Fraction no. 3 consisted of the alcohols, thymol and carvacrol, already identified in fraction no. 2, and four new compounds: linalool, α-terpineol, borneol, and 1-terpinen-4-ol.

Borneol represents the main constituent of the essential oil. This is an interesting fact because in the literature no other variety of thyme was reported to contain more than 8% of borneol, and generally the level is around 2 to 3% of the total essential oil. Adulteration of Provence thyme by Moroccan thyme might be detectable by determining the quantity of borneol. We are now studying *T. satureioides* to establish whether the borneol content of all samples is consistently higher than 20%.

In fraction no. 4, two new compounds were identified: *trans*-4-thujanol and bornyl acetate.

Fraction no. 5 revealed peaks already known, and from fraction no. 6, camphor was characterized by its infrared spectrum. The presence of this compound was assumed by several workers, but to date, no evidence has been presented. It is noteworthy that this particular variety of thyme contains a large amount of camphor, 2.6%, giving a noticeable note of camphor to the essential oil.

In fraction no. 7, no new components were identified.

The chromatogram of the whole essential oil on a Carbowax 20M glass capillary column (Figure 2) shows that all the main constituents were identified. Analysis of several other samples of *T. satureioides* revealed this same chromatographic pattern.

LITERATURE CITED

Bouche, J., Verzele, M., *J. Gas Chromatogr.* **6**, 501 (1971).
Brown, H., Geoghegan, P., Jr., *J. Am. Chem. Soc.* **89**, 1522 (1967).
Debuigne, G., "Dictionnaire des plantes qui guérissent", Librairie Larousse Ed., D.42, Paris, 1972, pp 242–243.
Derdiles, R., Thèse Doctorat Université, Faculté Sciences, Montpellier, 1967, pp 1–178.
Granger, R., Passet, J., *Phytochemistry* **12**, 1683 (1973).
Guenther, E., "The Essential Oils", Vol. 3, Robert E. Krieger Publishing Co., Huntington, N.Y., 1972, pp 744–763.
Hérisset, A., Jolivet, J., Rey, P., *Plant. Med. Phytother.* **7**(1), 37 (1973a).
Hérisset, A., Jolivet, J., Rey, P., *Plant. Med. Phytother.* **7**(2), 114 (1973b).
Jennings, W. G., *Ind. Alim. Agric.* **87**(5), 537 (1970).
Lagriffe, L., "Le livre des épices, condiments et aromates : leur histoire, leur rôle médicinal et leurs vertus gastronomiques", Marabout service Ed., HS 193, Verviers, Gérard Cie., 1968, pp 241–245.
Messerschmidt, W., *Planta Med.* **12**(4), 501 (1964).
Messerschmidt, W., *Planta Med.* **13**(1), 56 (1965).
Naves, Y. R., *Fr. Parfums* **2**, 23 (1959).
Russell, G. F., Jennings, W. G., *J. Agric. Food Chem.* **18**, 733 (1970).
Russell, G. F., Olson, K. V., *J. Food Sci.* **37**(3), 405 (1972).
Schratz, E., Horster, H., *Planta Med.* **19**(2), 160 (1970).

Received for review August 18, 1975. Accepted March 22, 1976.

Volatile Flavor Components of Beef Boiled Conventionally and by Microwave Radiation

Glesni MacLeod* and Belinda M. Coppock

Using a modified Likens and Nickerson extraction procedure followed by low temperature/high vacuum distillation, representative samples of aroma volatiles were obtained from beef both while boiling by microwave radiation and by conventional means. Separation of the components of the isolates was achieved by gas chromatography and the majority of the components identified using combined gas chromatography–mass spectrometry. Odor assessments were made of the separated volatile components. A comparative study was undertaken of the effect on the volatile components of boiling for different periods of time conventionally and by microwave heating.

The flavor of meat has been extensively studied and the literature frequently reviewed (Dwivedi, 1975; Herz and Chang, 1970; Patterson, 1974; Rhodes, 1974; Wasserman, 1972). Many factors pre-slaughter, at slaughter, and post-mortem (including cooking procedure) may influence the final flavor (Herz and Chang, 1970; Patterson, 1974). Uncooked meat has little odor and only a blood-like, metallic taste and it is generally agreed that desirable meat flavor is developed during cooking. Two types of cooked meat flavor have been distinguished, a "meaty" flavor believed to originate from the lean and a "species" flavor which characterizes different animal species and which derives from the fat tissue. Nevertheless, lean meat contains 4–6% intramuscular fat which is sufficient to give rise to the characteristic species flavor (Patterson, 1974).

Department of Food Science and Nutrition, Queen Elizabeth College, London W8 7AH, England.

The flavor of cooked beef, in particular, has been comprehensively researched. Numerous workers, using gas chromatographic techniques, have studied and characterized the volatile flavor components (Hirai et al., 1973; Liebich et al., 1972; Mussinan et al., 1973; Persson and von Sydow, 1973; Watanabe and Sato, 1971a, 1972; Wilson et al., 1973) and lists of over 300 volatile constituents of the aroma of heated beef have been compiled (Coppock, 1975; Dwivedi, 1975; van Straten and de Vrijer, 1973). These include members of at least 18 different chemical classes. Heating methods used for flavor development have included conventional boiling and roasting at atmospheric pressure, frying, pressure cooking, and retort heating.

In contrast to this wealth of literature, only two references can be traced to the use of modern methods (e.g., gas chromatography and/or mass spectrometry) in the flavor analysis of any food cooked in the microwave oven (MacLeod and MacLeod, 1970; Walradt et al., 1970), neither of which relates to meat.

The theory and use of microwave ovens for cooking and heating have been described in many publications (Copson, 1962; Goldblith, 1972; MacLeod, 1972; Proctor and Goldblith, 1951). In summary, and considering the specific case of a frequency of 2450 MHz (the frequency of the oven used in this work), microwaves are absorbed by "lossy" substances such as water, which has molecules possessing a permanent dipole moment. The heating effect is a result of dipole rotation as the dipoles attempt to align themselves with the alternating electric field. In lossy materials with uniform distribution of polar molecules, a dramatic heating effect is almost instantaneous throughout the volume of material. Most foods which are not pure fats contain water and therefore are good absorbers of microwaves at 2450 MHz. Because microwaves heat very quickly, overcooking can readily occur and this should be remembered in any comparisons. Visible browning of cooked surfaces occurs to a limited extent only, if at all, during the short cooking times and it has been suggested that this leads to a lack of many of the aroma compounds attributed to nonenzymic browning (Reynolds, 1970).

A study of the effect of these influences, characteristic of microwave heating, on the composition of boiled beef aroma was the aim of the present work.

EXPERIMENTAL SECTION

The meat used for all experimental comparisons was standardized as far as possible on breed, age, sex, feeding regime, and slaughtering conditions. Longissimus dorsi muscles from Friesian steers of similar age and history were used. The beef was frozen immediately after dissection and stored at −16 °C until required, when it was allowed to thaw at room temperature. All visible fat and connective tissue were removed and the lean meat minced (aperture 4 mm). Removal of visible fat avoided differences in the composition of meat samples used for each experiment. To eliminate errors further, for any one comparison the mince was halved, one portion used for conventional heating analysis and the other half for microwave heating.

The following limitations were imposed on the choice of aroma isolation procedure: the technique had to be applicable to both methods of heating; for use in the microwave oven there should be no metal parts; the penetration depth of the waves into the food (2.5–3 in. at 2450 MHz (Copson, 1962)) placed a limit on the mass of meat used for each experiment; since two different heating methods were being compared, it was essential that no additional heating of the microwave cooked meat or of any extract or isolate should take place; equally since different cooking times were to be compared, aroma isolation should take place during cooking; an isolation procedure which gave a sufficiently concentrated isolate during the short microwave cooking times was demanded; it was considered essential to keep the isolation process as simple as possible, to avoid artefact formation, loss of volatiles, and contamination of samples and also to obtain an isolate representative of boiled beef aroma.

Headspace and solvent extraction procedures best fulfil these criteria and evaluation experiments indicated the procedure detailed below to be the most efficient and reproducible.

Aroma Isolation. Aroma volatiles were extracted from 1 kg of lean minced beef during conventional boiling by means of a Likens and Nickerson (1964) extraction apparatus modified according to MacLeod and Cave (1975) and using 20 ml of twice distilled 2-methylbutane as solvent. For extracting the volatiles from the microwave boiled beef, the same procedure was used but only the meat flask was contained within the microwave oven cavity, because of the heating effect which would otherwise occur on the cooling water in the extractor. The latter was situated on top of the oven and was connected to the meat flask by glass tubing via a hole bored in the roof of the oven (Hirst Microwave Industries Oven, 2450 MHz). Using standardized rates of heating, warm-up times to extraction were 30 min (conventional) and 5 min (microwave). For both heating methods, the meat sample was brought to a boil and the volatiles extracted for varying periods of time: conventional extraction times—15 min (sample C_2), 30 min (C_3), 1 h (C_4), 1.5 h, 4 h; microwave extraction times—5 min (sample M_1), 15 min (M_2), 30 min (M_3), 1 h (M_4). Thus, total heating time = warm-up time + extraction time.

Concentration of Extracts. Extracts were concentrated by a low temperature/high vacuum distillation procedure, whereby the bulk of the solvent was removed. The extract, cooled to −70 °C in solid carbon dioxide/acetone, was distilled slowly under reduced pressure (<0.3 mmHg) into a receiver at liquid nitrogen temperature until a constant volume of ~400 μl remained.

Gas Chromatographic Analysis. The concentrated extracts were separated into their individual components by means of routine gas chromatography, using a Pye Unicam Series 104 chromatograph equipped with a flame ionization detector. Polar and nonpolar stationary phases were evaluated and PEG 20M was found to give by far the best resolution. An 18 ft × 0.25 in. o.d. glass column packed with 20% PEG 20M on acid-washed 100–120 BSS mesh Celite was used with a nitrogen carrier gas flow of 30 ml/min. The oven temperature was increased from an initial temperature of 70 °C at the rate of 6 °C/min to 115 °C which was held for 40 min and then raised at 12 °C/min to a final temperature of 150 °C. Gas chromatograms were obtained for each isolate at different attenuations and using different injection volumes in order to achieve accurate measurements of samples of different strengths. Peak areas were calculated and normalized to a standard injection volume and attenuation for all runs. No area was recorded where the peak was too small to measure accurately. Total peak areas were calculated and for samples extracted up to 1 h each peak area was expressed as a percentage of the total peak area, i.e. relative percentage abundance (RPA).

Odor port assessment of some of the peaks was accomplished by sniffing each component on elution from a splitter (split ratio 10:1, unity in the direction of the detector) placed between the end of the gas chromatographic column and the detector. A small heater at 220 °C situated in the analyzer oven wall at the odor port outlet prevented condensation of the separated components as they eluted. Many peaks were too minor to be assessed. Also, as the temperature of the column increased above 130 °C an unaccountable background odor described as sweet/rubbery emerged which prevented sensory appraisal of all peaks emerging subsequently. A new column, without sample injection, also gave this odor and so it did not derive from the sample.

Identification of Aroma Components. Identification of the volatile flavor components was achieved by means of combined gas chromatography/mass spectrometry using a PYE 104/AEI MS30 instrument equipped with a heated membrane separator interface and using a sample obtained by pooling together all extracts, followed by further concentration by low temperature/high vacuum distillation. Mass spectrometric conditions of analysis were as follows: helium carrier gas, 30 ml/min; interface tem-

perature varied, 100 to 200 °C; ionization potential, 20 eV; ionizing current, 300 μA; source temperature, 200 °C; resolution, 1000; scan speed, 10 s/decade. Verification of identities, when mass spectral identification was not absolutely positive, was achieved by relative retention measurements of reference compounds when readily available from commercial suppliers. Internal standards were used and the retention of any one peak was measured relative to the nearest standard, the retention of which had in turn been standardized relative to all other standards. Elemental compositions of many compounds were subsequently obtained by means of an AEI DS50 data acquisition system equipped with double beam accurate mass measurement facility (resolution 1500) attached to the MS30 which enabled many identifications to be as positive as possible.

RESULTS AND DISCUSSION

The overall aroma of the extracts was characteristic and representative of boiled beef aroma, although their individual aroma qualities varied. Approximately 100 volatile components were separated by gas chromatography and a number of the major components were identified (see Table I). It is appreciated that some of the minor unidentified components may be potent odorants and would thus contribute to characteristic beef aroma and be important in changes observed in the cooking variations described herein. Many of the aroma compounds identified have been previously reported in flavor isolates of beef (Coppock, 1975). However, some are identified for the first time in beef aroma. These are: 2-methylheptane, dimethylbutene*, hex-1-ene, hept-1-ene, oct-1-ene, non-1-ene, dec-1-ene, undec-4-ene*, dodec-1-ene, tridec-1-ene, heptadec-1-ene*, two branched C_{20} alkenes (mol wt 280; branch at C_{13}), a C_{20} branched alkadiene (mol wt 278; branch at C_{13}), a methylpyridine (probably 2-methyl-), a C_3 pyridine (probably 2-*n*-propyl-), pyrrole, a methylpyrrole (probably 2-methyl-), a dimethylpyrrole (2,4- or 2,5-)*, a C_5 saturated substituted pyrazine (possibly methylisobutyl-)*, 2-ethylfuran, *m*-toluonitrile, ethylbenzaldehyde, and tetrachloroethane (an asterisk indicates tentative identification).

The hydrocarbons probably derive from the thermal oxidation of fats (Roth and Rock, 1972), a reaction catalyzed by heme compounds such as hemoglobin and myoglobin (Brodnitz, 1968; Sink, 1973). Relatively few of the other well-known products of fat oxidation were identified, e.g., the alcohols, aldehydes, ketones, acids, lactones, and esters (Forss, 1972; Sink, 1973), probably due to the low fat content of the beef used (Hornstein and Crowe, 1960). Those aldehydes and ketones which were identified are likely products of Strecker degradation, caramelization, and Maillard reactions (Hodge, 1967; Reynolds, 1970; Self et al., 1963).

The pyridines and pyrazines almost certainly derive from Maillard browning reactions (Ferretti and Flanagan, 1971) and the presence of such classes of compounds in meat aroma has hitherto been particularly associated with roasted rather than boiled meat (Watanabe and Sato, 1971a).

Only two pyrrole compounds have previously been reported in beef aroma (Liebich et al., 1972; Watanabe and Sato, 1972). Although proline and myoglobin may act as precursors for the pyrroles, they are also known Maillard products.

The furans may derive from sugar degradation or from sugar–amine interactions (Reynolds, 1969) or from fat (Persson and von Sydow, 1973).

Ethylbenzaldehyde probably derives from pyrolysis of benzenoid amino acids (Wasserman, 1972) or thermal degradation of sugars (Heyns et al., 1966).

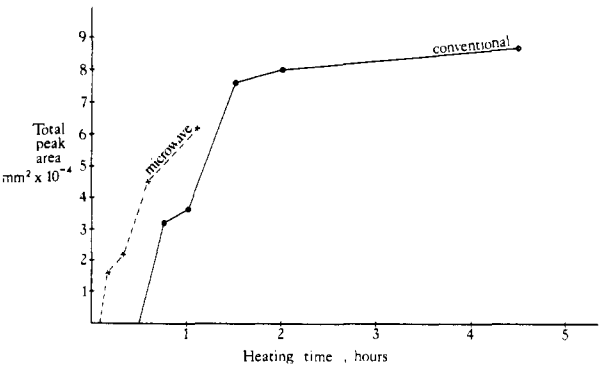

Figure 1. Effect of heating time on total organic volatiles extracted.

The tetrachloroethane probably does not contribute to beef flavor and may possibly have derived from chlorinated hydrocarbons in the animal feed. Such compounds are known to inhibit rumen methanogenesis (Gutcho, 1973) and may be prescribed by a veterinarian. Chlorinated pesticides are usually not completely biodegraded and if ingested will persist intact or only partly degraded in the animal tissue unless excreted. This compound may also have derived from contamination of the beef with chlorine-containing refrigerants during freezer storage.

The C_{20} alkenes are of interest and may originate from the decarboxylation of a C_{21} carboxylic acid (Kolattukudy, 1970). Naturally occurring high molecular weight fatty acids are synthesised by bacteria in the rumen. Although such acids are fairly rare, they also occur in fish oils and in certain plants, notably the *Brassica* (which are common constituents of animal feed), e.g. sprout tops and rape (Altman and Dittmer, 1972; Hudson and Karis, 1974). Mechanisms for chain elongation and even desaturation exist in leaves (Kolattukudy, 1970). Alternatively they may have derived from high energy fat, another common constituent of animal feed. The fat for this comes largely from raw soap stock produced as a by-product from alkali refining of vegetable oils (Gutcho, 1973). Once ingested, such long-chain hydrocarbons (the acid may be decarboxylated in the rumen) could be absorbed unchanged into the animal tissue unless excreted.

Table II (see paragraph at end of paper regarding supplementary material) presents values for absolute area and relative percentage abundance (RPA) of each peak in each aroma isolate. These figures are based on the flame ionization detector response of the gas chromatograph, since response factors to relate area to mass for each individual component are not required for a comparative survey such as this.

Figure 1 shows the effect of variation in heating time on the total organic volatiles extracted (i.e., the total area of the gas chromatograms) for both the conventional and microwave series of boiled beef samples. The production of aroma volatiles increases relatively rapidly during the early heating stages and reaches a plateau which is maintained between 1.5 and 4 h of heating conventionally. Figure 2 shows that this initial rapid increase in total organic volatiles extracted is due largely to increased production of the benzenoid compounds, alkenes, alkanes, furans, pyrazines, and aliphatic aldehydes with only very minor contributions from the pyridines, alcohols, ketones, and pyrroles. The plateau effect was shown to be due to exhaustion of precursors in the meat, rather than a saturation of the solvent with volatiles and it is therefore a true representation of reduced rate of production of the

Table I. Volatile Flavor Components of Beef Isolates

Peak no.	Rel t_R, min	Identity	Certainty[a] of MS ident.	Int. stand.	Rel t_R ref, min	Aroma as eluted from column
1	5.7	Dimethylbutene[b]	*			Pleasant, sweet
2	6.4	Hex-1-ene[b]	**			Dull, cardboard
3	7.1	*n*-Heptane	***		7.1	Cooked meat
4	8.5			*n*-Heptane at retn 7.1		
5	9.3	Hept-1-ene[b]	***			Sulfurous
6	10.5	2-Methylheptane[b]	***			Solvent-like
7	12.8	*n*-Octane	***			Meaty
8	14.3	Acetone	***		14.3	Dull, meat-broth
9	16.5	Oct-1-ene[b] (+ ethyl-methyl sulfide ??)	***		15.4 EMS	Strong garlic, onions
10	18.0					Faint, pleasant, animal-like
11	18.5	2-Methylfuran	*		18.6	Pleasant, slightly sulfurous, meaty
12	18.8					
13	20.5	*n*-Nonane	***		20.5	Strong, sour, harsh, burnt, unpleasant
14	21.2	Butanone	***		21.2	Strong, grassy, sweet, sickly, doughy
15	22.4	3-Methylbutanal	**		22.5	Meaty, sulfurous
16	23.7	Non-1-ene[b]	***			Strong, grassy, onions, rancid
17	24.5	Benzene + 2-ethylfuran[b]	***	Benzene at retn 24.5	Benzene 24.5	Grassy, solvent-like
18	25.4	2-Methylthiophen ??	*			Very strong garlic, sweet
19	26.4	Pentan-2-one	***		26.3	Buttery, sweet, sickly, meaty
20	26.9	*n*-Decane + a methylbutanol	***			Strong, pungent, sweet, solvent-like
21	28.5					Strong, sweet, sickly, buttery, fruity
22	29.0	Tetrachloroethane[b]	***			Strong, sweet
23	30.6	Dec-1-ene[b]	***			Strong, cardborad
24	32.1	Toluene	***		32.3	Strong, fruity, becoming dank, batter
25	33.3					Rancid
26	34.9					Sour, slightly burnt
27	36.4	*n*-Undecane + unknown (prob. N containing)	***		36.4 Undecane	Strong, very unpleasant
28	37.4	Dimethyl disulfide	***		37.3	Very strong, sulfurous, garlic, onions
29	39.5	*n*-Hexanal	**	*n*-Undecane at retn 36.4	40.0	Strong, rancid, unpleasant
30	41.0	Undec-1-ene	***			Unpleasant, old meat
31	42.8	*p*-Xylene	***		43.4	Fruity, solvent-like, sickly, fatty
32	43.4					
33	44.6					
34	45.6	Undec-4-ene[b]	*			
35	46.8					Sweet, sulfurous, faint meaty
36	48.0					Rancid, rotting vegetables
37	50.1	Unknown, not dodecane at 50.2				
38	52.0	*o*-Xylene	***		52.0	Sweet
39	52.9	A methylpyridine[b] (prob. 2-methyl-)	***		54.0	
40	55.9	*n*-Propylbenzene + 2-*n*-pentylfuran	***	Pr. benz. at retn 55.9	Pr. benz. 56.0	
41	57.5					
42	60.5	A C_3 benzene	***			
43	63.4	Dodec-1-ene[b]	***			Medicinal
44	66.0	Styrene	***			
45	68.5	Methylpyrazine	*	Styrene at retn 66.0	68.3	
46	69.3	A C_3 pyridine[b] (prob. 2-*n*-propyl-)	**			Background smell, sweet, green peppers, rubber
47	70.5					
48	72.8	Tridec-1-ene[b]	***			
49	74.6	A dimethylpyrazine (prob. 2,6-)	***	2,6-Dimethyl-pyrazine at retn 74.6	74.5	
50	75.7	Ethylpyrazine	**		75.6	
51	76.2					
52	76.5					
53	77.5					
54	78.2					
55	79.3					
56	80.5	*n*-Tetradecane + unknown	***			

Table I (*Continued*)

Peak no.	Rel t_R, min	Identity	Certainty[a] of MS ident.	Int. stand.	Rel t_R ref, min	Aroma as eluted from column
57	81.5	An ethylmethylpyrazine (prob. 2-Et-6-Me-)	***			
58	81.7	An ethylmethylpyrazine (prob. 2-Et-5-Me-)	***			
59	83.3	A trimethylpyrazine (prob. 2,3,5-)	***			
60	83.8	Long-chain hydrocarbon	***			
61	87.4	Tetradec-1-ene + unknown	***			
62	90.7	A dimethylethylpyrazine (prob. 2,3-diMe-5-Et-)	***			
63	91.6			*n*-Pentadecane		
64	93.3	A dimethylethylpyrazine (prob. 2,5-diMe-3-Et-)	***	at retn 97.2		
65	94.3					
66	97.2	*n*-Pentadecane	***		97.2	
67	97.8					
68	100.0					
69	102.5	Pyrrole[b]	***		102.8	
70	106.0					
71	107.0	Benzaldehye	***		106.5	
72	109.0	Nonanol	***		108.0	
73	113.0	A methylpyrrole[b] (prob. 2-methyl-)	***			
74	116.0					
75	119.0					
76	122.0	*n*-Hexadecane	***			
77	130.0	Hexadec-1-ene	**			
78	136.5					
79	138.5					
80	142.5	A dimethylpyrrole[b] (2,4- or 2,5-)	*			
81	147.5	A C_5 satd. subst. pyrazine[b] (poss. methyl isobutyl-)	*			
82	157.0	*n*-Heptadecane	***		157.0	
83	161.0	Phenol	*		160.0	
84	166.0	*m*-Toluonitrile[b]	**		166.0	
85	169.5	Heptadec-1-ene?[b]			168.0	
86	179.0					
87	187.5	Ethylbenzaldehyde[b]	***			
88	191.5					
89	201.0	A C_{20} branched alkene[b] (mol wt 280, branch at C_{13})	***			
90	208.0			*n*-Heptadecane		
91	218.0			at retn 157.0		
92	226.5					
93	237.0					
94	245.0					
95	258.0	A C_{20} branched alkene[b] (mol wt 280; branch at C_{13})	***			
96	266.0					
97	278.0					
98	285.0					
99	300.0					
100	311.0	A C_{20} branched alkadiene[b] (mol wt 278; branch at C_{13})	***			
101	345.0					
102	356.0					

[a] ***, denotes mass spectral identification as positive as possible from good mass spectra; **, denotes very slight doubt due to one uncertainty in the spectrum; *, denotes tentative identification since mass spectrum shows a number of discrepancies. [b] Denotes compounds identified for the first time in beef aroma.

volatile flavor components. However, the experiments involving microwave cooking were not prolonged beyond a heating period of 1 h 5 min since the product after this amount of cooking in the microwave oven was excessively cooked. It is unknown therefore where the "exhaustion plateau" would have been reached in this instance.

When comparing equal heating times of up to 1 h for the two heating methods (see Figure 1) greater amounts of volatiles are extracted from the microwave samples, due to the rapid initial rise in temperature (boiling point being reached in 5 min compared with 30 min of conventional heating, for the given system). Thus, if the liberation of any particular volatile is the result of temperature rise, then it should be produced far more rapidly by microwave radiation than during conventional cooking as long as the precursors are not exhausted. These results agree with similar work on cabbage (MacLeod and MacLeod, 1970).

Graphs of total organic volatiles extracted vs. extraction time for the two heating methods follow each other quite closely. Thus, after the initial differential warm-up periods

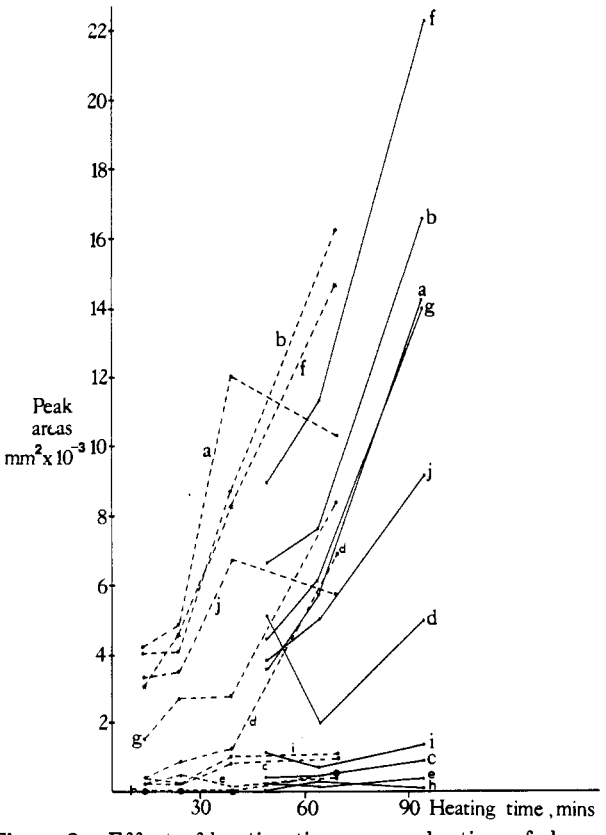

Figure 2. Effect of heating time on production of classes of volatile components: (—) conventional; (---) microwave; (a) alkanes; (b) alkenes; (c) alcohols; (d) aldehydes; (e) ketones; (f) benzenoids; (g) furans; (h) pyrroles; (i) pyridines; (j) pyrazines.

to extraction, shown in Figure 1, the rate of production of volatiles during any given period up to 1 h is approximately equal for the two heating methods.

When comparing acceptably cooked products for the given mass of meat (samples C_4 and M_2 conventional and microwave respectively, see Table II (supplementary material) and Figure 1) there is a quantitative difference as far as the total organic volatiles are concerned (2.2×10^4 mm^2, microwave; 7.6×10^4 mm^2, conventional). Criteria used to judge acceptability by both trained and untrained judges were appearance, texture, and flavor. Thus, boiled beef cooked to an acceptable degree of doneness by microwave and conventional cooking produces different amounts of volatiles, the microwave boiled beef having liberated only approximately one-third of the total volatiles of the conventional product. This illustrates the importance of time for the production of some volatiles at least. In microwave cooking, the initial increased rate of production of volatiles shown in Figure 1 is not sufficient to make up for the shorter cooking time as far as the total volatiles production is concerned.

Figure 2 shows that variations in the rate of production, both with heating time within each series and with mode of heating, are different for the various classes of volatile components and even decreased amounts (in absolute terms) of some volatiles occur with increased heating time. This may be because products of initial reactions undergo secondary reactions but also possibly because they may decompose forming very volatile components which cannot be collected (water, carbon dioxide, ethylene, etc.) or may further react to give nonvolatile compounds. This variation in production rates will lead to differences in the relative proportions of the constituent volatiles of the isolates (see Table II) which in turn will lead to variations

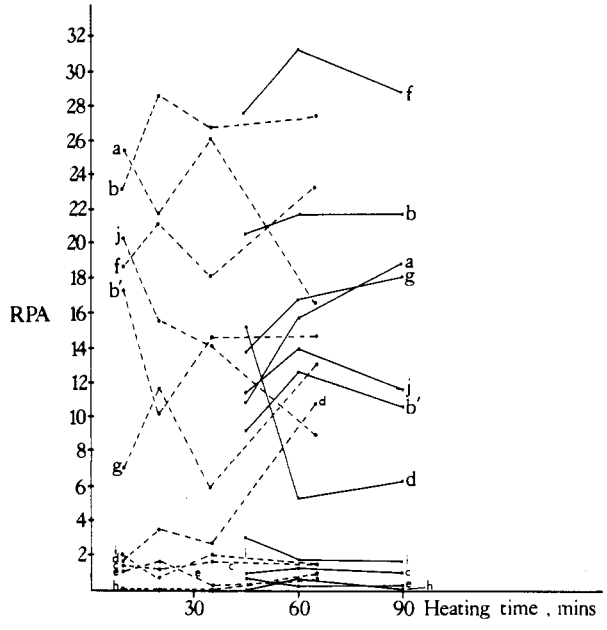

Figure 3. Effect of heating time on RPA of classes of volatile components: (—) conventional; (---) microwave; (a) alkanes; (b) alkenes; (b′) alkenes < C_{20}; (c) alcohols; (d) aldehydes; (e) ketones; (f) benzenoids; (g) furans; (h) pyrroles; (i) pyridines; (j) pyrazines.

Table III. Compounds Generally Present in Greater Concentration in Conventional Boiled Beef Samples than in Microwave Boiled Beef Samples

Benzenoid compounds	Benzene, *p*-xylene, *n*-propylbenzene,[a] styrene, phenol, ethylbenzaldehyde
Aliphatic aldehydes	3-Methylbutanal, *n*-hexanal
Furans	2-Methylfuran, 2-ethylfuran, 2-*n*-pentylfuran[a]
High mol wt hydrocarbons	*n*-Pentadecane, *n*-heptadecane
Alkenes	The two C_{20} branched alkenes (peaks 89 and 95) and also
	2-ethyl-6-methylpyrazine,
	2,3,5-trimethylpyrazine,
	2-*n*-propylpyridine,
	2-methylthiophen (?), pyrrole,
	9 unknowns

[a] The composite peak representing *n*-propylbenzene and 2-*n*-pentylfuran was the largest peak in the majority of the conventional boiled beef samples, representing up to 12% of the total organic volatiles.

in the sensory properties of the samples. For this reason, Figure 3 shows the effect of heating time on the RPA of the various classes of volatile components identified in the conventional and microwave boiled beef isolates.

As a generalization, classes of components which represent greater proportions of the isolates for the *conventional* boiled beef are: the benzenoids (particularly propylbenzene and ethylbenzaldehyde), the aldehydes (particularly 3-methylbutanal), and the furans (particularly 2-*n*-pentyl- and 2-ethyl-). A single representative of any class need not, of course, follow the generalized trend for that class and therefore specific compounds which, as a generalization for the entire series, are present to a greater extent in the conventional samples than in the microwave samples are given in Table III. It is highly unlikely that any nonthermal chemical reactions occur by microwaves specifically because the quantum energies concerned are insufficient for chemical bond scission (Rosen, 1972). Therefore, two factors relevant to the present discussion which affect the rates of chemical reactions in general are

Table IV. Compounds Generally Present in Greater Concentration in Microwave Boiled Beef Samples than in Conventional Boiled Beef Samples

n-Alkanes	(Lower mol. wt. members in particular) *n*-heptane, 2-methylheptane[a] *n*-octane, *n*-nonane, *n*-decane, *n*-undecane[b]
Alkenes	(Lower mol. wt. members in particular) dimethylbutene, hex-1-ene, hept-1-ene, undec-1-ene, undec-4-ene, dodec-1-ene
Alcohols	A methylbutanol, 1-nonanol[a]
Pyrazines	Methylpyrazine,[a] 2,6-dimethylpyrazine, ethylpyrazine, 2,3-dimethyl-5-ethylpyrazine, 2,5-dimethyl-3-ethylpyrazine,[a] 3-methyl-2-isobutylpyrazine, and also the C_{20} alkadiene, tetrachloroethane, acetone, dimethylpyrrole,[a] 18 unknowns of which 9 are marked[a]

[a] Indicates that the components were present in measurable amounts only in the microwave samples. However, the samples compared here involved total heating times of up to 90 min. These components were in fact present in conventional samples heated for 4 h. [b] The composite peak representing *n*-undecane plus a N-containing unknown was consistently the largest peak in all the microwave boiled beef isolates, representing up to 19% of the total organic volatiles.

time and temperature. The components present in greater concentrations in the conventional samples are probably representative of compounds formed by chemical reactions which have relatively low rates of reaction and therefore time is important for their production in significant amounts.

Classes of compounds which in general occur in greater amounts in the *microwave* boiled beef isolates are: the alkanes (particularly undecane), the alkenes below C_{20}, the alcohols, and the pyrazines. Alkanes and alk-1-enes are produced when meat is γ irradiated (Merritt et al., 1966) and it has been suggested that increased concentration of hydrocarbons in foods is unique to the effects of γ irradiation. This is obviously incorrect, since the present work shows that this is also caused by microwave irradiation. Only five alk-1-enes have previously been reported in beef aroma (Hirai et al., 1973; Watanabe and Sato, 1971b). The higher RPA figures for the pyrazines in the microwave boiled samples are interesting and were unexpected. Although some pyrazines can be produced by heating certain aminohydroxy compounds, e.g., ethanolamine, glucosamine, serine, or threonine in air (Wang and Odell, 1973), pyrazines in general are typical products of Maillard browning and visible browning occurs to only a limited extent during microwave cooking. However, Maillard reactions can take place in a microwave oven; suitable conditions do exist, i.e. a temperature of ~90 °C and a degree of dryness (Wasserman, 1972). Nevertheless Smith (1972) reports that this reaction requires time and may not occur to any considerable extent until after 30 min. Reineccius et al. (1972) showed that pyrazine formation occurred at time–temperature relationships as low as 30 min at 70 °C while Copson et al. (1955) found that raw food coated with different mixtures of Maillard browning pairs browned in a short time by microwave radiation. Dehydrating agents aided the development of browning. The present study shows that the pyrazines were present in high concentrations (~20%) after only 10 min of microwave heating time and slowly decreased in concentration in the aroma to ~12% after 45 min (the time when the first conventional isolate was obtained). This may be due to the fact that in microwave heating, rapid volatilization of water causes slight surface drying and this would cause the correct conditions of dryness faster than with conventional boiling. Evolution of pyrazines would lead to a decrease in RPA values with continued heating unless further surface drying took place, which was inhibited by the recycling conditions of the extraction procedure. Specific compounds which as a generalization for the entire series are present to a greater extent in the microwave samples than in the conventional samples are shown in Table IV. Chemical reactions in general are strongly temperature dependent and the rapid initial temperature rise (idealistically uniform) in microwave cooking has to be contrasted with the slower conventional process of conduction where a high temperature gradient initially exists throughout the food. The compounds present in higher concentrations in the microwave samples are probably representative of compounds formed by reactions which have relatively high rates of reaction and are thus formed in significant amounts in the relatively short microwave cooking times.

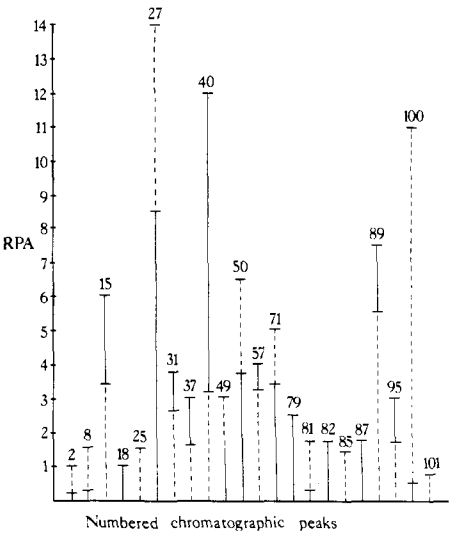

Figure 4. Selected peak variations for two samples of equivalent doneness: (—) conventional, C_4; (---) microwave, M_2.

In addition to the above generalizations, comparisons were made of selected conventional and microwave boiled beef samples on the basis of equivalent and acceptable doneness, equal heating time, and equal extraction time.

Equivalent and Acceptable Doneness Comparisons. The two samples selected for comparison on the basis of equivalent and acceptable degree of doneness for 1 kg of beef were samples C_4 and M_2 (conventional and microwave, respectively; see Table II and Figure 3). The alkanes, alcohols, and pyrazines have a higher RPA in the microwave sample while the benzenoid compounds, aldehydes, pyrroles, pyridines, and furans are in greater concentrations in the conventional sample. Figure 4 shows selected peak variations. These results agree with the generalizations earlier made in the series comparisons and with the specific compounds then quoted.

Equal Heating Time Comparisons. The two samples selected for comparison on the basis of equal heating time (1 h) were samples C_3 and M_4 (conventional and microwave, respectively; see Table II and Figure 3). It is immediately obvious that a greater degree of similarity in relative concentrations of individual components exists between these two samples than was evident in the acceptable doneness comparisons. Once more, from Figure 3, components present in greater concentration in the conventional sample are the benzenoid compounds, furans,

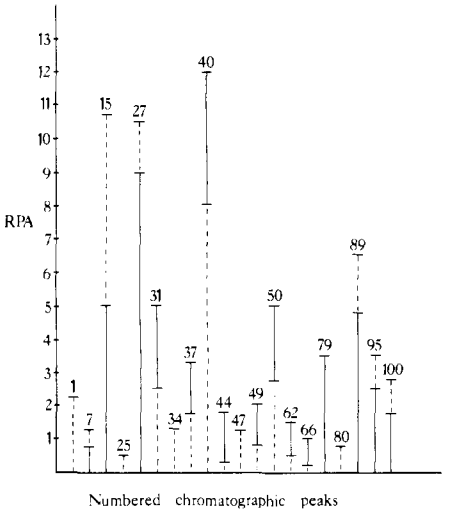

Figure 5. Selected peak variations for two samples of equal heating time, 1 h: (—) conventional, C_3; (---) microwave, M_4.

and pyridines but not the aldehydes and pyrroles. However, this time the pyrazines are included in this group. This fits with previous deductions that the pyrazines are readily formed compounds. Components present in greater concentration in the microwave sample are, as before, the alkanes and alkenes (relatively low molecular weight members in particular) and the alcohols but also this time the carbonyls and pyrroles. Figure 5 shows selected peak variations.

Equal Extraction Time Comparisons. Three pairs of samples can be compared on the basis of equal extraction times. These are C_2 vs. M_2 (15 min), C_3 vs. M_3 (30 min), and C_4 vs. M_4 (60 min); see Table II and Figure 3. Comparing C_2, C_3, and C_4 with M_2, M_3, and M_4 the total number of peaks observed and measured were 41, 56, 54 and 49, 59, 65, respectively. This reflects the overall increase in the number of volatile components produced as heating continued, the increase being particularly marked in the microwave series, possibly because 60-min heating by microwaves is excessively long by usual standards and this overcooking would be expected to cause increased decomposition of flavor volatiles and precursors. For the lower extraction time samples (C_2 and M_2) classes of compounds present to a greater extent in the microwave sample are again the alkanes and alkenes (low molecular weight members in particular), alcohols, and pyrazines. But as heating and extraction continue many alkanes (particularly the higher molecular weight members) and pyrazines are less evident in this group and are present in greater concentrations in the conventional samples. Conversely, compounds present to a greater extent in the low extraction time conventionally cooked samples are the benzenoid compounds, the furans, the C_3 pyridine, pyrrole, 3-methylbutanal, and 2-methylthiopen (tentative identification). However, as heating continues, the same effect is observed for the benzenoids and furans but the 3-methylbutanal, the pyridine, and the pyrroles are present in greater concentrations in the microwave samples. Concentration differences of the various components decrease both in size and number with longer heating time, due to the trends just described. This suggests an effect characteristic of the different warm-up periods and that after 1 h extraction the microwave sample had "caught up" with the conventional.

CONCLUSION

In summary, classes of components characteristically present in higher concentrations in *microwave* boiled beef aroma include: alkanes (in particular low molecular weight members and *n*-undecane); alkenes (especially low molecular weight members) and alcohols. Conversely, classes of components characteristically present in higher concentrations in the *conventional* boiled beef aroma include: benzenoid compounds (especially *n*-propylbenzene) and furans (especially 2-*n*-pentylfuran). Additionally, carbonyl compounds (particularly 3-methylbutanal), pyrroles, and pyridines (particularly the C_3 pyridine) are characteristic of a high degree of cooking, whether by microwaves or conventionally, while pyrazines appear to be representative of a low degree of cooking, i.e. relative underdoneness.

These relative concentration differences are presented in isolation at this stage. However, the significance of these in relation to the sensory properties of the samples is currently being studied. Closely allied to this is the information obtained regarding the aroma of peaks on elution from the GC column. Table I shows that, as other workers have found, no individual compounds were indicated as being uniquely responsible for the characteristic beef flavor, although some peaks were described as "meaty". Many of the odor descriptions of Table I agree with those given by Persson and von Sydow (1973) and by Watanabe and Sato (1968), particularly the occurrence of many sickly, sweet, and green notes. It is interesting to note at this stage that several of the alkanes, alkenes, and alcohols present in high concentrations in the microwave boiled beef aroma are associated with relatively undesirable odor port descriptions such as sour, harsh, unpleasant, pungent, and dull cardboard; equally, the benzenoid compounds, furans, and 3-methylbutanal present in high concentrations in the conventionally boiled beef aroma are characterized by more desirable terms such as pleasant, grassy, solvent, fruity, and meaty. This confirms other findings in this laboratory that time appears to be an important factor as far as desirable beef flavor production is concerned.

ACKNOWLEDGMENT

Grateful appreciation is due to the U.K. Meat and Livestock Commission for supplying "standard" meat supplies and to J. W. Letcher, A. G. Wilkinson, and A. J. MacLeod for expertise in the operation of the GC–MS and data processing system.

Supplementary Material Available: Table II, absolute peak areas and relative percentage abundances of boiled beef aroma isolates (5 pages). Ordering information is given on any current masthead page.

LITERATURE CITED

Altman, P. L., Dittmer, D. S., Ed., "Biology Data Book", Publication of the Federation of American Societies for Experimental Biology, Washington, D.C., 1972.
Brodnitz, M. H., *J. Agric. Food Chem.* **16**(6), 994 (1968).
Coppock, B. M., Ph.D. Thesis, University of London, 1975.
Copson, D. A., "Microwave Heating", Avi Publishing Co., Westport, Conn., 1962.
Copson, D. A., Neumann, B. R., Aaron, L. B., *J. Agric. Food Chem.* 3(5), 424 (1955).
Dwivedi, B. A., *Crit. Rev. Food Technol.*, 487 (April, 1975).
Ferretti, A., Flanagan, V. P., *J. Agric. Food Chem.* **19**(2), 245 (1971).
Forss, D. A., *Prog. Chem. Fats Other Lipids* **13**(4), 177 (1972).
Goldblith, S. A., *Microwave Energy Applications Newsl.* **5**, 3 (1972).
Gutcho, M. H., "Feeds for Livestock, Poultry and Pets", Food Technology Review No. 6, Noyes Data Corp., Park Ridge, New Jersey, 1973.
Herz, K. O., Chang, S. S., *Adv. Food Res.* **18**, 1 (1970).
Heyns, K., Stute, R., Paulsen, H., *Carbohyd. Res.* **2**, 132 (1966).

Hirai, C., Herz, K. O., Pokorny, J., Chang, S. S., *J. Food Sci.* **38**, 393 (1973).
Hodge, J. E., in "Chemistry and Physiology of Flavors", Schultz, H. W., Day, E. A., Libbey, L. M., Ed., Avi Publishing Co., Westport, Conn., 1967, pp 465–491.
Hornstein, I., Crowe, P. F., *J. Agric. Food Chem.* **8**(6), 494 (1960).
Hudson, B. J. F., Karis, I. G., *J. Sci. Food Agric.* **25**, 1491 (1974).
Kolattukudy, P. E., *Lipids* **5**, 259 (1970).
Liebich, H. M., Douglas, D. R., Zlatkis, A., Muggler-Chavan, F., Donzel, A., *J. Agric. Food Chem.* **20**(1), 96 (1972).
Likens, S. T., Nickerson, G. B., *Proc. Am. Soc. Brew. Chem.*, 5 (1964).
MacLeod, A. J., Cave, S. J., *J. Sci. Food Agric.* **26**, 351 (1975).
MacLeod, A. J., MacLeod, G., *J. Food Sci.* **35**, 744 (1970).
MacLeod, G., *Food Proc. Ind.*, 27 (March, 1972).
Merritt, C., Jr., Angelini, P., Bazinet, M. L., McAdoo, D. J., *Adv. Chem. Ser.* **No. 56**, 225 (1966).
Mussinan, C. J., Wilson, R. A., Katz, I., *J. Agric. Food Chem.* **21**(5), 871 (1973).
Patterson, R. L. S., in "Meat", Cole, D. J. A., Lawrie, R. A., Ed., Butterworths, London, 1974, p 359.
Persson, T., von Sydow, E., *J. Food Sci.* **38**, 377 (1973).
Proctor, G., Goldblith, S. A., *Adv. Food Res.* **3**, 119 (1951).
Reineccius, G. A., Keeney, P. G., Weissberger, W. J., *J. Agric. Food Chem.* **20**(2), 202 (1972).
Reynolds, T. M., in "Symposium on Foods", Schultz, H. W., Cain, R. F., Wrolstad, R. W., Ed., Avi Publishing Co., Westport, Conn., 1969.
Reynolds, T. M., *Food Technol. Aust.* **22**(11), 610 (1970).
Rhodes, D. N., in Proceedings of the I.F.S.T. (U.K.) Tenth Anniversary Symposium on Meat and Meat Products, 1974, p 5.
Rosen, C. G., *Food Technol.*, 36 (July, 1972).
Roth, H., Rock, S. P., *Baker's Dig.*, 38 (Aug, 1972).
Self, R., Casey, J. C., Swain, T., *Chem. Ind. (London)*, 863 (1963).
Sink, J. D., *J. Am. Oil Chem. Soc.* **50**(11), 470 (1973).
Smith, D. P., *Microwave Energy Applications Newsl.* **5**(4), 2 (1972).
van Straten, S., de Vrijer, F., Rapport NR. R4030, "Lists of Volatile Compounds in Food", 3rd ed, Centraal Instituut voor Voedingsonderzoek, Zeist, Netherlands, 1973.
Walradt, J. P., Lindsay, R. C., Libbey, L. M., *J. Agric. Food Chem.* **18**(5), 926 (1970).
Wang, P. S., Odell, G. V., *J. Agric. Food Chem.* **21**(5), 868 (1973).
Wasserman, A. E., *J. Agric. Food Chem.* **20**(4), 737 (1972).
Watanabe, K., Sato, Y., *Agric. Biol. Chem.* **32**(11), 1318 (1968).
Watanabe, K., Sato, Y., *J. Agric. Food Chem.* **19**(5), 1017 (1971a).
Watanabe, K., Sato, Y., *Agric. Biol. Chem.* **35**(5), 756 (1971b).
Watanabe, K., Sato, Y., *J. Agric. Food Chem.* **20**(2), 174 (1972).
Wilson, R. A., Mussinan, C. J., Katz, I., Sanderson, A., *J. Agric. Food Chem.* **21**(5), 873 (1973).

Received for review December 24, 1975. Accepted March 22, 1976. This project was financed by the U.K. Meat and Livestock Commission and the University of London Central Research Fund.

Study of Meat Volatiles Associated with Aroma Generated in a D-Glucose–Hydrogen Sulfide–Ammonia Model System

Takayuki Shibamoto and Gerald F. Russell*

The volatile compounds produced by heating a model system of D-glucose–hydrogen sulfide–ammonia were entrained on Porapak Q and subsequently desorbed and transferred to a glass capillary column for separation and identification. Gas chromatographic and mass spectrometric methods were used to identify 34 of the major components. The compounds identified included a thiol, sulfides, thiophenes, thiazoles, and furans. Thiophene and furan derivatives were the major volatile constituents of this reaction mixture which gave roast beef-like aroma.

Over 100 volatile components have been identified in cooked meat extracts. These have included aliphatic aldehydes, esters, ketones, hydrocarbons, and some nitrogen- and sulfur-containing compounds (Nonaka et al., 1967; Persson and von Sydow, 1973). Recently, pyrazine compounds have also been found in cooked meat (Watanabe and Sato, 1971). Some thiazole, oxazole, thiazoline, and oxazoline compounds have been identified in cooked meat and some of these possess a meaty flavor (Mussinan et al., 1975).

Many studies using simulated cooking conditions in model systems have been conducted to investigate meat flavor precursors (Mulders, 1973; Mussinan and Katz, 1973; Kato et al., 1973). Wood (1961) reported that heating an aqueous solution of fresh ox muscle extract with glucose produced a meaty flavor and browning, but neither was observed when the glucose was omitted. Mussinan and Katz (1973) reported the formation of sulfur-containing compounds in model systems consisting of hydrolyzed vegetable protein (HVP)–L-cysteine·HCl–D-xylose–water and L-cysteine–xylose–water. They found in their reaction mixtures some thiophenes and sulfides which have also been identified in meat. van den Ouweland and Peer (1975) reported the formation of thiophene derivatives from the reaction of a sugar degradation product, 4-hydroxy-5-methyl-3(*H*)-furanone, and hydrogen sulfide.

Carbonyl compounds (aldehydes, ketones, and diketones) are known to be formed as degradation products of sugars (Nodzu, 1935; Carson, 1953). Carbonyls present in food include glucosamine, dehydroascorbic acid, and pyruvaldehyde. Diketones are also formed from thermal degradation of glucose (Shibamoto, 1974). Takken et al. (1975) obtained heterocyclic compounds such as oxazoles, thiazoles, and thiazolines which had a meaty flavor (Mussinan et al., 1975) from a model system consisting of α-dicarbonyls or aldehydes, hydrogen sulfide, and ammonia. These experiments suggest that compounds which give characteristic meat aroma are formed from the reaction of carbonyl compounds, hydrogen sulfide, and ammonia, and it appears that the compounds associated with characteristic meat aroma include hydrogen sulfide, ammonia, carbonyls, pyrazines, thiophenes, thiazoles,

Department of Food Science and Technology, University of California, Davis, California 95616.