Table V. Analysis of Mixture of Known Composition of Purified Natural and Purified Synthetic Vanillins of Known δ_{PDB} ^{13}C

	δ_{PDB} ^{13}C	% composition in mixture	weighted average
natural	−20.6	33.6	−6.9
synthetic	−26.8	66.4	−17.8
		calcd	−24.7
		found	−24.6

other syntheses of vanillin are possible, the cost would not be competitive at this time.

From this data (see Table IV), it is clear that an isolated vanillin sample more negative than δ_{PDB} ^{13}C −21.0 would be considered to contain vanillin from a source other than vanilla beans. Since the mean for Madagascar vanillin is δ_{PDB} ^{13}C −20.4 (standard deviation, 0.2), this can be stated with a confidence of 99.9%. The remainder of the naturals are the same as or less negative than the Madagascar vanillin; Mexican mean δ_{PDB} ^{13}C −20.3, Javan mean δ_{PDB} ^{13}C −18.7, and Tahitian mean δ_{PDB} ^{13}C −16.8. The commercially available synthetics are found to have a mean δ_{PDB} ^{13}C −27.0. The vanillins synthesized from clove oil eugenol and guaiacol were found to be more negative than this at means of δ_{PDB} ^{13}C −30.8 and −32.7, respectively.

The application of this technique was confirmed by mixing isolated and purified crystals of natural Madagascar vanillin of known δ_{PDB} ^{13}C with synthetic vanillin of known δ_{PDB} ^{13}C and experimentally obtaining a value which can be predicted by calculation (see Table V). Thus, this ratio is an intrinsic property dependent on the vanillin composition. Furthermore, evaluation of an altered extract fell in the correct range for predicting sophistication. Vanillin has also been extracted from a liquor and the addition of synthetic vanillin confirmed, indicating the techniques' application to vanilla products.

In addition, geographic origin of the beans or extracts can be determined at the 97% confidence level, differentiating between natural Tahitian, Javan, and Mexican–Madagascar.

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Nonacidic Constituents of Volatiles from Cooked Mutton

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The nonacidic volatiles from cooking mutton have been analyzed by gas chromatography–mass spectrometry, both as ether extracts and by adsorption onto porous polymer traps. Of the 93 compounds identified (some tentatively), 56 have not previously been reported in volatiles from cooked ovine tissues and 15, including the new compound 3,6-dimethyl-1,2,4,5-tetrathiane, have not been previously identified in cooked meats.

Sheep meats find low consumer acceptance in many countries. This has been attributed to the distinctive cooking odor and to the flavor of the cooked meat (Batcher et al., 1969; Weidenhamer et al., 1969; Wasserman and Talley, 1968). Previous studies in this laboratory established the contribution of 4-methyloctanoic acid to mutton flavor (Wong et al., 1975b) and examined the novel constituents of the acidic portion of the steam distillate from cooking mutton (Wong et al., 1975a). It was also suggested that additional contributors to mutton odor are present in the nonacidic fraction of the distillate.

Recently reports have appeared concerning the basic (Buttery et al., 1977) and neutral (Caporaso et al., 1977) portions of the volatiles from cooked ovine adipose tissue. This paper reports the results of our studies of the nonacidic volatiles from cooking mutton mince.

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EXPERIMENTAL SECTION

Details of materials, combined gas chromatography–mass spectrometry, and sensory evaluation procedures have been described (Wong et al., 1975a). Gas chromatography was carried out using a Hewlett Packard 7620A gas chromatograph (all glass system). After cooking and distillation, components of extracts were separated on 2.5 m × 2 mm i.d. glass columns of 10% (w/w) stabilized polyethylene glycol adipate (EGA, Analabs Ltd.) on 100–120 mesh Gas-Chrom Q (Applied Science Laboratories) and 10% (w/w) methyl silicone OV-101 (Applied Science Laboratories) on the same support. The more volatile contents of the porous polymer traps were separated on a 2.5 m × 3.2 mm o.d. stainless steel column of Tenax (Applied Science Laboratories). In addition, an

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Table I. Volatile Nonacidic Components of Mutton Mince

compound	fraction[a]	concn[b]	identification[c]	GC retention[d]
hydrocarbons				
hexane	1, 2, 3, 4, 5	H	MS, RT, C	600
heptane	1, 2, 3, 4, 5	M	MS, RT, C	700
octane, branched	1, 2, 3, 4	S	(MS), RT, C	757
nonane	1, 2, 3, 4	M	MS, RT, C	900
decane	1, 2, 3, 4	M	MS, RT, C	1000
dodecane	1, 2	S	MS, RT	1200
tridecane	1, 2	M	MS, RT	1300
tetradecane	1, 2, 3	M	MS, RT	1400
pentadecane, branched	1, 2	S	(MS), (RT)	1490
pentadecane	1, 2	S	(MS), (RT)	1500
hexadecane, branched	1, 2	S	(MS), (RT)	1590
hexadecane	1, 2	H	MS, RT	1600
heptadecane, branched	1, 2	S	(MS), (RT)	1690
heptadecane	1, 2	H	(MS), (RT)	1700
octadecane	1, 2	S	MS, RT	1800
eicosane	1, 2	S	MS, RT	2000
benzene	1, 2, 3, 4, 5	M	MS, RT, C	650
toluene	1, 2, 3, 4, 5	M	MS, RT, C	730
xylene, 2 isomers	1, 2, 3, 4, 5	M	MS, RT, C	843, 852
ethylbenzene	1, 2, 3, 4, 5	M	(MS), (RT), C	877
trimethylbenzene, 3 isomers	1, 2, 3, 4	S	(MS), C	916, 920, 924
ethyldimethylbenzene, 3 isomers	1, 2, 3, 4	S	(MS), C	972, 984, 1000
propylbenzene	1, 2, 3, 4	S	(MS), C	1050
butylbenzene	1, 2, 3	VS	(MS), C	1100
naphthalene	2	S	MS, RT, C	1162
methylnaphthalene[e,f]	2	VS	(MS)	1271
isoprenoid $C_{16}H_{34}$[e,f]	2	M	U, S	1465
phyt-1-ene[e,f]	2	H	MS, RT, S	1791
cis-phyt-2-ene[e,f]	2	M	MS, RT, S	1835
trans-phyt-2-ene[e,f]	2	M	MS, RT, S	1847
ketones				
acetone	T	H	MS, RT	
2-butanone	T	S	MS, RT	
2-decanone	3	S	MS, RT	1172
2-undecanone	3, 4	M	MS, RT	1277
2-dodecanone	3	S	(MS), (RT)	1378
2-tridecanone	3, 4	M	MS, RT	1482
2-pentadecanone	2, 3, 4	H	(MS), (RT)	1685
2-hexadecanone	3	S	U, (RT), S	1787
2-heptadecanone	3, 4	M	(MS), (RT)	1890
biacetyl	T	M	MS	
2,3-octanedione[e]	5	M	MS, RT	923
3-hydroxyoctan-2-one[e,f]	5	VH	[MS], RT, S	1073
alcohols				
methanol[e]	T	M	MS, RT	
ethanol[e]	T	M	MS, RT	
propanol[e]	T	VS	MS	
2-propanol[e]	T	VS	MS	
butanol[e]	T	S	MS, RT	
pentanol[e]	5	M	MS, RT	770
3-methylpentanol[e]	T	S	(MS)	
hexanol	A	M	MS, RT	870
1-penten-3-ol[e]	T	S	(MS)	
1-octen-3-ol[e]	A	S	MS, RT	982
phenol[e]	A	S	MS, RT	1135
cresol[e]	A	VS	MS, RT	1200
aldehydes				
acetaldehyde	T	H	MS, RT	
2-methylpropanal[e]	T	S	(MS), (RT)	
2-methylbutanal[e]	T	M	MS, RT	
3-methylbutanal[e]	T	M	MS, RT	
hexanal	T	H	MS, RT	787
heptanal	A	M	(MS), (RT)	885
nonanal	A	M	MS	1085
2-butenal[e,f]	T	S	(MS)	
2-methylbut-2-enal	T	VS	(MS)	
2-nonenal	A	M	(MS), (RT)	1130
2-decenal	A	M	(MS), (RT)	1235
2-undecenal	A	M	(MS), (RT)	1340
2,4-hexadienal[e,f]	T	S	MS, RT	
2,4-heptadienal	A	S	(MS), (RT)	1000
2,4-octadienal[e]	A	S	U, (RT), S	1100
2,4-nonadienal[e]	A	M	MS, RT	1200
2,4-decadienal	A	S	MS, RT	1300
2,4-undecadienal[e]	A	S	(MS), (RT)	1400

Table I. (*Continued*)

compound	fraction[a]	concn[b]	identification[c]	GC retention[d]
benzaldehyde[e]	T, 2, 3, 4	S	MS, RT	944
phenylacetaldehyde[e]	2, 3	S	MS, RT	960
esters				
methyl hexanoate[e,f]	A	S	MS, RT	910
ethyl myristate[e]	5	S	MS, RT	
ethyl palmitate[e]	5	S	MS, RT	
butyl palmitate[e,f]	5	S	MS, RT	
butyl stearate[e,f]	5	S	MS, RT	
lactones				
δ-octalactone	5	S	(MS), (RT)	1270
γ-dodecalactone	5	S	(MS, Watanabe and Sato, 1971), (RT), S	1720
δ-dodecalactone	5	S	MS, RT, S	1670
4-hydroxy-*cis*-6-enoic acid lactone	5	S	(MS, Park et al., 1974)	1640
γ-tetradecalactone	5	S	U, (RT), S	1920
δ-tetradecalactone	5	S	(MS, Watanabe and Sato, 1971), (RT), S	1870
γ-hexadecalactone[e]	5	S	U, (RT), S	2120
δ-hexadecalactone	5	S	U, (RT), S	2070
furan derivatives				
furan[e,f]	T	S	MS, RT	
2-methylfuran[e]	T	S	MS, RT	
furfural[e]	5	S	MS, RT	750
furyl methyl ketone[e]	5, T	S	MS, RT	890
5-methyl-2-furaldehyde[e]	5	S	(MS)	944
amines				
ammonia[e]	T	H	(MS)	
trimethylamine[e]	T	H	MS, RT	
sulfur compounds				
hydrogen sulfide	T	VH	(MS)	
dimethyl sulfide[e]	T	S	MS, RT	
dimethyl disulfide[e]	T	S	MS, RT	
ethylene sulfide[e]	T	VS	(MS)	
ethane-1,1-dithiol[e,f]	2	S	U[g], S	810
bis(1-mercaptoethyl) sulfide[e,f]	4	S	(MS, Boelens et al., 1974)	1024
3,5-dimethyl-1,2,4-trithiolane[e]	2, 3, 4, 5	H	MS, RT	1100
thialdine[e]	4	VH	(MS, Brinkman et al., 1972)	1150
3,6-dimethyl-1,2,4,5-tetrathiane[e,f]	2, 3, 4, 5	S	MS, RT	1325, 1344
1, 2, 3, 5, 6-pentathiepane[e,f]	4, 5	VS	(MS, Morita and Kobayashi, 1966)	1616
bis(mercaptomethyl) sulfide[e,f]	2, 3	S	U, S	960
(2 or 3)-methylthiophene[e]	T	S	(MS)	
thiophene-2-carboxyaldehyde[e]	5	S	MS, RT	918

[a] Arabic numerals refer to subfractions produced by silica gel chromatography. A indicates compound only present in extract prepared by method A. T signifies compound identified in contents of porous polymer trap, on Tenax GC column. [b] Semiquantitative indication only. VH = >20%, H = 10-20%, M = 1-10%; S = 0.1-1%; VS = <0.1% of total extract. [c] MS = mass spectrum agrees with spectrum of authentic sample, and with published data (Stenhagen et al., 1974). (MS) = mass spectrum agrees with published spectrum (Stenhagen et al., 1974, unless otherwise indicated). [MS] = mass spectrum agrees with authentic sample. RT = GC retention matches that of authentic standard. (RT) = GC retention consistent with retention predicted from plot of homologous compounds. U = tentative assignment, based solely on interpretation of MS. C = also detected in extract from blank determination. S = mass spectral details listed in Supplementary Material. [d] Linear indices on OV-101. [e] Not previously observed in sheep meat volatiles. [f] Not previously observed in meat volatiles. [g] Mass spectrum and GC identical with that of product of reaction between H_2S and acetaldehyde (not isolated), cf. Boelens et al. (1974).

SF96 (Applied Science Laboratories) 150 m × 0.7 mm i.d. stainless steel open tubular column (Mon, 1971) was used for both extracts and trap contents. Temperature programming at 2 °C/min was employed for all columns over the following temperature ranges: EGA and OV-101, 50 to 220 °C; Tenax and SF96, 30 to 180 °C.

Infrared spectra were determined on a Perkin-Elmer 137 instrument as solutions in CCl_4. NMR spectra were measured on a Varian Associates T-60 instrument, also in CCl_4.

Cooking and Distillation of Mutton Mince. Ether extracts of the volatiles from cooked mutton mince were prepared by two methods.

Method A. Mince (4.5 kg) was refluxed with water (2 L) under N_2 for 3 h. The added water was then distilled off, the residue was filtered, and the fatty filtrate was steam distilled at reduced pressure and this distillate was extracted with ether as previously described (Wong et al., 1975a).

Method B. Mince (3.3 kg) consisting of adipose tissue and lean meat (1:9, w/w) with added water (330 mL) was distilled and extracted with ether (100 mL) for 4 h in a continuous steam distillation/ether extraction apparatus (Likens and Nickerson, 1964).

Sample Preparation. Acidic components were removed by extraction with 10% Na_2CO_3 (3×, total 100 mL), followed by passage through a column of anhydrous Na_2CO_3 (10 g) ground with NaCl (30 g), the nonacidic components being eluted with ether (100 mL). The extract was concentrated to 5 mL and fractionated on silica gel (Watanabe and Sato, 1971) (Mallinckrodt Silic AR CC-7; 2.5 cm i.d., 160 g). Five fractions, designated 1 to 5 (see Table I) were eluted with 150-mL portions of solvent. Fraction 1 was eluted with petroleum ether (bp 30–40 °C). Fractions 2–4 were eluted with petroleum ether/diethyl ether mixtures (fraction 2, 9:1; fraction 3, 4:1; fraction 4, 7:3). Fraction 5 was eluted with diethyl ether. Fractions were reduced to approximately 8 mL (rotary evaporator) before concentration to 0.4 mL under a stream of N_2 prior to analysis. A control extraction and fractionation was carried out in an identical manner, the only exception being the omission of the mince.

During the preparation of the distillate, it was obvious that some odorous components were escaping collection in the apparatus described by Likens and Nickerson (1964). Porous polymer traps, similar to those used by Murray (1977) for the collection and concentration of airborne volatiles, were therefore employed to collect these components. Stainless steel traps, similar to those used previously for two-dimensional GC (Wong et al., 1975a) but containing Chromosorb 105 (Applied Science Laboratories; 100 mg), were conditioned by purging with N_2 (20 mL min^{-1}) while heating to 180 °C, initially for 1 week or, before re-use, overnight (cf. Murray 1977). Traps were then attached to the top of the condensor of the Likens and Nickerson apparatus. Breakthrough of H_2S became obvious after 2–3 h, but other odors were contained. When collection was completed, PTFE caps were fitted to the traps, which were then stored at −10 °C. The contents were introduced into the GC as previously described (Wong et al., 1975a) or were flushed out for smelling by briefly heating the trap to 120 °C and then purging with N_2 (15 mL min^{-1}).

Synthesis of 3,6-Dimethyl-1,2,4,5-tetrathiane. Liquid H_2S (6 mL) and acetaldehyde (3 mL) were reacted in a sealed tube at room temperature for 3 days (Boelens et al., 1974). The crude reaction mixture, which GC–MS showed to be chiefly ethane-1,1-dithiol [*m/e* 96 (3), 95 (1), 94 (29), 61 (100), 60 (42), 59 (42), 45 (33), 35 (17), 34 (17)], was diluted with EtOH (50 mL) and treated with excess ethanolic iodine and then poured into water (250 mL) and extracted with ether (3×, 100 mL total). The ethereal solution was dried (Na_2SO_4), concentrated, and chromatographed on alumina (Woelm neutral, 60 × 2.5 cm). Elution with $CHCl_3/CCl_4$ (1:1) gave an oil (3 g) which on preparative GC (OV-101, 80–120 °C, programmed 4 °C min^{-1}) gave *3,5-dimethyl-1,2,4-trithiolane* (two isomers by analytical GC, IR, NMR, and MS in agreement with published data; Tjan et al., 1972) and *3,6-dimethyl-1,2,4,5,-tetrathiane* (homogeneous by analytical GC): IR 2940, 1450, 1370, 1200, 1170, 1125, 1065 cm^{-1}; NMR δ 1.75 (6 H, d, J = 7 Hz, 2 CH_3), 4.72 (2 H, q, J = 7 Hz, 2 CH); MS *m/e* 186 (2), 185 (1), 184 (12), 154 (0.5), 153 (0.3), 152 (5), 126 (2), 125 (1), 124 (16), 121 (0.5), 120 (0.3), 119 (5), 94 (0.5), 93 (0.8), 92 (6), 88 (4), 87 (3), 76 (3), 66 (3), 65 (1), 64 (27), 62 (2), 61 (7), 60 (58), 59 (100), 58 (19), 57 (8), 56 (2), 55 (4), 47 (1), 46 (1), 45 (28). Found M^+ 183.9509 ($C_4H_8S_4$ requires 183.9508). GC indicated that the tetrathiane was initially formed as two isomers but was converted to a single isomer during chromatography on alumina.

RESULTS AND DISCUSSION

In this study, the ethereal extract of the volatiles of mutton mince was prepared by two methods: by extraction of the steam distillate of the filtrate obtained by filtering the cooked mince (Wong et al., 1975a) (method A) or by continuous steam distillation/ether extraction of the cooking mince in a Likens and Nickerson apparatus (method B). The essential difference between these two methods was the considerable reduction in volume and consequent possible overheating during the first distillation in method A. The sensory properties of the two extracts and of corresponding fractions obtained from them were very similar, although differences were observed in their chemical composition.

The total ether extract prepared by either method possessed an odor distinctly reminiscent of its ovine origin, as judged informally by the authors. The odors of both acidic and nonacidic portions could be recognized as contributing in different ways to this overall "sheepy" odor. The odors of the fractions prepared by silica gel chromatography of the nonacidic portions were less distinctly sheepy, although the combined fractions 2 to 5 had an odor very similar to that of the total nonacid fraction. Fraction 1 had little odor. Fractions 2–5 had odors described as follows: 2, green, sulfurous-onionlike, oily; 3, green, oily, musky; 4, oily, musky, sweet (lactonelike), sharp-smoky; 5, oily, sweet (lactonelike), floral, musky.

The compounds identified are listed in Table I. Although carefully redistilled analytical grade solvents were used for all extractions, a number of compounds identified in the extract were also detected at similar levels in the control extraction and are therefore due to solvent impurities. These included benzene, toluene, several alkylbenzenes, and naphthalene. The possibility that these compounds may also be formed from the sample material is not excluded. Toluene and naphthalene have previously been reported as flavor constituents of ovine adipose tissue (Caporaso et al., 1977). Apart from these compounds, 93 components are identified. Of these, 56 have not previously been reported in the volatiles of sheep meats and 15 have not been identified in meat products. One compound, 3,6-dimethyl-1,2,4,5-tetrathiane, has not been previously reported. The identification of the three phytene isomers as major components is interesting in view of their nondetection in the earlier study (Caporaso et al., 1977).

The importance of the unsaturated lactone, 4-hydroxydodec-*cis*-6-enoic acid lactone, to the flavor of lambs fed diets supplemented with polyunsaturated lipids has been demonstrated (Park et al., 1974). It has been suggested (Hornstein and Crowe, 1963; Caporaso et al., 1977) that the carbonyl compounds are important contributors to the characteristic mutton flavor. Caporaso et al. listed 14 "key" compounds of which ten were aldehydes and three were ketones. Our work indicates that these aldehydes may be of only minor importance to this characteristic flavor since their formation depends on the method of sample formation. The extracts prepared by methods A and B had similar odors, both being distinctly sheepy. However, while the extract produced by method A contained all the ten "key" aldehydes of Caporaso et al., they were not detected in the extract produced by method B.

This conclusion is supported by their occurrence in beef volatiles (Dwivedi, 1975), again dependent on the method of sample preparation (Chang, 1976).

Ammonia, trimethylamine, and hydrogen sulfide are all odorous compounds identified as major components. As such they will be important to the overall odor. Their major role, however, will be as intermediates in the formation of other odor compounds (Boelens et al., 1974; Schutte and Koenders, 1972; Brinkman et al., 1972; Shibamoto and Russell, 1976, 1977).

Several of the sulfur-containing compounds identified in this study, in particular thialdine and 3,5-dimethyl-1,2,4-trithiolane, are well-known constituents of beef volatiles (Dwivedi, 1975). It seems probable that the remaining five compounds have not been detected in beef volatiles because of variations in experimental conditions and component concentrations. Of these five compounds, ethane-1,1-dithiol and bis(1-mercaptoethyl) sulfide have been shown to be formed in vitro from the same precursors (acetaldehyde and hydrogen sulfide) as thialdine and the trithiolane (Boelens et al., 1974), while a similar method was used in the synthesis of the new compound 3,6-dimethyl-1,2,4,5-tetrathiane. Bis(mercaptomethyl) sulfide and 1,2,3,5,6-pentathiepane could be formed by a similar mechanism. Although these compounds may be common to both beef and mutton volatiles, quantitative differences

are likely. Cooking sheep meats have been shown to evolve hydrogen sulfide in greater quantities, and at a greater rate, than beef (Kunsman and Riley, 1975). In view of the low odor thresholds of these sulfur-containing compounds (Shankaranarayana et al., 1974; Schutte, 1974), the quantitative differences in these and other, highly odorous sulfur-containing compounds may be of importance in the distinctive species odors.

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Supplementary Material Available: A table of mass spectral details of indicated compounds (2 pages). Ordering information is given on any current masthead page.

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Protease Activity of Water- and Acid-Reconstituted Grain Sorghum

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Protease activity of grain sorghum as affected by variety, reconstitution with water, and reconstitution with propionic acid was determined. Varieties having smaller kernel size had higher levels of protease activity than a similar variety with larger size kernels. Protease activity was highest on dry grain and declined during anaerobic reconstitution. Reconstitution with 2% w/w propionic acid accelerated the rate of decline of protease activity. Protease activity increased during aerobic reconstitution. The level of soluble nitrogen in propionic acid reconstituted sorghum was greater ($P < 0.05$) than water-reconstituted grain. This increase in protein solubility of propionic acid reconstituted grains indicates that either initial levels of protease are adequate or that solubilization of proteins is due to factors other than proteases in grain.

Sorghum is the fourth leading cereal grain produced in the world and serves as a major feed constitutent for livestock. Although comparable in nutrient content to other feed grains, digestibility coefficients of sorghum nutrients are generally lower (NRC, 1970). The reduced availability of sorghum nutrients may be due to the physical arrangement of nutrients within the kernel. In the peripheral endosperm of sorghum, the starch granules are embedded in a protein matrix that is poorly digested by ruminants (Sullins et al., 1971; Walker and Lichtenwalner, 1977). This structural arrangement of starch granules and protein is under genetic control. In nonwaxy or normal sorghum, the peripheral endosperm starch granules are small and tightly packed in a proteinaceous matrix. In the genetic mutant, waxy sorghum, the starch granules are larger and less tightly packed (Sullins and Rooney, 1974). This may account for the increased digestibility of waxy sorghums.

Processing of sorghum improves nutrient availability and reconstitution consumes less energy than heat processing (Lipper et al., 1976). Reconstitution is the process of adding sufficient moisture back to grain and sealing the moist grains from the environment so fermentation can occur. In some instances, propionic acid is added to the grain at time of reconstitution to deter fungal growth. Sullins et al. (1971) noted in a histological study that the protein matrix of reconstituted grains was partially disrupted. This is consistent with the earlier proposal that degradation of the protein matrix accounts for the increased feed efficiency of cattle fed reconstituted grain

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