CHANGES IN THE NITROGENOUS CONSTITUENTS OF BEEF AS INDUCED BY PRE-HEATING, IRRADIATION AND STORAGE FOR TWO HUNDRED DAYS

by

ROBERT HUGH THOMPSON

A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1959

APPROVED:

Associate Professor of Food and Dairy Technology

in Charge of Major

Head of Department of Food and Dairy Technology

Chairman of School Graduate Committee

Dean of Graduate School

Date thesis is presented May 15, 1959

Typed by Patricia Malango

ACKNOWLEDGEMENTS

A number of people deserve special notice for their various contributions, labor, encouragement and suggestions which altogether have helped to make this work possible.

Dr. Robert F. Cain has been most helpful with his direction, encouragement and keen analysis of the problem encountered. The author expresses his sincere thanks to Dr. Cain for the unending faith he has had in the eventual completion of the problem.

Special thanks should also be alloted to Miss Flordeliza R. Bautista for her labors during the preparation of the samples, as well as her many suggestions in the techniques to be employed for the various chemical analyses.

The author would like to extend his appreciation to Professor Russel O. Sinnhuber for his valuable suggestions with the chemical analyses and his constant encouragement.

Thanks go to Dr. Allen Anglemier and to Messrs. Artar, Crawford, Johnson, Korten, and Wilson for their cooperation in the Laboratory.

Last but certainly not least much credit is due my wife, Hille, for her help with the preparation of the samples and the task of compiling the many tables. Her vast patience and constant encouragement have been greatly appreciated.

DEDICATION

This work is dedicated to the memory of my grandfather, Mr. Will Gibbs.

TABLE OF CONTENTS

LIST OF TABLES

CHANGES IN THE NITROGENOUS CONSTITUENTS OF BEEF AS INDUCED BY PRE-HEATING, IRRADIATION, AND STORAGE FOR TWO HUNDRED DAYS

INTRODUCTION

Since the beginning of the atomic age man has dreamed of harnessing nuclear energy to do a multitude of tasks. By 1953 it became evident that ionizing radiations might be utilized to preserve food products by killing food spoilage organisms. This new method of food preservation has been under comprehensive investigation through the endeavors of the U. S. Atomic Energy Commission, the Department of Defense and the Quartermaster Food and Container Institute.

Two advantages for using ionizing radiations seem apparent: (1) an atomic waste product can be utilized from reactors and (2) preservation of foods without high temperature processing would seem attractive. The latter would seem particularly alluring to meat processors since a cook of 240-250°F. for a period of ninety minutes is often necessary for canned meat products in order to insure freedom from Clostridium botulinum. The consequences of a high temperature cook to the fresh flavor, color, and texture of meat are quite obvious.

Morgan (36, p. 24) found that an irradiation dosage of 4.8 megarad was needed to kill the spores of C. botulinum. This complies with public health requirements and

establishes a degree of commercial sterility. However, it has been known since 1941 from work by Tytell and Karsten (48, p. 525) that a ten fold increase in dosage over 4.8 megarad is needed to inactivate the proteolytic enzymes of foods. This becomes important since it is known from observations by the author that proteolytic activity can literally consume an otherwise sterile meat product, while the effects of a 50 megarad dosage of irradiation would be just as disastrous to fresh meat characteristics and sensory appeal.

It has become apparent that the utilization of irradiation alone would be undesirable for the preservation of fresh meats. The use of moderate heating along with irradiation, however, could present an approach to the problem. Following this reasoning, Cain, Anglemier, Sather, Bautista, and Thompson (12, p. 603-610) found that beef which had been heated to 160°F. and irradiated at 1.86 and 2.79 megarad was still acceptable to a taste testing panel after storage at 72°F. for eight months.

With the above results in mind the author speculated on the outcome of an attempt at using pre-irradiation heating temperatures below 160 F., in conjunction with sterilization dosages of gamma irradiation, to retard proteolytic activity in beef during long term storage. This then became the problem which resulted in the work represented here.

REVIEW OF LITERATURE

In the animal body dietary proteins are one of the essential foods needed for growth and maintenance. They play an important part in many activities of living cells. Enzymes which catalyze most of the chemical reactions needed for the normal physiological functions of cells are usually made up partially or wholly of proteins. Proteins are also conspicuous in antibodies, hormones, muscle fibers and blood cells. It could be stated that proteins are the basis of all earthly life.

Amino acids are the building blocks of proteins. It has long been known (3, p. 156) that a protein consumed by an animal will be broken down to amino acids which can go to form new body protein. It is interesting to note that the ultimate source of the proteins man and all animals need is from the plant world. Only green plants can synthesize certain "essential" amino acids needed by animals. The ten amino acids that Rose (44, p. 112-130) found to be essential in the diet of the rat are arginine, histidine, isoleucine, leucine, tryptophan, lysine, methionine, phenylalanine, threonine, and valine. The word "essential" is used for an amino acid when an animal does not synthesize enough of that component to satisfy bodily needs and must rely upon its dietary intake. The work of Rose (44, p. 112-130) has been collaborated by Albanese (1, p. 249) and

Mitchell (34, p. 95).

The quality of a protein is usually associated with its ability to be used and incorporated in an animal body. Mitchell (33, p. 874) has described the "biological value" of proteins as the percentage of nitrogen retained in an animal body of the total protein nitrogen consumed in a particular food.

Block (8, p. 123-130) notes that the best source of dietary protein for man is from the flesh of animals. In general, cereals contain much less protein and often do not have all the essential amino acids needed as reported by Morgan and Kern (35, p. 377) and Newton et al. (37, p. 589).

Alexander and Elvehjem (2, p. 708), Schweigart et al. (45, p. 23-26), and Block (8, p. 124) have determined the amino acid content of beef muscle, using microbiological techniques. These results may be seen in Table 1. Today many analyses of this type would be accomplished using the technique of paper chromatography. Chromatographic techniques for the separation of amino acids and other nitrogenous constituents has been reviewed in great detail by Block and Bolling (9) and Block, et al. (11).

Animal Proteins: Changes During Storage

A very remarkable change takes place in muscle protein during the first few hours after the death of an animal. This phenomenon, which is known as "rigor mortis", is

TABLE 1

AMINO ACID COMPOSITION OF BEEF MUSCLE PROTEIN

(Expressed in gms./100 gms. of protein)

Amino Acids	Block (8, p. 124)	Schweigert et al. (45, p. 23-26)	Alexander and Elvehjem (2, p. 708)
Aspartic Acid	6.0	8.75	10.0
Glutamic Acid	15.4	14.35	15.8
Serine	5.4	3.77	4.4
Glycine	5.0	7.11	4.6
Threonine	4.6	4.04	4.4
Alanine	4.0	6.40	6.2
Methionine	3.3	2.32	2.4
Tyrosine	3.4	3.24	3.4
Valine	5.0	5.71	5.2
Phenylalanine	4.9	4.02	4.3
Leucine	7.7	8.40	7.7
Lysine	8.1	8.37	8.7
Cystine	1.3	1.35	0.9
Proline	6.0	5.40	3.8
Arginine	7.7	6.56	6.7
Histidine	2.9	2.94	3.5
Tryptophan	1.3	1.10	1.3
Isoleucine	6.3	5.07	5.7

evidenced by the contraction of fibrils in the muscle fibers. According to Huxley (26, p. 66), as the supply of adenosine triphosphate is exhausted in the muscle region the protein myosin seems to combine chemically with the protein actin. The resulting continued contraction exists for 24-72 hours during which time the flesh takes on a firmer characteristic. Wierbicki, et al. (50, p. 86), (49, p. 511) have found that during the first 12-15 days of storage there is an ion exchange mechanism in existance which is thought to have a considerable effect upon the tenderness of the flesh. Magnesium and sodium ions are lost from the muscle fibers while a large amount of potassium ions are gained. The net change allows a positive charge to be accumulated by the muscle protein. This may directly result in increased hydration of the protein which has been correlated with tenderness in meat.

Gliken and Loewy (20, p. 505), Chen and Bradley (13, p. 164) and Hertzman and Bradley (23, p. 240) attributed the increase in tenderness after rigor as being due primarily to autolysis. However, it appears from more recent work by Wierbicki (49, p. 511) and Husaini (24, p. 316) that the amount of autolysis (proteolysis) in fresh beef which occurs in 12-15 days of storage at 3.5°C. is extremely small. There are no data in the literature concerning proteolysis as associated with long term storage of irradiated meats which are bacteriologically sterile.

A complete study of the proteolysis of muscle has been needed for some time. Recent work by Zender, et al. (51, p. 305-326) has begun to shed some light on this little known and rather precarious subject. By using very extreme methods they retained the aseptic condition of muscle without resorting to the use of preservatives. Under aseptic and anaerobic conditions rabbit and lamb muscle was allowed to undergo proteolytic processes for 150 days at 25°C. and for 15 days at 38°C. A steady increase in the level of the free amino acids and a decrease of glycine soluble protein was noted during these periods. According to electrophoretic studies it appeared that the protein first split into large fractions and later into amino acids. The cathepsin enzymes of muscle were thought to be responsible for the proteolysis noted. In Table 2 it can be seen how slowly proteolytic breakdown to amino acids occurs. Storage at 38°C. resulted in a much greater breakdown in the same time period than that which occured at 25°C. It would seem that proteolytic activity of meat in storage is associated with certain enzyme systems already present within the flesh of an animal.

Animal Proteins: Changes Induced by Heat

Daniel and McCollum (16, p. 18) and Ingvaldsen (27, p. 98) found that heat effected the nutritive value of protein in fish meals. According to Maynard, et al. (31,

TABLE 2

RELEASE OF AMINO ACIDS FROM LAMB MUSCLE DURING 70 DAYS STORAGE AT 25°C.*

Days Storage	Tyrosine Index OD at 279 mu
0	6.5
1	6.8
2	5.5
10	5.8
20	7.2
33	6.1
50	9.5
70	11.8

* After Zender et al. (51, p. 310)

p. 602) vacuum-dried menhaden had the highest protein nutritive value, while steam-dried ranked somewhat less and flame-dried was much the lowest. It was thought that certain of the essential amino acids were reduced during the heating process above 195°F. Maynard and Tunison (30, p. 1170-1171) believed cooking and pressing caused loss of soluble proteins in the drip.

Morgan and Kern (35, p. 373) found that beef proteins were also lowered in biological value as a result of heating. Biological value was found to be inversely proportional to the severity of heating.

Ginger, et al. (19, p. 410-416) determined that a four to thirty fold decrease in the total soluble nitrogen of cooked beef was normal as compared with raw beef. As meat was heated above 185°F. Newton, et al. (37, p. 589) found the loss of water soluble proteins was over fifty percent. While investigators report varying amounts of protein lost due to heating, which depend largely upon their particular methods, it can be stated that the nutritive value of meat protein is lowered by heating and this amount appears to be of considerable significance.

Certain amino acids of casein appear to be affected by heat processing. Greaves, et al. (21, p. 126), Rice and Beuk (43, p. 223-279) and Evans and Butts (17, p. 420) reported that when casein was heated at 140°F. for 30

minutes the first amino acid destroyed was lysine while histidine was next. Upon destruction, these two amino acids were found not to be nutritionally available. According to Baldwin, et al. (5, p. 117) phenylalanine, leucine, isoleucine, and threonine were not effected by temperatures slightly above boiling, while arginine, tryptophan and methionine were greatly reduced in quantity. The reduction of the nutritive values for valine, methionine, arginine, tryptophan, and histidine occasioned by the use of heat has been reported by Block et al. (11, p. 300) and Kon and Markuze (28, p. 1483). There appears to be little doubt of the ability of heat to generally lower the nutritive value of animal proteins.

Animal Proteins: Changes Induced by Irradiation

Some effect upon the nutritional value of certain proteins has been noted when proteins are subjected to sterilization dosages of ionizing radiations (38, p. 56). Metta and Johnson (32, p. 489) found the biological value of irradiated milk proteins was lowered by eight percent. Beef proteins that were subjected to sterilization doses of radiation retained their digestibility and nitrogen content as well as biological activity.

Haddock fillets exposed to sterilization dosages of cathode rays by Proctor and Bhatia (40, p. 359) were found

to retain most of the eight essential amino acids present. Pure amino acids in solution were found to be much more easily destroyed by sterilizing doses of cathode rays (7, p. 552) (41, p. 537) (42, p. 2). Deamination of histidine, cystine, phenylalanine, tyrosine, and tryptophan all occurred in varying amounts with ammonia being released.

It was found by Scheffner, et al. (46, p. 460) that no particular destruction of essential amino acids in beef proteins occurred after being irradiated by gamma rays at 1.89 megarad. This is shown and compared to that occurring in thermal processing of beef as reported by Scheffner, et al. (46, p. 457):

Amino Acid mg/g.	Control Unprocessed	Irradiation At 1.89 Megarad	Thermal Processed 240°F. for 161 Minutes
Methionine	10.0	9.3	7.0
Cystine	1.1	1.1	0.32
Phenylalanine	11.6	9.5	5.1
Tyrosine	6.5	5.7	3.1
Tryptophan	7.0	5.3	2.1
Threonine	27.1	27.4	17.5
Lysine	3.4	3.1	1.9

In general, it would appear that the amino acids in intact proteins are much less susceptible to breakdown due to irradiation than are the pure amino acids in solution.

The effect of ionizing radiations upon meat pigments has been discussed by Fox, et al. (18). It was observed that irradiation of myoglobin in meat resulted in the

formation of a red and a green pigment. The red pigment was formed when oxygen was not present, while the green pigment was formed when oxygen was present. The green pigment was identified as sulfmyoglobin, which was formed during irradiation of meat by the reaction of myoglobin, hydrogen peroxide and a sulfide which was liberated from certain sulfhydryl bearing substances. The green pigment has an intact heme moiety but a much altered globin fraction.

Other cases of possible protein changes induced by irradiation have been observed by Fox, et al. (18). Brown metmyoglobin can be formed from red oxymyoglobin and vice versa depending upon the substrate available in the meat at the time of irradiation. At present little is known of these particular reactions.

Animal Proteins: Changes Induced by Heating, Irradiation and Storage

A thesis entitled "Changes in the Nitrogenous Constituents of Beef as Induced by Pre-Heating and Irradiation and Storage for Eighty Days" was reported by Flordeliza Bautista (6). A review of her conclusions for the work with beef using storage temperatures of 34°F. for eighty days after pre-irradiation heating and irradiation will be given here. The following is taken from the work by Bautista (6, p. 95-98).

1. Pre-irradiation heating temperatures of 150°F. or lower prior to irradiation at 0.1 and 5.0 megarad did not inactivate the agents responsible for proteolytic activity in beef. Proteolysis was evidenced by the increase in total soluble nitrogen, non-protein nitrogen (TCA-soluble), amino nitrogen and free amino acids.

2. Slight increases in total soluble nitrogen and certain of the free amino acids were evidence of some slight amount of change in the protein of meats which had been precooked to 195°F.

3. The meats irradiated at 0.1 megarad generally reacted no differently than the non-irradiated samples. Both showed the same pattern of changes of the nitrogenous constituents analyzed during the course of the storage period.

4. Increase in gamma radiation dosage to 5.0 megarad apparently exposes the various constituents of the protein and apparently increased the proteolytic activity.

5. Increase in storage time resulted in an increase in total soluble nitrogen, non-protein nitrogen, amino nitrogen and free amino acids. The increase was governed by the pre-heating temperatures and the gamma irradiation dosage employed.

6. The proteins were not significantly destroyed. The values obtained for the free amino acids were too low

to substantiate significant destruction during eighty days storage.

7. All the free amino acids determined quantitatively increased with an increase in gamma radiation dosage, and storage time, except for aspartic acid. No free aspartic acid was found in irradiated meat after sixty days of storage.

EXPERIMENTAL METHODS

Preparation of Samples

Each of three Hereford bull carcasses was selected for approximate uniformity. After a minimum of time, usually twenty-four hours, the *longissimus dorsi* muscles of each carcass were excised and stripped of all visible fat. The pair of muscles from one animal was used as a replicate. Therefore, there were three replications.

After chilling each muscle for three hours at -18°F. so as to firm the meat, it was sliced mechanically in cross section in slices of $\frac{1}{4}$ to $\frac{3}{8}$ of an inch in thickness. Each sample slice was weighed in order to conform to a 40-50 gram weight. All the samples were placed individually into polyester bags and heat-sealed, with as much air as possible being pressed out by hand before sealing. At this time, all the bags of meat were randomized within a replication to prevent any effects of the longitudinal variations of the muscle.

Pre-irradiation Heating

The bags of meat were heated in a steam-heated water bath whose temperature was automatically controlled. Samples were heated to internal temperatures of 100°, 110°, 120°, 130°, 140°, 150°, and 195°F. Unheated samples were

kept as controls. The water bath temperature was maintained from one to two degrees above the desired temperature of the samples. The meat was heated in the water bath until the internal temperature, as indicated by thermocouples in representitive samples, was that which was desired. This usually took from six to eight minutes depending on the temperature required. At the time when the required temperature was attained the bags of meat were placed in a cold water bath in order to stop all heating. Then, the bags were put into previously labelled half pound flat (307 x 202) "C" enamel cans and closed mechanically. It should be noted that one bag of meat was placed into one can. The cans were then frozen at -18°F. before they were shipped to the Materials Testing Reactor for irradiation. Some samples were analyzed immediately upon heating in order to serve as controls for the various chemical determinations.

Shipment and Irradiation of Samples

All samples except those to be non-irradiated were packed in dry ice and shipped in insulated containers to the Materials Testing Reactor, Idaho Falls, Idaho. During the eight day interval between shipment and receipt the samples were kept frozen at 0°F. or under dry ice, except during the irradiation period. The non-irradiated samples were kept at 0°F. storage throughout this time.

Irradiation was accomplished by exposing the cans to a gamma grid until a dosage of 5.0 megarad had been acquired. According to the reactor technicians a period of two hours and fifty-two minutes was needed for the 5.0 megarad samples. The samples were then frozen, re-packed in dry ice and returned to Corvallis where they were put into the particular storage environments.

Storage of Samples

The 5.0 megarad samples were divided with half being stored at 70°F. and half at 100°F. and scheduled for analysis at 0, 15, 30, 45, 60, 80, 120, 160 and 200 days of storage.

Quantitative analysis for total nitrogen, moisture, total soluble nitrogen, trichloroacetic acid-soluble nitrogen, amino nitrogen, and free amino acids was carried out for each of the samples.

Total Nitrogen Determination

One to two grams of meat were weighed on low nitrogen weighing paper. The method of Hiller, Van Slyke, and Plazin (24, p. 1402-1420) was used, while the indicator used was that developed by Ma and Zuazaga (29, p. 280-282). "Kel-Pak" catalyst containing 10.0 grams of potassium sulfate was used instead of that used by Hiller, et al.

Total nitrogen was reported for each sample on a dry weight basis.

Moisture Determination

Meat was cut into small pieces and put into numbered, weighed aluminun pans and dried for twenty-four hours at 70°C. and 28 inches vacuum. After drying, the pans were cooled in a desiccator and re-weighed. The moisture content of each sample was calculated from the weight lost.

Sample Preparation for Amino Nitrogen, Total Soluble Nitrogen and Trichloroacetic Acid-Soluble Nitrogen

Ten grams of meat was blended with 25 mls. of distilled water for one minute in a micro-blender. The samples and blender were cooled to 40°F. before blending. The mixture was filtered using Whatman #12 filter paper and the filtrate collected was used for the following three determinations.

Amino Nitrogen Determination

Five mls. of the extract as prepared above were diluted to 100 mls. with distilled water. An aliquot of five mls. of the diluted solution was used for the amino nitrogen determination of Peters and Van Slyke (39, p. 385). The final results were calculated in mgs. percent amino nitrogen on a dry weight basis.

Total Soluble Nitrogen Determination

The microkjeldahl method of the A. O. A. C. (4, p. 805) with the following modifications was used for the total soluble nitrogen determination. One ml. of the extract prepared as above under sample preparation was digested with 2 mls. of concentrated sulfuric acid with two selenium-coated Hengar granules acting as the catalyst. Distillation was carried out with the use of a Kirk-type continuous microkjeldahl unit. 25 mls. of 4% boric acid was used as the receiving solution. Tenth normal sulfuric acid was used to titrate the ammonia in the receiving solution. The indicator used was that suggested by Ma and Zuazaga (29, p. 280-282). The results were calculated in mgs. percent on a dry weight basis.

Trichloroacetic Acid-Soluble Nitrogen

The protein precipitation method of the A. O. A. C. (4, p. 227) was used except that a 25% trichloroacetic acid solution was used to precipitate out protein instead of phosphotungstic acid. The extract used was that as indicated under the sample preparation above. One ml. of the TCA-soluble filtrate was digested using the microkjeldahl method of the A. O. A. C. (4, p. 805). The trichloroacetic acid-soluble nitrogen was calculated in mgs. percent of the dry sample.

Sample Preparation for Amino Acids

A. Free Amino Acids: The free amino acid samples were prepared using the method of Block, et al. (10, p. 84). Ten gram samples of the meat were blended with ethyl alcohol and extracted with chloroform. The extracts were stored at room temperature in half ounce bottles after being brought up to 10 mls. volume with 80% alcohol.

B. Total Amino Acids: Samples were acid hydrolyzed using the method of Block, et al. (10, p. 64). This method was adequate for all amino acids analyzed except tryptophan. The alkaline hydrolysis method of Block, et al. (10, p. 83) was used for the liberation of tryptophan. Raw, non-irradiated, unstored meat samples were analyzed for total amino acids as well as raw, irradiated meat stored for 200 days.

Determination of Amino Acids

Seventeen amino acids were analyzed qualitatively and quantitatively using one-dimensional paper chromatography. Buffered and unbuffered methods were used. The amino acids were analyzed in six groups depending on the solvent system used. Groups I through V were suggested by Hackman (22, p. 282-288) and Group VI by Subramanian and Lakshminarayan Rao (47, p. 566-570). Each group contained the following amino

acids: Group I: aspartic acid, glutamic acid, serine, glycine, threonine, and alanine; Group II: methionine, valine, phenylalanine and leucine; Group III: lysine and arginine; Group IV: histidine, and proline; Group V: tryptophan; and Group VI: cystine and tyrosine. The solvent systems used were as follows: Group I: phenol (74 percent w/w) and a buffer of pH 10.0 (0.053 M boric acid and potassium chloride, 0.047 M sodium hydroxide); Group II: n-butanol (77 percent v/v), acetic acid (6 percent v/v), and water (17 percent v/v); Group III: pH 7.0 buffer (0.040 M Na_2HPO_4, 0.027 M KH_2PO_4) using 60 percent (v/v) acetone plus 40 percent (v/v) phosphate buffer pH 7.0; Group IV: 60 percent (v/v) aqueous acetone; Group V: same as Group II but classed differently because another color reagent was used; and Group VI: 7 mls. of pH 1.0 buffer (50 mls. of 0.2 M potassium chloride solution plus 97 mls. of 0.2 M hydrochloric acid) added to 50 mls. of distilled phenol. When a buffer was used in a solvent system the paper was also buffered using the same buffer.

Two pyrex baking dishes, 10 inches by 15 inches were ground so that when fitted together an air tight chamber would result. Twelve such units were built to act as chromatographic chambers.

Sheets of Whatman #1 filter paper 8½ x 13 inches were

used as the chromatograms. They were held in the chambers by means of glass rods so that only one end of the paper would be dipped in the solvent.

The chambers were held in place by wooden racks each of which was provided with a tilting mechanism. This allowed the papers to be equilibrated before dipping if necessary for a particular solvent system. The chromatography chambers were kept in a room at a constant temperature of 24°C. Each chamber was run using 25 mls. of solvent.

Spotting was done using micro-pipettes spacing eight spots on one paper. Several runs of samples were made in order to determine the amount to be spotted for a particular storage time. The amounts actually spotted are as follows for the various storage times and temperatures: 70°F. storage, 0, 15, 30, 45 days at 20 microliters; 60 days, 10 microliters; 80 days, 5 microliters; 120, 160, 200 days, 2 microliters. Those stored at 100°F. for 0, 15, 30, 45 days were spotted at the rate of 20 microliters; 60 days, 5 microliters; 80 days, 2 microliters; and 120, 160, 200 days, 1 microliter. Chromatograms developed with the Group I solvent system required a 24 hour equilibration before being dipped into the solvent, while none of the others required equilibration.

Chromatograms were developed for color by spraying with the following solvents: Group I: 2 percent ninhydrin

in ethanol with 2 percent acetic acid; Group II: 1 percent ninhydrin in ethanol with 0.25 percent triethylamine; Group III: 2 percent ninhydrin in ethanol: Group IV: (proline-1 percent isatin in ethanol), (histidine-diazotized sulphanilamide solution); Group V: 1 gm. of p-dimethylaminobenzaldehyde in 90 mls. of acetone with 10 mls. of concentrated hydrochloric acid; and Group VI: 0.4 percent ninhydrin in ethanol containing 4 percent acetic acid.

All chromatograms were air-dried. The quantity of an amino acid was determined by the density of its spot at the time of greatest density using a Photovolt electronic densitometer Model 525 with a #47 Wratten filter.

Calculations were carried out using the method of Block, et al. (10, p. 68-70). All free amino acids were calculated to mgs. per 100 gms. of dry meat by using ratio and proportion methods. The reading for the density of a sample spot was compared with the reading for a known standard which was run on the same paper. The total amino acids were calculated as the percentage of protein.

Statistical Analysis

All data for the amino nitrogen, trichloroacetic acid-soluble nitrogen, and total soluble nitrogen were statistically analyzed by analysis of variance as suggested by Cochran and Cox (915, p. 455).

RESULTS AND DISCUSSION

The results consist of the effects of the following variables: the pre-irradiation heating temperature; the storage time; and the storage temperature on the release of amino nitrogen, trichloroacetic acid-soluble nitrogen, total soluble nitrogen and free amino acids in beef muscle irradiated at 5.0 megarad.

It should be noted that the results for all constituents analyzed are averages of three replications.

Total Amino Acids

In Table 3 the total amino acid composition for 17 amino acids is shown for the longissimus dorsi muscle of beef. The results are in approximate agreement with those as indicated in Table 1 which were assayed using microbiological methods. This would indicate a general agreement between the methods of paper chromatography and microbiology for the total amino acid content of beef muscle.

Changes in the Nitrogenous Constituents of Beef as Induced by Pre-Irradiation Heating, Irradiation at 5.0 Megarad and Storage for Two Hundred Days at 70°F. and 100°F.

It should be noted that all the data to eighty days storage for the amino nitrogen, total soluble nitrogen, trichloroacetic acid-soluble nitrogen, leucine,

TABLE 3

THE AMINO ACID COMPOSITION OF BEEF MUSCLE PROTEIN [1]

Amino Acids	gms./100 gms. Protein
Aspartic Acid	8.68
Glutamic Acid	13.83
Serine	3.20
Glycine	7.26
Threonine	4.36
Alanine	6.15
Methionine	2.39
Tyrosine	3.37
Valine	5.64
Phenylalanine	4.50
Leucine	8.56
Lysine	8.40
Cystine	1.02
Proline	5.17
Arginine	6.68
Histidine	2.99
Tryptophan	1.25

1. All amino acids except tryptophan analyzed by Bautista (6, p. 31)

phenylalanine, valine, methionine, alanine, threonine, glycine, serine, glutamic acid and aspartic acid are those of Bautista (6).

Amino Nitrogen

It can be seen from Table 4 that the interactions of storage temperature, storage time and pre-irradiation heating temperature on beef are significant. However, the storage temperature assumed the greatest importance in the increase of amino nitrogen. The storage time was next in importance, but it was little more so than the pre-irradiation heating temperature.

The effect of the above three variables on the amino nitrogen of irradiated beef can be seen in Tables 5a and 5b. In Table 5 it should be noted how the pre-irradiation heating temperature of 195°F. (cooked) resulted in a greatly retarded development of the amino nitrogen irrespective of the storage temperature. The pre-irradiation heating temperature of 150°F. did keep the amino nitrogen value at about half that of the raw sample, but was not nearly as effective as complete cooking (195°F.). It would appear that the meat which was cooked, then irradiated, and stored for 200 days was at about the same point in protein breakdown as the raw meat stored for 15-45 days regardless of storage temperature. The meat which was

TABLE 4

ANALYSIS OF VARIANCE FOR AMINO NITROGEN IN BEEF IRRADIATED AT 5.0 MEGARAD AS AFFECTED BY PRE-IRRADIATION HEATING TEMPERATURE, STORAGE TIME AND TEMPERATURE

Variation due to	Degrees of Freedom	Mean Square	F
Total	431		
Replication	2	1.9622	3.77*
Storage Temperature	1	592.8634	1138.15**
Storage Time	8	146.8568	281.93**
Pre-Irradiation Temperature	7	142.1594	272.91**
Stg. Temp. x Stg. Time	8	24.0243	46.12**
Stg. Temp. x Pre-Temp.	7	17.9449	34.45**
Stg. Time x Pre-Temp.	56	3.5276	6.77**
Stg. Temp. x Stg. Time x Pre-Temp.	56	1.8602	3.57**
Error	286	0.5209	

* Significant at 5% level.

** Significant at 1% level.

TABLE 5

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE AMINO NITROGEN CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD

(Values expressed as mgs. percent dry weight sample)

a. Stored at 70°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200	Pre-Irradiation Temperature Mean
Raw	250	340	370	440	500	570	349	532	897	472
100	230	330	360	430	490	580	432	532	760	460
110	230	320	340	390	460	510	403	304	815	419
120	250	320	330	340	410	470	355	502	737	412
130	190	300	290	320	450	450	356	388	598	371
140	180	270	290	300	420	410	455	417	572	364
150	150	280	250	300	320	390	320	164	607	309
195	100	80	100	90	70	80	129	189	422	140
Storage time Mean	198	280	287	326	390	432	349	378	676	

b. Stored at 100°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200	Pre-Irradiation Temperature Mean
Raw	250	470	810	850	970	1250	979	772	1176	836
100	230	470	680	820	1070	1190	438	861	1070	758
110	230	470	690	860	970	1140	638	951	1054	778
120	250	380	690	800	790	1010	482	799	950	683
130	190	350	480	630	720	840	572	755	968	611
140	180	320	490	630	700	820	470	631	644	542
150	150	320	390	520	710	700	316	437	634	464
195	100	100	100	90	90	90	108	201	552	159
Storage time Mean	198	357	462	650	750	870	500	675	881	

cooked and stored for 120 days at 100°F. (Table 5b) had apparently not formed much more amino nitrogen than the cooked, unstored meat. Meat that has been heated to 150°F. and stored at 100°F. for 160 days would seem to have little more breakdown of the protein to amino nitrogen than the raw meat stored for 15 days at 100°F.

The fact that the increase of amino nitrogen during the storage of irradiation sterilized meat is inversely proportional to the pre-irradiation heating temperature has been indicated previously by the work of Bautista (6, p. 41) who reported on similarly treated samples held at 34°F. The work as represented here would seem to be consistent with the earlier work of Bautista.

The two way table indicating the effect of time of storage and storage temperature is presented in Table 6. There was about a four fold increase in the amino nitrogen content irrespective of pre-irradiation heating or storage temperature. It is interesting to note that as the pre-irradiation heating temperature increased, less amino nitrogen could be quantitated but this was further modified by both the time and temperature of storage. An increase in the latter two variables almost always resulted in a greater quantity of the amino nitrogen.

TABLE 6

THE EFFECT OF STORAGE TIME AND TEMPERATURE ON THE AMINO NITROGEN CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD

(Values expressed as mgs. percent dry weight sample)

Storage Temperature $0^{o}F.$	Storage time (days)									Storage Temperature Mean
	0	15	30	45	60	80	120	160	200	
70	199	279	287	325	391	433	349	377	640	364
100	199	357	540	649	751	878	500	675	881	603
Storage Time Mean	199	318	413	487	571	655	424	526	760	

Trichloroacetic Acid-Soluble Nitrogen

Although a three way interaction between the storage temperature, storage time and pre-irradiation heating temperature was not indicated from Table 7 the storage time would appear to have had the greatest influence upon the release of TCA-soluble nitrogen. The storage temperature was second in prominence, while the pre-irradiation heating temperature was somewhat less important. The interactions between the storage temperature and storage time were significant as were the other two way interactions.

The effect of the three variables mentioned above on the TCA-soluble nitrogen of irradiated beef can be seen in Tables 8a and 8b. As can be seen from Table 8 irrespective of the storage temperature the pre-irradiation heating temperature of 195°F. (cooked) resulted in a slow increase of TCA-soluble nitrogen. As can be seen from the means in Table 8, the TCA-soluble nitrogen values for samples pre-heated to 195°F. are about half those of samples pre-heated to 150°F. It can be seen that the meat that was pre-heated 195°F., then irradiated, and stored for 200 days had nearly the same TCA-soluble nitrogen content as did the raw meat stored for 60 to 80 days.

It would appear that the release of TCA-soluble nitrogen during the storage of irradiated meat will increase with decreasing pre-irradiation heating temperatures.

TABLE 7

ANALYSIS OF VARIANCE FOR TCA-SOLUBLE NITROGEN IN BEEF IRRADIATED AT 5.0 MEGARAD AS AFFECTED BY PRE-IRRADIATION TEMPERATURE, STORAGE TIME AND TEMPERATURE

Variation due to	Degrees of Freedom	Mean Square	F
Total	431		
Replication	2	0.0072	0.208(N.S.)
Storage Temperature	1	6.5539	189.419**
Storage Time	8	22.0405	637.008**
Pre-Irradiation Temperature	7	3.2956	95.248**
Stg. Temp. x Stg. Time	8	1.1694	33.7976**
Stg. Temp. x Pre-Temp.	7	0.0924	2.6705**
Stg. Time x Pre-Temp.	56	0.1928	5.572**
Stg. Temp. x Stg. Time x Pre-Temp.	56	0.0446	1.289(N.S.)
Error	286	0.0346	

** Significant at 1% level.

TABLE 8

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE TCA-SOLUBLE NITROGEN CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD

(Values expressed as mgs. percent dry weight sample)

a. Stored at 70°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200	Pre-Irradiation Temperature Mean
Raw	280	290	390	530	1250	1430	1780	1280	1830	1006
100	270	280	380	480	1180	1380	1570	1770	1980	1032
110	270	260	320	460	1010	1270	1500	1230	1850	907
120	260	250	330	430	910	1100	1340	1290	1730	848
130	240	260	320	370	800	1020	1170	930	1690	755
140	230	240	290	360	800	870	1470	970	1610	760
150	210	220	260	280	790	810	950	770	1680	663
195	80	100	120	90	80	90	720	490	1480	361
Storage time Mean	230	238	303	375	852	996	1312	1091	1731	

b. Stored at 100°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200	Pre-Irradiation Temperature Mean
Raw	280	320	400	650	1260	1690	2580	1410	2980	1285
100	270	340	380	650	1130	1550	2230	1960	2830	1260
110	270	300	400	620	1110	1480	2540	2020	2540	1253
120	260	280	380	620	990	1130	1950	1820	2660	1121
130	240	260	310	500	920	950	2050	1730	2790	1083
140	230	250	300	420	860	940	2360	1590	2190	1015
150	210	210	280	350	880	740	1860	1320	1760	845
195	80	90	90	90	110	80	1050	780	1650	446
Storage time Mean	230	256	317	487	907	1070	2077	1578	2425	

TABLE 9

THE EFFECT OF STORAGE TIME AND TEMPERATURE ON THE TCA-SOLUBLE NITROGEN CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD

(Values expressed as mgs. percent dry weight sample)

Storage Temperature $0^{o}F.$	Storage time (days) 0	15	30	45	60	80	120	160	200	Storage Temperature Mean
70	230	238	303	378	855	998	1314	1082	1734	792
100	230	255	318	489	908	1071	2076	1578	2425	1038
Storage Time Mean	230	246	310	433	881	1023	1695	1330	2079	

A two way table showing the effect of storage time and storage temperature is shown in Table 9. Irrespective of pre-heating temperature, the quantity of TCA-soluble nitrogen released in meat stored at 100°F. was about thirty percent greater than that released at 70°F. storage. This can be seen from the storage temperature means in Table 9.

From Table 9 the importance of storage time on the release of TCA-soluble nitrogen should be noted. Irrespective of the pre-irradiation heating temperature or storage temperature there was about a ten fold increase during 200 days of storage.

An increase in storage time or storage temperature always resulted in higher TCA-scluble nitrogen values irregardless of the pre-heating temperature employed. However, the pre-irradiation heating temperature of 195°F. did drastically reduce the release of TCA-soluble nitrogen.

Total Soluble Nitrogen

While all interactions between storage temperature, storage time and pre-irradiation heating appear significant in Table 10 for their effect on total soluble nitrogen, the pre-irradiation heating temperature was the most important. The storage temperature was next in importance while the storage time was a slightly less critical variable. The two way interactions of storage temperature and storage time,

TABLE 10

ANALYSIS OF VARIANCE FOR TOTAL SOLUBLE NITROGEN IN BEEF IRRADIATED AT 5.0 MEGARAD AS AFFECTED BY PRE-IRRADIATION HEATING TEMPERATURE, STORAGE TIME AND TEMPERATURE

Variation due to	Degrees of Freedom	Mean Square	F
Total	431		
Replication	2	0.1000	5.405**
Storage Temperature	1	2.2360	120.8648**
Storage Time	8	1.6852	91.0918**
Pre-Irradiation Temperature	7	7.7105	416.7837**
Stg. Temp. x Stg. Time	8	0.2315	12.5135**
Stg. Temp. x Pre-Temp.	7	0.0450	2.4324*
Stg. Time x Pre-Temp.	56	0.1261	6.8162**
Stg. Temp. x Stg. Time x Pre-Temp.	56	0.0372	2.0108**
Error	286	0.0185	

* Significant at 5% level.

** Significant at 1% level.

storage temperature and pre-irradiation heating, as well as storage time and pre-irradiation heating were all significant.

In Tables 11a and 11b can be seen the effects of the above three variables on the total soluble nitrogen in irradiated beef. The pre-irradiation heating temperature of 195°F. kept the soluble nitrogen value at a lower level than did the other pre-heating temperatures. As can be seen from the pre-temperature means in Table 11 the pre-heating temperature of 150°F. held down the release of soluble nitrogen, but was not as effective as cooking at 195°F. Meat that was cooked and irradiated, then stored for 200 days at 100°F. had about the same soluble nitrogen content as raw meat stored under the same conditions for 60 to 80 days. Meat which was cooked, irradiated, then stored for 200 days at 70°F. was similar in total soluble nitrogen content to raw meat stored at 70°F. for 45 days.

It would appear from Table 11 that the increase in total soluble nitrogen is greater as the pre-irradiation heating temperature decreases.

The two way table with the effect of storage time and storage temperature is indicated in Table 12. The amount of total soluble nitrogen released in irradiated meat stored at 100°F. was slightly greater than that released at 70°F. storage irrespective of storage time or pre-heating.

TABLE 11

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE TOTAL SOLUBLE NITROGEN CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD

(Values expressed as mgs. percent dry weight sample)

a. Stored at 70°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200	Pre-Irradiation Temperature Mean
Raw	676	1460	1760	1910	2010	2060	2480	1920	2840	1901
100	670	1310	1700	1906	2040	2040	1990	2220	2730	1845
110	640	1000	1613	1820	1920	1940	2160	1950	2690	1748
120	530	940	1660	1713	1723	1870	2060	1980	2540	1668
130	520	770	1496	1613	1630	1890	1770	1580	2300	1507
140	350	603	1193	1267	1473	1647	1950	1620	1850	1328
150	330	500	980	960	1140	1223	1900	1260	2350	1182
195	286	270	570	460	793	960	900	710	1950	776
Storage time Mean	500	851	1371	1456	1591	1703	1912	1655	2405	

b. Stored at 100°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200	Pre-Irradiation Temperature Mean
Raw	676	1403	1790	1880	1883	2200	2680	1870	3010	1932
100	670	1453	1673	1790	2020	2140	2790	2600	2800	1992
110	640	1460	1760	1930	1970	2010	2550	2360	2760	1937
120	530	1360	1740	1847	1730	2003	2490	2110	2720	1836
130	520	753	1413	1550	1700	1920	2400	2190	2850	1699
140	350	720	1241	1460	1500	1780	2430	1830	2190	1500
150	330	540	1140	1126	1240	1173	1860	1770	2180	1262
195	286	420	670	770	850	990	1350	1140	2050	947
Storage time Mean	500	1013	1428	1544	1611	1777	2318	1983	2507	

TABLE 12

THE EFFECT OF STORAGE TIME AND TEMPERATURE ON THE TOTAL SOLUBLE NITROGEN CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD

(Values expressed as mgs. percent dry weight sample)

Storage Temperature °F.	Storage time (days)									Storage Temperature Mean
	0	15	30	45	60	80	120	160	200	
70	501	856	1372	1452	1591	1704	1912	1653	2405	1494
100	501	1014	1430	1545	1611	1777	2353	1984	2563	1642
Storage Time Mean	501	935	1401	1496	1601	1742	2132	1818	2484	

This can be noted from the means for storage temperature in Table 12.

The effect of storage time in the release of total soluble nitrogen is also shown in Table 12. There was about a five fold increase in the constituent during 200 days of storage, irrespective of the storage temperature or pre-heating temperature.

As the pre-irradiation heating temperature increased, less total soluble nitrogen was found in meat but this was modified to some extent by the storage time and storage temperature. As the storage temperature and time increased the total soluble nitrogen nearly always increased, irrespective of the pre-irradiation heating temperature.

Free Amino Acids

Leucine. The amount of free leucine released in beef during storage after pre-irradiation heating and irradiation at 5.0 megarad is shown in Tables 13a and 13b. For the meat stored at 70°F., there was a steady increase with time in the free leucine in the raw samples and those heated at 100°, 110° and 120°F. Beef pre-heated to 130° and 140°F. showed only traces of free leucine at the beginning of the storage period but as the storage time increased free leucine was found in increasing amounts. Beef pre-heated to 150°F. reacted much the same as that heated to 140°F.

TABLE 13

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE LEUCINE CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD
(Values expressed as mgs. percent dry weight sample)

a. Beef irradiated at 5.0 megarad and stored at 70°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0.7	3.4	3.2	3.9	4.6	6.6	26.7	17.2	12.8
100	0.6	3.2	3.2	4.1	4.3	5.8	35.3	17.2	17.1
110	0.5	3.4	4.1	4.1	4.6	6.4	17.9	13.7	13.7
120	0.5	1.6	3.1	4.0	3.7	5.5	16.8	13.2	13.8
130	T	1.5	2.1	3.2	3.8	5.6	8.6	10.8	15.5
140	T	1.4	2.1	3.4	3.4	4.9	6.2	8.6	7.6
150	0	1.1	1.2	1.7	3.3	3.4	2.7	5.9	2.8
195	0	T	0.5	0.9	1.6	2.9	0	T	0

b. Beef irradiated at 5.0 megarad and stored at 100°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0.7	3.5	3.9	9.6	8.9	29.6	27.1	29.5	91.7
100	0.6	3.8	3.6	10.6	10.6	32.9	32.0	25.5	63.1
110	0.5	4.0	3.9	9.8	10.4	49.1	25.6	22.9	50.6
120	0.5	3.8	4.3	10.9	10.8	38.5	26.6	21.3	48.3
130	T	2.4	2.6	9.1	10.7	37.7	17.4	24.6	50.9
140	T	2.0	2.0	6.1	11.9	29.6	18.4	16.3	32.6
150	0	1.2	1.3	6.4	11.5	16.5	16.8	10.3	13.0
195	0	T	3.0	1.0	10.5	17.6	T	T	T

but the amount of free leucine found was considerably less. Free leucine was found in certain stored samples which had been pre-heated to 195°F. but at 120, 160 and 200 days storage only traces were found at the concentrations spotted. Attempts were made to increase the quantity of material applied to the paper so that these trace amounts could be quantitated. This was not successful since the other amino acids in the mixture were present in such large amounts that they obliterated the amino acid in question due to overrun.

When the pre-heated and irradiated beef was stored at 100°F. the amount of free leucine was greatly increased. It appeared as if the only effect of the storage temperature was that of an increase in the splitting of the amino acid from its parent protein.

The effect of storage time on the release of free leucine of pre-heated and irradiated beef was to increase the amount found as the storage increased. However, at 70°F. storage there was a decrease after 120 days after a previous increase in free leucine. There may have been a deamination mechanism active during the later storage times.

Phenylalanine. The release of free phenylalanine during the storage of beef after pre-irradiation heating and irradiation at 5.0 megarad can be seen in Tables 14a and 14b. Considering the meat stored at 70°F., there was a continuous increase with time in the free phenylalanine in

TABLE 14

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE FREE PHENYLALANINE CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD

(Values expressed as mgs. percent dry weight sample)

a. Beef irradiated at 5.0 and stored at 70°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	T	2.3	2.8	3.3	5.1	18.8	11.4	6.4	6.4
100	T	2.1	2.6	2.8	4.2	16.9	8.7	8.4	7.0
110	T	1.8	2.9	2.8	5.1	17.0	7.3	7.4	6.5
120	T	3.3	4.0	3.9	8.9	13.9	6.3	3.7	8.6
130	0	0.7	3.5	3.2	5.0	14.0	5.6	2.8	6.3
140	0	0.5	3.1	3.0	4.6	9.7	4.3	2.9	3.6
150	0	T	1.0	1.7	3.1	4.3	T	T	T
195	0	0	T	0.7	1.7	4.1	T	T	0

b. Beef irradiated at 5.0 megarad and stored at 100°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	T	2.3	2.7	5.6	6.8	7.4	16.2	10.6	14.1
100	T	2.3	2.3	5.8	6.9	7.2	17.2	13.8	13.7
110	T	1.4	1.6	5.0	7.3	14.7	15.9	13.0	13.9
120	T	1.4	1.9	5.7	5.5	14.4	14.4	12.0	7.9
130	0	1.1	1.4	4.5	5.6	7.0	8.4	9.1	13.9
140	0	0.5	0.9	2.6	5.1	7.0	12.0	5.3	8.0
150	0	T	0.3	2.7	4.1	6.5	7.8	T	T
195	0	0	0	T	3.4	6.6	0	0	0

the raw samples and those pre-heated to 100°, 110°, and 120°F. Beef that was pre-heated to 130° and 140°F. showed no free phenylalanine at the start of the storage period but as the storage time increased free phenylalanine was quantitated in increasing amounts. The beef pre-heated to 150°F. and 195°F. reacted much the same as that heated to 140°F. but the amount released was much less. However at 120, 160 and 200 days storage only traces of free phenylalanine were found in beef pre-heated to 150°F., while no free phenylalanine was found in some of the meat pre-heated to 195°F. with the concentrations spotted. Any attempt to increase the quantity of material spotted resulted in the complete obliteration of the spot area with streaks from other amino acids.

The pre-heated and irradiated beef stored at 100°F. had greater amounts of free phenylalanine released. The effect of the storage temperature increase was to increase separation of the amino acid from the protein.

As the storage time increased an increase in the free amino acid was noted up to 80 to 120 days, when either a decrease or a leveling off was observed. There could have been some loss of free phenylalanine through deamination during the latter part of the storage time.

Valine. In Tables 15a and 15b is shown the amount of free valine released during storage of beef samples after pre-irradiation heating and irradiation at 5.0 megarad.

TABLE 15

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE FREE VALINE CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD
(Values expressed as mgs. percent dry weight sample)

a. Beef irradiated at 5.0 megarad and stored at 70°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0.7	4.3	4.9	4.0	5.1	10.5	18.3	13.6	14.1
100	0.2	3.7	4.0	4.6	4.7	7.7	20.0	15.0	16.5
110	0.2	3.7	4.5	4.7	5.1	8.6	9.9	12.2	11.5
120	T	3.5	3.9	4.8	4.5	7.4	13.8	11.0	11.6
130	T	1.1	3.8	4.4	5.0	7.4	8.6	9.9	11.2
140	T	0.8	3.3	3.9	4.1	5.9	4.1	5.2	8.4
150	0	T	1.3	1.6	2.5	4.6	T	4.2	5.6
195	0	T	0.8	1.3	1.2	2.2	0	0	0

b. Beef irradiated at 5.0 megarad and stored at 100°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0.7	4.4	4.8	7.3	7.7	29.6	20.4	26.7	45.3
100	0.2	4.1	4.0	7.6	8.3	30.9	26.3	23.4	42.0
110	0.2	4.3	4.2	6.9	8.4	35.0	16.2	25.9	42.6
120	T	3.9	4.4	6.9	8.0	25.2	22.1	20.0	38.3
130	T	1.7	2.2	6.2	7.8	24.7	16.2	21.9	34.0
140	T	1.0	2.0	4.0	6.8	17.6	13.2	13.4	12.2
150	0	0.4	1.1	4.1	8.1	6.5	6.7	11.9	8.7
195	0	T	0.4	0.7	6.9	9.9	0	0	0

For meat stored at 70°F., there was a steady increase with storage time in the free valine in raw samples and those pre-heated to 100°, 110°, 120° and 130°F. Beef pre-heated to 140°F. showed only traces of free valine at the beginning of storage but as the storage time increased the free valine was found also to increase. Beef pre-heated to 150°F. was found to react quite like that pre-heated at 140°F. except that a much smaller amount of the free valine was quantitated. Free valine was found in some stored samples which had been pre-heated to 195°F. but at the 120, 160 and 200 day storage periods no free valine was found at the concentrations spotted. Any attempt to increase the concentration of the spotting was unsuccessful due to the presence of larger amounts of other amino acids which blotted out the separation areas of free valine.

The pre-heated and irradiated beef that was stored at 100°F. had greatly increased amounts of free valine. The effect of increased storage temperature was to cause an increase in the splitting of valine from the protein.

The effect of storage time on the release of free valine of the pre-heated and irradiated beef was to increase the amount quantitated as the storage time increased. However, there appeared to be a leveling off of the release of free valine after 80 days storage. Either less free valine appeared or some of it was broken down.

Methionine. The amount of free methionine released during storage of beef after pre-irradiation and irradiation 5.0 megarad can be seen in Tables 16a and 16b. When the beef was stored at 70°F. there was a steady increase with time in the free methionine content in raw samples and those pre-heated to 100°, 110°, 120°, 130° and 140°F. up to 80 days storage when a decrease was noted. Beef pre-heated to 150°F. reacted much the same as that heated to 140°F. but at the 120, 160 and 200 days storage periods very little of the free amino acid was quantitated. Free methionine was found in some samples pre-heated to 195°F. but in very low quantities. At 120, 160 and 200 days storage no free methionine was found at the quantities spotted. Higher amounts could not be spotted due to interference from other amino acids in higher concentration.

As pre-heated and irradiated beef was stored at 100°F. the amount of free methionine increased greatly over that stored at 70°F. The free methionine content increased to 80 days storage then decreased noticeably. The possibility of deamination of the free methionine or loss in some way should not be discounted.

Alanine. Tables 17a and 17b can be consulted for the amount of free alanine released in pre-irradiation heated and irradiated beef during storage. Considering the beef stored at 70°F., there was a steady increase with time in

TABLE 16

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE FREE METHIONINE CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD
(Values expressed as mgs. percent dry weight sample)

a. Beef irradiated at 5.0 megarad and stored at 70°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0	2.9	3.1	3.7	4.5	10.2	10.7	5.6	5.5
100	0	2.1	4.0	4.1	4.6	10.5	9.8	3.9	4.6
110	0	2.7	3.4	3.4	3.8	10.0	7.3	5.9	10.5
120	0	2.6	3.0	3.3	3.7	8.4	4.7	3.2	2.4
130	0	0.9	3.1	2.9	3.6	8.4	6.3	3.9	4.8
140	0	0.6	3.8	3.7	4.0	11.3	4.7	3.3	2.3
150	0	0.4	2.9	2.8	3.8	6.3	2.3	0.7	0.5
195	0	T	T	T	1.4	5.4	0	0	0

b. Beef irradiated at 5.0 megarad and stored at 100°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0	3.8	4.5	8.1	8.2	37.0	9.3	10.5	14.6
100	0	3.4	3.9	7.2	9.6	30.3	9.9	10.5	11.9
110	0	3.2	3.2	5.7	8.8	30.7	7.4	9.8	14.4
120	0	3.1	3.4	6.0	9.4	30.1	10.6	5.9	13.7
130	0	2.7	3.1	6.5	8.9	16.4	7.3	9.9	12.0
140	0	1.6	1.8	4.2	8.1	23.5	7.2	6.0	7.2
150	0	0.8	1.2	5.2	8.5	21.7	6.2	T	T
195	0	T	0.4	1.7	7.3	9.9	0	0	0

TABLE 17

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE FREE ALANINE CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD
(Values expressed as mgs. percent dry weight sample)

a. Beef irradiated at 5.0 megarad and stored at 70°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	14	30	45	60	80	120	160	200
Raw	0.6	1.9	2.4	3.1	4.2	35.0	18.6	44.3	12.7
100	0.5	1.3	2.1	3.7	5.4	34.0	25.4	48.0	9.4
110	0.2	1.2	2.1	2.8	4.2	30.6	11.4	24.6	9.2
120	T	0.9	1.2	2.0	2.8	25.9	6.6	37.4	9.3
130	T	0.5	1.1	1.4	2.0	22.4	12.4	11.3	14.1
140	T	T	0.5	0.5	1.8	21.3	6.6	20.0	11.2
150	0	T	T	T	1.4	6.2	2.2	9.7	3.6
195	0	0	0	T	1.1	3.3	2.6	3.1	T

b. Beef irradiated at 5.0 megarad and stored at 100°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0.6	2.6	3.2	16.1	17.6	30.8	46.3	65.9	65.6
100	0.5	2.7	2.8	17.0	20.7	42.4	56.0	67.2	56.3
110	0.2	2.9	3.6	17.1	18.4	33.8	37.2	59.7	58.9
120	T	2.1	2.7	17.2	17.4	36.1	34.4	47.8	44.3
130	T	2.0	2.2	16.2	17.2	35.3	43.2	49.4	42.0
140	T	1.1	1.3	17.0	14.2	17.6	22.5	47.7	46.0
150	0	T	T	12.3	10.3	16.3	8.8	26.6	23.4
195	0	0	0	2.9	3.4	9.9	T	T	3.9

the free alanine in raw samples and those pre-heated at 100°, 110°, 120°, 130° and 140°F. Beef pre-heated to 150°F. showed no free alanine at the beginning of storage but as the storage time increased free alanine was quantitated in small amounts. Free alanine was found in very small amounts in samples pre-heated to 195°F. but at 200 days storage only a trace could be quantitated at the concentration spotted. Any attempt to increase the quantity of material spotted was met with failure due to the interference of other amino acids.

As pre-heated and irradiated beef was stored at 100°F. the amount of the free amino acid greatly increased over that at 70°F. storage.

As the storage time increased a greater release of free alanine was noted up to 160 days storage at 70°F. when a decrease was noted. The amount of free alanine increased steadily during storage at 100°F.

Threonine. The release of free threonine during the storage of beef after pre-irradiation heating and irradiation at 5.0 megarad can be seen in Tables 18a and 18b. There was a steady increase with time in the free threonine content of beef pre-heated to 100°, 110°, 120°, 130°, 140° and 150°F. and stored at 70°F. Beef pre-heated to 195°F. was found to release small quantities of free threonine but at 120 and 160 days storage the difficulties of spotting

TABLE 18

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE FREE THREONINE CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD

(Values expressed as mgs. percent dry weight sample)

a. Beef irradiated at 5.0 megarad and stored at 70°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0	0.6	1.2	3.6	3.8	25.1	8.1	14.1	6.0
100	0	0.6	2.1	3.1	5.3	24.4	9.4	12.4	7.6
110	0	T	1.8	2.4	3.8	15.3	7.0	6.1	10.1
120	0	T	T	2.6	3.4	14.3	8.9	4.0	4.0
130	0	T	T	1.8	2.5	11.2	15.2	T	6.0
140	0	0	T	T	2.3	8.9	5.9	5.1	6.0
150	0	0	0	T	1.2	5.7	1.7	1.8	3.0
195	0	0	0	T	1.0	5.5	0	T	1.6

b. Beef irradiated at 5.0 megarad and stored at 100°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0	1.1	2.1	13.5	14.7	29.6	13.8	14.9	7.9
100	0	1.2	2.1	13.9	16.7	31.1	12.4	17.2	14.2
110	0	T	0.7	14.1	15.7	23.1	7.4	14.0	19.9
120	0	T	T	14.2	15.3	25.7	T	20.4	14.8
130	0	T	T	13.4	14.7	23.2	2.6	16.1	3.6
140	0	T	T	7.8	7.7	10.1	4.0	13.2	11.5
150	0	0	0	5.8	9.1	13.9	1.7	7.3	8.0
195	0	0	0	T	4.3	14.1	0	0	T

with interference from some other amino acids did not allow the use of greater concentrations. A quantitative amount was read at 200 days, however, and a noticeable decrease was noted from 80 days storage which had been the last samples quantitated at the pre-heating temperature of 195°F.

As the pre-heated and irradiated beef was stored at 100°F. the amount of free threonine quantitated was greater than that found in beef which had been stored at 70°F. It may be that the only effect of the storage temperature was that of an increase in the amount of threonine to split from the protein.

The effect of storage time on the release of free threonine in pre-heated and irradiated beef was to increase the amount found as the time of storage increased to 80 days. After 80 days storage there is a noticeable decrease in free threonine. This may be due to a loss of the free threonine content by deamination or some other form of breakdown.

Glycine. In Tables 19a and 19b can be seen the amount of free glycine released during the storage of pre-heated and irradiated beef. Raw meat and that pre-heated to 100°, 110°, 120°, 130° and 140°F. and stored at 70°F. was found to have a steady increase in the release of free glycine with time. Beef pre-heated to 150°F. was found to react much the same as that pre-heated at 140°F. but with considerably less being quantitated. As can be seen from Table

TABLE 19

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE FREE GLYCINE CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD

(Values expressed as mgs. percent dry weight sample)

a. Beef irradiated at 5.0 megarad and stored at 70°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	T	2.2	2.5	5.3	7.7	21.1	6.8	10.0	3.2
100	T	2.0	2.2	8.1	7.9	15.4	9.0	6.7	3.1
110	T	1.5	2.2	3.7	6.1	20.6	4.0	3.2	4.7
120	T	0.7	1.4	4.0	5.4	13.3	T	2.6	2.0
130	0	0.6	1.7	3.5	5.7	14.9	4.0	1.2	2.6
140	0	T	0.7	3.4	4.1	14.2	1.1	2.1	3.2
150	0	0	T	T	4.5	6.9	T	0.6	2.0
195	0	0	0	T	2.5	6.6	0	0	T

b. Beef irradiated at 5.0 megarad and stored at 100°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	T	4.8	5.7	7.0	9.7	29.6	10.1	6.1	16.4
100	T	5.1	5.8	7.2	11.8	36.3	5.3	11.5	13.1
110	T	4.4	5.0	7.4	10.5	25.0	5.0	9.4	12.1
120	T	2.2	4.3	7.2	8.6	25.4	9.6	9.8	7.6
130	0	1.5	1.8	6.8	9.8	25.4	8.8	8.2	5.2
140	0	1.4	2.0	6.1	8.2	15.5	6.0	7.6	8.8
150	0	T	T	6.2	9.1	9.1	T	2.2	T
195	0	0	0	T	6.5	13.2	0	0	1.0

19a, the meat that was pre-heated at 195°F. was found to release some free glycine but only in relatively small quantities. It should be noted that where traces appear in the tables all efforts to quantitate the free amino acid failed, as was the case in the preceeding amino acids studied.

At 100°F. storage the free glycine was released in higher amounts than at 70°F. storage. It would seem that the higher temperature allowed more of the glycine to be released from the native protein.

The effect of storage time was to increase the amount of free glycine found as the storage time increased. However, after 80 days storage there was a sharp decrease in the free glycine quantitated. The possibility of the deamination of glycine exists.

Serine. In Tables 20a and 20b is shown the amount of free serine released during the storage of pre-heated and irradiated beef. The samples stored at 100°F. had over two times the free serine that like samples had which were stored at 70°F.

The effect storage time had on the release of the free amino acid can be seen easily from the tables. The free serine reached a peak amount at 80 days storage after which it declined slightly to 200 days of storage. This

TABLE 20

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE FREE SERINE CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD

(Values expressed as mgs. percent dry weight sample)

a. Beef irradiated at 5.0 megarad and stored at 70°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0	0.7	1.6	3.7	6.0	12.5	5.5	11.4	5.2
100	0	0.6	4.0	5.6	6.6	15.0	7.5	6.7	6.4
110	0	T	2.5	4.1	5.1	12.2	4.7	4.1	6.1
120	0	T	T	1.3	3.4	10.3	T	T	4.7
130	0	0	T	1.5	2.5	7.4	7.3	T	4.0
140	0	0	T	T	1.5	5.9	2.5	2.1	3.2
150	0	0	0	T	1.7	5.7	1.1	T	4.1
195	0	0	0	T	0.6	5.5	0	0	5.3

b. Beef irradiated at 5.0 megarad and stored at 100°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0	0.7	1.6	8.6	11.1	29.6	8.9	8.8	9.3
100	0	0.8	2.6	8.5	11.7	14.5	8.2	16.4	14.3
110	0	0.4	0.8	7.1	15.0	29.5	7.6	10.6	12.0
120	0	T	0.8	8.7	9.2	28.8	8.3	6.0	9.9
130	0	T	0.3	8.6	9.7	14.1	6.1	5.4	6.4
140	0	0	T	4.4	6.3	25.4	3.7	7.7	9.0
150	0	0	0	4.8	7.1	13.0	1.1	8.6	8.2
195	0	0	0	T	6.1	13.2	0	0	1.0

phenomenon of the decrease of free serine after a buildup has also been noted with several other amino acids.

The effect the pre-irradiation heating had on the amount of free serine released is quite evident. The higher pre-irradiation heating temperatures held down the release of the free amino acid quite well. At the 100°F. storage temperature the samples heated to 150°F. and stored for 200 days had the same amount of free serine as the raw samples stored for 45 days.

Glutamic Acid. The release of free glutamic acid during the storage of pre-heated and irradiated beef can be seen from Tables 21a and 21b. The release of free glutamic acid in 100°F. storage was several times that for the samples stored at 70°F.

Storage time was an important variable in the release of free glutamic acid. It steadily increased during storage with the peak coming at 80 days for the 70°F. storage samples and at 200 days for those stored at 100°F. There was a slight decrease of the free amino acid after 80 days storage at 70°F.

The effect of pre-irradiation heating on the release of this amino acid is apparent. Very little if any free glutamic acid was found in the cooked samples, while very high amounts were found in those which were raw. Samples

TABLE 21

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE FREE GLUTAMIC ACID CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD
(Values expressed as mgs. percent dry weight sample)

a. Beef irradiated at 5.0 megarad and stored at 70°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	1.4	3.0	3.5	4.2	5.1	18.8	10.7	24.4	14.5
100	1.1	3.1	2.8	4.6	6.6	23.2	13.3	16.0	7.7
110	0.9	2.0	2.5	4.1	5.1	18.4	14.7	7.4	12.6
120	T	1.2	2.0	4.4	5.3	18.5	10.2	7.4	11.3
130	T	1.1	1.9	3.9	4.4	17.9	8.7	7.3	8.6
140	T	0.3	1.1	1.9	2.3	7.1	8.5	6.7	4.8
150	0	0.4	0.7	1.1	1.7	6.9	4.7	3.8	4.9
195	0	0	0	T	0.6	0.9	4.3	T	6.4

b. Beef irradiated at 5.0 megarad and stored at 100°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	1.4	4.0	4.8	10.6	11.6	22.2	28.1	24.6	46.9
100	1.1	4.3	5.1	11.3	12.8	40.0	27.8	39.5	21.9
110	0.9	4.3	4.9	11.6	13.2	44.2	32.2	27.5	31.8
120	T	2.5	3.0	12.0	12.7	28.8	13.0	18.1	24.2
130	T	2.5	2.4	11.7	13.7	19.7	17.9	14.9	20.6
140	T	0.3	1.7	5.2	9.5	14.1	9.1	10.6	23.3
150	0	T	T	5.1	6.4	13.0	2.8	9.0	12.8
195	0	0	0	T	3.6	6.6	1.1	T	T

heated to 150°F. and stored for 160 days at 100°F. were equivalent in the amount of free glutamic acid they contained to raw samples stored for 45 days. Efforts at quantitating free glutamic acid in samples where only traces were noted failed due to the interfering affects of other amino acids.

Aspartic Acid. The release of free aspartic acid of pre-heated, irradiated beef is shown in Tables 22a and 22b. In 70°F. storage only the 30, 45 and 60 day storage periods showed quantitative amounts of the free amino acid. In 100°F. storage only the 30 day samples showed significant amounts in the free state. After 60 days of storage there was not even a trace of free aspartic acid in the samples. The amino acid had either been broken down or it had recombined with the protein in some way.

Only the raw samples and those heated to lower temperatures had free aspartic acid in quantitative amounts. This might indicate that enzymic activity had some function in its release and disappearance.

The raw samples stored at 100°F. for 30 days had the highest amount released in the free state.

Lysine. The results for the release of free lysine in pre-heated and irradiated beef can be seen in Tables 23a and 23b. In 70°F. storage the release of a quantitative amount of free lysine was obtained only in the 80

TABLE 22

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE FREE ASPARTIC ACID CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD
(Values expressed as mgs. percent dry weight sample)

a. Beef irradiated at 5.0 megarad and stored at 70°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0	T	1.6	2.5	2.2	0	0	0	0
100	0	T	1.4	2.5	2.7	0	0	0	0
110	0	T	1.5	2.4	1.0	0	0	0	0
120	0	T	T	1.3	T	0	0	0	0
130	0	0	T	T	T	0	0	0	0
140	0	0	T	T	T	0	0	0	0
150	0	0	0	T	T	0	0	0	0
195	0	0	0	T	T	0	0	0	0

b. Beef irradiated at 5.0 megarad and stored at 100°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0	T	3.6	0	0	0	0	0	0
100	0	T	1.7	0	0	0	0	0	0
110	0	T	1.8	0	0	0	0	0	0
120	0	T	T	0	0	0	0	0	0
130	0	T	T	0	0	0	0	0	0
140	0	0	T	0	0	0	0	0	0
150	0	0	0	0	0	0	0	0	0
195	0	0	0	0	0	0	0	0	0

TABLE 23

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE FREE LYSINE CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD
(Values expressed as mgs. percent dry weight sample)

a. Beef irradiated at 5.0 megarad and stored at 70°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0	0	0	0	T	1.1	0	T	T
100	0	0	0	0	T	0.7	0	T	T
110	0	0	0	0	T	T	0	T	T
120	0	0	0	0	0	0	0	T	T
130	0	0	0	0	0	0	0	0	0
140	0	0	0	0	0	0	0	0	0
150	0	0	0	0	0	0	0	0	0
195	0	0	0	0	0	0	0	0	0

b. Beef irradiated at 5.0 megarad and stored at 100°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0	T	0.9	3.0	2.4	T	0	T	13.0
100	0	T	0.8	3.3	2.8	T	0	5.3	5.4
110	0	T	1.0	2.6	2.6	T	0	T	5.5
120	0	T	1.1	2.6	2.0	T	0	T	5.2
130	0	0	0.5	1.8	1.7	0	0	T	3.3
140	0	0	0.5	1.2	1.5	0	0	0	T
150	0	0	T	1.0	1.6	0	0	0	0
195	0	0	0	0.6	0.9	0	0	0	0

day samples. Traces were noticed in several others, but only in the raw samples or those heated to lower temperatures. Much more free lysine came out in the 100°F. storage samples. It started to be released after 15 days of storage and then peaked at 45 days. After 45 days there was a slow decline until no free lysine was detected at 120 days storage. At 160 days of storage the free amino acid again appeared and at 200 days had built up to its second and highest peak. Most of the efforts in attempting to secure quantitative results for the 80 and 120 day samples were fruitless due to the previously discussed interferences of other amino acids.

The effects of cooking and of the 150°F. pre-irradiation heating temperature were particularly noticeable since little or no free lysine appeared in those samples.

Tyrosine. In Tables 24a and 24b can be seen the release of free tyrosine during storage of pre-heated and irradiated beef. Free tyrosine was much more evident in the samples stored at 100°F. which had practically twice as much as the corresponding 70°F. storage samples.

The increase in free tyrosine was steady throughout and peaked at 200 days storage. A large increase occurred in the 100°F. samples between 30 and 60 days, and a huge increase was noticed after 80 days storage. For the samples stored at 70°F. there was a large increase in free

TABLE 24

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE FREE TYROSINE CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD
(Values expressed as mgs. percent dry weight sample)

a. Beef irradiated at 5.0 megarad and stored at 70°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0.7	1.6	2.4	5.0	7.8	12.6	31.2	54.9	74.5
100	0.6	1.4	3.0	5.8	6.6	11.8	29.0	48.8	68.0
110	0.5	0.6	2.6	4.9	5.5	9.8	24.5	49.4	67.7
120	T	1.3	2.2	5.3	5.2	9.8	19.6	34.9	64.1
130	T	0.6	1.9	4.4	4.1	7.4	15.9	30.7	51.2
140	T	0.7	1.5	3.0	2.6	5.4	5.9	19.4	49.9
150	T	0.3	1.1	1.8	2.5	3.0	T	T	16.0
195	T	T	0.5	0.6	1.0	0	0	0	T

b. Beef irradiated at 5.0 megarad and stored at 100°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0.7	1.9	4.5	14.5	25.1	28.4	93.3	122.0	142.0
100	0.6	1.5	4.7	12.9	27.6	27.8	107.0	138.0	131.0
110	0.5	1.9	3.6	12.6	23.3	36.9	80.9	126.0	128.0
120	T	1.4	3.4	11.6	23.9	27.7	83.0	94.4	114.0
130	T	1.6	2.4	7.1	12.2	25.3	34.5	49.2	99.7
140	T	1.3	1.8	7.5	11.7	9.4	34.0	34.7	99.2
150	T	0.6	1.1	3.4	4.2	3.2	15.7	22.5	24.4
195	T	0	0.6	2.7	2.1	0	0	0	0

tyrosine between 80 and 120 days with the increase continuing on to 200 days.

Pre-irradiation heating was an important factor in preventing the release of free tyrosine. The cooked samples had very little if any of the free amino acid present even after 200 days of storage at 100°F., while the raw samples had very large amounts present. With the very large amount of the free amino acid that was released in the 160 and 200 day samples it becomes easier to understand how tyrosine crystals could appear on the surface of raw, irradiated beef stored for 4 to 6 months (12, p. 606).

With the very steady increase in free tyrosine noted over 200 days of storage it could become quite useful as an index of proteolysis for the long term storage of irradiated meats.

Histidine. The results for the release of free histidine in pre-heated and irradiated beef can be seen in Tables 25a and 25b. Somewhat more free histidine was liberated under 100°F. storage conditions than was released at 70°F. storage. The increase of the amino acid was steady throughout the entire 200 day storage period. The increase was large between 30 and 60 days storage at both 70°and 100°F.

There was considerably less free histidine released from cooked samples as compared with raw samples, but the amount released in the former was much higher than that of

TABLE 25

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE FREE HISTIDINE CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD
(Values expressed as mgs. percent dry weight sample)

a. Beef irradiated at 5.0 megarad and stored at 70°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0	0.8	0.8	11.2	27.8	40.1	65.8	66.0	81.0
100	0	0.6	1.2	11.9	28.9	42.6	66.5	62.8	66.8
110	0	0.7	0.7	12.8	25.9	33.7	66.2	56.2	61.3
120	0	0.5	0.5	11.5	20.1	30.3	43.8	58.8	77.8
130	0	0.3	0.5	8.0	19.8	22.6	57.6	55.9	49.6
140	0	T	0.3	5.2	14.5	17.5	28.5	38.8	39.2
150	0	0	T	2.5	5.5	8.5	13.1	17.1	15.8
195	0	0	0	0.9	2.3	3.7	4.6	4.4	4.6

b. Beef irradiated at 5.0 megarad and stored at 100°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0	1.7	4.9	23.2	41.0	61.3	73.0	78.7	124.0
100	0	1.8	4.4	23.5	41.7	55.1	77.9	84.8	118.0
110	0	1.9	4.9	22.7	41.8	66.4	72.4	84.1	114.0
120	0	1.2	4.8	20.7	41.3	42.8	75.4	80.9	131.0
130	0	0.5	2.9	15.8	26.6	33.3	52.3	38.5	117.0
140	0	0.5	2.0	10.8	19.8	25.2	39.1	32.7	55.6
150	0	T	1.1	4.5	11.8	15.4	20.5	22.3	21.2
195	0	0	0.4	1.4	3.7	4.7	8.4	9.5	9.0

other free amino acids. The cooked samples stored for 200 days at 100°F. had about the same free histidine content as did the raw samples stored for 30 to 45 days. Histidine was apparently one of the interfering amino acids which had caused much trouble in the attempts to quantitate certain amino acids in cooked samples.

Arginine. The release of free arginine during the storage of pre-heated and irradiated beef can be seen in Tables 26a and 26b. This amino acid was released steadily and in large amounts. Three or four times more free arginine was found in the 100°F. storage samples than in those stored at 70°F.

The increase in free arginine in 200 days was from eight to thirty fold.

The cooked samples were found to have very little of the amino acid in the free form. The effect of the 150°F. pre-irradiation heating temperature was evident in holding down the release of free arginine in the 70°F. storage samples. An attempt to quantitate trace values found for some cooked samples met with failure due to the previously discussed problem of spotting and interference.

Cystine. No free cystine was found. If any was released during storage it immediately broke down and was not recovered as cystine.

TABLE 26

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE FREE ARGININE CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD
(Values expressed as mgs. percent dry weight sample)

a. Beef irradiated at 5.0 megarad and stored at 70°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	3.2	3.3	3.6	3.6	3.1	14.6	23.0	29.2	24.0
100	2.8	3.4	3.3	3.5	3.6	15.0	25.0	26.2	22.4
110	2.7	2.9	3.2	3.6	2.9	16.6	20.4	29.4	25.6
120	2.8	2.7	2.8	3.2	2.2	13.2	15.4	21.0	23.8
130	2.4	2.4	2.5	2.0	2.3	8.3	12.1	13.1	24.9
140	1.7	1.8	2.0	1.6	1.6	3.9	6.2	11.9	22.2
150	1.3	1.2	1.4	1.0	1.5	3.5	3.5	5.6	8.5
195	0.5	0.5	0.7	0.6	0.3	2.0	2.5	T	1.5

b. Beef irradiated at 5.0 megarad and stored at 100°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	3.2	5.5	11.2	20.2	22.1	32.2	53.8	58.3	109.0
100	2.8	5.1	10.4	19.4	24.6	33.6	55.2	49.9	96.6
110	2.7	5.8	11.1	20.9	22.2	36.9	51.1	48.7	100.0
120	2.8	4.6	8.9	16.9	19.2	30.6	42.3	58.8	79.1
130	2.4	4.3	6.0	13.6	15.5	21.8	17.6	47.7	68.5
140	1.7	2.8	4.0	10.3	11.0	8.1	10.5	30.5	46.0
150	1.3	1.5	3.2	7.9	7.2	5.7	7.1	8.3	35.9
195	0.5	0.7	1.0	1.2	1.6	T	T	T	T

Total cystine was, however, present in the meat but only in small amounts (Table 29).

Proline. The release of free proline during the storage of pre-heated and irradiated beef can be seen in Tables 27a and 27b. For the meat that was stored at 70°F., there was a steady increase with storage time in the free proline in raw samples and those pre-heated to 100°, 110°, 120° and 130°F. Beef pre-heated to 140°, 150° and 195°F. reacted much the same as that heated to 130°F. but at 80, 120, 160 and 200 days storage there often were only traces and sometimes no free proline could be found at the concentrations which were spotted.

When the pre-heated and irradiated beef was stored at 100°F. the quantity of free proline released was greatly increased over those stored at 70°F. It would seem that the higher storage temperature allowed a greater amount of proline to split from its original protein source.

Tryptophan. The results for the release of free tryptophan during the storage of pre-heated and irradiated beef can be seen in Tables 28a and 28b. Only a small amount of free tryptophan could be quantitated and then only at 45 to 80 days storage. There must have been an actual decrease in the free tryptophan after 80 days storage since the method for the separation of free tryptophan is not bothered

TABLE 27

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE FREE PROLINE CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD
(Values expressed as mgs. percent dry weight sample)

a. Beef irradiated at 5.0 megarad and stored at 70°F.

Pre-Irradiation Temperature	Storage time (days)								
0°F.	0	15	30	45	60	80	120	160	200
Raw	1.8	1.5	2.1	2.5	3.2	4.5	3.1	11.0	22.1
100	1.4	1.7	2.0	2.2	3.3	5.1	4.0	11.0	19.3
110	1.5	1.6	1.9	2.0	2.5	4.0	4.9	9.2	18.5
120	1.1	1.3	1.6	1.5	2.0	3.2	2.9	6.2	14.3
130	0.6	1.0	1.1	1.4	2.0	2.4	3.0	3.0	15.0
140	0.3	0.8	0.8	0.8	0.9	1.8	0	T	6.9
150	0.2	0.3	0.2	0.2	0.6	T	0	0	T
195	0.2	0.6	0.2	T	0.5	0	0	0	0

b. Beef irradiated at 5.0 megarad and stored at 100°F.

Pre-Irradiation Temperature	Storage time (days)								
0 F.	0	15	30	45	60	80	120	160	200
Raw	1.8	2.6	6.4	11.8	13.1	15.4	17.5	20.6	52.6
100	1.4	2.6	5.6	10.3	13.2	13.0	18.6	20.7	45.2
110	1.5	2.6	2.9	4.1	10.6	10.1	15.0	18.4	44.0
120	1.1	2.2	2.2	3.1	6.1	9.3	10.0	17.0	24.6
130	0.6	1.5	1.7	2.1	4.5	4.7	7.4	18.7	27.7
140	0.3	0.8	1.2	1.8	2.0	T	T	7.5	20.8
150	0.2	0.3	0.5	1.4	1.5	0	0	1.6	T
195	0.2	0.2	0.5	1.0	1.0	0	0	0	0

TABLE 28

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-IRRADIATION HEATING, STORAGE TIME AND TEMPERATURE ON THE FREE TRYPTOPHAN CONTENT OF BEEF IRRADIATED AT 5.0 MEGARAD
(Values expressed as mgs. percent dry weight sample)

a. Beef irradiated at 5.0 megarad and stored at 70°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0	0	0	0	2.0	3.2	T	T	T
100	0	0	0	0	1.7	2.5	T	T	T
110	0	0	0	0	1.2	2.4	T	T	T
120	0	0	0	0	0.6	1.4	T	T	T
130	0	0	0	0	T	T	0	0	0
140	0	0	0	0	T	T	0	0	0
150	0	0	0	0	T	T	0	0	0
195	0	0	0	0	T	0	0	0	0

b. Beef irradiated at 5.0 megarad and stored at 100°F.

Pre-Irradiation Temperature °F.	Storage time (days) 0	15	30	45	60	80	120	160	200
Raw	0	0	T	1.5	3.4	T	T	T	T
100	0	0	T	1.9	3.2	T	T	T	T
110	0	0	T	1.6	3.1	T	T	T	T
120	0	0	T	7.5	2.5	T	0	T	T
130	0	0	0	T	1.4	0	0	T	T
140	0	0	0	T	1.1	0	0	0	0
150	0	0	0	0	1.1	0	0	0	0
195	0	0	0	0	0.6	0	0	0	0

by the interference of other amino acids. Up to ten times the normal concentration spotted for the various storage times in question did not yield more than a trace.

It would appear that the pre-heating temperatures of 195°, 150° and 140°F. had the effect of holding down the release of free tryptophan during storage of irradiated beef.

Proline, arginine, histidine and tyrosine were released in steadily increasing amounts during 200 days of storage. The other amino acids were found in steadily increasing amounts up to 60 to 120 days when they either decreased in amount or stayed at about the same level. Cystine was not found in the free state.

The maximum release of free amino acids, irrespective of storage time, storage temperature or pre-irradiation heating temperature as the percentages of the total amino acids is as follows: aspartic acid, 0.05; glutamic acid, 0.37; serine, 1.02; glycine, 0.55; threonine, 0.79; alanine, 1.20; methionine, 1.71; tyrosine, 4.65; valine, 0.89; phenylalanine, 0.42; leucine, 1.18; lysine, 0.17; proline, 1.12; arginine, 1.80; histidine, 5.03; and tryptophan, 0.66 (Table 29).

To see if any appreciable change in the amounts of the total amino acids had taken place during storage a raw sample stored at 100°F. for 200 days was analyzed. The quantitative results of the analysis for the total amino acids

TABLE 29

THE MAXIMUM RELEASE OF AMINO ACIDS IN BEEF MUSCLE DURING STORAGE

(Values expressed as mgs./gm. nitrogen)

Amino Acid	Total Amount	Highest Amount Released	Percent Released
Aspartic Acid	542.5	0.249	0.05
Glutamic Acid	864.4	3.236	0.37
Serine	200.0	2.044	1.02
Glycine	453.8	2.509	0.55
Threonine	272.5	2.151	0.79
Alanine	384.4	4.639	1.20
Methionine	149.4	2.555	1.71
Tyrosine	210.6	9.799	4.65
Valine	352.5	3.131	0.89
Phenylalanine	281.3	1.188	0.42
Leucine	535.0	6.329	1.18
Lysine	525.0	0.897	0.17
Cystine	63.8	0.00	0.00
Proline	323.1	3.630	1.12
Arginine	417.5	7.522	1.80
Histidine	186.9	9.402	5.03
Tryptophan	78.1	0.519	0.66

in the 200 day sample are given in Table 30. It can be noted that the difference between the initial analysis (0 days storage) and the final analysis (200 days storage) is very slight and probably only reflects experimental error. This would indicate that there is no large loss of amino acids during storage by the actual breakdown of a free amino acid. There could be a small amount of breakdown but using the limited methods of this research it could not be detected.

The very small release of free amino acids in irradiated beef found during storage confirms that previously observed by Proctor and Bhatia (40, p. 359) and Bautista (6, p. 91).

Further work with the use of radioactive tracers may give more knowledge about some of the phenomena found here.

Physical Characteristics of Beef After Pre-Irradiation Heating, Irradiation of 5.0 Megarad and Storage at 70°and 100°F.

All the beef stored for 160 to 200 days at 100°F. was crumbly, foul smelling and dark in color. The cooked as well as the raw samples were in this generally decomposed state. Cooking, irradiation at 5.0 megarad and storage at 100°F. for 200 days may have so altered the structural proteins that even though microbiologically sterile

TABLE 30

THE TOTAL AMINO ACIDS OF BEEF MUSCLE AS AFFECTED BY IRRADIATION AT 5.0 MEGARAD, AND STORAGE AT 100°F. FOR TWO HUNDRED DAYS

(Values expressed as mgs./gm. nitrogen)

Amino Acid	Raw, non-stored Total Amount	Raw, stored Total Amount	Difference - or +
Aspartic Acid	542.5	531.8	-10.7
Glutamic Acid	864.4	810.0	-54.4
Serine	200.0	197.5	-02.5
Glycine	453.8	405.6	-48.2
Threonine	272.5	261.2	-11.3
Alanine	384.4	387.5	+03.1
Methionine	149.4	138.1	-10.3
Tyrosine	210.6	208.1	-02.5
Valine	352.5	350.0	-02.5
Phenylalanine	281.3	281.2	-00.1
Leucine	535.0	533.7	-01.3
Lysine	525.0	522.5	-02.5
Cystine	63.8	65.6	+01.8
Proline	323.1	325.0	+01.9
Arginine	417.5	415.6	-01.9
Histidine	186.9	181.8	-05.1
Tryptophan	78.1	71.2	-06.9

and inactive enzymatically the meat became quite undesirable. A slight increase in soluble exudate was noticed as the storage time increased. Off odors may have come from the action of irradiation on the plastic bag used to hold the meat samples.

The meat stored at 70°F. for 160 to 200 days was much better in general appearance. Colors were more natural with the cooked samples appearing brown and the raw samples red. Some darkening was noticed in raw and lightly heated samples but this did not appear to the author as undesirable. These samples were firm and had fairly good texture qualities. The off odor that was noticeable in raw and lightly heated samples was absent in those cooked or pre-heated to 150°F.

It is the view of this author that once meat is heated sufficiently to inactivate the proteolytic enzymes and sterilized by gamma irradiation the factors involved in storage which may cause undesirable physical properties are enhanced by high storage temperatures. It may be that non-enzymic chemical changes are taking place in the meat during long term storage when it has been previously sterilized with ionizing radiations.

There should be much more work done on the enzymic as well as non-enzymic proteolysis of beef using pre-irradiation cooking, irradiation sterilization and long term storage at higher than room temperatures.

SUMMARY AND CONCLUSIONS

1. As the time of storage increased irrespective of storage temperatures and pre-irradiation heating temperatures there was an almost continuous increase of the amino nitrogen, trichloroacetic acid-soluble nitrogen, total soluble nitrogen, free tyrosine, free arginine, free histidine and free proline. Twelve other amino acids were released in steadily increasing amounts up to 60 to 120 days of storage when they either decreased in amount or stayed at about the same level. Free aspartic acid disappeared altogether after 60 days of storage, while no free cystine was found in the meat at all. A slightly larger amount of free exudate was noted with an increase in storage time.

2. The meat stored at 100°F. all had higher amounts of the constituents analyzed than did the samples stored at 70°F. All long term (over 100 days storage) samples stored at 100°F. were crumbly, foul smelling and dark in color. It is possible that the odor may have resulted from the use of plastic containers.

3. A pre-irradiation heating temperature of 150°F. kept the release of all constituents analyzed at lower levels than did the pre-irradiation temperatures of 140°F. and below. Pre-irradiation cooking at 195°F. kept the release

of the constituents analyzed at very low levels. The slight changes noted in the cooked samples may be attributed possibly to non-enzymic proteolysis. The odor of meat pre-heated to 150°F. or 195°F. and stored for 200 days at 70°F. was good, while those pre-heated at 140°F. and less had some off odor.

4. Only a minimum of difference in the total amino acids before and after storage was found.

5. The percentages of the following amino acids calculated on the scale of mgs. of amino acid per gm. of nitrogen released at their highest concentration of the total in the meat are: aspartic acid, 0.05; glutamic acid, 0.37; serine, 1.02; glycine, 0.55; threonine, 0.79; alanine, 1.20; methionine, 1.71; tyrosine, 4.65; valine, 0.89; phenylalanine, 0.42; leucine, 1.18; lysine, 0.17; proline, 1.12; arginine, 1.80; histidine, 5.03; and tryptophan, 0.66.

6. Cystine was not found in the free state.

7. Tyrosine was released at such a steady rate it might be useful as an index of proteolysis during long term storage.

8. From this study it is recommended that a pre-irradiation heating temperature somewhat above 150°F. and possibly below 195°F. be used in order to lessen the rate of

proteolytic activity in irradiated beef during storage. The irradiated beef should be stored at 70°F. or less if possible in order to insure good physical qualities.

BIBLIOGRAPHY

1. Albanese, Anthony A. The amino acid requirements of man. Advances in Protein Chemistry 3:227-293. 1947.

2. Alexander, J. C. and C. A. Elvehjem. Chemical constituents of meat; isolation and identification of nitrogenous components in meat. Agricultural and Food Chemistry 4:708-711. 1956.

3. Allison, James B. Biological evaluation of proteins. Advances in Protein Chemistry 5:155-200. 1949.

4. Association of Official Agricultural Chemists. Official Methods of Analysis. 8th ed. Washington D. C., The Association, 1955. 1008 p.

5. Baldwin, R. R., J. R. Lowry and R. Thiessen, Jr. Some effects of processing on the nutritive values of proteins. Food Research 16:107-117. 1951.

6. Bautista, Flordeliza R. Changes in the nitrogenous constituents of beef as induced by pre-heating and irradiation and storage for eighty days. Master's thesis. Corvallis, Oregon State College, 1959. 106 numb. leaves.

7. Bhatia, D. S. and B. E. Proctor. Effects of high-voltage cathode rays on aqueous solutions of histidine monohydrochloride. Biochemical Journal 49:550-553. 1951.

8. Block, R. J. Amino acid composition of food proteins. Advances in Protein Chemistry 2:119-134. 1945.

9. Block, R. J. and D. Bolling. The amino acid composition of proteins and foods. Springfield, Illinois, Charles C. Thomas, 1945. 396 p.

10. Block, R. J., E. L. Durrum and G. Zweig. A manual of paper chromatography and paper electrophoresis. New York, Academic Press, 1955. 484 p.

11. Block, R. J. et al. The effects of baking and toasting on the nutritional value of proteins. Archives of Biochemistry 10:295-301. 1946.

12. Cain, R. F., A. F. Anglemier, Lois A. Sather, Flor R. Bautista, and R. H. Thompson. Acceptability of fresh and precooked radiated meats. Food Research 23:603-610. 1958.

13. Chen, K. K., and H. C. Bradley. The studies of autolysis X. The autolysis of muscle. Journal of Biological Chemistry 50:151-164. 1924.

14. Chick, H. J. et al. The biological value of proteins. VII. The influence of the variation in the level of protein in the diet and of heating the protein on its biological value. Biochemical Journal 20:1712-1719. 1935.

15. Cochran, William G. and Gertrude M. Cox. Experimental designs. New York, John Wiley and Sons, Inc., 1950. 458 p.

16. Daniel, E. P. and E. V. McCollum. Studies of the nutritive value of fish meals. 1931. 19 p. (U. S. Dept. of Commerce. Bureau of Fisheries. Investigation Report no. 2, vol. 1)

17. Evans, R. J. and H. A. Butts. Heat inactivation of the basic amino acids and tryptophan. Food Research 16:415-421. 1951.

18. Fox, J. B. et al. Studies on myoglobin and related heme pigments in muscle tissue. In: American Meat Institute Foundation's Annual Report, Chicago, 1958. p. 27. (Bulletin No. 36)

19. Ginger, I. D. et al. Effect of aging and cooking on the distribution of certain amino acids and nitrogen in beef muscle. Food Research 19:410-416. 1954.

20. Gliken, W., and A. Loewy. The autolytic and hydrolytic decomposition of protein under normal and pathlogical conditions. Biochemische Zeitschrift 10:498-505. 1908.

21. Greaves, E. O., A. F. Morgan and M. K. Loveen. The effect of amino acid supplements and of variations in temperature and duration of heating upon the biological value of heated casein. Journal of Nutrition 16:115-128. 1938.

22. Hackman, R. H., and M. Lazarus. Quantitative analysis of amino acids using paper chromatography. Australian Journal of Biological Sciences 9:281-292. 1956.

23. Hertzman, A. B., and H. C. Bradley. The studies of autolysis. XIII. The kinetics of the autolytic mechanism. Journal of Biological Chemistry 62:231-243. 1924.

24. Hiller, A. J., J. Plazin and D. D. Van Slyke. A study of conditions for Kjeldahl determination of nitrogen in proteins. Journal of Biological Chemistry 176: 1401-1420. 1948.

25. Husaini, S. A. et al. Studies on meat. I. The biochemistry of beef as related to tenderness. Food Technology 4:313-316. 1950.

26. Huxley, H. E. The contraction of muscle. Scientific American 199(5):66-82. 1958.

27. Ingvaldsen, T. Fish meals. Part 1. The effect of the high temperature employed for drying, on the nitrogen partition in fish meals. Canadian Journal of Chemistry and Metallurgy 13:97-99. 1929.

28. Kon, S. K. and Z. Markuze. The biological values of the proteins of breads baked from rye and wheat flours alone or combined with yeast or soyabean flour. Biochemical Journal 25:1476-1484. 1931.

29. Ma, T. S. and G. Auazaga. Microkjeldahl determination of nitrogen. Industrial and Engineering Chemistry, Analytical Edition 14:280-282. 1942.

30. Maynard, A. L. and A. V. Tunison. Influence of drying temperatures upon the digestibility and biological value of fish proteins. Industrial and Engineering Chemistry 24:1168-1171. 1932.

31. Maynard, A. L., R. C. Bender and C. M. McCay. Vitamin A and protein content of various fish meals. Journal of Agricultural Research 44:591-603. 1932.

32. Metta, V. C. and B. C. Johnson. The effect of radiation sterilization on the nutritive value of foods. 1. Biological value of milk and beef proteins. Journal of Nutrition 59:479-490. 1956.

33. Mitchell, H. H. A method of determining the biological value of protein. Journal of Biological Chemistry 58:873-903. 1924.

34. Mitchell, Phillip H. A textbook of biochemistry. 2d ed. New York, McGraw-Hill, 1950. 695 p.

35. Morgan, A. F. and L. E. Kern. The effect of heat upon the biological value of meat protein. Journal of Nutrition 7:367-379. 1934.

36. Morgan, Bruce H. Current status of radiation preservation of foods. Food Processing, June, 1957. p. 24-28.

37. Newton, R. C. et al. Review of literature on meat for 1936. Food Research 2:581-605. 1937.

38. Nutritive value of proteins. Nutrition Reviews 16: 56-58. 1958.

39. Peters, John P. and Donald D. Van Slyke. Quantitative clinical chemistry. Vol. 2. Baltimore, Williams and Wilkins, 1932. 957 p.

40. Proctor, B. E. and D. S. Bhatia. Effects of high-voltage cathode rays on amino acids in fish muscle. Food Technology 4:357-361. 1950.

41. Proctor, B. E. and D. S. Bhatia. Effects of high-voltage cathode rays on aqueous solutions of tryptophan, tyrosine, phenylalanine and cystine. Biochemical Journal 51:535-538. 1952.

42. Proctor, B. E. and D. S. Bhatia. Mode of action of high-voltage cathode rays on aqueous solutions of amino acids. Biochemical Journal 53:1-3. 1953.

43. Rice, Eldon and Jack F. Beuk. The effects of heat upon the nutritive value of protein. Advances in Food Research 4:233-279. 1953.

44. Rose, William C. The nutritive significance of the amino acids. Physiological Reviews 18:109-136. 1938.

45. Schweigert, B. S. et al. Amino acid content of processed meats. Journal of the American Dietetic Association 28:23-26. 1952.

46. Sheffner, A. L., R. Adachi and H. Spector. The effect of radiation processing upon the *in vitro* digestibility and nutritional quality of proteins. Food Research 22:455-461. 1957.

47. Subramanian N., and M. V. Lakshminarayan Rao. Behaviour of tyrosine and cystine on paper chromatograms and their quantitative estimation. Journal of Scientific and Industrial Research 14B:566-570. 1955.

48. Tytell, A. A. and H. Karsten. Effect of soft X-rays on urease and catalase. Proceedings of the Society for Experimental Biology and Medicine 48:521-525. 1941.

49. Wierbicki, Eugen *et al.* The relation of tenderness to protein alterations during postmortem aging. Food Technology 8:506-511. 1954.

50. Wierbicki, Eugen *et al.* Post-mortem changes in meat and their possible relation to tenderness together with some comparisons of meat from heifers, bulls, steers and diethylstilbestrol-treated bulls and steers. Food Technology 10:80-86. 1956.

51. Zender, R. *et al.* Aseptic autolysis of muscle: Biochemical and microscopic modifications occurring in rabbit and lamb muscle during aseptic and anaerobic storage. Food Research 23:305-326. 1958.