and less astringent than the other fractions. In all cases, including the most astringent fraction (IV), the fractions were more bitter than astringent.

LITERATURE CITED

Amerine, M. A., Ough, C. S., "Wine and Must Analysis", Wiley, New York, 1974.
Arnold, R. A., Noble, A. C., *Am. J. Enol. Viticult.* **29**, 150 (1978).
Bate-Smith, E. C., *Phytochemistry* **12**, 907 (1973).
Joslyn, M. A., Goldstein, J. L., *Adv. Food Res.* **13**, 179 (1964).
Kramling, T. E., Singleton, V. L., *Am. J. Enol. Viticult.* **20**, 86 (1969).
Lea, A. G., Arnold, G. M. *J. Sci. Food Agric.* **29**, 478 (1978).
Lea, A. G. H. Bridle, P., Timberlake, C. F., Singleton, V. L., *Am. J. Enol. Viticult.* **30**, 289 (1979).
Lea, A. G., Timberlake, C. F., *J. Sci. Food Agric.* **25**, 1537 (1974).
Ough, C. S., *Am. J. Enol. Viticult.* **20**, 93 (1969).
Rossi, J. A., Singleton, V. L., *Am. J. Enol. Viticult.* **17**, 240 (1966).
Singleton, V. L., Draper, D. E., Rossi, J. A., *Am. J. Enol. Viticult.* **17**, 206 (1966).
Singleton, V. L., Esau, P., *Adv. Food Res. Suppl.* **1**, 282 (1969).
Singleton, V. l., Noble, A. C., *ACS Symp. Ser.* **26**, 47 (1976).
Singleton, V. L., Rossi, J. A., *Am. J. Enol. Viticult.* **16**, 144 (1964).
Singleton, V. L., Sieberhagen, H. A., de Wet, P., van Wyk, C. J., *Am. J. Enol. Viticult.* **26**, 62 (1975).
Su, C. T., Singleton, V. L., *Phytochemistry* **8**, 1553 (1969).

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Analysis of Malted Barley for *N*-Nitrosodimethylamine

Four procedures for isolation of *N*-nitrosodimethylamine (NDMA) from direct-fired kiln dried malted barley were compared: Soxhlet extraction with dichloromethane, direct aqueous extraction, steam distillation, and vacuum distillation. A gas chromatograph coupled to a thermal energy analyzer was used to estimate levels of NDMA. All the isolation procedures gave similar results with the exception of the Soxhlet extraction which gave one-fourth the NDMA content of the other three methods. Grinding the malt did not significantly affect the level of NDMA. It was also determined that, without addition of approximately 70% of the sample weight in water, the NDMA concentration found by vacuum distillation was significantly reduced.

European beer has been reported to contain *N*-nitrosodimethylamine (NDMA) at an average concentration of 2.7 ppb (Spiegelhalder et al., 1979). More recently, Goff and Fine (1979) and Scanlan et al. (1980) reported similar levels of NDMA in beer produced in the U.S.

Recent work in our laboratory indicates that malted barley produced by direct-fired kilning is the major source of NDMA in beer. Since initial analyses of malted barley produced confounding data, we undertook work to determine which methods of NDMA isolation produced the best estimate of the NDMA level in malted barley.

REAGENTS

N-Nitrosodipropylamine (NDPA) and NDMA standards were made gravimetrically in hexanes. Dichloromethane (DCM) was all-glass distilled, and hexanes were nanograde. All other reagents were analytical reagent or better and blanks were run on each new reagent lot.

PROCEDURE

Three kilograms of direct-fired kiln dried malted barley was obtained from a commerical maltster. The malt was screened and throughly mixed to insure homogeneity. The NDMA was isolated from the malt by direct solvent extraction, direct aqueous extraction (Congress Wort), steam distillation, and vacuum distillation. Additionally, the sample was analyzed whole kernel after 30 s of grinding in ca. 250 mL of liquid nitrogen (LN_2) in a commercial blender or after grinding in a laboratory malt mill (Table I).

The direct solvent extraction was carried out on 25-g samples in a Soxhlet apparatus (AOAC, 1975) using DCM as the extracting solvent. Whole kernel, blender ground (LN_2), and blender ground (LN_2) with 10 g of anhydrous sodium sulfate samples were extracted for 16 h. The resulting DCM extract was washed with 3 N hydrochloric acid (25 mL), 1.5 N sodium hydroxide (25 mL), dried by passing through anhydrous sodium sulfate, and concentrated as outlined below.

Table I. *N*-Nitrosodimethylamine Content of a Commercially Malted Barley Analyzed by Various Procedures

method	concn[b]
I. Soxhlet extraction	
A. whole malt	22
B. LN_2, blender ground	27
C. LN_2, blender ground (Na_2SO_4)	30
II. Direct aqueous extraction	
A. malt mill ground	115
III. steam distillation	
A. LN_2, blender ground	118
IV. vacuum distillation	
A. LN_2, blender ground	114
B. dry, blender ground	117
C. malt mill ground	99
D. unground malt	105

[a] Values are the average of two-three independent assays. [b] Parts per billion.

Laboratory mill ground malt was prepared by the fine grinding procedure and was analyzed after preparing a direct aqueous extract (American Society of Brewing Chemists, 1976). A 100-g aliquot of the resulting direct aqueous extract was extracted with DCM (1 × 100 mL followed by 2 × 50 mL); the DCM was dried by passing

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through anhydrous sodium sulfate and concentrated as given below.

Blender ground malt was steam distilled by a procedure similar to that of Goodhead and Gough (1975). A 50-g sample of ground malt was suspended in 400 mL of distilled water, 180 g of sodium chloride, 5 g of ammonium sulfamate and 30 mL of 1 N sulfuric acid. A steam generator was attached and 600 mL of distillate was collected. The distillate was acidified to pH 3, saturated with sodium sulfate, and extracted with DCM (3 × 50 mL). The combined extracts were washed with 1.5 N sodium hydroxide (1 × 40 mL), dried by passing through anhydrous sodium sulfate, and concentrated as outlined below.

Ground and unground malt was vacuum distilled from mineral oil by a modification (Hotchkiss et al., 1980) of the procedure of Fine et al. (1975). Fifty grams of malt, 5 g of ammonium sulfamate dissolved in 40 mL of 1 N sulfuric acid, and 50 mL of pharmaceutical grade mineral oil were placed in a 1-L, round-bottom distillation flask fitted with a thermometer well. A vacuum of greater than 100 μ was applied, and the distillate was trapped in two vacuum vapor traps connected in series and cooled in LN_2. The flask was heated to 100 °C over a 1-h period. The distillate was thawed and each trap rinsed with distilled water (3 × 40 mL) followed by DCM (1 × 40 mL). The aqueous phase was acidified (sulfuric acid, pH 2), saturated with sodium sulfate, and extracted with the combined DCM rinses. The aqueous phase was further extracted with DCM (2 × 40 mL), and the combined DCM extracts were washed with 1.5 N sodium hydroxide (1 × 40 mL) and dried by passing through anhydrous sodium sulfate. The DCM fractions were first concentrated to ca. 3 mL in a Kuderna-Danish concentrator, then further concentrated to 1 mL under a stream of nitrogen using a micro-Snyder column.

Percent recovery was determined for the vacuum distillation procedure by adding 3 μg each of NDMA and NDPA in 1 mL of hexane to 50 g of LN_2 ground malt which had been dried in an all electric kiln and did not contain NDMA above 0.5 μg/kg.

All samples were quantitatively analyzed against external standards by injecting 4–8-μL portions into the gas chromatograph–thermal energy analyzer (GC–TEA) (Fine and Rounbehler, 1975). The GC–TEA parameters were as follows; injector, 180 °C; column, 3.7 m × 3.18 mm o.d. stainless steel packed with 10% Carbowax 20M on 60/120 Chromosorb G-AW, 180 °C isothermal; furnace, 400 °C; vacuum, 5 mm; trap, −160 °C.

RESULTS AND DISCUSSION

The recoveries of NDMA and NDPA from ground malt by the vacuum distillation procedure averaged 83 and 95%, respectively. These values are similar to values reported for other foods (Fine et al., 1975; Havery et al., 1978). Because NDMA consistently gave lower recoveries than NDPA, the use of the percent recovery of NDPA to correct for NDMA losses during sample workup may underestimate the actual value. For this reason the values given here are uncorrected although NDPA was added to most samples.

Table I gives the NDMA content of a commercial, gas-fire kilned, malted barley analyzed by several different procedures. Each value represents the average of two or three independent assays. The low values produced by Soxhlet extraction, regardless of sample preparation, are surprising in view of the high partition coefficient of NDMA in DCM/water (Singer et al., 1977). Subsequent analysis of the residue left from the Soxhlet extraction by vacuum distillation showed that sufficient NDMA could be recovered to bring the total concentration to near the levels found by the other methods. This indicated that direct extraction by DCM is not an efficient method for removing NDMA from malt.

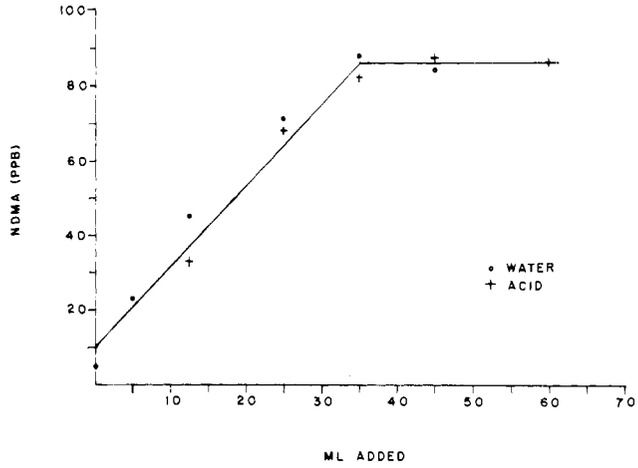

Figure 1. *N*-Nitrosodimethylamine content of a malted barley analyzed with different amounts of water or 1 N sulfuric acid added to the vacuum distillation flask.

Direct aqueous extraction of the ground malt resulted in values which compared favorably to those obtained by steam or vacuum distillations. However, because only 100 g of extract (50 g of malt + 400 g of water) is assayed, the sensitivity of the method is one-fourth that of the others. Additionally, the tendency of DCM/direct aqueous extraction to form emulsions necessitated centrifugation of each extract.

Grinding in a high shear blender with or without LN_2 did not effect the isolation of NDMA by vacuum distillation. Samples which were blended 5 min without the addition of LN_2 gave an average NDMA content of 117 ppb, while malt ground with LN_2 gave an average value of 114 ppb. Mill ground malt averaged slightly lower values, although mill ground malt analyzed by preparation of a direct aqueous extraction gave higher values. Unground malt, when analyzed by vacuum distillation gave an average only slightly lower than the LN_2 ground malt. This indicates that NDMA may be concentrated in the outer layers of the malt kernal.

The possible formation of NDMA during the vacuum distillation or workup was investigated by adding 2.5 mg of diethylamine to 50 g of ground malt and analyzing for *N*-nitrosodiethylamine (NDEA). NDEA was not found; however, 112 ppb NDMA was found as expected. This indicates that NDMA is not an artifact of analysis. The sulfuric acid caused some charring toward the end of the vacuum distillation procedure. Ammonium sulfamate and sulfuric acid were added to guard against the possibility of artifactual nitrosamine formation (Hotchkiss et al., 1980); however, samples of malt analyzed with and without these reagents gave very similar results by the vacuum distillation procedure (see Figure 1).

Fine et al. (1975) in their original description of a similar vacuum distillation procedure added 4 mL of 0.1 N base to a 20-g sample before distillation. Havery et al. (1978) did not add any water prior to distillation. We have found the addition of water to be critical for the removal of NDMA from the ground malt by the vacuum distillation procedure. As shown in Figure 1, when less than 35 mL of water or 1 N sulfuric acid was added to the malt prior to distillation, the values for NDMA in the ground malt decrease sharply. Without the addition of water, a value of only 5 ppb was obtained.

The importance of water in the vacuum distillation method may be related to the failure of DCM to extract the NDMA from solid malt, while the direct aqueous extraction was successful. The low moisture content of dried malted barley and the polarity of NDMA may allow NDMA binding to some molecular constituent in the malt. Apparently nonpolar DCM fails to release the bound NDMA, whereas the addition of a highly polar solvent such as water is necessary for efficient release. Further work is underway to elucidate the nature of the NDMA binding.

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LITERATURE CITED

American Society of Brewing Chemists, "Methods of Analysis", 7th ed, Malt-4, American Society of Brewing Chemists, St. Paul, MN, 1976, p 4-6.
Association of Official Agricultural Chemists, "Official Methods of Analysis", 12th ed, 7.045, Washington, DC, 1975, p 135.
Fine, D. H., Rounbehler, D. P., *J. Chromatogr.* **109**, 271 (1975).
Fine, D. H., Rounbehler, D. P., Oettinger, P. E., *Anal. Chim. Acta* **78**, 383 (1975).
Goff, E. U., Fine, D. H., *Food Cosmet. Toxicol.*, in press (1979).
Goodhead, K., Gough, T. A., *Food Cosmet. Toxicol.* **13**, 307 (1975).
Havery, D. C., Fazio, T., Howard, J. W., *J. Assoc. Off. Anal. Chem.* **61**, 1374 (1978).
Hotchkiss, J. H., Libbey, L. M., Scanlan, R. A., *J. Assoc. Off. Anal. Chem.*, in press (1980).
Scanlan, R. A., Barbour, J. F., Hotchkiss, J. H., Libbey, L. M., *Food Cosmet. Toxicol.*, in press (1980).
Singer, G. M., Taylor, H. W., Lijinsky, W., *Chem-Biol. Interact.* **19**, 133 (1977).
Spiegelhalder, B., Eisenbrand, G., Preussmann, R., *Food Cosmet. Toxicol.* **17**, 29 (1979).

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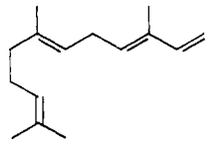
Characterization of a New Citrus Component, *trans,trans*-α-Farnesene. Isolation of α-Farnesene Isomers from Dehydration of Farnesol

trans,trans-α-Farnesene, which had never been found in citrus, was identified as a component in cold-pressed Valencia orange oil and distilled lime oil. Although *trans*-β-farnesene is a relatively common food constituent, only rarely has an α-farnesene isomer been identified in any food. Dehydration of farnesol and separation of the crude product by gas chromatography afforded an authentic sample of *trans,trans*-α-farnesene, as well as the other three α-farnesene isomers and the two β-farnesene isomers. This dehydration reaction had been reported to yield *trans*-β-farnesene as the only isolatable farnesene isomer.

Economic importance of citrus oils and food safety for the consumer depend upon composition of natural flavor components being determined as thoroughly as possible. Therefore, research efforts are continuing to identify and evaluate new constituents of citrus flavor fractions. The many studies identifying citrus components from each type of fruit were reviewed by Shaw (1977a,b). *trans*-β-Farnesene [(*E*)-7,11-dimethyl-3-methylenedodeca-1,6,10-triene] has been identified in cold-pressed orange oil and lemon oil (Shaw, 1977a) and has been quantitated in orange oil (Shaw and Coleman, 1974), but none of the other five possible isomers have been identified in any citrus oil or essence. Anet (1970) has synthesized all six isomers of α- and β-farnesene by acid-catalyzed dehydration of nerolidol and has assigned configurations to the two β-farnesenes and the four α-farnesenes thus found.

In the current study, the volatile compound, *trans,trans*-α-farnesene (1) [(3*E*,6*E*)-3,7,11-trimethyldodeca-1,3,6,10-tetraene] was isolated from distilled lime oil and



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from cold-pressed Valencia orange peel oil. It is being reported for the first time as a constituent of citrus. Acid-catalyzed dehydration of farnesol afforded an authentic sample of *trans,trans*-α-farnesene, as well as other α-farnesene isomers. This dehydration reaction has been reported to yield *trans*-β-farnesene as the only isolatable farnesene isomer (Naves, 1966; Brieger et al., 1969).

EXPERIMENTAL SECTION

Separation Procedures. Samples of distilled Mexican lime oil and cold-pressed Valencia orange oil were analyzed by gas–liquid chromatography (GLC) on packed columns on a Perkin-Elmer Model 900 gas chromatograph equipped with a thermal conductivity detector, using 0.10 in. i.d. × 20 ft stainless steel columns packed with either 10% Carbowax 20M or 10% UCW-98 on 60–80 mesh Gas-Chrom P. The following operating conditions were used: helium flow, 36 mL/min; injection port temperature, 250 °C; detector temperature, 275 °C; column temperature, 80–210 °C at 2 °C/min. Individual compounds were collected as they were eluted from the polar (20M) column and reinjected onto the nonpolar (UCW-98) column for further purification when necessary. Separations on a glass capillary column (60 m) coated with Carbowax 20M were carried out with a Hewlett-Packard Model 5840A gas chromatograph equipped with an injection port splitter (100:1). Operating conditions were as follows: injection port temperature, 250 °C; FID detector temperature, 300