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PRODUCTION AND PREVENTION OF IRRADIATED ODOR IN BEEF*

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Over the past several years the Quartermaster Corps has been conducting a research program to determine whether radiation can supplement or replace refrigeration as a means of food preservation. With beef, the dose necessary for sterilization is responsible for what has been widely characterized as an "irradiated" or "wet dog" odor (10, 15). Until recently there has been no general agreement regarding the component or components in beef from which the odor arises (1, 3, 16). Similarly, there is no agreement regarding the nature of the components responsible for the odor (1, 5, 12).

The intent of this study was to determine the component or components of beef from which the irradiated odor arises, the chemical nature of the odor, and a means of preventing its production. The approach was to fractionate the aqueous extract produced by refluxing the beef previous to irradiation. Characterization of the implicated fraction could then be pursued.

EXPERIMENTAL

Preparation of fractions from beef. Twelve hundred grams of defatted beef chuck was diced and refluxed overnight with 1200 ml of a 10% saline solution. The extract was collected by filtration, concentrated to 250 ml under reduced pressure, and dialyzed, first with 10% saline, and then with water for several days with periodic changes. The dialyzable fraction was concentrated, the non-dialyzable fraction recovered from the tubing, and both fractions lyophilized. An undialyzed fraction was also lyophilized. From 1200 g of meat, 35 g of non-volatile solids were obtained. Approximately $\frac{2}{3}$ of the solids were non-dialyzable. In another study, the same fractionation was carried out on beef at 4° C.

Chemical analyses of the non-dialyzable fraction

a. *Nitrogen.* Protein nitrogen was determined by the Kjeldahl procedure and amino nitrogen by the Frame method (9).

b. *Phosphorus.* Phosphorus was determined gravimetrically as $Mg_2P_2O_7$ according to the A.O.A.C. official methods of analysis.

c. *Ash.* Magnesium nitrate was added to the sample prior to ashing at 590° C.

d. *Quantitative amino acid analysis.* A 5 mg, moisture-free sample was hydrolyzed with 6 N HCl at 105° C for 48 hr in a sealed tube which had been flushed with nitrogen. A hydrolysate was also prepared from a sample which had been irradiated. The hydrolysates were adjusted to pH 2.2, applied to a Dowex 50-x-5 ion exchange column and chromatographed according to the procedure of Moore and Stein (13). Fractions containing 2.5 ml were collected by means of a volume principle fraction collector, the tubes stoppered, refrigerated, and analyzed at convenience with ninhydrin after the method of Moore and Stein (14). Identity of the peaks was confirmed by preparation and chromatography of the 2,4-dinitrophenyl derivatives from pooled fractions obtained from the column (11).

e. *Bound iron.* A sample was hydrolyzed with 6 N HCl in a sealed tube at 105° C for 48 hr. The hydrolysate was placed on a IR-120 cation exchange column in the acid

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stage. The column was successively washed with de-ionized water to remove anions, with 2 N NH₃ to remove amino acids and other ampholytes, and with 3 N HCl to recover any cations present. The acidic eluant was then concentrated and an aliquot determined colorimetrically by the thiocyanate method. Corrections were made for the iron content of the HCl and NH₃.

Electrophoretic studies. The electrophoretic pattern of the sample before and after irradiation was obtained in veronal buffer at pH 8.7 and an ionic strength of 0.1.

Irradiation procedures

a. *General procedures.* Irradiation was performed in the gamma facility at Argonne National Laboratory, Lemont, Illinois. Unless otherwise stated, samples were sealed in evacuated metal cans and given a dose of 5 megarads. (A rad is defined as the absorption of 100 ergs of radiant energy in 1 g of the material being irradiated.) Samples were kept frozen at all times except during exposure in the facility. The samples were irradiated both as a 33% slurry and in the dry state.

b. *Multiple exposure.* Aqueous slurries were irradiated at from 1 to 10 megarads. After evaluation, they were dialyzed with 10% saline and with distilled water for several days employing periodic changes. The samples were then given an additional exposure to irradiation of 5 megarads.

c. *Addition of compounds prior to irradiation.* One and 2% concentrations of a number of compounds were added to the 33% slurry prior to irradiation.

d. *Removal of irradiated odor with additives.* After irradiation, sufficient compound was added to the sample to give a concentration of 1% of the total volume. Preliminary chromatographic studies on extracts from the irradiated non-dialyzable fraction

a. *Ammonium salts.* Aqueous, irradiated and non-irradiated protein samples were extracted with methylal and the extract made alkaline with ammonia. The ammonium salts were evaporated to dryness and aliquots chromatographed on Whatman No. 1 paper with an isopropanol-water-ammonia solvent mixture (200/20/10:v/v/v). Chromatograms were then developed with a series of diagnostic color reagents.

b. *Dinitrophenyl derivatives.* Methylal extracts, obtained as described, were treated with 1-fluoro-2,4-dinitrobenzene according to the method of Levy (11). The dinitrophenyl derivatives, found to be soluble in acid solution, were chromatographed on Whatman No. 1 paper employing a n-butanol-water-acetic acid system (60/25/15:v/v/v). The dinitrophenyl derivatives were also prepared by treating the sample directly with the reagent. They were then separated from the sample by dialysis prior to chromatography.

c. *Other derivatives.* Methylal extracts from aqueous, irradiated and non-irradiated protein samples were reacted with 3,5-dinitrobenzoyl chloride and also with 1-anthraquinonesulfonic acid. The 3,5-dinitrobenzoates were chromatographed on Whatman No. 1 paper employing a methanol-water-pyridine solvent mixture (160/40/8:v/v/v). The paper was then developed by spraying with a mixture of *α*-naphthylamine and potassium hydroxide. The 1-anthraquinonesulfonates were also chromatographed on Whatman No. 1 paper employing the n-butanol-acetic acid-water solvent system (60/15/25:v/v/v). The paper was developed by spraying with a mixture of chloroplatinic acid, potassium iodide and hydrochloric acid (Toennies reagent).

RESULTS AND DISCUSSION

Irradiation of the fractions. After irradiation of the fractions with 5 megarads, the samples were evaluated by an informal odor panel and the following conclusions were made. As compared with the non-irradiated controls, the undialyzed extract and the non-dialyzable fraction developed the irradiated odor while the dialyzable fraction developed only a slight off-odor characterized as being "grassy." The residue from the extraction also developed a slight amount of the irradiated odor. This was expected because no attempt had been made to extract the meat exhaustively. It therefore appears that the irradiated odor comes primarily from the non-dialyzable fraction. These results are summarized in Figure 1.

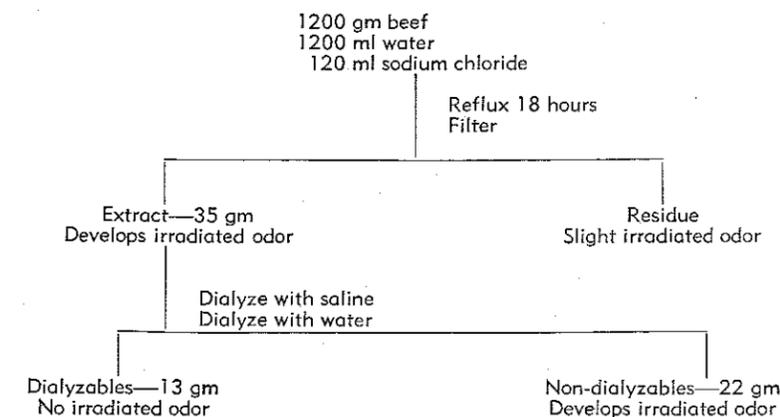


Figure 1. Irradiation of fractions from beef.

In another experiment in which the extraction was carried out at 4° C, the results obtained were only slightly altered. Compared with the non-irradiated controls, the original extract and the non-dialyzable fraction developed the irradiated odor. The odor of the dialyzable fraction seemed improved upon irradiation while the extracted residue again developed a small amount of the irradiation odor.

These results indicate that the non-dialyzable fraction is largely responsible for the development of the odor. It should be emphasized, however, that other off-odors are developed in beef upon irradiation. These may arise from interaction of this fraction with the fat or with the low molecular weight material, or from one of these fractions independently. Furthermore, the fractionation procedure may have altered or degraded the system so that additional odor producing reactions cannot occur.

Chemical and physical characterization of the non-dialyzable fraction. Since the non-dialyzable fraction was expected to consist mainly of protein, several analyses were performed for confirmative purposes. By Kjeldahl procedure the sample analyzed for 16.04% nitrogen which would be 100.25% protein assuming a conversion factor of 6.25. The amino nitrogen accounted for 13.6% of the total sample weight. The sample was found to contain 0.76% ash. Of this, 0.21% was phosphorus and 0.021% iron. Assuming a calculated average N/P ratio, approximately 5% of the sample was nucleoprotein. The detection by chromatographic methods of hypoxanthine in the sample volumes for these peaks and comparative corrected relative retention volumes served as additional evidence for the presence of nucleoproteins.

The iron was also assumed to be bound to the protein. The amount found would be expected with muscle proteins.

Quantitative amino acid analysis. The non-dialyzable fraction was irradiated and subsequently subjected to acid hydrolysis and analysis according to the Moore and Stein procedure. Compared with the non-irradiated control, several changes in ninhydrin reactive compounds were noted; however, no new compounds were found. Cysteine and methionine could no longer be detected, while the content of histidine decreased by approximately 25% as a result of irradiation of the protein. The content of serine was increased by

TABLE 1
Amino acid content, moles x 10⁻⁴/gm

| Peak no | Probable identity | Control | Irradiated | Peak no | Probable identity | Control | Irradiated |
|---------|-------------------|---------|------------|---------|-------------------|---------|------------|
| 1 | Cysteic acid | 0.35 | 0.35 | 11 | Glycine | 2.05 | 1.80 |
| 2 | Taurine | 4.25 | 3.80 | 12 | Alanine | 4.40 | 4.60 |
| 3 | Unknown | 1.45 | 1.25 | 13 | Unknown | 2.55 | 2.40 |
| 4 | Unknown | 1.55 | 1.60 | 14 | Valine | 3.50 | 3.40 |
| 5 | Hydroxy proline | 1.60 | 1.75 | 15 | Cysteine | 0.80 | 0.00 |
| 6 | Aspartic acid | 8.00 | 8.20 | 16 | Methionine | 0.75 | 0.00 |
| 7 | Threonine | 9.25 | 9.30 | 17 | Leucine | 0.45 | 0.30 |
| 8 | Serine | 6.40 | 7.20 | 18 | Aromatics | 5.95 | 5.45 |
| 9 | Proline | 0.85 | 0.65 | 19 | Lysine | 5.15 | 4.85 |
| 10 | Glutamic acid | 2.30 | 2.25 | 20 | Histidine | 2.60 | 1.95 |
| | | | | 21 | Arginine | 2.95 | 3.15 |

Tryptophan not analyzed.

12.5%. Proline and the aromatic amino acids were decreased by less than 10% and the remaining amino acids were not markedly affected (Table 1).

These observations confirm previous reports such as those by Dale and Davies (6) on the lability of cysteine, by Schwartz and Clark (17) on the lability of methionine and proline, and by Drake, Giffie, Johnson and Koenig (7) on the lability of histidine.

Electrophoresis of the fraction. The sample was also studied electrophoretically before and after irradiation and was found to contain at least 2 proteins, one of which was increased by irradiation, apparently at the expense of the other peak which was noticeably decreased. Whether this represents an actual formation of one protein from the other or merely a conversion to a third protein which migrates at the same rate under the conditions employed is not known.

This pattern change would not be unexpected as it has been shown that when irradiated chicken plasma protein is subjected to paper electrophoresis, spots appeared which were not present in the original protein (4). Both fragmentation and polymerization of proteins by ionizing radiation have been reported. Svedberg and Brohult (18), using radon as a source of alpha particles, observed that hemocyanin was split in two parts. Fernau and Pauli (8) noted decreases in viscosity with irradiated proteins. This decrease in viscosity, as well as bleaching in color, has been noted in the non-dialyzable fraction which is the subject of this report.

Effect of irradiation in the dry state on development of the irradiated odor. When the lyophilized sample was subjected to 5 megarads of irradiation, little or no odor was present. However, when water was added to the sample, the typical odor developed. That irradiated proteins release their odor upon addition of water has also been shown with globin, bovine plasma albumin, egg albumin, blood albumin and sodium caseinate (2). The mechanism responsible for release of the odor is not understood.

Multiple exposure to radiation. A series of samples (33% slurries) was subjected to increasing doses of irradiation ranging from 1 to 10 megarads at one megarad intervals. When the odors of these samples were evaluated by an informal panel the maximum odor was judged to be produced by 3 or 4 megarads. There was an increase in odor with increasing dose from

1 to 3 megarads, and at 10 megarads the character of the odor had changed and was judged to be no more, and possibly less, offensive.

It was found that the irradiated odor could be removed by dialysis in 10% sodium chloride solution. Therefore, in order to determine whether production of odor was finite, this series of samples was exhaustively dialyzed and reirradiated with 5 megarads. Results showed that the sample previously given a dose of only one megarad produced the most odor and that the sample previously given 2 megarads produced a small amount of irradiated odor. The rest of the samples exhibited little or no odor. These data provide presumptive evidence that only a finite amount of odor is produced from this source.

Quenching of odor by additives. The irradiated protein (a 33% slurry) and the non-irradiated control were treated with a number of reagents to determine whether the odor could be quenched. Of those employed, only mercuric acetate, sodium p-chloromercuribenzoate, n-ethyl maleimide and sodium hydroxide decreased the odor. Hydrochloric acid, ninhydrin and 2,4-dinitrophenylhydrazine did not quench the odor. When cysteine was added to the irradiated sample which had been made odorless with sodium p-chloromercuribenzoate, part of the original odor returned. These data suggest that compounds with active hydrogens, probably sulfur containing, were responsible for the odor.

Prevention of irradiated odor formation. A number of compounds were added to the protein slurries prior to irradiation in an attempt to learn whether the production of odor could be prevented or masked. Table 2

TABLE 2
Effect of additives¹ to beef protein slurries on development of irradiation odor 5 megarads

| Additive | Result |
|-------------------------------------|---|
| Cystine..... | No improvement, butyl mercaptan |
| Cysteine..... | No improvement, H ₂ S odor |
| Methionine..... | No improvement, oxidized methional (?) odor |
| Ascorbic acid..... | Acid odor, "sour" |
| Maleic acid..... | Reduced odor, becomes "tallowy" |
| Hydroquinone..... | Reduced odor |
| Sodium p-chloromercuribenzoate..... | Odor removed except for slight background |
| n-Ethyl-maleimide..... | Odor removed except for slight background |
| Mercuric acetate..... | Odor removed except for slight background |

¹ 1 and 2% of the total volume.

summarizes the additives studied and the results which were observed. No apparent differences were observed if the additive concentration was raised from 1 to 2% of the total volume. It therefore seems unlikely that further increases in the additive concentration would measurably affect the results obtained under the conditions employed. It cannot be determined from these data whether the effective compounds actually protected the sample from irradiation damage or merely combined with the odor compounds as they were formed.

Preliminary chromatographic studies. Aliquots of an ammonium salt mixture prepared from a methylal extract of the irradiated non-dialyzable

fraction were chromatographed and treated with diagnostic color reagents. The irradiated sample did not give a positive diazotized sulfanilic acid reaction indicating the absence of aromatic amines and phenols. The 2,6-dichloroquinone chloroimide test for thiol imidazoles was also negative. The aroyl glycine test for substituted hippuric acids and related compounds gave a faint red spot at R_f 0.20 with the irradiated sample only. The ninhydrin test was negative. Chromatographic studies on preparations obtained by treatment of the sample with 1-fluoro-2,4-dinitrobenzene, 3,5-dinitrobenzoyl chloride and 1-anthraquinonesulfonic acid demonstrated that irradiation has altered the protein in such a manner as to make available for reaction at least two additional compounds which were not available in the original sample. Efforts to isolate and identify these compounds are in progress.

SUMMARY

The irradiation odor of beef has been shown to arise chiefly from a water soluble, non-dialyzable fraction. This fraction is a mixture of at least two electrophoretically separable proteins. It appears that the odor is associated with sulfhydryl or closely related compounds since cysteine and methionine could no longer be detected in the protein after irradiation and since the odor is quenched by sulfhydryl reagents. The protein was found to contain small amounts of iron and phosphorus.

It was shown that the irradiated odor was not released until water was added to the protein irradiated in the dry state. Evidence is presented that only a finite amount of odor can be produced from the water-soluble protein fraction.

Paper chromatographic studies have demonstrated that reagents such as 1-anthraquinonesulfonic acid, silver nitrate-bromphenol blue, 1-fluoro-2,4-dinitrobenzene, 3,5-dinitrobenzoyl chloride, and platinum iodide formed derivatives with compounds present in the irradiated protein but not in the original sample.

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