Isolation of Precursors of Mutton Odor

Water-soluble constituents of lean mutton have been fractionated and odor significant fractions have been subjected to systematic separation by a combination of adsorption, ion exchange, and paper chromatographic steps, with sensory assessment of fractions being carried out concomitantly. A single fraction has been obtained which on heating to 180 °C produced odor notes reminiscent of cooked mutton.

Mutton has a characteristic odor and flavor which is distasteful to many people. A sweaty flavor note in the meat of sheep and goats has been correlated with the presence of 4-methyloctanoic acid (Wong et al., 1975a,b). Other volatile constituents are considered also to contribute to mutton odor and flavor, although specific sensory correlations with other chemical components have not yet been made (Buttery et al., 1977; Caporaso et al., 1977; Nixon et al., 1979). Wasserman and Spinelli (1972) have shown that precursors of the characteristic odor of heated lamb adipose tissues were present in the "lipids" extracted with chloroform–methanol, and that water-washing of the extract removed the components involved in the formation of distinctive odor into the aqueous phase. This and other studies have led to the general belief that species-specific odors are produced by the action of heat on precursors present in the adipose tissues of meat from different animal species (Dwivedi, 1975; Sink and Caporaso, 1977). Everyday experience, however, tells us that "lean" sheep meat can generally also be distinguished by its distasteful odor and flavor and it seems possible that these are produced by the action of heat on precursor substances present in situ in lean meat. This work reports the isolation of mutton odor precursors from lean sheep meat and describes the method devised for their fractionation and separation from other meat constituents.

EXPERIMENTAL SECTION

Extraction. Lean mutton without visible fat (500 g) was minced and blended with chloroform–methanol (2:1) (2 L) and the extract washed with an equal volume of water. The aqueous methanol phase was concentrated in vacuo and freeze-dried (yield of solids, 1.6%). The defatted meat residue was then further extracted with methanol–water (1:3) (1 L) to give a second batch of solid extractives (1.07%), which was later shown to be closely similar to the first lot of solids in terms of both sensory and chemical properties.

Ion-Exchange Chromatography. The above extractives were separated into crude fractions conveniently designated as bases (B), amino acids (aa), acids, and neutrals, by ion-exchange chromatography in a method modified slightly from that of Jarboe and Mabrouk (1974). Three columns containing Amberlite IRC-50 (H^+ form), Amberlite IR-120 (H^+ form), and Dowex 1-X4 (OH^- form) were used in series for adsorption, and elution was carried out separately with 2 N NH_3. The solid material from each fraction was recovered by freeze-drying (yield: B 10.7%, aa 26.1%, acids 19.3%, neutrals 17.0%).

Odor Assessment. Sensory significance for these fractions, as for other fractions obtained in all subsequent fractionation steps, was determined from the odor produced by heating an aqueous solution of the sample (ca. 0.5 mL, 20 mg/mL) in a sealed tube in a 180 °C oven for 1.5 h. Odor assessment was made informally by the authors, with the help of two other individuals also attuned to the distasteful nature of mutton odor.

Charcoal Adsorption. The aa fraction was found to be the chief source of the character-impact odor on heating. Treatment of an aqueous solution of the aa fraction (1–3%) with activated charcoal (Norit SG1) (5 mL/g of aa), effectively removed the odor precursors from solution, and these were subsequently recovered by elution with ammoniacal aqueous ethanol (concentrated NH_3–H_2O–EtOH, 1:59:40).

Chromatography on Anion-Exchange Column. The above aa-adsorbate fraction was next applied to a weak anion-exchange column, Amberlite IR-45 (OH^- form), which was then eluted successively with (1) H_2O, (2) 0.2 M ammonium acetate buffer (Hirs et al., 1952), pH 5.1, (3) H_2O again, and (4) 1 N formic acid. The eluate in each of the four fractions was recovered by freeze-drying direct from the eluant solution. Analysis revealed that the bulk of the amino acids (52%) was located in fraction 1 (neutral aa's) and fraction 2 (acidic aa's, mainly glutamic acid). Sensory assessment revealed that fraction 3 (yield 31%) contained the unpleasant odor precursors.

Paper Chromatography. Further fractionation of fraction 3 from above was carried out by means of preparative paper chromatography in 1-butanol–acetic acid–water (12:3:5) (BAW) or in H_2O. The whole chromatogram was cut up into ten strips each corresponding to an R_f zone and eluted with water, and the eluates were freeze-dried.

RESULTS AND DISCUSSION

One of the problems encountered in working with samples of precursor material was that odors tend to be formed spontaneously on keeping. Thus the crude 25% methanol solid extractive, after storage at 0 °C for 6 months, was found to have a distinct roast mutton aroma. These "preformed" odors tend to cause confusion in the assessment of sensory relevance of individual fractions at a subsequent stage of fractionation. In both of the ion-exchange chromatographic steps, the early fractions containing nonadsorbable materials were generally highly odorous, but since the odors were also detectable without the mediation of heat, they were taken to represent preformed odors from the original sample rather than odors formed de novo from precursors present in the particular fraction.

Sensory assessment revealed that the "amino acid" fraction of mutton extracts was the chief repository of the character-impact odor precursors. This fraction was found to consist chemically of a complex mixture of amino acids, most of which have previously been studied (Mabrouk and Holmes, unpublished). The situation was made simpler with the finding that the charcoal treatment step selectively concentrated the odor precursors. The bulk (75–80%) of the amino acid constituents was thus removed from the fraction of interest (the aromatic amino acids tyrosine and phenylalanine by contrast were concentrated into this fraction). Later it was shown that this aa-absorbate fraction can be more conveniently obtained by carrying the charcoal adsorption step first (yield 16% of total extractive) to be followed by ion-exchange chromatography on the IR-120 column with prior adsorption on the IRC-50

column (yield of adsorbate-aa fraction, 5.0% of total extractive).

Paper chromatography proved to be the effective final step in locating the odor precursor substance. The bulk of the material was recovered from zones 4 and 5 (representing respectively regions R_f 0.3–0.4 and R_f 0.4–0.5) in BAW and zones 6 and 8 in H_2O. In terms of sensory significance, the area of most interest was found to be zone 5 from BAW and zone 8 from H_2O. Further study revealed that the zones of interest coincided closely with two UV-absorbing components, identified from their spectral properties (UV, IR, NMR) to be the purine derivatives inosine and hypoxanthin. Relevant R_f values for these two compounds are given below:

	BAW	H_2O
inosine	0.33	0.72
hypoxanthin	0.46	0.55
tyrosine	0.45	0.76

The two-dimensional chromatographic data revealed that the precursor substance is not inosine or hypoxanthin, although in one-dimensional chromatography in each of the two solvent systems used it coincided closely with one or other of the two purines. The two-dimensional chromatographic location of the precursor substance appeared to coincide closely with that of tyrosine, found also as a component of fraction 3. Heating of authentic tyrosine, inosine, and hypoxanthin under the standard conditions, or of samples of the two purines recovered from two-dimensional chromatography, did not reproduce the unpleasant odors previously recognized (inosine gave a characteristic burnt caramel odor and deposited copious black precipitates, while hypoxanthin was not affected by heating). Due to lack of material it was not possible to establish any chemical characteristics for this precursor substance to aid in its physical detection. Sensory assessment remained the only means of monitoring its existence.

This work establishes that precursor substances contributing to the characteristic odor of mutton are present in the lean meat, and these can be isolated and fractionated by techniques applicable to the fractionation of aromatic amino acids and N-heterocyclics. The chemical nature of the precursor(s) remains to be elucidated.

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Induction of Hepatic Mixed Function Oxidases by Photomirex

Various parameters associated with the hepatic mixed function oxidase system were studied in hepatic microsomal preparations of male rats exposed to a dietary level of 50 ppm photomirex for 15 days. Hydroxylation of pentobarbital and hexobarbital was increased in excess of twofold while hydroxylation of aniline was increased approximately 1.5-fold. N-Demethylation of aminopyrine was elevated approximately threefold while that of ethylmorphine was only slightly increased. Components of the electron transport system and substrate binding to microsomal protein were also found to be significantly increased by exposure to photomirex. These results suggest that photomirex is similar to mirex and Kepone in its potential for altering hepatic function.

Components of the hepatic drug metabolizing enzyme system are readily induced by numerous environmental contaminants. Examples of this phenomenon are provided by the potent induction of the hepatic mixed function oxidases by mirex (Baker et al., 1972; Mehendale et al., 1973) and its structural analogue Kepone (Mehendale et al., 1977; 1978) in both male and female rats. Mirex has been widely employed in the southeastern United States as the insecticide of choice for control of the fire ant (Shapley, 1971). Ivie et al. (1974) demonstrated that several photodegradation products appear following exposure of this fully chlorinated, ten-carbon compound to sunlight.

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