EFFECT OF CURRY LEAVES (Murray asp.) AND PEPPERMINT (Menth asp.) PASTE ON SHELF LIFE OF IRRADIATED CHICKEN TIKKA

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Thesis submitted in partial fulfilment of the requirement for the degree of

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Dedicated To My

Beloved Aai And

Parents

DECLARATION

I hereby declare that this thesis entitled **"EFFECT OF CURRY LEAVES (*Murraya sp.*) AND PEPPERMINT (*Mentha sp.*) PASTE ON SHELF LIFE OF IRRADIATED CHICKEN TIKKA"** 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.

Mannuthy **AHIRE GIRISH SURESHRAO**

Date:

CERTIFICATE

Certified that this thesis, entitled "**EFFECT OF CURRY LEAVES (*Murraya sp.*) AND PEPPERMINT (*Mentha sp.*) PASTE ON SHELF LIFE OF IRRADIATED CHICKEN TIKKA**" is a record of research work done independently by **AHIRE GIRISH SURESHRAO,** under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to him.

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We, the undersigned members of the Advisory Committee of **Ahire Girish Sureshrao**, a candidate for the degree of Master of Veterinary Science in Livestock Products Technology, agree that the thesis entitled "**EFFECT OF CURRY LEAVES (*Murraya sp.*) AND PEPPERMINT (*Mentha sp.*) PASTE ON SHELF LIFE OF IRRADIATED CHICKEN TIKKA**" may be submitted by **AHIRE GIRISH SURESHRAO**, in partial fulfillment for the degree.

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INTRODUCTION

1. INTRODUCTION

In developed and developing countries demand for ready-to-cook/eat minimally processed meat products is ever increasing. Urban Indian markets offer several such indigenous meat products like chicken chilly, chicken tikka, mutton shammi kababs and mutton sheek kababs, etc. Considering the perishable nature of these products has to be marketed only in the frozen state, but freezing facilities are expensive and inadequate. Storage of these products under chilling is advantageous in terms of convenience, economy and energy saving. Such chilled products can be marketed to a limited geographical area and the shelf life would be shorter. Technologies that allow several fold extension of the shelf-life are required for such products without compromising on microbiological safety and organoleptic quality.

Irradiation of meat and meat products has been approved by several countries (Molins *et al.,* 2001) and authorized by such international and governmental organizations such as the World Health Organisation (WHO), Food and Agriculture Organization (FAO) and the Food and Drug Administration (FDA). Quality parameters, such as oxidative changes, colour, stability and organoleptic attributes are decisive factors for the wide acceptance of radiation processed meat products.

Irradiation is known to accelerate lipid peroxidation of meat. Lipid oxidation is responsible not only for the loss of quality in meat but also microbiological deterioration. It occurs during processing and storage of meat and meat products which adversely affect the colour, flavour and texture. Controlling these changes is a prerequisite for better product development. One of the simplest means of ensuring oxidative stability of irradiated meat is by addition of synthetic antioxidants like BHA, BHT, etc.

Herbs such as garden mint (*Mentha spicata* L.), curry leaves (*Murraya koenigii*) are widely used as a flavour enhancer in several culinary

preparations. These herbs are reported to have strong antioxidant activity. (Ali *et al.* 2002; Tachibana *et al.* 2003). Consumer always prefers natural antioxidants over synthetic antioxidants in any food item.

Chicken tikka is one of the very popular ethnic meat products available throughout India with a keeping quality of very short duration. Gamma radiation of meat and meat products are permitted in India for extending the shelf life and to destroy pathogenic organisms. In order to assess the effect of irradiation and to evolve suitable technology to reduce the undesired effects of irradiation on chicken tikka by incorporating natural antioxidants like curry leaf paste and peppermint paste, this study was conducted to,

1) Assess the effect of irradiation on shelf life and organoleptic quality changes of the product.

2) Assess the effect of curry leaf and peppermint paste and their mixture on quality parameters of ready-to-eat chicken tikka under chiller storage (1 to 4^0C).

3) Study the changes in the proximate composition of chicken tikka by addition of the marinade containing curry leaf and peppermint paste.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Chicken tikka is one of the indigenous meat products commonly available as a fast food. Considering the perishable nature of the product, it has to be stored in frozen state to prevent multiplication of microorganisms and spoilage. Even this storage will not destroy bacteria and fungi *in toto*. Many of the meat preservation methods except canning does not destroy total microbial load present in meat and nobody can say meat is totally wholesome. Gamma radiation at sufficient dose destroys pathogens and spoilage organisms without affecting many of the qualities. Considering the wholesomeness of meat, irradiation of meat and meat products is recognized as a method of meat preservation.

2.1. RADIATION PRESERVATION OF FOOD

2.1.1. Recommendation of Irradiation in Food

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

In India, the Ministry of Health and Family Welfare amended the Prevention of Food Adulteration Act, 1954 through a special Gazette dated 06-04-1998 and meat and meat products including chicken are permitted for irradiation at dose of 2.5 to 4.0 kGy to extend shelf life and to control pathogens (PFA Rules, 1998).

In 1990 Food and Drug Administration and in 1992 United States Department of Agriculture approved irradiation at the dose range of 1.5 to 3.0

kGy for destroying pathogenic bacterial organisms. The USDA approved the dose up to 4.5 kGy (WHO, 1999).

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

Frenzen *et al.* (2000) reported that the Federal Government permitted food manufacturers to irradiate raw meat and meat products to control pathogenic microorganisms in the year 2000 and stated that consumer acceptance of irradiated foods could reduce public health hazard because many food borne illnesses occur when consumers handle or eat meat or poultry contaminated by microbial pathogens.

About 55 countries have approved and are using food irradiation technologies to ensure food safety and 29 countries have given clearance for irradiation of raw poultry and meat. Countries such as Belgium, China, France, Indonesia, the Netherlands, Thailand and United States have implemented irradiation of meat into commercial use (http://nucleaus.iaea.org., 2003).

2.1.2. Food Safety

The FAO / IAEA / WHO / Joint Committee on Irradiated foods at its meeting in Geneva in 1980 came to a conclusion that foods irradiated in the range of up to 10 kGy are toxicologically as well as microbiologically safe and nutritionally adequate and that no health hazard resulted from consuming such irradiated foods (WHO, 1981).

Dempster (1985) stated that low dose irradiation or radurisation eliminated most of the parasites in pork and very particularly, salmonella organisms in poultry and red meat. It increased the shelf life of poultry meat, red meat and meat products significantly.

The use of ionizing radiation as a method of food preservation has been studied since 1940. The major applications of food irradiation include

sterilization, pasteurization, disinfections, disinfestations, shelf life extension and product development (Nagai and Moy, 1985).

In a study conducted by Katusin–Razem *et al.* (1992) they stated that radiation induced chemical changes in irradiated foods are generally very less and usually difficult to observe in egg products. They also stated that irradiation at 2.5 kGy could be used for microbial decontamination in eggs and egg products which was more feasible than heat pasteurization.

Irradiation is a safe, efficient, environmentally clean, not tainted with chemical residue and energy efficient process being particularly valuable as end product decontamination procedure (Farkas, 1998).

Olson (1998) reported that low doses of radiation can kill at least 99.9% of *Salmonella* in poultry and an even higher percentage of *Escherichia coli* O157:H7 in ground beef and also mentioned that United States Department of Agriculture approved medium dose irradiation (1.0 to 10.0 kGy) for decontamination of raw meat and poultry.

Doyle (1999) stated that irradiation readily killed the most non spore forming bacteria and parasites in food. In general, Salmonella and Listeria were more resistant than *E. coli* and Staphylococcus. Species of Yersinia, Vibrio, Arcobacter, Aeromonas and Campylobacter were the most sensitive.

A joint FAO/IAEA/WHO Study Group on high dose irradiation met in Geneva from 15th to 20th September 1997 concluded and clearly established the wholesomeness of any food irradiated up to an overall average dose of 10 kGy (WHO, 1999). As far as India is concerned, even now the PFA Act has not amended and dose rate of 2.5 to 4 kGy is still continuing.

The Research Co-ordination Meeting on Radiation processing for safe, shelf-stable and ready-to-eat food (2003) reported that irradiation is widely recognized as an effective control measure for inactivating pathogenic bacteria

and parasites from solid food, especially those which are eaten raw or minimally processed. Global production of irradiated food, still in small quantities, is increasing steadily with some 2.5 lakhs tonnes in 1999.

Food irradiation is one set of processing technology that increased microbial safety and enhance shelf life of food and in combination with other processes enhanced the safety of minimal processed foods, hence food irradiation if properly carried out is a safe process (Lee, 2004).

Smith and Pillai (2004) reported that irradiation of food is a beneficial technology to control pathogens, increase shelf life and maintain food quality. It can be used in food without posing any human health hazard.

Kuttinarayanan *et al.* (2006) stated that the treatment of meat with ionizing radiation is an effective method to reduce or to eliminate several food borne pathogens and larvae of parasites. They also stated that lower doses of irradiation could reduce the growth of spoilage organisms, which helps in increasing the shelf life of meat.

According to Thayer (2007), radioactivity cannot be induced in foods by treatment with gamma rays from ^{137}Cs or ^{60}Co. Irradiation can inactivate protozoan or helminth parasites and significantly decrease the probability of viable food-borne bacterial pathogens in fish, poultry, and red meats.

Corliss *et al*. (2008) stated that research for more than 100 years on food irradiation had demonstrated that radiation will make food safer and improve the shelf life of irradiated foods. Typical gram-negative spoilage organisms are very sensitive to irradiation and their destruction leads to a significant increase in the acceptable shelf life. In addition, the destruction of these normal spoilage organisms did not provide a competitive growth advantage for irradiation injured food pathogens.

Kume e*t al.* (2009) reported, a total quantity of 4.05 lakh ton of food items were irradiated in 2005, out of which 1.86 lakh ton of spices and dried vegetables, 0.82 lakh ton of grains and fruits 0.32 lakh ton of meat and fish, 0.88 lakh ton of garlic and potato and 0.17 lakh ton of other food items including honey. The purpose of irradiation was mainly to enhance the shelf life.

2.2. NATURAL ANTIOXIDANTS

2.2.1. Use of Natural Antioxidants in Irradiated Meat and Meat Products

Kanatt *et al.* (1998) reported that chicken meat treated with tocopherol resulted in retardation of oxidative rancidity. Addition of tocopherol prior to irradiation showed a synergistic effect in decreasing free fatty acid content.

Sesamol, quercetin and BHT (Butylated Hydroxyl Toluene) retarded the lipid oxidation in both irradiated raw and cooked pork during 7 days of storage, whereas rosemary, oleoresin and rutin were effective only in irradiated raw pork up to 3 days. Generation of off odour volatiles was reduced by sesamol and querectin (Chen *et al*., 1999).

Nam *et al.* (2002a) reported that addition of antioxidant combinations viz., sesamol with tocopherol and gallate with tocopherols and aerobic packaging were effective in controlling lipid oxidation, reducing sulfur volatiles, which are responsible for irradiation off odour in irradiated pork patties.

Sebranek *et al.* (2005) reported that the rosemary extract was more effective than BHA (Butylated Hydroxyl Anisole) or BHT for preventing higher thiobarbituric acid reacting substances (TBARS) values or loss of red colour in raw frozen sausage.

Chitosan alone and in combination with either rosemary or α-tocopherol had good antioxidative effect compared to individual compound. Better results were obtained with the combination of chitosan and rosemary on frozen beef burgers. Chitosan added individually or in combination with either rosemary or α-

tocopherol also had a significant effect on the burger's appearance as it contributed to red colour retention for a much longer period compared all other treatments and the controls (Georgantelis *et al*., 2007).

2.2.2. Curry Leaves in Food Processing

Rahman *et al.* (2005) reported that a benzoisofuranone derivative, 3n-(1n-hydroxyethyl)-7-hydroxy-1-isobenzofuranone, and a dimeric carbazole alkaloid, 3,30- [oxybis(methylene)]bis(9-methoxy-9H-carbazole), along with six known carbazole alkaloids and three known steroids were isolated from the stem bark of *Murraya koenigii* and their antimicrobial property were assessed and the minimum inhibitory concentrations (MIC) of these compounds were found to be in the range 3.13–100 lg/ml.

Biswas *et al*. (2006) reported that the lipid oxidation was effectively inhibited in both raw and cooked samples treated with curry spice mix and curry leaf powder in which the latter had a potent antioxidative effect in raw meat than cooked patties.

According to Rao *et al*. (2007), oleoresin of curry leaves obtained using acetone, was evaluated for its antioxidant activity using β-carotene–linoleic acid model system along with the other extracts obtained using methanol, water and volatile oil. The oleoresin showed maximum activity of 83.2% at 100 ppm among all other extractives in comparison to a synthetic antioxidant, viz., BHA which exhibited 90.2% activity at the same concentration.

Ningappa *et al*. (2008) reported that the *in vitro* antioxidant properties of different extracts (water, alcohol, alcohol:water, hexane or chloroform extract) of curry leaves (*Murraya koenigii* L.) were evaluated using various assays. The alcohol:water (1:1) extract of curry leaves showed the highest antioxidant and free radical scavenging activity. It inhibited membrane lipid peroxidation by 76%, at 50 lg/ml, scavenged 93% of superoxides at 200 lg/3 ml and scavenged approximately 90% of hydroxyl and 1,1-diphenyl-2-picrylhydrazyl radicals at 4–

5-fold lower concentrations compared to the other tested extracts. In addition, the alcohol:water extract reduced cytochrome c and ferric ion levels, chelated ferrous ions and inhibited ferrous sulfate:ascorbate-induced fragmentation and sugar oxidation of DNA.

2.2.3. Peppermint in Food Processing

Marinova and Yanishlieva (1997) conducted the experiment on antioxadative activity of extracts of *Melissa oficinalis* L., *Mentha piperita* L., *Mentha spicata* L., *Ocimum basilicurn* L., *Origanum vulgare* L. and *Saturejae hortensis* L. on sunflower oil at 100°C. It was found that the extracts from *Ocimum basilicurn* L. and *Origanum vulgare* L. do not improve the oxidation stability of sunflower oil. The ethanol extracts from the other four spices have proved to be the most active in retarding the autoxidation process.

Iscan *et al*. (2002) investigated that essential oils of peppermint *Mentha piperita* L. (Lamiaceae), which were used for flavours, fragrances, pharmaceuticals and antimicrobial properties against 21 human and plant pathogenic microorganisms. The bioactivity of the oils, menthol and menthone was compared using the combination of in vitro techniques such as microdilution, agar diffusion, and bioautography. Using the bioautography assay, menthol was found to be responsible for the antimicrobial activity of these oils.

Murcia *et al*. (2004) conducted the experiment on the antioxidant properties of seven dessert spices (anise, cinnamon, ginger, licorice, mint, nutmeg, and vanilla) which were compared with antioxidants like BHA, BHT and propyl gallate. The influence of irradiation process on antioxidant activity was also evaluated. Mint and cinnamon exhibited a higher percentage of inhibition of oxidation than the other spices and compounds as tested by the lipid peroxidation assay.

Kanatt *et al*. (2007) conducted the study on the effectiveness of mint leaves (*Mentha spicata* L.), as a natural antioxidant for radiation-processed lamb

meat. Mint extract (ME) retarded lipid oxidation, monitored by TBARS, in radiation-processed lamb meat. TBARS values of ME containing irradiated meat stored at chiller temperatures were significantly lower ($p<0.05$) than samples without ME. After 4 weeks of chilled storage, TBARS in irradiated meat containing ME (0.1%) was half of that in untreated irradiated.

2.3. EFFECT OF CURRY LEAVES, PEPPERMINT AND IRRADIATION ON MEAT AND MEAT PRODUCTS

2.3.1. Shelf Life

According to Dempster (1985) low dose irradiation can destroy microorganisms of public health significance and extend the shelf life of meat products.

The lamb meat chunks irradiated at 1.0 kGy and 2.5 kGy remained in acceptable condition for 3 and 5 weeks respectively, and the shelf life of irradiated minced meat at 1.0 kGy and 2.5 kGy was acceptable for 2 and 4 weeks respectively at 0 to 3^{o}C storage. In contrast, non-irradiated meat chunks and minced meat were spoiled within one week at the same storage condition (Paul *et al.,* 1990).

Extended chiller storage was observed by Roberts and Weese, (1998) in ground beef patties for 14, 21 and 42 days when irradiated at 1.0, 3.0 and 5.0 kGy, respectively.

Johnson *et al.* (2004) reported that irradiated frankfurters did not have a detrimental effect on consumer acceptance and sensory characteristics and were acceptable up to 32 days under refrigeration compared to 14 days in case of non-irradiated samples.

Irradiated spices, packaging material (10.0 kGy) and luncheon meat (2.0 kGy) kept at refrigeration temperature (1-4^{o}C) for 12 months showed that gamma

radiation increased the shelf life by decreasing the microbial count of spices, packaging material and packed products (Al-Bachir, 2005).

In a study conducted by Kanatt *et al.* (2005) found that 3 kGy was optimal for shelf life extension of some ethnic Indian meat products like chicken chilly, mutton shammi kababs and pork salami. The shelf life was extended by more than 2 weeks at 0 to 3^{o}C compared to corresponding non-irradiated samples.

Jenifer (2006) found that irradiation of minced beef at 1.0, 2.0 and 3 kGy had increased the keeping quality up to 10, 25 and 33 days respectively at chiller temperature.

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

2.3.2. Physical Qualities

According to Narshimharao and Sreenivasmurthy (1986), when the shelf life of meat was assessed by considering sensory parameters such as discolouration and odour, unacceptable odour in fresh meat developed at 6 days of refrigerated storage (4 ± 1^{o}C).

Paul *et al.* (1990) observed freshly ground mutton irradiated at 2.5 kGy had a better colour, odour and microbiological acceptability than non-irradiated or irradiated mutton at 1.0 kGy. The meat chunks irradiated at 1.0 and 2.5 kGy remained in acceptable conditions for 3 and 5 weeks, respectively. Whereas, the shelf life of minced and irradiated product was 2 and 4 weeks, respectively. In contrast, non-irradiated meat chunks and minced spoiled within one week of storage.

Grant and Patterson (1991) observed that microbiological population of irradiated pork was mainly composed of lactic acid bacteria, which were responsible for 'sour' or 'dairy' odours.

Rosdriguez *et al.* (1993) suggested that low dose irradiation (2.0 kGy) could be a reliable preservation method to obtain an organoleptically stable retail fresh beef products, since, it reduced naturally occurring spoilage microflora and enhanced the shelf life under refrigeration.

Zhao *et al.* (1996) observed that pork in air permeable packages the odour scores were high initially then decreased after 2 weeks of storage. Wherein, the odour scores between irradiated and non-irradiated samples were indifferent after 2 weeks of storage.

Badr (2004) reported that panelists preferred both irradiated and non-irradiated rabbit meat samples, as the samples were having high acceptance as judged by appearance and odour until rejection. Non-irradiated samples were rejected due to appearance of mould growth, slime formation and off odours by 6^{th} day while irradiated sample showed off odour and mould growth by 12^{th} to 21^{st} day of refrigerated storage (4 ± 1^{0}C).

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

2.3.3 Physicochemical Qualities

2.3.3.1. Proximate Analysis

Sakala *et al.* (1987) reported that carbohydrates, lipids, proteins and amino acids were affected to a minimal degree as a result of low to medium dose of irradiation.

Heath *et al.* (1990) showed that there was no difference in moisture content of non-irradiated (65.0 %) and irradiated (64.0 %) chicken meat at 100, 200, 300 k rads.

Katta *et al.* (1991) found that chicken carcass irradiated at various dose levels ranging from 0 to 3 kGy using gamma radiation and stored in refrigerator conditions did not show any variation in their fatty acid profile.

The proximate composition of ground beef patties was studied and it was found that fat and moisture percentage were not affected by irradiation. They also observed no significant difference in the values of proximate composition between irradiated and non-irradiated patties up to 5 weeks in chiller storage (Wheeler *et al.*, 1999).

Du *et al.* (2001) studied the cooked chicken patties packed in oxygen permeable or impermeable bags, irradiated at 0 or 3 kGy which on analysis revealed that average moisture, fat and pH were unaffected by irradiation.

Daoud *et al.* (2002) studied effect of gamma radiation (0, 3, 5, 7 and 9 kGy) on the chemical and microbial qualities of chilled minced beef and noted that irradiation with different doses resulted in slight changes in chemical composition. During the study, moisture and protein content decreased as storage period extended, whereas fat and ash per cent increased with storage and irradiation doses.

The trace components of food such as essential amino acids, essential fatty acids, minerals and elements were unaffected under practical irradiation conditions although some vitamins such as vitamin C and thiamine were partially lost (Lee, 2004).

Smith and Pillai (2004) reported that macronutrient (protein, lipid and carbohydrate) and mineral content were unaffected by irradiation.

Al-Bachir (2005) reviewed that the luncheon meat on irradiation at 2 kGy and kept for 12 months in refrigerator storage (1-4^{0}C) showed no significant difference in moisture, protein, fat, pH value, total acidity and volatile basic nitrogen.

Rana Raj (2006) observed irradiation at different doses did not significantly affect proximate composition like moisture, ether extract, protein, crude fibre, ash, nitrogen free extract, gross energy, calcium and phosphorus content of intermediary moisture pet food.

Shijin (2008) reported that moisture, fat, protein and ash were not significantly affected either due to irradiation or chitosan application in chicken fry.

2.3.3.2. pH

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

Basker *et al.* (1986) found that pH of non-irradiated chicken leg meat generally increased on storage at 4^{o}C by perhaps 0.5 units in a month, probably as a result of microbiological activity. Irradiation at 2 and 3 kGy had no effect on increase of pH, whereas 3.75 and 4.5 kGy had an effect.

Lee *et al.* (1996) did not observe any difference in pH up to 7 days of storage in irradiated (2.0 kGy) and non-irradiated beef samples, irrespective of storage temperature at 15^{o}C and 30^{o}C. However, after 14 days, pH of the irradiated samples stored at 30^{o}C was the lowest, because of growth of lactic acid bacteria after 7 days.

Increase in pH value during storage of cooked pork patties containing rosemary, ginseng and BHA/BHT as added antioxidant was reported. (McCarthy *et al*., 2001).

Daoud *et al.* (2002) studied effect of gamma radiation (0, 3, 5, 7 and 9 kGy) on the chemical qualities of chilled minced beef and found that the pH values of irradiated samples were lower than those of non-irradiated samples.

Pexara *et al.* (2002) noted a drop in pH during storage in cured, cooked and smoked turkey breast filets at 4 and 10^{o}C. The decrease in pH occurred more rapidly in samples stored at 10^{o}C. They also observed that decrease in pH of sausage was less than in filets due to low fermentable carbohydrate in sausage than in filets.

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

Irradiation of luncheon meat at 2 kGy had not revealed any significant difference in pH value of 1 to 4^{o}C for 12 months (Al- Bachir, 2005).

According to Biswas *et al.* (2006), there was no significant difference found among samples treated with different combinations of spice mix and curry leaf powder but the pH value of chicken patties increased significantly on day 7 and onwards.

Salke (2007) conducted study on preservation of meat cutlet employing gamma radiation under different packaging systems and observed that pH values of the beef cutlet were non significantly increased from 0 to 10th day of storage and thereafter it was gradually reduced.

2.3.3.3. Thiobarbituric Acid Reactive Substances (TBARS)

Dempster *et al.* (1985) reported that doses of 1.03 and 1.54 kGy irradiation of vacuum packaged beef burger gave significantly higher peroxide value than for raw control.

Kanatt *et al.* (1997) irradiated chicken, lamb and buffalo meat by low-dose gamma radiation (2.5 kGy) and stored at 0–3^0 C and found that irradiated meat showed slight increase in thiobarbituric acid (TBA) number on storage as compared to non-irradiated meat.

Kanatt *et al.* (1998) found that TBA values for irradiated samples of ground chicken meat were higher than for non-irradiated samples. Addition of antioxidants tocopherol (natural) or BHT (synthetic) resulted in retardation of oxidative rancidity ($p<0.05$). Meat treated with antioxidants prior to irradiation had lower TBA values as compared to untreated irradiated counterparts.

According to Murano *et al.* (1998) ground beef patties irradiated (2 kGy) and stored under air and those irradiated under vacuum and stored under air, showed a higher degree of lipid oxidation (TBA value) compared with samples irradiated and stored under vacuum or non-irradiated.

Alasnier *et al.* (2000) determined the changes in composition and amount of free fatty acids and TBARS in chicken breast and thigh muscle between 1 and 14 days of storage at 4°C and reported that lipolysis did not promote lipid oxidation.

Du *et al.* (2001) reported that TBARS value of aerobic packed cooked chicken meat patties after 5 days of storage were higher than that of day 0. Irradiation effect on TBARS of both vacuum and aerobic packaged cooked meat was not as significant and consistent as that of day 0, indicating that irradiation had only a minor impact on the oxidation of cooked meat lipid during storage.

Du *et al.* (2001a) reported that at day 0, the TBARS of aerobically packaged turkey and pork patties was significantly higher than those of vacuum packaged, but not for beef. Aerobic packaging significantly increased TBARS in cooked turkey, pork and beef patties after seven day storage, but vacuum packaging was very effective in preventing lipid oxidation, irradiation had only a minor effect.

Nam and Ahn (2002) reported that under vacuum condition, lipid oxidation of irradiated (1.5 kGy) raw turkey breast patties did not increase during 10 days storage, while lipid oxidation in non-irradiated increased during storage.

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

Du *et al.* (2003) conducted a study on quality characteristics of irradiated chicken breast roll and stated that after irradiation the total amount of volatiles in chicken rolls doubled compared with the initial values. Volatiles induced by irradiation include alkenes, aldehydes and sulphur compounds.

Lee *et al.* (2003) reported that addition of sesamol and tocopherol or gallate and tocopherol lowers TBARS values and aldehydes in irradiated turkey meat, especially under aerobic conditions.

Aerobic packaging and irradiation both increased the lipid oxidation of turkey breast patties, but presence of oxygen was a more critical factor than irradiation on lipid oxidation during storage. The TBARS of meat was the highest with aerobic packaging, the lowest with vacuum packaging and in the middle with double packaging (Nam and Ahn, 2003).

Irradiation (2 kGy) and storage of turkey breast rolls (vacuum packaged shortly after cooking) increases the TBARS value from 0.104 to 0.175 mg mal (malonaldehyde)/kg, while in non-irradiated it increased from 0.029 to 0.183 mg mal/kg at 0 to 28 days, respectively because of presence of residual oxygen or oxygen permitting packaging material during storage. However, due to vacuum packaging TBARS did not change significantly at day 0 or 14 days of refrigerated storage (Zhu *et al.,* 2004).

Kanatt *et al.* (2005) found that non-irradiated control samples showed lower TBARS than irradiated samples. Increase in TBARS was dose dependent in case of mutton shammi kabab and pork salami. However, in case of chicken chilly the increase in TBARS values of irradiated samples was not significant, probably due to spices used in the preparation that are known to have antioxidant activity.

Nam *et al.* (2006) found that rosemary and α-tocopherol combination at 0.05% and 0.02% of meat weight, respectively, showed potent antioxidant effects in reducing both TBARS values and the amounts of volatile aldehydes in irradiated raw and cooked pork loins.

2.3.3.4. Tyrosine Value (TV)

Cessation of the proteolytic reaction due to bacteria or endogenous enzymes during frozen storage caused slight decrease in tyrosine values of plate frozen meat cuts and minced meat (Ziauddin *et al.*, 1993).

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

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

There was no significant difference in tyrosine value among aerobic (8.89 mg/100g), vacuum (9.25 mg/100g) and modified atmospheric (8.59 mg/100g) packaging methods when the samples were stored at 4 ± 1^{o}C (Jayanthi, 2003).

Kuttinarayanan *et al.* (2005) reported that proteolytic changes as estimated by tyrosine value have not shown any significant change between control and irradiated turkey breast samples initially. As the period enhanced from 0 to 25th

day a non significant increase in tyrosine value was noticed, which is an expected biochemical change in refrigerated meats.

In aerobically packed chicken Balamatsia *et al.* (2006) found that volatile amines, both trimethyl amine nitrogen (TMA-N) and total volatile basic nitrogen (TVB-N) values for non-irradiated samples increased steeply, whereas irradiated samples showed lower TMA-N and TVB-N values ($P<0.05$) during refrigerated storage of 21 days.

Jenifer (2006) reported that irradiation treatment of minced beef had no significant effect on tyrosine values compare to control samples at day 0. As storage days increased, tyrosine value increased with significant change among the treatments. For irradiation dose of 3 kGy it was increased from 3.04 to 5.95 mg/100g of sample.

Shijin (2008) observed that tyrosine value, indicating the proteolytic changes in meat showed a comparatively higher value in control non-irradiated samples (6.96 mg/100g) compared to the treatment groups with the lowest in chitosan treated irradiated (6.05 mg/100g) followed by irradiated samples (6.14 mg/100g) of chicken fry.

2.3.4. Microbiological Analysis

2.3.4.1. Aerobic Plate Count (APC)

Basker *et al.* (1986) showed that irradiation of raw whole chicken carcass by 2 to 4.5 kGy reduce the initial total aerobic mesophilic count by a factor of 10^3 to 10^4, while in subsequent storage at 4°C for 30 days the total count gradually rose to the initial value of non-irradiated samples.

Thayer (1993) reported that the irradiation dose required for inactivating 90 per cent of the Colony Forming Unit (CFU) of common food borne pathogens associated with meat and meat products was in the range of 1.0 to 4.0 kGy.

Naik *et al.* (1993) suggested a dose of 2.5 kGy would reduce the mesophilic count of buffalo meat samples immediately by 2 to 3 log cycles and observed that after 3 weeks of storage at 0-3^{o}C, the CFU of irradiated meat was equivalent to the initial CFU of control and had a shelf life of 4 weeks.

Mcateer *et al.* (1995) observed that low dose irradiation (2 and 3 kGy) reduced the number of microorganisms in the meat to less than 100 per g and microbial growth did not occur during storage (2-3^{o}C for 15 days).

Decrease in total plate count by 3 log cycles in buffalo and 2 log cycles each in chicken and lamb meat observed when subjected to low dose gamma radiation (2.5kGy). Enterobacteriaceae and fecal coliforms were not detected in irradiated buffalo meat throughout the storage period however, they were present in the control samples and their numbers increased on storage at 0-3^{0}C (Kanatt *et al.,* 1997).

Murano *et al.* (1998) observed that the microbial quality of irradiated ground beef patties was better than that of non-irradiated, with 2 to 3 log reduction in total viable count immediately after irradiation. Non-irradiated patties reach a load of 10^{7} cells per g after 8 days, whereas irradiated patties reached 10^{7} cells per g after 55 days of storage at 4^{o}C.

Lewis *et al.* (2002) observed that irradiation dose of 1.0 and 1.8 kGy reduced the mean count of 4.6 log CFU per 200 ml of rinsate in boneless skinless chicken breast to 2.23 and 1.62 log CFU per 200 ml of rinsate, respectively.

Quattara *et al.* (2002) evaluated the combined effect of gamma radiation and incorporation of naturally occurring antimicrobial compounds on microbial and biochemical characteristics of ground beef. Irradiation of ground beef patties significantly reduced the total aerobic plate counts (APC). Irradiation doses of 1, 2, and 3 kGy produced immediate reduction of 2, 3, and 4 log units of APCs, respectively. Shelf-life periods were higher for ground beef samples containing

ascorbic acid alone and ascorbic acid coated with the protein-based coating containing spices.

Kanatt *et al.* (2005) reported that in a refrigerated storage (0-3^{o}C) of irradiated (3 kGy) ethnic Indian meat products, non-irradiated chicken chilly had counts greater than 6 log CFU per g in less than 14 days, while in irradiated, it did not reach the number even after 28 days of storage.

Chouliara *et al.* (2006) noted the TVC of 6 log CFU per g in meat or fat trimmings used for Greek dry salami was reduced by irradiation at a dose of 2 kGy (4.8 log CFU per g) and 4 kGy (3.9 CFU per g) with *Pseudomonas* showing the highest sensitivity while yeast were the most resistant followed by lactic acid bacteria. Both of these doses reduced population of *Enterobacteria*, *Enterococci* and pathogenic *Staphylococci* to 1, 2 and 2 log CFU per g, respectively while *Listeria* were undectable.

Hassan *et al.* (2007) investigated the microbial quality as well as the effect of gamma radiation (dose level of 2.0, 3.0, 3.5 and 4.0 kGy) on the microbial population of ready-to-eat meat, frozen beef kofta, vegetarian kofta, beef burgers and vegetarian burgers. The microbial reduction increased as the dose level of irradiation increased, whereas irradiation of meat product samples at 2 kGy dose reduced aerobic counts and inactivated *Staphylococcus aureus, Escherichia coli* and Enterobacteriaceae.

2.3.4.2. Psychrotrophic Count

Niemand *et al*. (1983) reported that *Pseudomonas* spp., and *Enterobacteriaceae* could not be detected throughout the entire storage period since radurization of minced beef at dose of 2.5 kGy completely eliminated them.

Mattison *et al*. (1986) observed that irradiated (100 krad) vacuum packaged pork loins showed less psychrotrophic count of 0.7 to 1.9 log CFU per g

than non-irradiated samples 0.7 to 3.6 CFU per g from 0 to 21 days of storage at 4^{o}C, as storage time increased difference became greater.

Lambert *et al.* (1992) showed that irradiation of fresh pork at 1.0 kGy reduced psychrotrophic and mesophilic bacterial populations by two log cycles and inactivated *Enterobacteriaceae*, whereas lactic acid bacteria were largely unaffected regardless of packaging atmosphere.

Lewis *et al.* (2002) indicated that in boneless skinless chicken breast the mean psychrotrophic count was 1.92 log CFU per 200 ml of rinsate in control and was not detected when the samples were subjected to an irradiation dose of 1.0 to 1.8 kGy.

Gomes *et al.* (2003) reported that psychotropic counts were higher for non-irradiated samples in mechanically deboned chicken meat up to 8th day in refrigeration than irradiated samples. However, psychrotrophic count exceeded the recommended limit of 6.48 log CFU per g after 6 days in non-irradiated, while in irradiated (3.0 and 4.0 kGy) it was only after 12 days of storage.

Irradiation doses of 1.5 and 3.0 kGy reduced the counts of aerobic mesophilic bacteria, psychrophilic bacteria, yeast and mould and prolonged the shelf life of refrigerated rabbit meat samples to 12 and 21 days compared to 6 days for non-irradiated controls (Badr, 2004).

Chouliara *et al*. (2006) found that the count of *Pseudomonas*, *Enterococci* and pathogenic *Staphylococci* and *Enterobacteria* in meat and fat trimmings used for sausage production was reduced to less than 2 and 1 log CFU per g by irradiation at 2.0 and 4.0 kGy, respectively. Wherein, *Pseudomonas* showed the highest sensitivity with reduction of more than 3.4 log CFU per g for either doses and natural contamination of *Listeria* spp. was eliminated.

Salke (2007) observed a significant reduction ($P<0.05$) in psychrotrophic count due to irradiation and vacuum packaging of beef cutlets under chiller storage.

2.3.4.3. Yeast and Mould Count

On irradiation with 2.5 kGy, Monk *et al.* (1995) observed reduction in yeast population on the chicken breast from 5×10^2 CFU per g to 3.2×10^1 CFU per g. They also reported that *Sporobolmyces roseus* exhibited the least resistance whereas, *Trichosporon* and *Candida* show maximum resistance towards gamma radiation.

Abu-Tarboush *et al.* (1997) reported that yeasts of genera *Candida*, *Saccharomyces* and *Alternaria* started to grow on 12^{th} day in chicken stored at 4^oC treated with less than 5.0 kGy for 21 days of storage, but not in samples treated with more than 5 kGy.

Even though moulds, yeasts and sulphite reducing *Clostridia* were the most resistant species, irradiation at 10.0 kGy led to optimum sanitization of red paprika (Nieto-Sandoval *et al.*,2000).

Balamatsia *et al.* (2006) studied the effect of low dose radiation on the microbiological characters of chicken meat stored aerobically at 4^oC and they found that *Pseudomonas* spp*., Enterobacteriaceae*, yeast and moulds were highly sensitive to gamma radiation and were completely eliminated at 2 kGy.

Chouliara *et al.* (2006) reported that yeast were the most resistant followed by lactic acid bacteria and their reduction is dose dependent. Yeast did not show any major growth due to injury caused by irradiation (2 and 4 kGy) but survived without death.

Kuttinarayanan *et al.* (2006b) observed a 97 per cent reduction with respect to yeast and mould count in minced beef by irradiation at 2.0 kGy and 95-98 per cent in other meat and meat products (Kuttinarayanan, 2007).

2.3.5. Organoleptic Evaluation

2.3.5.1. Colour

Kropf (1980) suggested that colour is probably the single greatest appearance that determines whether the meat cut would be purchased.

> Darmadji and Izumimoto (1994) opined that addition of chitosan to meat resulted in better sensory attributes and had good effect on the development of red colour of meat during storage. There was an increase in a* and b* values of all chitosan added meat samples during storage.

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

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

Alur *et al.* (1998) showed that radicidation dose of 2.5 kGy in pork meat products did not cause any adverse effect on texture, odour, flavour and pigments of products.

Chen *et al.* (1999) conducted the study on irradiated (4.5 kGy) pork patties with antioxidants (sesamol, quercetin, rutin, BHT, and rosemary oleoresin) stored at 4°C and observed the effects of antioxidants on colour changes of raw pork patties were minor and inconsistent.

Zhu *et al.* (2003) reported that irradiation up to 2 kGy has limited effects on colour and oxidation of vacuum packaged commercial turkey ham.

According to Smith and Pillai (2004), irradiation at a dose less than 3 kGy causes no difference in flavour, texture or colour of ground beef.

Shijin (2008) reported that there was significant improvement in colour by chitosan coating, irradiation and their combination (8.35, 8.36, and 8.39 respectively) than control non-irradiated (8.31) samples of chicken fry on the day of preparation.

Stetzer *et al.* (2009) conducted the study on the effect of citric acid and rosemary extract on colour of an irradiated beef myoglobin model system and observed that rosemary extract and citric acid maintained colour lightness and redness. It was also observed that surface application of these antioxidants had the potential to preserve red meat colour during irradiation.

2.3.5.2. Flavour

Niemand *et al.* (1981) observed a higher ranking throughout storage period for both appearance and odour evaluation in radurized samples. On the day of irradiation, experienced person could detect a faint but typical irradiation odour in radurized samples although it was not found to be objectionable. When evaluated for aroma and taste, radurized samples showed a low score in fourth week and higher score at eighth week than control.

Heath *et al.* (1990) reported that irradiation produced a detectable odour in raw chicken thigh after exposure to 100, 200 and 300 krads and in cooked thigh after exposure to 200 and 300 krads. No odour was found in cooked thighs after irradiation at 100 krads and was dependant on fat content of the sample.

Hashim *et al.* (1995) observed that irradiating uncooked chicken meat produced a characteristic bloody and sweet aroma that remained even after cooking the meat.

According to Patterson and Stevenson (1995), α-Tocopherol and ascorbic acid induced stability in tissues *in vivo* and *post mortem.* The use of enhanced

concentrations of these two vitamins in combination in the diet of poultry may provide a means of controlling development of off-odour in irradiated raw chicken, thus improving the consumer acceptability. Yields of irradiation volatiles from the tissues of these birds were very much reduced compared to yields from similar tissues from birds fed unsupplemented diets.

Zhao *et al.* (1996) showed that odour of irradiated products was as less desirable than non-irradiated but score did not change during 4 weeks of storage. For non-irradiated pork in air permeable packages, odour score was high initially, then decreased after 2 weeks of storage. Score between irradiated and non-irradiated remained the same after two weeks of storage.

Du *et al.* (2001) reported that dietary conjugated linoleic acid treatment had no effect on the odour of irradiated cooked chicken meat but, irradiation produced relatively small significant odour difference in cooked chicken meat patties.

According to Nam and Ahn (2002), for short term storage, irradiation of turkey breast meat in which lipid oxidation is 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 reduced under aerobic conditions.

Ahn and Lee (2005) observed that irradiation of ready-to-eat turkey breast rolls at 3 kGy showed irradiation odour in treated samples was two times higher than those of non-irradiated samples and irradiation had no effect on colour and texture of ready-to-eat turkey breast rolls.

Arthur *et al.* (2005) reported when chilled carcasses were subjected to low dose irradiation, there was no difference in flavour of irradiated (1 kGy) and non- irradiated ground beef patties samples.

Kanatt *et al.* (2005) reported that irradiation of Indian ethnic meat products like chilly chicken, mutton shammi kabab and pork salami either at 1, 2 or 3 kGy did not impart any detectable odour.

Shijin (2008) reported that, in chiller stored chicken fry samples significant reduction of flavour score was noticed with enhanced storage period.

Brewer (2009) showed that irradiating fresh meat, even at low doses, can result in off-odours and flavours which have been described as rotten egg, bloody, fishy, barbecued corn, burnt, sulfur, metallic, alcohol or acetic acid and suggested methods to decrease the detrimental effects of irradiation include oxygen exclusion (vacuum packaging), replacement with inert gases (nitrogen), addition of protective agents (antioxidants), and post-irradiation storage to allow flavour to return to near normal levels .

2.3.5.3. Juiciness

Luchsinger *et al.* (1996) evaluated acceptance of fresh or frozen irradiated boneless pork chops (1.5, 2.5 and 3.85 kGy) using a trained panelist and consumers. No differences in acceptance, meatiness, freshness or juiciness of products irradiated at 2.5 kGy or below were observed by them.

Irradiation doses (2.5 to 10.0 kGy) had little effect on the sensory acceptability (appearance, odour, texture and taste) of both raw and cooked chicken. Moreover, juiciness and tenderness of cooked chicken were only slightly affected by irradiation. (Abu-Tarboush *et al.*, 1997).

According to Murano *et al.* (1998), ground beef patties irradiated under vacuum and tasted one day later demonstrated increased juiciness, while those irradiated under vacuum but stored aerobically showed increased tenderness. Samples evaluated after seven days of storage showed no difference in any sensory attributes.

In an experiment by Ohene-Adjei *et al.* (2004) reported that irradiation neither affected juiciness of ground pork and the loin chops, nor the texture or mouth feel of the ground pork but decreased the tenderness of loin chops. An increased juiciness was noted in irradiated ground pork when supplemented with vitamin E.

Johnson *et al.* (2004) showed that overall acceptance, juiciness and tenderness of non-irradiated diced chicken and frankfurters were significantly lower than irradiated (1, 2, and 3 kGy) at day 18 and day 32, respectively at 4^{o}C.

According to Shijin (2008), irradiated samples had maximum score (8.42) followed by chitosan coated irradiated samples (8.41) compared to control non-irradiated samples (8.14) of chicken fry on the day of preparation.

2.3.5.4. Tenderness

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

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

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

Murano *et al.* (1998) studied the changes in flavour, texture and juiciness of ground beef patties after either 2 or 7 days of storage at 25^{o}C prior to cooking. It was noted that, irradiated air or vacuum packed samples were more tender,

irradiated vacuum packed samples were more moist and irradiated air samples had the least aftertaste.

Ohene-Adjei *et al.* (2004) reported that irradiation (1.5 kGy) of loin chops decreases the tenderness, which might be due to weakened texture of meat system due to irradiation that caused loss of moisture through drip or purge loss.

Arthur *et al.* (2005) suggested that on low dose irradiation (1 kGy) of ground beef patties the tenderness and juiciness were not dose related and ratings decreased with increased duration of frozen storage.

2.3.5.5. Overall Acceptability

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

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

The consumer acceptance of irradiated poultry cooked products based on colour, appearance, flavour, mouth feel and overall acceptability using a nine point Hedonic scale, 73 per cent participants gave the product a minimum rating of 7.0. (Hashim *et al.*, 1995).

Spoilage changes of non-irradiated and irradiated beef burger and beef kabab samples could be in the form of souring, stickiness and disintegration. Colour and appearance were good but decrease in odour, texture and overall acceptability were noticed in irradiated and non-irradiated kababs on storage (Sawant, 1998).

Wheeler *et al.* (1999) indicated that hamburgers made from ground beef patties irradiated under usual conditions would encounter little consumer acceptance problems at the 3.0 kGy dose and only slightly more problems at 4.5 kGy dose.

Badr *et al.* (2004) studied that the samples of fried burgers prepared from both irradiated and non-irradiated fresh rabbit meat had similar high score for odour, taste, texture and juiciness. This indicated that irradiation of rabbit meat at 1.5 and 3.5 kGy doses did not significantly affect the sensory quality of cooked meat.

Johnson *et al.* (2004) reported that although quality of the irradiated samples decreased with increasing storage time, the overall acceptance of flavour, juiciness, tenderness and mouth feel of non-irradiated diced chicken and frankfurter were significantly lower than irradiated (1, 2 and 3 kGy) at day 18 and 32, respectively.

Kanatt *et al.* (2005) reported that overall sensory scores for appearance, flavour and texture of irradiated samples (1, 2 and 3 kGy) of three meat products (chicken chilly, mutton shammi kababs and pork salami) were different from their non-irradiated controls and were acceptable immediately after irradiation.

Kuttinarayanan (2005) studied consumer acceptance of irradiated cutlet, beef and minced beef and revealed that 20 to 22 per cent consumer responded, 72.5 per cent were willing to purchase irradiated cutlet and 37 per cent were ready to pay more to irradiated product since it can be kept at chiller conditions. Majority of them did not notice any peculiar smell or taste difference in the products due to irradiation.

2.4. COST OF PRODUCTION

Sangilimadan (1997) calculated the cost of ready-to-cook duck meat sticks prepared by two different recipes and obtained Rs. 5.63 and Rs. 5.73 as the cost of one duck meat stick correspondingly.

Murugan (1998) conducted study on the assessment of the quality of tenderized chicken meat pickle and found that the cost of production of one kg chicken pickle prepared using non tenderized and tenderized meat as Rs. 60.27 and Rs. 60.45 respectively.

The cost of production of low fat turkey loaf was calculated by Naseera (2007) and obtained Rs. 160.00, Rs. 179.00, Rs. 167.00 and Rs. 164.00 for added fat product, low fat product, low fat with carrageenan and low fat with non fat dry milk respectively.

Shijin (2008) observed during the study of effect of hurdle technology, chitosan and gamma radiation on quality parameters of chicken fry, the cost of production was Rs. 109.83 and Rs. 114.21 per kg for the control and chitosan treated groups respectively.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

A study on the effect of curry leaves (*Murraya sp.*) and peppermint (*Mentha sp.*) paste on shelf life of irradiated chicken tikka was conducted at the Department of Livestock Products Technology, Mannuthy.

Seven batches of chicken tikka were prepared, packaged, irradiated at 2.5 kGy and stored at chiller (1-4^{o}C). Samples were analysed for physical, physicochemical, microbiological and organoleptic evaluation on the day of preparation and on day 5, 10, 15, 20, 25, 30, 45 and 60 of chiller storage or until spoilage, whichever was earlier. The samples were analysed for proximate composition on the day of preparation.

3.1. PREPARATION OF CHICKEN TIKKA

Broiler chicken of 1.9 to 2.0 kg were procured from the local market, slaughtered and dressed under hygienic conditions at the Department of Livestock Products Technology. The cleaned and washed carcasses were made into cuts of uniform size of about 20 to 25 mm cubes. Gizzard, liver, spleen and skin were not included with the cuts.

The flow chart for preparation of chicken tikka is given in the figure 1. The marinade (Table 1) was prepared by mixing the ground spices with other ingredients. Chicken cubes were uniformly coated with the marinade and kept overnight at chiller temperature and these marinated cuts were steam cooked for 10 minutes on low flame. The batter (Table 2) was prepared using stock water and the partially cooked cuts were mixed uniformly in batter followed by deep fat frying in double refined deodourised vegetable oil.

3.1.1. Addition of Curry Leaf Paste (CL) and Peppermint Paste (PL)

Curry leaf paste at the rate of one percent (chicken + marinade + batter) was added in the control group. Similarly the peppermint paste at a rate of one percent was added to another set. To a third set one percent each CL and PL paste was also added.

Table 1. Composition of the marinade for chicken tikka

Ingredients	Quantity(g)
Chicken	1000
Turmeric powder	3
Salt	8
Pepper powder	3
Clove powder	1
Red Chilly powder	3
Garlic paste	5
Tomato puree	100

Table 2. Composition of the batter for chicken tikka

Ingredients	Quantity(g)
Refined wheat flour	85
Corn flour	60
Bengal gram flour	25
Salt	6
Pepper powder	5
Red Chilly powder	4
Tomato puree	30

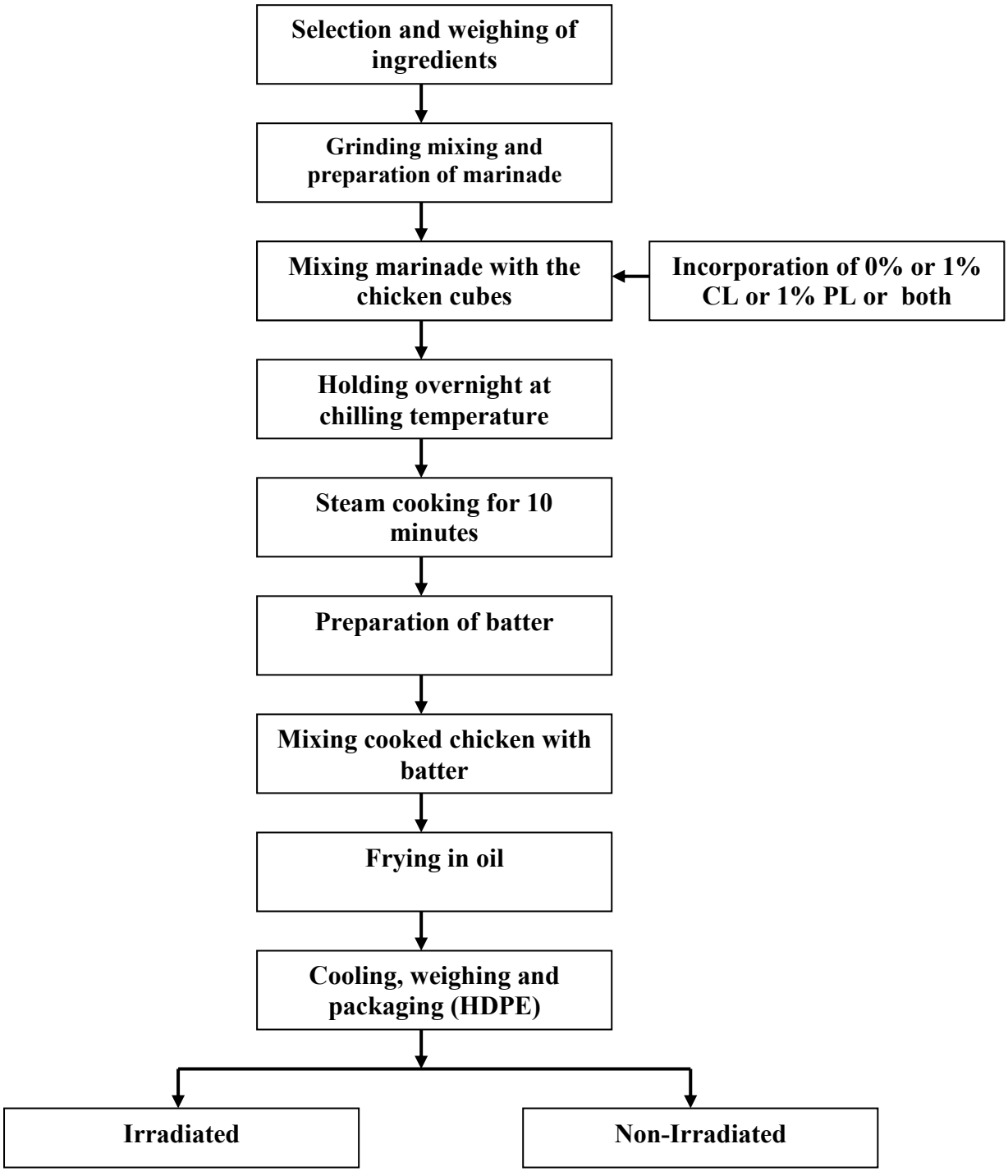

Figure 1. Flow chart for chicken tikka preparation

3.2. GAMMA RADIATION

Immediately after packaging gamma radiation of the product was carried out at melting ice temperature in Gamma Chamber 5000, (BRIT-DAE, Mumbai) where ^{60}Co is the source of radiation.

The non-irradiated control samples were designated as C-NR, irradiated samples as C-IR, curry leaf treated as CL-NR, curry leaf treated irradiated samples as CL-IR, peppermint treated as PL-NR, peppermint treated irradiated as PL-IR and combination of curry leaf paste and peppermint paste treated as CLPL-NR, combination of curry leaf paste and peppermint paste treated irradiated as CLPL-IR.

The samples were stored at chiller temperature (1-4^{0}C) and were analysed on the day of preparation and on 5, 10, 15, 20, 25, 30, 45 and 60 day or until spoilage which was assessed with physical qualities, *viz*., odour and colour, consistency, slime formation and mould growth. The proximate analysis of the product was conducted on the day of preparation. The non spoiled samples were analysed for the following parameters.

3.3. PHYSICAL QUALITIES

Chicken tikka packets stored at the chiller storage were opened on 0, 5, 10, 15, 20, 25, 30, 45 and 60 days of preparation and examined for signs of spoilage, *viz*., change in colour, odour, consistency, slime formation and mould growth.

3.4. PHYSICOCHEMICAL QUALITIES

3.4.1. Proximate Composition

Chicken tikka was analysed for its proximate composition, *viz*., moisture, fat, protein and ash content on the day of preparation. The composition was expressed as percentage of the chicken tikka.

3.4.1.1. Moisture

The moisture content of the chicken tikka was analysed as per (AOAC, 1990). A 30 g sample in an evaporating dish was kept in a hot air oven at 100ºC to 102ºC for 16 to 18 h. The weight of the dry samples was taken after cooling in a desiccator. The difference in the weight was recorded as the moisture content and expressed as percentage.

3.4.1.2. Fat

Fat was estimated as per AOAC (1990). Fat content of three grams of moisture free sample was extracted in petroleum ether (boiling range 40-60ºC) using Socs Plus Solvent Extraction System (Pelican Equipments, India). Ether extract obtained was dried to a constant weight at 100ºC, cooled and weighed. The difference in weight is the total fat content of sample and expressed as percentage of the chicken tikka.

3.4.1.3. Protein

The Copper Catalyst Kjeldal method was used to determine the protein content of the samples (AOAC, 1990). The analysis was conducted in Kel Plus Nitrogen Estimation System (Pelican Equipments, India). The total nitrogen estimated was converted to percentage of protein by multiplying with the constant.

$$\text{Protein \%} = 6.25 \text{ X \% Nitrogen.}$$

3.4.1.4. Ash

Ash is the total mineral content of a sample. Five grams of sample was placed in a silica crucible and kept in a muffle furnace at 600±20ºC for 2.5 hours. Then the sample was transferred to a desiccator, allowed to cool and weighed immediately. The resultant weight is the total mineral content of the sample and ash content was converted to wet matter basis (AOAC, 1990).

3.4.1.5. Energy Calculation

The energy content of chicken tikka was determined as per FAO (2002) on wet matter basis.

Energy (kcal) = (fat per cent x 9) + (protein per cent x 4) + (carbohydrate per cent x 4).

3.4.2. pH

The pH of irradiated and non-irradiated, samples stored at chilling temperature was recorded by using a digital pH meter (µ pH system-Systronics, India). About 10 g of chicken tikka sample was homogenized with 50 ml of distilled water and the electrode was inserted into the supernatant liquid. The pH was recorded and the probe was thoroughly rinsed with deionised distilled water before each measurement. The pH meter was standardized using pH 4 and pH 7 buffer solutions at weekly intervals.

3.4.3. Thiobarbituric Acid Reactive Substances (TBARS)

The Thiobarbituric Acid Reacting Substances (TBARS) were determined as per Alasnier *et al.* (2000).

Two g of sample were mixed with butyl hydroxyl toluene (BHT) in ethanol (10µg BHT/ g of lipids) and 16 ml of trichloroacetic acid (TCA 5%). Samples were homogenized for 20 s at 20,000 rpm and then filtered through Whatman filter (No.4). Two ml of filtrate was added to two ml thiobarbituric-acid solution (TBA 20 mM). The tightly closed tubes were heated at 70^0C for 30 min and then cooled and centrifuged at 8000 rpm for 10 min to obtain a clear supernatant. The absorbance of the pink coloured supernatant was measured at 532 nm against a blank containing 2ml distilled water and 2ml 20 mM thiobarbituric acid solution in UV-Vis Spectrophotometer 119 (Systronics, India). By reference to the standard graph (Figure 1.) the TBARS was calculated and expressed as mg of malonaldehyde/ kg of chicken tikka.

3.4.3.1. Standard Graph for Thiobarbituric Acid Reactive Substances (TBARS)

Five micro litre malonaldehyde was dissolved in 5% trichloroacetic acid and butyl hydroxyl toluene (BHT) in ethanol (10µg BHT/ g of lipids) in a 500 ml volumetric flask and then solution was made up to the mark with water. The following volumes of malonaldehyde solution were then added to a series of 100 ml volumetric flasks: 0, 2, 3, 4, 5, 6, 7, 8, 9, 10 ml. Each was made up to the mark with double distilled water and mixed. Two ml of each solution were shaken with two ml thiobarbituric-acid solution (TBA 20 mM). The tightly closed tubes were heated at 70^0C for 30 min then treated as described as for the determination above. The standard graph (Figure 1) was prepared by plotting optical density against mg of malonaldehyde/ g of sample (assuming that 4.0g were used).

3.4.4. Tyrosine Value (TV)

The tyrosine values of the samples were estimated as per the method described by Pearson, D. (1968) as follows.

Two grams of sample were weighed and 40 ml of 5% trichloroacetic acid solution were added. After homogenization for 2 min the sample was filtered and the filtrate was collected. The filtrate, termed TCA extract was used in the estimation of tyrosine value. To 2.5 ml of TCA extract, equal quantity of distilled water was added in a test tube and shaken with 10 ml of 0.5 N NaOH and 3 ml of diluted Folin and Ciocalteu's phenol (FC) reagent (1 ml of concentrated FC reagent and 2 ml of distilled water). After mixing, the contents were allowed to stand for 5 min at room temperature, the optical density was measured at 660 nm in UV-Vis Spectrophotometer 119 (Systronics, India) using a blank containing 2.5 ml of 5% TCA, equal quantity of distilled water was added in a test tube and shaken with 10 ml of 0.5 N NaOH and 3 ml of diluted Folin and Ciocalteu's phenol (FC) reagent (1 ml of concentrated FC reagent and 2 ml of distilled water) for comparison. By reference to the standard graph (Figure 2) the TV was calculated and expressed as mg/100 g of chicken tikka.

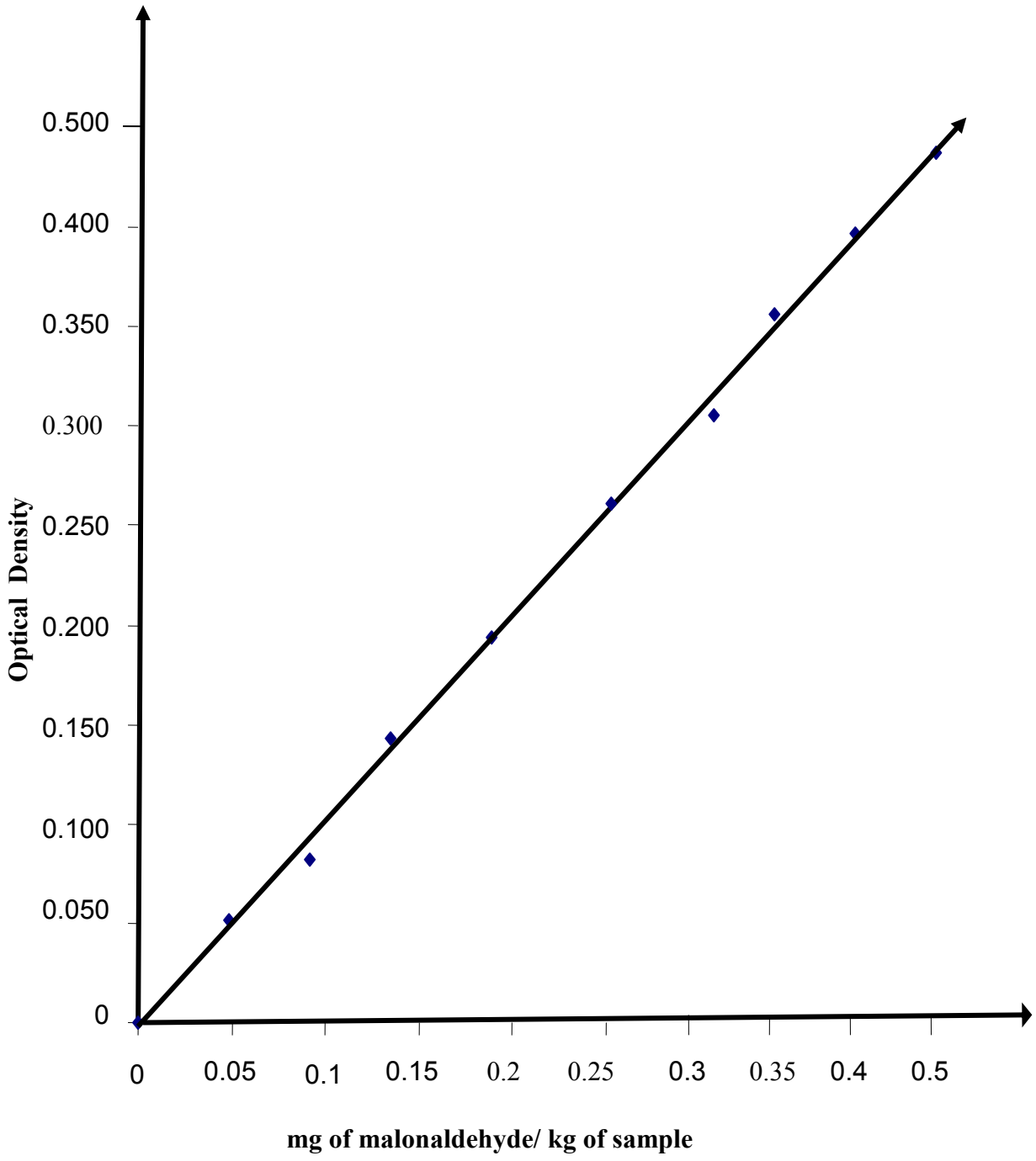

Figure 1. Standard graph for Thiobarbituric Acid Reactive Substances (TBARS)

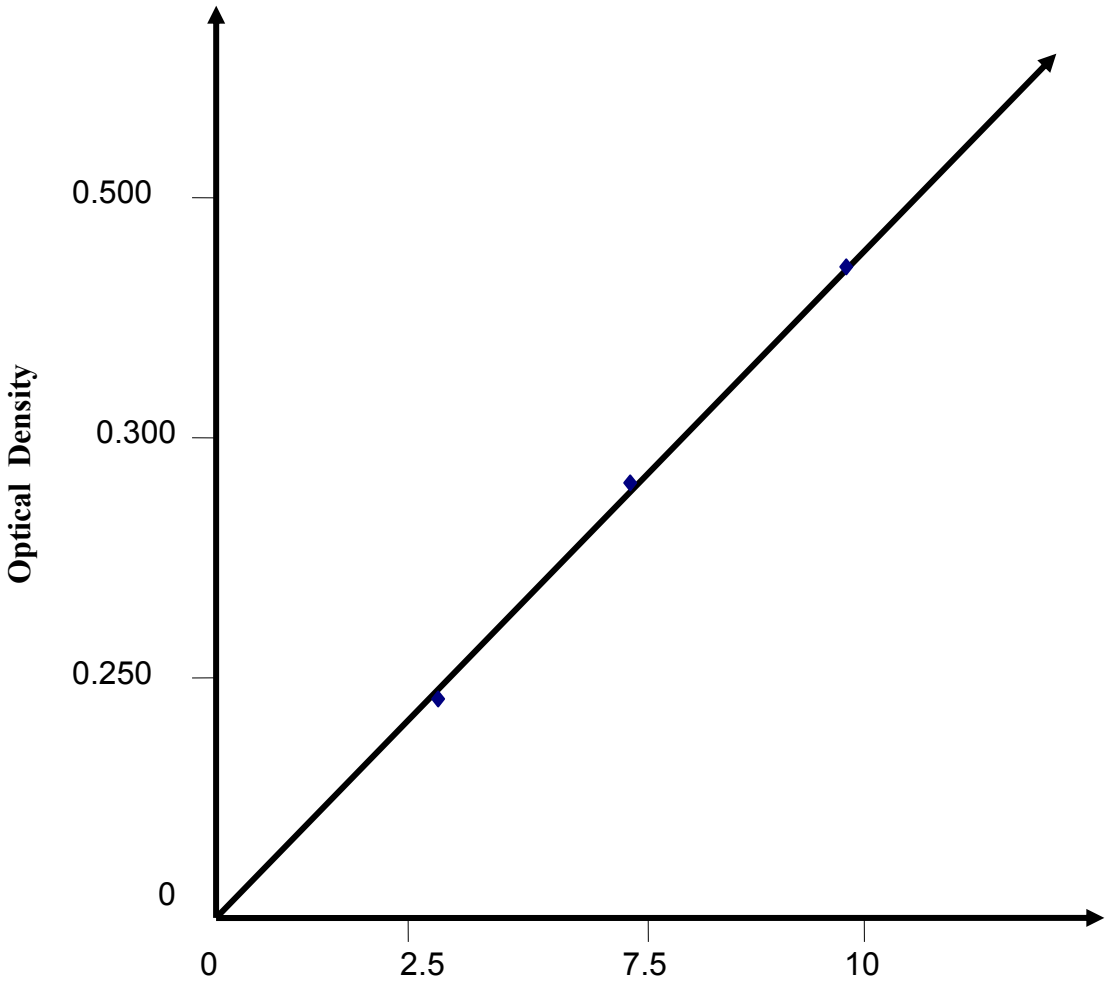

Figure 2. Standard graph for Tyrosine value

3.4.4.1. Standard Graph for Tyrosine Value

0.1 g tyrosine were dissolved in 5% trichloroacetic acid in a 500 ml volumetric flask and then solution was made up to the mark with water. The following volumes of tyrosine solution were then added to a series of 100 ml volumetric flasks: 0, 1, 3, 5, 7, 10, 12, 15, 20 ml. Each was made up to the mark with double distilled water and mixed. 5 ml of each solution were shaken with sodium hydroxide solution and diluted Folin and Ciocalteu's reagent and then treated as described as for the determination above. The standard graph (Figure 2) was prepared by plotting optical density against mg tyrosine/100 g sample (assuming that 2g were used).

Recoveries were checked by adding known amounts of tyrosine dissolved in trichloroacetic acid solution.

3.5. MICROBIOLOGICAL ANALYSIS

Sealed packets of chicken tikka were opened under aseptic precautions and 25 g of the sample was homogenized for 30 seconds at 230 rpm with sterile 225 ml of 0.01 per cent peptone water (diluent) in a stomacher (Seward Stomacher® 400 circulator) so as to form one in 10 dilution of the sample. Further serial 10 fold dilutions were prepared by transferring one millilitre of inoculum to nine millilitre of the diluents. Selected serial dilutions were used to estimate the count of aerobic bacteria, psychrotrophic bacteria, yeast and mould and converted and expressed as $\log_{10}$ cfu (colony forming units)/g of sample.

3.5.1. Aerobic Plate Count (APC)

Aerobic plate count (APC) of each sample was estimated by pour plate technique, as described by Mortan (2001). From the selected dilution of each sample, 1 ml of inoculum was transferred in labeled duplicate petri dishes of size 100×17 mm. To each of these inoculated plates, about 15-20 ml sterile

molten Standard Plate Count Agar (HiMedia, Mumbai) maintained at 45ºC was poured and mixed with the inoculum by gentle clockwise, anticlockwise, forward and backward movements. The inoculated plates were allowed to solidify at room temperature and incubated at 37°C for 24 hours in inverted position. At the end of the incubation period, the plates having colonies between 20 and 200 were selected and counts were taken with the help of a digital colony counter (Royal, India). The number of cfu per g of the sample was calculated by taking the average of duplicate plates and multiplied by the dilution factor and converted to $\log_{10}$ cfu/g of sample.

3.5.2. Psychrotrophic Count

Psychrotrophic count was assessed as per Cousin *et al.* (2001). Inoculated agar plates by pour plate method, prepared as in the case of aerobic plate count were incubated at 7±1ºC for 10 days in BOD incubator (Rotec, India). At the end of the incubation period, petri dishes with a bacterial count between 20 and 200 colonies were selected and the colony counts were taken with the help of a digital colony counter (Royal, India). The number of cfu per g of the sample was calculated by taking the average of duplicate plates and multiplied by the dilution factor and converted to $\log_{10}$ cfu/g of sample.

3.5.3. Yeast and Mould Count

Method described by Beuchat and Cousin (2001) was followed for estimation of yeast and mould count per gram of the sample. Potato Dextrose Agar (HiMedia, Mumbai) was used for the estimation of yeast and mould count by pour plate technique. From the selected dilutions of each sample 1 ml of inoculum was transferred on to duplicate plates. To each plate 15 to 20 ml of sterile molten media at 45ºC was added mixed well and allowed to solidify. The plates were incubated at 25-27ºC for 3 days. After incubation colonies were counted with the help of a digital colony counter (Royal, India) and average count was multiplied with the dilution factor and expressed as $\log_{10}$ cfu/g.

3.6. ORGANOLEPTIC EVALUATION

Taste panel assessment of the non spoiled chicken tikka was conducted with the help of semi trained taste panelists drawn from the Department of Livestock Products Technology, Mannuthy. Uniform amount of the product from each group was selected and was heated to 65ºC. The panelists were served with coded samples and a score card was also provided (Table 3). They were asked to rate in the nine point Hedonic scale (Badr, 2004). The individual scores were recorded and the average was taken as the score for the particular attribute.

3.7. COST OF PRODUCTION

The cost of production of chicken tikka was calculated based on the prevailing cost of chicken and other ingredients used for the preparation.

3.8. STATISTICAL ANALYSIS

The data obtained on physicochemical, microbiological and sensory evaluation of samples were statistically analysed by one-way analysis of variance up to 25 days of storage period and thereafter by *t*-Test (Two samples assuming equal variances), Kruskal-Wallis test using SPSS soft ware as per Snedecor and Cochran (1994).

Table 3. Score card for taste panel evaluation

Name of the Product: **Chicken Tikka** Date: Sample No:

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

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

Specify comments if any:

Name and designation: Signature:

RESULTS

4. RESULTS

Seven batches of chicken were procured, slaughtered under hygienic precautions at the Department of Livestock Products Technology, Mannuthy. Chicken tikka was prepared incorporating ingredients as shown in table 1 and 2. The same was packed in HDPE (50μ) packets and sealed. Half of the packets in all treatment groups and control were subjected to irradiation at 2.5 kGy. These samples were kept at chiller condition for further studies. Samples were analysed on the day of preparation for proximate composition like moisture, protein, fat, ash and carbohydrates. The stored samples up to spoilage were assessed for physicochemical qualities like pH, TBARS, TV, microbiological parameters like aerobic plate count, psychrotrophic count and yeast and mould count and organoleptic evaluation.

4.1. PHYSICAL QUALITIES AND SHELF-LIFE

The samples kept in chiller were examined frequently for the presence of signs of spoilage, *viz*., odour, colour, slime formation and mould growth. The spoiled samples were discarded and were not subjected to any further analysis. The date of spoilage was recorded. The appearance of meat and meat product is the principal characteristic by which the consumer accepts or rejects the product. The shelf life assessed with these physical qualities are shown in table 4 and presented in figure 4.

It was observed that certain packets were organoleptically spoiled in due course of storage. The minimum storage life was noted in non-irradiated (27-30 days) control group, which was assessed based on physical signs of spoilage. The maximum storage life was noticed in PL-IR samples (68-70 days). All other samples had storage life in between. On an average the irradiated samples had two times the keeping quality than non-irradiated samples.

The non spoiled samples were subjected to various analyses on day 5, 10, 15, 20, 25, 30, 45 and 60 or till its spoilage whichever was earlier.

Table 4. Shelf life of chicken tikka based on physical signs of spoilage (Days)

Treatment Groups	Non Irradiated (Days)	Irradiated (Days)
Control	27-30	61-63
Control +Curry leaf paste	31-33	64-66
Control + Peppermint paste	35-38	68-70
Control +Curry leaf paste + Peppermint paste	32-35	65-68

4.2. PHYSICOCHEMICAL QUALITIES

The physicochemical qualities like pH, TBARS and TV of chicken tikka stored in chiller were assessed on the day of preparation and on day 5, 10, 15, 20, 25, 30, 45 and 60 or till its spoilage whichever was earlier. The proximate composition was estimated only on the day of preparation.

4.2.1. Proximate Composition

Ready-to-eat chicken tikka was analysed for proximate composition, *viz*., moisture, protein, fat and ash on the day of preparation. The carbohydrates and other components were assessed by subtracting the sum of these from 100.00. Data is shown in table 5 and presented in figure 5.

The moisture percentage varied from 50.68±0.43 (PL-IR) to 53.71±0.32 (CL-NR) on the day of preparation. Compared to fat, protein, ash and carbohydrate, moisture had shown significant ($P<0.05$) differences among some

Table 5. Proximate composition of chicken tikka.

Treatment		% Proximate Composition					kcal/100g
		Moisture	Fat	Protein	Ash	CHO	Energy
C	NR	53.33^{cd} ±0.45	15.05^{a}±0.35	25.34^{ab}±0.26	1.5±0.05	4.77±0.02	239.55^{ab}±3.48
	IR	52.28^{bc}±0.43	15.90^{ab}±0.26	25.51^{ab}±0.37	1.56±0.07	4.75±0.07	245.13^{bc}±2.88
CL	NR	53.71^{d}±0.32	15.18^{a}±0.35	24.74^{a}±0.26	1.57±0.07	4.80±0.03	235.56^{a}±2.75
	IR	52.97^{cd}±0.18	15.52^{a}±0.42	25.07^{a}±0.28	1.60±0.08	4.83±0.04	239.99^{ab}±2.80
PL	NR	51.89^{b}±0.32	16.59^{b}±0.27	25.17^{a}±0.16	1.51±0.06	4.84±0.04	250.03^{cd}±2.38
	IR	50.68^{a}±0.43	16.77^{b}±0.20	26.12^{b}±0.40	1.66±0.08	4.77±0.03	255.4^{d}±2.79
CLPL	NR	53.68^{d}±0.14	15.26^{a}±0.11	24.79^{a}±0.17	1.57±0.07	4.71±0.03	236.48^{a}±1.04
	IR	52.79^{bcd}±0.34	15.88^{ab}±0.30	24.98^{a}±0.26	1.55±0.07	4.80±0.04	242.81^{abc}±2.43

Means bearing same alphabates in the column do not indicate significant difference (P<0.05).
C-Control; **CL**-Curry leaves; **PL**-Peppermint Leaves; **CLPL**- Curry leaves and Peppermint Leaves.
NR-Non Irradiated; **IR**-Irradiated; **S**-Spoiled.

of the groups. The fat percentage varied from 15.05±0.35 in C-NR to 16.77±0.20 in PL-IR group and the value was significantly ($P<0.05$) different. Almost similar trend was noticed in case of protein percentage on wet matter basis. The values varied from 24.74±0.26 in CL-NR to 26.12±0.40 in PL-IR group, later was significantly ($P<0.05$) different from almost all other treatment groups.

Percentage of ash present in different treatment groups was not significantly different. The values varied from 1.50±0.05 in C-NR to 1.66±0.08 in PL-IR samples. Similarly carbohydrate did not reveal any significant difference between treatment groups.

The least energy content was noticed in CL-NR group (235.56±2.75 kcal/100g) and with maximum energy level in PL-IR group (255.40±2.79 kcal/100g).

4.2.2. pH

The pH value of the different treatment groups were assessed up to day 60 or till spoilage whichever was earlier. The pH showed a decreasing trend throughout the storage period except that in case of curry leaf added non-irradiated and irradiated samples. The data is given in table 6. The highest pH of 6.18±0.02 was observed in CLPL-NR samples on the day of preparation. Even though the initial value was lower than the highest on 60th day, as storage period enhanced it reached the highest pH recorded 6.36±0.01 in case of CL-IR sample on day 60. The trend in pH variation during the storage period is shown in figure 6a and 6b.

On 60th day of storage the highest value was noticed in CL-IR samples followed by PL-IR, CLPL-IR and C-IR samples which were significantly ($P<0.05$) lower than the initial value and between the storage periods. It was also noticed that the trend of pH was downward except in case of curry leaf applied samples during the storage period until day 60 or spoilage whichever was earlier.

Table 6. pH of chicken tikka

Treatment		Days of storage								
		0	**5**	**10**	**15**	**20**	**25**	**30**	**45**	**60**
C	**NR**	6.14^{ab}±0.03	6.09^{abc*}±0.03	6.06^{ab*}±0.03	6.00^{ab*}±0.03	5.89^{a*}±0.02	5.79^{a*}±0.02	S	S	S
	IR	6.12^{ab}±0.03	6.08^{ab*}±0.03	6.04^{ab*}±0.02	5.99^{a*}±0.03	5.95^{b*}±0.03	5.90^{b*}±0.03	5.87^{b*}±0.03	5.81^{a*}±0.02	5.73^{a*}±0.01
CL	**NR**	6.11^{ab}±0.02	6.15^{c*}±0.02	6.19^{c*}±0.02	6.24^{c*}±0.02	6.27^{c*}±0.02	6.30^{d*}±0.02	6.34^{d*}±0.01	S	S
	IR	6.07^{a}±0.02	6.11^{abc*}±0.02	6.15^{c*}±0.01	6.20^{c*}±0.02	6.22^{c*}±0.01	6.24^{c*}±0.01	6.27^{c*}±0.01	6.31^{b*}±0.01	6.36^{c*}±0.01
PL	**NR**	6.11^{ab}±0.02	6.08^{abc*}±0.02	6.06^{ab*}±0.02	6.02^{ab*}±0.02	5.97^{b*}±0.02	5.90^{b*}±0.02	5.82^{ab*}±0.02	S	S
	IR	6.07^{a}±0.01	6.05^{a*}±0.02	6.02^{a*}±0.01	5.99^{a*}±0.01	5.95^{b*}±0.01	5.92^{b*}±0.02	5.87^{b*}±0.02	5.82^{a*}±0.01	5.77^{b*}±0.01
CLPL	**NR**	6.18^{b}±0.02	6.14^{bc*}±0.01	6.09^{b*}±0.02	6.06^{b*}±0.02	5.98^{b*}±0.01	5.91^{b*}±0.01	5.79^{a*}±0.01	S	S
	IR	6.13^{ab}±0.02	6.09^{abc*}±0.02	6.06^{ab*}±0.02	6.03^{ab*}±0.02	5.98^{b*}±0.02	5.93^{b*}±0.02	5.87^{b*}±0.02	5.80^{a*}±0.01	5.75^{ab*}±0.01

Means bearing same alphabets in the column do not indicate significant difference ($P<0.05$).
* represents significance difference between storage periods.
C-Control; **CL**-Curry leaves; **PL**-Peppermint Leaves; **CLPL**- Curry leaves and Peppermint Leaves.
NR-Non Irradiated; **IR**-Irradiated; **S**-Spoiled

4.2.3. Thiobarbituric Acid Reactive Substances (TBARS)

The values of TBARS in mg of mal per kg are shown in table 7. The trend of change in TBARS value is shown in figure 7a and 7b.

On the day of preparation the lowest value of 0.10±0.00 was observed in PL-NR samples, with the highest value of 0.20±0.01 in C-IR samples which was significantly ($P<0.05$) different from few of the treatment groups. During the storage period, PL-IR samples maintained the lowest value and reached its maximum 0.29±0.01 on 60th day of storage, whereas the other irradiated samples had shown a significantly ($P<0.05$) higher value than this. It was also observed among treatment groups, on the day of preparation irradiation did not accelerate oxidative rancidity as revealed by TBARS value, which was non-significant between IR and NR samples in all treatment groups. It was also noticed that on day 25 where full set of samples were perfectly normal and started to spoil beyond 25th day only whereas, storage had significant influence on enhancing the oxidative rancidity even in non-irradiated samples.

4.2.4. Tyrosine Value (TV)

The data of tyrosine value of chicken in mg/100 g of chicken tikka is shown in table 8. On the day of preparation irradiation of the samples revealed less tyrosine compared to non-irradiated counterparts with the least value of 4.57±0.04 in PL-IR samples. The maximum value recorded was 5.90±0.07 in case of control samples. Storage had significant effect on enhancing the value of tyrosine. The trend of increase in tyrosine value is shown in figure 8a and 8b. Throughout the storage period PL-IR samples revealed lower value compared to all other treatment groups, even though uniform increase was noticed due to storage. The highest value of 7.97±0.03 was observed on 25th day in C-NR samples, which was significantly higher in terms of days of storage as well as treatment groups.

Table 7. TBARS values of chicken tikka (mg malonaldehyde /kg)

Treatment		Days of storage								
		0	**5**	**10**	**15**	**20**	**25**	**30**	**45**	**60**
C	**NR**	0.19^{d} ±0.01	0.22^{d*}±0.01	0.27^{d*} ±0.01	0.30^{e*} ±0.01	0.33^{e*} ±0.01	0.36^{e*}±0.01	S	S	S
	IR	0.20^{d} ±0.01	0.24^{d*}±0.01	0.28^{d*} ±0.00	0.32^{f*} ±0.01	0.36^{f*} ±0.01	0.38^{e*}±0.01	0.42^{d*}±0.01	0.46^{c*}±0.01	0.51^{d*}±0.01
CL	**NR**	0.13^{bc}±0.01	0.15^{bc*}±0.01	0.17^{bc*}±0.01	0.20^{cd*}±0.01	0.22^{cd*}±0.01	0.25^{cd*}±0.01	0.28^{c*}±0.01	S	S
	IR	0.15^{c} ±0.01	0.16^{c*}±0.01	0.19^{c*} ±0.01	0.21^{d*} ±0.01	0.24^{d*} ±0.00	0.27^{d*}±0.01	0.30^{c*}±0.01	0.32^{b*}±0.00	0.35^{c*}±0.01
PL	**NR**	0.10^{a} ±0.00	0.12^{a*}±0.00	0.13^{a*} ±0.01	0.15^{a*} ±0.01	0.17^{a*} ±0.01	0.19^{a*}±0.01	0.22^{a*}±0.01	S	S
	IR	0.11^{ab}±0.01	0.13^{ab*}±0.01	0.14^{a*} ±0.01	0.17^{b*} ±0.01	0.19^{b*} ±0.01	0.21^{ab*}±0.01	0.23^{ab*}±0.01	0.26^{a*}±0.01	0.29^{a*}±0.01
CLPL	**NR**	0.12^{ab}±0.01	0.13^{ab*}±0.01	0.15^{ab*}±0.01	0.18^{bc*}±0.01	0.20^{bc*}±0.01	0.22^{bc*}±0.01	0.25^{b*}±0.01	S	S
	IR	0.13^{bc}±0.01	0.15^{bc*}±0.01	0.17^{bc*}±0.01	0.19^{cd*}±0.01	0.22^{cd*}±0.01	0.24^{cd*}±0.01	0.28^{c*}±0.01	0.30^{b*}±0.01	0.32^{b*}±0.01

Means bearing same alphabates in the column do not indicate significant difference ($P<0.05$).
* represents significance difference between storage periods.
C-Control; **CL**-Curry leaves; **PL**-Peppermint Leaves; **CLPL**- Curry leaves and Peppermint Leaves.
NR-Non Irradiated; **IR**-Irradiated;**S**-Spoiled

Table 8. Tyrosine values of chicken tikka (mg/100 g)

Treatment		Days of storage								
		0	**5**	**10**	**15**	**20**	**25**	**30**	**45**	**60**
C	**NR**	$5.90^{g}\pm0.07$	$6.21^{g*}\pm0.09$	$6.69^{g*}\pm0.09$	$7.14^{g*}\pm0.09$	$7.57^{f*}\pm0.05$	$7.97^{f*}\pm0.03$	S	S	S
	IR	$5.14^{de}\pm0.08$	$5.39^{de*}\pm0.07$	$5.71^{de*}\pm0.06$	$5.96^{de*}\pm0.05$	$6.28^{d*}\pm0.05$	$6.52^{c*}\pm0.06$	$6.83^{c*}\pm0.06$	$7.14^{d*}\pm0.06$	$7.59^{d*}\pm0.05$
CL	**NR**	$5.49^{f}\pm0.08$	$5.73^{f*}\pm0.06$	$6.02^{f*}\pm0.05$	$6.29^{f*}\pm0.06$	$6.70^{e*}\pm0.04$	$7.09^{e*}\pm0.03$	$7.62^{f*}\pm0.03$	S	S
	IR	$4.89^{bc}\pm0.05$	$5.12^{bc*}\pm0.04$	$5.45^{bc*}\pm0.03$	$5.72^{bc*}\pm0.04$	$5.96^{bc*}\pm0.05$	$6.18^{b*}\pm0.05$	$6.43^{b*}\pm0.06$	$6.83^{c*}\pm0.04$	$7.32^{c*}\pm0.05$
PL	**NR**	$4.99^{cd}\pm0.07$	$5.26^{cd*}\pm0.08$	$5.55^{cd*}\pm0.08$	$5.83^{cd*}\pm0.07$	$6.11^{c*}\pm0.07$	$6.58^{c*}\pm0.05$	$7.09^{d*}\pm0.05$	S	S
	IR	$4.57^{a}\pm0.04$	$4.82^{a*}\pm0.03$	$5.12^{a*}\pm0.03$	$5.41^{a*}\pm0.02$	$5.72^{a*}\pm0.05$	$5.98^{a*}\pm0.04$	$6.25^{a*}\pm0.06$	$6.49^{a*}\pm0.05$	$6.96^{a*}\pm0.05$
CLPL	**NR**	$5.25^{e}\pm0.10$	$5.50^{e*}\pm0.08$	$5.81^{e*}\pm0.09$	$6.08^{e*}\pm0.09$	$6.38^{d*}\pm0.08$	$6.85^{d*}\pm0.04$	$7.44^{e*}\pm0.04$	S	S
	IR	$4.75^{ab}\pm0.04$	$5.02^{b*}\pm0.06$	$5.30^{b*}\pm0.03$	$5.57^{ab*}\pm0.03$	$5.83^{ab*}\pm0.02$	$6.06^{ab*}\pm0.03$	$6.28^{a*}\pm0.04$	$6.68^{b*}\pm0.04$	$7.15^{b*}\pm0.02$

Means bearing same alphabates in the column do not indicate significant difference ($P<0.05$).
* represents significance difference between storage periods.
C-Control; **CL**-Curry leaves; **PL**-Peppermint Leaves; **CLPL**- Curry leaves and Peppermint Leaves
NR-Non Irradiated; **IR**-Irradiated; **S**-Spoiled

4.3. MICROBIOLOGICAL ANALYSIS

4.3.1. Aerobic Plate Count (APC)

The aerobic plate count of chicken tikka expressed in $\log_{10}$ cfu/g during the storage period is shown in table 9.

On the day of preparation the control samples had the highest count of 2.55 ± 0.09 $\log_{10}$ cfu/g of chicken tikka. Irradiation had significantly ($P<0.05$) reduced the count. The lowest count of 1.29 ± 0.10 $\log_{10}$ cfu/g was observed in PL-IR group. Throughout the entire study period PL-IR treatment group had the lowest count comparing to the other samples on a particular day of storage. This was followed by CLPL-IR and CL-IR. Even on the verge of spoilage, samples had very low count. The highest count of 4.62 ± 0.08 $\log_{10}$ cfu/g was obtained in C-NR on 25^{th} day of storage whereas, in irradiated group even upto 60^{th} day of storage such higher count was not observed.

The trend of growth of aerobic organisms of chicken tikka during storage is shown in figure 9a and 9b. The count had significant influence between storage periods in different treatment groups.

4.3.2. Psychrotrophic Count

Psychrotrophic count of chicken tikka stored under chiller condition during storage period is given in table 10.

On the day of preparation the highest count of 2.53 ± 0.08 $\log_{10}$ cfu/g was observed in C-NR group. Irradiation had significantly ($P<0.05$) brought down the count. The lowest count of 0.51 ± 0.25 $\log_{10}$ cfu/g was recorded in PL-IR samples. Throughout the storage period this treatment group revealed the lowest psychrotrophic load. The trend of psychrotrophic count on storage is shown in figure 10a and 10b. It is almost similar to that of aerobic plate count. Storage of the product in chiller had significant ($P<0.05$) effect in enhancing the psychrotrophic count of the product. The highest count of 3.95 ± 0.07 $\log_{10}$ cfu/g

Table 9. Aerobic plate count of chicken tikka . (log $_{10}$ cfu/g)

Treatment		Days of Storage								
		0	**5**	**10**	**15**	**20**	**25**	**30**	**45**	**60**
C	**NR**	$2.55^{d}\pm0.09$	$2.98^{e*}\pm0.09$	$3.35^{e*}\pm0.11$	$3.71^{d*}\pm0.11$	$4.16^{e*}\pm0.11$	$4.62^{d*}\pm0.08$	S	S	S
	IR	$1.79^{b}\pm0.08$	$2.03^{b*}\pm0.09$	$2.35^{b*}\pm0.10$	$2.61^{b*}\pm0.10$	$2.92^{b*}\pm0.10$	$3.20^{b*}\pm0.10$	$3.46^{b*}\pm 0.09$	$3.82^{b*}\pm0.08$	$4.16^{c*}\pm 0.04$
CL	**NR**	$2.32^{cd}\pm0.09$	$2.68^{d*}\pm0.05$	$2.98^{d*}\pm0.07$	$3.25^{c*}\pm0.09$	$3.64^{d*}\pm0.10$	$4.01^{c*}\pm0.11$	$4.47^{d*}\pm 0.09$	S	S
	IR	$1.59^{b}\pm0.13$	$1.85^{ab*}\pm0.10$	$2.14^{ab*}\pm0.11$	$2.44^{ab*}\pm0.10$	$2.69^{ab*}\pm0.11$	$2.93^{ab*}\pm0.12$	$3.20^{ab*}\pm 0.12$	$3.55^{ab*}\pm0.10$	$3.93^{b*}\pm0.06$
PL	**NR**	$2.13^{c}\pm0.06$	$2.42^{c*}\pm0.05$	$2.67^{c*}\pm0.04$	$3.01^{c*}\pm0.05$	$3.33^{c*}\pm0.05$	$3.74^{c*}\pm0.06$	$4.09^{c*}\pm 0.08$	S	S
	IR	$1.29^{a}\pm0.10$	$1.61^{a*}\pm0.10$	$1.92^{a*}\pm0.08$	$2.22^{a*}\pm0.08$	$2.51^{a*}\pm0.09$	$2.71^{a*}\pm0.11$	$2.96^{a*}\pm 0.12$	$3.27^{a*}\pm0.09$	$3.66^{a*}\pm0.07$
CLPL	**NR**	$2.25^{c}\pm0.07$	$2.62^{cd*}\pm0.05$	$2.91^{cd*}\pm0.07$	$3.26^{c*}\pm0.06$	$3.56^{cd*}\pm0.09$	$3.91^{c*}\pm0.11$	$4.29^{cd*}\pm 0.11$	S	S
	IR	$1.58^{b}\pm0.09$	$1.80^{ab*}\pm0.08$	$2.10^{a*}\pm0.08$	$2.42^{ab*}\pm0.09$	$2.69^{ab*}\pm0.09$	$2.90^{ab*}\pm0.11$	$3.11^{a*}\pm 0.12$	$3.48^{a*}\pm0.10$	$3.90^{b*}\pm 0.05$

Means bearing same alphabates in the column do not indicate significant difference ($P<0.05$).
* represents significance difference between storage periods.
C-Control; **CL**-Curry leaves; **PL**-Peppermint Leaves; **CLPL**- Curry leaves and Peppermint Leaves.
NR-Non Irradiated; **IR**-Irradiated; **S**-Spoiled

Table 10. Psychrotrophic count of chicken tikka. (log_{10} cfu/g)

Treatment		Days of storage								
		0	**5**	**10**	**15**	**20**	**25**	**30**	**45**	**60**
C	**NR**	$2.53^{e}\pm0.08$	$2.88^{f*}\pm0.04$	$3.23^{f*}\pm0.06$	$3.46^{f*}\pm0.08$	$3.70^{f*}\pm0.08$	$3.95^{f*}\pm0.07$	S	S	S
	IR	$1.65^{c}\pm0.06$	$2.02^{c*}\pm0.06$	$2.33^{c*}\pm0.03$	$2.62^{c*}\pm0.01$	$2.81^{c*}\pm0.03$	$2.96^{c*}\pm0.05$	$3.13^{c*}\pm0.07$	$3.33^{b*}\pm0.09$	$3.66^{c*}\pm0.06$
CL	**NR**	$2.38^{de}\pm0.03$	$2.73^{ef*}\pm0.04$	$2.97^{e*}\pm0.04$	$3.25^{e*}\pm0.06$	$3.49^{e*}\pm0.07$	$3.67^{e*}\pm0.06$	$3.79^{e*}\pm0.07$	S	S
	IR	$1.55^{c}\pm0.09$	$1.89^{bc*}\pm0.07$	$2.09^{b*}\pm0.06$	$2.36^{b*}\pm0.05$	$2.53^{b*}\pm0.04$	$2.67^{b*}\pm0.04$	$2.88^{b*}\pm0.04$	$3.01^{a*}\pm0.05$	$3.43^{b*}\pm0.05$
PL	**NR**	$2.11^{d}\pm0.05$	$2.40^{d*}\pm0.04$	$2.65^{d*}\pm0.03$	$2.94^{d*}\pm0.03$	$3.19^{d*}\pm0.04$	$3.40^{d*}\pm0.06$	$3.58^{d*}\pm0.06$	S	S
	IR	$0.51^{a}\pm0.25$	$1.43^{a*}\pm0.07$	$1.74^{a*}\pm0.05$	$2.02^{a*}\pm0.04$	$2.23^{a*}\pm0.05$	$2.46^{a*}\pm0.05$	$2.68^{a*}\pm0.05$	$2.84^{a*}\pm0.05$	$3.12^{a*}\pm0.05$
CLPL	**NR**	$2.27^{de}\pm0.04$	$2.60^{e*}\pm0.04$	$2.88^{e*}\pm0.03$	$3.13^{e*}\pm0.05$	$3.37^{e*}\pm0.05$	$3.56^{de*}\pm0.06$	$3.72^{de*}\pm0.07$	S	S
	IR	$1.14^{b}\pm0.22$	$1.72^{b*}\pm0.10$	$1.97^{b*}\pm0.08$	$2.24^{b*}\pm0.06$	$2.44^{b*}\pm0.05$	$2.61^{ab*}\pm0.05$	$2.77^{ab*}\pm0.05$	$2.94^{a*}\pm0.04$	$3.29^{b*}\pm0.05$

Means bearing same alphabates in the column do not indicate significant difference ($P<0.05$).
* represents significance difference between storage periods.
C-Control; **CL**-Curry leaves; **PL**-Peppermint Leaves; **CLPL**- Curry leaves and Peppermint Leaves.
NR-Non Irradiated; **IR**-Irradiated; **S**-Spoiled

was noticed on 25th day of storage in control samples whereas, such count was not noticed in all the four irradiated samples even on day 60.

4.3.3. Yeast and Mould Count

The yeast and mould count of chicken tikka stored in chiller upto 60 days is given in table 11. The count was showing similar trend to that of aerobic plate count and psychrotrophic count, had the highest value 1.54±0.07 in C-NR samples on day of preparation compared to other treatment groups. The lowest count of 0.14±0.14 $\log_{10}$ cfu/g was recorded in PL-IR group and throughout the study period it maintained lower count than other treatment groups. In all the cases irradiation had beneficial effect in reducing yeast and mould count of product.

The trend of growth of yeast and mould in chicken tikka under chiller storage is shown in figure 11a and 11b. Storage had significant effect on growth of these organisms as revealed by significant ($P<0.05$) difference between storage periods. The highest count of 3.29±0.05 $\log_{10}$ cfu/g was observed in C-NR group on 25th day whereas, such a higher count was not observed even up to 60th day of storage in irradiated samples since irradiation had significantly ($P<0.05$) reduced the count in the concerned treatment groups.

4.4. ORGANOLEPTIC EVALUATION

The organoleptic qualities *viz*., colour, flavour, juiciness, tenderness and overall acceptability of the product were evaluated with the help of nine point Hedonic scale.

4.4.1. Colour

The samples on organoleptic analysis by the semi-trained panelists showed that irradiated samples had higher score when compared to non-irradiated counterparts on the day of preparation. The highest score of 8.74 was observed in PL-IR samples compared to non-irradiated control sample which scored 8.33. Both CL and PL paste incorporation increased the colour score where as a

Table 11. Yeast and Mould count of chicken tikka. (log_{10} cfu/g)

Treatment		Days of storage								
		0	**5**	**10**	**15**	**20**	**25**	**30**	**45**	**60**
C	**NR**	$1.54^{d}\pm0.07$	$1.92^{e*}\pm0.06$	$2.24^{g*}\pm0.05$	$2.61^{f*}\pm0.03$	$2.90^{e*}\pm0.03$	$3.29^{c*}\pm0.05$	S	S	S
	IR	$0.61^{b}\pm0.22$	$1.42^{c*}\pm0.07$	$1.69^{cd*}\pm0.06$	$1.92^{c*}\pm0.09$	$2.13^{b*}\pm0.10$	$2.34^{a*}\pm0.12$	$2.55^{a*}\pm0.11$	$2.81^{*}\pm0.11$	$3.10^{b*}\pm0.07$
CL	**NR**	$1.41^{cd}\pm0.09$	$1.79^{de*}\pm0.07$	$2.10^{fg*}\pm0.06$	$2.41^{ef*}\pm0.06$	$2.70^{de*}\pm0.06$	$2.85^{b*}\pm0.08$	$3.07^{b*}\pm0.09$	S	S
	IR	$0.33^{ab}\pm0.21$	$1.26^{bc*}\pm0.08$	$1.55^{bc*}\pm0.06$	$1.85^{bc*}\pm0.07$	$2.11^{b*}\pm0.09$	$2.31^{a*}\pm0.09$	$2.53^{a*}\pm0.10$	$2.69^{*}\pm0.11$	$2.96^{ab*}\pm0.09$
PL	**NR**	$1.09^{c}\pm0.06$	$1.44^{cd*}\pm0.06$	$1.83^{de*}\pm0.06$	$2.15^{d*}\pm0.08$	$2.41^{c*}\pm0.09$	$2.66^{b*}\pm0.08$	$2.87^{b*}\pm0.09$	S	S
	IR	$0.14^{a}\pm0.14$	$0.76^{a*}\pm0.20$	$1.37^{a*}\pm0.05$	$1.59^{a*}\pm0.08$	$1.82^{a*}\pm0.08$	$2.08^{a*}\pm0.08$	$2.34^{a*}\pm0.08$	$2.50^{*}\pm0.09$	$2.75^{a*}\pm0.08$
CLPL	**NR**	$1.26^{cd}\pm0.08$	$1.66^{de*}\pm0.06$	$1.97^{ef*}\pm0.07$	$2.26^{de*}\pm0.09$	$2.56^{cd*}\pm0.09$	$2.73^{b*}\pm0.09$	$2.96^{b*}\pm0.09$	S	S
	IR	$0.14^{a}\pm0.14$	$1.08^{b*}\pm0.06$	$1.44^{ab*}\pm0.05$	$1.70^{ab*}\pm0.06$	$1.97^{ab*}\pm0.09$	$2.22^{a*}\pm0.09$	$2.42^{a*}\pm0.08$	$2.62^{*}\pm0.11$	$2.85^{ab*}\pm0.09$

Means bearing same alphabates in the column do not indicate significant difference ($P<0.05$).
* represents significance difference between storage periods.
C-Control; **CL**-Curry leaves; **PL**-Peppermint Leaves; **CLPL**- Curry leaves and Peppermint Leaves.
NR-Non Irradiated; **IR**-Irradiated; **S**-Spoiled

combination of theses two had substantially reduced the colour score. The data is shown in table 12.

The trend of reduction in colour score is shown in figure 12a and 12b. It was observed that there was no significant difference due to storage between day 0 and 5 whereas, from day 5 onwards storage had significantly ($P<0.05$) reduced the color score of chicken tikka. On day 60^{th} the highest score of 7.46 was obtained in PL-IR with the lowest of 6.83 in CLPL-IR group.

4.4.2. Flavour

The flavour score of chicken tikka is shown in table 13. Initially the non-irradiated control samples had the lowest score of 8.30 as against 8.74 in peppermint paste applied irradiated (PL-IR) samples. In case of flavour score also irradiation had slightly increased the score on the day of preparation. 5^{th} day of storage had significantly ($P<0.05$) decreased the score only in case at CL-NR & CLPL-IR groups whereas storage in chiller up to day 5 had no significant effect on other treatment groups.

From 5^{th} day onwards flavour score of the product was significantly ($P<0.05$) reduced and the trend of reduction of flavor score is shown in figure 13a and 13b. During the storage period a uniform reduction was noticed both in control as well as different treatment groups. Throughout the storage period the PL-IR group had recorded the highest score on various days of storage and the same group revealed 7.47 score on 60^{th} day of preparation.

4.4.3. Juiciness

The juiciness score of chicken tikka (in nine point Hedonic scoring system) is given in table 14. The control sample had initial score of 8.46 due to irradiation the same was increased to 8.60. The highest score of 8.61 was obtained for PL-IR group. Due to storage under chiller condition up to 5^{th} day the

Table 12. Colour score of chicken tikka.

Treatment		Days of storage								
		0	**5**	**10**	**15**	**20**	**25**	**30**	**45**	**60**
C	**NR**	8.33	8.21	7.86*	7.59*	7.33*	6.90*	S	S	S
	IR	8.54	8.36	8.21*	8.11*	7.73*	7.71*	7.51*	7.36*	7.17*
CL	**NR**	8.39	8.21	8.14*	7.74*	7.57*	7.43*	6.99*	S	S
	IR	8.46	8.36	8.36	8.04*	7.86*	7.71*	7.53*	7.43*	7.26*
PL	**NR**	8.41	8.29	8.07*	7.87*	7.64*	7.57*	7.24*	S	S
	IR	8.74	8.57	8.43*	8.34*	8.11*	7.93*	7.66*	7.57*	7.46*
CLPL	**NR**	8.11	8.07	7.79*	7.56*	7.36*	7.14*	6.71*	S	S
	IR	8.34	8.21	8.14	7.80*	7.71*	7.57*	7.46*	7.14*	6.83*

* represents significance difference between storage periods.
C-Control; **CL**-Curry leaves; **PL**-Peppermint Leaves; **CLPL**- Curry leaves and Peppermint Leaves.
NR-Non Irradiated; **IR**-Irradiated; **S**-Spoiled

Table 13. Flavour score of chicken tikka.

Treatment		Days of storage								
		0	**5**	**10**	**15**	**20**	**25**	**30**	**45**	**60**
C	**NR**	8.30	8.21	7.86*	7.64*	7.40*	6.79*	S	S	S
	IR	8.64	8.36	8.29*	7.93*	7.80*	7.71*	7.51*	7.36*	6.91*
CL	**NR**	8.41	8.21*	8.00*	7.87*	7.57*	7.36*	6.99*	S	S
	IR	8.50	8.36	8.21*	8.09*	7.93*	7.71*	7.49*	7.43*	7.20*
PL	**NR**	8.50	8.36	8.07*	7.89*	7.73*	7.57*	7.31*	S	S
	IR	8.74	8.64	8.43*	8.31*	8.04*	7.86*	7.74*	7.64*	7.47*
CLPL	**NR**	8.36	8.21	8.07*	7.63*	7.57*	7.43*	7.20*	S	S
	IR	8.67	8.43*	8.21*	8.04*	7.93*	7.86*	7.61*	7.57*	7.15*

* represents significance difference between storage periods.
C-Control; **CL**-Curry leaves; **PL**-Peppermint Leaves; **CLPL**- Curry leaves and Peppermint Leaves.
NR-Non Irradiated; **IR**-Irradiated; **S**-Spoiled

Table 14. Juiciness score of chicken tikka.

Treatment		Days of storage								
		0	**5**	**10**	**15**	**20**	**25**	**30**	**45**	**60**
C	**NR**	8.46	8.21*	7.86*	7.61*	7.33*	6.71*	S	S	S
	IR	8.60	8.50	8.43	8.09*	7.93*	7.79*	7.53*	7.29*	6.76*
CL	**NR**	8.43	8.21*	8.07*	7.80*	7.54*	7.43*	7.17*	S	S
	IR	8.53	8.43	8.29*	8.16*	7.86*	7.86*	7.66*	7.57*	7.21*
PL	**NR**	8.50	8.36	8.14*	7.89*	7.79*	7.43*	7.29*	S	S
	IR	8.61	8.57	8.36	8.23*	8.09*	7.93*	7.93*	7.64*	7.37*
CLPL	**NR**	8.34	8.14	8.14	7.99*	7.64*	7.36*	7.09*	S	S
	IR	8.57	8.36*	8.36*	8.13*	7.87*	7.86*	7.63*	7.43*	7.13*

* represents significance difference between storage periods.
C-Control; **CL**-Curry leaves; **PL**-Peppermint Leaves; **CLPL**- Curry leaves and Peppermint Leaves.
NR-Non Irradiated; **IR**-Irradiated; **S**-Spoiled

score was significantly ($P<0.05$) reduced in case of C-NR, CL-NR and CLPL-IR treatment groups.

The trend of reduction of juiciness during storage is shown in figure 14a and 14b. From 15th day onwards the score had significantly reduced in all treatment groups with the lowest score of 6.76 in C-IR group on 60th day and with the highest score of 7.37 in PL-IR group.

4.4.4. Tenderness

The tenderness score of the chicken tikka during storage period is shown in table 15. Almost all the treatment groups and control group obtained a score of above 8. On the day of preparation with maximum of 8.67 in case of PL-IR group followed by 8.54 in CL-IR group. Irradiated samples in different treatment groups had higher score than their counterpart. In case of CL-NR, CL-IR and CLPL-NR treatment groups the score was significantly ($P<0.05$) reduced from that of day 0.

The trend of reduction in tenderness score is shown in figure 15a and 15b. It was observed from 5th day onwards until the spoilage the score had reduced showing storage period had significant ($P<0.05$) effect in reducing the tenderness of product. Even on 60th day irradiated samples had comparatively good score of 7.00 or above 7.00 indicating a good tenderness of the product.

The highest score of 7.37 was recorded in PL-IR group and the samples had storage life beyond 60 days.

4.4.5. Overall acceptability

Overall acceptability score of the chicken tikka indicates the general acceptability of the product by the consumer and is the product of all the sensory attributes and not the sum of the individual attributes. The product was acceptable throughout the study period as revealed by score of more than 7.00. The data is given in table 16. A score of 8.34 was obtained in control samples and it was

Table 15. Tenderness score of chicken tikka

Treatment		Days of storage								
		0	**5**	**10**	**15**	**20**	**25**	**30**	**45**	**60**
C	**NR**	8.24	8.14	7.86*	7.57*	7.33*	6.86*	S	S	S
	IR	8.50	8.36	8.29*	8.09*	7.94*	7.79*	7.56*	7.43*	7.00*
CL	**NR**	8.50	8.29*	8.14*	7.89*	7.66*	7.43*	7.13*	S	S
	IR	8.54	8.36*	8.14*	8.04*	7.93*	7.79*	7.60*	7.43*	7.29*
PL	**NR**	8.43	8.36	8.14*	7.99*	7.80*	7.43*	7.29*	S	S
	IR	8.67	8.57	8.43*	8.27*	8.04*	7.93*	7.79*	7.57*	7.37*
CLPL	**NR**	8.41	8.14*	8.07*	7.83*	7.59*	7.36*	7.09*	S	S
	IR	8.50	8.43	8.14*	8.04*	7.87*	7.79*	7.51*	7.43*	7.21*

* represents significance difference between storage periods.
C-Control; **CL**-Curry leaves; **PL**-Peppermint Leaves; **CLPL**- Curry leaves and Peppermint Leaves.
NR-Non Irradiated; **IR**-Irradiated; **S**-Spoiled.

Table 16. Overall acceptability score of chicken tikka.

Treatment		Days of storage								
		0	**5**	**10**	**15**	**20**	**25**	**30**	**45**	**60**
C	**NR**	8.34	8.14	7.93*	7.63*	7.40*	6.86*	S	S	S
	IR	8.54	8.29	8.21*	8.04*	7.94*	7.79*	7.56*	7.43*	7.00*
CL	**NR**	8.41	8.14*	7.93*	7.87*	7.64*	7.36*	7.13*	S	S
	IR	8.54	8.43	8.29*	8.09*	7.86*	7.71*	7.54*	7.43*	7.26*
PL	**NR**	8.50	8.29	8.07*	7.89*	7.73*	7.36*	7.20*	S	S
	IR	8.61	8.57	8.43*	8.27*	8.04*	7.93*	7.81*	7.57*	7.40*
CLPL	**NR**	8.41	8.14*	8.07*	7.81*	7.57*	7.36*	7.13*	S	S
	IR	8.54	8.43	8.36*	8.04*	7.93*	7.79*	7.53*	7.43*	7.26*

* represents significance difference between storage periods.
C-Control; **CL**-Curry leaves; **PL**-Peppermint Leaves; **CLPL**- Curry leaves and Peppermint Leaves.
NR-Non Irradiated; **IR**-Irradiated; **S**-Spoiled.

substantially improved to 8.54 by the process of irradiation. Application of PL had significant ($P<0.05$) effect in improving overall acceptability of 8.61 (PL-IR) on the day of preparation.

The trend of reduction in overall acceptability score is shown in figure 16a and 16b. Storage had significant influence as noticed in other organoleptic qualities and reduced the score significantly ($P<0.05$). From 5th day onwards it was significantly ($P<0.05$) reduced and reached to 7.00 in case of C-IR samples on 60th day. The highest score of 7.40 was obtained on 60th day in case of PL-IR samples, which retained the highest score right from 0th day to 60th day of investigation.

4.4.6. Kruskal-Wallis Rank Score

The Kruskal-Wallis (KW) rank score analysis of the organoleptic qualities of the product during the storage period were analysed and it was found from day 5th onwards the values were significant ($P<0.05$) from each other. The analysis showed that among treatments during the storage period PL-IR sample recorded the highest score in the entire study period and was significantly ($P<0.05$) higher than other values.

4.5. COST OF PRODUCTION

The cost of production of chicken tikka was calculated for both the control and treatment groups and is presented in the table 17. The cost of production was Rs.122.30, Rs.123.30, Rs.125.30 and Rs.126.30 per kg for the control, CL, PL and CLPL treatment groups respectively.

Table 17. Cost of production of one kg ready-to-eat chicken tikka.

Item	Treatment Groups			
	C	**CL**	**PL**	**CLPL**
Chicken	90.30	90.30	90.30	90.30
Marinade mix	6.25	6.25	6.25	6.25
Batter mix	7.00	7.00	7.00	7.00
Oil	18.75	18.75	18.75	18.75
CL	-	1	-	1
PL	-	-	3	3
Cost per kg of final product	**122.30**	**123.30**	**125.30**	**126.30**

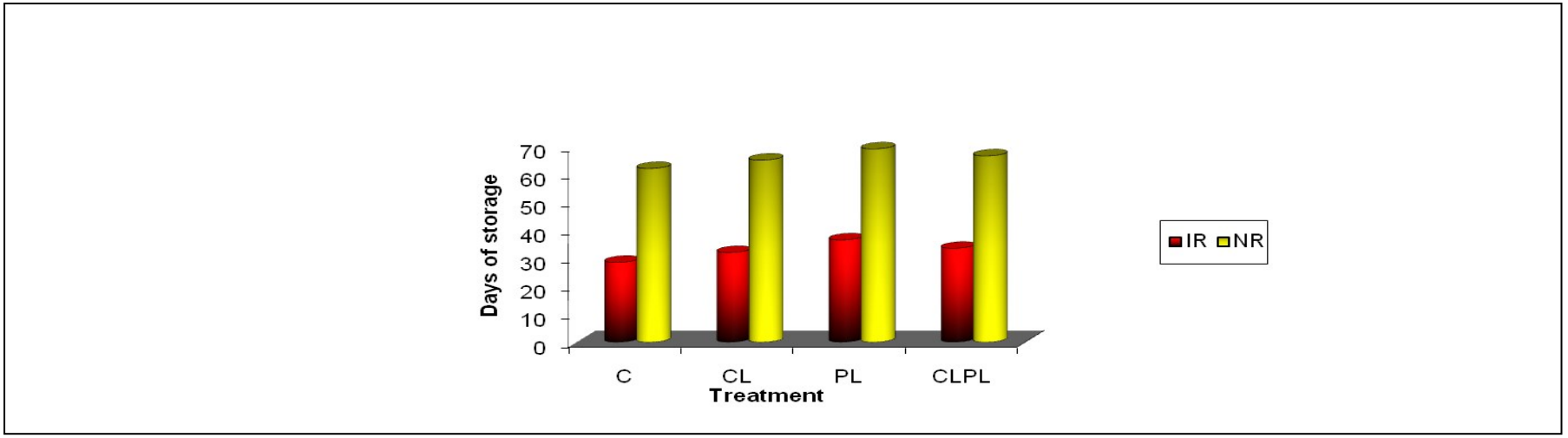

Figure 4. Shelf life of chicken tikka in chiller storage

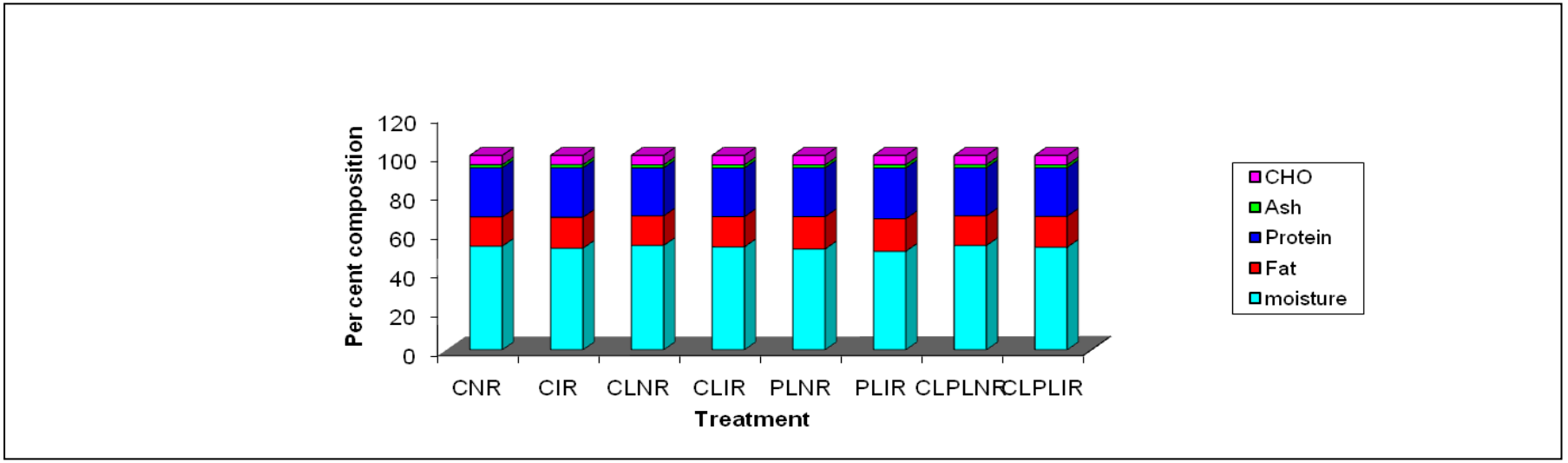

Figure 5. Proximate composition of chicken tikka in chiller storage

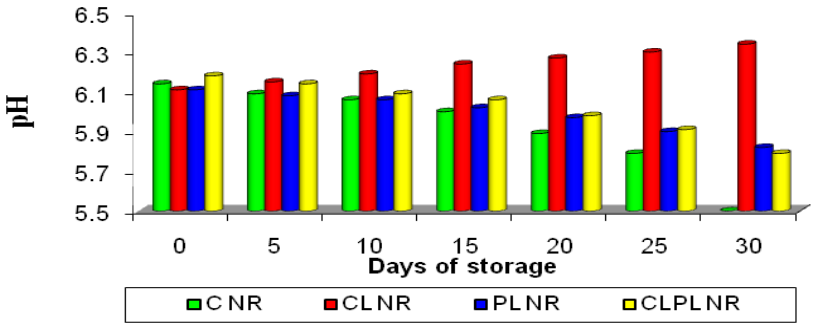

Figure 6a. pH values of non-irradiated chicken tikka in chiller storage

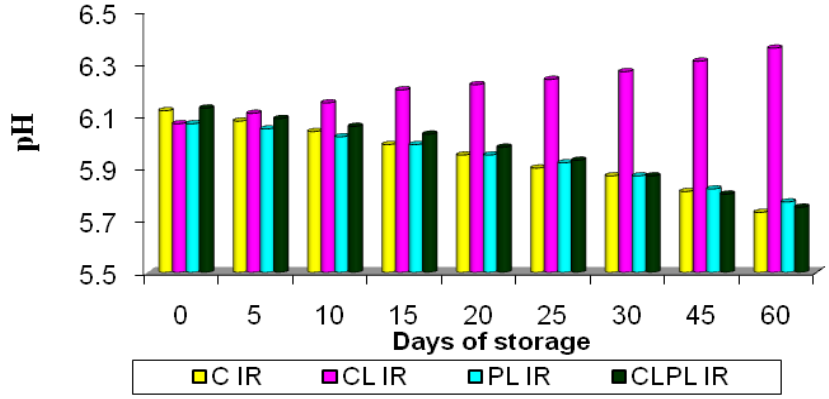

Figure 6b. pH values of irradiated chicken tikka in chiller storage

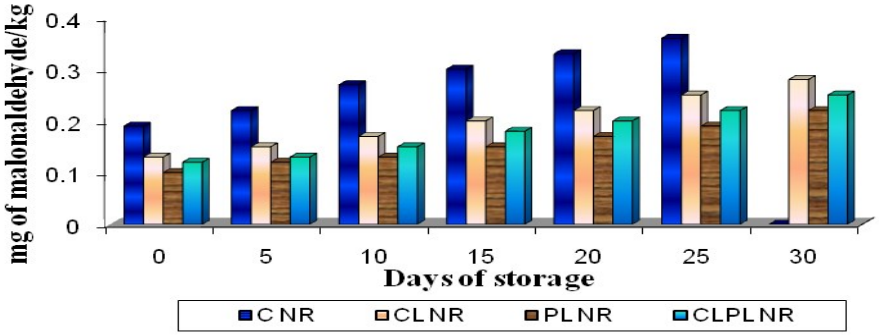

Figure 7a. TBARS values of non-irradiated chicken tikka in chiller storage

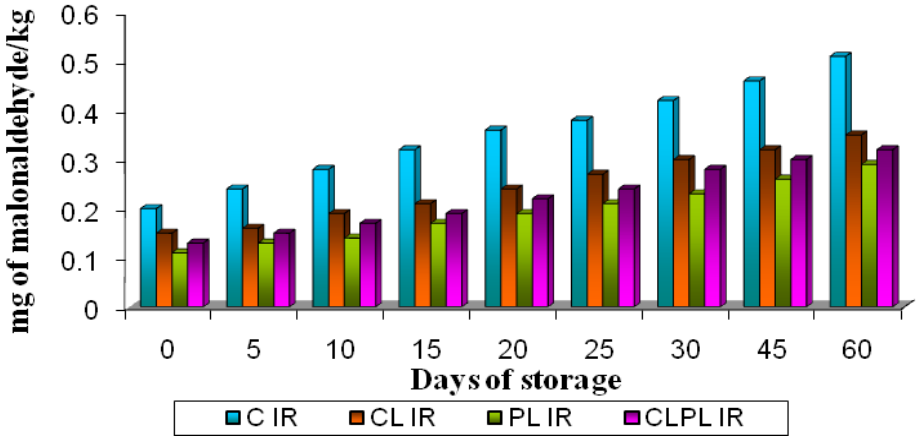

Figure 7b. TBARS values of irradiated chicken tikka in chiller storage

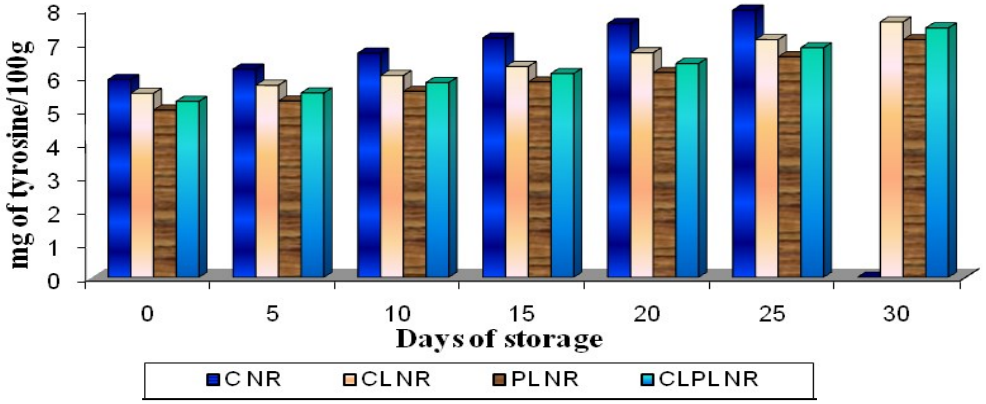

Figure 8a. Tyrosine values of non-irradiated chicken tikka in chiller storage

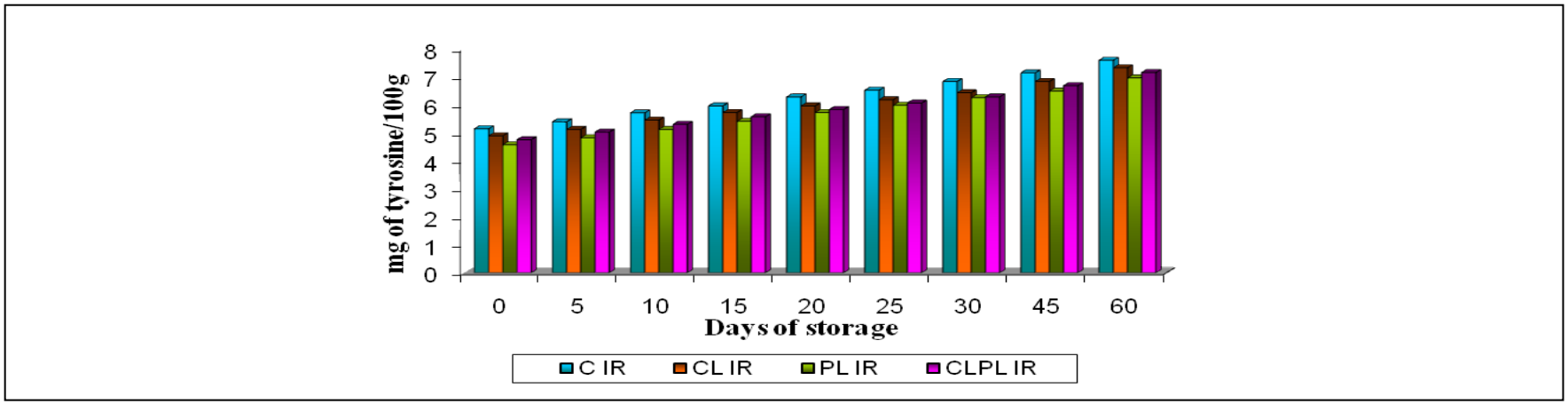

Figure 8b. Tyrosine values of irradiated chicken

Figure 9a. Aerobic plate count of non-irradiated chicken tikka in chiller storage

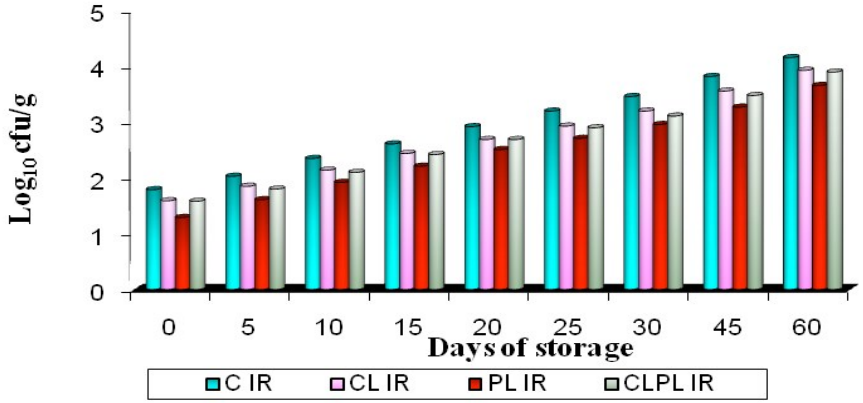

Figure 9b. Aerobic plate count of irradiated chicken tikka in chiller storage

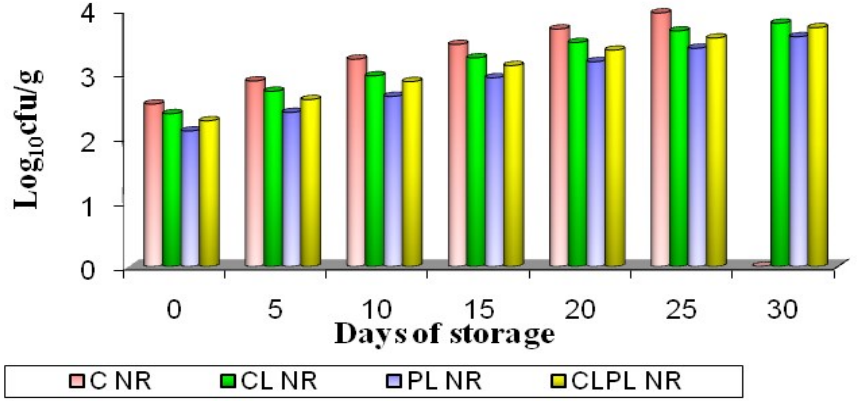

Figure 10a. Psychrotrophic count of non-irradiated chicken tikka in chiller storage

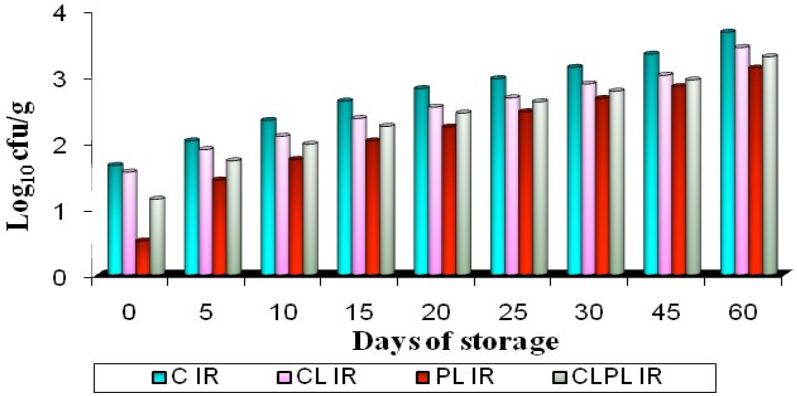

Figure 10b. Psychrotrophic count of irradiated chicken tikka in chiller storage

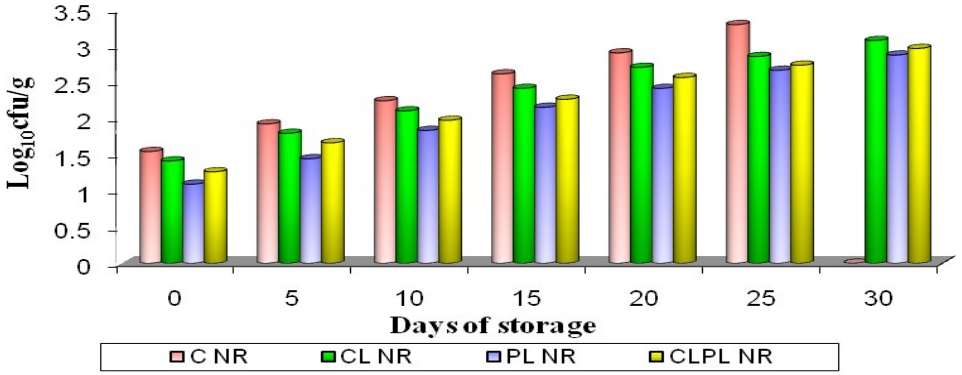

Figure 11a. Yeast and Mould count of non-irradiated chicken tikka in chiller storage

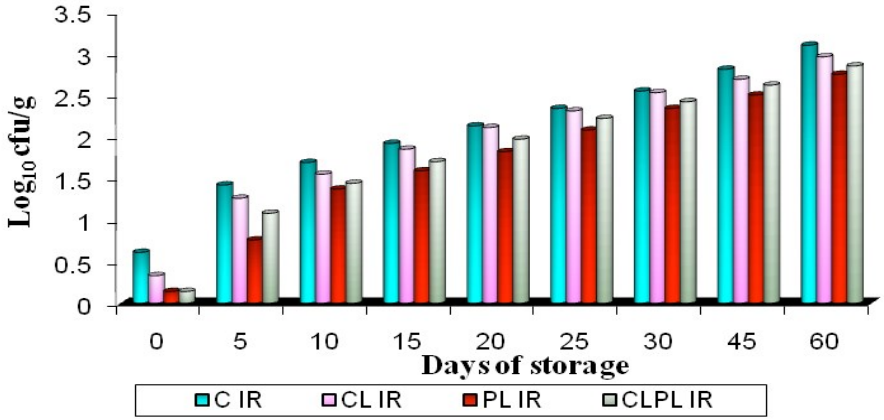

Figure 11b. Yeast and Mould count of irradiated chicken tikka in chiller storage

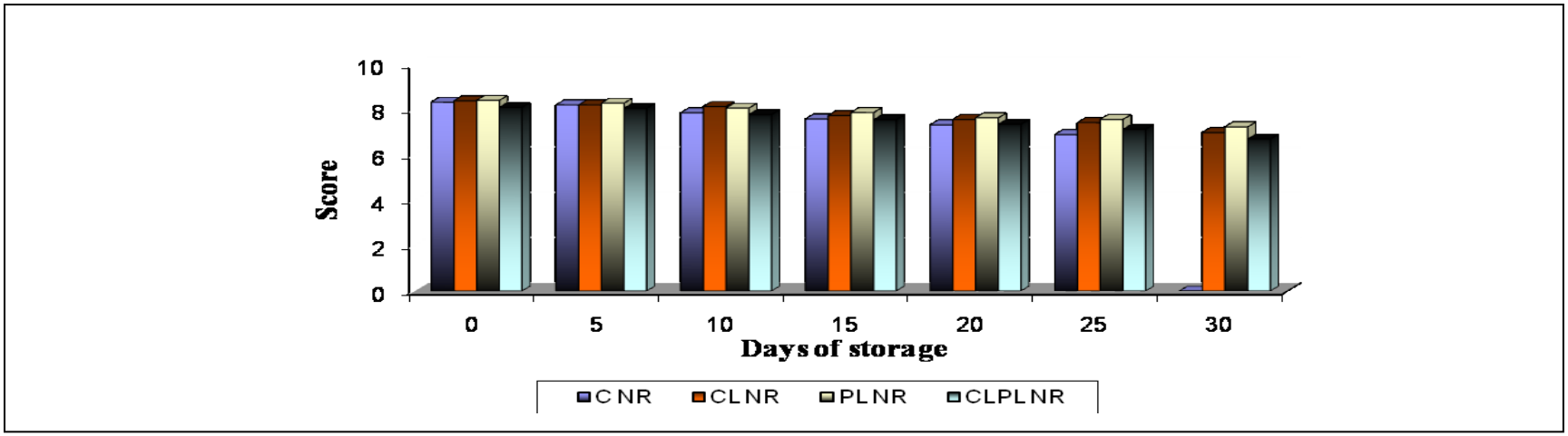

Figure 12a. Colour score of non-irradiated chicken tikka in chiller storage

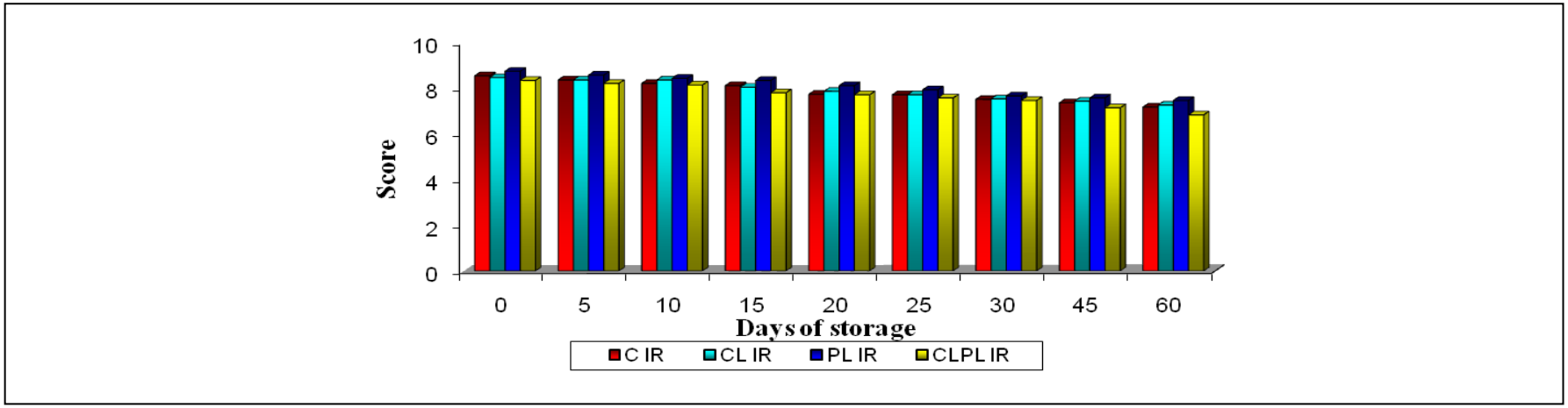

Figure 12b. Colour score of irradiated chicken tikka in chiller storage

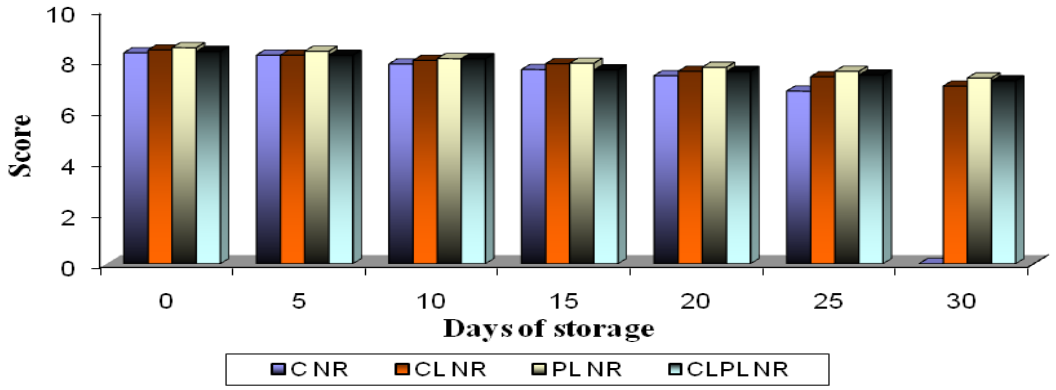

Figure 13a. Flavour score of non-irradiated chicken tikka in chiller storage

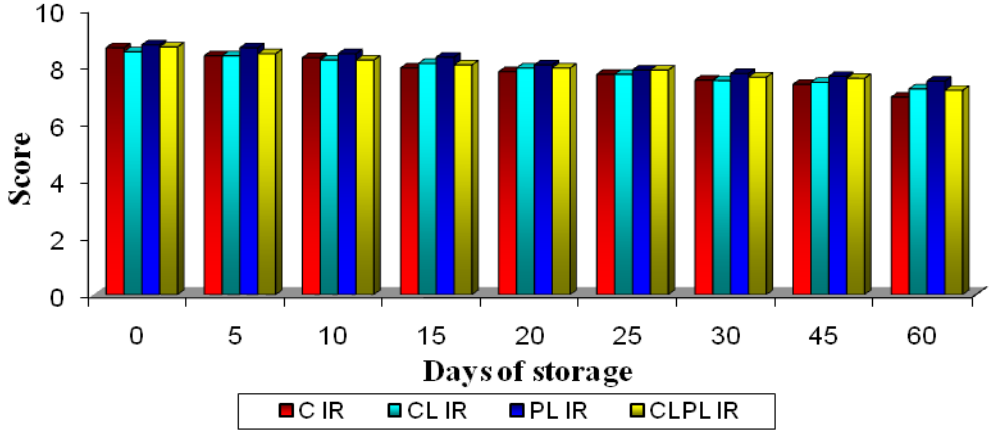

Figure 13b. Flavour score of irradiated chicken tikka in chiller storage

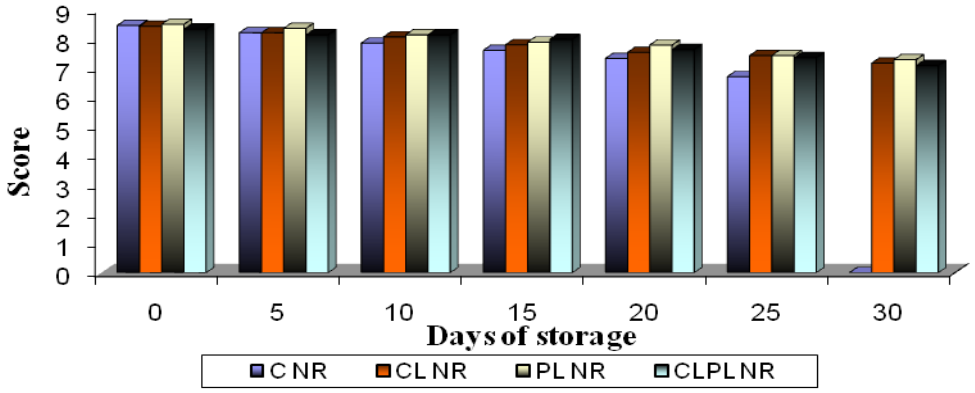

Figure 14a. Juiciness score of non-irradiated chicken tikka in chiller storage

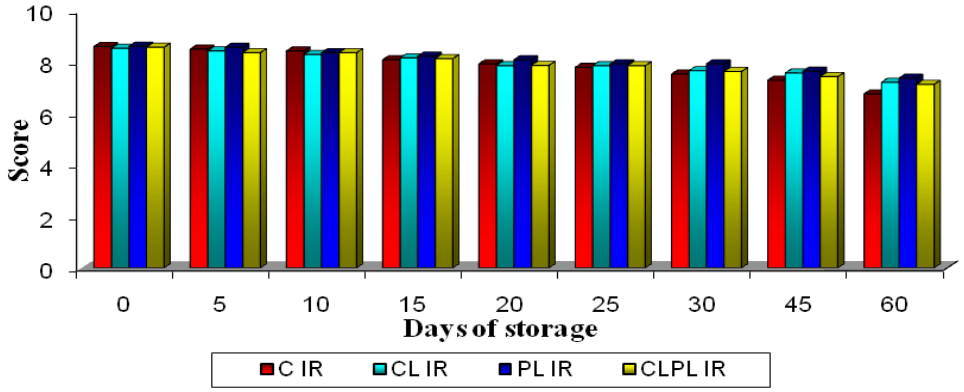

Figure 14b. Juiciness score of irradiated chicken tikka in chiller storage

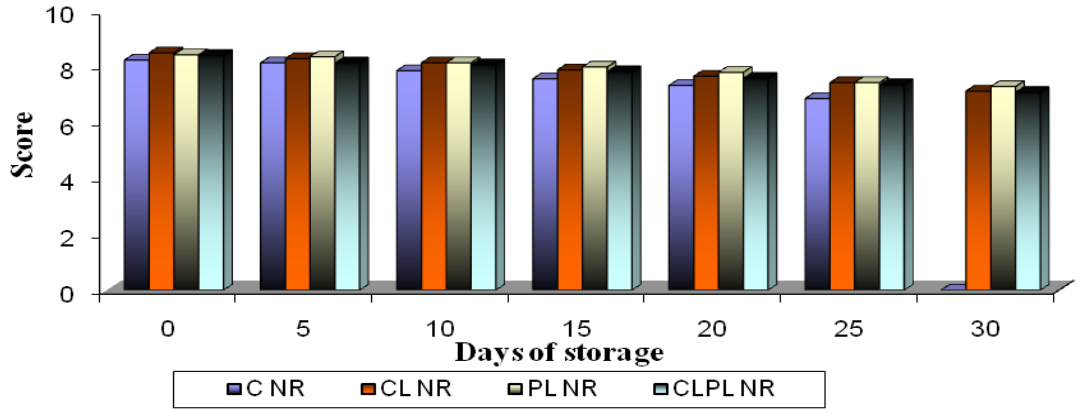

Figure 15a. Tenderness score of non-irradiated chicken tikka in chiller storage

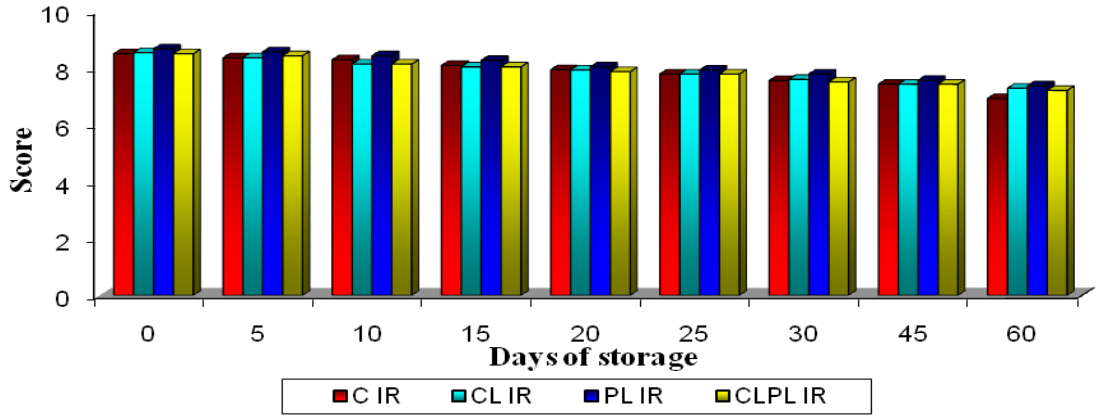

Figure 15b. Tenderness score of irradiated chicken tikka in chiller storage

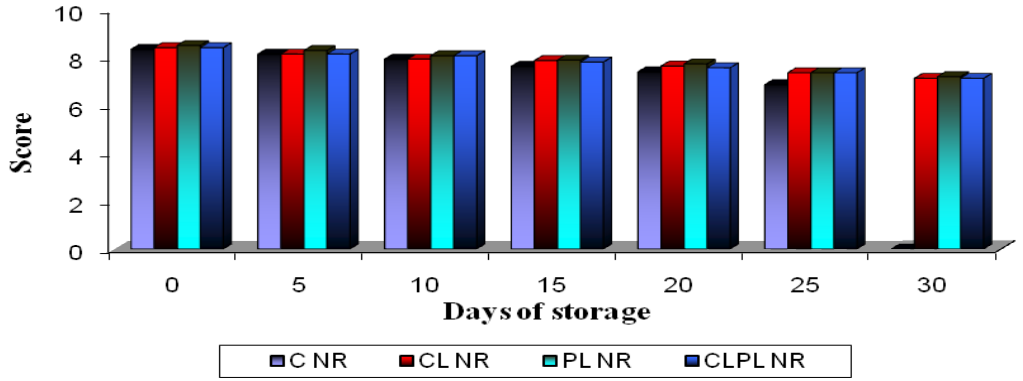

Figure 16a. Overall acceptability score of non-irradiated chicken tikka in chiller storage

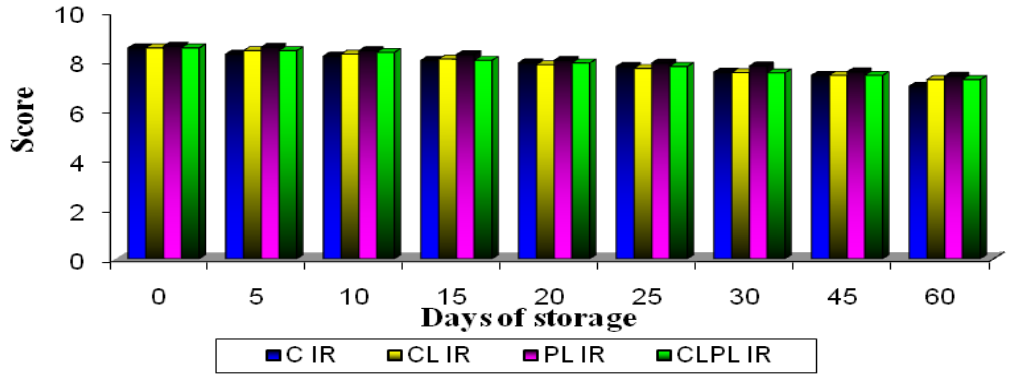

Figure 16b. Overall acceptability score of irradiated chicken tikka in chiller storage

DISCUSSION

5. DISCUSSION

In order to prepare chicken tikka broiler chicken having uniform weight of approximately 2.0 kg were procured and brought to the Department of Livestock Products Technology, College of Veterinary and Animal Sciences Mannuthy. They were slaughtered under hygienic conditions, and the whole chickens were cut into uniform size of about 20-25 mm cubes. The marinade was prepared and applied on the cubes and these were kept in chiller. The marinated chicken (control, 1% CL, 1% PL and both) were cooked separately for 10 min in low flame. The batter was prepared in stock solution and the partially cooked cuts were mixed uniformly in the batter. The tikka prepared was subjected to deep fat frying and after cooling it was packed in HDPE packets. Half number of packets were subjected to irradiation at 2.5 kGy and analysed for various physical, physicochemical, microbiological and organoleptic evaluation on the day of preparation. Sufficient number of packets prepared was kept at chiller temperature (1-4^{0}C). The stored samples were assessed for signs of spoilage and the spoiled samples were discarded, the non spoiled samples were subjected to various analyses.

5.1. PHYSICAL QUALITIES AND SHELF-LIFE

The maximum shelf life of 68-70 days was observed in PL added chicken tikka which was subjected to irradiation at 2.5 kGy. Compared to non-irradiated samples irradiated samples had approximately two times shelf life in all four groups.

Paul *et al.* (1990) reported an extended storage life of 2 and 4 weeks under chiller storage at 1.0 and 2.5 kGy respectively in lamb meat chunks. Similarly Roberts and Weese (1998), Johnson *et al.* (2004) reported extended storage life due to irradiation in different meat products. Kanatt *et al.* (2005) reported only two weeks extended storage at 3 kGy irradiation in ethnic meat products whereas, another very popular ethnic meat product chicken tikka had an extended storage life of 4-5 weeks due to irradiation. Application of CL, PL and

combination of these two extended the shelf life by 3-4 days, 7-8 days and nearly 5 days respectively. The maximum effect was noticed in PL applied samples. The usage of CL, PL and their effect in inhibiting the microbial and chemical spoilage were reported by Marinova and Yanishlieva (1997), Iscan *et al.* (2002), Biswas *et al.* (2006), Rao *et al.* (2007) and Ningappa *et al*. (2008) due to the antioxidant, antimicrobial activities of various active ingredients of these leaves. As a simple and effective treatment method for extending the shelf life of chicken tikka, application of CL and PL can be advocated.

The colour and odour were not significantly different between various treatment groups both in irradiated and non-irradiated samples. Still it was more appealing in control and PL applied samples compared to other treatment groups both in irradiated and non-irradiated samples.

5.2. PHYSICOCHEMICAL QUALITIES

5.2.1 Proximate Composition

Ready-to-eat chicken tikka was analysed for proximate composition *viz*., moisture, protein, fat and ash on the day of preparation. The carbohydrates and other components were assessed by subtracting the sum of these from 100.00. The control samples had very good protein and fat percentage of 25.34±0.26 and 15.05±0.35 respectively and provided 239.55±3.38 kcal/100g of chicken tikka as energy level. The proximate compositions were not significantly affected due to irradiation. Non significant effect due to irradiation was already reported by Heath *et al.* (1990), Katta *et al.*(1991), Wheeler *et al.* (1999), Du *et al*. (2001), Daoud *et al.* (2002), Smith and Pillai (2004) in various meat and meat products by different dosage of irradiation and results of present study are in agreement with earlier reports. Daoud *et al*. (2002) reported slight changes in proximate composition during study period by different dosage of irradiation in chilled minced beef as lower moisture and protein content. In the present study it was observed that moisture was slightly reduced whereas, protein content has non significantly improved by 2.5 kGy irradiation. Some of the proximate principles were significantly ($P<0.05$) affected by application of CL, PL or their

combination but with respect to fat, ash and carbohydrate remained unchanged in all these treatment groups. Compared to control samples slight difference was noticed in case of moisture, protein and energy level and might be due to extra quantity of leaf paste added.

The effect of CL, PL as an antioxidant in meat and meat products were studied by various authors but their effects on proximate composition were not investigated in previous works. The samples obtained an energy level of 235.56 to 255.40 kcal/100g of chicken tikka having the least in CL-NR and maximum in PL-IR treated samples.

5.2.2. pH

On the day of preparation CLPL-NR groups of significant ($P<0.05$) difference in pH than that of control samples. The significant change in pH was not noticed due to irradiation in different treatment groups on day 0. Tarkowski *et al.* (1984), Basker *et al.* (1986), Lee *et al.* (1996), Nam and Ahn (2002), Al-Bachir (2005), Salke (2007) did not observed significant change due to irradiation in different meat and meat products and the present study is also in agreement with the previous reports. As storage period enhanced the pH had significantly ($P<0.05$) reduced except in CL-NR and CL-IR group. Lee *et al*. (1996) observed significant change in pH in irradiated samples. Pexara *et al.* (2002) in smoked turkey fillets reported storage had an effect on reducing the pH. McCarthy *et al.* (2001) reported an increase in pH due to addition of an antioxidant, whereas, in the present study none of the non-irradiated sample containing CL, PL and both showed any difference in pH up to 5^{th} day of storage whereas, from 10^{th} day onwards the CL applied samples had significantly ($P<0.05$) higher pH both in NR and IR samples up to spoilage. Biswas *et al.* (2006) reported spice mix and curry leaves treated samples showed increase in pH from day 7 onwards in chicken patties. In present study all the other combinations were showing a downward trend of pH where as CL-NR and CL-IR group showed an upward trend of pH.

5.2.3. Thiobarbituric Acid Reactive Substances (TBARS)

On the day of preparation the control sample value of 0.19±0.01 was significantly (P<0.05) lowered by the addition of CL, PL and CLPL in non-irradiated samples. The TBARS values were non-significantly affected due to irradiation on day of preparation in various treatment groups. Du *et al.* (2001a) reported a non significant change on the day of preparation in different meat products. The results of present study are in agreement with their findings. As storage period enhanced, in all treatment groups had significant (P<0.05) effect in enhancing TBARS value and findings are in agreement with Kanatt *et al.* (1997), Murano *et al.* (1998), Nam and Ahn (2002) and Zhu *et al.* (2004).

Addition of CL, PL and CLPL alone had significant effect in reducing the TBARS compared to control samples both the NR and IR groups. Kanatt *et al.* (2005) reported the difference was not significant due to irradiation in chicken chilly since it contains spice used in preparation. In present study also addition of these two combinations reduced TBARS and maintained significantly (P<0.05) lower value compared to control sample which did not have any of these paste. This might be the reason to have significantly (P<0.05) higher value of 0.51±0.01 in C-IR group on day 60. On the same day it was only 0.29±0.01 in PL-IR group. There are reports which showed addition of synthetic or natural antioxidant had significantly (P<0.05) reduced the TBARS values under aerobic packing condition (Lee *et al.* 2003, Nam *et al.* 2006, Shijin 2008). Biswas *et al.* (2006), Rao *et al.* (2007) and Ningappa *et al.* (2008) (Curry leaves), Marinova and Yanishlieva (1997), Murcia *et al*. (2004) and Kanatt *et al*. (2007) (Peppermint leaves) were shown antioxidant property in reducing TBARS value in different meat and meat products.

From above results it can be inferred than even though irradiation increases the TBARS value non significantly, it can be significantly (P<0.05) reduced by addition of CL, PL and CLPL and keeps its value at lower level in the entire storage period. Among these three combinations PL was better than CL and CLPL.

5.2.4. Tyrosine Value (TV)

The tyrosine value of a meat product indicates the breakdown of protein subjected to storage or any other treatment. Similar to TBARS value the addition of CL, PL and CLPL had significantly ($P<0.05$) reduced the tyrosine value on day of preparation. Irradiation at 2.5 kGy also significantly ($P<0.05$) reduced the tyrosine value compared to the control samples. Combined effect of irradiation and leaf paste application was noticed on the day of preparation. Lawrie (1998) reported irradiation for retaining quality and retarding proteolysis in meat. Here, in the present study irradiated samples had lower tyrosine value than non-irradiated samples, similar observation was noticed by Shijin (2008). Kuttinarayanan *et al*. (2005) and Jenifer (2006) did not observe significant changes due to irradiation whereas, Balamatsia *et al*. (2006) reported higher total volatile basic nitrogen in non-irradiated samples. As storage period enhanced the tyrosine value was significantly ($P<0.05$) increased in all the samples. The increase was in comparison with the initial tyrosine value. The effect of storage on tyrosine value was reported by Kuttinarayanan *et al.* (2005) as a normal biochemical change which is expected in refrigerated meat and meat products. There are reports about the beneficial effect of CL and PL in meat whereas, such reports on inhibitory effect on proteolysis are scanty. In present study control sample had always higher tyrosine value and this was true up to 60^{th} day of storage under chiller condition. Among the three combinations PL had significant ($P<0.05$) effect in reducing the tyrosine value. Even on the 60^{th} day of storage, the C-IR samples had significantly ($P<0.05$) higher value of 7.59±0.05 compared to other treatment groups *viz*., 7.32±0.05, 6.96±0.05 and 7.15±0.02 in CL-IR, PL-IR and CLPL-IR respectively. The content of tyrosine can be one of the criteria to say whether the sample is spoiled or not as evidenced by its higher value in the spoiled sample or on the verge of spoilage.

5.3. MICROBOLOGICAL ANALYSIS

5.3.1. Aerobic Plate Count (APC)

In India, irradiation of meat and meat products is aimed to destroy the microorganisms and to extend the shelf life as envisaged in PFA. Chicken tikka prepared by adding various natural antioxidants had shown their effect on the microbial population combined with effect of irradiation. Irradiation alone significantly reduced the microbial load, similar reports were already reported by Basker *et al.* (1986) in chicken, Thayer (1993) in various meat and meat products, Mcateer *et al.* (1995) in meat, Murano *et al.* (1998) in ground beef patties. Kanatt *et al.* (2005) reported 3 kGy irradiation significantly reduced the microbial load and extended the shelf life of ethnic meat products which contains substantial quantity of spices and condiments. In the present study also, the batter as well as marinade contains spices and condiments and had shown their individual effects over and above the irradiation in reducing or preventing the multiplication of aerobic organisms. As evident in the results, the storage period had significant effect on increasing the number of organisms but the count was significantly ($P<0.05$) lower than that of control samples (C-NR) in all treatment groups. It was also evident by day 25 onwards C-NR samples had shown the signs of spoilage whereas, CL-NR, PL-NR and CLPL-NR had shown the signs of spoilage only after day 30 of storage.

Iscan *et al.* (2002) reported the antimicrobial activity of oils extracted from peppermint and this might be the reason having the lowest microbial load in PL-NR as well as PL-IR samples from day 0 till spoilage compared to all other treatment groups. Even keeping the samples up to 60^{th} day, none of the IR samples had a count higher than 4.16 ± 0.04 $\log_{10}$ cfu/g. Compared to many other reports the reduction in APC was non comparable since the tikka prepared was having moisture per cent around 50 only. It is quite clear that the effect of irradiation will be higher in food items containing higher per cent of moisture.

5.3.2. Psychrotrophic Count

The ready-to-eat chicken tikka prepared contained 2.53±0.08 log_{10} cfu/g count initially. Samples containing PL only had a count significantly ($P<0.05$) lower than that of the control samples. Even though numerical reduction was noticed in CL and CLPL groups, irradiation had significantly ($P<0.05$) reduced the psychrotrophic count compared to C-NR in all other treatment groups. Among the treatment groups also irradiation had significant effect and that was continued up to 60th day and thereby extended the shelf life of the product. Irradiation combined with PL application had synergistic effect in reducing the count. Effect of irradiation in reducing psychrotrophic count was already reported by Neimand *et al.* (1983) in minced beef, Mattison *et al.* (1986) in packed pork loins who tried different doses to obtain significant reduction and to extend the shelf life. In the present study 2.5 kGy alone or in combination with CL and PL had also significantly ($P<0.05$) reduced the psychrotrophic count. Gomes *et al.* (2003) reported psychrotrophic count exceeded the recommended limit after 6 days in non-irradiated and after 12 days of storage in irradiated chicken meat which was considerably higher than the present study count even up to 60th day of storage. Compared to some of the earlier reports in which 100 per cent reduction in irradiation occurred, was not obtained might be due to the lower water per cent in the chicken tikka.

5.3.3. Yeast and Mould Count

The yeast and mould count of chicken tikka had followed the same trend that of APC and psychrotrophic counts. Initially the samples had count of 1.54±0.07 log_{10} cfu/g. This was numerically reduced by application of CL and CLPL and significantly ($P<0.05$) reduced by PL treatment. CL and PL application followed by irradiation and irradiation alone significantly ($P<0.05$) reduced the yeast and mould count of the chicken tikka.

It was already reported that the effect of irradiation on reducing the yeast and mould count at different dose levels in different meat foods (Monk *et al.* 1995, Balamatsia *et al.* 2006 and Abu-Tarboush *et al.* 1997). Kuttinarayanan *et*

al. (2006b) and Kuttinarayanan (2007) reported 95-98 percent reduction of yeast and mould count in various meat and meat products. In present study the significant ($P<0.05$) reduction of the count was observed but not to that extend. This might be due to the per cent of moisture present in the product. Storage had significant ($P<0.05$) effect in increasing the count of the chicken tikka. The non-irradiated samples spoiled by less than 30 days whereas, leaf paste applied samples had an extended period up to 45th day and the counts were non-significant. The irradiated samples had an extended storage period beyond 60 days having the maximum count in C-IR group compared to CL-IR, PL-IR and CLPL-IR.

Storage had significant effect in increasing the yeast and mould count under chiller condition. Irradiation alone or in combination with leaf paste had extended storage life within the limit of microbial load. Among all the treatment groups PL applied irradiated chicken tikka had the lowest microbial load initially as well as at the verge of spoilage. This was followed by CLPL-IR group, CL-IR group and C-IR group indicating that all treatments had beneficial effect in reducing the yeast and mould count as well as extending the storage of ready to eat chicken tikka.

5.4. ORGANOLEPTIC EVALUATION

5.4.1. Colour

The sensory evaluation of the chicken tikka was conducted with the help of nine point Hedonic scale. The purchaser always goes for a product by its appearance and colour and any changes in these attributes will adversely affect in its marketing channel. The control samples on the day of preparation recorded a very good score of 8.33. This was significantly ($P<0.05$) improved by the process of irradiation, CL and PL application and followed by irradiation. Whereas, the score was numerically reduced by application of mixture of CL and PL at 1 per cent level. Such a reduction was counterchecked by the process of irradiation attaining its original value (8.33 and 8.34). The colour score remained non significant up to 5th day of storage and from there onwards the colour score had

significantly (P<0.05) reduced. Lefebvre *et al.* (1994), Fu *et al.* (1995), Murano *et al.* (1998), Zhu *et al.* (2003), Smith and Pillai (2004) and Shijin (2008) reported no change or higher score due to irradiation in various meat products. Chen *et al.* (1999) reported inconsistent or minor change to irradiation along with addition of antioxidants. In the present study irradiation and application of CL or PL had improved the colour score. Similarly, Darmadji and Izumimoto (1994) reported effect of antioxidants like chitosan in improving the sensory attributes which are in agreement with results of present study. As storage period enhanced there was significant (P<0.05) reduction in colour score from 5th day onwards. Still irradiated samples beyond 60 days maintained a score of above 7 in case of CL-IR, PL-IR samples, but a significantly (P<0.05) lower value was obtained in CLPL-IR samples.

5.4.2. Flavour

The combined perception received by the sense of taste and smell is recorded as flavour of a product. In case of flavour of the score was increased by all type of treatments. Irradiation alone improved the flavour score and that was retained till its spoilage even though storage had significant (P<0.05) effect on the same. Neimand *et al.* (1981), Ahn and Lee (2005) were in agreement with results of present study. There are reports that irradiation had imparted less desirable flavour (Zhao *et al.* 1996 and Brewer *et al.* 2009). Application of CL and PL also had beneficial effect in improving the flavour. Murcia *et al*. (2004) reported the effect of mint and cinnamon in controlling oxidation of lipids in meat and meat products. In the present study mixing of either CL or CLPL had some beneficial effect whereas, PL-IR samples had better score than that of C-NR and C-IR samples. Up to 5th day of storage, there was no significant effect on flavour score except in case of CL-NR, CLPL-IR samples. From 5th day onwards, there was significant (P<0.05) reduction in flavour score. Nam and Ahn (2002) conducted aerobic packaging for turkey meat for short term storage and observed that aerobic packaging had reduced off odour production. In the present study aerobic packaging with respect to chicken tikka retained the safe and acceptable flavour

score throughout the study period. Reduction in flavour score might be due to various biochemical changes that might be taken place under chiller storage. Similar results were obtained by Shijin (2008) in chicken fry where significant ($P<0.05$) difference in flavour score was obtained on storage.

5.4.3. Juiciness

The control samples on the day of preparation had a very good score of 8.46. Application of different treatments like CL and CLPL had numerically reduced the score, while PL application had significantly ($P<0.05$) improved the score. Irradiated samples had better score than non-irradiated samples in all groups with the highest in PL-IR and C-IR groups. Score of juiciness was increased significantly ($P<0.05$) by irradiation and Murano *et al.* (1998), Johnson *et al.* (2004) and Shijin (2008) reported higher acceptance and improved juiciness in irradiated samples. According to Luchsinger *et al.* (1996), Abu-Tarboush *et al.* (1997) and Ohene-Adjei *et al.* (2004) reported little effect of irradiation on juiciness in different products. In the present study all treatment groups obtained a significant effect due to irradiation. As storage period enhanced the score was significantly ($P<0.05$) reduced by 5^{th} day of storage in C-NR, CL-NR and CLPL-NR groups only.

From 10^{th} day to 60^{th} day, the score showed a downward trend in all the days of assessment, the PL-IR group recorded a significant ($P<0.05$) higher score with the least in C-IR group followed by CLPL-IR group. Thus it can be inferred that juiciness can be maintained to the satisfaction of the customer by application of PL alone and irradiate the samples so that product can be stored for maximum days without affecting the juiciness score of the chicken tikka.

5.4.4. Tenderness

Ready-to-eat chicken tikka on the day of preparation had very good score of 8.24 and was improved significantly ($P<0.05$) by addition of CL (8.50), PL (8.43) and CLPL (8.41). All these rating were significantly ($P<0.05$) improved by the process of irradiation indicated irradiation had significant effect in improving

tenderness of product or reducing toughness. Hashim *et al.* (1995), Murano *et al.* (1998) and Arthur *et al.* (2005) reported increased tenderness due to irradiation and the present study results are in agreement with them. Coleby *et al.* (1961) reported shrinkage of collagen as the cause of immediate softness and tenderness in meat foods. This may be the reason for a significantly ($P<0.05$) higher tenderness score obtained for this product. Whereas, Ohene-Adjei *et al.* (2004) reported a decrease in tenderness due to irradiation. Application of different pastes had significant role in increasing tenderness. It was also observed during storage period that leaf paste applied samples had better score than control. From 5^{th} day onwards tenderness score was significantly ($P<0.05$) reduced due to storage.

The reduction in juiciness score may be the reason for reduction in tenderness score since both of these attributes are interrelated. Compared to the different treatment groups the PL-IR samples had significantly ($P<0.05$) higher value of tenderness than other treatment groups. Even beyond 60 days of storage the lowest score of 7.00 was noticed in C-IR group. All other treatment groups retained significantly ($P<0.05$) higher score. This indicates irradiation of chicken tikka will extend the shelf life with very good tenderness score beyond 60 days under chiller storage. Application of PL in marinade was highly beneficial.

5.4.5. Overall Acceptability

The overall acceptability is the product of the individual sensory qualities. The control samples on the day of preparation had very good score of 8.34. This was significantly ($P<0.05$) improved by various treatments like application of CL, PL and CLPL. Irradiation alone improved the score since, many of the scores like colour, flavour, juiciness and tenderness were improved significantly ($P<0.05$), the overall acceptability of the product also improved by different treatments. Naik *et al*. (1994), Johnson *et al*. (2004) and Kanatt *et al*. (2005) reported similar trend in irradiated product. Kuttinarayanan (2005) reported that many of the buyers did not observe any particular smell or taste difference to the products due to irradiation. In the present study also, the irradiation had significant ($P<0.05$)

effect under chiller storage the samples maintained very good score even though reduction was noticed.

The Kruskal Wallis rank score analysis showed from day 5th onwards the values were significant from each other at 5 per cent level indicating storage had significant ($P<0.05$) effect on reducing the score.

Storage had significant ($P<0.05$) effect as noticed in all organoleptic qualities. Many of the quality changes either due to irradiation or due to storage can be taken care of by addition of preferably PL or CL or CLPL. From the results, it was observed that PL-IR sample retained better score with respect to all organoleptic qualities. In terms of reduction in TBARS value, TV, and microbial count (aerobic plate count, psychrotrophic count and yeast and mould count), it was also observed that PL applied IR samples had maintained its supremacy over other treatments. The synergistic effect of CL and PL was not promising hence, it is better to apply either one of these, preferably PL.

5.5. COST OF PRODUCTION

The cost of production of any ready-to-serve or ready-to-cook food items in Kerala are highly varying since many of the raw materials are coming from outside the state. The cost of ready-to-eat chicken tikka prepared in this study had a cost of Rs.122.30 in case of control samples. In case of CL applied samples it was increased by Rs.1.00, PL application by Rs.3.00 and by combined mixture of CLPL by Rs.4.00. The difference in cost of treatment samples was due to the cost difference of curry leaves and peppermint leaves. The cost of control samples was comparatively higher than that of the previous reports since the cost of raw materials in the earlier studies were considerably low. The chicken cost itself was about Rs.90.30 for the production of one kg ready-to-eat chicken tikka.

From the above results it can be inferred that ready-to-eat chicken tikka can be prepared and preserved by addition of CL or PL or CLPL. Mere application of these pastes had resulted in extended shelf life up to 8 days in case of non-irradiated samples. Application of treatment combined with irradiation of

product had resulted in extended shelf life of 68-70 days under chiller storage. Any product having shelf life of 70 days under chiller condition can be marketed to many of the areas since the product will be sold within this period. The cost of refrigeration can considerably reduced by the process of chilling alone. The product is stored under chiller condition, it is a highly convenient ready-to-use for any occasion in the nuclear families. The process of irradiation destroys many of the spoilage bacteria and fungi including the pathogenic organisms and hence the product is safe and can be popularised. The undesired effects especially in increasing the TBARS values can be controlled by addition of CL or PL or CLPL, preferably PL alone.

SUMMARY

6. SUMMARY

Shelf stable ready-to-eat meat products prepared and preserved by canning or retort pouch and are not affordable to many middle class families even though they are very fond of these products. Low moisture meat products can be stored for shorter duration at chiller without much change in their qualities. Irradiation of meat and meat products including chicken is permitted in India and studies have shown that irradiation enhances the keeping quality. Even though irradiation destroys many pathogens and spoilage causing organisms, it may accelerate lipid oxidation in processed meat and meat products. Many of these changes can be controlled by addition of synthetic antioxidants whereas, consumers always prefers natural antioxidants. Application of many of herbal antioxidants is tried in various food items, limited work has been carried out in meat and meat products.

In order to assess the effect of curry leaves (*Murraya sp.*) and peppermint (*Mentha sp.*) paste and gamma irradiation on the shelf life of chicken tikka this study was conducted at Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy. The most popular and convenient chicken preparation, the ready-to-eat chicken tikka was prepared using ingredients *viz*., chicken, spices, condiments, flour, salt etc. Chicken tikka was prepared containing zero per cent, 1.0% CL, 1.0% PL or both. After frying in oil and cooling the tikka was packed in HDPE (50µ) packets. Half of the packets in each treatment group were subjected to irradiation at 2.5 kGy using Gamma Chamber 5000 and stored at chiller temperature (1–4^{0}C). The irradiated and non-irradiated chicken tikka of various treatment groups under chiller storage were analysed for different quality parameters *viz*., physical, physicochemical, microbiological analysis and organoleptic evaluation on the day of preparation and on days 5, 10, 15, 20, 25, 30, 45 and 60 or until spoilage whichever was earlier. The samples were subjected to proximate analysis on the day of preparation.

The spoilage of the product was assessed on the basis of physical signs like changes in colour, odour, consistency, slime formation and mould growth. Irradiated samples had approximately two times shelf life than that of non-irradiated samples in all treatment groups under chiller storage. The C-IR and PL-IR samples had storage life of 61-63 days and 68-70 days respectively in chiller storage whereas, the C-NR samples had storage life of 27-30 days. It indicated that application of PL and irradiation had significant effect in extending the shelf life of the product under chiller storage.

The proximate composition like moisture, fat, protein, ash and carbohydrates of the samples were analysed on the day of preparation. Irradiation did not significantly affect any of the proximate composition. Moisture, fat and protein were significantly affected by application of CL, PL and their combination. It was noticed that PL-IR samples recorded higher percentage of fat, protein and carbohydrate as 16.77±0.22, 26.12±0.40 and 4.77±0.03 respectively. The highest energy content of 255.40±2.79 kcal/100g was recorded in PL-IR group. The pH of chicken tikka samples did not show any significant difference due to irradiation in different treatment groups on the day of preparation. As storage period enhanced pH had significantly ($P<0.05$) reduced in all treatment groups except in CL-NR and CL-IR groups pH had significantly increased.

The TBARS values were not significantly ($P<0.05$) affected due to irradiation on the day of preparation in various treatment groups. Addition of CL, PL and CLPL alone had significant ($P<0.05$) effect in reducing the TBARS compared to control samples in both NR and IR groups. As storage period increased, the TBARS values had significantly ($P<0.05$) increased indicating the oxidative rancidity changes under chiller conditions. Among these three combinations PL was better than CL and CLPL to control these types of biochemical changes in meat products.

TV indicating the proteolytic changes in meat showed a comparatively higher value of 5.90±0.07 in C-NR samples compared to the other treatment

groups with the lowest value of 4.57±0.04 in PL-IR samples. Storage had significant ($P<0.05$) effect in increasing tyrosine value. The initial aerobic plate count of 2.55±0.09 $\log_{10}$ cfu/g on the day of preparation was reduced by 30 per cent due to irradiation. The combined effect of application of PL and irradiation significantly ($p<0.05$) reduced the count and the least value of 1.29±0.10 $\log_{10}$ cfu/g was observed in PL-IR group compared to all other treatment groups on the day of preparation. On the day of preparation the highest psychrotrophic count of 2.53±0.08 $\log_{10}$ cfu/g was noticed in C-NR group. Irradiation had significantly ($P<0.05$) reduced the count. Irradiation in combination with PL application had the lowest count of 0.51±0.25 $\log_{10}$ cfu/g. Storage of product in chiller condition had significant ($P<0.05$) effect in enhancing the psychrotrophic count.

The yeast and mould count was showing similar trend to that of aerobic plate count and psychrotrophic organisms, had the highest value of 1.54±0.07 $\log_{10}$ cfu/g in C-NR samples on day of preparation which was reduced by about 60 per cent due to irradiation. Under chiller storage, the count had gradually increased and the increase was significant in each period of investigation.

The organoleptic qualities of the product were assessed with the help of nine point Hedonic scale. Irradiated samples had a higher score when compared to non-irradiated counterparts on the day of preparation. The maximum score of 8.74 out of 9 was recorded for PL-IR samples. Both CL and PL paste incorporation increased the colour score whereas CLPL had substantially reduced the score. It was observed that from day 5 onwards storage had significantly reduced the colour score. Even on the 60th day of storage, the PL-IR samples recorded a fairly good score of 7.46 indicating that sample was good. In case of flavour score irradiation slightly increased the score on the day of preparation. The maximum score of 8.74 was obtained in PL-IR samples. From 5th day onwards score of the product was significantly ($P<0.05$) reduced. Juiciness of the product was increased due to irradiation. The highest score of 8.61 was obtained in PL-IR group on the day of preparation. From 15th day onwards the score had significantly ($P<0.05$) reduced in all treatment groups. Similar to other

organoleptic qualities tenderness score of the product was maximum in PL-IR group on the day of preparation. It was also observed that from 5th day onwards storage had a significant ($P<0.05$) effect in reducing the tenderness. Even on 60th day irradiated samples had comparatively good score of 7.00 or above 7.00 indicating a good tenderness of the product, with the highest score of 7.37 in PL-IR group.

The overall acceptability was increased due to irradiation. Application of PL had significant effect in improving overall acceptability at 8.61 (PL-IR) on the day of preparation. From 5th day onwards storage had significantly ($P<0.05$) reduced the overall acceptability score. The Kruskal-Wallis (KW) rank score showed that among treatments during storage period, PL-IR samples recorded the highest score in entire study period and was significantly ($P<0.05$) higher than other values. The cost of production was Rs. 122.30, Rs 123.30, Rs 125.30 and Rs 126.30 per kg for the control, CL, PL and CLPL treatment groups respectively.

Ready-to-eat chicken tikka can be prepared and stored by chiller storage for shorter duration. The shelf life of the product can be extended by the process of irradiation which destroys the pathogenic and spoilage causing organisms in meat and meat products. The undesired changes brought about by irradiation can be controlled by application of various natural antioxidants present in herbal pastes. It was observed that, incorporation of peppermint paste at one per cent level in the marinade will reduce many of the changes especially that produced in lipids present in meat. It was also found that, incorporation of curry leaf paste at one per cent level and mixture of curry leaf and peppermint paste at one per cent level is having desired effect but, not to that of peppermint paste alone. Hence, it can be inferred that chicken tikka can be prepared with peppermint paste at one per cent level in the marinade and after preparation of the product packed in HDPE packets followed by irradiation at 2.5 kGy will extend the shelf life up to 68-70 days without affecting qualities.

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EFFECT OF CURRY LEAVES (Murray asp.) AND PEPPERMINT (Menth asp.) PASTE ON SHELF LIFE OF IRRADIATED CHICKEN TIKKA

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ABSTRACT

To study the beneficial effects of irradiation, application of curry leaves (*Murraya sp.*) and peppermint (*Mentha sp.*) paste in the marinade of chicken tikka, the present study was conducted at Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy. Chicken tikka was prepared incorporating zero per cent, 1.0% CL, 1.0% PL or both and it was fried separately. After cooling, tikka was packed in HDPE (50µ) packets. Half of the packets in each treatment group were subjected to irradiation at 2.5 kGy using Gamma Chamber 5000 and stored at chiller temperature (1–4^{0}C). The irradiated and non-irradiated chicken tikka of various treatment groups under chiller storage were analysed for different quality parameters *viz*., physical, physicochemical, microbiological analysis and organoleptic evaluation on the day of preparation and on days 5, 10, 15, 20, 25, 30, 45 and 60 or until spoilage whichever was earlier. The samples were subjected to proximate analysis on the day of preparation. Shelf life of chicken tikka was assessed based on the physical signs of spoilage.

The non-irradiated control samples had a shelf life of 27-30 days in chiller storage. Application of CL, PL and both had extended the shelf life of the product by 3-4 days, 7-8 days and nearly 5 days respectively. Irradiated samples had approximately two times shelf life than that of non-irradiated samples in all treatment groups under chiller storage. The C-IR and PL-IR samples had storage life of 61-63 days and 68-70 days respectively in chiller storage.

Irradiation did not significantly affect any of the proximate composition. Moisture, fat and protein were significantly affected by application of CL, PL and their combination. The highest energy content of 255.40±2.79 kcal/100g was recorded in PL-IR group. The pH of chicken tikka samples did not show any significant difference due to irradiation in different treatment groups on the day of preparation. As storage period enhanced pH had significantly ($P<0.05$) reduced in all treatment groups except in CL-NR and CL-IR groups where, pH had significantly ($P<0.05$) increased.

The TBARS values were non significantly increased due to irradiation on the day of preparation in various treatment groups. Addition of CL, PL and CLPL alone

in the marinade had a beneficial effect in reducing the TBARS compared to control samples in both NR and IR groups. TV showed decreasing trend due to irradiation as well as application of CL, PL and CLPL. Storage had significant ($P<0.05$) effect in increasing both these physicochemical properties.

Aerobic plate count, Psychrotrophic count and yeast and mould count were significantly ($P<0.05$) reduced due to irradiation and combination of irradiation with CL, PL and CLPL. Whereas, extend of reduction due to application of CL, CLPL alone was not up to the level of PL alone to non-irradiated groups. PL-IR samples had recorded the lowest counts among all treatment groups throughout the storage period. As storage period enhanced the counts were significantly ($P<0.05$) increased.

The organoleptic qualities were assessed with help of nine point Hedonic scale. The colour, juiciness, tenderness and overall acceptability of the product were improved by irradiation as well as addition of CL, PL and CLPL. A gradual decrease in organoleptic qualities was observed only after 5^{th} day of storage in most of the samples. Even on 60^{th} day of chiller storage, the samples had an overall acceptability score of above 7 indicating the samples are preferred by the consumers. The cost of production was Rs. 122.30, Rs. 123.30, Rs. 125.30 and Rs. 126.30 per kg for the control, CL, PL and CLPL treatment groups respectively.

Irradiation of ready-to-eat chicken tikka was beneficial for enhancing the keeping quality of the product under chilling condition without affecting qualities. Addition of herbal pastes containing natural antioxidants in the marinade for the preparation of chicken tikka was found to be beneficial in reducing many of undesirable effects. Among the herbal pastes *viz.,* peppermint paste, curry leaf paste and their combination, it was found that peppermint paste had better effect than the other two. The microbial counts were significantly ($P<0.05$) reduced due to irradiation at 2.5 kGy, the lowest limit prescribed by PFA.

Considering extended shelf life, wholesomeness of the product, reducing the microbial load and energy saving aspects, preparation of chicken tikka incorporating peppermint paste in the marinade and followed by irradiation can be advocated as a suitable method for preparation of ready-to-eat value added meat products.