TECHNICAL REPORT
FD-32

STUDIES OF BEEF IRRADIATION FLAVOR USING A CONCURRENT RADIATION-DISTILLATION TECHNIQUE

by

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Cambridge, Massachusetts

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December 1965

U. S. Army Materiel Command
U. S. ARMY NATICK LABORATORIES
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FOREWORD

Preservation of meat foods by ionizing radiation successfully sterilizes the products and prevents microbial spoilage during prolonged storage. An unwanted result of this treatment is the production of a characteristic off-flavor which reduces the acceptance of the preserved meat. Identification of the components of this off-flavor is necessary for an understanding of the mechanism of their production and for the development of techniques to mitigate the unwanted off-flavor.

The work covered in this report, performed by the Division of Sponsored Research, Massachusetts Institute of Technology under Contract No. DA 19-129-AMC-87(N), represents the concluding phases of an intensive study of the contribution of volatile components to the irradiation off-odor in irradiated, enzyme inactivated beef. The investigator was Professor E.L. Wick.

The U. S. Army Natick Laboratories Project Officer was A.S. Henick and the Alternate Project Officer was J.G. Kapsalis, both of Food Chemistry Branch, Food Division.

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ABSTRACT

Progress in the identification of beef components indispensable to the production of irradiation off-flavor is described. Evidence is presented that methional, n-nonanal, and phenylacetaldehyde are the major indispensable components.

INTRODUCTION

The goal of this research was to establish the contribution to irradiation off-odor of the volatile components of irradiated enzyme-inactivated beef. This was accomplished by 1) estimation of the quantitative composition of irradiated and non-irradiated beef odor concentrates, 2) selection of those constituents which are major contributors to off-odor, 3) recombination of these constituents in enzyme-inactivated non-irradiated beef slurries, and 4) sensory comparison of the resulting synthetic "irradiated samples" with freshly irradiated beef slurries.

Concurrently with the above activities work was continued on the identification of the remaining unknown volatile constituents.

EXPERIMENTAL PROCEDURES AND RESULTS

Enzyme inactivation and irradiation. Approximately 15-lb batches of ground raw lean beef were vacuum packed in sardine cans and enzyme-inactivated (Chiambalero et al., 1959) by bringing the beef to an internal temperature of 175°F for 9 min.

Irradiation of canned enzyme-inactivated beef at 5 megarad was either carried out in the MIT cobalt-60 source or concurrent radiation-distillation at 5 megarad was carried out by procedures previously described (Wick et al., 1961).

Preparation and investigation of beef odor distillates.

Aqueous slurries (8 liters) of very finely ground lean beef (1 part beef to 3 parts distilled water) were circulated through an irradiation chamber beneath the electron tube of a 1 Mev GE Resonant Transformer, to a flash evaporator for the removal of volatile components, and back through the irradiation chamber. In this manner slurries which contained from 10 to 15 lb (4.5 to 6.8 kg) of beef were irradiated at 5 megarad doses, and at almost the same time volatile components were distilled from the slurry at pressures of about 25 mm Hg. The average temperature of the circulating mixture was 32-36°C, and the rate of distillation was approximately 6 liters per hour. When radiation was carried out prior to distillation, cans of enzyme-inactivated beef were subjected to 5 megarad in the MIT cobalt-60 source. They were then opened either immediately or after six months storage at ambient temperature , the beef was slurried, and the distillation carried out as previously described (Wick et al., 1963 and 1965). Non-irradiated beef slurries were processed in exactly the same manner as were periodic "blank" distillations of distilled water to allow detection of contaminants or artifacts contributed by the distillation apparatus.

The oxidizable carbon content of aliquots of the total

aqueous distillates (approximately 8 liters) was determined (Gertner et al., 1954). The distillates were saturated with sodium chloride and extracted with diethyl ether (2.9 volumes of distillate to 1 volume of ether). The ether extracts were dried over anhydrous sodium sulfate and then concentrated by careful distillation to the minimum practical volume (about 1 ml). The yields of the resulting odor concentrates on an ether-free basis were estimated gas chromatographically. These yields and the oxidizable carbon content of the aqueous distillates from which they were derived are summarized in Table I.

Informal odor evaluation of the odor concentrates and distillates from freshly irradiated beef confirmed that they exhibited typical irradiation off-odor. The same fractions from six-month stored irradiated beef were bland in odor. Analogous non-irradiated beef fractions had typical bland meatlike odors.

The odor concentrates were stored at 0°C in the dark and in ether solution for periods of 3 to 4 weeks without deterioration (as judged by gas chromatographic analysis under standard conditions (see below)).

Gas chromatographic separation of odor concentrates and examination of the resulting fractions. Separation of odor

concentrates was carried out on two instruments. One apparatus was constructed in this laboratory. The detector was a thermal conductivity cell employing a matched pair of 100,000 ohm thermistors (type A-177, Victory Engineering Corp., Union, N. J.). It was operated at 12 ma. bridge current and maintained at 202°C. The chromatograms shown in Figures 1 and 2 were obtained with this instrument.

The other apparatus was an F & M Model 1609 Gas Chromatograph fitted with a flame ionization detector and fraction delivery line. Use of this instrument made possible detection of minor components not previously found as well as collection of individual fractions. Chromatograms thus obtained showed the presence of a total of 50 to 60 components instead of the 25 to 35 detected with the less sensitive instrument. The additional compounds were eluted after the peaks shown in Figures 1 and 2.

Preparative separations of 40- or 50- µl samples were obtained on a 2-meter, 4-mm, i.d., stainless steel column packed with 20% Carbowax 20 M on Chromosorb P (60-65 mesh). The column was programmed from approximately 70 to 196°C at rates of 1 degree or 2.4 degrees per min.

Fractions were trapped at the detector outlet in glass U-tubes chilled in liquid nitrogen. The tubes were sealed and stored in a freezer until further investigations could be made.

Infrared spectra of individual fractions were determined by means of a Beckman IR-5 spectrophotometer equipped with a 5X KBr lens-type beam condenser. Fractions were transferred to a type D sodium chloride cavity cell (Connecticut Instrument Co., Wilton, Conn.) of nominal path length 0.05 mm. When quantities permitted, pure liquid as well as carbon tetrachloride solution spectra were obtained. Infrared spectra of selected reference compounds were obtained from samples which had been purified by chromatography. Based on the identity of infrared spectra and retention data with those of authentic reference compounds, the identifications listed in Tables II, III, & IV were made.

To obtain information about minor components not detectable in the infrared spectra and about components which remained unknown in May 1964 (peaks 10,11,13,15,23,25, and 27 in Figure 1b and peaks 2,3,22, and 23 in Figure 2b) mass spectra were obtained as components of beef odor concentrates were eluted from a 10 foot, 1/8 inch 5% Carbowax 20M column programmed from 20 to 160°C at 1°/minute. This work was made possible through the kindness of Dr. Charles Merritt, Analytical Laboratory, U. S. Army Natick Laboratories. Professor Phillip Issenberg of the M.I.T. Department of Nutrition & Food Science carried out the mass spectrometric analyses on

a modified Model 14 Bendix Time-of-Flight mass spectrometer. Electron energy was set at 70 ev and spectra were scanned from m/e 14 to 200 in 6 seconds. Mass spectra of the following authentic reference compounds were obtained in the same manner: n-alcohols (C_2 - C_9), iso-alcohols (C_3-C_5), acetoin, diacetyl, methional, benzaldehyde, phenylacetaldehyde, C_3-C_9, C_{11} 2-ketones, and C_4, C_7-C_{11} n-aldehydes.

Interpretation of spectra of irradiated beef components and comparison with known spectra resulted in confirmation of the presence of all substances listed in Tables II, III, and IV, and, in addition, showed the presence of n-tetradecane, 1-tetradecene, n-pentadecane, 1-pentadecene, n-hexadecane, 1-hexadecene, methional and phenylacetaldehyde among fractions shown in Figure 2b in the Traps isolate. Because of sensory evidence (described below) that no significant contribution to irradiation odor was made by substances eluted after those shown in Figures 1 and 2 or by the several fractions still remaining unknown, further mass spectral analyses were not carried out.

Estimation of Yields and the Quantitative Composition of Odor Concentrates.

All preparations of odor concentrates from both irradiated and non-irradiated beef, carried out since the beginning of the research project have been reviewed. All those which

could be compared on a fair analytical basis have been evaluated and their results summarized in this report. A summary of the yields of odor concentrates obtained from non-irradiated beef, from beef subjected to concurrent radiation-distillation, to irradiation prior to distillation, and to distillation after 6 months storage at ambient temperature, is presented in Table I. Review of these data shows that the quantity of beef processed in any single batch varied from 5 to about 14 kg (11-31 lb.). Odor concentrates from non-irradiated beef were isolated in yields ranging from 25.1 to 36.4 ppm. Radiation prior to distillation gave odor concentrates in quantities averaging 36.2 $\pm$ 12.8 ppm. Odor concentrates from concurrent radiation-distillation averaged 43.3 $\pm$ 7.8 ppm in yield. In all cases concentrates from irradiated beef strongly exhibited typical irradiation off-odor while concentrates from non-irradiated beef had normal beef odor. A significantly smaller yield (9.7 $\pm$ 0.2 ppm) of odor concentrate was obtained from irradiated beef that had been stored 6 months at ambient temperature.

Variations in the yields isolated are believed to result from experimental difficulties inherent in measuring, on the one hand, kilograms and liters of materials and, on the other hand, very small volumes of volatile ether solutions.

The quantitative composition of odor concentrates has been estimated from chromatograms analogous to and including those shown in Figures 1 and 2. An assumption was made that all important components had been eluted from the column. This was justified by the fact that when all the fractions shown on irradiated beef chromatograms were collected in a single trap, the trap exhibited irradiation off-odor. Based on this same observation it was concluded that the 25 components eluted after the last fractions shown in Figures 1 and 2, and only detected on chromatograms obtained with a flame ionization detector, did not contribute significantly to irradiation off-odor. Thus only components in chromatograms like those in Figures 1 & 2 determined by thermal conductivity detection were investigated intensively.

Based on the method of internal normalization of peak areas, the percentage composition (less the diethyl ether solvent shown as the initial very large component in the Figures) of odor concentrates is given in Tables II and III. These tables contain the average composition (with standard deviations) of samples of odor isolates from individual preparations as well as from different preparations of the same type, i.e., concurrent or non-concurrent procedures. All preparations carried out since the research began, and which can be compared on a fair analytical basis (same gas chromatographic

detector, column and conditions) are included. Tables II and III supercede and replace all previous tables since they include all available data rather than only a portion. Both the complete and incomplete data, however, point to the same conclusions.

Table II shows the average composition of non-irradiated and irradiated distillate odor concentrates (similar to those shown in Figure 1). The non-irradiated concentrate is noteworthy in that 40.14 ± 2.11% is accounted for by acetoin and n-octanal. n-Hexanal (13.30 ± 0.61% is the next largest component followed by benzene (9.70 ± 0.55%), n-pentanol (7.68 ± 0. 10%) and n-heptanol (7.02 ± 0.14%). The remaining approximately 22% is distributed among 18 components.

Comparison of the composition of irradiated distillate concentrates obtained by non-concurrent and concurrent procedures is of interest. Very striking quantitative differences in composition exist in spite of the fact that both exhibited typical irradiation odor. For example, concurrent radiation-distillation resulted in methional being the largest component (29.89 ± 0.33%). n-Nonanal was next largest (11.81 ± 0.02%) and a mixture of n-octanal and acetoin was next with 10.84 ± 1.43%. The non-concurrent procedure gave a concentrate in which acetoin and n-octanal accounted for the greatest proportion (27.25 ± 3.85%). It differed primarily from a

non-irradiated concentrate in the large amount (23.79 ± 2.94%) of methional and the relatively decreased proportion of n-hexanal (1.94 ± 0.57%), n-octanal and acetoin present. A relatively smaller total percent of n-alkanals (24.50%) was present in non-concurrent concentrates--in contrast to the amount (36.05%) in concurrent radiation-distillation produced isolates.

Review of Table III containing the average composition of non-irradiated and irradiated odor concentrates derived from the dry ice-ethanol cooled traps (separated in the manner shown in Figure 2) points out additional gross compositional differences. A large proportion (51.84%) of the non-irradiated isolate is accounted for by n-hexanal (32.42 ± 0.93%) and 2-butanol (19.42 ± 1.96%). The isolate from concurrent radiation-distillation contains roughly 76% n-alkanals and 6% hydrocarbons. In sharp contrast the non-concurrently irradiated isolate contains only about 21% alkanals and 48% hydrocarbons. Both isolates contain methional.

A rough estimation of the composition of total volatile organic substances (Traps plus Distillate) in odor concentrates from the various meat preparations was obtained by combining the data already presented in Tables II and III. Results of the summation of these data are given in Table IV. They represent the best available estimation of the composition of the total volatile odor concentrates from each processing method studied.

The non-irradiated isolates contained about equal amounts of n-hexanal (22.86%) and the mixture of n-octanal and acetoin (22.90%). Alcohols accounted for 32.25%. The irradiated isolates were similar in that they contained about the same proportion of methional, but differed greatly in overall composition. The concurrently irradiated and distilled concentrates contained 55.24% aldehydes, 16.58% alcohols, and 3.67% hydrocarbons. The non-concurrent isolates, on the other hand, contained 23.36% aldehydes, 18.30% alcohols, and 24.04% hydrocarbons.

Sensory Estimation Of Components Indispensable To Irradiation Off-Odor

Selection of indispensable components was aided by the observation that when the substances listed within brackets in Table IV (representing all fractions eluted after n-heptanal) were collected together in a single trap, the trap exhibited irradiation off-odor. This was particularly true when samples of "Distillate" isolate were separated, though weak off-odor was recognized when "Traps" isolates were examined. An arbitrary decision was thus made to consider as potential indispensable components only those compounds present in irradiated "Distillates" eluted after n-heptanal. This automatically eliminated hydrocarbons from consideration, though they were recognized as products of the irradiation process. Substances remaining for consideration

are shown in Table V in percentages found in the various beef isolates and in proportions calculated relative to phenylacetaldehyde. Validity of the relative quantities was checked by rough determination of gas chromatographic detector response to the compounds shown in Table VI. No differences great enough to greatly affect the composition of the odor concentrates, or of any synthetic mixtures, in any practical way, were noted.

Synthetic mixtures of irradiated beef components, based on the relative quantities given in Table V, were prepared by adding appropriate volumes of dilute aqueous ethanolic (5%) stock solutions of each compound to 100 g of enzyme-inactivated ground beef slurried in 75 ml water. Stock solutions containing 238 ppm were prepared by dissolving the gas chromatographically purified compound (13 mg.) in ethanol (3.7 ml) and adding 51.7 ml of distilled water. They were kept in dropping bottles so that synthetic mixtures could easily be made based on the relative quantities shown in Table V. This method for preparing "synthetic" irradiated beef slurries was suggested by the director of flavor development of an industrial concern who indicated that in dilute solution reactions such as acetal formation or instability of individual compounds did not generally occur.

The odor of the resulting "synthetic" irradiated beef slurries was compared with an equal quantity of freshly irradiated and slurried beef. Comparisons were made informally by persons experienced in sensory evaluation and familiar with irradiation off-odor. It became obvious almost immediately that some of the compounds in Table V made little or no contribution to irradiation odor, while others affected it very greatly. Trial and error mixing of compounds and concurrent odor evaluation led to the opinion that when added to a slurry of 100 g of enzyme-inactivated beef in 75 ml distilled water, the following mixture caused very close approximation of irradiation off-odor.

		Relative Amount
Methional	3.0 ppm	20
n-Nonanal	.30 ppm	2
Phenylacetaldehyde	.15 ppm	1

Since the enzyme-inactivated beef used as substrate contributes volatile components of non-irradiated beef (see Table IV), the three substances added are not completely responsible for the resulting odor. They are, however, believed to be the most important contributors, since even when added to water a good representation of irradiation odor is obtained. The quantities necessary in aqueous solution were

		Relative Amount
5.0 ppm	Methional	20
.5 ppm	n-Nonanal	2
.25 ppm	Phenylacetaldehyde	1

In both beef slurries and aqueous solutions the relative proportion of added components was methional (20): n-nonanal (2): phenylacetaldehyde (1). Reference back to experimentally determined relative quantities shown in Table V indicates that the non-concurrently processed isolate resembled the synthetic mixtures, i.e., methional (14.8): n-nonanal (2.8): phenylacetaldehyde(1). The concurrent isolate was very different: methional (44.7): n-nonanal (72.9): phenylacetaldehyde(1) .

Statistical evaluation of the degree to which the "synthetic" sample (prepared by addition of the above three compounds to non-irradiated enzyme-inactivated beef) differed from enzyme-inactivated irradiated beef, was determined based on a multiple comparison procedure using a reference sample (Maloney et al., 1957). Using the score sheet given in Figure 3 panel members evaluated the odor quality of a standard sample (S) and two coded samples (one of which was identical with the standard) and indicated the degree of difference noted. Two sets of samples were tested in a single session. One had irradiated beef slurry as the standard sample (Set I, Table VIIa). The other had a "synthetic" irradiated slurry as standard (Set II, Table VIIb). Panelists were allowed to take as long as they wished and to re-evaluate samples if they so desired. Samples (10 g) were presented in glass-stoppered bottles (1 oz) covered with aluminum foil so that appearance did not affect the evaluations.

A total of 44 persons ranging in age from 17 to 43, all students or staff in the Department of Nutrition & Food Science, initially participated in the sensory evaluations. As months passed the number dwindled to a group of 17 experienced persons who could be relied upon to make daily evaluations.

Results of evaluations of "synthetic" vs. irradiated samples

are given in Tables VIIa & VIIb. Review of these data show that even though sample S' in each set was the same as the standard reference sample, panel members thought it differed to a slight degree in odor quality since the means of all scores for S' were 1.61 (Set I) and 1.74 (Set II). Observations of the two samples in each set were therefore paired and the differences between means for each judge were determined. The hypothesis that no difference existed within each pair, i.e., that there was no difference between samples, was then evaluated using a t-test for correlated pairs. As shown in Tables VIIa & VIIb this hypothesis had to be rejected for both sets. A significant difference did exist in both cases. However, the degree of these differences ($\bar{d}$=1.51 in Set I; $\bar{d}$=1.97 in Set II) was described by the panel to be less than slight. It was therefore concluded that major responsibility for the production of irradiation odor in enzyme-inactivated beef slurries could be assigned to the presence of methional, n-nonanal, and phenylacetaldehyde.

Statistical evaluation of the threshold of significant effect of each of these three compounds in enzyme-inactivated beef slurries was determined in a similar manner. A score sheet like the one shown in Figure 3 but providing for evaluation of three rather than two samples was used. Panel members evaluated

the odor quality of a standard sample(s) and three coded samples, one of which was identical with the standard. The other two samples contained the test compound at increasing concentration levels.

Results of evaluations are given in Tables VIII, IX, and X. A threshold of significant effect was considered to have been determined when a significant difference in odor quality from that of enzyme-inactivated non-irradiated beef slurry was noted. As before, a t-test for correlated pairs was used. Based on the evidence shown in Tables VIII, IX, & X the following quantities of methional, n-nonanal, and phenylacetaldehyde were required.

Threshold of Significant Effect of Individual Compounds

Methional	6 ppm
Phenylacetaldehyde	0.94 ppm
n-Nonanal	greater than 7.6 ppm

Higher concentrations of n-nonanal were not tried since 7.6 ppm was already about twenty-five times greater than the amount needed in a mixture with methional and phenylacetaldehyde to cause irradiation off-odor to be recognized.

Discussion

Investigation of components of irradiated odor concentrates by mass spectrometric analyses resulted in identification in the Traps isolate, of methional, phenylacetaldehyde, n-tetradecane,

1-tetradecene, n-pentadecane, 1-pentadecene, n-hexadecane, and 1-hexadecene. The presence of methional and phenylacetaldehyde in small quantities had been suspected because the Traps isolate exhibited irradiation off-odor though with weaker intensity than the Distillate isolate. The presence of C_{14}-C_{16} hydrocarbons was also expected since they had been previously found by Merritt (1965).

Mass spectral evidence for the presence of additional minor unknown components was obtained. However, identification of these substances or of components of odor concentrates obtainable in very small quantity (such as from stored irradiated beef) will require the development of columns having greatly improved resolving power, and will depend upon exclusive use of mass spectrometric analyses. Isolation of components in amounts large enough for infrared analyses cannot be expected unless distillation procedures are greatly scaled-up.

Meaningful consideration of odor concentrate yield and composition data presented in Tables I, II, and III requires an understanding of the following factors. The yields given in Table I represent the total organic material isolatable by ether extraction of two aqueous condensates, i.e., material from dry ice-ethanol cooled "Traps" (Figure 2) and from an ice-water cooled carboy called "Distillate" (Figure 1). These isolates

differ widely in qualitative as well as quantitative composition although many compounds are common to both. The relative amount isolated as "Traps" or "Distillate" depends upon conditions existing during distillation, i.e., the vacuum in the apparatus and efficiency of trapping. Thus yields have been reported as the total of both. They represent the amount isolated and not necessarily the amount present in beef.

The isolation of two odor concentrates, i.e., "Traps" and "Distillate", from each meat preparation was advantageous for the separation and identification of volatile components, since each was a relatively less complex mixture than a combination of the two would be. The "Traps" contained the relatively more volatile substances and the "Distillate" the less volatile components. Review of Tables II and III shows that the composition of these isolates <u>within</u> a given type of preparation (concurrent vs. non-concurrent, etc.) was remarkably reproducible considering the many opportunities for loss during each investigation Some of these opportunities resulted from 1) the limited amounts of isolate available which required both qualitative and quantitative information to be obtained from each sample, 2) the fact that when fractions were trapped for qualitative analyses, several days might elapse between separations, 3) the need to protect isolates from decomposition by storage in dilute ether

solution and then to remove the excess ether before analysis yielding a concentrate of possibly different composition.

In view of the above considerations and the results given in Tables II and III it was concluded that within a given method of meat preparation (non-irradiated, concurrent or non-concurrent irradiation) the composition of odor concentrates was reasonably constant. The composition of isolates from different processing methods, however, was strikingly different in spite of the fact that both types of irradiated isolates clearly exhibited typical irradiation odor. The possibility that this major difference resulted because the gas chromatographic detector used had not been calibrated for its response to different classes of compounds was dismissed for the following reason. The chromatograms on which compositions were based were obtained on the same column, the same thermal conductivity detector, and under as similar conditions as possible (though perhaps days apart). Thus there was no reason why they could not be compared even though in the absolute sense the percentages of individual compounds might be incorrect due to differences in the detector response to different compounds. Detector calibration would not change the fact that great compositional differences existed for isolates which exhibited typical irradiation odor.

In view of the fact that irradiation odor was exhibited by mixtures which varied widely in the relative proportions of methional, n-nonanal, and phenylacetaldehyde, it was somewhat puzzling to determine that irradiation odor production in enzyme-inactivated non-irradiated slurries depended upon the addition of these compounds in 20:2:1 amounts, respectively. Although no attempt was made to determine whether these proportions could be varied within certain limits, the general impression was that variations probably would not result in irradiation odor. It is therefore suspected that factors such as masking effects by other components must be operative in the concurrently processed isolates to allow them to exhibit irradiation odor.

There is no doubt that widely differing sensory effects result from the presence alone and in a mixture of methional, n-nonanal, and phenylacetaldehyde in meat slurries. This was illustrated by the finding that much greater amounts of these compounds were needed to cause a sensory difference when added individually to slurry than when present in a mixture.

	Individual Threshold	Quantity for Irradiation Odor
Methional	6.12 ppm	3 ppm
Phenylacetaldehyde	0.94 ppm	0.15 ppm
n-Nonanal	>7.6 ppm	0.30 ppm

The significance of sub-threshold concentration as important contributors to aromas has been noted and discussed by others (Guadagni et al., 1963; Nawar and Fagerson, 1962; Day et al., 1961). Although it is clear that much more must be learned before the relationship between chemical structure, concentration and sensory effect can be understood, it is believed that the major contributors to irradiation off-odor have been determined.

REFERENCES

Chiambalero, C. J., D.A. Johnson, and M.P. Drake. 1959. A time-temperature relationship for heat-enzyme inactivation of radiation-sterilized beef and pork. J. Agr. Food Chem. 7: 782

Day, E.A. and D.A. Lillard. 1961. Autoxidation of milk lipids. II. The Relationship of Sensory to Chemical Methods for Measuring the Oxidized Flavor of Milk Fats. J. Dairy Sci. 44: 623

Gertner, A., and H. Ivecovic. 1954. Determination of total organic carbon in aqueous solution. Z. Anal. Chem. 142: 36

Guadagni, D.G., R.G. Buttery, S. Okano, and H.K. Burr. 1963. Additive Effect of Sub-Threshold Concentrations of Some Organic Compounds Associated with Food Aromas. Nature, 200: 1288

Mahoney, C.H., H.L. Stier, and E.A. Crosby. 1957. Evaluating Flavor Differences in Canned Foods. Food Technol. 11 (9): 37

Merritt, C. Jr., and P. Angelini. 1965. Irradiation Damage in Lipids and Its Effect on Flavor. Presented at 149th National Meeting of the American Chemical Society, Detroit, Mich.

Nawar, W.W., and I.S. Fagerson. 1962. Direct Gas Chromatographic Analysis as an Objective Method of Flavor Measurement. Food Technol., 16: 107

Wick, E.L., T. Yamanishi, L.C. Wertheimer, J.E. Hoff, B.E. Proctor, and S.A. Goldblith. 1961. An investigation of some volatile components of irradiated beef. J. Agr. Food Chem. 9: 289

Wick, E.L., J.E. Hoff, S.A. Goldblith, and B.E. Proctor. 1961. The application of radiation-distillation to the production and isolation of components of beef irradiation flavor. J. Food Sci. 26: 258

Wick, E.L. 1963. Volatile components of irradiated beef. In "Exploration in Future Food-Processing Techniques" (Goldblith, S.A., ed.) MIT Press, Cambridge, Mass.

Wick, E.L., Koshika, M., Mizutani, J. 1965. Effect of Storage at Ambient Temperature on the Volatile Components of Irradiated Beef. J. Food Sci. 30: 433

Table I. Carbon Content of Distillates and Yield of Odor Concentrates

	Preparation	Quantity (kg)	MgC/kg	Total mg Odor Concentrate	Yield (ppm)
A.	1. Unirradiated	6.8	9.0	170.8	25.1
	2. Unirradiated	5.0	32.4	182	36.4
				Average	30.8 ± 7.9
B.	Radiation prior to distillation				
	1. Irradiated	6.8	10.6	204	30.0
	2. Irradiated	4.1	31.0	213.1	52.0
	3. Irradiated	6.8	-	266.6	39.2
	4. Irradiated	7.0	-	165.9	23.7
	Water blank	0.0	-	30	-
				Average	36.2 ± 12.8
C.	Stored 6 months				
	1. Irradiated	5.4	15.8	53.3	9.9
	2. Irradiated	14.1	-	135.8	9.6
				Average	9.7 ± 0.2
D.	Concurrent radiation-distillation				
	1. Irradiated	5.4	32.2	184	34.1
	2. Irradiated	4.1	-	201.3	49.1
	3. Irradiated	6.4	-	298.4	46.6
	Water blank	0.0	-	7.7	-
				Average	43.3 ± 7.8

Table II continued

iso-Pentanol	14	1.33	0.34	-	-	-	15	2.79	0.74
n-Heptanal	15	0.88	0.04	15	4.50	0.12	15		
n-Pentanol	16	7.68	0.10	16	1.70	0.03	16	2.17	0.18
Acetoin	17	40.14	2.11	17	10.84	1.43	17	27.25	3.85
n-Octanal	17			17			17		
n-Hexanol	18	1.04	0.01	18	1.85	0.17	18	1.00	0.17
Unknown	19	1.17	0.13	-	-	-	-	-	-
n-Nonanal	20	1.04	0.14	20	11.81	0.02	20	2.05	0.44
n-Heptanol	21	7.02	0.14	21	6.61	1.19	21	2.57	0.32
Methional	22	0.51	0.03	22-22a	29.89	0.33	22	23.79	2.94
n-Decanal	23	0.68	0.26	-	-	-	23	1.08	0.08
Benzaldehyde	24	1.27	0.33	24a	4.10	0.09	24	2.25	0.28
n-Octanol	24			24b	3.26	0.02	24		
n-Undecanal	25	2.10	0.17	25	1.20	0.06	25	0.97	0.17
Phenyl-acetaldehyde	26	0.80	0.20	26	0.74	0.12	26	2.45	0.58
		100.12			99.86			99.99	

[1]Average of two analyses carried out on one day from Prep. A, 1, Table I.

[2]Average of two analyses carried out 10 days apart on a single concentrate.

[3]Average of five analyses: two from Prep. B, 1, Table I done on same day and three from Prep. B, 4, Table I carried out over an 18 day period.

Table II. Comparison of Composition of Odor Concentrates (Distillates) From Non-Irradiated and Irradiated Beef

Compositions are based on internal normalization of all except the ether peak in chromatograms like those shown in Figure 1.

Compound	Fraction	Non-Irradiated[1] (%)	(S.D.)	Fraction	Concurrent[2] Irradiation (%)	(S.D.)	Fraction	Non-Concurrent[3] Irradiation (%)	(S.D.)
Ethyl acetate	-	-	-	4	2.33	0.03	4	1.94	0.32
n-Butanal	-	-	-	4			-	-	-
2-Butanone	4	2.22	0.00	5	4.45	0.18	5	5.76	1.24
Ethanol	5	0.57	0.07	6	4.85	0.30	6	4.26	1.17
iso-Propanol	6	0.55	0.01	6			6		
Benzene	7	9.70	0.55	7	0.64	0.15	7	4.50	3.34
n-Pentanal	8	1.23	0.07	8	1.63	0.10	8	0.92	0.06
2-Butanol	9	4.65	0.30	9	1.45	0.13	9	9.14	0.61
Unknown	10	0.48	0.02	10	0.30	0.12	-	-	-
Unknown	11	-	-	-	-	-	11	0.49	0.08
n-Hexanal	12	13.30	0.61	12-13	4.32	0.09	12-13	1.94	0.57
n-Butanol	13	1.66	0.90	14	3.39	0.08	14	2.67	0.07

Table III continued

1-Dodecene	-	-	-	16	2.33	0.86	16	8.65	0.69
n-Octanal	12	5.67	1.31	17	9.03	1.67	17	6.97	1.68
Acetoin	12			17			17		
n-Hexanol	12a	1.83	0.17	18			18	4.01	0.04
n-Tridecane	-	-	-	19	1.97	0.69	19		
1-Tridecene	-	-	-	19			19	6.23	0.27
n-Nonanal	13	3.99	0.51	20	42.07	1.63	20	4.75	1.17
n-Heptanol	14	3.52	0.49	21	2.44	0.21	21	0.95	0.02
Methional				22			22		
n-Tetradecane				22	3.05	1.48	22	12.37	1.11
1-Tetradecene				22			22		
n-Decanal				23			23		
n-Pentadecane									
1-Pentedecene		1.35	0.41		2.84	1.27		9.05	4.48
n-Octanol	15			23			23		
Benzaldehyde	16								
n-Hexadecane									
1-Hexadecene		0.82	0.02	-	-	-	-	-	-
Phenylacetaldehyde									
		99.98			99.92			100.06	

[1]Average of three analyses carried out over 4 days on Prep. A, 1 Table I.

[2]Average of two analyses done over 12 day period on a single concentrate.

[3]Average of four analyses: two from Prep. B, 1, Table I done on a single day and two from Prep. B, 4, Table I done on a single day.

Table III. Comparison of Composition of Odor Concentrates(Traps)

From Non-Irradiated and Irradiated Beef

Compositions are based on internal normalization of all except the ether peak in chromatograms like those shown in Figure 2.

Compound	Fraction	Non-Irradiated[1] (%)	(S.D.)	Fraction	Concurrent Irradiation[2] (%)	(S.D.)	Fraction	Non-Concurrent Irradiation[3] (%)	(S.D.)
2-Butanone	4	7.33	2.44	4	0.99	0.06	4	2.43	1.51
Unknown	5	3.70	0.89	-	-	-	-	-	-
n-Nonane	-	-	-	-	-	-	5	2.96	0.66
Ethanol	6	6.82	0.34	6	1.97	0.45	6	2.96	0.66
1-Nonene	-	-	-	-	-	-	7	2.93	1.38
n-Pentanal	7	2.95	0.59	8	0.71	0.31	8	0.93	0.10
2-Butanol	8	19.42	1.96	-	-	-	-	-	-
n-Decane	-	-	-	9	0.40	0.00	9	10.83	0.28
1-Decene	-	-	-	10	1.25	0.93	10	4.45	0.72
n-Hexanal	9	32.42	0.93	11	15.29	0.09	11	2.30	0.04
n-Butanol	9a	1.79	0.37	12	1.16	0.22	-	-	-
n-Undecane	-	-	-	12			12	4.53	0.10
1-Undecene	-	-	-	13	0.50	0.11	13	2.64	0.52
n-Heptanal	10	4.85	1.72	14	12.02	0.98	14	5.23	0.97
n-Pentanol	11	3.52	1.12	15	1.90	0.27	-	-	-
2 Unknowns									
n-Dodecane	-	-	-	-	-	-	15	4.89	0.93

Table IV. Comparison of the Average Composition of Total Odor Concentrates from Non-Irradiated and Irradiated Beef

Compound	Non-Irradiated (%)	Concurrent Irradiation (%)	Non-Concurrent Irradiation (%)
Ethyl acetate	-	~.83	0.97
n-Butanal	-	~.83	-
2-Butanone	4.78	2.72	4.09
Unknown	1.85	-	-
Ethanol	3.70	~2.48	~2.54
iso-Propanol	0.28	~1.49	~1.06
n-Nonane	-	-	1.48
1-Nonene	-	-	1.46
Benzene	4.85	0.32	2.25
n-Pentanal	2.09	1.17	0.92
2-Butanol	12.04	0.72	4.57
Unknown	0.24	0.15	0.24
n-Decane	-	0.20	5.42
1-Decene	-	0.62	2.22
n-Hexanal	22.86	9.80	2.12
n-Butanol	1.73	~1.98	1.33
iso-Pentanol	0.66	-	~0.69
n-Undecane	-	~0.29	2.26
1-Undecene	-	0.25	1.32
n-Heptanal	2.86	8.26	~3.31

Table IV--cont

n-Pentanol, 2 unknowns	5.60	1.80	1.08
n-Dodecane	-	-	2.44
1-Dodecene	-	1.66	4.32
Acetoin n-Octanal	22.90	9.93	17.11
n-Hexanol	1.98	~1.25	2.50
Unknown	0.58	-	-
n-Tridecane 1-Tridecene	-	0.65	3.12
n-Nonanal	2.52	26.99	3.40
n-Heptanol	5.27	4.52	1.71
Methional n-Tetradecane 1-Tetradecene	0.26	16.47	18.08
n-Decanal n-Pentadecane 1-Pentadecene	0.34	~0.71	0.54
n-Octanol	0.99	~2.34	~2.82
n-Undecanal	1.05	0.60	0.48
Benzaldehyde n-Hexadecane 1-Hexadecene	0.78	2.05	~2.82
Phenylacetaldehyde	0.40	0.37	1.22

Table V. Relative Proportions of Possible Indispensable Off-Odor Components in Non-Irradiated and Irradiated Beef (from Table IV)

Compound	Non-Irradiated		Concurrent		Non-Concurrent	
	% Found	Relative Amount	% Found	Relative Amount	% Found	Relative Amount
n-Pentanol	5.60	14.0	1.80	4.9	1.08	0.88
Acetoin + n-Octanal	22.90	57.2	9.93	26.8	17.11	14.0
n-Hexanol	1.98	4.9	~1.25	3.4	2.50	2.1
n-Nonanal	2.52	6.3	26.99	72.9	3.40	2.8
n-Heptanol	5.27	13.2	4.52	12.2	1.71	1.4
Methional	0.26	0.6	16.47	44.5	18.08	14.8
n-Decanal	0.34	0.8	~0.71	1.9	0.54	0.44
n-Octanol	0.99	2.5	~2.34	6.3	~2.82	2.3
n-Undecanal	1.05	2.6	0.60	1.6	0.48	0.39
Benzaldehyde	0.78	1.9	2.05	5.5	~2.82	2.3
Phenyl-acetaldehyde	0.40	1.0	0.37	1.0	1.22	1.0

Table VI. Rough Estimation of Detector Response to Certain Beef Components

Compound	Density g/ml	Response	
		Per Unit Volume (Found)*	Per Unit Weight (Calculated)
n-Nonanal	0.827	1.00	1.21
n-Octanal	0.821	1.10	1.34
Phenylacetaldehyde	1.03	1.11	1.07
Benzaldehyde	1.05	1.11	1.06
n-Octanol	0.825	1.10	1.33
Methional	1.05	0.99	0.94
n-Dodecane	0.751	1.01	1.34
1-Dodecene	0.762	0.74	0.97
n-Undecane	0.741	0.67	0.91

*Relative response based on the average peak area of three 1 µl samples run on the 20% CW 20 M column and detector used on the beef odor concentrates.

Table VIIa Results of Observations of Degrees of Difference in Irradiation Odor (85 Evaluations, 17 Judges)

Set I.

Standard: Irradiated Sample

Judge No.	Means of Differences from Standard S'(Irradiated) $\bar{x}_1$	Synthetic $\bar{x}_2$	Difference in Means $(\bar{x}_2 - \bar{x}_1) = d_i$
1	1.00	2.20	+1.20
3	2.50	3.25	0.75
8	1.66	3.00	1.34
11	1.00	2.00	1.00
13	2.00	2.57	0.57
15	1.83	3.66	1.83
18	1.71	1.71	0.00
19	1.33	4.00	2.67
20	2.66	1.66	-1.00
21	1.00	5.66	4.66
25	2.22	1.20	-1.02
22	2.00	2.14	0.14
14	1.00	3.50	2.50
16	1.00	5.50	4.50
12	1.00	5.50	4.50
10	2.00	1.77	-0.23
26	1.50	3.77	2.25
	Mean.....1.61	Mean.....3.12	Mean Difference..... 1.51*

* For Ho, $\bar{d} = o$; $t = 3.44$, $P \leq 0.01$

Table VIIb. Results of Observations of Degrees of Difference in Irradiation Odor (85 Evaluations, 17 Judges)

Set. II.

Standard: "Synthetic" Sample

Judge No.	Means of Differences from Standard S' (Synthetic) $\bar{x}_1$	Irradiated $\bar{x}_2$	Difference in Means $(\bar{x}_2 - \bar{x}_1) = d_i$
1	1.20	2.00	+0.80
3	1.22	4.00	2.78
8	1.33	1.33	0.00
11	1.00	3.00	2.00
13	2.00	3.29	1.29
15	3.83	3.66	-0.17
18	1.00	1.57	+0.57
19	1.50	4.17	2.67
20	1.33	4.33	3.00
21	1.50	6.00	4.50
25	1.60	2.40	0.80
22	1.43	2.86	1.43
14	1.50	4.33	2.83
16	1.00	5.83	4.83
12	1.00	5.50	4.50
10	2.50	3.25	0.75
26	4.75	5.75	1.00
	Mean.....1.74	Mean.....3.60	Mean Difference.....1.97*

* For Ho, $\bar{d} = o$; $t = 5.13$, $P \leq 0.01$

Table IX. Results of Observations of Degrees of Difference in Sensory Effect: Phenylacetaldehyde in Enzyme-Inactivated Beef Slurry

(61 Evaluations, 16 Judges)

Standard: Enzyme-Inactivated Beef Slurry

Judge No.	Means of Difference from Standard			Difference in Means	
	S' $\bar{x}_1$	Level 1 (0.94 ppm) $\bar{x}_2$	Level 2 (1.56 ppm) $\bar{x}_3$	Level 1 $x_2 - x_1 = d_1$	Level 2 $\bar{x}_3 - \bar{x}_1 = d_2$
1	1.00	3.00	4.00	2.00	3.00
3	1.00	7.40	6.80	6.40	5.80
10	1.80	3.20	2.60	1.40	0.80
11	1.80	1.80	2.40	0.00	0.60
12	1.00	1.50	3.50	0.50	2.50
13	2.00	2.80	3.40	0.80	1.40
14	1.00	4.25	4.25	3.25	3.25
15	1.20	3.40	5.20	2.20	4.00
16	3.50	4.50	4.00	1.00	0.50
18	1.40	4.60	4.20	3.20	2.80
19	1.33	2.66	3.00	1.33	1.67
20	2.00	4.50	3.00	2.50	1.00
21	1.66	4.33	5.00	2.67	3.37
22	1.33	3.00	3.00	1.67	1.67
25	1.00	1.60	1.60	0.60	0.60
26	3.00	4.00	4.75	1.00	1.75
Mean....	1.62	Mean.. 3.53	Mean..3.79	Mean...1.91*	Mean... 2.17

* For Ho, $\bar{d} = o$; $t = 4.96$, $P \leqslant 0.01$

Table VIII. Results of Observations of Degrees of Difference in Sensory Effect: Methional in Enzyme-Inactivated Beef Slurry

(58 Evaluations, 15 Judges)

Standard: Enzyme-inactivated beef slurry

Judge No.	Means of Differences from Standard			Difference in Means	
	S' $\bar{x}_1$	Level 1 (6 ppm) $\bar{x}_2$	Level 2 (9 ppm) $\bar{x}_3$	Level 1 $\bar{x}_2 - \bar{x}_1 = d_1$	Level 2 $\bar{x}_3 - \bar{x}_1 = d_2$
1	1.40	3.20	3.00	1.80	1.60
3	1.25	1.50	2.25	0.25	1.00
10	1.20	3.80	4.80	2.60	3.60
11	1.50	2.50	2.75	1.00	1.25
12	2.00	3.00	5.00	1.00	3.00
13	2.00	2.50	2.50	0.50	0.50
14	1.25	3.25	4.25	2.00	3.00
15	1.75	3.25	3.50	1.50	1.75
16	1.00	3.33	5.00	2.33	4.00
18	1.00	4.40	4.00	3.40	3.00
19	2.66	2.33	4.00	-0.33	1.34
21	2.00	4.75	5.00	2.75	3.00
22	1.00	3.00	2.00	2.00	1.00
25	1.40	3.20	3.20	1.80	1.80
26	3.00	4.25	4.75	1.25	1.75
Mean.....	1.62	Mean.. 3.21	Mean.. 3.73	Mean.... 1.59*	Mean 2.11

* For Ho, $\bar{d} = o$; $t = 6.03$, $P \leqslant 0.01$

Table X. Results of Observations of Degrees of Difference in Sensory Effect:

n-Nonanal in Enzyme-Inactivated Beef Slurry

(71 Evaluations, 15 Judges)

Standard: Enzyme-Inactivated Beef Slurry

Judge No.	Means of Difference from Standard			Difference in Means	
	S' $\bar{x}_1$	Level 1 (6.12 ppm) $\bar{x}_2$	Level 2 (7.6 ppm) $\bar{x}_3$	Level 1 $\bar{x}_2 - \bar{x}_1 = d_1$	Level 2 $\bar{x}_3 - \bar{x}_1 = d_2$
1	1.00	1.00	1.50	0.00	0.50
3	1.40	2.40	2.40	1.00	1.00
10	1.83	2.33	2.66	0.50	0.83
11	1.66	2.33	2.00	0.67	0.34
12	2.00	4.25	4.50	2.25	2.50
13	2.40	2.40	2.40	0.00	0.00
14	1.00	1.66	1.66	0.66	0.66
15	1.20	1.80	2.00	0.60	0.80
16	1.40	1.60	3.00	0.20	1.60
18	1.17	2.00	2.33	0.83	1.16
19	3.33	2.17	2.17	-1.16	-1.16
20	2.00	2.60	2.20	0.60	0.20
22	1.33	2.66	1.66	1.33	0.33
25	2.17	2.33	1.50	0.16	-0.67
26	5.50	3.00	3.50	-2.50	-3.00
Mean....	1.95	Mean.. 2.30	Mean... 2.36	Mean.. 0.34*	Mean.... 0.34*

* Not significant

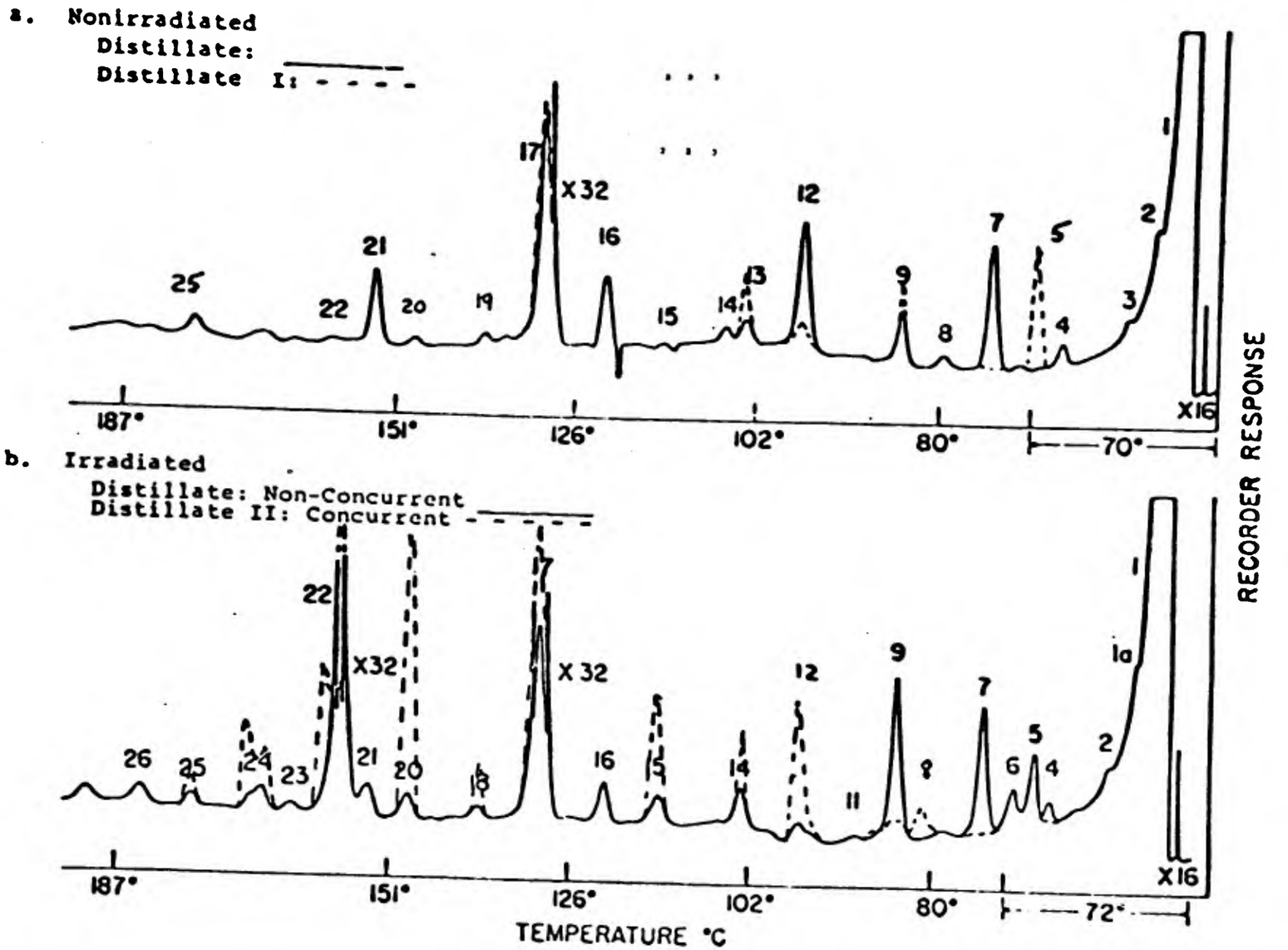

Figure 1. Temperature programmed separation of isolates from non-irradiated and irradiated beef on a 20% Carbowax 20 M column.

a. Nonirradiated Traps

b. Irradiated
Traps: Non-Concurrent ———
Traps: Concurrent - - -

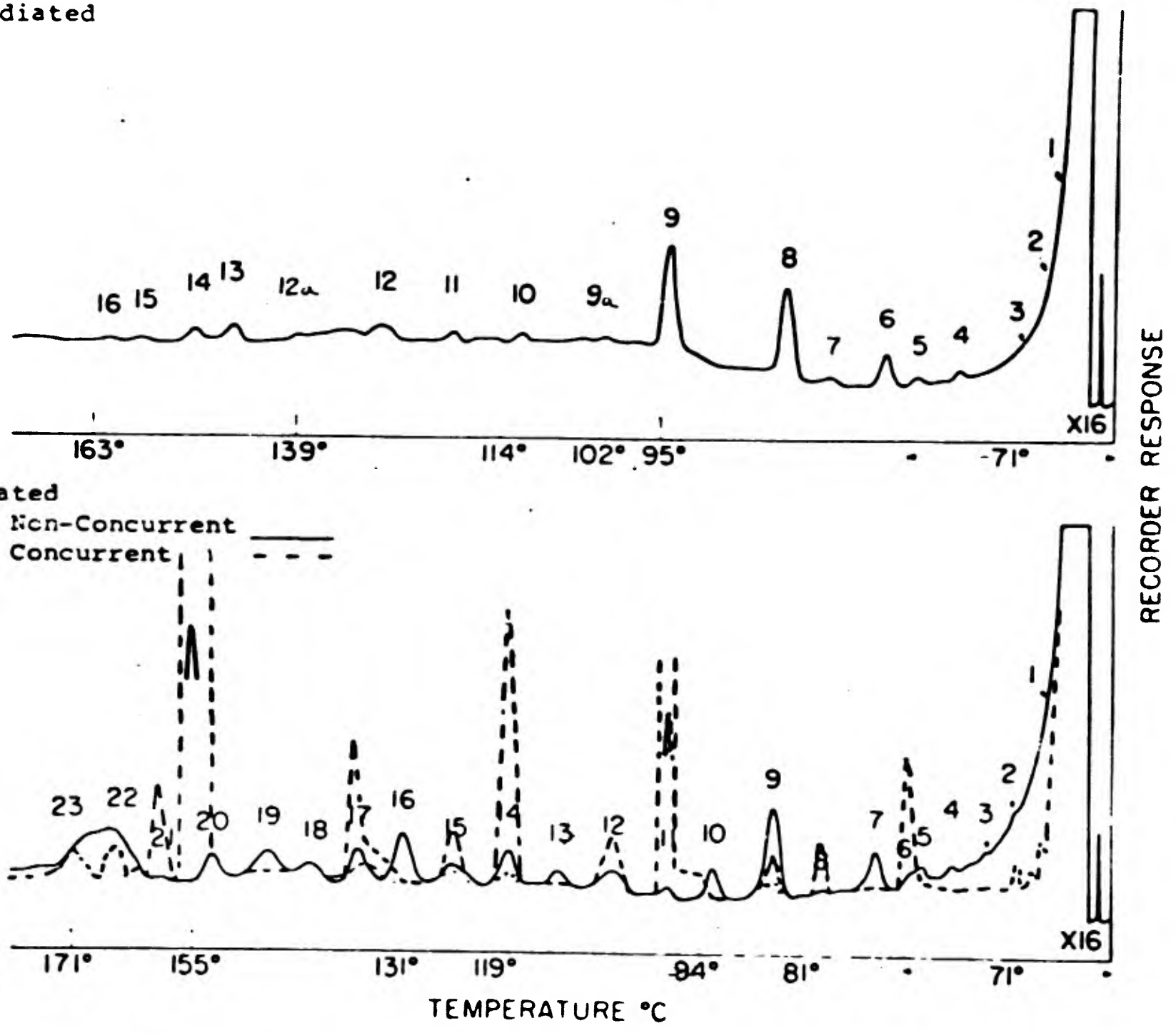

Fig 2 Temperature Programmed Separation of Isolates from Non-irradiated and Irradiated Beef on a 20% Carbowax 20M Column

Figure 3

Odor Difference Evaluation

NAME:

DATE:

Compare the odor of the numbered samples with that of the reference sample "S." Indicate the degree of difference, if any, from the odor of "S" by checking the appropriate box opposite the term which best describes the degree of odor difference. Take as much time as you need. Base your judgement on odor quality and not intensity.

	Sample number		Score
	____	____	
None.....	☐	☐	1
Between None & Slight.....	☐	☐	2
Slight.....	☐	☐	3
Between Slight & Moderate.....	☐	☐	4
Moderate.....	☐	☐	5
Between Moderate & Large.....	☐	☐	6
Large.....	☐	☐	7
Between Large & Extreme.....	☐	☐	8
Extreme.....	☐	☐	9

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13 ABSTRACT

Progress in the identification of beef components indispensable to the production of irradiation off-flavor is described. Evidence is presented that methional, n-nonanal, and phenylacetaldehyde are the major indispensable components.

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