sodium gentisate was added also showed no oxidized flavor development during the entire 6-month period. With copper added, an oxidized flavor became apparent at the fourth month of storage at the 0.15, 0.1, 0.05, and 0.01% levels of sodium gentisate. A comparison of these results with those obtained by the use of ethyl hydrocaffeate indicates that sodium gentisate is slightly more effective as an antioxidant in frozen whole milk even in the presence of added copper.

The trials with Ionol were discontinued at the end of the first month, since, under the conditions of the present experiment, the compound imparted a peculiar flavor to the milk and also had a strong destabilizing effect on the milk proteins.

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MEAT IRRADIATION

The Role of Glutathione and Methionine in the Production of Hydrogen Sulfide and Methyl Mercaptan during Irradiation of Meat

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Experiments designed to determine the origin of the major volatile sulfur compounds formed during the irradiation of meat were carried out by adding to ground meat S^{35}-DL-methionine and S^{35}-glutathione, and irradiating with gamma rays at dosages from 2 to 10 megarads. Most of the methyl mercaptan appeared to be formed directly from free methionine. Some mercaptan was produced during the irradiation of glutathione, possibly indirectly or as a secondary product. Although H_2S was found in both cases as a product of the irradiation of methionine and glutathione, the observed isotope dilutions indicated that most of the H_2S apparently originated from other sulfur-containing precursors in meat.

THE PRINCIPAL COMPOUNDS in the volatile fraction of the off-odor material produced by irradiation of beef have been shown to be methyl mercaptan and hydrogen sulfide (*1, 11*). Studies (*4*) on the irradiation of amino acids with gamma rays showed that, with increased dosage, increased amounts of mercaptans are produced from methionine, while H_2S is apparently the result of secondary reactions, since the amount produced is not correlated with dosage. The radiation degradation of S^{35}-labeled methionine in solution has been followed (*6*), and the products containing S^{35} have been identified as methyl mercaptan, inorganic sulfate, methionine sulfoxide, methionine sulfoximine, methionine sulfone, homocysteine, and homocysteic acid. At a total dosage of about 5.6×10^6 rads, about 3 to 4 times as much sulfate as methyl mercaptan was produced. A similar production of sulfate was observed in in vivo studies on S^{35}-methionine in irradiated rats (*8*).

Production of H_2S by the irradiation of cysteine and glutathione has also been studied (*3, 12*). However, the interrelationship between the formation of mercaptan, H_2S, and the degradation of glutathione and cysteine, was not developed in these investigations.

The present investigation was undertaken to ascertain whether methyl mercaptan or hydrogen sulfide, found in the off-odor material in meat during irradiation, originated from free methionine or from the sulfur-containing amino acids in peptide linkage. In principle, an isotope dilution method has been employed. Since the radiation reaction results in a number of different products, the amount of isotope recovered as labeled methyl mercaptan or H_2S gives a measure of the extent of each particular reaction. It may be assumed that the specific activity per gram-atom of S^{35} will not change, if the product is formed exclusively from the original radioactive component. Therefore, any decrease in molar specific activity should indicate the degree of formation of mercaptan or H_2S from sources other than the labeled material.

Experimental

Experiment 1, Single Dosage. Samples consisted of 20.0 grams of uncooked, lean ground beef muscle to which 100 to 250 μg. of S^{35}-DL-methionine, in a solution containing about 50 μg. of methionine per ml., was added. Samples were refrigerated and irradiated at 4° C. with Co^{60} (ca. 234,000 rads per hour) to a total dosage of 5×10^6 rads. Unirradiated controls were held in a refrigerator at 4° C. during the time the samples were irradiated.

After irradiation, both the control and the sample were rapidly transferred with 60 ml. of H_2O to ebullition tubes for determination of CH_3SH and H_2S. The procedure for mercaptan was that described by Sliwinski and Doty (*10*), in which a trapping solution of mercuric

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Table I. Composition and Activity of Control Samples before Irradiation[a]

Samples	S^{35} Methionine Added, μmoles	Total Methionine, μmoles	10^4 C.P.M.[b]	M.S.A.[c] 10^4 C.P.M./μmole
1	1.50	24.1	1005	42.0
2	1.50	25.5	886	34.7
3	1.50	26.0	867	33.3
4	0.63	26.6	2280	85.7
5	0.63	23.1	2324	100.6
6	1.27	23.3	1874	80.4
7	0.95	23.2	1373	59.2
8	0.95	23.3	1388	59.6

[a] μm. H_2S found = 0.03 ± 0.02. μm. CH_3SH found = 0.001 ± 0.002.
[b] Counts per minute.
[c] Molar specific activity.

Table II. Effect of Irradiation[a] on S^{35}-DL-Methionine in Meat Extract

Product	μmoles, %	Total Radioactivity, %	Original M.S.A., %
Methionine	96.1 ± 2.11[b]	91.93 ± 0.73	96.0 ± 2.55
H_2S	2.69 ± 0.28[b]	0.19 ± 0.028	7.7 ± 0.85
CH_3SH	2.03 ± 0.37[b]	1.79 ± 0.26	86.7 ± 6.86

[a] 5 × 10^6 rads.
[b] Relative to μmoles methionine present in control.

Table III. Isotope Dilution Effects in H_2S and CH_3SH Produced from S^{35}-DL-Methionine and S^{35}-Glutathione in Meat Irradiated at Various Dosages

Dosage 10^6 Rads	Per Cent Original M.S.A.[a] [b] H_2S	CH_3SH
	From Methionine	
0	0	0
2	0	15.0
6	5.5	22.8
10	21.8	8.7
	From Glutathione	
0	0	0
2	14.8	27.6
6	19.8	38.7
10	19.8	91.0

[a] Specific activity.
[b] Per cent dilution from other sources may be calculated by subtracting figures shown from 100.

acetate was employed. For H_2S, the procedure of Marbach and Doty (*9*) was modified by substituting the bismuth nitrate trapping solution of Koren and Gierlinger (*7*) for the cadmium hydroxide. Bismuth nitrate solution was capable of removing H_2S completely, without absorption of methyl mercaptan, even at the low levels encountered here. An absorption train was set up for trapping H_2S and CH_3SH, using bismuth nitrate and mercuric acetate solutions in sequence. The entire amount of trapping solution was used, in each case, for the colorimetric determination of H_2S and CH_3SH with *N,N* - dimethyl - *p* - phenylenediamine.

For counting purposes, a small aliquot was removed from each of the solutions used for the colorimetric determinations. These aliquots were neutralized with sodium carbonate and brought to a final dilution of 1 to 5. Planchets were prepared by evaporating 0.5 ml. of this final solution to dryness under an infrared lamp. Counts were corrected for the decay of S^{35} and for self-absorption by counting, with each set of samples, standards identical to the samples in composition, thickness, and age.

The meat residue and solution remaining in the ebullition tubes after volatiles had been removed were boiled for 5 minutes and filtered to remove the coagulated protein. The filtrate was brought to a volume of 100 ml. In order to obtain a suitable solution for counting, a 1- to 50-dilution of the final extract was made. Plating was carried out as above, using an aliquot of 0.5 ml.

The methionine content of the extract was determined by Bolling's modification of the Sullivan-McCarthy method (*2*), with the exception that 1.0 ml. of 1% nitroprusside reagent was used instead of the 0.3 ml. of 10% nitroprusside specified by Bolling. The extract was diluted 1 to 2 for use in this determination.

Calculations. To convert the raw data into meaningful figures, the amounts of methionine, H_2S, and CH_3SH found by chemical means were expressed as μmoles. Since each compound contained 1 gram-atom of sulfur, all samples were directly comparable on a micromolar basis. Specific activity was therefore calculated as counts per minute per μmole, or molar specific activity (M.S.A.). Since the only source of radioactivity was S^{35}-DL-methionine, the dilution effects could be expressed as per cent of the original molar specific activity:

$$\% \text{ M.S.A.} = \frac{\text{M.S.A. of product}}{\text{M.S.A. of original methionine}} \times 100$$

Yields of volatile products were based on total radioactivity:

$$\% \text{ Yield} = \frac{\text{counts per minute in volatile product}}{\text{total counts per minute in control}} \times 100$$

Experiment 2, Various Dosages. Two meat samples were prepared—one contained S^{35}-labeled glutathione, and the other, S^{35}-labeled DL-methionine. The same procedures outlined for Experiment 1 were followed, with the exceptions that methionine was determined microbiologically without hydrolysis of the sample, and glutathione was determined by the nitroprusside method of Grunert and Phillips (*5*). Samples were irradiated at dosages of 2, 6, and 10 megarads.

Results and Discussion

Table I gives the control data for the eight samples used in Experiment 1. These samples represent four groups of varying specific activity, as indicated. Table II shows the chemically determined amounts, in per cent, of H_2S and CH_3SH formed, and of methionine remaining after irradiation of the corresponding samples. In addition, the per cent radioactivity for each product, and the per cent change in the original molar specific activity are tabulated. All figures represent the mean of eight individual percentages plus or minus the standard deviation of the mean.

It is evident from the chemical determination of methionine that about 1 μmole was lost on irradiation. The loss of specific activity of methionine may be due to dilution with methionine from another source. However, this dilution effect is of the order of the standard deviation and may not be real.

The production of 0.5 μmole of methyl mercaptan appeared to be associated with the decrease in methionine. Furthermore, most of the mercaptan apparently came from methionine, since the molar specific activity of the mercaptan was only slightly lower than that of methionine. These data show that about 13% of methyl mercaptan came from sources other than free methionine. However, on the basis of the specific activity data, only 8% of the H_2S produced on irradiation originated from free methionine, with more than 90% coming from other sulfur-containing material present in the meat.

The amount of methyl mercaptan produced (as μg. per gram of meat) was in agreement with that found by Sliwinski

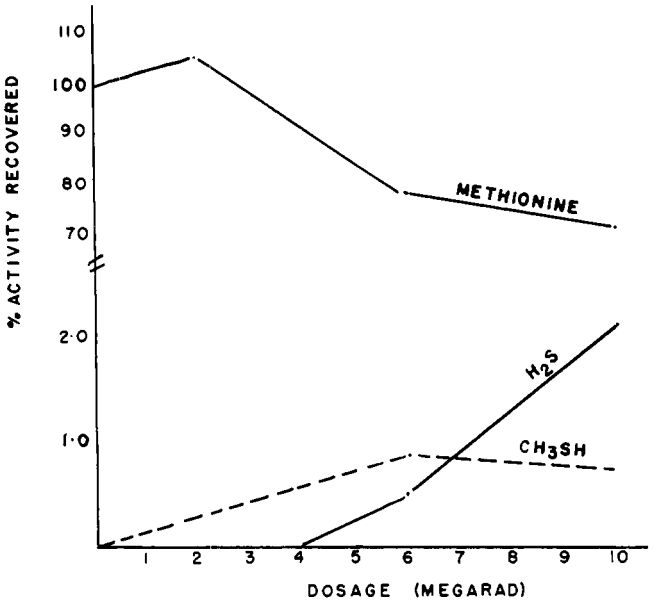

Figure 1. Formation of H_2S and CH_3SH by irradiation of S^{35}-methionine in meat

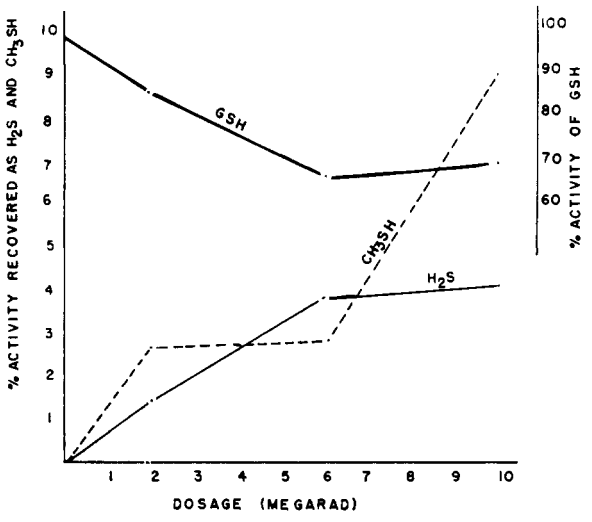

Figure 2. Formation of H_2S and CH_3SH by irradiation of S^{35}-glutathione in meat

and Doty (*10*) for equivalent dosages. Kopoldová *et al.* (*6*) have stated that 2.5% of the total radioactivity of aqueous DL-S^{35}-methionine solutions appears as volatile compounds, mainly methyl mercaptan, after irradiation at 3.6×10^{20} e.v. per ml. (about 5×10^6 rads). This figure is essentially the same as that obtained in the present study when meat was used. Since 1.8% of the total radioactivity was recovered as methyl mercaptan and 0.2% as hydrogen sulfide, the total recovered radioactivity was 2.0% of the original. Other volatile sulfur compounds which were not estimated in this study would increase this value, and bring it into even closer agreement with that of Kopoldová.

The per cent radioactivity, recovered as H_2S and CH_3SH, is equivalent to the product yield, and may be compared with the figures obtained by Duran and Tappel (*4*), i.e., 0.42% CH_3SH and 0.11% H_2S. The yield of mercaptan was approximately four times as great, and the yield of H_2S about twice as great, as that obtained by Duran and Tappel, at the same dosage. In addition, the amount of H_2S relative to CH_3SH was 10.5% as compared to 26%, calculated from Table II. While this would give further weight to the argument that H_2S is a secondary product of the irradiation of methionine, discrepancies in these values cannot be easily reconciled. However, two factors must be considered as influencing product yield. First, the concentration of methionine in meat is of the order of 1 m*M*, as compared to the 100 m*M* solutions of pure methionine used by Duran and Tappel. Furthermore, the presence of large amounts of other substances in meat may affect the course of the reactions of methionine during irradiation.

Since the data for Experiment 2 were limited and differed quantitatively from Experiment 1, only general conclusions could be drawn. Qualitatively, the results were not in disagreement, and the effects of dosage were clearly shown. The product yield, based on radioactivity, and the corresponding decomposition of S^{35}-DL-methionine and S^{35}-glutathione are shown in Figures 1 and 2. In the case of methionine, mercaptan formation appeared to be the predominating reaction at dosages of less than 6 megarads. At higher dosages, H_2S became the more abundant reaction product. Since the increase in H_2S was accompanied by a slight decrease in mercaptan, the former was probably produced from secondary reactions in the irradiation of methionine at higher dosages. The high loss of specific activity of methionine, observed in this series, may be due in part to the detection, by biological assay for free methionine, of methionine sulfoxide or similar products from protein-bound methionine. This would lead to abnormally high values for methionine and to low specific activity figures. This effect is minimized in Experiment 1 by the more specific chemical determination of free methionine.

The data obtained when glutathione was the source of labeled sulfur did not clearly indicate whether H_2S or CH_3SH was the primary product below dosages of 6 megarads. At higher dosages, methyl mercaptan was definitely produced in greater quantity, apparently at the expense of H_2S. There is no question that S^{35} from the glutathione was incorporated in CH_3SH at all dosages. Table III, which shows the isotope dilution effects for H_2S and CH_3SH from methionine and glutathione, indicates that, in both instances, more radioactive sulfur was contained in the mercaptan than in H_2S. A mechanism must therefore exist for the direct or indirect production of methyl mercaptan by the irradiation of glutathione as well as methionine.

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