LOW DOSE GAMMA IRRADIATION ON THE KEEPING QUALITY OF MINCED BEEF

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DECLARATION

I hereby declare that this thesis, entitled **"LOW DOSE GAMMA IRRADIATION ON THE KEEPING QUALITY OF MINCED BEEF"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"LOW DOSE GAMMA IRRADIATION ON THE KEEPING QUALITY OF MINCED BEEF"** is a record of research work done independently by **P.Jenifer**, under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Introduction

1. INTRODUCTION

Our nation has been bestowed with world's largest livestock population, whereas we are in the fifth rank with respect to meat production of 5.9 million tonnes per annum. The important meat-producing animals include cattle, buffalo, sheep, goat, pig and poultry. Preservation technology comes into picture when there is a difference between supply and demand. For centuries, man has been preserving meat by natural methods such as drying, smoking, salting, curing etc. Many of the preservation techniques are having an adverse effect on sensory qualities. By the advent of refrigeration system, chilling has become one of the most important technique in meat preservation. Even though there are different techniques available for meat preservation many of them are inadequate to render the food free of pathogens and spoilage bacteria.

The joint FAO/ IAEA/ WHO Expert Committee on the technical basis for Legislation for Irradiated Food met in Rome in April, 1964, stated that tests conducted in animals and human volunteers have shown no indication of adverse effect of any kind, and no evidence that the nutritional value of irradiated food is affected in any important way (WHO, 1999).

Considering the advantages of food preservation by irradiation, organizations like United States Department of Agriculture, World Health Organization, the National Food Processors Association, Food and Drug Administration and several other private and university based food research institutes have endorsed irradiation as a technique to preserve foods to maintain microbiological safety (Monk, 1995). In India, the Prevention of Food Adulteration (1958) has amended by special Gazette in 1998 and approved irradiation of meat and meat products including poultry to destroy pathogens and to extend shelf life at a dose rate of 2.5 to 4 kGy.

The radiation can be basically classified as ionizing and non ionizing radiation. Food irradiation are carried out utilizing ionizing radiations, which are having a wavelength of 2000^0 A or less. Gamma rays are emitted from the excited nucleus of elements such as Cobalt-60 (^{60}CO) and Cesium-137 (^{137} CS) are of major importance in food preservation. This is the cheapest form of radiation and these rays are having excellent penetration power (Jay, 1996). Ionizing radiation is capable of causing a variety of chemical changes in microorganisms.

The effect of irradiation of food depends on time, temperature and dose rate of irradiation. The low dose irradiation of food is known as radurization and is conducted to extend the shelf life of the products, usually at a dose rate of 1.0 to 4.0 kGy. The gram-negative nonspore forming rods are the most radiosensitive of all bacteria, and they are the principal spoilage organisms of meat and meat products. The major drawback associated with irradiation of meat and meat products is the dose dependent formation of off- flavours due to free radicals, induced lipid oxidation and radiolytic breakdown of proteins and lipids, which affect the organoleptic qualities (Kim *et al.,* 2002a).

In order to make the meat industry more economically viable, value addition is indispensable. There are different fast moving value added meat products in the market among which, the minced products are very important. The lion share of the meat products are comminuted meat products. Mincing and other processing techniques definitely leads to increased microbial load of the final product, hence it is highly perishable. Although the contemporary processing and preservation technologies are judged satisfactory for the marketing of ground beef, the use of ionizing radiation treatment could be beneficial especially to destroy pathogens like *E.coli* (Halkman, 2004)

In India much work has not been conducted in the field of meat irradiation. In order to evaluate the effect of low dose irradiation on minced beef, this study was undertaken to assess the shelf life, physicochemical, microbiological and organoleptic qualities of the product.

2. REVIEW OF LITERATURE

Food irradiation, one of the beneficial applications of atomic energy, is an important innovation in food preservation. Low dose gamma irradiation substantially reduces the microbial population, increases the shelf life, and eliminates parasites such as Trichinae and Cysticerci in meat and meat products. Thus the preservation technique plays an important role in safe guarding health of the public and reduces meat-borne diseases. In addition, irradiation will not impart any residual material in food, which is not possible in many of the preservation techniques.

2.1 HISTORY OF FOOD IRRADIATION

The usage of irradiation to control the spoilage of food was demonstrated in the early decades of the 20^{th} century. However, no commercial development occurred due to various reasons (Urbain, 1989).

In 1905, United States and British patents were issued for the use of ionizing radiation to kill bacteria in foods. Many research works were conducted on the physical, chemical and biological effects of ionizing radiation (ACSH, 1988).

Thayer *et al.* (1986) stated that from 1940 through 1953, exploratory research in food irradiation in United States was sponsored by the Department of Army, the Atomic Energy Commission and private industry.

In 1981, the use of irradiation was approved by the FAO/ IAEA/ WHO joint committee on the wholesomeness of irradiated food. Since then significant progress was made by using irradiation doses lower than 10.0 kGy to control the growth of pathogenic and spoilage bacteria (Giroux *et al.*, 2001).

2.2 ACTION

Ionizing radiation is capable of causing a variety of chemical changes in microorganisms. It is generally assumed that DNA is the most critical target of

ionizing radiation and that the inactivation of microorganisms by ionizing radiation can affect DNA either directly by energy deposition in the surrounding water leading to the formation of diffusive primary radicals, including hydrogen electrons. The hydroxyl radical is the most important; which formed in the hydration layer around the DNA molecule are responsible for 90 per cent of the damage. Consequently, in living cells, the effect is especially significant (WHO, 1999).

2.3 APPROVALS

The meeting of the Joint Expert Committee (JEC), convened in 1976, recommended the unconditional acceptance of irradiated food items, including chicken. This paved the way for the development of Draft International General Standards on Irradiated Foods and a Draft International Code of Practice for the Operation of irradiation facilities used for the treatment of foods through the Codex Alimentarius Commission (WHO, 1977).

In 1990, FDA and in 1992, USDA approved irradiation at the dose range of 1.5-3.0 kGy for destroying pathogenic bacterial organisms. The USDA approved the dose up to 4.5 kGy in 1999 (WHO, 1999).

In December 1997, FDA approved irradiation for red meat to control food borne pathogens and to extend shelf life. In February 1999, USDA allowed the proposal of irradiation of raw meat and meat products (Buzby and Morrison, 1999).

In India, the Ministry of Health and Family Welfare amended the Prevention of Food Adulteration Rules (1954) through a Gazette notification dated August 9, 1994, permitting irradiation of onion, potato and spices. In 1998, meat and meat products including chicken were permitted for irradiation at dose of 2.5-4.0 kGy to extend shelf life and to control pathogens (PFA, 1998).

2.4 SHELF LIFE OF IRRADIATED MEAT AND MEAT PRODUCTS

Niemand *et al.* (1981) reported a doubling in the shelf life of vacuum packaged beef cuts irradiated at 2.0 kGy when compared to nonirradiated samples.

The control had an acceptable shelf life of approximately three weeks, whereas the irradiated samples had a shelf life of more than eleven weeks when stored at 4^{0} C.

Dempster (1985) noticed that low dose irradiation destroyed microorganisms of public health significance and extended the shelf life of meat products.

Paul *et al.* (1990) reported that the lamb meat chunks irradiated at 1.0 kGy and 2.5 kGy remained in acceptable condition for 3 and 5 weeks respectively, whereas the shelf life of irradiated minced meat (1.0 kGy and 2.5 kGy) was 2 and 4 weeks respectively at 0-3°C storage. In contrast, unirradiated meat chunks and mince were spoiled within one week at the same storage condition.

Rodriguez *et al.* (1993) suggested that low dose gamma irradiation (2.0 kGy) could be a reliable preservation tool to obtain an organoleptically stable retail fresh beef products, by reducing naturally occurring spoilage microflora and enhancing the shelf life under refrigeration.

Low dose gamma irradiation had efficiently protected whole chicken carcasses from bacterial spoilage by inactivating more than 99 per cent of the microbial load at an irradiation dose of 2.0 kGy destroying a majority of microorganisms (Katta *et al.*, 1991)

Thayer and Boyd (1993) found that a dosage of 1.0 to 4.0 kGy inactivated 90 per cent of the colony forming units (cfu) of the common food borne pathogens associated with meat and meat products.

Lee *et al.* (1995) suggested that the application of gamma radiation up to a dose level of 10.0 kGy could eliminate a number of food spoilage microorganisms.

Patterson (1996) observed that packing pork chops in an atmosphere of 25 per cent Carbon dioxide and 75 per cent nitrogen followed by irradiation at 1.75 kGy was effective in controlling microbial growth. Treated samples had a shelf life of 12 days at 4°C when compared to 3 days in nonirradiated samples.

In a study conducted by Roberts and Weese (1998) observed extended shelf life of 14, 21 and 42 days for ground beef patties when irradiated at 1.0, 3.0, and 5.0 kGy respectively at chiller storage.

Lacroix *et al.* (2000) irradiated air and vacuum packaged fresh pork loins samples at a dose of 6.0 kGy and reported that irrespective of packaging and dose rate of radiation, all pork samples could be stored at 4±1°C without bacterial spoilage for 43 days.

Irradiation has reduced the normal spoilage microorganisms and extended the shelf life of soft fruit, meat and fish (Lee, 2004).

Balamatsia *et al.* (2006) opined that the low dose irradiation (0.5 kGy and 1 kGy) in combination with aerobic packaging extended the shelf life of fresh chicken fillets by 4 to 5 days whereas irradiation at 2.0 kGy extended the shelf life by 15 days at 4°C.

The keeping quality of gamma irradiated beef fry was studied by Kuttinarayanan *et al.* (2006b) and the irradiated samples showed an enhanced shelf life of 28 to 32 days whereas control samples spoiled organoleptically by 7 to 9 days of storage in the chiller.

2.5 PHYSICOCHEMICAL PROPERTIES

2.5.1 pH

Niemand *et al.* (1981) reported that a dose of 2.0 kGy had little effect on the lactobacilli and the metabolites produced from these bacteria lowered the pH.

Irradiation did not influence the pH of filet americain, but pH values of samples stored at 3°C increased slightly by 0.2 to 0.4 pH units (Tarkowski *et al.*, 1984)

Lefebvre *et al.* (1994) opined that irradiation contributed to a diminution of pH in ground beef samples at 1.0, 2.5 and 5.0 kGy. Gram-negative bacteria, which

increase the pH by the production of ammonia and amines, were more sensitive to irradiation than that of gram-positive bacteria.

Lee *et al.* (1996) observed that the pH values were not different upto 7 days of ageing in irradiated (2.0 kGy) and nonirradiated beef samples, irrespective of storage temperature at 15° C and 30° C. However, after 14 days, pH of the irradiated samples stored at 30° C was lowest, because of growth of lactic acid bacteria after 7 days.

Karthikeyan *et al.* (2000) found out that there was no significant difference in the pH values between the control and acidulant and humectant treated chevon keemas whereas, storage at room temperature gradually increased the pH of treated keema and there was a decrease in the pH of untreated keema.

The pH values were not found to be significantly affected by the addition of sodium chloride either in the case of minced beef or pork, although the values should be slightly lowered with the increase in sodium chloride concentration in both types of meats (Medynski *et al.*, 2000).

Irradiation had not shown any significant effect on the pH of vacuum packaged turkey breast meat samples at 1.5 kGy on day 0 but increased slightly after 10 days of storage at 4° C (Nam and Ahn, 2002).

2.5.2 Water Holding Capacity (WHC)

Grau and Hamm (1957) assessed the WHC by calculating the area of water diffused from the meat on to a filter paper under the influence of a standardized but manually applied pressure. The area of the fluid obtained around the meat film was proportional to the amount of free water in the meat.

Irradiation caused some protein denaturation that increased on storage especially at high temperature. The resultant loss in WHC caused considerable exudation (Schweigert, 1959).

Van Laack and Smulders (1992) suggested that the degree of protein denaturation was an important determinant of the WHC of meat and more protein denaturation generally resulted in lower WHC. They also reported that lowering the temperature would slow down the pH fall and resulted in less protein denaturation and thereby a better WHC.

Ziauddin *et al.* (1993) reported better WHC for minced beef when plate freezing followed by thawing at chiller temperature, while blast frozen samples had a better WHC when it was thawed in running water.

Roserio *et al.* (1994) opined that a drastic fall in muscle pH decline denatured the sarcoplasmic and myofibrillar proteins and increased the tendency of actomysin to contract, thus the amount of fluid free to enter extra cellular spaces was affected.

Kristensen and Purslow (2001) reported that WHC of pork, which decreased post mortem, was found to be increased during subsequent ageing. The degradation of the cytoskeleton proteins weakened the linkage between the myofibrils allowing inflow of previously expelled water, so that WHC increased in later periods of storage.

The overall mean of WHC of aerobically packaged chevon, was lesser compared to vacuum and modified atmosphere packaging at 4 ± 1°C storage (Jayanthi, 2003).

Melody *et al.* (2004) reported that variation in water holding capacity was due to differences in post mortem degradation of intermediate filament protein like desmin. They also found that *psoas major* had more degradation with a lower drip loss compared to *longissmus dorsi* and *semi membranous*.

Zhu *et al.* (2004a) reported that irradiation increase the centrifugation loss in pork loins at 1.5 and 2.5 kGy. The increase in water loss might be related to structural damage of muscle fibers and denaturation of muscle proteins.

Huff-Lonergan and Lonergan (2005) found out that the early postmortem events including rate and extent of pH decline, proteolysis and even protein oxidation are the key factors in influencing the ability of meat to retain water. Much of the water in the muscle is entrapped in structures of the cell, including the intra and extramyofibrillar spaces; therefore, changes in the intracellular architecture of the cell influence the ability of muscle cells to retain water.

Rajkumar *et al.* (2005) reported that the mean WHC of turkey meat samples packaged under modified atmosphere increased up to the third day of storage at 4 °C and thereafter reduced upto the 21st day.

Karakaya *et al.* (2006) assessed the WHC of different meat in pre rigor and post rigor stages and reported that the WHC values of mutton and chevon in pre and post rigor stages were higher than those of beef and rabbit. These higher values were attributed to the higher pH values.

2.5.3 Thiobarbituric Acid Reacting Substances (TBARS)

The fat content and the composition of fatty acids in the lipid of meat patties were important in determining the development of lipid oxidation of aerobic packaged broiler and pork loins during storage (Ahn *et al.*, 1996).

In an experiment, Ahn *et al.* (1998) found that under oxygen permeable packaging conditions, the TBARS of patties from *longissmus dorsi* muscle of pork increased by 10 fold from day 0 to day 14 of storage at 4°C. Even though irradiation cause accelerated lipid oxidation, oxygen exposure was an important factor than irradiation in catalyzing lipid oxidation of raw meat patties during storage.

Murano *et al.* (1998) studied TBA values of irradiated ground beef patties under different packaging and storage systems. They reported that beef patties irradiated and stored under air and those irradiated under vacuum and stored under air, showed a higher degree of lipid oxidation compared with products irradiated and stored under vacuum or nonirradiated.

Irradiated muscle strips produced more TBARS than nonirradiated in aerobic packaging during storage (Ahn *et al.*, 2000a).

Ahn *et al.* (2000b) reported that TBARS of vacuum packaged frozen and refrigerated patties was not influenced by irradiation and storage time. However, with aerobic packaging, TBARS of refrigerated pork patties increased with storage time.

Irradiated meat produced more volatiles and higher levels of TBARS than nonirradiated meat regardless of animal species, but correlated with irradiation dose (Kim *et al.*, 2002a).

According to Quattara *et al.* (2002), the TBARS and free sulfydryl contents were stabilized during post irradiation storage for samples containing ascorbic acid coated with protein based film immobilized spice powders in ground beef.

Gomes *et al.* (2003) reported that nonirradiated mechanically deboned chicken meat showed lower TBARS values than irradiated samples with a correlation between the TBARS values and oxidation odour in irradiated samples. The samples irradiated with dose of 3.0 and 4.0 kGy were acceptable for 10 and 6 days respectively with TBARS value below or equal to 3.9 mg malonaldehyde per kilogram.

However, in a study conducted by Zhu *et al.* (2003) the increase in TBARS values after irradiation was small due to the vacuum packaging conditions of turkey hams during irradiation and storage.

Ahn and Nam (2004) found out that as the storage time increased, the overall lipid oxidation increased in faster rate in irradiated ground beef than nonirradiated. However, the effect of ascorbic acid in ground beef was more distinct as the TBARS values were lesser than that of irradiated controls.

Irradiation of rabbit meat samples significantly increased the amounts of TBARS on storage (Badr, 2004). Zhu *et al.* (2004b) reported that the TBARS

values of ready to eat turkey breast did not change significantly at 0 day or after 14 days of refrigerated storage, because vacuum packaging prevented lipid oxidation.

Irradiating beef biltong at doses up to 10.0 kGy under vacuum had no effect on lipid oxidation. The propagation of fatty acid free radicals and formation of oxygen free radicals on irradiation were limited in an oxygen free environment. The low fat content of the biltong samples probably also contributed to the low degree of oxidation. (Nortje *et al.*, 2005).

Thiobarbituric values for nonirradiated and irradiated (aerobically packaged) chicken samples were less than one mg of malonaldehyde per kg of muscle during refrigerated storage for 21 days (Balamatsia, 2006).

2.5.4 Tyrosine Value

The mean tyrosine values were higher in electrically stimulated mutton samples than their controls on chiller storage (Kuttinarayanan, 1988). The mutton carcasses obtained from old sheep over 7 years had the lowest mean values of 7.79 mg per 100 g of meat compared to that of 3 to 5 years age group with mean values of 12.43 mg per 100 g of meat when stimulated at 220 V.

In plate frozen meat cuts and minced meat, tyrosine values were slightly decreased during storage, since the proteolytic reaction due to bacteria or endogenous enzymes was ceased during frozen storage (Ziauddin *et al.*, 1993).

The irradiation of meat at 1-10 kGy could be useful in retaining quality since proteolysis by endogenous enzymes would be diminished (Lawrie, 1998).

A higher protein degradation was observed by Karthikeyan *et al.* (2000) in keema when stored at ambient temperature. The unusual higher tyrosine values noted in treated keema was due to proteolysis of added soy protein isolates and skim milk powder when compared to that of untreated keema.

Dushyanthan *et al.* (2001) observed that mutton packed in multilayered material and under vacuum revealed lower mean tyrosine values of 20.54 mg and 21.35 mg per 100g of meat respectively. Anaerobic environment and barrier property of multilayered material for oxygen led to lower proteolysis and hence the lowest tyrosine values.

The tyrosine values of chevon samples were noticed in different packaging methods by Jayanthi (2003). There was no significant difference between aerobic (8.89mg/ 100g), vacuum (9.25mg/100g) and modified atmospheric (8.59 mg/ 100g) packaging methods when the samples were stored at 4±1° C.

2.5.5 Colour

Gamma irradiation converted the brown metmyoglobin to a red myoglobin pigment, which was similar but not identical to oxymyoglobin. The formation of red pigment was accelerated under nitrogen atmosphere and inhibited in the presence of oxygen (Satterlee *et al.,* 1971).

Upon irradiation, the free binding sites of myoglobin reacted with free radicals such as hydroxyl (OH) and sulfuryl (-SH) radicals to form metmyoglobin and sulfmyoglobin, respectively. The metmyoglobin was associated with brown colour of meat and sulfmyoglobin for green colour (Judge, 1989).

Millar *et al.* (1995a) studied the effect of ionizing radiation on colour of beef samples and reported that L* values of irradiated beef increased with storage. The* and b* values were significantly lower than control on storage. The mean L*a*b* values of lightness, redness and yellowness on irradiated stored (4°C) samples were 45.06, 10.80 and 6.64 respectively.

The development of red or pink colour of irradiated poultry meat was due to ferrous myoglobin derivatives such as carboxymyoglobin, nitricoxide myoglobin other than oxymyoglobin (Millar *et al.*, 1995b).

Ahn et *al.* (1998) reported that L* values of irradiated aerobic packaged pork patties increased to the highest levels after 7 days of storage and then decreased after 14 days. When irradiation was combined with aerobic condition further reduction in a* values were observed with higher b* values on chiller storage than the controls.

Irradiation did not affect the colour of the ground beef samples, with differences only due to packaging atmosphere. Samples stored under vacuum were darker and redder than samples under air with mean L*a*b* values of 37.90, 4.74 and 8.0 at vacuum packaging (Murano *et al.*, 1998).

Giroux *et al.* (2001) observed reduced lightness, redness and yellowness in beef patties when irradiated at doses between 2.0 and 4.0 kGy. The mean L*a*b* values at 2.0 kGy were 44.31, 12.35 and 17.42 respectively

Kim *et al.* (2002b) assessed the effect of irradiation on colour of beef samples under different packaging. The L* values of aerobically irradiated beef decreased significantly (36.10 from 43.99) after 7 days of storage with increased a* values (19.77 from 15.00). In vacuum packaging the L* values remained unaffected with decreased a* values and the b* values increased regardless of packaging and storage.

The increased red colour was resulted from the formation of carboxy myoglobin and carboxyhaemoglobin in mechanically deboned chicken meat (MDCM) (Gomes *et al.*, 2003). These pigments were associated with carbon monoxide developed during irradiation and also due to the incorporation of bone marrow in MDCM.

Usually light meat produced pink colour due to carboxyl heme pigments while dark meat became brown or gray after irradiation. The production of carbon monoxide in red meat was similar to that of light meat, but colour changes are different due to high pigment content in red meat (Nam and Ahn, 2003).

Zhu *et al.* (2003) observed that irradiation up to 2.0 kGy had minor effect on the colour of turkey ham. Irradiation decreased colour L* values and increased a* values on storage at 4°C.

Brewer (2004) suggested that rapid generation of large amounts of metmyoglobin when irradiating under oxygen containing environment was due to accelerated oxidation of myoglobin.

Irradiation initially resulted in a darker (decreased L* values), redder (increased a* values) and less yellow (decreased b* values) ground pork patties due to formation of metmyoglobin from oxymyoglobin. As the display time increased to 8 days, L* and b* values were stabilized and a* values were decreased (Ohene-Adjeri *et al.*, 2004).

During refrigerated storage, a* values of 1.0 and 2.0 kGy irradiated ready to eat turkey breast increased significantly at 7 and 14 day, compared with that at day 0 (Zhu *et al.*, 2004b).

2.5.6 Cooking Loss

Niemand *et al.* (1981) observed that cooking loss of the beef cuts irradiated at 2.0 kGy were higher (25.1%) than their controls (24.5%) throughout the storage period of 8 weeks at 4°C.

When the proteins of muscle were exposed to heat, they lose their native structure and undergo several changes in configuration. This denaturation of protein followed by an aggregation or clumping of the protein molecules was indicated by loss in protein solubility (Forrest *et al.*, 1975).

Bendall and Restall (1983) reported that structure of muscle proteins was altered according to temperature of heat treatments. Expulsion of water from individual myofibers was slow at 40-53°C, but was rapid at 60°C as the collagen of the basement membrane was shrinking. At 64-90°C, shrinkage of the endomysial, perimysial and epimysial collagens were noticed decreasing the myofibre diameter.

Prolonged heating converted the collagen to gelatin and concomitant tenderizing occurs.

Cooking loss of buffalo samples were studied by Ziauddin *et al.* (1993) on two different freezing methods. He reported that cooking loss decreased during the second month of storage and increased during the third month of storage in plate frozen samples. However in blast frozen samples a marked increase in second month and decrease in third month of storage were also noticed.

Ziauddin *et al.* (1994) reported higher percentage of cooking loss and thermal shrinkage in *biceps femoris* and *longissimus dorsi* muscles of old animals compared to young animals.

The control beef samples exhibited less cooking loss than the samples marinated with sodium chloride and calcium chloride solutions (Aktas *et al.*, 2003). The samples marinated with calcium chloride held less water with more cooking loss than the sodium chloride samples as the pH was nearer the isoelectric point of myofibrillar proteins.

Yoon (2003) observed that irradiated chicken breast had more cooking loss (26.4 %) than unirradiated samples (23.81 %) throughout the 14 days storage period. Gamma irradiation caused significant textural toughening with contraction of the sarcomere width and physical disruption in myofibrils.

The myofibrillar proteins that are extracted into the water phase during comminution and blending was a important factor for the quality of protein network or gel formation. The myofibrillar proteins on heating created a dense protein network which held water efficiently by capillary forces (Tornberg, 2005).

Beef and rabbit meat had higher cooking loss values in the pre rigor stage due to low pH and WHC than chevon and mutton (Karakaya *et al.*, 2006).

2.6 MICROBIOLOGICAL ANALYSIS

2.6.1 Aerobic Plate Count

Naik *et al.* (1994) suggested a dose of 2.5 kGy would reduce the mesophilic count of buffalo samples immediately by 2-3 log cycles. After 3 weeks of storage at 0-3°C, the colony forming units (cfu) of irradiated meat was equivalent to the initial cfu of irradiated control and had a shelf life of 4 weeks.

Alur *et al.* (1998) subjected the frozen processed pork meat products to gamma radiation at 2.5 kGy and observed 3 to 4 log reduction in mesophilic count.

Murano *et al.* (1998) observed that the microbial quality of irradiated ground beef patties were better than that of nonirradiated controls, with 2 to 3 log reduction in total viable count immediately after irradiation. The shelf life for the product was extended by 55 days at 4°C regardless of the method of packaging.

Giroux *et al.* (2001) found that irradiation at a level of 1.0 kGy after one day of storage produced a 1.78 log unit reduction of aerobic plate count in beef patties samples without ascorbic acid and 3.77 log reduction in samples containing ascorbic acid. Bacterial growth was below the detectable level when the samples were irradiated at 2.0 kGy.

Aziz *et al.* (2002) found that the immediate effect of exposing beef samples to a dose level of 5.0 kGy was the reduction in the number of bacteria by 2-3 log cycles.

Irradiation doses of 1.5 and 3.0 kGy significantly reduced the counts of aerobic mesophilic bacteria, psychrophilic bacteria, yeast and moulds and prolonged the shelf life of refrigerated rabbit meat samples from 12 to 21 days (Badr, 2004).

Kuttinarayanan *et al.* (2005b) observed the total plate count was reduced by 95 per cent on turkey breast when irradiated at 2.5 kGy on the day of preparation.

2.6.2 Coliforms and *Escherichia coli*

Lefebvre *et al.* (1992) reported that coliforms were not found in any of the irradiated samples of ground beef at any time during storage whereas the control samples had a initial count of 1.6×10^3 cfu/g on the day 0 and reached over 3.0×10^6 cfu/g by day 13 of storage at 4°C.

A significant reduction in the *Enterobacteriaceae* population was attained at 2.0 kGy irradiation on beef samples (Rodrigueq *et al.*, 1993).

Ninety per cent of the viable *E. coli* in chicken meat was eliminated by doses of 0.27 kGy at + 5 ^{0} C and 0.42 kGy at – 5^0 C (Thayer and Boyd, 1993)

E.coli O157:H7 had a significantly higher D 10 values when irradiated at – 17 to – 15 ^{0}C than at 3 to 5°C in raw ground beef patties (Clavero *et al.*, 1994).

Naik *et al.* (1994) observed that buffalo meat samples irradiated with 2.5 kGy were completely free of *Enterobaceriaceae* when stored at 0-3°C for five weeks.

Olson (1998) suggested that a minimum dose of 1.5 kGy was sufficient to reduce 6 log cycles of *E. coli* O157: H7, which had a D10 value of about 0.24 kGy when muscle foods were irradiated.

The ground chicken samples when irradiated at 2.0 kGy and 4.0 kGy reduced the *E.coli* population by 3 log cycles and 6 log cycles respectively in relation to the control. However, the doses of 6.0 and 8.0 kGy reduced *E.coli* population below the detectable level (Spoto *et al.*, 2000).

Chirinos *et al.* (2002) observed that a dose of 1.08 kGy was sufficient to reduce *E.coli* O157: H7 contamination to 4 log cycles, without affecting the sensory attributes of the hamburgers.

Satin (2002) reported that a relatively low irradiation dose of 1.5 kGy was sufficient to cause a 4 log reduction in the number of *E.coli* O157: H7 at 5°C in processed meat.

Enterobacteriaceae were reduced by 97.8 per cent in 1.5 kGy irradiated rabbit meat samples and at 3.0 kGy, the count was below the detectable level (Badr, 2004).

Halkman (2004) noticed that *E.coli* O157: H7 and total coliforms were twice sensitive to the irradiation when compared with *E.coli* type 1 in minced beef at doses up to 1.5 kGy.

Kuttinarayanan *et al.* (2005a) reported 100 percent reduction with respect to coliform and *E.coli* in beef, minced beef and cutlet by 2.0 kGy irradiation.

2.6.3 Faecal Streptococci

Graham (1980) found that the approximate minimal doses of gamma irradiation to destroy *Streptococcus faecalis* in minced beef was around 0.38 Mrad.

Streptococcus faecalis in minced chicken meat at 4°C had a D10 value of 0.651, 0.702, and 0.697 in air, carbon dioxide and vacuum packaging respectively (Patterson, 1988).

Enterococcus faecium R53 was the most resistant of non-spore forming bacteria to irradiation (Jay, 1996).

Rabbit meat samples subjected to 1.5 kGy irradiation significantly decreased the counts of *Enterococcus faecalis* by 74 per cent and to 3.0 kGy by 96.3 per cent (Badr, 2004).

Kuttinarayanan *et al.* (2005a) noticed 100 per cent reduction in faecal streptococcal and staphylococci count in irradiated beef, minced beef and cutlet at 2.0 kGy.

2.6.4 Staphylococci

Monk *et al.* (1994) predicted a dose of 2.5 kGy would be sufficient to kill 5.12 $\log_{10}$ *Staphylococcus aureus* per gram of ground beef and the D 10 values ranged from 0.435 to 0.453 kGy. Neither the fat content of beef nor the temperature during irradiation treatment influenced the inactivation rates.

Thayer *et al.* (1997) concluded that *Staphylococcus aureus* could be eliminated in bison, ostrich, alligator and caiman meats when irradiated at dose range of 1.5 to 3 kGy.

The staphylococcal count of the processed pork meat was reduced by 3-4 logs when irradiated at 2.5 kGy (Alur *et al.*, 1998).

Spoto *et al.* (2000) observed that a dose of 2.0 kGy reduced *Staphylococcus aureus* in approximately 4 log cycles and a dose of 4.0 kGy reduced 6-log cycles. Colonies were not detected in the refrigerated ground chicken meat samples irradiated by 6.0 and 8.0 kGy.

In a study, Lamb *et al.* (2002) noticed that irradiation was an effective method for reducing *Staphylococcus aureus in* ready to eat ham sandwiches. Sandwiches irradiated with 5.9 kGy did not reveal *Staphylococcus aureus* growth at any time, and irradiated with 3.85 kGy showed a 6.18 log reduction after 13 days.

The mean initial *Staphylococcus aureus* count was found to be 3.982 cfu/g in rabbit meat samples. Subjecting these samples to 1.5 kGy reduced the count by 93 per cent and at 3 kGy below the detectable levels (Badr, 2004).

Kanatt *et al.* (2005) noticed that mutton shammi kababs and pork salami irradiated at 1.0 kGy reduced the *Staphylococcal* count by 2 log cycles. And when

these samples were irradiated at 2.0 and 3.0 kGy these organisms were not detected throughout the storage period.

2.6.5 Salmonellae

The effects of low temperature (+5°C and – 18°C) and irradiation on number of *Salmonellae* were studied by Mulder (1982). Irradiation at chill temperature resulted in *Salmonella* negative samples after one month of storage while irradiation after freezing resulted in *Salmonella* negative samples after a three months storage period.

Thayer *et al.* (1991) found that a radiation dose of 1.5 kGy killed 2.04, 2.49, 2.85, 3.13 or 3.33 log colony forming units (cfu) of *Salmonella typhimurium* at irradiation temperature at -20, -10, 0, +10, or $+20^0$C, respectively. The same radiation dose applied at the same irradiation temperature followed by heating for 3 minutes at 60°C killed 9.17, 9.82, 10.18, 10.26 or 10.06 log cfu of *S. typhimurium.*

Grant and Patterson (1992) noticed that a dose level of 2.5 kGy reduced 3 to 8 log cycle of *Salmonella* in roast beef meal.

Clavero *et al.* (1994) reported that D 10 values ranged from 0.618 to 0.800 kGy for *Salmonellae* and an applied dose of 2.5 kGy would be sufficient to destroy $10^{3.1}$ *salmonellae* in raw ground beef patties.

Monk *et al.* (1995) opined that among the gram negative pathogens *Salmonella* species was the most resistant organism and any irradiation process designed to elimininate *Salmonellae* would eliminate other gram-negative bacteria.

The radicidation dose of 2.5 kGy reduced the *Salmonella* count by more than 4 log cycles in processed pork meat products (Alur *et al.*, 1998). However 4.0 kGy could eliminate *Salmonella* completely below the limit of detection.

Farkas (1998) reported that radiation treatment at doses of 2.0 to 7.0 kGy could effectively eliminate potentially pathogenic bacteria like Salmonella, *Staphylococcus aureus,* Campylobacter, *Listeria monocytogens* or *Escheriachia coli* O157: H7 from suspected food products without affecting sensory, nutritional and technical qualities.

In fresh poultry carcasses, an irradiation dose of 2.5 kGy would elimininate *Salmonella* and extends the shelf life of the food by a factor of about two if the post irradiation storage temperature was maintained below 5° C (Gracey *et al.*, 1999).

Badr (2004) subjected rabbit meat samples to 3.0 kGy and found that Salmonella were below the detectable level.

2.7 SENSORY EVALUATION

Collagen shrinks when irradiated wet (Perron and Wright, 1950) and, indeed, irradiation caused softness and tenderness of texture as an immediate effect (Coleby et al., 1961).

When beef was irradiated with 1.0 kGy prior to the addition of mayonnaise sauce showed no significant taste difference when compared to nonirradiated samples (Tarowski *et al.*, 1984).

Lefebvre *et al.* (1994) conducted the sensory evaluation of irradiated ground beef (1.0, 2.5 and 5.0 kGy) and found that odour and flavour of the irradiated cooked ground beef was slightly disliked while no difference was perceived in the colour and texture. The lower the dose of irradiation, the better the taste appreciated.

Naik *et al.* (1994) showed that after 2 weeks of storage the control samples had an acceptability score of less than 5 with off odour and signs of spoilage in buffalo meat. In contrast, irradiated meat (2.5 kGy) showed high sensory scores of above 7.5 and had an overall acceptability score higher at 6.5 even at the end of five weeks.

Luchsinger *et al.* (1996) evaluated acceptance of fresh or frozen irradiated boneless pork chops (1.5, 2.5 and 3.85 kGy) using a trained panelist and consumers. They did not observe any significant differences in acceptance, meatiness, freshness or juiciness of products irradiated at 2.5 kGy or below.

Sensory evaluation of ground beef patties revealed significant increase in texture and juiciness and a decrease in aftertaste in samples irradiated under vacuum, regardless of storage atmosphere when compared to nonirradiated controls (Murano *et al.*, 1998). They also suggested that lack of oxygen in vacuum packaging and unavailability of water in the frozen state caused changes in the meat tissue, which favourably affected tenderness and perceived juiciness.

In the sensory panel conducted by Wheeler *et al.* (1999) in hamburgers prepared with 4.5 kGy treated ground patties rated lower score in taste than their control and hamburgers prepared with 3 kGy treated patties.

Irradiation odour intensity increased in dose dependent manner in vacuum packaged frozen pork patties, which lasted for three months (Ahn *et al.*, 2000a).

Irradiation had no negative effect on the acceptance of raw meat and approximately 70 per cent of sensory panelist characterized irradiation odour as barbecued corn like odour (Ahn *et al.*, 2000b)

In an experiment by Ohene-Adjei *et al.* (2004), reported irradiation neither affected juiciness of ground pork and the loin chops, nor the texture or mouth feel of the ground pork but decreased the tenderness of loin chops.

Kanatt *et al.* (2005) reported that appearance, flavour and texture of irradiated samples of three meat products (chicken chilly, mutton shammi kababs and pork salami) were different from its nonirradiated controls and were acceptable immediately after irradiation. Mutton shammi kababs after two weeks of chilled storage and pork salami after one week of storage were also found to be acceptable.

Nortje *et al.* (2005) irradiated beef biltong at 2.0 and 4.0 kGy and reported that it was liked significantly more than nonirradiated samples, indicating non-oxidative, irradiation induced flavour changes would contribute to flavour development in the bland moist biltong.

Materials and Methods

3. MATERIALS AND METHODS

A study on the effect of low dose gamma irradiation on the keeping quality of minced beef was carried out in the Department of Livestock Products Technology, Mannuthy.

Fresh beef samples collected from six beef carcasses slaughtered at the department during the period from September 2005 to July 2006 were utilized in the present study. After removing the connective tissue, the samples were minced in a meat mincer (Mado Junior, Italy) through 13 mm grinder plate the day after collection. The minced beef samples were packaged 200g each in High Density Polyethylene (HDPE) pouches and kept in a chiller prior to irradiation.

3.1 GAMMA IRRADIATION

The chilled samples were divided randomly into four groups and considering one group as control (C), the other groups were subjected to irradiation at melting ice temperature at different doses, either at 1.0 (R1), 2.0 (R2) and 3.0 (R3) kGy. Irradiation was done using Gamma Chamber 5000 (BRIT- DAE, Mumbai) where the source of irradiation is Cobalt-60. After irradiation the C, R1, R2, and R3 samples were stored at chiller (4 ± 1^0C).

The nonirradiated and irradiated minced beef samples were evaluated for physicochemical, microbiological properties and sensory evaluation on day 0, 7, 15, 21, 30, 37, 44, 51 and 58 or until organoleptic detection of spoilage was noticed.

3.2 PHYSICOCHEMICAL PROPERTIES

3.2.1 pH

The pH of irradiated and nonirradiated samples was reported by using a digital pH meter (μ pH system-Systronics, India) as described by O'Halloran *et al.*

(1997). About 50 g of minced beef was packed in a glass beaker and the electrode was inserted into the sample without entrapping any air space around the bulb of the electrode. The pH was recorded and the probe was thoroughly rinsed with distilled water before each reading. The pH meter was standardized using 4 and 7 pH buffer solutions at weekly interval.

3.2.2 Water Holding Capacity

Water holding capacity (WHC) of the samples was determined by adopting the centrifugation method as per Wardlaw *et al.* (1973) with slight modification. An accurately weighed 5 g of packaged sample was placed in the calibrated centrifuge tube and 7.5 ml of 0.6 M sodium chloride solution was added. The contents were stirred for one min with a glass rod. After holding it for 15 min at 4°C, the meat slurry was again stirred for 1 min and immediately centrifuged at 6000 rpm for 15 min in the research centrifuge. The volume (v) of the supernatant was recorded. The amount of added solution retained by the meat was reported as the WHC in ml per 100 g meat and was calculated as follows

$$\text{WHC (ml/100 g)} = \frac{(7.5\text{-}v)\ 100}{5}$$

3.2.3 Thiobarbituric Acid Reacting Substances (TBARS)

Thiobarbituric acid reacting substances (TBARS) produced from lipid peroxidation were determined using the method of Witte *et al.* (1970). Twenty gram sample was blended with 50 ml chilled extracting solution containing 20 per cent trichloroacetic acid in 2M phosphoric acid for 1.5 to 2 min. The resultant solution was transferred to a 100 ml volumetric flask. Then the sample was made up to 100 ml using deionised distilled water. This solution was filtered using Whatman No.1 filter paper. Five milliliter filtrate was transferred to a screw capped vial followed by the equal quantity of 2-thiobarbituric acid solution (0.005M in distilled water). The solution was mixed by inverting the vial and it is kept for 15 h in darkness at room temperature. The absorbance of this solution was determined at

530 nm against blank containing 5 ml distilled water and 5 ml 2- thiobarbituric acid solution (0.005M) in UV-Vis Spectrophotometer 119 (Systronics, India). The absorbance was converted to TBARS values and was expressed as milligram malonaldehyde per kilogram of meat.

3.2.4 Tyrosine Value

Tyrosine value of meat sample was estimated as per the method described by Strange *et al.* (1977) with some modifications.

3.2.4.1 Preparation of trichloro acetic acid (TCA) extract

Twenty grams of sample was blended in 50 ml of cold 20 per cent tricholoroacetic acid for 2 min. The blended contents were rinsed with 50 ml of distilled water, mixed together and filtered through the Whatman No.1 filter paper and the filtrate was collected. The filtrate, termed the trichloroacetic acid extract was used in the estimation of tyrosine value.

3.2.4. 2 Estimation of tyrosine value

To 2.5 ml of TCA extract, equal quantity of distilled water was added in a test tube. To this 10 ml of 0.5N sodium hydroxide was added followed by 3 ml of diluted Folin and Ciocalteu's phenol (FC) reagent (1 ml of concentrated FC reagent and 2ml of distilled water). After mixing, the contents were allowed to stand for 15 min. at room temperature. The developed colour was measured as absorbance at 660 nm in UV-Vis Spectrophotometer 119 (Systronics, India) using a blank for comparison. By reference to a standard graph tyrosine value was calculated as mg tyrosine per 100 g of sample.

3.2.4.3 Standard graph for estimation of tyrosine value

Hundred milligram of L-tyrosine was dissolved in 500 ml of 5 per cent TCA in a volumetric flask. The following volumes of the above solution were then transferred to a series of 100 ml volumetric flasks: 0, 1, 3, 5, 7, 10, 12 and 15 ml and were made up to the mark with distilled water and mixed thoroughly. To 5 ml of each of the resultant solutions, 10 ml of 0.5 NaOH and 3 ml of diluted Folin and Ciocalteu's phenol reagent were added and then treated as described for tyrosine value. The standard graph was prepared with the known concentration of L-tyrosine in the solutions and their corresponding absorbance values (Fig. 1).

3.2.5 Colour

Colour of the meat samples was determined using Hunterlab Miniscan XE plus Spectrophotometer (Virginia, USA) with diffuse illumination as outlined by Boakye and Mittal (1996). The instrument was set to measure Hunter 'L', 'a' and 'b' using illuminant 45/0 and 10° standard observer with an aperture size of 2.54 cm. It was calibrated using black and white tiles, and colorimeter score recorded with 'L' of black =0 and 'L' of white 100, 'a' of green = (-80) and 'a' of red =100 and 'b' of blue = (-50) and 'b' of yellow =70. The colour coordinates 'L' (lightness), 'a' (redness) and 'b' (yellowness) of the sample was measured thrice and the average values were taken.

3.2.6 Cooking Loss

According to Boccard *et al.* (1981) 70-100g sample was placed in high-density polyethylene pouch and sealed in moderate vacuum to remove the trapped air between the sample and the wall of the bag. The bag was kept in the water bath at 75°C for 50 min. Then the pouch was placed in the running tap water for 40 min, after which the cooked meat was taken out from the bag, mopped dry and weighed. The percentage of cooking loss was assessed as follows

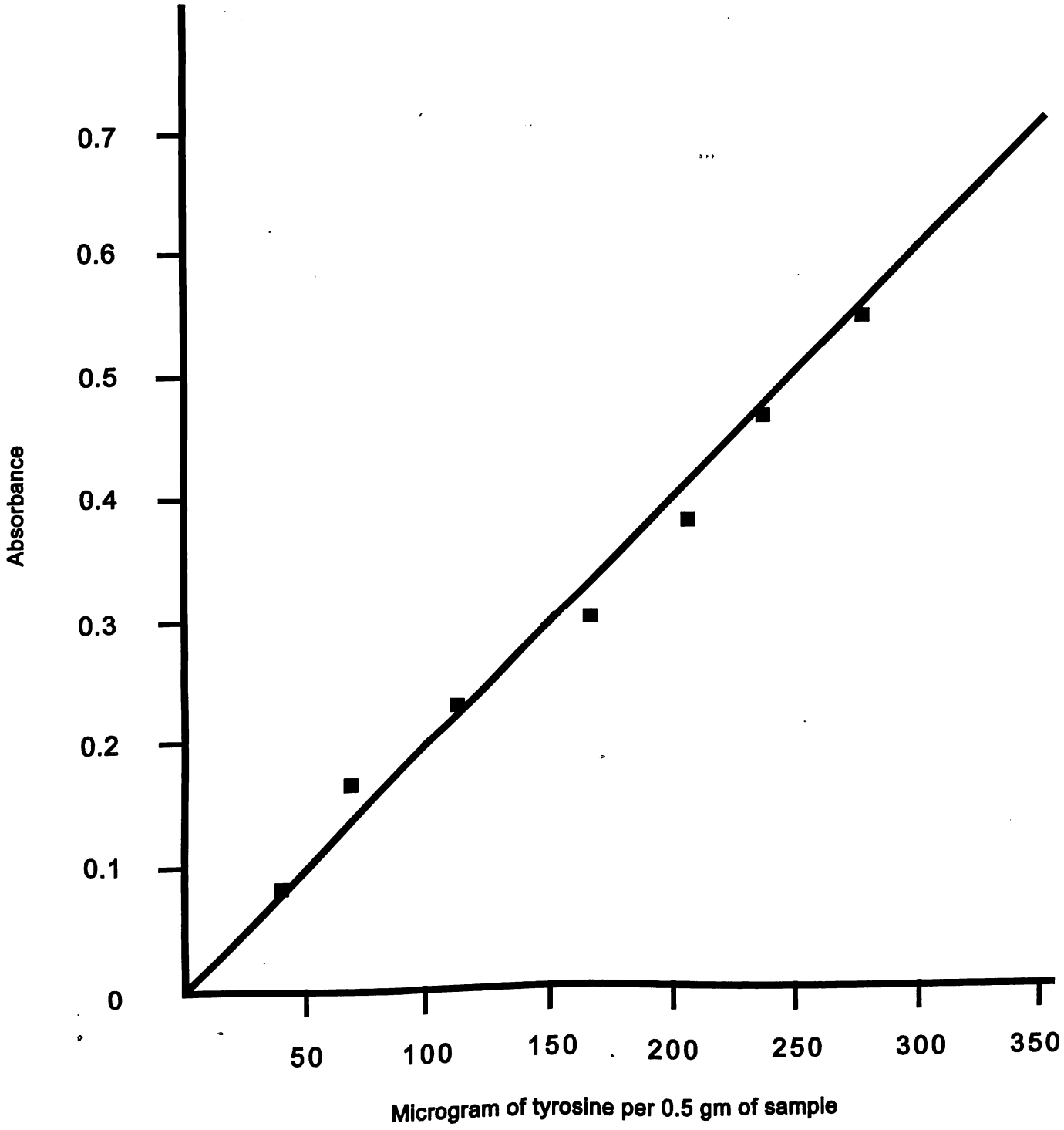

Fig. 1 Standard graph for Tyrosine value

$$\text{Cooking loss (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100$$

3.3 MICROBIOLOGICAL ANALYSIS

3.3.1 Processing of samples

The sealed pouches were opened near a burner taking all aseptic precautions. Twenty five gram of sample was weighed and transferred to the stomacher bag containing 225 ml of 0.1 per cent peptone water and blended in the stomacher (Seward stomacher® 400 circulator) for 30 sec. so that it forms 1 in 10 dilution. Further 10 fold dilutions were prepared by transferring one milliliter of the inoculum into nine milliliters of the diluent of 0.1 per cent peptone water. From serial dilutions, selected dilutions were used for assessing various microbial counts.

3.3.2 Bacterial Counts

3.3.2.1 Aerobic plate count

Aerobic plate count (APC)) of each sample was estimated by pour plate technique, as described by Mortan (2001). From the selected dilution of each sample, one milliliter of inoculum was transferred in labeled duplicate petri dishes. To each of these inoculated plates, about 15-20 ml sterile molten Standard Plate Count agar (HiMedia, Mumbai) maintained at 45°C was poured and mixed with the inoculum by gentle rotatory, forward and backward movements. The inoculated plates were allowed to solidify at room temperature and incubated at 37°C for 24 hours in inverted position. At the end of the incubation period, petri dishes with a bacterial count between 20 and 200 colonies were selected and the colony counts were taken with the help of a digital colony counter (Royal, India). The number of colony forming units (cfu) per gram of the sample was calculated by taking the average of duplicate plates and multiplied by the dilution factor and converted to $\log_{10}$ cfu/ g of sample.

3.3.2.2 *Coliform* count *and Escherichia coli count*

Coliform count was estimated according to the procedure described by Kornaks and Johnson (2001). From selected dilution, 1 ml of the inoculum was transferred in to the sterile duplicate petri plates and 15-20 ml Eosin Methylene Blue agar (HiMedia, Mumbai) maintained at 42 to 45°C was poured, mixed and allowed to solidify. These plates were incubated in inverted position at 37°C for 24 h. At the end of incubation, purplish red colonies with diameter at least 0.5 mm, surrounded by a reddish zone of precipitate were counted as coliforms.

The colonies, which are showing greenish black metallic sheen on deflected light, were counted as *Escherichia coli* and the counts were expressed in $\log_{10}$ cfu/ g and further confirmed by IMViC test.

3.3.2.3 *Faecal streptococcal count*

Fecal streptococcal count was estimated as per the Hartman *et al.* (2001). From the selected dilutions of each sample, 1 ml of the inoculum was transferred on to duplicate plates. Fifteen to twenty milliliters of Karl Friedrich Streptococcal agar (HiMedia, Mumbai) maintained at 45°C was added, mixed well and allowed to solidify. The plates were incubated in inverted position at 37°C for 48 h. After the period of incubation, pink to dark red colonies with a diameter 0.5 and 3 mm and surrounded by a narrow whitish zone were counted as faecal streptococci and expressed as $\log_{10}$ cfu/g.

3.3.2.4 *Staphylococcal count*

The staphylococcal count of the samples was estimated by the method described by Lancete and Bennet (2001). One ml of the selected dilution was inoculated into the petri dishes and Baird Parker agar (HiMedia, Mumbai) maintained at 45°C was poured. After mixing, the plates were allowed to solidify at room temperature and were then incubated at inverted portion at 37°C for 24 hours. At the end of incubation, colonies showing characteristic appearance with circular,

smooth, convex, greyish black to jet black, frequently with light-coloured margin were counted with the help of colony counter and the counts were converted to $\log_{10}$ cfu /g.

3.3.2.4 Detection of salmonella

The presence of samonella was estimated by the method described by Andrews *et al.* (2001). Twenty five gram of aseptically weighed sample was blended in 225 ml of lactose broth and pre enriched at 37°C for 24 h. Ten milliliters portion of the preenrichment culture was transferred to 100 ml of Tetrathionate Brilliant Green broth and to 100 ml of Rappaport Vassiliadis medium. Incubate the broths for 24 h at 43°C. At the end of incubation a loopful of the culture from each enrichment broth was inoculated on the duplicate plates of Brilliant Green (BGA) agar (HiMedia, Mumbai) and Salmonella and Shigella (SS) agar and incubated at 37°C for 24 h. Colonies with colourless pinkish white opaque to translucent colonies with a diameter of about 1-2 mm, surrounded on a pink or red hue on BGA plates and colourless colonies with black center on SS agar plates, were indicative of *Salmonella*

3.4 SENSORY EVALUATION

Taste panel assessment of the nonirradiated and irradiated samples was done on day 0, 7, 15, 21, 30, 37, 44, 51, and 58 or until spoilage whichever was earlier. Uniform amount of samples were taken and cooked for about 20 min in the boiling water (100 °C). The cooked samples were cooled to room temperature and served to trained panelists drawn from the in the Department of Livestock Products Technology, College of Veterinary and Animal Science, Mannuthy. Nine point Hedonic scale score card (appended) was provided to the panelist to assess colour, flavour, juiciness, tenderness and overall acceptability of the cooked product.

SCORE CARD FOR TASTE PANEL EVALUATION

Name of The Product: Cooked Minced Meat date: sample no:

	Colour	Flavour	Juiciness	Tenderness	Overall acceptability	
	Extremely Appealing	Delicious	More Juicy	Very Tender	More Acceptable	9
						8
						7
	Appealing	Desirable	Juicy	Tender	Acceptable	6
						5
						4
	Less appealing	Not so desirable	Less Juicy	Tough	Less Acceptable	3
						2
						1

Guide lines for giving judgement: If you feel that the colour of the product given to you for taste panel evaluation is extremely appealing, put a tick mark in any one of the three boxes against colour. Lower box signifies that it is less appealing and a tick in the central box signifies that it is for appealing. Similarly mark for the other characters viz., flavour, juiciness, tenderness and overall acceptability.

Specify comments if any:

Name and designation: Signature:

3.5 STATISTICAL ANALYSIS

The data obtained with respect to physicochemical, microbiological and sensory evaluation of nonirradiated and irradiated samples were analysed using one way analysis of variance and paired t - test (Snedecor and Cochran, 1994).

4. RESULTS

Beef samples were collected from cattle slaughtered at the Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy. The excess connective tissue were trimmed off and minced with the help of meat mincer. The minced beef was packed in 200 g each in HDPE packets and kept in chiller. Half of the number of packets prepared were subjected to irradiation either at 1.0, 2.0, or 3.0 kGy at melting ice temperature. The irradiated and nonirradiated samples were kept in chiller for further studies. It was observed that certain packets were organoleptically spoiled in due course of storage. The maximum storage life obtained in the case of 3.0 kGy treated sample was spoiled by 32-33 days. The non spoiled samples were analysed for its physicochemical, microbiological and sensory qualities on the day of preparation and on day 7, 15, 21, 30 since none of the sample had a keeping quality up to 37 days. The keeping quality of control and different treatment groups were shown in Table 1.

Table 1. Keeping quality of minced meat

Treatment groups	Days of spoilage
Control	2-3
1.0 kGy	8-10
2.0 kGy	22-25
3.0 kGy	32-33

4.1 PHYSICOCHEMICAL PROPERTIES

4.1.1 pH

The mean pH values of irradiated and nonirradiated samples at different days of storage are shown in Table 2. The fall of pH in samples during the storage period is shown in Fig. 2. It was observed that there was no significant difference in the pH values on the day of preparation among the control and treatment groups. As time passed a uniform decrease in pH was noticed in the storage period. It was also noticed that there is no significant difference in the pH values due to irradiation by different doses.

Table 2. pH values of minced beef at different days of storage

Days of storage	Dose of gamma irradiation			
	control	1.0 kGy	2.0 kGy	3.0 kGy
0	5.54 ± 0.02	5.54 ± 0.02	5.53 ± 0.03	5.51 ± 0.03
7	S	5.50 ± 0.02	5.49 ± 0.02	5.45 ± 0.03
15	S	S	5.45 ± 0.02	5.43 ± 0.03
21	S	S	5.40 ± 0.03	5.36 ± 0.03
30	S	S	S	5.36 ± 0.02

S: denotes samples spoiled.

4.1.2 Water Holding Capacity

The mean and standard error of WHC of minced beef is shown in Table 3. It was observed nonsignificant reduction of WHC in 1.0 kGy, and 2.0 kGy irradiated meat samples but a significant reduction was noticed in 3.0 kGy compared to control samples (Fig. 3). Similarly on 7^{th} day of storage the picture was similar to that of 0^{th} day of preparation. It was also noticed a nonsignificant and

uniform decrease in WHC on 15th and 21st days (2.0 kGy) and on 15th, 21th, 30th days of storage (3.0 kGy) of treated samples. The original WHC was reduced by 50 per cent in case of 3.0 kGy treated and 30 days stored minced meat. The effect might be due to either storage or due to treatment. The maximum WHC recorded was 15. 01 ± 0.38 in control sample on the day of preparation.

Table 3. Water holding capacity of irradiated and control minced beef (ml/100g of meat)

Days of storage	Dose of gamma irradiation			
	control	1.0 kGy	2.0 kGy	3.0 kGy
0	15.01 ± 0.38[a]	14.71 ± 0.49[a]	13.82 ± 0.47[ab]	13.15 ± 0.53[b]
7	S	12.65 ± 0.72[a]	11.48 ± 0.38[ab]	10.95 ± 0.45[b]
15	S	S	9.45 ± 0.38	8.84 ± 0.28
21	S	S	8.79 ± 0.22	8.15 ± 0.31
30	S	S	S	7.59 ± 0.25

S: denotes samples spoiled.
Identical superscripts in same row do not differ significantly.

4.1.3 Thiobarbituric Acid Reacting Substances

The mean and standard error of TBARS values of control and irradiated minced beef are shown in Table 4. The change in TBARS due to irradiation and/or storage is shown in Fig. 4. The TBARS values were ranged from 0.13 ± 0.03 (control) to 0.41 ± 0.06 (3.0 kGy). The values of control and that of the treated were nonsignificant on the day of preparation. A similar trend was noticed during the different days of storage among treatment groups. A uniform increase was

noticed on various days of storage studied. Even though the treatment effect was not significant, it was also observed a nonsignficant increase in the TBARS values between 2.0 kGy and 3.0 kGy treated samples on subsequent storage.

Table 4. TBARS values of irradiated minced beef at different days of storage (mg malonaldehyde/kg of meat)

Days of storage	Dose of gamma irradiation			
	control	1.0 kGy	2.0 kGy	3.0 kGy
0	0.13 ± 0.03	0.13 ± 0.03	0.13 ± 0.03	0.13 ± 0.03
7	S	0.17 ± 0.07 [a]	0.19 ± 0.07 [ab]	0.21 ± 0.01 [b]
15	S	S	0.24 ± 0.14	0.27 ± 0.18
21	S	S	0.30 ± 0.04 [a]	0.36 ± 0.09 [b]
30	S	S	S	0.41 ± 0.06

S: denotes samples spoiled.
Identical superscripts in same row do not differ significantly.

4.1.4 Tyrosine Value

The mean tyrosine values of the minced beef for different treatments were shown in Table 5. Similar to that of TBARS, the tyrosine values were also nonsignificant compared to control samples and treatment groups (Fig. 5). On 7th day of storage, the samples treated by 1.0 kGy were significantly ($P< 0.05$) higher than that of 3.0 kGy samples. A similar trend was also noticed in the course of storage. The control samples on the day of preparation contained 3.11 ± 0.16 mg of tyrosine per 100 g of sample. It has gradually increased during the storage to 5.95 ± 0.08 by 30th day of storage in irradiated samples.

Table 5. Tyrosine values of irradiated and control minced beef (mg/100g of sample)

Days of storage	Dose of gamma irradiation			
	control	1.0 kGy	2.0 kGy	3.0 kGy
0	3.11 ± 0.16	3.09 ± 0.15	3.08 ± 0.16	3.04 ± 0.16
7	S	4.10 ± 0.18 [a]	3.88 ± 0.12 [ab]	3.49 ± 0.13 [b]
15	S	S	5.04 ± 0.16 [a]	4.16 ± 0.15 [b]
21	S	S	5.51 ± 0.17	5.14 ± 0.11
30	S	S	S	5.95 ± 0.08

S: denotes samples spoiled.
Identical superscripts in same row do not differ significantly.

4.1.5 Colour

The colour measured with the help of Hunterlab Miniscan XE Plus Spectrophotometer are shown as 'L' values (Table 6), 'a' values (Table 7) and 'b' values (Table 8). On the day of preparation the colour 'L' values was 32.17 ± 1.64. It was observed a nonsignificant reduction due to 1.0 kGy irradiation on the day of preparation and on 7 th day of storage, whereas 2.0 and 3.0 kGy irradiation did not reveal significant increase on the day of preparation compared to control sample (Fig. 6). It was also observed a nonsignificant increase due to irradiation and storage in 'L' values of irradiated and stored samples. It reached 34.39 ± 1.72 in the case of 3.0 kGy treated 30 days stored sample, which was significant ($P< 0.05$) compared to zero day control sample. The changes in 'L' values shows that the meat was lighter after 30 days of storage in HDPE packets.

Table 6. Colour 'L' values of irradiated minced beef at different days of storage

Days of storage	Dose of gamma irradiation			
	control	1.0 kGy	2.0 kGy	3.0 kGy
0	32.17 ± 1.64	31.33±1.71	32.43 ±1.50	32.92 ± 1.49
7	S	31.92±1.44	32.22 ±1.44	32.95 ±1.95
15	S	S	32.98 ±1.75	33.56 ±1.68
21	S	S	33.48 ± 1.80	34.06 ± 1.68
30	S	S	S	34.39 ±1.72

S: denotes samples spoiled.

The 'a' value, which denotes redness of the meat has shown a decreasing trend in the case of irradiated as well as stored samples. Initially the control sample had 'a' value of 11.92 ± 0.45 and a nonsignificant reduction due to irradiation on the day of preparation was noticed. The reducing trend of values was shown in (Fig.7). From 11.92 ± 0.45 (control value) it has reached to 9.73 ± 0.28 by 3.0 kGy irradiation and storage up to 30 days.

The 'b' value of Hunterlab Miniscan shows yellowness of meat. Initially the sample had 'b' value of 8.21± 0.25 and irradiated samples had nonsignificant lower values on the day preparation. It was also observed a lowering trend due to storage and irradiation in the course of study. The 3.0 kGy treated and 30 days stored samples recorded a significantly ($P< 0.05$) lower value than that of control and reached to 7.89 ± 0.25. The decreasing trend of the yellowness in different storage period is shown in Fig. 8.

Table 7. Colour 'a' values of irradiated minced beef

Days of storage	Dose of gamma irradiation			
	control	1.0 kGy	2.0 kGy	3.0 kGy
0	11.92 ± 0.45	11.85±0.39	11.69 ±0.39	11.58 ± 0.41
7	S	11.39±0.40	11.15 ±0.40	10.89 ±0.39
15	S	S	10.58 ±0.43	10.37 ±0.37
21	S	S	10.43 ± 0.35	10.06 ± 0.35
30	S	S	S	9.73 ± 0.28

S: denotes samples spoiled.

Table 8. Colour 'b' values of irradiated minced beef

Days of storage	Dose of gamma irradiation			
	control	1.0 kGy	2.0 kGy	3.0 kGy
0	8.21± 0.25	8.19±0.25	8.17 ±0.25	8.14 ± 0.25
7	S	8.15±0.25	8.11 ±0.25	8.08 ±0.24
15	S	S	8.06 ±0.24	8.01 ±0.24
21	S	S	8.04 ± 0.24	7.95± 0.24
30	S	S	S	7.89 ± 0.25

S: denotes samples spoiled.

4.1.6 Cooking Loss

Minced meat samples after irradiation were subjected to cooking and the cooking loss was calculated and expressed as percentage cooking loss. The values were compared with that of control samples and shown in Table 9. On the day of preparation the cooking loss among the different treatment group and control were not significantly different. It ranged from 23.37 ± 0.69 to 24.61 ± 0.43 percentage. As the days of storage increased there was uniform increase in cooking loss and reached 31.41 ± 0.33 per cent in 3.0 kGy irradiated 30 days stored minced meat samples. The cooking loss in samples irradiated with 1.0 and 2.0 kGy on day 7 and on day 21 respectively, were significantly ($P< 0.05$) lower than that of 3.0 kGy treated samples. The increasing trend of cooking loss of irradiated samples on different days of storage was shown in Fig. 9.

Table 9. Cooking loss values of minced beef (percentage)

Days of storage	Dose of gamma irradiation			
	control	1.0 kGy	2.0 kGy	3.0 kGy
0	23.37± 0.69	23.97±0.44	24.11 ± 0.38	24.61 ± 0.43
7	S	25.60±0.37 [a]	27.32 ± 0.49 [b]	27.46 ± 0.38 [b]
15	S	S	28.44 ± 0.35	29.36 ± 0.42
21	S	S	29.19 ± 0.33 [a]	30.42 ± 0.38 [b]
30	S	S	S	31.41 ± 0.33

S: denotes samples spoiled.
Identical superscripts in same row do not differ significantly.

4.2 MICROBIOLOGICAL ANALYSIS

4.2.1 Aerobic Plate Count

The aerobic plate count estimated in control and irradiated samples on different days of storage are shown in Table 10. The control samples recorded a 4.88 ± 0.05 $\log_{10}$ cfu/g of minced beef. Due to irradiation, it was observed a significant ($P< 0.05$) reduction in bacterial count at different doses of irradiation. It was also observed a uniform increase in the count during the storage period. The initial $\log_{10}$ count of 2.17 ± 0.21 in 3.0 kGy irradiated minced beef samples have increased to 5.21 ± 0.03 $\log_{10}$ cfu/g by 30 days of storage. The effect of storage on bacterial growth under chiller condition has shown in Fig.10

Table 10. Aerobic plate count of minced beef at different days of storage ($\log_{10}$ cfu/g)

Days of storage	Dose of gamma irradiation			
	control	1.0 kGy	2.0 kGy	3.0 kGy
0	4.88± 0.05 [a]	3.42±0.08 [b]	2.65± 0.21 [c]	2.17± 0.21 [d]
7	S	3.91±0.15 [a]	3.39± 0.17 [b]	3.04 ± 0.17 [c]
15	S	S	4.24± 0.22	3.76± 0.11
21	S	S	4.93± 0.05	4.63± 0.09
30	S	S	S	5.21 ± 0.03

S: denotes samples spoiled.
Identical superscripts in same row do not differ significantly.

4.2.2 Coliforms and *E.coli* Count

The control sample analysed on the day of preparation has obtained a coliform count of 2.78 ± 0.07 log_{10} cfu/g of the sample and 1.047 ± 0.04 log_{10} cfu/g in the case of sample irradiated at 1.0 kGy. This count was raised to 1.73 ± 0.12 log_{10} cfu/g by 7 days of storage. All other irradiation treatments have totally destroyed coliform organisms. During the storage period 2.0 kGy and 3.0 kGy treated samples were free of coliform organisms.

None of the control samples or treated samples recorded *E.coli* organisms per gram of sample.

4.2.3 Fecal Streptococcal Count

The control sample on the day of preparation recorded the fecal streptococci count of 2.85 log_{10}cfu/g of sample. Treatment of samples at 1.0 kGy, the count has significantly ($P< 0.05$) reduced to 1.37 log 10 cfu/g and the storage of samples by 7 day recorded an increase of organisms to 2.14 log_{10} cfu/g. The treatment of minced meat at 2.0 kGy and 3.0 kGy totally destroyed the faecal streptococci and none of the sample had faecal streptococci during the storage period in case of 2.0 and 3.0 kGy treated minced beef.

4.2.4 Staphylococcal count

The mean staphylococci count of the control and irradiated sample were shown in Table 11. The initial count of 3.04 ± 0.06 log_{10} cfu/g of staphylococci has been significantly ($P< 0.05$) reduced due to irradiation by 1.0, 2.0 and 3.0 kGy. On storage it was found that the organisms have multiplied and the number has been increased. Similar to that of Coliform count a uniform increase in log count was noticed during the period of storage. The pattern of increase count has been shown in Fig.11.

4.2.5 Salmonella

The minced beef samples either the control or the irradiated were free from salmonella on the day of preparation and its subsequent storage period.

Table 11. Staphylococcal count of irradiated minced beef ($\log_{10}$ cfu/ g)

Days of storage	Dose of gamma irradiation			
	control	1.0 kGy	2.0 kGy	3.0 kGy
0	3.04± 0.06 [a]	2.02±0.08 [b]	1.90± 0.07 [c]	1.64± 0.11 [d]
7	S	2.26±0.06 [a]	2.14± 0.06 [ab]	1.97 ± 0.08 [b]
15	S	S	2.58± 0.06	2.33± 0.05
21	S	S	3.00 ± 0.28	2.91± 0.23
30	S	S	S	3.55 ± 0.28

S: denotes samples spoiled.
Identical superscripts in same row do not differ significantly.

4.3 SENSORY EVALUATION

4.3.1 Colour

The sensory evaluation studied with help of nine point Hedonic scale with respect to colour is shown in Table 12. Initially irradiated samples recorded slightly higher nonsignificant score compared to control. During the storage period the colour of 2.0 and 3.0 kGy treated sample had uniformly reduced and reached 6.36 ± 0.30 by 30 days of storage for 3.0 kGy treated samples.

4.3.2 Flavour

The most important sensory attributes of any product evaluated are flavour. Similar to that of colour score the irradiated sample showed a slightly nonsignificant higher score on the day of preparation than compared to control samples (Table 13). The flavour attributes of the cooked product were uniformly

Table 12. Colour score of irradiated minced beef

Days of storage	Dose of gamma irradiation			
	control	1.0 kGy	2.0 kGy	3.0 kGy
0	8.57± 0.07	8.70±0.07	8.66 ± 0.05	8.66± 0.07
7	S	8.19±0.20	8.32 ± 0.19	8.38 ± 0.16
15	S	S	7.66 ± 0.13	7.40 ± 0.21
21	S	S	6.74 ± 0.22	6.68 ± 0.28
30	S	S	S	6.36 ± 0.30

S: denotes samples spoiled.

Table 13. Flavour score of irradiated minced beef

Days of storage	Dose of gamma irradiation			
	control	1.0 kGy	2.0 kGy	3.0 kGy
0	8.63± 0.06	8.66±0.07	8.69 ± 0.05	8.69± 0.05
7	S	8.36±0.07	8.29 ± 0.16	8.48 ± 0.08
15	S	S	7.75± 0.18	7.59 ± 0.17
21	S	S	7.02 ± 0.20	6.97 ± 0.18
30	S	S	S	6.52 ± 0.18

S: denotes samples spoiled.

reduced during storage period and reached 6.52 ± 0.18 in 3.0 kGy treated and 30 days stored minced beef. The values among treatment group were nonsignificant during the storage period.

4.3.3 Juiciness

The juiciness score of the control and irradiated minced beef is shown in Table 14. It was observed a significant ($P< 0.05$) increase of juiciness due to 2.0 and 3.0 kGy compared to control (8.44 ± 0.06). A similar trend was noticed on 7th day of storage compared to 1.0 kGy, whereas the control samples were already spoiled. From day 0, both 2.0 kGy and 3.0 kGy treated samples has shown a uniform decrease in juiciness and finally reached 6.17 ± 0.26 by 30 days of storage in case of 3.0 kGy treated samples.

Table14. Juiciness score of control and irradiated minced beef

Days of storage	Dose of gamma irradiation			
	control	1.0 kGy	2.0 kGy	3.0 kGy
0	8.44± 0.06^{a}	8.56±0.07^{a}	8.71 ± 0.04^{b}	8.71± 0.05^{b}
7	S	7.97±0.07^{a}	8.32± 0.06^{b}	8.37 ± 0.07^{b}
15	S	S	7.67± 0.15	7.43± 0.16
21	S	S	6.74 ± 0.30	6.69± 0.23
30	S	S	S	6.17 ± 0.26

S: denotes samples spoiled
Identical superscripts in same row do not differ significantly.

4.3.4 Tenderness

The picture of tenderness of minced beef was similar to that of juiciness (Table 15). The 2.0 kGy and 3.0 kGy treated sample had a significant ($P< 0.05$) increase in tenderness compared to control and 1.0 kGy treated samples. A similar trend was noticed in day 7 even though the tenderness of sample reduced. During storage the tenderness of minced beef reduced uniformly and finally reached 6.28 ± 0.28 on 30^{th} day.

Table 15. Tenderness score of irradiated minced beef

Days of storage	Dose of gamma irradiation			
	control	1.0 kGy	2.0 kGy	3.0 kGy
0	8.44± 0.06 [a]	8.56±0.07 [a]	8.71 ± 0.05 [b]	8.74± 0.04 [b]
7	S	7.97±0.07 [a]	8.28± 0.06 [b]	8.30 ± 0.06 [b]
15	S	S	7.67± 0.15	7.43± 0.16
21	S	S	6.81 ± 0.28	6.69± 0.23
30	S	S	S	6.28 ± 0.28

S: denotes samples spoiled
Identical superscripts in same row do not differ significantly.

4.3.5 Overall acceptability

The mean and standard error of overall acceptability score of control and irradiated sample on different days of storage is shown in Table 16. The values were nonsignificant on the day of preparation between treatment and control. The maximum score of 8.77 ± 0.04 was recorded by 3.0 kGy treated samples on the day of preparation. Uniform decrease was noticed in the overall acceptability and has

shown in Fig. 12. The score was reduced to 6.14 ± 0.28 by 30 days storage in case of 3.0 kGy treated minced beef.

Table 16. Overall acceptability score of control and irradiated minced beef

Days of storage	Dose of gamma irradiation			
	control	1.0 kGy	2.0 kGy	3.0 kGy
0	8.60 ± 0.06	8.66 ± 0.07	8.76 ± 0.05	8.77 ± 0.04
7	S	8.05 ± 0.07	8.25 ± 0.06	8.36 ± 0.06
15	S	S	7.61 ± 0.15	7.58 ± 0.16
21	S	S	6.66 ± 0.28	6.53 ± 0.23
30	S	S	S	6.14 ± 0.28

S: denotes samples spoiled.

Minced beef prepared and packed in 200 g quantity in HDPE packets and subjected to irradiation studies revealed that 3.0 kGy irradiated samples could be stored upto 30 days without any signs of spoilage under chiller conditions. It was observed that pH was unaltered due to irradiation. The WHC, TBARS, TV and colour values showed variation either due to irradiation or by storage. The organoleptic qualities are unaltered due to different dose of radiation, where as storage has reduced these qualities. It was also observed that the juiciness and tenderness were increased due to irradiation initially. The irradiation has reduced the microbiological load of minced beef significantly, whereas storage under chiller condition had a significant effect. The 3.0 kGy treated samples were totally free from coliforms, *E. coli,* faecal streptococci and salmonella and with a significant reduction in aerobic plate count and staphylococci.

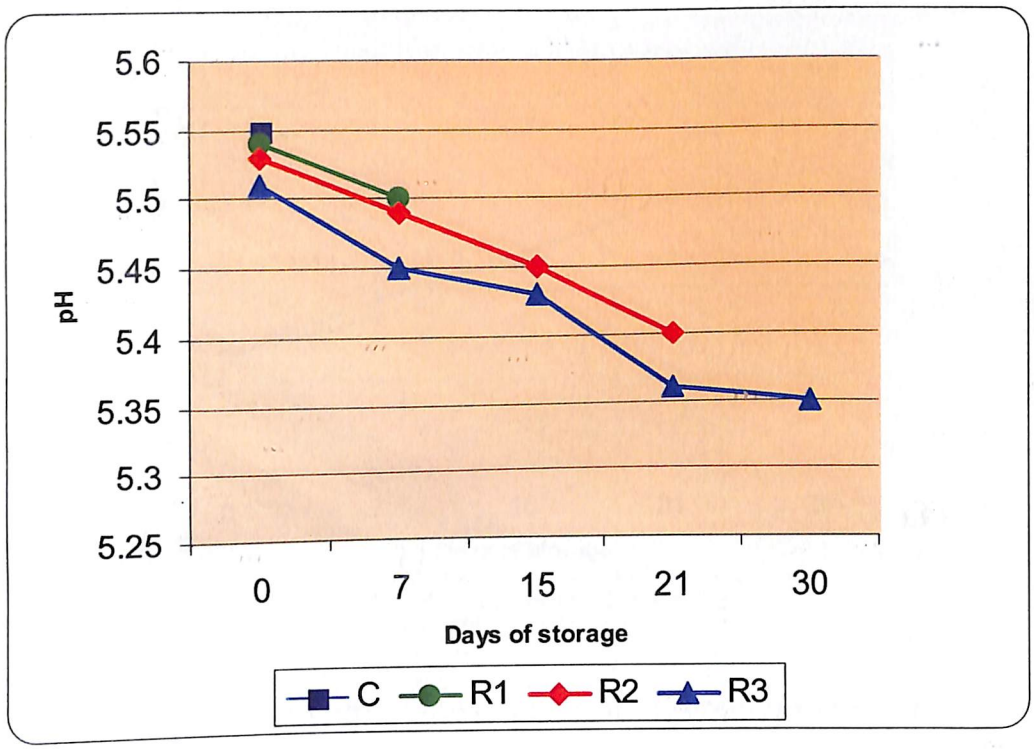

Fig. 2. pH values in irradiated minced beef

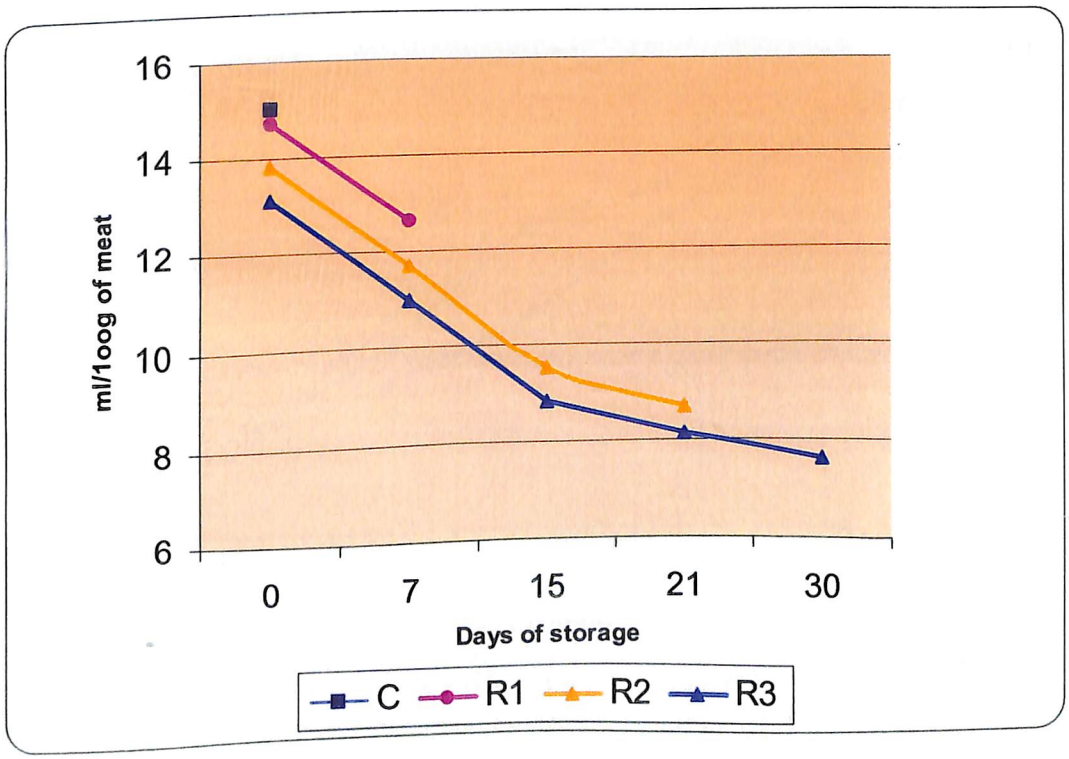

Fig. 3. The WHC of minced beef at different doses of irradiation

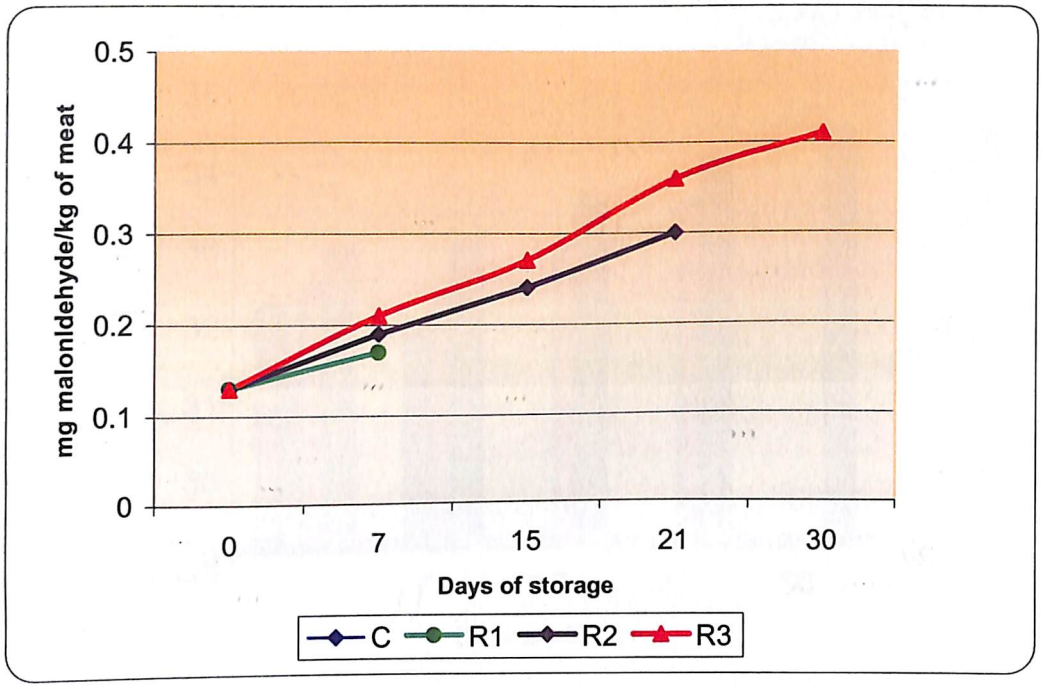

Fig. 4. TBARS values of minced beef at different doses of irradiation

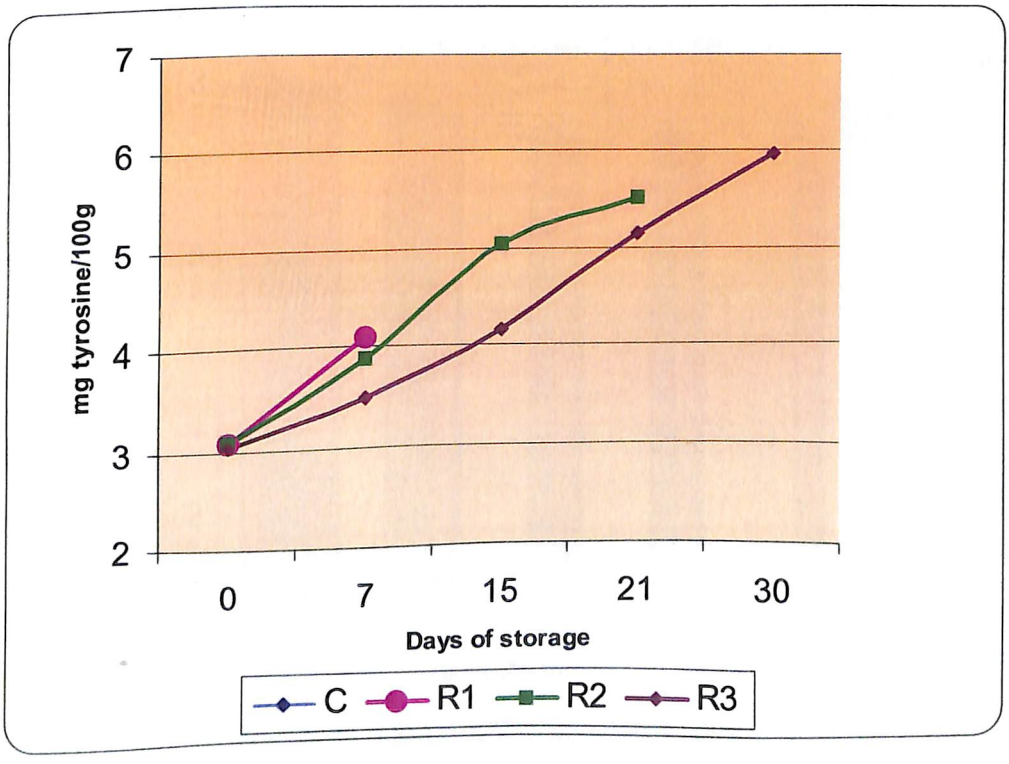

Fig. 5. Tyrosine values of irradiated minced beef

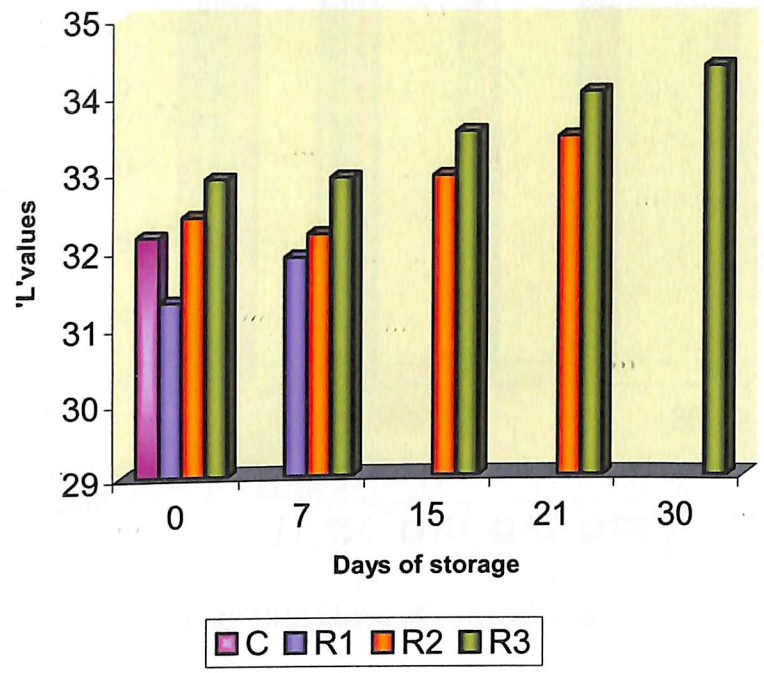

Fig.6. Colour 'L' values of irradiated and stored minced beef

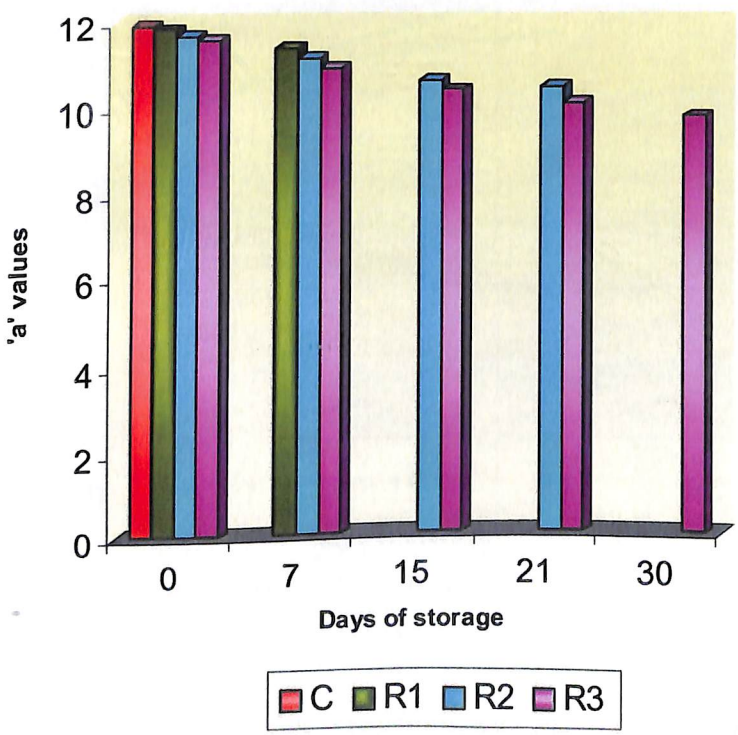

Fig. 7. Colour 'a' values of control and irradiated minced beef

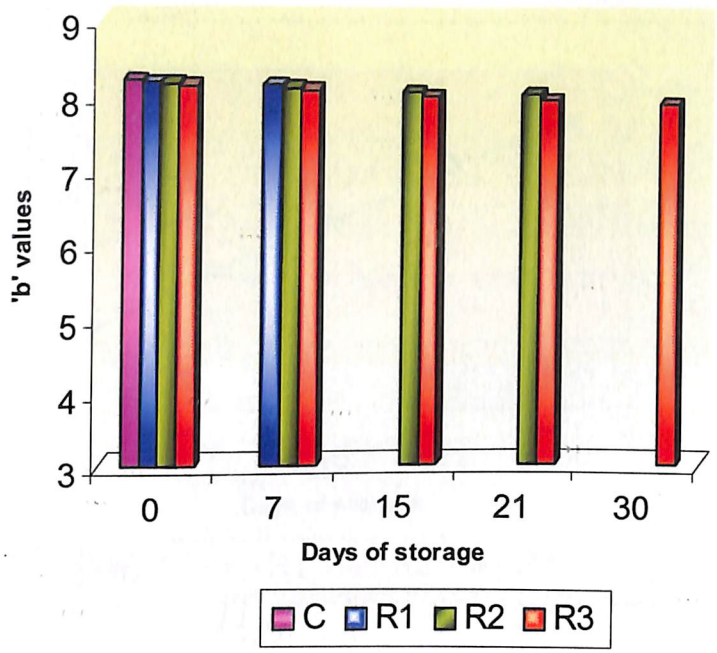

Fig. 8. Colour 'b' values of control and irradiated minced beef

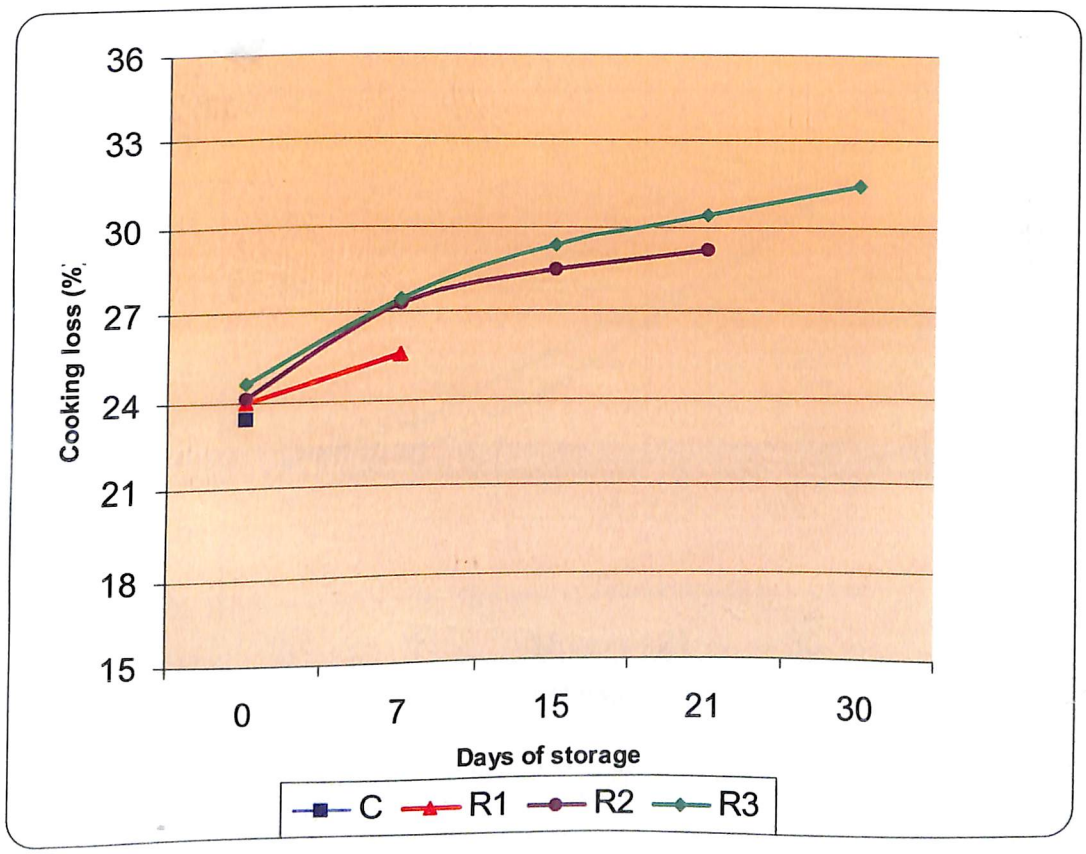

Fig. 9. Cooking loss of irradiated and control minced beef

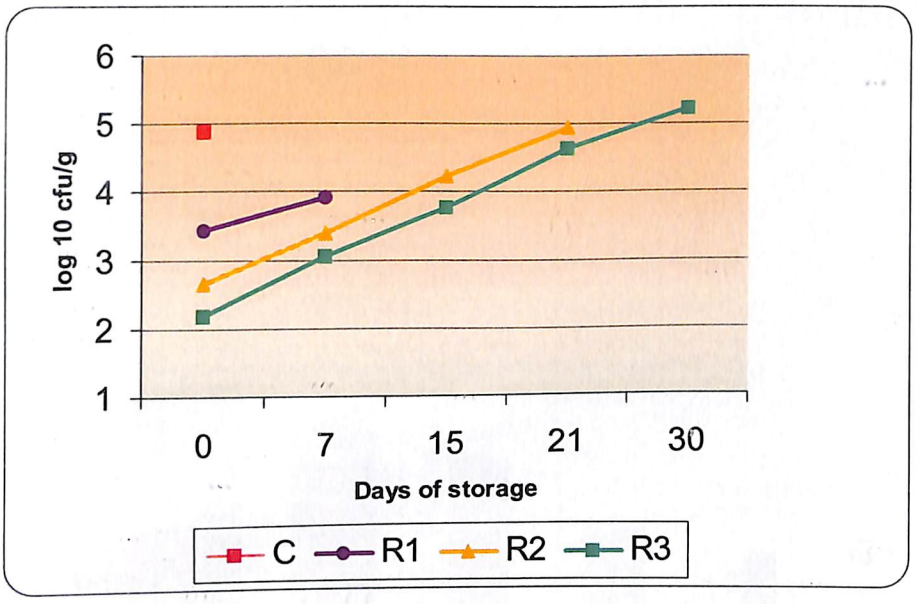

Fig. 10. Aerobic plate count of irradiated minced beef

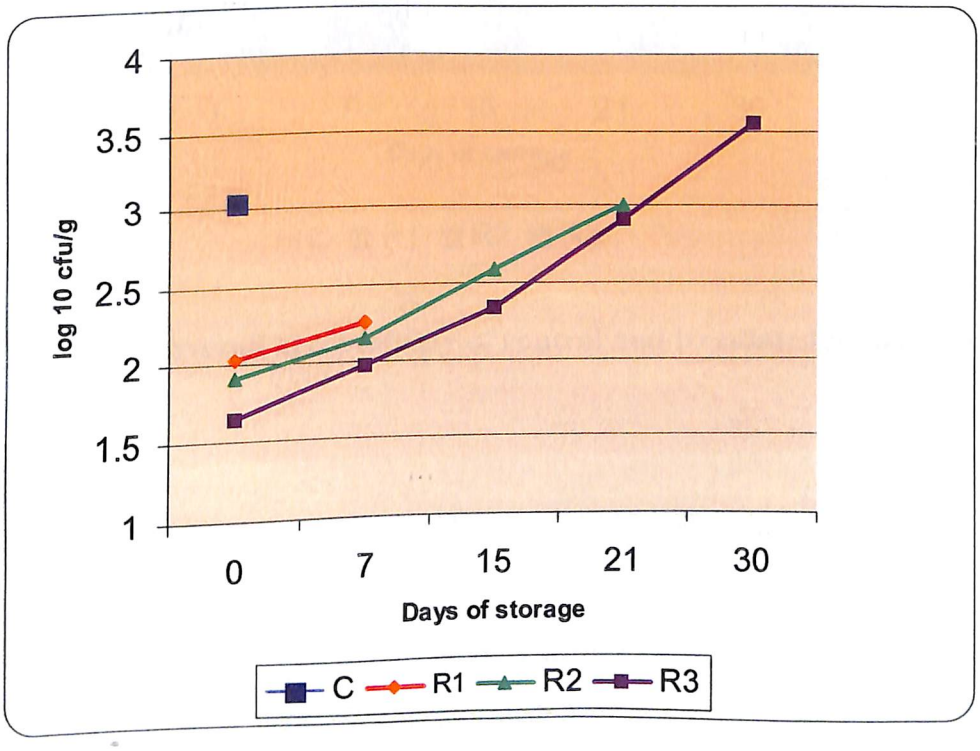

Fig. 11. Staphylococcal count of irradiated minced beef

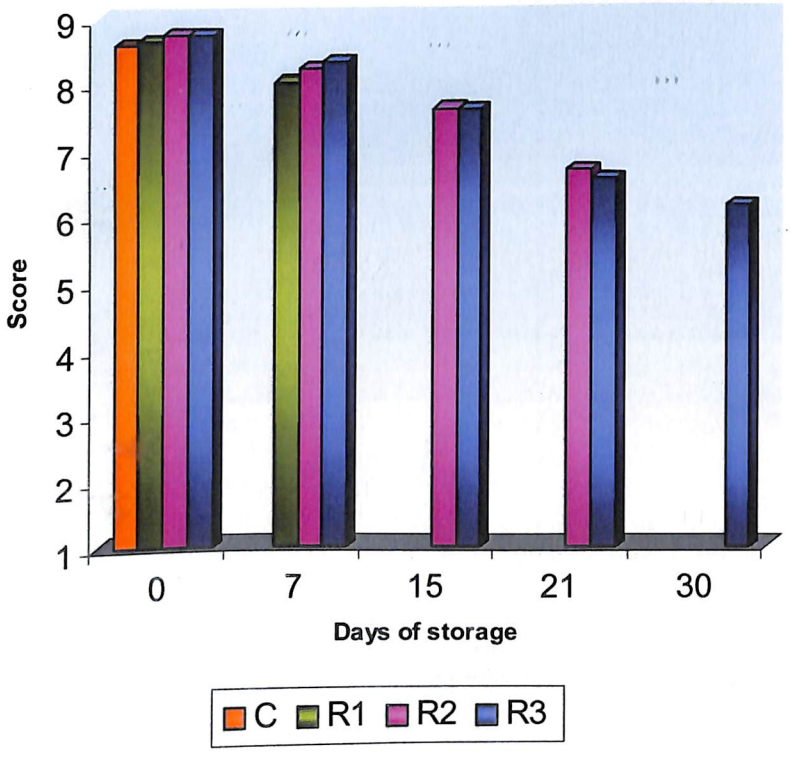

Fig. 12. The overall acceptability of control and irradiated minced beef

5. DISCUSSION

Beef samples collected from the Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy, were used to prepare minced beef and packed in 200 g each in HDPE packets. Half the number of packets were subjected to irradiation either at 1.0, 2.0 or 3.0 kGy at melting ice temperature and the samples were kept in chiller (+4°C). The maximum number of storage life was obtained in 3.0 kGy irradiated samples and extended by 32-33 days. Niemand *et al.* (1981) reported a doubling of shelf life in beef cuts by irradiation at 2.0 kGy. In the present study the control samples were spoiled by 2-3 days and 2.0 kGy treated samples got an extended shelf life by 22-25 days. Paul *et al.* (1990) reported an extended shelf life of 4 weeks in the case of 2.5 kGy treated samples. Rosdriguez *et al.* (1993) reported an extended shelf life of retail fresh beef products by 2.0 kGy. In the present study 2.0 kGy treated samples obtained the shelf life of 3- 4 weeks and 3.0 kGy obtained nearly 5 weeks while the nonirradiated minced meat samples were spoiled within one week. Roberts and Weese (1998) have reported a shelf life of 21 days in 3.0 kGy treated samples and 14 days in 1.0 kGy treated samples, which were in agreement with the present study. The initial microbial load of the minced beef prepared was comparatively lower hence 2.0 kGy and 3.0 kGy treated samples obtained a better storage life when compared to many of the earlier reports.

5.1. PHYSICOCHEMICAL PROPERTIES

5.1.1. pH

The effect of irradiation and storage period on pH values has shown a nonsignificant effect due to irradiation either at 1.0, 2.0 or 3.0 kGy. As time passes from 0 to 30th day, like any meat the minced beef attained its ultimate pH and from there it slightly decreased. Lefebvre *et al.* (1994) reported a fall in pH due to irradiation at 1.0, 2.5 and 5.0 kGy. Lee *et al.* (1996) reported a nonsignificant pH

values up to 7 days in 2.0 kGy irradiated samples and the present data of 2.0 kGy also were nonsignificant with control samples up to 21 days of storage. Nam and Ahn (2002) reported a significant decrease in pH due to irradiation in turkey breast. In the present study, meat samples were collected and it was minced on the next day, by the time it attained its ultimate pH and due to radiation either at 1.0, 2.0, or 3.0 kGy has changed the pH than that of the control samples. It was also observed a nonsignificant difference between treatment and between storage periods, which was in agreement with many other reports.

5.1.2. Water Holding Capacity

The WHC of the nonirradiated sample was 15.01 ± 0.38 ml/100g of meat. Due to irradiation at 1.0 and 2.0 kGy, the WHC was nonsignificantly reduced whereas 3.0 kGy treatment had a significant reduction. As storage period enhanced the WHC significantly reduced from the day of preparation. Schweigert (1959) reported that radiation caused some protein denaturation, which resulted in lowering the WHC in meat. Zhu *et al.* (2004a) in pork and Kuttinarayanan *et al.* (2006a) in minced beef reported a similar reduction in WHC, which is in agreement with the present observation. The storage also played a role in reducing WHC from 13.15 ± 0.53 to 7.59 ± 0.25 ml in 3.0 kGy treated and 30 day stored samples. Huff- Lonergan and Lonergen (2005) reported pH decline, proteolysis and protein oxidation are the important factors influencing the ability of meat to retain its water. In the present study, the meat is subjected to comminution and radiation, which had contributed to changes in the intracellular architecture of muscle cells, thereby the ability of muscle to retain its water, was lost.

5.1.3. Thiobarbituric Acid Reacting Substances

The TBARS were measured in terms of milligram malonaldehyde per kg of meat. It was observed that due to irradiation on the day of preparation, there was no significant difference in the TBARS values. As the days of storage enhanced TBARS values are increased and showed a significant treatment effect. Both storage as well as irradiation has influenced these values in 3.0 kGy treated 30 days of stored samples. The values have gone 3 times higher than that of original value. Murano *et al.* (1998) reported a higher value and high lipid oxidation compared to nonirradiated samples. Ahn *et al.* (2000b) reported that vacuum packaging had taken care of increase in TBARS values. The findings of Kim *et al.* (2002a) were in agreement with present observations. The present study has not utilized any antioxidants and Quattara *et al.* (2002) reported that ascorbic acid is having a stabilizing effect on TBARS due to irradiation and storage. Ahn and Nam (2004) reported a similar effect due to irradiation. Generally in aerobic packing addition of antioxidants had a beneficial effect in reducing the TBARS values in irradiated samples. The present study has not used any such protective materials might be the reason for the higher content of TBARS in irradiated and stored minced beef.

5.1.4. Tyrosine Values

The tyrosine value indicates the protein breakdown of meat and meat products, which is subjected to storage or any type of treatment. The present study revealed a similar trend in tyrosine values like that of TBARS. The treatment had no significant effect on tyrosine value compared to control samples. As storage days increased, it was observed significant changes among different treatments. The initial content of tyrosine values was 3.04 ± 0.16 mg per 100 g of sample in 3.0 kGy treated minced beef and has significantly increased to 5.95 ± 0.08 mg by storage. Kuttinarayanan

(1988) reported a higher content in stored mutton. Karthikeyan *et al.* (2000) reported that proteolysis was the factor for increased tyrosine values in ambient temperature stored minced chevon. Jayanthi (2003) reported that aerobic, vacuum or modified atmospheric packaging had little effect on tyrosine values. In the present study the meat was subjected to comminution and radiation, might have increased the activities of natural enzymes of meat leading to protein degradation and hence increased tyrosine values in stored samples even when the microbial load of the product was well within the range.

5.1.5. Colour

The various attributes of colour like 'L' value, 'a' value and 'b' value were measured with the help of Hunterlab Miniscan XE plus Spectrophotometer showed a nonsignificant value in 1.0, 2.0, and 3.0 kGy treated samples compared to control. The treatment values were nonsignificantly higher (in case of 'L') compared to control samples and nonsignificantly lower compared to control in case of 'a' and 'b' values. Miller *et al.* (1995a) reported significant values in beef samples. Murano *et al.* (1998) stated a nonsignificant effect due to irradiation in ground beef samples with respect to 'L' 'a' 'b' values whereas, packaging had significant effect in darkness or redness of meat. Kuttinarayanan *et al.* (2005b) reported a nonsignificant variation and the samples values were maintained up to 25 days of storage in case of 2.5 kGy irradiated turkey breast at chiller storage.

5.1.6. Cooking Loss

The cooking loss of control and irradiated meat samples were expressed in percentage basis. It was observed a nonsignificant increase in cooking loss and similar result was observed by Niemand *et al.* (1981). Among the treatments there were no difference on day of preparation whereas on 7^{th} day of observation 2.0 and 3.0 kGy

treated samples had higher values than that of 1.0 kGy. A similar trend on 21st day among 2.0 and 3.0 kGy treated samples was also noticed. As storage period increased, the 3.0 kGy treated sample had higher per cent of loss (31.41 ± 0.3). Yoon (2003) also reported a higher cooking loss in irradiated chicken throughout the storage period. Tornberg (2005) reported myofibrillar proteins on heating created a dense protein network, which held water, firmly by capillary force. In the present study, the comminution and blending might be the probable reason for reduction in cooking loss during storage.

5.2. MICROBIOLOGICAL ANALYSIS

5.2.1. Aerobic Plate Count

Initially the minced beef samples had an aerobic plate count of 4.88 ± 0.05 $\log_{10}$ cfu/ g of meat. This was significantly reduced by treating the samples at 1.0, 2.0, and 3.0 kGy. The 3.0 kGy treated samples had reduced more than 50 per cent of initial count. Naik *et al.* (1994) reported 2-3 $\log_{10}$ cycle reduction by irradiating buffalo samples at 2.5 kGy. The values at 1.0 kGy irradiation are in agreement with Giroux *et al.* (2001) who reported 1.78 log reduction due to irradiation in beef patties. In the present study 1.46 log reduction was noticed at the same dose. The 2.0 kGy irradiation has completely destroyed the bacterial growth but in the present study, the reduction was only 54 per cent. Contradictory to previous statement, Aziz *et al.* (2002) reported 2-3 log reduction in the case of 5.0 kGy irradiation which shows that the present study obtained a better reduction even by 3.0 kGy treatment. The present values are lower than that of Kuttinarayanan *et al.* (2005) who reported 95 per cent reduction by 2.5 kGy treatment.

As storage enhanced, a proportionate increase in colony count was noticed and by 30 days of storage the bacterial population of 3.0 kGy treatment samples has

overgrown than that of 0 day control samples showing the definite role of chiller storage in bacterial population.

5.2.2. Coliforms and *E.coli*

Coliforms were detected only in the control and 1.0 kGy treated minced beef. The reduction was about 63 per cent compared to initial count and this remaining organisms has multiplied and reached to 1.73 log $_{10}$ cfu/g by 7 day of storage in same treated sample. Several authors have reported reduced coliform count due to irradiation (Rodriquez, 1985; Lefebvre, 1992; Naik, 1994; Badr 2004). The treatment of minced beef by 2.0 kGy and 3 .0 kGy has totally destroyed the coliforms as well as *E.coli* organisms and none of the samples revealed any coliforms and *E.coli* count throughout the study period. Similarly results were also reported by Spoto *et al.* (2000), Satin *et al.* (2002) and Kuttinarayanan *et al.* (2005). Since the initial count of control samples was comparatively lower, a treatment of 2.0 or 3.0 kGy was sufficient to reduce coliforms and *E.coli* count to non-detectable level. This clearly shows that the most pathogenic *E.coli* O157: H7 can be destroyed to certain extend in minced beef by the process of irradiation as reported by Olson (1998).

5.2.3. Faecal Streptococci Count

The control samples recorded a log count of 2.85 cfu/g on the day of preparation. About 50 per cent reduction was noticed by treating samples at 1.0 kGy and none of the other treated groups revealed any faecal streptococci. This clearly shows that the treatment of minced beef by 2.0 kGy totally destroyed faecal streptococci provided the initial counts are within the range. Badr (2004) reported 75 per cent reduction by 1.5 kGy and 93.6 per cent reduction by 3.0 kGy in rabbit meat samples. The present values are better than earlier report. Kuttinarayanan *et al.* (2005a)

also recorded 100 per cent reduction in faecal streptococci in minced beef by 2.0 kGy treatment, which is in agreement with the present findings.

5.2.4. Staphylococcal Count

The minced meat samples had recorded an initial count of 3.04 ± 0.06 log $_{10}$ cfu/g of meat. The treatment at 1.0, 2.0 and 3.0 kGy significantly reduced the count and reached 1.64 ± 0.11 log $_{10}$ cfu/g by 3.0 kGy treatment accounting to 47 per cent reduction. As storage period enhanced under chiller condition it reached 3.55 ± 0.28 log $_{10}$ cfu/g by 30 days. Lefebvre *et al.* (1992) reported that 1.0 kGy treatment had totally destroyed Staphylococcal spp. in ground beef. Thayer *et al.* (1997) reported that a dose of 1.5 to 3.0 kGy was required for total destruction of Staphylococci. The present study even at 3.0 kGy treatment has not totally destroyed the organisms. Alur *et al.* (1993) reported 2-4 log reduction by 2.5 kGy treatment. Badr (2004) reported 93 per cent reduction in 3.0 kGy treatment in rabbit meat samples. In the present study the reduction was only 47 per cent. Even with hurdle technology combined with irradiation at 1.0 kGy, only 2 log reduction was noticed and by 2.0 and 3.0 kGy complete destruction was observed by Kanatt *et al.*, 2005. The present sample was minced meat that might be the reason for total nondestruction of organisms by these doses.

5.2.5. Salmonellae

Like that of *E.coli*, none of the samples (control and irradiated) has revealed Salmonella. Grant and Patterson (1992) reported 3-8 log cycle reduction by 2.5 kGy and Badr (2004) reported 3.0 kGy for total destruction of salmonella in rabbit meat. As in the case of Staphylococci if the initial count was considerably high, the low dose irradiation might not be sufficient for total destruction of Salmonella in minced beef.

5.3. Sensory evaluation

The sensory evaluation of the cooked product was analysed and evaluated with the help of nine point Hedonic scale. Like that of 'L' 'a' 'b' values, there was no panelist difference on the day of preparation at different doses of irradiation. Paul *et al.* (1990) reported an acceptable colour for minced meat in case of irradiated mutton. As storage enhanced the colour score of irradiated meat (3.0 kGy) has reduced and reached from 8.66 ± 0.07 to 6.36 ± 0.03 by 30 days of storage showing a significant effect due to storage. The minced meat samples were packaged in HDPE packets which is not an oxygen barrier film might be reason for reduced colour score for stored samples. Kanatt *et al.* (2005) reported an acceptable colour for irradiated meat products immediately after irradiation.

A similar trend was noticed in case of flavour score with a slightly higher score for irradiated samples on the day of preparation. As storage period enhanced, there was a reduction in the flavour score of irradiated meat samples, by the time control samples were already spoiled. Lefebvre *et al.* (1992) reported a slightly disliked flavour for irradiated ground beef at 1.0, 2.0 and 5.0 kGy and also reported that lower dose of irradiation,better the taste appreciated. Luchsinger *et al.* (1996) did not observe any significant difference due to irradiation of pork chops at 2.5 kGy or below. Even in the present study, there was no significant difference noticed due to irradiation in minced beef.

The juiciness of cooked minced beef was altered due to irradiation especially at 2.0 and 3.0 kGy and observed significant higher values than that of the control. Even 1.0 kGy treatment has an increased score. As storage period enhances, there existed a treatment effect even though the score was lower. By 30 days of storage the score has significantly reduced from 8.71 ± to 6.17. Luchsinger *et al.* (1996) did not observe any significant difference in juiciness by 2.5 kGy irradiation. Ohene-Adjei (2004) reported

a nonsignificant difference on juiciness of ground pork whereas, in the present study the juiciness was enhanced significantly due to irradiation. It was also observed on increasing the dose the juiciness increases since a similar effect was noticed in tenderness.

Irradiation at different doses (2.0 and 3.0 kGy) significantly increased the tenderness compared to control samples. The 1.0 kGy treated samples also recorded a higher score than that of control. During storage period, the tenderness slightly decreased, however treatment effect was noticed throughout the study period. In case of irradiation, collagen shrinks in its dry state and become soluble in water if irradiated wet and indeed irradiation causes softness and tenderness of texture as an immediate effect (Coleby et al., 1961). Murano *et al.* (1998) reported an increased texture in irradiated ground beef patties whereas, Ohene-Adjei (2004) reported a decrease in tenderness in loin chops. Minced meat which was already subjected to mincing process, while on irradiation leads to changes in the collagen and thereby a reduction in background toughness of meat might be the cause of increased tenderness in irradiated samples compared to control.

Overall acceptability of the product was unaffected by the process of irradiation on the day of preparation. Even though numerical difference was noticed due to irradiation, storage has brought lowering the score from 8.77 ± 0.04 (3.0 kGy, 0 day) to 6.14 ± 0.28 by 30 days storage. Lefebvre *et al.* (1994) observed a better taste for irradiated ground beef at low dose irradiation. Naik *et al.* (1994) reported better acceptability score of 6.5 even at the end of 5 weeks storage in irradiated buffalo meat and the findings are in agreement with the present study. Luchsinger *et al.* (1996) did not observe any significant difference in the acceptance of irradiated pork chops at 2.5 kGy or below. Ahn *et al.* (2000b) reported radiation has no negative effect on acceptance of meat and in the present study overall acceptability score was higher than

that of control samples and even by 30 days it maintained 6.4 showing that the taste is appreciated.

From the above results, it can be inferred that the irradiated minced beef can be kept at chiller temperature (+4^0 C) without any signs of spoilage up to 8-10 days in 1.0 kGy irradiation, 22-25 days by 2.0 kGy irradiation and 32-33 days by 3.0 kGy irradiation. Minced beef kept in the chiller can be readily processed further and no thawing is required whereas if the product is kept under deep freezer it requires high energy for freezing and hours together is required for thawing the product. That means energy and time can be saved without affecting the quality of the product. Many of the dreadful organisms like coliform, *E.coli,* salmonellae can be completely destroyed and organism like staphylococcus and other aerobic organism can be significantly reduced without affecting organoleptic and nutritional qualities of the product.

Summary

6. SUMMARY

Minced beef was prepared from the samples collected from cattle slaughtered at the Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy. The samples were packed in 200 g each in HDPE packets and randomly divided into four groups and considering one group as control, the other packets were subjected to irradiation at melting ice temperature at either doses of 1.0, 2.0 and 3.0 kGy. The effect of irradiation on the keeping quality of minced beef was assessed with respect to physicochemical, microbial properties and sensory qualities on different days of storage.

The irradiation process has preserved the minced beef by 8-10 days, 22-25 days and 32-33 days at 1.0, 2.0 and 3.0 kGy respectively. The control samples were spoiled by two to three days. The pH of the irradiated samples did not vary significantly on the day of preparation and on storage there was a uniform decrease in pH values. The WHC of the minced beef was altered by higher dose of irradiation as 3.0 kGy treated samples had significantly reduced WHC compared to control, 1.0 and 2.0 kGy samples on day 0. As storage period enhanced the WHC, has significantly lowered and reached 50 per cent of the original value in 3.0 kGy irradiated and 30 days stored samples.

The oxidative rancidity changes of the sample were measured by TBARS values. The values on the day of preparation were nonsignificant due to irradiation. As the days of storage enhanced, the TBARS values were increased and showed a significant treatment effect. As radiation treatment doses increased the TBARS values were correspondingly increased in different storage periods. The value has gone three times than that of original value in 3.0 kGy irradiated 30 days stored samples.

The tyrosine value will indicate the protein breakdown of the meat and meat products. The treatment had no significant effect on tyrosine values compared to

control samples. As storage increased the tyrosine values increased with significant change among the treatments.

The colour 'L' values had a nonsignificant reduction due to 1.0 kGy irradiation on the day of preparation and on 7th day of storage whereas, 2.0 and 3.0 kGy irradiation revealed a nonsignificant increase compared to control samples. The lightness of the minced beef increased due to irradiation and storage. The redness ('a' values) and yellowness ('b' values) showed a nonsignificant decreasing trend in case of irradiated as well as stored sample.

On the day of preparation the cooking loss among the different groups was nonsignificant and on 7th day, the 1.0 and 2.0 kGy irradiated samples were significantly different from 3.0 kGy irradiated samples. On storage the cooking loss increased with significant difference between the treatments (2.0 and 3.0 kGy) and comminution and blending might be probable reasons for its increase. The control samples on the day of preparation recorded 23.37 ± 0.69 per cent cooking loss and by 30 day it reached 31.41 ± 0.33 in case of 3.0 kGy irradiated samples.

Irradiation had significantly reduced the aerobic plate count of minced beef at different doses. There was 1.46, 2.23 and 2.71 log $_{10}$ cfu/g reduction was noticed at 1.0, 2.0 and 3.0 kGy irradiated samples. On storage the count increased and reached 5.21 ± 0.03 log $_{10}$ cfu/g in 3.0 kGy and 30 days stored samples. The coliform count was recorded only in the control and 1.0 kGy treated samples and reduction was about 63 percent in 1.0 kGy samples compared to that of control. The remaining organisms reached to 1.73 log$_{10}$ cfu/ g by 7 days of storage. The treatments with 2.0 and 3.0 kGy have totally destroyed coliform organisms and these irradiated samples were free from coliforms thorough out the storage period. *E.coli* and salmonella organisms were not detected in any of the control as well as irradiated samples. Similar to Coliform count only the control and 1.0 kGy irradiated samples recorded the faecal streptococci and about 50 per cent reduction was noticed due to 1.0 kGy irradiation. None of other treated groups revealed any faecal streptococcal organisms on the day of preparation and on subsequent storage.

The staphylococcal count of minced beef had been significantly reduced due to irradiation by 1.0, 2.0 and 3.0 kGy. The maximum reduction of 47 per cent was noticed in 3.0 kGy treated sample. On storage it was found that staphylococci have multiplied and reached 3.55 ± 0.28 $\log_{10}$ cfu/g in 3.0 kGy treated and 30 days stored samples from initial 3.04 ± 0.06 $\log_{10}$ cfu/g.

The sensory evaluation was carried out with the help of nine point Hedonic scale for cooked mined beef for colour, flavour, juiciness, tenderness and overall acceptability. The colour and flavour did not change significantly due to irradiation. The scores were reduced on storage from 8.57 to 6.36 and from 8.63 to 6.52 for colour and flavour respectively. The juiciness and tenderness was significantly increased due to irradiation at 2.0 and 3.0 kGy compared to control and 1.0 kGy treated samples. During storage the juiciness and tenderness values reduced uniformly and reached a score around six. The overall acceptability of the product remained unaffected by the process of irradiation with decrease in scores due to storage. The panelist did not detect any unobjectable odour or taste due to on irradiation of minced beef.

The minced beef can be preserved by chilling employing gamma irradiation without affecting the quality of the products thereby energy and time can be saved. In addition to preservation this technique also plays an important role in safeguarding the public by destroying many pathogenic organisms like coliforms, *E. coli*, streptococcus, salmonellae along with reducing the number of staphylococcus and other aerobic organisms in meat and meat products.

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LOW DOSE GAMMA IRRADIATION ON THE KEEPING QUALITY OF MINCED BEEF

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ABSTRACT

Prevention of Food Adulteration Act (1954) as amended in 1998 has permitted irradiation at a dose rate of 2.5 to 4.5 kGy to control pathogenic microorganisms and to extend the shelf life of meat and meat products including poultry products. A study was conducted to evaluate the effect of low dose dose gamma irradiation on the various quality parameters of minced beef. The minced beef was prepared and packed in 200 g each in HDPE packets were subjected to irradiation either at 1.0, 2.0 and 3.0 kGy at melting ice temperature and kept at +4° C for further analysis on day 0, 7, 15, 21 and 30 or till spoilage whichever was earlier. The non spoiled samples were analyzed for its physicochemical, microbiological and sensory qualities. The keeping quality of minced beef was extended by 8-10 days at 1.0 kGy, 22-23 days at 2.0 kGy and 32-33 days at 3.0-kGy treatment, whereas the control samples were spoiled by 2- 3 days.

With respect to physicochemical parameters, pH had no significant difference due to irradiation at different doses. On storage pH values decreased slightly without any significant difference between treatments. The WHC was significantly reduced in 3.0 kGy irradiated samples compared to control, 1.0 and 2.0 kGy samples on day of preparation and on storage by 7 days. The ability of minced meat to retain its water decreased gradually on storage and reached 50 per cent of its original value in 3.0 kGy treated and 30 days stored samples.

The TBARS values which were nonsignificant due to irradiation on the day of preparation, increased as days of storage enhanced. The uniform increase with respect to higher dose of irradiation was noticed. The irradiation treatment had no significant effect on tyrosine values compared to control samples on day 0. As storage days increased, tyrosine values increased with significant changes among different treatments. The colour 'L'values of the irradiated samples were nonsignificant compared to that of control and increased slightly as storage period extended. The 'a' and 'b' values were nonsignificantly decreased due to irradiation and storage. On the day of preparation the cooking loss among the different treatment groups were not significant and increased uniformily on storage.

Irradiation had a beneficial effect on microbiological qualities of the minced beef. There was significant reduction in aerobic plate count at different doses of irradiation (1.0, 2.0 and 3.0 kGy). The initial counts gradually increased on storage. The coliform organisms were isolated from control and 1.0 kGy irradiated samples. There was about 63 per cent reduction in 1.0 kGy treated sample compared to that of control samples. All other irradiation doses (2.0 and 3.0 kGy) have totally destroyed coliform organisms in the minced beef and could not be detected throughout the storage period. None of the control samples or treated samples recorded *E.coli* and salmonella organisms. Like that of coliforms the faecal streptococci were found only in control and 1.0 irradiated samples. The treatment of minced beef meat by 2.0 and 3.0 kGy has totally destroyed the fecal streptococci and none of the samples had faecal streptococci during the storage period. The staphylococcal count was significantly reduced due to irradiation by 1.0, 2.0 and 3.0 kGy. It was found that organisms have multiplied and the number has increased as storage period enhanced.

The organoleptic qualities of irradiated samples recorded a slightly higher nonsignificant scores compared to control for colour and flavour. The juiciness and tenderness were significantly higher in irradiated samples compared to control and 1.0 kGy treated samples. On storage all the sensory attributes scores were decreased and reaches the acceptable score around 6 in 30 days stored and 3.0 kGy irradiated samples.

The irradiation preservation of minced beef was beneficial in saving the energy and time as the product can be chiller stored rather than in freezer. In addition irradiation could effectively control food borne illness by destroying the major pathogenic organisms without affecting the sensory and nutritional qualities of the product.