

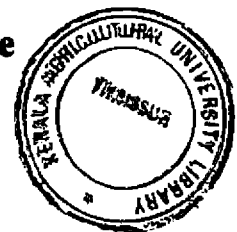
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EFFECTS OF PRESLAUGHTER STRESS AND IRRADIATION ON PHYSICOCHEMICAL QUALITIES OF MEAT

VIVEK. A. K.

**Thesis submitted in partial fulfillment of the
requirement for the degree of**



Master of Veterinary Science

**Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur**

2006

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DECLARATION

I hereby declare that the thesis entitled **“EFFECTS OF PRESLAUGHTER STRESS AND IRRADIATION ON PHYSICOCHEMICAL QUALITIES OF MEAT”** 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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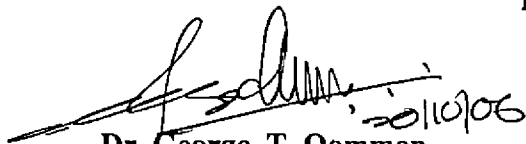

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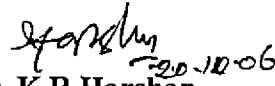
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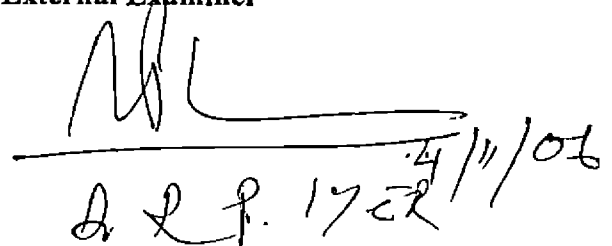


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Introduction

INTRODUCTION

Animals slaughtered in Kerala are being brought from the neighbouring states under stressful conditions which may deleteriously affect the quality of meat and also its shelf life. Stress is defined as a non specific response in an animal attempting to resist or adapt to maintain homeostasis *i.e.*, the tendency for the internal environment of the body to be maintained constant and in equilibrium (Selye, 1974). There are two main reactions of an animal to stress, the alarm or emergency reaction, mediated through sympathetic nervous system resulting in outpouring of catecholamines, nor adrenaline and adrenaline into blood stream and the general adaptive syndrome mediated through hypothalamus-pituitary-adrenal axis which brings about the production of corticosteroids. The physiological changes caused by the reactions described above occur when an animal is stressed and it can have a very significant effect on the quality of the meat if it occurs in the period prior to slaughter.

The prelaughter stress can result in adverse effects like mortality, weight loss, immunosuppression and reduced carcass and meat qualities. In cattle, this can result in a condition called dark cutting beef which is characterised by a high post mortem pH, increased water holding capacity and sticky texture and the fact that it will not bloom when exposed to air. The high ultimate pH makes the meat highly vulnerable to spoilage. So in order to make the meat more acceptable to the consumer and to prevent economic loss we have to think appropriate preservation technique without heavy economic inputs and having little undesirable effect on the meat quality.

Consumption of fresh food is preferable, but not always possible. The seasonal nature of production, long distance between production and consumption centers and the rising gap between demand and supply have made the need of preservation technique more relevant today. Meat preservation

involves application of measures to delay or check certain changes that make meat unsafe as a food or which lower some quality aspects of it. The concept of employing ionising radiation to preserve food has been developed since 1940 (Lawrie, 1998). According to Prevention of Food Adulteration (PFA) Act 1954 and its amendments in 1998 irradiation is permitted as an approved technique that can be used for the preservation of meat. Food irradiation is the process of exposing food to a controlled source of ionising radiation for the purpose of reducing the microbial load, destruction of pathogens and extension of product shelf life. Ionising radiation can damage the nucleic acids and ultimately kill the microbes by direct or indirect hits. Gamma rays, X rays and high energy electron beams are different forms of ionising radiation which are capable of knocking electrons out of the normal orbits in atoms or molecules. The amount of radiation energy absorbed is measured in units of grays (or kilograys). One gray equals one joule per kilogram of heat energy absorbed. The process of food irradiation is often called as cold pasteurisation because it kills most bacteria without increasing the temperature of the product. The process does not involve any addition or deletion of agents to meat and meat products.

On the perusal of this literature, it was observed that only limited works have been carried for evaluating the effects of stress, irradiation, and their combined effect on meat. Under these circumstances, the present study will be important in evaluating the physicochemical changes observed in the meat as a result of stress and irradiation and the main objectives of the study include:

- a) assessment of the effect of stress on the blood parameters and physicochemical qualities of meat and
- b) assessment of the effect of irradiation and stress on the qualities of fresh meat.

Review of Literature

REVIEW OF LITERATURE

Stress is a reflex reaction that occurs in an animal when exposed to adverse environmental conditions and which causes many unfavourable consequences in animal, ranging from discomfort to death. Conditions like transportation, loading and unloading, mixing of new group, sexual behaviour *etc.*, can cause stress in animals which are awaiting slaughter. These can lead to changes in blood picture as well as in quality of meat obtained from such animals.

2.1. HAEMATO-BIOCHEMICAL PARAMETERS DURING STRESS

Crookshank *et al.* (1979) found out that trucking of calves produced a definite increase in cortisol level while weaning produced a small increase in it. The cortisol level returned to the initial level within 4 to 7 days in the weaned and trucked calves. Weaning slightly increased Creatine Kinase (CK) but trucking had no effect on it.

A study conducted by Mc Veigh and Tarrant (1982) showed that there was a massive increase in plasma CK activity immediately after mixing of young bulls, which declined slowly during the recovery period and on day 4, it was still significantly elevated above the mean resting value. By day 7, plasma CK activity had returned to the resting level. There was a substantial glycogen break down in muscle tissue and the recovery to resting value was a comparatively slow process.

Tarrant *et al.* (1988) concluded that the high stocking density adversely affected animal welfare and lowered carcass quality when compared with the medium and low stocking densities. Plasma cortisol and glucose increased with stocking density, as did plasma CK activity and carcass bruising.

Tarrant (1990) reported that transport of cattle was inevitably associated with a degree of quantifiable stress, but distress should not occur. Distress might be avoided by observing statutory rest periods on long journeys, good animal handling, considerate driving technique, and by using correctly designed pens, loading ramps and stock crates.

Transport normally led to poor welfare in calves as evidence from mortality rate, heart rate, adrenal activity, enzyme changes, immunological effects and carcass quality. (Trunkfield and Broom, 1990)

Mohan Raj and Moss, (1992) suggested that mixing of cattle before slaughter led to increased physical activity and the homosexual behaviour, teasing and mounting, were detrimental to meat quality.

Tarrant *et al.* (1992) transported Friesian steers by road for 24 h at low, medium and high stocking density and reported that plasma cortisol and glucose were elevated after transport particularly at high stocking density. The white blood cell count and neutrophil numbers increased and the numbers of lymphocytes and eosinophils decreased. Packed Cell Volume (PCV) and red blood cell count increased, as did the concentration of total protein, hemoglobin and fibrinogen. The results showed that stocking densities above 550 kg/m² were unacceptable for animals in this weight range (about 600kg) on long journeys.

The experiment done by Shackelford *et al.* (1994b) demonstrated that there was genetic variation in the incidence of the DFD condition; however, genetic variation was small relative to environmental variation. Control of antemortem stress remained paramount to the elimination of variation in beef lean colour.

In a study conducted on plasma and muscle cortisol measurements as indicators of meat quality and stress in pigs, Shaw and Trout (1995) observed a strong positive correlation between plasma and muscle cortisol concentrations.

In older cattle, cortisol concentrations increased in response to loading, unloading and during the first portion of a journey, but it might decrease in case of transport of repeated or long duration as a result of habituation. The CK tended to increase with duration of journey, as did albumin, total plasma proteins, and osmolality. Free fatty acids, urea, and β -hydroxybutyrate also increased. In the case of long-distance transport, recovery to pretransport levels was slow because of the disruption of eating cycles and water deprivation. Recovery to normal level took as long as 5 days after transport (Warriss *et al.*, 1995).

McNally and Warris, (1996) reported that there was a strong relationship between animal welfare, stress, bruising and meat quality.

Grandin (1997), in a study on the assessment of stress during handling and transport, suggested that animals could be stressed by either psychological stresses (restraint, handling or novelty) or physical stresses (hunger, thirst, fatigue, injury or thermal extremes). Cortisol was found to be a useful indicator of short-term stress from handling or husbandry practices.

The studies of Schaefer *et al.* (1997) suggested that the use of electrolyte therapy using fluid similar in constituents to interstitial fluid seemed to attenuate the physiological changes due to stress, resulting in improved live and carcass weights as well as a reduction in meat quality degradation in transported cattle.

Jacobson and Cook, (1998) subjected calves for 40 min of transport, which was repeated three times, (with 1 day rest between each) found that the physical demands of transport did not contribute greatly to peak heart rate response during transport, and that calves could learn to cope with psychologically with the novelty and stress of short term transport.

Knowles *et al.* (1999) found a negative correlation between age at transport and mortality, indicating that transport mortality is comparatively less in mature cattle.

The relationships between farmers' behaviour towards veal calves, their responses to handling and transport, and the meat quality were assessed by Lensink *et al.* (2001) and found that a positive behaviour towards calves during rearing was likely to decrease the calves' fear and stress responses when they were transported for slaughter.

Nigil Mathew (2002) observed that low density stock group and high density stock group had a significant increase in biochemical parameters like blood glucose level, concentration of cortisol, Blood Urea Nitrogen (BUN) and CK activity immediately after transport. Many of them returned to pre-transport level after 18 hours in low density stock group whereas not in high density stock group even after giving 18 hours of rest.

According to Swanson and Morrow-Tesch (2002) physiological measures indicated that transport of cattle could result in immune suppression, which could lead to increased susceptibility to disease and might result in increased pathogen shedding.

Tasco-supplemented Boer goats were transported for 6h to impose stress, held in pens overnight without feed and slaughtered on two different days. It was observed that the dietary treatment did not influence plasma cortisol, glucose concentrations and CK activity. Plasma cortisol and glucose increased due to transportation, but decreased significantly after holding. Plasma CK activities increased during transportation and holding, and peaked significantly at 24h sampling, might be due to agonistic activities during feed deprivation. (Galipalli *et al.*, 2004)

In an experiment conducted by Kuchenmeister *et al.* (2005), pigs were subjected to stress of two kinds (immobilisation by a nose snare and the use of

an electrical goad) prior to slaughter and compared with non stressed animals and found that a stress before slaughter had a significant influence on meat quality.

Knowles and Warriss (2005) suggested that stress during transport could be indicated by cortisol for fear/arousal, PCV for both fear/arousal and dehydration, CK activity for physical exertion and urea for food deprivation.

Guardia *et al.* (2005) found out that the risk of Dark Firm Dry (DFD) increased with high stocking density, lairage time, and with on-farm fasting times longer than 22 h. Their results revealed that lowering the stocking density from 0.37 to 0.50 m² per 100 kg pig during transport would increase the risk of DFD pork by 11 per cent.

2.2 . IRRADIATION

According to Prevention of Food Adulteration Act (PFA) 1954 and its amendments in 1998, irradiation is permitted as an approved technique for the preservation of meat. Exposing food to a controlled source of ionising radiation can cause reduction of microbial load, destruction of pathogens and extension of product shelf life.

Fox *et al.* (1995) studied about the loss of thiamin and riboflavin due to gamma irradiation of beef, lamb and pork *longissimus dorsi*, and turkey breast and leg muscles. Thiamin losses averaged 11 per cent/kiloGray (kGy) and riboflavin losses 2.5 per cent /kGy above three kGy. Any detriment from such slight losses would seem to be more than compensated by the advantage of controlling bacteriological contamination by irradiation processing.

Lakritz *et al.* (1995) studied the effect of gamma radiation on levels of α -tocopherol in red meat and turkey and found that irradiation resulted in decrease in α -tocopherol levels in all of the meats studied. But there were no

significant differences in the rate of loss of α -tocopherol due to species, with exception of turkey breast.

Based on results for tenderness, chemical, visual, and microbiological effects Lee *et al.* (1996) suggested that, irradiation in conjunction with modified atmospheric packaging containing 25 per cent carbon dioxide and 75 per cent nitrogen could be used for an accelerated ageing process of beef at 30°C for 2 days.

Radiation treatment at doses of 2.0 to 7.0 kGy, depending on condition of irradiation and the food, could effectively eliminate potentially pathogenic nonsporeforming bacteria including both long-time recognised pathogens such as *Salmonella* and *Staphylococcus aureus* as well as emerging or “new” pathogens such as *Campylobacter*, *Listeria monocytogenes* or *Escherichia coli* O157:H7 from suspected food products without affecting sensory, nutritional and technical qualities. (Farkas, 1998)

Ground beef patties (irradiated with 2.0 kGy and nonirradiated) were packaged using oxygen permeable (polyolefin) or oxygen impermeable material (polyethylene). A 3 log₁₀ reduction in total aerobic counts was detected immediately after irradiation. No difference in lipid oxidation was found within the first week of storage, regardless of packaging atmosphere. Shelf life of ground beef patties was extended up to 55 days at 4°C. (Murano *et al.*, 1998)

A minimal dose of 1.0 kGy was sufficient to increase the shelf life of fresh pork loins although variations in initial pork contamination were found to be the determining factor accounting for the effectiveness of the treatment. (Dogbevi *et al.*, 1999)

Park *et al.* (2000) studied the influence of refrigeration, freezing, repetitive freezing, thawing and irradiation on meat quality by detecting DNA damage to beef muscle tissues using single cell gel electrophoresis assay

(Comet assay) and found out that irradiation from 1.0 to 10.0 kGy caused the most serious DNA damage among the treatments compared.

In a study conducted by Ahn *et al.* (2001), the DFD meat was very stable and resistant to oxidative changes in both irradiated and non irradiated pork during storage, suggesting that irradiation could significantly increase the utilisation of raw pork and greatly benefit the pork industry.

Aziz *et al.* (2002) concluded that combined treatments of gamma rays (3.0 kGy) and microwave (20s exposure) had the potential to greatly enhance the shelf life of beef products and its safety with little effect on its chemical and sensory quality.

Consumer studies conducted by Fox (2002) indicated that counteracting negative information about irradiation could positively affect purchase decisions and that marketing efforts should focus on women

Based on the results of the study conducted by Lewis *et al.* (2002) electron beam irradiation was an effective means of eliminating bacteria from breast fillets even at the minimum dose of 1.0 kGy. An irradiation dose of 1.0 kGy was also effective in improving the colour of the breast fillets.

DFD pork, which had a shorter shelf life than the others, could be benefited from irradiation because the shelf life of DFD meat could be extended significantly by both the methods of vacuum packaging and irradiation. (Nam *et al.*, 2002)

Satin (2002) opined that irradiation was not a stand-alone process that could guarantee safe food. It must be integrated as part of an overall good manufacturing practice programme.

Ahn and Nam (2004) reported that the addition of ascorbic acid at 0.1 per cent or seasmol along with α -tocopherol, each at 0.01 per cent level, to ground beef prior to irradiation were effective in reducing lipid oxidation and

sulphur containing volatiles. As storage time increased, however, the antioxidant effect of seasmol and α -tocopherol in irradiated ground beef was superior to that of ascorbic acid.

Smith and Pillai (2004) reported that irradiation technology had been proven beneficial for not only controlling pathogens, but also increasing shelf life and maintaining food quality. Irradiation to ensure food safety was to be implemented as a part of an overall HACCP plan and was not meant to replace existing control measures.

Kuttinarayanan (2005) conducted a consumer acceptance study for irradiated beef products and found that majority of the consumers (72.5 per cent) liked to purchase irradiated and 37.5 per cent were ready to pay more for the irradiated product since it could be kept under chiller condition. Majority did not observed any peculiar smell or taste difference in irradiated meat and meat products compared to non irradiated products. Nobody had reported unwillingness to consume irradiated meat and meat products.

The descriptive analysis conducted by Nayga *et al.* (2005) suggested that information about the nature and benefits of food irradiation led to positive changes in consumers' perception and buying decisions.

Kuttinarayanan *et al.* (2006 b) reported that the judicious use of the dose of radiation and other techniques like irradiating at lower temperature, absence of oxygen , chemical additives and proper packaging would help most foods to be treated with minimal negative effects.

2.3 . PHYSICOCHEMICAL QUALITIES OF MEAT

Physicochemical qualities of meat can be measured by using both objective and subjective methods. There are various preslaughter and post slaughter conditions which may affect the physicochemical properties of meat.

Stress in animals awaiting slaughter and irradiation of fresh meat are the two among other factors.

2.3.1. pH of meat

Korkeala *et al.* (1986) determined the pH of meat electrometrically using different electrode systems and different presentations of samples and found that no pH measurements methods could be considered better than the others.

Kuttinarayanan (1988) found that there was a drastic decline in pH in electrically stimulated meat samples and the difference in value remained significant up to 8 hours of storage. At 24 and 48th hours of storage there was no difference in pH between control and stimulated samples.

Lefevbre *et al.* (1994) found out irradiation clearly contributed to a diminution of the pH and an increase in peroxides of ground beef. The ten non-expert taste panelists indicated a change in the odour and colour of the raw product as a result of irradiation.

In a study, Page *et al.* (2001) used an ultimate pH of 5.87 as an appropriate cut off value between normal and dark cutting carcasses and found out that L*, a* and b* values were negatively correlated to muscle pH, showing that as muscle pH increased muscle colour values also decreased.

In a study on the changes of morphological and biochemical properties of gamma irradiated bovine muscle before rigor onset, a rapid decrease of pH and breakdown of ATP were observed in irradiated muscle. (Yook *et al.*, 2001)

Nam and Ahn, (2002a) studied the effect of double packaging and acid combination on the quality of irradiated raw turkey patties and found that irradiation had no effect on the pH of vacuum packaged meat.

Effect of ionising radiation on the quality characteristics of vacuum packaged normal, Pale Soft Exudate (PSE) and DFD pork (after attaining ultimate pH) was studied by Nam *et al.* (2002) and found that the original ultimate pH value of normal, PSE and DFD meat showed that irradiation had no effect on the pH of the three pork types.

Maria *et al.* (2003), conducted a study using three transport times (30 min, 3 or 6 h) and found no differences in ultimate pH between transport groups, which reflected the lack of variation in other variables such as Water Holding Capacity (WHC) and most measurements of texture and colour.

According to Pipek *et al.* (2003), colour (lightness) and tenderness (Warner Bratzler shear test) values were influenced by the ultimate pH value.

Hambrecht *et al.* (2004a) showed that preslaughter stress level affected the pH and temperature of both blood and muscle. They also found that rapid chilling could not compensate for the detrimental effect of stress on drip loss, filter paper moisture absorption and meat colour.

Tenderness and colour properties of chevon were found to be highly dependent on post-mortem pH and temperature as well as the ultimate pH attained by the carcasses. (Simela *et al.*, 2004)

Okeudo and Moss, (2005) opined that serum cortisol level was negatively correlated with initial pH and positively correlated with intramuscular fat in castrates, the same correlations in other sex-types were not significant.

2.3.2. Water holding capacity (WHC) of meat

Irradiation causes some protein denaturation and this increase during storage, especially at high temperature and the resultant loss in WHC causes considerable exudation (Cain *et al.*, 1958; Schweigert, 1959).

Offer and Trinick (1983) conducted a study on the mechanism of water holding in meat and opined that myofibrils could shrink by a substantial amount in response to factors such as pH fall, to rigor formation or cooking which reduced the WHC.

Dark cutting beef is a condition associated with high ultimate pH always above 6 and sometimes above 6.5 which is considerably above the isoelectric point of myofibrillar proteins and so the water molecules will be tightly bound to them (Kauffman and Marsh, 1987).

Urbain and Campell (1987) opined that unlike the comparable thermal process of sterilisation, irradiation did not alter the water holding capability of meat.

Kuttinarayanan, (1988) used electrical stimulation as a method of tenderising the meat of older animals and found that the WHC was not affected by either voltage or treatment and a better value for WHC was obtained at zero hour than that at 8, 24 and 48h of keeping.

Two studies were conducted by Lesiak *et al.* (1997) to determine 1) the effect of post-mortem time before chilling on hot boned prerigor breast muscle WHC and 2) post-mortem temperature effect on sarcomere length and drip loss of uncooked breast, and shear force and WHC of turkey breast muscle that was hot-boned, marinated, and cooked and concluded that the post-mortem time before chilling had an influence on uncooked and cooked muscle WHC, but the differences found in uncooked muscle drip loss were not reflected in WHC or shear force of cooked muscle with water, salt, and sodium tripolyphosphate added.

Diminution of the *in vivo* WHC is manifested by exudation of fluid known as “weep” in uncooked meat that has not been frozen, as “drip” in thawed uncooked meat and as “shrink” in cooked meats, where it is derived from both aqueous and fatty sources. (Lawrie, 1998)

Kristensen and Purslow, (2001), found that the WHC of pork decreased post mortem but had been shown to increase during subsequent ageing. These observations were consistent with the hypothesis that degradation of the cytoskeleton slowly removed the linkage between lateral shrinkage of myofibrils and shrinkage of entire muscle fibres, so removing the force that caused flow into the extracellular space. Inflow of previously expelled water was then possible, so increasing WHC as observed in later periods of storage

Zhu *et al.* (2004) reported that irradiation increased centrifugation loss which might be related to the structural damage in membrane during irradiation in pork loins.

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

2.3.3. Cooking loss

A scanning electron microscopic study of heat induced alterations in bovine connective tissue revealed that epimysium did not show large alterations after cooking; however, the perimysium and endomysium becomes granular and at 60°C and gelatinised at 80°C (Wu *et al.*, 1985)

Kauffman *et al.* (1986) conducted an experiment on the comparison of methods to estimate the WHC in post rigor porcine muscle and found out that cooking loss tests failed to differentiate between PSE and normal samples. The transmission, imbibition and pressed fluid methods did not always distinguish between DFD and normal meat.

Denaturation during cooking caused structural changes such as the destruction of cell membranes, transverse and longitudinal shrinkage of muscle fibres, the aggregation of sarcoplasmic proteins and shrinkage of the connective tissue. All these events, particularly the connective tissue changes, resulted in cooking losses in meat. (Honikel, 1998)

Cooking loss depended much more on the endpoint temperature and the speed of heating. The higher the final temperature and the slower the velocity of heating, the higher was the cooking losses. Between 60 and 70°C, there was nearly a linear relationship between the final temperature and cooking losses. Cooking losses did not depend on shortening or on the PSE condition that could lead to high drip losses. This could be due to the fact that heating caused denaturation of the proteins and disintegration of membranes, which had a tremendous influence on the bulk phase water so that shortening or the amount of extracellular space became unimportant. (Honikel and Hamm, 1999)

Whole meat toughness was found to increase in two separate phases upon cooking from 40 to 50°C and again from 60 to 80°C. The changes in whole meat toughness at temperatures below 60°C were found to correspond to changes in the mechanical properties of the perimysial connective tissue, whereas changes of whole meat toughness at temperatures above 60°C were found to correspond to increase breaking strength of single muscle fibers. (Christensen *et al.*, 2000)

Tornberg (2005) reported that most of the sarcoplasmic proteins aggregated between 40 and 60 °C, but for some of them the coagulation could extend up to 90 °C. For myofibrillar proteins in solution unfolding started at 30 to 32 °C, followed by protein to protein association at 36 to 40 °C and subsequent gelation at 45 to 50 °C. At temperatures between 53 and 63 °C the collagen denaturation occurred, followed by collagen fibre shrinkage, which decreased the WHC of meat leading to cooking loss.

Irradiated chicken breasts had more cooking loss and higher shear force than non irradiated chicken breast. Transmission electron microscopy showed significant differences in size of myofibril units (sarcomere) between irradiated and non irradiated breast. Shrinkage in sarcomere width (myofibril diameter) and disruption of myofibrils in irradiated breast meat were also noticed when compared with non irradiated breast meat. (Yoon, 2003)

Okeudo and Moss (2005) opined that serum cortisol level was negatively correlated with cooking loss in all sex-types of crossbred lambs and also significantly related to fatty acid profile.

2.3.4. Tenderness

Collagen shranked when irradiated in a dry state and became soluble in water if irradiated wet (Perron and Wright, 1950), and indeed irradiation caused softness and tenderness of texture as an immediate effect (Coleby *et al.*, 1961). The effect probably might be due to the destruction of some of the hydrogen bonds which hold together the triple helix.

As the growth rate decreased and the animal matured the collagen fibres, initially stabilised by intermolecular head to tail cross links, form a stable network *via.*, multivalent cross links. The greater the proportion of these crosses links the tougher the meat would be. Therefore the quality, rather than the quantity of the collagen determined its contribution to the texture of meat. (Bailey, 1985)

The effects of early post mortem glycolytic rate on beef tenderness was studied by Marsh *et al.* (1987) and suggested that the goal of maximising the early post mortem rate of pH decline in bovine muscle was misguided and if attained, would cause suboptimal tenderness.

Purchas (1990) reported that the effects of ultimate muscle pH on the shear force values were similar for beef from the *longissimus dorsi* of bulls and

steers, with a shear force maximum at a pH between 6.0 and 6.2. The toughening effect of increases in pH up to 6.2 appeared to be at least partly due to decrease in sarcomere length.

The lower tenderness of *longissimus dorsi* muscle of *Bos indicus* cattle, compared with *Bos taurus* cattle, was apparently due to reduced post mortem proteolysis in myofibrillar proteins in *Bos indicus* which was associated with higher activity of calcium dependant protease inhibitor in this cattle (Whipple *et al.*, 1990).

According to Jeremiah *et al.* (1991) the segregation of beef carcasses with ultimate *longissimus* pH values between 5.8 and 6.19 appeared to be an easy, non destructive, practical means to effectively remove the majority of tough carcasses (shear value >6.0) in all sex groups, regardless of breed.

The course of rigor mortis (rigor), ageing and tenderness had been evaluated for three beef muscles; *biceps femoris* (BF), *semimembranosus* (SM) and *semitendinosus* (ST), when entering rigor at constant temperatures of 15 and 37°C respectively, with and without electrical stimulation (85 V, 14 Hz and 32 s). Even though tenderness was measured after ageing (15 days post mortem), shortening during rigor seemed to be more important for toughness when rigor mortis occurred at 37°C than any suggested tenderising effect due to increased proteolysis in this temperature region (Hertzman *et al.*, 1993).

Kendall *et al.* (1993) concluded that although m-calpain and calpastatin activities decreased with increasing ionic strength, they might be active in influencing the loss of structural integrity of the myofibrils in postmortem muscle even at elevated ionic strengths and decreased pH.

The results of study conducted by Pike *et al.* (1993) suggested that the effect of early post mortem glycolytic rate was of paramount importance to beef *longissimus thoracis et lumborum* tenderness.

In a study conducted by Shackelford *et al.* (1994a) three-hour post-mortem muscle pH was not an accurate indicator of tenderness for cattle slaughtered and processed under commercial or laboratory conditions.

In a study conducted to determine whether modification of early post mortem muscle pH and control of post mortem ageing time could be used to improve tenderness, Eliers *et al.* (1996) found out that lower values of 24h pH of the *longissimus dorsi* were associated with increased tenderness and greatest tenderness was achieved by 12th day. The use of electrical stimulation increased the rate of post mortem muscle pH decline and decreased shear force of *longissimus dorsi* steaks.

Marsh (1997) opined that two muscle components, collagen and the contractile apparatus determined tenderness of meat. The collagen contribution to toughness was due to the presence of intermolecular cross-links, which with increasing animal age, became more thermally resistant and thus less readily broken during cooking. By contrast, toughness due to the contractile proteins was determined by condition during the first few post mortem hours.

The results of study conducted by O'Halloran *et al.* (1997) suggested that the rate of post-mortem pH fall played an important role in proteolysis and tenderisation.

Taylor and Koohmaraie (1998) conducted experiments to examine ultrastructural changes in *longissimus* from normal and callipyge lamb during 14 day of postmortem storage at 4°C and concluded that the major factor responsible for the toughness of meat from callipyge *longissimus* was the postmortem stability of myofibrils.

The *longissimus thoracis et lumborum* muscle from bulls with normal ultimate pH (<5.7), bulls with unacceptably high ultimate pH (>5.7) and steers were compared after storage at 15°C and found that high ultimate pH samples had higher shear force at 120h post mortem than the other samples. The activity

of μ calpain was low at all times and appeared to be involved in the increased toughness of the sample. (Thomson *et al.*, 1999)

In a study conducted by Lee *et al.* (2000) for understanding the effects of irradiation on myofibrillar proteins, found that gamma irradiation caused conformational changes to the major contractile muscle protein, myosin. Myosin solution became increasingly turbid with increasing dose. The hydrophobicity of myosin solution was also increased by irradiation. Electrophoretic patterns showed that the myosin heavy chain disappeared and new bands were generated at higher molecular weight ranges.

Yook *et al.* (2001) conducted experiments on the changes of morphological and biochemical properties of gamma irradiated bovine muscle before rigor onset and concluded that the destruction of muscle bundles was faster in the gamma irradiated than in the non-irradiated muscle and shear force was decreased by irradiation.

There was no significant effect of transport time (30 min, 3 or 6 h) on Warner Bratzler (WB) values of maximum load or toughness, which were similar for all journey times and significantly lower at 14 days for all journeys and also suggested that ageing could potentially attenuate any negative effects of transport. (Maria *et al.*, 2003)

Observations made by Maddock *et al.* (2005) provided new evidence that rates of pH decline and increases in ionic strength were the important variables to be considered while examining the variations in calpain induced proteolysis of meat proteins in postmortem muscle.

2.3.5. Colour

The results of spectral studies on the role of ionising radiation in colour changes of radappertised beef showed that ionising radiation reduced the heme iron of the brown pigment of cooked meat (globin myohemichromogen) to an

unstable red pigment (globin myohemochromogen), which upon exposure to air, reverted of the original ferric brown pigment (Kamarei *et al.*, 1979)

Dark cutting beef muscle would turn red if mitochondrial respiration was inhibited, allowing myoglobin at muscle surfaces to remain oxygenated. (Egbert and Cornforth, 1986)

Kauffman and Marsh (1987) explained that the ultimate pH above the isoelectric point of myofibrillar proteins would make the water molecules tightly bound to them and the resulting modified structures reflected less of the incident light and the meat appeared unusually dark. Also due to the high pH the meat would be more prone to bacterial spoilage.

The effects of vacuum packaging, chilling rate (slow or fast) and fat cover thickness (≤ 4 mm or 7 to 8 mm) on the colour parameters of beef *longissimus dorsi* muscle were assessed during ageing and found that ageing time influenced all colour parameters, while chilling rate affected lightness (*L*), yellowness (*b*) and *hue angle* difference whereas fat cover thickness influenced *L* and *b* only. (Boakye and Mittal, 1996).

The results of the study of Kannan *et al.* (1997) suggested that higher preslaughter stress levels in broilers could influence the colour of thigh meat, although overall meat quality was not affected under the conditions of their study.

Wulf *et al.* (1997), found out that the correlation of colour measurements were higher than correlations of marbling score with tenderness measurements, also of the three colour measurements, *b** value showed the highest correlation with shear force value and taste panel tenderness rating.

The colour changes induced by irradiation were different depending on the animal's species, muscle type, irradiation dose and packaging type. (Ahn *et al.*, 1998)

Wulf and Wise (1999) used the main advantage of the L* categorisation system over the b* system since the L* value was less sensitive to bloom time. The main advantage of the b* categorisation system over the L* system was that the b* system was slightly more precise at segregating carcasses based upon corresponding differences in muscle pH.

Millar *et al.* (2000) irradiated beef (obtained from carcasses after 24 h post mortem) and found out that L* values of irradiated beef increased significantly with storage and a* values for non irradiated samples decreased significantly with storage. Irradiation resulted in significantly higher hue angle (h⁰) values and the a*, b* and C* values were significantly higher on the exterior than freshly cut surface.

Abril *et al.* (2001) concluded that pH had a significant effect on colour development, and the colour development in pH \geq 6.1 group was less visually appreciable than in pH $<$ 6.1 group.

Irradiation increased stable pink colour in raw turkey breasts with both aerobic and vacuum packaging for which carboxymyoglobin could be responsible. Volatiles increased with irradiation dose, aerobic packaging and storage time. (Nam *et al.*, 2001)

L* value of irradiated beef decreased significantly after 7days of storage and irradiated beef had a lower L* value than non irradiated after 7days of storage. At storage zero days, there was a decrease in a* value due to irradiation regardless of packaging type but on storage for 7 days beef in aerobic packaging showed slightly increased colour a* values, while a* values decreased in vacuum packaging. In beef, b* values decreased at zero day which increased on storage. (Kim *et al.*, 2002a)

Nam and Ahn (2002a) found out that the irradiation of precooked , vacuum packaged turkey breast resulted in increased pink colour which was

stable during frozen storage and found that carboxy heme pigments could be responsible for the colour.

Nam and Ahn, (2002b) studied the mechanism of pink colour formation in irradiated precooked turkey breast meat in which the reflectance of meat and the absorption spectra of myoglobin solution supported the assumption that denatured carboxy myoglobin was the pigment in irradiated precooked turkey breast.

Nam *et al.* (2002) subjected pork *longissimus dorsi* muscle to ionising radiation and found out that irradiation¹ increased the redness of vacuum packaged normal, PSE and DFD pork.

Nam and Ahn (2003a) reported that usually light meat produced pink colour while dark meat became brown or gray after irradiation. The production of carbon monoxide and changes of oxidation – reduction potential in red meat by irradiation were similar to those of light meat, but colour changes were different from those of light meat due to high pigment content in red meat.

Nam and Ahn, (2003b) reported that ageing, because of consumption of reductants and inactivation of reducing systems due to accumulation of metabolic by-products, could have significant effects on colour of beef. Ageing increased L* value of beef, a change which was exacerbated by irradiation.

Irradiation decreased the redness of ground beef and visible colour of beef changed from a bright red to a green/brown depending on the age of meat. Colour L* value increased as the ageing time of beef increased. During storage after irradiation, L* values of ground beef also showed an increasing trend as the storage time increased. (Ahn and Nam, 2004)

Hambrecht *et al.* (2004 b) found out that high stress resulted in inferior pork quality attributes including reflectance, electrical conductivity, filter paper moisture drip loss and L*value.

Brewer, (2004), opined that colour changes in irradiated fresh meat occurred because of the inherent susceptibility of the myoglobin molecule, especially the iron, to alterations in the chemical environment and to energy input. Increasing ageing time prior to irradiation increased L* value and decreased a* value. It was suggested that maintenance of ideal meat colour during the process of irradiation could be enhanced by various combinations of preslaughter and post slaughter handling of meat.

Lee and Ahn, (2004), studied about the sources and mechanisms of carbonmonoxide production by irradiation and the found that the production of carbonmonoxide, carbondioxide and methane in all samples were dose dependent and the amounts of carbonmonoxide produced were large enough to react with most of the heme pigments present in poultry breast and pork loin.

2.3.6. Sensory evaluation

2.3.6.1. Colour

Kropf (1980) reported that colour is probably the single greatest appearance factor that determines whether a meat cut will be purchased.

Sensory evaluation by Fu *et al.* (1995) of raw beef steaks and ground beef irradiated at 2.0 kGy reported no significant difference in colour, but detected off-odours that quickly dissipated after opening vacuum packages

The appearance of the meat surfaces to the consumer depends, however, not only on the quantity of myoglobin present but also on the type of myoglobin molecule, on its chemical state and on the chemical and physical conditions of other compounds in the meat. A high ultimate pH of muscle makes it aesthetically unpleasant due to its poor appearance and enhances bacterial growth (Lawrie, 1998)

2.3.6.2. Flavour

In the view of development of flavour which accompanies conditioning it is of interest that hypoxanthine or its precursor inosinic acid, was reported to enhance flavour when added to meats. (Kodama, 1913)

The components of meat that are responsible for flavour and aroma has not been completely identified but some evidence shows that inosine monophosphate (IMP) and hypoxanthine enhance flavour or aroma. Since IMP and hypoxanthine are break down products of ATP, it is obvious that muscle with large energy stores would have a more pronounced flavour. (Forrest *et al.*, 1975)

Ahn *et al.* (1998) suggested that irradiation produced many unidentified volatile products that could be responsible for the off odour in irradiated raw meat.

Flavour is a complex sensation. It involves odour, taste, texture, temperature and pH, of these, odour is the most important. (Lawrie, 1998)

Beef flavour was not characterized by lipid oxidation products but by degradation products of thiamin. Thiamin might well prove to be the key constituent of beef, yielding the characteristic flavour. Recent work suggestions that beef flavour might be common to all species if immobilised by lipid contributions. (Reineccius, 1999)

Mottram (1998) found that sulphur-compounds, derived from ribose and cysteine, seemed to be particularly important for the characteristic aroma of meat. In meat, the main sources of ribose were IMP and other ribonucleotides.

Ahn (2002), reported that the sulphur compounds produced from the side chains of methionone and cysteine were the most important volatiles for off odour production in irradiated meat and the contribution of methionone to the irradiation odour was far greater than that of cysteine.

The majority of newly generated and increased volatiles by irradiation were sulphur compounds, indicating that sulphur amino acids were the most susceptible to changes by irradiation. More than one site in the monoacid side chains were liable to free radical attack and many volatiles were produced by the secondary chemical reactions after the primary radiolytic degradation of side chains. (Ahn and Lee, 2002)

Gorraiz *et al.* (2002) observed a gender specific sensory quality in meat as beef from bulls had stronger livery odour and flavour and bloody flavour than that from heifers. Ageing of beef between 4 and 7 days caused an improvement in flavour, originating stronger characteristic flavour and after taste.

Kim *et al.* (2002b) conducted an experiment on the volatile profiles , lipid oxidation and sensory characteristics of irradiated meat from different animal species and found out that irradiation not only produce many new volatiles not found in non irradiated meats but also increased the amounts of some volatiles found in non irradiated meats. The amount of volatiles in aerobically packaged irradiated meats decreased with storage while those of non irradiated meats increased.

For short term storage, irradiation of turkey breast meat in which lipid oxidation was not a great problem, aerobic packaging would be more beneficial than vacuum-packaging, because sulphur volatile compounds responsible for the irradiation off-odour could be significantly reduced under aerobic conditions. After exposure to aerobic conditions for 3 days, the irradiated meat should be kept in vacuum conditions to minimise lipid oxidation for long term storage. (Nam and Ahn, 2002a)

Lee and Ahn, (2003) studied the production of volatiles from fatty acids and oils by irradiation and found out that the amount of aldehydes, the indicator of lipid oxidation, in oil emulsion did not increase by irradiation, and the

volatiles from lipids account for only a small part of the off odour in irradiated meat.

Nam and Ahn (2003a) tried a combination of aerobic and vacuum packaging to control lipid oxidation and off odour volatiles of irradiated raw turkey breast and found out that irradiating and storing turkey breast meat for 1 to 3 days under aerobic conditions and then storing under vacuum conditions could minimise irradiation off flavour by volatilising S-volatile compounds and lipid oxidation products compared with vacuum and aerobically packaged meats.

Sensory analysis conducted by Zhu *et al.* (2003), indicated that sulphury odour increased as irradiation dose increased and the contents of sulphur compounds in irradiated ready to eat turkey hams were higher than those in non irradiated samples. However, overall quality changes of such products were minor.

Nortje *et al.* (2005) reported that although lean moist beef biltong could be irradiated to doses up to 8.0 kGy without adversely affecting the sensory acceptability, low dose irradiation (≤ 4.0 kGy) was most feasible to optimise the sensory quality.

2.3.6.2. Juiciness

Juciness has two organoleptic components. First one is the impression of wetness during the first few chews which is produced by the rapid release of meat fluid and the second is one of sustained juciness, largely due to the stimulatory effect of fat on salivation.(Weir, 1960)

Jeremiah *et al.* (1971) noticed chronological age had no significant effect on either marbling or juciness score.

Berry *et al.* (1981) stated that hot-boned roasts from *semimebranosus* and *semitendinosus* muscles had higher shear force values, higher amount of

connective tissue, lower tenderness and higher juiciness score than cold boned cooked roasts when served as cubes.

If meat is consistently and acceptably tender and there is no off flavour, then juiciness becomes the sensory characteristic that is the primary determinant of meat quality. The only reliable and consistent measure of juiciness is achieved using sensory methods. (Winger and Hagyard, 1999)

2.3.6.3. Tenderness

Perception of tenderness has been described in terms of the following conditions of the meat during mastication such as softness to tongue and cheek, resistance to tooth pressure, ease of fragmentation, mealiness, adhesion and residue after chewing. (Forrest *et al.* 1975)

Of all the attributes of eating quality, texture and tenderness are presently rated most important by the average consumer and appear to be sought at the expense of flavour or colour. The degree of tenderness can be related to three categories of protein in muscle - those of connective tissue (collagen, elastin, reticulin, muco polysaccharides of the matrix), of the myofibril (actin, myosin, tropomyosin) and of the sarcoplasm (sarcoplasmic proteins and sarcoplasmic reticulum). (Lawrie, 1998)

The effects of irradiation of refrigerated and frozen chicken on sensory properties were investigated on skinless, boneless breast (white) and leg quarters (dark) and found that cooked irradiated frozen dark meat had more chicken flavour and cooked irradiated refrigerated dark meat was tenderer than controls (Hashim *et al.*, 1995)

Fernandez *et al.* (1996) reported a reduced tenderness score for transported and feed deprived calves.

Wulf *et al.* (2002) found out that low glycolytic potential was associated with DFD beef which resulted in substantially less palatability in cooked steaks with more off flavours and less tender than that of normal beef.

Villarroel *et al.* (2003) reported that transport time of 30 min, 3 or 6 h affected sensory meat quality in terms of tenderness and overall liking. However, as in other studies, the effect was weak and possibly not enough to convince producers that improper transport could decrease their income by decreasing the sensorial quality of the product.

2.3.6.5. Overall acceptability

Dempster *et al.* (1985) showed no sensory differences between irradiated and non irradiated raw beef burgers up to 11 days after irradiation (1.5 kGy) under vacuum.

The results of study conducted by Wheeler *et al.* (1999) implied that hamburgers made from ground beef patties irradiated under certain conditions of would encounter little, if any, consumer acceptance problems at the 3.0 kGy dose and only slightly greater problems at the 4.5 kGy dose.

Sensory evaluation revealed that electron beam irradiation resulted in a decrease in the texture, flavour, and overall acceptability of the product as the storage period increased. The observed decrease in product quality can be attributed to increased level of oxidation due to irradiation. (Lewis *et al.*, 2002)

Kuttinarayanan *et al.* (2006a) evaluated the organoleptic qualities of minced beef with the help of nine point hedonic scale in terms of colour, flavour, juiciness, tenderness, and overall acceptability of cooked product and observed a slight increase in tenderness of the irradiated samples and on an average no significant difference was noticed with respect to control, irradiated and salted samples on zero and 4th day of investigation. It was found that

irradiated minced beef had an extended storage life of more than 20 days at chiller temperature of 4⁰C compared to 4 days in non irradiated groups.

Kuttinarayanan *et al.* (2006c), conducted a study on the keeping quality and organoleptic studies of beef fry preserved by employing gamma radiation and found that physicochemical characters (like pH, thiobarbituric acid reacting substance and tyrosine value) and organoleptic evaluation (with respect to colour, flavour, tenderness, juiciness and overall acceptability) using nine point hedonic scale didn't reveal any marked difference between irradiated and non radiated sample even after 28 days of storage at chiller temperature.

Materials and Methods

MATERIALS AND METHODS

3.1. EXPERIMENTAL PROCEDURE

3.1.1. Animals

Adult cattle culled from University Livestock Farm (ULF), Mannuthy, Cattle Breeding Farm (CBF), Thumboormozhi and Kerala Livestock Development Board (KLDB), Mattupetti and Kulathupuzha, for the reasons of low productivity and infertility, brought for slaughter at Department of Livestock Products Technology (LPT), College of Veterinary and Animals Sciences, Mannuthy, were utilised for the present study. The animals were having an age of 4.41 ± 0.58 years and a weight of 351.07 ± 39.57 kg. Geographically, ULF, CBF and KLDB Mattupetti and Kulathupuzha are livestock farms located 200m, 50 km, 163 km, and 258 km respectively, away from the Department. All the animals were grouped into three, having nine animals in each.

Group I, comprised of animals from ULF, was considered as control groups which were having only a very short distance (less than 200m) journey and were slaughtered immediately after the arrival at the Department. Group II comprised of animals from CBF, which were transported by trucking during early morning, for 2 hours, to reach the Department. These animals were slaughtered immediately after transport.

Group III comprised of animals from the farms of KLDB, Kulathupuzha and Mattupetti, which were having 6 to 7 hours of journey. Trucking of these animals started in morning and reached the Department by the evening of the same day. The animals were given rest in lairage for 18 hours without feed but

with *ad libitum* water. Each animal was given a floor space allowance of 3m² in the lairage.

3.1.2. Collection of blood

From all the animals of group I, 10 ml of blood sample was collected with and without anticoagulant (heparin 20U/ml) by jugular vein puncture before they were slaughtered in the Department. In case of group II animals, the first blood collection was done at CBF and was repeated immediately after transport at the Department. In case of group III animals, blood collection was done at their respective farms, then immediately after the arrival at the Department and another collection was done after giving 18 hours of rest, just before slaughter.

The blood samples collected with anticoagulant were subjected for the estimation of Packed Cell Volume (PCV). From blood samples collected without anticoagulant, serum was separated by centrifuging at 3000 rpm for 20 min and stored at -20⁰C until used for further analysis.

3.1.3. Transporting conditions:

A truck (IVECO with crane) of 4.1m x 2.16 m dimensions was used for transportation. The floor space in the truck was divided into two halves using iron grill and a floor space allowance of 1.77 m²/animal was provided. The vehicle was well ventilated and the animals were allowed to stand in the direction facing the movement of air. Loading and unloading of the animals were done using a ramp of approximately 0.8 m height.

3.1.4. Collection of meat samples:

All the animals were slaughtered in morning hours of the day. Animals were stunned using captive bolt pistol and the neck of the animals was severed to bleed until death. About 1kg of *longissimus dorsi* muscle was taken from the left side of each carcass (region between 9th and 12th thoracic vertebrae)

immediately after dehiding. The muscle was trimmed off from visible fat and connective tissue and was divided into samples for control (C) and irradiated (R). The samples were then packaged in High Density Polyethylene (HDPE) pouches and kept for irradiation.

3.1.5. Irradiation of the samples

The packaged samples (500g) were irradiated with 2.0kGy, at melting ice temperature, using a Gamma Chamber 5000 (BRIT, DAE, Mumbai), where the source of irradiation was Cobalt 60 (^{60}Co). The packaged non irradiated samples were designated as control samples. After irradiation, the control and irradiated samples were stored at 4°C for further studies.

3.2 .ESTIMATION OF HAEMATO-BIOCHEMICAL PARAMETERS

3.2.1. Packed Cell Volume (PCV)

Blood sample collected with anticoagulant was used for the estimation of PCV as per standard procedure (Shastri, 1998).

3.2.2. Serum cortisol concentration

Concentration of serum cortisol was estimated using the Gamma coat [^{125}I] cortisol radioimmunoassay kit (M/s Diasorin, Minnesota, USA) based on the competitive binding principles of radioimmunoassay.

3.2.3. Activity of Creatine Kinase (CK)

Creatine kinase activity was estimated by spectrophotometric determination of NAD coupled glucose-6- PO_4 oxidation using CK NAC, Labkit (M/s Merck Limited, Mumbai, India). One international unit (IU) is the amount of coenzyme that transforms 1 μmol of substrate per minute in standard conditions. The concentration is expressed in units per litre of the sample (U/l).

3.2.4. Estimation of Blood Urea Nitrogen (BUN)

The BUN level was estimated by spectrophotometric determination of urea according to the Urease GLDH (Glutamate dehydrogenase) method (kinetic UV test) using Ecoline kit (M/s Merck Limited, Mumbai, India)

3.2.5. Estimation of creatinine level

Estimation of creatinine was done with spectrophotometric determination of creatinine based on the Jaffe's kinetic method without deproteinisation, using Merkotest kit (M/s Merck Specialities Private Limited, Mumbai, India)

3.3. DETERMINATION OF MUSCLE QUALITY PARAMETERS

3.3.1. pH

About 50g of samples was taken in a beaker and was incised with a scalpel blade. The glass electrode of the digital pH meter (micro pH System 362, Systronics, India) was immersed approximately 2 inches into the muscle without entrapping any air pockets around the bulb of the electrode and reading was taken. The pH of the sample was read directly immediately after collection and then at 6, 12, and 24h post mortem. The probe was thoroughly rinsed with distilled water before each reading (O'Halloran *et al.*, 1997). The standardisation the pH meter was done once in a week using known pH buffers of 4 and 7.

3.3.2. Water Holding Capacity (WHC)

Water Holding Capacity of the fresh meat was determined by using a centrifugation technique modified from Wardlaw *et al.* (1973). Weighed 5g of the sample and was minced in a calibrated centrifuge tube. It was mixed with 7.5 ml of 0.6M sodium chloride and was stirred for 1 min with a glass rod. After holding 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 centrifuge. The volume (V) of supernatant layer was determined. From this the WHC of meat samples was calculated as

$$\text{WHC (in ml/100g)} = (7.5 - V) / 5 \times 100$$

WHC of each sample was calculated at 0, 6, 12 and 24 h post mortem.

3.3.3. Cooking Loss

Cooking loss was estimated using the method explained by Boccard *et al.* (1981). Weighed 80g of sample and was cut into small pieces of approximately equal size (1 inch cube). Then it was placed in a HDPE pouch and sealed in a moderate vacuum to remove the trapped air between the sample and the wall of the pouch. The pouch was then placed in water at 75°C for 50 min and then placed in running tap water at about 15°C for 40 min. Then the meat was taken from the pouch, mopped dry and weighed. Cooking loss was calculated in percentage immediately after collection and 24h post mortem for each sample.

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

3.3.4. Shear Force

The tenderness of the meat samples was determined using the method outlined by (Wheeler, 1997). The meat samples were kept in water bath and cooked to an internal temperature of 80°C, monitored using a thermometer. After cooking, the steaks were refrigerated overnight at 2°C (±2°C) before coring. On the next day, 6 to 8 cylindrical cores with 1.27 cm diameter were removed from the centre of each sample using a hand driven core borer, parallel to the longitudinal axis of muscle fibres. Cores were sheared at the centre by using Universal Testing Machine (UTM) having Warner Bratzler (WB) Shear attachment (Shimadzu Texture Analyser model EZ TEST, Japan), with a

crosshead speed of 200 mm/min and the values were expressed in kilogram force.

3.3.5. Colour

Colour of the meat samples was determined at zero and 24 h of storage post mortem with a Hunterlab Miniscan XE plus spectrophotometer, USA, with diffuse illumination. The instrument was set to measure Hunter L , a and b using illuminant 45/0 and 10° standard observer with an aperture size of 2.54cm. It was calibrated using black and white tiles, and colorimeter scores were recorded for the meat samples.

From the above values of L , a , b the following parameters were also calculated total colour change (ΔE), *hue* angle (h^0) and *Chroma* (C) (Mac Dougall, 1999)

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

$$h^0 = \tan^{-1}(b/a)$$

$$C = (a^2 + b^2)^{1/2}$$

where ΔL denotes the difference in L value, Δa denotes the difference in a value, and Δb denotes difference in b value.

3.3.6. Sensory evaluation

Taste panel assessment of the samples was conducted immediately after collection and 24h post mortem. Cubes of approximately equal size were taken in sufficient number and cooked in boiling water bath (100°C) for 20 min. Codified samples were served to the trained panelists drawn from Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy. They were provided with a scorecard of nine point ascending hedonic scale (appended) to assess colour, flavour, juiciness, tenderness and overall acceptability.

3.4. STATISTICAL ANALYSIS

The data recorded were statistically analysed in order to compare the effect of transport within the group by students paired *t* test and between groups by students unpaired *t* test. Analysis of variance was carried out to compare the groups (Snedecor and Cochran, 1994)

SCORE CARD FOR TASTE PANEL EVALUATION

Name of The Product: Cooked Meat

date:

sample no:

	Colour		Flavour		Juiciness		Tenderness		Overall acceptability																															
Extremely Appealing	<table border="1" style="width: 30px; height: 30px; border-collapse: collapse;"> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> </table>							Delicious	<table border="1" style="width: 30px; height: 30px; border-collapse: collapse;"> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> </table>							More Juicy	<table border="1" style="width: 30px; height: 30px; border-collapse: collapse;"> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> </table>							Very Tender	<table border="1" style="width: 30px; height: 30px; border-collapse: collapse;"> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> </table>							More Acceptable	<table border="1" style="width: 30px; height: 30px; border-collapse: collapse;"> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> </table>							9 8 7
Appealing	<table border="1" style="width: 30px; height: 30px; border-collapse: collapse;"> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> </table>							Desirable	<table border="1" style="width: 30px; height: 30px; border-collapse: collapse;"> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> </table>							Juicy	<table border="1" style="width: 30px; height: 30px; border-collapse: collapse;"> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> </table>							Tender	<table border="1" style="width: 30px; height: 30px; border-collapse: collapse;"> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> </table>							Acceptable	<table border="1" style="width: 30px; height: 30px; border-collapse: collapse;"> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> </table>							6 5 4
Less appealing	<table border="1" style="width: 30px; height: 30px; border-collapse: collapse;"> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> </table>							Not so desirable	<table border="1" style="width: 30px; height: 30px; border-collapse: collapse;"> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> </table>							Less Juicy	<table border="1" style="width: 30px; height: 30px; border-collapse: collapse;"> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> </table>							Tough	<table border="1" style="width: 30px; height: 30px; border-collapse: collapse;"> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> </table>							Less Acceptable	<table border="1" style="width: 30px; height: 30px; border-collapse: collapse;"> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> <tr><td style="width: 15px; height: 15px;"></td><td style="width: 15px; height: 15px;"></td></tr> </table>							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:

Results

RESULTS

A total 27 culled cattle were transported from different farms. They were grouped into three categories, *viz.*, group I comprising of nine animals which were not subjected to transport (control group); group II with other nine animals which were transported from farm about 50 km away and were subjected to 2 hour transport to reach the Department of LPT. They were slaughtered immediately after the transport. The third group, comprising of 9 animals, was subjected to 6 to 7 hours of journey to the Department and were given rest in lairage for 18h. All these animals were slaughtered under hygienic precautions.

4.1. HAEMATO-BIOCHEMICAL PARAMETERS DURING STRESS

In all the groups, blood samples were collected on three occasions namely, prior to transport, immediately after bringing to the Department in group II and III and immediately before slaughter (after rest) in group III animals. The blood parameters studied *viz.*, cortisol, CK, BUN, creatinine and PCV prior to transport of the animals in the three groups are shown in Table 1 and the variations of the same has been shown in Figure 1. It was found from the analysis of data prior to transport, that there was no significant difference between the various parameters studied. It was observed that important parameters of stress like cortisol and CK were within the range and varied non significantly in all the groups.

The blood parameters investigated were repeated immediately after transport in case of group II animals and immediately after transport and before slaughter in group III animals. The data are given in Table 2, Table 3 and Table 4. It was observed that there was a significant increase in case of cortisol ($P < 0.01$), CK activity ($P < 0.01$) and BUN ($P < 0.05$), whereas creatinine and PCV did not show any significant change in group II, due to transport.

Table 1: Certain haemato-biochemical parameters in cattle before transport

	PCV ^{NS} (%)	Cortisol ^{NS} (nmol/L)	Creatine kinase ^{NS} (U/L)	BUN ^{NS} (mg/dl)	Creatinine ^{NS} (mg/dl)
Group I	35.09±0.93	20.91±1.53	84.78±2.36	22.89±0.96	2.33±0.17
Group II	36.05±0.83	21.29±1.39	85.00±2.31	22.56±1.61	2.44±0.18
Group III	35.94±1.63	22.44±1.58	86.11±2.19	21.89±1.37	2.56±0.24

Table 2: Certain haemato-biochemical traits in cattle (group II) before transport and after transport

	PCV (%)	Cortisol (nmol/L)	Creatine kinase(U/L)	BUN (mg/dl)	Creatinine (mg/dl)
Before tr	36.05±0.83	21.29±1.39	85±2.31	22.56±1.61	2.44±0.18
After tr	36.74±0.58	39.04±1.22	196.±10.4	24.56±1.12	2.78±0.15
t stat	1.09 ^{NS}	11.33**	11.13**	2.31*	1.41 ^{NS}

** indicates the observation in that column are significantly ($P < 0.01$) different

* indicates the observation in that column are significantly ($P < 0.05$) different

^{NS} indicates the observations in that column are not significantly ($P > 0.05$) different

BUN - Blood Urea Nitrogen

PCV - Packed Cell Volume

Table 3: Certain haemato-biochemical traits in cattle (group III) before transport, after transport and after rest

	PCV (%)	Cortisol (nmol/L)	Creatine kinase(U/L)	BUN (mg/dl)	Creatinine (mg/dl)
<i>Bt</i>	35.94±1.63	22.44±1.58	86.11±2.19	21.89±1.37	2.56±0.24
<i>At</i>	37.69±1.51	72.14±6.72	443.11±42.12	26.11±1.64	3.44±0.18
t stat	1.92 ^{NS}	8.04**	8.79**	2.41*	2.53*
<i>At</i>	37.69±1.51	72.14±6.72	443.11±42.12	26.11±1.64	3.44±0.18
<i>Bs</i>	36.61±1.55	31.51±2.63	292.33±43.95	28.44±2.19	2.78±0.28
t stat	1.68 ^{NS}	7.74**	6.46**	0.68 ^{NS}	1.79 ^{NS}
<i>Bt</i>	35.94±1.63	22.44±1.58	86.11±2.19	21.89±1.37	2.56±0.24
<i>Bs</i>	36.61±1.55	31.51±2.63	292.33±43.93	28.44±2.19	2.78±0.28
t stat	0.49 ^{NS}	3.21**	4.78**	2.55*	0.69 ^{NS}

At- after transport

Bt- before transport

Bs- before slaughter

Table 4: Effect of transport on certain haemato-biochemical traits in group II and III cattle

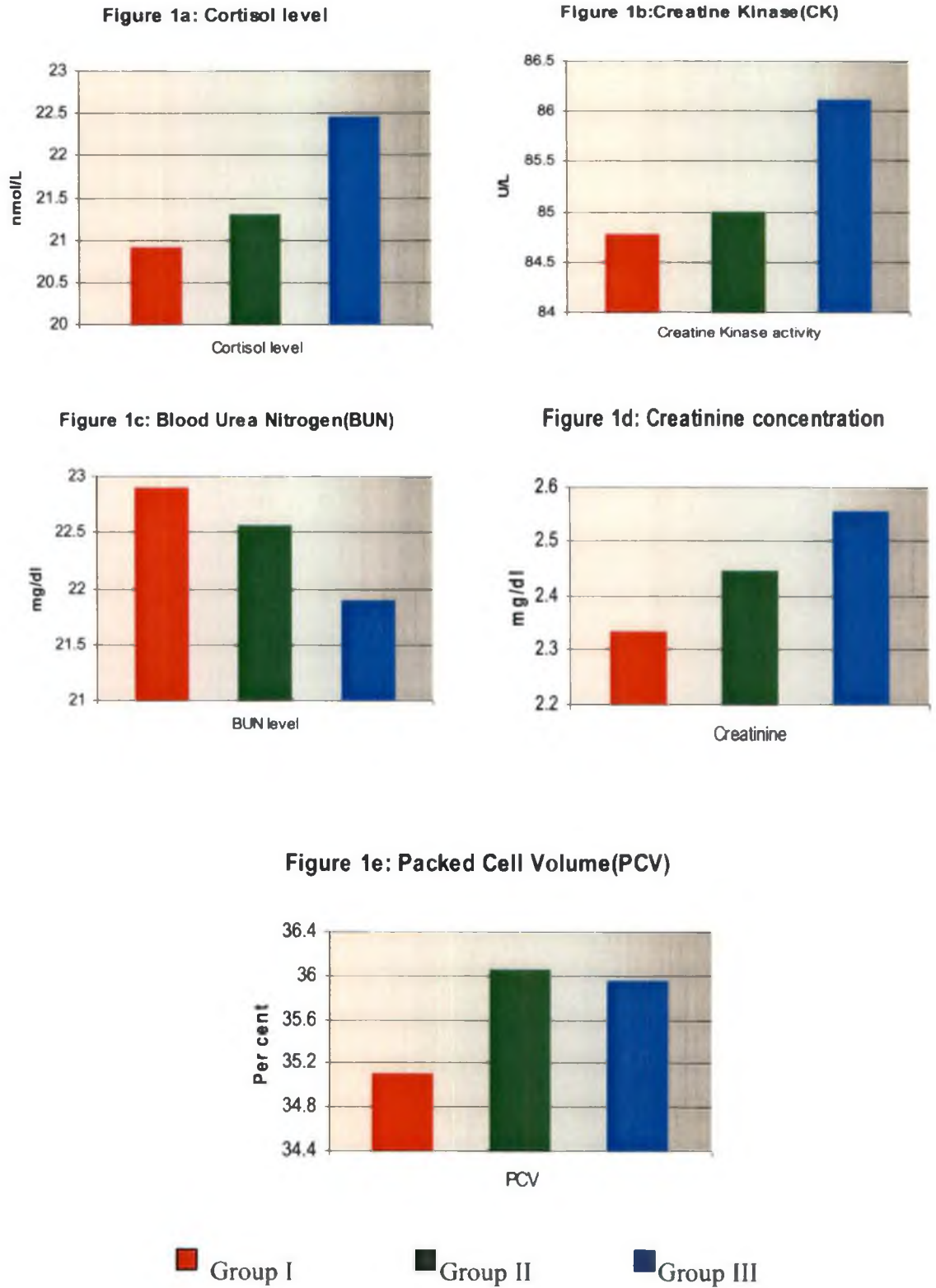
	PCV (%)	Cortisol (nmol/L)	Creatine kinase(U/L)	BUN (mg/dl)	Creatinine (mg/dl)
<i>At_{II}-Bt_{II}</i>	0.69±0.64	17.76±1.57	111±9.97	2±0.87	0.33±0.24
<i>At_{III}-Bt_{III}</i>	1.75±0.91	49.7±6.18	357±40.62	4.22±1.99	0.89±0.35
t stat	0.95 ^{NS}	5.01**	5.88**	1.02 ^{NS}	1.31 ^{NS}

** indicates the observation in that column are significantly (P<0.01) different

* indicates the observation in that column are significantly (P<0.05) different

^{NS} indicates the observations in that column are not significantly (P>0.05) different

Figure 1. Haemato-biochemical traits in cattle before transport



The values immediately after slaughter were significantly higher with respect to cortisol ($P<0.01$), CK ($P<0.01$), BUN ($P<0.05$) and creatinine ($P<0.05$) due to transport in case of group III animals. It was also observed from the data that a sufficient rest of 18h caused a significant ($P<0.01$) reduction in case of values of cortisol and CK. A similar reduction was not noticed in case of BUN, creatinine and PCV, even after 18h rest.

Even after 18h rest, the values of cortisol, CK and BUN did not return to its basal level whereas creatinine and PCV were having values which were non significantly different from the before transport values. The after transport values were compared in case of group II and III animals and found that the increase in cortisol and CK values of group III were significantly ($P<0.01$) higher than that in group II whereas BUN, creatinine and PCV values were non significant.

4.2. PHYSICOCHEMICAL QUALITIES OF MEAT

4.2.1. pH of meat

Meat samples collected in HDPE packets, one sample from each animal, were subjected to irradiation employing gamma rays at 2.0kGy using Cobalt 60 as source. The control and irradiated samples were subjected to estimation of pH immediately after collection and then at 6, 12 and 24h storage at 4°C. The data have been shown in Table 5. The fall of pH in control and irradiated samples are presented in Figure 2. It was observed that the values among groups were non significant during the storage period as well as in irradiated groups except the control meat samples of group III at 6 and 12 h storage.

The reduction of pH due to storage in case of control and irradiated samples were significantly ($P<0.05$) different (Table 6). A significant difference was seen in the case of pH fall at zero to 6h, 6 to 12h and 12 to 24 h of control and irradiated

Table 5: pH of meat at different storage periods (control and irradiated)

	0 th		6 th		12 th		24 th	
	Control	Irradiated	Control	Irradiated	Control	Irradiated	Control	Irradiated
Group I	6.71±0.04 ^a	6.65±0.05 ^a	5.98±0.02 ^{ab}	5.88±0.03 ^a	5.71±0.02 ^a	5.64±0.02 ^a	5.58±0.01 ^a	5.57±0.02 ^a
Group II	6.7±0.06 ^a	6.67±0.06 ^a	5.95±0.04 ^a	5.87±0.02 ^a	5.75±0.02 ^a	5.69±0.02 ^a	5.59±0.02 ^a	5.57±0.03 ^a
Group III	6.73±0.06 ^a	6.71±0.06 ^a	6.05±0.03 ^b	5.91±0.03 ^a	5.84±0.03 ^b	5.71±0.03 ^a	5.64±0.02 ^a	5.6±0.02 ^a

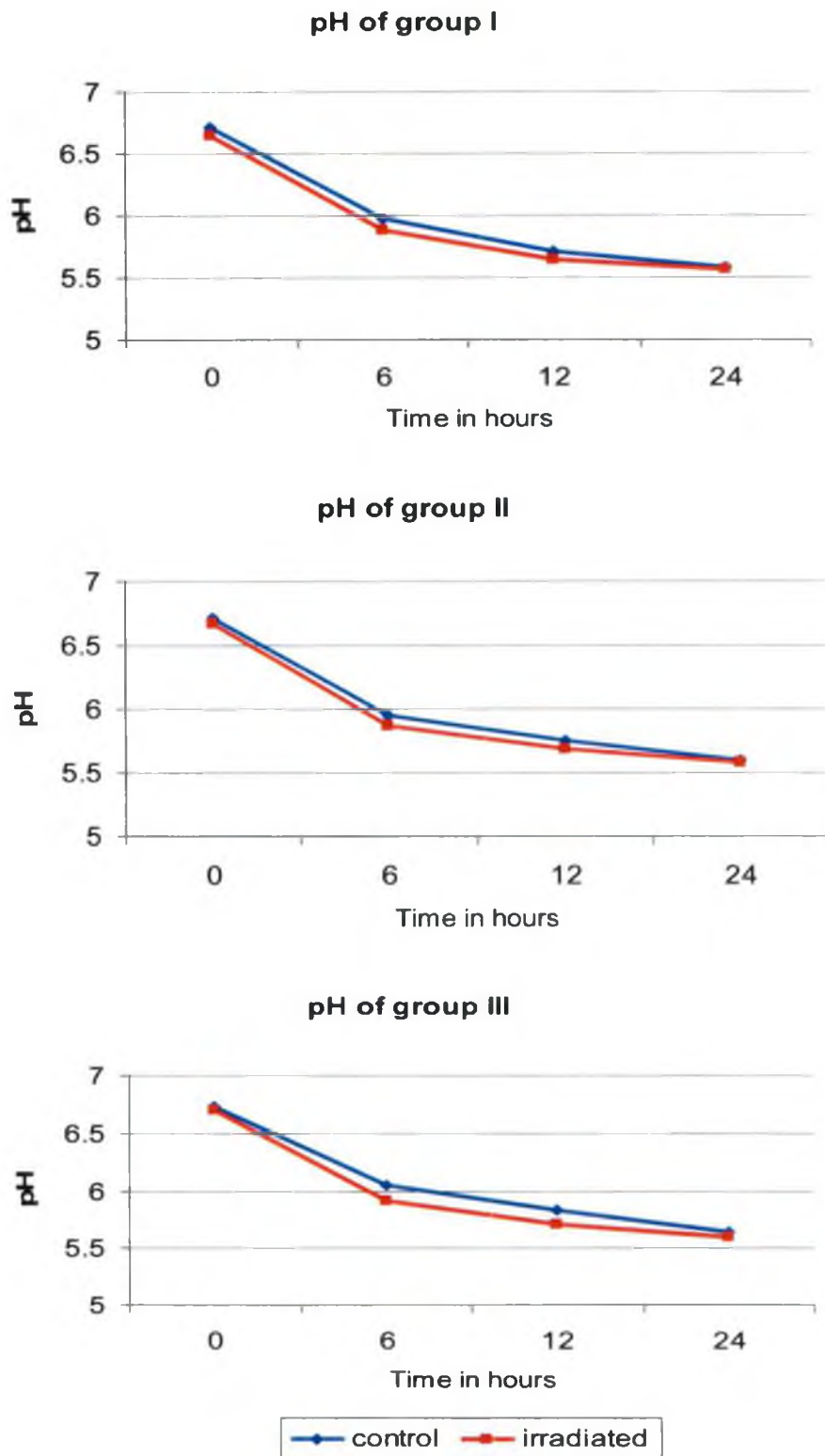
Observations having same superscript in the same column are not significantly ($P>0.05$) different

Table 6 : Fall of pH in control and irradiated samples during storage

	0-6	6-12	12-24	mean
Control	0.72 ^b	0.22 ^c	0.16 ^c	0.37±0.03 ^a
Irradiated	0.79 ^a	0.21 ^c	0.1 ^d	0.36±0.04 ^a
Mean	0.76±0.02 ^a	0.22±0.01 ^b	0.13±0.01 ^c	

Observations having same superscript in the same rows and/or column are not significantly ($P>0.05$) different

Figure 2. Fall of pH in control and irradiated meat samples at different storage periods



samples. The group means were non significant in case of control and irradiated samples.

The angular measurements of tangent of pH decline at different storage periods were taken. The angular measurement at zero to 6h was found to be less than the subsequent angular measurements at 6 to 12h, 12 to 24h of storage. This indicated that the fall in pH from zero to 6h was a steep fall which was followed by a gradual fall up to 24 h.

4.2.2. Water holding capacity

The WHC of the meat sample (control and irradiated) was done immediately after collection of the sample and then at 6, 12 and 24 h of storage at 4°C. A similar trend like that of pH was observed (Table 7). The data are graphically presented in Figure 3. In the case of control and irradiated samples among groups, no significant difference was noticed during the entire period of storage. It was observed that there was a significant ($P<0.05$) reduction in WHC during storage (Table 8). Such a variation was not observed between control and irradiated samples. The group means were not significant with respect to control and irradiated samples, whereas group means were significantly ($P<0.05$) different during storage period.

Initially, a highest WHC of 26.67 ± 1.25 ml/100g and 25.56 ± 1.59 ml/100g in group III control and irradiated samples respectively were observed immediately after collection. By 24h of storage, the values were significantly reduced to 11.56 ± 0.8 ml/100g and 10.00 ± 0.94 ml/100g under chiller conditions. In all the groups, as the storage period enhanced, the WHC reduced, whereas no significant difference was observed between the groups.

4.2.3. Cooking loss

The cooking loss of meat of control and irradiated samples of group I, II and group III animals were conducted immediately after collecting the sample

Table 7: Water holding capacity (ml/100g) at different storage periods (control and irradiated)

	0 th		6 th		12 th		24 th	
	Control	Irradiated	Control	Irradiated	Control	Irradiated	Control	Irradiated
Group I	25.56±1.48 ^a	23.78±0.91 ^a	19.78±0.52 ^a	16.22±0.62 ^a	14.22±0.78 ^a	11.78±0.7 ^a	10.22±0.62 ^a	9.33±0.82 ^a
Group II	26.44±1.24 ^a	24.00±1.00 ^a	19.56±0.80 ^a	16.44±0.73 ^a	14.67±0.88 ^a	11.78±0.78 ^a	11.11±0.8 ^a	9.78±0.94 ^a
Group III	26.67±1.25 ^a	25.56±1.59 ^a	20.67±0.82 ^a	17.78±0.7 ^a	15.33±0.67 ^a	12.67±0.94 ^a	11.56±0.8 ^a	10.00±0.94 ^a

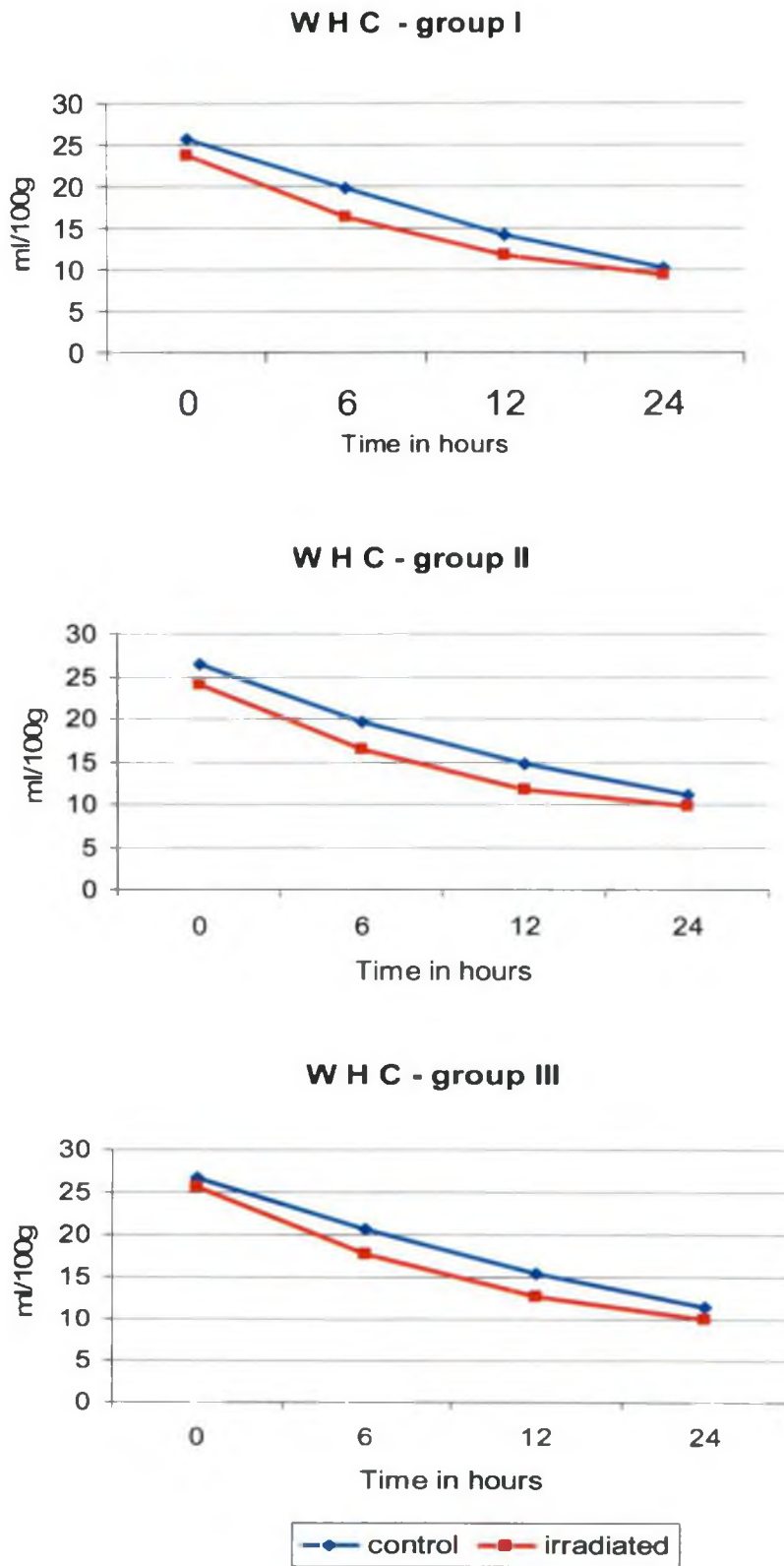
Observations having same superscript in the same column are not significantly ($P>0.05$) different

Table 8: Fall of WHC in control and irradiated samples during storage

	0-6	6-12	12-24	mean
Control	6.22 ^{ab}	5.26 ^{bc}	3.78 ^{cd}	5.09±0.34 ^a
Irradiated	7.63 ^a	4.74 ^{bc}	2.37 ^d	4.91±0.36 ^a
Mean	6.93±0.47 ^a	5±0.32 ^b	3.07±0.31 ^c	

Observations having same superscript in the same rows and/or column are not significantly ($P>0.05$) different

Figure 3. Fall of water holding capacity in control and irradiated meat samples at different storage periods



and at 24h of storage in chiller condition (4°C). The data are presented in Table 9. The changes in cooking loss of control and irradiated samples at different storage period have been shown in Figure 4.

The highest cooking loss of 28.13 ± 0.72 per cent was observed in group III irradiated meat sample at zero hour and 34.37 ± 0.56 per cent in 24 h stored sample. The values among groups in control and irradiated samples were not significantly different. The cooking loss observed at zero hour was not significantly different between control and irradiated samples whereas a significant ($P < 0.05$) increase in cooking loss was observed in irradiated samples at 24h compared to the control samples (Table 12A and B). As storage period increased from zero to 24h the values were significantly ($P < 0.01$) increased both in control and irradiated group (Table 12) with a non significant difference among groups. The lowest values recorded among the groups were 26.19 ± 0.74 per cent in control and 26.9 ± 0.94 per cent in irradiated meat samples of group I at zero hour.

4.2.4. Shear force

The shear force values of the meat sample were determined by using UTM having WB shear attachment and expressed in kilogram force. The data are given in Table 10. The Figure 5 shows a comparison of shear force required to shear a uniform core of meat sample. A significant difference was observed in shear force value in control samples of group I than that of group II and III at zero hour. Similarly such a difference was noticed in case of irradiated samples both at zero and 24h of observations. It was also observed a significant ($P < 0.01$) increase in shear force value from 8.46 ± 0.1 kgf to 11.95 ± 0.2 kgf by 24h of storage at chiller conditions (Table 12). The group I irradiated sample recorded the lowest shear force of 7.79 ± 0.18 kgf and the highest value was 9.25 ± 0.15 kgf in group III control meat samples. Among the storage periods, the control

samples had a significantly ($P<0.01$) higher value than that of its counter part in irradiated meat sample through out the study (Table 12A and B).

4.2.5. Colour values

The colour values of meat samples were determined immediately after collection and irradiation and at 24h of storage using Hunterlab Miniscan XE plus spectrophotometer.

4.2.5.1. Colour *L* values

The group I control sample had a significantly ($P<0.05$) higher *L* value compared to group II and group III. Similarly group III had a lower value than the other two groups. Such a difference among groups was not noticed in case of irradiated samples (Table 12). The irradiated samples at zero hour were having a significantly ($P<0.01$) higher *L* values than the control samples and on storage this difference became non significant (Table 12A and B). As storage period increased the colour faded significantly ($P<0.01$) as evidenced by comparison of colour between zero and 24h post mortem (Table 12 and Figure 6).

4.2.5.2. Colour *a* value

The mean and standard error of colour *a* values are shown in (Table 13), compared with the help of a graph (Figure 7). It was observed that there was no significant difference at zero hour among the control and irradiated meat samples between the groups. Group III control and irradiated meat samples were significantly ($P<0.05$) different from other groups at 24h of observation and became paler. The highest score was 13.27 ± 0.9 in group II irradiated meat samples and the lowest was 8.99 ± 0.6 in control meat samples obtained from group III animals. On comparison of colour *a* values, it was observed that as storage time enhanced from zero to 24h a significant ($P<0.01$) increase in redness was noticed due to storage in meat samples (Table 12).

Table 9: Cooking loss of meat samples (control and irradiated) in per cent

	0 th hour		24 th hour	
	Control	Irradiated	Control	Irradiated
Group I	26.19±0.74 ^a	26.90±0.94 ^a	33.78±0.32 ^a	34.06±0.46 ^a
Group II	27.30±0.65 ^a	27.79±0.65 ^a	33.17±0.55 ^a	33.60±0.76 ^a
Group III	27.92±0.68 ^a	28.13±0.72 ^a	33.49±0.63 ^a	34.37±0.56 ^a

Table 10: Shear force values of meat samples (control and irradiated) in kgf

	0 th hour		24 th hour	
	Control	Irradiated	Control	Irradiated
Group I	8.21±0.18 ^a	7.79±0.18 ^a	11.60±0.29 ^a	10.99±0.51 ^a
Group II	8.93±0.13 ^b	7.96±0.25 ^{ab}	11.97±0.36 ^a	11.27±0.46 ^{ab}
Group III	9.25±0.15 ^b	8.61±0.26 ^b	13.29±0.37 ^b	12.55±0.52 ^b

Table 11: Colour *L* values of meat samples (control and irradiated)

	0 th hour		24 th hour	
	Control	Irradiated	Control	Irradiated
Group I	25.91±1.11 ^a	28.36±1.27 ^a	31.30±0.81 ^a	31.54±1.60 ^a
Group II	23.26±0.97 ^b	27.79±1.40 ^a	30.63±0.89 ^a	31.40±1.32 ^a
Group III	22.15±0.85 ^b	25.03±1.33 ^a	27.00±1.03 ^b	28.17±1.36 ^a

Observations having same superscript in the same column are not significantly ($P>0.05$) different

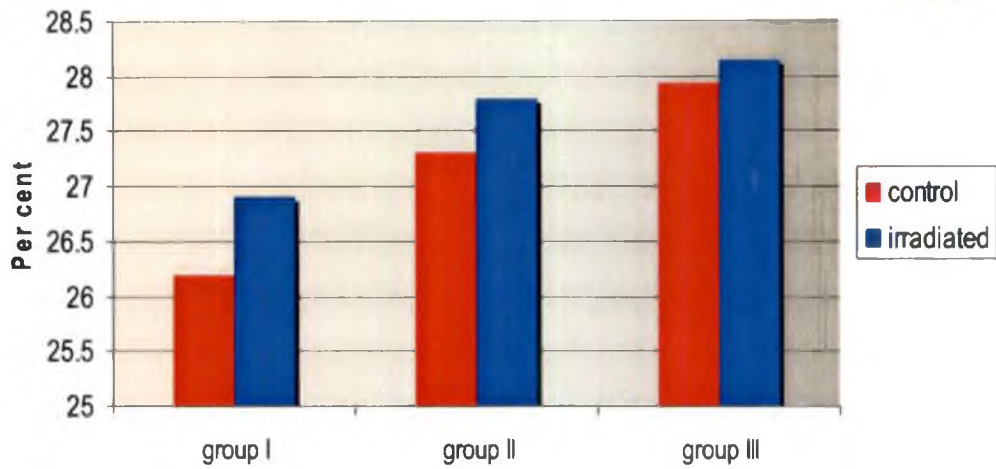
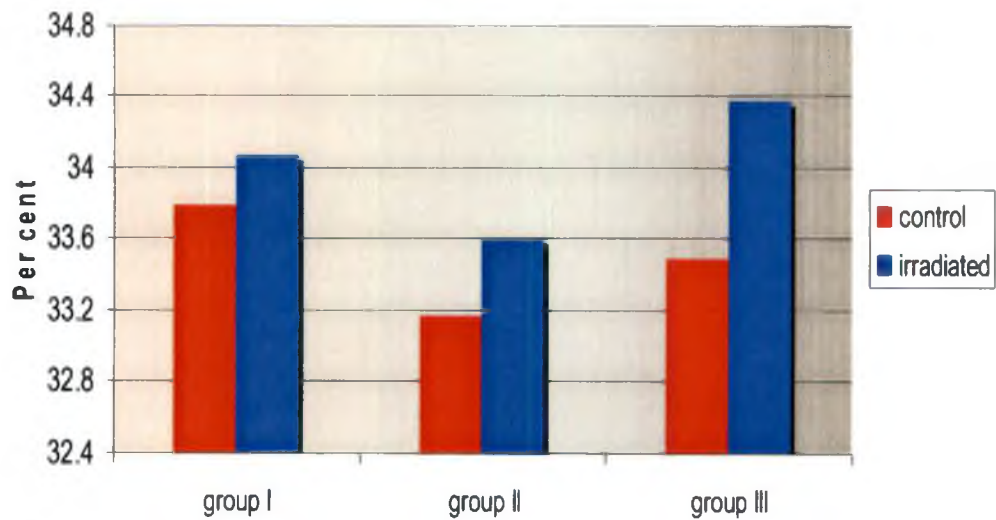
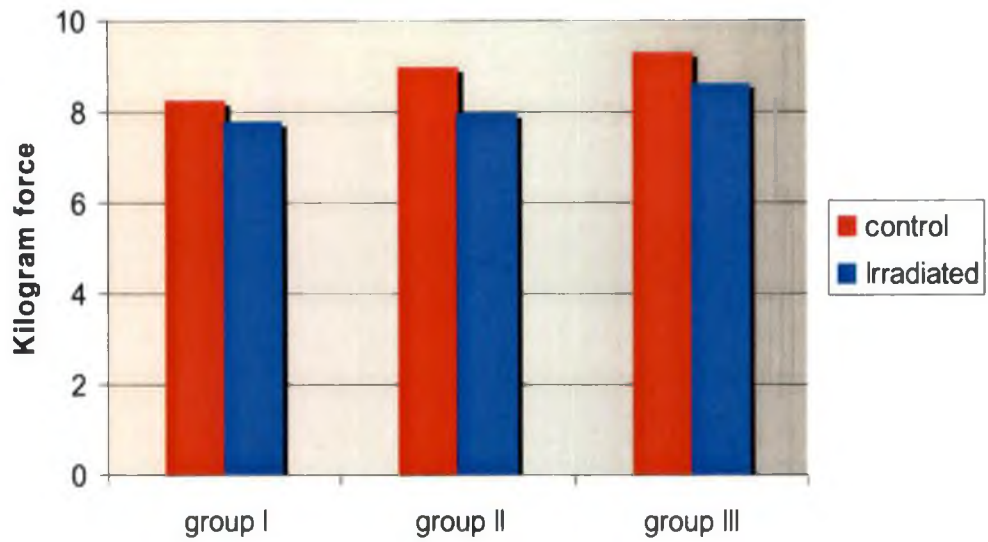
Figure 4. Cooking loss of meat samples at zero and 24 hour**Cooking loss at zero hour****Cooking loss at 24 hour**

Figure 5. Shear force (SF) values of meat samples at zero and 24 hour

S F values at zero hour



S F values at 24 hour

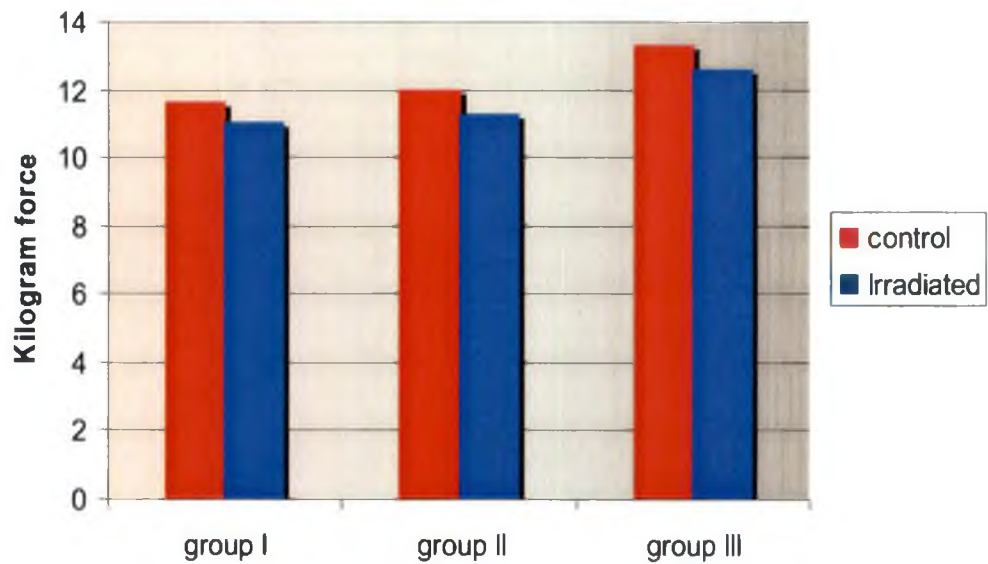


Figure 6. Colour *L* values of meat samples at zero and 24 hour

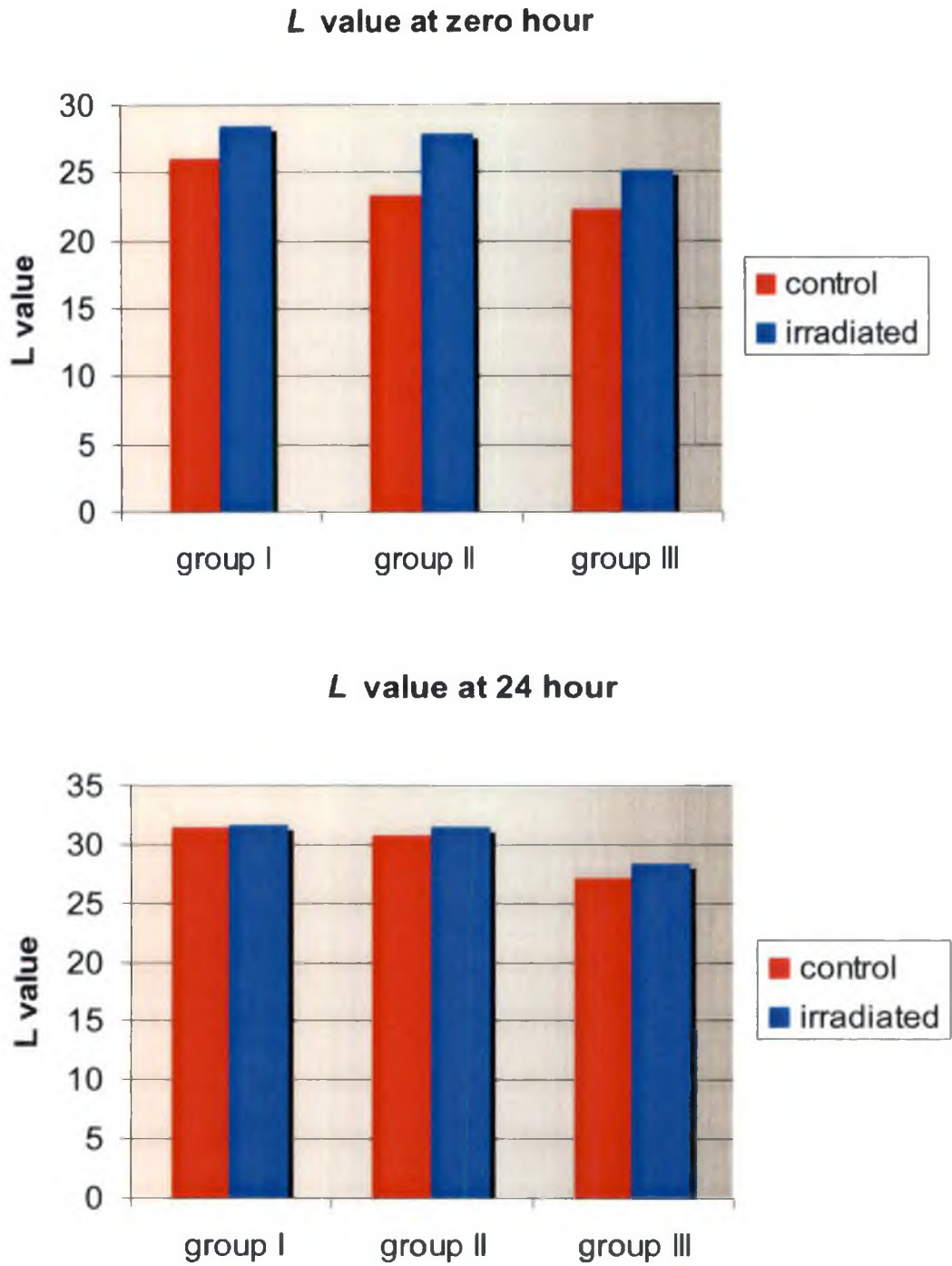


Table 12: Comparison between 0 and 24 hours values of different parameters

	Cooking loss	Shear force	<i>L</i> value	<i>a</i> value	<i>b</i> value	<i>C</i> value	<i>hue angle</i>
0 th hour	27.37±0.01	8.46±0.00	25.42±0.07	10.29±0.00	5.79±0.00	11.82±0.01	28.98±0.04
24 th hour	33.74±0.00	11.95±0.00	30.01±0.06	11.9±0.01	7.54±0.00	14.11±0.01	32.15±0.04
t stat	20.95**	26.18**	11.64**	5.33**	11.71**	7.09**	7.65**

** indicates the observations in that column are significantly ($P < 0.01$) different

Table 12A : Comparison of parameters between control and irradiated meat samples at zero hour

	Cooking loss	Shear force	L value	a value	b value	C value	hue angle
Control	27.14±0.03	8.80±0.00	23.77±0.11	9.56±0.01	5.12±0.00	10.86±0.02	27.92±0.18
Irradiated	27.61±0.04	8.12±0.00	27.06±0.31	11.01±0.01	6.46±0.00	12.79±0.02	30.04±0.12
t stat	1.42 ^{NS}	5.68 ^{**}	5.74 ^{**}	3.71 ^{**}	5.72 ^{**}	4.37 ^{**}	3.67 ^{**}

Table 12B : Comparison of parameters between control and irradiated meat samples at 24th hour

	Cooking loss	Shear force	L value	a value	b value	C value	hue angle
Control	33.48±0.01	12.29±0.00	29.65±0.13	11.93±0.01	7.35±0.00	14.03±0.02	31.36±0.06
Irradiated	34.01±0.01	11.60±0.01	30.37±0.34	11.86±0.05	7.73±0.01	14.19±0.07	32.94±0.22
t stat	2.23 [*]	3.67 ^{**}	1.58 ^{NS}	0.16 ^{NS}	1.85 ^{NS}	0.37 ^{NS}	2.52 [*]

** indicates the observations in that column are significantly (P<0.01) different

* indicates the observations in that column are significantly (P<0.05) different

^{NS} indicates the observations in that column are not significantly (P>0.05) different

4.2.5.3. Colour *b* value

The data of *b* value recorded in control and irradiated meat samples are shown in Table 14. The comparison among groups at zero and 24 h of control and irradiated samples are shown in Figure 8. Among groups, it was observed that a significantly ($P<0.01$) higher yellow value in irradiated samples compared to the control samples at zero hour which became non significant by 24 h of storage (Table 12A and B). As time of storage enhanced, the yellowness of both irradiated and control samples increased significantly ($P<0.01$) as shown in Table 12. The lowest *b* value was recorded, like *L* value and *a* value, in group III animals.

4.2.5.4. Colour *C* value

The colour Chroma (*C*) value was obtained from the calculation as follows

$$C = (a^2 + b^2)^{1/2}$$

The values are shown in (Table 15). It was observed that similar to that of *L*, *a* and *b* values, a lowest score was recorded in group III. Among groups, I and II were significantly ($P<0.05$) different from that of group III in control at zero hour post mortem. The values of group III, both control and irradiated, were significantly ($P<0.05$) different from that of group I and II at 24h of storage. Comparison of values between zero and 24h storage (Table 12) showed a significantly ($P<0.01$) higher value at 24h indicating better *Chroma*. Between the control and irradiated samples, irradiated samples had a significantly ($P<0.01$) higher *Chroma* at zero hour which became non significant at 24h of storage.

Table 13: Colour *a* values of meat samples (control and irradiated)

	0 th hour		24 th hour	
	Control	Irradiated	Control	Irradiated
Group I	10.14±0.86 ^a	11.34±0.6 ^a	12.47±0.61 ^a	12.61±0.75 ^a
Group II	9.54±0.29 ^a	11.44±0.5 ^a	12.52±0.48 ^a	13.27±0.9 ^a
Group III	8.99±0.6 ^a	10.25±0.47 ^a	10.8±0.53 ^b	9.7±0.33 ^b

Table 14: Colour *b* values of meat samples (control and irradiated)

	0 th hour		24 th hour	
	Control	Irradiated	Control	Irradiated
Group I	6.05±0.42 ^a	6.97±0.39 ^a	7.96±0.38 ^a	8.19±0.47 ^a
Group II	4.95±0.15 ^b	6.84±0.43 ^a	7.76±0.26 ^a	8.52±0.48 ^a
Group III	4.35±0.36 ^b	5.59±0.43 ^b	6.32±0.35 ^b	6.48±0.29 ^b

Table 15: Colour *C* values of meat samples (control and irradiated)

	0 th hour		24 th hour	
	Control	Irradiated	Control	Irradiated
Group I	11.83±0.68 ^a	13.32±0.49 ^a	14.81±0.51 ^a	15.07±0.56 ^a
Group II	10.76±0.28 ^b	13.34±0.63 ^a	14.75±0.47 ^a	15.79±0.99 ^a
Group III	9.99±0.69 ^b	11.7±0.59 ^a	12.52±0.60 ^b	11.7±0.3 ^b

Observations having same superscript in the same column are not significantly ($P>0.05$) different

Figure 7. Colour *a* values of meat samples zero and 24 hour

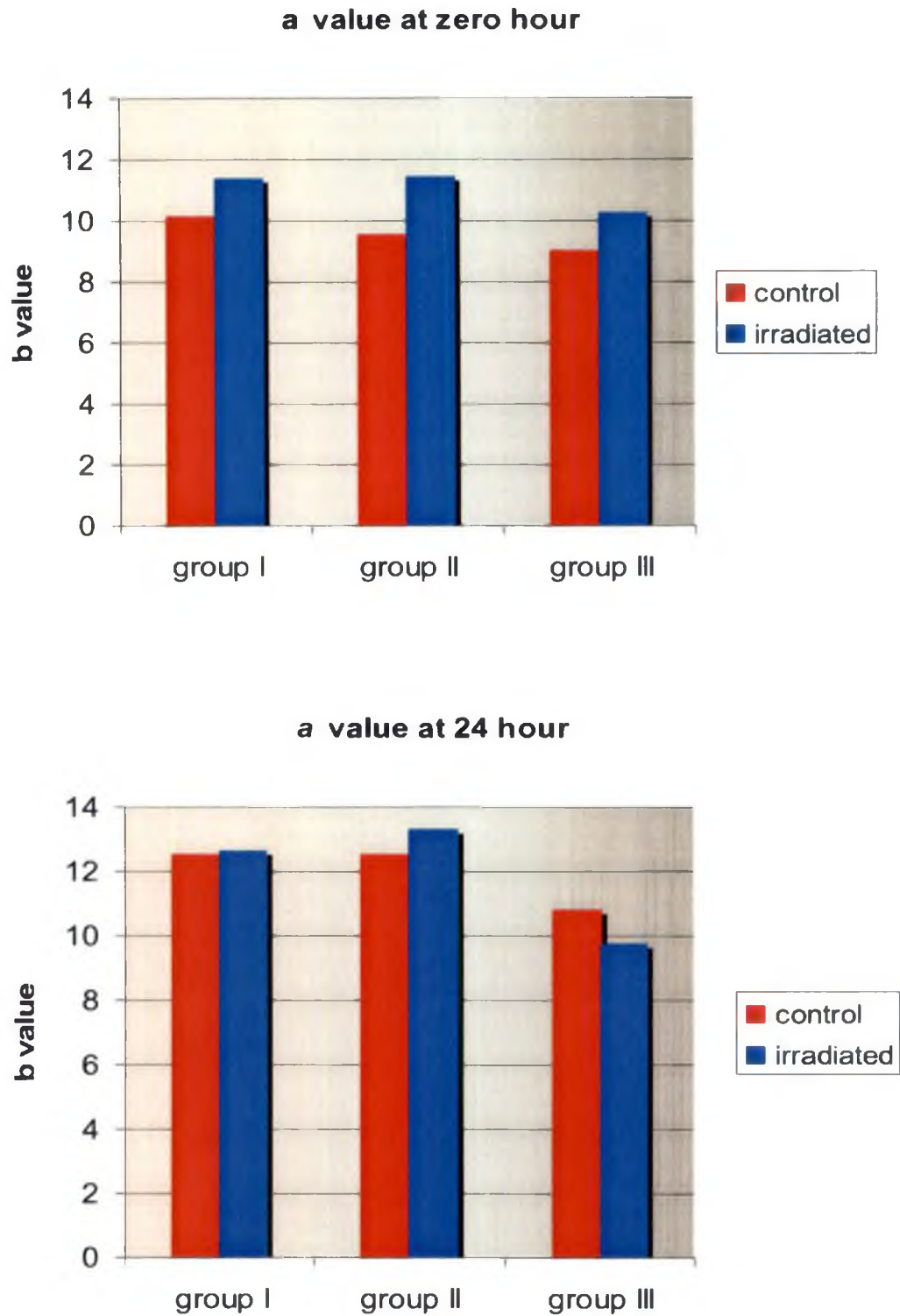
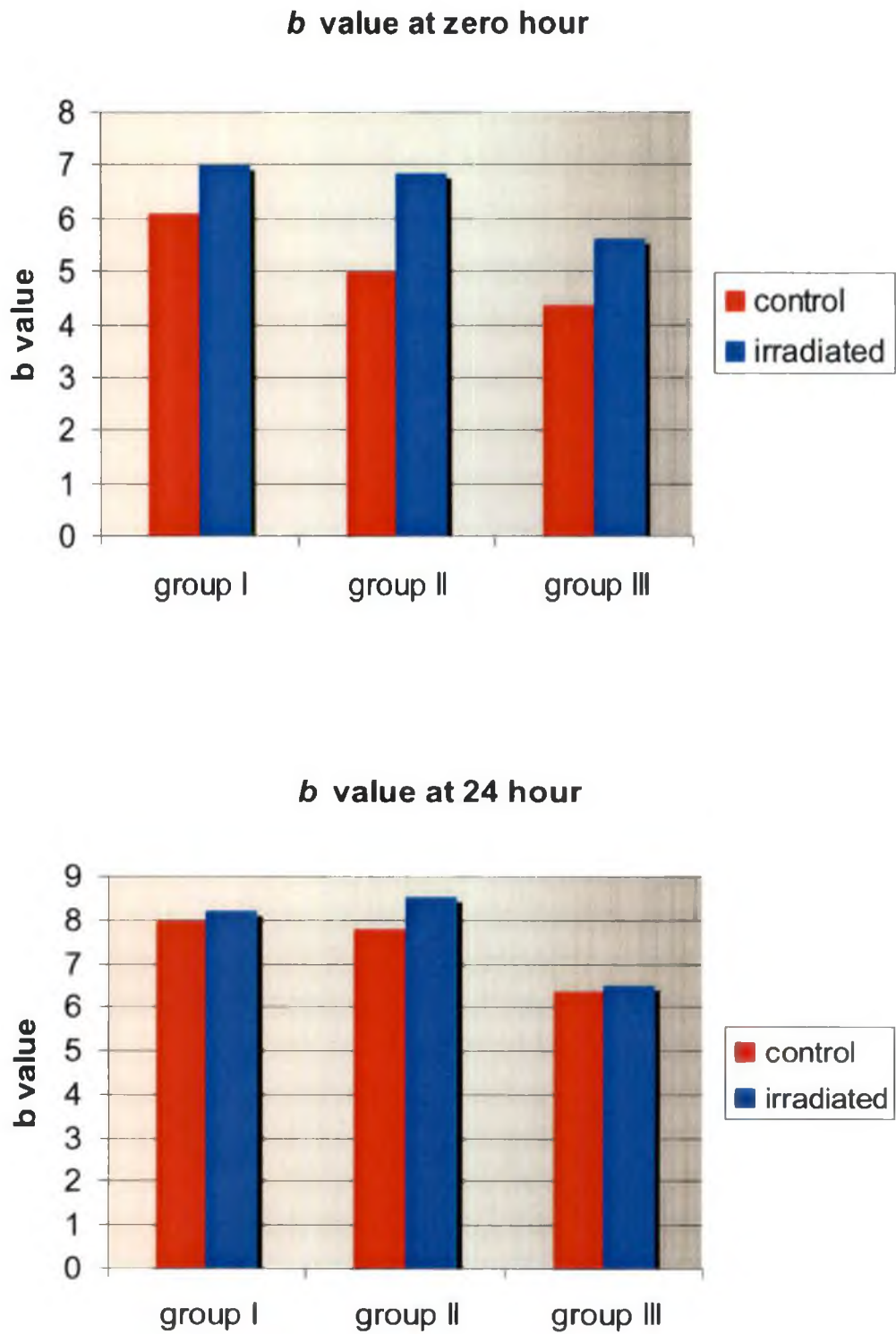


Figure 8. Colour *b* values of meat samples at zero and 24 hour



4.2.5.5. Colour hue angle values

The *hue* value of the meat was designed as *hue angle* and abbreviated as h° . It was calculated by the formula $\tan^{-1}(b/a)$. The values of *hue angle* are as shown in Table 16. Among groups, group I control was significantly different ($P < 0.05$) from that of II and III at zero hour and there was no significant difference among the groups in control and irradiated samples at 24h of storage. Between control and irradiated samples, irradiated samples had a significantly higher value at zero hour ($P < 0.01$) and 24h ($P < 0.05$) of storage than that of corresponding control samples (Table 12A and B).

4.2.5.6 Total colour difference

The total colour difference between control and irradiated samples at zero and 24 hour (Table 17) showed that t statistic values were significantly ($P < 0.01$) different in group I animals. There was a higher difference between control and irradiated samples at zero hour than that at 24 h of storage.

A comparison of total colour difference between zero and 24h of storage in control and irradiated samples is shown in (Table 18). As evidenced from statistical analysis ('t' test), the values were significantly ($P \leq 0.05$) different between control and irradiated samples at zero and 24h storage in all the groups.

The comparison of total colour changes in groups between control and irradiated samples and the total colour difference between groups are shown in Table 19 and 20 respectively. It was observed that there was no group difference in Group I-II, Group II-III and Group I-III at zero and 24h with respect to irradiation. Total colour differences between groups were non significant except between Group II-Group III and Group I-Group III of control samples at 24h of storage.

Table 16: Colour *hue angle* values of meat samples (control and irradiated)

	0 th hour		24 th hour	
	Control	Irradiated	Control	Irradiated
Group I	30.8±1.02 ^a	31.37±0.86 ^a	32.32±0.68 ^a	32.8±1.25 ^a
Group II	27.38±0.86 ^b	30.51±0.85 ^{ab}	31.62±0.99 ^a	32.61±0.85 ^a
Group III	25.58±0.76 ^b	28.22±1.11 ^b	30.15±0.85 ^a	33.4±1.51 ^a

Observations having same superscript in the same column are not significantly (P>0.05) different

Table 17: Comparison total colour (ΔE) difference between irradiated and control samples at 0th and 24th hours

	Group I	Group II	Group III
ΔE_{R-C} 0 th	4.37±0.61	5.57±1.1	3.96±0.96
ΔE_{R-C} 24 th	2.42±0.45	3.00±0.86	3.36±0.51
t stat	2.57*	1.83 ^{NS}	0.56 ^{NS}

Table 18: Comparison total colour (ΔE) difference between 0th and 24th hour in control and irradiated meat

	Group I	Group II	Group III
ΔE_{0-24} C	6.43±0.56	8.61±0.85	5.9±0.66
ΔE_{0-24} R	4.58±0.59	5.93±0.93	3.83±0.66
t stat	2.25* ✓	2.12* ✓	2.20* ✓

* indicates the observation in that column are significantly (P<0.05) different

^{NS} indicates the observations in that column are not significantly (P>0.05) different

Table 19: Comparison of total colour (ΔE) changes in groups between control and irradiated meat

	0 th			24 th		
	G _I -G _{II}	G _{II} -G _{III}	G _I -G _{III}	G _I -G _{II}	G _{II} -G _{III}	G _I -G _{III}
ΔE_C	4.57±0.74	3.91±0.67	5.68±0.91	3.53±0.44	6.14±0.84	6.1±0.93
ΔE_R	4.28±0.88	6.53±1.54	6.17±1.25	5.53±1.14	7.81±1.6	7.71±1.23
t stat	0.26 ^{NS}	1.56 ^{NS}	0.31 ^{NS}	1.63 ^{NS}	0.92 ^{NS}	1.05 ^{NS}

^{NS} indicates the observations in that column are not significantly different

Table 20: Total colour (ΔE) difference between groups

	0 th hour		24 th hour	
	Control	Irradiated	Control	Irradiated
G _I - G _{II}	4.57±0.74 ^a	4.28±0.88 ^a	3.53±0.44 ^a	5.53±1.14 ^a
G _{II} - G _{III}	3.91±0.67 ^a	6.53±1.54 ^a	6.14±0.84 ^b	7.81±1.6 ^a
G _I - G _{III}	5.68±0.91 ^a	6.17±1.25 ^a	6.1±0.93 ^b	7.72±1.23 ^a

Observations having same superscript in the same column are not significantly (P>0.05) different

4.2.6. Organoleptic qualities

Organoleptic quality assessment of the cooked product was conducted at zero hour and 24h storage. The test was conducted with the help of a nine point hedonic scale score card to assess the colour, flavour, juiciness, tenderness and overall acceptability of the meat samples. The data are shown in (Table 21) and the statistical analysis of the data revealed a significant ($P < 0.05$) difference among few characteristics.

Colour of group III, both control and irradiated, was different from Group I and Group II at zero and 24 h of storage. Apparently there was no significant difference between control and irradiated at zero and 24h of storage. The flavour attributes were non significant among groups and treatments at zero and 24h. The flavour had numerically increased and shown a significant difference between control and irradiated samples at 24 h of storage.

Juiciness score showed a reducing trend even though many of the values were not significant. The lowest score was 7.36 ± 0.09 for group II irradiated meat samples, compared to the highest value of 7.84 ± 0.01 obtained at zero hour in group III.

A similar trend was noticed in case of tenderness score. The values varied between 7.71 ± 0.09 in zero hour irradiated to 7.22 ± 0.1 in 24h control samples. Even though there was no significant difference between control and irradiated samples, the data revealed a higher value for irradiated samples compared to control samples both at zero and 24 h of observations.

The overall acceptability scores of the cooked product are shown in Table 21. It was observed a non significant difference among groups, between control and irradiated samples at zero and 24h of observations.

The Kendall test results (Table 22) showed the highest colour score for 24h group III irradiated sample followed by group III 24 h control and group III

Table 21: Organoleptic values of control and irradiated meat

	0 th hour		24 th hour	
	Control	Irradiated	Control	Irradiated
Colour				
G _I	7.36±0.09 ^a	7.42±0.07 ^{ab}	7.53±0.06 ^{abcd}	7.51±0.07 ^{abcd}
G _{II}	7.36±0.11 ^a	7.36±0.08 ^a	7.47±0.05 ^{ab}	7.49±0.08 ^{abc}
G _{III}	7.64±0.10 ^{bcd}	7.67±0.1 ^{bcd}	7.76±0.07 ^{cd}	7.78±0.11 ^d
Flavour				
G _I	7.44±0.06 ^a	7.56±0.06 ^{ab}	7.6±0.06 ^{abc}	7.67±0.05 ^{abcd}
G _{II}	7.44±0.09 ^a	7.56±0.07 ^{ab}	7.80±0.07 ^{cd}	7.84±0.09 ^d
G _{III}	7.51±0.08 ^a	7.58±0.09 ^{abc}	7.78±0.05 ^{bcd}	7.80±0.08 ^{cd}
Juiciness				
G _I	7.73±0.09 ^{bc}	7.69±0.11 ^{bc}	7.53±0.09 ^{ab}	7.53±0.07 ^{ab}
G _{II}	7.69±0.10 ^{bc}	7.64±0.09 ^{abc}	7.38±0.08 ^a	7.36±0.09 ^a
G _{III}	7.84±0.10 ^c	7.76±0.11 ^{bc}	7.62±0.06 ^{abc}	7.56±0.04 ^{abc}
Tenderness				
G _I	7.58±0.09 ^{bc}	7.71±0.09 ^c	7.42±0.11 ^{abc}	7.47±0.09 ^{abc}
G _{II}	7.56±0.06 ^{bc}	7.60±0.08 ^{bc}	7.38±0.08 ^{ab}	7.44±0.08 ^{abc}
G _{III}	7.53±0.10 ^{bc}	7.56±0.14 ^{bc}	7.22±0.1 ^a	7.31±0.11 ^{ab}
Overall acceptability				
G _I	7.56±0.09 ^a	7.60±0.09 ^a	7.64±0.10 ^a	7.64±0.11 ^a
G _{II}	7.47±0.07 ^a	7.49±0.04 ^a	7.58±0.04 ^a	7.62±0.08 ^a
G _{III}	7.39±0.12 ^a	7.44±0.12 ^a	7.53±0.11 ^a	7.58±0.14 ^a

Observations having same superscript in the same set of table are not significantly ($P < 0.05$) different

Table 22: Kendall's test of organoleptic values

	Colour	Flavour	Juiciness	Tenderness	Overall acceptability
Group I 0h Control	4.17	3.83	8.11	7.61	6.33
Group I 0h irradiated	5.06	5.33	7.22	9.83	7.22
Group II 0h Control	5.11	4.06	7.72	7.28	5.17
Group II 0h irradiated	4.11	5.28	6.89	8.17	6.06
Group III 0h Control	7.22	4.83	9.22	7.67	4.61
Group III 0h irradiated	7.78	5.72	8.39	7.89	5.83
Group I 24h Control	6.94	5.83	5.39	5.00	7.78
Group I 24h irradiated	6.56	7.11	5.50	5.89	7.67
Group II 24h Control	5.72	8.94	3.61	5.17	7.33
Group II 24h irradiated	6.28	9.17	3.17	5.89	7.78
Group III 24h Control	9.50	9.06	7.06	3.17	6.22
Group III 24h irradiated	9.56	8.83	5.72	4.44	6.00

zero hour irradiated. The 24h group II irradiated samples scored the highest flavour and the lowest was for group I zero hour control meat samples. The top score in juiciness was for group III zero hour control samples followed by group III zero hour irradiated samples. The maximum tenderness was noticed in zero hour irradiated samples in group I animals, followed by the similar samples from group II animals. The maximum overall acceptability was recorded in group I, 24h control and group II 24 h irradiated samples.

In order to verify whether there was any differential response due to irradiation for the stress given to the animals, two factor Complete Randomised Design (CRD) analysis was carried out for all the parameters over zero and 24 h observations with that of radiation (control and 2.0 kGy) and groups as factors. None of the interactions were found to be statistically significant leading to the results that irradiation conditions did not have any impact on meat obtained from stressed animals.

Discussion

DISCUSSION

Twenty seven culled cattle brought from different farms, under the control of KAU and KLDB farms at Kulathupuzha and Mattupetti, were grouped into three, each comprising of nine animals. Group I animals belonged to ULF, Mannuthy which were not subjected to any type of stress. Group II animals comprising of nine animals were subjected to transportation and were slaughtered immediately in the stress period. The third group of animals was subjected to transportation of about 6 to 7 hours and was brought to the Department. They were rested in the lairage for 18 hours and subjected to slaughter. The blood samples of these animals were collected and analysed for haemato-biochemical parameters like cortisol, CK, BUN, creatinine and PCV at different stages. Meat from slaughtered animals were collected, packed and subjected to irradiation studies and the results were compared with the non irradiated samples.

4.1. HAEMATO-BIOCHEMICAL PARAMETERS DURING STRESS

The animals brought from different farms were subjected for the estimation of haemato-biochemical parameters like cortisol, CK, BUN, creatinine and PCV. Even though they were from different farms, no significant differences were observed in blood values before transport indicating that there was no group difference between the groups and as such there was no influence of animal groups in the present study.

A significant effect was observed in the certain haemato-biochemical parameters due to transport in group II and group III animals as evidenced by the 't' statistic values (Table 2), where the after transport values were significantly

higher than the before transport values. Trunkfield and Broom (1990) observed an increased enzymatic and adrenal activity due to transportation, in calves. Tarrant *et al.* (1992) reported an increase in plasma cortisol and glucose level due to transportation. Warris *et al.* (1995) reported that there was an increase in CK activity, plasma protein, osmolality, urea and β -hydroxybutyrate levels due to long distance transport. Knowles and Warriss (2005) had suggested that the stress during transportation can be estimated by using cortisol for fear/arousal, PCV for fear and dehydration, CK activity for physical exertion and urea for starvation. In the present study many of the parameters investigated had shown similar result in accordance with these reports. Even two hour transport caused a significant increase with respect to cortisol ($P < 0.01$), CK ($P < 0.01$) and BUN ($P < 0.05$) whereas such an effect was not noticed in PCV and creatinine after transport. In case of group III animals, all the values were significantly increased in cortisol ($P < 0.01$), CK ($P < 0.01$), BUN ($P < 0.05$) and creatinine ($P < 0.05$) whereas PCV did not show a significant increase after the transport. In these animals, which were subjected to sufficient rest, the cortisol and CK reduced significantly ($P < 0.01$) with respect to after transport and before slaughter values. This clearly indicated that cortisol and CK could be considered as more sensitive indicators of stress than BUN, creatinine and PCV. It was observed that even after 18h rest, the values did not return to its original levels with respect to cortisol, CK and BUN, whereas creatinine and PCV returned to values not significantly different from its original values.

Crookshank *et al.* (1979) reported that cortisol would return to its initial level within 4 to 7 days after weaning and trucking in calves. Mc Veigh and Tarrant (1982) reported that CK activity would return only by 7 days to its original level. In the present study, it was observed that cortisol and CK had not returned to its original values by giving proper rest for 18 h. Similarly Warriss *et*

al. (1995) reported that 5 days after transport were required for the blood values like cortisol, CK, total proteins, free fatty acids, urea, *etc.*, to return to its original level. Nigil Mathew (2002) reported a non significant effect of pretransport groups and transported animals had become to its normal level after 18 hours of rest. In the present study, only cortisol, CK and BUN have showed such a difference by 18 hours whereas creatinine and PCV remain unchanged, between before transport and before slaughter values. Between transported groups, it was noticed that in group III animals, the increase in cortisol and CK due to transport were significantly ($P<0.01$) more than that in group II animals whereas, no such difference was observed between group II and III in case of BUN, creatinine and PCV (Table 4). Hence from the above fact it was clear that cortisol and CK were the better indicators for assessing transport stress as well as the efficiency of resting period in lairage before slaughter.

4.2. PHYSICOCHEMICAL QUALITIES OF MEAT

4.2.1. pH of meat

The meat obtained from the animals of the three groups was packed in HDPE packets and half of the samples were subjected to radiation at 2.0 kGy and stored up to 24h. The pH in all the three groups were compared immediately after collection and storage at 6, 12 and 24 h, it was observed that there was no much significant difference in samples at different storage periods (Table 5). The effect of storage on the fall of pH has been shown in Table 6 and observed a significantly ($P<0.01$) higher fall in pH both in control and irradiated sample at zero to 6h which was followed by 6 to 12 and 12 to 24h observations. At the same time, it was also observed that there was a non significant difference in fall of pH between control and irradiated samples.

A decrease or diminution of pH due to irradiation reported by Lefevbre *et al.* (1994) and Yook (2001) was in accordance with the fall of pH in the present study due to irradiation in all the three groups. The 24h pH in the present study did not show any significant difference between control and irradiated samples of all the groups and the data were in accordance with the study conducted by Maria *et al.* (2003), where 30 min, 3h or 6h transport did not reveal any difference in the ultimate pH. Page *et al.* (2001) reported a cut off pH of 5.87 for dark cutting beef and normal meat. None of the group in the present study has shown a pH of 5.87 or above even in group II animals which were slaughtered immediately after transport.

4.2.2. Water Holding Capacity (WHC)

The WHC of the meat at zero, 6, 12 and 24 h for control and irradiated samples for the various groups have been shown in Table 7. There was no significant difference between groups both for control and irradiated samples through out the study. It was also noted that there was a numerical difference between control and irradiated samples in all the observation periods. Compared to the decrease in pH, the changes in WHC between control and irradiated samples were high in the present study. Cain *et al.* (1958), Schweigert (1959) and Lawrie (1998) reported a considerable decrease in WHC due to irradiation in meat. Zhn *et al.* (2004) reported an increased centrifugal loss due to irradiation in pork and opined that structural damage in the membrane may be the cause. In the present study, WHC at zero hour was higher compared to other storage periods and this was in accordance with the findings of Kuttinarayanan (1988) where a higher WHC was reported at zero hour both in control as well as electrically stimulated samples. The fall of the WHC noticed in the present study between zero to 6, 6 to 12 and 12 to 24h were significantly ($P < 0.05$) different with maximum fall in zero to 6h. The fall between control and irradiated samples

among the time intervals were non significant (Table 8). Huff-Lonergan and Lonergan (2005) opined that early post mortem events including rate and extent of pH decline, proteolysis and even protein oxidation caused changes in intracellular architecture of cell which could influence the ability of muscle cell to retain water.

Maria *et al.* (2003) observed transport times of 30 min, 3h and 6h didn't show any significant effect on the WHC. The present study results were also in agreement with them since the WHC values between groups were not significant through out the study with different storage and treatment groups.

4.2.3. Cooking loss

The cooking loss of the control and irradiated samples collected from three groups of animals were determined as per the standard method prescribed by Boccard *et al.* (1981). It was observed that there was no significant difference between the cooking loss values of group I, II and III. The cooking loss at zero hour of storage was not significantly different between control and irradiated samples whereas a significant ($P<0.05$) increase for cooking loss was noticed in irradiated samples at 24h of storage in chiller condition. The cooking loss of the meat sample at zero hour was significantly ($P<0.01$) lower than that of 24 hour stored samples.

Yoon (2003) reported a more cooking loss in irradiated chicken breasts compared to that of non irradiated samples while, Okeudo and Moss (2005) opined that serum cortisol level was negatively correlated with cooking loss. In the present study, there was no group difference in cooking loss even though a group difference was seen in cortisol level. As time passed from zero to 24 hour storage, a significant ($P<0.01$) increase was observed in cooking loss. This was in

agreement with the reduction of WHC noticed irrespective of the group differences.

4.2.4. Shear force values

The objective measurement of tenderness of the control and irradiated samples among the three groups was obtained with the help of UTM and the values were expressed in kgf. Contradictory to other parameters like pH, WHC and cooking loss, there was a significant ($P < 0.05$) difference between group I to that of group II and III which were having higher values at zero hour control samples. Maria *et al.* (2003) reported that transport time of 30min, 3h or 6h had little effect on WB shear values and was not in agreement with the observations of the present study where the group II and III animals subjected to slaughter and measurement of WB shear revealed some group differences.

In case of irradiated samples at zero hour, group III had a higher shear force value than that of group I where as group II was in between. The trend had continued up to 24 h of storage in all the three groups. The force required to cut a uniform core of meat at zero hour and 24 hour was significantly ($P < 0.01$) different, as the irradiated samples required less force compared to that of control samples. Irradiation in moist condition made collagen soluble in water (Perron and Wright, 1950) and caused softness and tenderness of texture as an immediate effect (Coleby *et al.*, 1961). Yook *et al.* (2001) reported a decreased shear force values in irradiated muscles and attributed the cause as morphological and biochemical properties of irradiated bovine muscles. In the present study, a similar reduction was observed in shear force values in irradiated meat compared to control samples at zero and 24 h of storage.

4.2.5. Colour

Among the colour values, lightness of the meat measured with the help of *L* value in Hunterlab Miniscan XE plus spectrophotometer showed that the control samples in group I at zero hour had a better colour (lightness) than that of group II and III, whereas the observations of irradiated samples at zero and 24 hour did not show a significant difference between groups. The control samples of group I and II at 24h storage showed a significant ($P<0.05$) difference with that of group III (Table 11). By 24 h of storage, the colour *L* value were increased from 25.42 ± 0.04 to 30.01 ± 0.06 showing a significant ($P<0.01$) increase and indicated that by 24 h the meat became lighter. Similarly, irradiated samples recorded a significantly ($P<0.01$) lighter colour than that of control samples at zero hour which became non significant by 24h of storage (Table 12A and 12B).

Almost similar picture was obtained in case of *a* values which denotes the redness of meat. It was also observed that there was no significant difference in control and irradiated samples between groups at zero hour of observation. At 24h of storage, the group III animals recorded a significantly ($P<0.05$) lower value than that of group I and II, indicating lower redness. A significant ($P<0.01$) difference was observed between the control sample and the irradiated sample at zero hour of observation and by 24 h of storage it changed to a non significant value.

The *b* values which denotes the yellowness of meat which is mainly contributed by the fat percentage showed that the group I animals were significantly ($P<0.05$) different from that of group II and III in case of control samples. The effect due to irradiated was significant ($P<0.01$) as evidenced by a higher value showing high yellowness (Table 12A). By 24h storage, the difference between control and irradiated samples reduced and became non

significant (Table 12A and 14). The changes due to transportation maintained the trend through out the study period.

The C values of the meat samples denotes the chroma which was calculated as $C = (a^2 + b^2)^{1/2}$ and denotes the colourfulness in relation to the brightness of the surroundings (Mac Dougall, 1999). There existed a significant ($P < 0.01$) difference between the control samples of group I at zero hour with the other two groups whereas, irradiation process had nullified such an effect. But at 24h of storage, only group III animal had a significantly ($P < 0.05$) lower value than that of group I and II in control and irradiated samples. A significant ($P < 0.01$) difference was observed between control and irradiated sample at zero hour of storage (Table 12A) and a non significant difference at 24h of storage (Table 12B).

The *hue angle* values of the meat colour denote that attribute described in colour names red, green, purple etc. (Mac Dougall, 1999). It was observed that there was a significant ($P < 0.05$) difference among groups at zero hour control samples and this nature was absent at 24h of storage. A significant increase in hue value was observed with 24h storage and irradiated samples had a higher hue value at zero and 24h post mortem.

The total colour difference measured as $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$ showed that the total colour difference between control and irradiated samples at zero and 24h of storage were significantly ($P < 0.05$) different in group I animals (Table 17) and the total colour difference between the zero hour and 24h storage in control samples were significantly ($P \leq 0.05$) different from the irradiated samples in all the groups.

Millar *et al.* (2000) observed the L value of irradiated beef increased significantly with storage and was in accordance with the present observations

and a higher *hue* angle and *a,b,C* values were significantly higher than that of freshly cut beef surface. The findings of Kim *et al.* (2002b) showed irradiated beef had a significantly decreased *L** value after 7 days of storage and both *a** and *b** values decreased at zero day of storage. It was observed on zero hour of observation *a* value increased and by the time bloom has developed values were non significantly different (table 12A and B). Brewer (2004) reported an increased yellowness (*b* value) in irradiated meat of all species and the present study also observed an increased and significant *b* value in both zero and 24 h of observation compared to the control.

4.2.6. Organoleptic evaluation

The organoleptic study of the cooked product was conducted at zero and 24h of irradiation and compared with control samples. The test was conducted with the help of nine point ascending hedonic scale. It was observed that group III animals' colour observations were significantly ($P<0.05$) different at zero and 24h among control and irradiated samples. In case of flavour, a similar trend was noticed at 24h of observation. As far as flavour was concerned, there was no significant difference between control and irradiated samples at zero and 24h of observations. Juiciness of samples decreased numerically due to irradiation so also with storage. It was observed that irradiation increased tenderness slightly and the tenderness observed by the panelist agreed with the changes noticed in WB shear value. Absolutely no significant difference was observed in overall acceptability of the control and irradiated samples.

Fu *et al.*(1995) observed no significant difference between the colour of irradiated and non irradiated samples. Here in the present study also the values were non significant between groups, even in animals subjected to stress. Ahn *et al.* (1998) reported the production of unidentified volatile compounds due to

irradiation and production of off odour in irradiated raw meat. Yook *et al.* (2001) reported a higher content of IMP due to irradiation in meat and so a higher flavour. In the present study, the irradiated samples had non significant higher values than that of control samples in different storage periods and Norjite *et al.* (2005) reported a low dose irradiation (≤ 4.0 kGy) as the most feasible dose to optimise sensory qualities. Juiciness observed in the present study, the values were non significant among control and irradiated samples during the zero and 24h of storage. The WHC capacity obtained in the study was non significant during the hours of observation even though the fall of WHC from zero to 24h was significant. Such a numeric reduction was noticed in case of juiciness in zero and 24h samples.

Tenderness also followed a similar pattern and as time passed from zero to 24h, there was a numerical difference between control and irradiated samples a similar trend as noticed in case of WB shear values. Lawrie (1998) reported that the degree of tenderness can be related to the three categories of proteins as connective tissue, myofibrillar and sarcoplasmic proteins. The background toughness of meat is mainly due to the connective tissue, especially the collagen. In case of irradiation, collagen shrinks in its dry state and become soluble in water if irradiated in wet (Perrón and Wright, 1950) and indeed irradiation cause softness and tenderness of texture as an immediate effect (Coleby *et al.*, 1961). The probable reason of increased tenderness in irradiated samples might be due to this collagen shrink and higher solubility of collagen in irradiated meat compared to the control.

The overall acceptability of the control and irradiated sample at zero and 24h were non significant and irradiated samples were rated comparatively higher value through out the study. Wheeler *et al.* (1999) had not reported any consumer acceptance problem at 3.0kGy dose. Dempster *et al.* (1985) did not observed any

significant difference between irradiated and non irradiated beef burger. Kuttinarayanan *et al.* (2005) had not observed any significant difference on organoleptic evaluation by irradiation preservation of beef fry. Kuttinarayanan *et al.* (2006a) observed no significant difference in colour, flavour, juiciness, tenderness and overall acceptability of minced beef on zero and 24 days of investigation are in agreement with the present observations.

In the present study, in general, colour, flavour, juiciness, tenderness and overall acceptability were not much altered due to irradiation at 2.0 kGy in case of all the groups. The Kendall test results showed that the excellent score with respect to colour was for group III 24h irradiated samples; flavour group III 24h irradiated sample; juiciness group III zero hour control; tenderness in group I zero hour irradiated and overall acceptability for group II 24h irradiated and group I 24h control samples. This indicated that irradiated samples had a higher score than that of control samples irrespective of groups.

Summary

SUMMARY

In the absence of organised and centralised abattoirs, most of the animals are being subjected to long distance transport and inhumane handling before they are being slaughtered. This can lead to the production of low quality meat with reduced shelf life and aesthetic value, leading to economic loss. In order to assess the effect of stress on the haemato-biochemical parameters and physicochemical qualities of meat and the effect of radiation on such meat this study was undertaken, since Prevention of Food Adulteration (PFA) Act 1954 and its amendments in 1998 has permitted irradiation as an approved technique that can be used for the preservation of meat and meat products including chicken to destroy pathogens and to extend shelf life. The objectives of the study include the assessment of the effect of stress on the blood parameters and physicochemical qualities of meat and effect of irradiation and stress on the qualities of fresh meat.

Twenty seven adult cattle culled from University Livestock Farm, Mannuthy (ULF), Cattle Breeding Farm (CBF) Thumboormozhi and Kerala Livestock Development Board (KLDB), Mattupetti and Kulathupuzha, having an age of 4.41 ± 0.58 years and a weight of 351.07 ± 39.57 kg were utilised for the present study. All the animals were grouped into three, having nine animals each. Group I comprised of animals procured from ULF, Mannuthy which had not been subjected to any type of stress and considered as control animals. Group II comprising of animals having a journey of 2h and were slaughtered immediately after transport. Group III animals were transported for 6 to 7h and were given a rest of 18h before slaughter.

From all the groups, blood samples were collected prior to transport, immediately after bring to the Department in group II and III and before slaughter (after rest) in group III animals, to study the blood parameters studied *viz.*, cortisol, CK, BUN, creatinine and PCV. Cortisol was estimated using RIA technique. CK, BUN and creatinine were estimated using commercially available diagnostic kits. PCV was estimated using haematocrit tubes. From the analysis of data prior to transport, it was found that there was no significant difference between the various parameters studied. There was a significant increase in case of cortisol ($P<0.01$), CK ($P<0.01$) and BUN ($P<0.05$) immediately after transport in group II animals. In case of group III animals, the similar values increased significantly in the all the parameters starting from cortisol (22.44 ± 1.58 to 72.14 ± 6.72 nmol/L), CK (86.11 ± 2.19 to 443.11 ± 42.12 U/L), BUN (21.89 ± 1.37 to 26.11 ± 1.84 mg/dl), creatinine (2.56 ± 0.24 to 3.44 ± 0.18 mg/dl) except in PCV (35.94 ± 1.63 to 37.69 ± 1.51 per cent). The increase in cortisol and CK due to transport in group III animals were significantly ($P<0.01$) more than that in group II animals. By giving 18h rest, there was a significant ($P<0.01$) reduction in cortisol and CK. But even after giving 18 h rest, cortisol ($P<0.01$), CK ($P<0.01$) and BUN ($P<0.05$) values were significantly higher than the original values.

Meat samples were collected from each animal and half of the samples were subjected to irradiation using Gamma Chamber 5000 at 2kGy using cobalt 60 as source. Characters like pH and WHC were determined immediately after collection, then at 6, 12 and 24h post mortem. Cooking loss, shear force (WB shear), colour (Hunter lab) and organoleptic qualities were assessed immediately after collection and 24h post mortem. The pH and WHC were estimated as per standard procedure. Cooking loss was estimated and expressed on percentage basis. The tenderness of the meat sample was determined using UTM having WB shear attachment and expressed in kilogram force.

Colour of the meat sample was determined with Hunterlab Miniscan XE plus spectrophotometer to get *L*, *a* and *b* values, from this total colour change, *chroma* and *hue angle* values were calculated. Organoleptic qualities of meat were determined for colour, flavour, juiciness, tenderness and overall acceptability using nine point ascending hedonic scale.

The pH and WHC showed a similar trend through out the study, where between groups the values were non significant, with a higher value immediately after slaughter. A drastic fall in pH and WHC was observed in first six hours of storage, followed by 6 to 12h and comparatively reduced fall at 12 to 24 h, reaching the ultimate pH. The decline of pH and WHC did not show any significant difference in control and irradiated samples. Cooking loss values were non significant between groups in control and irradiated samples through out the study. At zero hour, there was no significant difference between control and irradiated samples. As storage time increased, the cooking loss increased significantly ($P<0.01$) (33.74 ± 0.00 per cent) as compared to the zero hour (27.37 ± 0.01 per cent) samples and irradiated sample showed significantly ($P<0.05$) higher cooking loss at 24 h storage when compared to the control samples. At 24h storage, group III animals were having a significantly ($P<0.05$) higher shear force values than the other two groups in all the samples through out the study, while the values of group I samples were significantly ($P<0.05$) different from the other two groups in zero hour control samples. Irradiated samples were having a significantly ($P<0.01$) reduced shear force through out the study.

Colour *L* values of meat samples were homogenous between the groups through out the study except in group I control samples at zero hour storage and group III control samples at 24h storage. Colour *L*, *a*, *b*, *C* and *hue angle* values were significantly ($P<0.01$) greater for irradiated samples at zero hour storage. As the storage period increased, this difference increased significantly ($P<0.01$) from

that of zero hour and only *hue angle* values of irradiated samples maintained the significant ($P < 0.05$) difference with control samples. The total colour difference between control and irradiated samples at zero hour was significantly ($P < 0.05$) different from that at 24h in group I meat samples, whereas the total colour difference between zero hour and 24h storage in control samples were significantly ($P \leq 0.05$) different from that in irradiated samples in all the groups.

In case of organoleptic evaluation, the irradiated sample had a slightly higher colour score even though the difference was not significant. The flavour of irradiated meat was numerically greater and storage period slightly increased the flavour. Juiciness score was in accordance with the WHC of the meat, as it was greater in control samples and storage decreased juiciness. Irradiated samples had comparatively more tenderness and meat became tougher on 24h storage, which was in accordance with the shear force values. Overall acceptability did not show any significant difference between control and irradiated samples.

From the above study, it was observed that compared to various blood parameters investigated, cortisol and CK were better indicators of stress than BUN, creatinine and PCV, since animals in the stress group had higher value with these parameters. Irradiation of meat obtained from various groups showed varying level of characters. The differential response due to irradiation on the meat obtained from stressed and normal animals showed that there was a non significant effect due to irradiation on the various physicochemical, organoleptic, colour and tenderness attributes of meat from different group of animals.

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**EFFECTS OF PRESLAUGHTER STRESS AND
IRRADIATION ON PHYSICOCHEMICAL
QUALITIES OF MEAT**

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ABSTRACT

The effect of stress on the blood parameters and physicochemical qualities of meat as well as effect of irradiation on the qualities of fresh meat from stressed cattle were investigated in the present study.

A total of 27 culled adult cattle from different farms were grouped into three, having nine animals in each group. Group I animals were considered as control while group II animals were transported for 2 h and slaughtered immediately after transport. Group III animals were transported for 6 to 7 h and given rest for 18 h before slaughter. Blood samples were collected prior to transport in all the animals, immediately after transport in group II and III and after 18 hours rest, before slaughter, in group III animals. Meat samples were collected from all the animals in which half of the samples were subjected to irradiation at 2.0kGy with gamma irradiation, as Cobalt 60 as source. The samples were stored at 4⁰C for further studies.

Animals in all the groups were having homogenous blood values prior to transport in cortisol, CK, BUN, creatinine and PCV. After transport, group II had a significant increase in cortisol ($P<0.01$), CK ($P<0.01$) and BUN ($P<0.05$), while group III had a significant increase in cortisol ($P<0.01$), CK($P<0.01$), BUN ($P<0.05$) and creatinine ($P<0.05$) except PCV, in which the increase in cortisol and CK were significantly ($P<0.01$) greater than that in group II. By giving rest, even though there was significant ($P<0.01$) reduction in cortisol and CK values, the after rest values of cortisol ($P<0.01$), CK ($P<0.01$) and BUN ($P<0.05$) were significantly higher than that of the before transport values.

In the case of pH and WHC, the trend was almost similar, with a higher value at zero hour and the decline was drastic in zero to 6 h, followed by 6 to 12 and 12 to 24 h and the control and irradiated samples did not show any significant difference in the decline. Cooking loss of the control and irradiated samples did not have a significant difference at zero hour storage, while the 24h storage brought a significant ($P<0.01$) increase in cooking loss in all the samples and irradiated samples had a significantly ($P<0.05$) higher cooking loss

at 24h. Shear force values were significantly ($P<0.01$) less for irradiated samples while storage significantly ($P<0.01$) increased the values of all the samples.

The Hunterlab colour values, *L*, *a*, *b*, *C* and *hue angle*, were significantly ($P<0.01$) higher in irradiated samples at zero hour storage. At 24h storage, the values were significantly ($P<0.01$) higher than that at zero hour, and only the *hue angle* values were significantly ($P<0.05$) higher in irradiated samples. Even though there existed a slight difference between control and irradiated samples in various organoleptic qualities like colour, flavour, juiciness and tenderness, there was no significant difference in overall acceptability between control and irradiated samples.

In the present study, the differential response due to irradiation for the stress given to the animals, found to be statistically non significant leading to the result that irradiation conditions did not have an impact on the meat obtained from stressed animal.