Directly or indirectly, these two essential amino acids will contribute to the welfare of mankind.

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IRRADIATION EFFECTS IN MEAT

Production of Carbonyl Compounds during Irradiation of Meat and Meat Fats

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Investigation of the chemical changes that occur when meat and meat products are subjected to gamma radiation for sterilization indicated the formation of carbonyl compounds. Extraction procedures and the behavior of the compounds with various chromatographic solvent systems suggested that those obtained from irradiated meat differ from those obtained from irradiated fat. Carbonyl compounds increase in both meat and fat with increasing irradiation dosages.

PREVIOUS STUDIES in this laboratory on irradiated meat related to changes that occur in the meat pigment myoglobin (*4, 5*), sulfur-containing constituents (*1, 7*), proteolytic enzyme activity (*2*), and peroxide and free fatty acid production in meat fats (*10*). In the latter paper, reference was made to preliminary experiments which showed that an increased amount of carbonyl compounds was produced during irradiation of meat fat at 2×10^6 rep of gamma ray treatment. These changes have also been observed in model systems. Mead and associates (*8, 9*) stated that irradiation causes induction of autoxidation of methyl linoleate, and Dugan and Landis (*3*) reported an increase in peroxides and carbonyl compounds from the irradiation of methyl oleate.

Although the products from the fat in irradiated meat do not contribute directly to the off-odors, meats with a high fat content do not develop off-odors to the same extent as do leaner meats. The possibility of carbonyls from fat being involved in the suppression of off-odors merited further investigation of these compounds.

In this study, experiments have been extended to determinations of carbonyl compounds extracted with benzene from beef and pork fats, and those extracted by acidic solutions and/or benzene from ground beef muscle irradiated under various conditions and dosages.

The results indicate that carbonyl compounds produced in irradiated ground beef muscle differ from those obtained from irradiated beef or pork fat. The amounts of the carbonyl compounds were shown to increase with the use of increasing irradiation dosage levels.

Experimental

The benzene and alcohol used in these experiments were carbonyl free. The 2,4-dinitrophenylhydrazine was twice recrystallized from carbonyl-free methanol.

Benzene Extraction. A 10-gram sample of irradiated meat or fat, mixed in a Waring Blendor for 2 minutes with 90 ml. of benzene, was filtered and washed with sufficient benzene to make 100 ml. of the mixture. Then 2-ml. aliquots were transferred to a 25-ml. volumetric flask and 3 ml. of 4% trichloroacetic acid in benzene and 3 ml. of a saturated benzene solution of 2,4-dinitrophenylhydrazine were added. This mixture was maintained at 60° C. for 30 minutes in a water bath. After cooling to room temperature, 5 ml. of 4% potassium hydroxide in ethyl alcohol was added and the solution was brought to volume with ethyl alcohol. The absorbance values were determined with a Beckman spectrophotometer at 430 mμ. The 2,4-dinitrophenylhydrazone of *n*-heptaldehyde in appropriate concentrations was used for the standard curve.

Acid-Salt Extraction. A 10-gram sample of irradiated meat or fat was mixed in a Waring Blendor for 2 minutes with 60 ml. of cold 3% metaphosphoric acid and 20 ml. of cold distilled water. Then 35 grams of sodium chloride was added and the solution was mixed for 1 minute. The solution was filtered and brought to volume (100 ml.) with a saturated sodium chloride solution in water. Aliquots of 2 ml. were transferred to test tubes and 2 ml. of a saturated solution of 2,4-dinitrophenylhydrazine in methanol was added. The tubes were placed in a water bath for 30 minutes at 50° C. and then cooled to room temperature; 8 ml. of a 10% potassium hydroxide solution in methanol was added (final volume of 10 ml. was due to evaporation of the alcohol on the water bath). The solutions were filtered (to remove precipitated sodium chloride) directly into cuvettes and read on a Coleman Jr. spectrophotometer at 480 mμ. Calculations were based on the $E_{max.}$ value at this wave length according to Lappin and Clark (*6*).

Preparation of Samples for Irradiation. The meat and fat used for these experiments were purchased at local markets. Beef suet and pork fat were ground three times in a food chopper fitted with a fine plate, weighed, and packed in regenerated cellulose casing (Visking), saran casing, or cork-stoppered glass vials. Samples were refrigerated at 5° C. until irradiated.

Irradiation Sources. Two sources were used; one at the Argonne Cancer Research Hospital, University of Chicago, which is a cobalt-60 source, with a dose rate of approximately 350,000 rep per hour and refrigerated to 5.5° ± 2° C., and the other one at the Argonne National Laboratory, Lemont, Ill. with a dose rate of approximately 10^6 rep per hour, in which the samples are irradiated at ambient temperatures (about 20–22° C.).

Results. Preliminary studies on pork and beef fat irradiated at 2×10^6 rep indicated that an increase occurred in the amount of carbonyl compounds over that present in the control samples. Spectral curves of the 2,4-dinitrophenylhydrazine derivatives from the irradiated fats were obtained. These showed a maximum of around 428 mμ. The 2,4-dinitrophenylhydrazine derivatives of various aliphatic aldehydes were then prepared and their absorbance curves obtained by the same method used for the fat samples. The derivative of *n*-heptaldehyde was found to have a strong peak at 425 mμ similar to that observed for the carbonyl compounds from the samples of fat. This compound was then selected for the preparation of the standard curve which was used for the subsequent determinations of the carbonyl content of irradiated and nonirradiated fat. The solution obtained from fat followed Beer's law with increasing concentration of sample. While the limitations of this procedure are recognized, it was necessary to have some basis for comparison of the values obtained between irradiated samples and controls.

Table I gives the values obtained from beef and pork fat when irradiated in saran (oxygen-impermeable) casing, and in a regenerated cellulose (oxygen-permeable) casing (Visking). These data indicate that an increase in carbonyl compounds occurs on irradiation.

A large increase in carbonyl compounds, extractable by acid-salt solution from irradiated ground round of beef muscle, was observed with increasing dosages (Table II). These data suggested the possibility that the carbonyls extracted by the acid-salt solution from irradiated meat were different from those obtained from irradiated meat fat by benzene extraction, inasmuch as the carbonyl values were lower for the meat samples having a higher fat content.

Several experiments were run, therefore, with fat added to samples of pork and beef muscle and irradiated at various dosage levels. The same basic amount (10 grams) of meat was used for each sample and fat was added to the percentage indicated in Tables III and IV. According to these results, fat contributes little to the amount of carbonyl compounds, produced during irradiation, that are extracted by acid-salt solution.

Comparative experiments were run on the same batch of meat and fat using the two methods of extraction. The carbonyl values obtained from irradiated beef by the acid-salt extraction are high

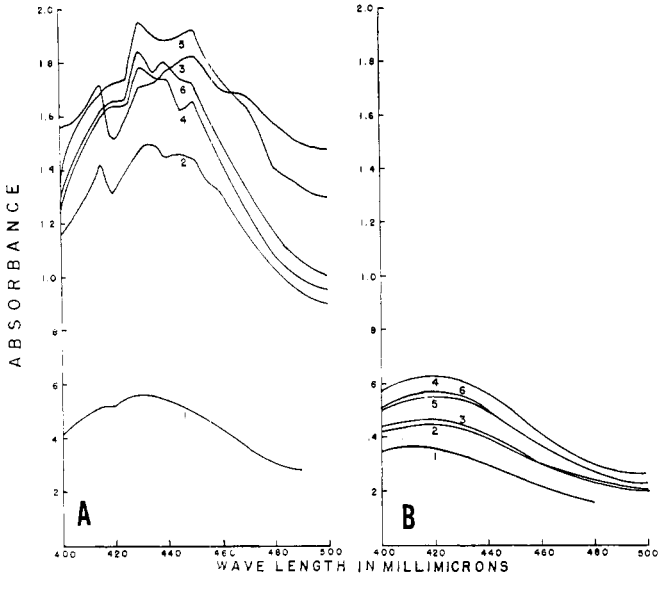

Figure 1. Absorption curves of 2,4-dinitrophenylhydrazine derivatives of carbonyl compounds extracted with benzene from irradiated pork fat (A) and irradiated ground round of beef (B)

1. Nonirradiated
2. 2×10^6 rep
3. 4×10^6 rep
4. 6×10^6 rep
5. 8×10^6 rep
6. 10×10^6 rep

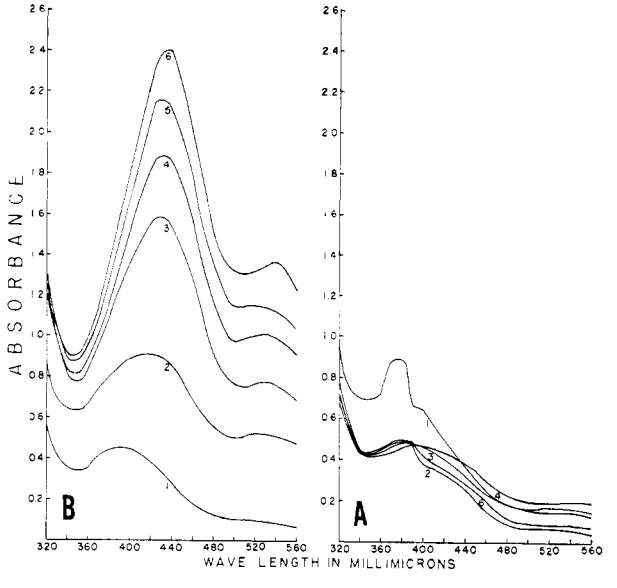

Figure 2. Absorption curves of 2,4-dinitrophenylhydrazine derivatives of carbonyl compounds extracted with acid-salt solution from irradiated pork fat (A) and irradiated ground round of beef (B)

1. Nonirradiated
2. 2×10^6 rep
3. 4×10^6 rep
4. 6×10^6 rep
5. 8×10^6 rep
6. 10×10^6 rep

Table I. Carbonyl Content of Irradiated (2×10^6 rep) Fat

Type of Fat	Packaging Material	× 10^{-5} M Carbonyl/G. Fat Expt. 1	Expt. 2
Pork (non-irradiated)	.....	0.31	0.27
Pork	Saran	0.40	0.47
Pork	Regenerated cellulose[a]	0.46	0.54
Beef (non-irradiated)	.....	0.22	0.33
Beef	Saran	0.44	0.57
Beef	Regenerated cellulose[a]	0.48	0.55

[a] Visking.

Table II. Carbonyl Content of Acid-Salt Extract of Irradiated Ground Round with Varying Fat Content as Affected by Irradiation Dosage

Irradiation Dosage (× 10^6 rep)	% Fat in Meat 6	8	13	23.3
	× 10^{-5} M Carbonyl/G. Meat			
0	1.27	3.22	0.98	0.92
2	4.31	4.93	2.13	2.30
4	6.60	8.74	3.40	3.57
6	8.63	11.50	4.65	5.06
8	11.20	12.88	6.46	7.25
10	11.50	10.35	7.42	4.95

Table III. Effect of Fat (Beef Suet) Added to Beef Prior to Irradiation on Production of Carbonyl Compounds Extracted by Acid-Salt Solutions

Irradiation Dosage (×10^6 rep)	% Fat Added to Beef 0[a]	5	10	15	20	25
	×10^{-5} M Carbonyl/G. Meat					
0	1.15	1.13	1.21	1.50	1.27	1.27
2	4.49	4.20	4.20	4.26	4.03	3.97
4	7.02	7.65	7.48	...	6.67	6.21
6	9.43	8.97	8.91	8.51	8.86	8.11
8	11.50	10.64	10.24	9.43	10.01	9.32
10	11.50	11.50	12.08	10.35	11.27	10.35

[a] Contained 6.4% fat.

Table IV. Effect of Pork Fat Added to Pork Tenderloin Prior to Irradiation on the Amount of Carbonyl Compounds Produced and Extracted by Acid-Salt Solutions

Irradiation Dosage (×10^6 rep)	% Fat Added to Pork 0[a]	5	10	15	20
	×10^{-5} M Carbonyl/G. Meat[b]				
0	0.91	1.03	1.09	1.09	1.21
2	7.02	7.13	6.61	5.52	6.90
4	10.24	10.47	9.66	8.05	9.43
6	12.65	11.50	12.08	10.35	12.31
8	19.55	17.25	18.40	18.40	16.68

[a] Contained 9% fat.
[b] Samples irradiated at 10×10^6 rep were estimated at 23×10^{-5} *M* carbonyl/g. meat.

Table V. Influence of Irradiation Dosage and Method of Extraction on Carbonyl Content of Ground Beef and Pork Fat

Radiation Dosage (×10^6 rep)	Ground Beef Acid-salt extraction	Ground Beef Benzene extraction	Pork Fat Acid-salt extraction	Pork Fat Benzene extraction
	×10^{-5} M Carbonyl/G. Meat			
0	1.44	0.18	0.28	0.30
2	6.47	0.24	0.43	0.79
4	10.35	0.25	0.73	0.91
6	13.23	0.34	1.05	0.92
8	14.95	0.29	1.15	1.00
10	17.25	0.30	1.12	0.99

when compared to those obtained by benzene extraction (Table V). Although the values obtained from fat are similar for the two methods, as was previously indicated, the addition of fat to meat caused no apparent increase in carbonyls obtained by acid-salt extraction (Table III and IV).

Absorption spectra were run on the 2,4-dinitrophenylhydrazine derivatives of the carbonyl compounds obtained by acid-salt, and by benzene extractions of both meat and fat. These curves are presented in Figures 1 and 2. The curves obtained from fat by benzene extraction are typical of several experiments; however, smooth curves with a single absorption maximum around 430 mμ were observed in earlier experiments. The curves obtained by acid-salt extraction of fat did not show a peak in this region. The curves obtained by acid-salt extraction of meat showed peaks in the 420- to 430-mμ region, while the benzene extracts of meat showed maxima at around 420 mμ, probably because of concentration effect as there is a definite shift in the maxima of the peaks with higher concentration in the acid-salt extracts of meat.

Although the maxima in benzene-extracted meat and fat and the acid-salt extracts of meat have their maxima in the 420- to 430-mμ. region, the acid-salt extract of fat did not have a maximum in this vicinity. Of further interest is the curve obtained by acid-salt extract of the nonirradiated fat which has a peak in the 380-mμ area. This peak tends to disappear in the irradiated samples, which suggests that some compound present in fat is altered by irradiation. These and other data indicate that different carbonyl compounds are obtained from irradiated meat and meat fat by the different extraction methods used.

Chromatographic procedures are being used to separate the 2,4-dinitrophenylhydrazine derivatives from both meat and fat. Preliminary results (*11*) indicate that at least four fractions are present in irradiated fat and in irradiated beef muscle. The behavior observed with these compounds, in the various solvent systems used, further strengthens the contention that different carbonyl compounds are obtained by the two methods of extraction.

Although the carbonyl compounds produced by irradiation of meat and meat fats probably do not directly contribute to the off-odors produced in irradiated beef, they may possibly have a role in decreasing the apparent off-odors by reacting with the compounds that do contribute—i.e., with sulfhydryl compounds and amines. These reaction products would then be of more importance than the residual, unreacted carbonyls. When sufficient amounts of these carbonyl compounds are isolated for identification,

their significance in relation to flavor and odor changes in irradiated meat may be evaluated.

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AMINO ACIDS IN FERMENTATION

Utilization of C^{14} Leucine and C^{14} Glycine by *Saccharomyces cerevisiae*

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The utilization of leucine and glycine by *Saccharomyces cerevisiae* was investigated using leucine-2-C^{14} and glycine-2-C^{14} in microfermentations. The amino acids were separated by monodimensional paper chromatography. The distribution of the tagged carbon was traced by radioautographs. The carbon skeleton of leucine, unlike that of glycine, is not utilized to any appreciable extent in the synthesis of other amino acids.

RECENT WORK (*4*) verifies that *Saccharomyces cerevisiae* deaminates and decarboxylates α-amino acids to form alcohols as indicated by Ehrlich and Neubauer and Formherz.

Thorne (*5,6*) has shown that differences exist in the utilization of amino acids, not only when tested individually, but when tested in the presence of other amino acids. He concluded that amino acids were integrated intact into yeast protein.

The authors (*4*) found that a relatively constant amount of leucine was unaccounted for in a leucine balance under their standardized conditions. While Gilvarg and Bloch (*2*) have shown that the carbon skeletons of some amino acids come from acetate carbon and some from glucose, during yeast metabolism, it is not improbable that the carbon skeleton of one amino acid is utilized in the synthesis of another amino acid.

For the investigation of the utilization of radioactively tagged amino acids by yeast, leucine and glycine were chosen, as they represent a well utilized and a poorly utilized amino acid (*6*).

Table I. Media for Test Tube Fermentations

Component	Medium 1, G.	Medium 2, G.
Dextrose	126	126
Monopotassium phosphate	0.055	0.055
Potassium chloride	0.0425	0.0425
Calcium chloride	0.0125	0.0125
Magnesium sulfate	0.0125	0.0125
Ferric chloride	0.0002	0.0002
Manganese sulfate	0.0002	0.0002
Yeast extract[a]	50 ml.	.....
Yeast culture	30 ml.	30 ml.
Demineralized water, make vol. to	1 liter	1 liter

[a] 80 grams of Difco yeast extract per liter of demineralized water.

Experimental

Materials and Equipment. Chromatographic chambers, one 28 × 28 × 28 inch and one 14 × 28 × 28 inch cabinet.

Chromatographic paper, Whatman No. 1, $18^1/_4 \times 22^1/_2$ inches.

Solvent systems (3). Phenol and water in the ratio of 4 to 1 and with 20 mg. of 8-quinolinol per 500 ml. of solvent. A beaker containing 0.3% ammonia was placed in the cabinet when this solvent was used.

2-Butanol and 3% ammonia in the ratio of 3 to 1.

2-Butanol, water, and formic acid in the ratio of 120 to 40 to 1.

Densitometer, Macbeth Ansco Model 12.

Film, Kodak, medical x-ray film, No-Screen, 11 × 14 inches.

Xerograph, Xerox, Model D, Haloid Co., Rochester, N. Y.

Leucine-2-C^{14}, Tracerlab, Inc., Boston, Mass.

Glycine-2-C^{14}, Tracerlab, Inc., Boston, Mass.

Fermentation. A series of fermentations was conducted in test tubes using 5 ml. of fermentation medium. The composition of the media used is given in Table I. The dextrose and mineral salts were dissolved, combined, and sterilized. The yeast extract was sterilized separately. The mineral salts were in the same ratio as in the previous work with 10- and 1-liter fermentations (*4*), but the concentration was cut in half. Tests indicated that yeast growth was not impaired at this lower mineral level. This reduction was merely a precaution to minimize the possibility of salt effects at high hydrolyzate concentrations in subsequent chromatographic analyses.

Sterilized stock solutions of leucine and of glycine were prepared. Aliquots of either the leucine or glycine solutions were pipetted into sterilized test tubes to give the desired amino acid levels, as shown in Table II. A standard solution of leucine-2-C^{14} was prepared, having an activity of 17.7×10^3 disintegrations per second per milliliter. The activity of a comparable glycine-2-C^{14} solution was 1.48×10^5 disintegrations per second per milliliter. Two milliliters of radioactive leucine were added to each tube in the leucine fermentation runs, while radioactive glycine was substituted in the glycine tests.