Volatile Medium Chain Fatty Acids and Mutton Flavor

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Mutton fat, cooked with or without meat added, produced a host of steam volatile fatty acids in trace amounts. These have been identified by GC–MS as medium chain normal, branched, unsaturated, oxygenated, and aromatic acids belonging to 11 homologous series. Odor properties of these acids have been evaluated and results indicate that the branched chain and unsaturated acids having 8 to 10 carbon atoms contribute to the undesirable flavor of cooked mutton. The 4-methyl branched C_9 and C_{10} acids in particular are considered primarily responsible for the sweaty odor note described in Chinese as "SOO".

The low consumer acceptance of sheep meats (lamb and mutton) in many countries has been attributed to the objectionable cooking smell and to the flavor of the cooked meat (Batcher et al., 1969; Weidenhamer et al., 1969). The pertinence of this mutton flavor factor is perhaps best underscored by citing the existence of the Chinese word "SOO" used in that language specifically to describe the characteristic flavor of sheep meats.

In an attempt to define the distasteful flavor and odor of mutton in sensory and chemical terms, we have isolated from mutton fat, cooked either with or without lean meat added, steam distillates which possess unpleasant smells strongly reminiscent of their ovine origin. Separation of the distillate into acids and nonacids produced in turn fractions with unpleasant smells which could be recognized as contributing in different ways to mutton flavor and odor. The sensory and chemical studies of the acid fraction from such distillates form the subject of this paper. A preliminary account of this work has previously appeared (Wong et al., 1975).

EXPERIMENTAL SECTION

Materials. Sheep carcasses were purchased from local commercial sources and kept frozen at −10° until required. Fatty tissues were trimmed off and bulked and the residual meat without visible fat was taken as the lean meat fraction.

Cooking and Distillation of Mutton Mince. Mince (4.5 kg) consisting of lean meat and adipose tissues (9:1, w/w) was refluxed with 2 l. of water under N_2 for 3 hr. The water (2 l.) was then distilled off in a stream of N_2 at a temperature below 110°. The distillation residue was filtered through glass wool and the fatty filtrate was then steam distilled at 110° under reduced pressure (5 cmHg) until 1 l. of aqueous distillate had collected. The distillate was saturated with NaCl and extracted continuously for 24 hr with ether. After reduction in volume to 100 ml the ether extract was separated into acids and nonacids by extraction with 5% Na_2CO_3 (3×, total 100 ml). Acidification of the carbonate solution and reextraction into ether yielded the acid fraction which after drying was reduced in volume to a stock solution of 400 μl in ether.

Cooking and Distillation of Mutton Adipose Tissues. To 4.5 kg of finely divided adipose tissues was added 500 ml of water and the mixture was refluxed for 2 hr under N_2. Water was then distilled off under N_2 until the temperature of the fat reached 140°. The fat was then steam distilled at 120° under reduced pressure (8 cmHg) until 500 ml of aqueous distillate was collected. This distillate was ether extracted and separated into acids and nonacids as described for the mince distillation. The acid fraction was obtained as a stock solution in 400 μl of ether.

Alkaline Hydrolysis of Mutton Fat. Mutton adipose tissues (1 kg) were extracted with chloroform (3×, total 3 l.) in a Waring Blendor and the extract after cooling with ice was washed with 1 l. of Na_2CO_3 to remove traces of free acids. The bulk of the chloroform was then removed under reduced pressure at 60° and N_2 was bubbled through the fat at 70° for 3 hr to remove the last traces of the solvent. Four liters of 10% aqueous KOH was then added to the fat and this mixture was stirred overnight at 80° under N_2. After cooling, the mixture was acidified with 1:1 H_2SO_4 and the fatty acid mixture then steam distilled at atmospheric pressure until 1 l. of distillate was collected. The distillate was ether extracted and separated into acid and nonacid as before. The final acid solution was reduced to 400 μl in ether.

Gas Chromatography. The fatty acids were separated on a 2.5 m × 3.2 mm o.d. stainless steel column of 10% (w/w) stabilized polyethylene glycol adipate (EGA, Analabs Ltd.) on 100–120 mesh Gas-Chrom Q (Applied Science Labs) with temperature programming from 50 to 200° at 2°/min. Carrier gas was N_2 at a flow rate of 50 ml/min. Methyl esters, prepared by refluxing with BF_3–methanol, were analyzed on the EGA column or a similar column containing OV-101 silicone oil. Hewlett Packard 7620A and Pye 104 gas chromatographs were used.

Collection of Trace Components and Rechromatography. For the resolution and subsequent GC–MS identification of methyl esters of the trace acids occurring in much lower proportion than the *n*-acids, two-dimensional gas chromatography was carried out in which esters appearing in intermediate regions of the chromatogram after separation on EGA were trapped and rechromatographed on OV-101 or vice versa.

Stainless steel collection tubes (150 mm × 3.2 mm o.d.), packed to half their lengths with the adipate column material (conditioned for 1 hr at 200° with a slow flow of carrier gas through them) and cooled with Dry Ice, were used for preparative collections of the esters. Collected fractions were reinjected into a second column through a modified septum retainer that had a swagelok thread attached to it so that the collection tube could be inserted into the injection port. The column used for the reinjection chromatography was attached to the chromatograph by a large i.d. tube column insert liner (for Hewlett Packard Model 80 pyrolysis unit); carrier gas flow through the inlet was reduced from 50 to 10 ml/min during transfer of the sample onto the cooled column (initial short length covered with Dry Ice) while the sample was flushed from the tube by a flow of carrier gas (~45 ml/min) passing through it.

Combined Gas Chromatography and Mass Spectrometry. Mass spectra of esters after second dimension gas chromatography were obtained with an AEI MS-30 double

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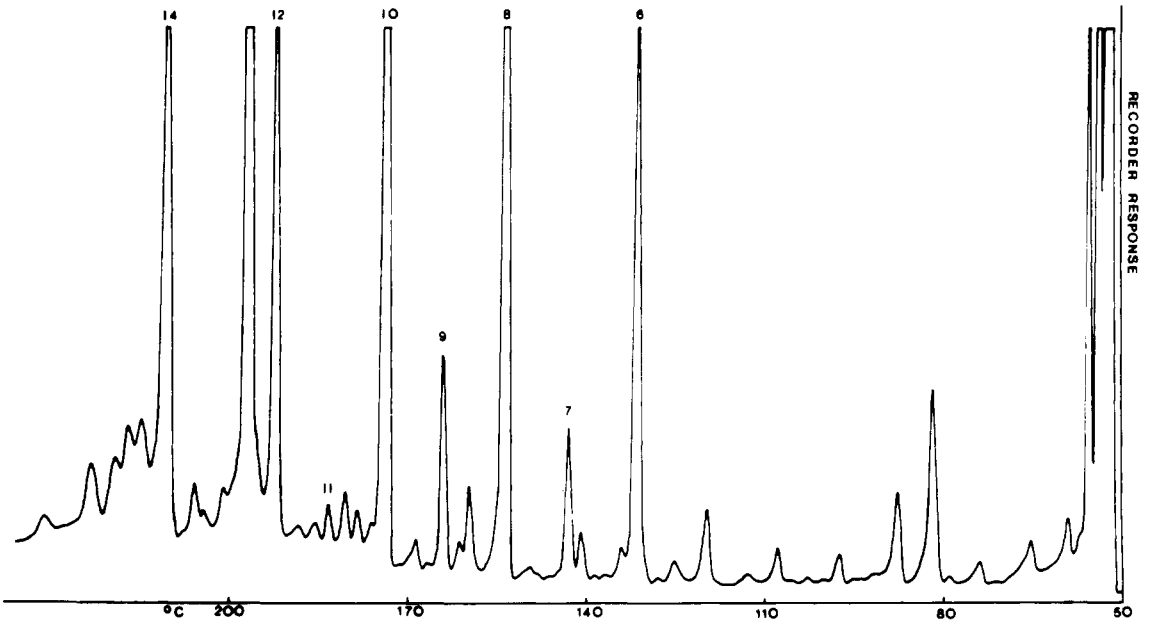

Figure 1. Gas chromatogram of the volatile fatty acids from mutton mince cook-up.

beam instrument coupled to the PYE 104 gas chromatograph with a silicone elastomer membrane interface. Helium was used as the carrier gas at a flow rate of 40 ml/min.

Sensory Evaluation after Gas Chromatography. For the odor evaluation of acids, an all-glass gas chromatographic system was used (Packard, Model 803). Eighty percent of the effluent was split to an exit port to which close fitting glass capillaries could readily be attached and held by friction. The effluent passing through the capillary could be sniffed by nose during a run, or by cooling the capillary in Dry Ice, the individual peaks or combination of peaks could be trapped and evaluated at the end of the GC run.

Identification of Acids of Sensory Significance. The acids implicated as contributing to mutton flavor after appraisal by nose were collected on a silver oxide trap and converted to methyl esters in situ with methyl iodide (Johnson and Wong, 1975). The esters were then introduced directly into the GC–MS system for chemical identification.

Synthesis of 4-Methyloctanoic and 4-Methylnonanoic Acids. 4-Methyloctanoic and 4-methylnonanoic acids were synthesized from ethyl levulinate and the appropriate Grignard reagent according to the general method described by Cason et al. (1944). Treatment of the resulting γ,γ-disubstituted butyrolactone with thionyl chloride and ethanolic HCl resulted in the branched unsaturated ester. Hydrogenation and subsequent hydrolysis yielded the required 4-methyl substituted acids. Physical properties of the acids and their synthetic intermediates are available on microfilm (see paragraph at end of paper regarding supplementary material).

RESULTS

Chemical. A typical gas chromatogram of the volatile fatty acids from mince cook-ups is shown in Figure 1. The bulk of this acid fraction, which generally amounts to well under 0.01% of the fat used, is made up of the even-numbered normal acids from C_6 to C_{12}, identifiable by gas chromatograph comparisons with authentic samples. The remaining acids vary in proportion in different preparations but collectively generally constitute not more than 10–20% of the volatile acid fraction.

The chemical identities of these acids as determined by combined gas chromatography–mass spectrometry of their methyl esters are given in Table I. GC retention data for the esters, expressed in equivalent chain length (ECL) (Ackman, 1972), are also given.

Mass spectrometry allows unequivocal structure determination of nearly all of the acids (esters) studied. In the case of the unsaturated acids, the position of the double bond (other than that in α,β-unsaturated acids) cannot be established from the mass spectrum (Bieman, 1962). That the unsaturated acids from mutton volatiles belong to three homologous series (designated a, b, and c in Table I), however, can be inferred from the plot of elution temperature vs. carbon number for these acids (Figure 2).

Sensory. The overall smell of the volatile acid fraction from all preparations was most unpleasant. When a drop of the solution was allowed to evaporate over a period of time, the smell changed in character from fecal to sweaty, sweaty-coconut-like, coconut-like, to fertilizer-like. The sweaty-coconut-like or sweaty-sour odor note was recognized by one of us (E.W.) as corresponding to that described in Chinese as "SOO".

Smelling of the effluents after gas chromatography of the acids in an all-glass system provided a range of odor characteristics essentially similar to that detected batchwise with time. Sheepy odors were found to be associated predominantly with the trace acids lying between n-C_8 and n-C_{10}. The "SOO" odor note was associated in particular with the regions centered at ECL of about 8.6 and 9.7. As with quantities, the odor qualities and intensities of the various GC regions vary somewhat in different preparations. An additive odor interaction of the trace acids, however, was indicated on collecting and smelling the combined effluents of the different GC regions. With most samples studied, a combination of all the acids with ECL from 7.6 to 9.9 gave an overall "SOO" odor reminiscent of the distasteful flavor of cooked mutton.

Chemical and Sensory Correlation. Although the acids in the volatile fraction have collectively been identified via their methyl esters (Table I), correlation with mutton odor requires that the acids in the SOO and associated smelly regions be individually identified. This was accomplished by trapping the acids from the appropriate GC regions, converting them in situ into the methyl esters, and introducing these into the GC–MS system for identification. By this means, the SOO region at ECL $\simeq$ 8.6 was found to contain 4-methyloctanoic acid (4-Me-9) overlapping with octenoic acid and that at ECL $\simeq$ 9.7 contained 4-methylnonanoic acid (4-Me-10) overlapping with nonenoic acid. Identities of other acids in the senso-

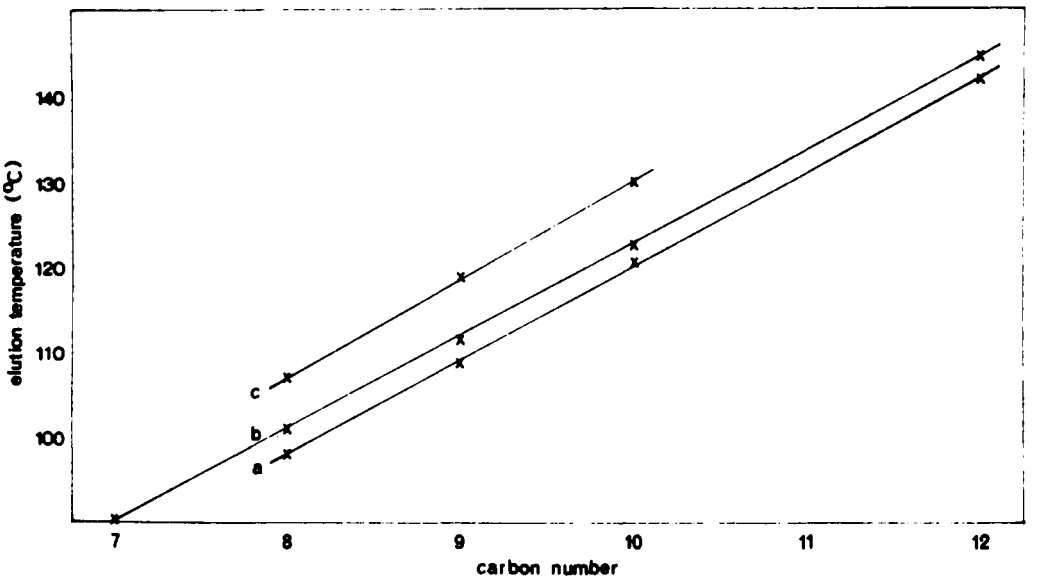

Figure 2. Retention data for unsaturated volatile acids from mutton fat. (Positions of double bond not established for series a and b. Series c identified as α,β-unsaturated acids.)

Table I. Volatile Acids from Cooked Mutton Mince

Identity of acid[a]	ECL of methyl ester EGA	ECL of methyl ester OV-101	Identity of acid	ECL of methyl ester EGA	ECL of methyl ester OV-101
Valeric (*n*-5)	5.00	5.00	Capric (*n*-10)	10.00	10.00
Caproic (*n*-6)	6.00	6.00	Benzoic	~10.2	7.64
2-Methylhexanoic (2-Me-7)		6.42	2-Nonenoic (9:1c)	10.30	9.49
4-Methylhexanoic (*ai*-7, 4-Me-7)	6.70	6.71	Decenoic (10:1a)	10.38	9.94
n-Heptanoic (*n*-7)	7.00	7.00	2-Methyldecanoic (2-Me-11)		10.39
2-Methylheptanoic (2-Me-8)		7.40	4,6-Dimethylnonanoic (4,6-Me_2-11)	10.41	10.55
6-Methylheptanoic (*i*-8, 6-Me-8)	7.55	7.63	9-Methyldecanoic (*i*-11)	10.52	10.66
			Decenoic (10:1b)	10.55	~10
Heptenoic (7:1b)	7.79	~7	8-Methyldecanoic (*ai*-11)	10.65	10.70
Caprylic (*n*-8)	8.00	8.00	Undecanoic (*n*-11)	11.00	11.00
2-Methyloctanoic (2-Me-9)		8.42	2-Decenoic (10:1c)	11.26	10.50
Octenoic (8:1a)	8.40	7.91	4,8-Dimethyl- and 6,8-dimethyldecanoic (4,8-Me_2-12 and 6,8-Me_2-12)	11.26	11.40
4-Methyloctanoic (4-Me-9)	8.52	8.58			
6-Methyloctanoic (*ai*-9, 6-Me-9)	8.66	8.69			
Octenoic (8:b)	8.67	~8	4,6-Dimethyldecanoic (4,6-Me_2-12)	11.44	11.54
n-Nonanoic (*n*-9)	9.00	9.00			
2-Octenoic (8:1c)	9.22	8.44	10-Methylundecanoic (*i*-12)	11.56	11.65
4,6-Dimethyloctanoic (4,6-Me_2-10)	9.36	9.45	Branched 4-oxooctanoic (4-oxo-8 br)	~11.6	8.52
Nonenoic (9:1a)	9.38	8.89	Branched 4-oxooctanoic (4-oxo-8 br)	~11.6	8.60
4-Methylnonanoic (4-Me-10)	9.51	9.61			
Nonenoic (9:1b)	9.53	~9	Phenylacetic	11.73	8.41
8-Methylnonanoic (*i*-10)	9.57	9.65	Lauric (*n*-12)	12.00	12.00

[a] Major mass spectral peaks of methyl esters of these acids appear in the supplementary material.

rily significant region ECL = 7.6-9.9 can be found in Table I, the ECL values of their esters on EGA being indicative of the relative retentions of the acids on the same phase.

Synthesis. The above results strongly suggested that the 4-methyl-substituted C_9 and C_{10} acids are the ones primarily responsible for the SOO odor note. This view was confirmed by synthesis. Racemic 4-methyloctanoic and 4-methylnonanoic acids were synthesized and found to correspond in GC, MS, and olfactory properties with the compounds identified from mutton fat.

Comparison of Cooking Methods. Cooking of mutton fat alone with H_2O gave a range of steam volatile acids essentially similar to that described above for the mince distillates. Distillation of the volatile acids produced by alkaline hydrolysis of mutton fat at 70° again yielded a complex mixture of trace acids, with all the different series of branched and unsaturated acids in Table I being represented. 4-Methyloctanoic and 4-methylnonanoic acids were produced by these treatments.

DISCUSSION

We believe from the results of the sensory and chemical studies that the branched chain and unsaturated acids having 8 to 10 carbon atoms contribute to the undesirable flavor of cooked mutton. The characteristic SOO odor

note is primarily associated with the 4-methyl-substituted C_9 and C_{10} acids (4-methyloctanoic and 4-methylnonanoic acids). Other components of mutton odor are present in the nonacid fractions obtained in the mince and fat cook-ups and these will form the subject of a subsequent publication.

The acids from mince cook-ups listed in Table I belong to 11 homologous series, comprising *n*, 2-Me, 4-Me, ante-iso (*ai*), iso (*i*), 4,6-dimethyl, α,β-unsaturated, unsaturated (series a and b), oxo, and aromatic acids. That the fat is the most likely source of the volatile acids produced can be inferred from the fact that the same series of acids appears to be produced on heating mutton fat with or without lean meat added. Furthermore, the production of the same branched and unsaturated medium chain acids by chemical hydrolysis alone indicates that these acids are probably normal constituents of mutton triglycerides. Partial hydrolysis would easily account for their production under the cook-up conditions used in this study.

Mutton fat is notorious for the complexity of the mixture of its fatty acid components, with many long chain branched acids and positional geometrical isomers of unsaturated acids being characteristic (Shorland, 1963; Ackman et al., 1972). The presence of medium chain odd and even normal acids and certain short branched chain acids has also previously been reported (McInnes et al., 1956). Very recent work has shown that a variety of monomethyl-substituted acids, of chain length 10–17 and with the methyl substituent in the 2, 4, 6, or 8 position, occur in the triglycerides of lamb fed on a diet rich in barley or other cereals (Garton et al., 1972; Duncan et al., 1974). Dimethyl-substituted acid has also been detected by these workers. The branched chain acids reported in the present work fall into the same structural patterns, but are novel and complementary in that they represent the medium chain members linking the short and long chain series previously reported. Medium chain unsaturated and oxygenated acids have not previously been recorded as constituents of mutton fat.

The ultimate origin of the 4-methyl-substituted and other branched medium chain acids in mutton tissues, here implicated as contributors of the peculiar mutton flavor, logically lies most likely in the characteristic metabolic processes in the rumen of the sheep animal. Garton et al. (1972) have recently suggested that branched acids with methyl substituents at even numbered carbon atoms result from the incorporation of methylmalonyl-CoA (arising from propionate metabolism) in the place of malonyl-CoA during chain lengthening. The site of this branched-chain acid synthesis is considered to be the liver. The 4-methyl-substituted acids (and 2-methyl and 4,6-dimethyl acids) found in the present work most probably represent medium chain examples of products of this same metabolic pathway.

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Supplementary Material Available. A listing of physical properties of intermediates in the synthesis of 4-methyloctanoic and 4-methylnonanoic acids and major mass spectral peaks of methyl esters of volatile acids from cooked mutton mince will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JAFC-75-495.

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