Identification of Volatile Constituents Responsible for Characteristic Molasses Aroma by Unconventional Gas Chromatography

Mary A. Godshall,* Earl J. Roberts, and Michael G. Legendre

A novel instrumental method was utilized for direct gas chromatographic analysis (GC) without solvent extraction of low molecular weight molasses volatiles. Volatile constituents were identified on the basis of GC retention time, peak enhancement, sensory evaluation, and mass spectrometry. Twenty-three compounds, some not previously reported in molasses, were identified. Dimethyl sulfide was found to be a major contributing factor to the characteristic molasses odor.

Knowledge about the volatile constituents responsible for the aroma profile of any food is an important adjunct to the formulation of a successful product. This information is especially important when the product undergoes much processing. With gas chromatographic/mass spectrometric (GC/MS) techniques, it has become possible to refine and extend the information available about the kinds and amounts of volatile constituents present in foods. Most methods of the collection of volatiles require time-consuming distillation or extraction procedures. With these methods the low molecular weight (<100) volatile constituents may be partially lost or not present in an amount proportionate to that of the original material. The development of an external sample inlet for direct elution of volatiles onto a GC column (Legendre et al., 1979) has helped to overcome these difficulties.

In the present study, the volatiles in molasses were examined by the direct-sampling technique in conjunction with GC and GC/MS. Many studies have been done to characterize different molasses fractions, and a large number of volatile and nonvolatile components that contribute to the molasses flavor and aroma have been reported (Bobbio, 1959; Ito et al., 1965; Shirasaki et al., 1965; Hashizume et al., 1967, 1968; Hrdlicka and Janicek, 1968; Yokota and Fagerson, 1971; Ito, 1976). Many of these constituents are responsible for the sweet aroma notes of molasses (Ito, 1976). However, it does not seem likely that the identified constituents are responsible, alone or in combination, for the very characteristic "green" or "grasslike" aroma of molasses.

EXPERIMENTAL SECTION

The molasses used in this study was a South American blackstrap molasses, 75% solids, obtained from the New York Sugar Trade Laboratory.

Source of Standards. 2-Methyl-3-oxotetrahydrofuran was synthesized by the method of Gianturco et al. (1964). All other chemicals were purchased from commercial sources.

Gas Chromatographic Conditions. Comparison of retention times and sensory evaluation were carried out on a Hewlett-Packard Model 5750 gas chromatograph equipped with dual-flame ionization detectors. The column was stainless steel, 15 ft long, 0.085 in. i.d., packed with Tenax-GC 35/60 mesh, coated with approximately 6% poly-MPE. Helium was used as the carrier gas at a flow rate of 40 mL/min. The detector temperature was 290 °C.

Sensory evaluation of the compounds was accomplished by blowing out the flame just as a peak began to emerge on the chromatogram and noting the odor of the compound as it emerged from the detector. The major contributing volatiles were immediately detectable in this manner.

Elution of Volatiles. The gas chromatograph was fitted with an external injection port based on the method of Legendre et al. (1979). For standard GC operation, the condenser was omitted. (It is, however, necessary for use with GC/MS.) The inlet was modified for use on the HP-5750 GC to accommodate a larger sample by using $4^3/_4$ in. × $^1/_2$ in. o.d. glass tubing to hold the sample; a $^3/_8$ in. × 4 in. stainless-steel pipe nipple held the glass tubing in place. This assembly was wrapped with heater tape to heat the sample uniformly during collection of volatiles.

Sampling was by the method of Fore et al. (1979). One hundred to 300 mg of molasses was evenly coated onto a $2^1/_2$ in. × $^5/_{32}$ in. glass rod. The coated rod was lightly wrapped with a thin layer of volatile-free glass wool, put inside the glass holder, and held in place with glass wool plugs. The sample was placed into the external inlet system and sealed in place with the septum-lined pipe cap. The six-port rotary valve was set to the "inject" position, and the entire assembly was heated with heating tape at 135–145 °C for 12–30 min. During this elution time, helium was swept through the sample, depositing the volatiles on the head of the GC column, which was cooled to 30 °C by a stream of air. This technique allowed the volatiles to collect at the head of the column. After the elution period, the rotary valve was switched to the "run/purge" position, and the volatiles were separated with temperature programming from 30 °C to 210 °C at 4 °C/min. The glass liner with sample was removed, and the inlet assembly purged with air to the atmosphere during the GC run.

Authentic compounds, used for determining retention times, were sampled in a similar manner: A small amount of glass wool was placed inside a glass liner and a dilute solution of standard was injected into the glass wool with a 10-μL syringe. The sample was then subjected to the same elution conditions as the molasses samples.

Because retention time alone is not always a reliable method of identifying compounds, especially in the case of closely eluting peaks, a peak enhancement technique that avoided the uncertainties of relying on retention time alone was worked out. A sample of molasses was prepared in the manner described above, but the sample was enhanced by injection of a dilute standard solution into the glass wool layer surrounding the molasses. The sample was run in the usual manner, and the enhancement of the peak in question, relative to a previous unenhanced sample, constituted satisfactory confirmation of identity when coupled with sensory and mass spectral data.

Cane Sugar Refining Research Project, Inc., P. O. Box 19687, New Orleans, Louisiana 70179 (M.A.G., E.J.R.), and the Southern Regional Research Center, Science and Education Administration, U.S. Department of Agriculture, P. O. Box 19687, New Orleans, Louisiana 70179 (M.G.L.).

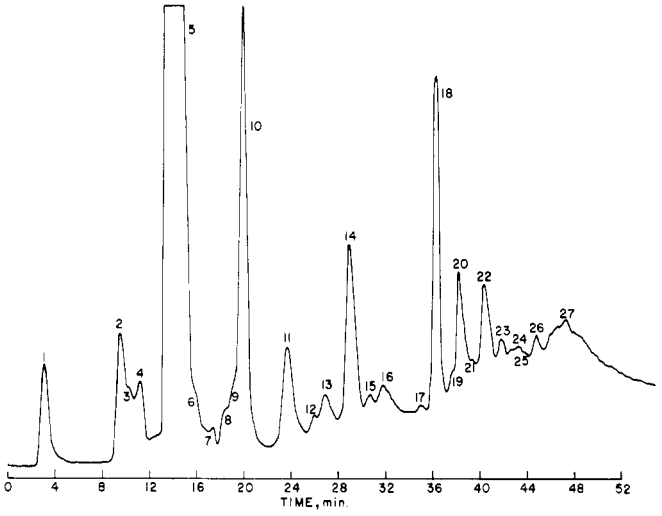

Figure 1. Gas chromatogram of molasses volatiles. Peak numbers refer to compounds in Table I.

Mass Spectrometry. Direct GC/MS analyses of molasses volatiles were conducted on a Tracor-222 gas chromatograph equipped with dual hydrogen flame detectors. A column packed with Tenax-GC coated with 6–7% poly-MPE, 8 ft long × $^1/_8$ in. o.d., was used. The chromatograph was interfaced with a helium silicone membrane separator to a Hewlett-Packard 5930A mass spectrometer. The operating parameters were 70 eV ionizing potential and scan range 21–350 amu in 2 s. An INCOS 2000 mass spectrometer data system was utilized.

Samples of molasses were analyzed in a manner similar to that already described for GC, except that volatile components were swept from the sample through a condenser packed with Na_2SO_4-coated glass wool to eliminate moisture before entering the mass spectrometer. Inlet temperature was 140 °C; elution time was 15 min. After the elution period, the rotary valve was put into the "run/purge" position, the GC/MS interface valve was opened, and the GC oven was heated rapidly to 80 °C. Once at 80 °C, the ion source and data system were turned on and the GC oven programmed to 220 °C at 4 °C/min. With the GC/MS run in progress, the condenser was heated at 150 °C while being purged with helium gas to the atmosphere and recooled in preparation for the next sample.

RESULTS AND DISCUSSION

Figure 1 shows a typical chromatogram of the molasses volatiles. Tenax GC coated with poly-MPE is very well suited to the analysis of food volatiles because it can separate a wide range of alcohols, aldehydes, and esters, is inert to water, and has a very low bleed rate (Novotny et al., 1975). It does lack high efficiency and thus the resolution was not ideal. However, good reproducibility was obtained over a volatile collection period ranging from 12 to 30 min with only a slight increase in relative quantity of volatiles collected with increased time. It was necessary to maintain the inlet temperature at a narrow range, 135–145 °C. If the temperature was lower, reproducibility from sample to sample was poor, with the small peaks often not detectable.

Table I lists the 23 compounds identified. Although identified by their mass spectra with the aid of mass chromatograms, two pairs of compounds, 2- or 3-methylfuran/diacetyl and 2,5- or 2,6-dimethylfuran/2,3-pentanedione, could not be individually confirmed by GC retention data and peak enhancement because they had identical retention times. However, their corresponding peaks were individually enhanced by each compound. Diacetyl and 2,3-pentanedione were additionally identified by their powerful odors at the exit port.

Table I. Volatile Constituents Identified in Molasses

peak no.[a]	constituent[b]	method of identification: MS	GC retention & enhancement	sensory
1	methanol		+	+
2	acetaldehyde	+	+	+
3	unknown			
4	unknown sulfur dioxide[c]	+		
5	ethanol	+	+	+
6	unknown			
7	furan	+	+	
8	acetone	+	+	
9	carbon disulfide	+	+	+
10	dimethyl sulfide	+	+	+
11	2-methylpropanal hexane[c]	+	+	+
12	2- or 3-methylfuran	+	+	
12	diacetyl	+	+	+
13	ethyl acetate	+	+	+
14	2-methylbutanal	+	+	+
15	2,5- or 2,6-dimethylfuran	+	+	
15	2,3-pentanedione	+	+	+
16	acetoin	+	+	
17	unknown			
18	2-methyl-3-oxo-tetrahydrofuran	+	+	+
19	furfural	+	+	+
20	furfuryl alcohol	+	+	
21	unknown (43, 86, 73, 116)	+	+	
22	2,5- or 2,6-dimethylpyrazine	+	+	
23	substituted furan (81, 53, 126, 80)	+		
24	furyl methyl ketone (2-acetylfuran)	+	+	
25	substituted furan (81, 98, 43, 140)	+		
26	furyl ethyl ketone	+	+	
27	unknown (134, 74, 105, 50)	+		

[a] Compounds with same peak number had the same retention time. Peak numbers correspond to peak numbers in Figure 2. [b] Numbers in parentheses refer to major ions of the mass spectrum. [c] Seen on MS only.

The compounds identified in the molasses possessed many different odors. In order to determine which ones contributed most qualitatively to the molasses aroma, a Vigreaux column low holdup distillation of a 50% solution of the molasses was done, as a supplementary procedure. The distillate had a typical molasses aroma. The constituents in the distillate were identified by GC and MS to be acetaldehyde, dimethyl sulfide, 2-methylpropanal, diacetyl, 2-methylbutanal, 2,3-pentanedione, and 2-methyl-3-oxotetrahydrofuran. It can be assumed that these compounds play a major role in the aroma complex of molasses. Methanol and ethanol were also major components of the distillate, but these alcohols have high flavor thresholds (Sheldon et al., 1971; Bomben et al., 1973) and are thought to contribute only slightly to the overall impression.

During this distillation, it was noted that a powerful molasses aroma was escaping from the vent. A short length of rubber tubing was attached to the vent, and the vent vapors were collected in 1 mL of water cooled in an ice

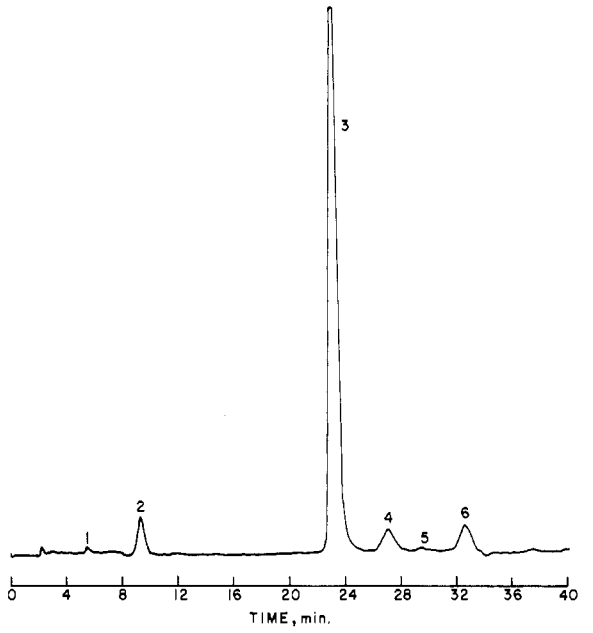

Figure 2. Gas chromatogram of dimethyl sulfide in vapors from molasses distillation: collected in ice water for 20 min. 1, methanol; 2, acetaldehyde; 3, dimethyl sulfide; 4, 2-methylpropanal; 5, diacetyl; 6, 2-methylbutanal.

bath. After 20 min of collecting, a GC run (Figure 2) showed the major component trapped in the ice water was dimethyl sulfide. This provided a strong indication of its importance in the molasses aroma complex.

In 1953, Binkley and Wolfrom isolated a volatile extract from cane molasses with strong molasses odor whose infrared spectrum indicated the possibility of a sulfur function. This finding was not pursued further, and, as far as can be determined, the present report is the first identification of dimethyl sulfide as a component of molasses. Furan and seven substituted furans were identified as well as two substituted furans whose structure could not be further elucidated. These compounds have mild, indistinct, faintly sweet odors in low concentrations and are not expected to contribute significantly to the aroma of molasses, with the exception of furfural, which has an odor threshold in water of 3 ppm (Bomben et al., 1973) and is used as a caramellic food flavoring in the 1–30-ppm range.

Yokota and Fagerson (1971) identified seven furans in their study on the volatile components of cane molasses. This study confirmed their findings of furfural, 2-acetylfuran, and furfuryl alcohol. The other furans found in the present study have not been reported previously in molasses.

Dimethylpyrazine was found in the volatiles and tentatively identified as the 2,5-dimethyl derivative although 2,6-dimethylpyrazine could not be ruled out because of a similar fragmentation pattern on MS and identical retention on GC. Wiggins and Wise (1955) observed that acid-hydrolyzed molasses heated with ammonia gave a variety of pyrazine derivatives. Shibamoto and Bernhard (1978) showed the formation of 35 pyrazine derivatives from the reaction of L-rhamnose with ammonia, and Hodge (1953) discussed the formation of pyrazines and furans from sugar–amine reactions.

Many of the constituents identified in this study are the result of carbohydrate degradation through a variety of pathways (acid, thermal, and Strecker degradation, as well as nonenzymic browning) with a large number of possible products. It is unlikely, therefore, that molasses from different sources will contain exactly the same constituents because of the variety of conditions under which molasses is produced.

This study has shown that molasses aroma consists of two major factors. One is the sweet component which is contributed by constituents such as diacetyl, acetoin, furfural, and the aldehydes. The other component is the powerful "grassy" or "green" note contributed by dimethyl sulfide, which gives molasses its characteristic odor. These two components in combination are responsible for molasses aroma. The other minor constituents may help round out the odor to give the full-bodied aroma readily recognizable as molasses.

LITERATURE CITED

Binkley, W. W., Wolfrom, M. L., Scientific Report Series, No. 15, Sugar Research Foundation, Inc., New York, 1953.
Bobbio, F. O., *Rev. Quim. Ind. (Rio de Janeiro)* **28**, 21 (1959); *Chem. Abstr.* **53**, 2260d.
Bomben, J. L., Bruin, S., Thijssen, H. A. C., Merson, R. L., *Adv. Food Res.* **20**, 1 (1973).
Fore, S. P., Fisher, G. S., Legendre, M. G., Wadsworth, J. I., *Peanut Sci.* **6**, 58 (1979).
Gianturco, M. A., Friedel, P., Giammarino, A. S., *Tetrahedron* **20**, 1763 (1964).
Hashizume, T., Kikuchi, N., Sasaki, Y., Sakata, I., *Agric. Biol. Chem.* **32**, 1306 (1968).
Hashizume, T., Yamagami, T., Sasaki, Y., *Agric. Biol. Chem.* **31**, 324 (1967).
Hodge, J. E., *J. Agric. Food Chem.* **1**, 928 (1953).
Hrdlicka, J., Janicek, G., *Sb. Vys. Sk. Chem.-Technol. Praze Potraviny E* **21**, 77 (1968).
Ito, H., *Agric. Biol. Chem.* **40**, 827 (1976).
Ito, H., Kagabu, K., Kamoda, M., *Proc. Res. Soc. Jpn. Sugar Refineries' Technol.* **15**, 56 (1965).
Legendre, M. G., Fisher, G. S., Dupuy, H. P., Rayner, E. T., *J. Am. Oil Chem. Soc.* **56**, 552 (1979).
Novotny, M., Hayes, J. M., Bruner, F., Simmonds, P. G., *Science* **189**, 215 (1975).
Sheldon, R. M., Lindsay, R. C., Libbey, L. M., Morgan, M. E., *Appl. Microbiol.* **22**, 263 (1971).
Shibamoto, T., Bernhard, R. A., *J. Agric. Food Chem.* **26**, 183 (1978).
Shirasaki, T., Ito, H., Kamoda, M., *Proc. Res. Soc. Jpn. Sugar Refineries' Technol.* **16**, 44 (1965).
Wiggins, L. F., Wise, W. S., *Int. Sugar J.* **57**, 435 (1955).
Yokota, M., Fagerson, I. S., *J. Food Sci.* **36**, 1091 (1971).

Received for review June 22, 1979. Accepted April 8, 1980. Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.