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Registry No. *all-trans*-Lycopene, 502-65-8; 2-methyl-2-hepten-6-one, 110-93-0; pseudoionone, 141-10-6; 6-methyl-3,5-heptadien-2-one, 1604-28-0; geranial, 141-27-5; neral, 106-26-3; citral, 5392-40-5; 5-hexen-2-one, 109-49-9; hexane-2,5-dione, 110-13-4; geranyl acetate, 105-87-3.

Identification of Pyrazines in Maple Syrup

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Commercial samples of Quebec maple syrup (1, amber; 3, dark) were extracted successively with diethyl ether, an acid solution, a basic solution, and then finally dichloromethane. Capillary gas chromatographic analysis of the dichloromethane extract indicated the presence of methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, and trimethylpyrazine. Gas chromatography–mass spectrometry confirmed the presence of the six pyrazine compounds and also suggested the presence of the 2-ethyl-6-methylpyrazine.

Pyrazines are heterocyclic, nitrogen-containing compounds found both in many processed foods as well as naturally in many plant foods (Maga, 1982; Maga and Sizer, 1973). These compounds contribute characteristic flavor and aroma to foods. For example, alkylmethoxypyrazines have been reported to be responsible for the "earthy" or "potato-like" odors of many vegetables and plants (Gallois and Grimont, 1985) while the contribution of alkylpyrazines to aroma, flavor, and color of a large number of thermally processed or cooked foods has been reported by several workers (Shibamoto 1986; Fors and Erickson, 1986; Masuda and Mihara, 1986). Previous reports have been reviewed by Maga (1982).

The formation of pyrazine compounds in many thermally processed foods results from Maillard-type reactions between reducing sugars and free amino acids or amides (Koehler and Odell, 1970; Koehler et al., 1969). The mechanisms by which pyrazines are formed have been proposed (Shibamoto et al., 1979; Newel et al., 1967; Koehler et al., 1969). These reports have been reviewed by Maga (1982).

Traditionally, the conversion of maple sap to maple syrup involves concentration by boiling of the sap for several hours. The presence of small quantities of reducing sugars (Jones and Alli, 1987) and free amino acids and amides, particularly aspartic acid, glutamic acid, glutamine, asparagine, and citrulline, has been reported (Morselli and Whelan, 1986; Kallio, 1988). Although the conditions are favorable for formation of pyrazine compounds, a systematic attempt to identify pyrazine compounds in maple syrup has not been reported in the literature. Nevertheless, there have been sporadic reports indicating some relationship between maple flavor and pyrazine compounds. Masuda and Mihara (1986) indi-

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Table I. Gas Chromatographic and Mass Spectrometric Data of Pyrazine Standards and Pyrazines in Maple Syrup

pyrazine	origin	ret time, min	peaks in the EI spectra of pyrazines: m/z (rel intens)
2-methyl	maple syrup	18.86	95 (6), 94 (100), 67 (45), 53 (16), 42 (11), 41 (7), 40 (17), 39 (16)
	standard	18.82	95 (6), 94 (100), 67 (51), 57 (8), 53 (17), 42 (15), 41 (14), 40 (21), 39 (21)
2,5-dimethyl	maple syrup	23.64	108 (100), 81 (10), 57 (18), 49 (23), 42 (90), 41 (26), 40 (28), 39 (32), 36 (25)
	standard	23.59	108 (100), 81 (17), 52 (4), 42 (90), 40 (24), 39 (33)
2,6-dimethyl	maple syrup	24.22	109 (9), 108 (100), 67 (8), 42 (75), 41 (4), 40 (44), 39 (33)
	standard	24.24	109 (7), 108 (100), 67 (8), 42 (87), 41 (10), 40 (48), 39 (39)
ethyl	maple syrup	24.99	108 (72), 107 (100), 84 (12), 80 (21), 53 (18), 52 (12), 49 (20), 39 (9), 36 (14)
	standard	24.85	108 (68), 107 (100), 80 (22), 56 (11), 53 (17), 52 (12), 39 (14)8, 36 (2)
2,3-dimethyl	maple syrup	25.90	108 (100), 67 (95), 52 (9), 49 (19), 45 (42), 40 (25), 36 (18)
	standard	25.82	108 (100), 67 (95), 52 (8), 42 (25), 41 (21), 40 (23), 39 (14)
trimethyl	maple syrup	31.65	122 (79), 81 (12), 57 (14), 54 (10), 42 (100), 41 (23), 40 (15), 39 (19)
	standard	31.64	122 (68), 81 (17), 57 (5), 54 (9), 42 (100), 41 (7), 40 (12), 39 (21)

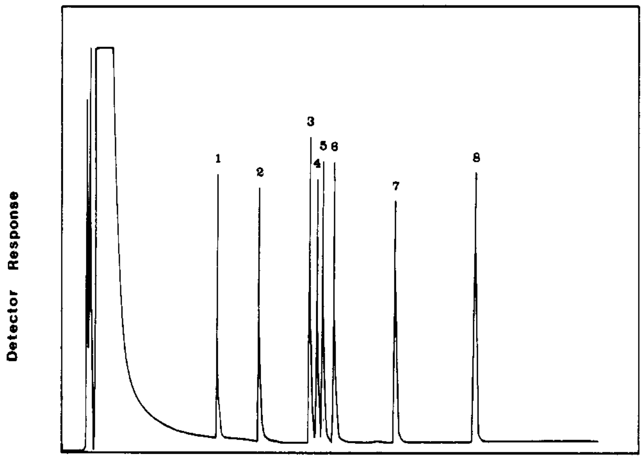

Figure 1. Gas chromatogram of a mixture of pyrazine standards: 1, pyrazine; 2, methylpyrazine; 3, 2,5-dimethylpyrazine; 4, 2,6-dimethylpyrazine; 5, ethylpyrazine; 6, 2,3-dimethylpyrazine; 7, 2,3,5-trimethylpyrazine; 8, tetramethylpyrazine.

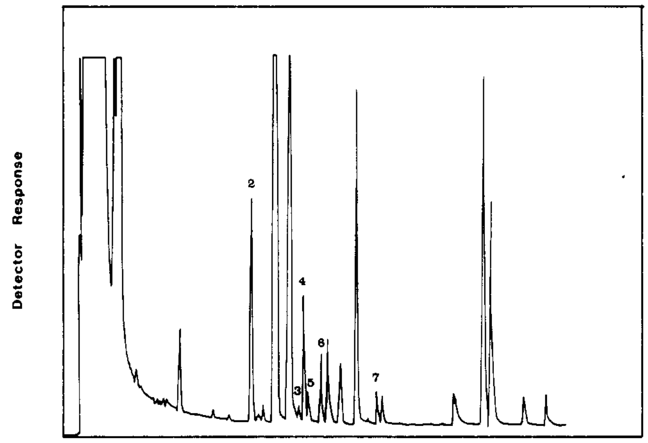

Figure 2. Gas chromatogram of the dichloromethane extract of maple syrup: 2, methylpyrazine; 3, 2,5-dimethylpyrazine; 4, 2,6-dimethylpyrazine; 5, ethylpyrazine; 6, 2,3-dimethylpyrazine; 7, trimethylpyrazine.

cated that 5-isopropyl-2,3-dimethylpyrazine gave a sweet, maplelike, brown odor while Winter et al. (1972) reported that 2,5-dimethyl-3,6-diisobutylpyrazine demonstrated maplelike flavor characteristics. Kallio (1988) reported that 2,6-dimethylpyrazine was present in the volatiles extracted from birch syrup. A non-pyrazine compound, 2-hydroxymethylcyclopent-2-en-1-one, a minor component of maple syrup flavor extract, was considered to have the characteristic maple flavor (Filipic et al., 1965). The present study was carried out to identify the presence of pyrazine compounds in commercial samples of maple syrup.

MATERIALS AND METHODS

Materials. Commercial samples of maple syrup (1, amber; 3, dark) were obtained from The Maple Sugar Producers of Quebec, Plessisville, Quebec, Canada. The pyrazine standards pyrazine, methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, 2,3,5-trimethylpyrazine, and tetramethylpyrazine were obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI).

Extraction of Pyrazine Compounds from Maple Syrup. The extraction procedure described by Reineccius et al. (1972) for cocoa beans was used with some modifications. Maple syrup (400 g) was extracted (25 °C, 3 h) with diethyl ether (150 mL) with continuous stirring. The mixture was allowed to stand overnight for complete separation of the aqueous and organic phases. The ether layer was extracted (5 × 20 mL) with a solution (pH 1.0) containing 100 g of NaCl and 6 mL of concentrated HCl/L of water. The aqueous extracts were combined, washed (2 × 40 mL) with diethyl ether, and then adjusted to pH 8.3 by dropwise addition of KOH solution (1 M). The aqueous extract was extracted (5 × 20 mL) with dichloromethane. The dichloromethane extracts were combined, dried over $MgSO_4$, and then concentrated to approximately 0.15 mL under a stream of nitrogen.

A mixture of the following pyrazines containing 1 mg of pyrazine/mL of dichloromethane was prepared and used as standards for identification of the pyrazines in the maple syrup extracts: pyrazine, methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, trimethylpyrazine, and tetramethylpyrazine.

Gas Chromatography. A sample (1 μL) of the concentrated dichloromethane extract obtained from the maple syrup was analyzed for pyrazines on a Varian Model 3700 gas chromatograph equipped with a flame ionization detector. Conditions of chromatography were as follows: fused silica capillary column, 30-m length × 0.32-mm i.d., Supelcowax 10 (Supelco, Inc.); temperature program, initial temperature 40 °C, program rate 1 °C/min, final temperature 90 °C; nitrogen carrier gas flow rate, 0.6 cm^3/min; injection port temperature, 200 °C; detector temperature, 250 °C; splitless injection mode. Chromatograms were recorded by means of a Hewlett-Packard Model HP 3390A integrator. The pyrazines in the dichloromethane extracts of the maple syrup were identified by comparison with retention times of the standard pyrazines.

Gas Chromatography–Mass Spectrometry. GC–MS experiments were carried out on a HP-5890 gas chromatograph with a direct inlet to a VG-7070-EHF medium-resolution mass spectrometer equipped with a VG-11-250 data system linked on-line to NBS data library. The ionization energy was 70 eV. Helium was used as carrier gas. The analytical conditions were similar to those used for gas chromatography. GC retention indices (Supelcowax 10) and EI fragmentation data were measured and accumulated in a database for computerized retrieval.

RESULTS AND DISCUSSION

Figure 1 shows the gas chromatogram of the mixture of eight pyrazine standards; the pyrazines were well separated under the conditions utilized. Mass spectrometry confirmed the identity of eight peaks as the pyrazine standards. Figure 2 shows the chromatogram of the dichloromethane extract obtained from a sample of no. 3 dark maple syrup; qualitatively, the chromatograms of no. 1 amber maple syrup were similar to that of the no. 3 dark maple syrup. The fractions labeled 2–7 were identified as pyrazines. This identification was made by comparing the retention times (Table I) of the standard pyrazines with those of the peaks in the sample chromatogram; confirmation of the identity of the peaks was done by comparison of EI fragmentation patterns of the standard pyrazines with those found in the maple syrup. A library search was done by comparing the eight largest peaks of each library entry with the unknown pyrazine identified by retention time data. The search proceeded with direct comparison between the data from the pyrazine in the maple syrup and the library data.

By use of the combination of retention times and GC–MS data (Table I), the following pyrazines were confirmed in the maple syrup, in order of magnitude of their response: methylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, ethylpyrazine, and trimethylpyrazine. In addition, 2-ethyl-6-methylpyrazine was identified by GC–MS using the resident data bank only. The seven identified pyrazines are commonly found in numerous thermally processed (heated, cooked, roasted, baked) foods (Maga, 1982) and are therefore not unique to maple syrup. However, this represents the first report on their identification in maple syrup. The identified pyrazine compounds have also been identified as products from the heating of reaction mixtures containing glucose, hydrogen sulfide, ammonia, and water (Shibamoto and Russel, 1977); in these reactions methylpyrazine is the major pyrazine identified. The 2,6-dimethylpyrazine has been identified in the pentane–diethyl ether extract of birch syrup but not of maple syrup (Kallio, 1988).

The results indicate that the extraction procedures was not specific for pyrazines. The GC–MS data system revealed the presence of 3-hydroxybutanone, 3-hydroxy-2-propanone, and possibly 3-methyl-2,5-furandione as important non-pyrazine compounds in the dichloromethane extract. However, Reineccius et al. (1972) obtained primarily pyrazine compounds using this extraction procedure for roasted cocoa beans. It is likely that these compounds could also contribute to maple syrup flavor; Filipic et al. (1965) suggested that 2-hydroxymethylcyclopent-2-en-1-one, a minor component of maple syrup, contributed the characteristic maple flavor.

CONCLUSION

The present work has demonstrated that pyrazine compounds, commonly found in numerous cooked or heated foods, are present in maple syrup. At least seven pyrazines were identified. These are methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, 2-ethyl-6-methylpyrazine, and trimethylpyrazine. On the basis of the current knowledge of pyrazine compounds in general, it is likely that these compounds are formed from reactions between amino acids and reducing sugars during the heat processing of maple sap to maple syrup.

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Registry No. Methylpyrazine, 109-08-0; 2,6-dimethylpyrazine, 108-50-9; 2,3-dimethylpyrazine, 5910-89-4; 2,5-dimethylpyrazine, 123-32-0; ethylpyrazine, 13925-00-3; trimethylpyrazine, 14667-55-1; 2-ethyl-6-methylpyrazine, 13925-03-6; 3-hydroxybutanone, 513-86-0; 3-hydroxy-2-propanone, 116-09-6; 3-methyl-2,5-furandione, 616-02-4.