CHEMICLEARANCE: PRINCIPLE AND APPLICATION TO IRRADIATED MEATS

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INTRODUCTION

CHEMICLEARANCE, the approach by which irradiated foods would be evaluated primarily on chemical data, is based on a straightforward principle: that if one food within a generic class of foods is wholesome when irradiated, then all members of that class would be chemically and toxicologically equivalent when similarly irradiated. Two key relationships underlie this principle. First, that wholesomeness is directly related to the types and amounts of radiolysis products formed. This relationship is phenomenological. Second, that the formation of such products depends on the composition of the food and on the irradiation conditions, particularly dose (Merritt et al. 1978b; Taub et al. 1979a). This relationship is a consequence of the chemistry in an irradiated food component being independent of other components and of the radiation energy being apportioned according to the weight fraction of each component. This consistent chemistry leads to common products and allows yields to be predicted.

Proper application of the chemiclearance approach to irradiated meats involves in part demonstrating this commonality of chemistry in relevant systems. One must demonstrate that reactions occur in which common intermediate species from specific components participate and ultimately lead to a general set of stable products. Results should be obtained from model systems of food-related compounds and compared with results on foods, where these components appear in a more complex state of organization. The intermediates could be detected and characterized; of the several techniques available, the most effective is electron spin resonance spectrometry. Final products are best separated and identified by GC/MS and high pressure liquid chromatographic (HPLC) techniques. For such a study, model systems of proteins and lipids are appropriate and should be compared with actual foods.

Proper application of chemiclearance also involves in part demonstrating the predictability of the products on the basis of food composition. One must demonstrate for products derived directly from general or specific components of the food that their yields are correlatable with the proportion of the precursor components in the food. Results from quantitative chromatographic measurements should be obtained from homogenized foods of different composition that have been irradiated under carefully controlled dose and temperature conditions. Experiments of this type have been carried out on model systems of myofibrillar proteins (particularly myosin) and of lipids (particularly tripalmitin and ethyl palmitate) and on enzyme-inactivated chicken, ham, pork, and beef to illustrate and validate the approach. These meats in practice would be irradiated at -40°C to about 4 Mrads to give them long-term shelf-stability. The results have lead to establishing radiolysis mechanisms and to demonstrating the radiolytic similarity in meats. As a consequence, it is justified to treat these meats as a generic class and eventually to evaluate pork, ham, and beef by comparing with chicken, which is currently undergoing extensive wholesomeness testing.

MATERIALS AND METHODS

SAMPLES of model systems and meats to be irradiated and subsequently examined by ESR or analyzed chromatographically were prepared in standard ways. Models for proteins include myosin and myofibrils extracted from beef and chicken. Models for lipids include tripalmitin and ethyl palmitate, used directly or purified by preparative chromatography. Meats formulated and enzyme-inactivated according to methods developed for radiation preservation (Wierbicki et al. 1975; Heiligman et al. 1979) include beef, pork, ham, and chicken. Fats from these beef, pork, and chicken preparations were extracted using chloroform. Samples for ESR examination were made into frozen rods 3mm x 15mm. Samples for chromatographic analyses were homogenized prior to irradiation and packed in evacuated tinplate containers.

Electron spin resonance measurements were made using an *in situ* irradiation/detection system described by Halliday et al. (1979). It utilizes a 10 MeV Linac and a specially modified Bruker 420R spectrometer. Free radicals can be examined under conditions at which they are stable or at which they decay with lifetimes on the order of 10 ms or more.

Chromatographic analyses of volatile compounds have been described by Merritt (1970; 1972) and of nonvolatiles by Vajdi et al.(1979). The volatiles are vacuum fractionated, separated by GC, and identified mass spectrometrically. The nonvolatiles are separated by HPLC size exclusion techniques and in some cases further separated and analyzed by GC/MS. Advances in the HPLC methodology allows one to characterize high molecular weight products derived from lipids.

RESULTS AND DISCUSSION

IRRADIATION of protein and lipid components of meats either as isolated models or in combination in the meats leads to similar free radicals that are precursors of the final degradation products. The radicals derive from specific radiolytic processes, and certain types predominate in proteins and lipids. A major consequence for

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the proteins is degradation and not aggregation. Degradation of the triglycerides comprising the lipids produces fatty acids, propane dioldiesters, CO_2, H_2, hydrocarbons, and adduct compounds in highest yields. The products correlate with the precursor components in the food. Some of the basic concepts, some results from ESR measurements on myosin, tripalmitin, and fats, and representative results from analyses of products from ethyl palmitate and the meats will be given and discussed.

Basic Radiolytic Concepts. Several concepts have to be kept in mind when considering the radiolysis of these systems. First, there are direct and indirect effects. The former relate to the radiolytic consequences of the energy being deposited in a particular component; the latter to consequences of a free radical formed from a major component reacting with substituents of lower abundance. Secondly, the amount of a product will increase linearly with dose (Taub et al. 1979a), the slope of the yield-dose dependence being related to "G-value," the number of molecules formed per 100 electron volts (eV) of energy absorbed in the system. Thirdly, the G-value will be significantly influenced by phase and temperature, particularly if the product is due to indirect effects (Taub et al. 1978; Merritt et al. 1980b).

ESR Measurements on Proteins (Myosin). The results obtained for myosin, the major myofibrillar protein, are representative for fibrous proteins. Upon irradiation at -40°C, the ESR spectra for myosin show a prominent doublet signal with smaller outer lines (Taub et al. 1979b). Based on experiments with acetyl amino acids and dipeptides (Sevilla et al. 1978; Taub et al. 1979b), these spectra are attributed to a collection of peptide radicals formed by the loss of a hydrogen from the CHR group in the peptide chain. The mechanism for forming these radicals involves cationic, anionic, and excited species. An anion radical having an electron attached to the carbonyl group undergoes a scission reaction leading to a deamidation radical, which subsequently abstracts a hydrogen from the protein to form the peptide radical. ESR measurements at -10°C show that the radical can then decay slowly; it does not persist in samples that have been thawed. Electrophoretic examination of unirradiated and irradiated myosin shows the loss of the myosin moiety, but no formation of a high molecular weight aggregate. Both the mechanism that is consistent with the ESR results and the electrophoretic evidence indicate that some degradation will take place, but that little if any dimerization will occur. Similar results have been obtained for the myofibrils from beef and chicken; similar ESR spectra are seen and similar changes in the electrophoretic pattern occur. The chemistry is independent of the state of organization of the protein molecules.

ESR Measurements on Tripalmitin. The results obtained for tripalmitin are representative for triglycerides. Upon irradiation between 0°C and 40°C, the ESR spectrum for tripalmitin shows an asymmetric five line spectrum. Based on studies with fatty acids and related compounds, this spectrum is attributed to the radical formed by the abstraction of a hydrogen alpha to the carbonyl group of a palmitic acid moiety in the tripalmitin. If the irradiation is carried out at -125°C, a broad, singlet-like spectrum with additional outer lines is observed. If the sample is warmed, the spectrum changes, and at -25°C reflects the contribution of other radicals including the abstraction radical (Halliday and Taub, 1980). The mechanism for forming these radicals also involves cationic, anionic, and excited species. The anionic route can be described as follows (where $RCH_2O(C{=}O)CH_2-(CH_2)_{13}CH_3$ stands for tripalmitin and in which R is the ethane dioldipalmitate moiety):

$$e^- + RCH_2O(C{=}O)CH_2(CH_2)_{13}CH_3 \longrightarrow RCH_2O(\dot{C}O^-)CH_2(CH_2)_{13}CH_3 \longrightarrow R\dot{C}H_2 + {}^-O_2CCH_2(CH_2)_{13}CH_3 \quad (1)$$

$$R\dot{C}H_2 + RCH_2O(C{=}O)CH_2(CH_2)_{13}CH_3 \longrightarrow RCH_3 + RCH_2O(C{=}O)\dot{C}H(CH_2)_{13}CH_3 \quad (2)$$

Reaction 1 implies the formation of palmitic acid and $R\dot{C}H_2$, the precursor of the propane dioldiester. Reaction 2 shows only one fate for $R\dot{C}H_2$, namely, formation of the abstraction radical; another fate could be the cross-combination with the abstraction radical to form an adduct. At higher temperatures, the abstraction radical decays, presumably leading to a dimer. Major aspects of the overall radiolysis of the triglycerides are indicated by these findings and the corresponding interpretation.

ESR Measurements on Meat Fats. Results on chicken, beef, and pork fat irradiated below -130°C and warmed until the spectra disappear show the conformity of the chemistry in the fats to the chemistry of tripalmitin and the similarity of the chemistry in all three meat fat systems. Each fat system shows the same conversion of a broad singlet at low temperature to a more complex spectrum at an intermediate temperature and ultimately to a five line spectrum at the highest practical temperature, above which the fat becomes less viscous and the radicals decay. Figure 1 shows the spectra for all three fats at about -40°C, each of which corresponds to a collection of radicals comparable to those observed in tripalmitin. These spectra are similar but not identical. The collection of radicals in each sample could differ slightly, primarily because each sample was held at different temperatures for different times. (A similar comparison for whole meat samples of chicken, pork, ham, and beef was reported by Taub et al. (1979a) in which all spectra are superimposable.) By implication, the same basic reactions of the triglycerides are occurring in these fats despite the different fatty acid composition and despite the slightly different physical environment.

Product Analyses of Irradiated Lipids. Chromatographic analyses of the volatiles and higher molecular weight radiolysis products of tripalmitin and ethyl palmitate substantiate the involvement of precursor radicals and provide evidence for specific routes of reaction. These analyses were done on samples irradiated over a wide range of dose to establish the linear dependence of products on dose and to obtain sufficient levels of minor products for detection and quantitation.

Tripalmitin products. Upon irradiation at -40°C and +25°C tripalmitin degrades by routes that produce palmitic acid, H_2, CO_2, pentadecane, propane dioldiester, palmitone, and as yet unspecified adduct compounds among the major compounds. The palmitic acid and propane dioldiester can be accounted for by reactions 1 and 2. The formation of pentadecane and CO_2 can be postulated as a decomposition of an acyloxy radical followed by abstraction of hydrogen from tripalmitin as follows:

$$CH_3(CH_2)_{14}CO_2\cdot \longrightarrow CH_3(CH_2)_{13}\dot{C}H_2 + CO_2 \quad (3)$$

$$CH_3(CH_2)_{13}\dot{C}H_2 + RCH_2O(C{=}O)CH_2(CH_3)_{13}CH_3 \longrightarrow CH_3(CH_2)_{13}CH_3 + RCH_2O(C{=}O)\dot{C}H(CH_2)_{13}CH_3 \quad (4)$$

Combination of the pentadecanyl radical with the tripalmitin abstraction radical could lead to one of the adduct compounds. More detail on this system will be reported elsewhere (Vajdi et al. 1980).

Ethyl palmitate products. Upon irradiation below and above the melting point, ethyl palmitate produces H_2, CO_2, palmitic acid, ethane, pentadecane, CO, ethanol, and adduct compounds. In the representative compound shown in reactions 1 and 2, the R group should be taken as CH_3 to correspond to ethyl palmitate. Thus, the ethane formed is a counterpart to the propane dioldiester. The ratio of products appears to depend on the phase and/or temperature, indicating the involvement of competitive pathways for reaction of some of these intermediates. Details of this study will appear elsewhere (Merritt et al. 1980b).

Comparison of Products in Irradiated Meats. Chemical analyses of volatiles and higher molecular weight compounds formed in irradiated meats, particularly beef and chicken, show that they are similar and consistent with model system studies. A compilation of the volatiles identified in beef and the levels observed for a ∿4.5 Mrad dose has been published (Merritt, 1972; Merritt et al. 1978b; Vajdi et al. 1979); a similar list is being developed for chicken. The volatiles are normally found in the 1-30 ppb range. Some of the less volatile compounds from beef have been reported elsewhere (Merritt et al. 1978a); the major ones are found in the ppm range. The differences in the levels of these products (normalized to the same dose) among the different meats can be correlated with the total fat content, fatty acid composition, and distribution of fatty acids comprising the triglycerides. Illustrative and representative results are presented below for randomly selected enzyme-inactivated samples of chicken, pork, ham, and beef that had been prepared for wholesomeness testing. A proximate analysis of these samples is given in Table 1.

Correlation of products with total fat. Products that are formed with equivalent probability in reactions common to all the triglycerides should correlate with the total fat content. Hydrocarbon compounds with 8 or fewer carbon atoms are formed from all the component fatty acids and would conform to this relationship. Hexane and hexene derived from the meats given in Table 1 show this conformity as Fig. 2 indicates. Similar results for octene have been published previously (Taub et al. 1979a). The yields of all volatile hydrocarbons have been shown to increase with increasing fat content by using beef samples having different fat contents (Merritt et al. 1978b). Consequently, the yields of hydrocarbons in a particular meat would be predictable knowing the dose and the fat content.

Correlation of products with fatty acid composition. Products formed in reactions that are specific to a fatty acid moiety of the triglycerides should correlate with the fraction of that fatty acid in the total fat. For the samples used in this study, the fatty acid composition is given in Table 2. Since most of these are similar, the best test of the correlation would be for a product from linoleate, which differs significantly between beef and chicken. One particular product derivable from linoleate, by decarboxylation of the acyloxy radical indicated in reaction 4, is heptadecadiene. Provided that the precursor radical is formed with equal probability in all systems and that the irradiation is carried out under similar conditions, the heptadecadiene yield (normalized to the same fat content) will depend on the percent linoleate. Results for beef, pork, and chicken are consistent with this relationship, about six times as much heptadecadiene being found in chicken as compared to beef (Merritt et al. 1980b).

Correlation of products with triglyceride composition. Products formed in reactions involving specific positions of the fatty acids on the glycerol backbone of the triglyceride should correlate with the abundances of

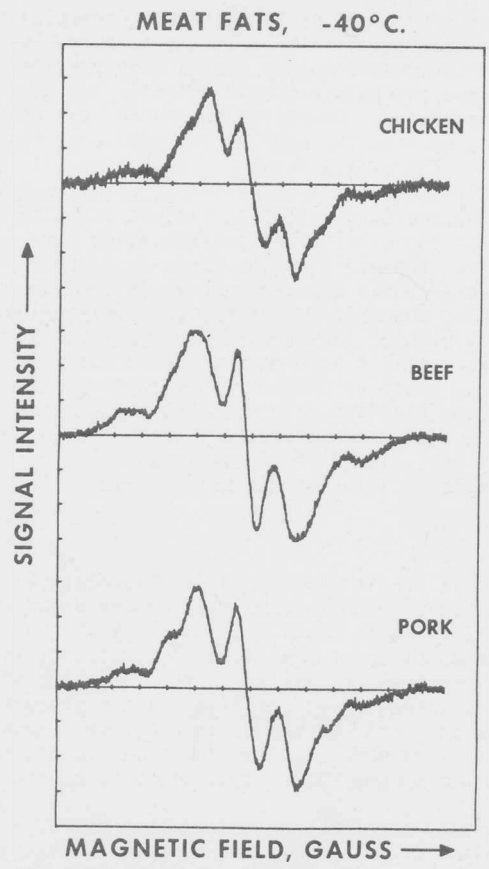

Fig. 1. ESR Spectra of Meat Fats at ∿40°C. Samples received ∿5 Mrads at temperatures between -135°C and -80°C and were all warmed at different rates to temperatures near -40°C.

Table 1. Proximate Analysis of Meat Samples

Sample	% Composition Fat	Water	Protein	NaCl	Ash
Pork	14.3	62.2	21.8	0.8	1.9
Ham	7.3	68.3	20.8	2.7	4.0
Chicken	11.7	65.9	21.1	0.8	1.9
Beef	15.4	59.5	23.4	0.9	2.0

Table 2. Fatty Acid Composition of Meat Fats

Fatty Acid	% of Total Fat Pork	Ham	Chicken	Beef
16:0 Palmitic	23.1	23.2	21.7	25.4
16:1 Palmitoleic	3.2	3.2	5.2	5.1
18:0 Stearic	11.3	10.9	6.3	12.5
18:1 Oleic	47.5	47.4	35.3	44.4
18:2 Linoleic	10.8	11.0	26.1	3.8

particular triglycerides in the fat. Formation of a propane dioldiester with palmitic acid involves loss of a fatty acid moiety from a triglyceride having at least two palmitic acid moieties, two of which remain unaffected. Depending on whether the moiety is eliminated from the ends or the middle of the molecule, a 2,3 or 1,3 propane dioldiester will be formed. Provided that this elimination is random and that appropriate weighting factors are used to account for the statistical possibilities for elimination, the yield of the isomeric propane dioldiesters should depend linearly on the sum of the weighted abundance of precursor triglycerides. Measured yields of the propane dioldiesters having either two palmitic acid moieties or a palmitic acid and an oleic acid moiety show this relationship, though statistically computed rather than measured triglyceride abundances were used in the graphical analysis (Merritt et al. 1980b). Further work on this relationship is being pursued.

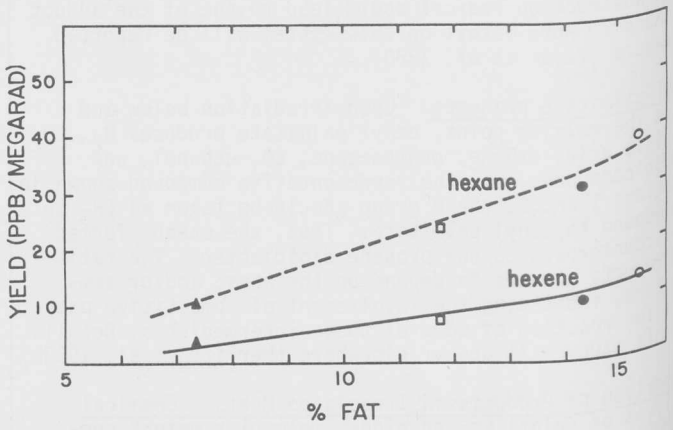

Fig. 2. Normalized Yield of Hexane and Hexene in Meats Irradiated at -40°C. Values shown correspond to the slopes of linear yield-dose plots for the meats. Symbols: ▲, ham; □, chicken; ●, pork; ○, beef. Dashed line corresponds to hexane; solid line to hexene.

CONCLUSION

COMMONALITY, predictability, and extrapolation characterize the conclusions that can be drawn from the results of this study. Commonality of the radiation chemistry for individual components in all the foods, which results in the formation of a limited number of key products from proteins and lipids, has been established. Predictability of the product yields from components, independent of their physical distribution in the food, on the basis of gross composition has been demonstrated. Extrapolation in a valid manner of results from model systems to actual foods, from one food within a generic class to the other foods in that class, and from one set of conditions to another (see also Taub et al. 1976) has been illustrated.

These results and the conclusions that follow from them support the chemiclearance efforts being pursued internationally. Clearing irradiated meats (including bacon) in the United States could follow a clearance given to irradiated chicken, by relying on the chemistry to interrelate all these meats. Clearing all major classes of foods irradiated up to a specified average dose, which is to be considered by the Joint Expert Committee on Food Irradiation in Geneva in October, rests largely on findings such as these. Not only are data now available on meats as a class, but similar data treating starches as a class are also available. Expectations are that chemiclearance will facilitate and promote the acceptance and commercial utilization of the irradiation process.

REFERENCES

HALLIDAY, J.W., Caspersen, J.M., Nickerson, C.L., Rees, C.W., and Taub, I.A. 1979. ESR studies of transient free radicals in irradiated food components. IEEE Trans. Nuc. Sci. NS-26: 1771.

HALLIDAY, J.W. and Taub, I.A. 1980. ESR characterization of free radicals formed in tripalmitin irradiated between -140°C and +40°C. (In preparation.)

HEILIGMAN, F., Wierbicki, E., Cohen, J.S., and Mason, V. 1979. Industrial production and quality of whole carcass beef rolls used in wholesomeness testing of irradiated beef. J. Food Proc. Preserv. (In press.)

MERRITT, C., Jr. 1970. Combination of gas chromatography with mass spectrometry. Appl. Spect. Rev. 3:263.

MERRITT, C., Jr. 1972. Qualitative and quantitative aspects of trace volatile components in irradiated foods and food substances. Radiat. Res. Rev. 3:353.

MERRITT, C., Jr., Angelini, P., and Nawar, W.W. 1978a. Chemical analysis of radiolysis products relating to the wholesomeness of irradiated food. "Food Preservation by Irradiation," Wageningen, Netherlands, IAEA, Vienna, 1:37.

MERRITT, C., Jr., Angelini, P., and Graham, R.A. 1978b. Effect of radiation parameters on the formation of radiolysis products in meat and meat substances. J. Ag. and Food Chem. 26:29.

MERRITT, C., Jr., Vajdi, M., and Nawar, W.W. 1980a. Quantitative study of the pathways involved in the formation of radiolysis products in ethyl palmitate. (In preparation.)

MERRITT, C., Jr., Angelini, P., and Vajdi, M. 1980b. A quantitative comparison of the yields of radiolysis products in various meats and their relationship to precursors. (In preparation.)

SEVILLA, M.D., D'Arcy, J.B., and Morehouse, K.M. 1979. An electron spin resonance study of γ-irradiated frozen aqueous solutions containing dipeptides: Mechanisms of radical reaction. J. Phys. Chem. 83:2887.

TAUB, I.A., Angelini, P., and Merritt, C., Jr. 1976. Irradiated food: Validity of extrapolating wholesomeness data. J. Food Sci. 41:942.

TAUB, I.A., Kaprielian, R.A., and Halliday, J.W. 1978. Radiation chemistry of high protein foods irradiated at low temperature. "Food Preservation by Irradiation," Wageningen, Netherlands, IAEA, Vienna. 1:371.

TAUB, I.A., Kaprielian, R.A., Halliday, J.W., Walker, J.E., Angelini, P., and Merritt, C., Jr. 1979a. Factors affecting radiolytic effects in food. Radiat. Phys. Chem. 14:639.

TAUB, I.A., Halliday, J.W., and Sevilla, M.D. 1979b. Chemical reactions in proteins irradiated at subfreezing temperatures. "Proteins at Low Temperatures," O. Fennema, Ed., Adv. Chem. Ser. 180:109.

TAUB, I.A., Robbins, F.M., Simic, M.G., Walker, J.E., and Wierbicki, E. 1979c. Effect of irradiation on meat proteins. Food Tech. 33:184.

VAJDI, M., Nawar, W.W., and Merritt, C., Jr. 1979. Identification of radiolytic compounds from beef. J. Am. Chem. Soc. 56:611.

VAJDI, M., Nawar, W.W., and Merritt, C., Jr. 1980. Effect of radiation parameters on the formation of radiolysis products in triplamitin. (In preparation.)

WIERBICKI, E. et al. 1975. Preservation of meats by ionizing radiation. 21st EMMRW, Berne.