was 4 minutes. This resulted in a sharp peak.

A 10-μl. Hamilton microsyringe was employed in the injection of the samples. Over-injection occurred owing to the "flashing-off" of a portion of the solution in the syringe needle. This laboratory, as well as Goodwin *et al.* (*3*), has found that a standardized procedure makes this increment "flashing off" reproducible, so that allowance can be made when the syringe is filled to obtain an accurate aliquot.

The gas chromatographic method was compared with the diazotization method. Forty-seven samples of grape juice obtained from grape seedlings were analyzed by both methods, and results are shown in Figure 2. The range of concentration was between 0 and 6.8 p.p.m. The data were subjected to the student's t test (*8*). The value for t ($t_{92d.f.}$ = 4.368) was larger than the critical 1% value of t. It was concluded that the observed difference of means, 0.078 p.p.m. methyl anthranilate, is not reconcilable with the zero difference one would expect if both analyses have the same bias. These analyses show that the results of the diazotization method may be expected to be high relative to those of the gas chromatographic method by an amount between 0.04 and 0.11 p.p.m.

Literature Cited

(1) Assoc. Offic. Agr. Chemists, Washington, D. C., "Methods of Analysis," 9th ed., p. 101, 1960.
(2) Clark, S. J., Abstracts, 140th Meeting ACS, Chicago, Ill., 1961.
(3) Goodwin, E. S., Goulden, R., Reynolds, J. G., *Analyst* **86,** 697 (1961).
(4) Lovelock, J. E., *Anal. Chem.* **33,** 1958 (1961).
(5) Lovelock, J. E., Lipsky, S. R., *J. Am. Chem. Soc.* **82,** 431 (1960).
(6) Mattick, L. R., Barry, D. L., Antenucci, F. M., Avens, A. W., J. AGR. FOOD CHEM. **11,** 54 (1963).
(7) Shaulis, N., Robinson, W. B., *Proc. Am. Soc. Hort. Sci.* **62,** 214 (1953).
(8) Snedecor, G. W., "Statistical Methods," 5th ed., p. 45, Iowa State College Press, Ames, Iowa, 1956.
(9) Wheelco Instrument Manual, "Model 10 Gas Chromatography Ionization Detection System," p. 29, Barber-Colman Co., Rockford, Ill., 1959.

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MEAT FLAVOR

Components of the Flavor of Lamb

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Components of lamb flavor were studied in the volatile and water-soluble fractions. In the volatile materials from roasting lamb, carbonyl compounds were found to be important contributors to aroma. These components were further investigated by precipitation as 2,4-dinitrophenylhydrazones from vapors of simmering lamb. The 2,4-DNPHs were separated by column chromatography and characterized by infrared and melting-point analysis. Monocarbonyls present were identified as *n*-alkanals of two- to 10-carbon atoms, 2-alkanones of five- to 10-carbon atoms, and possibly 2-methylcyclopentanone. Polycarbonyls are undergoing fractionation and identification. Carbonyls collected from simmering lamb were saturated compounds. Water-soluble compounds of raw and cooked lamb were separated by dialysis and ion exchange. These included glucose, fructose, and inositol, and 19 amino-containing components. Among three breeds of sheep (Southdown, Hampshire, and Columbia), no differences were evident in analyses of volatile or soluble components.

RECENT STUDIES of the chemistry of meat flavor have included those of Pippen and coworkers (*19–21*) and Spencer (*22*) on chicken flavor; of Batzer *et al.* (*2*), Hornstein *et al.* (*11*), and Yueh and Strong (*25*) on beef; and of Hornstein and Crowe (*10*) and Witting and Schweigert (*24*) on pork. An abstract of a current study by Hornstein and Crowe (*9*) indicates that another report on the chemical nature of lamb flavor will be forthcoming.

The consumption of lamb and mutton has remained at a very low level. During the last 10 years, lamb has accounted for only 2.2 to 2.6% of the total meat used annually in the United States (*1*). The objective of this study was to determine the components of lamb flavor, the knowledge of which may suggest methods of making this meat more attractive to the consumer.

The experiments reported here were a part of a larger study including sensory evaluations of lamb varying in breed, age, sex, and feeding management (*12, 23*), and cookery methods (*7, 8*).

VOLATILE COMPONENTS FROM ROASTING LAMB

Experimental

Collection and Concentration. Methods of collection, concentration, and fractionation of the volatile components of roasting lamb were developed in this laboratory. The meat analyzed was obtained from the University flock of known breed and controlled management and feeding. Most of the meat came from animals 9 months of age. The ground samples were a mixture of the lean with its marbling and 1/4 inch or less of subcutaneous fat. The ground lamb was roasted for 7 hours under 20 inches of vacuum and at 80° C., the temperature of well-done lamb. Volatile materials were swept by air through specially constructed traps, two in ice-salt and four in dry ice–alcohol freezing mixtures (Figure 1). In each run, 7 pounds of meat were roasted in four pyrex pans. Condensates of all traps as collected from three runs, totaling about 2 liters, were concentrated for each analysis.

Table I. Infrared Spectral Interpretations of Concentrates of Volatiles of Roasting Lamb

Fraction	Microns	Interpretation
1 (3 peaks)	3.36, 3.40	C—H
	5.81	C=O [acid, aldehyde, ketone(?)]
2 (5 peaks)	3.36, 3.50	C—H (saturated aliphatic)
	4.93	Small peak, found repeatedly
	5.81	C=O (aldehyde, ketone, acid, or ester)
	5.98	Unsaturated ketone, aldehyde, or acid
	6.23	Conjugated C=C
	6.78, 7.2	Aliphatic chain
	9.00	Possibly C—O

By the most effective method of concentration, condensates were saturated with salt (3 parts distillate to 1 part NaCl) and extracted with diethyl ether (1/2 volume of distillate). Distillation of the ether layer in a Vigreaux column at 40° C. yielded a yellow oily residue. The concentrate was dried with anhydrous sodium sulfate before fractionation by gas chromatography. Previously, several methods of concentrating the distillate were tried and discarded. Fractional distillation by means of a modified 40-plate Oldershaw column (*4*) was not effective for the small quantities of distillate (approximately 2100 ml.) collected from each series of three runs. The volatile compounds were probably lost by distribution throughout the relatively large apparatus. In other trials, extraction was made with isopentane, which was then allowed to evaporate rapidly from an open container at room temperature. A large portion of the volatile material was lost with the solvent. Further, adsorption of the distillates on activated carbon (NuChar C-190) proved ineffective because sequential elutions with hexane, ether, and methanol did not remove the strongly held components from the carbon. Lyophilization was found to be ineffective because highly volatile materials were lost into the traps.

Fractionation of Concentrates. Aliquots of yellow oily concentrates (75 to 100 μl.) were separated into several fractions by gas chromatography, using an instrument equipped with a thermal-conductivity detection unit. The most satisfactory column substrate was tri-*ortho*-tolyl phosphate (30% by weight on Chromosorb W), but polypropylene glycol also yielded useful separations. Conditions at time of injection were 35° to 40° C. column temperature with helium flow of 30 to 40 ml. per minute. After collection of the first fraction, the column temperature was gradually increased to a maximum of 115° C. Collection tubes (Figure 2) were designed to decrease contamination of fractions with water from frost above the dry ice-alcohol freezing bath. After collection, each tube-unit was stoppered, then centrifuged and held in the freezing bath for infrared analysis.

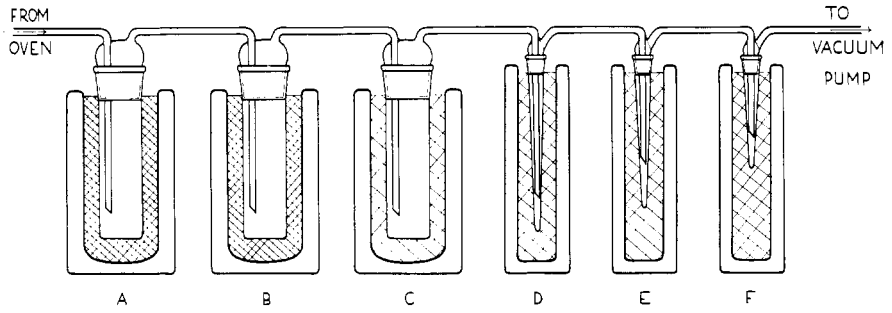

Figure 1. Traps used to collect volatile materials from roasting lamb

(*A* and *B*, ice-salt; *C*, *D*, *E*, and *F*, dry ice–alcohol)

Esterification prior to chromatography did not improve separation, indicating that free fatty acids were probably not components of concentrates.

Identification of Fractions. Infrared analyses were performed and interpreted by personnel of the Washington State University Division of Industrial Research. Spot tests (*5*) for aldehydes and ketones, hydrogen sulfide, ammonia, fatty acids, primary and secondary amines, and methyl ketones were also performed on the concentrates. Paper chromatographic tests (*3*) of concentrates for fatty acids, amino compounds, and certain carbonyls were made.

Results

Separation in the gas chromatograph of the yellow oily concentrate yielded eight component peaks in addition to those associated with the diethyl ether used as solvent. Individual volumes of these components were too small to afford successful infrared analysis, so it was decided to collect them as two fractions. Infrared analysis of these is shown in Table I.

Infrared analysis of the concentrates showed the presence of both aliphatic and conjugated carbonyl compounds. Exposing the freshly formed volatile materials to moistened filter paper gave positive indications of hydrogen sulfide (lead acetate) and ammonia (silver nitrate–manganese nitrate). Aliphatic aldehydes were shown to be present in the concentrates by positive tests with *o*-dianisidine; aromatic aldehydes were also present, according to tests with azobenzenephenylhydrazine sulfonic acid. Tests for fatty acids (formation of iron hydroxamate) and for primary and secondary amines (conversion to dithiocarbamate) were negative. Paper chromatographic tests of the concentrate were also negative for fatty acids (butanol–ammonium hydroxide) and for amino compounds (butanol–acetic acid–water).

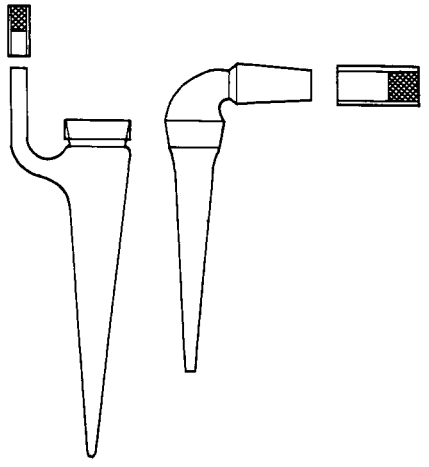

Figure 2. Tube-unit used to collect fractions from gas chromatograph

CARBONYLS FROM SIMMERING LAMB

Experimental

Collection of Carbonyls. Since infrared analysis confirmed the presence of carbonyls as major components of the volatile materials of lamb, the carbonyls were further studied by precipitating as 2,4-dinitrophenylhydrazones (DNPHs).

Ten-pound lots of ground lamb were gently simmered in 4.5 liters of water for 5 hours in a 22-liter boiling flask set into a heating mantle. The air-entrainment procedure of Pippen *et al.* (*21*) was followed, in which volatile carbonyls were precipitated as 2,4-dinitrophenylhydrazones. Air was drawn by aspiration through a preliminary trap, the simmering flask, and three precipitation flasks at a rate of five bubbles per second. Upon completion of each run, the 2,4-DNPHs were filtered, washed with water, dried at 60° C. under 5 inches of vacuum, and stored under desiccation until further analyzed. Two runs were made on lamb from each of three

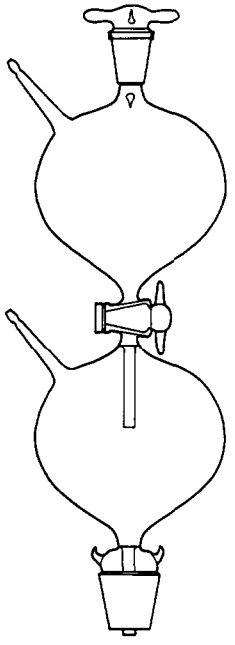

Figure 3. Pressure-feed apparatus used for column chromatography

breeds of sheep (Southdown, Hampshire, and Columbia) and on an additional sample from a mixed source.

Fractionation of 2,4-Dinitrophenylhydrazones. The DNPHs obtained from the seven lots of lamb were individually washed from the filter papers with hot ethanol and chloroform, digested, and filtered through a single No. 50 Whatman filter. It had previously been suggested (*17*) that polycarbonyls, being less soluble in hot ethanol and chloroform, could be separated from monocarbonyls by collecting on the filter in this way. Actually, however, it was discovered in later separation of the monocarbonyl fraction that a large amount of the polycarbonyls had gone through the filter with the monocarbonyls. The filtrates (monocarbonyls and some polycarbonyls) were collected in seven separate flasks and evaporated to dryness on a steam bath.

The insoluble polycarbonyl DNPHs were washed from the filter with near-boiling nitrobenzene and allowed to crystallize, then were refiltered, washed with ethanol, and dried under vacuum at 100° C. for 24 hours.

Monocarbonyl DNPHs in each of the filtrates were chromatographed on columns according to the method of Gordon *et al.* (*6*), modified by Pippen *et al.* (*21*). The adsorbent (2 silicic acid: 1 Celite) was packed to a height of 21 cm. in standard two-section, 38 × 230 mm. chromatographic tubes. Each tube was joined at the top to a double-reservoir feed-apparatus designed for use with nitrogen pressure (Figure 3). The gradient solvent system used was diethyl ether in petroleum ether, beginning with 2% diethyl ether and increasing 1% with each addition of 200 ml. of solvent to a maximum of 15%. With 5 pounds of nitrogen pressure, seven or eight preliminary bands were eluted for each sample, and two very slow bands were cut from the column itself. The solvents were evaporated in air; the fractions were recrystallized from ethanol. Comparable bands from all seven columns were combined according to elution order and melting point, but were kept separate according to breed of lamb represented by the samples. These combined fractions were recrystallized for infrared analysis.

Table II. Infrared Spectral Interpretations of DNPHs of Monocarbonyls in Volatiles from Lamb

Fractions[a] in Order of Elution	Probable Compound
1	*N*-Hexaldehyde (or straight chain aldehydes of 4- to 10-carbon atoms)
2	Methyl isopropyl ketone (or methyl ketones of 5- to 10-carbon atoms)
3	2-Methylcyclopentanone(?)
4	Propionaldehyde
5	Acetaldehyde

[a] Each compound was found in all three breeds of sheep.

Identification of DNPHs. The alcoholic-alkali test of Newberg and Strauss (*18*) was used to differentiate monocarbonyl DNPHs (red color) from those having two or more adjacent carbonyl groups (violet color).

Melting points were determined on authentic DNPHs as prepared by the method of Pippen *et al.* (*21*) and upon fractions from the various unknowns by means of a Fisher-Johns melting-point apparatus.

Paper chromatography was carried out by the procedure of Lynn *et al.* (*15*), with the exception that Whatman No. 1 strips were used.

Results

Of the eight bands eluted from each chromatographic column, five were shown by melting-point behavior and by the alcoholic-alkali test (*18*) to consist of monocarbonyl derivatives. Interpretation of infrared analysis of these five fractions (bands from all seven columns combined according to elution order and melting point but separated by breed) is shown in Table II.

Acetone was not found, nor any evidence of unsaturation. All other fractions of monocarbonyls (unseparated fractions as indicated by depressed melting points) were composited to search for any possible unsaturated linkages; none were found in the infrared spectrum.

The polycarbonyls, expected to be retained on the filter because of their reported (*17*) insolubility in hot alcohol and chloroform, had mostly gone through the No. 50 Whatman filter. In each of the column chromatographs, the last four or five fractions were shown to be polycarbonyls by the alcoholic-alkali test. These fractions plus that collected on the filter await further characterization. A method of separating the polycarbonyls is being investigated (*14*).

WATER-SOLUBLE COMPONENTS FROM RAW AND COOKED LAMB

Experimental

Extraction and Concentration. Duplicate 150-gram samples of ground raw lamb were dialyzed according to the procedure of Spencer (*22*). Each sample was blended with 140 ml. of glass-distilled water for 10 seconds and the slurry transferred to seamless cellulose dialysis tubing ($1^{7}/_{8}$ inch flat width) with 10 ml. of water for rinsing. This was immersed in a liter-size graduated cylinder containing 400 ml. of glass-distilled water. The cylinder was capped with aluminum foil and dialysis allowed to proceed for 24 hours at 33° to 34° F.

In a parallel experiment, duplicate 300-ml. aliquots of simmered lamb and broth were taken from the mixture remaining in the flask after the 5-hour simmering runs in which volatile carbonyls were collected. The slurries were dialyzed against 400 ml. of glass-distilled water for 36 hours at 33° to 34° F.

To effect concentration, dialyzates were lyophilized and reconstituted to 60 ml. for raw samples and 80 ml. for cooked meat. Concentrates were held frozen until fractionated by ion exchange.

Separation of Dialyzates by Ion Exchange. Concentrates of dialyzates as prepared above were separated by the following procedures into an ion-free portion (for analysis of sugars) and a portion free of inorganic cations (for amino acid chromatography). Chromatographic columns used were 10 × 300 mm., fitted at the top with separatory funnels as supply reservoirs. Sixty milliliters of concentrate from raw lamb were first put through a column of strong cation-exchange resin (Amberlite IR-120), and the effluent was placed on a column of weak anion exchanger (Amberlite IR-45). The resulting ion-free solution was lyophilized, and reconstituted 50:1 (grams original meat : ml. concentrate). The cation-exchange column was eluted with 0.1*N* ammonium hydroxide to remove amphoteric amino acids, the column retaining the strongly held inorganic cations. This eluate was evaporated to dryness under reduced

pressure at 35° C., then reconstituted to a 50:1 ratio with a 1:9 mixture of 4N HCL and 10% ethanol. The reconstituted samples were held frozen until analyzed by paper chromatography. With dialyzates from cooked meat and broth, only 20 ml. of each concentrate were put through the columns because of their higher concentration. In other respects, the ion-exchange procedure was the same for concentrates of dialyzates from both raw and cooked lamb.

Identification of Components of Dialyzates by Paper Chromatography. Dialyzates of raw and cooked lamb were tested for sugars and amino acids by the paper chromatographic methods of Block *et al.* (*3*) but using the solvent systems of Ma *et al.* (*16*). All chromatograms were placed on Whatman No. 1 chromatographic sheets. The chromatographic chamber used was a 12 × 12 × 24-inch cylindrical borosilicate glass jar, fitted for descending chromatography and with a plate glass cover. This jar was covered with a large insulated cardboard box to minimize temperature fluctuations and air currents.

Concentrates of ion-free fractions of dialyzates were chromatographed against standards of known sugars, using a solvent system of butanol–acetic acid–water (200:50:250, v./v.), and allowed to descend 40 hours before development by the silver nitrate method (*3*). Concentrates containing the amino acid fraction were chromatographed against known acids using a two-dimensional method. A butanol–acetic acid–water (250:50:250) solvent system was employed in descending chromatography for 30 hours. The papers were dried, formed into a cylinder, and allowed to ascend in phenol–water–ammonium hydroxide (100:20:0.1, v./v.) for 6 hours. The color was developed by dipping in 0.25% ninhydrin in acetone (*3*).

Results

Paper chromatographic tests of the reconstituted dialyzates from both raw and cooked lamb showed the presence of glucose, fructose, and inositol. There was no difference among the three breeds studied (Southdown, Hampshire, and Columbia) with respect to sugars present. Amino acid determinations showed the presence of 19 ninhydrin-positive spots in both raw and cooked lamb dialyzates. These have been tentatively identified as leucine, isoleucine, phenylalanine, valine, methionine, tryptophan, tyrosine, alanine, threonine, glutamic acid, glycine, serine, aspartic acid, taurine, arginine, lysine, histidine, and cysteine. Proline, hydroxyproline, and cystine may be present. There was no difference among breeds in apparent identity of those amino acids which were characterized.

Discussion

The uniformity of the identity of sugars and amino acids among breeds and samples suggests that these soluble components are not responsible for important differences in lamb flavor, although they doubtless contribute to the meaty flavor as suggested by Spencer (*22*).

The typical lamb flavors may be found to result from variations in the carbonyl compounds. Studies are under way in this laboratory involving the regeneration of carbonyls from their 2,4-dinitrophenylhydrazones (*13*). Organoleptic analysis may also indicate the contribution of individual carbonyl compounds to the total lamb flavor.

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Literature Cited

(1) "Agriculture Statistics," U. S. Department of Agriculture, Washington, D. C., 1960.
(2) Batzer, O. F., Santoro, A. T., Tan, M. C., Landmann, W. A., Schweigert, B. S., J. AGR. FOOD CHEM. **8,** 498 (1960).
(3) Block, R. J., Durrum, E. L., Zweig, G., "Paper Chromatography and Paper Electrophoresis," 2nd ed., pp. 23–37, 110–26, 147–54, 170–205, 215–20, Academic Press, N. Y., 1958.
(4) Dimick, K. P., Simone, M. J., *Ind. Eng. Chem.* **44,** 2487 (1952).
(5) Feigl, F., "Spot Tests in Organic Analysis," pp. 77, 91, 197, 210–18, 223, 237–40, 257–70, Elsevier Publishing Co. (Van Nostrand, New York, U.S.A. Distributors), 1956.
(6) Gordon, B. E., Wopat, F., Jr., Burnham, H. D., Jones, L. C., Jr., *Anal. Chem.* **23,** 1754 (1951).
(7) Headley, M. E., Jacobson, M., *J. Am. Dietet. Assoc.* **36,** 337 (1960).
(8) Hofstrand, J., Jacobson, M., *Food Sci.* **25,** 706 (1960).
(9) Hornstein, I., Crowe, P. F., Abstracts, p. 22a, 140th Meeting, ACS, Chicago, Ill., September, 1961.
(10) Hornstein, I., Crowe, P. F., J. AGR. FOOD CHEM. **8,** 494 (1960).
(11) Hornstein, I., Crowe, P. F., Sulzbacher, W. L., J. AGR. FOOD CHEM. **8,** 65 (1960).
(12) Jacobson, M., Weller, M., Galgan, M. W., Rupnow, E., *Wash. State Agr. Expt. Sta. Tech. Bull.* **40,** 1–22, (1962).
(13) Keeney, M., *Anal. Chem.* **29,** 1489 (1957).
(14) Lindsay, R. C., Day, E. A., Sandine, W. E., *J. Food Sci.* **27,** 210 (1962).
(15) Lynn, W. S., Jr., Steele, L. A., Staple, E., *Anal. Chem.* **28,** 132 (1956).
(16) Ma, R. M., Matlack, M. B., Hiner, R. L., *J. Food Sci.* **26,** 485 (1961).
(17) Malmberg, E. W., *J. Am. Chem. Soc.* **76,** 980 (1954).
(18) Newberg, C., Strauss, E., *Arch. Biochem.* **7,** 211 (1945).
(19) Pippen, E. L., Eyring, E. J., *Food Tech.* **9,** 53 (1957).
(20) Pippen, E. L., Nonaka, M., *Food Sci.* **25,** 764 (1960).
(21) Pippen, E. L., Nonaka, M., Jones, F. T., Stitt, F., *Ibid.*, **23,** 103 (1958).
(22) Spencer, J. V., Ph.D. thesis, Purdue University, Lafayette, Ind., 1961.
(23) Weller, M., Galgan, M. W., Jacobson, M., *J. Animal Sci.*, **21,** 927 (1962).
(24) Witting, L. A., Schweigert, B. S., *J. Am. Oil Chemists' Soc.* **35,** 413 (1958).
(25) Yueh, M. H., Strong, F. M., J. AGR. FOOD CHEM. **8,** 491 (1960).

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