Effect of Radiation Parameters on the Formation of Radiolysis Products in Meat and Meat Substances

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Analytical chemical methods, employing gas and liquid chromatography for separation and mass spectrometry for identification, have been used to study the formation of radiolysis products in various meats such as beef and pork and in fats and proteins derived from meat. In this study the dependency of the amount of product formed in beef is evaluated as a function of various parameters such as radiation dose, dose rate, temperature of irradiation, precursor concentration, and various other factors. Statistical analysis of data accumulated from a large number of samples is provided by means of a laboratory automation computer. The significance of the data is assessed with particular regard to the wholesomeness of the irradiated product.

The papers presented previously in this symposium have been concerned with the radiation effects on various components of food, i.e., lipids, proteins, and amino acids, carbohydrates, nucleic acids, etc. In this paper, some quantitative aspects of the parameters which affect the formation of radiolytic products in meat and related substances are discussed. Although the various studies which have been described in the foregoing presentations contribute greatly to the understanding of the mechanisms involved in the formation of radiolytic products from various precursors, in practice it is also necessary to have direct analytical data about the composition of radiolysis products in order to assess the wholesomeness of irradiated food and to convince the health authorities that irradiated food is safe for consumption.

Analytical studies of the compounds formed during irradiation of meat have been in progress on a continuing basis for more than 15 years (Batzer et al., 1955, 1957; Burks et al., 1959; Champagne and Nawar, 1969; Dubravcic and Nawar, 1968; LeTellier and Nawar, 1972a,b, 1974; Merritt et al., 1959, 1965, 1966a,b, 1967a,b, 1972, 1975; Monty et al., 1961; Nawar et al., 1969, 1972; Scribney et al., 1955; Wick et al., 1961, 1963, 1967), and accordingly the qualitative aspects of composition are very well known. A summary of some of the compounds identified is seen in Table I. These components are those which can be isolated as volatile compounds. Although recent studies (Vajdi, 1976) have been concerned with less volatile compounds, these data are incomplete. This discussion is, therefore, confined to the volatile components. The compounds found are those predicted by studies of model systems, i.e., various hydrocarbons and carbonyl compounds from lipids, sulfur, and aromatic compounds from the protein.

A detailed summary of the qualitative composition of various radiolysis products obtained from beef, pork, lamb, and chicken was presented at the symposium on Recent Advances in the Chemistry of Food Irradiation held at the National Meeting of the American Chemical Society in Los Angeles and is published in *Radiation Research Reviews* (Merritt, 1972). At that time a comparison was made between the compounds formed by irradiation and those which occur naturally in other foods, such as fruit, cheese, eggs, and beverages such as coffee and cocoa. It could easily be shown that no volatile compounds are produced in meat by irradiation that are not found similarly to occur in other foods and in amounts as great or greater than those in the irradiated product.

Table I. Compounds in Irradiated Meat Volatiles

C_1-C_{20} RH	C_2-C_6 RCHO
C_2-C_{20} RC=CH_2	C_3-C_6 R_2CO
C_2-C_{20} RC≡CH	C_1-C_4 RSH
C_1-C_6 ROH	C_2-C_6 RSR, RSSR
C_4-C_{20} alkadienes	

For the past 3 or 4 years the Natick Laboratories have been conducting a long-range feeding study on the wholesomeness of irradiated beef. In this connection a parallel study of the composition of the radiolysis products is also in progress, so the feeding study data may ultimately be correlated with the chemical composition.

EXPERIMENTAL SECTION

The analytical methods employed have been described in several previous publications (Angelini et al., 1967; Merritt et al., 1959, 1964, 1967, 1970, 1972, 1974) but a brief review may provide some background for those who are unfamiliar with the work.

The trace volatile compounds formed by radiolysis are separated from the meat by a high vacuum distillation into a receiver at liquid nitrogen temperature. The total condensate thus collected is further fractionated by high vacuum distillation at −80 °C into a distillate called the carbon dioxide fraction and a raffinate called the water fraction.

The mixtures of volatile components isolated by vacuum distillation are ultimately separated and analyzed by combined gas chromatographic and mass spectrometric techniques. A programmed temperature gas chromatograph is coupled directly to a fast scanning mass spectrometer which is operated in repetitive scan mode. The mass spectrum output is digitized on line by a minicomputer, and the digitized spectra are subsequently transferred to a larger computer which is used to provide automatic component identification. The gas chromatographic output is also digitized by means of an integrator which in turn provides the quantitative data for the mixtures.

RESULTS AND DISCUSSION

The data automation computer has permitted acquisition and processing of a prodigious amount of mass spectral data and has provided the basis for statistical treatment of the analytical results from the meat samples obtained from the wholesomeness feeding study. These data have demonstrated the remarkable reproducibility and, acordingly, the striking predictability of radiation effects. The reproducibility of formation of radiolysis

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Table II. Volatile Compounds Isolated from Meat Substances[a]

Beef protein	Beef fat	Beef lipoprotein
Methyl mercaptan (l)	C_1-C_{12} *n*-alkanes (l)	C_1-C_{14} *n*-alkanes (l)
Ethyl mercaptan (s)	C_2-C_{15} *n*-alkenes (l)	C_2-C_{14} *n*-alkenes (l)
Dimethyl disulfide (m)	C_4-C_8 *i*-alkanes (s)	Dimethyl sulfide (s)
Benzene (m)	Acetone (m)	Acetone (m)
Toluene (m)	Methyl acetate (t)	
Ethylbenzene (s)		
Methane (s)		
Carbonyl sulfide (s)		
Hydrogen sulfide (s)		

[a] l = large; m = moderate; s = small; t = trace.

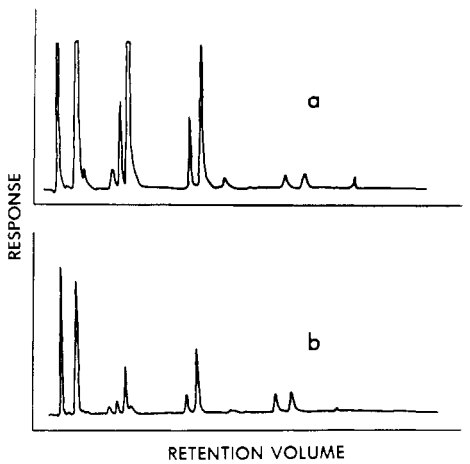


Figure 1. Gas chromatograms of volatile compounds obtained from irradiated beef: (a) irradiated Feb 1960; (b) irradiated Nov 1960.

compounds has been known for many years from comparison of chromatograms. For example, as seen in Figure 1, the chromatograms of the volatiles obtained from two samples of meat irradiated in 1960 show that the component composition is identical and that the relative amounts of each are likewise the same. The current statistical analysis of data confirms the chromatographic observations in every respect and provides several additional insights as well.

More than 100 compounds have been identified among the products of irradiation in beef. These have been tabulated elsewhere (Merritt, 1972) but the basic composition can be summarized as seen in Table II. The principle products are hydrocarbons, i.e., alkanes, alkenes, alkynes, and alkadienes. Alkanes and alkenes are most abundant and constitute more than 95% of the total volatile compounds. The hydrocarbons are derived from the fat. Oxygenated compounds such as alcohols and carbonyl compounds are less abundant, and only relatively small amounts (less than 1% of the total) of the sulfur compounds and aromatic hydrocarbons obtained from the protein are found.

All of the data obtained from the analyses is stored in the computer. Table III is a printout of the composition of the volatile compounds identified in a carbon dioxide fraction. It gives the replicate values of the amount of each component determined for samples which were stored for varying periods of time.

Figure 2 shows how these data may be presented as control charts to demonstrate their reproducibility. In the wholesomeness feeding study the irradiated beef has been procured and processed in several production lots. Moreover, since the beef is used over a period of time, its storage characteristics are important. Figure 2A shows a computer generated plot of the values of the amount of heptane as a function of storage time. The individual

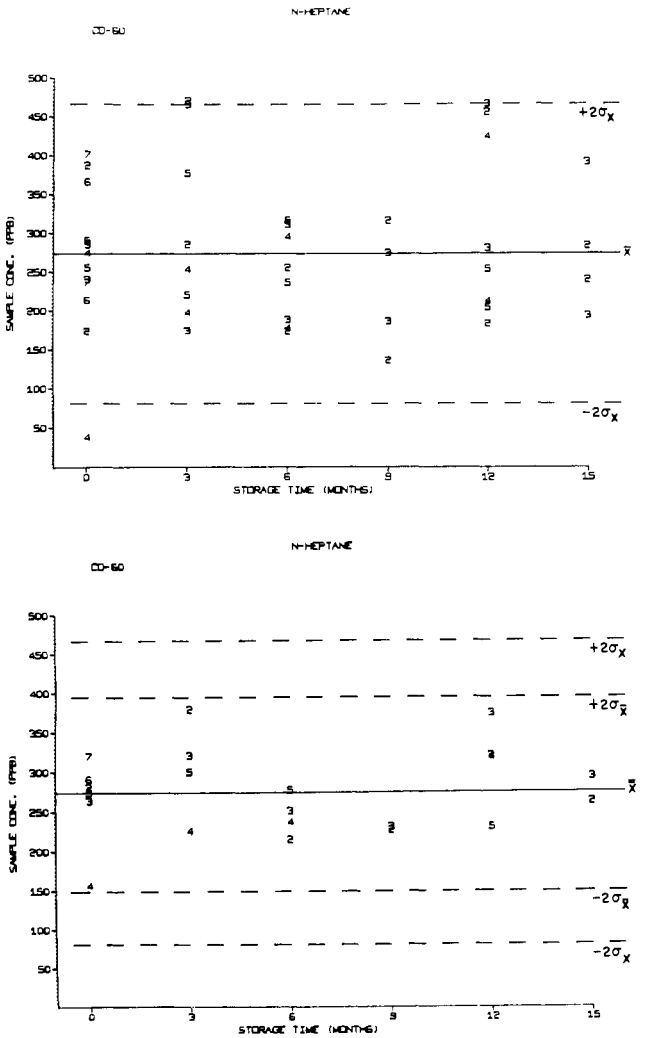


Figure 2. Control charts of data for the amount of *n*-heptane obtained from various production lots of ^{60}Co-irradiated beef as a function of storage time.

values are indicated by numbers which correspond to the production lot. It is sometimes difficult to read because of overprints, but there are two values for lots 2, 3, 4, etc. for each of the storage times. The central line indicates the mean or grand averge, $\bar{X}$, which is calculated for all the values. The dashed lines show the limits of values falling within $\pm 2\sigma$. Except for an occasional random deviation, as with one of the samples in lot 4 at zero time, all the values fall within the limits of $\pm 2\sigma$, which from the point of view of statistics represents a narrow range. These data are for beef samples which were irradiated at 3.7 Mrad with ^{60}Co.

It has been convenient, instead of plotting the individual values separately, to plot the average of the two determinations made for each procurement lot at each storage

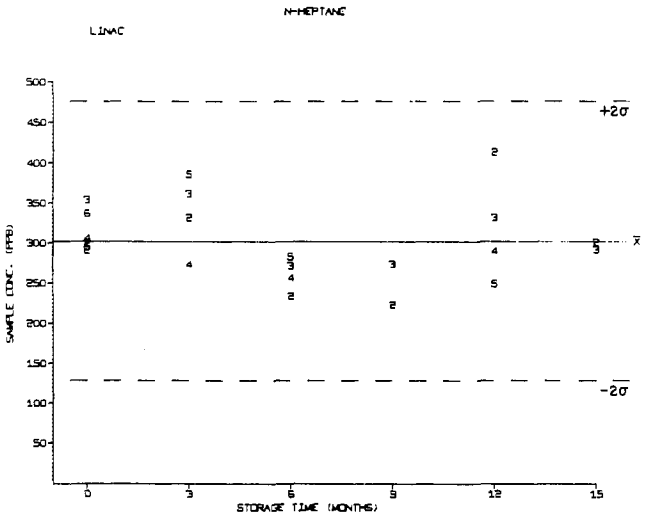


Figure 3. Control chart of data for the amount of *n*-heptane obtained from various production lots of electron-irradiated beef as a function of storage time.

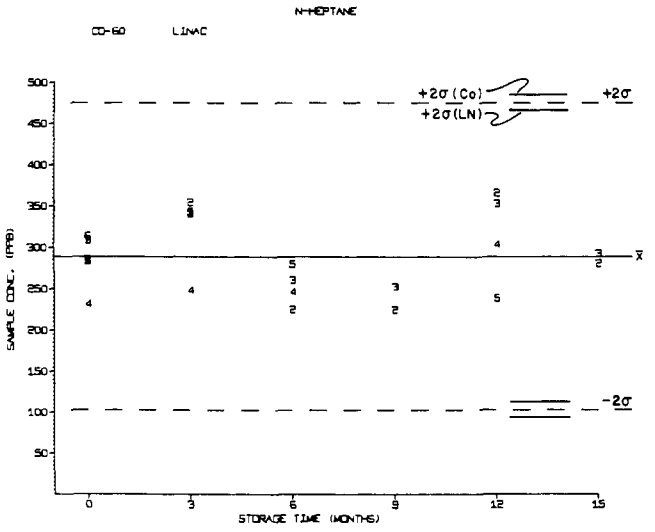
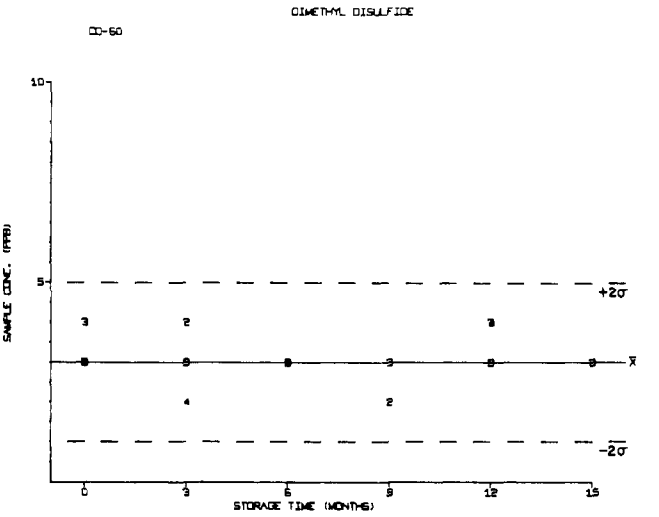


Figure 4. Control chart of data for the amount of *n*-heptane obtained from various production lots of ^{60}C- and electron-irradiated beef as a function of storage time.

time. Figure 2B shows such a plot for *n*-heptane from ^{60}Co irradiated samples of beef. The grand average, $\bar{X}$, is the same as previously, but if the standard deviation is computed for averages, the control limits, i.e., $\pm 2\sigma_{\bar{X}}$, appear to be much closer than when the standard deviation is computed from individual values, i.e., $2\sigma_{\bar{X}}$.

In addition to the samples prepared for the feeding study by irradiation with ^{60}Co, another group is irradiated at the same nominal dose, i.e., 3.7 Mrad, by means of a linear accelerator. Figure 3 shows the data for the LINAC irradiated beef. The amount of heptane produced in the electron irradiated beef is essentially the same as that produced in beef irradiated with γ radiation. In this case the mean is about 300 ppb, whereas as we saw in Figure 2, the mean for ^{60}Co irradiated beef was about 275 ppb, and the standard deviations show that these values are statistically the same.

This can be demonstrated more rigorously by treating the values for ^{60}Co and LINAC irradiated beef as members of the same population as seen in Figure 4. Here all the values are averaged for both ^{60}Co and LINAC for each production lot at each storage time. The mean is about 290 ppb, and the standard deviation calculated from individual values is shown by the dashed lines. The limits



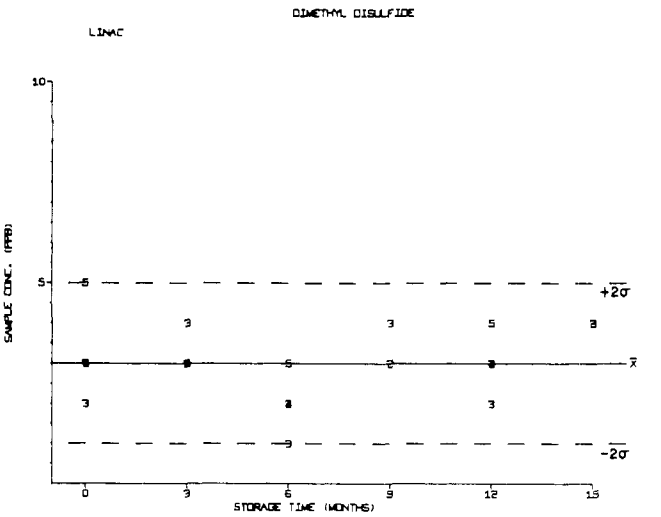


Figure 5. Control charts of data for the amount of dimethyl sulfide obtained from various production lots of ^{60}Co- and electron-irradiated beef as a function of storage time.

for twice the standard deviation also computed separately for both the ^{60}Co and LINAC samples are shown beside the control line. The variations are insignificant and we may conclude that the formation of heptane by radiolysis of beef with electrons or γ radiation is quantitatively the same.

The same conclusion can be shown for other radiolysis products. Figure 5 shows the data graphs for dimethyl sulfide from ^{60}Co and LINAC irradiated beef; the mean for the various production lots, and storage time up to 15 months, is 4 ppb in both cases although the deviation of the values for LINAC samples is slightly greater. A graph of ^{60}Co and LINAC values averaged together shows that the amount of dimethyl sulfide formed is consistently the same regardless of the type of radiation employed. In fact, this can be shown for all the components produced.

Despite the fact that there are a large number of radiolysis products (there are about 49 in the carbon dioxide fraction alone), the treatment of the voluminous data is relatively easy by means of the computer generated control charts.

Although the behavior of the individual components is easily depicted, the amount of the data is at times cumbersome. It is also possible to treat the data in the aggregate. For example, the compounds may be grouped according to functionality. Figure 6A shows alkenes

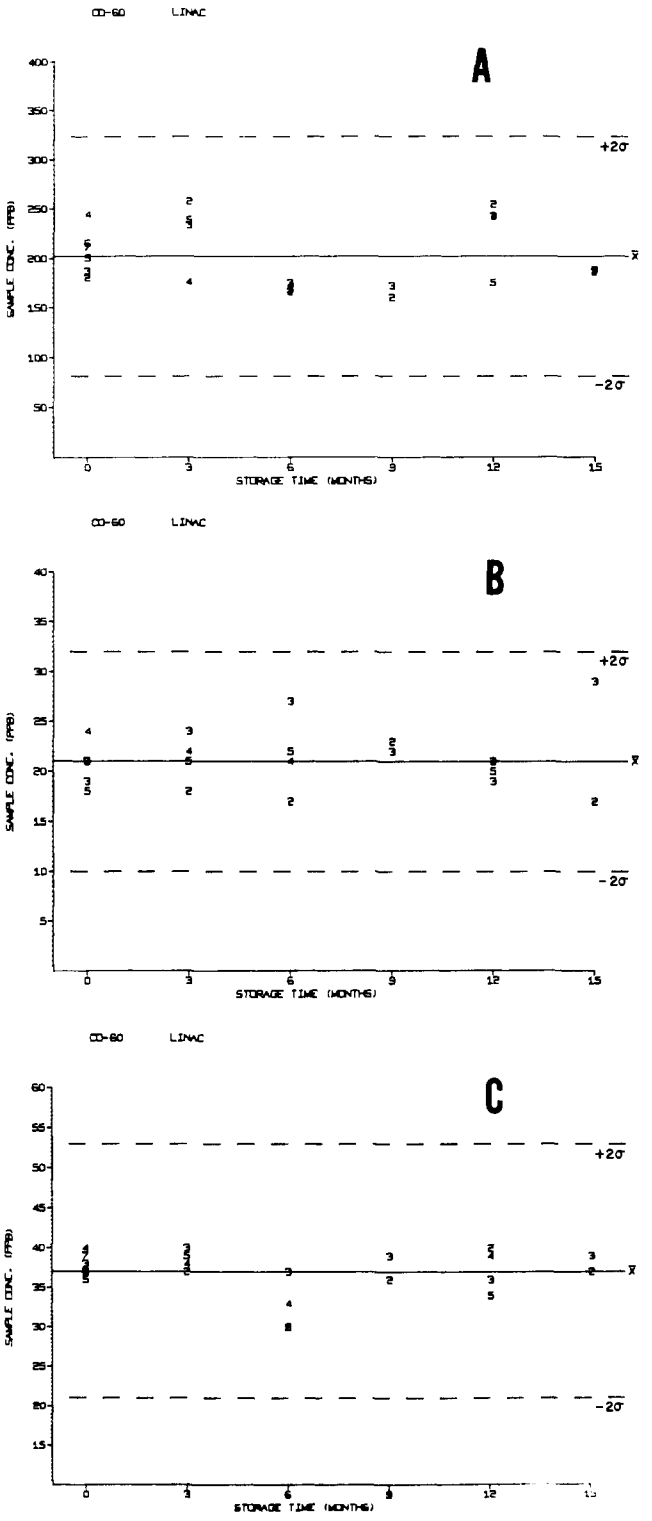


Figure 6. Control charts of data for the amounts of certain compounds obtained from various production lots of ^{60}Co- and electron-irradiated beef as a function of storage time: (A) alkenes, (B) aldehydes, (C) thiaalkanes.

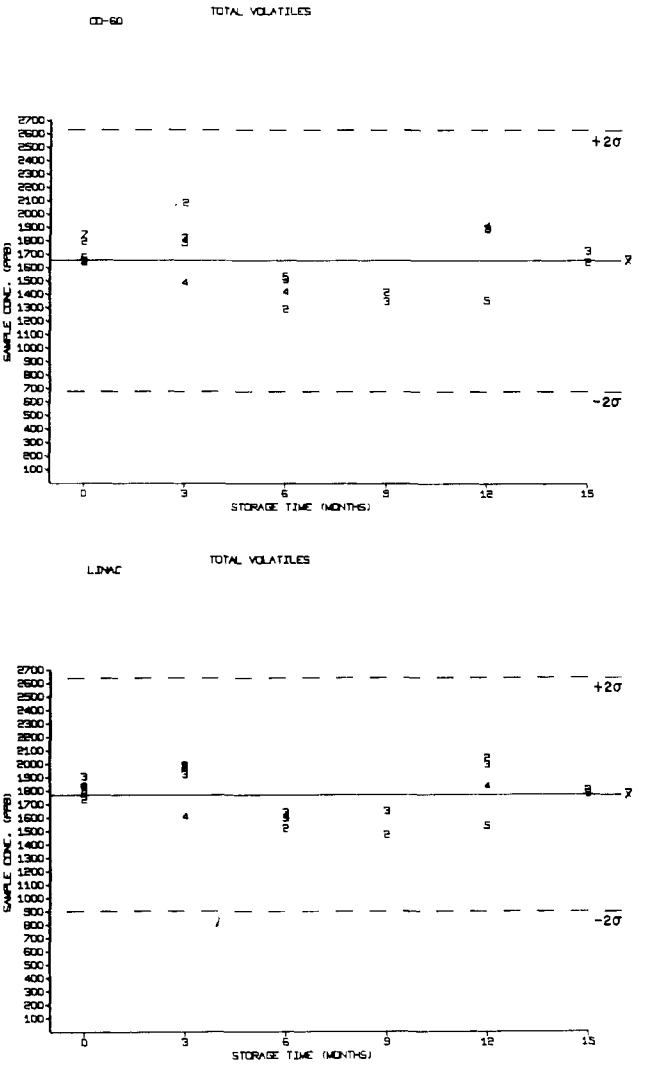


Figure 7. Control charts of data for the total amount of volatile compounds obtained from various production lots of ^{60}Co- and electron-irradiated beef as a function of storage time.

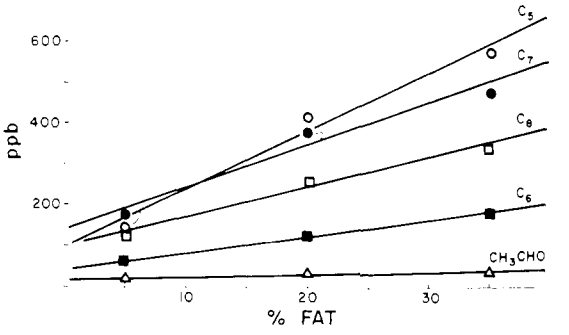


Figure 8. Graph of the amount of various radiolysis products formed in beef as a function of fat content: C_5, *n*-pentane; C_6, *n*-hexane; C_7, *n*-heptane; C_8, *n*-octane. Temperature, −25 °C; radiation dose, 3.7 Mrad.

treated as the sums of their amounts. The values for the ^{60}Co and electron irradiated sample are also combined. Figure 6B shows a similar plot for the aldehydes, and Figure 6C shows the graph for thiaalkanes. In fact, the amounts of all the components may be added and the data presented as total volatiles as seen in Figure 7.

As we have seen throughout the presentation of the data in Figures 2–7, the statistical variation for a given set of irradiation conditions is very small. The data provide two other important conclusions. Irradiation by electrons or by γ radiation is indistinguishable for a given dose and there are no changes brought about by long-term storage.

There are several other important parameters which can effect the quantitative composition of the radiolysis products. It has long been established that the most abundant components such as the hydrocarbons are formed by cleavage reactions of the triglycerides (Merritt, 1972; Nawar, 1972). One would expect, therefore, the amount of these components to vary with the fat content of the beef.

Table III. Computer Printout of Analytical Data from Wholesomeness Study of Irradiated Beef

FILE: P2CO2 SAMPLE: CO=60=HEAT STORAGE TIME=MONTHS SAMPLE CONCENTRATION=PPB
(+ = 0 TO 1 PPB, 0 = COMPOUND NOT FOUND IN SAMPLE,
- = NO DATA OBTAINED FOR SAMPLE,

NO.	COMPOUND	TIME:	0		3		6		9		12		15	
		SAMPLE:	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2
2	PROPANE		83	37	90	54	51	35	71	31	96	38	63	53
3	N-BUTANE		173	77	185	111	107	73	154	66	190	76	128	108
4	ISOBUTANE		37	17	41	25	24	16	34	14	40	16	27	23
5	N-PENTANE		235	105	259	157	155	105	205	89	272	110	177	149
6	ISOPENTANE		6	3	6	4	3	2	5	2	7	3	4	4
7	N-HEXANE		280	126	313	189	186	126	249	107	331	133	214	182
8	2-METHYLPENTANE		14	6	16	10	8	5	12	5	16	6	10	8
10	N-HEPTANE		388	174	472	286	256	174	317	137	456	184	284	240
11	N-OCTANE		391	175	439	265	250	170	305	131	466	188	292	248
12	METHYLHEPTANE		14	6	17	11	10	7	14	6	17	7	12	10
13	N-NONANE		37	17	45	27	26	18	35	15	47	19	31	27
14	N-DECANE		8	4	4	5	5	3	6	3	7	3	5	5
20	BUTENE		1	1	22	14	12	8	15	7	23	9	15	13
21	ISOBUTENE		2	1	3	2	1	1	2	1	3	1	1	1
22	PENTENE		3	1	4	2	+	•	1	+	4	2	2	1
23	HEXENE		3	1	4	2	1	•	2	+	4	2	2	2
24	HEPTENE		62	28	70	42	39	27	54	24	74	30	47	39
25	OCTENE		23	11	34	20	18	12	27	11	43	17	22	18
26	NONENE		22	10	24	14	14	10	20	8	29	11	16	14
33	BENZENE		19	9	22	14	12	8	17	7	24	10	14	12
34	TOLUENE		70	32	77	47	45	31	61	27	81	33	54	45
35	XYLENE		9	2	5	3	3	2	2	1	7	3	3	3
36	TRIMETHYLBENZENE		26	?	?	0	0	0	0	0	0	0	0	0
37	METHYL ALCOHOL		47	11	15	9	13	9	21	9	23	9	21	17
38	ETHYL ALCOHOL		134	60	97	59	74	50	80	34	123	49	74	62
40	ACETONE		123	64	162	98	117	79	108	72	180	46	119	101
41	BUTANONE		10	55	76	46	67	45	103	45	96	38	101	85
45	2-METHYL PENTANAL		14	6	10	6	11	7	17	7	16	6	11	9
47	CARBONYL SULFIDE		3	1	2	1	2	2	2	+	3	1	2	2
48	HYDROGEN SULFIDE		2	1	2	1	2	1	2	•	2	1	2	1
49	METHYL MERCAPTAN		+	+	•	•	•	•	+	+	+	+	+	+
50	ETHYL MERCAPTAN		12	5	9	5	6	4	9	4	12	5	8	7
51	DIMETHYL SULFIDE		6	3	6	4	4	3	5	2	6	3	4	4
52	DIMETHYL DISULFIDE		4	2	5	3	3	2	3	1	4	2	3	3
53	ETHANE NITRILE		4	2	3	2	4	3	4	2	3	1	4	4

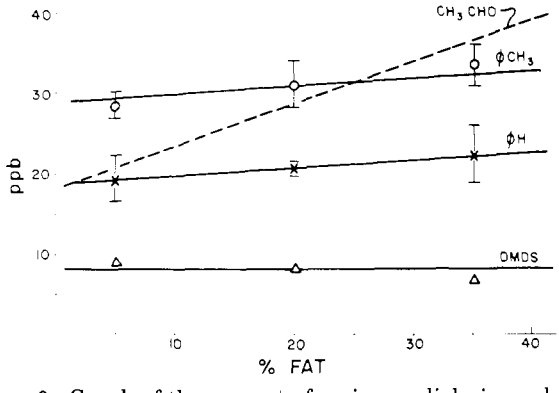


Figure 9. Graph of the amount of various radiolysis products formed in beef as a function of fat content: DMDS, dimethyl disulfide; temperature, −25 °C; radiation dose, 3.7 Mrad.

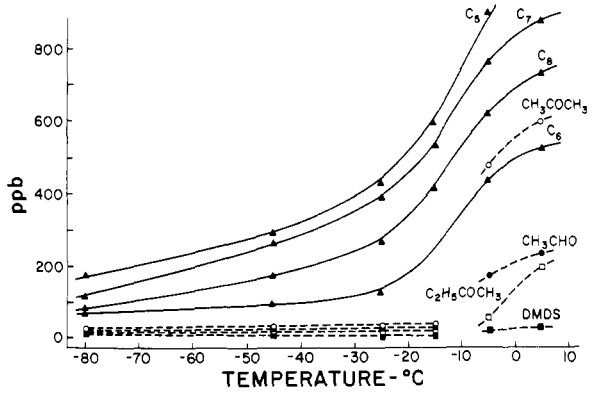


Figure 10. Graph of the amount of various radiolysis products formed in beef as a function of temperature during irradiation: C_5, *n*-pentane; C_6, *n*-hexane; C_7, *n*-heptane; C_8, *n*-octane; DMDS, dimethyl disulfide; radiation dose, 3.7 Mrad; fat content, 20%.

Figure 8 shows a graph of the amount of various alkanes produced as a function of the fat content of the beef. These components are some of the low carbon number alkanes found in the carbon dioxide fraction and are not as abundant as the larger compounds such as heptadecene and pentadecane found in the water fraction, but it is clear, nevertheless, that the amount increases with fat content. The plot for acetaldehyde is also shown, but on this scale the fact that its concentration also increases is not easy to observe.

Figure 9 is a graph of the behavior of some of the less abundant components as function of fat content. On this scale it may be clearly seen that acetaldehyde does increase with fat content. The amounts of benzene, toluene, and dimethyl disulfide do not increase. Although there is some scatter of the data for benzene and toluene, when the deviations are treated statistically by linear least-squares regression, and in addition compared with the overall variation established from the computer analysis of production lot and storage time data, it is clear that the amount of benzene and toluene does not depend on the fat content. These results are, of course, consistent with the concept that these components are derived from the radiolysis of the protein.

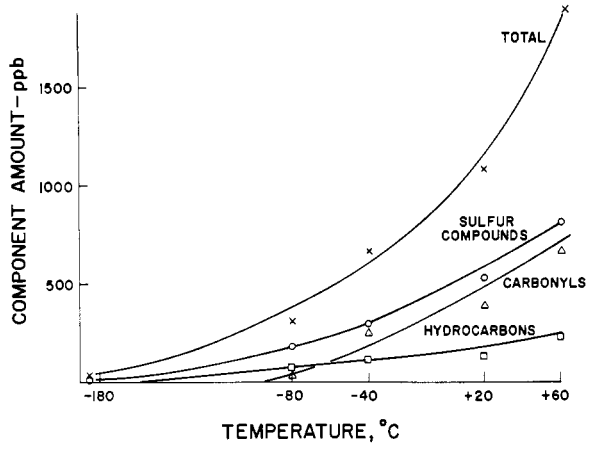


Figure 11. Graph showing relative amounts of component produced in beef irradiated with 4.5 Mrad as a function of temperature.

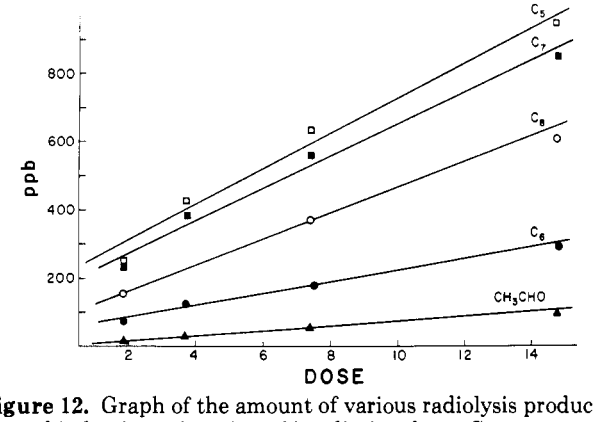


Figure 12. Graph of the amount of various radiolysis products formed in beef as a function of irradiation dose: C_5, *n*-pentane; C_6, *n*-hexane; C_7, *n*-heptane, C_8, *n*-octane; temperature, −25 °C; fat content, 20%.

Another important parameter is the temperature at which irradiation takes place. Figure 10 shows the changes in the amount of various components as a function of temperature. The temperature range covered here is mainly cryogenic. The reason for this is due to processing considerations, since it has been found that a product of superior quality is obtained by irradiating at low temperature. The hydrocarbons are seen to increase slightly up to about −15 °C. The changes in the amounts of carbonyl and sulfur compounds are negligible below −15 °C. At temperatures near 0 °C and above, the amounts of all the components tend to increase greatly.

Figure 11 shows some additional data on the effect of temperature, but for the components grouped according to functionality and for a wider temperature range than given in Figure 10. The increase in the amount of radiolysis products in beef irradiated at temperatures above freezing is consistent with the poor organoleptic quality of such products (Merritt et al., 1975).

Probably the most important of all the irradiation parameters is dose. It has been well established from much prior work that the amount of radiolysis product is a linear function of dose, but additional current data for several typical hydrocarbons and for acetaldehyde are shown in Figure 12. There is some variation among the values, but all the lines have been established by least-squares regression, and correlation coefficients of 0.99 or higher are obtained for the linear fit. It may be noted these data go up to 14 Mrad which is more than three times the nominal sterilizing dose.

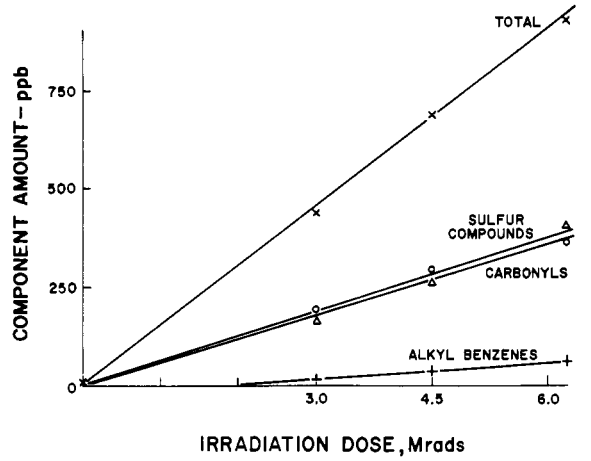


Figure 13. Graph showing relative amounts of component produced in beef irradiated as a function of dose at −40 °C.

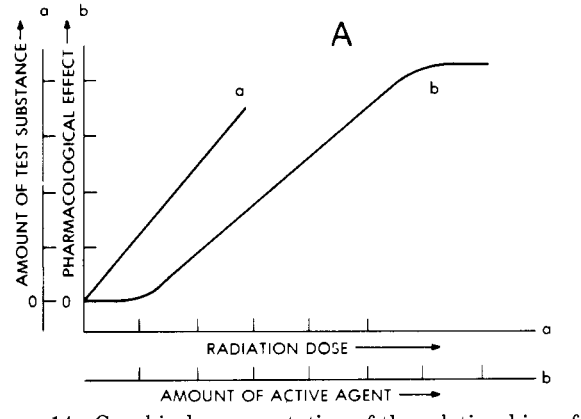


Figure 14. Graphical representation of the relationships of a pharmacological response to an active agent and of concentration of radiolytic products to dose: Plot a represents the linear radiation dose dependence for formation of a hypothetical radiolytic product having physiological activity. Curve b represents a typical pharmacological response of a test subject to an effect-inducing substance.

Figure 13 shows additional dose dependency data for the components as functional groups. In all cases, including a grouping as total volatiles, the amount increases linearly with dose. The linear dose dependency has in every instance been established rigorously by computing linear least-squares regression characteristics and correlation coefficients.

The establishment of linear dose dependency is extremely important for the purpose of correlating or evaluating biological activity and especially in extrapolating chemical data to assess the wholesomeness of products which are irradiated at doses which are different from those used in feeding studies. The situation may be depicted in Figure 14. Plot A shows the typical linear dose response for the formation of a radiolytic products, and plot B, a hypothetical biological response to an effect-producing substance. Typically, in the latter case there is an induction period, or threshold level, before the effect is observed, followed by a linear response which eventually levels off. If a biological response is observed, it is related to the amount of the antagonistic substance and can be correlated with that amount.

In practice, in a recent case (Taub et al., 1976), the question arose concerning the wholesomeness of food irradiated at a dose less than that at which the feeding studies were made. In this instance, as repeatedly demonstrated previously, the amount of the radiolysis products formed increases linearly with dose. However,

none of the feeding studies at 0.2, 0.6, 3, or 5.8 Mrad showed any adverse effects. Clearly, if there is no effect at a higher dose, there should be none at a lower dose.

This rationale is very important in consideration of meeting standards for acceptance of irradiated food products, for many of the products now undergoing feeding tests are being irradiated at higher doses than may be used later. It is with these problems in mind that the current studies have been undertaken to elucidate the behavior of the formation of the various radiolytic products in meat.

ACKNOWLEDGMENT

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Trace Analysis of Etrimfos and Two Degradation Products in Corn and Alfalfa

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A method was devised for determining residues of etrimfos [*O,O*-dimethyl *O*-(6-ethoxy-2-ethyl-4-pyrimidinyl)phosphorothioate], its O analogue, and its hydrolysis product in corn and alfalfa at levels of about 10 ppb or less. Salient elements of the procedure include Soxhlet extraction of the residues overnight with dichloromethane–10% methanol, preliminary cleanup on a column of Sephadex LH-20, separation of the three compounds on a silica gel column, and further cleanup of the hydrolysis product by liquid–liquid partitioning. The parent and O analogue fractions are separately analyzed by using gas chromatography with either a flame photometric detector sensitive to phosphorus or a rubidium-sensitized nitrogen–phosphorus detector. The hydrolysis product is analyzed directly by high-pressure liquid chromatography or converted to a pentafluorobenzoate derivative and assayed by electron-capture gas chromatography. Ancillary analytical chemical data and information concerning the efficiency of extracting field-weathered residues from corn were obtained and anticholinesterase activity and lethality data for etrimfos and the O analogue are also presented.

Etrimfos [formula I, *O,O*-dimethyl *O*-(6-ethoxy-2-ethyl-4-pyrimidinyl)phosphorothioate (also known as SAN 197, ENT 29126, and Ekamet), Sandoz, Inc., Homestead, Fla.] is a new nonsystemic, nonpersistent insecticide with a relatively low mammalian toxicity (acute oral LD_{50} for male rat is 1800 mg/kg). It is recommended for control of chewing, sucking, and biting pests on most crops (Knutti and Reisser, 1975).

Analytical methodology for the compound was required in our studies concerning relationships between chemical

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