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Roasted Lamb Fat: Basic Volatile Components

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The basic fraction from the steam volatile oil obtained from roasted lamb fat has been analyzed by the combination of glass capillary column gas chromatography and mass spectrometry. Twelve alkyipyridines, twelve alkyipyrazines, and two alkyithiazoles were characterized. The major component of the basic fraction was 2-pentylpyridine. Other unusual compounds characterized included 5-methyl-2-pentylpyridine, 5-ethyl-2-pentylpyridine, 2-butylpyridine, 2-hexylpyridine, and 3-pentylpyridine.

The volatile flavor components of meats have been reviewed by Dwivedi (1975). Lamb flavor has probably been one of the least studied.

Major work in this area has, however, been recently carried out by Wong et al. (1975a,b) and Park et al. (1974, 1975, 1976). It has generally been considered that the main part of the flavor of lamb originates in the lamb fat. Most of the previous studies have been concentrated on the nonbasic components of lamb fat. The present study was aimed at characterizing components of the basic fraction.

EXPERIMENTAL SECTION

Materials. Lamb adipose tissue was obtained from the renal area of 4-7-month old, 80-120 lb lambs from ewes of Targhee and Finn-Targhee crosses sired by Suffolk Rams. This was kindly provided by the Animal Science Department, University of California, Davis. It was stored at -40 °C. Lamb fat and ground lamb were also obtained from a local market. These were refrigerated and used within a day or two of the purchase.

Authentic Chemical Samples. These were obtained commercially or synthesized by well-established methods. 2-Butylpyridine, 2-pentylpyridine, and 2-hexylpyridine were synthesized from 2-methylpyridine using sodium amide in liquid ammonia and the appropriate alkyl bromide (cf. Vogel, 1962). 3-Pentylpyridine was synthesized in a similar way from 3-methylpyridine. 5-Methyl-2-pentylpyridine and 5-ethyl-2-pentylpyridine were synthesized, also using a similar procedure, from 2,5-di-

methylpyridine and 5-ethyl-2-methylpyridine, respectively. Authentic samples of pyrazines were synthesized by established procedures (Flament et al., 1967).

Isolation of Volatile Oil. Two main methods of isolation were used. With the first method lamb fat (4 kg) was placed in a three-necked, 12-L flask. Attached to the central neck of the flask was a special type of Likens-Nickerson steam distillation continuous extraction head (cf. Nickerson and Likens, 1966) modified so that the condensed water did not return directly to the 12-L flask but was led off into a 1-L flask from which it was pumped, using a zenith pump, back into the 12-L flask through one of its side arms. Freshly distilled diethyl ether was used in the solvent side arm of the Likens-Nickerson head. The lamb fat was gradually heated to 150 °C, and the aqueous distillate (normal adipose tissue moisture) was collected in the 1-L flask. The distillate was then pumped back into the fat at a rate of about 8 mL/min. This rate allowed the temperature of the fat to be kept at 150 °C. This was continued for 3 h. The ether was then dried over Na₂SO₄ and concentrated to give the whole volatile oil (60 μL). This oil was then again taken up in ether (100 mL) and extracted with 3 N HCl (2 × 10 mL). The acid extract was washed with ether (1 × 20 mL, the ether washing being discarded) and then covered with ether (100 mL) and neutralized with excess NaHCO₃. The ether layer was separated and the neutralized aqueous solution extracted further with ether (2 × 50 mL). The ether extracts of the neutralized solution were then combined and dried over sodium sulfate and the ether removed to give the lamb fat basic fraction (10 μL).

In the second method of isolation, lamb fat (1.4 kg) was roasted in shallow metal pans at 175 °C oven temperature

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Table I. Identities of Constituents of Basic Fraction of the Steam Volatile Oil of Roasted Lamb Fat

Pyridines ^{a, b}	Pyrazines ^{a, b}
(10) Pyridine MS, IR, RT (1-5%)	(16) 2-Methylpyrazine MS, RT (1-5%)
(15) 2-Methylpyridine MS, RT (2%)	(22) 2,5-Dimethylpyrazine MS, IR, RT (4-5%)
(19) 3-Methylpyridine MS, RT (2-3%)	(23) 2,6-Dimethylpyrazine MS, RT (3-4%)
(18) 2-Ethylpyridine MS, RT (2-16%)	(25) 2,3-Dimethylpyrazine MS, RT (1%)
(28) 3-Ethylpyridine ^e MS, RT (2-7%)	(28) 2-Ethyl-6-methylpyrazine ^e MS, RT (2-7%)
(33) 5-Ethyl-2-methylpyridine MS, RT (1%)	(29) 2-Ethyl-5-methylpyrazine MS, RT (2-3%)
(38) 2-Butylpyridine MS, RT (2%)	(31) Trimethylpyrazine MS, RT (4%)
(47) 2-Acetylpyridine MS, IR, RT (0.6%)	(34) 2-Ethyl-3,5-dimethylpyrazine ^e MS, RT (1%)
(51) 2-Pentylpyridine MS, IR, RT (13-16%)	(35) 2-Ethyl-3,6-dimethylpyrazine ^e MS, IR, RT (4-5%)
(63) 2-Hexylpyridine MS, RT (1%)	(39) Tetramethylpyrazine ^c MS (1-2%)
(64) 3-Pentylpyridine MS, RT (1-2%)	(40) 2,3-Diethyl-5-methylpyrazine MS, RT (1%)
(65) 5-Methyl-2-pentylpyridine MS, IR, RT (2-4%)	(41) 2,6-Diethyl-5-methylpyrazine ^c MS (1%)
(72) 5-Ethyl-2-pentylpyridine MS, IR, RT (2%)	
Other nitrogen compounds ^{a, b}	Tentatively identified compounds ^d
(27) 2,4,5-Trimethyl-2-thiazoline ^e MS, RT	1-Pyrolline
(28) 2,4,5-Trimethylthiazole ^e MS, RT	2,5-Dimethylpyridine
(35) 5-Ethyl-2,4-dimethylthiazole ^e MS, RT	3,5-Dimethylpyridine
(67) Thialdine (2,4,6-trimethylperhydro-1,3,5-dithiazine) MS, RT (1-8%)	2-Ethyl-5,6-dimethylpyrazine
(86) Benzothiazole MS, RT (1%)	2-Propylpyridine

^a MS, IR, RT = mass spectral, infrared spectral, and GLC retention evidence, respectively. ^b Evidence cited consistent with that of an authentic sample unless otherwise indicated. ^c No authentic sample available but spectra consistent with published data. ^d Mass spectra similar to that indicated but apparently of mixtures. ^e Only part of a peak, no separate quantitative analysis possible.

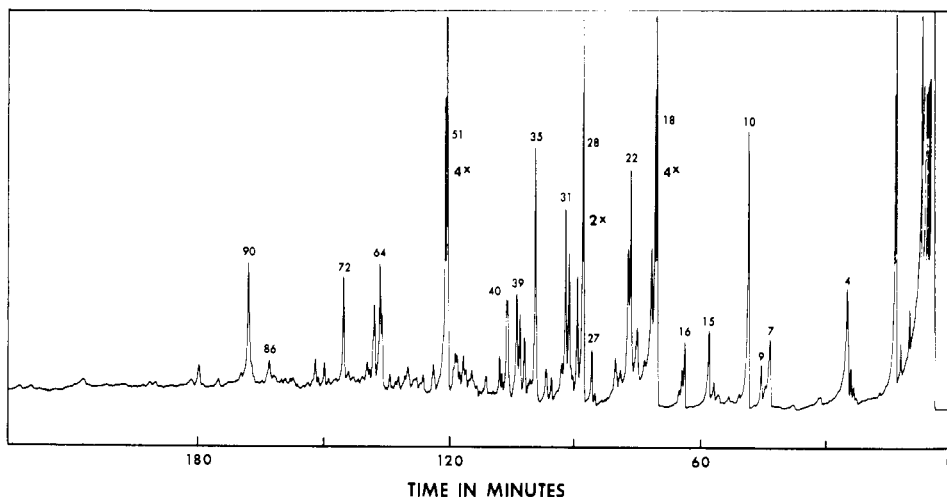


Figure 1. Capillary GLC analysis of the basic fraction of roasted lamb. See text for GLC conditions.

for 1 h. After transfer of the roasted fat to a 12-L flask together with 5 L of water, the volatile oil was isolated using a standard Likens-Nickerson steam distillation continuous extraction head at atmospheric pressure with diethyl ether as solvent. The extraction was continued for 3 h. The basic fraction was isolated as for the first method.

Ground lamb was also placed in shallow metal pans and baked at 175 °C for 1.5 h and the volatile oil isolated using the standard Likens-Nickerson extraction head. The basic fractions from these oils was separated in a similar way to that described above.

Capillary GLC-Mass Spectral Analysis. The GLC column used was a 150 m × 0.75 mm i.d. Pyrex glass capillary coated with Tween 20 containing 5% Igepal CO-880. After injection the column was kept at 25 °C for 10 min and then programmed from 50-170 °C at 1 °C/min and held at the upper limit. The end of the column was coupled to a modified Consolidated 21-620 cycloidal type mass spectrometer using a silicone membrane molecular separator.

Packed Column GLC-Infrared Spectral Analysis. Samples were separated for IR spectra using a 3 m × 0.64 cm stainless steel column packed with 80-100 mesh Chromosorb G coated with 2% Tween 20. The column

was temperature programmed from 50-170 °C at 2 °C/min. Spectra were measured using ultramicrocavity cells and a reflecting beam condenser with a Perkin-Elmer Model 237 instrument.

RESULTS AND DISCUSSION

The lamb adipose tissue used in this work was obtained from the back of the lamb near the kidneys, it contained no significant amount of red meat. The adipose tissue, however, contains some white protein tissue in addition to the predominant triglycerides.

Two main methods were used for the isolation of the volatiles from roasted lamb fat. The first method (Teranishi et al., 1977) carried out the roasting in a 12-L flask with the fat at 150 °C. The volatiles were obtained, during the roasting, by gradually pumping water through the molten fat and collecting the volatiles using a modified Likens-Nickerson steam distillation continuous extraction head. The amount of total volatile oil obtained using this procedure was 15 parts per million (ppm) of the original fat. The basic fraction amounted to 17% of this oil (2.6 ppm of the fat).

In the second main method used, the fat was roasted in shallow pans in an oven at an oven temperature of 175 °C

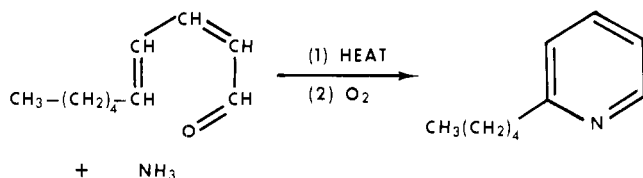


Figure 2. Possible mechanism for the formation of 2-pentylpyridine.

for 1 h. The volatiles were then removed using a standard Likens-Nickerson steam distillation continuous extraction head. The amount of volatile oil (14 ppm) and basic fraction (3 ppm) obtained was similar to the first method. The first method was capable of processing greater quantities of fat more conveniently.

The GLC patterns obtained for the basic fractions from both methods of isolation were similar. Figure 1 shows that obtained for the basic fraction obtained using the second method. Table I lists the components characterized in the basic fraction with peak numbers corresponding to the peaks in Figure 1 shown in parentheses immediately before the compounds name. Some idea of the range of relative percent of the compounds found in the basic fraction with different samples is also listed.

The alkyprazines found are qualitatively similar to those found in other meats and other roasted products (cf. Dwivedi, 1975). The longer chain alkyprazines are more unusual although 2-pentylpyridine had been previously found in pan fried beef (Watanabe and Sato, 1971) and in roasted peanuts (Walradt et al., 1971). In the samples studied 2-pentylpyridine was the major component of the basic fraction. The pyridines may be formed from the reaction of aldehydes with ammonia or other amino compounds. It is well known (Noller, 1957) that 5-ethyl-2-methylpyridine is formed in good yield from the reaction of paraldehyde and ammonia at 220 °C. Figure 2 outlines how 2-pentylpyridine might be formed from deca-2,4-dienal and ammonia by first condensing to the aldimine ($\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}=\text{CHCH}=\text{NH}$), followed by ring closure to the 1,2-dihydropyridine which could be oxidized by air to the pyridine.

As far as the authors can determine, 5-methyl-2-pentylpyridine and 5-ethyl-2-pentylpyridine had not been previously reported in nature or synthesized before. The mass spectra for these and 3-pentylpyridine and 2-hexylpyridine are listed below (two major ions each 14 mass units above m/e 34, intensities in parentheses, molecular ion in dark type).

5-Methyl-2-pentylpyridine: 39 (16), 41 (9); 51 (5), 53 (4); 65 (7), 66 (4); 77 (9), 79 (6); 92 (3), 93 (5); 106 (15), 107 (100); 120 (24), 121 (7); 134 (19), 134 (4); 148 (1); 162 (1), 163 (0.7).

5-Ethyl-2-pentylpyridine: 39 (12), 41 (9); 51 (6), 53 (4); 63 (1), 65 (5); 77 (12), 78 (5); 91 (5), 93 (3); 106 (36), 107 (6); 121 (100), 122 (11); 134 (31), 135 (8); 148 (24), 149 (3); 162 (1); 176 (2), 177 (2).

3-Pentylpyridine: 39 (49), 41 (36); 51 (14), 57 (22); 65 (36), 66 (7); 77 (7), 79 (6); 92 (100), 93 (95); 106 (22), 107 (6); 120 (3), 121 (1); 134 (2); 149 (43), 150 (4).

2-Hexylpyridine: 39 (15), 41 (10); 51 (9), 52 (6); 65 (9), 66 (5); 78 (9), 79 (6); 93 (100), 94 (12); 106 (37), 107 (8); 120 (31), 121 (3); 134 (5); 148 (2); 162 (2), 163 (1).

In separate experiments it was found that higher temperatures of roasting of the lamb fat (>200 °C) gave

higher concentrations of pyridine compounds relative to pyrazines. On the other hand, whole ground lamb (where the fat was not separated from the meat), roasted at a moderate temperature (175 °C oven temperature), gave a relatively higher concentration of alkyprazines. This latter fact is probably due to the higher level of protein providing precursors for pyrazine formation.

Contribution to Baked Lamb Fat Odor. The odor threshold of a number of the alkyprazines had been determined by some of the authors previously (Guadagni et al., 1972; Teranishi et al., 1974) in both water and oil. Of these 2-ethyl-3,6-dimethylpyrazine had the lowest threshold (0.4 parts per 10^9 in water, 24 parts per 10^9 in vegetable oil). This compound is also one of the major alkyprazines in baked lamb fat. The odor threshold of 2-pentylpyridine determined in the present work in water was 0.6 (0.5–0.7) parts per 10^9 parts of water. In the authors opinion, in dilute solutions 2-pentylpyridine has a fatty- or tallowy-like odor.

The odors of the alkyprazines are generally associated with pleasant roasted foods (e.g., chocolate, coffee, roasted nuts). The odors of the pyridines are less pleasant, and it is possible that the alkypridine content of lamb fat is the reason for its rejection by some consumers.

The authors hope to carry out further studies on the determination of odor thresholds of the lamb fat volatile components in the future.

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LITERATURE CITED

- Dwivedi, B. K., *CRC Crit. Rev. Food Technol.*, 487 (1975).
 Flament, I., Willhalm, B., Stoll, M., *Helv. Chim. Acta* 50, 2233 (1967).
 Guadagni, D. G., Buttery, R. G., Turnbaugh, J. G., *J. Sci. Food Agric.* 23, 1435 (1972).
 Nickerson, G. B., Likens, S. T., *J. Chromatogr.* 21, 1 (1966).
 Noller, C. R., "Chemistry of Organic Compounds", W. B. Saunders, Philadelphia, Pa., 1957, p 627.
 Park, R. J., Ford, A. L., Ratcliff, D., *J. Food Sci.* 40, 1217 (1975).
 Park, R. J., Ford, A. L., Ratcliff, D., *J. Food Sci.* 41, 633 (1976).
 Park, R. J., Murray, K. E., Stanley, G., *Chem. Ind. (London)*, 380 (1974).
 Teranishi, R., Buttery, R. G., Guadagni, D. G., *Ann. N.Y. Acad. Sci.* 237, 209 (1974).
 Teranishi, R., Buttery, R. G., Ling, L. C., 173rd National Meeting of the American Chemical Society, Abstract AGFD No. 64, New Orleans, La., March 20, 1977.
 Vogel, A. I., "Practical Organic Chemistry", 3rd ed, Wiley, New York, N.Y., 1962, p 846.
 Walradt, J. P., Pittet, A. O., Kinlin, T. E., Muralidhara, R., Sanderson, A., *J. Agric. Food Chem.* 19, 972 (1971).
 Watanabe, K., Sato, Y., *J. Agric. Food Chem.* 19, 1017 (1971).
 Wong, E., Johnson, C. B., Nixon, L. N., *N.Z. J. Agric. Res.* 18, 261 (1975a).
 Wong, E., Nixon, L. N., Johnson, C. B., *J. Agric. Food Chem.* 23, 495 (1975b).

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