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IRRADIATION EFFECTS IN MEAT

Detection of Amines Produced on Irradiation of Beef

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The volatile bases in beef were studied in an effort to identify the compounds responsible for the characteristic unpleasant odor of beef preserved by irradiation. The beef used in this work received 2.33 and 3.72 megarads of gamma radiation, and its odor was due in part to volatile bases that were measured quantitatively by the Conway-Byrne microdiffusion method and by gas chromatography. The amine fraction of the volatile bases was estimated quantitatively by a colorimetric method and by gas chromatography. Methylamine and ethylamine were identified by gas and paper chromatography as the major components of the amine fraction, with at least four other amines being detected also.

THIS STUDY was undertaken to identify the organic compounds responsible for the characteristic unpleasant odor of beef preserved by gamma radiation. Prior to this work, several groups of workers had found chemical changes that were significant, but amines had not been reported as a cause of the objectionable odor. Batzer and Doty (*2*) found abnormally large amounts of volatile sulfur compounds in irradiated beef. Sribney, Lewis, and Schweigert (*11*) found carbonyl compounds and peroxides. Sharpless, Blair, and Maxwell (*10*) found ethylamine in irradiated aqueous solutions of alanine. Tolbert and Noller (*12*) found that irradiation of dry glycine produced methylamine. Because amines have such powerful unpleasant odors it seemed important to determine whether they are components of the undesirable odor of irradiated beef.

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Experimental Methods

Preparation of Irradiated Beef. The beef used was boneless sirloin butt that had been trimmed of excess fat and ground to ensure thorough mixing. Each batch of meat was packed in No. 2 cans, frozen in dry ice, and shipped to the Materials Testing Reactor, Arco, Idaho, where one third of the cans received an irradiation dose of 3.72 megarads, and another third received 2.33 megarads of gamma radiation from waste fission products. The remaining third of the cans constituted the control samples, receiving identical treatment with regard to shipping and temperature, but no exposure to irradiation. All samples subsequently remained in dry ice until they were opened for examination.

Detection of the Volatile Bases in the Odor. The contribution of the volatile bases to the odor of irradiated meat was appraised by acidifying the condensate obtained from lyophilization of the meat. The condensate from irradiated meat had the usual wet-dog or burnt-hair odor which could be modified considerably by acidification to pH 2 with hydrochloric acid. The acidified condensate from irradiated meat still had a stronger odor than the condensate from unirradiated meat, but much of the recognizable unpleasant odor had been eliminated by the addition of acid.

Measurement of Total Volatile Bases. The Conway-Byrne (*3, 4*) microdiffusion technique was used to measure the total volatile bases in irradiated and unirradiated beef. The moisture in the original meat was determined by the AOAC method (*1*). Samples for the study were taken from two entirely separate batches of meat and from different cans within each batch.

Extracts were prepared by chopping 100 grams of meat with 200 ml. of water for 5 minutes in a Waring Blendor. The resulting mixture was centrifuged and then filtered. The filtrate was diluted with an equal volume of water, and 1.0 ml. of the resulting solution was placed in the Conway-Byrne unit using saturated potassium carbonate solution to liberate the volatile bases. The major

portion of the bases distilled over in 3 hours, but the process was continued for a total of 5 hours at 27° C. Table I lists the results and the standard deviations of the Conway-Byrne analyses. The figures shown for microequivalents per gram of whole meat were calculated from the measured total volatile bases.

Isolation of Volatile Bases as Hydrochlorides. The volatile basic and neutral components of beef were separated from the nonvolatile ones by lyophilization. The procedure was as follows: Ground beef, 200 grams, was chopped in a Waring Blendor with 200 ml. of water for 5 minutes, and the slurry was clarified by centrifuging. To 100 ml. of the clear, red solution was added 4 ml. of saturated potassium carbonate solution. The solution was divided among three 100-ml. round-bottomed flasks and frozen in dry ice. Lyophilization at 10 to 50 microns pressure required 6 to 10 hours. The thawed distillate was a colorless solution of the volatile basic and neutral components in water. It was acidified with dilute hydrochloric acid to make the pH less than 2 and it was lyophilized again. The residues from this procedure were white crystalline solids weighing 6 to 25 mg. Table II lists the yields from isolations performed under similar conditions, and comparison shows that the yield increased with increasing radiation.

Comparison of the data in Tables I and II shows that lyophilization yielded 40 to 60% of the total available volatile bases.

Identification of Ammonia in the Volatile Bases. Infrared spectra and x-ray diffraction patterns of the unknown base hydrochlorides were essentially identical with those of ammonium chloride. Other compounds present were not detected positively by either of these methods.

Colorimetric Estimation of Amines in the Volatile Bases. The method of Perry (*5*, *9*) and his associates was used to estimate the amines in the volatile bases of irradiated and normal meat. The procedure adopted was as follows: The hydrochloride, 5 mg., was dissolved in 10.0 ml. of isopropyl alcohol. A 1.0-ml. aliquot of the solution was placed in a 10-ml. volumetric flask with 5 ml. of pyridine which had been purified by distilling first from phthalic anhydride and then from solid potassium hydroxide. Ninhydrin solution, 2.0 ml., was added and the solution was diluted to 10.0 ml. with isopropyl alcohol. This ninhydrin solution had been prepared by dissolving 0.2 gram of recrystallized ninhydrin in 20.0 ml. of pyridine and diluting to 100 ml. with isopropyl alcohol. The final solution was heated for 7 minutes at 85° C., then cooled in tap water. The spectra of the solutions were recorded within 30 minutes on a Beckman DK2 spectrophotometer. A representative set of curves is shown in Figure 1. Although the peaks in the absorbance curves of the hydrochlorides from meats did not correspond exactly to those in the spectrum of methylamine hydrochloride, there was so much similarity in the curves that methylamine was used as a standard to estimate the amount of primary amines in the volatile bases (Table III).

Table I. Total Volatile Bases of Irradiated and Unirradiated Beef

	Wet Basis		Dry Basis
	Microequivalents/gram	P.P.M. calculated as NH_3	P.P.M., Calculated as NH_3
Unirradiated[a]			
Av. (7)	7.05	120	364
Std. dev.	0.25		
2.33 megarads			
Av. (7)	8.14	139	421
Std. dev.	0.50		
3.72 megarads			
Av. (8)	8.52	145	443
Std. dev.	0.84		

[a] Number of determinations in parenthesis.

An attempt was made to use fluorodinitrobenzene as a colorimetric reagent. McIntire, Clements, and Sproull (*8*) have proposed a method which involves conversion of amines to yellow dinitrophenylamines. Primary and secondary dinitrophenylamines produce slightly different absorption peaks. The procedure was modified to suit the quantities involved in the base hydrochlorides of meat, but there was considerable variation between samples in spectral absorbance, location of the main peaks, and background intensity.

Paper Chromatography of the Base Hydrochlorides. The mixed base hydrochlorides, as isolated from meat by lyophilization, were not suitable for chromatography on paper because of the excessive quantity of ammonium chloride

Table II. Yields of Base Hydrochlorides Obtained from Beef by Lyophilization

Sample No.[a]	Yields of Salts: As obtained, mg.	Yields of Salts: Wet meat, mg./g.	Yields of Salts: Dry meat, mg./g.	Calculated as NH_3, Dry Basis, P.P.M.
Unirradiated				
Av.	10.4	0.17	0.45	140
Std. dev.	5.0			
2.33 megarads				
Av.	13.0	0.21	0.57	180
Std. dev.	4.5			
3.72 megarads				
Av.	18.1	0.30	0.71	260
Std. dev.	6.3			

[a] Each average was taken from seven measurements.

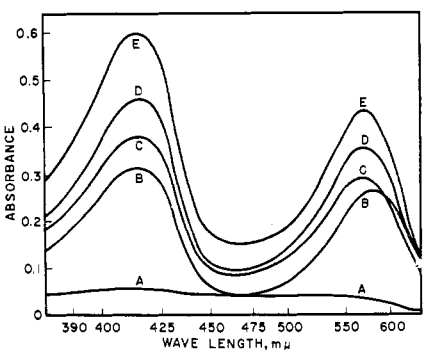

Figure 1. Spectra of amine-ninhydrin reaction products

A. 0.9 mg. of base-HCl from unirradiated meat
B. 5×10^{-7} mole of Ch_3NH_3Cl
C. 0.8 mg. of base-HCl from meat irradiated with 2.33×10^6 rad
D. 0.6 mg. of base-HCl from meat irradiated with 2.33×10^5 rad
E. 2.0 mg. of base-HCl from meat irradiated with 3.72×10^6 rad

Table III. Colorimetric Estimation of Volatile Bases in Beef by Reaction with Ninhydrin

Sample Number	Base HCl, Mg.[a]	Calculated Amines[b] in Base HCl, %	Amines, P.P.M.[c]
Unirradiated			
1	12.1	0.28	0.70
2	12.4	0.29	0.75
3	9.3	0.46	0.89
Av.	11.3	0.3	0.8
2.33 megarads			
1	13.7	6.45	18.3
2	9.4	4.95	9.64
3	11.5	3.05	7.29
4	19.1	2.21	8.72
Av.	13.4	4.2	11.0
3.72 megarads			
1	21.9	1.82	8.3
2	25.3	4.61	24.2
3	22.7	2.54	12.9
Av.	23.3	3.0	15.1

[a] Isolated from 61 grams of meat.
[b] Calculated as methylamine hydrochloride from a calibration curve prepared using solutions of methylamine hydrochloride.
[c] Calculated as methylamine in the dry weight of meat. These figures are listed as obtained. Correction for the 40 to 60% yield of the lyophilization step would make them twice as high, and would provide an estimated value for comparison with Tables I and II.

present. The ratio of amines to ammonia was increased by extracting the mixed hydrochlorides, for 5 minutes, with 1.0 ml. of dry isopropyl alcohol at 80° C. The resulting solution could be chromatographed without streaking.

Four different solvents were used to chromatograph the amine hydrochlorides. They were:

n-Butyl alcohol–acetic acid–water (4-1-5 by volume), upper layer

n-Butyl alcohol saturated with water

n-Butyl alcohol–isopropyl alcohol–water (3-5-2 by volume)

Phenol saturated with water

Known compounds were chromatographed simultaneously with the hydrochlorides from meat. Two of the unknown spots coincided consistently with those of methylamine and ethylamine hydrochlorides (Table IV). In addition, there were four or more other spots which were seen in some chromatograms but were not correlated with known compounds.

When chromatograms were run with comparable quantities of material, the spots from irradiated meat were invariably much darker than those from unirradiated meat. When disproportionately large quantities of extract from the unirradiated samples were chromatographed in order to reveal the methylamine and ethylamine, spots appeared which were not seen in the irradiated material. This explains the larger number of spots listed in Table IV for the unirradiated samples.

In addition to chromatograms in the four solvents listed above, the mixed base hydrochlorides were converted to the N-substituted 2,4-dinitrophenylamines and chromatographed. Again, spots were obtained corresponding to methylamine and ethylamine (Table IV). As a further check, an irradiated mixture was chromatographed as the hydrochlorides in *n*-butyl alcohol–acetic acid–water, and the two areas corresponding to methylamine and ethylamine were cut out and eluted separately with dilute hydrochloric acid. The resulting solutions were converted to the dinitrophenylamines and chromatographed separately with known compounds. Correlation was again satisfactory between methylamine, ethylamine, and the two unknown amines.

The dinitrophenylamine derivatives were prepared by reaction with fluorodinitrobenzene by an adaptation of the method of McIntire (*8*). The reagents were purified as follows: The fluorodinitrobenzene (Eastman White Label) was recrystallized from ethyl alcohol. The cyclohexane was shaken successively with concentrated sulfuric acid, water, and sodium hydroxide solution, dried over anhydrous potassium carbonate, decanted, and distilled. Absolute ethyl alcohol was refluxed with potassium hydroxide and distilled. Dioxane was purified according to Fieser's procedure (*6*) using hydrochloric acid and sodium. The following procedure for the preparation of the dinitrophenyl derivative was used: A solution containing 250 γ or less of the base hydrochloride in 0.05 ml. of water was mixed with 0.027 ml. of an ethyl alcohol solution of fluorodinitrobenzene (0.15 ml. of fluorodinitrobenzene in 2 ml. of ethyl alcohol). A sodium bicarbonate solution, 1*M*, 0.030 ml., was added, and the resulting solution was kept at 60° C. for 20 minutes. Sodium hydroxide, 1.0 ml. of 0.25*N* solution in 60% aqueous dioxane, was added and the solution was kept at 60° C. for 1 hour. The resulting solution was diluted to 5 ml. with water and shaken vigorously with 5 ml. of cyclohexane. The extraction was repeated three times with fresh cyclohexane. The cyclohexane was evaporated, at room temperature, in a stream of nitrogen, and the residue was dissolved in acetone for spotting on the chromatograms. The best solvent system after many trials was Skellysolve C (largely *n*-heptane)–methanol–water in the proportions of 10-8-2 by volume, the upper layer being used. It was found that 0.01 to 0.02 μmole (2 to 4 γ), but no more, could be chromatographed from a small spot without streaking, and this amount was readily visible. The ethylamine derivative tended to streak less than the methylamine derivative.

Gas Chromatography of the Volatile Bases. Gas chromatography was far less sensitive than paper chromatography in revealing the amines in the meat. However, when sufficiently large samples were used, gas chromatography provided supporting information on the number, identity, and quantity of the amines present. First, it was necessary to find a column-packing material that would separate ammonia from amines. Fatty alcohols and paraffin on kieselguhr, as used by James, Martin, and Smith (*7*), did not adequately separate the compounds.

All experiments were performed with the Burrell Kromo-Tog, Model K-1. Eventually an acceptable separation of the ammonia from the amines was obtained in a column packed with triethanolamine on C-22 firebrick. The conditions selected were: column length, 2 meters; column temperature, 60° C.; flow rate, 50 ml. per minute; carrier gas, helium; cell current, 130 ma. To handle the solid hydrochlorides an inverted T-tube was mounted in the gas stream just ahead of the column. The weighed sample was placed in a depression of the T-tube, the gas flow was started, and an excess of concentrated sodium hydroxide solution was added to the hydrochloride by means of a hypodermic syringe, inserted through a silicone-rubber diaphragm in the upper stem of the inverted T-tube. The bases were rapidly set free by applying heat. Table V lists the

Table IV. Paper Chromatography of the Hydrochlorides of the Volatile Amines from Beef

	Average R_f Values				
	Amine Hydrochlorides				
	n-Butyl alcohol–acetic acid–water	*n*-Butyl alcohol–water	*n*-Butyl alcohol–isopropyl alcohol–water	Phenol-water	DNP derivative
Methylamine	0.23	0.10	0.25	0.62	0.35
Ethylamine	0.33	0.16	0.34	0.74	0.55
n-Butylamine	0.59	0.36	0.56	0.90	
n-Amylamine	0.71	0.48	0.59	0.92	
Phenylethylamine	0.69	...	0.59	...	
Unirradiated					
Spot 1	0.22	0.10	0.30	None	None
Spot 2	0.34	0.18	0.37		
2.33 megarads					
Spot 1	0.21	0.11	0.28	0.57	0.34
Spot 2	0.34	0.17	0.37	0.73	0.55
3.72 megarads					
Spot 1	0.21	0.12	0.28	0.60	None
Spot 2	0.35	0.19	0.36		
Unirradiated					
Other spots	0.03	None	0.03	None	None
	0.07		0.08		
	0.17		0.15		
	0.40		0.46		
	0.48		0.68		
2.33 megarads					
Other spots	0.07	None	0.09	0.45	None
			0.16		
			0.45		
			0.68		
3.72 megarads					
Other spots	0.08	None	None	None	None

data taken on a number of samples studied. Methylamine and ethylamine were not separated but could be measured as a mixture. The unirradiated sample evidently contained 99.9% of ammonia and 0.1% of an unknown with less retention time than ammonia. The irradiated sample contained at least three compounds having retention times less than that of ammonia and amounting to about 2.3% of the hydrochloride. In addition, the irradiated sample contained 4.4% of material which corresponded to a mixture of methylamine and ethylamine. These results agreed reasonably well with the results obtained with paper chromatography and colorimetry.

Results and Discussion

The volatile bases are partial contributors to the odor of irradiated beef as demonstrated by the fact that acidification changed the odor of the condensate obtained by lyophilizing irradiated beef. However, nonbasic compounds are also important, because acidification did not deodorize the condensate.

The excess of volatile bases in irradiated beef was measured quantitatively, and the difference between the irradiated and unirradiated samples proved to be significant at the 1% level. The results are listed in Table I. The amounts of volatile bases in the samples ranged from 120 to 145 p.p.m., calculated as ammonia.

Lyophilization was a convenient and effective method of separating the volatile from the nonvolatile components of beef for the purpose of studying the odor components. By this means the volatile bases were isolated as their hydrochlorides. The yield of the bases was less than desired, 40 to 60%; but lyophilization evidently produced a representative collection of the volatiles, because the odor of the condensate was identical to the odor of the original meat. Table II lists the yields of the base hydrochlorides obtained from the irradiated and unirradiated beef.

Ammonia was 92 to 95% of the total volatile bases in the irradiated beef. The detailed data are in Tables I, III, and V. Paper and gas chromatography data, as summarized in Tables IV and V, showed the bases other than ammonia to consist of at least six amines, the two major components being methylamine and ethylamine. The amines were estimated colorimetrically, and the results are given in Table III. The irradiated samples contained 10 to 20 times more volatile amines than the unirradiated samples.

It now seems evident that many different compounds are responsible for the odor of irradiated beef. Some compounds may have definite effects on the over-all odor when they are in combination with similar compounds although each may be present in a concentration that would be undetectable if the compound were alone. The unidentified amines may be important components of the over-all contribution of the bases. Some of the unidentified amines may have intense odors and thereby contribute more to the odor than those identified.

Direct evidence was not obtained as to the nature of the precursors of the amines in irradiated beef. However, there is some information that has a bearing on this subject. Amines have been isolated as products of the irradiation of amino acids (*10, 12*), but no reports have been found where amines have been detected as the products of the irradiation of pure proteins. The formulas show that an amine can be set free from an amino acid by the breaking of one bond whereas at least two bonds must be broken to release amines from proteins. Thus it is reasonable to suspect that the nonprotein nitrogen compounds are the principle source of the free amines in the irradiated meat.

Table V. Gas Chromatography of Known Bases and of the Bases Isolated from Beef

(Column packing, triethanolamine on C-22 firebrick)

Sample of Base Hydrochlorides[a]	Peak No.	Identity	Retention Time, Minutes	Total[b] Area, %
Known	1	Ammonia	2.3	
	2	Methylamine and ethylamine	8.8	
	3	*n*-Butylamine	23.0	
	1	Trimethylamine	1.8	
	2	Ammonia	2.3	
	3	Dimethylamine	4.7	
	1	Ammonia	2.3	
	2	Dimethylamine	4.7	
	1	Ammonia, triethylamine	2.3	
	1	Ammonia	2.3	
	2	Diethylamine	7.1	
	1	Ammonia	2.3	
	2	Methylamine	6.5	
Unirradiated[c]	1	Unidentified	1.8	0.1
	2	Ammonia	2.2	99.9
Irradiated[c]	1	Unidentified	1.2	1.1
	2	Unidentified	1.5	0.03
	3	Unidentified	1.9	1.2
	4	Ammonia	2.3	93.0
	5	Methylamine and ethylamine	8.4	4.4

[a] Sample sizes ranged from 39 to 48 mg.
[b] Authorities differ on the question of whether per cent of total area is more closely related to mole per cent or weight per cent. Observations on the knowns of this series placed area per cent between mole per cent and weight per cent. Area per cent was finally assumed to be the same as weight per cent for the sake of simplicity, and because the lack of precision in the lyophilization made small differences at this point insignificant.
[c] The hydrochlorides from beef were composites of several lyophilizations. Portions of the irradiated sample had received 2.33 megarads and the remainder received 3.72 megarads.

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RADIATION PROCESSING OF MEATS

A Time-Temperature Relationship for Heat-Enzyme Inactivation of Radiation-Sterilized Beef and Pork

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Irradiated raw meat products are not stable upon extended storage at ambient temperatures, because of residual enzyme activity, particularly proteolytic enzyme activity. Control of enzyme activity is possible by the use of heat, and this study defines the parameters of time and temperature—from 140° to 170° F.—required for inactivation of proteolytic enzymes in 5-megarad irradiated beef and pork meats. Data indicate that the irradiation causes no significant sensitization of the enzymes to heat at the temperatures used.

THE ATTAINMENT OF A RAW product which may be stored for extended periods of time without refrigeration was an original objective for the use of ionizing radiation in meat processing. Studies have indicated, however, that such irradiated raw meat products are not stable upon extended storage at nonrefrigerated temperatures (*1*, *2*, *4*). Heat is at the present time the only practical method known for control of proteolytic enzyme activity in a meat product.

To design processing procedures for radiation-stabilized meat products, the relationship of time and temperature which would be required to inactivate tissue proteolytic enzymes in radiation-stabilized meats was studied. The proteolytic enzymes were chosen for this testing, because their activity correlated with the deterioration of radiation-stabilized meats on storage. Tissue proteolytic enzymes are relatively heat-stable, while most of the other enzymes present in the system are inactivated. Catalase was inactivated in samples which still had proteolytic enzyme activity.

Initially, the proteolytic enzyme was measured by use of a standard hemoglobin substrate and the Folin-Ciocalteau reagent. This approach was abandoned, because the assay was not sensitive enough at low levels of enzyme activity. Any data so obtained would require checking for application to muscle enzymes *in situ*.

The data herein reported have been obtained on muscle proteolytic enzyme *in situ*. Thin slices of meat in a flexible packaging material were used to minimize the heat transfer effects in the water bath. Incubated storage increased the sensitivity of the assay. Irradiation was used pre- or postheating in order to determine whether the enzymes were sensitized to heat inactivation by the irradiation. An increase in the sensitivity of *Clostridium botulinum* to heat after irradiation has been reported (*3*).

An irradiation dose of 5 megarad was used in these studies because investigations in other parts of the program have indicated that a dose in this range would be necessary to achieve a sterilizing radiation process with the same factor of microbiological safety as is used in thermal processing.

Experimental

Beef. Ten pounds of choice grade, frozen beef chuck were sliced into sections approximately 2 mm. thick, and circular pieces of meat 2 inches in diameter were cut from the lean portion of these sections. Each of these circular portions of meat was placed in a 2.5-inch packet made of a polyethylene-coated polyester film. (Scotchpak, Minnesota Mining & Mfg. Co., was chosen because of its resistance to changes upon irradiation and low gas transfer rate.) The packets were air evacuated and heat sealed. The meat was kept frozen during both the cutting and packaging procedures. The packets were arranged by weight in sets of seven. The weights between sets varied from 2.1 to 5.3 grams, but the maximum variation within each set was 200 mg. The sets of lower weight were used at higher inactivation temperatures—where exposure time was short—in order to minimize the heat penetration time.

Temperatures of 140°, 150°, 155°, 160°, 165°, and 170° F. were used where the heat treatment was prior to irradiation and temperatures of 140° and 160° F. where the heat treatment was post-irradiation. At each of these temperatures, six heating intervals were investigated. The intervals were chosen so that three would be shorter than the ones requiring complete inactivation (as determined from preliminary experiments). For each interval at a given temperature there were seven packets; three were maintained frozen for controls and four were incubated for 6 weeks at 100° F. The incubated samples were held in tightly sealed jars in order to avoid loss of moisture during the storage period.

The temperature-controlled water bath used was of large volume. All of the samples for each specific temperature were thawed and immersed together. The packets at each heating interval were then removed and cooled rapidly in ice water. For irradiation, the packets were frozen, canned, and shipped in frozen