Nature of Undesirable Odors Formed by Gamma Irradiation of Beef

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Precipitates were obtained by trapping the effluent gases from irradiated meat in solutions of lead acetate, zinc acetate, and mercuric cyanide. Analyses of these precipitates indicated sulfur compounds as the source of some undesirable odors in irradiated meat. Glutathione determinations indicated a considerable reduction of this and/or other sulfhydryl compounds during irradiation. Fractionation of beef to locate the source of some of the off-odors suggested that they are formed from some water-soluble compound(s).

THE POSSIBLE USE of high-energy beta or gamma-ray sources for the cold sterilization of food products has aroused considerable interest (*5*, *8*, *11*, *12*). Sterile products with excellent keeping qualities have been obtained in the absence of refrigeration, but, off-odors and colors have been undesirable by-products of this process. This work was done in an attempt to elucidate the nature of some of these off-odors and the compounds from which they are formed.

Radiation Source and Materials Used

The radiation used in these experiments came from a cobalt-60 irradiation source with an energy output of ~97,000 rep. per hour, refrigerated to 5.5° ± 2°C. The meat used was triple-ground sirloin (unless otherwise specified), purchased routinely from a local retail market. Approximately 160 grams of the ground meat packed in saran casing constituted the usual irradiation sample. A nonirradiated control was prepared in the same manner and stored at equivalent temperature conditions for the irradiation period, usually 16.5 hours (~1.6×10^6 rep.). This level falls within the generally accepted range for sterilization.

Results

Preliminary experiments showed that the undesirable odoriferous gases from irradiated meat could be trapped with dilute alkali or solutions of heavy metal salts. Precipitates were obtained with the heavy metal salts and the effluent vapors from the trapping solutions were completely free from the undesirable irradiation odor. To obtain the precipitates for additional study, nitrogen of high purity was bubbled through an irradiated meat slurry (150 grams of meat, 300 ml. of distilled water) and the effluent was passed through 3 to 5 ml. of a 4% solution of lead acetate, zinc acetate, or mercuric cyanide at 4 to 6° C. for 24 hours. A nonirradiated meat slurry and distilled water controls were run simultaneously with the irradiated meat sample.

With lead acetate solution a black precipitate was obtained from irradiated meat. This precipitate formed methylene blue when treated with 1 ml. of a 0.5% solution of *p*-dimethylaminoaniline in concentrated hydrochloric acid and 1 ml. of Reisner's solution (*10*). This indicated the presence of sulfide ion. Lead acetate paper gave a positive test. The odor of hydrogen sulfide was evident, but the predominant odor was characteristic of organic sulfides. The gases from irradiated meat prior to the heavy metal precipitation did not give these tests for sulfide ion.

With zinc acetate solution, a yellowish white precipitate was obtained which gave the same reactions as the lead precipitate.

Somewhat better yields resulted with mercuric cyanide solution. The precipitates obtained were a mixture of yellow and black, in proportions that varied from run to run. When precipitates were partially separated by repeated sedimentation in distilled water, the black precipitate settled first. Both were insoluble in strong mineral acids, but dissolved slowly in aqua regia. Both were very soluble in sodium sulfide solution. Ammonium sulfide changed the yellow precipitate to red, but had no observable effect on the black precipitate. In an effort to extract any mercury salts of organic sulfides, various organic solvents (methanol, ethanol, benzene, carbon disulfide, ethyl acetate, and acetone) were used without success. After these treatments the odor of organic sulfide still emanated from the precipitate when treated with aqua regia.

When the precipitate was treated with a hydriodic acid solution prepared by dissolving 5 grams of potassium iodide in 12 ml. of 10% sulfuric acid and diluting to 25 ml. (*7*), the precipitates dissolved readily with the evolution of an odor different from that obtained with aqua regia.

Approximately 0.2 mg. of heavy metal precipitate could be obtained from 150 grams of irradiated meat. No similar precipitate was ever obtained from the nonirradiated meat or distilled water blank.

Mercury salts of methyl and ethyl mercaptans (methane- and ethanethiol) were prepared for odor comparison by the addition of the appropriate mercaptan to 4% solutions of mercuric cyanide. The white salts were recrystallized once from hot ethanol. On addition of 10% sulfuric acid to these salts and comparison of the odors to those obtained from the lead and zinc precipitates, it was found that the odor from the mercury methyl mercaptide was most similar to that from precipitates obtained from irradiated meat. On treatment of this salt with hydriodic acid solution (prepared as previously described), the odor obtained was similar to that of the mercury precipitate from irradiated meat when subjected to the same treatment. This odor, in turn, was compared with

Table I. Influence of Irradiation (1.6 × 10^6 Rep.) on Glutathione Content of Beef

Condition of Meat	Pretreatment	Sample No.	Glutathione, Mg./100G. Meat: Nonirradiated	Glutathione, Mg./100G. Meat: Irradiated
Ground	None	1	15.36	5.96
		2	17.50	9.00
		3	25.00	11.75
		4	17.75	6.86
		5	22.00	3.75
		6	23.00	10.00
		7	23.25	8.12
		8	20.00	8.63
		9[a]	20.63	12.75
		10[b]	17.75	16.00
Intact pieces	None	11	23.00	10.00
		12	22.50	10.00
Ground	Evacuated and stored at 3° C. for: 5 days	13	23.75	6.25
	7 days	14	25.88	7.38
	12 days	15	23.75	6.25

[a] Fatty meat (ca. 15% fat).
[b] Excessively fatty meat (ca. 20% fat).

that of dimethyl sulfide and dimethyl disulfide; it was very similar to that of the latter.

As these findings indicated that the off-odors in irradiated meat are formed from sulfur-containing compounds, various methods were tested in an attempt to detect changes in the sulfur-containing amino acid content of irradiated meat: Block and Bolling's use of the Winterstein-Folin reaction for cystine (*2*), Bolling's modification of the Sullivan-McCarthy method for methionine (*2*), Lavine's method for methionine (*9*), and an adaptation of a method for the determination of glutathione (*6*). Only the last mentioned gave reproducible and meaningful results.

To decrease the possibility of errors due to the nonhomogeneity of the meat, a relatively large sample of meat was treated by cutting 10 grams of meat in a Waring Blendor for 1 minute with 60 ml. of cold 3% metaphosphoric acid and 20 ml. of cold distilled water. Enough sodium chloride (~30 grams) was added for saturation and the mixture was cut for 1 additional minute. The solution was centrifuged for 10 minutes, and the supernatant was filtered into a 100-ml. volumetric flask and made to volume with saturated sodium chloride solution. This procedure was carried out in the cold (4° to 6° C.). For the determinations, 4-ml. aliquots were pipetted into test tubes; 4 ml. of saturated sodium chloride solution was added to each tube, and allowed to equilibrate at room temperature for 10 minutes. One milliliter of 2% sodium nitroprusside solution and 1 ml. of 1.5*M* sodium carbonate–0.67*M* sodium cyanide solution were added. Each tube was read immediately in a Coleman Junior spectrophotometer at 520 mμ. Two per cent metaphosphoric acid solution saturated with sodium chloride was used as a blank. The standard curve was prepared from the appropriate concentrations of reduced glutathione in a 2% metaphosphoric acid–saturated sodium chloride solution. Absorbance values were proportional to the amount of glutathione in the range 0 to 200 γ of glutathione in 10 ml. of final colored solution.

Duplicate glutathione determinations were made on 30 different meat samples, with and without irradiation (Table I). The irradiated meat usually contained less than half as much glutathione as corresponding untreated samples. The amount of glutathione was reduced by irradiation in intact pieces as well as in ground meat. Removal of air (20-minute evacuation with a laboratory oil vacuum pump) and storage in sealed borosilicate glass tubes for several days did not prevent loss of glutathione during irradiation.

Table II. Influence of Irradiation (1.6 × 10^6 Rep.) on Glutathione Added to Ground Beef

Sample	Glutathione, Mg./100 G. Meat
Nonirradiated	17.50
Nonirradiated + 7.5 mg. GHS/100 g.	24.75
Irradiated	9.00
Irraidated[a] + 7.5 g. GHS/100 g.	12.00

[a] Glutathione added before irradiation.

The addition of glutathione to meat prior to irradiation noticeably enhanced the off-odor produced by irradiation. A recovery experiment (Table II) showed that the added glutathione, as well as that originally present in the meat, was destroyed by irradiation. Earlier work done in this laboratory on some of the nitrogenous constituents indicated small but significant differences between nonirradiated and irradiated meat (Table III). Creatinine nitrogen and the nonprotein nitrogen were increased by irradiation, while the soluble protein nitrogen fraction in general tended to decrease. Creatine, sulfhydryl, and volatile sulfur compounds were not consistently affected by irradiation. Although the changes induced were not great, the data suggest protein breakdown.

In accordance with this reasoning, various fractions of ground beef were separated and irradiated to determine the possible sources of some of the off-odors, following the general procedure outlined in Figure 1. The water extract, A, was concentrated by pervaporation (the solution was suspended in cellophane casings and placed in a current of dry air) prior to irradiation. After irradiation the usual off-odor was present. By using the ebullition procedure and trapping with mercuric cyanide solution, a precipitate was obtained. When this was treated with hydriodic acid solution, the usual undesirable odor was obtained. Residue B was washed several times with distilled water, centrifuged, and irradiated in the wet state. The undesirable irradiation odor resulted but was considerably weaker than in the original extract. A smaller amount of precipitate was obtained with mercuric cyanide; it gave the usual result with hydriodic acid.

When ammonium sulfate was added to extract A to 80% saturation and the resulting precipitate (residue D) was irradiated, the usual irradiation odor was produced but hydrogen sulfide was also present. The precipitate obtained with mercuric cyanide yielded hydrogen sulfide when treated with hydriodic acid. If residue D was dialyzed against running water for 24 hours (to a negative barium chloride test) to remove ammonium

Table III. Influence of Radiation on Some Nitrogenous and Sulfurous Constituents of Beef

Sample No.	Creatinine Nitrogen		Creatine Nitrogen		Soluble Protein Nitrogen		Nonprotein Nitrogen		—SH		Volatile Sulfur	
	Nonirradiated	Irradiated	Nonirradiated	Irradiated	Nonirradiated	Irradiated	Nonirradiated	Irradiated	Nonirradiated	Irradiated	Nonirradiated	Irradiated
	Milligrams per 100 Grams											
1	5.9	7.4	125	117	667	650	334	336	...	...	1.85	1.79
2	4.8	5.9	127	130	728	687	352	370	170	161	1.62	1.76
3	5.9	7.4	144	162	765	764	505	491	177	180	...	1.06
4	4.8	5.6	123	126	567	554	294	318	177	171	1.15	1.30
5	5.6	6.3	128	131	589	574	305	329	170	171	...	...
6	3.7	4.1	119	119	790	774	284	302	175	175	...	...
7	5.0	5.4	136	132	640	678	468	472	170	173	...	...

Samples 1–3 received 1.6×10^6 rep.
Samples 4–6 received 0.5×10^6 rep.
Sample 7 received 45,000 rep.

sulfate and then irradiated, a sweet burnt odor was present. In some cases the usual irradiation odor was also detectable. After dialysis and irradiation no precipitate was obtained with mercuric cyanide.

Supernatant C, dialyzed for 24 hours against running water, concentrated by pervaporation, and irradiated, gave a strong burnt odor. No precipitate was obtained with mercuric cyanide. In another experiment supernatant C was dialyzed against distilled water for 6 hours and dialyzate E was concentrated and irradiated. This solution had a strong odor of hydrogen sulfide, and gave a black precipitate with mercuric cyanide which, on treatment with hydriodic acid, gave off hydrogen sulfide. Supernatant C was further dialyzed against running water for 18 hours, pervaporated, and irradiated (residue F). A strong burnt odor was present. No precipitate with mercuric cyanide was obtained.

In later experiments, residue B was extracted with diethyl ether, and the extract, G, was evaporated and irradiated. The usual off-odor was not detected and no precipitate was obtained. Residue H was extracted several times with 10% sodium chloride solution, washed with distilled water, and irradiated in the moist state. No off-odor was observed and no precipitate formed with mercuric cyanide. Extract I was dialyzed against running water for 24 hours (negative silver nitrate test), concentrated by pervaporation, and irradiated. A different odor than heretofore observed was the result. No precipitate was obtained with mercuric cyanide.

Discussion

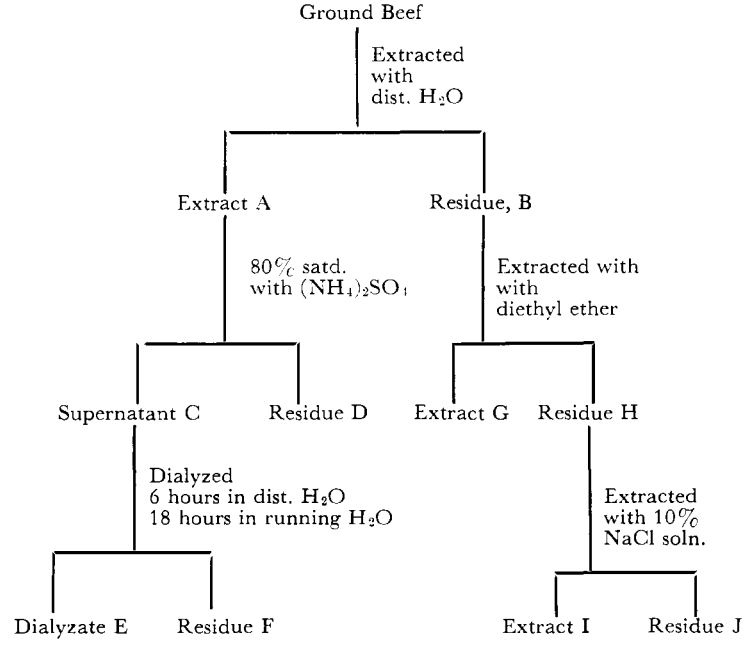
The nature of the heavy metal precipitates obtained with gases from irradiated meat indicates that sulfur-containing compounds contribute to some of the off-odors that develop in meat during irradiation. The results obtained indicate hydrogen sulfide as one of the components and, on the basis of subjective odor comparisons, methyl mercaptan as another. Because of the peculiar nature of the mercury precipitates, and the fact that attempts to form methylene blue directly from the effluent gases with *p*-dimethylaminoaniline were unsuccessful, it is necessary to consider the possible existence of another compound which releases hydrogen sulfide, methyl mercaptan, and other simple sulfur compounds on contact with the reagent used to obtain these precipitates. If the mercaptan were present as the straight mercury mercaptide in the precipitate, it should be extractable with ethanol. This further suggests a mercury complex of unknown nature. Efforts are now being made to obtain enough precipitate to resolve these problems.

The results obtained with glutathione determinations show a considerable loss of glutathione and/or some other sulfhydryl compound. This qualification is made on the basis of results obtained with preliminary chromatographic procedures. Though the presence of glutathione in the test extract is verified, other materials of a peptide nature are also present. Whether these substances have any effect on the determinations has not as yet been demonstrated.

Consistent with the results of these determinations are reports (*3*, *4*) of the formation of hydrogen sulfide from irradiated solutions of glutathione and cysteine. Two experiments in which solutions of glutathione were irradiated verified this. In one (*3*), evidence indicates that hydrogen sulfide formation from cysteine is independent of the presence of oxygen. The results obtained with the evacuated samples are in accord with this. The susceptibility of sulfhydryl compounds to radiation is covered in a report by Barron (*1*).

Methionine would appear to be the logical source of the mercaptan. However, the methods tried so far for determining this amino acid required hydrolysis with 20% hydrochloric acid or 5*N* sodium hydroxide. Recovery ex-

Figure 1. Diagram of fractionation procedure used to determine possible source of undesirable irradiation odors



periments showed extensive destruction of methionine by these procedures, which might account for the inconclusive results obtained.

The results obtained with the nitrogenous components, and those with the meat fractionation procedure, strongly suggest that the major odor-forming reactions occurring during irradiation involve the water-soluble proteins in meat. The appearance of hydrogen sulfide in the irradiated dialyzate, E, is in agreement with the results obtained with irradiated glutathione solutions, because glutathione should appear in this fraction. However, another alternative must be considered—that this fraction is necessary in the water extract, A, in conjunction with other substances to produce the usual off-odor observed there. This would also be compatible with the previous suggestion that the off-odor results from a compound that breaks down on contact with the trapping reagents to give the products obtained.

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ALFALFA CAROTENE

Effect of Added Animal Fats and Vegetable Oils on Stability of Carotene in Dehydrated Alfalfa Meal

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The effect of adding animal fats or vegetable oil to dehydrated alfalfa meal both in the laboratory and at an alfalfa dehydrator was studied. Increasing amounts of fat or oil from 1 to 5% increased the stability of carotene, reduced dustiness, and gave a greener appearing meal, but had little effect on the stability of green color. The use of fat and oil containing 5, 15, and 40% of free fatty acid had essentially the same effect on carotene and color retention as materials which contained little of these acids. Added fat or oil may increase carotene stability by bringing carotene and naturally occurring antioxidants of alfalfa into mutual solution, thus allowing the stabilizers to operate more effectively.

LARGE SUPPLIES of low-priced animal fats have stimulated interest in their use in animal feeds. Nutritional studies with poultry and cattle have shown that incorporation of low-grade animal fats in feeds, up to 5% of the ration consumed, results in efficient use of the fat with no apparent ill effects (*4*, *8*). More recently, it has been shown that the stability of vitamin A added to feeds as fish liver oil was increased when 6% of stabilized animal fats were also added (*10*). Earlier studies by this laboratory demonstrated that the stability of crystalline carotene added to feed ingredients was greatly improved by the addition of unstabilized oils (*1*). Mitchell *et al.* (*5*) showed that carotene retention during storage is influenced by amount of oil used in application of antioxidant to a meal. The present report on carotene stabilization in dehydrated alfalfa indicates also that additions of animal or vegetable fat without added stabilizers enhance the stability of the carotene in the meal during storage at room temperatures.

Experimental Procedures

For laboratory studies, the oils or fats were incorporated into the alfalfa meal by dissolving them in a minimum amount of petroleum ether and spraying on the meal in a mixing chamber (*11*).

Preparation of Mixtures For plant scale studies, an oil-metering unit (*12*) was employed. The oil was added to the chopped dried alfalfa as it came from the dryer prior to grinding, thus assuring thorough mixing in the hammer mill (*12*).

Storage Tests For storage studies, 2-gram samples of the meals were weighed into open shell vials (20 × 70 mm.). Samples were stored at either 25° or 65° C. (77° or 149° F.) (*11*). A petroleum ether extract of alfalfa meal dissolved in oil solution and crystalline carotene in oil solution were employed in several experiments for comparison with the alfalfa meal samples. They were stored under the same conditions as were the meal samples in shell vials. Details of the preparation and storage of such carotene solutions have been presented (*2*).

Laboratory Scale Application

To study the relative effectiveness of several vegetable oils for stabilizing carotene in alfalfa meal, 5% by weight of oil was added as indicated above to each in a series of meal samples. The meals were stored in a constant-temperature room at 25° C. After 4 months of