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

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

INTRODUCTION

The use of ionizing radiations as a means of food preservation was recognized and suggested as a fruitful area of research early in 1953. Through the efforts of the U. S. Atomic Energy Commission, the Department of Defense and the Quartermaster Food and Container Institute, various aspects of the new field of food preservation have been under extensive investigation.

This method of preservation is attractive in that it may utilize a waste product, from atomic reactors, as the source of ionizing energy and that it may accomplish sterilization with little or no rise in the temperature of the foods subjected to the energy. It is possible to preserve foods without subjecting them to the conventional method of heat sterilization and without the attending changes in the character of the foods brought on by rather long exposure to high temperatures. In short, this is a method of "cold sterilization".

In heat processing, temperatures in the range of 240^{o} to 250^{o}F. at times are utilized, especially in meat products, of up to 90 minutes duration. The necessity for these conditions is brought about by the fact that any process must be sufficient to kill the spores of *Clostridium botulinum*.

Like heat processing, there is a prime factor to consider what radiation dosage is necessary to effect preservation. Morgan (52, p. 24) reported that a dose of 4.8 megarad is necessary for *Clostridium botulinum* to satisfy public health requirements and to achieve commercial sterility.

Of equal importance, particularly in food products, is that of inhibiting the action of enzymes naturally present in the foods. All methods of food preservation not only consider the microbiological approach but are clearly defined as to their effects upon the enzymatic systems. In canning, prior to the final sterilization by heat, a somewhat lower level of heat may be used to inactivate the enzymes which may cause various difficulties if allowed to remain active. With ionizing radiations, the use of 4.8 megarad radiation dose does not inactivate the enzymes and while the product may be bacteriologically sterile, spoilage may ensue through the continued action of the enzymes. Tytell and Kersten (73, p. 525) reported that gamma dosages in the order of 10 times that required for bacterial sterilization is required to inactivate the enzyme found in various tissues.

The objection to the use of increased radiation dosage is the adverse effect it produces, not only on the color and flavor, especially in beef, but also on the texture as reported by Schnautz (67, p. 1). In view of this, some method or combinations of methods must be utilized to not only inactivate the enzymes but also provide a commercially sterile product. It follows that this must be accomplished while maintaining an acceptable level of the usual characteristics of the particular food product.

Enzymes exhibit greater resistance to ionizing radiations than bacteria while the reverse is true when heat sterilization is considered. Consequently, it appears that a combination of these two methods of preservation could be used in the final process. This would allow (1) the inactivation of enzymes by heat, and (2) the use of a low level of irradiation to kill the organisms. With the latter, the use of low levels of radiation would be expected to not have the extreme effect on flavor as would be experienced by radiation levels necessary to inactivate the enzyme systems.

Research on this phase, as reported by Cain, Anglemier, Sather, Bautista and Thompson (13) showed that beef heated to 160°F. prior to irradiation at 1.86 or 2.79 megarad did not develop undesirable changes during storage at 72°F. during an eight months storage test. The meat was judged as still acceptable to a large flavor panel at the end of the storage period.

These results attracted the author to determine if pre-heating at temperatures less than 160°F. would effect stabilization of the agents responsible for increasing the nitrogenous constituents in irradiated beef when stored at various temperatures for 80 days.

REVIEW OF LITERATURE

Proteins are one of the classes of food materials associated with an animal's growth, maintenance, repair, reproduction and resistance to diseases. Mitchell (48, p. 95) stated that proteins are indispensable components of the food of animals and that their absence is fatal. Dietary proteins are known to furnish the amino acids which Allison (3, p. 156) reported to be the building blocks of the body proteins, in turn forming the basic configuration of the living system.

Since proteins are of such vital importance to both human beings and animals, it appears that the quality of a dietary protein could only be evaluated in terms of its being incorporated and utilized in the body. Mitchell (45, p. 874) defined this as the "biological value" of the protein or the percentage of the absorbed nitrogen retained in the body of the animal.

Investigations on the nutritive value of the proteins were initiated by Rose (66, p. 112-130) who found that mixtures of amino acids could replace proteins in the diets of the animals. It was reported that at least ten amino acids, arginine, histidine, isoleucine, leucine, tryptophan, lysine, methionine, phenylalanine, threonine and valine must be included in optimum quantities in the diet for normal growth in the rat. These were called the essential amino acids which could not be synthesized by the animal in sufficient quantities to meet growth requirements. Albanese (1, p. 249) and Mitchell (48, p. 95) also gave the same list of essential amino acids and in addition, the non-essential amino acids.

The most important source of protein is that of animal origin (7, p. 123-130). Morgan and Kern (51, p. 377) and Newton, Piskur, Ramsbottom, Robinson and McLean (53, p. 589) reported that beef has a high biological value as compared to cereals and vegetables.

The analyses of the constituents of proteins are quite complex. They include methods such as the Kjeldahl nitrogen method, chromatography, electrophoretic and counter-current distribution methods for partition of constituents. Martin and Synge (39, p. 1-83), Block and Bolling (8), and Block, Durrum and Zweig (11) have extensively reviewed and discussed the methods of protein analysis, especially those for the amino acids. There is no standard method of presenting the results of the analyses of proteins but the most widely used has been in terms of grams of amino acid per 100 grams of protein. For the determination of protein nitrogen, the use of both micro- and macrokjeldahl methods are almost universal. These methods do not show the composition of the proteins. When the protein in a food becomes degraded, that is, denaturation or chemical breakdown occurs, the total nitrogen content would be the same, unless ammonia is lost, but this would not reveal the extent of degradation of the protein.

For a thorough analysis of the proteins, the amino acid composition is the ultimate goal of the determination. Modern chromatographic techniques for the analysis of the specific amino acids have replaced the precipitation and colorimetric determinations (9). The use of both column and paper chromatography has been extensively reviewed and discussed by Block and Bolling (8) and Block *et al*. (11). The microbiological methods of determination are still widely employed.

The amino acid content of beef muscle had been determined by Schweigert, Bennett, McBride and Guthneck (69, p. 23-26), Alexander and Elvehjem (2, p. 708) and Block (7, p. 124). Their results, shown in Table 1, were obtained by the use of microbiological methods. Alexander and Elvehjem (2, p. 708) were able to increase the percentage of nitrogen from 87 to 100 per cent by analyzing for additional nitrogenous constituents such as carnitine, creatine, creatinine, purines and vitamins.

Effect of Heat on Proteins

Heat is a very important agent in modifying the nutritive value of the protein. This was substantiated by several investigators (5, p. 117; 51, p. 377; 71, p. 524; 57, p. 374-376; 20, p. 420; 55, p. 437; 49, p. 790-792; 26, p. 325; 24, p. 103; 50, p. 354), who showed that heat altered the character of the protein in such a way that the nutritive value was either impaired or improved.

A review of Melnick and Oser (43, p. 69) pointed out that heat processing of foods had a profound effect on the nutritive value of the protein without affecting the protein content, the essential amino acid composition or the degree of protein digestibility. Thus, even if methionine was made nutritionally available by heat processing, and the value of lysine degraded, the quantitative analysis of these amino acids were found to be the same.

The sensitivity of cereal proteins to heat has been known since the experiments of Morgan (49, p. 790-792) and Morgan and King (50, p. 354). Growth experiments with rats showed that the proteins of cereals subjected to dry heat or toasting at 150° to 200°F for 30 to 45 minutes or to similar processes during manufacture did not provide factors for growth. Mitchell and Block (46, p. 610) and Stewart, Hensley and Peters (71, p. 524) have

TABLE 1

AMINO ACID COMPOSITION OF BEEF MUSCLE PROTEIN
(Expressed in mgs./100 gms. of protein)

Amino Acids	Block (7, p.124)	Schweigert *et al.* (69, 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	6.20
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
Isoleucine	6.3	5.07	5.7
Tryptophan	1.3	1.10	1.3

reported a marked reduction in the digestibility and nutritive value of a cereal breakfast food submitted to the puffing process.

Lysine appears to be particularly heat susceptible. Waisman and Elvehjem (74, p. 111) reported that protein-bound lysine is inactivated by heat so that it is not nutritionally available. Greaves, Morgan and Loveen (28, p. 126), Rice and Beuk (64, p. 223-279), and Evans and Butts (20, p. 420) reported that lysine was the first amino acid destroyed when casein was heated at 140°C. for 30 minutes, and histidine, the second. Baldwin, Lowry and Thiessen (5, p. 117) reported arginine to be particularly susceptible, with tryptophan and methionine, next, then histidine and valine. Phenylalanine, leucine, isoleucine and threonine were not affected to any appreciable extent during heating at temperatures of 212° to 250°F. The susceptibility of arginine, tryptophan, methionine, histidine and valine to heat was confirmed by Block, Cannon, Wissler, Steffee, Straube, Frazier and Woolridge (12, p. 300), Kon and Markuze (36, p. 1483-1484) and Chick, Boas-Fixen, Hutchinson and Jackson (14, p. 1718).

In the case of leguminous seeds the effect of heat was usually to improve the digestibility in navy beans (75, p. 17), velvet beans (76, p. 294) and soyabeans (31, p. 231). In these cases, evidently the intensity of heat treatment and the time of application determine the degree of improvement. Too severe treatment may nullify the favorable effects of a milder treatment. Evans and McGinnis (21, p. 459-461), Evans, McGinnis and St. John (22, p. 671-672) and Riesen, Clandinin, Elvehjem and Cravens (65, p. 149) demonstrated that moderate heating of raw soybean oil meal increased its nutitive value and supported chicks' growth, while autoclaving the oil meal at 130°C. for one-half to one hour decreased the value. Similarly, Clandinin, Cravens, Elvehjem and Haplin (15, p. 399) showed that soybean meal autoclaved at 15 pounds pressure for 4 minutes resulted in a meal which supported the growth of chicks, while the same meal autoclaved at the same pressure but for one to 4 hours did not support normal growth. Lysine and methionine had to be supplemented.

Mitchell, Hamilton and Beadles (47, p. 24) investigated the digestibility and biological value of the proteins of raw, partly exploded and totally exploded soyabean flours compared to that encountered in beef protein. They reported that the digestibility and biological value of the soyabean protein could be increased by heat processing. The biological value of partially exploded soyabean flour was similar to that of beef protein while that of the totally exploded flour was 4 units lower, and that of the raw material, 16 units lower.

Further effects of heat treatment were carried out on fish meals by Daniel and McCollum (17, p. 18) and Ingvaldsen (34, p. 98-99) as cited by Maynard, Bender and McCay (41, p. 602). They concluded that the differences in heat treatments were responsible for the nutritive differences found in the fish meals. Maynard et al. (41, p. 602) reported that vacuum-dried white meal menhaden ranked first in nutritive value followed by steam-dried menhaden and the flame-dried menhaden, the least. They considered that temperatures above 195°F. reduced certain essential amino acids and the Vitamin A content, which in turn affected the biological values. Schneider (68, p. 731) confirmed the work of Maynard et al. (41, p. 602), with nitrogen-balance studies. Maynard and Tunison (40, p. 1170-1171) added that cooking and pressing resulted in the loss of soluble proteins.

Aware of the importance of meat as a source of dietary proteins, Morgan and Kern (51, p. 377) studied the effects of various methods of preparation on the nutritive value of beef. They found that biological value decreased with the severity of the heat treatment. Thus, the biological value of raw beef was 67 while that of beef boiled at ordinary pressure to an internal temperature of 85°C., boiled for 7 minutes at 15 lbs. pressure and boiled for one hour at 15 lbs. pressure were 60, 62, and 56 respectively.

Newton _et al_. (53, p. 589) reported a loss of water-soluble proteins which ranged from 50 to 70 per cent when meat was heated from 185° to 248°F. Ginger, Wachter, Doty, Schweigert, Beard, Pierce and Hankins (25, p. 410-416) reported a 4- to 30-fold decrease in the soluble protein nitrogen of raw beef when heated.

With almost all of the foods reviewed above, heat seems to be the primary agent in lowering the digestibility and nutritional value of the proteins.

Effect of Irradiation on Proteins

The advent of "cold sterilization" methods through the use of ionizing radiations provided the opportunity of using little or no heat in the sterilization of foods. Interests of the scientists were stimulated to develop a practical and safe method of food sterilization. As in other methods of food sterilization, certain problems associated with the nutritional value, color, flavor and other attributes are encountered.

The nutritional value of irradiated foods may be lowered by the destruction of the vitamins and other nutrients (54, p. 56). It is to be noted, however, that the loss of these constituents is also encountered in heat processing of foods. Thus, the nutritional value of irradiated foods may be no different than that of heat-processed foods. Hannan (30, p. 71-98) extensively reviewed the effects of irradiation on proteins and amino acids, pure or in their native form. Metta and Johnson (44, p. 489) found no change in the nitrogen content or digestibility of milk or beef proteins when irradiated at 2.79 megarad. The biological value of beef protein was not affected by irradiation, but that of milk protein was reduced 8 per cent by radiation treatment and 6 per cent by heat sterilization.

Proctor and Bhatia (60, p. 359) examined the effects of high-voltage cathode rays on the amino acids in haddock-fillets. At sterilizing doses of radiation, (2.25 megarad), the ten essential amino acids were not significantly destroyed. However, later studies by these investigators (6, p. 552; 61, p. 537-538; 62, p. 2) showed that irradiation of pure aqueous solutions of amino acids resulted in deamination in the order of histidine > cystine > phenylalanine > tyrosine > tryptophan. The ammonia evolved upon irradiation of histidine was considered to be contributed both by the nitrogen in the imidazole ring and α-amino group. The evolution of hydrogen sulfide upon irradiation of cystine gave evidence to the sulfur linkage as the site of attack. With the three aromatic amino acids, tryptophan, tyrosine, and phenylalanine, breakage of the benzene ring was indicated.

Scheffner, Adachi and Spector (70, p. 460) did not find any significant destruction of the essential amino acids in milk, turkey or beef protein, following gamma irradiation of 1.86 megarad.

This marked difference between the influence of a specified amount of irradiation on a pure amino acid or a simple peptide in an aqueous solution and that of a similar dosage applied to an intact protein like an enzyme or a whole food was attributed by Fox and Ise (23, p. 16) to the proportionately larger amount of free radicals in a dilute solution, hence a greater destruction. The meat samples that were used were considered to be much more concentrated, having a moisture content of 56 per cent, as opposed to the 1 M concentration of the individual amino acids used.

Fox and Ise (23, p. 17) listed the following pure amino acids in solution which may be destroyed readily by the ionizing radiations: arginine, histidine, lysine, methionine, phenylalanine, threonine, tyrosine and valine. Their data showed that methionine was altered to the extent of 14 per cent, and tyrosine, 8 per cent, following an irradiation dose of 1.86 megarad. The other amino acids did not show apparent destruction, though arginine showed some loss.

All the protein and amino acid degradation previously reviewed were attributed mainly to the effect of ionizing radiations, usually at several levels of radiation dosages and determined immediately after irradiation. Kraybill (37, p. 194) discussed the effects of radiation sterilization on the nutritive value of foods from a different point of view. He pointed out that possibility of protein degradation may occur during storage due to the fact that enzymes are not stabilized at sterilization dosages of radiation (63, p. 174). This had been substantiated in intact foods sterilized by irradiation (13; 35, p. 346; 59, p. 497; 19, p. 23; 18, p. 63; 63, p. 174; 27, p. 255-256). All of these investigators pointed to protein degradation of stored irradiated foods through enzyme actions. Doty and Wachter (18, p. 63), Cain *et al.* (13), and Drake, Giffee, Ryer and Harriman (19, p. 23) reported protein degradation in stored raw meats irradiated at 2.79 megarad. Although the meats were bacteriologically sterile, proteolytic activity occurred during the storage period, as evidenced by the development of supposedly tyrosine crystals and changes in certain other nitrogenous constituents.

Effect of Aging and Storage on Proteins

Aging caused an increase in the amino nitrogen content of the non-protein nitrogen fraction of both raw and cooked meats as reported by Ginger *et al.* (25, p. 410-416). Analyses revealed that total arginine, leucine and tyrosine contents of the samples were not materially affected. However, a greater percentage of these amino acids was found in the drippings and non-protein nitrogen fractions after 2 weeks' aging. Bound forms of leucine, tyrosine, glutamic acid and lysine were also present in the drippings and the non-protein nitrogen fraction of the beef.

An increase of 41 per cent in total soluble nitrogen and 14 per cent in non-protein nitrogen of beef muscle, aged 29 days at 34°F., was found by Olson and Whitehead (56, p. 182). They associated these changes with palatability and tenderness of the meat. Their data shows the effect of aging upon the nitrogenous constituents of the *longissimus dorsi* in beef. The values below are expressed as the percentage of the total nitrogen.

Days	0	29
Ammonium nitrogen (including α-amino nitrogen)	0.33	0.38
Glutamine nitrogen (includimg α-amino nitrogen)	0.16	None
Asparagine nitrogen	0.30	None
TCA precipitated nitrogen	0.37	2.41
Basic nitrogen	6.05	8.76
Total soluble nitrogen (not including the TCA precipitated nitrogen)	8.28	11.65

At a much lower storage temperature of 15° and 18°F., Hiner, Gaddis and Hankins (33, p. 228-229) observed a small amount of proteolysis in raw, frozen, and frozen stored beef. This was evidenced by the slight increases in the percentage of non-protein nitrogen, soluble nitrogen and amino nitrogen. However, they concluded that these chemical changes were insignificant when compared to the changes in the fats and the apparent high correlation between the fat changes and organoleptic evaluations.

EXPERIMENTAL METHODS

Sample Preparation

Three Hereford bull carcasses especially selected for uniformity were used in this study. The carcasses were aged 24 hours before the *longissimus dorsi* muscles, stripped of fat, were excised. Each pair of muscles from a single carcass was used as a replicate. Thus, three replications were involved. The muscles were chilled for three hours at -18°F. prior to sampling. Then they were mechanically sliced in cross section into samples of 1/4 to 3/8 inch thick. The samples weighed between 40 and 45 grams each. The slices of meat were placed in polyester, heat-sealable bags. As much air as possible was removed from the bags before they were heat sealed. The bags containing the meat were randomized in order to nullify the effect of the longitudinal variation in the muscle.

Heat Treatments

The sealed bags of meat were heated to internal temperatures of 100°, 120°, 130°, 140°, 150°, and 195°F. Unheated samples were also prepared for irradiation. A steam-heated water bath, equipped with temperature controls, was used. The water bath was controlled to 5°F. higher than the above indicated temperatures and the meat was immersed for a period of 6 to 8 minutes. In order to determine when the internal temperature had been reached, representative bags containing meat were equipped with thermocouples which served to indicate the attainment of the desired temperature of the experimental samples. As soon as the desired temperature was attained, the bags of meat were immediately removed and immersed in cold water to stop any further heating. The bags were then fitted into half pound flat (307 x 202) "C" enamel cans. The cans were mechanically closed and stored at -18°F. prior to irradiation. A single bag was placed in a can.

Samples of meats treated similarly except for irradiation were immediately analyzed and served as controls for the chemical determination.

Shipment

The samples which were to be irradiated were packed under dry ice and shipped in insulated containers to the Materials Testing Reactor, Idaho Falls. Eight days elapsed between shipment to and receipt from the radiation source. The samples were kept at 0°F. or under dry ice during all intervals except the actual irradiation period.

The non-irradiated samples were likewise treated to serve as controls but were kept frozen at all times.

Irradiation

The cans of meat were exposed to the gamma grid in order to attain a dose of either 0.1 or 5.0 megarad. The flux intensity was 1.74×10^6 rad per hour. On this basis, 3 minutes and 27 seconds were required for the 0.1 megarad samples and 2 hours and 52 minutes for the 5.0 megarad samples.

Storage Conditions

Upon receipt of the meat samples from the Materials Testing Reactor, all the non-irradiated, those irradiated at the 0.1 megarad level, and certain of those irradiated at the 5.0 megarad level were placed in storage at 34°F. The balance of the 5.0 megarad samples were divided for 70° and 100° storage.

Samples stored at 34°F. were analyzed at 15 day intervals up to and including 60 days. Those stored at 70° and 100°F. were analyzed at the same time interval as indicated above, and in addition, an 80 day storage period was included.

The samples were quantitatively analyzed for total moisture, total nitrogen, total soluble nitrogen, non-protein nitrogen, amino nitrogen, and free and total amino acids.

Moisture Determination

The meat was cut into very small pieces and approximately 3-5 grams were accurately weighed into tared aluminum pans. The pans were then placed in oven at 28 inches vacuum at 70°C. for 24 hours, cooled in a desiccator and weighed. The loss in weight was reported as the moisture content.

Total Nitrogen Determination

Approximately 1.5 to 2.5 grams of meat were accurately weighed on low nitrogen weighing paper for the total nitrogen determinations. The method of Hiller, Van Slyke, and Plazin (32, p. 1402-1420) was followed using the indicator developed by Ma and Zuazaga (38, p. 280-282). Instead of using the catalysts suggested by Hiller *et al*. (32, p. 1402-1420) "Kel-Pak" catalysts containing 10 gm. potassium sulfate and 0.3 gm. copper sulfate per package were used. Total nitrogen content was reported on a dry weight basis.

Preparation of the Samples for Soluble Nitrogen, Non-protein Nitrogen, and Amino Nitrogen Determinations

Ten grams of meat were blended for one minute with 25 mls. of distilled water in a micro-blender. The mixture was filtered through Whatman #12 paper and the filtrate was used for the following determinations:

Total Soluble Nitrogen Determination. The microkjeldahl method (4, p. 805) was used in the determination of soluble nitrogen. One ml. of the meat filtrate preparation above was digested with 2 mls. of concentrated sulfuric acid with one selenium-coated Hengar granule added as catalyst. Distillation was carried out in the Kirk-type microkjeldahl apparatus with 25 mls. of 4 per cent boric acid as the receiving solution. A 0.100 N sulfuric acid solution was used to titrate the ammonia evolved. The same indicator was used as previously reported for the total nitrogen method of determination. Total soluble nitrogen was reported in mgs. per cent on a dry weight basis.

Non-protein Nitrogen Determination. The AOAC method (4, p. 227) was followed in this determination, except that a 25 per cent trichloroacetic acid solution was used as the precipitant instead of the sodium phosphotungstate. One ml. of the trichloroacetic acid filtrate was used to determine the non-protein nitrogen by the microkjeldahl method as in soluble nitrogen determination. Non-protein nitrogen was reported in mgs. per cent on a dry weight basis.

Amino Nitrogen Determination. Five mls. of the meat extract were diluted to 100 mls. and an aliquot of 5 mls.

was used for amino nitrogen determination by the Van Slyke method (58, p. 385). Results were reported in mgs. per cent amino nitrogen on dry weight basis.

Amino Acid Determinations

Preparation of Samples.

1. Total amino acids: The sample was prepared by acid hydrolysis according to the method of Block, Durrum and Zweig (11, p. 64).
2. Free amino acids: The free amino acids were likewise prepared according to the method of Block *et al*. (11, p. 84). The samples were ground with absolute ethanol and extracted with chloroform and stored in half ounce bottles at room temperature.

Chromatographic Determinations. The amino acids were separated using one-dimensional buffered and unbuffered paper chromatography as suggested by Hackman and Lazarus (29, p. 282-288) (cf., McFarren, 1951). The amino acids determined were aspartic acid, glutamic acid, serine, glycine, threonine, and alanine (Group I); and methionine, valine, tyrosine, phenylalanine and leucine (Group II).

Sheets of Whatman # 1 filter paper, 8½" x 13" were used as chromatograms while Pyrex baking dishes of suitable dimensions served as chromatogram chambers. Two baking dishes with ground edges were placed together in such a manner that one served as the cover while the other one served as the solvent container. Two glass rods were glued across the solvent container near to the ends to serve as supports for the filter paper during the analysis. The part of the filter paper which dipped in the solvent was held in place by a heavy glass rod bent at right angles at its end to facilitate its handling.

The chambers were placed on a rack provided with a lever so it could be tilted to an angle of 30°. This facilitated dipping the papers into the solvents without opening the chambers after the papers were equilibrated. The chambers were kept in a fairly constant temperature room at 25°C. Thirty mls. of the required solvent were used for each development. For those amino acids in Group I, phenol-buffer pH 10 (74:26, w/w) was used as the solvent system. Those amino acids in Group II were developed with n-butanol-acetic acid-water solvent system (77:6:17, v/v).

Eight spots of the amino acid solutions were placed on the paper; one for the unknown, one for the standard mixture and the balance for the standard single amino acids in question. The center of the spots were one inch apart on a straight line made by folding the paper $2\frac{1}{2}$ inches from one end. Preliminary runs were made to determine how much of the unknown free amino acids should be spotted. Thus, twenty μl. were found necessary to spot, except for those samples irradiated at 5.0 megarad and stored for 60 days at 70° and 100°F. which were spotted 10 μl. The 80 day samples were spotted at the rate of 2 μl. for those stored at 70°F. and 1 μl. for those stored at 100°F. One μl. was spotted for all the standards which contained 2 μg. of the amino acid per μl. For the unknown total amino acids two levels of 1 and 2 μl. were spotted.

Chromatograms of Group I series required 24 hours of equilibration while those of Group II were equilibrated for 12 hours.

For color development, the buffered chromatograms (Group I) were sprayed with one per cent ninhydrin in 95 per cent ethyl alcohol containing 2 per cent glacial acetic acid. The unbuffered papers (Group II) were sprayed with one per cent ninhydrin in 95 per cent ethyl alcohol containing 0.25 per cent triethanolamine.

The chromatograms were air-dried and the density of the amino acids were measured after 8 hours. The Photovolt electronic densitometer Model 525 was used to measure the density of the spots.

The calculations involved for the determinations of the total amino acids were those suggested by Block _et al_. (11, p. 68-70). The total amino acids were reported as grams per 100 grams portein on a dry basis. The free amino acids were calculated by ratio and proportion. The density of the spots were measured and compared with the density of the spots of the standards whose concentrations were known. Owing to the small amounts of the free amino acids, they were reported in mgs. per 100 gms. of dried meat.

Statistical Analysis

The data obtained for total soluble nitrogen, non-protein nitrogen, and amino nitrogen were submitted to statistical analysis by analysis of variance (16, p. 455).

The data for free amino acids were likewise analyzed. Since no free amino acids could be demonstrated in certain samples, in others only traces were observed and in still other samples the free amino acids could be quantitated, the analysis of variance was impossible. Hence, the preheating temperatures were used as the criteria for analysis. If no free amino acids occurred in any of the samples, a value of 0 was assigned. If free amino acids occurred either in quantitative amounts or in traces in the raw samples, the value 1 was assigned; 2, if they occurred in the samples pre-heated to 100°F., and so on up to 195°F. to which was assigned a value of 8.

RESULTS AND DISCUSSION

The results of the effects of:

(1) the temperatures attained in pre-heating prior to irradiation,

(2) the gamma radiation dosage employed,

(3) the storage time, and

(4) the storage temperature

on the release of free amino acids were investigated. Changes in total soluble nitrogen, non-protein nitrogen and amino nitrogen content of beef as affected by these variables were classified into two categories:

I - Effects of the temperature attained in pre-heating of beef prior to irradiation at 0.1 and 5.0 megarad and storage for 60 days at 34°F.

II - Effects of the temperature attained in pre-heating beef irradiated at 5.0 megarad and stored for 80 days at 70° and 100°F.

In this study the term pre-temperature may be defined as the temperature attained in heating the meats prior to irradiation. Post-temperature refers to the storage temperature of the meats.

The individual values in the tables for each of the nitrogenous constituents are averages of three replications.

Total Amino Acids

The quantitative determination of the total amino acids, expressed in mgs. per 100 gms. of protein, of the longissimus dorsi of beef are shown in Table 2.

These data agree closely with those reported by Schweigert et al. (69, p. 23-26). However, variance within 3 mgs. per 100 gms. protein was noted for those values reported by Alexander and Elvehjem (2, p. 708) for aspartic acid, serine, glycine and proline. Block and Mitchell (10, p. 249) suggest that disagreement between chemical and biological determinatioms for amino acids may be due to the following: It is known that the composition of the protein, the availability of the amino acids, and the nutritive value of the proteins are correlated. When the amino acids are determined microbiologically and certain specific amino acids which are required for growth are not made available to the organism during the assimilation process, little or no growth of the organism occurs and the nutritive value of the protein is lowered.

Effects of the Temperature Attained in Pre-heating of Beef Prior to Irradiation at 0.1 and 5.0 Megarad and Storage for 60 Days at 34°F.

TABLE 2

THE AMINO ACID COMPOSITION OF BEEF MUSCLE PROTEIN

Amino Acids	Mgs./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
Isoleucine	5.75

Total Soluble Nitrogen. It may be seen in Table 3 that the predominating effect responsible for the changes in the total soluble nitrogen was the storage time, despite the fact that significant interactions occurred among the three main effects, i.e., temperature attained in pre-heating, irradiation dosage and storage time. The temperature to which the meat was pre-heated is an important secondary source of variation. The irradiation dose was significant but was not as dominating and influential as the other two main effects.

Tables 4a, b and c show the changes which occurred in the total soluble nitrogen content of beef when pre-heated to different temperatures, subjected to irradiation at 0.1 and 5.0 megarad, and stored for 60 days at 34°F. Non-irradiated samples subjected to the variables of pre-heating and storage are also included. It may be seen from these data that the values for total soluble nitrogen gradually decreased with an increase in the pre-heating temperatures used. Pre-heating to an internal temperature of 150°F. did not stop the increase in the total soluble nitrogen content over the 60-day storage interval. In all cases, the samples heated to 195°F. showed a definite decrease in the total soluble nitrogen. This is consistent with the previous reports (25, p.

TABLE 3

ANALYSIS OF VARIANCE FOR TOTAL SOLUBLE NITROGEN IN BEEF AS AFFECTED BY IRRADIATION DOSAGE, PRE-HEATING TEMPERATURES AND LENGTH OF STORAGE AT 34°F.

Variation due to	Degrees of freedom	Mean square	F
Total	359		
Replication	2	0.0264	11.00*
Radiation	2	0.9104	379.33*
Storage time	4	11.0368	4598.60*
Pre-temperature	7	3.6920	1538.33*
Rad. x Stg. Time	8	0.6210	258.75*
Rad. x Pre-Temp.	14	0.0287	11.96*
Stg. Time x Pre-Temp.	28	0.2902	120.92*
Rad. x Stg. x Pre-Temp.	56	0.0288	12.00*
Error	238	0.0024	

* Significant at the 5% level

TABLE 4

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME ON THE TOTAL SOLUBLE NITROGEN CONTENT OF BEEF STORED AT 34°F.

(Values expressed as mgs. % dry weight sample)

a. Non-irradiated beef

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	Pre-temperature Mean
Raw	576	786	790	1590	1670	1082
100	576	703	760	1573	1651	1052
110	616	620	766	1570	1703	1055
120	583	630	690	1553	1690	1029
130	523	590	666	1453	1396	925
140	603	533	537	1266	1223	832
150	336	410	530	1206	787	653
195	293	383	360	247	393	335
Storage Time Mean	513	581	637	1307	1314	

b. Beef irradiated at 0.1 megarad

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	Pre-temperature Mean
Raw	633	840	820	1560	1686	1107
100	583	753	840	1600	1690	1093
110	553	583	810	1596	1653	1039
120	540	510	800	1463	1580	978
130	517	460	796	1416	1393	916
140	480	380	580	1406	1120	793
150	340	383	490	610	650	495
195	293	310	360	320	406	338
Storage Time Mean	492	527	687	1246	1272	

c. Beef irradiated at 5.0 megarad

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	Pre-temperature Mean
Raw	676	736	1546	1710	1706	1274
100	670	690	1610	1620	1666	1251
110	640	586	1520	1690	1640	1215
120	530	630	1440	1640	1560	1160
130	520	530	1323	1590	1540	1160
140	350	420	1340	1240	1306	931
150	330	390	1220	920	880	748
195	286	313	360	380	466	361
Storage Time Mean	500	537	1294	1348	1345	

410-416; 53, p. 589; 40, p. 1170-1171) that heating results in a decrease of the soluble protein nitrogen of beef.

These data in Tables 4a, b and c show the two-way effects of storage time and pre-heating temperature for the non-irradiated, 0.1 and 5.0 megarad samples. The non-irradiated samples and those irradiated at 0.1 megarad did not show any great differences in total soluble nitrogen as shown by the mean values for the specific heat treatments over the storage period involved. The main effect of the irradiation dosage is shown in the mean values for total soluble nitrogen at the 5.0 megarad level.

In Table 4c, it may be noted that there was a significant difference between the mean values for total soluble nitrogen of the 5.0 megarad samples at the 30-day period and the mean values of the 0.1 and non-irradiated samples for the same period. It may also be seen that the total soluble nitrogen did not greatly increase beyond 45 days in the non-irradiated and 0.1 megarad samples. Between 30 and 45 days a two-fold increase in total soluble nitrogen was experienced in these samples. The two-fold increase in total soluble nitrogen was experienced during the 15-to-30 day storage period for those samples irradiated at 5.0 megarad.

The effect of the temperature to which the meat was pre-heated and of the irradiation dosage given the meat is shown in Table 5. It may be seen that the main difference in the mean values for irradiation dosage lies in the values for the 5.0 megarad samples. Furthermore, the clear-cut effect of pre-heating temperature in decreasing the total soluble nitrogen in irradiated beef is shown.

Non-protein Nitrogen. The analysis of variance for non-protein nitrogen as shown in Table 6 shows the same main effects as shown for total soluble nitrogen. The data show that although significant interactions occurred among the three main effects, the predominating effect responsible for the changes in the non-protein nitrogen was the storage time. The temperature to which meat was pre-heated gave a secondary effect, while the irradiation dosage, though significant, was not as important a source of variation as the other two effects.

Table 7a shows the non-protein nitrogen in the non-irradiated meats subjected to the variables of pre-heating and storage. Tables 7b and 7c also show the changes which occurred in the non-protein nitrogen content of beef when pre-heated to different temperatures, subjected

TABLE 5

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING AND IRRADIATION DOSAGE ON THE TOTAL SOLUBLE NITROGEN CONTENT OF BEEF STORED FOR 60 DAYS AT 34°F.

(Values expressed in mgs. % dry weight sample)

Irradiation Dose (megarad)	Pre-temperature (°F.) Raw	100	110	120	130	140	150	195	Irradiation Dose Mean
N.I	1080	1050	1040	1029	926	833	654	328	867
0.1	1107	1094	1040	979	917	794	495	338	841
5.0	1275	1251	1217	1164	1101	931	749	362	1006
Pre-temperature Mean	1142	1131	1099	1057	981	853	633	343	

TABLE 6

ANALYSIS OF VARIANCE FOR NON-PROTEIN NITROGEN IN BEEF AS AFFECTED BY IRRADIATION DOSAGE, PRE-HEATING TEMPERATURES AND LENGTH OF STORAGE AT 34°F.

Variation due to	Degrees of freedom	Mean square	F
Total	359		
Replication	2	0.0005	0.45 (N.S.)
Radiation	2	0.0258	23.45*
Storage Time	4	2.3905	2173.18*
Pre-Temperature	7	0.6726	611.45*
Rad. x Stg. Time	8	0.0119	10.82*
Rad. x Pre-Temp.	14	0.0019	1.73*
Stg. Time x Pre-Temp.	28	0.1042	91.73*
Rad. x Time x Pre-Temp.	56	0.0036	3.27*
Error	238	0.0011	

* Significant at the 5% level

TABLE 7

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME ON THE NON-PROTEIN NITROGEN CONTENT OF BEEF STORED AT 34°F.

(Values expressed as mgs. % dry weight sample)

a. Non-irradiated beef

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	Pre-temperature Mean
Raw	230	290	340	380	980	444
100	230	280	330	360	940	428
110	230	250	320	370	720	378
120	230	270	330	360	710	380
130	220	250	320	350	640	356
140	190	240	270	290	500	298
150	50	210	230	270	390	230
195	70	90	100	90	90	88
Storage Time Mean	181	235	280	309	621	

b. Beef irradiated at 0.1 megarad

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	Pre-temperature Mean
Raw	290	280	340	360	970	448
100	280	280	340	350	970	444
110	270	270	340	350	940	434
120	260	250	330	340	830	402
130	250	260	280	330	660	356
140	260	250	260	310	390	294
150	190	210	230	260	290	236
195	80	100	90	90	90	90
Storage Time Mean	235	238	276	299	643	

c. Beef irradiated at 5.0 megarad

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	Pre-temperature Mean
Raw	280	290	340	430	1000	468
100	270	280	340	410	950	450
110	270	270	320	430	870	432
120	260	260	330	390	820	412
130	240	230	280	350	780	376
140	230	210	280	320	650	338
150	210	200	240	280	460	278
195	80	90	100	100	100	100
Storage Time Mean	230	229	280	339	704	

to irradiation at 0.1 and 5.0 megarad and stored for 60 days at 34°F. It may be seen from these data that the non-protein nitrogen values gradually decreased with an increase in the pre-heating temperatures employed. This is consistent with previous reports (25, p. 410-416; 53, p. 589) that heating results in a decrease of the non-protein nitrogen of beef. Pre-heating to an internal temperature of 150°F. did not prevent the increase in the non-protein nitrogen values during the 60-day storage interval. All the samples heated to 195°F. showed a definite decrease in non-protein nitrogen when compared with samples pre-heated at lower temperatures. The non-protein nitrogen in the samples pre-heated to 195°F. did not increase to as great an extent, during storage, as that of the samples pre-heated at lower temperatures.

Tables 7a, b and c show approximately the same pattern of change in the non-protein nitrogen content of beef, although a higher initial content of non-protein nitrogen was exhibited in the 0.1 megarad samples. Non-protein nitrogen was considerably higher at the 45-day storage period in the meat irradiated at 5.0 megarad than in those samples which were not irradiated or irradiated at 0.1 megarad.

The effect of irradiation, Table 8, shows that the non-protein nitrogen values increased with an increase in

TABLE 8

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING AND IRRADIATION DOSAGE ON THE NON-PROTEIN NITROGEN CONTENT OF BEEF STORED FOR 60 DAYS AT 34°F.

(Values expressed as mgs. % dry weight sample)

Irradiation Dose	Pre-temperature (°F.)								Irradiation Dose
(megarad)	Raw	100	110	120	130	140	150	195	Mean
N.I	445	488	389	384	355	298	230	90	328
0.1	448	443	435	402	357	291	238	89	338
5.0	471	451	432	413	375	337	278	96	357
Pre-temperature Mean	455	441	419	400	362	309	249	92	

the irradiation dose. The mean values for the specific heat treatments over the storage period involved is also shown and clearly demonstrate the reduction in non-protein nitrogen as the temperature to which the beef was pre-heated was increased.

Amino Nitrogen. It may be noted in the analysis of variance in Table 9 that although significant interactions occurred among the three main effects, the predominating effect responsible for the changes in the amino nitrogen content of beef was the temperature to which the beef was heated. The length of storage time was shown to be of secondary importance while the irradiation dose, although significant, was not as important as the other two main effects.

Table 10a shows the non-irradiated samples subjected to the variables of pre-heating and storage time. It may be seen from these data that the values for amino nitrogen decreased with increase in pre-heating temperatures used. As in the case of total soluble nitrogen and non-protein nitrogen, this decrease in amino nitrogen is consistent with the previous reports (25, p. 410-416; 53, p. 589) that heating results in a decrease of the amino nitrogen of beef. Pre-heating to an internal temperature of 150°F. did not prevent the increase in amino nitrogen

TABLE 9

ANALYSIS OF VARIANCE FOR TOTAL AMINO NITROGEN IN BEEF AS AFFECTED BY IRRADIATION DOSAGE, PRE-HEATING TEMPERATURES AND LENGTH OF STORAGE AT 34°F.

Variation due to	Degrees of freedom	Mean square	F
Total	359		
Replication	2	0.080	3.07*
Radiation	2	6.370	235.00*
Storage Time	4	16.210	623.46*
Pre-Temperature	7	28.650	1101.92*
Rad. x Stg. Time	8	1.090	41.92*
Rad. x Pre-Temp.	14	0.360	13.40*
Stg. Time x Pre-Temp.	28	0.390	15.00*
Rad. x Time x Pre-Temp.	56	0.100	3.846*
Error	238	0.026	

* Significant at the 5% level

TABLE 10

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME ON THE AMINO NITROGEN CONTENT OF BEEF STORED AT 34°F.

(Values expressed as mgs. % dry weight sample)

a. Non-irradiated beef

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	Pre-temperature Mean
Raw	2600	2800	3100	3200	3400	3020
100	2500	2600	3000	3100	3200	2880
110	2800	2600	2800	3100	3200	2900
120	2500	2600	2800	3200	3500	2920
130	2200	2200	2500	3200	3100	2640
140	2100	2000	2400	2800	2800	2420
150	1800	2100	2400	2800	2500	2320
195	900	900	1000	1100	1100	1000
Storage Time Mean	2170	2220	2500	2810	2850	

b. Beef irradiated at 0.1 megarad

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	Pre-temperature Mean
Raw	2600	3200	4000	4300	4300	3680
100	2600	2600	3500	3700	4100	3300
110	2500	2500	3300	3500	4000	3160
120	2300	2500	3200	3400	3800	3040
130	2300	2300	3200	3300	3400	2900
140	2100	2400	3000	3400	3200	2820
150	1900	2300	2900	2800	2900	2560
195	1100	800	1000	800	900	920
Storage Time Mean	2170	2320	3010	3150	3330	

c. Beef irradiated at 5.0 megarad

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	Pre-temperature Mean
Raw	2500	3700	3800	4500	4700	3840
100	2300	3900	4000	3500	4400	3620
110	2300	3400	3600	4100	4200	3520
120	2500	2200	3500	3400	4000	3320
130	1900	2800	3000	3300	4000	3000
140	1800	2600	2700	3000	3700	3760
150	1500	2700	2700	3100	3300	2660
195	1000	900	900	1100	700	920
Storage Time Mean	1980	2900	3020	3250	3620	

content over the 60-day storage interval. The samples heated to 195°F. showed a very slight increase or a definite decrease in the amino nitrogen content for their respective irradiation dose when compared with samples pre-heated at lower temperatures.

A two-way effect of storage time and pre-heating temperatures for the non-irradiated samples and those irradiated at 0.1 and 5.0 megarad is also shown in Tables 10a, b and c. It may be noted from these data that as the irradiation dose increased there was an increased rate of amino nitrogen formation for each specific pre-heating temperature other than 195°F. For those samples irradiated at 0.1 megarad, a definite increase in amino nitrogen content may be noted during the 15- to 30-day storage period, and for those samples irradiated at 5.0 megarad, during the 0- to 15-day storage period.

The effect of pre-heating temperature and irradiation dosage on the meats is shown in Table 11. A definite increase in amino nitrogen with increase in irradiation dosage can be seen in the mean values for irradiation while the effects of pre-heating temperatures in decreasing the amino nitrogen content of irradiated beef is also shown to be of great significance.

TABLE 11

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING AND IRRADIATION DOSAGE ON THE TOTAL AMINO NITROGEN CONTENT OF BEEF STORED FOR 60 DAYS AT 34°F.

(Values expressed as mgs. % dry weight sample)

Irradiation Dose (megarad)	Pre-temperature (°F.)								Irradiation Dose Mean
	Raw	100	110	120	130	140	150	195	
N.I	3010	2910	2940	2940	2640	2420	2310	980	2520
0.1	3650	3290	3150	3040	2880	2830	2550	950	2790
5.0	3850	3710	3530	3320	3020	2780	2670	970	2980
Pre-temperature Mean	3500	3300	3210	3100	2850	2680	2510	970	

Effects of the Temperatures Attained in Pre-heating Beef Irradiated at 5.0 Megarad and Stored for 80 Days at 70° and 100°F.

Total Soluble Nitrogen. The analysis of variance for the main effects of pre-temperature, storage time, and storage temperature for the meats irradiated at 5.0 megarad level is shown in Table 12. It is to be pointed out that while significant interactions between the effects are noted as in the previous analysis of variance (Table 3), the storage time and the temperature to which the meats were subjected prior to irradiation were the main significant effects.

Tables 13a and 13b show the results of the effects of storage time and pre-temperature on the total soluble nitrogen content of the meat irradiated at 5.0 megarad and stored at 70° and 100°F. The data indicate the same basic trend as previously discussed for the meats irradiated at 5.0 megarad, stored at 34°F. (Table 4c). However, it is to be pointed out that the rate of change was significantly greater at 100°F. storage temperature. It is to be noted that a two-fold increase of total soluble nitrogen was observed at the 0- to 15-day period for the meats stored at 100°F. At 34°F., the two-fold increase was noted during the 15- to 30-day period

TABLE 12

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

Variation due to	Degrees of freedom	Mean square	F
Total	287		
Replication	2	0.0054	1.74 (N.S.)
Storage Temperature	1	0.3220	103.87*
Storage Time	5	10.5905	3416.29*
Pre-Temperature	7	5.0471	1628.10*
Stg. Temp. x Stg. Time	5	0.0376	12.13*
Stg. Temp. x Pre-Temp.	7	0.0322	10.39*
Stg. Time x Pre-Temp.	35	0.1131	36.48*
Stg. Temp. x Stg. x Pre-Temp.	35	0.0148	4.77*
Error	190	0.0031	

* Significant at the 5% level

TABLE 13

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

(Values expressed as mgs. % dry weight sample)

a. Stored at 70°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	80	Pre-temperature Mean
Raw	676	1460	1760	1910	2010	2060	1646
100	670	1310	1700	1906	2040	2040	1611
110	640	1000	1613	1820	1920	1940	1488
120	530	940	1660	1713	1723	1870	1406
130	520	770	1496	1613	1630	1890	1319
140	350	603	1193	1267	1473	1647	1088
150	330	500	980	960	1140	1223	855
195	286	270	570	460	793	960	556
Storage time Mean	500	851	1371	1456	1591	1703	

b. Stored at 100°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	80	Pre-temperature Mean
Raw	676	1403	1790	1880	1883	2200	1638
100	670	1453	1673	1790	2020	2140	1624
110	640	1460	1760	1930	1970	2010	1628
120	530	1360	1740	1847	1730	2003	1535
130	520	753	1413	1550	1700	1920	1309
140	350	720	1241	1460	1500	1780	1175
150	330	540	1140	1126	1240	1173	925
195	286	420	670	770	850	990	664
Storage time Mean	500	1013	1428	1544	1611	1777	

(Table 10c).

These data show that the pre-heating temperatures employed did not prevent the solubilization of the proteins. This occurred at 195°F. just as it occurred in the meat pre-heated at lower temperatures, but at a much slower rate. Increase of the temperature to which the meats were precooked prior to irradiation decreased the amount of soluble nitrogen, and an increase in the temperature and duration of storage increased the soluble nitrogen, irrespective of the pre-heating temperatures employed. The effects of the storage time and temperature are clearly shown in Table 14.

Thus, minimum increases in the quantity of soluble nitrogen are obtainable through increasing the temperature to which the meat is pre-heated prior to irradiation and storing the meat at temperatures as low as practical. Increased length of storage simply results in greater solubility of the protein irrespective of irradiation dose and the pre-heating temperatures employed.

Non-protein Nitrogen. Table 15 shows the analysis of variance for the main effects of pre-temperature, storage time and post-temperature on the non-protein nitrogen content of beef irradiated at 5.0 megarad. It can be seen from the table that while significant

TABLE 14

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

Storage temperature (°F.)	Storage time (days)					Storage temperature Mean
	0	15	30	45	60	
34	501	538	1296	1350	1346	1006
70	501	856	1372	1452	1591	1154
100	501	1014	1430	1545	1611	1220
Storage time Mean	501	803	1366	1449	1516	

TABLE 15

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

Variation due to	Degrees of freedom	Mean square	F
Total	287		
Replication	2	.0043	1.79 (N.S.)
Storage Temperature	1	.1467	61.12*
Storage Time	5	5.8182	2424.25*
Pre-Temperature	7	1.4611	608.79*
Stg. Temp. x Stg. Time	5	.0515	21.46*
Stg. Temp. x Pre-temp.	7	.0089	3.71*
Stg. Time x Pre-temp.	35	.1821	75.87*
Stg. Temp. x Stg. Time x Pre-temp.	35	.0048	2.00*
Error	190	.0024	

* Significant at the 5% level

interactions occurred among the different effects as in previous analyses of variance, dominant effects were shown in the order of storage time, pre-temperature, and post-temperature.

The two-way tables on the effects of storage time and pre-temperature on the non-protein nitrogen content of beef irradiated at 5.0 megarad and stored at 70° and 100°F. are shown in Tables 16a and 16b, respectively. The data show the same basic trend as previously discussed for the non-protein nitrogen in meats irradiated at 5.0 megarad and stored at 34°F. (Table 7c). However, it is to be noted that the rate of increase in non-protein nitrogen was faster at the higher storage temperatures. This increase can be noted following the 45-day storage period for meats stored at 70° and 100°F.

These data show that the pre-heating temperatures employed up to 150°F. did not prevent the increase in the non-protein nitrogen content of beef during the period of storage. At 195°F. pre-cooking temperature the agents responsible for increasing the non-protein nitrogen content of beef seemed to be stabilized as shown by the values which did not increase over the storage period involved. This seems contrary to the increase in total soluble nitrogen of meat heated to 195°F. It is to be

TABLE 16

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

(Values expressed as mgs. % dry weight sample)

a. Stored at 70°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	80	Pre-temperature Mean
Raw	280	290	390	530	1250	1430	695
100	270	280	380	480	1180	1380	661
110	270	260	320	460	1010	1270	600
120	260	250	330	430	910	1100	546
130	240	260	320	370	800	1020	501
140	230	240	290	360	800	870	465
150	210	220	260	280	790	810	428
195	80	100	120	90	80	90	93
Storage time Mean	230	238	303	375	852	996	

b. Stored at 100°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	80	Pre-temperature Mean
Raw	280	320	400	650	1260	1690	767
100	270	340	380	650	1130	1550	720
110	270	300	400	620	1110	1480	696
120	260	280	380	620	990	1130	610
130	240	260	310	500	920	950	530
140	230	250	300	420	860	940	500
150	210	210	280	350	880	740	445
195	80	90	90	90	110	80	90
Storage time Mean	230	256	317	487	907	1070	

noted, however, that the increase in total soluble nitrogen of meat heated at 195°F. was very low compared to those pre-heated at temperatures of 150°F. and lower.

The effects of the storage time and temperature are clearly shown in Table 17.

Amino Nitrogen. Table 18 shows the analysis of variance for the main effects of pre-temperature, storage time and post-temperature for the meats irradiated at 5.0 megarad. The data also show that significant interactions occurred on the three main effects. However, post-temperature showed a predominating effect responsible for the changes in the amino nitrogen content. This is contrary to those samples stored at 34°F. wherein storage time was shown as the most dominant effect. For the meats stored at 70°F. and 100°F., storage time was only a secondary source of variation, and pre-temperature, though significant, was not as influential as post-temperature and storage time.

Tables 19a and 19b show the results of the effects of storage time and pre-temperature on the amino nitrogen content of beef irradiated at 5.0 megarad and stored at 70° and 100°F. The data indicate the same pattern of change in amino nitrogen values for those stored at 34°F. However, it may be noted in Table 20 that at the higher

TABLE 17

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

Storage temperature (°F.)	Storage time (days) 0	15	30	45	60	Storage temperature Mean
34	230	228	280	338	708	356
70	230	238	303	378	855	401
100	230	255	318	489	908	440
Storage time Mean	230	240	300	402	823	

TABLE 18

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

Variation due to	Sum of squares	Degrees of freedom	Mean square	F
Total	22.0654	287		
Replication	.0016	2	0.080	2.66 (N.S.)
Storage Temp.	4.2705	1	427.100	10903.3*
Storage Time	6.7048	5	134.000	4366.0*
Pre-Temperature	6.5813	7	94.000	3133.0*
Stg. Temp. x Stg. Time	1.7689	5	35.400	1180.0*
Stg. Temp. x Pre-Temp.	0.8800	7	12.600	420.0*
Stg. Time x Pre-Temp.	1.3612	35	3.800	126.6*
Stg. Temp. x Stg. Time x Pre-Temp.	.4320	35	1.230	41.0*
Error	.0651	190	0.034	

* Significant at the 5% level

TABLE 19

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

(Values expressed as mgs. % dry weight sample)

a. Stored at 70°F.

Pre-temperature	Storage time (days)						Pre-temperature
°F.	0	15	30	45	60	80	Mean
Raw	2500	3400	3700	4400	5000	5700	4110
100	2300	3300	3600	4300	4900	5800	4030
110	2300	3200	3400	3900	4600	5100	3750
120	2500	3200	3300	3400	4100	4700	3530
130	1900	3000	2900	3200	4500	4500	3330
140	1800	2700	2600	3000	4200	4100	3060
150	1500	2800	2500	3000	3200	3900	2810
195	1000	800	1000	900	700	800	860
Storage time Mean	1980	2800	2870	3260	3900	4320	

b. Stored at 100°F.

Pre-temperature °F.	0	15	30	45	60	80	Pre-temperature Mean
Raw	2500	4700	8100	8500	9700	12500	7660
100	2300	4700	6800	8200	10700	11900	7430
110	2300	4700	6900	8600	9700	11400	7210
120	2500	3800	6900	8000	7900	10100	6530
130	1900	3500	4800	6300	7200	8400	5350
140	1800	3200	4900	6300	7000	8200	5230
150	1500	3200	3900	5200	7100	7000	4620
195	1000	1000	1000	900	900	900	950
Storage time Mean	1980	3570	4620	6500	7500	8700	

TABLE 20

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

Storage temperature (°F.)	Storage time (days) 0	15	30	45	60	Storage temperature Mean
34	1990	2900	3040	3200	3680	2980
70	1990	2790	2870	3250	3910	2960
100	1990	3570	5400	6490	7510	4990
Storage time Mean	1990	3090	3770	4350	5030	

storage temperatures, there was a faster rate of increase in amino nitrogen content as shown by the mean values for storage temperature.

These data showed that there was no stabilization of the agents responsible for the changes in amino nitrogen increase when the meats were pre-heated at varying temperatures up to 150°F. Storage time resulted in increased solubility of the proteins of the irradiated meats which had been pre-heated at temperatures lower than 195°F.

As in the case for total soluble nitrogen and non-protein nitrogen, if the increase in the amino nitrogen content of beef is to be retarded, higher pre-cooking temperatures and a lower storage temperature are necessary. However, even if these conditions are used, amino nitrogent content of the meat will increase somewhat during the storage period.

Free Amino Acids

Since the analysis of variance for the free amino acids used the pre-heating temperature as the criterion for analysis the main effects tested were: (1) irradiation dose, (2) storage time and (3) storage temperature.

Table 21 shows the analysis of variance for the free aspartic acid in non-irradiated meat and for those irradiated at 0.1 and 5.0 megarad, and stored for 60 days at 34°F. It may be seen from the data that although irradiation dosage and storage time showed significant interactions, the storage time was more influential than the irradiation treatment. This effect is typical of all the analysis of variance for the free amino acids determined, except that for phenylalanine which showed irradiation dosage to be more influential in contributing to the increase of free phenylalanine than storage time although the interaction was significant.

A typical analysis of variance for aspartic acid is shown on Table 22 with respect to storage temperatures of 34°, 70° and 100°F. and storage time of 60 days. This analysis of variance is typical for all the free amino acids determined in the meats irradiated at 5.0 megarad. It may be seen in Table 22 that both storage time and temperature were important sources of variations despite the highly significant interaction. An extended period of 20-days storage time for those samples stored at 70° and 100°F. showed the same effects on the release of the free amino acids with the exception of aspartic acid. In the case of the free aspartic acid, the storage temperature was the predominating effect rather than the storage time. These data can be seen in the analysis of variance in Table 23.

TABLE 21

ANALYSIS OF VARIANCE FOR ASPARTIC ACID CONTENT OF BEEF AS AFFECTED BY IRRADIATION DOSAGE AND STORAGE TIME

Variation due to	Degrees of freedom	Mean square	F
Total	44		
Replication	2	0.0222	0.0318 (N.S.)
Radiation	2	13.3555	191.34*
Storage Time	4	67.4222	965.93*
Rad. x Stg. Time	8	1.0222	14.64*
Error	28	0.0698	

* Significant at the 5% level

TABLE 22

ANALYSIS OF VARIANCE FOR ASPARTIC ACID CONTENT OF BEEF AS AFFECTED BY STORAGE DURING 60 DAYS AND STORAGE TEMPERATURES OF 34°, 70° AND 100°F.

Variation due to	Degrees of freedom	Mean square	F
Total	44		
Replication	2	0.1555	1.849 (N.S.)
Storage Temperature	2	44.2889	526.62*
Storage Time	4	48.7444	579.06*
Stg. Time x Stg. Temp.	8	19.3444	230.02*
Error	28	0.0841	

* Significant at the 5% level

TABLE 23

ANALYSIS OF VARIANCE FOR ASPARTIC ACID CONTENT OF BEEF AS AFFECTED BY STORAGE DURING 80 DAYS AND STORAGE TEMPERATURES OF 70° and 100°F.

Variation due to	Degrees of freedom	Mean square	F
Total	35		
Replication	2	0.1111	2.20 (N.S.)
Storage Temperature	1	72.2500	1430.69*
Storage Time	5	41.0944	813.75*
Stg. Temp. x Stg. Time	5	22.1166	437.95*
Error	22	0.0505	

* Significant at the 5% level

Aspartic Acid. Tables 24a, b, c, d and e show the pattern of release of aspartic acid. It can be seen from the data that aspartic acid was released very slowly. Free aspartic acid did not appear in the non-irradiated meat until the 60-day storage period, although traces showed up during the 30- and the 45-day storage period in the raw samples and those pre-heated at 100° and 110°F. (Table 24a). A gradual increase of free aspartic acid was observed with increase in irradiation dosage (Tables 24b and c). However, with the increase in the storage temperature, aspartic acid was found to disappear during the later part of the storage period. This is noted in samples irradiated at 5.0 megarad and stored at 70° and 100°F. (Tables 24d and e). From this behavior of free aspartic acid it may be deduced that decarboxylation occurred in this amino acid and formation of free alanine was favored. A sudden increase in free alanine may be noted during the 45- to 60-day storage period for those samples stored at 70°F. (Table 29d) and during the 30- to 45-day storage period for those stored at 100°F. (Table 29a) corresponding to the loss of free aspartic acid during the same storage intervals. The disappearance of free aspartic acid also accounts for the behavior of this amino acid in the analysis of variance in Table 20 which shows that the storage temperature has a predominating

TABLE 24

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE FREE ASPARTIC ACID CONTENT OF BEEF

(Values expressed as mgs. % dry weight sample)

a. Non-irradiated beef, stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0[1]	0	T[2]	T	3.85
100	0	0	T	T	3.71
110	0	0	T	T	7.47
120	0	0	0	T	4.28
130	0	0	0	0	T
140	0	0	0	0	T
150	0	0	0	0	0
195	0	0	0	0	0

[1] 0 denotes no value obtained

[2] T denotes traces
These designations are used hereafter.

b. Beef irradiated at 0.1 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0	0	T	T	3.92
100	0	0	T	T	6.05
110	0	0	T	T	3.84
120	0	0	0	T	4.96
130	0	0	0	0	3.86
140	0	0	0	0	T
150	0	0	0	0	0
195	0	0	0	0	0

c. Beef irradiated at 5.0 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0	T	1.33	1.21	2.42
100	0	T	1.11	1.21	2.36
110	0	T	T	0.64	0.94
120	0	0	T	T	0.84
130	0	0	T	T	0.96
140	0	0	0	T	T
150	0	0	0	0	T
195	0	0	0	0	T

Table 24, continued
THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE ASPARTIC ACID CONTENT OF BEEF

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

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	80
Raw	0	T	1.69	2.53	2.22	0
100	0	T	1.48	2.53	2.73	0
110	0	T	1.50	2.48	1.09	0
120	0	T	T	1.34	T	0
130	0	T	T	T	T	0
140	0	0	T	T	T	0
150	0	0	0	T	T	0
195	0	0	0	T	T	0

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

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	80
Raw	0	T	3.61	0	0	0
100	0	T	1.73	0	0	0
110	0	T	1.80	0	0	0
120	0	T	T	0	0	0
130	0	T	T	0	0	0
140	0	0	T	0	0	0
150	0	0	0	0	0	0
195	0	0	0	0	0	0

effect on the release of aspartic acid, contrary to the other free amino acids determined. In the latter cases, the storage time was the more influential factor.

Glutamic Acid. Glutamic acid was released much earlier than aspartic acid as shown by Tables 25a, b, c, d and e. Measurable amounts and traces of this amino acid occurred irrespective of the variables. Thus, free glutamic acid was found in the non-irradiated raw meat (Table 25a). An increase in the irradiation dose resulted in a slight increase in raw meat irradiated at 0.1 megarad and a noticeable increase in meat irradiated at 5.0 megarad (Tables 25b and 25c). Increase in the storage temperature definitely released the glutamic acid at a much faster rate as shown in Tables 25d and 25e. The data for glutamic acid show that an increase in the temperature to which the meat is pre-heated results in a decrease of the free glutamic acid. No free glutamic acid was found in those samples heated at 195°F. except some traces during the 45-days storage interval for the 5.0 megarad samples stored at 70° and 100°F. and some quantitative amounts in the same samples during the 60- and 80-days storage intervals.

Serine. Quantitative amounts of serine were not obtained in the non-irradiated meats and those irradiated

TABLE 25

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE FREE GLUTAMIC ACID CONTENT OF BEEF

(Values expressed as mgs. % dry weight sample)

a. Non-irradiated beef stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	1.10	1.18	1.70	1.92	4.70
100	1.01	1.04	1.37	2.19	3.30
110	T	T	1.29	1.50	4.35
120	T	T	1.01	1.17	4.76
130	0	T	T	0.98	2.10
140	0	0	T	T	0.82
150	0	0	0	0	0
195	0	0	0	0	0

b. Beef irradiated at 0.1 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	1.13	1.50	1.13	1.84	4.35
100	0.85	1.29	1.25	2.21	4.23
110	T	0.97	1.15	1.54	5.12
120	T	T	T	1.30	4.72
130	0	T	T	0.76	2.90
140	0	0	T	T	0.75
150	0	0	0	0	T
195	0	0	0	0	0

c. Beef irradiated at 5.0 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	1.46	1.95	2.62	3.21	4.73
100	1.18	1.45	1.55	2.59	3.48
110	0.98	0.93	0.75	2.25	3.45
120	T	T	0.80	1.42	2.19
130	T	T	T	0.77	2.90
140	T	T	T	T	1.21
150	0	0	0	T	T
195	0	0	0	0	T

Table 25, continued
THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE GLUTAMIC ACID CONTENT OF BEEF

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

Pre-temperature °F.	Storage time (days)					
	0	15	30	45	60	80
Raw	1.46	3.05	3.59	4.21	5.17	18.85
100	1.18	3.18	2.88	4.63	6.65	23.20
110	0.98	2.09	2.50	4.10	5.13	18.44
120	T	1.25	2.06	4.47	5.33	18.54
130	T	1.16	1.96	3.90	4.42	17.93
140	T	0.39	1.19	1.94	2.31	7.12
150	0	0.40	0.77	1.15	1.73	6.93
195	0	0	0	T	0.64	0.99

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

Pre-temperature °F.	Storage time (days)					
	0	15	30	45	60	80
Raw	1.46	4.09	4.88	10.68	11.62	22.22
100	1.18	4.38	5.13	11.30	12.83	40.00
110	0.98	4.38	4.97	11.60	13.25	44.26
120	T	2.58	3.00	12.00	12.79	28.89
130	T	2.59	2.42	11.73	13.78	19.79
140	T	0.35	1.70	5.23	9.50	14.14
150	0	T	T	5.15	6.40	13.05
195	0	0	0	T	3.68	6.60

at 0.1 and 5.0 megarad, stored at 34°F., until the 45- and the 60-day storage period. These data may be found in Tables 26a, b and c, where the significant effect of irradiation can be seen in those samples irradiated at 5.0 megarad, which show traces of serine much earlier. The non-irradiated meats and those irradiated at 0.1 megarad did not show apparent differences in the release of free serine. However, increase in the storage temperature resulted in a large increase of this amino acid as may be seen in Tables 26c, d and e. The effect of pre-heating temperatures was a decrease in free serine content of the samples.

Glycine. The release of glycine (Tables 27a and 27b) in the non-irradiated meats and those meats irradiated at 0.1 megarad was approximately the same as that for serine. However, a greater release and measurable amounts of free glycine were obtained in those samples irradiated at 5.0 megarad and stored at 34°F. (Table 27c). Tables 27d and 27e show the data for the 5.0 megarad samples stored at 70° and 100°F. which indicate that more free glycine are released at higher storage temperatures and higher irradiation dosage. All data in Table 27 show that increase in the pre-heating temperatures decreased the amount of free glycine.

TABLE 26

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE FREE SERINE CONTENT OF BEEF

(Values expressed as mgs. % dry weight sample)

a. Non-irradiated beef stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0	0	T	1.53	3.08
100	0	0	T	1.51	4.23
110	0	0	T	T	2.42
120	0	0	T	T	1.71
130	0	0	0	T	T
140	0	0	0	0	T
150	0	0	0	0	0
195	0	0	0	0	0

b. Beef irradiated at 0.1 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0	0	T	1.47	4.27
100	0	0	T	1.39	4.99
110	0	0	T	T	3.70
120	0	0	T	T	3.04
130	0	0	0	T	0.91
140	0	0	0	0	T
150	0	0	0	0	T
195	0	0	0	0	0

c. Beef irradiated at 5.0 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0	T	1.49	1.39	2.32
100	0	T	T	0.39	2.26
110	0	0	T	T	1.50
120	0	0	T	T	0.74
130	0	0	0	T	T
140	0	0	0	T	T
150	0	0	0	0	T
195	0	0	0	0	T

Table 26, continued
THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE SERINE CONTENT OF BEEF

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

Pre-temperature °F.	Storage time (days)					
	0	15	30	45	60	80
Raw	0	0.76	1.63	3.79	6.05	12.57
100	0	0.62	4.04	5.69	6.65	15.04
110	0	T	2.50	4.14	5.13	12.29
120	0	T	T	1.34	3.43	10.30
130	0	0	T	1.56	2.54	7.47
140	0	0	T	T	1.54	5.93
150	0	0	0	T	1.73	5.77
195	0	0	0	T	0.64	5.54

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

Pre-temperature °F.	Storage time (days)					
	0	15	30	45	60	80
Raw	0	0.77	1.60	8.62	11.11	29.63
100	0	0.81	2.62	8.50	11.79	14.54
110	0	0.43	0.83	7.14	15.05	29.51
120	0	T	0.83	8.76	9.26	28.89
130	0	T	0.34	8.69	9.73	14.14
140	0	0	T	4.47	6.39	25.40
150	0	0	0	4.88	7.11	13.05
195	0	0	0	T	6.12	13.21

TABLE 27

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE FREE GLYCINE CONTENT OF BEEF

(Values expressed as mgs. % dry weight sample)

a. Non-irradiated beef stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0	T	T	T	4.62
100	0	T	T	T	5.56
110	0	0	T	T	4.04
120	0	0	T	T	4.03
130	0	0	0	0	1.51
140	0	0	0	0	T
150	0	0	0	0	T
195	0	0	0	0	0

b. Beef irradiated at 0.1 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0	T	0.50	1.47	4.70
100	0	T	T	T	6.45
110	0	0	T	T	4.84
120	0	0	T	T	5.09
130	0	0	0	T	3.09
140	0	0	0	0	0.97
150	0	0	0	0	T
195	0	0	0	0	0

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

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	T	0.78	1.31	1.55	2.71
100	T	0.69	1.09	1.94	2.26
110	T	T	0.75	1.58	1.80
120	T	T	1.00	1.46	1.86
130	0	T	T	0.50	1.08
140	0	T	T	T	T
150	0	0	0	0	T
195	0	0	0	0	T

Table 27, continued
THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE GLYCINE CONTENT OF BEEF

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

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	80
Raw	T	2.29	2.57	5.31	7.78	21.12
100	T	2.08	2.22	8.19	7.99	15.47
110	T	1.51	2.25	3.73	6.15	20.65
120	T	0.73	1.48	4.02	5.48	13.35
130	0	0.69	1.76	3.55	5.79	14.94
140	0	T	0.71	3.49	4.16	14.24
150	0	0	T	T	4.51	6.93
195	0	0	0	T	2.56	6.65

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

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	80
Raw	T	4.85	5.78	7.05	9.77	29.63
100	T	5.12	5.89	7.29	11.86	36.36
110	T	4.49	5.04	7.48	10.52	25.00
120	T	2.21	4.33	7.24	8.66	25.46
130	0	1.52	1.86	6.85	9.84	25.45
140	0	1.40	2.04	6.16	8.28	15.55
150	0	T	T	6.27	9.15	9.13
195	0	0	0	T	6.56	13.21

Threonine. Table 28 shows the pattern of release of threonine with respect to irradiation dosage, pre-heating temperatures, storage temperatures and storage time. In the non-irradiated meat and that irradiated at 0.1 megarad, no free threonine was quantitated until the 45-day storage interval and then only in the raw meat and that pre-heated to 100°F. (Tables 28a and 28b). Free threonine was quantitated at the 30-day storage interval in raw meat irradiated at 5.0 megarad and stored at 34°F. (Table 28c). An increase in threonine can be observed in meat irradiated at 5.0 megarad as the temperature of storage was increased. This is shown in Tables 28c, d and e. The traces of threonine indicated in those samples stored at 34°F. for any particular condition could be quantitated when the meat was stored at 70° and 100°F. As in the free amino acids discussed previously, the effect of pre-heating temperatures was to decrease the amount of free threonine.

Alanine. Irradiation did not result in an increased rate of release of alanine in beef as shown for the non-irradiated samples and those irradiated at 0.1 and 5.0 megarad levels when analyzed before the start of the storage interval. Tables 29a, b and c show these data. However, an increase in the storage temperature of meat

TABLE 28

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE FREE THREONINE CONTENT OF BEEF

(Values expressed as mgs. % dry weight sample)

a. Non-irradiated beef stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0	0	T	1.53	5.77
100	0	0	T	2.03	4.45
110	0	0	T	T	1.62
120	0	0	0	T	2.57
130	0	0	0	T	T
140	0	0	0	0	T
150	0	0	0	0	T
195	0	0	0	0	0

b. Beef irradiated at 0.1 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0	T	T	1.47	5.48
100	0	0	T	1.55	5.65
110	0	0	T	T	3.84
120	0	0	0	T	3.97
130	0	0	0	T	2.32
140	0	0	0	0	1.12
150	0	0	0	0	T
195	0	0	0	0	0

c. Beef irradiated at 5.0 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0	T	1.34	1.55	3.87
100	0	T	T	1.55	2.26
110	0	0	T	T	1.50
120	0	0	T	T	T
130	0	0	0	T	T
140	0	0	0	0	T
150	0	0	0	0	T
195	0	0	0	0	T

Table 28, continued
THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE THREONINE CONTENT OF BEEF

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

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	80
Raw	0	0.64	1.22	3.64	3.89	25.14
100	0	0.66	2.16	3.16	5.32	24.49
110	0	T	1.88	2.48	3.85	15.36
120	0	T	T	2.68	3.43	14.32
130	0	T	T	1.85	2.54	11.21
140	0	0	T	T	2.31	8.90
150	0	0	0	T	1.29	5.77
195	0	0	0	T	1.07	5.54

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

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	80
Raw	0	1.15	2.17	13.59	14.75	29.63
100	0	1.22	2.11	13.95	16.70	31.17
110	0	T	0.72	14.16	15.78	23.18
120	0	T	T	14.21	15.39	25.79
130	0	T	T	13.45	14.76	23.23
140	0	T	T	7.84	7.74	10.10
150	0	0	0	5.88	9.15	13.98
195	0	0	0	T	4.37	14.15

TABLE 29

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE FREE ALANINE CONTENT OF BEEF

(Values expressed as mgs. % dry weight sample)

a. Non-irradiated beef stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0.51	0.65	1.56	1.82	3.85
100	0.21	0.34	1.47	1.88	4.45
110	T	0.34	1.28	1.25	3.23
120	T	T	0.62	0.97	2.14
130	T	T	T	0.68	0.76
140	0	0	T	0	0.37
150	0	0	0	0	T
195	0	0	0	0	T

b. Beef irradiated at 0.1 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0.53	1.01	1.75	1.75	3.92
100	0.31	0.67	1.21	1.93	3.30
110	T	0.45	0.61	1.61	4.36
120	T	T	0.54	0.98	2.53
130	T	T	T	0.64	1.41
140	T	T	T	T	1.29
150	0	0	0	0	T
195	0	0	0	0	T

c. Beef irradiated at 5.0 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0.61	0.80	1.12	1.72	6.88
100	0.59	0.67	0.78	0.86	6.83
110	0.29	0.35	0.75	1.32	6.99
120	T	T	0.40	0.86	6.83
130	T	T	T	0.77	7.67
140	T	T	T	T	7.87
150	0	0	T	T	6.36
195	0	0	0	0	5.29

Table 29, continued
THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE ALANINE CONTENT OF BEEF

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

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	80
Raw	0.61	1.91	2.45	3.16	4.24	35.07
100	0.59	1.30	2.16	3.79	5.44	34.03
110	0.29	1.25	2.19	2.80	4.20	30.60
120	T	0.91	1.24	2.01	2.86	25.96
130	T	0.52	1.17	1.48	2.08	22.41
140	T	T	0.59	0.58	1.89	21.36
150	0	T	T	T	1.41	6.24
195	0	0	0	T	1.17	3.32

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

Pre-temperature °F.	Storage time (days) 0	15	30	45	60	80
Raw	0.61	2.60	3.28	16.15	17.66	30.86
100	0.59	2.74	2.83	17.01	20.75	42.42
110	0.29	2.90	3.60	17.14	18.41	33.81
120	T	2.15	2.72	17.21	17.40	36.11
130	T	2.06	2.20	16.29	17.23	35.34
140	T	1.16	1.39	17.07	14.20	17.67
150	0	T	T	12.30	10.37	16.31
195	0	0	0	2.94	3.47	9.91

irradiated at 5.0 megarad level resulted in more alanine appearing during the storage intervals irrespective of the pre-heating temperatures used (Tables 29c, d and e). Extending the storage time resulted in a release of alanine even in the cooked meats, as may be seen in Table 29. Of all the free amino acids determined by the phenol-buffer system, alanine and glutamic acid were released at a quantitatively greater rate during the storage intervals employed in this experiment.

Methionine. Table 30 shows the pattern of release of methionine in beef samples subjected to the different variables employed in this experiment. A great difference can be noted in the effects of storage temperatures on the release of methionine. At 34°F., the non-irradiated meats and those irradiated at 0.1 and 5.0 megarad resulted in lower quantitative values and more traces of this free amino acid over the 60-day storage period (Tables 30a, b and c). When stored at 70° and 100°F., a great increase in free methionine content was observed. This was first observed at the 15-day storage interval (Tables 30d and 30e). The irradiation effect can also be seen from the data in Tables 30a, b and c which indicate that an increase in irradiation dosage resulted in an increase of free methionine, although this

TABLE 30

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE FREE METHIONINE CONTENT OF BEEF

(Values expressed as mgs. % dry weight sample)

a. Non-irradiated beef stored at 34°F.

Pre-temperature °F.	Storage time (days)				
	0	15	30	45	60
Raw	0	0	T	T	0.11
100	0	0	T	T	0.05
110	0	0	T	T	0.08
120	0	0	0	T	T
130	0	0	0	0	T
140	0	0	0	0	T
150	0	0	0	0	0
195	0	0	0	0	0

b. Beef irradiated at 0.1 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days)				
	0	15	30	45	60
Raw	0	0	T	0.56	2.90
100	0	0	T	T	2.48
110	0	0	T	T	1.79
120	0	0	0	T	1.41
130	0	0	0	T	T
140	0	0	0	0	T
150	0	0	0	0	T
195	0	0	0	0	0

c. Beef irradiated at 5.0 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days)				
	0	15	30	45	60
Raw	0	T	0.98	1.09	2.57
100	0	T	0.35	0.85	2.29
110	0	T	T	0.74	1.53
120	0	0	T	T	1.41
130	0	0	T	T	1.32
140	0	0	0	T	T
150	0	0	0	T	T
195	0	0	0	T	T

Table 30, continued
THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE METHIONINE CONTENT OF BEEF

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

Pre-temperature °F.	Storage time (days)					
	0	15	30	45	60	80
Raw	0	2.97	3.18	3.79	4.54	10.28
100	0	2.18	4.00	4.11	4.66	10.54
110	0	2.76	3.44	3.42	3.85	10.06
120	0	2.67	3.09	3.36	3.71	8.43
130	0	0.96	3.17	2.93	3.65	8.49
140	0	0.69	3.86	3.78	4.05	11.33
150	0	0.48	2.90	2.88	3.89	6.30
195	0	T	T	T	1.49	5.44

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

Pre-temperature °F.	Storage time (days)					
	0	15	30	45	60	80
Raw	0	3.88	4.51	8.13	8.20	37.03
100	0	3.41	3.91	7.29	9.66	30.30
110	0	3.23	3.29	5.70	8.84	30.74
120	0	3.19	3.47	6.06	9.43	30.10
130	0	2.79	3.10	6.56	8.96	16.49
140	0	1.63	1.83	4.22	8.18	23.56
150	0	0.86	1.29	5.28	8.54	21.75
195	0	T	0.43	1.73	7.35	9.91

effect is confounded with the storage interval. This interaction is apparent in all amino acid analyzed.

Valine. The pattern of change of valine when subjected to the different variables of pre-heating and irradiation and stored at various temperatures is shown in Table 31. Increase in pre-heating temperatures decreased the amount of free valine while increase in irradiation dosages resulted in an increase (Tables 31a, b and c) of this amino acid. As with the other amino acids previously discussed, the effect of an increase in storage temperature was an increase in free valine which can be clearly seen in Tables 31c, d and e. At 100°F. storage temperature, fewer traces and greater quantitative amounts of valine may be noted than when the meat was stored at 70°F.

Phenylalanine. Free phenylalanine showed the same pattern of release as that of valine with respect to the storage temperatures employed. However, no quantitative values of free phenylalanine occurred at the onset of the storage period. Traces were noted in all samples of meat irrespective of the irradiation. The typical effects, as reported for the other free amino acids, of irradiation dosages, pre-heating temperatures, and storage temperatures on the free phenylalanine content of beef may be

TABLE 31

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE FREE VALINE CONTENT OF BEEF

(Values expressed as mgs. % dry weight sample)

a. Non-irradiated beef stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0.08	0.10	0.11	0.14	0.34
100	0.02	0.03	0.05	0.11	0.18
110	T	T	0.04	0.10	0.20
120	T	T	T	0.05	0.29
130	0	T	T	T	0.09
140	0	0	T	T	0.08
150	0	0	0	T	T
195	0	0	0	0	T

b. Beef irradiated at 0.1 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0.75	0.87	1.30	1.31	3.62
100	T	0.58	1.23	1.30	2.73
110	T	T	0.53	1.10	2.07
120	T	T	0.51	0.56	2.44
130	0	T	T	T	1.28
140	0	0	T	T	1.72
150	0	0	0	T	0.52
195	0	0	0	0	T

c. Beef irradiated at 5.0 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0.73	0.73	1.25	2.32	3.10
100	0.24	0.64	0.98	2.07	2.77
110	0.23	0.25	0.49	1.58	2.00
120	T	T	0.53	0.97	1.49
130	T	T	T	T	0.51
140	T	T	T	T	T
150	0	0	T	T	T
195	0	0	0	T	T

Table 31, continued
THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE VALINE CONTENT OF BEEF

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

Pre-temperature	Storage time (days)					
°F.	0	15	30	45	60	80
Raw	0.73	4.32	4.98	4.06	5.19	10.55
100	0.24	3.79	4.05	4.61	4.79	7.73
110	0.23	3.77	4.52	4.79	5.13	8.60
120	T	3.59	3.90	4.89	4.57	7.41
130	T	1.11	3.84	4.44	5.08	7.47
140	T	0.88	3.32	3.99	4.16	5.93
150	0	T	1.30	1.68	2.59	4.62
195	0	T	0.87	1.30	1.28	2.21

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

Pre-temperature	Storage time (days)					
°F.	0	15	30	45	60	80
Raw	0.73	4.46	4.81	7.38	7.79	29.63
100	0.24	4.14	4.04	7.63	8.30	30.91
110	0.23	4.33	4.20	6.96	8.47	35.04
120	T	3.93	4.48	6.90	8.08	25.28
130	T	1.78	2.27	6.28	7.86	24.74
140	T	1.05	2.04	4.08	6.82	17.67
150	0	0.41	1.13	4.15	8.18	6.52
195	0	T	0.40	0.75	6.98	9.91

noted in Table 32.

<u>Leucine</u>. Of all the free amino acids analyzed by the butanol:acetic acid:water system, leucine appeared in the greatest amounts even in the non-irradiated meats when analyzed prior to storage. This may be seen in Table 33a. In the meats irradiated at 5.0 megarad and stored at 70° and 100°F., free leucine occurred throughout the storage period except in those meats heated at 150° and 195°F. and analyzed prior to storage (Tables 33d and 33e). Meats irradiated at 0.1 megarad showed the same pattern of traces of free leucine as the non-irradiated meats. Quantitative amounts of leucine were greater in meats irradiated at 0.1 megarad than in the non-irradiated samples (Tables 33a and 33b). Irradiation effect may be noted in Table 33c for the samples irradiated at the 5.0 megarad level compared to Tables 33a and 33b for the non-irradiated and those irradiated at 0.1 megarad.

<u>Tyrosine</u>. Unexpectedly, no tyrosine was found. This could be because (1) the procedure for the determination of the free amino acids destroyed the tyrosine, (2) tyrosine was not soluble in the solvent used, (3) the amount of free tyrosine was so small that it did not show in the chromatograms, or (4) that even though released,

TABLE 32

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE FREE PHENYLALANINE CONTENT OF BEEF

(Values expressed as mgs. % dry weight sample)

a. Non-irradiated beef stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0	0	0	1.04	1.75
100	0	0	0	T	1.01
110	0	0	0	T	0.73
120	0	0	0	T	0.66
130	0	0	0	0	T
140	0	0	0	0	T
150	0	0	0	0	0
195	0	0	0	0	0

b. Beef irradiated at 0.1 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0	T	T	0.99	2.06
100	0	0	T	T	2.35
110	0	0	0	T	1.44
120	0	0	0	T	1.65
130	0	0	0	0	0.81
140	0	0	0	0	0.62
150	0	0	0	0	T
195	0	0	0	0	T

c. Beef irradiated at 5.0 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	T	0.64	1.12	3.87	3.87
100	T	0.45	1.09	2.71	3.58
110	T	T	0.75	1.98	2.62
120	T	T	T	1.16	2.35
130	0	T	T	T	0.77
140	0	T	T	T	T
150	0	0	0	T	T
195	0	0	0	0	T

Table 32, continued
THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE PHENYLALANINE CONTENT OF BEEF

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

Pre-temperature °F.	Storage time (days)					
	0	15	30	45	60	80
Raw	T	2.36	2.86	3.32	5.19	18.85
100	T	2.12	2.60	2.84	4.25	16.92
110	T	1.82	2.93	2.80	5.13	17.05
120	T	3.31	4.08	3.93	8.96	13.90
130	0	0.72	3.53	3.24	5.08	14.01
140	0	0.57	3.17	3.05	4.63	9.79
150	0	T	1.04	1.73	3.11	4.33
195	0	0	T	0.79	1.71	4.16

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

Pre-temperature °F.	Storage time (days)					
	0	15	30	45	60	80
Raw	T	2.36	2.79	5.64	6.80	7.41
100	T	2.30	2.36	5.83	6.90	7.27
110	T	1.43	1.64	5.08	7.36	14.75
120	T	1.40	1.97	5.70	5.50	14.44
130	0	1.14	1.41	4.55	5.63	7.07
140	0	0.52	0.93	2.63	5.11	7.07
150	0	T	0.31	2.74	4.15	6.53
195	0	0	0	T	3.47	6.60

TABLE 33

THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE FREE LEUCINE CONTENT OF BEEF

(Values expressed as mgs. % dry weight sample)

a. Non-irradiated beef stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0.54	0.59	1.04	1.47	2.31
100	0.49	0.50	0.84	1.13	1.48
110	0.46	0.42	0.83	0.94	2.02
120	0.32	0.39	0.22	0.97	2.08
130	T	T	T	0.41	1.01
140	T	T	T	T	0.41
150	0	0	0	0	T
195	0	0	0	0	T

b. Beef irradiated at 0.1 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0.62	0.87	0.94	1.44	2.71
100	0.68	0.83	0.95	1.18	2.40
110	0.54	0.62	0.67	0.96	2.08
120	0.30	0.66	0.69	0.98	2.36
130	T	T	T	0.38	1.67
140	T	T	T	T	1.21
150	0	0	0	0	T
195	0	0	0	0	T

c. Beef irradiated at 5.0 megarad and stored at 34°F.

Pre-temperature °F.	Storage time (days) 0	15	30	45	60
Raw	0.73	0.80	1.12	1.79	2.15
100	0.68	0.73	1.16	1.66	1.68
110	0.53	0.58	0.90	1.20	1.31
120	0.57	0.53	0.86	0.66	1.18
130	T	T	0.37	0.44	1.02
140	T	T	T	0.27	0.91
150	0	T	T	T	0.37
195	0	0	0	T	0.34

Table 33, continued
THE EFFECT OF THE TEMPERATURE ATTAINED IN PRE-HEATING, IRRADIATION DOSAGE AND STORAGE TIME AND TEMPERATURE ON THE LEUCINE CONTENT OF BEEF

d. Beef irradiated at 5.0 megarad and stored at 70 F.

Pre-temperature °F.	Storage time (days)					
	0	15	30	45	60	80
Raw	0.73	3.43	3.29	3.98	4.67	6.60
100	0.68	3.20	3.28	4.19	4.39	5.80
110	0.53	3.43	4.11	4.10	4.62	6.45
120	0.57	1.65	3.11	4.03	3.78	5.56
130	T	1.58	2.13	3.24	3.81	5.60
140	T	1.42	2.12	3.49	3.47	4.98
150	0	1.16	1.29	1.73	3.31	3.46
195	0	T	0.58	0.95	1.60	2.99

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

Pre-temperature °F.	Storage time (days)					
	0	15	30	45	60	80
Raw	0.73	3.56	3.97	9.65	8.97	29.63
100	0.68	3.84	3.63	10.62	10.61	32.97
110	0.53	4.07	3.96	9.83	10.49	49.18
120	0.57	3.88	4.39	10.94	10.86	38.52
130	T	2.40	2.60	9.14	10.77	37.70
140	T	2.02	2.04	6.10	11.91	29.69
150	0	1.27	1.36	6.43	11.56	16.53
195	0	T	3.03	1.02	10.53	17.61

it combined with other constituents which rendered the tyrosine insoluble. No traces of tyrosine were moted in any of the samples. The crystals presumed to be tyrosine observed on stored irradiated meats by Drake et al. (19, p. 23) and Cain et al. (13) were not found on the raw irradiated meat during the 80-day storage interval used in this experiment.

All the free amino acids analyzed, except aspartic acid, showed an increase with an increase in irradiation dosage and storage time, and a decrease with an increase in the pre-heating temperatures employed prior to irradiation. These increases in free amino acid content show the unfolding of the protein as a result of the variables employed in this experiment. Table 34 shows the individual amino acids with their maximum amounts released during the course of the storage intervals. It could be seen from the data that the greatest increase is shown by free methionine which is only 1.77 mgs. per gram of nitrogen with respect to total methionine. The lowest value is that for aspartic acid which is 0.03 mgs. per gram nitrogen with respect to total aspartic acid.

This small increase in the free amino acid content of irradiated meats is substantiated by the report of Proctor and Bhatia (60, p. 359) as cited by Fox and Ise (23, p. 16) that although the protein may be unfolded by

Retitle

TABLE 34

PERCENTAGE RELEASE OF AMINO ACIDS AS AFFECTED BY PRE-HEATING TEMPERATURES, IRRADIATION DOSAGE, LENGTH OF STORAGE TIME AND STORAGE TEMPERATURE

(Values expressed as mgs./gm. nitrogen)

Amino Acid	Total Amount	Maximum Amount Free (~~Released~~)	Per cent Free (~~Released~~)
Aspartic Acid	542.5	0.181	0.03
Glutamic Acid	864.4	3.160	0.36
Serine	200.0	2.116	1.06
Glycine	453.7	2.597	0.57
Threonine	272.5	2.226	0.82
Alanine	384.4	3.030	0.80
Methionine	149.4	2.645	1.77
Valine	352.5	2.503	0.71
Phenylalanine	281.2	1.346	0.48
Leucine	535.0	3.513	0.65

Maximum amount of free amino acids determined during the course of the experiment.

irradiation, it is conceivable that the amino acid may be protected by the structure and the surrounding material of the protein, thus little of the amino acids are set free.

General Effects of Pre-heating, Irradiation, Storage Temperature and Storage Time on the Physical Conditions of the Meat.

All the meats became firmer and the change in color ranged from red to grayish-brown with increase in pre-heating temperatures. Generally, raw samples and those pre-heated to 100°F. developed a dark color during storage. Most of the samples of meat heated from 110° to 150°F. and irradiated at 5.0 megarad developed a pink color which readily turned brown on exposure to air. The pigment responsible for the pink color was identified as denatured globin hemochrome by Tappel (72, p. 411).

A very intense off-odor was noticed in the samples irradiated at 5.0 megarad and this became more intense with increase of storage time. This may be partly due to the effect of the plastic which was used to contain the meat or to the interaction between the volatiles produced by irradiation of the packaged meat.

The non-irradiated meats became slimy and dark with storage time. Those irradiated at 0.1 megarad did not show much difference from the non-irradiated samples.

Meats irradiated at 5.0 megarad lost their texture and became very crumbly. It was difficult to lift an intact sample out of the container.

The greatest amount of proteolytic activity occurred in the meats stored at 100°F. These meats had the largest amount of exuded fluids and the most intense off-odor. The samples stored at 34°F. did not have as much juice and a much less intense off-odor, except for those which had been irradiated at 5.0 megarad. These meats had, likewise, lost their texture.

SUMMARY AND CONCLUSIONS

Following is a summary of the changes in the nitrogenous constituents and physical conditions of beef muscle as affected by (1) the various temperatures attained in pre-heating prior to irradiation, (2) the gamma radiation dosage employed, (3) the storage time, and (4) the storage temperature. The nitrogenous constituents of meat analyzed were total soluble nitrogen, non-protein nitrogen, amino nitrogen and total and free amino acids.

1. Pre-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, amino nitrogen and free amino acids.

2. Slight increases in total soluble nitrogen and certain of the free amino acids was 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, gamma irradiation dosage and storage temperature employed.

6. Increase in storage temperatures definitely increased the rate of release of the nitrogenous constituents analyzed. The greatest release occurred in the meats stored at 100°F.

7. The proteins were not significantly destroyed. The values obtained for the free amino acids were too low to substantiate significant destruction.

8. All the free amino acids determined quantitatively increased with an increase in gamma radiation dosage, storage temperature and storage time, except for aspartic acid. No free aspartic acid was found in meat irradiated at 5.0 megarad after 60 days storage at 70°F. and 30 days at 100°F.

9. No free tyrosine was found.

10. The greatest amount of the individual amino acids released during the course of the storage interval,

calculated on the basis of mgs. per gram of nitrogen, was 0.03, 0.36, 1.06, 0.57, 0.82, 0.80, 1.77, 0.71, 0.48 and 0.65 per cent respectively of the total aspartic acid, glutamic acid, serine, glycine, threonine, alanine, methionine, valine, phenylalanine and leucine.

11. The meats became firmer as the pre-heating temperature was increased. When irradiated, those exposed to 5.0 megarad were considerably more tender. This effect was also more pronounced as the time and temperature of storage was increased.

12. Large amount of juices exuded from the meats with increase in storage time. Darker juice and darker meats were noticed in the raw samples and in those pre-heated to 100°F.

13. The non-irradiated meats became slimy and dark with increase in storage time at 34°F.

14. As the temperature of pre-heating was increased the color of the meats changed from red to grayish-brown. On storage of the irradiated meats they developed a pink color which has been identified by Tappel (72, p. 411) as globin hemochrome. This pigment readily turned brown on exposure to air.

15. Meats irradiated at 5.0 megarad developed a very intense off-odor which became more intense with increase in storage time. It is to be pointed out, however,

that the packaging film may be, in part, responsible for this change.

In general, increase in pre-heating temperatures reduced the amount and rate of release of the nitrogenous constituents of beef muscle, while increase in gamma radiation dosage and storage temperature employed caused an increase. Thus, if the increase in the nitrogenous constituents are to be retarded, higher pre-heating temperatures, lower irradiation dosages and lower storage temperatures are desired. These factors are all governed by the storage time involved which simply results in greater solubility of the proteins irrespective of the other variables of irradiation, pre-heating temperatures and storage temperatures employed.

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