feature in addition to appreciable amounts of the relatively rare neoeriocitrin can be used as a means of distinguishing sour orange juices from grapefruit juice.

CONCLUSION

Since there are several cultivars that contain naringin and can legally be present in commercial orange juice, the view that naringin alone can be used to detect the presence of grapefruit juice is clearly incorrect. Other flavanone glycosides such as neohesperidin and neoeriocitrin must also be taken into consideration. Naringin concentrations in grapefruit, sour orange, and K-Early juices are reasonably similar. However, neohesperidin concentration ranges were unique for each of the three naringin-containing cultivar groups. Therefore, the concentration profile of naringin and especially neohesperidin could be used to distinguish between juices that may be legally added to orange juice (sour orange, K-Early) from those that may not (grapefruit juice).

Registry No. Naringin, 10236-47-2; narirutin, 14259-46-2; neohesperidin, 13241-33-3; hesperidin, 520-26-3.

LITERATURE CITED

Albach, R. F.; Redman, G. H. "Composition and Inheritance of Flavanones in Citrus Fruit". *Phytochemistry* **1969**, *8*, 127–143.

Anis, M.; Aminuddin. "Flavonoid Patterns of Leaves of Some Citrus Species and their Hybrids". *J. Plant Biochem.* **1981**, *8*(1), 56–60.

Attaway, J. A.; Barron, R. W.; Blair, J. G.; Buslig, B. S.; Carter, R. D.; Dougherty, M. H.; Fellers, P. J.; Fisher, J. F.; Hill, E. C.; Huggart, R. L.; Maraulja, M. D.; Petrus, D. R.; Ting, S. V.; Rouse, A. H. "Some New Analytical Indicators of Processed Orange Juice Quality, 1971–72". *Proc. Fla. State Hortic. Soc.* **1972**, *85*, 192–203.

Drawert, F.; Leupold, G.; Pivernetz, H. "Quantitative Determination of Rutin, Hesperidin and Naringin in Orange Juice by Gas Chromatography". *Chem. Mikrobiol., Technol. Lebensm.* **1980**, *6*, 189–191.

Galensa, R.; Ara, V.; Siewek, F. "Investigations on the Content of Naringin in Orange Juice". *Fluess. Obst* **1986**, *53*, 454–456.

Greiner, G.; Wallrauch, S. "Naringin as Evidence for blending Grapefruit Juice with Orange and Tangerine Juices". *Fluess. Obst* **1984**, *12*, 626–628.

Horowitz, R. M. "Studies on the Structure and Bitterness of the Flavanone Glycosides of Citrus". In *Proceedings of Symposium on Biochemistry of Plant Phenolic Substances*; Johnson, G., Geissman, T. A., Eds.; Colorado State University: Fort Collins, CO, 1961; pp 1–8.

Kamiya, S.; Esaki, S.; Knoishi, F. "Flavonoids in Citrus Hybrids". *Agric. Biol. Chem.* **1979**, *43*, 1529–1536.

Park, G. L.; Avery, S. M.; Byers, J. L.; Nelson, D. B. "Identification of Bioflavonoids from Citrus". *Food Technol.* **1983**, *37*(12), 98–105.

Swingle, W. T.; Reece, P. C. "The Botany of Citrus and Its Wild Relatives". In *The Citrus Industry*; Webber, H. J., Betchelor, L. D., Eds.; University of California: Berkely, CA, 1943; Vol. 1, pp 129–174.

Tatum, J. H.; Berry, R. E.; Hearn, C. J. "Characterization of Citrus Cultivars and Separation of Nucellar and Zygotic Seedlings by Thin Layer Chromatography". *Proc. Fla. State Hortic. Soc.* **1974**, *87*, 75–81.

U.S. Food and Drug Administration "Frozen Concentrated Orange Juice". 21: CFR 146.146; U.S. GPO: Washington, DC, revised April 1984.

Young, R. "Fresh Fruit Cultivars". In *Fresh Citrus Fruits*; Wardowski, W. F., Nagy, S., Grierson, W., Eds.; Avi: Westport, CT, 1986; pp 101–126.

Received for review January 9, 1987. Accepted June 8, 1987.

Analysis of Volatile Heteroatomic Meat Flavor Principles by Purge-and-Trap/Gas Chromatography–Mass Spectrometry

John R. Vercellotti,* Jui-Chang W. Kuan, Ray H. Liu,[1] Michael G. Legendre,[2] Allen J. St. Angelo, and Harold P. Dupuy[3]

A simple purge-and-trap/gas chromatography–mass spectrometry (GC–MS) procedure was developed and proven effective for the comprehensive analysis of nitrogen-, oxygen-, and sulfur-containing volatile compounds in ground roast beef, commerical beef flavor concentrate, and beef meat powder. These compounds, as released from the sample heated in the evaporator flask of a rotary evaporator, were effectively trapped in a Tenax-containing glass liner under 5 psi vacuum. The trap was then heated in an external inlet port, and the volatiles were purged into a GC–MS system for identification and concentration estimation. More than 50 heteroatomic, mostly cyclic, compounds were identified in these samples.

It has been repeatedly suggested (Vernin, 1982; Shibamoto, 1980; Ohloff and Flament, 1979; Maga, 1975, 1981, 1982; Maga and Sizer, 1973, 1974; Wasserman, 1979; Dwivedi, 1975; Katz, 1981; KacLeod and Seyyedain-Ardebili, 1981) that heteratomic compounds containing oxygen, nitrogen, and sulfur, mostly Maillard reaction products with cyclic structures (Bailey, 1983), are the principal constituents of meat flavors and aromas. Numerous methods, as summarized in the reviews cited above, for the identification and isolation of these compounds have been developed. These procedures, in general, required kilograms of sample and involved laborious and time-consuming extraction steps, followed by concentration of large volumes of combined extracts (Min et al., 1979; Mussinan et al., 1976; Chang et al., 1968; Tonsbeek et al., 1969, 1971; Watanabe and Sato, 1971; Mussinan et al., 1973; Wilson et al., 1973; Hirai et al., 1973; van der Ouweland and Peer, 1975).

Southern Regional Research Center, U.S. Department of Agriculture—Agricultural Research Service, New Orleans, Louisiana 70179.

[1]Current address: Department of Criminal Justice, University of Alabama at Birmingham, Birmingham, AL 35294.

[2]Current address: USDA, APHIS, PPQ, P.O. Box 3209, Gulfport, MS 39505-3209.

[3]Visiting professor, Virginia Polytechnic Institute and State University, Blacksburg, VA 24601.

As part of on-going studies aimed at a better understanding of the chemical basis underlying the development of warmed-over flavor in meat and concomitant sensory effects (Vercellotti et al., 1985; St. Angelo et al., 1987; Dupuy et al., 1987), we are interested in the development of methodologies suitable for monitoring meat flavor principles and lipid oxidation products. This paper reports a simple and efficient method for the isolation, identification, concentration, and estimation of a wide range of volatile meat flavor principles and oxidation products of meats and meat-related products down to the parts per billion level. Although this approach can be used for the simultaneous analysis of both meat flavor principles and lipid oxidation products, only the results related to the former category of compounds will be reported.

MATERIALS AND METHODS

Procedure and Apparatus. The analysis includes three types of beef-related samples: cooked ground roast beef, beef flavor concentrate, and meat powder. Roast beef samples were prepared from ca. 0.5-kg chunks of commerical (Economical Supermarket, Metairie, LA) lean boneless top round raw beef. The oven was preheated to 163 °C, and the beef samples were roasted to internal temperatures of 85 and 95 °C. The weight changes of these two samples occurring in the 2-h roasting process were from 440 to 288 g and 389 to 232 g, respectively. The entire roast was ground, and 250- and 206-g aliquots were used for analysis. The beef flavor concentrate and beef meat powder samples were obtained from the Campbell Soup Co. (Camdem, NJ) and International Seasoning, Inc. (Torrance, CA). A 1-g portion of beef flavor concentrate (Campbell) and 5 g of beef meat powder (International Seasoning) were mixed, respectively, with 50 mL of water prior to the trapping process. For the purge-and-trap procedure, samples were placed in a 1-L round-bottom evaporator flask of a Buchi rotary evaporator. The condenser and the receiver flask of the rotary evaporator were replaced with a Soxhlet extractor in a reversed geometry. The exit end of the extractor was connected to one end of a glass liner that was packed with 200 mg (60/80 mesh) of Tenax GC (Teklab, Baton Rouge, LA) sandwiched between two plugs of volatile-free glass wool. A 5 psi vacuum was applied to the other end of the glass liner. The sample in the evaporator flask was immersed in a water bath preheated to 75 °C. The Tenax cartridge was preconditioned at 250 °C under helium for at least 24 h and cooled to ambient temperature prior to trapping volatiles generated from the heated sample for 3 h.

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis. A custom-built closed external inlet device (Legendre et al., 1979; Scientific Instrument Service, River Ridge, LA), attached to a Finnigan 4500 GC–MS system, was used for desorbing the volatiles from the Tenax cartridge. The cartridge end that faced the sample in the absorption step was placed toward the GC column. Volatiles were forced into the column by passing the helium carrier gas through the inlet port for 3 min at 200 °C. This desorption condition had been proven effective in quantitative removal of lipid oxidation products that existed in much higher abundance (Suzuki and Bailey, 1985). A 50-m SE-54 (cross-linked 5% phenylmethylsilicone) capillary column (0.31-mm i.d., 0.52-μm film thickness) was used for separation. The flow rate of the carrier gas was 1.2 mL/min with a split ratio of 5:1. The oven temperature was held at −30 °C for 3 min during sample purging and then programmed at 5 °C/min and held at the final temperature, 250 °C, for 20 min. The mass spectrometer was operated on EI mode at 70 eV, scanning from m/z 33 to 450 at 1 s/cycle. Comparisons of the mass spectra of the compounds identified with those in the NBS–EPA and user-generated libraries were made with the library program of the INCOS data system.

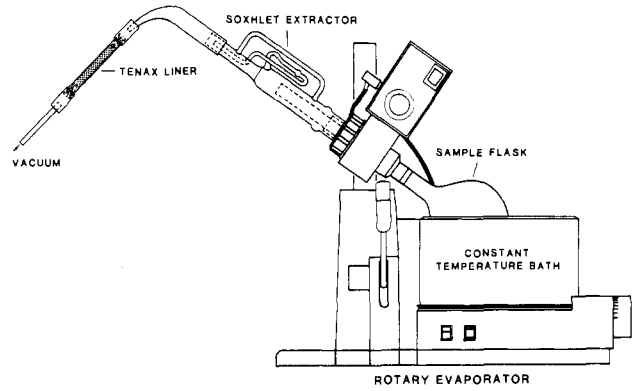

Figure 1. Apparatus for meat volatiles generation and condenser system for trapping on Tenax GC liner under diminished pressure.

Relative (mass spectral) response factors for selected compounds in the ion chromatogram were determined as follows. A known amount of reference compound was spiked with a known concentration of internal standard. The area counts for each peak were then determined from the ion chromatogram. The relative response factor (RRF) was then calculated by dividing the response of the reference compound by that of the internal standard:

$$\mathrm{RRF} = \frac{\left(\dfrac{\text{ion resp area counts}}{\text{concn}}\right)_{\text{ref}}}{\left(\dfrac{\text{ion resp area counts}}{\text{concn}}\right)_{\text{int std}}}$$

To ensure suitably similar intensities in the ion chromatogram response, the compound under study for relative response comparison was either identical with the substance to be quantitated or of comparably close molecular structure. Structures were selected to fragment into ions of intensity similar to the compound being determined. Quantification was based on relative response factors for the internal standard, pyridine-d_5. Reproducibility of response was checked with thiazole, 4-methylthiazole, pyrazine, and 2-methylpyrazine as references for typical heterocycles in meat flavor.

RESULTS AND DISCUSSION

Since the development of a direct GC procedure for the analysis of volatiles in vegetable oil (Dupuy et al., 1971), the same approach was extended to the analysis of aqueous systems with an adsorption/desorption device serving as an enrichment and water-filtering mechanism (Rayner et al., 1978). An external inlet device (Legendre et al., 1979) was further developed for use in GC–MS systems.

In the present study, the modified evaporator system shown in Figure 1 facilitated the concentration of trace volatile components from roast beef down to the parts per billion level (see results shown later). Meat flavor principles as well as oxidation products were effectively adsorbed onto the Tenax trap while the sample was heated under vacuum. Holding the column at −30 °C, while the volatile compounds were eluted from the Tenax trap, took advantage of cryofocusing techniques (Dupuy et al., 1985; Brettell and Grob, 1985) and allowed the concentration of the analytes onto the initial portion of the column. Since the volatiles were trapped moisture free under the vacuum trapping conditions, they were purged directly into

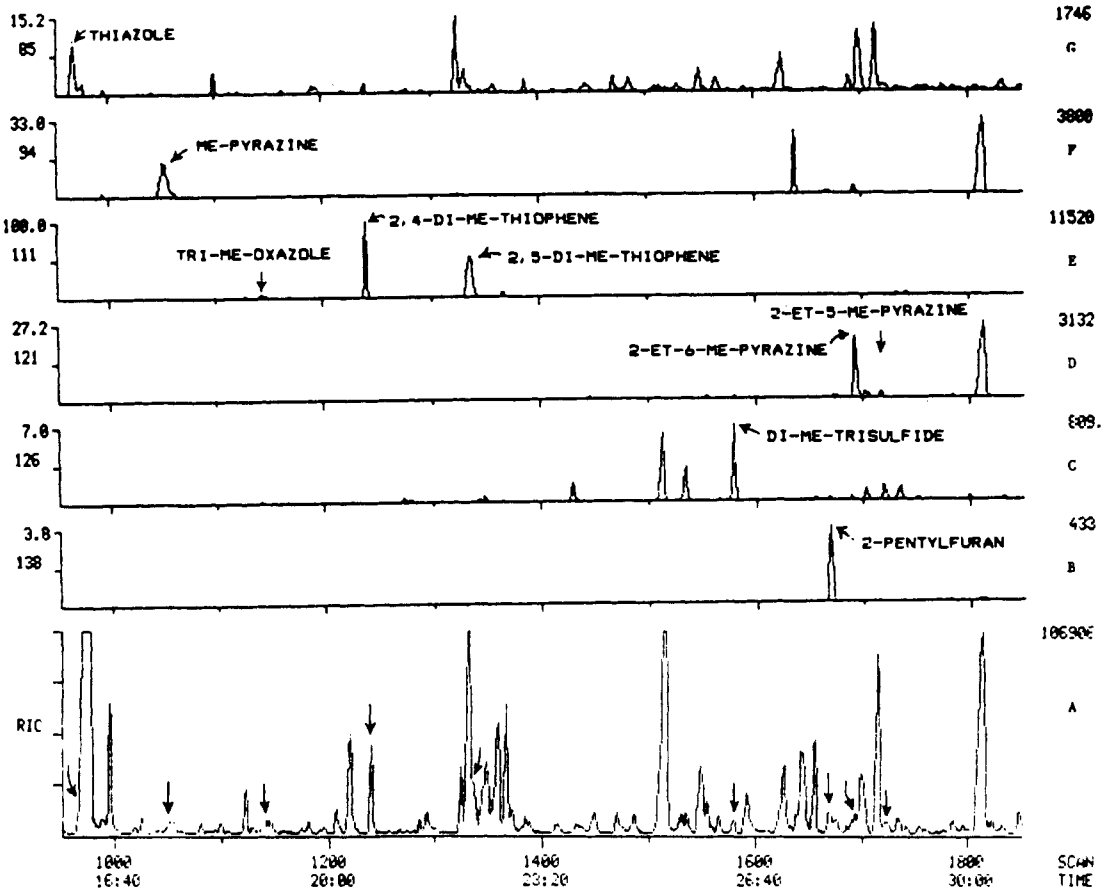

Figure 2. A. Portion of a typical ion chromatogram under conditions described. B–G. Selected ion displays used in screening for presence of target flavor compounds.

the GC–MS system. Moisture-free sample and subambient oven temperature programming allowed effective resolution of low molecular weight volatiles as well as high molecular weight ones. It should be emphasized that a successful experiment required a moisture-free sample. Under the cryofocusing conditions, moisture may occlude the capillary column, thus preventing the column from accepting the volatiles eluted from the trap in the external inlet. The occlusion may further nullify the separation capability of the column in the −30 to 0 °C temperature range in which the lower molecular weight volatiles would be most effectively resolved.

Qualitative Analysis. A portion of a typical chromatogram obtained under the described conditions is shown in Figure 2A. Selected ion displays (Figure 2B–G) were used for alerting the possible presence of target compounds. If their presence were confirmed, these single-ion intensities were integrated for quantitative information. Heteroatomic compounds detected in the three types of samples analyzed are listed in the first column of Table I. Compounds that were confirmed with authentic references are noted in the table. The confirmation process includes the comparison of chromatographic and mass spectrometric parameters of the test compounds and those of authentic standards submitted to the identical procedure. The identification of other compounds might be considered tentative (Christman, 1982); the mass spectra of these compounds matched well with those of assigned structures. Their relative chromatographic retention characteristics were also considered reasonable with respect to those compounds positively confirmed.

Quantitative Analysis and Detection Limit. The well-established internal standard method was used to estimate the concentrations of the compounds identified. A series of experiments were performed in which known amounts of authentic compounds were mixed with a known amount of the deuteriated internal standard, pyridine-d_5, and this mixture was analyzed under the same procedure used for experimental samples. The relative response factors of selected ions (cf., Materials and Methods and column 4 in Table I) for these compounds, with respect to the m/z 85 ion for the internal standard, pyridine-d_5, were calculated. These factors were then used as the basis for converting the response ratios observed in the samples into corresponding concentration values. The relative responses of selected compounds were studied and proven reproducible as shown in Table II. With these established precisions, the true concentrations of the compounds identified may vary 5.5–16% (1 standard deviation) from that listed in the main entries of Table I. The concentrations of many other compounds (where each quantification basis was designated as A–J) were estimated based on the relative response factors established for compounds of similar chemical structures. Assuming that these response factors may err up to 100%, the true concentrations of the corresponding compounds may deviate from those listed in Table I by as much as 116%. Noting that the analytes are in ppb levels, an error in the order of 100% (meaning half to twice the reported value) still represents an acceptable estimate.

The detection limits for the compounds listed in Table I vary with their relative response factors and the amount of samples used for analysis. With the ions selected (Table I) for concentration estimation, the relative responses of these compounds ranged from 0.30 (2-pentylfuran) to 2.5 (thiazole) with respect to the m/z 85 ion derived from the internal standard, pyridine-d_5. From the information (sample weight, amount of internal standard used, mass

Table I. Heteroatomic Compounds[a] Identified in Beef-Derived Samples

compound	RRT[b]	mol wt	quant ion	quant[c] basis	concentration[d] roast beef 85 °C	roast beef 95 °C	BFC[e]	BMP[f]
2-ethylfuran	0.4429	96	96	S	26	23	96	5.8
thiazole	0.4952	85	85	S	7.2	3.6	880	–
pyrazine	0.4978	80	80	S	46	9.1	300	–
2,3-dimethylthiirane	0.4997	88	88	A	–	–	410	–
2,5-dimethylfuran	0.5120	96	96	B	6.9	1.2	–	–
dimethyl disulfide	0.5120	94	94	S	71	54	350	37
1*H*-pyrrole	0.5417	67	67	C	–	–	670	1400
4,5-dimethyloxazole	0.5475	97	97	D	–	–	370	–
2-methylthiophene	0.5643	98	97	E	7.2	7.6	470	25
3-methylthiophene	0.5785	98	97	E	–	–	90	2.6
3-methylisothiazole	0.6385	99	99	E	2.7	–	320	–
dihydro-2-methyl-3(2*H*)-furanone	0.6431	100	100	B	–	3.7	2400	22
4-methylthiazole	0.6579	99	99	S	0.6	–	86	–
2-methylpyrazine	0.6792	94	94	S	52	82	6600	480
2-furancarboxaldehyde	0.6979	96	96	B	44	5.0	690	–
2-methylthiazole	0.7341	99	99	S	–	–	120	–
trimethyloxazole	0.7373	111	111	D	–	–	170	0.8
2,4-dimethylthiophene	0.8006	112	111	E	–	–	1600	14
2,5-dimethylthiophene	0.8625	112	111	E	–	–	1700	–
1-(2-furanyl)ethanone	0.8832	110	110	B	–	–	4700	30
2,3-dimethylpyrazine	0.8785	108	108	F	36	–	–	–
2,5-dimethylpyrazine	0.8858	108	108	S	41	38	4300	–
2-ethylpyrazine	0.8923	108	107	S	–	7.1	750	–
2,4-dimethylthiazole	0.8186	113	113	S	–	–	4300	–
dihydro-1-ethyl-1*H*-pyrrole-2,5-dione	0.9252	125	110	D	–	–	1200	–
methyl propyl disulfide	0.9284	122	122	A	–	–	78	5.2
2,6-dimethylpyrazine	0.9310	108	108	S	40	25	–	–
2,5-dimethylthiazole	0.9329	113	113	D	–	–	360	–
dimethyl trisulfide	1.0194	126	126	S	13	35	120	32
2-thioxo-4-imidazolidinone	1.0691	116	116	G	–	–	2000	–
2-pentylfuran	1.0775	138	138	S	110	14	410	34
2-furfuryl acetate	1.0872	140	140	B	–	–	110	–
4-ethyl-2-methylthiazole	1.0911	127	127	D	–	–	390	0.4
2-ethyl-6-methylpyrazine	1.0930	122	121	S	22	10	1500	210
2,3,5-trimethylpyrazine	1.1053	122	122	S	28	5.4	770	–
2-ethyl-5-methylpyrazine	1.1092	122	121	S	17	8.5	210	100
2-ethyl-5-methylthiazole	1.1105	127	127	S	–	–	1500	210
2-ethyl-3-methylpyrazine	1.1123	122	121	S	–	2.5	–	76
2-ethyldimethyl-1,3-oxathiane	1.1325	160	131	B	–	–	51	–
5-ethyl-4-methylthiazole	1.1376	127	127	D	–	–	40	–
2-ethenyl-6-methylpyrazine	1.1388	120	120	I	–	–	–	220
2-ethyl-2,6-dimethyl-1,3-oxathiane	1.2351	160	131	D	–	–	51	–
dipropyl disulfide	1.2570	150	150	A	–	–	33	–
1-methylethyl propyl disulfide	1.2577	150	150	A	–	–	33	–
5-ethyl-2,4-dimethylthiazole	1.2874	141	126	D	–	–	160	–
3-ethyl-2,5-dimethylpyrazine	1.2938	136	135	S	13	6.3	840	600
2-ethyl-3,5-dimethylpyrazine	1.3074	136	135	S	–	–	350	–
bis(1-methylethyl) disulfide	1.3629	150	150	A	–	–	71	13
3,4-dimethylpyrazolo[5,1-*c*][1,2,4]triazine	1.3655	148	148	F	–	–	130	–
2-propylthiophene	1.3971	126	125	D	–	–	230	–
2,5-diethyl-4-methylthiazole	1.4101	155	140	D	–	–	27	–
thiazolidinethione	1.4230	119	119	G	–	–	29	–
2-methyl-3,5-diethylpyrazine	1.4776	150	149	J	–	–	–	120
2,5-dimethyl-3-propylpyrazine	1.4851	150	149	J	–	–	–	34
2-phenylthiophene	1.5237	160	160	E	–	–	550	15
3,5-dimethyl-1,2,4-trithiolane	1.5502	152	152	D	–	–	41	–
2-*n*-butylthiophene	1.5863	140	140	D	–	–	23	–
benzothiazole	1.6393	135	135	S	1.7	0.5	190	6.4

[a] Common lipid oxidation products are not included. [b] Relative retention time with respect to benzaldehyde. RRT = $(T_t - T_c)/(T_b - T_c)$, where T_t, T_c, and T_b are retention times of the test compound, CO_2, and benzaldehyde, respectively. [c] Quantifications of the compounds identified were based on the response factors established for the authentic compound (S), dimethyl disulfide (A), 2-ethylfuran (B), pyrazine (C), 4,5-dimethylthiazole (D), 4-methylthiazole (E), 2,6-dimethylpyrazine (F), thiazole (G), 2-ethyl-3-methylpyrazine (H), 2-ethyl-5-methylpyrazine (I), and 2-ethyl-3,5-dimethylpyrazine (J). [d] Concentrations are listed in parts per billion with two significant figures. [e] Beef flavor concentrate from Campbell Soup Co. [f] Beef meat powder from International Seasoning, Inc.

Table II. Reproducibility Study on the Relative Responses of Selected Compounds

compound	ion *m/z*	relative response			mean	std dev	std dev/mean
pyridine-d_5	84	1.00	1.00	1.00			
thiazole	85	2.38	2.61	2.41	2.47	0.243	9.8%
4-methylthiazole	99	2.48	2.62	2.77	2.62	0.145	5.5%
pyrazine	80	0.470	0.402	0.530	0.467	0.0640	14%
2-methylpyrazine	94	0.290	0.285	0.377	0.317	0.0517	16%

Table III. Estimation of Detection Limits

parameter	roast beef 85 °C	roast beef 95 °C	BFC[a]	BMP[b]
sample wt, g	250	206	1.00	5.00
wt of int std, μg	1.05	1.05	1.05	1.05
concn int std, ppb	4.22	5.07	1050	210
response (area) of int std	401	1560	8963	45729
min detectable area	100	100	100	100
concn of analyte corresp to min detectable area, ppb				
rel resp of analyte, 0.3	3.5	1.1	39	1.5
rel resp of analyte, 2.5	0.42	0.13	4.7	0.18

[a] Beef flavor concentrate from the Campbell Soup Inc. [b] Beef meat powder from International Seasoning, Inc.

spectrometric response for the internal standard) listed in Table III and an integrated area of 100 for the selected ions of respective analytes as the limit of detection, the lowest concentrations of 2-pentylfuran (relative response 0.30) detectable in these four samples were estimated as 3.5, 1.1, 39, and 1.5 ppb, respectively. For thiazole (relative response 2.5), the corresponding detection limits were 0.42, 0.13, 4.7, and 0.18 ppb, respectively. The main entries (Table I) filled with hyphens stand for concentrations that are below these corresponding detection limits.

Entries in Table I indicate that more heteroatomic compounds (often with higher concentration) were found in the beef flavor concentrate and the beef meat powder. Since these two commercial products are concentrated forms of beef flavor, the higher concentrations of the heteroatomic compounds observed in these products were anticipated. Furthermore, the lower responses (401 and 1560 in Table III) observed for the internal standard in the roast beef samples are probably indications of an incomplete purging process associated with the large sample size. It is possible that purging conditions, such as temperature, vacuum level, moisture content, and pH, used in this study might not have contributed significantly toward the intended improvement of detection limits through the use of larger sample size. Since most of these test compounds exist in the roast beef samples at a lower level, many of them probably remain undetected and are not entered in Table I.

CONCLUSION

Selected MS ion intensities are commonly used as the basis for quantitative determination of compounds encountered in environmental samples (Liu et al., 1984). To our knowledge, this study represents the first comprehensive application of a selected MS ion intensity approach to the analysis of heteroatomic compounds in meat-related samples. The ability to estimate low levels of these compounds, as demonstrated by this study, is extremely informative and may be considered significant progress in the subject area. The merits of our approach are further substantiated by the ability to simultaneously analyze a wide range of compounds in a fraction of the time. Also, the meat sample size needed in our method is relatively much smaller than that required in other methods as described in the meat flavor literature.

Registry No. 2-Ethylfuran, 3208-16-0; thiazole, 288-47-1; pyrazine, 290-37-9; 2,3-dimethylthiirane, 4426-36-2; 2,5-dimethylfuran, 625-86-5; dimethyl disulfide, 624-92-0; 1*H*-pyrrole, 109-97-7; 4,5-dimethyloxazole, 20662-83-3; 2-methylthiophene, 554-14-3; 3-methylthiophene, 616-44-4; 3-methylisothiazole, 693-92-5; dihydro-2-methyl-3(2*H*)-furanone, 3188-00-9; 4-methylthiazole, 693-95-8; 2-methylpyrazine, 109-08-0; 2-furancarboxaldehyde, 98-01-1; 2-methylthiazole, 3581-87-1; trimethyloxazole, 20662-84-4; 2,4-dimethylthiophene, 638-00-6; 2,5-dimethylthiophene, 638-02-8; 1-(2-furanyl)ethanone, 1192-62-7; 2,3-dimethylpyrazine, 5910-89-4; 2,5-dimethylpyrazine, 123-32-0; 2-ethylpyrazine, 13925-00-3; 2,4-dimethylthiazole, 541-58-2; dihydro-1-ethyl-1*H*-pyrrole-2,5-dione, 128-53-0; methyl propyl disulfide, 2179-60-4; 2,6-dimethylpyrazine, 108-50-9; 2,5-dimethylthiazole, 4175-66-0; dimethyl trisulfide, 3658-80-8; 2-thioxa-4-imidazolidinone, 503-87-7; 2-pentylfuran, 3777-69-3; 2-furfuryl acetate, 623-17-6; 4-ethyl-2-methylthiazole, 32272-48-3; 2-ethyl-6-methylpyrazine, 13925-03-6; 2,3,5-trimethylpyrazine, 14667-55-1; 2-ethyl-5-methylpyrazine, 13360-64-0; 2-ethyl-5-methylthiazole, 19961-53-6; 2-ethyl-3-methylpyrazine, 15707-23-0; 2-ethyldimethyl-1,3-oxathiane, 110271-14-2; 5-ethyl-4-methylthiazole, 31883-01-9; 2-ethenyl-6-methylpyrazine, 13925-09-2; 2-ethyl-2,6-dimethyl-1,3-oxathiane, 110271-13-1; dipropyl disulfide, 629-19-6; 1-methylethyl propyl disulfide, 33672-51-4; 5-ethyl-2,4-dimethylthiazole, 38205-61-7; 3-ethyl-2,5-dimethylpyrazine, 13360-65-1; 2-ethyl-3,5-dimethylpyrazine, 13925-07-0; bis(1-methylethyl) disulfide, 4253-89-8; 3,4-dimethylpyrazolo[5,1-*c*]-[1,2,4]triazine, 6726-49-4; 2-propylthiophene, 1551-27-5; 2,5-diethyl-4-methylthiazole, 41981-71-9; thiazolidinethione, 41583-92-0; 2-methyl-3,5-diethylpyrazine, 18138-05-1; 2,5-dimethyl-3-propylpyrazine, 18433-97-1; 2-phenylthiophene, 825-55-8; 3,5-dimethyl-1,2,4-trithiolane, 23654-92-4; 2-*n*-butylthiophene, 1455-20-5; benzothiazole, 95-16-9.

LITERATURE CITED

Bailey, M. E. In *The Maillard Reaction in Food and Nutrition*; Waller, G. R., Feather, M. S., Eds.; ACS Symposium Series 215; American Chemical Society: Washington, DC, 1983; p 169.

Brettell, T. A.; Grob, R. L. *Am. Lab. (Fairfield, Conn.)* **1985**, *17*(11), 50.

Chang, S. S.; Hirai, C.; Reddy, B. R.; Herz, K. O.; Kato, A. *Chem. Ind. (London)* **1968**, 1639.

Christman, R. F. *Environ. Sci. Technol.* **1982**, *16*, 143A.

Dupuy, H. P.; Fore, S. P.; Goldblatt, L. A. *J. Am. Oil Chem. Soc.* **1971**, *48*, 876.

Dupuy, H. P.; Flick, G. J., Jr.; Bailey, M. E.; St. Angelo, A. J.; Legendre, M. G.; Sumrell, G. *JAOCS, J. Am. Oil Chem. Soc.* **1985**, *62*, 1690.

Dupuy, H. P.; Bailey, M. E.; St. Angelo, A. J.; Legendre, M. G.; Vercellotti, J. R. In *Warmed-Over Flavor of Meat*; St. Angelo, A. J., Bailey, M. E., Eds.; Academic: Orlando, FL, 1987; in press.

Dwivedi, B. K. *CRC Crit. Rev. Food Technol.* **1975**, *5*, 487.

Hirai, C.; Herz, K. O.; Pokorny, J.; Chang, S. S. *J. Food Sci.* **1973**, *38*, 393.

Katz, I. In *Flavor Research: Recent Advances*; Teranishi, R., Flath, R. A., Sugisawa, H., Eds.; Marcel Dekker: New York, 1981; p 217.

Legendre, M. G.; Fisher, G. S.; Schuller, W. H.; Dupuy, H. P.; Rayner, E. T. *J. Am. Oil Chem. Soc.* **1979**, *56*, 552.

Liu, R. H.; Ramesh, S.; Liu, J. Y.; Kim, S. *Anal. Chem.* **1984**, *56*, 1808.

Maga, J. A. *CRC Crit. Rev. Food Sci. Nutr.* **1975**, *5*, 153.

Maga, J. A. *CRC Crit. Food Sci. Nutr.* **1981**, *14*, 295.

Maga, J. A. *CRC Crit. Rev. Food Sci. Nutr.* **1982**, *15*, 153.

Maga, J. A.; Sizer, C. E. *J. Agric. Food Chem.* **1973**, *21*, 22.

Maga, J. A.; Sizer, C. E. *CRC Crit. Rev. Food Technol.* **1974**, *4*, 39.

MacLeod, G.; Seyyedain-Ardebili, M. *CRC Crit. Rev. Food Sci. Nutr.* **1981**, *14*, 309.

Min, D. B. S.; Ina, K.; Peterson, R. J.; Chang, S. S. *J. Food Sci.* **1979**, *44*, 639.

Mussinan, C. J.; Wilson, R. A.; Katz, I. *J. Agric. Food Chem.* **1973**, *21*, 871.

Mussinan, C. J.; Wilson, R. A.; Katz, I.; Hruza, A.; Vock, M. H. In *Phenolic, Sulfur, and Nitrogen Compounds in Food Flavor*; Charalambous, G., Katz, I., Eds.; ACS Symposium Series 26; American Chemical Society: Washington, DC, 1976; p 133.

Ohloff, G.; Flament, I. In *Progress in the Chemistry of Organic Natural Products*; Herz, W. Ed.; Springer-Verlag: New York, 1979; Vol. 36, p 231.

Rayner, E. T.; Wadsworth, J. I.; Legendre, M. G.; Dupuy, H. P. *J. Am. Oil Chem. Soc.* **1978**, *55*, 454.

St. Angelo, A. J.; Vercellotti, J. R.; Legendre, M. G.; Vinnett, C. H.; Kuan, J. W.; James, C., Jr.; Dupuy, H. P. *J. Food Sci.* **1987**, in press.

Shibamoto, T. *J. Food Chem.* **1980**, *28*, 237.

Suzuki, J.; Bailey, M. E. *J. Agric. Food Chem.* **1985**, *33*, 343.
Tonsbeek, C. H. T.; Koenders, E. B.; van der Zijden, A. S. M.; Losekoot, J. A. *J. Agric. Food Chem.* **1969**, *17*, 397.
Tonsbeek, C. H. T.; Copier, H.; Plancken, A. J. *J. Agric. Food Chem.* **1971**, *19*, 1014.
Van der Ouweland, G. A. M.; Peer, H. G. *J. Agric. Food Chem.* **1975**, *23*, 501.
Vercellotti, J. R.; St. Angelo, A. J.; Dupuy, H. P. Presented at the 190th National Meeting of the American Chemical Society, Chicago, IL, Sept 1985; paper CARB 12.
Vernin, G. In *Chemistry of Heterocyclic Compounds in Flavours and Aromas*; Vernin, G., Ed.; Ellis Horwood: Chichester, 1982; p 72.
Wasserman, A. E. *J. Food Sci.* **1979**, *28*, 237.
Watanabe, K.; Sato, Y. *J. Agric. Food Chem.* **1971**, *19*, 1017.
Wilson, R. A.; Mussinan, C. J.; Katz, I.; Sanderson, A. *J. Agric. Food Chem.* **1973**, *21*, 873.

Received for review August 28, 1986. Accepted May 20, 1987.

Strecker Degradation of Leucine and Valine in a Lipidic Model System

Anna Arnoldi,* Claudio Arnoldi, Olga Baldi, and Alberto Griffini

Leucine and valine were reacted with fructose in a mixture of cocoa butter and water at different temperatures. The rate of the Strecker reaction is higher in cocoa butter–water than in water. In the presence of cocoa butter the two amino acids give some aldehyde also without sugar. The participation of cocoa butter in the production of flavor during the roasting of cocoa beans is therefore proposed.

Chocolate flavor is the product of several operations among which fermentation and roasting of beans are of great importance for its quality.

Fermentation produces some of the flavor components and some indispensable precursors like glucose, fructose, and free amino acids, while during roasting at well-defined temperatures (generally in the range 115–140 °C) reducing sugars and amino acids react together and the principal compounds of the flavor are obtained.

The qualitative and quantitative determination of sugars in cocoa beans before and after fermentation was studied by Rohan and Stewart (1967b) and Reineccius et al. (1972a), and the production of free amino acids during fermentation, by Rohan and Stewart (1967a). The destruction of both amino acids and sugar during roasting is well documented (Rohan and Stewart, 1966, 1967c; Reineccius et al. 1972b).

Many groups analyzed volatile constituents of roasted beans or cocoa powder and identified, among others, carbonyl compounds (Van Praag et al., 1968; Van der Wal et al., 1971).

By gas chromatographic headspace analysis, Ziegleder (1972) studied some carbonyl compounds with the aim of using them as indicators during cocoa processing, and in particular he defined the optimal concentration of 3-methylbutanal.

Darsley and Quesnel (1972) by radioactive tracer techniques showed that carbonyl compounds derive from amino acids like leucine, phenylalanine, and threonine.

The main route for the formation of aldehydes during the process of roasting is the Strecker degradation (Hodge, 1953). In this reaction amino acids react with α-dicarbonyl and α-hydroxycarbonyl compounds (Nyhammar et al., 1983) coming from the Maillard reaction, forming Schiff bases that later enolize and decarboxylate. The new Schiff base, with one C atom less, is hydrolyzed to the amine and an aldehyde containing one carbon atom less than the starting amino acid.

As the study of the Maillard and Strecker reactions in foods is very complex, in order to obtain data on the effect of different components or conditions, many authors have studied them by model systems. They are mixtures of an amino acid, a sugar, and sometimes a few other compounds. In most cases water is used as a solvent, but in some cases mixtures of water with methanol (Eichener and Karel, 1972; Lee et al., 1984), ethanol (Ledl, 1982), diethylene glycol (Koehler and Odell, 1970), and octanol (Westphal and Cieslik, 1983) are used. To our knowledge this last one is the only lipophilic solvent used.

In choosing a model system of the roasting of cocoa beans, one should take into account that they contain about 50–60% of cocoa butter and are therefore a highly lipophilic medium. Moreover, the effect of cocoa butter on the products of the Maillard and Strecker reactions is not clear nor is its role in the production of the flavor.

As 3-methylbutanal and 2-methylpropanal (the Strecker aldehydes from leucine and valine) are considered important indicators during cocoa processing, we have studied their formation at different temperatures in a model system composed of fructose, leucine, or valine in a mixture of 4% water and 96% deodorized cocoa butter. The results were compared with those obtained in the same amount of water alone.

EXPERIMENTAL SECTION

Model Systems in Cocoa Butter. Cocoa butter (96 g) was melted in a 250-mL flask equipped with a reflux condenser, a good magnetic stirrer, a pressure-equalizing valve, and a rubber septum. L-Leucine (200 mg, 1.52 mmol) and undecane (internal standard, 44.40 mg) were added. The mixture was heated at the desired temperature, and fructose (1.37 g, 7.6 mmol) and distilled water (4 mL) were added. The temperature was kept constant for 2–3 h. Every 10–13 min samples of 1 mL of the vapor phase were drawn with a gas-tight syringe (Hamilton 1001) and injected in the gas chromatograph.

With the same procedure L-valine (200 mg, 1.7 mmol) and fructose (1.53 g, 8.5 mmol) were reacted. The chro-

Istituto di Biochimica e di Chimica, Facoltà di Agraria, Università di Milano, I-20133 Milano, Italy (A.A., O.B., A.G.), and Alivar, Corso Vercelli, I-28100 Novara, Italy (C.A.).

0021-8561/87/1435-1035$01.50/0