# A COMPARISON OF THE VOLATILE FRACTIONS FROM CURED AND UNCURED MEAT

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## SUMMARY

Gas chromatographic examination of the volatiles of cured and uncured ham showed that hexanal and valeraldehyde were present in appreciable quantities in the uncured product but were barely detectable in the volatiles of the cured meat. The differences were less pronounced in the contents of butyraldehyde, propionaldehyde, and acetaldehyde between cured and uncured ham volatiles, though these aldehydes tended to be more prevalent in the uncured ham. The branched-chain aldehydes (isobutyraldehyde, isovaleraldehyde, 2-methylbutyraldehyde) occurred to the same extent in both meats. Acetone was found to represent a major carbonyl constituent of the volatiles in both cured and uncured ham. The sulfur-containing fractions of the volatiles from both meats were found to comprise hydrogen sulfide and methanethiol.

## INTRODUCTION

During the last few years, an ever-increasing number of publications have dealt with the nature of the flavor precursors and flavor components of various types of meat.

It is generally agreed (Batzer *et al.*, 1960, 1962; Crocker, 1948; Hornstein *et al.*, 1960; Kramlich and Pearson, 1958) that raw meat has little or no flavor, that the flavor of a particular meat develops on heating, that flavor precursors can be extracted with water from raw meat, and that the characteristic odor of a meat can be produced by heating together the isolated precursors and the fat fraction. It has also been suggested (Hornstein and Crowe, 1960) that the odor derived by heating the water-soluble precursors is the same, regardless of the type of meat from which the precursors are obtained, and that the characteristic flavor differences are due to the contributions of volatiles derived from fat.

The volatile compounds from cooked meat or meat extracts were found (Bender and Ballance, 1961; Hornstein *et al.*, 1960; Yueh and Strong, 1960; Kramlich and Pearson, 1960) to comprise carbonyl compounds, organic acids and alcohols, sulfur compounds, and ammonia. The carbonyl fraction included acetone, methyl ethyl ketone, and diacetyl as well as a variety of normal and branched-chain aldehydes; the acid portion consisted of *n*-alkanoic acids ($C_1$-$C_4$) and isobutyric acid; methanol and ethanol represented the only alcohols; the sulfur-containing fraction included hydrogen sulfide, methanethiol, ethanethiol, and dimethyl sulfide. Of all these compounds, the carbonyls and the sulfur-containing substances are believed to be the predominant contributors to meat flavor.

Published reports on the constituents of meat flavor have dealt with various types of meat such as beef, chicken, pork and lamb, but very little information has become available on the flavor components of cured meats. Ockerman *et al.* (1964) reported very recently on the gas chromatography of the volatiles derived from dry-cured hams; the spectrum of compounds described was very similar to that recorded by other investigators for the volatiles of uncured meat. Our study was undertaken to determine the effects of curing on the volatile constituents of meat flavor.

## EXPERIMENTAL

A Perkin-Elmer 154D gas chromatograph with flame ionization detector was employed. The meat volatiles were identified by comparison of their retention times with those of known compounds on two packed columns. A 14-ft. copper tube (1/4 in. OD), filled with glass microbeads coated with 1% SE-30 silicone gum, was operated at 40°C and at 24 psig, the flow of helium being maintained at 40 cc per min. The second column consisted of a 6-ft. stainless-steel tube (1/4 in. OD) filled with UCON-LB-550X on diatomaceous earth; this column was operated at 60-80°C and 14 psig, the flow of helium being 35 cc per min. A U-shaped precolumn (Mackay *et al.*, 1959) consisted of a 4-in. copper tube (1/4 in. OD) packed with 1% DC-550 silicone oil on glass microbeads. This column was operated in conjunction with the gas-sampling valve. The gas chromatographic examinations were carried out for 40 min. Exploratory experiments for periods up to 1.5 hr. or at

increased column temperatures (80°C) did not reveal significant amounts of compounds eluted after hexanal.

The cured hams used in the experiments had been artery-injected with pickle to a level of 13%, and curing was carried out for 5 days. The semimembranosus muscle was dissected, canned, and cooked in a water-bath at 75°C until the internal temperature reached 70°C. The cooking time was approximately two hours, after which time the salt content of the ham muscle averaged 2.5%. The samples of uncured ham (semimembranosus muscle) as well as the other meats were canned within one day after slaughter, then cooked immediately in the same manner. All of the cans were stored at 3°C, and were used for examination of meat volatiles within several days after cooking.

Prior to gas chromatography, the volatiles from meat were concentrated by one of two different methods. In the first procedure, samples (40 g) of meat cubes (1/2 cm) were placed in a test tube fitted with a gas inlet leading to the bottom of the tube. The meat samples, kept at 60°C on a water bath, were purged for 15-20 min with a stream of nitrogen at 30-40 cc per min. The effluent gas then passed into the precolumn, which was cooled by powdered dry ice. The second method employed small cubes of meat (100-300 g) and water (150 ml) in a 500-ml round-bottom flask. While the flask and its contents were kept at 85-90°C, a stream of nitrogen was bubbled through the mixture at 20-25 cc per min for 4-5 hr. The emerging gas stream was then directed through various trapping solutions. Basic compounds were collected in 2N hydrochloric acid solution,

volatile carbonyl compounds were trapped in 2N hydrochloric acid solution saturated with 2,4-dinitrophenylhydrazine, and the sulfur compounds were precipitated in mercuric chloride or mercuric cyanide solution.

The hydrazones, after extraction with methylene chloride, were passed through a column of neutral alumina (Woelm grade 1) to remove unreacted 2,4-dinitrophenylhydrazine. The eluate was evaporated to dryness, the residue was then hydrolyzed with 20% sulfuric acid, and the liberated carbonyl compounds were introduced directly into the gas chromatograph. A portion of the mixture of hydrazones was examined by thin-layer chromatography according to a modification of a procedure described by Dhont and De Rooy (1961); the derivatives were chromatographed on silica gel G, using methylene chloride-Skelly F (4:1) as developing solvent. The sulfur compounds, precipitated by the mercuric salts, were centrifuged, washed, and dried. The resulting black solid was examined by infrared spectrophotometry, was analyzed for carbon and hydrogen, and was treated with hydriodic acid (Batzer and Doty, 1955) in order to liberate the sulfur compounds, which were subsequently collected in the precolumn.

## RESULTS AND DISCUSSION

When the volatiles from samples (100 g) of ham were passed through a 2N hydrochloric acid solution and this solution was then evaporated to dryness in a desiccator over potassium hydroxide, 0.4 mg of white solid remained as a residue. There was no difference between cured and uncured ham in the amount of residue isolated; all these residues gave strongly positive

tests with Nessler's reagent. Because ammonia is known to occur in the volatile fraction of cooked meat and since it does not contribute significantly to meat flavor (Pippen and Eyring, 1957), this basic fraction was not examined further.

Gas chromatographic examination of the volatile carbonyl compounds, obtained by hydrolysis of their 2,4-dinitrophenylhydrazones or by direct concentration from cooked meat, indicated (Fig. 1 and 2) that certain aldehydes were present to a much greater extent in uncured ham than in cured ham. These differences were particularly evident for hexanal and valeraldehyde, which were always major constituents of uncured-ham volatiles but were absent (or present in only minute quantities) in cured-ham volatiles. The differences in content of acetaldehyde, propionaldehyde, and butyraldehyde were not as marked, but these aldehydes do occur in larger amounts in uncured-ham than in cured-ham volatiles. The results were essentially the same when cured and uncured beef or chicken were compared. It seems reasonable to assume that hexanal, valeraldehyde, and, to a lesser extent, butyraldehyde are derived by oxidative cleavage of unsaturated fatty acid residues, probably from linoleate.

Branched-chain aldehydes were found to the same extent in the volatiles from cured and uncured ham. These aldehydes comprise isobutyraldehyde, isovaleraldehyde, and 2-methylbutyraldehyde, which are believed to be respectively derived from valine, leucine, and isoleucine. The branched-chain aldehydes occur in considerably smaller quantities

than the straight-chain aldehydes. The above results indicate that curing with nitrite does not affect the conversion of these amino acids to the corresponding aldehydes. Thin-layer chromatography of the 2,4-dinitrophenylhydrazones confirmed the data obtained by gas chromatography; hexanal and valeraldehyde were shown to be present in appreciable amounts in uncured ham but barely detectable in cured ham. Although gas chromatography did not provide a separation between acetone and propionaldehyde, thin-layer chromatography of the hydrazones indicated that acetone represented the larger portion of this mixture. It was concluded that acetone is a major component in the volatiles of cured and uncured ham; although it accounts for approximately 25% and 50% of the volatile carbonyls from uncured and cured meat respectively, the total amount of 2,4-dinitrophenylhydrazones obtained from cured ham is only half that isolated from uncured ham. Thus, acetone is present in about the same quantity in both meats. We have not been able to detect methyl ethyl ketone in meat volatiles.

Two different samples (300 g) of uncured ham yielded 9.8 and 7.3 mg of 2,4-dinitrophenylhydrazones, whereas equal amounts of three samples of cured ham provided 3.4, 4.5, and 5.2 mg of carbonyl derivatives. Hydrolysis, and subsequent gas chromatography of the aldehydes and acetone, gave the following respective results, expressed in weight percent:

| | Uncured | | Cured | | |
|---|---|---|---|---|---|
| Acetaldehyde | 24.8 | 32.7 | 33.2 | 44.1 | 39.4 |
| Propionaldehyde + acetone | 16.5 | 26.2 | 58.7 | 48.3 | 54.8 |
| n-Butyraldehyde | 0.8 | 0.7 | 0.5 | 0.4 | 0.6 |
| Isovaleraldehyde | 1.5 | 1.6 | 3.4 | 3.0 | 2.7 |
| 2-Methylbutyraldehyde | 0.7 | 1.1 | 2.0 | 1.8 | 1.5 |
| n-Valeraldehyde | 5.4 | 3.4 | 0.7 | 0.6 | 0.3 |
| Hexanal | 50.4 | 34.4 | 1.6 | 1.7 | 0.7 |

The volatiles, after passage through the 2,4-dinitrophenylhydrazine solution, were found to have the characteristic cured-ham aroma, regardless of whether cured or uncured ham was the source of the volatiles. Furthermore, the volatiles of cured or uncured chicken and beef, after having been stripped of carbonyl compounds by passage through 2,4-dinitrophenylhydrazine solutions, possessed an aroma very similar to that of cured ham. The carbonyl-free volatiles were then bubbled through 1% solutions of mercuric chloride or mercuric cyanide, and the effluent gas stream was found to be practically odorless. Thus, all the volatile components, which impart the characteristic odor to cured ham, were trapped by the mercury salts. Samples (250 g) of cured and uncured ham yielded approximately the same amounts (7-14 mg) of black precipitates, which were found to contain 0.4-0.6% carbon and 0.2-0.3% hydrogen. These analyses demonstrated that the precipitates contained only minute amounts of organic matter, and the bulk of the material

is considered to be mercuric sulfide. Treatment of the black mercury compounds with hydriodic acid, concentration of the resulting volatiles in the precolumn, and subsequent gas chromatography revealed only dimethyl disulfide. This compound presumably arose from methanethiol by oxidation with iodine during the hydriodic acid treatment.

These experiments show that the main difference between cured-ham and uncured-ham volatiles is the presence of large amounts of valeraldehyde and hexanal in the uncured-ham volatiles. The volatiles from cured and uncured ham contain appreciable quantities of shorter-chain carbonyls ($C_1$-$C_3$) but these do not seem to contribute significantly to the characteristic aroma of cured ham. Curing with nitrite does not seem to contribute any volatile compounds (other than nitrogen oxides) that are not present in cooked uncured meat. We consider that cured-ham flavor represents the basic meat flavor derived from precursors other than triglycerides, and that the different aromas of the various types of cooked meat depend on the spectra of carbonyl compounds derived by oxidation of fat. It has been reported (Tappel, 1961) that uncured-bacon extracts catalyzed the oxidation of methyl linoleate and that the rate of oxidation was proportional to the amount of extract used; hematin compounds such as hemin, hemoglobin, and cytochrome were found to exert a similar action, which could be inhibited by cyanide ions.

When a sample of uncured ham was injected with sodium

cyanide solution, the higher straight-chain aldehydes were completely absent in the volatiles of the cooked product. It is concluded that nitrite as well as cyanide interferes with the oxidation of unsaturated lipids, possibly by deactivating hematin catalysts.

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## RÉSUMÉ

L'étude par chromatographie en phase gazeuse des fractions volatiles du jambon saumuré et non-saumuré, a montré des teneurs appréciables en hexanal et en valéraldéhyde dans le produit non-saumuré, alors que ces aldéhydes sont tout juste détectables dans la viande saumuree. Les écarts entre les contenus en butyraldéhyde, propionaldéhyde et acétaldéhyde des fractions volatiles du jambon saumuré et non-saumuré, furent moins prononcés bien que ces aldéhydes eurent tendance à prédominer dans le jambon non-saumuré. Des quantités égales d'aldéhydes à branches ramifiées (isobutyraldéhyde, isovaléraldéhyde, 2-méthyle-butyraldéhyde) se présentèrent dans les deux viandes. L'acétone fut trouvé un constituant majeur des groupes carbonyles des fractions volatiles du jambon saumuré et non-saumuré. Les composés sulfurés des fractions volatiles des deux viandes comprenaient du sulfure d'hydrogène et du méthyle mercaptan.

Fig. 1. Gas chromatogram of volatile carbonyl compounds isolated from uncured ham.

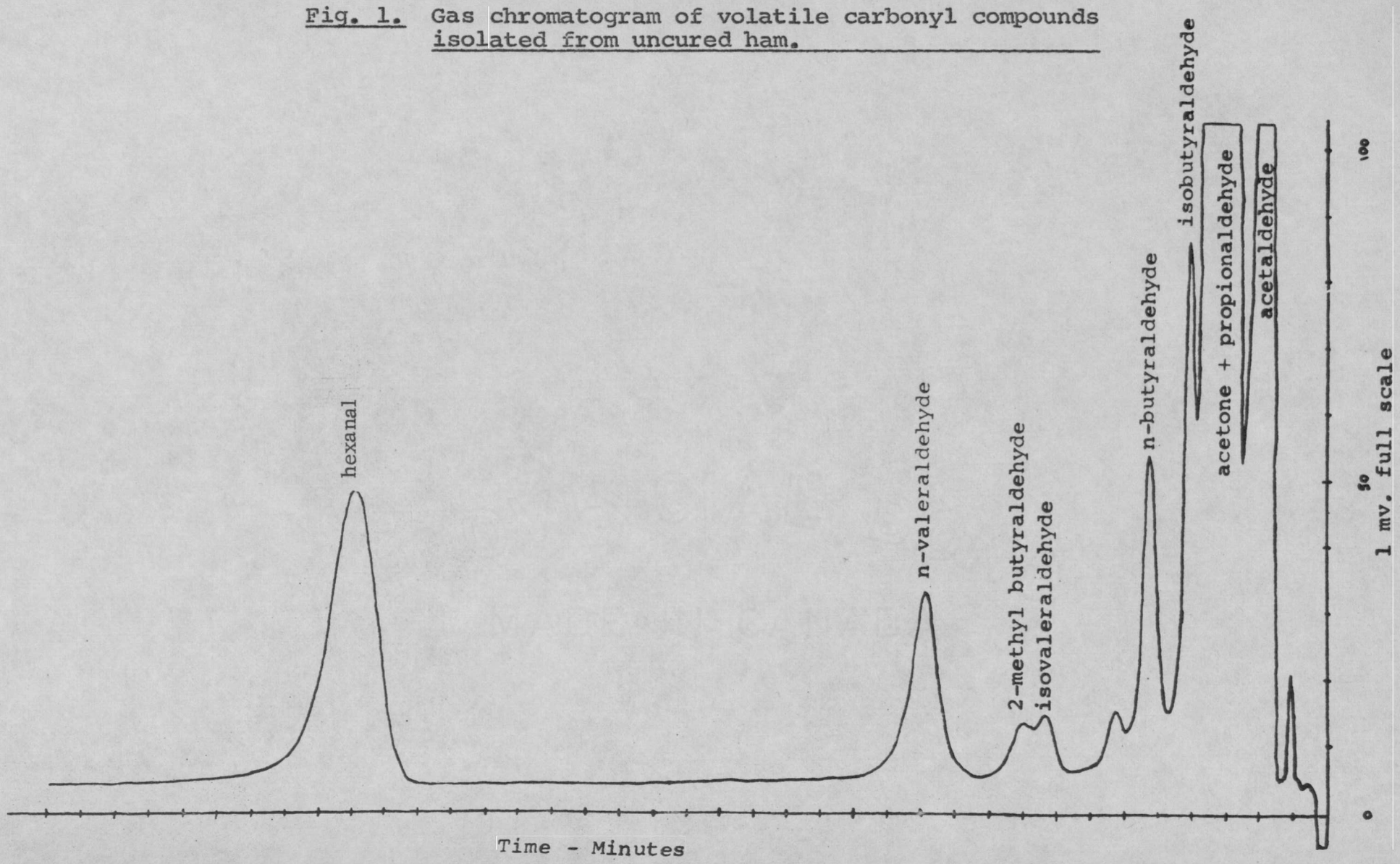

Fig. 2. Gas chromatogram of volatile carbonyl compounds isolated from cured ham.