could not be detected in the essential oil. The relatively lower content of *cis*-ocimene in the steam distillate than in the vacuum distillate could be attributed to its susceptibility to thermal change during the isolation procedure. Recently, β-myrcene, structurally analogous to *cis*-ocimene, was reported by Hayashi and Komae (1982) to undergo thermal polymerization, resulting in various monomers and dimers.

The UV spectra of the essential oil isolated by the high-vacuum distillation technique showed λ_{max}^{MeOH} at 222 nm, and its IR characteristics according to the order of strong to weak absorption were as follows: 3.85 (s), 11.01 (s), 10.05 (s), 6.02 (w), 6.25 (m), 6.85 (m), 7.15 μm (m). These analytical data, although determined from crude essential oil, resembled that of *cis*-ocimene identified earlier by Gholap and Bandyopadhyay (1977) in the latex of raw Alphonso mango. The foregoing results suggest that the mango-like aroma in *C. amada* was essentially due to the abundant occurrence of *cis*-ocimene, supplemented by car-3-ene hving a mango leave odor.

Registry No. α-Pinene, 80-56-8; car-3-ene, 13466-78-9; *cis*-ocimene, 27400-71-1.

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Headspace Sampling of Cooked Beef Aroma Using Tenax GC

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A novel technique was developed and optimized for isolating genuine cooked beef aroma volatiles, using a modified headspace sampling procedure involving adsorption of the headspace vapors onto Tenax GC. Rapid heat desorption transferred the volatiles directly into a gas chromatography column for separation. The aroma isolation, heat desorption, and gas chromatographic techniques were validated sensorially. By use of combined gas chromatography/mass spectrometry, 67 identifications were made, including 8 compounds tentatively identified for the first time in cooked beef aroma. The relevance of heterocyclic compounds, especially the thiazoles, is discussed. The isolates obtained can be directly analyzed sensorially, since there is no interference from solvent odor, and this enables complementary instrumental and sensory analyses of the desorbed aromas.

The aroma of cooked beef has been extensively studied, and about 600 volatile compounds from variously heated beef samples have been identified (Ching, 1979; Uralets and Golovnja, 1980; Yamaguchi et al., 1980; Lee et al., 1981; MacLeod and Seyyedain-Ardebili, 1981). Unfortunately, aroma isolation methods have usually involved multistage techniques including distillations, flash evaporation, cold trap condensation, solvent extraction, acid/base fractionation, and/or chemical derivatization. The long and complicated nature of several of these methods adds to the possibility of losses and artifact formation already inherent in them.

Recently indirect headspace methods, involving adsorption and simultaneous concentration of volatiles on porous polymers, have become widely used in the analysis of trace volatiles and food aromas. Surprisingly, only one publication relates to beef aroma, and in this study Bryant (1971) trapped the heat-generated volatiles of beef flavor precursors on Porapak Q. Advantages of adsorptive techniques are that the volatiles are concentrated on the basis of their relative volatilities rather than affinity for, or solubility in, a solvent and that, compared with an isolate obtained from solvent extraction, there is no solvent odor to interfere with sensory analysis (Tassan and Russell, 1974).

The sampling method described in this paper was developed such that it (i) provided mild treatment of the beef throughout cooking and isolation, (ii) was as simple as possible and resembled home cooking, (iii) captured and concentrated the aroma quickly and in one stage (*direct* heat desorption into the gas chromatographic column was also desirable), (iv) minimized artifact formation, (v) achieved an aroma isolate representative of the original sample, and (vi) achieved aroma isolates amenable to direct sensory analysis.

Tenax GC was chosen as the adsorbent for several reasons. It has a very high affinity for organic compounds, which it adsorbs reversibly (Micketts and Lindsay, 1974). It is relatively hydrophobic, which is important in view of the large volume of water vapor produced on heating meat. It is the preferred polymer in applications where relatively high boiling compounds are of interest (Boyko et al., 1978; Cole, 1980), and compared with the Porapaks and Chromosorbs it has the highest temperature limit of 375 °C (Micketts and Lindsay, 1974). When heat desorption is the chosen method for releasing aroma volatiles, Barnes et al. (1981) determined that Tenax (desorbed at 250 °C)

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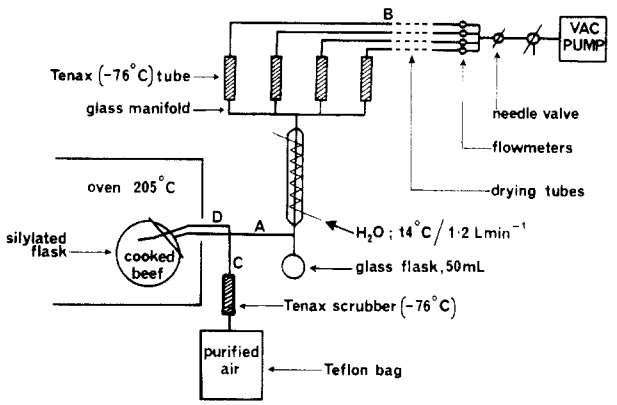

Figure 1. Idealized diagram of the headspace sampling system.

gave the highest recoveries, compared with a range of Chromosorbs, for a model system of some typical food aroma components; almost quantitative desorption occurred. Also, with sensory analysis in view, comparisons have established that Tenax gives superior sensory results to other polymers (Buckholz et al., 1980).

This paper reports a novel approach to the analysis of cooked beef aroma. The isolates obtained are suited to parallel instrumental and sensory analyses, for which there is a real need in meat flavor research.

EXPERIMENTAL SECTION

Preparation of Tenax Tubes and Blank Tenax Chromatograms. Tenax GC (60–80 mesh, Field Instruments Co., Ltd.) was conditioned by heating 20 g at 275 °C for 24 h in a dry N_2 flow of 50 mL min^{-1}. A 3-cm length of conditioned Tenax (0.09 g) was packed and plugged with glass wool into 20 cm long glass tubes (6-mm o.d.; 4-mm i.d.). A blank Tenax gas chromatogram was obtained by heat desorbing the Tenax into a gas chromatography (GC) column by using the procedure described under Gas Chromatography. Additional cleaning of the Tenax tubes was standardized by heat desorbing twice into the atmosphere.

Headspace Sampling System. An idealized diagram of the sampling system is shown in Figure 1. Glass connections were made by Swagelok unions. By means of the vacuum pump, a highly purified and dried air flow entrained the cooked beef headspace through each of the four "in-parallel" subambient Tenax tubes. Before each use oven glassware (disconnections having been made at A and D) were silylated by adding 1 mL of Silyl 8 (Pierce Chemical Co.) in 3 mL of acetone (dried over molecular sieve 5A) to the flask, which was then heated in the oven for 2 h at 205 °C in a 100 mL min^{-1} stream of N_2 (dried and purified by 50 g of molecular sieve 13X). The molecular sieve was regenerated immediately before each use.

Headspace Sampling Method. Preliminary experiments, the results of which are summarized briefly under Results and Discussion, led to the following optimized sampling method.

Before cooking, water at 14 °C was circulated through the condenser (42 cm) at 1.2 L min^{-1}, and disconnections were made at A and B (Figure 1). The Tenax temperature was equilibrated for 30 min at −76 °C in powdered solid CO_2, packed into glass cylinders, concentric with and surrounding the sampling tubes. During this time a 100 mL min^{-1} flow of N_2, purified and dried by using 300 g of molecular sieve 13X, was passed through the sampling apparatus from A to B.

The silylated flask, containing 1 kg of beef topside trimmed of all extramuscular fat, was placed in the oven and the apparatus disconnected at C. The flask atmosphere was flushed out, from C to A, by a highly purified air flow of 2 L min^{-1} for 10 min. This was achieved by using "high purity" air from a cylinder (British Oxygen Co., Ltd., Special Gases Division) and the flow was further "scrubbed" by passage through molecular sieve 13X (300 g), silica gel (200 g), a P_2O_5 drying trap, and a Tenax (0.25 g) scrubber equilibrated at −76 °C.

The N_2 and air flows were then stopped, the flask was connected to the sampling apparatus at A, and the Teflon tubing connection at D was closed with a Hoffman clip. The 50-L Teflon bag containing highly purified air (scrubbed as described above) was connected at C, and a clean Tenax scrubber at −76 °C was installed.

After a 1-h cooking period at 205 °C sampling began. The vacuum system was connected at B, and when the needle valve was slowly opened and the Hoffman clip at D was simultaneously released, purified air from the open-valved Teflon bag was entrained through the scrubber, the cooking flask, and the sampling apparatus at a rate of 500 mL min^{-1} through each of the four in-parallel Tenax tubes. Sampling continued for 5 min, after which the sampled Tenax tubes were removed and capped, ready for GC analysis.

The procedure was repeated by using a cooking period of 4 h before sampling.

Gas purifiers were regenerated/replaced before each use and a blank experiment (no meat) was performed before each sampling.

Heat Desorption of Aroma Volatiles. Aroma volatiles were directly transferred from the Tenax onto the GC column by rapid thermal desorption. The Tenax tube and a three-way valve were incorporated into the carrier gas line at the GC column inlet by means of Swagelok unions. By use of the valve, air was flushed out of the Tenax tube with the N_2 carrier gas for 5 s, to prevent Tenax decomposition on subsequent heating. The Tenax was rapidly heated to 250 °C and held for 1 min, while venting the N_2 to the atmosphere. The carrier gas was then rediverted through the Tenax tube and GC column, and simultaneously the GC temperature program was started.

Gas Chromatography. A Pye-Unicam Series 104 gas chromatograph with a heated fid was used, together with a glass column (5.5 m × 4 mm i.d.) packed with 20% PEG 20M coated on 100–120 BSS mesh acid-washed Celite, and a N_2 carrier gas flow rate of 30 mL min^{-1}. The best temperature program was 70 °C for 9 min, followed by a 10 °C min^{-1} increase to 175 °C until all peaks had emerged. Attenuation varied from 5×10^2 (i.e., 5×10^{-10} A fsd) to 2×10^4 (i.e., 2×10^{-8} A fsd) to achieve all peaks on scale. Peak areas were calculated and normalized to an attenuation of 5×10^2. Each peak area was expressed as a percentage of the total peak area, i.e., relative percentage abundance (RPA).

Sensory Validation of Sampling and GC Techniques. Four untrained assessors, who were experienced in sensory analysis, described the odor of an unresolved desorbed aroma isolate at an external GC odor port attached to an *empty* silylated and heated (175 °C) short glass column (30 cm × 4 mm i.d.). The aroma sample was split in a 100:1 ratio at the column exit, with the major portion passing through a silylated and heated (225 °C) line to the odor port.

This same procedure was then repeated, but this time desorbing into the empty column a Tenax tube obtained from previously trapping (at −76 °C at the end of a silylated and heated 100:1 outlet splitter) a total isolate chromatographed as described under Gas Chromatography. The odor of an unresolved chromatographed aroma

isolate was described by the assessors.

Pooling of Aroma Isolates. Aroma volatiles from four in-parallel sampled Tenax tubes were combined by a pooling technique before gas chromatography/mass spectrometry (GC/MS). Each of three sampled tubes was heat desorbed, as described above, onto the sampled end of the fourth sampled tube, held at −76 °C. This temperature was previously equilibrated in powdered solid CO_2 for 10 min with a 30 mL min^{-1} flow of dry N_2 through the Tenax.

Gas Chromatography/Mass Spectrometry. A Perkin-Elmer Sigma 3 gas chromatograph, linked via a single-stage heated all-glass jet separator interface to a Kratos MS25 mass spectrometer, was used. The system was connected on-line to a dedicated data processing system provided by a Data General Nova 2 computer using Kratos DS50-S software. The GC conditions were as described above, except that a helium carrier gas at 40 mL min^{-1} was used. Both electron ionization (EI) and chemical ionization (CI) mass spectrometry (MS) were performed on pooled isolates. Significant MS operational parameters during EI-MS were as follows: ionization voltage, 70 eV; ionization current, 100 μA; source temperature, 200 °C; resolution, nominal 600; scan speed, 1–3 s/decade (repetitive throughout GC run). For optimum sample transfer, an interface temperature of 250 °C was adopted. Identical conditions were used during CI-MS except for the following: reagent gas, methane; ionization potential, 125 eV; emission current, 5 mA. Data system facilities such as background subtraction, various scale expansions, manual deconvolution of unresolved GC peaks, and mass fragmentograms were extensively employed in evaluating the mass spectral data.

RESULTS AND DISCUSSION

Preliminary trials showed that certain experimental precautions were essential during and before sampling. Subambient Tenax traps were more efficient than ambient tubes for retaining the lower boiling aroma components. Temperature equilibration in a dry, purified N_2 flow prevented blockage of the traps by solidification of atmospheric water vapor. Under the optimized sampling conditions, no breakthrough occurred from the Tenax. Sampling efficiency decreased with sampling time—breakthrough then occurring—and this was not significantly decreased by using lower sampling flow rates. Hot oven headspace/laboratory atmosphere drawn through the sampling tubes caused gross contamination and GC drift. The cooked beef headspace was therefore confined in a glass flask, from which the laboratory atmosphere was flushed by using highly purified air. The latter was also essential as an entraining gas. Glass itself at oven temperature caused contamination, which was overcome by silylation. Resilylation of oven glassware and regeneration of molecular sieves immediately before use was essential.

An aroma isolate desorbed and assessed sensorially at the end of a short empty GC column was described as sweet, buttery, meaty, boiled beef aroma, oily, fatty, and musty (initially) and culminating as earthy, charred, smoky, burnt, toasted, pungent, and typical roast beef aroma. A good representative aroma isolate had therefore been obtained, showing that the Tenax had adsorbed and desorbed (under the analytical conditions used) volatile components that impart cooked beef aroma. The aroma isolation method and heat desorption techniques were therefore validated. Any possible changes occurring during chromatography were also checked. Identical odor descriptions were obtained for the desorbed aroma sample previously collected at the GC column exit after eluting through the 5.5-m PEG packed column. Changes (if any) were deemed insignificant, showing that the fid was receiving genuine cooked beef aroma components.

Table I. Components Identified in Cooked Beef Aroma Sampled on Tenax GC: Cooking Period 1 h[a]

component[b]	t_R, min	Kovats index	RPA[a]
air	3.8		0.02
carbon dioxide[e]	4.1		0.01 (0.10)
pentane	4.3	500	0.01
[hexamethyldisiloxane][e]	4.7		0.25 (1.00)
hexane[e]	5.0	600	0.50 (0.75)
acetaldehyde[e]	5.4	690	– (–)
[trimethylethoxysilane]	6.8		0.02
heptane	7.3	700	–
methanethiol	8.0		13.00
a hydrocarbon[c]	8.7		–
acetone[e]	14.8		10.00 (–)
carbon disulfide[e]	15.1		–
dimethyl sulfide[e]	15.8		6.50 (1.25)
a C_4 amine, M73[c]	19.4		0.20
propanal[e]	19.8	784	4.50 (7.00)
methylpropanal[e]	19.8	800	
methanol	20.8		–
ethanol	21.2	900	2.25
1,1,1-trichloroethane	21.2		
2-methylbutanal[e]	21.8	926	3.00 (5.50)
3-methylbutanal[e]	21.8	937	(30.00)
water[e]	22.2		0.10 (1.25)
butanedione (diacetyl)	22.6	963	–
[trimethylsilanol][e]	22.8		– (0.20)
pentan-2-one	23.1	969	0.25
but-2-enal (crotonal)	25.0		0.75
toluene	25.5	1066	0.25
hexanal	26.1	1084	1.00
dimethyl disulfide[e]	26.7	1081	1.25 (7.00)
butan-1-ol	27.5	1113	0.10
2,4,5-trimethyl-3-oxazoline[d,e]	28.3		0.05 (–)
a methylpentano-lactone[c,e] ?	29.6		2.00 (0.75)
[hexamethylcyclo-trisiloxane][e]	30.3		–
pentan-1-ol	30.8	1213	1.75
chlorobenzene[e]	32.8		0.20 (0.25)
[octamethylcyclo-tetrasiloxane][e]	34.2		0.50 (0.50)
3-hydroxybutanone (acetoin)[e]	36.1	1268	5.50 (0.75)
nonanal	43.3	1382	3.00
dimethyl trisulfide[e]	48.7	1400	12.00 (10.00)
octan-1-ol	57.1	1519	0.75
benzaldehyde[e]	61.7	1502	1.50 (0.20)

[a] A total of 40 GC peaks, representing approximately 30% of the total aroma isolate, remained unidentified. Brackets indicate silicon contaminants (identified also in blank experiments). RPA figures in parentheses relate to the 4-h sample; all RPA figures have been corrected as follows: >5% quoted to nearest 0.5%; 0.25–5% quoted to nearest 0.25%; <0.25% quoted as 0.25, 0.20, 0.10, 0.05, 0.02, and 0.01; (–) denotes no accurate peak area measurement possible although a trace amount was present. [b] Mass spectral identifications are positive unless indicated by footnote *c* or a question mark. [c] Partial characterization. [d] Reference mass spectrum from Mussinan et al. (1976); all others from "Eight Peak Index of Mass Spectra" (1974). [e] Also identified in cooked beef aroma sampled after a 4-h cooking period. ? = tentative identification.

Components identified by GC/MS in aromas sampled after cooking periods of 1 and 4 h are listed in Tables I and II, respectively, which present combined data from several GC/MS runs. In many cases, CI-MS was useful in defining the molecular weights of components, thus aiding the interpretation of conventional EI spectra. Literature (Jennings and Shibamoto, 1980) Kovats' re-

Table II. Additional Components Identified in Cooked Beef Aroma Sampled on Tenax GC: Cooking Period 4 h[n]

component[a]	ref MS[c]	t_R, min	Kovats index	RPA
carbonyl sulfide		6.0		1.00
trimethylamine		8.0		3.00
furan		11.8	786	–
ethanethiol		13.0		2.75
2-methylfuran		14.5	866	–
3-methylfuran		16.1		0.75
butanone		16.6	908	0.05
carbon tetrachloride		16.6		
2-ethylfuran		19.0	951	0.75
a methyl furoate[b] ?		22.7		–
2-methylthiophen		23.7	1123	0.10
2,4-dimethyl-3-oxazoline	*e*	25.3		–
ethyl methyl disulfide	*f*	25.8	1156	0.75
2,5-dimethylthiophen		28.0	1161	0.10
2-*n*-pentylfuran		28.4	1229	0.50
a methyl pyridine[b]		30.2		0.25
tridecane	*d*	30.2	1300	
2-methyltetrahydrofuran-3-one		31.7		– –
a $C_9H_{15}NO$ oxazole[b] (probably 2-isobutyl-4,5-dimethyl-)		33.1	1330	
2,6-dimethylpyrazine		34.8	1325	0.05
a $C_7H_{11}NS$ thiazole[b]		36.3		–
an ethyl methyl pyrazine[b]		38.3		0.25
acetic acid		39.2		0.10
a dimethylethylpyrazine[b]		43.1		0.50
a $C_8H_{13}NS$ thiazole[b]	*g*, *h*	44.2		0.75
furfural		46.0	1449	0.50
2,4-dimethyl-5-ethyl-thiazole	*h*, *i*	46.0		
2,6-dimethyl-3-ethyl-pyridine		47.0		–
decan-2-one		47.3	1480	0.20
a C_5 substituted pyrazine[b]		48.7		0.25
a benzenoid compound[b]		50.5		–
2-isopropyl-4-methyl-5-ethyl thiazole	*j*	51.0		0.50
2-propyl-4,5-dimethyl-thiazole	*d*	52.2	1500	1.00
butanoic acid		58.5		0.25
a thiazole, M183[b] ?	*j*	60.7		0.10
benzothiazole?	*k*	63.5		–
hexanoic acid		64.7		0.25
3-isopentyl-2,5-dimethylpyrazine	*l*	68.2		0.05
2-(hydroxymethyl)furan	*m*	75.3		0.10
dodecan-1-ol	*d*	101.8	1925	–

[a] Mass spectral identifications are positive unless indicated by footnote *b* or a question mark. [b] Partial characterization. [c] Reference mass spectrum from "Eight Peak Index of Mass Spectra" (1974) unless otherwise indicated. [d] Jennings and Shibamoto (1980). [e] Mussinan et al. (1976). [f] Cuer et al. (1979). [g] Goldman et al. (1967). [h] Pittet and Hruza (1974). [i] Buttery et al. (1973). [j] Maga (1975). [k] Vitzthum and Werkoff (1974). [l] Maga and Sizer (1973). [m] Flament et al. (1967). RPA figures corrected as in Table I. (-) See Table I footnote. ? = tentative identification. A total of 21 components listed in Table I and indicated by footnote *e* were also identified in this sample; they represented approximately 63% of the total isolate. A total of 16 GC peaks, representing approximately 20% of the total aroma isolate, remained unidentified.

tention indices of many components are also included in the tables. These values were determined on the same GC phase as employed in this project, and in comparison with the determined retention times, they serve as useful, but limited, confirmation of identity. Some GC peaks gave mass spectra that, on inspection indicated the presence of more than one component. Retrospective single ion monitoring confirmed these suspicions and distinguished the significant molecular and fragment ions of the individual compounds. Further manipulations of the data, using in particular the background subtraction facility of the data system, enabled generation of pure spectra of the components of the GC peak substantially free of contamination, hence facilitating interpretation. In this way, using a packed column it was possible to "recover" the qualitative resolution normally afforded by capillary columns but without loss of sample loading and hence sensitivity. By use of these techniques, three components eluting in one GC peak and differing overall by only five Kovats' indices have been resolved and identified in these laboratories. Interestingly, these three compounds also coeluted on glass capillary GC.

The majority of the compounds listed in Tables I and II have been identified previously in heated beef aromas; their precursors and possible formation pathways have been recently reviewed (MacLeod and Seyyedain-Ardebili, 1981). However, eight compounds are reported here for the first time in cooked beef aroma. These are trimethylamine, 3-isopentyl-2,5-dimethylpyrazine, 2,6-dimethyl-3-ethylpyridine, 2-isopropyl-4-methyl-5-ethylthiazole, 2-propyl-4,5-dimethylthiazole, chlorobenzene, carbon tetrachloride, and 1,1,1-trichloroethane. Some of these are worthy of further comment.

The chlorine-containing compounds are probably contaminants, possibly deriving from pesticide residues in the beef (Ho and Coleman, 1981). Other contaminants are the silicon compounds (Table I). These arose from heated glass or from the silylating agent. Unfortunately, it was impossible to eliminate them without creating many more contaminants derived from heated glass. Silylation replaces active hydrogen with a trimethylsilyl [$-Si(CH_3)_3$] group, thereby reducing the polarity of the reactant and decreasing the possibility of hydrogen bonding. Thus, by minimizing the number of reactive sites, stability of the silylated product is enhanced (Pierce Chemical Co., 1972). However, hydrolysis occurs even at low temperatures in dilute solution, and silanols result. In the presence of either base or acid, the corresponding siloxanes rapidly form (Barton and Ollis, 1979).

Trimethylamine has previously been identified in steam-distilled pork volatiles (Chang, 1972). It may have resulted from decarboxylation of amino acids or it may arise from lecithin. Enzymic conversion from trimethylamine oxide in fishery products has been well established (Tarr, 1940).

2,6-Dimethyl-3-ethylpyridine appears not to have been previously reported in any food aroma (van Straten et al., 1977). Pyridines can arise from reaction of alkanals with amino acids, followed by aldol-type condensation of the newly formed imine (Suyama and Adachi, 1980). Pyridines are also produced by the pyrolysis of cystine (Kato et al., 1973b) or β-alanine (Lien and Nawar, 1974), and by Maillard reaction, e.g., glucose/cysteine and glucose/cystine (Kato et al., 1973a; Scanlan et al., 1973). The reaction of proline and glucose under roasting conditions also produces pyridines (Tressl et al., 1979).

3-Isopentyl-2,5-dimethylpyrazine has been reported as a volatile flavor component of cocoa (Flament et al., 1967) and potato chips (Buttery et al., 1971). Pyrazines are formed from sugar/amino acid condensation reactions (Dawes and Edwards, 1966), by cyclization of hydroxy amino acids such as serine and threonine (Wang and Odell, 1973), and from sugar/NH_3 model systems (Shibamoto and Bernhard, 1977). Wasserman (1979) has suggested that when meat is first heated (pH 5.5), pyrazine formation by the former route is probably favored but, as heating

continues and more NH_3 is liberated, the increased pH is likely to favor the latter route.

Increasing the cooking time from 1 to 4 h caused a considerable increase in the concentration of aliphatic aldehydes, particularly 3-methylbutanal (the Strecker aldehyde of leucine), which was enhanced from about 3% to approximately 30% of the total isolate. Conversely, prolonged heating caused a significant decrease (from about 35% to approximately 20%) in the concentration of aliphatic sulfur compounds. This was largely accounted for by the absence of methanethiol. However, there was a simultaneous increased formation of thiazoles, and this confirms previously published evidence that the incorporation of sulfur into ring systems is favored by increased heating periods (Schwimmer and Friedman, 1972).

Seven thiazoles were identified—two for the first time in cooked beef aroma. Thiazoles may arise from thermal degradation of cystine (Kato et al., 1973b) and of thiamin (van der Linde et al., 1979) and by Strecker degradation of cysteine/cystine–pyruvaldehyde mixtures (Kato et al., 1973a). The Maillard reaction of glucose/cystine is also a possible pathway (Kato et al., 1973a). In particular, the 2,4-dimethyl-5-ethylthiazole is a reaction product of cysteine/cystine–ribose (Mulders, 1973). Reaction of α-dicarbonyls, aldehydes, NH_3, and H_2S also forms thiazoles (Takken et al., 1976), and suggested formation pathways have been recently summarized by MacLeod and Seyyedain-Ardebili (1981). Mussinan et al. (1976) have reported that thiazolines are less heat stable than the corresponding oxazolines, and this may explain the absence of thiazolines in the present study. Asinger et al. (1960) have shown that 3-thiazoline is thermally dehydrogenated in the presence of elemental sulfur to the corresponding thiazole. It has been reported previously that both meaty and roasted odors are associated with some thiazoles (Mussinan et al., 1976; Pittet and Hruza, 1974); also thiazoles and thiazolines have lower odor thresholds than the oxazoles and oxazolines (Mussinan et al., 1976) and are therefore likely to be more important contributors to roasted, meaty aromas. Increased substitution in thiazoles confers added nutty, roasted, and meaty qualities, and in general 5-substituted thiazoles are more sulfurous and roasted, and some are meaty (Pittet and Hruza, 1974). These structural features apply to the compounds identified here. In fact, 2,4-dimethyl-5-ethylthiazole has been described as nutty, roasted, and meaty, with an odor threshold value of 2 ppb (Mussinan et al., 1976).

CONCLUSION

Many heterocyclic compounds were identified in cooked beef headspace vapors sampled on Tenax GC. Several of these, e.g., the thiazoles, are likely to be important contributors, and additional components present in trace amounts remain to be identified. It is essential, however, in any eager search for characteristic trace volatiles to keep isolation methods simple and as representative as possible of normally cooked beef, so that artifacts do not cloud the issue. The headspace sampling procedure reported here is one such method. This incorporates the in-parallel concept of Zlatkis et al. (1973), which is particularly attractive in view of the high cost of meat. It furnished, from 1 kg of beef, four identical aroma isolates for instrumental and sensory comparisons; the sensory analyses will be reported separately.

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Registry No. Carbon dioxide, 124-38-9; pentane, 109-66-0; hexamethyldisiloxane, 107-46-0; hexane, 110-54-3; acetaldehyde, 75-07-0; trimethylethoxysilane, 1825-62-3; heptane, 142-82-5; methanethiol, 74-93-1; acetone, 67-64-1; carbon disulfide, 75-15-0; dimethyl sulfide, 75-18-3; propanal, 123-38-6; methylpropanal, 78-84-2; methanol, 67-56-1; ethanol, 64-17-5; 1,1,1-trichloroethane, 71-55-6; 2-methylbutanal, 96-17-3; 3-methylbutanal, 590-86-3; water, 7732-18-5; diacetyl, 431-03-8; trimethylsilanol, 1066-40-6; pentan-2-one, 107-87-9; but-2-enal, 4170-30-3; toluene, 108-88-3; hexanal, 66-25-1; dimethyl disulfide, 624-92-0; butan-1-ol, 71-36-3; 2,4,5-trimethyl-3-oxazoline, 22694-96-8; hexamethylcyclotrisiloxane, 541-05-9; pentan-1-ol, 71-41-0; chlorobenzene, 108-90-7; octamethylcyclotetrasiloxane, 556-67-2; nonanal, 124-19-6; dimethyl trisulfide, 3658-80-8; octan-1-ol, 111-87-5; benzaldehyde, 100-52-7; carbonyl sulfide, 463-58-1; trimethylamine, 75-50-3; furan, 110-00-9; ethanethiol, 75-08-1; 2-methylfuran, 534-22-5; 3-methylfuran, 930-27-8; butanone, 78-93-3; carbon tetrachloride, 56-23-5; 2-ethylfuran, 3208-16-0; ethyl methyl disulfide, 20333-39-5; 2-*n*-pentylfuran, 3777-69-3; tridecane, 629-50-5; 2,6-dimethylpyrazine, 108-50-9; acetic acid, 64-19-7; furfural, 98-01-1; 2,4-dimethyl-5-ethylthiazole, 38205-61-7; 2,6-dimethyl-3-ethylpyridine, 23580-52-1; decan-2-one, 693-54-9; 2-isopropyl-4-methyl-5-ethylthiazole, 53498-31-0; butanoic acid, 107-92-6; benzothiazole, 95-16-9; hexanoic acid, 142-62-1; 3-isopentyl-2,5-dimethylpyrazine, 18433-98-2; 2-(hydroxymethyl)furan, 98-00-0; dodecan-1-ol, 112-53-8.

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Growing Selenium-Enriched Tobacco

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Selenium levels in tobacco were successfully increased by growing the plants in soil, with added sodium selenite, or by spraying growing plants with an aqueous solution of the chemical. Plants were treated at 4–32 mg of selenium/plant levels. Plant treatments were 5–10 times more effective in increasing selenium levels than soil treatments. Thus, soil treatments at 32 mg/plant increased selenium contents of tobacco leaf lamina to about 2 ppm, while a single plant treatment at the 0.5-mg level produced tobacco with about 0.7 ppm of selenium. Pyrolyses of the cured tobacco showed that about 45% of the selenium could be transferred to tobacco smoke. The rationale for moderate increases in selenium levels in our tobacco smoking products is presented and discussed.

We have initiated research to increase the selenium (Se) content of tobacco as a possible way of reducing the hazards of smoking. This concept is based on the following findings. Twenty years ago, Se compounds were regarded not only as toxic contaminants in food and feed that adversely effected animals in seleniferous regions but also as possible carcinogens. This was the case for Se occurring in both large and small concentrations. Se is toxic at high concentrations but is now regarded as an essential trace element in food and feed. New data are being accumulated on the beneficial effects of trace concentrations of Se in our environment, indicating that Se has important metabolic functions (Harr, 1978) and possesses important protective characteristics. Results in numerous animal studies have indicated that Se significantly inhibited both carcinogens and mutagens (Wattenberg, 1978; Thompson and Becci, 1980; Jacobs, 1980; Adams et al., 1980; Schrauzer, 1976). Also, it was recently shown that low lung cancer countries had 3 times as much Se in their cigarettes as in U.S. cigarettes (Bogden et al., 1980, 1981). However, concentrations of possible tobacco smoke carcinogens, such as tar, nicotine, and polonium-210, were similar in tobaccos of both high- and low-incidence countries (Bogden et al., 1981). Therefore, since Se may act as an anticarcinogen in humans, it may be beneficial to increase the Se contents of tobacco products. Our initial efforts in growing some flue-cured tobacco with increased Se contents and then determining the transfer of Se to pyrolyzates are presented.

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EXPERIMENTAL SECTION

Soil Treatment. In 1981 and 2 weeks after being transplanted, young tobacco plants (variety NC 95) were fortified with solid sodium selenite. The chemical was sprinkled around each plant (four plants per treatment) in a radius of 5 in. and was incorporated into the soil with a garden rake to a depth of 2–3 in. Sodium selenite was applied at three rates: 17.5, 35.0, and 70.0 mg/plant, equivalent to 8, 16, and 32 mg of Se/plant, respectively. The plants were allowed to mature under normal conditions in the field and harvesting of mature leaves was begun 3 months after the first application. The tobacco was cured in the barn under standard conditions and then allowed to air-dry in the laboratory for 24 h. The leaf laminae were separated from the midribs and ground to pass through a 16-mesh sieve. The ground tobacco was then further dried in an oven (95 °C, 24 h) prior to Se analyses or conditioned at 60% relative humidity prior to pyrolysis. The experiment was repeated in 1982, but this time aqueous solutions of sodium selenite were sprayed onto the soil around the plants at rates of 3, 6, 12, and 24