking (Smith, 1981). Some drugs and pesticides are also secondary amines.

The most important nitrosating agent which participates in nitrosamine formation in food systems is probably nitrous anhydride ($N_2O_3$). Nitrous anhydride forms readily from nitrous acid in aqueous solution as depicted in the equilibrium:

$$2HONO \rightleftharpoons N_2O_3 + H_2O \qquad (1)$$

Nitrous anhydride reacts with the unshared pair of electrons on unprotonated secondary amines to form nitrosamines.

$$R_2\ddot{N}H + N_2O_3 \longrightarrow R_2N\text{-}N{=}O + HNO_2 \qquad (2)$$

From equilibrium (1), nitrous anhydride concentration is dependent on the square of the nitrous acid concentration, and this relationship is important to the understanding of the kinetics and mechanism of secondary amine nitrosation. Most secondary amines are nitrosated at moderate acidity by a third order reaction in which the rate of nitrosation is proportional to the amine concen-

tration and to the square of the nitrous acid concentration:

$$\text{Rate} = k_1[\text{amine}][\text{nitrite}]^2 \qquad (3)$$

At high acidity, the concentration of the nitrosating agent increases, while at low acidity the concentration of the free unprotonated amine increases. This inverse relationship between the concentration of the nitrosating agent and the free unprotonated amine results in an optimum pH for the nitrosation of each amine.

## Nitrosation of Tertiary Amines

N-Nitrosamine formation from tertiary amines in acetic acid at 40-50°C was reported by Wegler and Frank (1936). In 1967, Smith and Loeppky reviewed N-nitrosamine formation from tertiary amines and proposed a mechanistic scheme for the nitrosation reaction shown in Figure 1A. The nitrosating agent reacts with the unshared pair of electrons on the unprotonated tertiary amine; the nitrosammonium ion formed then underwent cis elimination of nitroxyl ion to form an immonium ion. The immonium ion obtained next underwent hydrolysis to yield a secondary amine intermediate and a carbonyl compound. Finally, the secondary amine was nitrosated to yield the N-nitrosamine.

The modified mechanism of Keefer (1979) for the nitrosation of a tertiary amine is given in Figure 1B.

In comparason, nitrosation of tertiary amines usually give

Figure 1. Proposed mechanisms for the nitrosative dealkylation of tertiary amines

A - The mechanism proposed by Smith and Loeppky (1967)

B - Mechanism proposed by Keefer (1979)

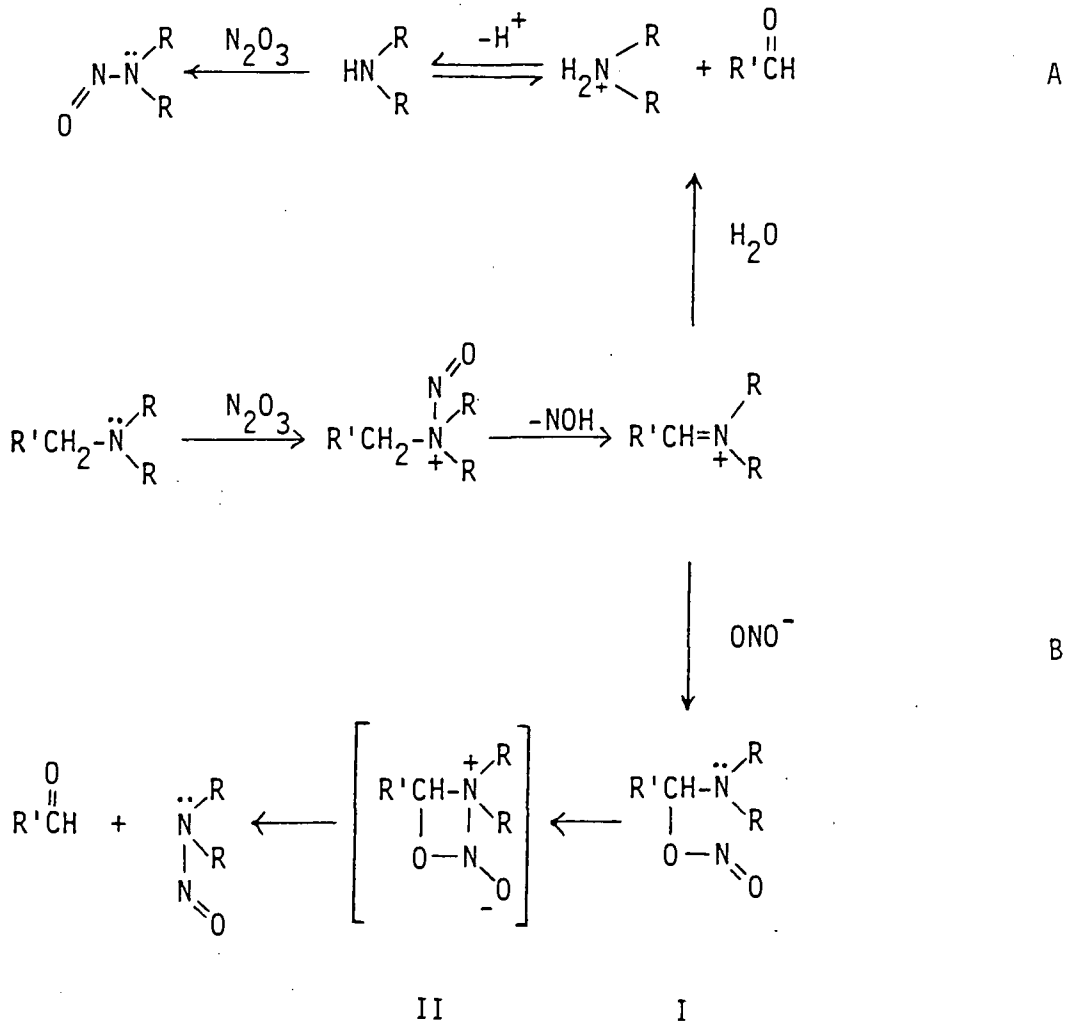



Figure 1

lower yields of N-nitrosamines than nitrosation of secondary amines, and the nitrosation of tertiary amines is more temperature dependent. The yield of NDMA from DMA was found to be about 10 times higher than from TMA, when equimolar concentrations of the two amines were reacted with nitrite in pH 5.6 buffer for 4 hr. at 78°C (Fiddler et al., 1972). However, tertiary amine nitrosation remains a point of interest since many products and food constituents are tertiary amines.

## Gaseous Nitrosating Agents

White and Feldman (1957) demonstrated the possibility of performing nitrosation without acid catalysis. Gaseous dinitrogentetroxide ($N_2O_4$) was used to nitrosate diethylamine in dichloromethane or ether solution. Reaction of amine and $N_2O_4$ occurred very rapidly. The thermal oxidation of ambient nitrogen produces nitrogen oxides which are considered to be nitrosating agents in direct-fired drying air. The equations are as follows (Mangino et al., 1981):

$$N_2 + O_2 \rightleftharpoons 2NO \quad (4)$$

$$2NO + O_2 \rightleftharpoons 2NO_2 \quad (5)$$

$$NO + NO_2 \rightleftharpoons N_2O_3 \quad (6)$$

$$NO_2 + NO_2 \rightleftharpoons N_2O_4 \quad (7)$$

Nitirc oxide (NO) itself has been shown to be very poor nitrosating agent (Challis and Kyrtopoulos, 1976). However, both $N_2O_3$ and $N_2O_4$ have been shown to be effective nitrosating agents in both neutral and alkaline solutions (Challis et al., 1978; Challis and Kyrtopoulos, 1978).

## C. Formation and Occurrence of Nitrosamines in Malt

The presence of NDMA was first reported in a significant proportion of samples of German beer (Spiegelhalder et al., 1979). A large scale survey of West German brewery products for the presence of volatile N-nitrosamines showed that 70% of 158 tested samples were contaminated with NDMA at a mean concentration of 2.7 µg/liter. The highest concentration for any individual sample was 68 µg/liter, and samples with a level below 0.5 µg/liter were taken as negative. Samples were analyzed by the most N-nitrosamine-specific method available: combined gas chromatography-thermal energy analysis (GC-TEA). In a sampling of mostly United States beers, Fazio et al. (1980) found that 62 of 64 samples were positive ( $>$ 0.2 µg/kg) for NDMA and the mean level was 2.8 µg/kg. In a survey of 25 U.S. beer samples, Scanlan et al. (1980) found that 23 samples were positive ( $>$ 0.1 µg/kg) and the mean level of NDMA was 5.9 µg/kg.

An important finding of the original analytical surveys indicated that NDMA was by far the predominant volatile N-nitros-

amine found in beer, which led to investigations of NDMA precursors.

## Sources of NDMA in Malt

Analyses of the raw materials of brewing, as well as of the NDMA content at different stages of beer production, have been reported by several groups (Kann et al., 1980; Spiegelhalder et al., 1980; Hardwick et al., 1981). Spiegelhalder et al. (1980) found that in all steps following the production of malt there was no increase in the NDMA content of beer. Invariably, the malting process was shown to be the source of NDMA in beer. Malting is the process in which barley is steeped, germinated and kilned to a product from which the brewer obtains the extract or "wort" used for the fermentation stage in beer production. Figure 2 shows the malting process used to produce malt. Raw barley is steeped in water to soften the outer hull so that the kernel will become permeable to water and air. The steeped barley is germinated for four days during which time enzymes are biosynthesized. A number of nitrogen-containing secondary metabolites are also biosynthesized. The germinated malt ("green malt") is then dried or kilned. During kilning, growth is stopped, enzyme activities are arrested, and desirable color and flavor changes are induced. The final product, called "clean malt" is obtained by passing kilned malt through a screw conveyor to remove the rootlets.

Analytical work carried out by the malting industry showed that raw barley and green malt contained negligible amounts of

Figure 2. Unit operations of the malting process

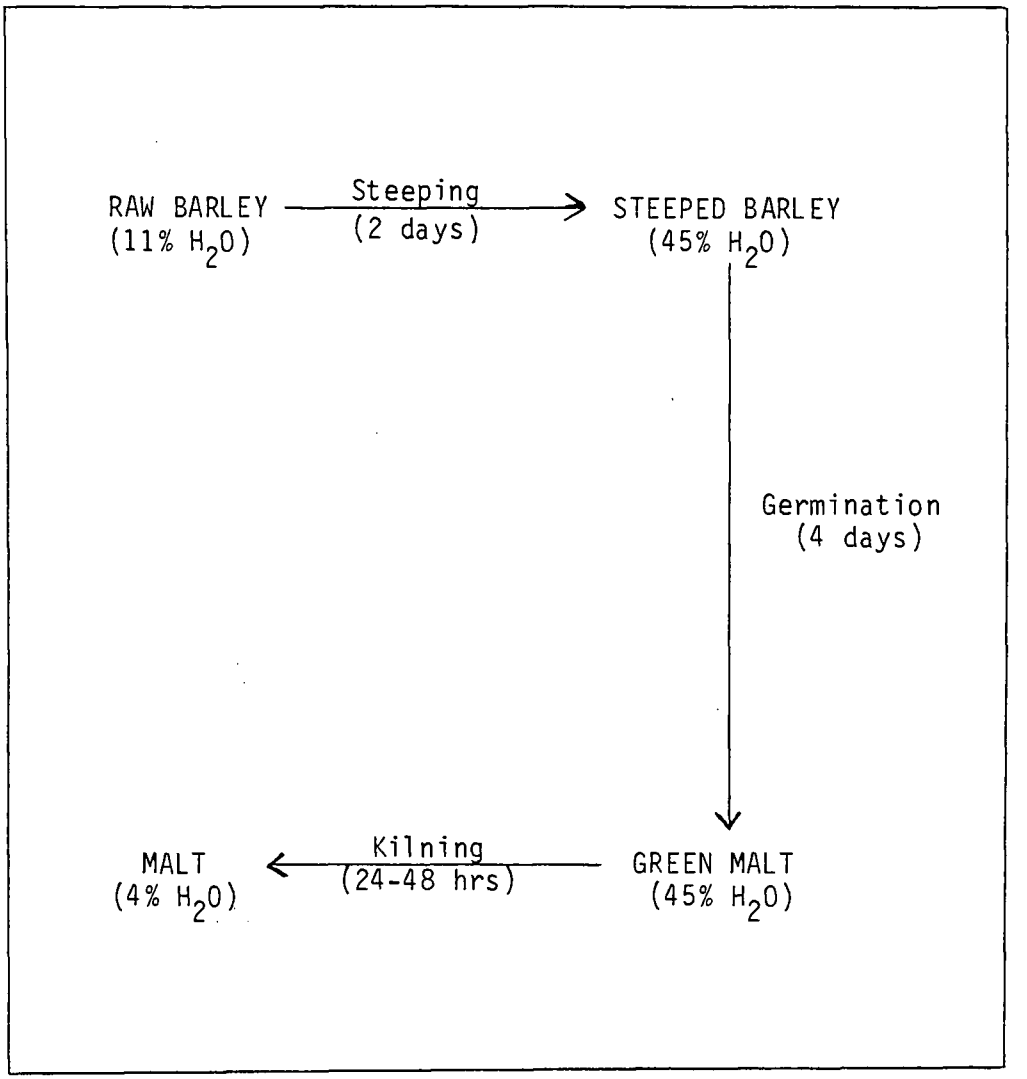

RAW BARLEY
(11% $H_2O$)
Steeping
(2 days)
STEEPED BARLEY
(45% $H_2O$)
Germination
(4 days)
GREEN MALT
(45% $H_2O$)
Kilning
(24-48 hrs)
MALT
(4% $H_2O$)

Figure 2

NDMA, but kilned malt did contain NDMA, often at a level in excess of 50 µg/kg (Hardwick, et al., 1981). These results revealed that NDMA formation occurred during the kilning operation. The thermal oxidation of ambient nitrogen in direct-firing produces nitrogen oxides $(NO)_x$ which form $N_2O_3$ and $N_2O_4$ during the kilning operation; both $N_2O_3$ and $N_2O_4$ have been shown to be effective nitrosating agents (Challis et al., 1978; Challis and Kyrtopoulos, 1978).

## D. Precursors of Nitrosamines in Malt

Knowing the identity of the NDMA precursors would be very useful in attempt at inhibiting the formation of NDMA by removing or reducing the amount of the precursors.

Several investigators have suggested that the compounds listed in Table 1 are formed during the germination of malt, and that these amines might serve as precursors for NDMA. Mangino and Scanlan (1982) suggested that the first four amines, shown in Table 1 (DMA, TMA, hordenine and gramine), must be considered as potential NDMA precursors.

DMA and TMA are both reported to be present in beer (Drews et al., 1957; Hrdlicka et al., 1964; Singer and Lijinsky, 1976). Drews et al. (1957) suggested that malt was the source of these volatile amines since they were reported not to be formed during fermentation. They found DMA in green malt, kilned malt, wort and raw barley. In later studies, Slaughter and Uvgard (1971) report-

TABLE[a] 1. Yield (%) of N-Nitrosodimethylamine upon Nitrosation of Potential Precursors[b]

| Amine | pH 4.4[c] | pH 6.4[d] |
|---|---|---|
| Dimethylamine (DMA) | 78 | 65 |
| Trimethylamine (TMA) | 8 | 0.8 |
| Hordenine | 11 | 2 |
| Gramine | 76 | 5 |
| N-Methyltyramine | 0.14 | - |
| Sarcosine | 0.09 | 0.15 |

[a]Values taken from Mangino and Scanlan (1982).

[b]Precursors: amine (0.1 M) reacted with 0.5 M sodium nitrite at 65 °C for 16 hr.

[c]Acetate buffer.

[d]Citrate-phosphate buffer.

ed DMA in both malt and beer as one of the major volatile amines; quantitative determinations of DMA in beer were in the range of 0.07-0.69 µg/ml. TMA, however, was not found in malt; it presumably was found in beer, but not as a major component. Mangino et al. (1981) estimated the DMA content of beer from previous reports, after correction for dilution to be 1-5 ppm.

Recently, French et al. (1982) used an acid extraction method to determine DMA in raw barley, green malt, kilned malt and malt roots. The values for DMA obtained by the acid extraction method were: 0.1, 1.4, 3.9, 1.8 and 54 µg/gm for raw barley, green malt, kilned malt, green malt roots and kilned malt roots, respectively. This study suggests that NDMA production occurs during the kilning operation.

Two secondary metabolites, hordenine and gramine, which are formed in malt by biosynthetic activity during germination might be potential precursors for NDMA. Hordenine, one of the major tertiary amine alkaloids found in malt, is formed biosynthetically from tyrosine. This biosynthesis leading to hordenine is reported to operate only in rootlets (Schneider and Wightman, 1974). Hordenine is the principal alkaloid formed in malt roots during germination.

In 1965, McFarlane carried out a quantitative study on hordenine in malt and malt fractions. Malt roots contained a high level of hordenine, 1600 ppm. The malt acrospires and clean malt also contained considerable levels of hordenine, 217 ppm and 67

ppm, respectively. Hordenine has been very frequently suggested as a NDMA precursor since large amounts have been found in malt and malt roots (Spiegelhalder et al., 1980; Kann et al., 1980; Slack and Wainwright, 1981). Spiegelhalder et al. (1980) proposed that the reaction between $(NO)_x$ gases in the drying air and either DMA, or a germination product such as hordenine, could lead to the formation of NDMA. Kann et al. (1980) suggested that hordenine is the more likely source of NDMA in malt.

Gramine is the other major tertiary amine alkaloid found in malt; it is found in malt acrospires after germination. Schneider and Wightman (1974) detected gramine in barley shoots.

Slack and Wainwright (1981) showed that gramine could be nitrosated to produce NDMA, but the yield was not reported. Mangino and Scanlan (1982) nitrosated gramine at 65°C for 16 hr. with nitrite in aqueous buffer solutions at pH 4.4 and pH 6.4 as shown in Table 1. The results indicated that gramine is extremely susceptible to nitrosation at pH 4.4.

The expected values of NDMA from the malt alkaloids hordenine and gramine were, however, far less than the total values for NDMA obtained from the nitrosation of the same malt (Poocharoen, 1984). This investigation on the precursors of NDMA in malt indicated that in addition to hordenine and gramine, DMA might be a potential precursor of NDMA. Therefore, experiments designed to determine the levels of DMA at each stage of the malting process, and to obtain the expected values of NDMA from DMA in the malt, are

the subjects of this thesis.

## E. Methods for Measurement of DMA in Malt

A number of analytical procedures, for example: colorimetric, spectrophotometric, and gas chromatographic methods, for the detection of secondary amines have been developed.

### Colorimetric Methods

A colorimetric method for the detection of secondary amines was developed by Dyer (1945). He determined DMA and TMA in fish muscle as the picrate salt; DMA and TMA can form the yellow colored picrate by mixing with picric acid reagent.

Another colorimetric method is based on the formation of blue-violet compounds when secondary aliphatic amines react with sodium nitroprusside and acetaldehyde. In 1957, Sweeley and Horning utilized these blue-violet derivatives for the qualitative identification of secondary amines. Lin and Wagner (1974) further developed this reaction into a simple, fast and specific method for the quantitative measurement of small amounts of secondary amines.

Because the colorimetric procedures described above have several problems with, for example; optimal color production, sensitivity, reproducibility and interference by primary and tertiary amines, it was not considered desirable to use a colorimetric method for measurement of DMA in malt.

## Atomic Absorption Spectrophotometric Methods

Atomic absorption spectrophotometry was used by Oles and Siggia (1973). The practical detection limit of this procedure is approximately 0.30 µl/mol of secondary amine per ml of solution. Dialkyldithiocarbamic acid (DTCH) was first produced by reaction of secondary amines with carbon disulfide, and then nickel dialkyldithiocarbamate [$Ni(DTC)_2$] was formed from DTCH and nickel. $Ni(DTC)_2$ was digested in a 1:1 mixture of $HNO_3$ and HCl. The resultant solution was then analyzed for nickel content by means of conventional atomic absorption spectrophotometry. Karweik and Meyers (1978) also used the DTC complex which forms a stable complex with metal ions.

The atomic absorption spectrophotometric method also has problems with sensitivity, reproducibility and interference from other amines.

## Gas Chromatographic Methods

In the analysis of DMA in malt, the low concentration and the probable complexity of the mixture indicated gas chromatography (GC) as the method of choice.

Practically, the analysis of amines by GC is not easy; for example these compounds are sometimes adsorbed by the column and they can cause severe tailing. Many investigators have modified the column packing to minimize these problems.

Alkali pretreatment of stationary phases played a major role in the development of satisfactory GC packings for the separation of aliphatic diamines (James, 1952; Smith, 1961). Decora and Dinneen (1959) coated extracted Tide with 10% by weight of potassium hydroxide (KOH) and obtained marked improvement in the separation of aliphatic amines. Ring and Riley (1958) used Flexol-8N8 on KOH-treated firebrick for amine separation, but this column bled excessively and could not be used on higher boiling diamines.

In 1974, Di Corcia and Samperi measured methylamine in water using a column consisting of 4% Carbowax + 0.8% KOH on Carbopack B (graphitized carbon black). Raulin (1980) analyzed nanogram levels of aliphatic amines using 6% Carbowax-20M and 0.8% KOH on Carbopack B; the reproducibility was good for ppm levels of aliphatic amines.

Although alkali-treated supports and liquid phases for GC column packing were developed to decrease peak tailing, the direct gas chromatography of alkylamines is still difficult due to their polar nature. Chemically derivatized alkylamines however, usually exhibit good peak shape and sensitivity (Ripley et al., 1982). In 1976, Singer and Lijinsky described a procedure for the analysis of naturally occurring secondary amines. Gas chromatography-mass spectrometry (GC-MS) was carried out after steam distillation and clean-up of tosylamide derivatives of the secondary amines. Derivatization reduces extreme volatility and allows facile analysis by GC and GC-MS. Hamano et al. (1981) also used derivati-

zation, and they established a simple GC method for determining naturally occurring secondary amines. Secondary amines separated from foods by extraction with hydrochloric acid were readily converted into the corresponding sulfonamides by reaction with benzene sulfonyl chloride under alkaline conditions. Gas chromatography was carried out on a capillary column coated with OV-101 and detection utilized a flame photometric detector. The limit of detection for DMA was 0.002 ppm.

Tilden and Van Middelem (1970) suggested that benzamide derivatives were easy to prepare, possessed thermal and chemical stability during GC analysis, and electron capture provided a sensitive method for detection. In 1982, Ripley et al. used the derivatization of mono- and dialkylamines using pentafluorobenzoyl chloride. An analytical method was described for the determination of DMA in raw barley, malt and beer. They obtained DMA by distilling a mixture of ground malt and 50% NaOH. The distillate was collected in 1 N HCl, after which DMA was converted to the pentafluorobenzamide derivative for analysis by GC. The DMA levels obtained represented more than the unbound (free or volatile) DMA, since the alkaline distillation method was reported to also release DMA from DMA-yielding compounds in the malt, such as hordenine and gramine.

French et al. (1982) used both alkaline distillation and acid extraction methods to determine DMA in raw barley, malt and malt

roots. For the acid extraction method, they extracted the malt sample with 0.1 N HCl. Following filtration the filtrate was then reacted with pentafluorobenzoyl chloride to yield the pentafluorobenzamide derivative for GC analysis. The values for DMA obtained from this method were considered as unbound or free DMA since the acid used would not release DMA from other compounds.

The results described above indicated that DMA can be derivatized to pentafluoro-N,N-dimethylbenzamide (DMA-PFB) which can be separated by GC and detected using an N-P detector.

It is now generally accepted that mass spectral analysis is essential for unequivocal confirmation of the identity of volatile amines (Andrzijewski et al., 1981) since it is difficult to identify them solely by their GC retention times. Mass spectrometry can also provide valuable structural information on amine derivatives by using characteristic fragmentation patterns (Saxby, 1968). Pentafluorobenzamide derivatives were examined by Ripley et al. (1982)using GC-MS and were found to be mono-substituted. They reported that the base peak in all cases was m/z 195, representing the pentafluorobenzoyl (PFB) group. Full-scan or selected-ion-monitoring of m/z 195 and the molecular ion of the derivatized amine constituted confirmation.

In conclusion, this review indicated that GC is the method of choice for the analysis of DMA in malt. It was concluded that it would be useful to utilize a modified GC packing (to minimize

tailing), and to derivatize the DMA prior to GC analysis. Confirmation of the DMA from malt would be by GC-MS.

# III. EXPERIMENTAL

## A. Samples and Reagents

The samples used in this study were raw barley, green and kilned malt and these were obtained from the Great Western Malting Co., Vancouver, Washington.

The following compounds and reagents were obtained from the Sigma Chemical Co.: gramine and hordenine hemisulfate. Sarcosine, dimethylamine hydrochloride, trimethylamine hydrochloride and pentafluorobenzoyl chloride were purchased from the Aldrich Chemical Co. Hydrochloric acid was purchased from the J.T. Baker Chemical Co. ("Baker Analyzed" grade), and benzene was obtained from the Burdick and Jackson Co. The methylethylamine used as the internal standard was purchased from the Eastman Kodak Co. Standard solutions of dimethylamine were prepared in 0.1 N HCl.

## B. Extraction and Derivatization of DMA from Malt

The method adopted in this study was developed based on modifications from procedures of previous reports (Ripley et al., 1982). The extraction and derivatization procedure is shown in Figure 3. A 10 gm sample of kilned malt was ground in an Osterizer blender, and then 100 ml of 0.1 N HCl was added. The mixture was shaken for 30 min., and then centrifuged for 10 min. at 1,000 x g. The centrifuged mixture was then filtered through a Whatman folded 2V filter paper; then 9 ml of benzene, 5 ml of 2 M $K_2CO_3$, and 1 ml

Figure 3. Schematic procedure for extraction and derivatization of DMA from malt

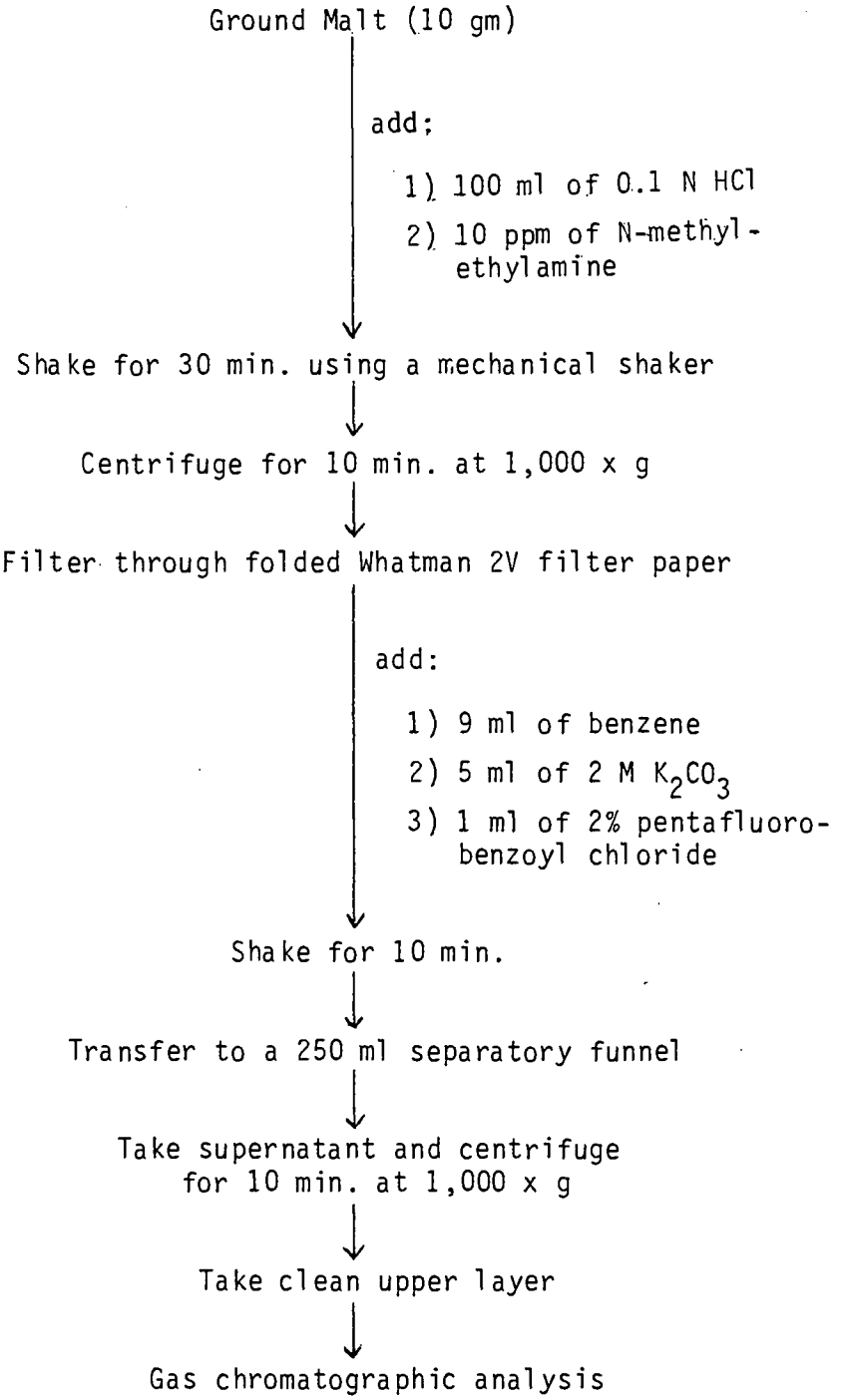

Ground Malt (10 gm)
add;
1) 100 ml of 0.1 N HCl
2) 10 ppm of N-methyl-ethylamine
Shake for 30 min. using a mechanical shaker
Centrifuge for 10 min. at 1,000 x g
Filter through folded Whatman 2V filter paper
add:
1) 9 ml of benzene
2) 5 ml of 2 M $K_2CO_3$
3) 1 ml of 2% pentafluoro-benzoyl chloride
Shake for 10 min.
Transfer to a 250 ml separatory funnel
Take supernatant and centrifuge for 10 min. at 1,000 x g
Take clean upper layer
Gas chromatographic analysis

Figure 3

of 2% pentafluorobenzoyl chloride in benzene were added to the filtrate. The flask containing the filtrate was stoppered and shaken for 10 min. on a wrist action shaker. The solution was transferred to a 250 ml separatory funnel, and the supernatant was removed for GC analysis. Standard solutions of known concentrations of DMA were prepared in 0.1 N HCl. These solutions were extracted and derivatized as described above.

## C. GC Determination of DMA

Gas chromatographic (GC) analyses were performed on a Varian model 3700 which was coupled to a thermionic N-specific detector. A 6 ft x 1/12 in. ID glass or nickel column packed with Ultra-Bond 20M was used. The glass column used was commercially available, but the nickel column was packed in the laboratory using commercially available packing. The analytical conditions for GC are shown in Table 2.

Gas chromatography-thermal energy analysis (GC-TEA) was carried out on a Varian model 1400 GC coupled to a Thermal Energy Analyzer model TEA-502 from the Thermo Electron Corp. The GC column was a 10 ft x 1/8 in. ID stainless steel column packed with 20% Carbowax 20M plus two per cent NaOH coated on 100/120 mesh Chromosorb W-AW. The column was operated isothermally at 170°C and the helium gas flow rate was 30 ml/min.

TABLE 2. Analytical Conditions for Gas Chromatography

| | |
|---|---|
| GC model | Varian 3700 |
| Column | 6 ft x 1/12 in. ID |
| Packing material | Ultra-Bond 20M |
| Carrier gas | Helium |
| Flow rate | He : 24 ml/min<br>$H_2$ : 3.63ml/min<br>air: 150 ml/min |
| Injection temperature | 250°C |
| Column temperature | 78°C |
| Detector | Thermionic specific detector |
| Sample size | 1 µl |

## D. Determination of Recovery

Freeze-dried Idaho Morex malt was used in this study. Freeze-dehydration of green malt was carried out in a Hull pilot scale freeze-dehydration unit. The temperature of the malt was approximately 18°C throughout the freeze-drying operation. One sample served as a blank, while the other three samples were spiked with standard dimethylamine at: 2 µg, 4 µg, and 10 µg/gm of sample. The added dimethylamine was recovered from the freeze-dried Idaho Morex malt using the extraction and derivatization procedure described in Section B above. The recovered dimethylamine from the spiked samples was quantitatively determined using the GC-thermionic N-specific detector in comparison with the peak height of the original standard solution. The amount of dimethylamine in each sample as well as the per cent recoveries were then calculated.

## E. Confirmation of Identity for DMA Extracted from Malt

Mass spectral confirmation was accomplished using a Finnigan 1015C quadrupole spectrometer interfaced to a Varian model 1400 GC. The GC-MS was interfaced to a Riber 400 data system. This system included a Digital Equipment Corp. PDP 8/E minicomputer, a Diablo 31 disk system, a Tektronix 4010-1 display terminal, and Complot digital plotter. The GC column was a 8 ft x 1/12 in. ID nickel column packed with Ultra-Bond 20M. The column was operated iso-

thermally at 105°C with a flow rate of 17 ml He/min., and an injection port temperature of 208°C. A Finnigan 1015C quadrupole mass spectrometer was used with the following conditions; 450 μA filament current; 2.9 KV electron multiplier setting and mass scans covered the range of m/z 25-270.

An electrically dried 81-Morex was used for this study. The sample was extracted and derivatized as described in Section B, EXPERIMENTAL. For GC-MS analysis, the final upper layer was evaporated under a stream of nitrogen gas to concentrate the derivative to about 100 μg in 0.5 μl. Standard solutions of DMA derivative in 0.1 N HCl were also evaporated under nitrogen to achieve a concentration of about 100 μg in 0.5 μl. Prior to GC-MS analysis, the evaporated samples were checked by GC to verify that they had a peak with the same retention time as the standard DMA derivative and also to see if the concentration was appropriate for GC-MS analysis.

## F. Quantitative Determination of Dimethylamine in Malt, Green Malt, Malt Roots and Raw Barley

### Part 1: Survey of Dimethylamine in Commercial Barley Malts

Commercial barley malts of Klages, Morex and 2 Row Blend were used in this experiment. Klages samples (See Table 5) were divided into 4-day and 5-day germination samples. Samples #1-18 were grown in South Idaho and #19-24 were grown near Klamath Falls.

All the samples were from different lots of a commercial malting process where $SO_2$ has been used to decrease the formation of NDMA and they were all "direct-fired" malted barleys. A 10 gm sample of ground malted barley was used for analysis, and DMA was extracted and derivatized according to the procedure described in Section B, EXPERIMENTAL.

Part 2: Determination of Dimethylamine Biosynthesized during the Malting Process

Three varieties of raw barleys were chosen; Klages, Morex and Piroline. The raw barleys were analyzed for DMA before germination, and then the raw barleys were carried through the pilot steeping, germination, and kilning procedures at the Great Western Malting Co. The following steps were carried out to prepare the samples used in this experiment.

Cage Malting Procedure

Experimental barley samples consisted of: two 1982 CB Klages (See Table 7, sample #1 and #2), one 1983 CB Morex (sample #3) and 1983 NW Piroline (#4). These samples were processed through the normal regime of production conditions in what were termed "cages" which were perforated stainless steel containers. Each cage was equipped with a chain so as to suspend it in the steep tank or in a compartment, thereby exposing the enclosed barley to conditions similar to those experienced by production barley. All the samples

were processed simultaneously under identical conditions.

## Steep

Cages were steeped for a total of 52 hr. with alternating steep (submerged), and couch (draining) periods. 'Steep' samples were obtained prior to placing the cages in the compartment house for germination.

## Germination

The steeped barleys were in germination for four days. On the first day, samples #2, #3 and #4 were treated with gibberellic acid at a concentration of 0.10 ppb on a dry weight basis for barley in the cage. The contents of each cage were manually aerated and watered to approximately 45% moisture. In regular production, this was accomplished automatically by water sprays and augers moving through the germinating bed in the compartment. As germination was completed, the cages were returned to the lab for pilot kilning. At this point germinated samples were obtained.

## Kilning

All four cages were kilned simultaneously in an indirect electric kiln for 16 hr. at 60°C, and then for an additional 4 hr. at 85°C until the moisture content was about four per cent. The resulting malts were "uncleaned", and therefore still had rootlets. This pilot kilning procedure approximated the commercial kilning

process, except that the malts were not exposed to nitric oxide gases $(NO)_x$ and were free of $SO_2$. Wet samples (both steep and germination) were frozen immediately upon receipt.

The rootlets of electrically dried malts were manually removed on a precision sieve (slotted, 0.09 x 9.75 in. ); this yielded clean malt. This manual removal did not totally separate husk and shoot from the rootlets. To remove remaining husk and shoot from the rootlets, a screen (opening, 0.0232 in.) was used. All moisture determinations were done gravimetrically using a two-stage oven method, except for the finished malt which employed a single stage oven treatment. Oven temperature was 103°C. Wet samples, (steep and germination), were frozen thoroughly in liquid nitrogen prior to removal of roots and grinding the sample.

Raw barley, steeped barley, clean germinated malt (green malt), green malt roots, kilned clean malt and kilned malt roots were analyzed for their DMA content according to the procedure described in Section B, EXPERIMENTAL. The values found for DMA were reported on a dry weight basis.

Part 3: Comparison of the Values of NDMA Obtained from the Nitrosation of Clean Malt with the Expected Values

Five varieties of barley, Winter, Morex, Klages, Piroline and Steptoe, which were grown under the same conditions of temperature, moisture, fertilizer, etc. were selected for this experiment.

The raw barleys were carried through the pilot steeping, germination and kilning procedure according to Part 2 above.

After the green malt for each variety was obtained, it was cooled with ice for shipment to the Department of Food Science and Technology where it was quickly blast frozen. The samples were then freeze-dried at room temperature until the moisture content was reduced to about four per cent. The second portion of each variety was dried by indirect electric heat, as described in Part 2 above, until the moisture content was also about four per cent.

These malts were free of $(NO)_x$ and $SO_2$. The levels of hordenine and gramine in these clean malts had been determined by Poocharoen (1984) utilizing high performance liquid chromatography (HPLC). These five varieties of clean malts were extracted and derivatized according to the procedure described in Section B, EXPERIMENTAL.

# VI. RESULTS AND DISCUSSION

## A. Extraction and Derivatization of DMA from Malt

The gas chromatograms obtained for DMA and N-methylethylamine are given in Figures 5, 6 and 7. These chromatograms indicated that the malt extracts obtained by this method were sufficiently clean so that separation, identification and quantitation could be done accurately by GC. The amounts of DMA extracted from a Klages malt are shown in Table 3. The mean value for five replications on this malt was 6.0 μg/gm for DMA. The variance and the standard deviation for DMA were both very small. This indicates that the method was capable of good reproducibility (See Table 3).

A variety of different derivatization procedures have been used to determine amines by GC. Typical analyses involve benzylation, dansylation and trifluoroacetylation. Lodge and barber (1961) prepared benzamides to determine aliphatic amines. Benzamides were chosen for this experiment, because they were found to be convenient to prepare, and also this derivative exhibited good thermal and chemical stability during GC analysis.

The results indicated that the malt extracts obtained by this method could be separated and quantitated accurately by GC.

## B. Determination of Recovery

The mean recovery for DMA over a range of concentrations is shown in Table 4; the recovery ranged from 95 to 99 per cent.

TABLE 3. Amounts of DMA Extracted from Different Samples of the Same Klages Malt

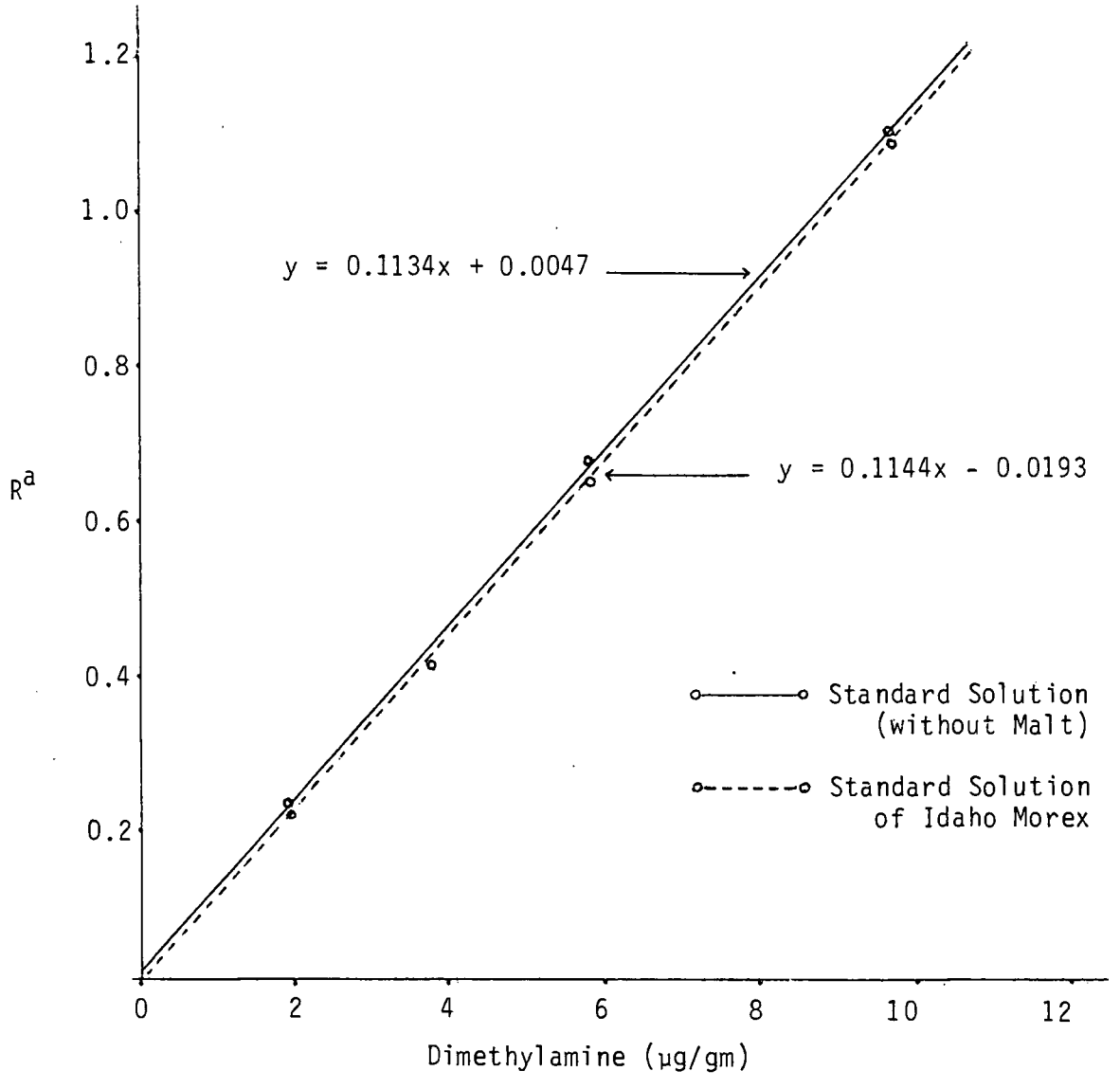
| Sample No. | DMA (µg/gm) |
|---|---|
| 1 | 5.9 |
| 2 | 6.0 |
| 3 | 6.3 |
| 4 | 6.0 |
| 5 | 5.8 |
| $\bar{X}^a$ | 6.0 |
| $S^{2b}$ | 0.04 |
| $S^c$ | 0.19 |

[a]Mean.

[b]Variance.

[c]Standard deviation.

Figure 4. Standard Curve for Dimethylamine




$$^aR = \frac{\text{Peak Value for Dimethylamine - Blank}}{\text{Peak Value for Methylethylamine (Internal Standard)}}$$

Figure 4

Figure 5. Gas chromatographic analysis using the pentafluorobenzoyl derivative for DMA from Morex malt extract

Conditions: Glass column packed with Ultra-Bond 20M[a]

A: Malt extract

B: Malt extract spiked with DMA

Peak assignment: #1 - Pentafluorobenzoyl-dimethylamine

[a]see Table 1

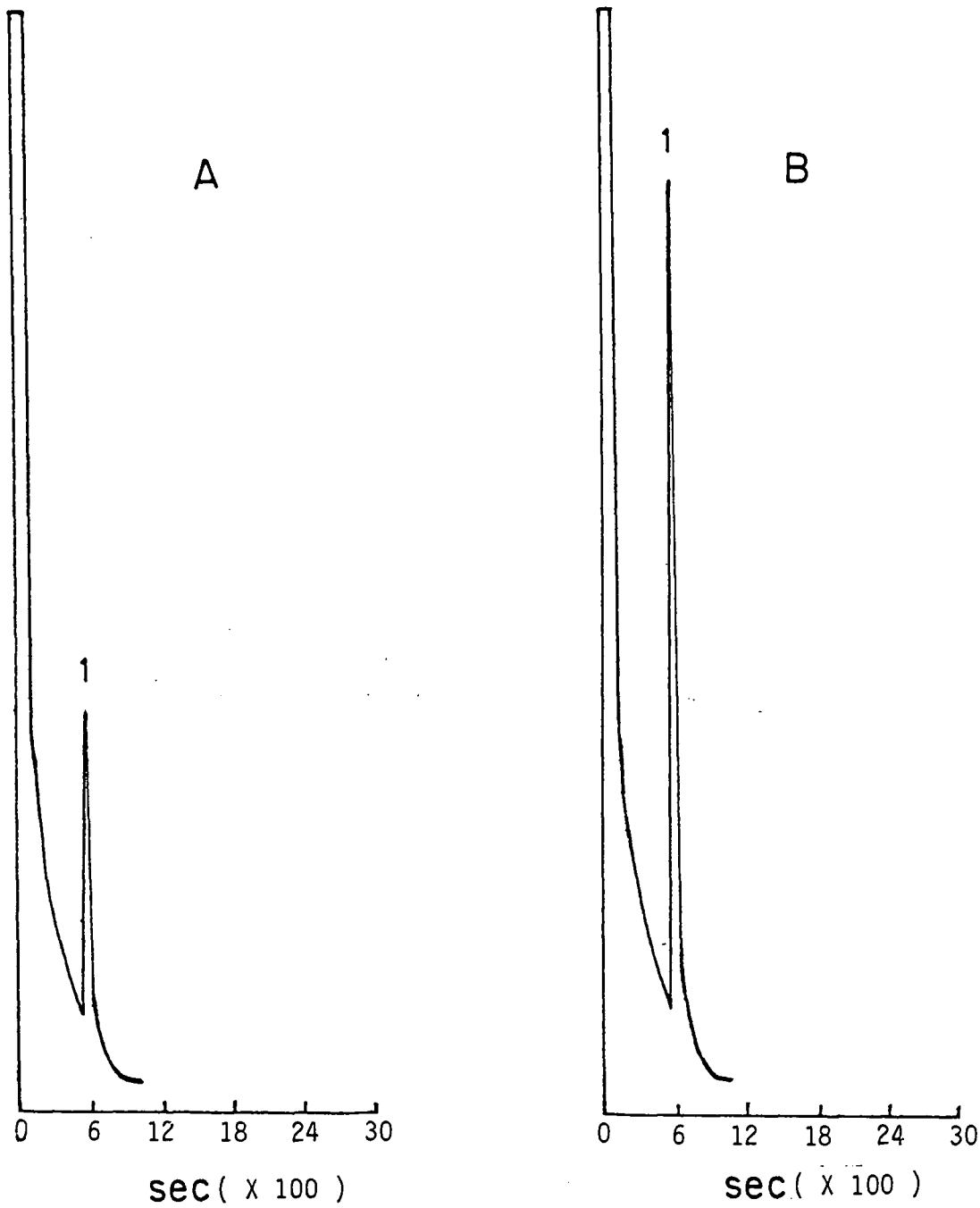



Figure 5

Figure 6. Gas chromatographic analysis using the pentafluorobenzoyl derivative for DMA and N-methylethylamine in Morex malt extract

Conditions: Glass column packed with Ultra-Bond 20M[a]

A: Malt extract

B: Malt extract spiked with N-methylethylamine (peak #2)

[a]see Table 1

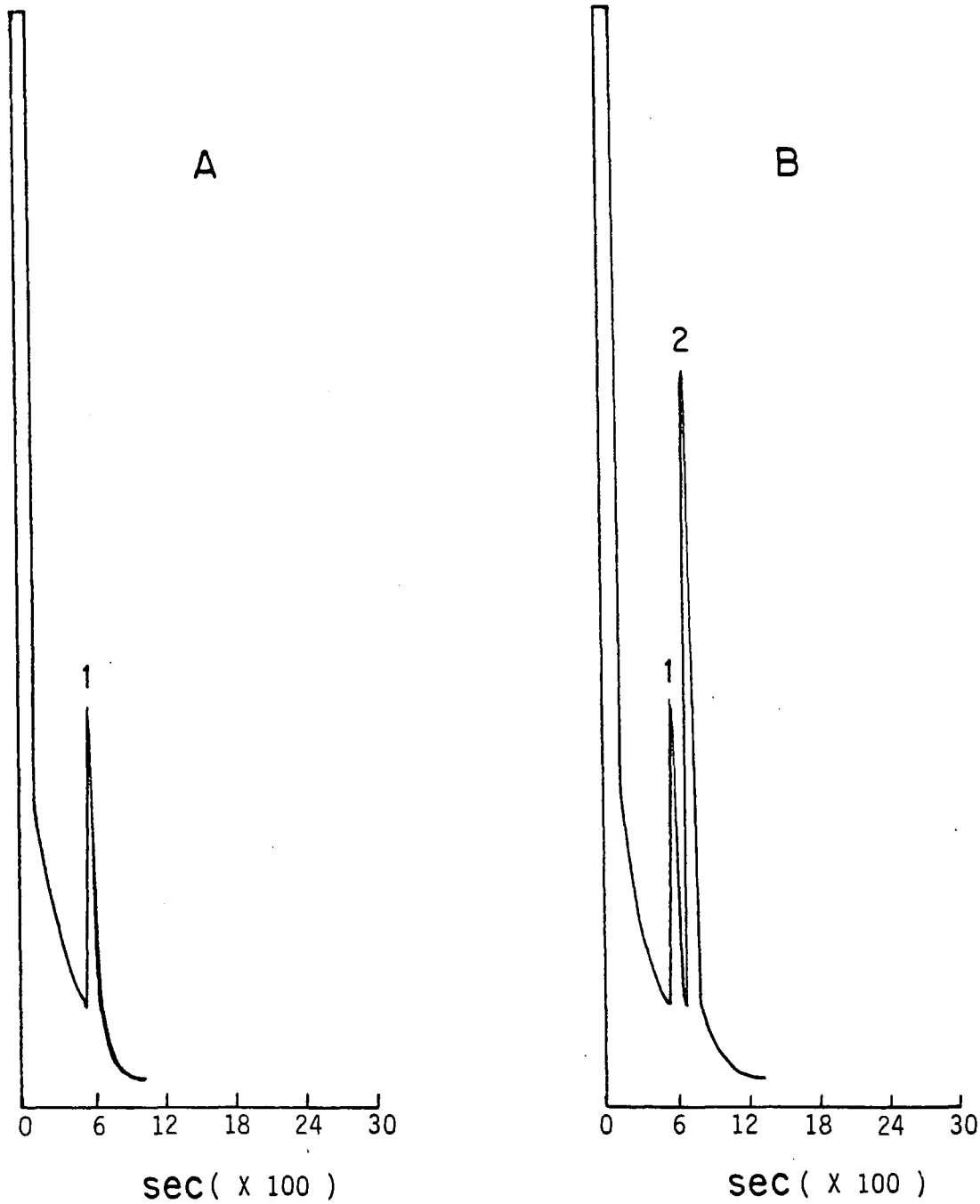



Figure 6

Figure 7. Gas chromatographic analysis using the pentafluorobenzoyl derivatives for DMA from Morex malt and standard solution of DMA and N-methylethylamine derivatives

Conditions: Glass column packed with Ultra-Bond 20M[a]

A: Standard DMA and N-methylethylamine solution

B: Malt extract spiked with N-methylethylamine (internal standard)

Peak assignment: #1 - Pentafluorobenzoyl-dimethylamine

#2 - Pentafluorobenzoyl-N-methylethylamine

[a]see Table 1

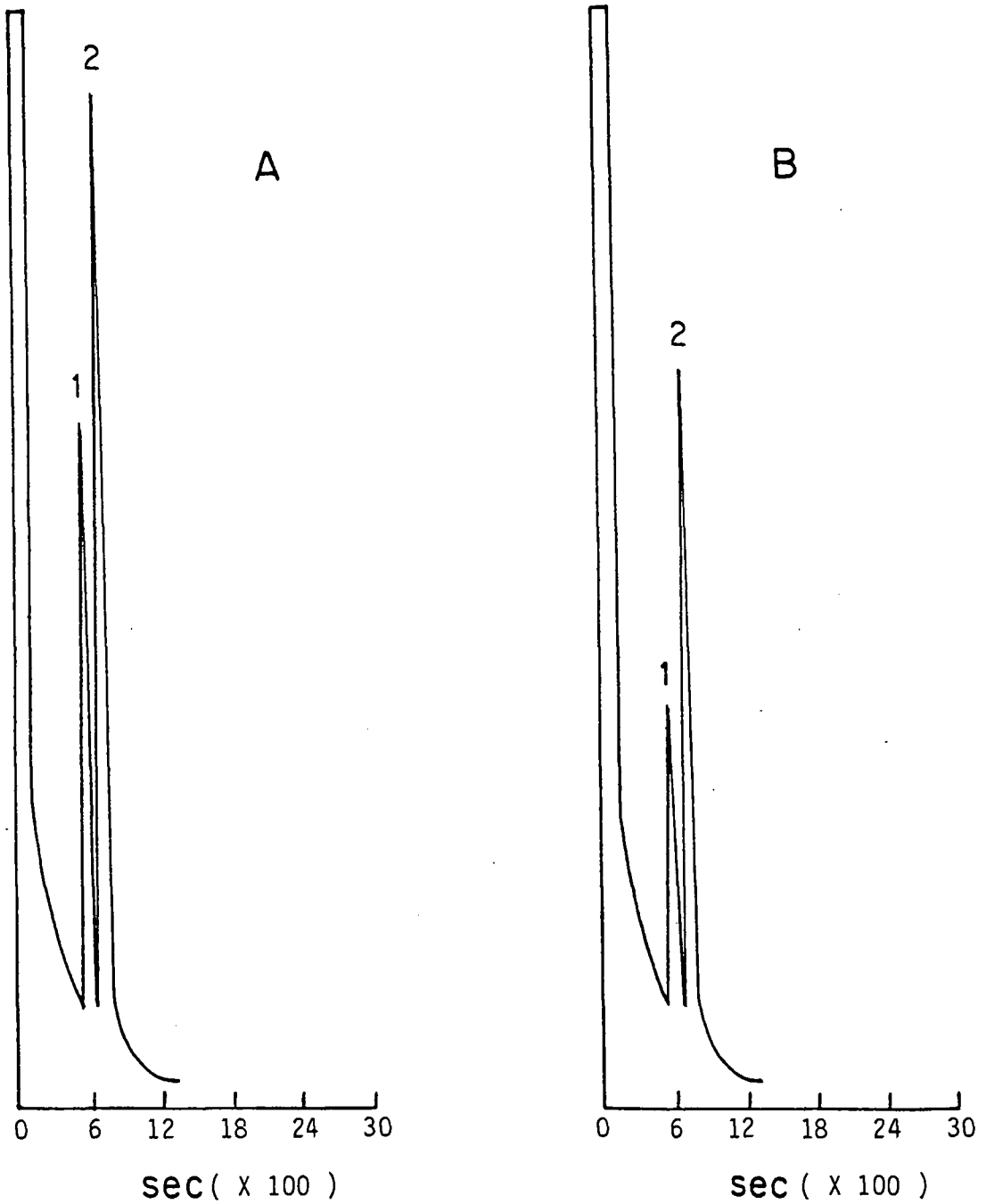



Figure 7

TABLE 4. Recovery of Added Dimethylamine from Idaho Morex Malt Using Acid Extraction

| Dimethylamine·HCl (µg) Added | Found | Recovery (%) |
|---|---|---|
| 0 | 1.8 | |
| | 1.7 | |
| | 1.8 | |
| | 1.8±0.1[a] | - |
| 2 | 3.9 | |
| | 3.7 | |
| | 3.6 | |
| | 3.7±0.2 | 95[b] |
| 4 | 5.5 | |
| | 5.7 | |
| | 5.6 | |
| | 5.6±0.1 | 95 |
| 10 | 11.7 | |
| | 11.5 | |
| | 11.8 | |
| | 11.7±0.2 | 99 |
| Average | | 96.3 |

[a] Mean ± standard deviation.

[b] $\frac{\text{Found (µg)} - \text{Blank (µg)}}{\text{Added DMA (µg)}} \times 100$

The recovery was estimated as shown in Table 4 (footnote b); the average recovery was 96.3 per cent.

Hamano et al. (1981) isolated secondary amines from foods by extraction with dichloromethane, re-extraction with hydrochloric acid, and conversion to the corresponding sulfonamides by reaction with benzenesulfonyl chloride under alkaline conditions. They used gas chromatography with a capillary column coated with OV-101 and flame photometric detection. The column temperature was programmed from 170 to 230°C at a rate of 5°C/min. Their mean recovery rates for DMA were; 71.3% (from miso), 74.1%(cod roe), 80.2%(baked ham), 82.8% (fish sausage), and 91.5% (spinach). In another study, DMA from minced red hake were analyzed by GC on a porous polymer packed column using a nitrogen-phosphorus specific flame ionization detector (Lundstrom et al., 1983). Recovery rates for DMA from red hake in the range; 60.0 to 64.5% (using N-amylalcohol as the organic solvent), and 99.7 to 109.7% (using benzene as the organic solvent). From this report, it is evident that benzene extraction gave a very high recovery rate.

Linearity curves (Figure 4) were plotted as the ratio of the component peak height to the internal standard peak height as a function of concentration to eliminate the influence of injection volume and detector sensitivity on absolute height measurements. Figure 4 shows the linearity of the thermionic N-specific detector response to DMA. Each data point represents the mean of triplicates. The range of 0-10 μg/gm approximates the range of DMA

which might be found in barley and barley malt. The linear regression equations were calculated; $y = 0.1134x + 0.0047$ (for standard DMA solution); $y = 0.1144x - 0.0193$ (Idaho Morex malt extract). Correlation coefficients for the regression lines were 0.999 (Standard DMA solution), and 0.996 (Idaho Morex malt extract); both coefficients were highly significant ($P \leqslant 0.001$).

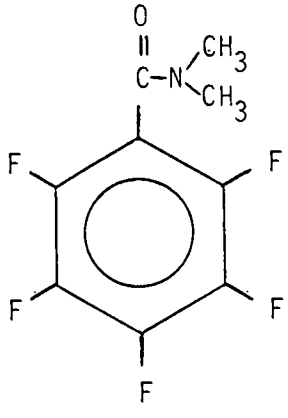
The recovery rates for this experiment indicated that for the procedure used in this study, DMA was extracted to a high degree and the method produced a reasonable recovery for DMA.

## C. Confirmation of Identity of DMA Extracted from Malt

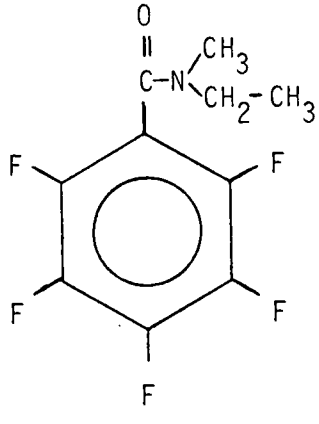
The identity of DMA found in samples was confirmed by GC and GC-MS. Retention times (RT) for DMA and N-methylethylamine (internal standard) could vary day to day, but were reproducible for each analysis on a single day. For the GC conditions in Table 2, the RT of DMA was 550 sec. and the RT for N-methylethylamine was 682 sec. The GC chromatograms given in Figure 5 for Morex malt extract show that the peak height was enhanced when spiked with DMA. Figure 6 shows malt spiked with N-methylethylamine which was the internal standard.

The mass spectra for the standard DMA derivative and for the malt-extracted DMA derivative are shown in Figure 9 and 10, respectively. GC-MS of the higher molecular weight derivative was easily accomplished. A flexible nickel column was prepared for GC-MS analysis because of some configurational changes in the GC

Figure 8. Structures for the pentafluorobenzoyl derivatives of dimethylamine and N-methylethylamine



Molecular weight
239



Molecular weight
253

Figure 8

Figure 9. Mass spectrum of standard dimethylamine derivative

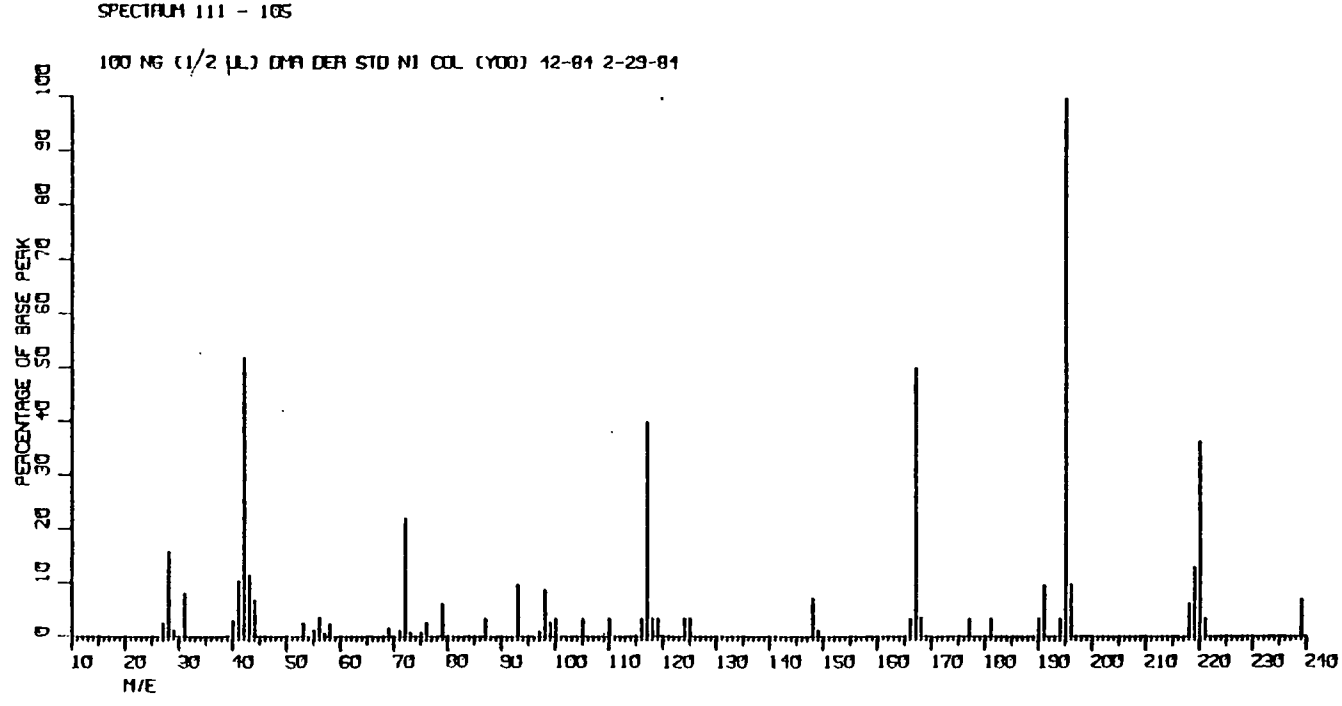

SPECTRUM 111 - 105
100 NG (1/2 µL) DNA DER STD N1 COL (Y00) 12-84 2-29-84
PERCENTAGE OF BASE PEAK
0
10
20
30
40
50
60
70
80
90
100
M/E
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240

Figure 9

Figure 10. Mass spectrum of dimethylamine derivative from malt extract

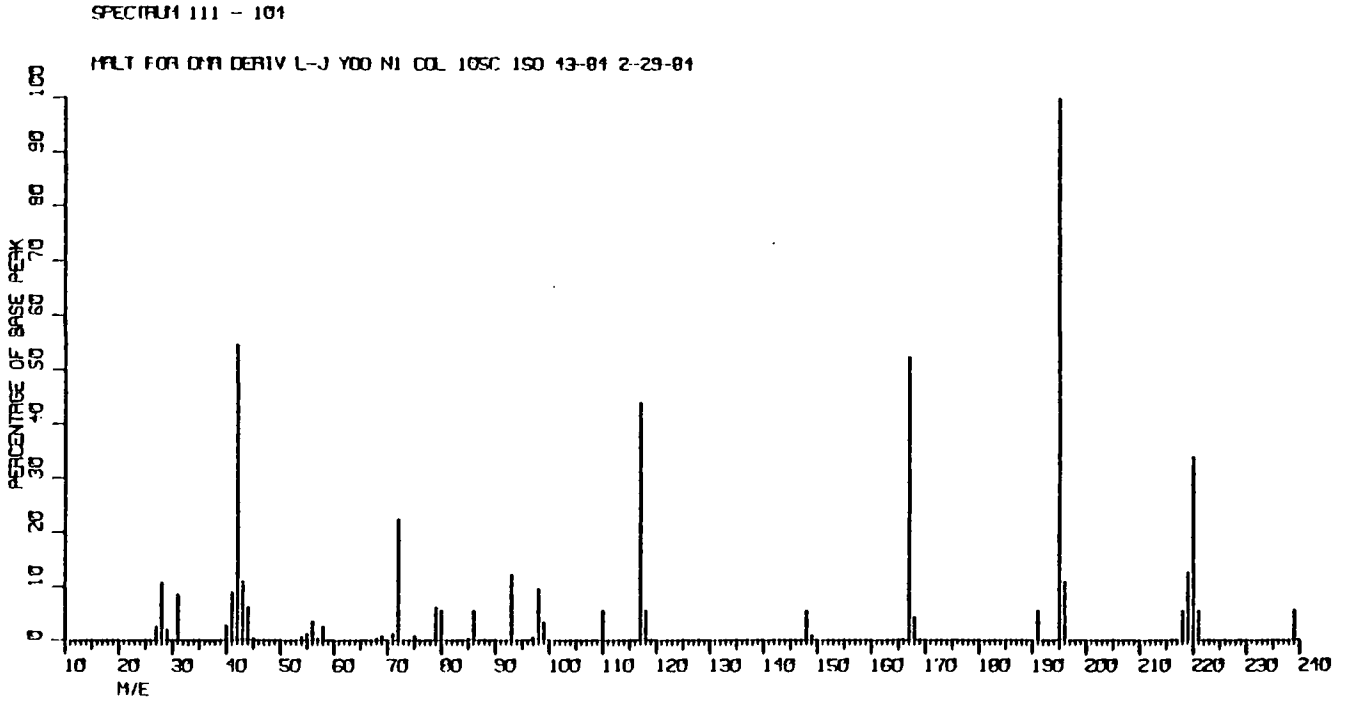

SPECTRUM 111 - 101
MALT FOR DYR DERIV L-J YOO NI COL 105C 15O 13-81 2-29-81
PERCENTAGE OF BASE PEAK
0
10
20
30
40
50
60
70
80
90
100
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
M/E

Figure 10

(Varian model 1400) connected to mass spectrometry. The nickel column was also used for GC in the Varian model 3700 to compare results with the glass column. The results indicated that the nickel column was also highly effective for GC analysis.

Ripley et al. (1982) also examined the mono-substituted DMA derivative. The base peak was m/e 195, representing the pentafluorobenzoyl (PFB) group; common fragment ions from PFB were observed at m/e 167 (-CO), 148, 136 and 117 (Figure 10). The derivative exhibited the expected molecular ion although all ion intensifies above m/e 195 were weak (less than 25%). The derivative fragmented as expected; it gave an ion for the loss of a single flourine atom (m/e 220) and it also gave an ion at m/e 195 ($M^{+}$-44) for the loss of the parent amine. The mass spectrum for the peak from malt extract (Figure 10) also indicated a molecular ion at m/e 239, a base peak at m/e 195, and other important fragment ions at m/e 167, 148 and 117 which agreed well with the spectra obtained from standard DMA (see Figure 9). These results very strongly suggest that the peak obtained from malt extract was DMA.

In conclusion, GC chromatograms and mass spectrometric analysis both verify that DMA exists in malt, comfirming earlier reports.

## D. Quantitative Determination of DMA in Malt, Green Malt, Malt Roots and Raw Barley

The extraction and derivatization method used for this study has been shown to be sufficiently precise and reproducible. The mass spectra of the DMA peak from malt extract verified that the peak obtained was indeed DMA. The method described above was then applied to the quantitative determination of DMA in malt, green malt, malt roots and raw barley.

### Part 1: Survey of DMA in Commercial Barley Malts

The purpose of this experiment was to determine the level of DMA in commercial malted barleys; DMA was considered to be a precursor of NDMA in malted barleys. Commercial barley malts of Klages, Morex and 2 Row Blend were picked for this study because of their importance in the malting and brewing industries. The levels of DMA in 4-day and 5-day germination Klages malt are shown in Table 5. Among the 5-day germination Klages malt, No. 13-18 samples from South Idaho and No. 19-24 from Klamath Falls were compared with each other. The mean value for DMA in Klages from South Idaho was 11.65 μg/gm; from Klamath Falls, 11.7 μg/gm. The DMA level in South Idaho Klages and Klamath Falls Klages were not significantly different at the 95% condidence interval.

DMA and NDMA levels of 4-day and 5-day germination Klages samples were also compared with each other. The DMA mean value of

TABLE 5. DMA and NDMA Values in Commercial Malt Samples of Klages

| 4-day Germination Klages Malt | | |
|---|---|---|
| Sample Number | DMA (µg/gm) | NDMA (ng/gm) |
| 1 | 3.6 | 1.3 |
| 2 | 3.1 | 1.0 |
| 3 | 3.1 | 1.1 |
| 4 | 5.7 | 1.7 |
| 5 | 4.8 | 1.0 |
| 6 | 7.2 | 1.3 |
| 7 | 2.6 | 0.5 |
| 8 | 3.8 | 0.4 |
| 9 | 4.4 | 0.8 |
| 10 | 7.3 | 2.9 |
| 11 | 8.2 | 2.9 |
| 12 | 5.5 | 2.0 |

| 5-day Germination Klages Malt | | |
|---|---|---|
| Sample Number | DMA (µg/gm) | NDMA (ng/gm) |
| 13 | 12.4 | 2.0 |
| 14 | 10.9 | 3.9 |
| 15 | 14.1 | 4.0 |
| 16 | 12.6 | 4.3 |
| 17 | 9.8 | 4.8 |
| 18 | 10.1 | 5.5 |
| 19 | 10.5 | 9.4 |
| 20 | 12.7 | 4.3 |
| 21 | 11.4 | 5.2 |
| 22 | 9.0 | 5.5 |
| 23 | 13.7 | 5.8 |
| 24 | 13.0 | 8.8 |

Samples 1-18 were grown in South Idaho and 19-24 were grown in Klamath Falls.

TABLE 6. Results of a Survey of DMA Levels in Commercial Malt Samples

| Sample | DMA (µg/gm) |
|---|---|
| 2 Row Blend (A) | 9.0 |
| 2 Row Blend (B) | 6.6 |
| Morex (C) | 7.5 |
| Morex (D) | 2.9 |
| Morex (E) | 2.9 |
| Morex (F) | 4.8 |
| Morex[a] (G) | 2.0 |
| Morex[a] (H) | 1.9 |

A,B and H refer to different malting lots.

[a]Freeze dried malt, not commercial samples.

2 Row Blend is a commercial sample of 2 row barley.

4-day was 4.9 µg/gm; for 5-day, 11.7 µg/gm and the NDMA mean value of 4-day was 1.5 ng/gm; for 5-day, 5.3 ng/gm. Both DMA and NDMA levels in 5-day germination Klages were significantly ($P < 0.05$) higher than in 4-day germination Klages.

Sulfur dioxide or the burning of elemental sulfur during direct-fired kilning was applied to reduce NDMA in malt samples used in this study. The use of $SO_2$ during malt kilning can retard NDMA formation by at least two modes of action. First, $SO_2$ dissolves in the aqueous phase on the surface of green malt thereby forming acid which lowers the surface pH of the malt by as much as two pH units (O'Brien et al., 1980). The lower pH leads to an increase in the level of protonated amines; only amines in the unprotonated form can be nitrosated. Secondly, $SO_2$ in solution is in equilibrium with the bisulfite ion ($HSO_3^-$). The bisulfite ion is a reducing agent in food systems and may chemically reduce nitrosating agents on the surface of malt.

The results of a survey of DMA levels in commercial malt samples are shown in Table 6. The mean value for Morex malt was 4.5 µg/gm. Freeze-dried Morex malt contains 2.0 µg/gm of DMA which is low compared to commercially kilned Morex malt. Commercial barley malts analyzed in this survey contain from 2-14 ppm levels of DMA.

Part 2: Determination of Dimethylamine Biosynthesized during the Malting Process

There were three objectives for this experiment;

1. To determine the levels of DMA in raw barley and in samples after the steeping, germination and kilning steps;
2. To see if the kilning step changed the level of DMA; and
3. To determine the effect of gibberellic acid on the level of DMA in the malting process.

The malted barleys used in this experiment were kilned without contacting $(NO)_x$ and were also free of $SO_2$. The results for the determination of DMA in each malt fraction are shown in Table 7. Relatively low levels of DMA were found in raw barley and steeped barley. Green and kilned malt, and rootlets from these samples, contained elevated levels of DMA. This strongly suggests that DMA was formed during germination.

When assessing the relative contribution of DMA in clean malt and DMA in malt roots to the total amount of DMA, it must be kept in mind that the malt roots represented only 6.2% of the dry weight of the malted barley.

French et al. (1982) also examined the levels of DMA in each of the malting steps. They reported 1.8 µg/gm of DMA in green malt roots and 1.4 µg/gm of DMA in green clean malt. Those are very

TABLE 7. Determination of DMA in Different Stages of the Malting Process (µg/gm)

| Variety | Raw Barley | Steeped Barley | Green Malt | | Kilned Malt | |
|---|---|---|---|---|---|---|
| | | | Kernel | Root | Kernel | Root |
| Klages[a] | 1.0±0.1 | 0.6±0.1 | 17.7±0.5 | 762±36 | 6.0±0.2 | 605±29 |
| Klages[b] | 1.0±0.1 | 0.6±0.1 | 11.0±0.8 | 453±37 | 6.6±0.4 | 571±10 |
| Morex[b] | 0.8±0.1 | 0.5±0.1 | 9.5±0.2 | 442±30 | 6.2±0.3 | 637±10 |
| Piroline[b] | 0.6±0.1 | 0.5±0.1 | 8.2±0.6 | 396±14 | 4.8±0.3 | 614±38 |

[a]Gibberellic acid was not used on this sample.

[b]0.10 ppb concentration of Gibberellic acid was treated at the first day of germination.

N = 3 repetitions for all the samples except roots of Green Malt and Kilned Malt (N = 2).

low levels compared to the results shown in Table 7. They mentioned an increase in the amount of DMA after kilning and suggested that gramine in the acrospire, hordenine in the roots, or some other natural product could be the source of DMA, either alone, or in combination.

The plant hormone, gibberellic acid, was applied to decrease the germination period, as it stimulates the germination of seedling. One of the Klages samples which was treated with a 0.1 ppb concentration of gibberellic acid had a lower level of DMA than the other Klages malt without the gibberellic acid treatment. Additional analyses of more samples will be necessary to determine the true effect of gibberellic acid on the level of DMA in malt.

The high levels of DMA in green and kilned malt strongly suggests that DMA was biosynthesized during germination (Table 7). An alternate interpretation is that some compound other than DMA is biosynthesized and this compound degrades to DMA under the condition of analysis. To check this possibility, sarcosine, gramine, and hordenine which are known to be present in malt, were exposed to the analytical method used for DMA, and the extract was examined by GC to see if any of these amines degraded to DMA. Sarcosine and hordenine failed to show a peak corresponding to DMA, while gramine had only a small DMA peak which corresponded to 3% of the original gramine concentration. If the malts contained gramine, a 3% conversion for gramine to DMA would make very little difference in the level of DMA in malt. For example, if malt

contained 3 μg/gm of gramine, only 0.03 μg/gm of DMA would result from gramine by the extraction and derivatization method used in this study. Furthermore, the malts used in this study (Klages, Morex and Piroline) were reported not to contain any gramine (Poocharoen, 1984).

The high levels of DMA in the green malt and kilned malts strongly suggests that DMA is biosynthetically formed during germination. This conclusion is reinforced by the very high levels of DMA in the rootlet samples. It is known that the rootlets are the site of enhanced biosynthetic activity during germination.

Part 3: Comparison of the Values of NDMA Obtained from the Nitrosation of Clean Malt with the Expected NDMA Values from Hordenine, Gramine and DMA in the Same Malt

The primary objective of this experiment was to determine the relative roles of hordenine, gramine and DMA as NDMA precursors in malt. The results from the nitrosation of these malts would reveal whether the two alkaloids and DMA were the sole NDMA precursors.

The expected values for NDMA from DMA was calculated using the per cent yield of DMA from nitrosation of the pure compounds. The per cent yields of NDMA from nitrosation were determined by Poocharoen (1984): 2.7% for hordenine, 87.9% for gramine, and 92.3% for DMA. HCl. As shown in Table 8 and 9, the percentage of the expected values of NDMA from hordenine, gramine and DMA ranged from 80-146% for green clean malt, and from 86-174% for kilned

TABLE 8. Comparison of the Values of NDMA Obtained from the Nitrosation of Green Clean Malts with the Expected Values from Hordenine, Gramine and DMA

| Variety | DMA (µg/gm)[b] | Expected NDMA (µg/kg) from Hordenine[a] | Gramine[a] | DMA[c] | Total | NDMA (µg/kg)[a] from Nitrosation |
|---|---|---|---|---|---|---|
| Winter | 3.4±0.1 | 220(3%)[d] | 2,710(32%) | 5,161( 61%) | 8,091( 94%) | 8,436 |
| Morex | 2.9±0.2 | 366(6%) | - | 4,402( 74%) | 4,768( 80%) | 5,981 |
| Klages | 3.4±0.1 | 430(8%) | - | 5,161( 97%) | 5,591(105%) | 5,316 |
| Piroline | 1.8±0.1 | 116(6%) | - | 2,732(133%) | 2,848(139%) | 2,053 |
| Steptoe | 2.4±0.1 | 152(4%) | 1,746(46%) | 3,643( 96%) | 5,541(146%) | 3,795 |

[a]Data adapted from: Poocharoen, (1984).

[b]Amount of DMA determined in malt on dry weight basis (N = 3 repetitions).

[c]Calculated from 92.3% yield of NDMA (Poocharoen, 1984)

$$\text{DMA (µg/gm)} \times 1000 \times \frac{74}{45} \times \frac{92.3}{100} = \text{NDMA (µg/kg)}.$$

[d]Percentage ratio of expected NDMA (µg/kg)/NDMA from nitrosation of malt.

TABLE 9. Comparison of the Values of NDMA Obtained from the Nitrosation of Kilned Clean Malts with the Expected Values from Hordenine, Gramine and DMA

| Variety | DMA (µg/gm)[b] | Expected NDMA (µg/kg) from Hordenine[a] | Gramine[a] | DMA[c] | Total | NDMA (µg/kg)[a] from Nitrosation |
|---|---|---|---|---|---|---|
| Winter | 5.4±0.4 | 169( 2%)[d] | 2,953(29%) | 8,196(82%) | 11,318(113%) | 10,012 |
| Morex | 2.3±0.2 | 382( 9%) | - | 3,491(78%) | 3,873( 86%) | 4,484 |
| Klages | 2.3±0.1 | 504(11%) | - | 3,491(78%) | 3,995( 89%) | 4,496 |
| Piroline | 5.0±0.2 | 263( 3%) | - | 7,589(96%) | 7,582(100%) | 7,878 |
| Steptoe | 2.3±0.4 | 270( 8%) | 2,497(69%) | 3,490(97%) | 6,257(174%) | 3,599 |

[a]Data adapted from: Poocharoen, (1984).

[b]Amount of DMA determined in malt on dry weight basis (N = 3 repetitions).

[c]Calculated from 92.3% yield of NDMA (Poocharoen, 1984)

$$\text{DMA (µg/gm)} \times 1000 \times \frac{74}{45} \times \frac{92.3}{100} = \text{NDMA (µg/kg)}.$$

[d]Percentage ratio of expected NDMA (µg/kg)/NDMA from nitrosation of malt.

clean malts.

For each sample in Table 8 and Table 9, considerably more NDMA was produced from DMA than from gramine and hordenine. Although the data in Tables 8 and 9 must be considered approximate, the results strongly indicate that DMA is likely the principal precursor of NDMA in malt.

# V. SUMMARY AND CONCLUSIONS

Dimethylamine (DMA), in addition to the alkaloids hordenine and gramine, has been suggested as a potential precursor of nitrosodimethylamine (NDMA) in malt. Analytical procedures were adopted and applied to extract and quantify DMA from malt, green malt, malt roots and raw barley. Ground malt was extracted at room temperature with 0.1 N HCl, and the extract was derivatized with 2% pentafluorobenzoyl chloride in benzene. After purification, the derivatized DMA was quantitated by gas chromatography with a thermionic N-specific detector. The method yielded a 96.3 per cent recovery for DMA, and exhibited a linear relationship between DMA concentration and GC peak height within a given range. The identity of the DMA derivative was confirmed by mass spectrometry.

Commercial barley samples of 4 and 5-day germination of Klages variety were analyzed. The mean value for the 4-day germination Klages was 4.9, and for the 5-day, 11.7 µg/gm. The DMA level of 5-day germination Klages were significantly higher than the 4-day ($P < 0.05$).

Levels of DMA in samples at each stage of the malting process were determined. DMA was found with a mean and range of 0.9 (0.6-1.0); 0.6 (0.5-0.6); 11.6 (8.2-17.7); 513 (396-762); 5.9 (4.8-6.6); and 607 µg/gm (571-637 µg/gm) on a dry weight basis for, respectively, raw barley, steeped barley, green malt, green malt roots, kilned malt and kilned malt roots. It was found that roots con-

tained the highest levels of DMA. These results indicate that DMA is biosynthetically formed during germination.

The nitrosation of 5 varieties of green and kilned malts (Winter, Morex, Klages, Piroline and Steptoe) was carried out to determine the significance of DMA as a precursor for NDMA. The amount of NDMA from DMA was compared to the amounts of NDMA from hordenine and gramine for each sample. In every sample the amount of NDMA from DMA was larger than the combined amount of NDMA from hordenine and gramine. The results strongly suggest that DMA is the primary precursor of NDMA in malt.

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