

- Maga, J. A. The Role of Sulfur Compounds in Food Flavor. Part II: Thiophenes. *CRC Crit. Rev. Food Sci. Nutr.* 1975, 241-270.
- Majlat, P.; Erdos, Z.; Takacs, J. Calculation and Application of Retention Indices in Programmed Temperature Gas Chromatography. *J. Chromatogr.* 1974, 91, 89-103.
- Matthews, R. F.; Scanlan, R. A.; Libbey, L. M. Autoxidation Products of 2,4-Decadienal. *J. Am. Oil Chem. Soc.* 1971, 48, 745-747.
- Michalski, S. T.; Hammond, E. G. Use of Labeled Compounds to Study the Mechanism of Flavor Formation in Oxidizing Fats. *J. Am. Oil Chem. Soc.* 1972, 49, 563-566.
- Nixon, L. N.; Wong, E.; Johnson, C. B.; Birch, E. J. Nonacidic Constituents of Volatiles from Cooked Mutton. *J. Agric. Food Chem.* 1979, 27, 355-359.
- Ohloff, G.; Flament, I.; Pickenhagen, W. Flavor Chemistry. *Food Rev. Int.* 1985, 1, 99-148.
- Patton, S.; Barnes, I. J.; Evens, L. E. n-Deca-2,4-dienal, Its Origin from Linoleate and Flavor Significant in Fats. *J. Am. Oil Chem. Soc.* 1959, 36, 280-283.
- Pokorny, J.; Janitz, W.; Viden, I.; Velisek, J.; Valentova, H.; Davidek, J. Reaction of Oxidized Lipids with Protein: Part 14. Aldolization Reactions of Lower Alkanals in Presence of Nonlipidic Substances. *Die Nahrung* 1987, 1, 63-70.
- Schieberle, P.; Grosch, W. Model Experiments About the Formation of Volatile Carbonyl Compounds. *JAOCs, J. Am. Oil Chem. Soc.* 1981, 58, 602-607.
- Shu, C. K.; Hagedorn, M. L.; Mookherjee, B. D.; Ho, C. T. pH Effect on the Volatile Components in the Thermal Degradation of Cysteine. *J. Agric. Food Chem.* 1985, 33, 442-446.
- Sims, R. J.; Fioriti, J. A. High Temperature Reaction of Fats with Amino Acids. *J. Am. Oil Chem. Soc.* 1975, 52, 144-147.
- Snyder, J. M.; Frankel, E. N.; Selke, E. Capillary Gas Chromatographic Analyses of Headspace Volatiles from Vegetable Oils. *JAOCs, J. Am. Oil Chem. Soc.* 1985, 62, 1675-1679.
- Ten Noever de Brauw, M. C.; Bouwman, J.; Tas, A. C.; La Vos, G. F. *Compilation of Mass Spectra of Volatile Components in Foods*; Central Institute for Nutrition & Food Research: Zeist, The Netherlands, 1983.
- Vernin, G.; Parkanyi, C. Mechanisms of Formation of Heterocyclic Compounds in Maillard and Pyrolyses Reactions. In *The Chemistry of Heterocyclic Flavoring and Aroma Compounds*; Vernin, G., Ed.; Ellis Horwood: Chichester, 1982; p 151.
- Vitzhum, O. G.; Werkhoff, P. Oxazoles and Thiazoles in Coffee Aroma. *J. Food Sci.* 1974, 39, 1210-1215.
- Zhang, Y.; Chien, M.; Ho, C.-T. Comparison of the Volatile Compounds Obtained from Thermal Degradation of Cysteine and Glutathione in Water. *J. Agric. Food Chem.* 1988, 36, 992-996.

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Selective Purge-and-Trap Method for the Analysis of Volatile Pyrazines

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A simple and selective purge-and-trap method for isolation, concentration, and fractionation of volatile pyrazines from a matrix with complex volatiles was developed. A dilute HCl aqueous solution was used to selectively trap pyrazines from total headspace components from a model system generated product and potato chips. After being titrated to pH 13, the acid-trapped pyrazines were then recovered by the headspace technique and analyzed by GC and GC-MS. Not only can the method quantify minor or trace pyrazines from a very small amount of sample with complex volatiles but also it is much less laborious and time-consuming compared with other traditional isolation, fractionation methods.

Alkylpyrazines have been considered important, characteristic, and essential trace flavor components present in many cooked, deep-fat fried, roasted, and toasted foods (Maga, 1982). Food products generally contain a large number of volatiles in various concentrations. In some cases, a direct determination of pyrazines of interest is possible. In most cases, the quantitative analysis of the pyrazines, which often are minor in quantity, is interfered with by the major constituents or by compounds with the same chromatographic properties. Physical or chemical fractionation after isolation is therefore required. Although there are no universal methods for isolation and fractionation, selective separation of volatile compounds based on functional groups is an approach that has been used for a long time. Pyrazines as well as other organic bases such as pyridines, pyrroles, and thiazoles can be separated from the solvent by extraction with dilute aqueous HCl. This is followed by transformation of the hydrochloride salts into free bases with 10% aqueous KOH (or NaOH) and

extraction with diethyl ether. However, this method normally requires a larger amount of material, and it is also quite laborious and time-consuming (Peyron, 1982).

Headspace analysis, by purge-and-trap on a porous polymer adsorbent, followed by thermal desorption or solvent elution, has been regarded as a simple and useful isolation method. The volatiles collected have an aroma note very close to that of the original sample (Chen et al., 1982). Maarse and Schaefer (1978) developed methods for collection and quantitative analysis of specific groups of compounds, such as pyrazines, phenols, acids, and carbonyls, in vapors above food products and in gases emitted during the processing of food. Pyrazines in the emission gases of a cocoa factory were collected by a trap with 10 mL of 1 N H₂SO₄. The collected pyrazines were then recovered by diethyl ether extraction after titrating the acid solution to pH 9. This method allowed the quantitative analysis of pyrazines occurring in low concentration, when the total chromatogram contained many interfering compounds. However, the method mentioned above still involved many procedures and was also laborious.

In this study, a simple and selective purge-and-trap method, using dilute HCl as a trap, was developed in order to isolate, concentrate, and fractionate pyrazines from samples containing complex volatiles. The method was applied for the determination of pyrazines generated from

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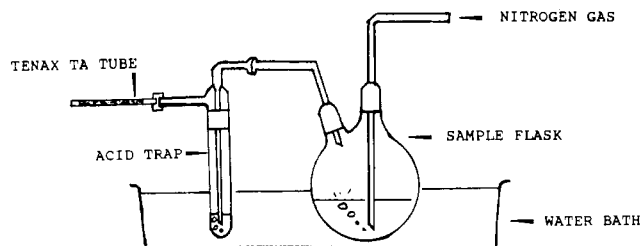


Figure 1. Apparatus for purge-and-trap isolation and fractionation.

a model system and potato chips.

MATERIALS AND METHODS

Materials. Corn gluten meal (CGM) was purchased from Sigma Chemical Co. (St. Louis, MO). Corn oil was obtained from a local supermarket. Two batches of potato chips manufactured by Frito-Lay were purchased from a local supermarket. Diethyl ether was obtained from Ace Scientific Co. (East Brunswick, NJ) and redistilled before use. Pentane, acetone, and tetramethylpyrazine were purchased from Aldrich Chemical Co. (Milwaukee, WI). Tenax TA (60–80 mesh), tridecane, and C_8 – C_{22} *n*-paraffins were ordered from Alltech Associates Inc. (Deerfield, IL).

Preparation of Defatted Corn Gluten Meal (DCGM). Commercially available corn gluten meal was subjected to Soxhlet extraction to remove the lipids using a gradient of solvents of different polarity. A total of 500 g of CGM was divided into five equivalent parts and individually wrapped with filter paper. The wrapped samples were placed in the Soxhlet extractor and extracted first with pentane for 24 h. The residue was dried in the hood overnight and then extracted with diethyl ether and acetone, respectively, in the same manner as described above.

Activation of Tenax TA Trap. Two-hundred milligrams of Tenax TA was packed in a silanized glass tube (12 cm \times 0.5 cm (i.d.)) and activated at 260 °C under a stream of nitrogen gas at a flow rate of 60 mL/min for 12 h prior to the purge-and-trap adsorption.

Conditions for Volatile Generation from a Model System. A combination of 50 g of DCGM, 4 g of corn oil, and 16.2 g of distilled water was mixed well, wrapped with two layers of aluminum foil, and baked in an oven at 185 °C for 30 min.

Pyrazine Fractionation by the Purge-and-Trap Method. The apparatus for the purge-and-trap fractionation is shown in Figure 1. The water bath temperature was 45 °C. Ten grams of sample from the heated DCGM/corn oil/water model system, together with 30 g of NaCl (Fisher Scientific, Malvern, PA) and 100 mL of distilled water, was placed in the sample flask. Tetramethylpyrazine (0.2136 mg) was added as an internal standard. Samples in the suspension were purged by nitrogen gas at a flow rate of 400 mL/min for 2 h. Due to their weak basic properties, pyrazines were trapped in 5 mL of 11.7% (w/v) HCl solution as salts while passing through the acid solution. Most of the nonbasic volatiles passed through the acid solution and were trapped by a Tenax TA glass tube. After the solution was purged for 2 h, the sample flask was removed and the acid solution was purged at the same flow rate for another 2 h to further remove the remaining nonbasic compounds in the acid trap. The acid solution containing trapped pyrazines was titrated with 30% NaOH solution to pH 13. The alkaline solution was then purged by nitrogen gas at the same flow rate for 2 h to trap the pyrazines in a separate Tenax TA tube.

Fifteen grams of ground potato chips was subjected to pyrazine fractionation by the same procedures described above. The total volatiles from both the model system and the potato chips were collected by the same conditions described above, without the acid trap. Tridecane (0.1012 mg) or tetramethylpyrazine (0.2136 mg) was added as an internal standard.

Thermal Desorption in a GC Capillary Column. The Tenax TA tube containing the adsorbed volatiles was placed in a modified GC packed-column injector warmed by a heating tape to 260 °C. One end of the injector had a screw cap, and the other end had a Luer lock connected to a 25-gauge, 2.5-in. needle. The GC carrier gas (He) was turned off while the tube was loaded and before the needle was punched through the septum of the GC

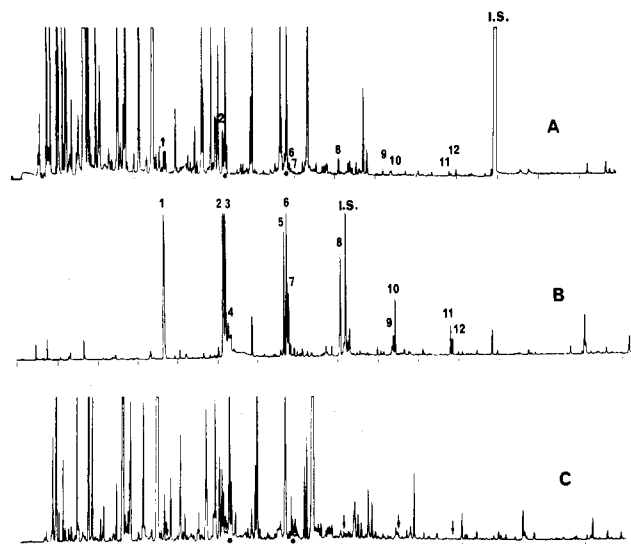


Figure 2. GC profiles of total volatiles (A), pyrazine fraction (B), and non-pyrazine fraction (C) from the model system generated product by purge-and-trap.

Table I. Pyrazines from the Model System Generated Sample

peak ^a	pyrazine	I_k (DB-1)	ppm ^b
1	2-methyl	801	12.2 \pm 0.20
2	2,5-dimethyl	890	15.4 \pm 0.94
3	2,6-dimethyl	894	8.1 \pm 0.59
4	2,3-dimethyl	896	2.2 \pm 0.04
5	2-ethyl-5-methyl	978	4.7 \pm 0.30
6	2-ethyl-6-methyl	982	7.1 \pm 0.55
7	2-ethyl-3-methyl	985	2.3 \pm 0.15
8	2-ethyl-3,6-dimethyl	1062	4.6 \pm 0.64
9	diethylmethyl	1137	0.9 \pm 0.28
10	diethylmethyl	1141	2.2 \pm 0.14
11	dimethylbutyl	1232	1.1 \pm 0.12
12	dimethylbutyl	1236	0.6 \pm 0.13

^a Numbers refer to Figure 2. ^b Average of two analyses.

injector. The carrier gas for thermal desorption was then supplied by helium gas from the modified injector rather than through the standard GC pressure regulator. During desorption, which lasted for 15 min, the oven temperature was set at -40 °C by putting dry ice in the GC oven to trap all the volatiles as a sharp band in the column. At the end of desorption, the needle was pulled out from the injector, and the carrier gas supply from the GC was resumed. A more complete description of this technique has been described by Hartman et al. (1987).

GC Analysis. A Varian 3400 gas chromatograph equipped with a flame ionization detector and a fused silica capillary column (60 m \times 0.32 mm (i.d.), d_f = 1.0 μ m, DB-1; J&W Scientific, Folsom, CA) was used to analyze the volatiles isolated by the purge-and-trap method. The operating conditions were as follows: injector temperature, 270 °C; detector temperature, 300 °C; He flow rate, 1 mL/min; oven temperature, -40 to +40 °C/min, 40–260 °C at 2 °C/min. Quantitative determinations were made without considering response factors and were carried out by using a Varian 4270 integrator. Linear retention indices were calculated against *n*-paraffins as references (Majlat et al., 1974).

GC-MS Analysis. Volatiles isolated by the purge-and-trap method were analyzed by GC-MS using a Varian 3400 gas chromatograph directly coupled to a Finnigan MAT 8230 high-resolution mass spectrometer. Mass spectra were obtained by electron ionization at 70 eV. The ion source temperature was 250 °C. The filament emission current was 1 mA, and spectra were recorded on a Finnigan MAT SS 300 data system. The GC column was the same as used in the previous section.

RESULTS AND DISCUSSION

Figure 2 shows the GC profiles of total (A), pyrazine (B), and non-pyrazine fractions (C) of the volatiles generated

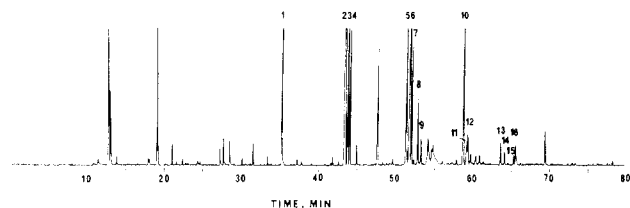


Figure 3. GC profile of pyrazines from potato chips by purge-and-trap fractionation.

Table II. Pyrazines from Potato Chips by Purge-and-Trap Fractionation

peak ^a	pyrazine	I_k (DB-1)	ppm ^b
1	2-methyl	801	3.9 ± 1.07
2	2,5-dimethyl	890	10.9 ± 0.04
3	2,6-dimethyl	894	1.5 ± 0.04
4	2,3-dimethyl	896	1.3 ± 0.30
5	2-ethyl-5-methyl	978	1.1 ± 0.10
6	2-ethyl-6-methyl	982	5.5 ± 0.04
7	2-ethyl-3-methyl	985	0.6 ± 0.06
8	2-methyl-5-vinyl	994	0.2 ± 0.04
9	2-methyl-6-vinyl	998	0.2 ± 0.04
10	2-ethyl-3,6-dimethyl	1062	2.2 ± 0.80
11	2-ethyl-3,5-dimethyl	1068	0.2 ± 0.04
12	2-ethyl-5,6-dimethyl	1069	0.3 ± 0.00
13	methyl- <i>n</i> -butyl	1120	0.1 ± 0.01
14	methyl- <i>n</i> -butyl	1126	0.1 ± 0.01
15	diethylmethyl	1137	0.1 ± 0.01
16	diethylmethyl	1141	0.1 ± 0.01

^a Numbers refer to Figure 3. ^b Average of two analyses.

from a model system of DCGM/cor oil/water. The identification and quantification of the fractionated pyrazines are listed in Table I. Three additional pyrazines, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, and 2-methyl-5-ethylpyrazine, were identified in the pyrazine fraction but could not be identified when the total volatiles were analyzed by GC-MS. Either they were too small in quantity to be detected or they were masked by other compounds in the same chromatographic area shown by asterisks in the GC profiles (Figure 2). The observed absence of pyrazines in the gas chromatogram of the non-pyrazine fraction indicated the effectiveness of the acid trap.

Figure 3 shows the GC profile of volatiles in a pyrazine fraction from commercially manufactured potato chips. The identification and quantification of the fractionated pyrazines are listed in Table II. Pyrazine compounds play a contributing role to the flavor of potato chips (Maga and Sizer, 1973). 2,5-Dimethylpyrazine, reported as reminiscent of potato chip flavor (Furia and Bellanca, 1975), was the major component among those in the pyrazine fraction collected by the developed purge-and-trap method. Except for methylbutylpyrazine and one of the two diethylmethylpyrazines, all the other identified pyrazines have been reported in potato chips in previous studies (Buttery et al., 1971; Deck et al., 1973). A number of thermally generated volatiles, such as aliphatic hydrocarbons, carbonyls, and acids, were from the oxidative degradation of fatty acids formed during deep-fat frying. Those thermal decomposition volatiles of frying fat, which were absorbed by the potato chips, were the major components (in quantity) of the aroma concentrate isolated by vacuum distillation (Dornseifer and Powers, 1965; Deck and Chang, 1965; Buttery et al., 1971). Without further fractionation after isolation, those volatiles from frying fat would certainly interfere with the identification and characterization of the flavor-important pyrazines that normally exist as

minor or trace volatile components of fried food. The simple and selective purge-and-trap method turned out to be an efficient isolation, concentration, and fractionation method. Not only can it quantify minor or trace pyrazines from a very small amount of sample containing complex volatiles but it is also much less laborious and time-consuming compared with other traditionally used methods. Once the correlation between flavor evaluation of products and quantitative data obtained from GC analyses can be obtained, this purge-and-trap method could be potentially used as a routine method for analyzing specific pyrazines of interest. In addition to pyrazines, other volatile organic bases such as thiazoles, oxazoles, and pyridines can also be selectively separated by this reported method.

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Registry No. 2-Methylpyrazine, 109-08-0; 2,5-dimethylpyrazine, 123-32-0; 2,6-dimethylpyrazine, 108-50-9; 2,3-dimethylpyrazine, 5910-89-4; 2-ethyl-5-methylpyrazine, 13360-64-0; 2-ethyl-6-methylpyrazine, 13925-03-6; 2-ethyl-3-methylpyrazine, 15707-23-0; 2-ethyl-3,6-dimethylpyrazine, 13360-65-1; diethylmethylpyrazine, 39723-60-9; dimethylbutylpyrazine, 97485-49-9; 2-methyl-5-vinylpyrazine, 13925-08-1; 2-methyl-6-vinylpyrazine, 13925-09-2; 2-ethyl-3,5-dimethylpyrazine, 13925-07-0; 2-ethyl-5,6-dimethylpyrazine, 15707-34-3; methyl-*n*-butylpyrazine, 106100-48-5; 2-methyl-5-ethylpyrazine, 13360-64-0.

LITERATURE CITED

- Buttery, R. G.; Seifert, R. M.; Guadagni, D. G.; Ling, L. C. Characterization of Volatile Pyrazines and Pyridine Components of Potato Chips. *J. Agric. Food Chem.* **1971**, *19*, 969-971.
- Chen, C. C.; Kuo, M. C.; Hwang, L. S.; Wu, J. S. B.; Wu, C. M. Headspace Components of Passion Fruit Juice. *J. Agric. Food Chem.* **1982**, *30*, 1211-1215.
- Deck, R. E.; Chang, S. S. Identification of 2,5-Dimethylpyrazine in the Volatile Flavor Compounds of Potato Chips. *Chem. Ind.* **1965**, 1343-1344.
- Deck, R.; Pokorny, J.; Chang, S. S. Isolation and Identification of Volatile Compounds from Potato Chips. *J. Food Sci.* **1973**, *38*, 345-349.
- Dornseifer, T. P.; Powers, J. J. Volatile Constituents of Potato Chips and Changes During Storage. *Food Technol.* **1965**, *19*, 877-879.
- Furia, T. E.; Bellanca, N. *Fenaroli's Handbook of Flavor Ingredients*, 2nd, ed.; CRC Press: Cleveland, OH, 1975.
- Hartman, T. G.; Rosen, R. T.; Ho, C.-T.; Rosen, J. D. A Simple and Inexpensive On-Column Injection System for GC Capillary Columns. *LC-GC* **1987**, *5*, 834-836.
- Maarse, H.; Schaefer, J. Quantitative Headspace Analysis: Total and Specific Group Analysis. In *Analysis of Food and Beverages—Headspace Techniques*; Charalambous, G., Ed.; Academic Press: New York, 1978; pp 17-35.
- Maga, J. A. Pyrazines in Foods: An Update. *CRC Crit. Rev. Food Sci. Nutr.* **1982**, *16*, 1-48.
- Maga, J. A.; Sizer, C. E. Pyrazines in Foods. A Review. *J. Agric. Food Chem.* **1973**, *21*, 22-30.
- Majalal, P.; Erdos, Z.; Takacs, J. Calculation and Application of Retention Indices in Programmed Temperature Gas Chromatography. *J. Chromatogr.* **1974**, *91*, 89-103.
- Peyron, L. Recent Techniques in the Analysis of Heterocyclic Aroma Compounds in Food. In *Chemistry of Heterocyclic Compounds in Flavors and Aromas*; Vernin, G., Ed.; Ellis Horwood Ltd.: Chichester, 1982; pp 262-304.

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